

**CAPTURING THE NUTRIENT OVERENRICHMENT-EUTROPHICATION-  
HYPOXIA CYCLE IN NEWTOWN CREEK**

A Final Report of the Tibor T. Polgar Fellowship Program

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## ABSTRACT

Nutrient loading in estuarine waters leads to alterations to biogeochemical cycling, decreased oxygen levels and massive fish and shellfish kills. In highly-urbanized areas, nutrient loads can be delivered by rain events containing raw sewage and allochthonous bacteria including pathogens. Past transect sampling of Newtown Creek, an urbanized tributary of the Lower Hudson River Estuary, has revealed that this tributary experiences a nutrient overenrichment-eutrophication-hypoxia (NOEH) cycle, throughout the summer, displaying high variability in chlorophyll and oxygen levels in surface waters. In order to assess the drivers behind this extreme variability, a major summer rain event was captured during a week of daily sampling on the creek. This sampling revealed that the tributary fluctuates between physical and biological extremes on a daily and even hourly basis in terms of variability in chlorophyll *a* concentrations, bacterial abundance, oxygen saturation and nutrient concentrations. A comparison of environmental conditions at surface and depth in the waters of this creek also underscores the importance of surface water processes in this body of water, where surface waters alternate between hypoxia and oxygen supersaturation within days. The combination of the establishment of a strong thermo/halocline and the persistent oil slick observed on the creek's surface waters in summer may result in surface waters disconnected from the physical processes of mixing and diffusion at the sea-air interface. A comparison of the ratio of autotrophic:heterotrophic microbial concentrations in surface waters to dissolved oxygen saturation during the week of sampling reveals a significant ( $p=0.00164$ ) relationship.

## TABLE OF CONTENTS

Abstract.....	II-2
Table of Contents.....	II-3
List of Figures.....	II-4
Introduction.....	II-5
Methods.....	II-8
Results.....	II-10
Discussion.....	II-17
Conclusions.....	II-20
Acknowledgments.....	II-22
Literature Cited.....	II-23

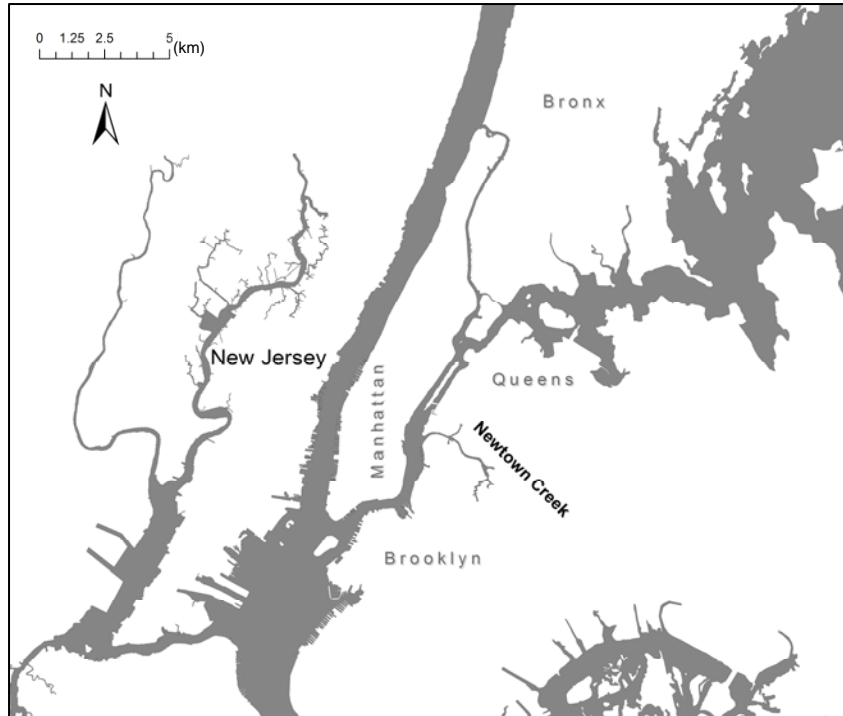
## LIST OF FIGURES

Figure 1 --The Lower Hudson River Estuary.....	II-6
Figure 2 -- Summer 2007 oxygen saturation of surface waters .....	II-6
Figure 3 – Proposed mechanisms driving oxygen levels in surface waters.....	II-7
Figure 4 – Map of Newtown Creek and sampling sites.....	II-8
Figure 5 -- Precipitation during the sampling period .....	II-11
Figure 6 -- Salinity and Temperature during sampling period .....	II-11
Figure 7 -- Transparency as determined by secchi disk .....	II-12
Figure 8 -- Bacterial abundances at each transect site .....	II-13
Figure 9 -- Dissolved oxygen saturation by site .....	II-13
Figure 10 -- Dissolved inorganic NH <sub>4</sub> , NO <sub>3</sub> , NO <sub>2</sub> , and PO <sub>4</sub> concentrations in surface and bottom waters at each site during sampling period.....	II-14
Figure 11 -- Surface Nitrogen:Phosphorus ratios.....	II-15
Figure 12 -- Chlorophyll <i>a</i> concentrations in transect surface waters .....	II-16
Figure 13 -- Chlorophyll:Bacteria ratio and oxygen saturation in surface waters...	II-17
Figure 14 – Microplankton relative abundances at NT2.....	II-18

