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Shannon Cook Tuesday May 8, 2018

Are We in Hot Water?:

Comparing Macroinvertebrate Communities and Water Quality over Time

By

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I must also give a big thanks to Kathleen Smith, who accompanied me while sampling in the cold and rain while the sun was setting to ensure that I survived with the necessary data, and who offered her insight on any puzzling findings I presented to her during our breakfast conversations. I would also like to Jeremy Pallant, for keeping me motivated and working with me to get through all of my thesis related problems.

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ABSTRACT

Freshwater ecosystems, which support a significant portion of the world's biodiversity, are often plagued by pollution, particularly excess nitrogen and phosphorus. Additionally, freshwater ecosystems are strongly impacted by climate change. In 2003, two macroinvertebrate studies were conducted at Mount Holyoke College to investigate the health of different sites according to biotic indices. This project repeats the macroinvertebrate surveys at four stream sites and two lakes sites to compare and analyze how the macroinvertebrate communities and abiotic factors of the freshwater ecosystems have changed over time. Abiotic water quality data has been collected every two weeks since 1996 using probes. Diversity and biotic indices were calculated from the macroinvertebrate samples and the changes in abiotic factors over time were examined. According to the general decrease in the biotic indices, the water quality has improved across all sites since 2003. The species richness was generally higher in the stream sites compared to the lake sites and the restored stream site. Since 1996, the average annual temperature and pH have both increased significantly. Although the health of the freshwater ecosystems on campus seem to be improving, there is some discrepancy between the different sites and the abiotic effects of climate change are alarmingly evident.

INTRODUCTION

Pollution

Fresh water is vital to all life on earth and is therefore one of the most valuable resources on our planet. Of all of earth's water, freshwater comprises only 2.5%, while saltwater accounts for 97.4% of the earth's water (USGS.gov., 2016). Additionally, of the world's total freshwater, only 1.2% is surface freshwater while the remaining 98.8% is contained in groundwater, glaciers, and ice caps, and only about 24% of the surface freshwater is accessible as lake, swamp, marsh, or river water, with the remaining 76% locked up in ground ice, permafrost, living things, the atmosphere, and soil moisture (USGS.gov., 2016). Due in part to its scarcity, freshwater is a valuable resource with more than half of the world's population living within three kilometers of freshwater (Kummu et al., 2012). Besides being an important natural resource, freshwater systems contribute significantly to global biodiversity by supporting approximately 6% of all described species, despite covering only 0.8% of the world's surface (Dudgeon et al., 2005; Gleick, 1996; Ormerod et al., 2012). However, freshwater ecosystems face a variety of threats including pollution, which can enter into a freshwater system through multiple points in the water cycle, and climate change, both of which can lead to species loss and extinction risks (Abell, 2002; Ormerod et al., 2010). Additionally, there is no standardized definition of freshwater ecosystem health that encompasses biological, chemical, and physical indicators, making freshwater management and accurate health assessments more challenging (O'Brien et al., 2016).

Many human created pollutants, including pollution from urban, agricultural, and industrial activities, end up in aquatic environments (Gilbert & Avenant-Oldewage, 2017). Agricultural runoff, urban runoff, eroded streambanks, leaking septic systems, sewage discharge, and dust from seed drilling machinery all bring pollution into waterways (USEPA, 2006; Bonmatin et al., 2015). Pollutants such as pesticides, herbicides, and fertilizers have a variety of impacts on different groups of aquatic macroinvertebrates (Buikema and Benfield, 1979; Harmon, 2008; Hering et al., 2009; Ischinger and Nalepa, 1975; Nalepa, 1975; Metcalfe, 1989). For example, amphipods tend to be sensitive to insecticides in that the insecticides alter behavior and more frequently lead to death, snails tend to concentrate herbicides more than other invertebrates via bioaccumulation though for unknown reasons (Buikema and Benfield, 1979), crayfish tend to accumulate minimal amounts of nitrate, but demonstrate reduced oxygen uptake after exposure to lead and loss of locomotor skills after exposure to cadmium (Camargo et al., 2005; Buikema and Benfield, 1979), and copepods are sensitive to pesticides and nitrogen from fertilizers (Di Lorenzo et al., 2014).

Nitrogen and phosphorus are some of the most abundant pollutants in freshwater streams in the United States, though this wasn't the case until they became commercially available in fertilizers relatively recently (USEPA, 2006). In the early 1900s, Fritz Haber developed the Haber-Bosch process for synthesizing ammonia which could then be used in fertilizer, and since then the use of fertilizer nitrogen has skyrocketed (Erisman et al., 2008). The use of phosphorus in fertilizers began in Germany in 1861 and spread to North America during World War 1 (Russell and Williams, 1977).

Nitrogen and phosphorus can be introduced to aquatic systems through runoff from fertilizer treated agricultural land (Bakken and Bleken, 1998; Cairns et al., 1972; Huang et al., 2017; Pant et al., 2004; Sharpley et al., 1998; Sharpley et al., 1999; Sharpley et al., 2001; Sharpley et al., 2003; Thorburn et al., 2003). Nitrogen can contribute to acidification (Cairns et al., 1972; Camargo and Alonso, 2006) and damages macroinvertebrate respiratory systems (Di Lorenzo et al., 2014). Phosphorous is linked to eutrophication which threatens biodiversity and therefore water quality (Huang et al., 2017; Pant et al., 2004; Sharpley et al., 1998; Sharpley et al., 1999; Sharpley et al., 2001; Sharpley et al., 2003).

Available nitrogen can limit primary producers in freshwater systems, but an excess of nitrogen contributes to eutrophication (Dodds and Welch, 2000; Thorburn et al., 2003). Additionally, once polluted by nitrogen, the affected ecosystem can take many years to return to a stable state, depending on the extent of the nitrogen pollution (Bakken and Bleken, 1998). Some of the most common forms of nitrogen in freshwater systems are nitrate (NO_3), which is toxic to aquatic life, and ammonium (NH_4) (Camargo et al., 2005). Nitrate ions interfere with the ability of pigments to carry oxygen in freshwater invertebrates (Camargo et al., 2005). Ammonium and hydroxyl ions are the result of ammonia (NH_3) reacting with water, causing an increase in pH, which allows for a greater ratio of ammonia to ammonium (Patil et al., 2014). Above a pH of 7.2, some free ammonia remains, though the pH continues to increase (Patil et al., 2014). Ammonia is toxic to fish, especially at high pH levels, in that it damages gills and can stunt growth (Patil et al., 2014).

Phosphorus is often a limiting factor for plant growth and is therefore used in agricultural fertilizer; however, agricultural run-off then pollutes freshwater systems with excess phosphorus, causing eutrophication and oxygen depletion (Pant et al., 2004). Orthophosphate (PO_4) is the most biologically active form of phosphorus and can overstimulate algae growth which leads to eutrophication (USEPA, 2011). Phosphorus primarily enters waterways through runoff, in part because excess phosphorus that provides no additional fertilizing may be present in agricultural soils until it is washed away, often by storms (Sharpley et al., 1999).

Besides nitrogen and phosphorus, other abiotic factors such as pH, dissolved oxygen, and temperature can indicate pollution and water quality. The acidity or basicity of a freshwater system can impact the species diversity of macroinvertebrates, with the highest levels of diversity occurring between pH values of 4.09 and 8.65 (Berezina, 1999). The buildup of organic carbon due to eutrophication can lead to a decrease in dissolved oxygen and an increase in pH

(Dodds and Welch, 2000). According to Simaika and Samways (2011), temperature and pH exert the strongest influence on assemblage structure, indicating the importance of heat and acidic pollution.

Climate Change

With their sensitivity to temperature, freshwater ecosystems have the potential to be strongly impacted by climate change, with macroinvertebrate species facing potential range shifts, increases or decreases in abundance, local extinction, and an overall change in diversity (Burgmer et al., 2007; Chessman, 2009; Daufresne et al., 2007; Domisch et al., 2011; Durance and Ormerod, 2007; Haidekker and Hering, 2007; Heino et al., 2009; Mouthon and Daufresne, 2006). One study which examined 38 benthic macroinvertebrate species from nine different orders found evidence suggesting that at least 97% of the species have shifted ranges in response to climate change (Domisch et al., 2011). Specifically, they found that species in streams with low mean annual air temperature experienced range contraction while species in streams with higher mean annual air temperature experienced range expansion (Domisch et al., 2011). Additionally, climate change may result in a change in species composition, specifically reducing the populations of cold-adapted headwater macroinvertebrate species, resulting in decreased genetic diversity, and increasing the abundance of non-native species (Burgmer et al., 2007; Daufresne et al., 2007; Domisch et al., 2011). Conversely, an increase in water temperature may result in an increase in macroinvertebrate richness, depending on the location (Mantyka-Pringle et al., 2014). Generally, with an increase in temperature, warm-water adapted taxa have increased while cold-water adapted taxa have decreased, which can lead to localized species loss as well as an overall decrease in diversity which renders the ecosystem less able to withstand changes (Burgmer et al., 2007; Chessman, 2009; Heino et al., 2009). These changes in community composition may also result in alterations of the proportions of functional groups such as certain types of feeders. Communities of macroinvertebrates have demonstrated low resilience to climate change and would therefore require a long period of time to recover from any damage to diversity or abundance, if they recovered at all (Daufresne et al., 2007; Mouthon and Daufresne, 2006).

Macroinvertebrates

Although water quality can be measured abiotically, all of these abiotic factors contribute to changes in the aquatic community, which then provides a realistic view of the impact of those factors when studied (Metcalfe, 1989). Macroinvertebrates prove to be excellent community representatives for such studies for a variety of reasons: (1) they are sensitive to various pollutants, (2) they are abundant and easy to collect, (3) they are generally sedentary and therefore representative of the local conditions, and (4) they have long enough lifespans to encompass a record of changes in the quality of the environment over time (Goodyear & McNeill, 1999; Masese et al., 2009; Metcalfe, 1989). The relative increases and decreases in abundance and diversity of different taxa can provide information about what factors are affecting the health of the freshwater system based on how the affected taxa are influenced by climate change or pollution. Additionally, a higher biodiversity indicates a more stable ecosystem since it is better able to recover from disturbances with the wider variety of uniquely adapted organisms.

