

**QUANTIFYING NITROGEN LOADING TO FROM SOUTHAMPTON
VILLAGE TO SURROUNDING WATER BODIES AND THEIR MITIGATION
BY CREATING A SEWER DISTRICT**



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EXECUTIVE SUMMARY

Located in the heart of the Village of Southampton, Lake Agawam is community focal point. For more than a decade, the Lake Agawam has experienced dense blue-green algal blooms that synthesize potent liver toxins and neurotoxins and represent a serious human and animal health threat. Since the NYSDEC began monitoring hundreds of lakes across New York in 2013, no lake has experienced blue-green algal blooms more frequently than Lake Agawam. Due to the extreme densities achieved by blue-green algal blooms, Lake Agawam can also experience low oxygen levels at night and has experienced massive fish kills as a result. While some blue-green algae are able to obtain or 'fix' nitrogen from the atmosphere and thus are fueled by phosphorus loading, the blue-green algae in Lake Agawam (*Microcystis*) are not able to fix nitrogen and more than a decade of research has repeatedly shown that blue-green algae biomass and toxins in Lake Agawam are fueled by excessive nitrogen loading. This study was undertaken to estimate the effect that the sewerage of a portion of the Village of Southampton would have on the total nitrogen loads to Lake Agawam and other neighboring water bodies as well as the water quality within these systems. Nitrogen loading models were constructed that considered nitrogen delivered to Lake Agawam, Old Town Pond, and Wickapogue Pond from three types of fertilizers, septic systems, the atmosphere, surface-run-off, storm drains, lake sediments, and birds. The models were run with and without the proposed sewer district, over 10-year and 50-year time frames, and with and without the upgrading of additional septic systems in the Village. The subsequent effects on water quality in the surrounding water bodies was quantified. The models demonstrated that wastewater was the largest source of nitrogen to Lake Agawam and Old Town Pond (69% and 73% of total nitrogen load), while fertilizer was the largest source to Wickapogue Pond (35%). The proposed sewerage of the Village would divert nearly 10,000 lbs of nitrogen away from Lake Agawam annually, reducing its nitrogen load by 20 to 50%, depending on the time frame considered (10 vs 50 years) and the fate of other septic systems in the Village (no action vs upgrades). Of the sources of nitrogen under the direct control of the Village (wastewater, fertilizer, run-off), these reductions in total nitrogen loading to Lake Agawam would represent between 20 and 65% again depending on the time frame considered (10 vs 50 years) and the fate of other septic systems in the Village (no action vs upgrades). Because the proposed sewer district is completely within the Lake Agawam sewer district, it would not affect Old Town Pond and Wickapogue Pond. A hypothetical expansion of building within the Village was considered whereby the additional growth without the sewage treatment plant would increase nitrogen loading by 33% whereas concurrently implementing the sewer district and the proposed growth connected to the sewage treatment plant would reduce nitrogen loads by 22% over a 10-year time frame. Upon the reduction of nitrogen loads to Lake Agawam, it is expected that the intensity of blue-green algae blooms would be reduced by 33 – 55% and that their toxicity would decline by 50 to 75%, again depending on time frames and management scenarios. Additional ecosystem benefits would include improved nighttime oxygen levels, improved water clarity, increases in submerged aquatic vegetation, and improved conditions for pelagic fish. Given that recent research at Stony Brook University has determined that waterfront or near-waterfront home values can be strongly effected by water clarity, improved water clarity and quality could financially benefit home owners in the region as well as the Village and associated tax revenues.

Task 1. SUMMARIZE THE CURRENT STATUS OF WATER QUALITY WITHIN LAKE AGAWAM AND KNOWN RELATIONSHIPS TO EXCESSIVE NITROGEN AND/OR PHOSPHORUS LOADING, AS WELL AS ALL PRIOR INFORMATION REGARDING NITROGEN AND PHOSPHORUS LOADING INTO LAKE AGAWAM.

Located at the center of the Village of Southampton, Lake Agawam is a focal point for the local and visiting community. Owing to the adjacent green, park, and sitting area, the seasonal Lake ferry, and near-by ocean beaches, much of the community life revolves around this Lake, particularly in summer. The excessive delivery of nutrients from land into coastal waters can lead to a host of environmental problems including algal blooms, hypoxic zones, and habitat loss. This occurrence is acutely apparent within freshwater bodies surrounding the Village of Southampton, particularly within Lake Agawam. Since monitoring of Lake Agawam began in 2003, dense, toxic blue-green algal blooms have been recorded each spring, summer, and fall. Blooms generally commence in May and persist through November. These blooms are comprised of genera of blue-green algae that synthesize biotoxins including microcystin, a gastrointestinal toxin, and the neurotoxin, anatoxin-a (Gobler et al 2007). Microcystin concentrations during these blooms often surpass the World Health Organization (WHO) safe drinking water standard of 1 µg/L and often exceed the recreational water limit of 20 µg/L (Chorus and Bartham, 1999). There are multitudes of examples of animal sicknesses and deaths associated with chronic, or even sporadic, consumption of water contaminated with cyanotoxins (O’Neil et al., 2012; Backer et al., 2014). Cyanotoxin exposure has been linked to mild and potentially fatal medical conditions in humans including gastrointestinal cancers (i.e. liver, colorectal; Chorus and Bartham 1999) and more recently, neurological disorders such as Alzheimer’s disease (Cox *et al.*, 2005).

The New York State Department of Environmental Conservation (NYSDEC) monitors hundreds of lakes and ponds in all 62 counties of the state and creates public warnings and lists specific water bodies on its statewide website when they experience a blue green algae bloom at an intensity exceeding its standard of 20 µg/L. During 2014 and 2015 and 2016, Lake Agawam was listed by NYSDEC more frequently than any other water body in NYS. Importantly, however,

Lake Agawam was not the only water body within the Village to be listed by NYSDEC. Since 2014, both Old Town Pond and Wickapogue Pond have been documented as having blue-green algae blooms beyond the NYSDEC threshold. Beyond blue-green algae, Lake Agawam has experienced additional, serious water quality impairments including low water clarity, low oxygen, and fish kills such as the event in 2006 when thousands of fish perished.

Careful research during the past decade has demonstrated that the blue-green algal blooms in Lake Agawam are being promoted by excessive nitrogen loading and, to a lesser extent, phosphorus loading (Gobler et al., 2007, unpublished; Davis et al., 2010). High levels of nitrogen promote both the growth and toxicity of blue-green algae in Lake Agawam (Gobler et al., 2007, unpublished; Davis et al., 2010). Prior experimental work has shown that reducing nitrogen loads into Lake Agawam could reduce the intensity of blue-green algal blooms there (Harke et al., 2008). Prior studies have also shown that groundwater from the region surrounding Lake Agawam and entering the lake is a significant source of nitrogen to Lake Agawam. Nitrogen loading is particularly acute within the Village of Southampton where the dense aggregation of cesspools and septic tanks from stores, restaurants, hotels, and homes surround Lake Agawam. All of these observations warrant consideration of a nitrogen loading mitigation strategy for the Village. The creation of a sewer district and the treatment of wastewater at a sewage treatment plant could be the most effective means for mitigating nitrogen loads and improving ecological conditions in Lake Agawam.

To date, there has been great confusion about many aspects of Lake Agawam and the Village with regard to the knowns and unknowns of nitrogen, phosphorus, and toxic algal blooms in Lake Agawam and neighboring lakes. Here, I summarize the current status of water quality within Lake Agawam and known relationships to excessive nitrogen and/or phosphorus loading, as well as all prior information regarding nitrogen and phosphorus loading into Lake Agawam. This summary includes prior nitrogen and phosphorus loading studies by Gobler, Nelson, Pope, and Vorhis, and Lombardo and Associates as well as more than a decade of research and monitoring by the Gobler Lab of Stony Brook University.

Gobler Lab monitoring of Lake Agawam Water Quality Characteristics since 2003

In 2004, the author of the present report, Christopher Gobler, reported on a study of Lake Agawam conducted in 2003. The purpose of the study was to assess the general water quality and health of this ecosystem. Samples were collected on a weekly to bi-weekly basis from four stations from April through November of 2003 and levels of chlorophyll *a*, dissolved oxygen, temperature, salinity, nutrients, water clarity and coliform bacterial levels were assessed. High levels of phosphorus and chlorophyll *a* were found throughout the year. Reduced bottom oxygen and elevated coliform bacteria levels were found during summer months. Storm drain runoff entering the northern extent of Lake Agawam had elevated levels of phosphorus and coliform bacteria, suggesting this was an important source of these constituents to this system. Low bottom oxygen levels during summer suggests Lake Agawam is most appropriately characterized as classification D of the New York State Department of Environmental Conservation water quality criteria which is the minimal classification needed to sustain viable fisheries. Based on US EPA classification, Lake Agawam's water quality characteristics fit the criteria of 'hypereutrophic' which is the lowest water quality ranking category (Table 1). Moreover, the total phosphorus concentrations suggest that the lake is currently unlikely to support Salmonid or Percid fisheries, but rather is more likely to host fish in the Centrarchid or Cyprinid families.

The Gobler laboratory monitoring of Lake Agawam has continued annual since that first study, with 2016 marking the 14th consecutive year of measurements. Figure 1 shows the annual average chlorophyll *a* and dissolved oxygen levels in Lake Agawam since 2003 and reveal that the Lake has remained hypereutrophic or at the high end of eutrophic every year since the first study. The high levels of algal biomass have contributed to high dissolved oxygen levels in the system, at least by day, as mean annual dissolved oxygen levels are consistently in a healthy range of ~10mg/L (Figure 1). The hidden side of algal blooms and dissolved oxygen can occur at night when respiration rates of algal blooms and/or bacteria feeding off of the organic matter associated with the algal blooms can lead to very low dissolved oxygen levels. The Gobler lab has recorded hypoxic oxygen levels at night and in 2006 observed a kill of thousands of white perch across Lake Agawam when nighttime oxygen levels dropped to < 1mg/L (Figure 2).

Two major genera of blue-green algae have been observed in Lake Agawam since 2004: *Microcystis* and *Anabaena*. While *Microcystis* is the most prevalent of the two blue-green algae

in this system *Anabaena* can sometimes be numerically dominant, particularly in early summer months (Figure 3). Importantly, these are toxic blue green algae that are capable of synthesizing the liver-toxin, microcystin, and neurotoxin, anatoxin-a, respectively (Figure 4). These compounds are well-known for causing serious illness and death in aquatic and terrestrial animals and were previously referred to as fast-death-factor and very-fast-death-factor, respectively (Carmichael et al., 2016). While anatoxin-a is only occasionally found in Lake Agawam, microcystin has been consistently present in the system for the past 14 years (Figure 5; Gobler Lab data, 2003 - 2016). Analyses by Dr. Greg Boyer from SUNY ESF in 2004 demonstrated that microcystin is also accumulating in both the fillet and viscera of carp and bass in Lake Agawam at levels exceeding the World Health Organization's allowable limits for fish (Figure 6; Chorus and Bartram, 1999). The presence of these toxins prompted the Southampton Town Trustees to first close Lake Agawam to recreation and fishing in 2004 (Figure 7). Similar closures have been implemented by Suffolk County's Department of Health Services for most of the summer since 2013 when they began their program of monitoring County-wide lakes.

The levels of microcystin present in Lake Agawam from 2013 through 2016 are shown in Figure 8. For reference, the World Health Organization (WHO) considers levels greater than 1 µg/L as a health risk for drinking water and 20µg/L a moderate recreational risk (Chorus and Bartram, 1999). Microcystin was detectable on every date sampled which is generally from May through November during these past four years (Figure 8). Of the nearly 100 samples collected from 2013 – 2016, 96% of the samples exceeded the WHO drinking water standard and 40% of samples exceeded the level of a moderate recreational risk (Figure 8). In 2013, the NYSDEC began a state-wide program to monitor blue-green algal blooms across NYS and to alert the public via a website and emails that blooms were present. In 2014 and 2015, data was compiled to compare the number of weeks different lakes across NYS were listed as having a bloom by NYSDEC (Figure 9,10). In both years, Lake Agawam was listed by NYSDEC for having blue-green algae blooms for 24 weeks of the year, more frequently than any other water body in NYS (Figure 9,10).

The very serious dangers of these 'cyanotoxins' are associated with the tendency of the blue-green algae to float to the surface of lakes during the day due to their procession of

photosynthetic gas vesicles (Figure 9; Chorus and Bartram, 1999). When persistent south winds develop off the Atlantic Ocean during the day in summer, this tends to blow surface accumulating blue-green algae along the northern extent of Lake Agawam (see cover photo), forming a shoreline scum (Figure 11). In 2016, samples were collected to contrast the whole Lake and shoreline scum levels of microcystin and showed that on dates when a shoreline scum was present along the Lake, microcystin levels were 50-times higher in the scum than within the Lake, at times reaching 2,000 µg/L, a level 50-times the recreational guideline for this toxin and 2,000-times the drinking water standard (Chorus and Bartram, 1999). Dogs may be most at risk from blue-green algal toxins that are concentrated at the shoreline. Backer et al (2014) compiled data through the US Center for Disease Control and reported on more than 400 cases of intoxication of dogs with blue-green algae toxins, in many cases leading to death. According to the NYSDOH, in 2012, a dog died upon drinking water or consuming shoreline scum from Georgica Pond in East Hampton and according to Suffolk County Department of Health Services, in 2015, two dogs were sickened drinking from Fort Pond in East Hampton that has experienced blue-green algal blooms.

Blue-green algae are promoted by excessive nutrient loading (Chorus and Bartram, 1999; Paerl et al., 2001; Gobler et al., 2016). Traditionally, it has been thought that algal growth is controlled by phosphorus in freshwater bodies based on the premise that nitrogen-fixing blue-green algae balance ecosystem N deficiencies (Schindler, 1974, 2008, 2012; Scott and McCarthy, 2010). As the total P concentration in many freshwater bodies has increased, a shift has been reported in phytoplankton assemblages toward blue-green algae dominance (Smith, 1983; Trimbee and Prepas, 1987; Watson et al., 1997). Over the past several decades, many lakes have been driven increasingly out of due to disproportionate anthropogenic inputs of N and P (Conley et al., 2009; Glibert et al., 2011; USEPA, 2015). Consequently, the literature is rich with examples of the importance of P (Schindler, 1977; Wetzel, 2001; Sterner et al., 2008; and references therein), and N (e.g. Gobler et al., 2007; Davis et al., 2010; Beversdorf et al. 2013, 2015) in controlling cyanobacteria blooms as well as with examples of N and P co-limitation (Elser et al 1990, 2007; Lewis and Wurtsbaugh 2008; Xu et al., 2010; Chaffin et al., 2013; Chaffin & Bridgeman, 2013; Davis et al., 2015). Concurrently, thought has evolved to recognition of the importance of controlling nitrogen to restrict phytoplankton assemblage structure and productivity (Conley et al., 2009; Glibert et al., 2011; USEPA, 2015).

There are several lines of evidence that demonstrate that nitrogen controls the growth and toxicity of blue-green algae in Lake Agawam. First, blue-green algae blooms in Lake Agawam are largely comprised of *Microcystis*, a blue-green alga that cannot extract gaseous forms of nitrogen and thus must rely on in-lake sources of nitrogen (Gobler et al., 2016). Gobler et al (2007) reported on the ability of nitrogen, but not phosphorus, to promote both the growth and toxicity of blue-green algal blooms in Lake Agawam (Figure 12, 13). Davis et al (2009) also showed that nitrogen, but not phosphorus, promoted the growth of *Microcystis* in Lake Agawam when temperatures were elevated. Davis et al (2010) and Gobler et al (2007) both showed that the ratio of inorganic nitrogen to inorganic phosphorus varied from high to low levels from spring to summer and that during summer, levels were far below 16, the ratio that provides the proper balance of nitrogen and phosphorus in the system, suggesting Lake Agawam was deficient in nitrogen at that time. Davis et al (2010) also showed that nitrogen almost always promoted the growth of total, toxic, and non-toxic forms of *Microcystis*. In the summer of 2014 and 2015, the Gobler laboratory returned to Lake Agawam to again assess the relative importance of nitrogen and phosphorus in controlling the growth of blue-green algae and found that, regardless of whether populations were incubated at ambient or elevated temperatures, nitrogen selectively promoted the growth of blue-green algae (Figure 14).

Harke, Davis, and Gobler, 2008

In 2008, Harke et al published a study of Lake Agawam that quantified nitrogen and phosphorus loads. These were volumetric loading budgets, but also included measurements of benthic flux and storm drain run-off. These nutrient budget calculations indicated that groundwater was the greatest contributor of inorganic nitrogen to the lake. DIN input from groundwater was calculated to be 718 mol day^{-1} (Table 2) which makes up 46% of the DIN supply (Figure 4). This was nearly twice the amount of DIN originating from benthic flux and effluent from the storm drain (354 mol day^{-1} (23%) and 439 mol day^{-1} (28 %) respectively) (Table 2, Figure 4). Atmospheric deposition and surface runoff were negligible when compared to these inputs. For organic fluxes of nitrogen, the benthos was the largest source, contributing 241 mol day^{-1} DON (Table 2) or 75% of the DON budget (Figure 4). Other source contributions were an order of magnitude less than this. Phosphorus inputs, both organic and inorganic, mainly originated from

the benthos (94% DOP, 83% DIP and 89% TDP) with inputs from the storm drain making up 12% of the DIP budget (Figure 4). In comparison, all other sources were minimal.

There exists several important caveats and weakness of this prior study that make its findings only generally comparable to the present study. Firstly, this study only considered dissolved nutrients and not particulate nutrients which in some cases are likely to be very substantial, for example when considering storm water run-off. In addition, benthic fluxes were overestimated. Rates were measured in mud only and at high temperatures and then were uniformly applied to the entire lake daily through a calendar year. More than half of Lake Agawam's sediments are sandy and thus likely have little-to-no benthic flux and benthic fluxes are temperature dependent and thus the warm water rates are likely applicable for summer months. Next, there have been improvement by the Village to the handling of stormwater since 2007 and thus outfall nutrient concentrations are now lower than previously measured. Finally, the groundwater loading was based on only a handful of measurements that were highly variable in concentrations. Hence, while these nutrient budgets provide useful context, they are in need of updating and improvement.

Harke et al (2008) also proved that experimental reductions of ambient nitrogen and phosphorus levels were capable of significantly reducing algal biomass during summer months. Nutrient dilution did not alter algal biomass in May, early June, September and October (Figure 15). However, during peak bloom months (mid-June through August) a reduction in nutrients by 50% reduced phycocyanin levels on average by half (Figure 15) and total algal biomass on average by 40%. In addition to the reductions of phycocyanin being larger than those in chlorophyll, during the late June experiment, phycocyanin levels decreased by 40% while chlorophyll levels were unchanged (Figure 15)

Nelson, Pope, and Voorhis study, 2009

In 2009, the consulting firm Nelson, Pope, and Voorhis published a study commissioned by the Village of Southampton entitled "Comprehensive Management Plan for Lake Agawam". The report summarized information generated regarding the Lake and generated a series of recommendations for the Lake. Since that study, the following recommendations have been at least partly implemented for Lake Agawam and its watershed:

- Examining municipally owned lakefront areas for improvement opportunities (control direct

stormwater overflow from paved surfaces in close proximity to the lake; establish lake front walking trails in areas where public access can be provided; provide public education and interpretive signage in appropriate lakefront areas).

- Providing educational opportunities in form of pamphlets, newsletters, web site information and other media tools through the Village of Southampton and the Lake Agawam Conservation Association.

- Intercepting and recharge stormwater runoff in higher elevations of the watershed.

- Maintaining catch basins and leaching pools on a regular basis by removing accumulated sediment.

- Maintaining roads on a regular basis through street sweeping to reduce potential for sediments to accumulate and/or enter the lake.

- Exploring potential for sewerage in areas of the watershed with commercial downtown development and shallow depth to groundwater .

- Ensure appropriate land use density within the Village and the watershed area for Lake Agawam, through coordination with SCDHS on the implementation of Article 6 of the SCSC; sanitary credit transfers to the Lake Agawam watershed area should be reviewed and limited based on nitrogen load.

- Encouraging and facilitating “pick up after your pet” practices

- Providing equipment to improve dissolved oxygen levels in the lake.

- Continuing water quality monitoring to determine effectiveness of implementation of management recommendations and track trends in water quality.

- Continuing monitoring for cyanotoxins levels and associated ecological-based studies (e.g. chemical, physical and biological factors) to further elucidate the factors which promote the presence of these toxins.

- Implementing an adaptive management approach for Lake Agawam as the understanding of cyanotoxins is expanded, and strive to improve water quality while minimizing health risks to humans and animals.

In contrast, it would seem the following recommendations have yet to be implemented.

- Controlling waterfowl populations through management practices.

- Encouraging homeowners to remove fertilizer dependent vegetation and establish native

planting areas.

- Placing shade trees near shore that will provide soil stability, biological uptake, and shading of surface water to maintain lower water temperatures allowing higher dissolved oxygen levels.

- Removing invasive vegetation in favor of natural habitat areas under controlled re-vegetation restoration programs.

- Examining potential for removal of existing hardened shorelines; discourage expansion of new hardened shoreline structures.

- Encouraging homeowners to regularly inspect and maintain sanitary systems in high groundwater areas and elsewhere in the watershed.

- Encourage and enforce when appropriate, upgrade of malfunctioning sanitary systems.

- Improving fish populations in the lake favoring native fish assemblages.

- Maintaining but not expanding areas of aquatic vegetation on the west side of the lake.

- Examining wetland biological treatment options for north end of lake near stormwater outfall

- Examining potential for removal of organically enriched surface sediments from the lake

Lombardo Associates Incorporated study, 2013

In 2013, Lombardo Associates completed their 'Lake Agawam Water Quality Restoration Action Plan that was commissioned by the Peconic Baykeeper. This report summarized the data available for Lake Agawam and its watershed, made some groundwater measurements, considered total maximum daily loads for the Lake and listed a series of water quality remediation options. These recommendations focused largely on addressing phosphorus loading to Lake Agawam as Lombardo Associates believed that the control of phosphorus only would be sufficient to improve water quality. Specific recommendations by Lombardo Associates included the use of phosphorus-free fertilizers in the watershed, the application of the chemicals alum or Phoslock to remove phosphorus from the water column of Lake Agawam and to prevent the release of phosphorus from sediments, Lake water extraction and treatment through a constructed wetland, dredging of sediments to eliminate the flux of phosphorus to the Lake, the construction of the stormwater treatment system using a constructed wetland, and a geese / waterfowl management plan. While sediments and storm water were estimated to be responsible for 89% and 8% of the total phosphorus load to the Lake, waterfowl were estimated as 1% of the phosphorus load.

Switching to the use phosphorus-free fertilizers would be of no cost but dredging was deemed too expensive by Lombardo Associates being estimated at \$55M. As such, the plan recommended pursuing the geese / waterfowl management plan that would cost \$215,000, a one-time Alum or Phoslock application what would cost \$500,000, and the construction of a treatment wetland that would cost up to \$7.3M. This constructed wetland would be designed to remove phosphorus from both stormwater and from lake water that would be pumped through the wetland. It is notable that these phosphorus removal projects could be performed in parallel with the construction of a sewer district within the Village to further improve water quality within Lake Agawam. Given the very small portion of the phosphorus load contributed by geese, their management may be considered a low priority. It is notable that the singular prior use of Phoslock on Long Island in Mill Pond in Water Mill, NY, was unsuccessful and did not mitigate blue-green algae blooms and led to the Town of Southampton terminating their contract with the consultants who were hired to apply this chemical. Finally, while the proposed constructed wetland is a costly endeavor, its ability to treat stormwater and lake water would hold the promise of removing a large amount of both nitrogen and phosphorus, including phosphorus from sediments since any phosphorus fluxed to the water column during summer and then retained within the wetland would not return to sediments.

Task 2. Develop a dynamic model for nitrogen loading rates and sources for the Village of Southampton to Lake Agawam.