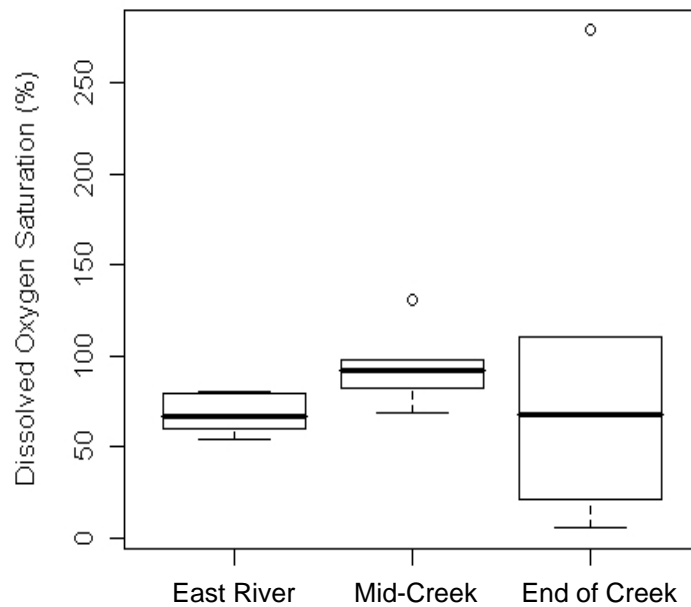
## INTRODUCTION

One of the major environmental factors influencing estuarine ecosystems in urbanized regions is the influx of nutrients from wastewater and other sources, which can result in dramatically increased levels of dissolved inorganic and organic material in the water column (Roman et al. 2000). Tributaries of the Hudson River Estuary have been implicated as major contributors to estuarine nutrient loading and sewage inputs in particular (Howarth et al. 2003; O'Mullan 2008). The overenrichment of estuarine nutrient cycles has a variety of effects, and perhaps the most acute from an ecological perspective is the decrease in oxygen levels due to eutrophication. Hypoxia (defined here as less than 50% oxygen saturation) in estuarine waters results from elevated microbial respiration during decomposition of algal blooms, and can result in fish and shell fish kills (National Research Council 2000).

Newtown Creek, a tributary that runs along the border between Brooklyn and Queens and empties into the East River (Fig. 1), sustains high levels of sewage input through both the largest Water Pollution Control Plant (WPCP) in New York City and Combined Sewer Outfalls (CSO's) located along its shores. Transect sampling in Summer 2007 revealed a system caught in a cycle of nutrient overenrichment-eutrophication-hypoxia (NOEH) and suffering wide fluctuations in surface oxygen levels (Fig. 2) (Dueker et al. 2008). Newtown Creek surface waters were routinely either oxygen supersaturated by massive phytoplankton blooms (saturation levels reaching as high as 270%) or oxygen-depleted by microbial processes (saturation levels reaching as low as 5%).



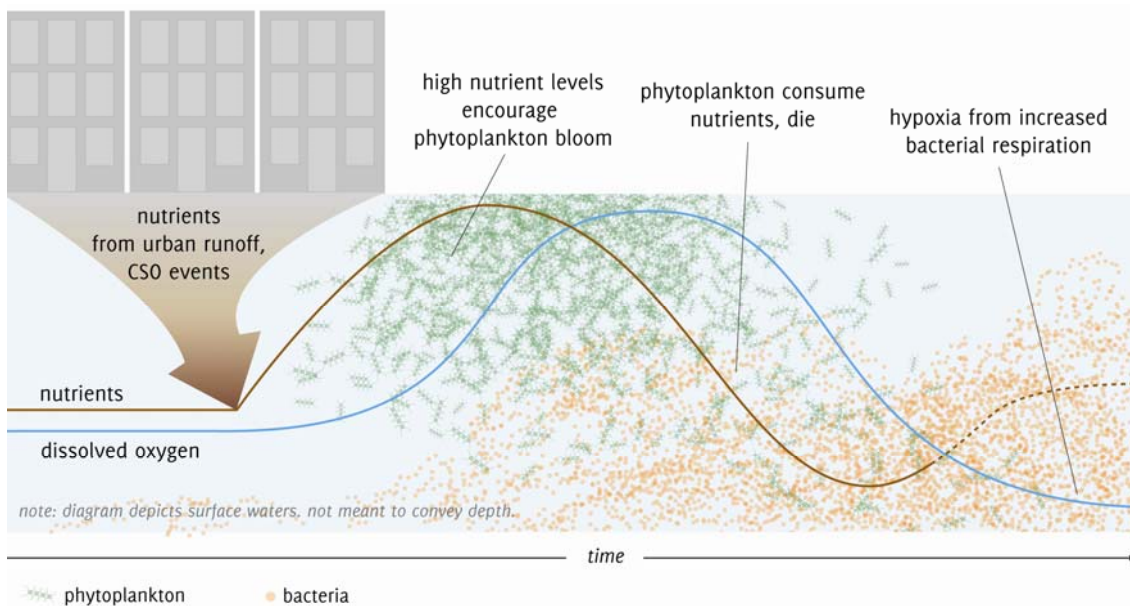
**Figure 1.** Location of Newtown Creek, running along the border between Brooklyn and Queens and emptying into the East River.



**Figure 2.** Boxplot of Summer 2007 surface dissolved oxygen saturation (%) along Newtown Creek transect.

The combination of summer heat, urban runoff, combined sewer overflows during rain events, and a persistent oil slick creates a unique microbial habitat in the surface waters of Newtown Creek. Surface waters are routinely out of equilibrium with the atmosphere in terms of oxygen content, with extreme high and low levels of oxygen sustained longer than expected through physical processes of diffusion (Dueker et al. 2008). This diffusion disconnect may indicate that the oil slick often covering these waters impedes diffusion and creates a gas exchange cap on surface waters.

This study tests the hypothesis that, much like biogeochemical cycling of nutrient pools, the oxygen levels in the surface waters of the creek are microbially-mediated (Fig. 3). As a heavily-nutrient enriched marine system, Newtown Creek microbial