Indicator taxa are focal taxonomic groups which indicate certain environmental conditions based on their tolerance of those conditions. The abundance of macroinvertebrate indicator taxa can serve as a proxy of the quality of the ecosystem and suggest imbalances in the system such as excess nutrients (Myslinski & Ginsburg, 1977). Freshwater macroinvertebrate indicator taxa include Oligochaetes which are tolerant of very low dissolved oxygen and higher temperatures, Chironomids which can withstand low dissolved oxygen (and indicate the level of dissolved oxygen in the water by the redness of their bodies, which is caused by hemoglobin when it stores oxygen) and moderate pollution, and Trichoptera, Ephemeroptera, and Plecoptera, all of which are intolerant of pollution with Plecoptera being the least tolerant (Myslinski & Ginsburg, 1977; Hellawell, 1986; Rosenburg & Resh, 1993). One way of quantifying a taxa’s tolerance of ecosystem quality, specifically pollution, is by assigning it a tolerance value which is then used to calculate a biotic index. Biotic indices are based on predetermined tolerance values and abundances of sampled macroinvertebrates to produce an approximate measure of overall freshwater quality (Hilsenhoff, 1977; Hilsenhoff, 1982; Hilsenhoff, 1987).

Study Rationale

The diverse freshwater systems in the northeastern United States have been manipulated by European settlers since the 17th century and consequently, these ecosystems have been receptacles of logging, industrial, and agricultural wastes for hundreds of years (USEPA, 2006). As of 2006, the Eastern Highlands had the highest levels of nitrogen and phosphorus in freshwater streams compared to the Plains and Lowlands, the West, and the overall national averages of the United States (USEPA). In the narrower ecoregion of the Northern Appalachians, which includes Massachusetts, 45% of the streams by length were in poor condition according to a macroinvertebrate index, and 19% of streams had lost at least half of their taxa compared to the expected taxa based on reference sites (USEPA, 2006). However, the lakes and ponds of New England appear to be on par with the rest of the nation in regards to taxa loss and a higher proportion of New England lakes and ponds boast good conditions in regards to nitrogen and phosphorus levels (NEIWPCC & EPA, 2010). These contradictory trends, in conjunction with the effects of climate change and the variety of human population densities render the freshwater systems of the northeast particularly interesting study sites.

In 2003, macroinvertebrate surveys were conducted at several sites along the local streams at Mount Holyoke College in South Hadley, Massachusetts, United States (Cooper & Baker, 2003; Moss & Baker, 2003). These investigations reported no significant differences in macroinvertebrate diversity or abundance between the sites, but found some differences in biotic indices between the sites, suggesting varying levels of pollution for different areas of the system (Cooper & Baker, 2003; Moss & Baker, 2003). Specifically, Lower Lake was significantly less polluted than the area near Morgan Street, but the site in between them, the Cathouse, was the most polluted, indicating an increase and then decrease in pollution from Morgan Street downstream to Lower Lake (Cooper & Baker, 2003). My study builds off of this previous work by repeating the macroinvertebrate surveys and conducting additional surveys at lake sites to investigate any changes in macroinvertebrate communities and use those changes and new surveys as indicators of the ecosystem’s health. It specifically seeks to investigate how the conditions of the ecosystem have changed in regards to temperature, species compositions, and pollution indicators, with additional sites added to provide a more comprehensive view of the system, act as a reference point for future studies, and contribute to the body of data evaluating Project Stream, which is an intensely studied restoration site.

MATERIALS AND METHODS

Macroinvertebrate Sampling

Aquatic macroinvertebrate samples were conducted at six total sites: four stream sites and two lake sites. The four stream sites were the Morgan Street site (site 1; 42º15’05”N 72º33’37”W), the Cathouse site (site 4; 42º15’24”N 72º34’18”W), the Below Lower Lake site (site 6; 42º15’09”N 72º34’23”W), and the Project Stream site (42º15’33”N 72º34’05”W) (Figure 1). The Project Stream site is located in a newly restored stream which flows south into the western portion of Upper Lake. Each lake had a single site with the Upper Lake site located along the northern border (42º15’32”N 72º34’03”W) of the lake and the Lower Lake site located along the northern shore by Prospect Hall (42º15’18”N 72º34’17”W).

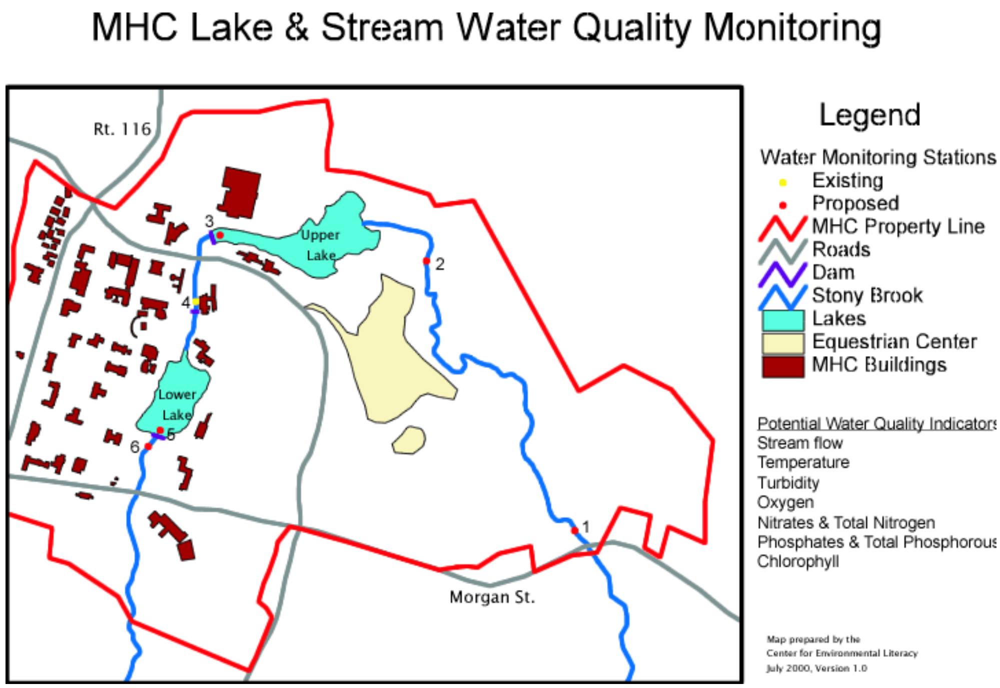

Figure 1. Water monitoring site locations at Mount Holyoke College.

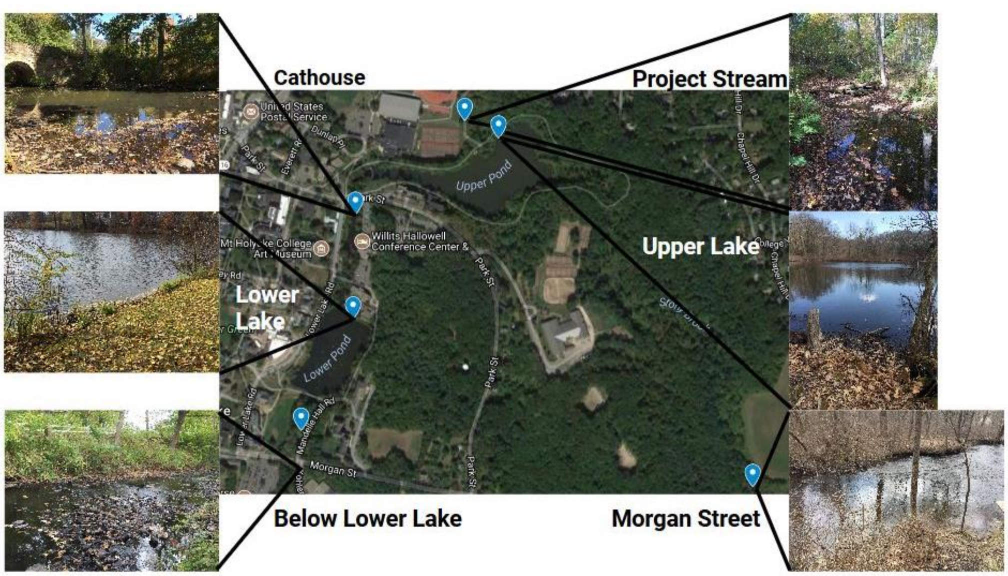

Figure 2. Sampling site locations at Mount Holyoke College.

The stream sites were sampled using the kick seine method. A Surber 500Mu Nitex Net with a 0.093 square meter quadrat at the front base of the net was placed in the stream so that the current flowing into the opening of the net (Figure 2). I spent one minute searching for and rinsing off rocks within the quadrat with my hands and then spent one minute disturbing the bottom of the stream in and in front of the quadrat using my hands and feet. If there were no rocks within the quadrat to process, I searched for some directly in front of the quadrat. All materials rinsed from the rocks or disturbed from the bottom of the stream were washed into the net by the current. After collecting the sample in the net, I transferred it to a container with some stream water and stored the container in a refrigerator with the lid open for later sorting and identification. Three samples were conducted at each site with three collections per sample. The Morgan Street site was sampled on September 30, 2017, the Cathouse site was sampled on October 3, 2017, the Below Lower Lake site was sampled on October 6, 2017, and the Project Stream site was sampled on October 14, 2017.