A model was developed to quantify the total dissolved nitrogen input into the waterbodies of Southampton Village was the Nitrogen Loading Model (NLM; Valiela et al., 1997) available through the Nitrogen load web-based modeling tool (nload.mbl.edu) described in Bowen et al. (2007) and used in Bowen and Valiela (2004) and recently in Kinney and Valiela (2011) among others. This model was then modified to utilize more accurate data sources, but the underlying assumptions and critical components were not altered. For example, originally average roof area was multiplied by the number of buildings to get an idea of total area of roofs in a watershed. With more accurate data the area of each roof in the watershed was calculated and then all the individual areas were summed together. The NLM uses information about land use in a defined watershed to predict both the amount of nitrogen that is released into the watershed from various sources and how much of it ends up in a corresponding waterbody. This

model requires accurate land-use and land cover information, such as area of agriculture, residential areas, and impervious surfaces as well as other environmental data gathered from scientific literature, GIS data, USGS reports, the Town of Southampton, and Suffolk County as described in Table 1.

The NLM is a good fit for watersheds such as the lakes and ponds within Southampton Village that are a mix of residential, forested, and agricultural lands (Monti and Scorca, 2003). The NLM assumes that the primary transport mechanism for nitrogen entering the lakes and ponds from each watershed is groundwater flow. This assumption is consistent with data available for the region as there is little inflow to the bays from streams and geologically, Long Island is composed of unconsolidated sands that allow for relatively easy transport of groundwater to coastal lagoons (PEP, 2001). The NLM assumes that all nitrogen entering the waterbodies from external sources originated from atmospheric deposition to the watershed, wastewater, or fertilizer. Valiela et al (1997) validated this model by comparing its nitrogen load prediction to empirically measured nitrogen levels. They found the NLM's results to be statistically indistinguishable from measured concentrations and that a linear relationship exists between the percent contributions from wastewater that the NLM predicted and the stable isotope signature for wastewater expected from known isotopic N values of nitrate in groundwater. The NLM is one of the most inclusive nitrogen loading models regarding the transformation and transport of nitrogen as it travels from watershed to estuaries (Bowen and Valiela, 2001). It is the model that NYSDEC has elected to use for their subwatersheds study of Long Island as part of the Long Island Nitrogen Action Plan.

The NLM utilizes multiple data inputs that were obtained or derived from Suffolk County and New York State data sources for the watersheds: number of people; number of people within 200 meters of shore; area of roofs; surface area of the watersheds; area of freshwater wetlands; area of agriculture; area of golf courses; lawn area on parks, athletic fields, and residential parcels; freshwater ponds; and, various impervious surfaces. The model also includes a list of constants assigned default values based on recommendations from NYSDEC's Long Island Nitrogen Action Plan subwatersheds study of Long Island.

Watershed delineation

The surface extent of the Agawam, Old Town Pond, and Wickapogue Pond watersheds were determined using a combination of H2M's groundwater travel time analysis and groundwater flow patterns, which have been previously found to generally follow hydraulic gradients established by surface topography (Schubert, 1998). Surface topography was determined using United States Geological Survey LiDAR data. Watersheds were limited on the northern edges by the 50-year travel time line provided by H2M. Watershed delineations were compared to and consistent with those drafted by the consulting firm CDM Smith's as part of the Suffolk County groundwater model.

Atmospheric Deposition of Nitrogen

Atmospheric nitrogen is delivered via precipitation (wet) or via dust (dry). Nitrogen that arrives in the Village watershed through wet and dry deposition may have a varied contribution to waterbody nitrogen load depending on where the nitrogen lands. Different land use types (impervious, vegetation, developed) alters the amount of nitrogen that makes it to the waterbody. Nitrogen landing on vegetation has time to be assimilated by plants and organisms in the soils, and/or may be denitrified in the aquifer. Nitrogen that lands on impervious surfaces can runoff directly into a stream, or bay. It may also flow through a municipal separate stormwater sewer system (MS4) where it eventually seeps into sandy soils and discharges into coastal zones. In general, significantly less of this atmospherically deposited nitrogen is removed when it lands on impervious surfaces.

Nitrogen runoff from driveways, roofs, and other impervious surfaces was attenuated because it first passes through turf and then into the sandy soils. All atmospheric depositions also go through denitrification in the aquifer. The atmospheric deposition of nitrogen is decreasing on Long Island and the Northeast in general, a trend expected to continue due to changes in industrial atmospheric discharge in the Midwest.

The land-use and land cover information used for the NLM was ascertained through the Suffolk County Land Use and Land Cover parcel dataset for all watersheds. This layer includes all taxable parcels, but areas like public roads are not covered. All inputs to the NLM and their

sources are referenced in Table 1. The areas of impervious land cover were estimated where the Normalized Difference Vegetation Index (NDVI) was low ($NDVI < 80$). The NDVI was created from the USGS's high resolution orthoimagery. Parcels that were known by land type to not have any impervious surfaces were removed to improve the accuracy. The removal included open water, vacant land, preserved/forested land, and agricultural land. Road area was estimated by limiting this impervious layer to areas where land parcels did not exist. Driveway areas were estimated by limiting the impervious layer to residential parcels and where the height of object on the properties was close to zero. The height of objects on properties (trees, buildings, decks, etc.) was determined by subtracting a Digital Elevation Model from a Digital Surface Model. These models were created from the same USGS LiDAR point cloud data. Total roof area was quantified by summing the area of each building footprint within the watershed. Footprint data was supplied by Suffolk County.

Wastewater

The contribution of nitrogen load to the bays from wastewater was calculated in the NLM by multiplying the nitrogen released per person by the number of occupants in the watershed. The number of occupants for most parcels in each watershed was determined by using H2M's model results. They determined that one residential parcel produces 300 gpd of sewage. Year-round residential properties were assigned 2.71 people per property which was comprised 2.46 of year round residency and 1.5 guests for two months of summer as reported by Suffolk County Planning Department for Southampton Town census data of seasonal residency. Seasonal occupancy properties were assigned 0.75 people assuming two months of occupancy and an average of 4.5 people per seasonal home as reported by Suffolk County Planning Department for Southampton Town census data of seasonal residency. Properties were determined as year round or seasonal based on the permanent address of the owner. Using Southampton Village census data obtained through Suffolk County it is known that there is a mean occupancy of 2.71 people per one year round residential parcel meaning that these 2.71 people are responsible for 300 gpd or 111 gpd per person. With this ratio and the modeled sewage output, the occupancy for other property types was determined based on known and/or permit wastewater flows. Most commercial and industrial properties included in H2M's study were assigned wastewater flow rates. The few remaining non-residential properties outside of H2M's study were handled

individually and treated largely as homes with an expected number of people based on the size of the dwelling. For Southampton Hospital, discharge volumes (~25,000 gallons per day) and nitrogen concentrations of the effluent (3.8 mg/L) were obtained from averages reported on USEPA Integrated Compliance Information System website from 2012 – 2016 and this discharge was treated as a point source of delivery to Old Town Pond as it discharges to ground <2,000 ft from the Pond.

Differing levels of nitrogen were then removed wastewater loading depending upon the type of on-site sewage disposal system (septic or cesspool) and the system's distance from shore as there is significantly less nitrogen removed when septic tanks and cesspools are within 200 m of coastal waters. **Error! Bookmark not defined.** Residential and commercial parcels have either an individual septic tank system or cesspool, which differ slightly in the fraction of nitrogen released to the underlying aquifer, with the less effective cesspools releasing more. In Suffolk County, a law was passed in 1973 requiring all newly constructed buildings to include a septic tank system instead of a cesspool. For this study, residential and commercial uses built before 1973 were assumed to have cesspools. The study area does not contain any municipal wastewater treatment facilities.

The NLM breaks down the nitrogen removal in septic tank and cesspool-based systems into three steps: removal in the tank, removal in leach fields, and removal in septic plumes. Cesspools on Long Island are typically composed of cylinders arranged vertically, eliminating any traditional leach field and the associated nitrogen removal therein. Although there is a disposal pit associated with these vertically structured cesspools systems and only a small amount of nitrogen is removed in this part of the system (<10%).

Fertilizer

The NLM considers fertilizer input from agricultural uses, golf courses, parks and athletic field lawns, and manicured residential lawns. The area of each type was calculated using ArcGIS processes; residential lawn areas was found by limiting high NDVI areas (NDVI>80) to residential parcels and to areas where the LiDAR height layer was near zero (height<0.05m). Golf courses were extracted from the Open Street Map and were further manually edited.

Agricultural land and was extracted from the Suffolk County Land Use and Cover dataset and manually verified with satellite imagery. Parks and athletic field parcels were also extracted from the Suffolk County Land Use and Land Cover dataset but were then further limited to lawn areas within those parcels with the same process used for residential lawns.

Additional loading factors

Beyond the loading rates determined by the altered N-Load model, rates of benthic fluxes, storm water run-off, and direct atmospheric deposition were also determined. The storm drain in Lake Agawam contained two outflow openings which measured 170 x 60 cm and 175 x 60 cm. During heavy rain events (precipitation > 2cm) both openings would be seen to release water. During light rain events (precipitation < 2cm) only the larger of the two openings (right facing) was seen to release water. There is a 20-minute delay from the initial start of the rain event to release of water from the storm drain, potentially due to a catch basin further up the pipe. During rain events, water samples were collected from outflow in acid-washed 250 ml bottles. Flow rates were measured using a General Oceanics Flowmeter (Model 2035). Flow depth was measured with a meter stick from the base of the opening to the middle of the flow to get an average depth. Rain start and end times were also recorded. Water samples were stored frozen and later analyzed for nitrate, ammonia, phosphate, DON and DOP (Valderrama 1981, Jones 1984, Parsons et al. 1984). Total storm drain contribution was also determined by considering the size of the Village watershed (approximated to be 4,839,243 m²) and estimating that 20% of this watershed was likely impervious and funneled into the storm drain system due to streets, parking lots, buildings and driveways (Civco et al. 2006). The volumetric contribution of the storm drain determined via this method was similar to volumes determined via several actual measurements extrapolated for the five-month study period.

To determine benthic flux, sediment core samples were obtained from three locations in the lake: one at the north sampling station, one at the longitudinal center of the lake and one near the southern portion of the lake. Cores were extracted using a box corer dropped from the side of the boat which was then brought to 0.3 m below the water surface. An acid-washed clear polycarbonate tube (length = 26.6 cm, diameter = 9.3 cm) was then inserted through the top of the corer to collect a sediment sample. While the tube was still in the sediment, a plastic cap was

placed on the bottom and then the top to capture the sediment sample and lake water immediately above the sediment. Cores were immediately placed in a cooler and transported back to the lab within one hour. A replicate and blank of the North End were also retrieved. Core samples were then incubated in similar light and temperature conditions to those measured at the lake bottom of each site. The samples were also aerated to achieve similar dissolved oxygen levels found in bottom waters of Lake Agawam using an aquarium air pump. Physical parameters were monitored using an Onset® temp/light monitor. Water samples were extracted using an acid-washed 60 ml syringe with 15 cm tubing attached to the end. Water was drawn up slowly from just above the sediment water interface and care was taken to not draw up sediment. Samples were placed in acid-washed 60 ml bottles and frozen. The incubation was allowed to run for 12 hours with a total of 5 samples obtained per core as a time course during the incubation. Samples were filtered on combusted GFF and analyzed for nitrate, phosphate, ammonia, DON and DOP (Valderrama 1981, Jones 1984, Parsons et al. 1984). As filtered lake water was not added to replace the volume extracted, a mass balance correction was applied using the equation $(C_0 - C_1) \times V_0 = \Delta m$ where C_0 is the starting concentration, C_1 is the ending concentration, V_0 is the starting volume and Δm is the mass change. This correction was applied to each time point in the series and the results were plotted against time. The resulting slope was used to determine the flux of nutrients out or into the sediment. Given that incubations were with mud and that sands generally do not provide benthic fluxes, flux rates were applied to only 75% of the bottom of the Lake, and the shoreline region which is at least 25% of the Lake is sandy. In addition, it was assumed that benthic fluxes cease during winter (December through March) when cold temperatures restrict this process.

Final processes considered were direct atmospheric deposition to the water bodies ($0.16 \text{ mole m}^{-2} \text{ yr}^{-1}$ as per Gobler and Stinnette, 2016) and waterfowl. Fleming, R. and H. Fraser (2001) reported the nitrogen content of Canadian geese droppings as: 3,168 mg/goose/day and 608 to 1,819 mg/bird/day. Bird populations of Lake Agawam, Wickapogue Pond, and Old Town Pond were estimated at 300, 50, and 50 birds, respectively, and a loading rate of 2,000mg/bird/day was used.

Nitrogen loading rates to Lake Agawam, Wickapogue Pond, and Old Town Pond

Recently, nitrogen loads have been quantified for many watersheds across Suffolk County. In most of these efforts, load calculations have been based exclusively on external

nitrogen loads from watersheds to the ecosystem and have not considered processes within the waterbody. For this study, both internal and external nitrogen loads to Lake Agawam, Wickapogue Pond, and Old Town Pond have been quantified. When considering all internal and external loads and the 50-year watersheds, total nitrogen loads to Lake Agawam, Old Town Pond, and Wickapogue Pond are 21,037, 4,048, and 2,714 kg N per year (Table 4). Wastewater was the largest source of nitrogen to Lake Agawam and Old Town Pond and represented 70%, 74% and 34% of the total nitrogen load to Lake Agawam, Wickapogue Pond, and Old Town Pond, respectively (Table 4; Figure 17 - 19). The next largest source of nitrogen was an internal source, specifically benthic fluxes that contributed 2884, 387, and 470 kg N per year and represented between 14%, 7%, and 18% of the total nitrogen loads to these systems (Table 4; Figure 17 - 19). Thereafter, fertilizer emanating from homes, golf courses, and public parks was the third largest source of nitrogen to Lake Agawam and Old Town Pond and was the largest source to Wickapogue Pond, contributing 8%, 12%, and 35% of the total nitrogen loads Lake Agawam, Old Town Pond, and Wickapogue Pond, respectively (Table 4; Figure 17 - 19). For Lake Agawam the storm drain at the north end of the lake which contributed 928 kg N per year represented 4% of the total load in this system Table 4; Figure 17 - 19). Atmospheric deposition was the only other major source of nitrogen to these systems, and when both direct depositions to the water bodies as well as watershed deposition that flows to the water bodies was considered, the atmosphere contributed between 5 and 11% of the total nitrogen loads to each system (Table 4; Figure 17 - 19). The Southampton Hospital sewage treatment plant discharges to groundwater which flows to Old Town Pond only, representing 3% of the total N load there (Table 4; Figure 18).

These distribution of nitrogen loads are similar to recent studies in Suffolk County with some important exceptions (Kinney and Valiela, 2011; Lloyd, 2014, 2016; Gobler and Stinnette, 2016). In 2016, the NYSDEC's Long Island Nitrogen Action Plan (LINAP) has made significant progress. One of the earliest actions of LINAP has been the formation of the Suffolk County Subwatersheds Study and committee. As part of that effort, individuals from US EPA, USGS, Cornell University, Stony Brook University, Suffolk County, NYSDEC, and The Nature Conservancy have been collaborating to consider the manner in which nitrogen from land is transported to bays, harbors, lakes, and estuaries in Suffolk County. Through that process, two

important and new consensus facts have been established. First, the existing cesspools and septic systems across Suffolk County have been found to be releasing significantly more nitrogen than had previously been thought. For example, in the original NLM model developed by Bowen et al., (2007) it was assumed that there was a 35% reduction in nitrogen within septic tanks, within leaching pits, and as groundwater traverses through the aquifer. While subsequent studies on Long Island began to reduce the removal rates for each step, LINAP has determined that the loss of nitrogen from each of these processes is between 5 and 10%, making wastewater a significantly stronger nitrogen source within the ecosystem (Figure 17 - 19). Another major change initiated by LINAP has been with regard to lawns. While NLM originally assumed lawns allowed 40% of nitrogen applied to enter groundwater, LINAP has compiled enough information to feel confident that the transmission rate is 20% (Table 3). Finally, although NLM had assumed there would be a large vadose zone removal of nitrogen applied to land surfaces, LINAP has concluded such a process does not exist on Long Island and thus it has been eliminated. This project used the most up-to-date information available regarding nitrogen loading on Long Island as developed by LINAP. As a result, the total nitrogen loads are higher since nitrogen is not being removed within the aquifer at the rates previously assumed but rather at much lower rates and more nitrogen is being transmitted by septic systems and lawns to groundwater. These changes were slightly larger for wastewater than for fertilizer, making the later process more important. Regardless, the findings of this study are generally consistent with recent studies that have found that wastewater is usually the largest source of nitrogen to a given watershed, although fertilizer can sometimes be larger (Kinney and Valiela, 2011; Lloyd, 2014, 2016; Gobler and Stinnette, 2016) as was the case for Wickapogue Pond.

Task 3. Use the dynamic model quantify how connecting different regions of Southampton Village to a sewage treatment plant or and/or alternative septic systems will alter nitrogen loading rates to Lake Agawam.

For task 3, the nitrogen loading model developed for the Village of Southampton was modified to consider the connection of a specific region of the downtown region to the sewage treatment plant to be built on Windmill Lane within the Lake Agawam watershed (Figure 16). When considering future wastewater loading scenarios, beyond the sewage treatment plant, the fate of on-site septic systems outside the sewer district is unclear. Given the recent passage of

Article 19 of the Suffolk County Health Code allowing alternative, high nitrogen removing septic systems to be installed and mandates already passed in Towns such as East Hampton to replace older septic systems with new, denitrifying systems it seems likely that regions outside of the sewer district are likely to upgrade their septic tanks in the future. Hence, when considered future nitrogen loading scenarios, two watersheds and multiple scenarios were considered for this task of the study. In addition to the 50-year watershed which encompasses all of the land draining into these water bodies, loading from within the 10-year watershed was also considered since there is more certainty regarding the coming decade than there is regarding decades thereafter. Calculations were also performed considering the upgrades of on-site systems within the watershed to alternative, denitrifying septic systems. Under Article 19 of the Suffolk County Health Code, such systems must reduce nitrogen loading to at least 19 mg N per liter. However, many such systems can reduce significantly below this level and systems being piloted by Stony Brook University's Center for Clean Water Technology can consistently reduce nitrogen levels to below 10 mg N per liter. To be conservative, for this task, a 15 mg N per liter discharge was considered for upgraded septic systems. To provide perspective on the nitrogen loads that were and were not under the control of the Village, nitrogen loads were divided between those that are controllable by the Village (wastewater, fertilizer, storm drain run-off) and those not in the control of the Village (benthic flux, birds, atmosphere). Finally, through watershed calculations it was determined that the region under consideration for sewerage falls entirely within the watershed of Lake Agawam, meaning that the proposed sewer district is not expected to have any effect on Wickapogue Pond or Old Town Pond and thus they are not considered within this task.

The proposed sewer district will have a substantial impact on nitrogen loading to Lake Agawam. Beyond the fact that the sewer district falls entirely within the Lake Agawam watershed, it is also important to note that the very large majority of nitrogen entering the Lake comes from wastewater, and hence a process such as treating a large fraction of this nitrogen load within a sewage treatment plant will have a significant impact on the total nitrogen load. Quantitatively, the nitrogen load that will be removed from Lake Agawam with the implementation of this sewer district within the 10-year watershed is 3,837 kg of N per year or more than 8,000 pounds of N (Figure 20; Table 5). This represents 30% of the total nitrogen load into Lake Agawam over the 10-year time horizon (Table 5; Figure 20). Given that the overwhelming majority of nitrogen load to the Lake is from wastewater, upgrading other dwellings within the 10-year watershed to

alternative septic systems would have a further effect on decreasing the total nitrogen load to this system by another 10% for a total of a 40% nitrogen reduction (Figure 20). Alternatively, these homes could be hooked up to the new sewage treatment plant for an even larger benefit given the proposed plant will reduce nitrogen loads to 10 mg N per liter. As stated above, there are certain nitrogen loads to Lake Agawam that are not in the control of the Village. Examining the nitrogen loads that are under direct control of the Village, the implementation of the sewer district would eliminate 40% of the nitrogen load the Village can control within the 10-year watershed and if septic systems within this watershed but not in the sewer district are upgraded, this would reduce the controllable nitrogen load by 50% (Figure 21).

If the complete 50-year watershed of Lake Agawam is considered, the creation of the sewage treatment plant still handles a large fraction of the total nitrogen load and the upgrading of additional system becomes even more important and potentially impactful. Quantitatively, the nitrogen load that will be removed from Lake Agawam with the implementation of this sewer district within the 50-year watershed is 4,152 kg of N per year or nearly than 10,000 pounds of N (Figure 22). This represents ~20% of the total nitrogen load into Lake Agawam over the 50 year time horizon (Table 5; Figure 20). Given that the overwhelming majority of nitrogen load to the Lake is from wastewater, upgrading other dwellings within the 50-year watershed to alternative septic systems would have an even larger effect on decreasing the total nitrogen load to this system by another 30% for a total of a 50% nitrogen reduction (Figure 22). Alternatively, these homes could be hooked up to the new sewage treatment plant for an larger benefit. As stated above, there are certain nitrogen loads to Lake Agawam that are not in the control of the Village. Examining the nitrogen loads that are under direct control of the Village, the implementation of the sewer district would eliminate 21% of the nitrogen load the Village can control within the 50-year watershed and if septic systems within this watershed not in the sewer district are upgraded, this would reduce the controllable nitrogen load by nearly 65% (Figure 23).

Task 3.1. Use the dynamic model quantify how connecting different regions of Southampton Village to a sewage treatment plant or and/or alternative septic systems and future growth will alter nitrogen loading rates to Lake Agawam.

With the implementation of the sewer district, nearly 10,000 lbs of nitrogen will be diverted from Lake Agawam annually (Figure 22). The creation of the sewage district will also allow the opportunity for an expansion of building within the Village with the newly constructed structures being connected to the sewage treatment plant which will treat the sewage to a 10 mg N per liter standard. Hence, for this added task, the 10-year watershed nitrogen loading model was run under four scenarios: Current nitrogen loading, nitrogen loading with the addition of 10 million square feet of commercial space within the Village, nitrogen loading with the implementation of the sewer district, and nitrogen loading with the addition of 10 million square feet of commercial space within the Village along with the implementation of the sewer district. It should be noted that this much building is unlikely to occur within the Village in the near future or potentially ever. This level was specifically chosen to represent a large growth scenario to assess how the implementation of the sewer district would affect future growth in the region.

As shown in figure 24, if 10 million square feet of commercial space was added to the Village without the sewer district, this would *increase* nitrogen loading rates to Lake Agawam by 33%, likely exacerbating environmental degradation of this water body. Alternatively, if the same growth occurred but the new structures were hooked up to the new sewage treatment plant, the net effect on Lake Agawam will be a 22% *reduction* in nitrogen loading to this water body (Figure 24). Hence, while increased building can lead to significant increases in nitrogen loading to coastal water bodies, when such growth occurs in parallel to the implementation of a sewer district, net nitrogen loading can be reduced.

Task 4. Project how connecting different regions of Southampton Village to a sewage treatment plant will improve water quality in Lake Agawam.

Introduction – the importance of nitrogen in fueling blue-green algal blooms

Toxic cyanobacteria are of worldwide concern because persistent blooms threaten drinking water supplies, recreation, tourism, and fisheries (Chorus and Bartram, 1999; World Health Organization, 2011, and references therein). Such blooms are commonly promoted by excessive nutrient loading (Wetzel, 1983, 2001; Paerl 1988; O’Neil et al., 2012). Thus, there is significant interest in implementing improved management actions to control the nutrients responsible for promoting blooms. The paradigm that primary production in freshwater is controlled by

phosphorus (P) (USNAS, 1969; Schindler, 1974) was established decades ago within oligotrophic lakes in Canada (e.g. Dillan and Rigler, 1974; Jones and Bachmann, 1976; Schindler, 1977). It is based largely on the premise that when inorganic nitrogen (N_i) levels are low, diazotrophic or N_2 -fixing cyanobacteria balance ecosystem N deficiencies (Schindler, 2008, 2012; Scott and McCarthy, 2010). As the total P concentration in many freshwater bodies has increased and total N:P ratios have decreased, a shift has been reported in phytoplankton assemblages toward cyanobacteria dominance (Smith, 1983; Trimbee and Prepas, 1987; Watson et al., 1997).