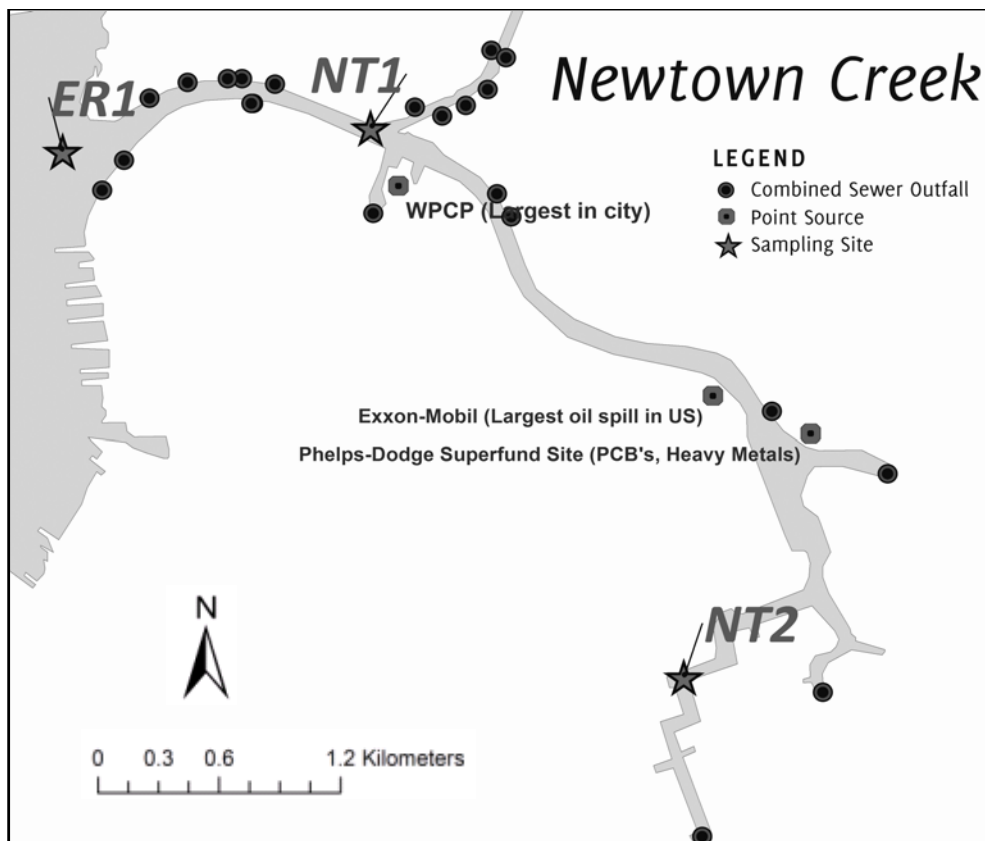
**Figure 3.** Diagram of proposed mechanisms creating surface water hypoxia in Newton Creek. (Diagram designed for this study by Jamie Stafford-Hill.)

communities are dominated by phytoplankton and bacteria, therefore, levels of oxygen in the water column should relate to the balance between autotrophy (creation of oxygen) and heterotrophy (respiration of oxygen) in surface waters.

Furthermore, biological control of oxygen levels in the surface waters of this tributary is predicted to result in additional environmental instability, with major implications for the maintenance of microbial community structure and diversity in this challenged tributary. To test these hypotheses, the NOEH cycle on the creek was monitored during a daily sampling regime conducted in the summer of 2008.

## METHODS

**Sample sites and timeframe.** The transect for this study spans Newtown Creek (Fig. 4), with sampling sites at its mouth (ER1), mid-point (NT1), and toward its farthest inland reach (NT2). Sampling was conducted at slack tide daily at each site from July



**Figure 4.** Newtown Creek with sampling sites, CSO locations and other pollution point sources identified.

20-23, 2008. An intensive daylight-hours observation of NT2 was conducted on July 24 to monitor the microbial activity in near-anoxic surface waters.

**Sample Collection.** Environmental transect samples were collected from shore using a Niskin bottle and a two-meter extension pole to reach into the Creek channel. At each site, samples were taken both at the surface and 0.2 meters above the Creek bottom. Oxygen levels were determined using a handheld Hach oxygen meter. Transparency was determined using a secchi disk. Samples for microbial community assessment were collected from the Niskin bottle and preserved. Ciliate, phytoplankton and dinoflagellate samples were preserved immediately with Acid Lugol's solution and stored in coolers for return to the lab. Samples were preserved for bacterial abundance counts using 1% formalin (final concentration). Samples for nutrient analyses (dissolved inorganic nitrogen (DIN), dissolved inorganic phosphorus (DIP)) were filtered through a 0.22  $\mu\text{m}$  Sterivex filter and frozen pending analysis. Nutrient analyses were performed at Horn Point Laboratory using colorimetric analysis protocols (Parsons et al. 1984). 300 ml of surface water from each site were filtered through 0.45  $\mu\text{m}$  pre-combusted glass fiber filters (GFF). These filters were immediately frozen for later analyses of chlorophyll *a* concentrations using an ethanol extraction and calibrated fluorometer.

**Ciliate, Phytoplankton and Dinoflagellate Enumeration.** Preserved samples (100 ml) treated with Acid Lugol's solution were counted within 3 months of collection. After initial settling, samples were transferred to 15 ml centrifuge tubes and gently centrifuged. The supernatant was removed to leave a pellet of 1 ml. 5-10 sub-samples (20-50  $\mu\text{l}$  drops) were then scanned and enumerated for ciliate, phytoplankton and dinoflagellate cells. All identifications were made to the lowest taxonomic level possible

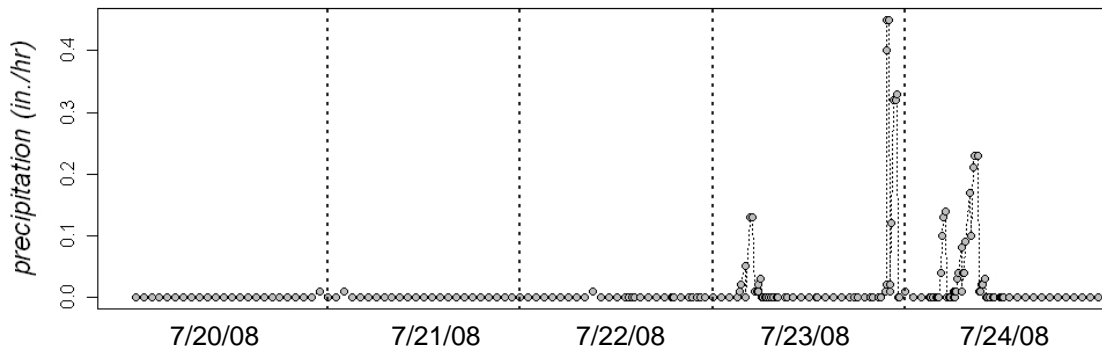
using descriptions in Lee et al. (2000) and Jahn et al. (1949). Size categories were recorded for organisms that could not be identified taxonomically.