Figure 3. The Surber 500Mu Nitex Net used in the kick seine method for sampling the stream sites.

The lake sites were sampled using the dip net method. A D-Frame Net was used to collect sediment from the bottom of the lake by placing the flat edge of the net on the lake bottom and pulling backwards approximately 2 feet (Figure 3). The collected sediment was transferred to a sieve bucket where it was strained to remove as much excess material as possible (Figure 4). The remaining sample was stored in a container with lake water in a refrigerator for later sorting and identification. Only one sample was collected at each lake site. The Upper Lake site was sampled on October 28, 2017 and the Lower Lake site was sampled on November 4, 2017.

Figure 4. The D-Frame Net used in the dip net method for sampling the lake sites.

Figure 5. The sieve bucket used to strain collected sediment in the dip net method when sampling the lake sites.

No more than 24-36 hours after sampling, I sorted the macroinvertebrates and preserved them in 70% ethanol. For each sample, I transferred a small amount of the material from the sample container along with some of the corresponding water into a petri dish and used a bright lamp and forceps to find and remove invertebrates from among the sediment and debris. I placed the invertebrates in vials containing ethanol, combining those similar in appearance in the same vial.

After collecting and sorting all samples, I consulted with Dr. Leszek Bledzki to identify the contents of each vial using primarily the *Key to Macroinvertebrate Life in the River* developed by the University of Wisconsin-Extension in cooperation with the Wisconsin Department of Natural Resources and the *Guide to Aquatic Insects and Crustaceans* by The Izaak Walton League of America (2006), with supplementary reference books and guides used as needed.

Abiotic Data

Water quality data has been collected on a biweekly basis at various sites since 1996 using a YSI 6600 V2 portable water quality sampling probe and data logger. These data (among other) include measurements of temperature, dissolved oxygen, pH, chlorophyll *a* levels, blue-green algae concentration, NO_3-N, NH_4-N, and PO_4-P (measured spectrophotometrically).

Analysis

Data analysis involved calculating and comparing biotic indices and diversity indices, as well as comparing water quality data between the sites from 2003 and 2017 in an effort to determine how the health of the ecosystem has changed over time and how the sites differ from each other currently. Biotic indices and diversity measurements will also be compared across sites within 2017 to examine quality variation throughout the ecosystem. The biotic index used was the Major Group Biotic Index which assigns a tolerance value to each major group and then uses the abundance of each group to produce a measure of pollution as indicated by the macroinvertebrate community (Dates, 1997). This same biotic index was calculated in the 2003 study for the Morgan Street site, the Cathouse site, the Below Lower Lake site, and an additional site located downstream of the Morgan Street site but just prior to the opening of the stream into Upper Lake, referred to henceforth as the Upper Lake Bridge site (Cooper and Baker, 2003; Moss and Baker, 2003). The diversity indices, which are used as additional indicators of ecosystem quality, include species richness (a direct count of the number of species present) and Shannon's diversity ($H' = -\sum p_i \ln(p_i)$ where p_i is the relative abundance of the *i*th species), and were calculated using the software EcoSim, which produces individual-based rarefaction curves using a method similar to bootstrapping (Gotelli and Entsminger, 2009). Diversity indices were only calculated for sites from 2017 because the samples were identified to different taxonomic levels than those from the 2003 sites. In the 2003 study, 11.3% of the specimens were identified down to the class level, 23.6% to the subclass level, 15.5% to the order level, 1.7% to the suborder level, and 47.9% to the family level, whereas in this study, 0.6% were identified to the phylum level, 9.7% to the class level, 3.0% to the subclass level, 45.1% to the order level, 5.5% to the suborder level, 35.7% to the family level, 0.2% to the genus level, and 0.3% to the species level. Additionally, I analyzed trends in water quality data over time, specifically trends in temperature, dissolved oxygen, pH, chlorophyll levels, blue-green algae concentration, NO_3-N, NH_4-N, and PO_4-N. All statistics were calculated in EcoSim and Rstudio.

RESULTS

Biotic Indices - Improvement from 2003 to 2017

In general, the biotic indices decreased over time for comparable sites, indicating an improvement in water quality between 2003 and 2017. The biotic index for the Cathouse site decreased by more than half since 2003, and the biotic index for the Morgan Street site decreased by a fair amount (Table 1). However, the biotic index for the Below Lower Lake site decreased minimally, suggesting very little, if any, actual change in quality (Table 1). In 2003, the most polluted site was the Cathouse site and as of last year the most polluted site was the Lower Lake site, though the Upper Lake Bridge site was not sampled last year and neither of the lake sites nor the Project Stream site were sampled in 2003 (Table 1). The Upper Lake Bridge site was not sampled in this study because since 2003, a beaver dam has been built above it which has altered the area from a flowing stream to a slow moving small pond, thus rendering it no longer comparable.

Table 1. The biotic indices for the lake sites and the Project Stream site and the average biotic indices for each of the stream sites. The indices from 2003 were reported in Moss and Baker (2003) and Cooper and Baker (2003). The scale for the biotic indices is as follows: 0-3.75 = Excellent, 3.76-4.25 = Very good, 4.26-5.00 = Good, 5.01-5.75 = Fair, 5.76-6.50 = Fairly poor, 6.51-7.25 = Poor, >7.26 = Very poor.

Site	Biotic Index	
	2003	2017
Morgan Street	5.554, Fair	3.333, Excellent
Cathouse	7.929, Very poor	3.646, Excellent
Below Lower Lake	4.143, Very good	4.114, Very good
Upper Lake Bridge	6.255, Fairly poor	-
Upper Lake	-	5.679, Fair
Lower Lake	-	7.167, Poor
Project Stream	-	5.882, Fairly poor

Diversity Indices - Streams are more diverse, lakes are less diverse

According to only species richness, the Cathouse and Below Lower Lake sites showed the most diversity while the lake sites and the Project Stream site lacked in diversity, comparatively (Table 2). However, when accounting for abundance, the Project Stream site did not significantly differ from the Morgan Street, Cathouse, Below Lower Lake, or Upper Lake sites in its species richness while the Lower Lake site differed significantly from all other sites (Table 3). When using Shannon's diversity, which accounts for both species richness and evenness, the Upper Lake site was the most diverse, though the Lower Lake and

Project Stream sites were still the least diverse (Table 4). All of the stream sites - Morgan Street, the Cathouse, Below Lower Lake, and Project Stream - formed a group with no significant differences between them, while the Upper Lake and Lower Lake sites were both significantly different from each and the other sites in their Shannon's diversity indices (Table 5).

Table 2. The average species richness per site as calculated by the software EcoSim using data from 2017.

Site	Average species richness
Morgan Street	15.511
Cathouse	19.816
Below Lower Lake	19.711
Upper Lake	14.615
Lower Lake	14.663
Project Stream	14.776

Table 3. Differences in species richness among sites. A dash (-) indicates no significant difference between the two sites and an asterisk (*) indicates a significant difference (p-value = 0.05).

	Morgan Street	**Cathouse**	**Below Lower Lake**	**Project Stream**	**Upper Lake**	**Lower Lake**
Morgan Street		*	*	-	*	*
Cathouse			-	-	-	*
Below Lower Lake				-	-	*
Project Stream					-	*
Upper Lake						*
Lower Lake						

Table 4. The average Shannon's diversity for each site as calculated by the software EcoSim using data from 2017.

Site	Average Shannon's diversity
Morgan Street	2.07847
Cathouse	2.18519
Below Lower Lake	2.12615
Upper Lake	2.31526
Lower Lake	1.80955
Project Stream	1.98743

Table 5. Differences in Shannon's diversity indices among sites. A dash (-) indicates no significant difference between the two sites and an asterisk (*) indicates a significant difference (p-value = 0.05).

	Morgan Street	**Cathouse**	**Below Lower Lake**	**Project Stream**	**Upper Lake**	**Lower Lake**
Morgan Street		-	-	-	*	*
Cathouse			-	-	*	*
Below Lower Lake				-	*	*
Project Stream					*	*
Upper Lake						*
Lower Lake						

Abiotic Data - Temperature and pH increase from 1996 to 2017

Analysis of the abiotic data showed significantly positive trends in average temperature and average pH over time (average temperature: t-value=5.646, p-value<0.001, R^2=0.6266; average pH: t-value=5.415, p-value=3.171×10^{-5}, R^2=0.6068; Figures 6 and 9). Between 1996 and 2017, the average temperature has increased approximately 6^{o}C between all sites and average pH has increased by about 1 (Figures 6 and 9). Time explained very little of the variation in all of the other abiotic factors ($0.0025 \leq R^2 \geq 0.2946$; Figures 7, 8, 10, 11, 12, 13, 14). Dissolved oxygen saturation has remained approximately stable with a slight decline in the concentration of dissolved oxygen, despite the increase in temperature which reduces water's capacity to hold dissolved oxygen (Figures 7 and 8). Chlorophyll *a* and the concentration of orthophosphate both declined until about 2007 and since then have been increasing somewhat (Figures 10 and 14). Blue-green algae concentration has been considerably varied over the years while the concentration of nitrate has generally increased, though again with much variation (Figures 11 and 12).