Over the past several decades, many lakes have been driven increasingly out of stoichiometric balance due to disproportionate anthropogenic inputs of N and P, or management efforts targeting reduction of one nutrient (usually P in freshwaters) but not the other (Conley et al., 2009; Glibert et al., 2011; Burkholder and Glibert, 2013; and references therein). Concurrently, thought has evolved from consideration of only one limiting nutrient to recognition of the importance of ecological stoichiometry in directly and/or indirectly controlling phytoplankton assemblage structure and productivity (Conley et al., 2009; Glibert et al., 2011; Burkholder and Glibert, 2013, and references therein). Consequently, the literature is rich with examples of the importance of P (Schindler, 1977; Wetzel, 2001; Sterner et al., 2008; and references therein), and N (e.g. Gobler et al., 2007; Davis et al., 2010; Beversdorf et al. 2013, 2015) in controlling cyanobacteria blooms as well as with examples of N and P co-limitation (Elser et al 1990, 2007; Lewis and Wurtsbaugh 2008; Xu et al., 2010; Chaffin et al., 2013; Chaffin & Bridgeman, 2013; Davis et al., 2015).

N limitation in freshwater systems has been most commonly reported during warmer months when planktonic cyanobacteria blooms are most common (Gobler et al., 2007; Xu et al., 2010; Chaffin et al., 2013; Chaffin & Bridgeman, 2013; Davis et al., 2015). Although N_2 fixation by cyanobacteria has been thought to minimize the role of N in controlling blooms, various physiological and ecological lines of evidence have indicated that the energetic demands of diazotrophy can restrict the extent to which N_2 fixation can offset N demands and limitation, particularly when concurrent rates of denitrification are considered (Scott and McCarthy, 2010). Moreover, some of the most common toxigenic genera of cyanobacteria, such as *Microcystis* and *Planktothrix* (Chorus and Bartram, 1999; World Health Organization, 2011), are not diazotrophs

but, rather, depend on exogenous N supplies for growth and toxin synthesis (Berman and Chava, 1999; Vézic et al., 2002; Davis et al., 2010; Monchamp et al., 2014, and references therein). A strong relationship between the growth of non-diazotrophic cyanobacteria and exogenous dissolved N supplies has commonly been reported. For example, in laboratory studies increased N_i has promoted the growth and toxicity of *Microcystis* (Watanabe and Oishi, 1985; Codd and Poon, 1988; Orr and Jones, 1998) and enhanced input of N_i (inorganic N) to systems with elevated P has led to succession from diazotrophs to non-diazotrophs (Bunting et al., 2007; Davis et al., 2010; Chaffin et al., 2013; Harke et al., 2015).

Seasonal cycles in N loading, P loading, and cyanobacterial blooms

Due to the seasonality of N and P inputs into freshwater ecosystems in temperate late summer cyanobacteria blooms occur when N_i delivery from rivers sources is often at an annual minimum (Turner et al 2003) and, thus, most likely to control the growth of primary producers. Similar trends have been observed in smaller lakes more influenced by groundwater flow than riverine input (Gobler et al., 2007). In Lake Erie, which has sustained major cyanobacterial blooms during the past two decades (Brittian et al., 2000; Conroy et al., 2005; Stumpf et al., 2012; Wynne and Stumpf, 2015), one of the dominant nutrient sources, the Maumee River, has an annual TN:TP minimum during the summer months when cyanobacteria blooms are most likely and summer N limitation has been demonstrated (Stow et al. 2015, Chaffin et al 2013, 2014).

Species and sources of N present in lakes can differ in their seasonal dynamics, which may also influence cyanobacterial blooms. Nitrate concentrations tend to be highest in winter-spring and decline to low levels as summer progresses in many north temperate lakes (Reynolds, 1984; Wetzel, 2001; Chaffin et al., 2011; Bridgeman and Chaffin, 2013). These low N conditions can be alleviated by periodic summer storms that deliver “new” N and/or by diazotrophic cyanobacteria that release (“leak”) amino acids and ammonia during N_2 fixation (Wetzel, 2001 and references therein) although nitrogen fixation has been shown to not offset N ecosystem level demands (Scott and McCarthy, 2010).

Lake sediments and porewaters are generally enriched in inorganic P (P_i) relative to the water column, although the extent to which sediments retain or export these nutrients varies

seasonally. The PO_4^{-3} ion binds preferentially with ferric oxides in sediments under oxygenated conditions, but during summer months as temperatures warm and microbial degradation of sedimentary organic matter accelerates, sediment and near-sediment oxygen levels are progressively depleted and often become anoxic (Wetzel, 2001, and references therein; Hupfer and Lewandowski, 2008). Under such conditions, PO_4^{-3} dissociates from ferric oxides and is released to the overlying water (Carlton and Wetzel, 1984) making anoxic sediments a substantial source of P_i during warm months, particularly in systems where benthic fluxes of P_i reach surface waters. Phosphate release from organic matter directly depends on rates of microbial decomposition which are typically temperature-dependent and, thus, also maximal during summer (Wetzel, 2001; Reitzel et al., 2007; Hupfer and Lewandowski 2008). Although NH_4^+ is also released from organic matter decomposition in sediments during warm periods, the N:P ratio of sedimentary fluxes can be enriched in P relative to N, particularly in eutrophic lakes where cyanobacteria blooms are common and anoxic sediments can promote P release and denitrification (Fukushima et al., 1991; Downing and McCauley, 1992; Søndergaard et al., 2003). Hence, maximal benthic fluxes during summer can be a stronger source of P relative to N, contributing toward N limitation, particularly in shallow, well-mixed systems.

Evidence for N control of toxic cyanobacteria blooms

Recently, N has been recognized as a key factor influencing cyanobacterial blooms. Kosten et al. (2012) assessed 143 lakes along a latitudinal transect ranging from subarctic Europe to southern South America, and found that temperature and TN concentrations were the strongest explanatory variables for cyanobacterial biomass. Similarly, Beaulieu et al. (2013) assessed cyanobacteria blooms in 1,147 lakes and reservoirs of differing trophic status across the U.S. and found that the best multiple linear regression model to predict these events was based on TN and water temperature. This finding is also consistent with the strong positive association between N_i concentrations and microcystin levels that has been reported across many U.S. lakes (Yuan et al. 2014). In 102 north German lakes, Dolman et al. (2012) found that the positive relationship between total cyanobacterial biovolume and P concentration disappeared at high TP concentrations, but continued to increase with increasing TN concentration. This may suggest that some cyanobacteria have higher N:P requirements and, thus, are potentially N limited within highly P-enriched lakes. Conversely, research in large experimental lake studies has shown that

reduction of N_i inputs can result in a decline in cyanobacterial abundance (Scott and McCarthy, 2011).

As other recent examples showing the importance of N in controlling cyanobacteria assemblages, Davis et al. (2010) compared N versus P influence on dense natural *Microcystis* blooms in a tidal (brackish) tributary and a eutrophic lake, and found that in both systems during nutrient amendment experiments, all *Microcystis* populations tested were stimulated by N more frequently than by P. Monchamp et al. (2014) assessed three shallow, mesotrophic to hypereutrophic lakes in southwestern Quebec, Canada, and found TN, NH_4^+ , and DON significantly influenced the cyanobacterial assemblage structure, and that the relative biomass of *Microcystis* spp. was significantly, positively related to DON concentrations. Davis et al. (2015) found that in blooms dominated by *Planktothrix agardhii/suspensa*, cyanobacterial growth and microcystin (MC) concentrations increased as inorganic N concentrations increased, and that loading of N_i combined with P_i most often lead to the highest MC concentrations.

Water-column N_i concentrations have also been shown to promote diazotroph-to-non-diazotroph succession in cyanobacteria assemblages. For example, based on two years of observations in highly eutrophic Lake Mendota, WI, USA, Beversdorf et al. (2013) reported that cyanobacteria assemblage changes were strongly correlated with dissolved N_i concentrations and that N_2 -fixation by the diazotroph *Aphanizomenon* provided N supplies for toxic *Microcystis*. *Microcystis* populations increased in cell density several days after the first significant N_2 -fixation rates were measured, and then *Microcystis* became dominant following a short period of low-DIN stress. In the year when N_2 -fixation rates were much greater, the MC concentrations were also higher. Importantly, this system was sufficiently eutrophic to support blooms of diazotrophic or non-diazotrophic cyanobacteria depending on prevailing conditions. Within a nutrient-enriched setting, temporary low-N stress can cause an initial decrease in non-diazotrophs which can subsequently form toxic blooms when provided N from diazotrophs.

Exogenous N influences on cellular toxin composition and quotas

Beyond N influence on the *occurrence* of cyanobacteria blooms, there is evidence

that the *toxicity* of blooms formed by non-diazotrophic cyanobacteria such as *Microcystis* is also highly influenced by N availability, beginning at the cellular level with toxin composition and cell quota. As noted by Glibert et al. (2015), various researchers have reported positive, direct relationships between N availability and toxin production in *Microcystis* and other toxigenic cyanobacteria (e.g., Lee et al., 2000; Vézic et al., 2002; Downing et al., 2005; Van der Waal et al., 2009; Harke and Gobler, 2013). The common cyanotoxins MCs, nodularins (NODs), cylindrospermopsins (CYNs), and saxitoxins (STXs) all either contain amino acids or require amino acid precursor(s) (Fig. 2) (Sivonen and Jones, 1999; Kellmann et al., 2008). Synthesis of amino acids, in turn, depends on N availability (Tapia et al., 1996; Van de Waal et al., 2010). Thus, N can play a central role in determining the quantity of toxins produced by cyanobacteria.

High levels of N_i are needed to synthesize the N-rich MCs, and high levels of exogenous N_i have been shown to promote higher cellular quotas of MCs in the non-diazotrophs *Microcystis* and *Planktothrix* (Lee et al., 2000; Vézic et al., 2002; Downing et al., 2005; Harke and Gobler, 2013; Horst et al., 2014; Van der Waal et al., 2010, 2014). One of the first studies to suggest a positive relationship between MC production in *Microcystis* and available external N_i supplies was by Long et al (2001), who found a positive correlation between N-dependent growth rates and the cellular MC quota, as well as MC production rates. Later research showed that cellular MC quota depends on cellular N availability and decreases when N_i is limiting (Downing et al., 2005; Van de Waal et al., 2009, 2014; Harke and Gobler, 2013; Horst et al., 2014). At the molecular level, the microcystin synthetase gene cassette (*mcy* genes) appears to be responsive to N supply. For instance, N-deprived cultures of *Microcystis* downregulated genes involved in peptide synthesis (*mcy*ABCDE) and a decrease in cellular quota of MC under N-deplete conditions (Harke and Gobler, 2013).

In the field, addition of NH_4^+ compared with NO_3^- has led to an increase in MC concentrations and bloom maintenance for a longer duration (Donald et al., 2011). During a survey of Hirosawa-no-ike Pond, Kyoto, Japan, the strongest correlations between MCs and nutrients were found at high concentrations of NO_3^- and NH_4^+ (Ha et al., 2009). Glibert et al. (2011) noted a common phenomenon among freshwater and brackish systems that had been subjected to P reductions but not N reductions in management efforts: In systems receiving

substantial NH_4^+ inputs, once the “sediment pump” of stored P began to increase P supplies to the overlying water, an interplay of P sequestration and NH_4^+ tolerance influenced shifts to new dominant taxa such as *Microcystis*. Under P limitation, N-rich toxins would be expected to be favored as a mechanism whereby N could accumulate in excess (Granéli and Flynn, 2006; Van der Waal et al., 2014, and references therein).

Recent research conducted by Beversdorf et al. (2015) is germane in this regard, and indicates that N supply and speciation can control MC synthesis: In Lake Mendota (WI, USA), the toxic phase of the annual cyanobacterial blooms occurred during a transition of high NO_3^- but declining NH_4^+ concentrations, coinciding with upregulation of the MC synthetase gene operon, and leading to high MC levels in the ecosystem. In addition, concentrations of MCs peaked at the same time as the TN/TP ratios, suggesting the importance of an elevated N supply in supporting MC production. These findings are consistent with prior laboratory studies, wherein MC production was tightly coupled to N-dependent growth rates (Long et al. 2001; Harke and Gobler, 2013) and field studies showing that N enrichment enhanced MC levels and the expression of peptide synthesis genes involved in MC production in *Microcystis* (*mcyBEG*; Harke et al., 2015).

Compared to the plethora of laboratory and field studies showing the strong link between MC synthesis and elevated nitrogen levels, two studies have reported an *increase* in cell quota of MCs, and/or MC synthetase gene expression under N limitation of *Microcystis* (Ginn et al., 2010; Pimentel and Giani, 2014). Such an apparently counterintuitive increase of MC synthesis may be linked to the putative role of MCs in protection against increased oxidative stress (Pimentel and Giani, 2014; Zilliges et al., 2011; Meissner et al., 2013, 2015). Thus, cellular MC quota depends on the relative availability of external bioavailable N, as N is needed for MC synthesis, but this dependency may in turn be affected by the function of MCs which determines when the compounds are required.

Intraspecific differences in N influence: toxic versus nontoxic strains

Consistent with studies of marine toxigenic algae, within a given genus of cyanobacteria, strains differ in toxin composition and cellular toxin quota, and nontoxic strains not only co-occur but can be major components of blooms (Burkholder and Glibert, 2006; and references therein).

In natural cyanobacteria populations, total cellular toxin quotas resemble average values for an entire population and strongly depend on the contribution of toxigenic versus nontoxic genotypes (Janse et al., 2005; Kardinaal et al., 2007; Briand et al., 2008; Davis et al., 2009, 2010; Orr et al., 2010; Burford et al., 2014). Shifts in the genotypic composition of a population will cause changes in both the average cellular toxin quota but also the toxin composition (Bittencourt-Oliveira et al., 2001; Ame and Wunderlin, 2005; Zurawell et al., 2005; Monchamp et al., 2014). While N can strongly influence the relative abundance of toxic versus nontoxic strains of cyanobacteria, intraspecific variation (strain differences) in toxin production are poorly understood but of paramount importance in the dynamics of overall bloom toxicity and N controls.

While environmental drivers such as N have been shown to influence cell quotas of MCs, most studies have shown only up to four-fold changes in such quotas (Sivonen and Jones, 1999; Horst et al., 2014; Harke and Gobler, 2013). During cyanobacterial blooms, however, changes in MCs and other cyanotoxins can often vary many times greater than four-fold (Chorus and Bartram, 1999; Zurawell et al., 2005; and references therein). Therefore, changes in community composition between cells with the genetic ability to produce cyanotoxins (i.e. toxigenic cells), and those lacking that capability (nontoxic cells; Davis et al., 2009) are likely to play a key role in influencing bloom toxicity.

Laboratory studies of *Microcystis* have shown that toxigenic (MC⁺) strains yield faster growth rates than nontoxic (MC⁻) strains at high N_i concentrations (Vézic et al., 2002, Zurawell et al., 2005). In contrast, MC⁻ strains of *Microcystis* require lower N_i concentrations to achieve maximal growth rates in comparison to MC⁺ strains (Vézic et al., 2002) and nontoxic *Microcystis* strains have been shown to outcompete MC⁺ strains when N_i concentrations are low (Vézic et al., 2002; Davis et al., 2010). In field research, bloom populations of *Microcystis* in a temperate, tidal (brackish) tributary and a eutrophic lake shifted from dominance of MC⁺ strains to MC⁻ strains as N_i concentrations decreased through the summer (Davis et al., 2010). Other researchers working in various lakes have observed a similar seasonal succession of toxic to nontoxic *Microcystis* populations (Fastner et al., 2001; Welker et al., 2007; Briand et al., 2009, Otten et al., 2012, Singh et al., 2015; Beversdorf et al., 2015) or have noted the dominance of MC⁻ strains during the peak of a *Microcystis* bloom event (Welker et al., 2003, 2007; Kardinaal et al., 2007). Since inorganic

nutrient levels are generally depleted by dense algal blooms (Wetzel, 2001, Sunda et al., 2006, and references therein), the predominance of MC⁻ strains in established (and senescing) blooms has been hypothesized to be a function of their ability to outcompete MC⁺ strains when nutrient levels are lower (Vézic et al., 2002; Davis et al., 2010). Thus, under low N conditions, MC⁺ strains would be succeeded by MC⁻ strains, and/or MC synthesis would be down-regulated. Overall, toxic *Microcystis* cells appear to have a higher N requirement than nontoxic cells (Vézic et al., 2002; Davis et al., 2010), likely related at least in part to the additional N requirements associated with the enzymes involved in MC synthesis (Tillet et al., 2000) and perhaps with additional light-harvesting pigments (Hesse et al., 2001). MC is a N-rich compound (average of 10 N atoms per molecule) and MC can represent up to 2% of cellular dry weight of toxic *Microcystis* cells (Nagata et al., 1997). Accordingly, in many eutrophic systems MC concentrations have more commonly been reported to increase in response to increasing N than increasing P (Gobler et al., 2007; Donald et al., 2011, Chaffin et al., 2013; Chaffin & Bridgeman 2013, Davis et al., 2015).

Interactions between N and other environmental factors further influence cyanotoxin concentrations (Chorus and Bartram, 1999; Zurawell et al., 2005; and references therein). For example, when MC⁺ strains dominate assemblages in the early bloom phase when N concentrations are high, there is lower overall biomass and, thus, higher average light intensities. MC⁺ strains have been shown to grow well under higher light intensities, better than their MC⁻ counterparts (Zilliges et al., 2011), while MC⁻ strains are better competitors at low light intensities characteristic of dense blooms (Kardinaal et al. 2007). Hence, while N plays a primary role in shaping the relative abundance of MC-producing cells in an ecosystem setting, other biotic and abiotic factors likely act and interact to influence these populations as well.

Expectations for Lake Agawam following nitrogen reductions

The primary water quality impairment within Lake Agawam is the annual occurrence of blue-green algal blooms that synthesize toxins and contribute toward low oxygen conditions. While blue-green algae are traditionally considered to be controlled by phosphorus, prior research has demonstrated that in Lake Agawam, their growth is stimulated by nitrogen loading (Gobler et al., 2007; in prep; Task 1, this report). Moreover, there is now a growing consensus among

scientists and US EPA that nitrogen can be as important in controlling blue-green algal blooms (Paerl and Otten, 2016; US EPA, 2015) and that nitrogen is the prime element controlling toxin synthesis by *Microcystis* and the other non-nitrogen fixing blue-green algae that dominate Lake Agawam for most of the year (Gobler et al., 2016).

Prior research in Lake Agawam has shown that the addition of nitrogen stimulates the growth of blue green algae (Gobler et al., 2007; in prep; Task 1, this report) and that reducing the amount of nitrogen entering the Lake can reduce the intensity of blooms (Harke et al., 2008). Relying on the findings of Harke et al (2008) and Harke and Gobler (2015), a model was constructed to relate the intensity and toxicity of blue-green algae blooms within Lake Agawam to nitrogen loading rates. The model is based on the principle that the relationship between nitrogen loading and blue-green algal biomass during summer months is 1:1.1 and thus the 30% reduction in nitrogen loading to the system is expected to reduce the intensity of blue-green algal blooms by 33% during the 10-year time frame (Figure 25). Alternatively, over the 50-year time horizon, if the sewer district was created and the septic systems within the watershed were upgraded to alternative system and/or connected to the sewage treatment plant, this could reduce blue-green algal bloom intensity by 55% (Figure 25). To put these values in perspective, in 2016, blue-green algae biomass levels in Lake Agawam forced NYSDEC to list the lake on their state-wide alert system for 86% of the weeks between June and November (Figure 26). With the implementation of the sewer district alone, after a decade it is expected that this percentage would drop to 68% of weeks (Figure 26). If the remaining septic systems in the Village were upgraded or attached to the sewage treatment plant, Lake Agawam would be not listed by NYSDEC more often than it was listed (Figure 26). All of these benefits are mostly likely to be realized during warmer months when temperatures are high and rates of freshwater flow and thus freshwater flow derived nutrients are lower.

Beyond reduced biomass of blue-green algae, the reduction in nitrogen loading will reduce the toxicity of these algae since they are consistently dominated by *Microcystis* which synthesizes microcystin, a liver-toxin that is rich in nitrogen (Gobler et al., 2016). Moreover, recent research has definitively shown that the nitrogen content of *Microcystis* is controlled by nitrogen loading (Gobler et al., 2007; Harke and Gobler, 2013; Horst et al., 2014; Van Der Waal et al., 2014; Davis et al., 2015; Gobler et al., 2016). In addition, a series of studies in ecosystems around the world

has shown that as nitrogen levels in a waterbody decline, the *Microcystis* community shifts from one dominated by cells that make microcystin to a community dominated by cells lacking the ability to synthesize the toxin (Davis et al., 2010, 2015; Horst et al., 2014; Gobler et al., 2016). Harke and Gobler (2013, 2015) demonstrated that when *Microcystis* is deprived of nitrogen, its microcystin content per cell declines by ~50%. Davis et al. (2009, 2010) found that the majority of *Microcystis* cells in Lake Agawam are non-toxic (50 – 99.99%) and that enhanced nitrogen loading promotes the growth of toxic cells whereas low nitrogen levels reduces it. A three-year study in Lake Erie recently demonstrated that in a drought year with a low river flow into the western basin of the Lake and low inorganic nitrogen concentrations in the Lake, the levels of microcystin were 90% lower and nearly undetectable (Gobler et al., 2016).

Given that lower nitrogen levels will reduce the biomass of *Microcystis*, the proportion of cells that synthesize microcystin, and the microcystin content per *Microcystis* cell, the reduction in microcystin levels associated with the sewerage of the Village of Southampton will be large. This study has demonstrated that total blue-green algal biomass should be reduced by 20 - 50%. Toxin content per cell could decline by up to 50% (Harke and Gobler, 2013, 2015). The percentage of toxic cells could be reduced to 0.01% of the total population (Davis et al., 2009). In Lake Erie, a year with low nitrogen delivery and low nitrogen concentrations yielded a >90% reduction in microcystin levels (Gobler et al., 2016). Given all of these facts, the levels of microcystin in Lake Agawam would be conservatively reduced by at least 50% by the proposed sewerage effort. However, given the observations in Lake Erie and the dynamics of toxic and non-toxic cells in response to reduced nitrogen loading a reduction, an even larger reduction may be likely. Being conservative and applying a 50% decline to the microcystin concentrations present in Lake Agawam in 2016, this decrease in toxin levels due sewer improvements of nitrogen would create a scenario whereby the lake would rarely exceed the WHO guideline for a moderate recreational risk for exposure to microcystin (Figure 27). Alternatively, over the 50-year time horizon, if the sewer district was created and the septic systems within the watershed were upgraded to alternative system and/or connected to the sewage treatment plant, this could reduce algal bloom toxicity by 75% (Figure 27). To put these value in perspective, in 2016, microcystin levels in Lake Agawam exceeded the level stated to be a recreational risk to humans by the World Health Organization (WHO; 4 micrograms per liter, Chorus and Bartram, 1999) for 75% of the weeks between June and November (Figure 28). With the implementation of the sewer district alone, after a decade it

is expected that this percentage would drop to 54% of weeks (Figure 28). If the remaining septic systems in the Village were upgraded or attached to the sewage treatment plant, Lake Agawam would exceed the World Health Organization recreational risk in only 21% of cases (Figure 28). All of these benefits are mostly likely to be realized during warmer months when temperatures are high and rates of freshwater flow and thus freshwater flow derived nutrients are lower.

Along with the reduction in the intensity of blue-green algae and microcystin levels in Lake Agawam, there are a host of additional ecosystem benefits to Lake Agawam that are likely. The at least 50% reduction in microcystin levels in Lake Agawam will create an ecosystem that is less toxic and less of an immediate and long-term health threat to animals and humans. Dogs will be significantly less likely to be poisoned from drinking lake water. Animals in the lake will be less likely to accumulate toxin, benefiting their health as well as the health of individuals consuming lake animals. This includes residents and visitors, as people have been known to regularly fish in Lake Agawam and consume the fish caught there despite the presence of microcystin levels in the fillets of fish in the lake that exceed guideline values set by the WHO (Chorus and Bartram, 1999).