**Bacterial Abundance.** Bacteria were counted using an epifluorescent enumeration method according to Patel et al. (2007). Briefly, within 2 months of collection, 250-750  $\mu\text{l}$  aliquots (volume depending on cell density) of formalin-preserved sample were filtered onto 0.22  $\mu\text{m}$  black polycarbonate filters (Nucleopore, Pleasanton, Calif.). 10  $\mu\text{l}$  of SYBR Green stain (causes bacterial DNA to fluoresce bright green) was applied to the filter for 15 minutes and then filtered through with a sterile phosphate-buffered saline wash. Filters were then mounted on a glass microscope slide, and a coverslip was applied over a 10 $\mu\text{l}$  drop of 50% glycerol, which acted as a cryoprotectant. The slides were immediately frozen until counting. At least 200 cells per filter were counted using epifluorescence microscopy.

**Statistical Analyses.** To test for significant relationships between physical parameters and microbial abundances I used linear models fitted using least squares regression in R statistical software (<http://www.r-project.org>).

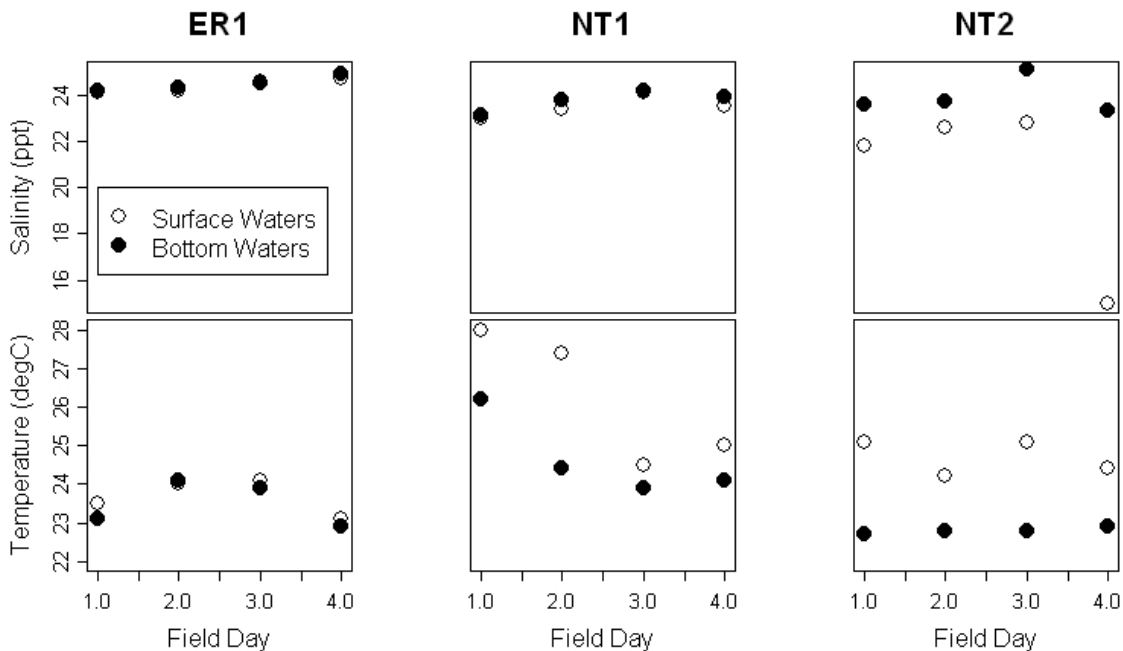
## RESULTS

**Precipitation, Temperature and Salinity.** The July sampling week began during the driest and hottest period of the NYC summer. A small rain event (0.2 in.) occurred early on the morning of July 23 to break this heat-wave, followed by an even larger rain event (over 1.5 inches) the evening of July 23, continuing into the morning of July 24 (Fig.5).



**Figure 5.** Precipitation during study (in./hr). Data from Central Park weather station.

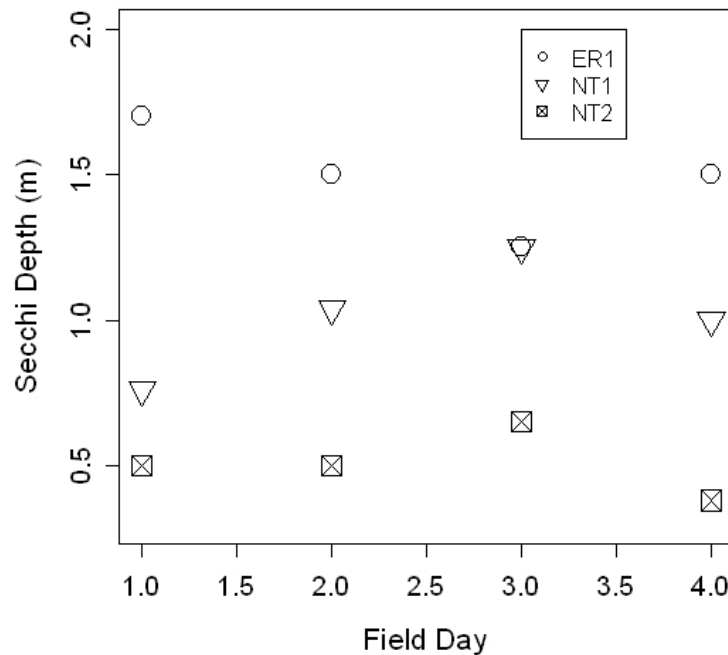
ER1 consistently had a well-mixed water column, with salinity and water temperature (Fig. 6) similar both at surface and depth, ranging from 23-24°C. NT1 had warmer (24-28°C) and fresher surface waters than bottom waters (24-26°C), indicating the establishment of a thermocline/halocline that could create a stable barrier between surface and bottom water processes (Fig. 6). NT2 was generally cooler than NT1



**Figure 6.** Salinity and temperature during sampling period, at depth (filled circles) and at surface (open circles). Field Day 1 = July 20, 2008.

(surface temperatures ranging from 24-25°C) and displayed the largest difference between surface and bottom water temperature and salinity (Fig. 6), indicating the establishment of a strong thermo/halocline in these waters over the time period studied, even through the rain event.