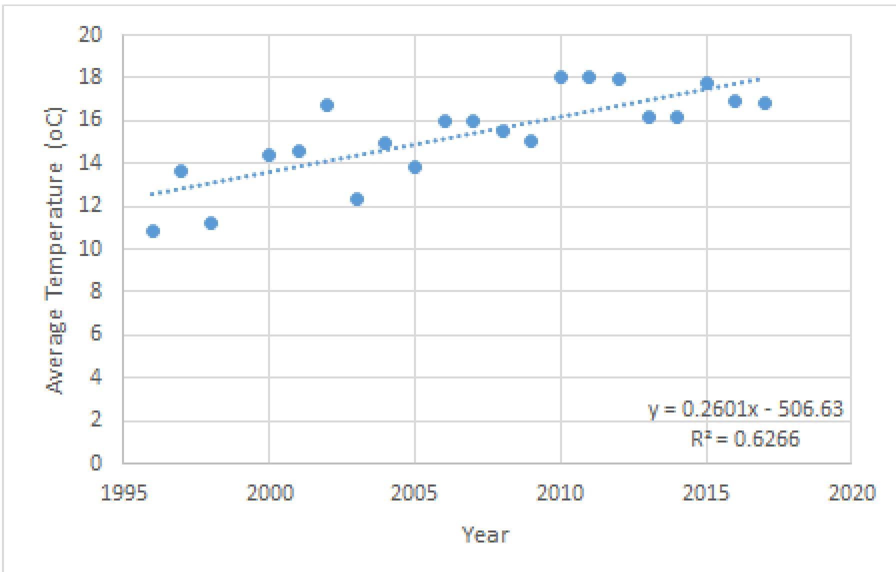

Figure 6. The average annual temperature (°C) across all available sites over time. No sites were measured in 1999 and the Project Stream and Morgan Street sites were not measured until the year 2000. Additionally, the Below Lower Lake site was not measured in 2001.

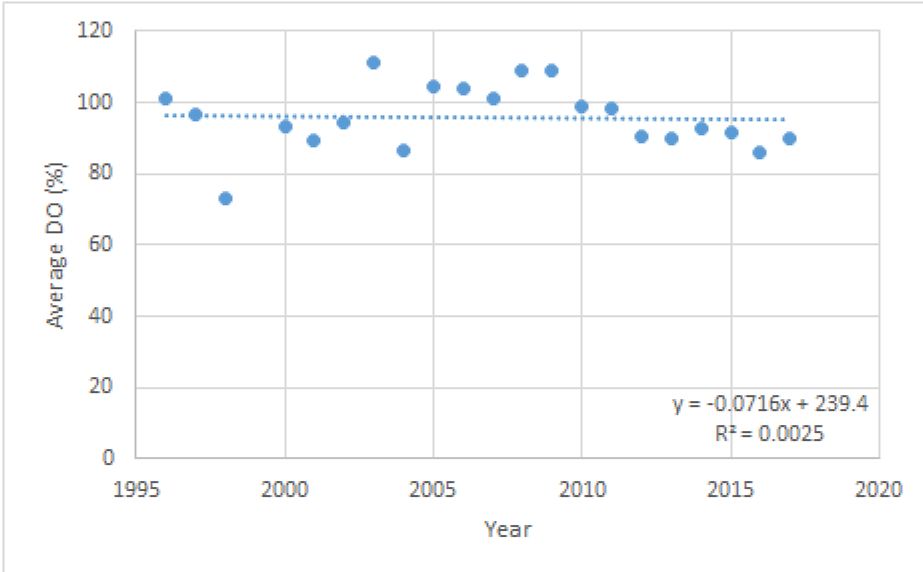

Figure 7. The average annual percentage of dissolved oxygen across all available sites over time. No sites were measured in 1999 and the Project Stream and Morgan Street sites were not measured until the year 2000. Additionally the Below Lower Lake site was not measured in 2001.

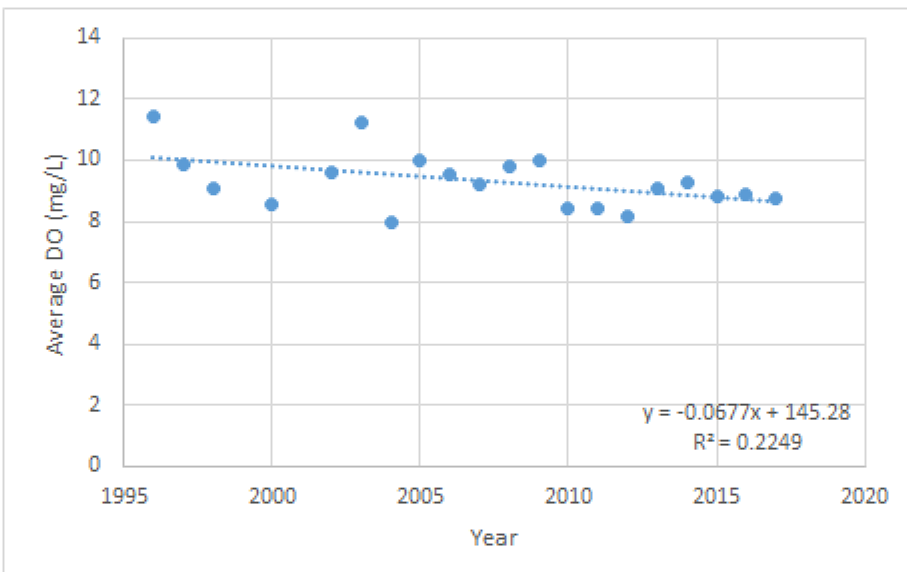

Figure 8. The average annual dissolved oxygen (mg/L) across all available sites over time. No sites were measured in 1999 or 2001 and the Project Stream and Morgan Street sites were not measured until the year 2000.

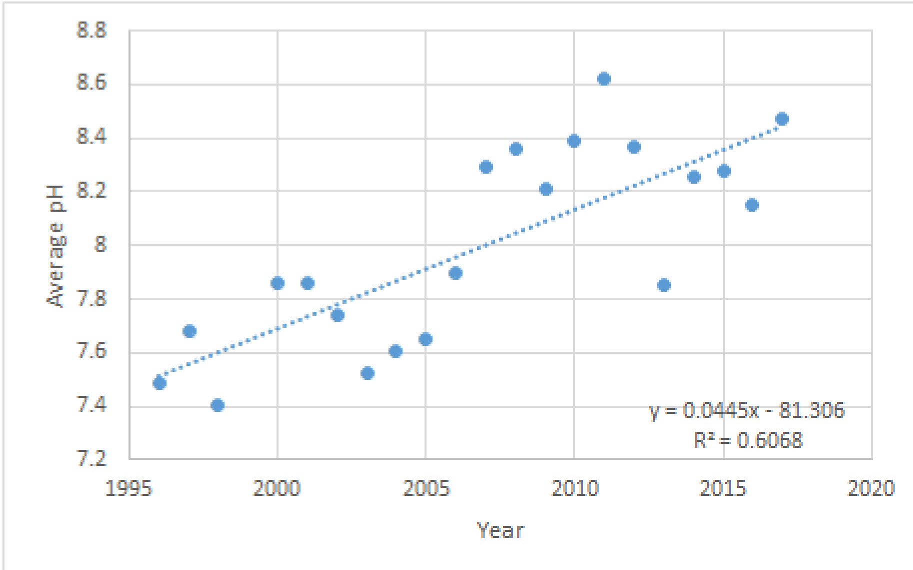

Figure 9. The average annual pH across all available sites over time. No sites were measured in 1999 and the Morgan Street and Project Stream sites were not measured at all prior to 2000.

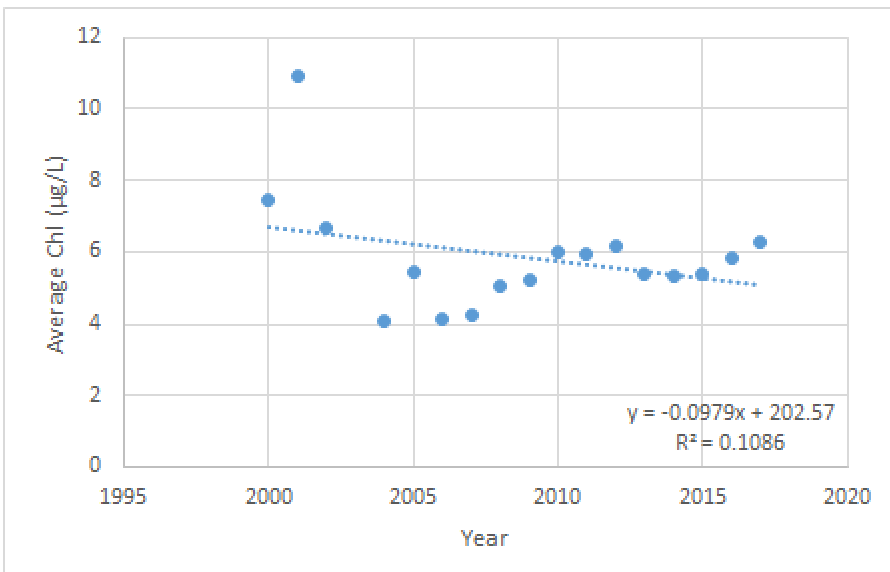

Figure 10. The average annual chlorophyll *a* (µg/L) across all available sites over time. No sites were measured in 2003.The Project Stream, Upper Lake, and the Cathouse sites were not measured in 2002 and the Below Lower Lake site was not measured in 2001 or 2002.

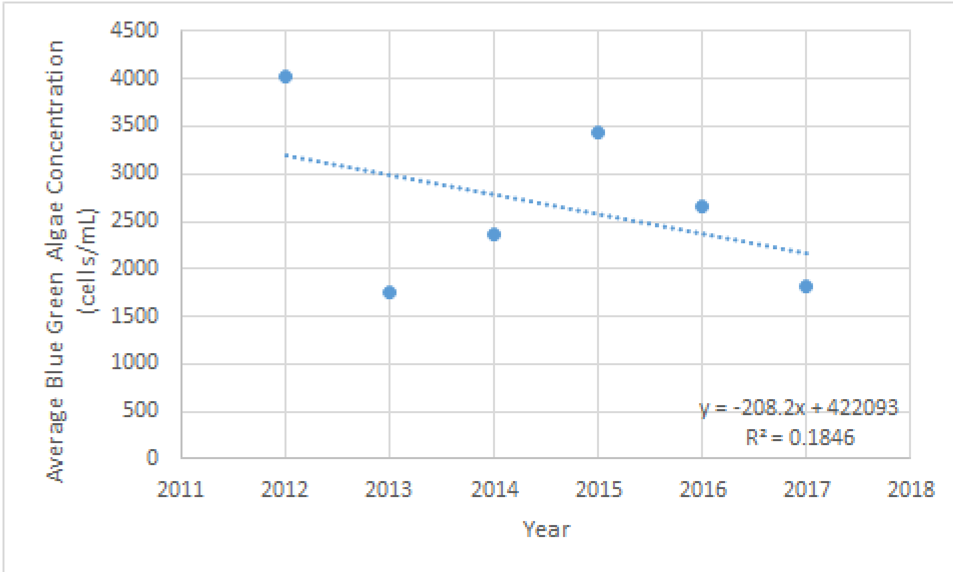

Figure 11. The average annual concentration of blue green algae (cells/mL) across all sites over time.