Reducing the intensity and toxicity of blue-green algae blooms in Lake Agawam, will have many other ecosystem benefits. The diversity of the phytoplankton community in Lake Agawam will likely increase. Blue-green algae compete with diatoms and green algae for dominance in Lake Agawam (Gobler lab, 2013-2016), and prior research has shown that nutrient reductions selectively reduce blue-green algae biomass more than other phytoplankton in general (Elser et al., 2007) and in Lake Agawam (Harke et al., 2008). This change will have whole ecosystem benefits. It is well-known that blue green algae are poorly grazed by zooplankton compared to other phytoplankton (Wilson et al., 2006; Ger et al., 2016) and during summer, blue-green algae bloom to the exclusion of other phytoplankton. Since zooplankton are the next step in aquatic food webs that ultimately yield fish, under current conditions, blooms of blue green algae are inhibiting the productivity of fish populations, especially pelagic fish that feed in the water (Downing and Plante, 1993). Hence, as nitrogen reductions begin to alter phytoplankton populations and reduce the prevalence of blue-green algae and enhance phytoplankton diversity, zooplankton populations should rebound, a change that will benefit pelagic fish populations.

Other changes wrought by a lowered intensity of blue-green algae blooms should include increased water clarity, improved dissolved oxygen levels, and enhanced levels of submerged

aquatic vegetation, and these changes are likely to have positive, synergistic effects on each other and fish populations. More than a decade of research in Lake Agawam has shown that water clarity is highly significantly correlated with the levels of algal biomass in the Lake ($p < 0.001$). Therefore, the 20 -50% reduction in blue-green algal biomass will significantly improve water clarity allowing more light to penetrate to the bottom of Lake Agawam. This should promote the growth of submerged aquatic vegetation in regions that previously were light-limited. Such vegetation can benefit fish populations whose juvenile forms may utilize the vegetation as a nursery habitat. These aquatic plants will also produce oxygen as they photosynthesize, thus enhancing oxygen levels in the Lake. Finally, the reduction in algal biomass from sewerage should also benefit the levels of nighttime dissolved oxygen in Lake Agawam. In September of 2006, there was a massive fish kill in Lake Agawam where 1,000s of white perch died overnight as a blue-green algae bloom was collapsing and dissolved oxygen levels declined to nearly zero. At night, in the absence of photosynthesis, dissolved oxygen levels are controlled by respiration rates which consume oxygen. These respiration rates are proportional to the total amount of algal biomass produced in the Lake which can directly respire or can result in bacterial respiration as the carbon from the algal biomass is consumed and/or degraded. In either scenario, reduced algal biomass from sewerage will reduce the incidence and likelihood of low dissolved oxygen levels and fish kills in Lake Agawam and thus should contribute toward a rebuilding of healthy fish stocks in this ecosystem.

Finally, there will be a financial benefit of sewerage the Village. Recent research at Stony Brook University has determined that waterfront or near-waterfront home values can be strongly effected by water clarity, with lowered water clarity leading to lowered home prices. Hence, the improved water clarity associated with 20 – 50% lower intensity algal blooms should financially benefit home owners in the region as well as associated tax revenues. Obviously, other benefits such as fewer fish kills and lower toxin levels will also likely improve home values as well as the number of visitors to Lake Agawam and the Village, occurrences that will have direct and indirect financial benefits for the Village and its residents.

References

- Ame, M.V., Wunderlin, D.A., 2005. Effects of iron, ammonium and temperature on microcystin content by a natural concentrated *Microcystis aeruginosa* population. *Water Air Soil Pollut.* 168, 235-248.
- Backer, L. C., Landsberg, J. H., Miller, M., Keel, K., & Taylor, T. K. (2013). Canine cyanotoxin poisonings in the United States (1920s–2012): Review of suspected and confirmed cases from three data sources. *Toxins*, 5(9), 1597-1628.
- Beaulieu, M., Pick, F., Gregory-Eaves, I., 2013. Nutrients and water temperature as significant predictors of cyanobacterial biomass in a 1147 lakes dataset. *Limnol. Oceanogr.* 58, 1736-1746.
- Beversdorf, L.J., Miller, T.R., McMahon, K.D., 2013. The role of nitrogen fixation in cyanobacterial bloom toxicity in a temperate, eutrophic lake. *PLoS ONE* 8, e56103. doi:10.1371/journal.pone.0056103.
- Beversdorf, L.J., Miller, T.R., McMahon, K.D., 2015. Long-term monitoring reveals carbon-nitrogen metabolism is key to microcystin production in eutrophic lakes. *Frontiers in Ecology* 6, 1-12.
- Berman, T., Chava, S., 1999. Algal growth on organic compounds as nitrogen sources. *J. Plankton Res.* 21, 1423-1437.
- Bittencourt-Oliveira, M.C., Cabral de Oliveira, M., Bolch, C.J.S., 2001. Genetic variability of Brazilian strains of the *Microcystis aeruginosa* complex (Cyanobacteria/Cyanophyceae) using the phycocyanin intergenic spacer and flanking regions (cpcBA). *J. Phycol.* 37, 810-818.
- Bowen, J. L., & Valiela, I. (2001). The ecological effects of urbanization of coastal watersheds: historical increases in nitrogen loads and eutrophication of Waquoit Bay estuaries. *Canadian Journal of Fisheries and Aquatic Sciences*, 58(8), 1489-1500.

- Bowen, J. L., Ramstack, J. M., Mazzilli, S., & Valiela, I. (2007). NLOAD: an interactive, web-based modeling tool for nitrogen management in estuaries. *Ecological Applications*, 17(sp5), S17-S30.
- Briand, E., Gugger, M., Francois, J.-C., Bernard, C., Humbert, J.-F., Quiblier, C., 2008. Temporal variations in the dynamics of potentially microcystin-producing strains in a bloom-forming *Planktothrix agardhii* (cyanobacterium) population. *Appl. Environ. Microbiol.* 74, 3839-3848.
- Briand, E., Escoffier, N., Straub, C., Sabart, M., Quiblier, C., Humbert, J.-F., 2009. Spatiotemporal changes in the genetic diversity of a bloom-forming *Microcystis aeruginosa* (cyanobacteria) population. *The ISME Journal* 3, 419-429.
- Bridgeman, T.B., Chaffin, J.D., 2013. Diversity of *Microcystis* across trophic gradients. Final Report to the Ohio Lake Erie Commission. Lake Erie Center and Department of Environmental Sciences, University of Toledo, Oregon, OH.
- Brittain, S.M., Wang, J., Babcock-Jackson, L., Carmichael, W.W., Rinehart, K.L., Culver, D.A., 2000. Isolation and characterization of microcystins, cyclic heptapeptide hepatotoxins from a Lake Erie strain of *Microcystis aeruginosa*. *J. Great Lakes. Res.* 26, 241-249.
- Bunting, L., Leavitt, P.R., Gibson, C.E., McGee, E.J., Hall, V.A., 2007. Degradation of water quality in Lough Neagh, northern Ireland, by diffuse nitrogen flux from a phosphorus-rich catchment. *Limnol. Oceanogr.* 52, 354-369.
- Burford, M.A., Davis, T.W., Orr, P.T., Sinha, R., Willis, A., Neilan, B.A., 2014. Nutrient-related changes in the toxicity of field blooms of the cyanobacterium, *Cylindrospermopsis raciborskii*. *FEMS Microbiol. Ecol.* 89, 135-148.
- Burkholder, J.M., Glibert, P.M., 2006. Intraspecific variability: An important consideration in forming generalizations about toxigenic algal species. *Afr. J. Mar. Sci.* 28, 177-180.

- Burkholder, J.M., Glibert, P.M., 2013. Eutrophication and oligotrophication. In: Levin, S. (Ed.), Encyclopedia of Biodiversity, 2nd edition, Vol. 3. Academic Press, Waltham, MA, pp. 347-371.
- Carlton, R.G., Wetzel, R.G., 1988. Phosphorus flux from lake sediments: effect of epipelagic oxygen production. *Limnol. Oceanogr.* 33, 562-570.
- Chaffin, J.D., Bridgeman, T.B., Heckathorn, S.A., Mishra, S., 2011. Assessment of *Microcystis* growth rate potential and nutrient status across a trophic gradient in western Lake Erie. *J. Great Lakes Res.* 37, 92-100.
- Chaffin, J.D., Bridgeman, T.B., Bade, D.L., 2013. Nitrogen constrains the growth of late summer cyanobacterial blooms in Lake Erie. *Adv. Microbiol.* 3, 16-26.
- Chaffin, J.D., Bridgeman, T.B., Bade, D.L., Mobilian, C.N., 2014. Summer phytoplankton nutrient limitation in Maumee Bay of Lake Erie during high-flow and low-flow years. *J. Great Lakes Res.* 40, 524-531.
- Chorus, I., and J. Bartram (Eds.), 1999. Toxic Cyanobacteria in Water – A Guide to Their Public Health Consequences, Monitoring and Management. E & FN Spon for the World Health Organization, New York, NY.
- Civco D, Chabaeva A, Hurd J (2006) A Comparison of Approaches to Impervious Surface Characterization. In: Chabaeva A (ed) Geoscience and Remote Sensing Symposium, 2006 IGARSS 2006 IEEE International Conference on, p 1398-1402
- Codd, G.A., Poon, G.K., 1988. Cyanobacterial toxins. In: Rogers, L.L., Gallon, J.R. (Eds.), Biochemistry of the Algae and Cyanobacteria. Proceedings of the Phytochemistry Society of Europe, Vol. 28. Oxford University Press, Oxford, UK, , pp. 283-296.
- Conley, Daniel J., Paerl, H.W., Howarth, R.W., Boesch, D.F., Seitzinger, S.P., Havens, K.E., Lancelot, C., Likens, G.E., 2009. Controlling eutrophication: nitrogen and phosphorus *Science* 323: 1014-1015.

- Conroy, J.D., Edwards, W.J., Pontius, R.A., Kane, D.D., Zhang, H., Shea, J.F., Richey, J.N., Culver, D.A., 2005. Soluble nitrogen and phosphorus excretion of exotic freshwater mussels (*Dreissena* spp.): potential impacts for nutrient remineralisation in western Lake Erie. *Freshwater Biol.* 50, 1146-1162.
- Cox, P. A., Banack, S. A., Murch, S. J., Rasmussen, U., Tien, G., Bidigare, R. R., ... & Bergman, B. (2005). Diverse taxa of cyanobacteria produce β -N-methylamino-L-alanine, a neurotoxic amino acid. *Proceedings of the National Academy of Sciences of the United States of America*, 102(14), 5074-5078.
- Davis, T.W., Berry, D.L., Boyer, G.L., Gobler, C.J., 2009. The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of *Microcystis* during cyanobacteria blooms. *Harmful Algae* 8, 715-725.
- Davis, T.W., Harke, M.J., Marcoval, M.A., Goleski, J., Orano-Dawson, C., Berry, D.L., Gobler, C.J., 2010. Effects of nitrogenous compounds and phosphorus on the growth of toxic and non-toxic strains of *Microcystis* during cyanobacterial blooms. *Aquat. Microb. Ecol.* 61: 149-162.
- Davis, T.W., Bullerjahn, G.S., Tuttle, T., McKay, R.M., Watson, S.B., 2015. Effects of increasing nitrogen and phosphorus concentrations on phytoplankton community growth and toxicity during *Planktothrix* blooms in Sandusky Bay, Lake Erie. *Environ. Sci. Technol.* 49, 7197-7207.
- Dillon, P.J., Rigler, F.H., 1974. The phosphorus-chlorophyll relationship in lakes. *Limnol. Oceanogr.* 19, 767-773.
- Donald, D.B., Bogard, M.J., Finlay, K., Leavitt, P.R., 2011. Comparative effects of urea, ammonium, and nitrate on phytoplankton abundance, community composition, and toxicity in hypereutrophic freshwaters. *Limnol. Oceanogr.* 56, 2161-2175.
- Downing, J. A., & Plante, C. 1993. Production of fish populations in lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, 50(1), 110-120.

- Downing, T.G., Sember, C.S., Gehringer, M.M., Leukes, W., 2005. Medium N : P ratios and specific growth rate co-modulate microcystin and protein content in *Microcystis aeruginosa* PCC7806 and *M. aeruginosa* UV027. *Microb. Ecol.* 49, 468-473.
- Elser, J.J., Marzolf, E.R., Goldman, C.R., 1990. Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North America: a review and critique of experimental enrichments. *Can. J. Fish. Aquat. Sci.* 47, 1468-1477.
- Elser, J.J., Bracken, M.E.S., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., Ngai, J.T., Seabloom, E.W., Shurin, J.B., Smith, J.E. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol. Lett.* 10, 1135-1142.
- Fastner, J., Erhard, M., von Dohren, H., 2001. Determination of oligopeptide diversity within a natural population of *Microcystis* spp. (Cyanobacteria) by typing single colonies by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *Appl. Environ. Microbiol.* 67, 5069-5076.
- Fleming, R. and H. Fraser, *The Impact of Waterfowl on Water Quality - Literature Review*, 2001, University of Guelph.
- Fukushima, T., Amano, K., Muraoka, K. 1991. Factors explaining sediment concentrations of 16 elements in 28 Japanese lakes. *Water Sci. Technol.* 23, 465-474.
- Ginn, H.P., Pearson, L.A., Neilan, B.A., 2010. NtcA from *Microcystis aeruginosa* PCC 7806 is autoregulatory and binds to the microcystin promoter. *Appl. Environ. Microbiol.* 76, 4362-4368.
- Glibert, P.M., Fullerton, D., Burkholder, J.M., Cornwell, J.C., Kana, T.M., 2011. Ecological stoichiometry, biogeochemical cycling, invasive species and aquatic food webs: San Francisco Estuary and comparative systems. *Rev. Fish. Sci.* 19: 358-417.
- Glibert, P.M., Wilkerson, F.P., Dugdale, R.C, Raven, J.A., Dupont, C., Leavitt, P.R., Parker, A.E., Burkholder, J.M., Kana, T.M., 2015. Pluses and minuses of ammonium and nitrate

- uptake and assimilation by phytoplankton and implications for productivity and community composition, with emphasis on nitrogen-enriched conditions. *Limnol. Oceanogr.*, DOI 10.1002/Ino.10203.
- Ger, K. A., Urrutia-Cordero, P., Frost, P. C., Hansson, L. A., Sarnelle, O., Wilson, A. E., & Lürling, M. (2016). The interaction between cyanobacteria and zooplankton in a more eutrophic world. *Harmful Algae*, 54, 128-144.
- Gobler, C.J., Davis, T.W., Coyne, K.J., Boyer, G.L., 2007. Interactive influences of nutrient loading, zooplankton grazing, and microcystin synthetase gene expression on cyanobacterial bloom dynamics in a eutrophic New York lake. *Harmful Algae* 6, 119-133.
- Gobler CJ, Burkholder JM, Davis TW, Harke MJ, Johengen T, Stow CA, Van de Waal DM. 2016. The dual role of nitrogen supply in controlling the growth and toxicity of cyanobacterial blooms. *Harmful Algae* 54, 87-97
- Gobler, CJ, Stinnette, I. 2016. Nitrogen Loading to the South Shore, Eastern Bays, NY: Sources, Impacts, and Management Options. Final Report to the NYS DOC South Shore Estuary Reserve Program
- Granéli, E., Flynn, K.J., 2006. Chemical and physical factors influencing toxin content, p. 229–241. In: E. Granéli, E., Turner, J.T. (Eds.), *Ecology of Harmful Algae*. Springer, Berlin, Germany, pp. 229-241.
- Harke, M.J., Gobler, C.J., 2013. Global transcriptional responses of the toxic cyanobacterium, *Microcystis aeruginosa*, to nitrogen stress, phosphorus stress, and growth on organic matter. *PLoS ONE* 8(7): E69834. doi:10.1371/journal.pone.0069834.
- Harke, M., Davis, T., Watson, S., Gobler, C.J., 2015. Nutrient-Controlled Niche Differentiation of Western Lake Erie Cyanobacterial Populations Revealed via Metatranscriptomic Surveys. *Environ. Sci. Technol.* DOI: 10.1021/acs.est.5b03931
- Heron, J., 1961. The seasonal variation of phosphate, silicate, and nitrate in waters of the English lake district. *Limnol. Oceanogr.* 6, 338-346.

- Hesse, K., Dittmann, E., Börner, T., 2001. Consequences of impaired microcystin production for light-dependent growth and pigmentation of *Microcystis aeruginosa* PCC 7806. FEMS Microbiol Ecol. 37. 39-43.
- Horst, G. P., Sarnelle, O., White, J.D., Hamilton, S.K., Kaul, R.B., Bressie, J.D., 2014. Nitrogen availability increases the toxin quota of a harmful cyanobacterium, *Microcystis aeruginosa*. Water Res. 54, 188-198.
- Hupfer, M., Lewandowski, J., 2008. Oxygen controls the phosphorus release from lake sediments – a long-lasting paradigm in limnology. Internat. Rev. Hydrobiol. 93, 414-432.
- Janse, I., Kardinaal, W.E.A., Kamst-van Agterveld, M., Meima, M., Visser, P.M., Zwart, G., 2005. Contrasting microcystin production and cyanobacterial population dynamics in two *Planktothrix*-dominated freshwater lakes. Environ. Microbiol. 7, 1514-1524.
- Jones, J., Bachmann, R., 1976. Prediction of phosphorus and chlorophyll levels in lakes. J. Wat. Pollut. Control Fed. 48, 2176-2184.
- Jones MN (1984) Nitrate reduction by shaking with cadmium: Alternative to cadmium columns. Water Research 18:643-646
- Kardinaal, W.E.A., Janse, I., Kamst-van Agterveld, M., Meima, M., Snoek, J., Mur, L.R., Huisman, J., Zwart, G., Visser, P.M., 2007. *Microcystis* genotype succession in relation to microcystin concentrations in freshwater lakes. Aquat. Microb. Ecol. 48, 1-12.
- Kellmann, R., Mihali, T.K., Jeon, Y.J., Pickford, R., Pomati, F., Neilan, B.A., 2008. Biosynthetic intermediate analysis and functional homology reveal a saxitoxin gene cluster in cyanobacteria. Appl. Environ. Microbiol. 74, 4044-4053.
- Kinney, E. L., & Valiela, I. (2011). Nitrogen loading to Great South Bay: land use, sources, retention, and transport from land to bay. Journal of Coastal Research, 27(4), 672-686.
- Kosten, S., Huszar, V.L.M., Bécares, E., Costa, L.S., van Donk, E., Hansson, L.-A., Jeppesen, E., Kruk, C., Lacerot, G., Mazzeo, N., de Meester, L., Moss, B., Lürling, M., Nöges, T.,

- Romo, S., Scheffer, M., 2012. Warmer climates boost cyanobacterial dominance in shallow lakes. *Global Change Biol.* 18: 118-126.
- Lee, S.J., Jang, M.-H., Kim, H.-S., Yoon, B.-D., Oh, H.-M., 2000. Variation on microcystin content of *Microcystis aeruginosa* relative to medium N:P ratio and growth stage. *J. Appl. Microbiol.* 89, 323-329.
- Lewis, W.M., Wurtsbaugh, W.A., 2008. Control of lacustrine phytoplankton by nutrients: erosion of the phosphorus paradigm. *Internat. Rev. Hydrobiol.* 93, 446-465.
- Lloyd, S. 2014. Nitrogen load modeling to forty-three subwatersheds of the Peconic Estuary . The Nature Conservancy Report. 20pp.
- Lloyd, S. 2016 Modeling nitrogen source loads on the north shore of Long Island. The Nature Conservancy Report. 36 pp.
- Lombardo Associates. 2013. LAKE AGAWAM WATER QUALITY RESTORATION ACTION PLAN VILLAGE OF SOUTHAMPTON, NY. 100pp
- Long, B.M., Jones, G.J., Orr, P.T., 2001. Cellular microcystin content in N-limited *Microcystis aeruginosa* can be predicted from growth rate. *Appl. Environ. Microbiol.* 67, 278-283.
- Meissner, S., Fastner, J., Dittmann, E., 2013. Microcystin production revisited: Conjugate formation makes a major contribution. *Environ. Microbiol.* 15, 1810-1820.
- Meissner, S., Steinhauser, D., Dittmann, E., 2015. Metabolomic analysis indicates a pivotal role of the hepatotoxin microcystin in high light adaptation of *Microcystis*. *Environ. Microbiol.* 17, 1497-1509.
- Monchamp, M.-E., Pick, F.R., Beisner, B.E., Maranger, R., 2014. Nitrogen forms influence microcystin concentration and composition via changes in cyanobacterial community structure. *PLoS ONE* 9, e85573. doi:10.1371/journal.pone.0085573.
- Monti, J., & Scorca, M. P. (2003). Trends in nitrogen concentration and nitrogen loads entering the South Shore Estuary Reserve from streams and ground-water discharge in Nassau and

- Suffolk counties, Long Island, New York, 1952-97. US Department of the Interior, US Geological Survey.
- Nagata, S., Tsutsumi, T., Hasegawa, A., Yoshida, F., Ueno, Y., Watanabe, M.F., 1997. Enzyme immunoassay for direct determination of microcystins in environmental water. *Journal of AOAC International* 80, 408-417.
- Nelson, Pope, and Voorhis. 2008. Comprehensive Management Plan for Lake Agawam
- O'Neill, J.M., Davis, T.W., Burford, M.A., Gobler, C.J., 2012. The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. *Harmful Algae* 14, 313-334.
- Orr, P.T., Jones, G.J., 1998. Relationship between microcystin production and cell division rates in nitrogen-limited *Microcystis aeruginosa* cultures. *Limnol. Oceanogr.* 43, 1604-1614.
- Orr, P.T., Rasmussen, J.P., Burford, M.A., Eaglesham, G.K., Lennox, S.M., 2010. Evaluation of quantitative real-time PCR to characterise spatial and temporal variations in cyanobacteria, *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya et Subba Raju and cylindrospermopsin concentrations in three subtropical Australian reservoirs. *Harmful Algae* 9, 243-254.
- Otten, T.G., Xu, H., Qin, B., Zhu, G., Paerl, H.W., 2012. Spatiotemporal patterns and ecophysiology of toxigenic *Microcystis* blooms in Lake Taihu, China: implications for water quality management. *Environ. Sci. Technol.* 46, 3480-3488.
- Paerl HW (1988) Nuisance phytoplankton blooms in coastal, estuarine, and inland waters. *Limnology and Oceanography* 33:823-847
- Paerl HW, Fulton RS, Moisander PH, Dyble J (2001) Harmful freshwater algal blooms with an emphasis on cyanobacteria. *The Scientific World* 1:76-113
- Paerl, H. W., & Otten, T. G. (2016). Duelling 'CyanoHABs': unravelling the environmental drivers controlling dominance and succession among diazotrophic and non-N₂-fixing harmful cyanobacteria. *Environmental microbiology*, 18(2), 316-324.

- Parsons TR, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for seawater analysis., Vol. Pergamon Press, Oxford
- Peconic Estuary Program (PEP). (2001). Comprehensive Conservation and Management plan.
- Pimentel, J.S.M., Giani, A., 2014. Microcystin production and regulation under nutrient stress conditions in toxic *Microcystis* strains. *Appl. Environ. Microbiol.* 80, 5836-5843.
- Reitzel, K., Ahlgren, J., Debrabandere, H., Waldebäk, M., Gogoll, A., Tranvik, L., 2007. Degradation rates of organic phosphorus in lake sediments. *Biogeochemistry* 82, 15-28.
- Reynolds, C.S., 1984. *The Ecology of Freshwater Phytoplankton*. Cambridge Studies in Ecology. Cambridge University Press, New York, NY.
- Schindler, D.W., 1974. Eutrophication and Recovery in Experimental Lakes: Implications for Lake Management. *Science* 184, 897-899.
- Schindler, D.W., 1977. Evolution of phosphorus limitation in lakes. *Science* 195, 260-262.
- Schindler, D.W., Hecky, R.E., Findlay, D.L., Stainton, M.P., Parker, B.R., Paterson, M.J., Beaty, K.G., Lyng, M., Kasian, S.E.M., 2008. Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37-year whole-ecosystem experiment. *Proc. Nat. Acad. Sci. (USA)* 105, 11254-11258.
- Schindler, D.W., 2012. The dilemma of controlling cultural eutrophication of lakes. *Proc. R. Soc. Lond. B* 279, 4322-4333.
- Schubert, C. E. (1998). Areas contributing ground water to the Peconic estuary, and ground-water budgets for the North and South forks and Shelter Island, Eastern Suffolk County, New York.
- Scott, J.T., McCarthy, M.J., 2010. Nitrogen fixation may not balance the nitrogen pool of lakes over timescales relevant to eutrophication management. *Limnol. Oceanogr.* 55, 1265-1270.
- Scott, J.T., McCarthy, M.J. 2011. Response to comment: Nitrogen fixation has not offset declines in the Lake 227 nitrogen pool and shows that nitrogen control deserves consideration in aquatic ecosystems. *Limnol. Oceanogr.* 56, 1548-1550.