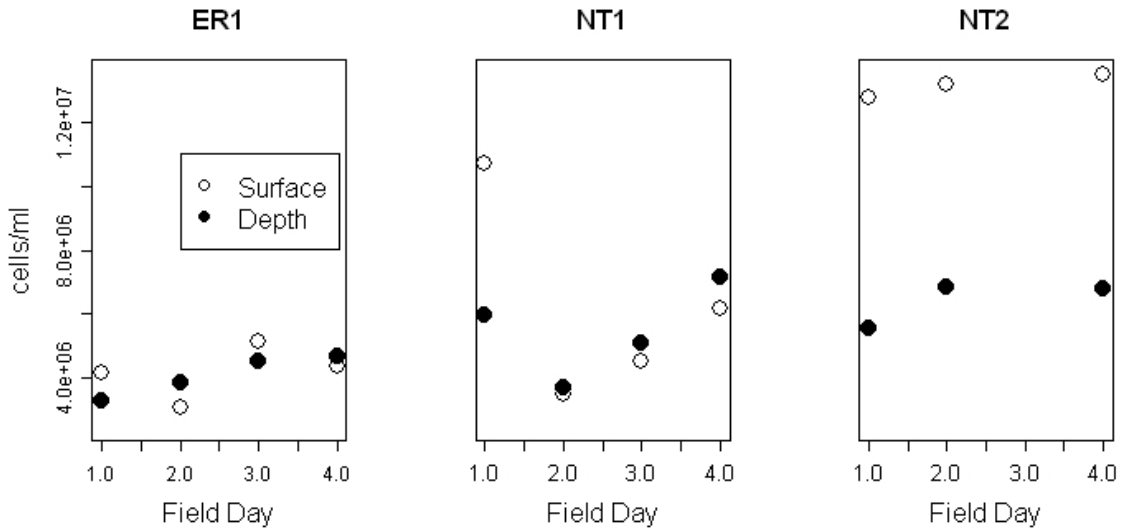
**Transparency.** The ER1 water column was consistently more transparent than creek waters (Fig. 7). NT2 had the lowest transparency throughout the week, decreasing along with NT1 during the rain event on day 4 (Fig. 7).



**Figure 7.** Water column transparency as determined by secchi disk measurements along the transect over the course of the study period.

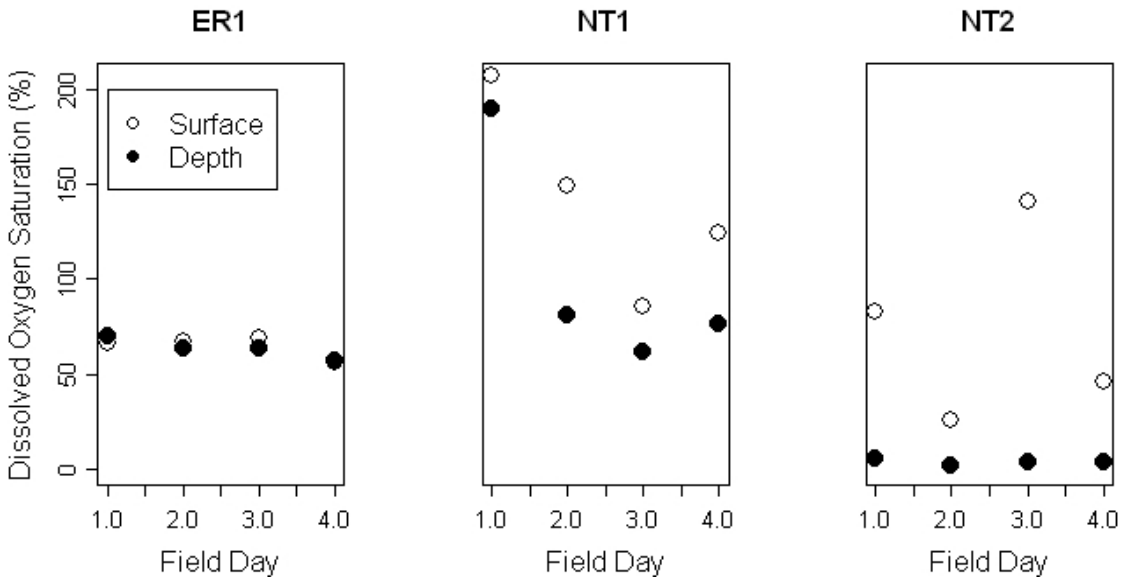
**Bacterial Abundance.** Bacterial abundances were highest at NT2 and lowest at ER1, both in surface and bottom waters (Fig. 8). Whereas the bottom and surface bacterial abundances were similar each day for ER1 and NT1, NT2 surface waters had almost an order of magnitude greater bacterial concentration than its bottom waters.

Bacterial abundance along the transect was significantly correlated with secchi depth ( $R^2=0.821$ ,  $p<0.0001$ ), but not with chlorophyll ( $R^2=-0.03204$ ,  $p=0.5778$ ).