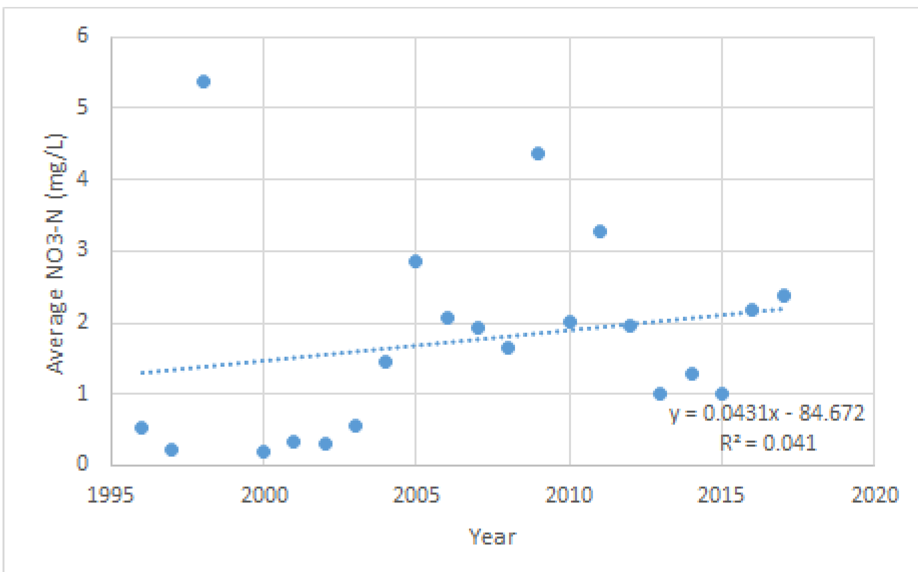

Figure 12. The average annual concentration of nitrate as nitrogen (NO_3-N) across all available sites over time. No sites were measured in 1999 and the Morgan Street and Project Stream sites were not measured at all prior to 2000.

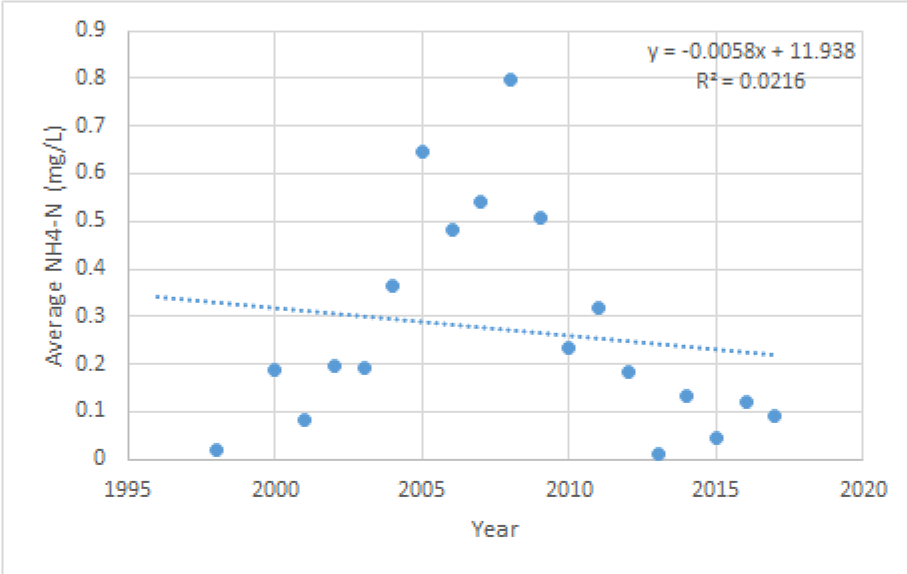

Figure 13. The average annual concentration of ammonium as nitrogen (NH_4-N) across all available sites over time. Prior to the year 2000, only the Upper Lake, Lower Lake, Cathouse, and Below Lower Lake sites were measured in 1998.

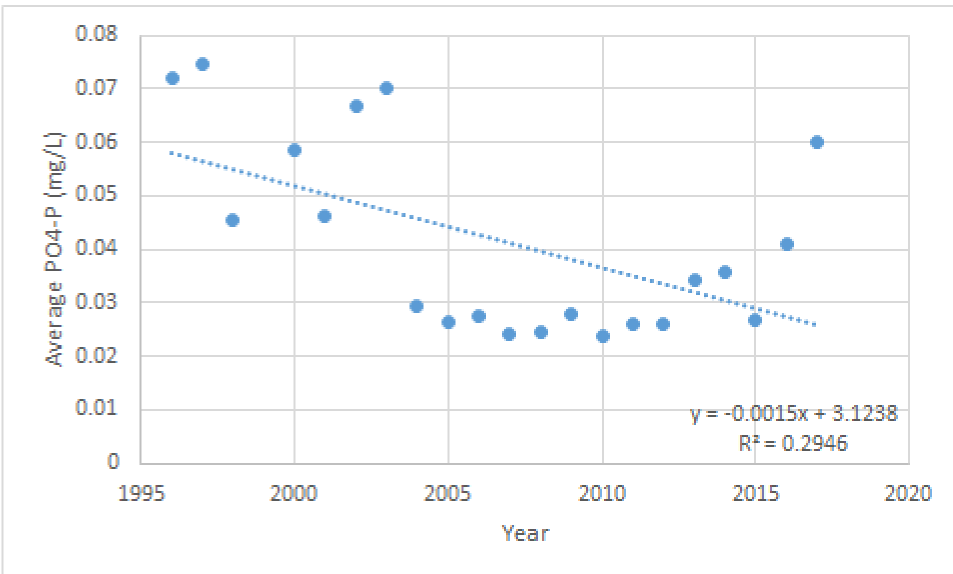

Figure 14. The average annual concentration of orthophosphate (PO_4-P) across all available sites over time. No sites were measured in 1999 and the Morgan Street and Project Stream sites were not measured at all prior to 2000.

DISCUSSION

Biotic Indices

According to the general decrease biotic indices for each site, the amount of pollution at each site has decreased since 2003 and the quality of the ecosystem has improved. Although the concentrations of orthophosphate and nitrate have declined since 2003, the concentration of ammonium has increased. The College did not use fertilizers from 1996 to 2003 or 2004, but then began applying fertilizer with slow release nitrogen twice a year until 2016, which should have produced the opposite trend: an increase in pollution (Michael Buckley, personal communication).

Each site showed different biotic indices with the Morgan Street, Cathouse, and Below Lower Lake sites having similar, lower indices, and the Upper Lake, Lower Lake, and Project Stream sites having similar, higher indices. These differences may be attributed to the type of site, extent of pollution, and relative location on campus. The indication of higher pollution in both of the lakes may be a result of pollution flowing into the lakes from a variety of sources including the streams. Project Stream is currently being restored and was selected as a site for restoration due to high levels of pollution from runoff from the athletic fields and golf course, which have likely not yet returned to ideal levels.

Within the group of more polluted sites, the Upper Lake site had the lowest biotic index, potentially due to the relative lack of activity, such as driving, walking pets and not cleaning up their waste, and maintaining landscapes, around Upper Lake, compared to Lower Lake, which is intensively used by birds especially during migration as well as daily by local flocks of ducks. Additionally, Upper Lake is more resistant to pollution due to its larger capacity and water retention time, which is about 4 days compared to 1.5 days for Lower Lake (Błędzki and Ellison, 2000). The higher water capacity dilutes pollutants and, depending on the type of pollution, the longer water retention time allows more opportunities for pollutants to break down or transform into less harmful forms. Among the group of less polluted sites, the Morgan Street site was less polluted than the Cathouse and Below Lower Lake sites, and is both relatively far from the main area of campus and therefore likely experiences less activity around it compared to the other two sites, and at the start of the system that flows through campus and thus may not have accumulated pollution produced by the campus.

Two potential issues with the biotic indices are the source of the tolerance values and the level of specificity used in calculating them. The tolerance values used in calculating the biotic indices originated from studies from the 1980s which were conducted in Wisconsin, and therefore may be outdated and inappropriate for our location. Since the samples were only identified down to the family level, the family index was used rather than the species index, which produces less reliable, accurate results because families contain a wide variety of species with potentially different tolerances to pollution. Similarly, due to the coarse level of identification, the use of indicator species may not be entirely reliable. With this in mind, there was a relatively high proportion of Oligochaetes at the Lower Lake site and Chironomids at the Morgan Street, Cathouse, Below Lower Lake, and Upper Lake sites, both of which supposedly indicate low dissolved oxygen, high temperatures, and moderate pollution (Myslinski & Ginsburg, 1977; Hellawell, 1986; Rosenburg & Resh, 1993). While all sites except the Morgan Street site had higher average temperatures than the overall average for 2017, none of the sites with high levels of Oligochaetes or Chironomids had particularly low dissolved oxygen according to our data. However, the sites were only monitored every second week, allowing for time in between samples when the dissolved oxygen could have dropped and impacted the macroinvertebrate community. Additionally, the Cathouse site boasted a relatively high proportion of Trichoptera and the Morgan Street and Below Lower Lake sites produced high proportions of Ephemeroptera, both of which are indicative of little pollution, as evident in the low biotic indices for those sites. However, Plecoptera, which is the least pollution tolerant of the three indicator species, was not particularly abundant at any sites.