- Singh, S., Rai, P.K., Chau, R., Ravi, A.K., Neilan, B.A., Asthana, R.K., 2015. Temporal variations in microcystin-producing cells and microcystin concentrations in two freshwater ponds. *Water Research* 69, 131-142.
- Sivonen, K., Jones, G., 1999. Cyanobacterial toxins, In: Chorus, I., Bartram, J. (Eds.), *Toxic Cyanobacteria in Water: A Guide to their Public Health Consequences, Monitoring and Management*. E. & F.N. Spon, London, UK, pp. 41-111.
- Smith, V.H., 1983. Low nitrogen to phosphorus ratios favor dominance by blue-green-algae in lake phytoplankton. *Science* 221: 669-671.
- Søndergaard, M., Jensen, J.P., Jeppesen, E., 2003. Role of sediment and internal loading of phosphorus in shallow lakes. *Hydrobiologia* 506, 135-145.
- Sterner, R.W., 2008. On the phosphorus limitation paradigm for lakes. *Internat. Rev. Hydrobiol.* 93, 433-445.
- Stow, C.A., Cha, Y.K., Johnson, L.T., Confesor, R., Richards, R.P., 2015. Long-term and seasonal trend decomposition of Maumee River nutrient inputs to western Lake Erie. *Environ. Sci. Technol.* 49, 3392-3400.
- Štrojsová, A., Vrba, J., Nedoma, J., Šimek, K. . 2005. Extracellular phosphatase activity of freshwater phytoplankton exposed to different in situ phosphorus concentrations. *Mar. Freshwat. Res.* 56, 417-424.
- Stumpf, R.P., Wynne, T.T., Baker, D.B., Fahnenstiel, G.L., 2012. Interannual variability of cyanobacterial blooms in Lake Erie. *PLoS ONE* 7(8): e42444.doi:10.1371/journal.pone.0042444.
- Sunda, W.G., Granéli, E., Gobler, C.J., 2006. Positive feedback and the development and persistence of ecosystem disruptive algal blooms. *J. Phycol.* 42, 963-974.
- Tapia, M.I., deAlda, J., Llama, M.J., Serra, J.L., 1996. Changes in intracellular amino acids and organic acids induced by nitrogen starvation and nitrate or ammonium resupply in the cyanobacterium *Phormidium laminosum*. *Planta* 198, 526-531.

- Tillett, D., Dittmann, E., Erhard, M., von Dohren, H., Borner, T., Neilan, B.A., 2000. Structural organization of microcystin biosynthesis in *Microcystis aeruginosa* PCC7806: an integrated peptide–polyketide synthetase system. *Chem. Biol.* 7, 753-764.
- Trimbee, A.M., Prepas, E.E., 1987. Evaluation of total phosphorus as a predictor of the relative biomass of blue-green-algae with emphasis on Alberta lakes. *Can. J. Fish. Aquat. Sci.* 44, 1337-1342.
- Turner, R.E., Rabalais, N.N., Justic, D., Dortch, Q., 2003. Global patterns of dissolved N, P and Si in large rivers. *Biogeochemistry* 64, 297-317.
- United States Environmental Protection Agency (U.S.EPA), 2000. Nutrient Criteria Technical Guidance Manual, Rivers and Streams. Report EPA-822-B-00-002. Office of Water, U.S. EPA, Washington, DC. Available at: <http://www.epa.gov/waterscience/criteria/nutrient/guidance/rivers>, last accessed in November 2015.
- United States National Academy of Sciences (USNAS) 1969. Eutrophication: causes, consequences, correctives. Proceedings of a Symposium. National Academy of Sciences Publication 1700. National Academy Press, Washington, DC.
- United States Environmental Protection Agency (U.S. EPA), 2015. Preventing eutrophication: scientific support for dual nutrient criteria. Office of Water, U.S. EPA, Washington, DC.
- Valderrama JC (1981) The simultaneous analysis of total nitrogen and phosphorus in natural waters. *Marine Chemistry* 10:109-122
- Valiela, I., Collins, G., Kremer, J., Lajtha, K., Geist, M., Seely, B., ... & Sham, C. H. (1997). Nitrogen loading from coastal watersheds to receiving estuaries: new method and application. *Ecological Applications*, 7(2), 358-380.
- Van de Waal, D.B., Verspagen, J.M.H., Lurling, M., Van Donk, E., Visser, P.M., Huisman, J., 2009. The ecological stoichiometry of toxins produced by harmful cyanobacteria: An experimental test of the carbon-nutrient balance hypothesis. *Ecol. Lett.* 12, 1326-1335.

- Van de Waal, D.B., Ferreruella, G., Tonk, L., Van Donk, E., Huisman, J., Visser, P.M., Matthijs, H.C.P., 2010. Pulsed nitrogen supply induces dynamic changes in the amino acid composition and microcystin production of the harmful cyanobacterium *Planktothrix agardhii*. FEMS Microbiol. Ecol. 74, 430-438.
- Van de Waal, D.B., Smith, V.H., Declerck, S.A.J., Stam, E.C.M., Elser, J.J., 2014. Stoichiometric regulation of phytoplankton toxins. Ecol. Lett. 17, 736-742.
- Vézie, C., Rapala, J., Vaitomaa, J., Seitsonen, J., Sivonen, K., 2002. Effect of nitrogen and phosphorus on growth of toxic and nontoxic *Microcystis* strains and on intracellular microcystin concentrations, Microb. Ecol. 43, 443-454.
- Visser, P.M., Passarge, J., Mur, L.R., 1997. Modelling vertical migration of the cyanobacterium *Microcystis*. Hydrobiologia 349, 99-109.
- Watanabe, M.F., Oishi, S., 1985. Effects of environmental factors on toxicity of a cyanobacterium (*Microcystis aeruginosa*) under culture conditions. Appl. Environ. Microbiol. 49: 1342-1344.
- Watson, S.B., McCauley, E., Downing, J.A., 1997. Patterns in phytoplankton taxonomic composition across temperate lakes of differing nutrient status. Limnol. Oceanogr. 42, 487-495.
- Welker, M., von Dohren, H., Tauscher, H., Steinberg, C.E.W., Erhard, M., 2003. Toxic *Microcystis* in shallow Lake Muggelsee (Germany) – dynamics, distribution, diversity. Arch. Hydrobiol. 157, 227-248.
- Welker, M., Šejnohová, L., Némethová, D., von Döhren, H., Jarkovský, J., Maršálek, B. 2007. Seasonal shifts in chemotype composition of *Microcystis* sp. communities in the pelagial and the sediment of a shallow reservoir. Limnol. Oceanogr. 52, 609-619.
- Wetzel, R. G., 1983. Limnology, 2nd edition. W. B. Saunders, Fort Worth, TX.
- Wetzel, R.G., 2001. Limnology: Lake and River Ecosystems, 3rd edition. Academic Press, San Diego, CA.

- Wilson, A.E., Sarnelle, O., Tillmanns, A.R., 2006. Effects of cyanobacterial toxicity and morphology on the population growth of freshwater zooplankton: Meta-analyses of laboratory experiments. *Limnology and Oceanography* 51(4), 1915-1924.
- World Health Organization (WHO), 2011. Management of cyanobacteria in drinking-water supplies: Information for regulators and water suppliers. WHO, Geneva, Switzerland. Available at: http://www.who.int/water_sanitation_health/dwq/cyanobacteria_in_drinking-water.pdf, last accessed in November 2015.
- Wynne, T.T., Stumpf, R.P. 2015. Spatial and temporal patterns in the seasonal distribution of toxic cyanobacteria in Western Lake Erie from 2002-2014. *Toxins (Basel)* 7, 1649-1663.
- Xu, H., Paerl, H.W., Qin, B.Q., Zhu, G.W., Gao, G., 2010. Nitrogen and phosphorus inputs control phytoplankton growth in eutrophic Lake Taihu, China. *Limnol. Oceanogr.* 55, 420-432.
- Yuan, L.L., Pollard, A.I., Pather, S., Oliver, J.L., D'Anglada, L., 2014. Managing microcystin: identifying national-scale thresholds for total nitrogen and chlorophyll *a*. *Freshwater Biol.* 59, 1970-1981.
- Zilliges, Y., Kehr, J.-C., Meissner, S., Ishida, K., Mikkat, S., Hagemann, M., Kaplan, A., Boerner, T., Dittmann, E., 2011. The cyanobacterial hepatotoxin microcystin binds to proteins and increases the fitness of *Microcystis* under oxidative stress conditions. *PLoS ONE* 6(3): e17615. doi:10.1371/journal.pone.0017615.
- Zurawell, R.W., Chen, H., Burke, J.M., Prepas, E.E., 2005. Hepatotoxic cyanobacteria: A review of the biological importance of microcystins in freshwater environments. *J. Toxicol. Env. Health (B)* 8, 1-37.

Table 1. A comparison of the total phosphorus levels (TP), secchi disc depth or water clarity depth (Secchi), and chlorophyll *a* thresholds US EPA uses to define the trophic status of lakes. By these definitions, Lake Agawam is a hypereutrophic waterbody.

	TP (ug / L)	Secchi (m)	Chlorophyll (ug / L)
Ultraoligotrophic	< 3	> 6	< 1
Oligotrophic	4 - 12	3 - 6	1 - 4
Mesotrophic	12 - 30	2 - 3	4 - 8
Eutrophic	30 - 100	0.8 - 2	8 - 25
<u>Hypereutrophic</u>	> 100	< 0.5	> 25
Lake Agawam,	120	0.46	110

Table 2. Nutrient contributions to Lake Agawam in mol day⁻¹ as reported by Harke et al (2008). DIN, DIP, DON, DOP, TDN, and TDP are dissolved inorganic nitrogen and phosphorus, dissolved organic nitrogen and phosphorus, and total dissolved nitrogen and phosphorus.

	DIN	DIP	DON	DOP	TDN	TDP
Surface Runoff	7	0	10	1	17	1
Groundwater	718	5	14	2	733	7
Benthic Flux	354	129	241	173	594	302
Storm Drain	439	19	44	7	483	26
ATM	35	2	11	1	47	3

Table 3. Constants and data used for the nitrogen loading models constructed for this study.

	Agawam	Old Town	Wickapogue	Units	Source
Watershed area	435.3	155.9	144.8	ha	ArcGIS®
Area of wetlands (freshwater)	0	0	0	ha	NYS freshwater wetlands maps
Area of agriculture	8.5	4.0	7.8	ha	Suffolk County Land Use and Land Cover dataset
Area of golf courses	2.7	0	0	ha	Open Street Map, Manual Delineation
Area of parks and athletic fields (fertilized)	2.3	3.9	4.3	ha	Suffolk County Land Use and Land Cover dataset
Impervious surfaces total	168.9	61.5	44.5	ha	Low NDVI created from USGS High Resolution Orthoimagery, open water areas removed.
Area of freshwater ponds	26.4	4.1	4.5	ha	Suffolk County Land Use and Land Cover dataset
Total Occupancy >200m of shore	2847	652	240	People	H2M Sewer Analysis, 2010 census + estimated seasonal population from Suffolk County. ²⁸
Total Occupancy <200m of shore	48	46	14	People	H2M Sewer Analysis, 2010 census + estimated seasonal population from Suffolk County. ²⁸
Percent of buildings with cesspools	50	50	50	%	Southampton GIS department (houses built before 1973 have cesspools) (SB, QB), estimate MB
Area of residential lawns	95.1	28.6	42.2		High NDVI (USGS HRO), limited to residential parcels, limited to areas where LiDAR height data was near zero. (USGS LiDAR)
Percent of parcels with fertilized lawns	90	90	90	%	NYSDEC, LINAP subwatersheds committee
Area of roof per building	42.9	14.6	9.1	ha	Suffolk County building footprint dataset, 2006
Area of Driveways	86.3	31.2	27.9	Ha	Impervious layer limited to developed parcels
Area of road	37.6	14.9	4.1	ha	Impervious layer limited to non-taxed parcels
Nitrogen inputs from wet and dry deposition	16.9	16.9	16.9	kg N ha ⁻¹ yr ⁻¹	Suffolk County Recommendations
Fertilizer applied to golf courses	146	146	146	kg N ha ⁻¹ yr ⁻¹	Suffolk County Recommendations
Fertilizer applied to parks and athletic fields	146	146	146	kg N ha ⁻¹ yr ⁻¹	Suffolk County Recommendations
Fertilizer applied to agriculture	136	136	136	kg N ha ⁻¹ yr ⁻¹	Suffolk County Recommendations
Denitrification in the aquifer	7.5	7.5	7.5	%	Suffolk County Recommendations

Table 4. Annual nitrogen loading rates from all sources to Lake Agawam, Old Town Pond, and Wickapogue Pond in kg nitrogen per year for the 50 year watershed with percentages of the total nitrogen load represented by each process also shown.

Total Nload (kg/yr)	Agawam, current	Old Town Pond	Wickapogue Pond	Agawam, current	Old Town Pond	Wickapogue Pond
Atmosphere, watershed	720	258	218	3%	6%	8%
Waste Water, sewered area	4,152	-	-	20%	0%	0%
Waste Water, unsewered	10,363	2,498	908	49%	62%	33%
Southampton Hospital	-	131	-	0%	3%	0%
Sewage Treatment Plant	-	-	-	0%	0%	0%
Storm drain	928	42	51	4%	1%	2%
Fert - Residential Lawns	1,159	348	514	6%	9%	19%
Fert - Agriculture	308	144	283	1%	4%	10%
Fert - Parks and Golf	170	128	143	1%	3%	5%
Fertilizer, all	1,637	620	939	8%	15%	35%
Benthic flux	2,884	387	470	14%	10%	17%
Bird, geese, swans	219	36	36	1%	1%	1%
Direct atmosphere	134	75	91	1%	2%	3%
Total	21,037	4,048	2,714	100%	100%	100%

Table 5. Annual nitrogen loading rates from all sources to Lake Agawam in kg nitrogen per year for the 10 year watershed with percentages of the total nitrogen load represented by each process also shown. Three scenarios are presented: Current, with the addition of the sewage treatment plant, and with the addition of the sewage treatment plant and septic upgrades.

Total Nload (kg/yr)	Agawam, current	Agawam with STP	Agawam with STP and upgrades	Agawam, current	Agawam with STP	Agawam with STP and upgrades
Atmosphere, watershed	425	425	425	4%	5%	6%
Waste Water, sewered area	3,837	-	-	33%	0%	0%
Waste Water, unsewered	1,911	1,911	573	17%	23%	8%
Southampton Hospital	-	-	-	0%	0%	0%
Sewage Treatment Plant	-	691	691	0%	8%	10%
Storm drain	928	928	928	8%	11%	13%
Fert - Residential Lawns	754	754	754	7%	9%	11%
Fert - Agriculture	308	308	308	3%	4%	4%
Fert - Parks and Golf	142	142	142	1%	2%	2%
Fertilizer, all	1,203	1,203	1,203	10%	14%	17%
Benthic flux	2,884	2,884	2,884	25%	34%	41%
Bird, geese, swans	219	219	219	2%	3%	3%
Direct atmosphere	134	134	134	1%	2%	2%
Total	11,542	8,396	7,058	100%	100%	100%

Figure 1. 14-year record of chlorophyll *a* and day-time dissolved oxygen in Lake Agawam.

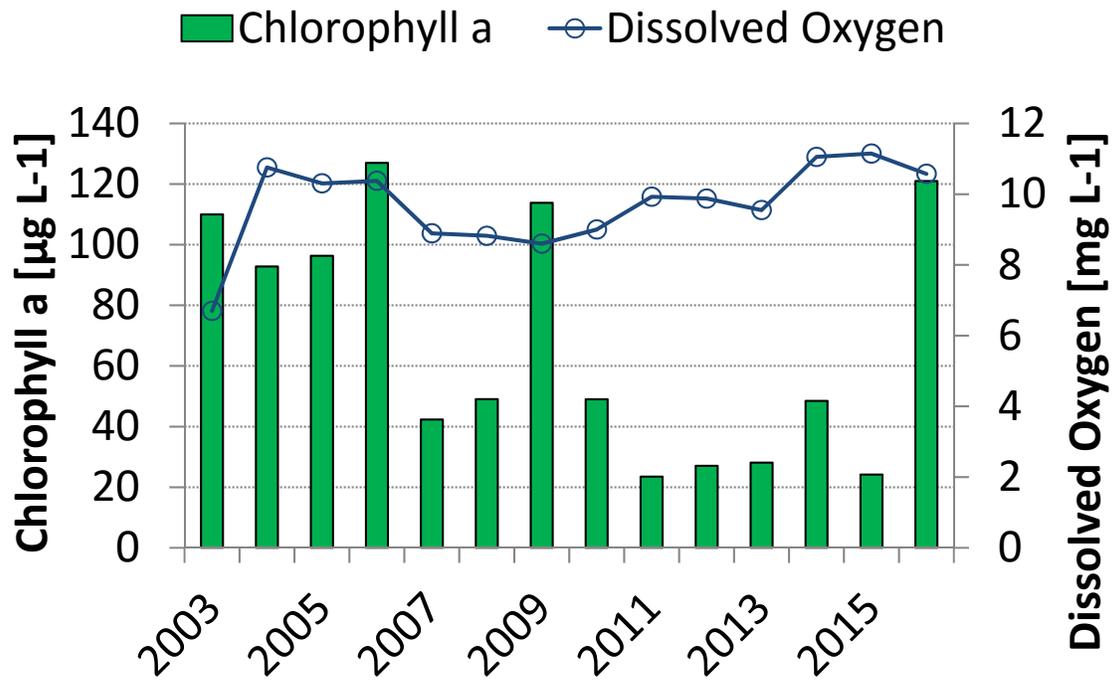


Figure 2. Blue-green algal bloom and fish kill in Lake Agawam. The fish kill occurred in September of 2006 when 1,000s of white perch died due to night-time low oxygen associated with the collapse of a blue-green algal bloom.



Blue green algae in Lake Agawam



2006 Fishkill in Lake Agawam

Figure 3. Dynamics of *Microcystis* and *Anabaena* in Lake Agawam in 2004 which are reflective of most years.

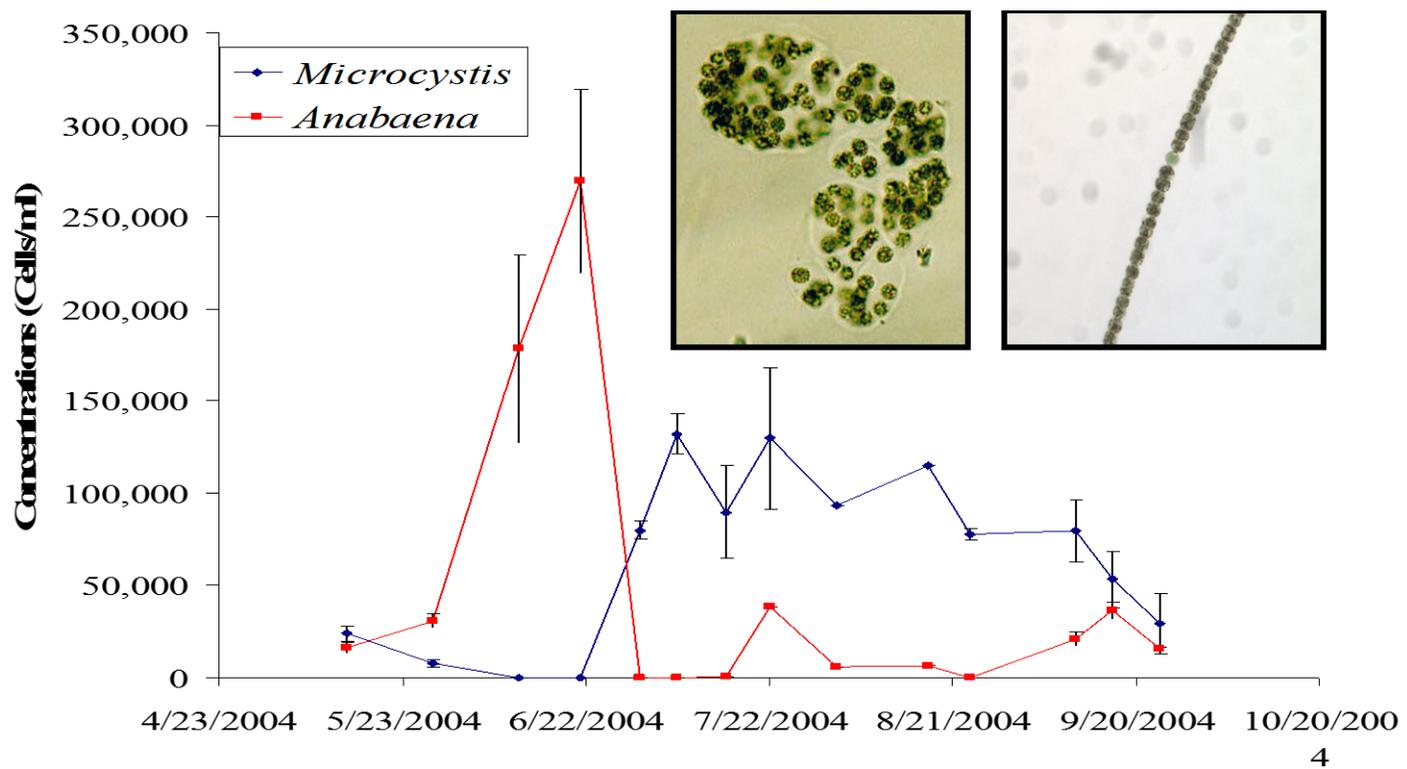


Figure 5. Dynamics of microcystin and anatoxin-a in Lake Agawam in 2004 which are reflective of most years.

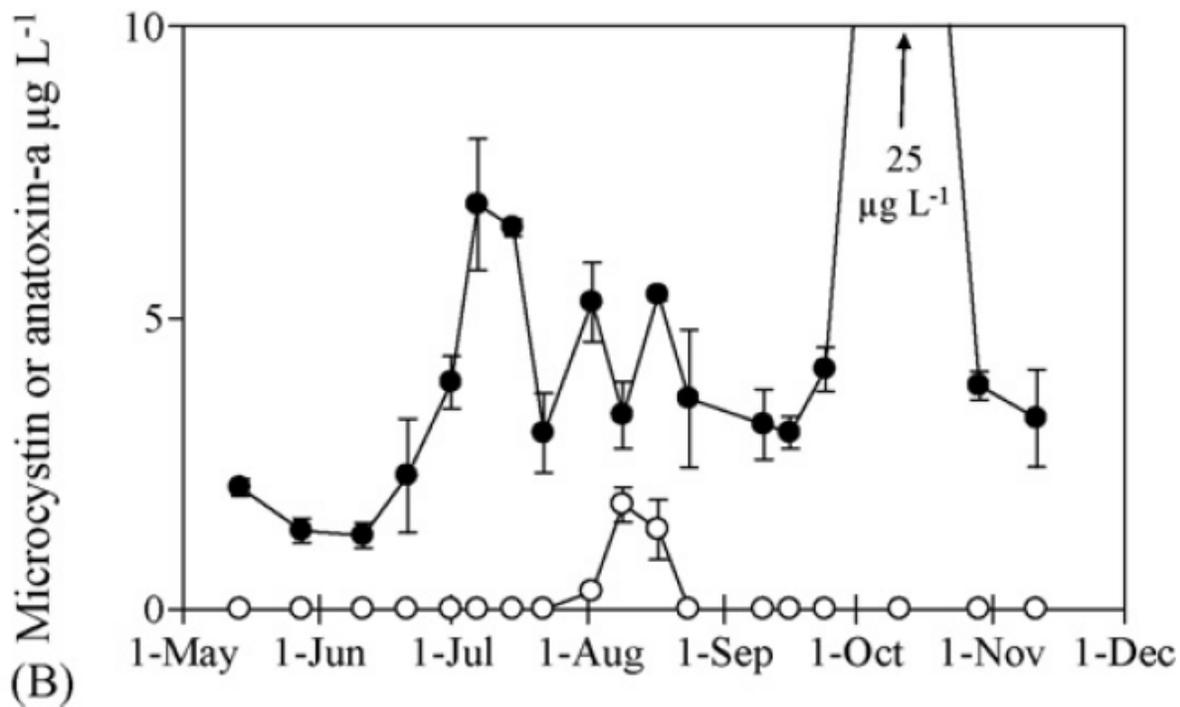


Figure 6. Levels of microcystin in the viscera and fillets of carp and bass in Lake Agawam.