**Figure 8.** Bacterial abundances along transect during study.

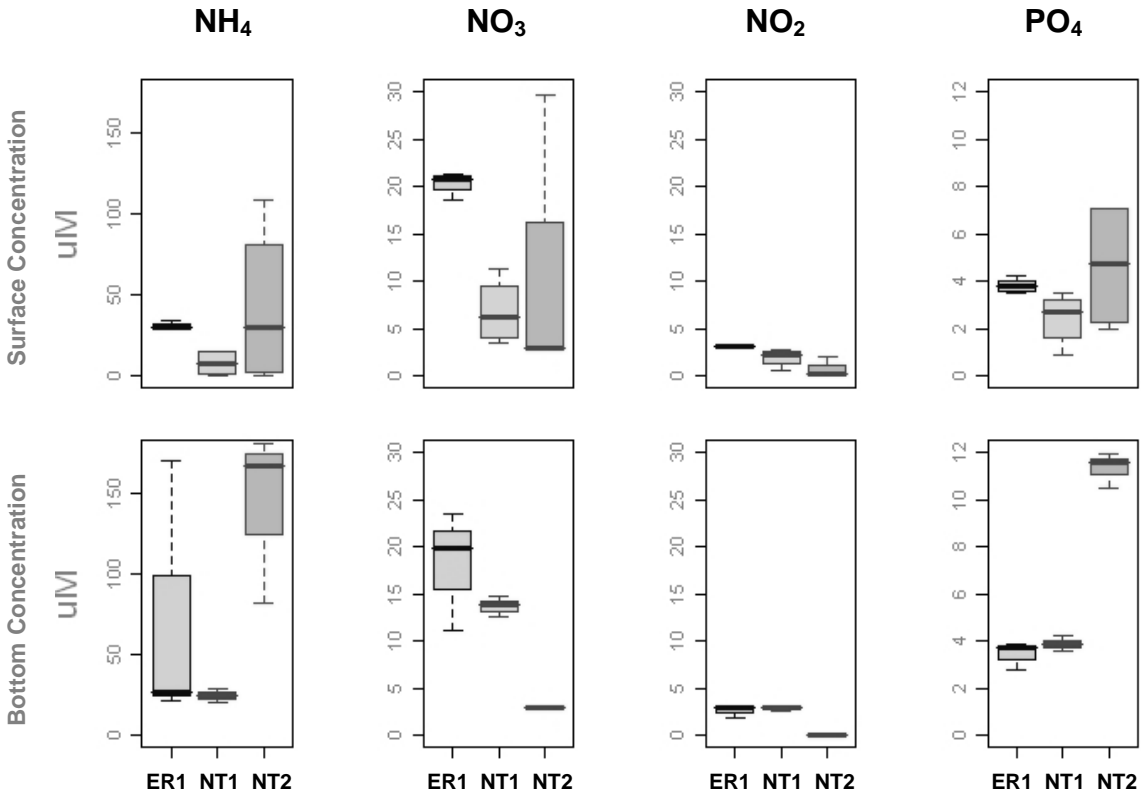
**Oxygen levels.** Oxygen levels at ER1 hovered at about 55% both at the surface and at depth throughout the week, with a slight decrease after the major rain event (Fig. 9). The mid-creek station (NT1) initially showed oxygen supersaturation at the surface



**Figure 9.** Dissolved oxygen saturation along transect during study.

and bottom waters that declined steadily until the July 23 (Field Day 4) rain event (Fig. 9). The inland station (NT2) had fluctuating surface oxygen levels during the week, oscillating daily from well-oxygenated to hypoxic conditions. The largest shifts occurred on the days with rain (3 and 4). Notably, the bottom waters of NT2 were consistently anoxic and, unlike the other stations, did not correspond with surface water conditions.

**Nutrient concentrations.** Nutrient concentrations in ER1 surface waters were relatively stable, in contrast to bottom water concentrations of  $\text{NH}_4$  and  $\text{NO}_3$ , which had high variability (Fig. 10). In general, ER1 surface waters maintained elevated  $\text{NO}_3$

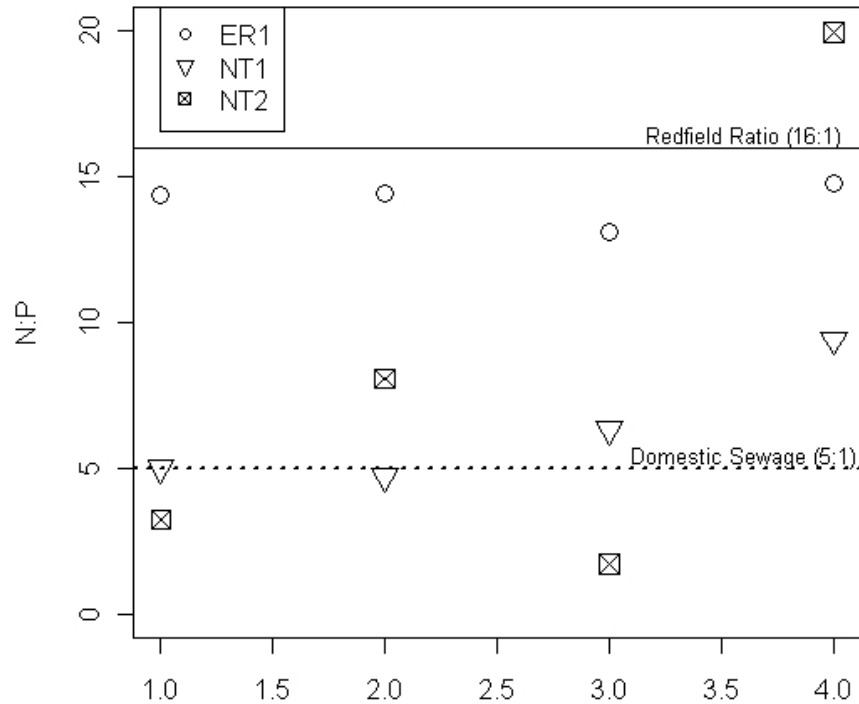


**Figure 10.** Boxplots of nutrient concentrations during study period by site. The top row shows surface water concentrations, the bottom row shows concentrations at depth.

concentrations (at about  $20 \mu\text{M}$ ) when compared to the inner creek sites. Nutrient concentrations in surface waters were more variable at inner creek sites, with NT2

displaying highest variability, particularly in the  $\text{NH}_4$ ,  $\text{NO}_3$ , and  $\text{PO}_4$  pools.  $\text{NO}_2$  levels were low both in surface waters and at depth at all sites. NT2 had significantly elevated  $\text{NH}_4$  and  $\text{PO}_4$  concentrations at depth. At NT2, bottom waters were notably depleted in  $\text{NO}_3$  and  $\text{NO}_2$ .