Diversity Indices

In regards to the diversity indices, the unusual differences in species richness, which is simply the number of total species or in this case taxonomic groups observed at a site, may be attributed to differences in the abundance of specimens collected at each site. Therefore, the Shannon's diversity index, which takes into account species evenness along with species richness (compared through the rarefaction method using EcoSim software, which allows for abundance standardization and comparison of species diversity across the sites at the same abundance), proves to be a more reliable and accurate measurement of diversity. The four stream sites were all significantly ($p=0.05$) more diverse than the Lower Lake site and significantly less diverse than the Upper Lake site. Streams and rivers tend to host a wider diversity of benthic macroinvertebrates than lakes due to their flowing water and substrate composition, which may explains the stream sites' relationship with the Lower Lake site (Horne and Goldman, 1994). Upper Lake is larger and has better developed littoral zones which support many invertebrate groups, compared to Lower Lake which is mostly lacking in littoral zones (Leszek Bledzki, personal communication). Additional differences between the sites may attributed to differences in their physical properties such as water depth, substrate composition, width or size of the body of water, current, surrounding vegetation, or amount of shade or sunlight.

The varying levels of taxonomic identification may have impacted the accuracy of the diversity indices. Although not all specimens were identified to the same taxonomic level, they were identified as specifically as possible and grouped as necessary to avoid potential overlap. For example, some Odonata were identified as either Epiprocta (dragonfly) or Zygoptera (damselfly), but I could not identify all specimens down to their suborder, so all were classified as Odonata to count towards a single taxonomic order. Therefore, the diversity indices are the most conservative estimates, and the actual measurements of diversity are greater. Ideally, all specimens would be identified down to a species level to produce the most accurate diversity indices and allow for more reliable and specific information to be gleaned from the indicator species.

Abiotic Data

Temperature and pH were the only abiotic factors which demonstrated a significant linear relationship over time. The increase in temperature is likely due in part to climate change to climate change, and in the case of the lakes is helped along by them slowly filling in and reducing the volume of water which is then easier to heat up, while the increase in pH mirrors the current trend of recovery from acidification in lakes in the United States, though this shift towards basicity may have been overshot in this case (Kahl et al., 2004; USEPA, 2006). Facilities Management has been spreading lime on campus since 1996 (Michael Buckley, personal communication), which is intentionally used to raise the pH to promote lawn health. Run-off from the lawns likely empties into the lakes, and the lime in the run-off probably contributed to the increase in pH. Although it is good that the lakes are not facing the previously common problem of acidification, the pH is approaching 8.65, which is the upper limit of the ideal range for supporting high levels of diversity (Berezina, 1999). The rise in temperature will likely produce a shift in species composition of the macroinvertebrate community which favors warm water adapted species (Burgmer et al., 2007; Daufresne et al., 2007; Domisch et al., 2011).

Among the more unusual trends amongst other abiotic factors were those for nitrogen and phosphorus concentrations. The concentrations of nitrogen, especially ammonium, generally increased until about 2007 or 2008 and since then has generally decreased. Meanwhile, phosphorus followed the opposite trend in that it primarily decreased until 2006 or 2007 and has been increasing since then. Compared to 2003, the average nitrate nitrogen concentration has increased, but the average ammonium nitrogen and phosphate phosphorus concentrations have decreased, which logically correlates to the lower biotic indices, indicating lower pollution levels and better quality ecosystems. The recent decline in ammonium may be attributed to the removal of beavers from Upper Lake in 2011 and the reduced number of birds using Lower Lake since 2002 when they were discouraged from utilizing the lake by a specially trained dog (Leszek Bledzki, personal communication). Though the changes in the average concentrations across all sites followed approximately the same pattern as the biotic indices, the same was not necessarily true for each individual site. At the Morgan Street and Below Lower Lake sites, the concentration of ammonium nitrogen decreased between 2003 and 2017, but the concentrations of nitrate nitrogen and phosphate phosphorus increased, albeit the change in orthophosphate concentrations was slight. The Cathouse, Upper Lake, Lower Lake, and Project Stream sites all experienced an increase in the concentration of nitrate nitrogen between 2003 and 2017 and a decrease in the concentrations of ammonium nitrogen and phosphate phosphorus. These contradictory patterns suggest that the decrease in overall pollution according to the lower biotic indices may be related to which pollutants are higher or lower in abundance as well as the magnitude of change in the concentrations over time. The biotic indices alone do not present comprehensive detailed information about the changes in pollution, but they reflect the response to pollution by macroinvertebrates.

The recommended average concentration of nitrate nitrogen to protect freshwater aquatic life is 3.0 mg/L and the maximum concentration is 32.8mg/L (Nordin and Pommen, 2009). While we are nowhere near the maximum concentration, with the highest average concentration in our system in the past three years being 2.47 mg/L at the Project Stream site in 2016, we have in the past surpassed the recommended average concentration and, if the current increasing trend continues, we will again go beyond the recommended average. The recommended average concentration of ammonia as nitrogen at a pH of 8.4 and a temperature of 17^{o}C, is 0.393 mg/L and the maximum concentration is 2.35 mg/L (Nordin and Pommen, 2009). Again, with the highest average in our system in the past three years being 0.16 mg/L at the Project Stream site in 2014, we are far from the maximum concentration, but we have previously exceeded the recommended average, though the current declining trend proves promising. According to the EPA, total phosphate phosphorus should not exceed 0.1 mg/L in streams, if eutrophication is to be controlled (Muller and Helsel, 1999). Fortunately, the average concentration of orthophosphates in the campus freshwater system has never exceeded this limit, but if the current increasing trend continues, we may reach 0.1 mg/L.

Future Studies

Given the relatively limited scope of this study, additional research is necessary to fully understand the processes and reasons behind the changes in pollution and macroinvertebrate community health of the Mount Holyoke College campus freshwater system. An experimental study investigating the correlations and ideally causal relationships between the abundance of certain species and trends in abiotic factors would allow for the development of more informative indicator species. A closer investigation of changes in the proportions of feeding roles in the macroinvertebrate community would provide a greater understanding of the changes in the community structure and function. An exploration of differences or the lack of differences in the morphology of macroinvertebrates from different sites or over time could provide additional information about the quality of conditions in regards to their growth and development. Besides studying the abiotic factors from this study, researching the sources of pollution and specifically the use of pesticides and herbicides on campus and in the freshwater system may offer additional insights regarding the composition of the macroinvertebrate communities and the extent of pollution. Ideally, this study will be repeated in several years (preferably with higher identification specificity) to analyze the progression of changes in pollution and the macroinvertebrate community, preferably with the addition of a reference site against which the on-campus sites may be compared. Moreover, depending on timing, future studies of the lakes should investigate the impact of dredging on pollution, since the College most recently dredged both lakes in 1985 and 1986. Further additional studies may be conducted in the surrounding region to investigate how trends in the characteristics of Mount Holyoke College's freshwater system compare to those in other parts of the local area.

Implications

While the streams on the Mount Holyoke College campus have shown some improvement in quality since 2003, the lakes could use additional assistance in reducing pollution and improving macroinvertebrate diversity, especially in the case of Lower Lake. Mount Holyoke College should take into account these findings when developing plans to increase campus sustainability and generally make the College more ecologically friendly. Several abiotic trends, namely the increase in temperature and pH and the decrease in nitrate concentrations, mirror the overall trends of lakes in the Northeastern United States (Kahl et al., 2004), though the macroinvertebrate biotic indices suggest that our freshwater system is healthier than the average stream in the Northeast (USEPA, 2006). Other environmentally conscious organizations may benefit from using this study as an inspiration to investigate the health of local freshwater systems and develop appropriate plans for addressing their needs. Although an individual's impact on fighting climate change is limited without policy reform, pollution is much more localized and may be monitored and reduced by individuals dedicated to improving the quality of freshwater systems. Since the implementation of policies such as the Clean Air Act and the Clean Water Act, freshwater systems and the ecosystems of the United States have improved overall (Kahl et al., 2004), though potential revocations of those policies pose a threat to this trend of improving health. However, individual or more local decisions to employ stricter policies or continue to follow rescinded policies, as in the case of many states pledging to follow the Paris climate agreement despite the federal government's opposing stance, is an important step towards ensuring the health of our environment. Therefore, restoring and cleaning up freshwater ecosystems will require the assistance of many people at all levels of power and ability.

Literature Cited

Abell, R. 2002. Conservation biology for the biodiversity crisis: A freshwater follow-up. Conservation Biology 16(5): 1435-7.

Bakken L and Bleken M. 1998. Temporal aspects of N-enrichment and emission of N2O to the atmosphere. Nutr Cycling Agroecosyst 52(2-3):107-21.

Berezina, NA. 2001. Influence of ambient pH on freshwater invertebrates under experimental conditions. Russian Journal of Ecology 32(5): 343-51.

Błędzki LA and Ellison AM. 2000. Effects of water retention time on zooplankton of shallow rheolimnic reservoirs. Verh.Int.Ver.Limnol. 27: 2865-2869.

Bonmatin JM, Giorio C, Girolami V, Goulson D, Kreutzweiser DP, Krupke C, Liess M, Long E, Marzaro M, Mitchell EA, and Noome DA. 2015. Environmental fate and exposure; neonicotinoids and fipronil. Environmental Science and Pollution Research, 22(1): 35-67.