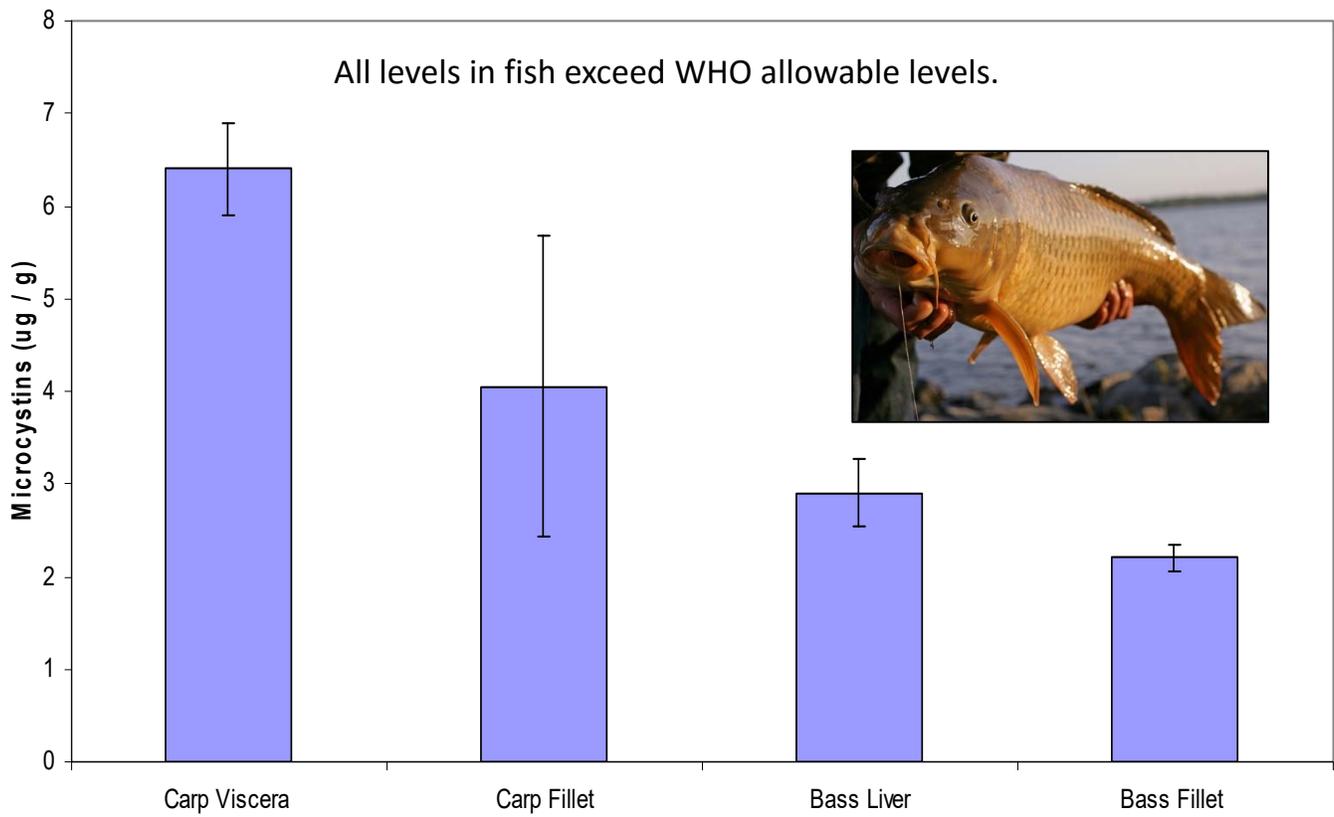


Figure 7. Sign from the first ever closure of Lake Agawam due to blue-green algal blooms in 2004. The Lake has been closed for part or most of the summer every year since.



Figure 8. Dynamics of microcystin in Lake Agawam from 2013 to 2016. Concentrations in the Lake and in shoreline scum are depicted. WHO has set a 1 ug/L threshold for safe drinking water and a 20ug/L threshold as a moderate recreational risk.

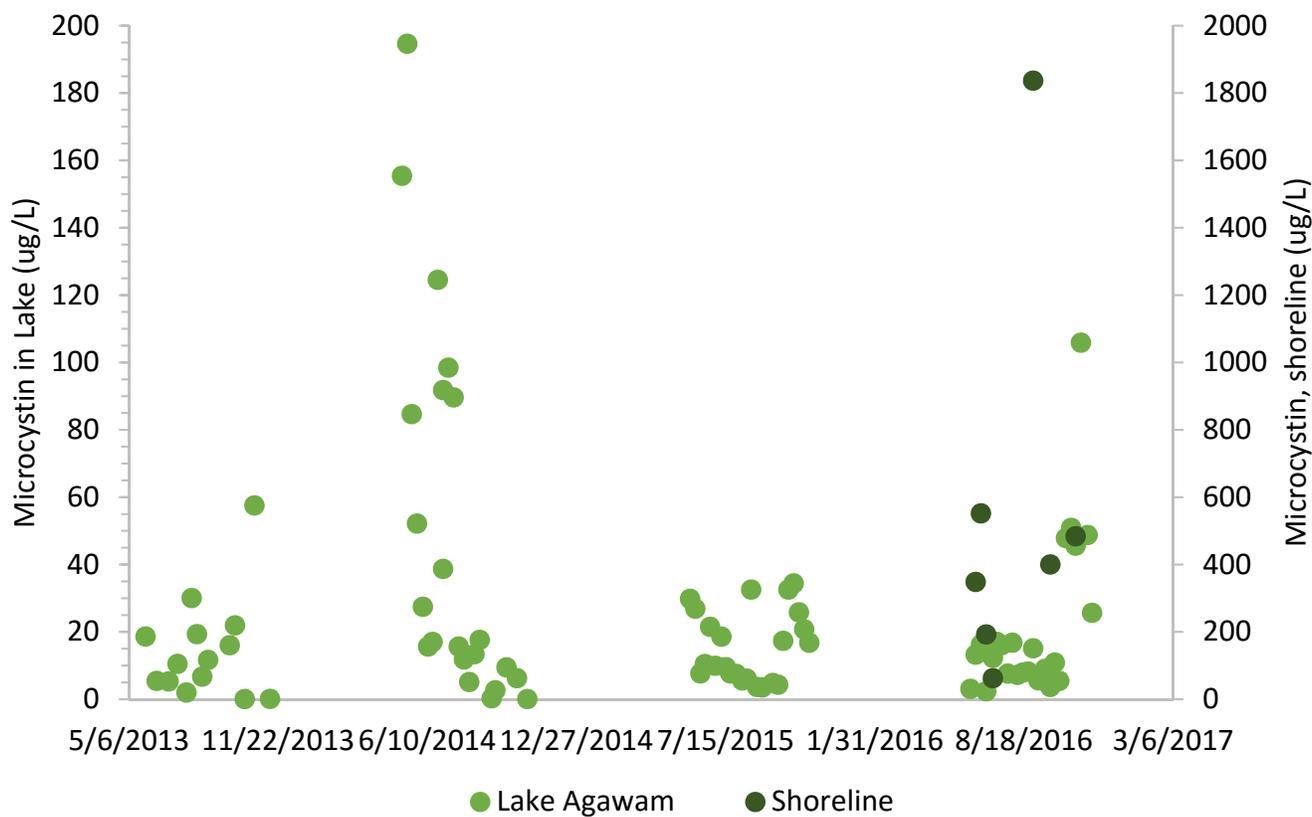


Figure 9. Top 10 lakes for blue-green algal bloom listings across NYS by NYSDC in 2014. No lake in NYS was listed more frequently than Lake Agawam.

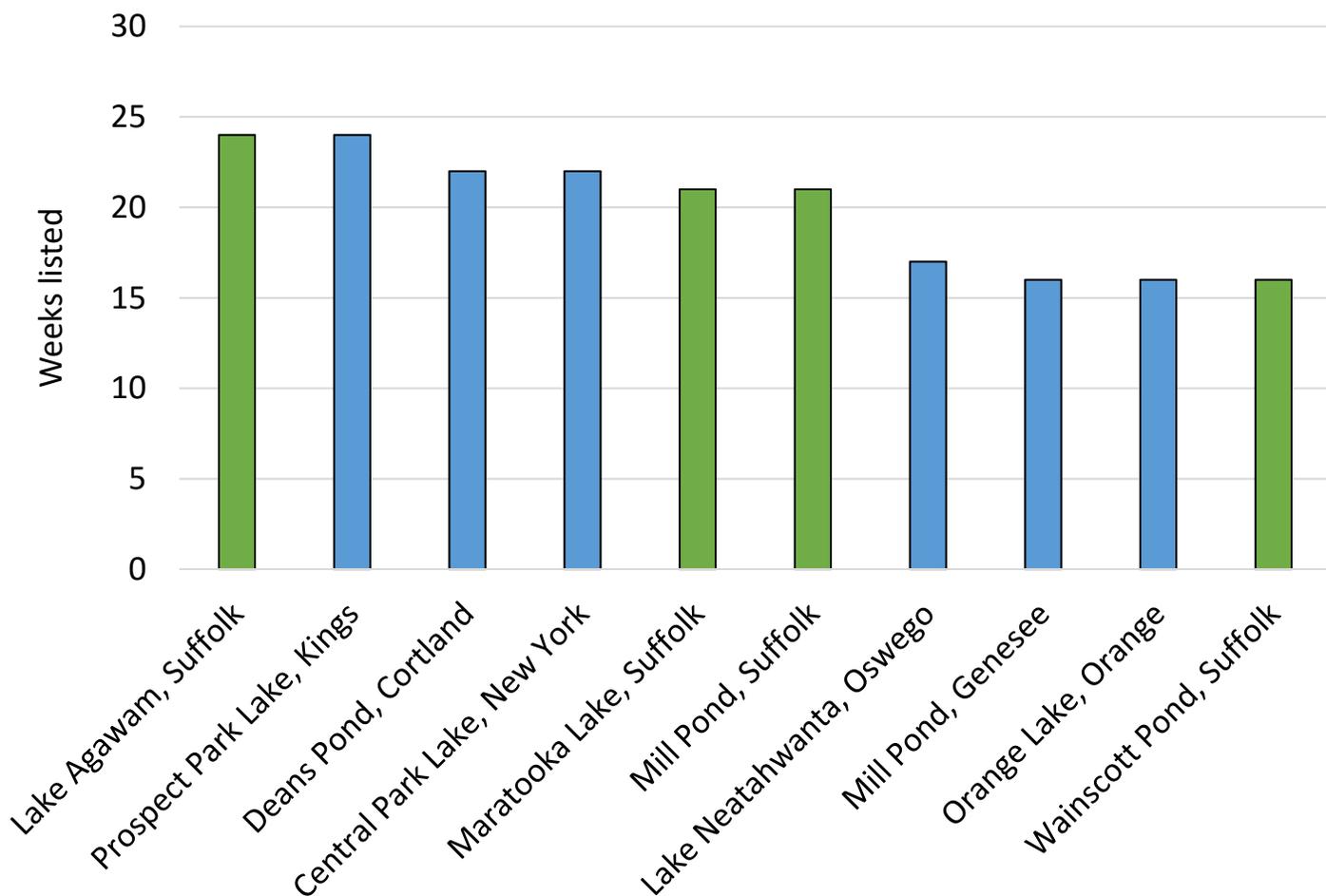


Figure 10. Top 15 lakes for blue-green algal bloom listings across NYS by NYSDC in 2015. No Lake in NYS was listed more frequently than Lake Agawam.

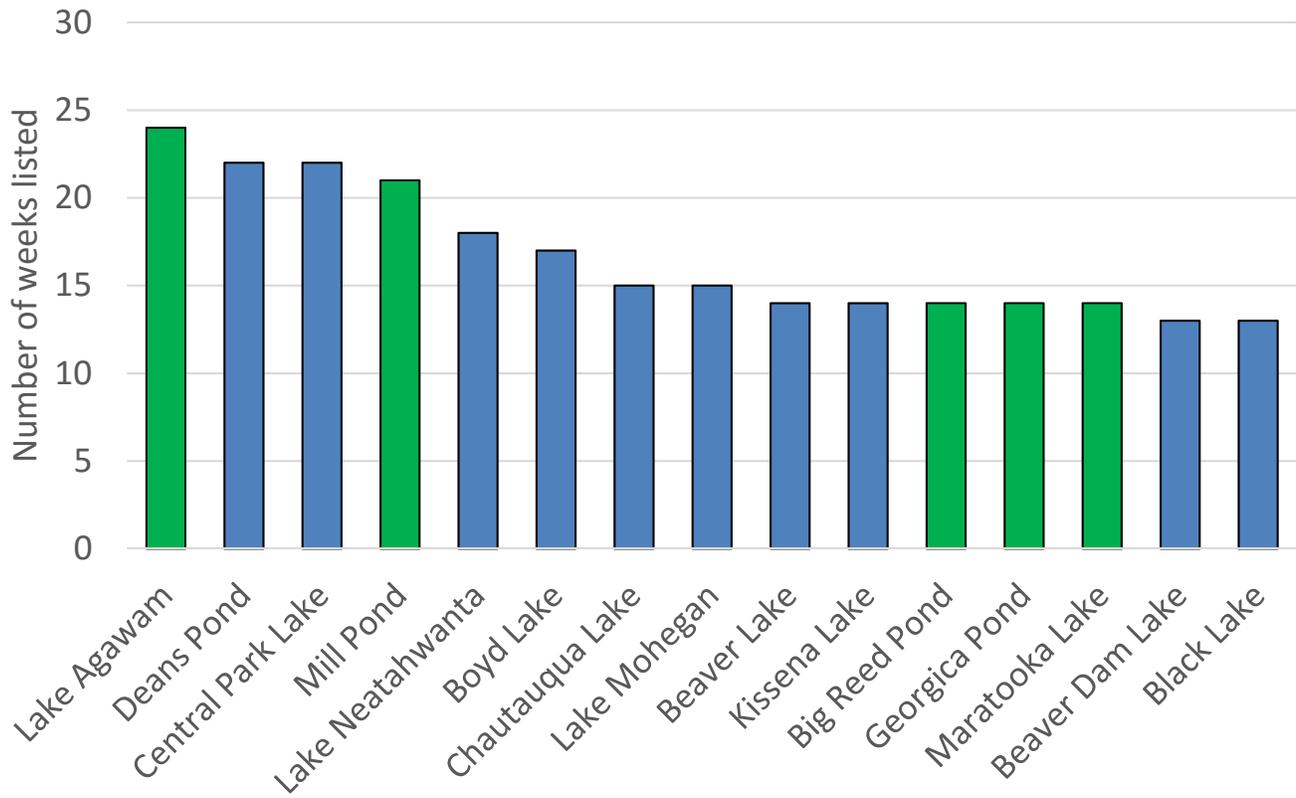


Figure 11. Illustration showing the process by which blue-green algae for a scum is concentrated on the shoreline of Lake Agawam, a threat to dogs and other animals.

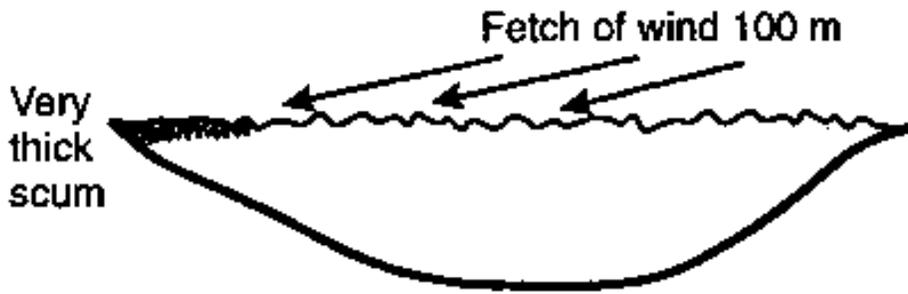


Figure 12. Response of *Microcystis* and algae in Lake Agawam to nitrogen and phosphorus, 2004 (Gobler et al., 2007).

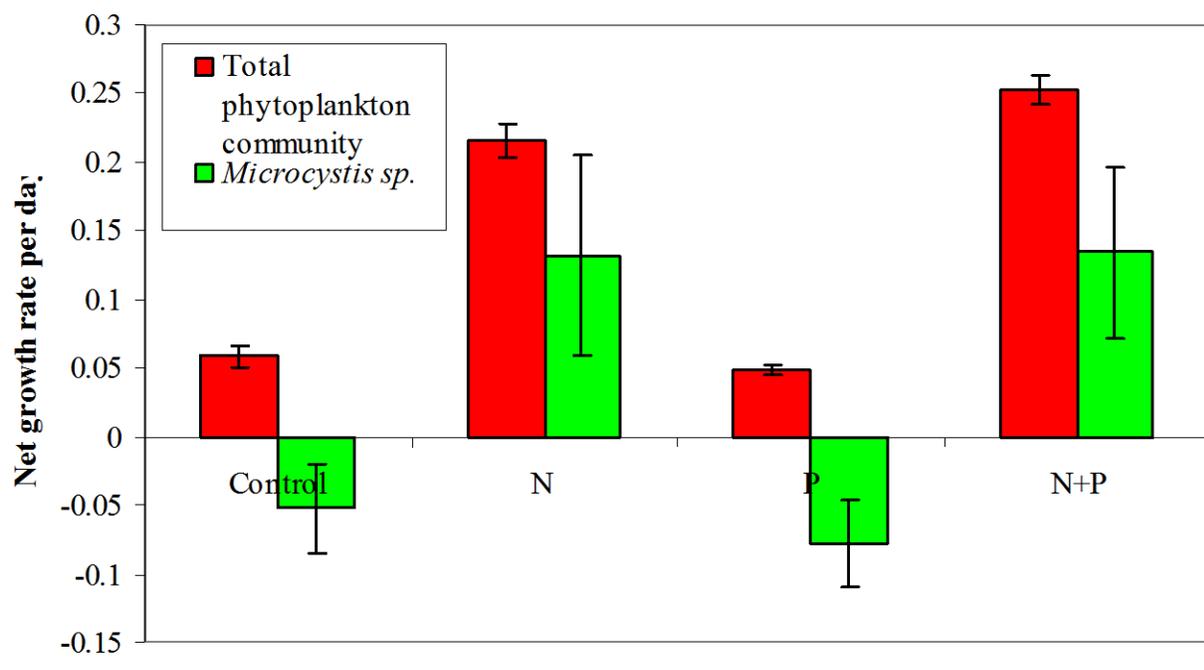


Figure 13. Response of blue-green algal toxins in Lake Agawam to nitrogen and phosphorus, 2004 (Gobler et al., 2007).

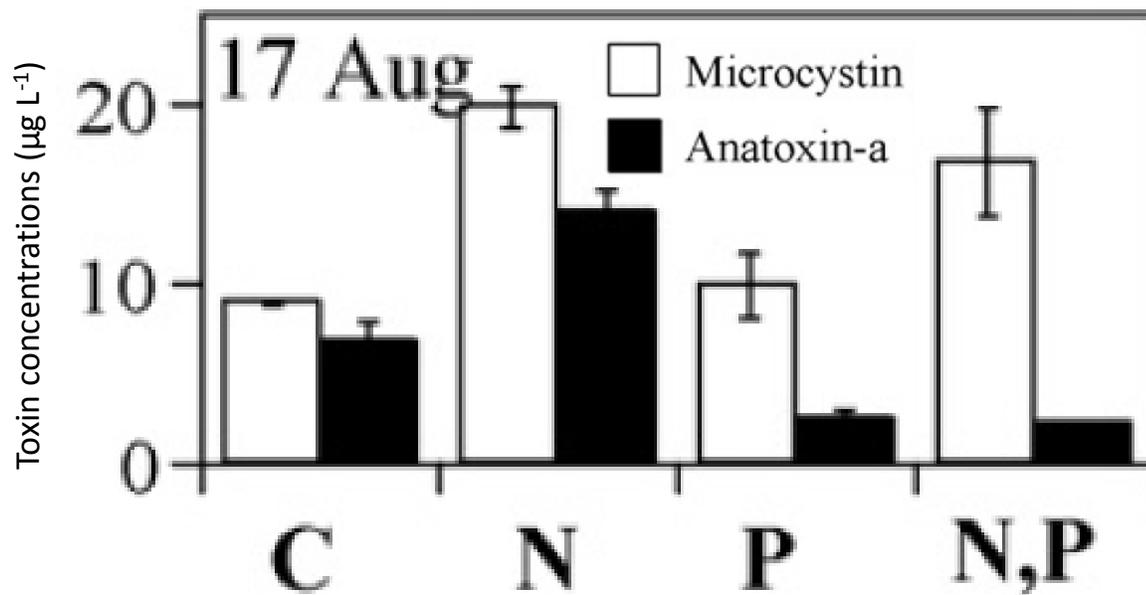


Figure 14. Response blue-green algae in Lake Agawam to nitrogen and phosphorus at two temperatures in 2015.

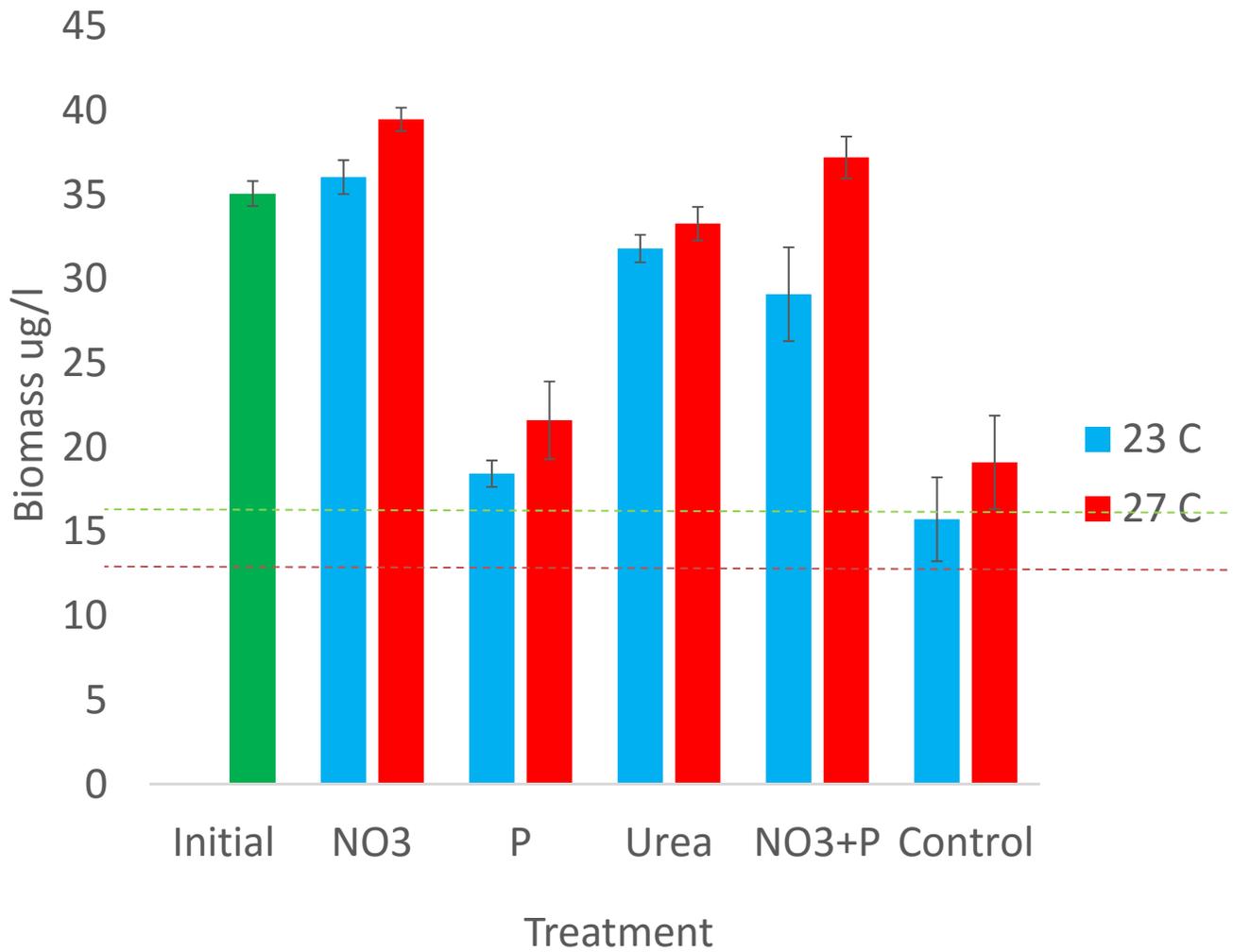


Figure 15. Response of blue-green algae in Lake Agawam a 50% reduction in nutrients (Harke et al., 2008).