All sites maintained surface water N:P ratios below the 16:1 Redfield ratio (solid line, Fig. 11) except after the major rain event on the evening of the third field day. ER1 maintained an N:P ratio just below 15:1 in its surface waters throughout the week (Fig. 11). The inner creek sites maintained low N:P levels, closer to the average N:P of

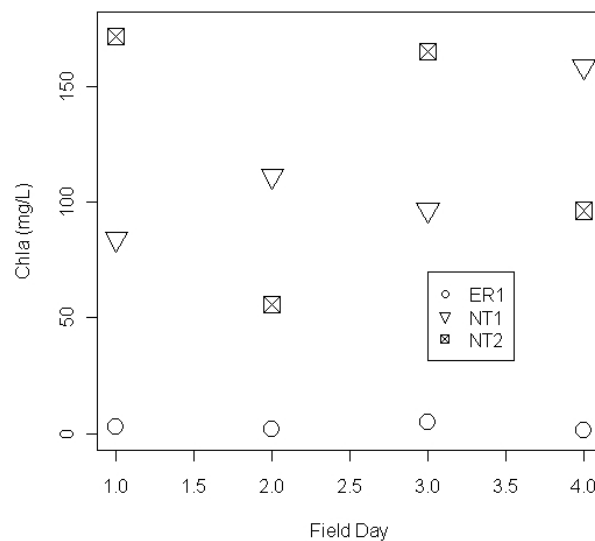


**Figure 11.** Nitrogen:Phosphorus ratios of surface waters along transect during study. Solid line denotes Redfield Ratio of 16:1, dotted line denotes domestic sewage ratio of 5:1).

domestic sewage, which is about 5 (Dunstan and Menzel 1971). NT1's N:P was 5 at week's start, but increased after the rain event. NT2 fluctuated wildly, with the biggest

variation occurring the day of the first small rain event, when it jumped from 1.75 to 20 (Fig. 11).

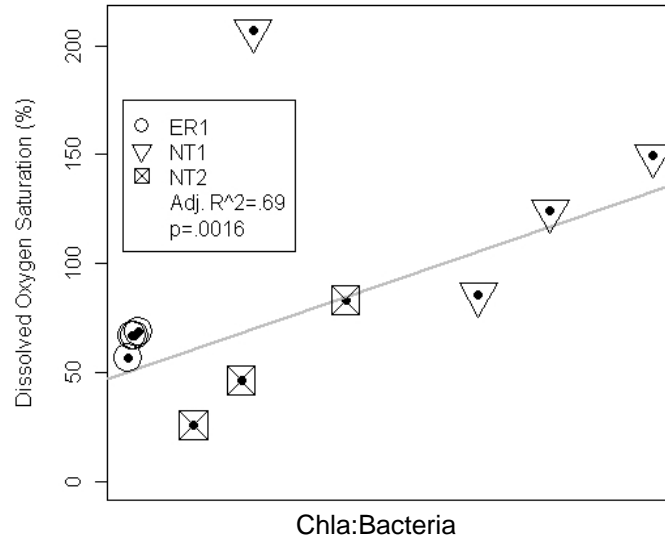
**Chlorophyll *a*.** ER1 had consistently lower Chl*a* concentrations than the mid and end-creek sites (Fig. 12). NT1 chlorophyll concentrations increased over the week, but levels fluctuated widely by day at NT2. NT2 chlorophyll concentrations peaked a day after NT1 concentrations peaked, suggesting possible advection of blooming phytoplankton from NT1 to NT2 surface waters.



**Figure 12.** Chl*a* concentrations in transect surface waters during study period.

Comparing the chlorophyll *a* concentrations:bacterial abundance ratio to the oxygen saturation in these surface waters revealed a significant relationship across the transect (Fig. 13). This excludes an outlier from NT1 on July 20.

**Surface water microplankton communities.** The microplankton communities (cells in the size range of 10  $\mu\text{m}$ -100  $\mu\text{m}$ ) in surface waters at NT2 varied greatly both in number and composition. In the five days NT2 was observed, total microplankton fluctuated greatly (Fig. 14). The phytoplankton communities were dominated by