Buikema A and Benfield E. 1979. Effects of pollution on freshwater invertebrates. Journal Water Pollution Control Federation 51(6):1708-17.

Burgmer T, Hillebrand H, Pfenninger M. 2007. Effects of climate-driven temperature changes on the diversity of freshwater macroinvertebrates. Oecologia 151(1):93-103.

Cairns J, Lanza G, Parker B. 1973. Pollution related structural and functional changes in aquatic communities with emphasis on freshwater algae and protozoa. Proc Acad Nat Sci Phila 124:79-127.

Camargo J, Alonso A, Salamanca A. 2005. Nitrate toxicity to aquatic animals: A review with new data for freshwater invertebrates. Chemosphere 58(9):1255-67.

Camargo JA and Alonso A. 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. Environ Int 32(6):831-49.

Chessman BC. 2009. Climatic changes and 13-year trends in stream macroinvertebrate assemblages in New South Wales, Australia. Global Change Biol 15(11):2791-802.

Cooper J and Baker K. 2003. Stony Brook's macroinvertebrates.

Dates, G. 1997. Living waters: using benthic macroinvertebrates and habitat to assess your river's health. River Watch Network.

Daufresne M, Bady P, Fruget J. 2007. Impacts of global changes and extreme hydroclimatic events on macroinvertebrate community structures in the French Rhone River. Oecologia 151(3):544-59.

Di Lorenzo T, Di Marzio WD, Saenz ME, Baratti M, Dedonno AA, Iannucci A, Cannicci S, Messana G, Galassi DMP. 2014. Sensitivity of hypogean and epigean freshwater copepods to agricultural pollutants. Environ Sci Pollut Res 21(6):4643-55.

Dodds WK and Welch EB. 2000. Establishing nutrient criteria in streams. Journal of the North American Benthological Society 19(1):186-196.

Domisch S, Jaehnig SC, Haase P. 2011. Climate-change winners and losers: Stream macroinvertebrates of a submontane region in central Europe. Freshwater Biol 56(10):2009-20.

Dudgeon, David, Angela H. Arthington, Mark O. Gessner, Zen-Ichiro Kawabata, Duncan J. Knowler, Christian Leveque, Robert J. Naiman, et al. 2006. Freshwater biodiversity: Importance, threats, status and conservation challenges. Biological Reviews 81(2):163-82.

Durance I and Ormerod SJ. 2007. Climate change effects on upland stream macroinvertebrates over a 25-year period. Global Change Biol 13(5):942-57.

Ellison AM and Błędzki LA. 1997. Biological and chemical characteristics of the lake and stream system on the campus of Mount Holyoke College. An assessment for The Orchards Golf Course. South Hadley, MA: 1-22.

Erisman JW, Sutton MA, Galloway J, Klimont Z, and Winiwarter W. 2008. How a century of ammonia synthesis changed the world. Nature Geoscience 1(10):636.

Gilbert BM and Avenant-Oldewage A. 2017. Parasites and pollution: The effectiveness of tiny organisms in assessing the quality of aquatic ecosystems, with a focus on Africa. Environ Sci Pollut Res 24(23):18742-69.

Gleick PH. 1996. Water resources. Encyclopedia of Climate and Weather (ed. S. H. Schneider). 817–823.

Goodyear K and McNeill S. 1999. Bioaccumulation of heavy metals by aquatic macro-invertebrates of different feeding guilds: A review. Sci Total Environ 229(1-2):1-19.

Gotelli NJ and Entsminger GL. 2009. EcoSim: Null models software for ecology. http://www.uvm.edu/~ngotelli/EcoSim/EcoSim.html, Acquired Intelligence Inc. & Kesey-Bear.

Haidekker A and Hering D. 2008. Relationship between benthic insects (Ephemeroptera, Plecoptera, Coleoptera, Trichoptera) and temperature in small and medium-sized streams in Germany: A multivariate study. Aquat Ecol 42(3):463-81.

Harmon SM. 2008. Effects of pollution on freshwater organisms. Water Environ Res 80(10):892-917.

Heino J, Virkkala R, Toivonen H. 2009. Climate change and freshwater biodiversity: Detected patterns, future trends and adaptations in northern regions. Biol Rev 84(1):39-54.

Hellawell JM. 1986. Biological Indicators of Freshwater Pollution and Environmental Management. Elsevier Applied Science, London.

Hering D, Schmidt-Kloiber A, Murphy J, Luecke S, Zamora-Munoz C, Jesus Lopez-Rodriguez M, Huber T, Graf W. 2009. Potential impact of climate change on aquatic insects: A sensitivity analysis for European caddisflies (Trichoptera) based on distribution patterns and ecological preferences. Aquat Sci 71(1):3-14.

Hilsenhoff WL. 1977. Use of arthropods to evaluate water quality in streams. Wisconsin Department of Natural Resources, Technical Bulletin No. 100.

Hilsenhoff WL. 1982. Using a biotic index to evaluate water quality in streams. Wisconsin Department of Natural Resources, Technical Bulletin No. 132.

Hilsenhoff WL. 1987. An improved biotic index of organic stream pollution. Great Lakes Entomologist 20:31-39.

Horne AJ and Goldman CR. 1994. Limnology. Second Edition. McGraw-Hill, Inc.

Huang J, Xu C, Ridoutt BG, Wang X, Ren P. 2017. Nitrogen and phosphorus losses and eutrophication potential associated with fertilizer application to cropland in China. J Clean Prod 159:171-9.

Ischinger L and Nalepa T. 1975. Freshwater macroinvertebrates. Journal Water Pollution Control Federation 47(6):1520-38.

Izaak Walton League of America. 2006. Guide to aquatic insects & crustaceans. Mechanicsburg (PA): Stackpole Books.

Kahl JS, Stoddard JL, Haeuber R, Paulsen SG, Birnbaum R, Deviney FA, Webb JR, Dewalle DR, Sharpe W, Driscoll CT et al. 2004. Have US surface waters to the 1990 clean air. Environ Science and Tech. 484-490.

Kummu M, de Hoel H, Porkka M, Siebert S, Varis O, and Ward PJ. 2012. Lost food, wasted resources: global food supply chain losses and their impacts on freshwater, cropland, and fertiliser use. Science of the Total Environ 438: 477-489.

Mantyka-Pringle CS, Martin TG, Moffatt DB, Linke S, Rhodes JR. 2014. Understanding and predicting the combined effects of climate change and land-use change on freshwater macroinvertebrates and fish. J Appl Ecol 51(3):572-81.

Masese FO, Muchiri M, Raburu PO. 2009. Macroinvertebrate assemblages as biological indicators of water quality in the Moiben River, Kenya. Afr J Aquat Sci 34(1):15-26.

Metcalfe J. 1989. Biological water-quality assessment of running waters based on macroinvertebrate communities - history and present status in Europe. Environ Pollut 60(1-2):101-39.

Moss S and Baker A. 2003. Under a rock, in a hard place: Stony Brook’s macroinvertebrates.

Mouthon J and Daufresne M. 2006. Effects of the 2003 heatwave and climatic warming on mollusc communities of the Saone: A large lowland river and of its two main tributaries (France). Global Change Biol 12(3):441-9.

Mueller DK and Helsel DR. 1999. Nutrients in the Nation's Waters--Too Much of a Good Thing? U.S. Geological Survey Circular 1136. National Water-Quality Assessment Program.

Myslinski E and Ginsburg W. 1977. Macroinvertebrates as indicators of pollution. Journal (American Water Works Association). 538-544.

MW Center for the Environment. Mount Holyoke College. (accession date - Jan 20, 2018). Water quality data: SB, UL, LL, ProjStr.

Nalepa T. 1978. Freshwater macroinvertebrates. Journal Water Pollution Control Federation 50(6):1301-13.

New England Interstate Water Pollution Control Commission and USEPA. 2010. Gauging the health of New England's lakes and ponds: a survey report and decision-making resource. RC Brayshaw & Co. Warner, NH.

Nordin RN and Pommen LW. 2009. Water quality guidelines for nitrogen (nitrate, nitrite, and ammonia). Water Stewardship Division, Ministry of Environment. Province of British Columbia.

O'Brien A, Townsend K, Hale R, Sharley D, and Pettigrove V. 2016. How is ecosystem health defined and measured? A critical review of freshwater and estuarine studies. Ecological Indicators 69: 722-9.

Ormerod SJ, Dobson M, Hildrew AG, and Townsend CR. 2010. Multiple stressors in freshwater ecosystems. Freshwater Biology 55: 1-4.

Patil A, Hatch G, Michaud C, Brotman M, Regunathan P, Tallon R, Andrew R, Murphy S, VerStrat S, Undesser P, et al. 2014. Ammonia Fact Sheet. Water Quality Association.

Pant HK, Adjei MB, Rechcigl JE. 2004. Agricultural catchments and water quality: A phosphorus enigma. J Food Agric Environ 2(1):355-8.

Rosenberg DM and Resh VH (Eds.). 1993. Freshwater Biomonitoring and Benthic Macroinvertebrates. Chapman and Hall, New York.

Sharpley A, Gburek W, Heathwaite L. 1998. Agricultural phosphorus and water quality: Sources, transport and management. Agric Food Sci Finland 7(2):297-314.

Sharpley AN, McDowell RW, Kleinman PJA. 2001. Phosphorus loss from land to water: Integrating agricultural and environmental management. Plant Soil 237(2):287-307.

Sharpley AN, Gburek WJ, Folmar G, Pionke HB. 1999. Sources of phosphorus exported from an agricultural watershed in Pennsylvania. Agric Water Manage 41(2):77-89.

Sharpley AN, Weld JL, Beegle DB, Kleinman PJA, Gburek WJ, Moore PA, Mullins G. 2003. Development of phosphorus indices for nutrient management planning strategies in the United States. J Soil Water Conserv 58(3):137-52.