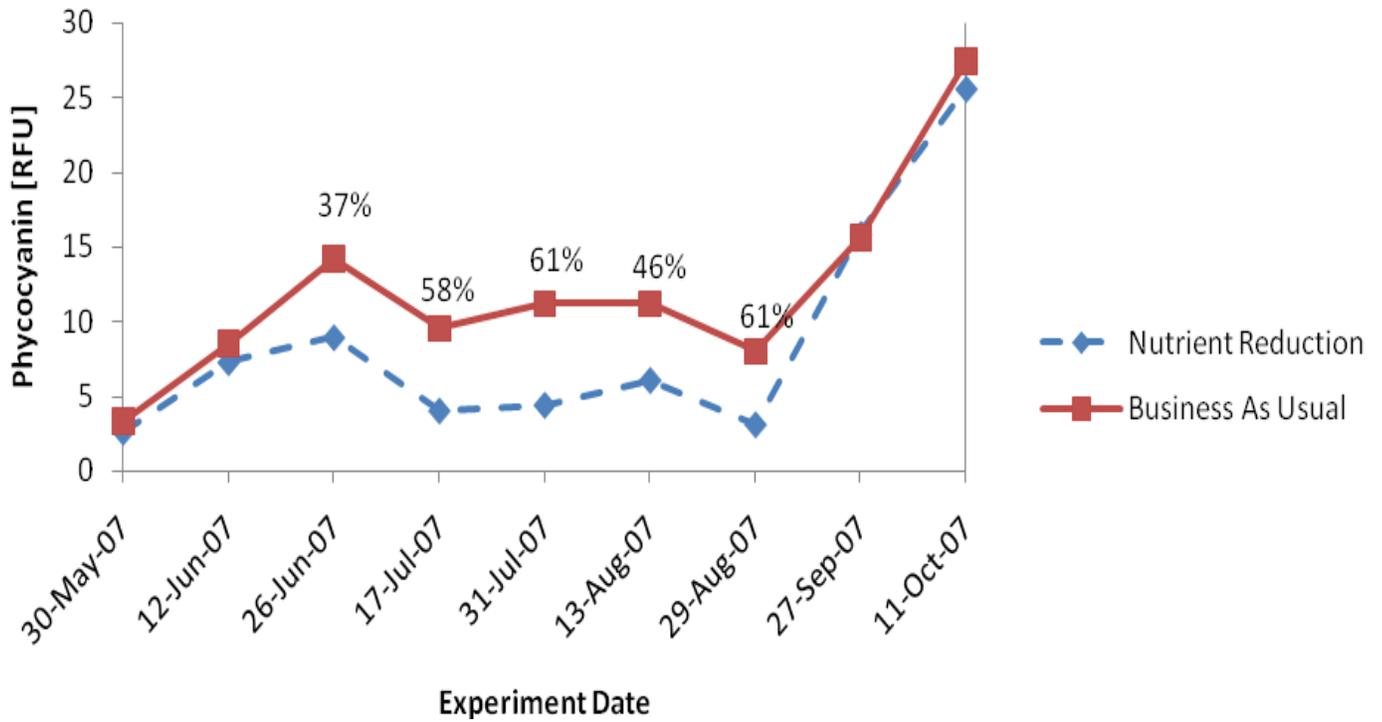


Figure 16. 50-year and 10-year (inset) watersheds for Lake Agawam, Old Town Pond, and Wickapogue Pond used in this study.

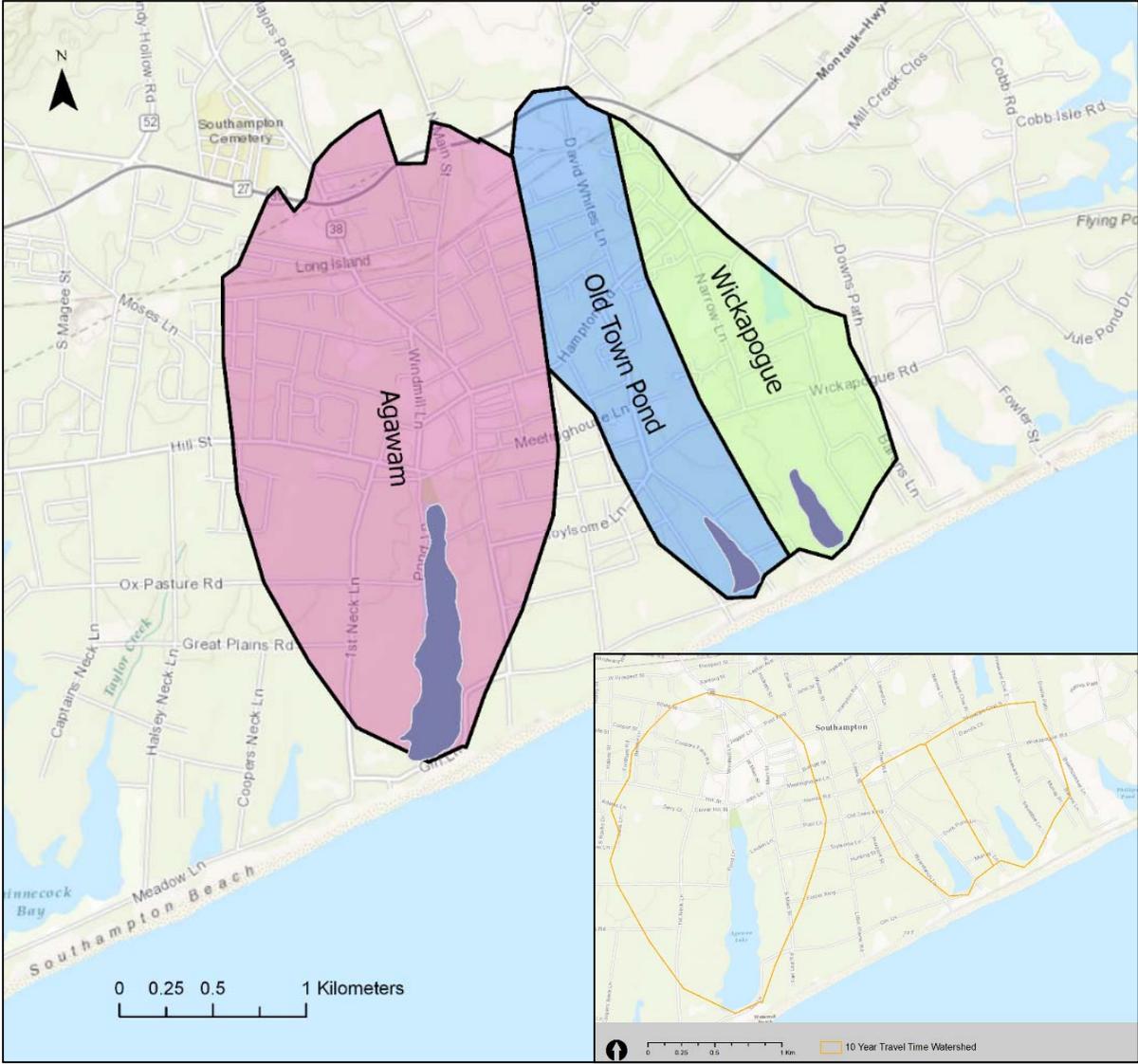


Figure 17. Relative contribution of various nutrient loading processes to the total nitrogen load to Lake Agawam for the 50 year watershed.

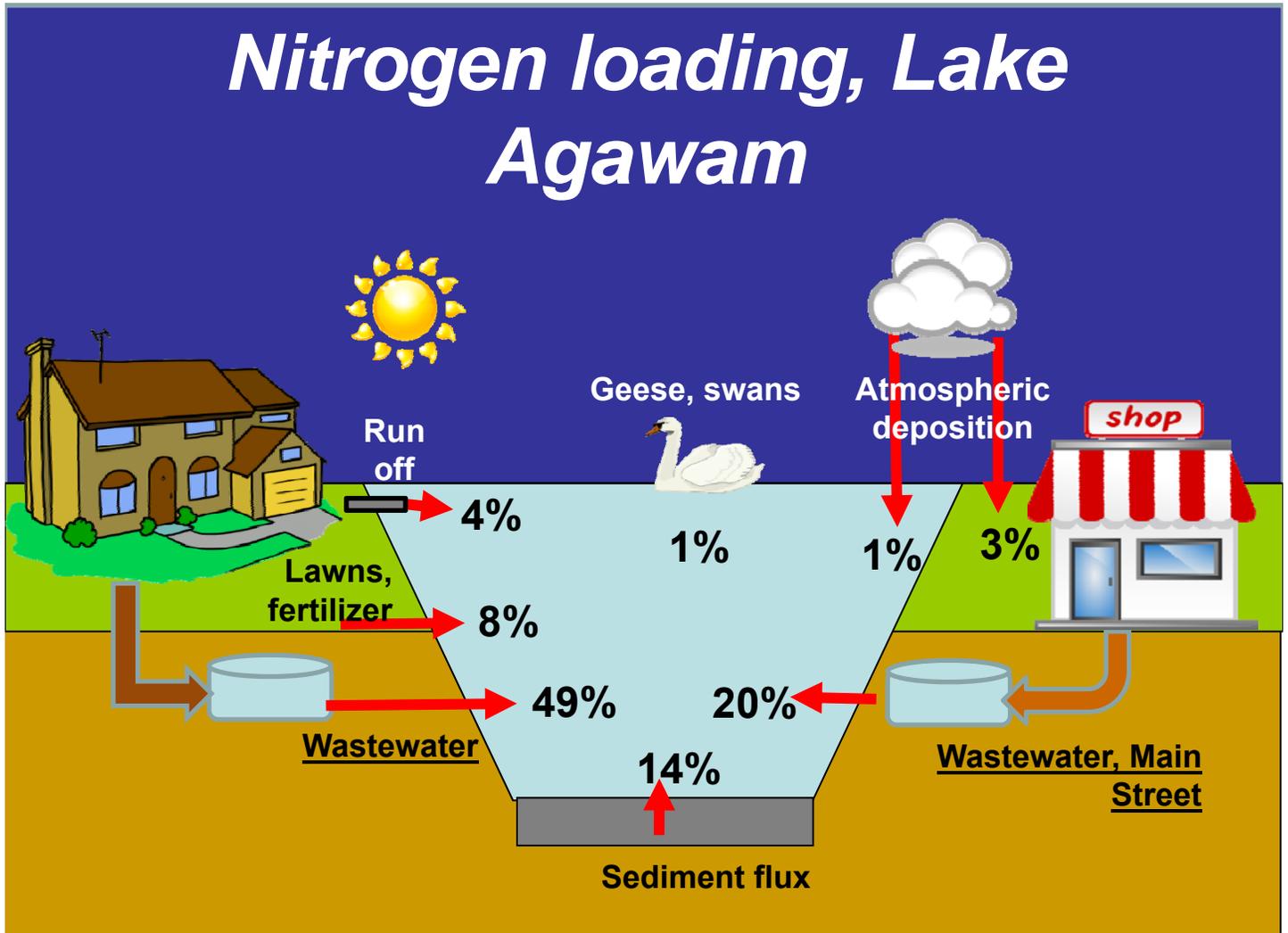


Figure 18. Relative contribution of various nutrient loading processes to the total nitrogen load to Old Town Pond for the 50 year watershed.

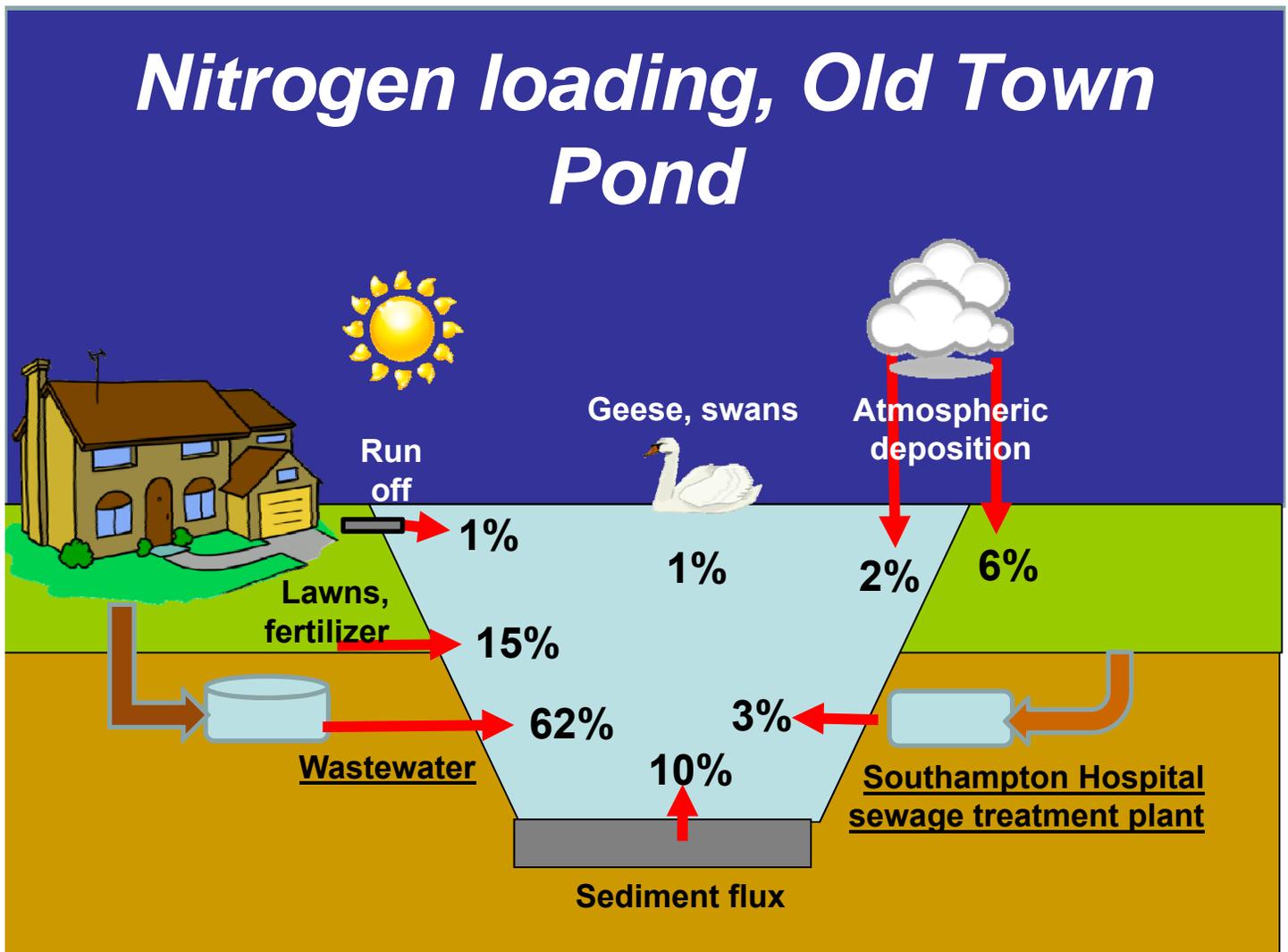


Figure 19. Relative contribution of various nutrient loading processes to the total nitrogen load to Wickapogue Pond for the 50 year watershed.

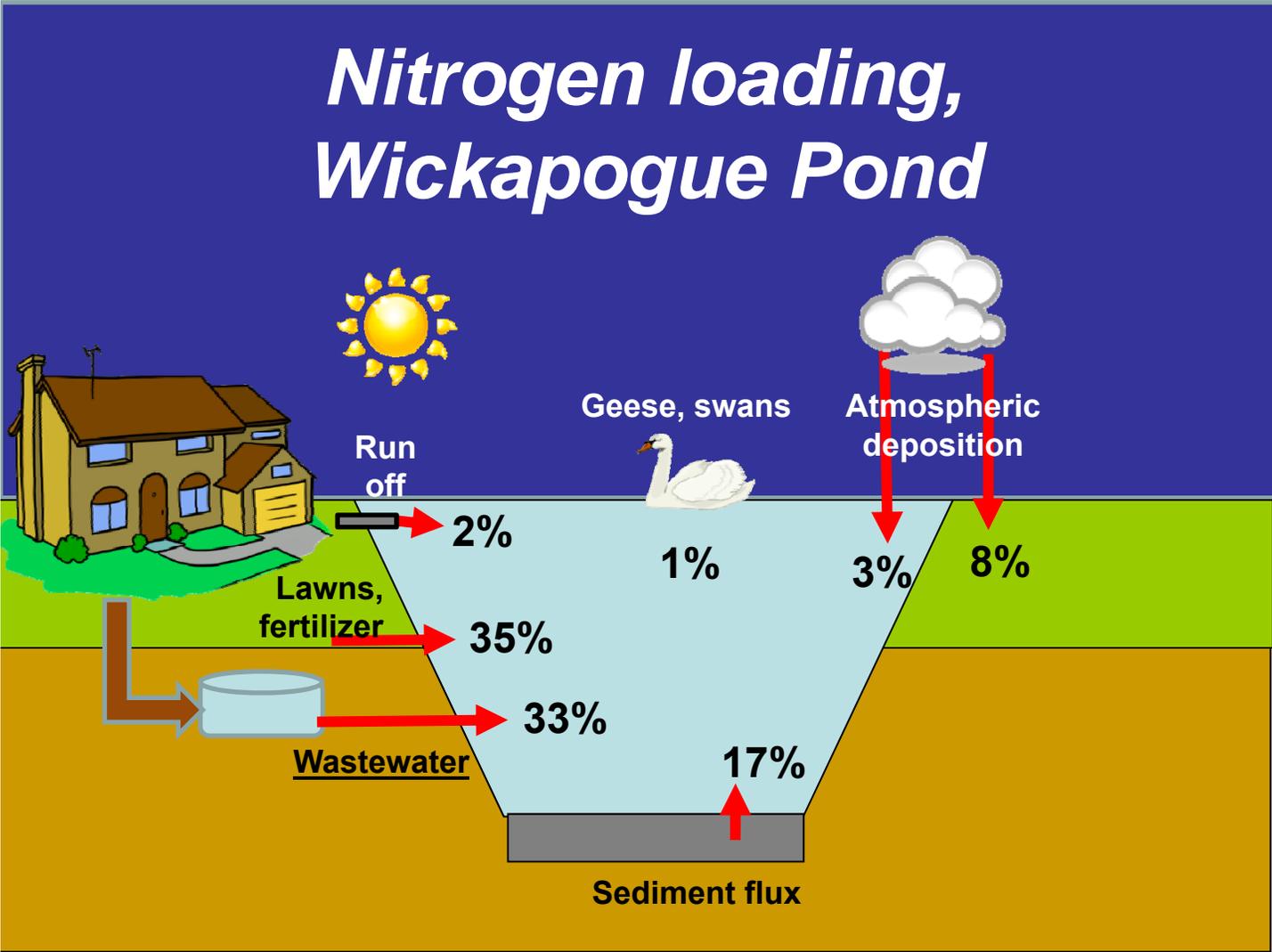


Figure 20. Contribution of various nitrogen loading processes to the total nitrogen load to Lake Agawam for the 10 year watershed under three wastewater management scenarios

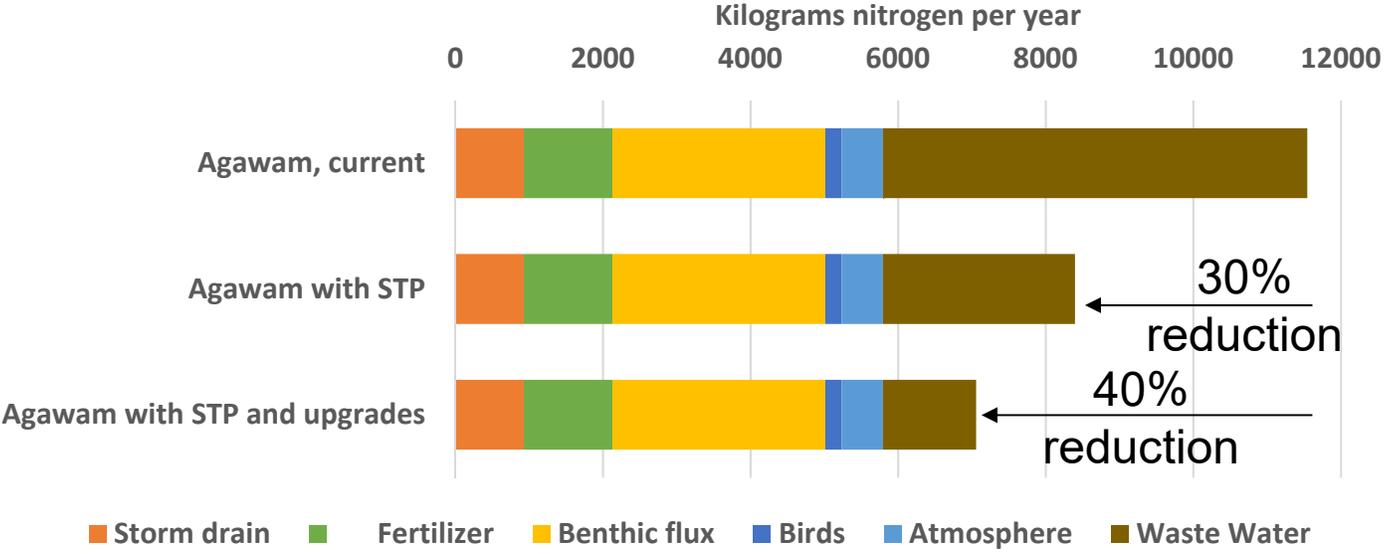


Figure 21. Nitrogen load to Lake Agawam controllable by the Village of Southamptom for the 10 year watershed under three wastewater management scenarios. Controllable nitrogen loads include wastewater, fertilizer, and the storm drain.