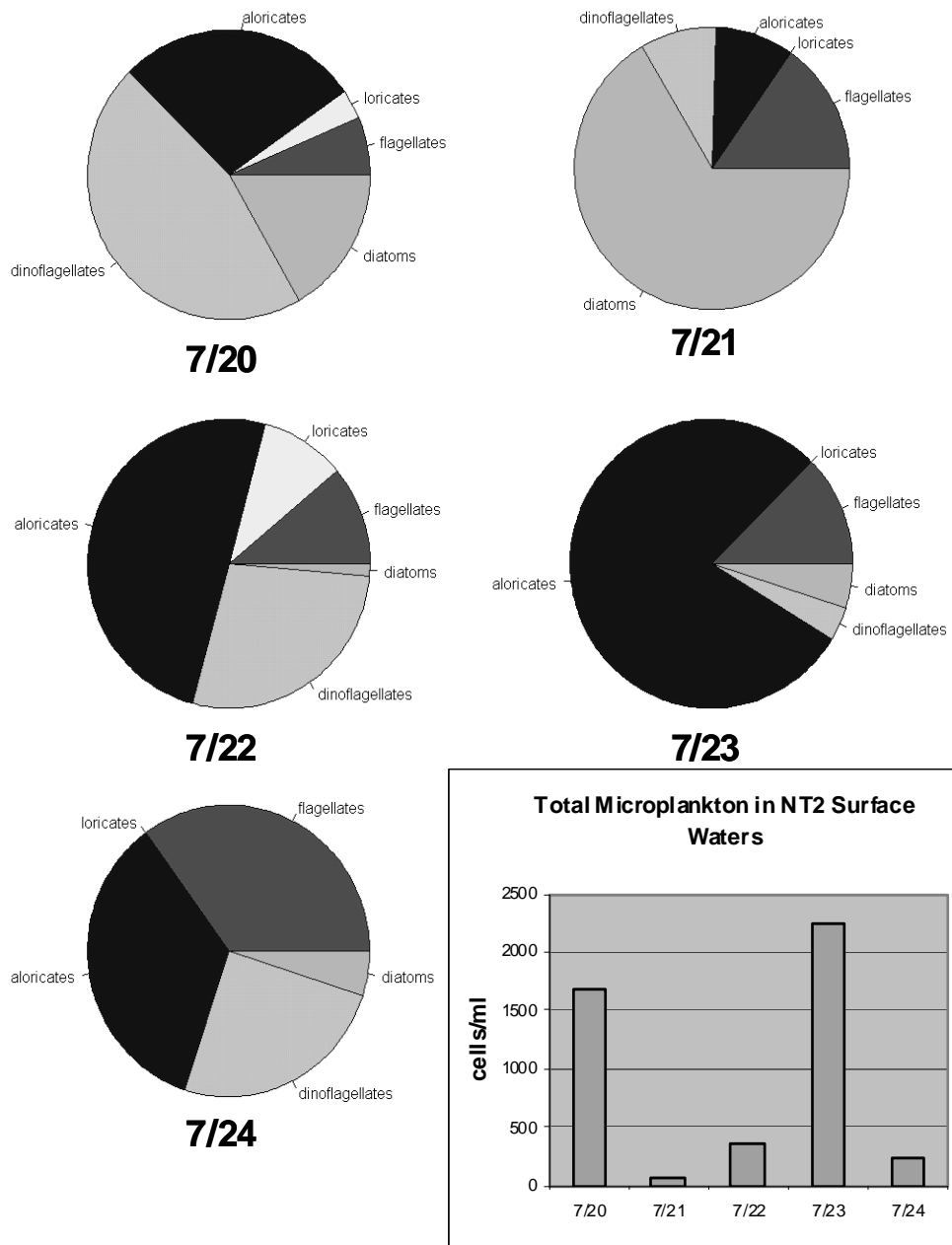


**Figure 13.** Dissolved Oxygen Saturation in transect surface waters regressed against the ratio of Chla concentrations: Bacterial concentrations. Grey line denotes linear model fitted using least squares regression.

euglenoids, dinoflagellates and centric diatoms. Small (30 - 40  $\mu\text{m}$ ) aloricate ciliates dominated the heterotrophic microplankton communities (Fig 14). Loricated ciliates were present only on days when phytoplankton were abundant in the microplankton communities (July 20 and July 23). The aloricate ciliate bloom community on July 23 was dominated by 30  $\mu\text{m}$  *Mesodinium rubrum*, a mixotroph which “enslaves” chloroplasts to make a living in eutrophic waters.

## DISCUSSION

Newtown Creek experienced considerable shifts in both physical and biological parameters during the week of sampling, presenting a complicated case study of the NOEH cycle. Low transparency in marine systems can often be attributed to enhanced mixing of particulate organic materials into the water column from depth, but the strong thermocline and halocline (which would reduce turbulent mixing) clearly developed at



**Figure 14.** Microplankton community structure and abundances in the surface waters of NT2 during the sampling period.

NT2 suggests that the addition of particulate matter to the water column is allochthonous (National Research Council 2000). While sorting out environmental drivers to microbial community change in this scenario is further complicated by unknown sewage-release practices and uncharacterized point sources, a few possible suspects do arise.

Nutrient levels in the creek were consistently below the Redfield ratio, closer to the average levels observed in domestic raw sewage (Fig. 11) (Dunstan and Menzel 1971). The consistently low N:P ratios measured underscore the immense human influence on this tidal creek through inputs of high-P sewage effluent. Studies have demonstrated a connection between low N:P ratios and phytoflagellate dominance in marine eutrophication scenarios (Hodgkiss and Ho 1997; Hodgkiss and Lu 2004). Dinoflagellates and euglenoid flagellates do play a major part in bloom communities in the creek (Fig. 14). When oxygen levels were low at NT2 (Fig. 9), bacterial abundances remained elevated (Fig. 8), but the grazer populations were fluctuating wildly (Fig. 14). This suggests a possible “disconnect” of functional relationships between grazer microplankton and bacterial populations during the NOEH cycle, resulting in the complete removal of top-down control of bacterial populations (Kirchman 2000).

High nutrient inputs translate to the potential for increased microbial activity, as demonstrated both by the high chlorophyll *a* concentrations and bacterial concentrations measured at NT1 and NT2 during the sampling period (Figs. 8, 12). NT1 maintained high phytoplankton activity throughout, as evidenced by oxygen supersaturation in the entire water column (Fig. 9). NT2, however, fluctuated wildly between high chlorophyll-high oxygen levels and low chlorophyll-low oxygen levels associated with the NOEH cycle resulting in hypoxia. Bacterial levels were consistently elevated at NT2 (almost an order of magnitude higher than ER1 counts) (Fig. 8), suggesting that variability in phytoplankton abundance, balanced against a consistently high bacterial oxygen demand, may regulate the large variability in oxygen levels observed there.

Increased microbial activity in surface waters, and in particular, blooms of certain species, creates a context where nutrient pools can shift quickly. The microbial mediation of these nutrient pools requires oxygen, as most processes in surface waters are assumed to be aerobic (Stumm 1996). Because Newtown Creek surface waters appear to be inhibited in physical processes of diffusion, oxygen levels become a competition between photosynthesis and respiration, an unusual situation for a surface water system.

I used the chlorophyll *a* concentrations:bacterial abundance ratio to represent an assessment of the autotrophic vs. heterotrophic dominance in surface waters of the transect. A significant correlation was detected between chlorophyll:bacteria and oxygen levels during this week-long observation (Fig. 13), which suggests that microbes mediate not only the nutrient cycling occurring in these waters, but also the oxygen saturation of the water column. The NT1 outlier from the first day's sampling occurred during conditions of oxygen supersaturation but relatively low chlorophyll *a* concentrations. This outlier may be a result of oils at the surface inhibiting diffusion, indicating that excess oxygen produced by phytoplankton is not able to escape to the atmosphere as predicted by diffusion processes.

## **CONCLUSIONS**

This study showed that Newtown Creek fluctuates between physical and biological extremes on a daily and even hourly basis in terms of variability in chlorophyll *a* concentrations, bacterial abundance, oxygen saturation and nutrient concentrations in its water column. Nutrient levels along the creek transect were consistently below the Redfield ratio, closer to the average levels observed in domestic raw sewage, highlighting

the immense human influence on this tidal creek through inputs of high-P sewage effluent.

A comparison of environmental conditions at surface and depth in the waters of this creek also underscores the importance of surface water processes in this body of water, where surface waters alternate between hypoxia and oxygen supersaturation within days. The combination of the establishment of a strong thermo/halocline and the persistent oil slick observed on the creek's surface waters in summer results in surface waters disconnected from the physical processes of mixing and diffusion at the sea-air interface. Surface microplankton community structure and abundance fluctuated wildly along with bacterial abundances in response to nutrient additions at NT2. This suggests that functional predator-prey relationships between grazers and bacteria are disrupted during hypoxic events. The control of surface dissolved oxygen levels by the relative abundances of phytoplankton and bacteria in these surface waters reveals an additional stressor to a system already challenged by extreme nutrient and bacterial loading from anthropogenic sources.

## **ACKNOWLEDGMENTS**

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