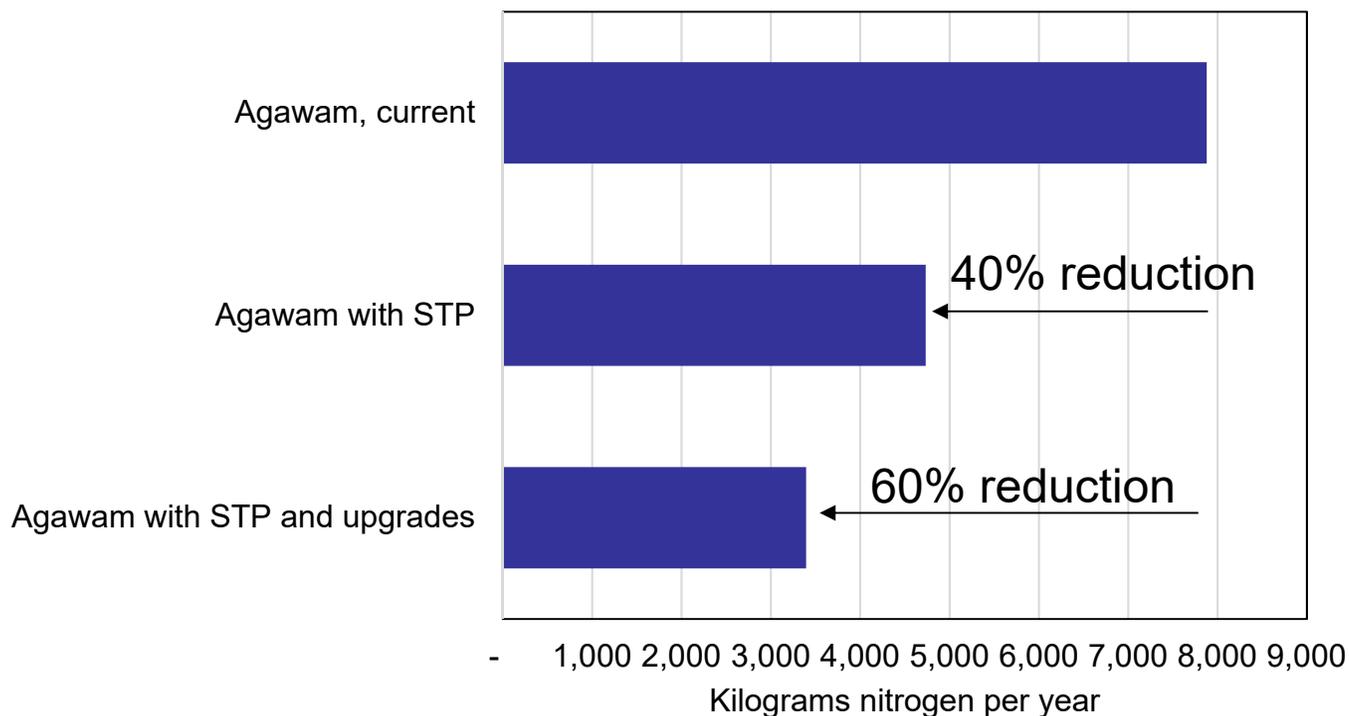


Figure 22. Contribution of various nitrogen loading processes to the total nitrogen load to Lake Agawam for the 50 year watershed under three wastewater management scenarios

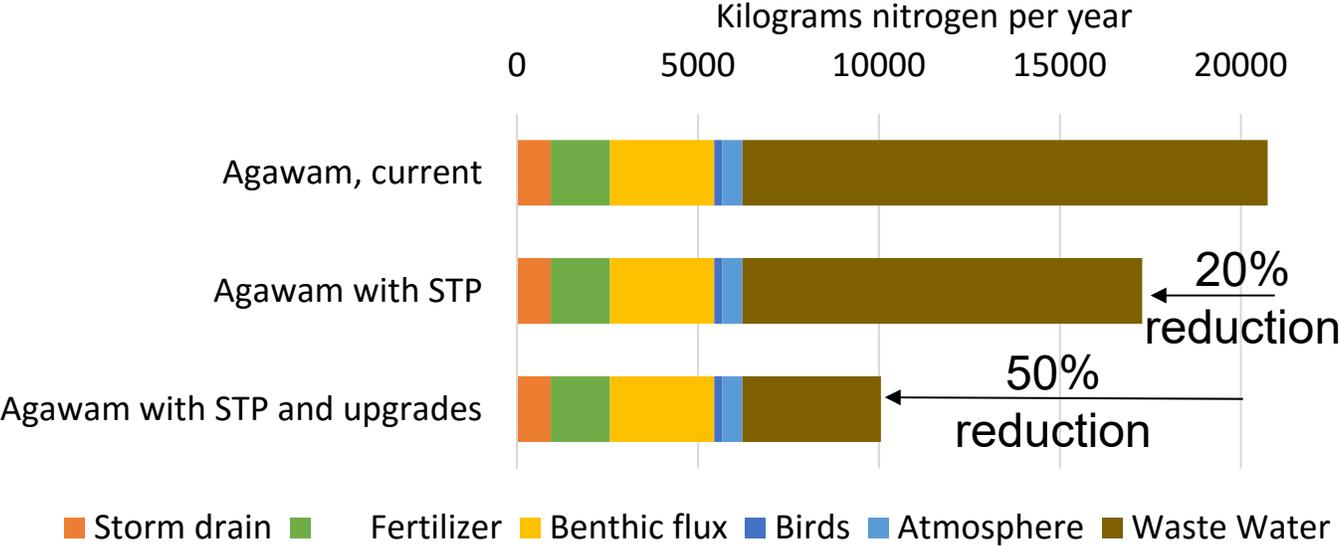


Figure 23. Nitrogen load to Lake Agawam controllable by the Village of Southamptn for the 10 year watershed under three wastewater management scenarios. Controllable nitrogen loads include wastewater, fertilizer, and the storm drain.

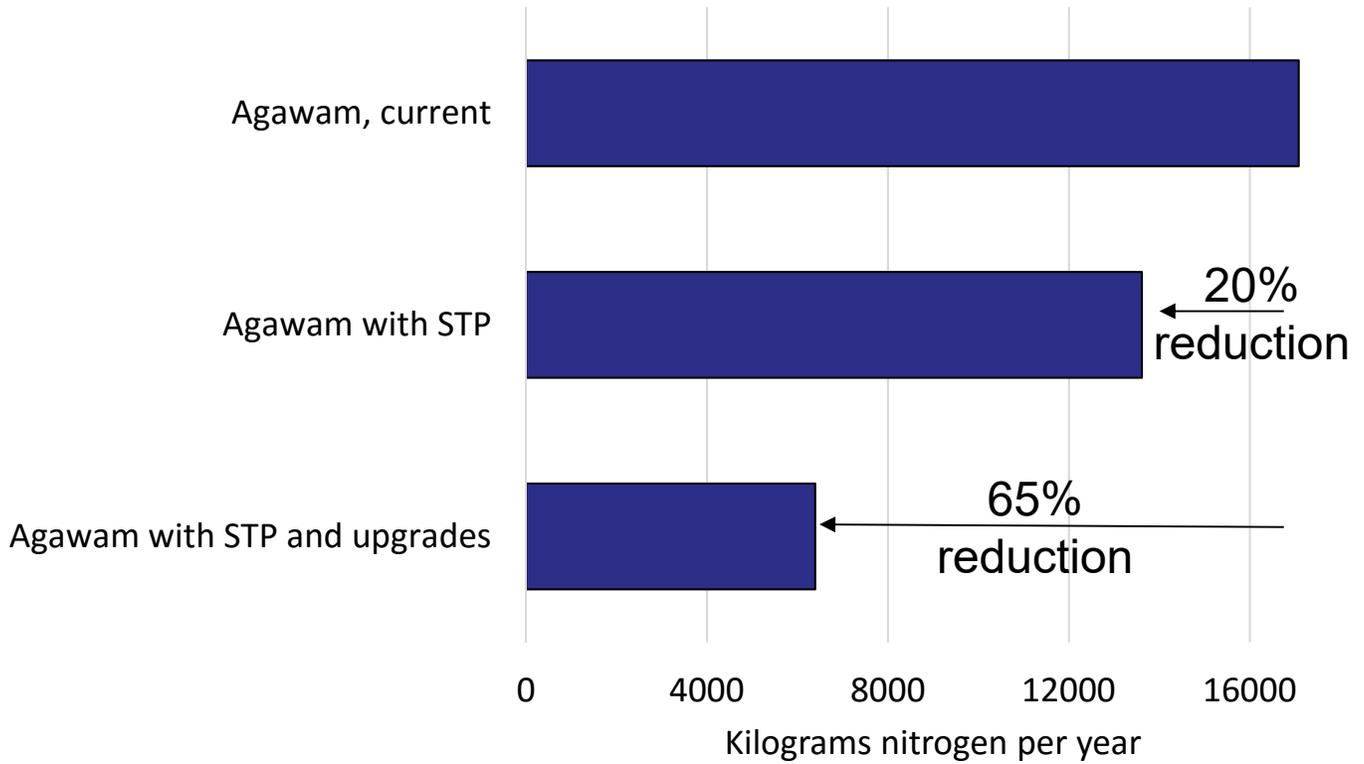


Figure 24. Nitrogen load to Lake Agawam under four scenarios: Current nitrogen loading within the 10-year watershed, growth of 10 million square feet of commercial space within the Village, the addition of the sewer district (STP), and the addition of the sewer district and 10 million square feet of commercial space within the Village. Dashed line shows baseline, current condition. Commercial flow was set at 0.07 gallons per square foot and 60 mg N per liter whereas the sewage treatment plant was calculated to perform at 10 mg N per liter.

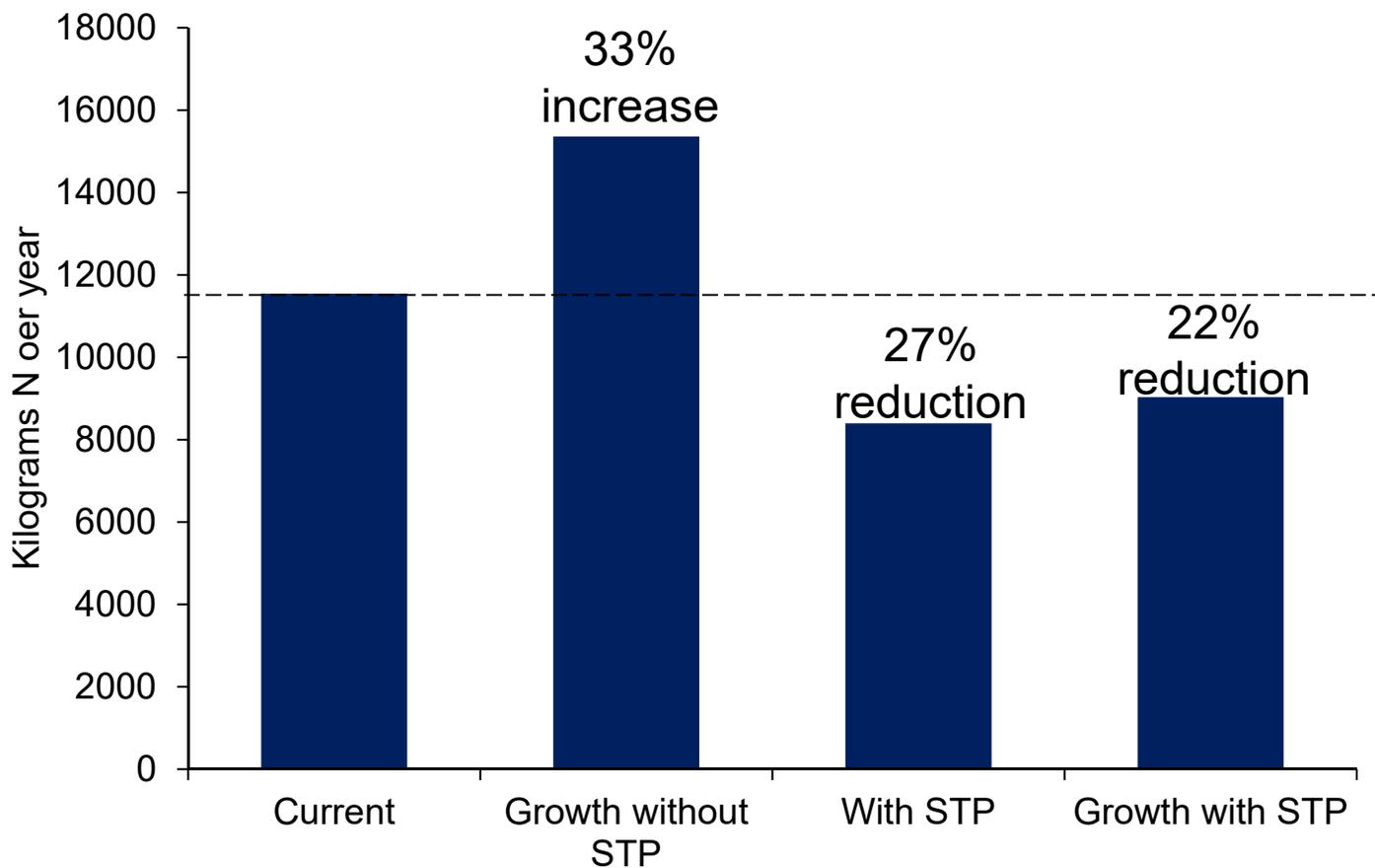


Figure 25. Comparison of blue-green algal bloom dynamics in Lake Agawam in 2016, with the implementation of the proposed sewer district over 10 years and with the sewer district and upgrades of septic tanks over 50 years.

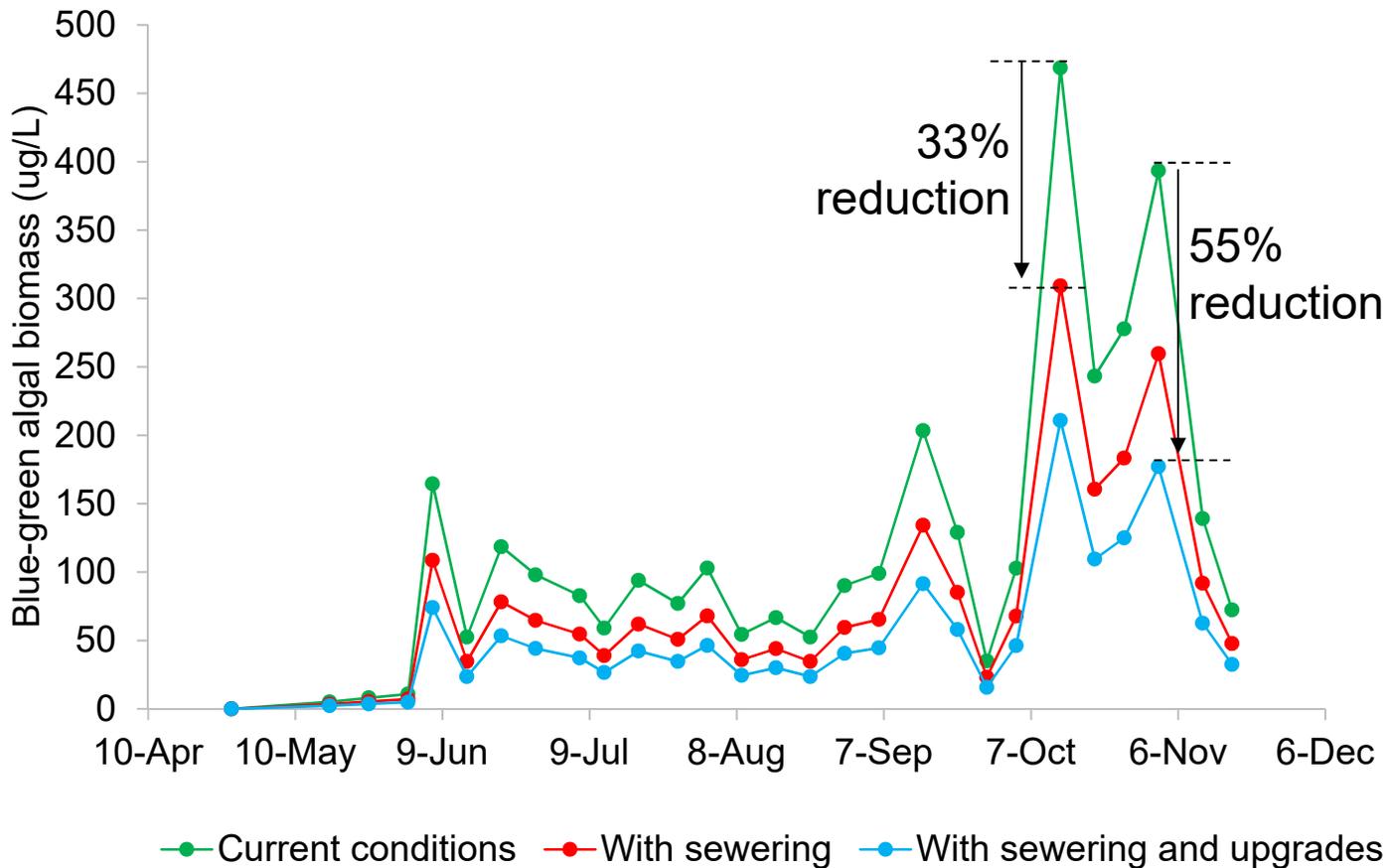


Figure 26. Percentage of 2016 observations in Lake Agawam exceeding the NYSDEC blue-green algal bloom threshold in 2016, with the implementation of the proposed sewer district over 10 years and with the sewer district, and upgrades of septic tanks over 50 years.

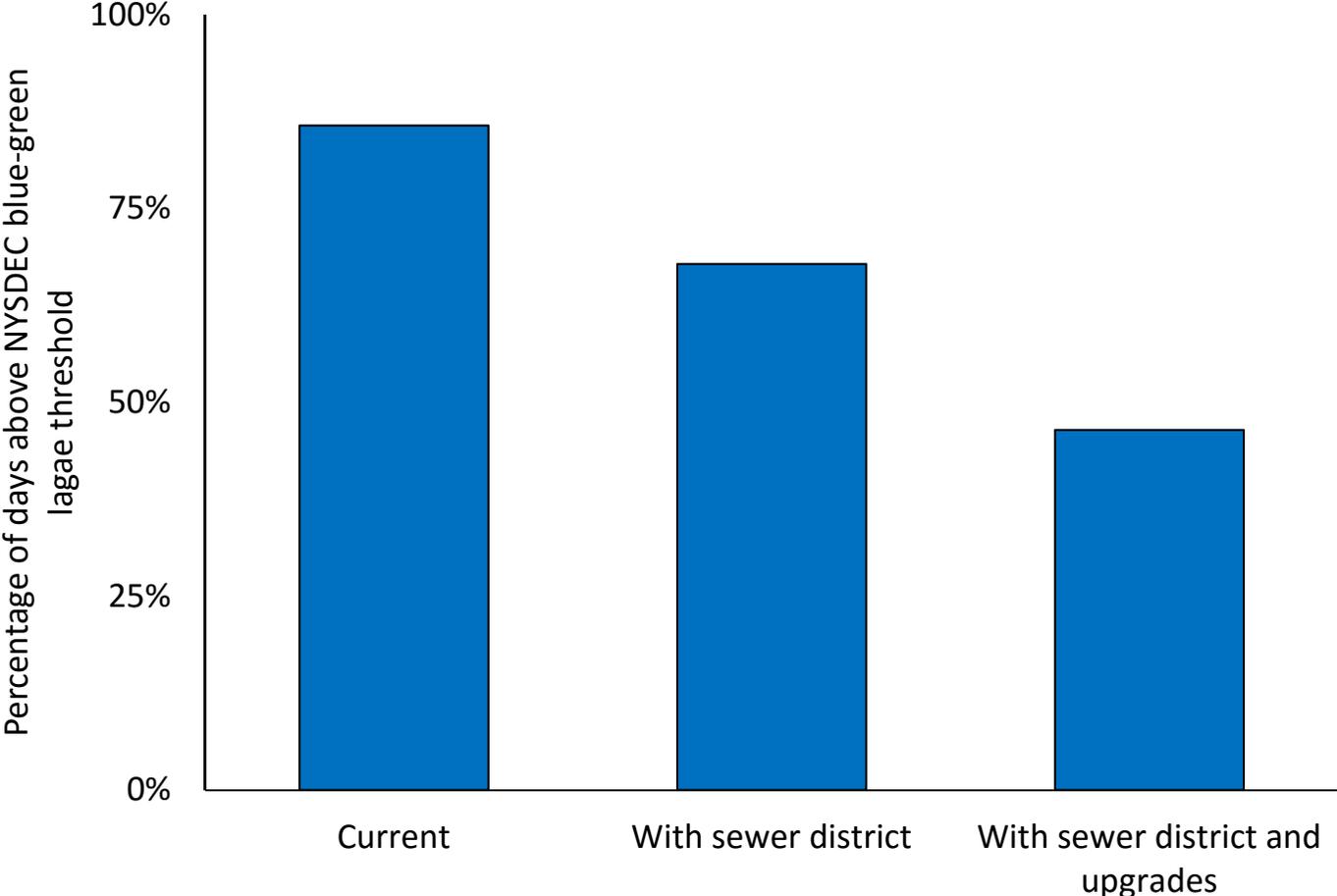


Figure 27. Comparison of microcystin dynamics in Lake Agawam in 2016, with the implementation of the proposed sewer district over 10 years and with the sewer district and upgrades of septic tanks over 50 years.

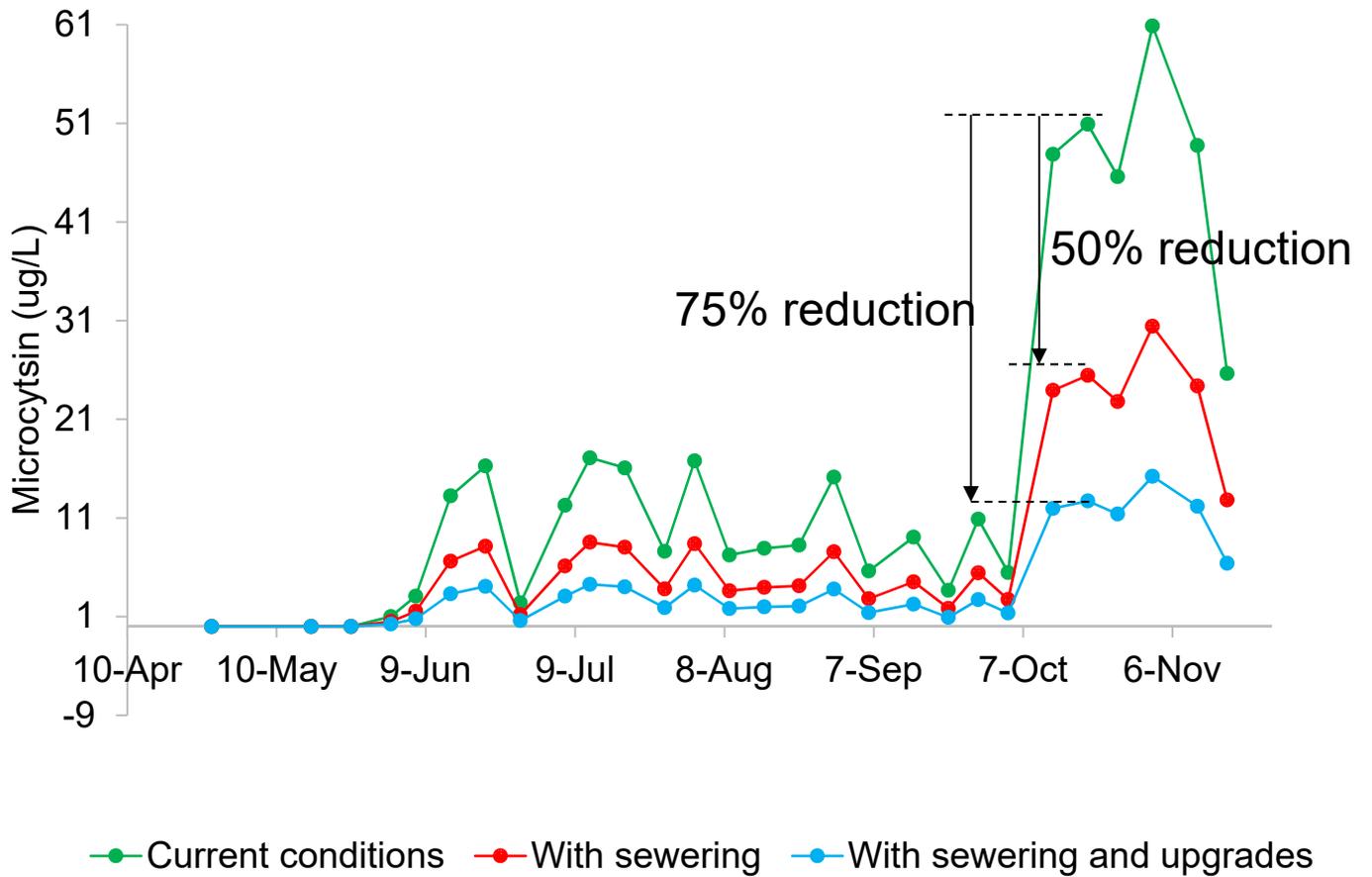


Figure 28. Percentage of 2016 observations in Lake Agawam exceeding the World Health Organization and NYSDEC low recreational risk threshold in 2016, with the implementation of the proposed sewer district over 10 years and with the sewer district, and upgrades of septic tanks over 50 years.