Simaika JP and Samways MJ. 2011. Comparative assessment of indices of freshwater habitat conditions using different invertebrate taxon sets. Ecological Indicators 11(2): 370-8.

Thorburn PJ, Biggs JS, Weier KL, Keating BA. 2003. Nitrate in groundwaters of intensive agricultural areas in coastal northeastern Australia. Agriculture Ecosystems & Environment 94(1):49-58.

USEPA. 2006. Wadeable streams assessment: a collaborative survey of the nation's streams. Washington, DC.

USEPA. 2011. Nitrogen and phosphorus in agricultural streams.

USGS.gov. 2016. The World's Water. US Department of the Interior. US Geological Survey.

APPENDIX A

Table A-1. The taxonomic level and taxa of all specimens collected and the sites where they were found.

Taxonomic Level	Taxa	Location
Order	Amphipoda	Morgan Street, Cathouse, Below Lower Lake, Upper Lake, Lower Lake
Class	Bivalvia	Morgan Street, Cathouse, Below Lower Lake, Upper Lake, Lower Lake
Family	Ceratopogonidae	Below Lower Lake, Upper Lake, Lower Lake
Family	Chironomidae	Morgan Street, Cathouse, Below Lower Lake, Project Stream, Upper Lake, Lower Lake
Genus	Cladocera - Diaphanosoma	Below Lower Lake
Genus	Cladocera - Scapholeberis	Upper Lake
Order	Coleoptera	Project Stream
Order	Copepoda - Cyclopoida	Project Stream
Order	Copepoda - Harpacticoida	Project Stream
Family	Culicidae	Cathouse, Below Lower Lake
Family	Dolichopodidae	Cathouse, Project Stream, Below Lower Lake
Family	Elmidae	Morgan Street, Cathouse, Below Lower Lake, Project Stream, Upper Lake, Lower Lake
Family	Empididae	Cathouse
Order	Ephemeroptera	Morgan Street, Cathouse, Below Lower Lake, Upper Lake, Lower Lake
Suborder	Epiprocta	Project Stream, Upper Lake, Lower Lake

Taxonomic Level	Taxa	Location
Subclass	Hirudinea	Lower Lake
Family	Hydropsychidae	Morgan Street, Cathouse, Below Lower Lake, Project Stream
Order	Isopod	Cathouse, Upper Lake, Lower Lake
Family	Lumbriculus	Morgan Street, Cathouse, Below Lower Lake, Project Stream, Upper Lake, Lower Lake
Phylum	Nematode	Project Stream
Order	Odonata	Morgan Street, Cathouse, Below Lower Lake, Lower Lake
Species	Orconectes rusticus	Below Lower Lake
Class	Ostracoda	Upper Lake
Family	Patellidae	Morgan Street, Cathouse, Below Lower Lake, Project Stream
Family	Physidae	Cathouse, Upper Lake, Lower Lake
Class	Planarian	Cathouse
Family	Planorbidae	Project Stream, Upper Lake, Lower Lake
Order	Plecoptera	Morgan Street, Below Lower Lake
Subclass	Prosobranchia	Project Stream, Upper Lake, Lower Lake
Suborder	Prostigmata	Morgan Street, Cathouse, Project Stream, Upper Lake, Lower Lake
Family	Psephenidae	Morgan Street, Cathouse, Below Lower Lake
Subclass	Pulmonata	Upper Lake
Family	Simuliidae	Morgan Street, Cathouse, Below Lower Lake
Family	Tipulidae	Morgan Street, Below Lower Lake, Project Stream

Taxonomic Level	Taxa	Location
Order	Trichoptera	Morgan Street, Cathouse, Below Lower Lake, Project Stream
Class	Turbellaria	Morgan Street, Cathouse, Below Lower Lake, Lower Lake
Suborder	Zygoptera	Morgan Street, Cathouse, Below Lower Lake, Project Stream, Lower Lake

APPENDIX B

Table B-1. Taxonomic groups and their corresponding tolerance values used to calculate biotic indices.

Taxonomic Group	Tolerance Value
Ephemeroptera	2
Plecoptera	1
Trichoptera	3
Chironomidae	7
Other Diptera	4
Odonata	5
Megaloptera	2
Coleoptera	4
Amphipoda	7
Isopoda	8
Decapoda	6
Gastropoda	7
Pelecypoda	7
Oligochaeta	9
Hirudinea	10

APPENDIX C

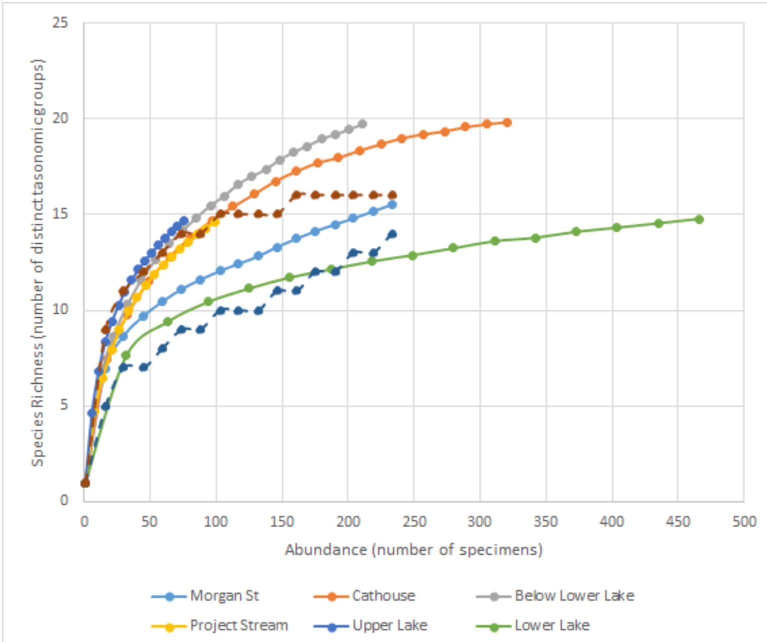

Figure C-1. Species richness at each site in relation to the number of specimens collected (abundance). The dashed red line is the upper limit and the dashed dark blue line is the lower limit of the 95% confidence interval for Morgan Street.

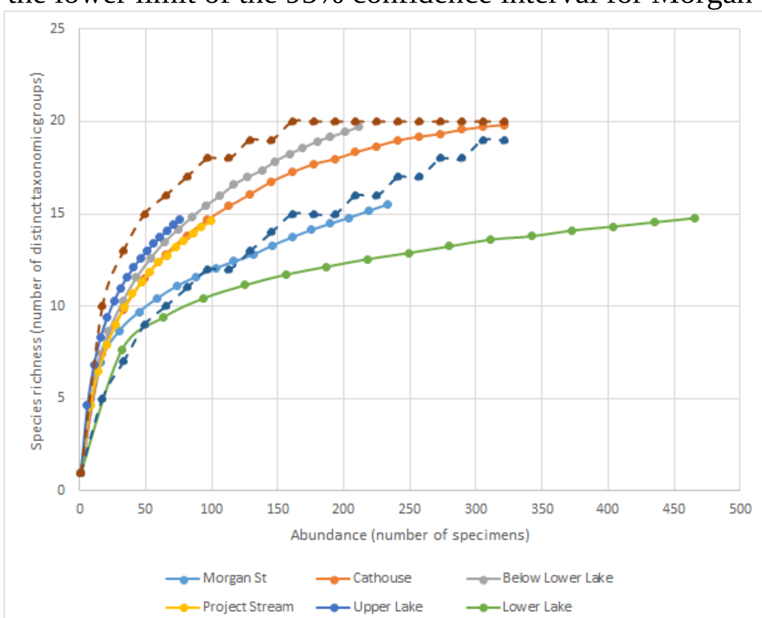

Figure C-2. Species richness at each site in relation to the number of specimens collected (abundance). The dashed red line is the upper limit and the dashed dark blue line is the lower limit of the 95% confidence interval for the Cathouse.

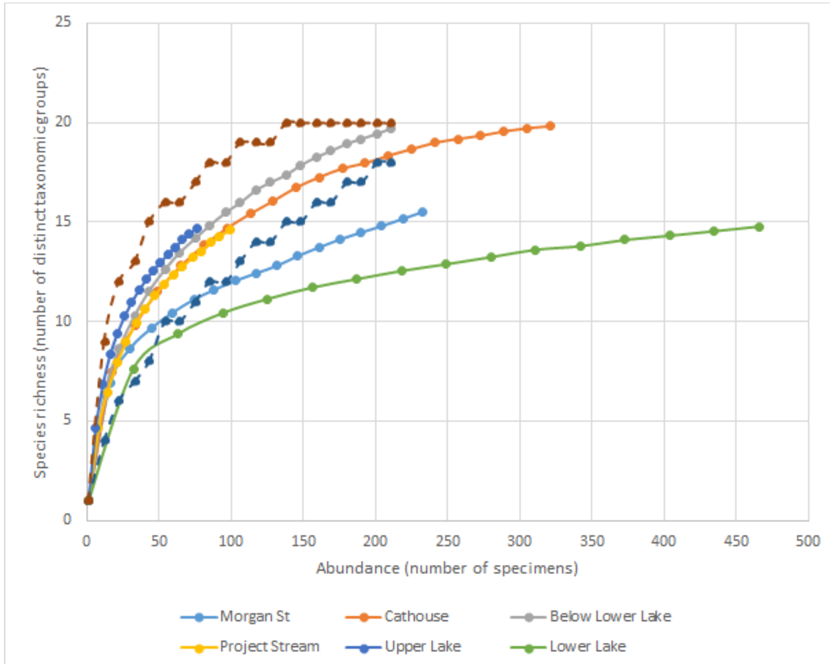

Figure C-3. Species richness at each site in relation to the number of specimens collected (abundance). The dashed red line is the upper limit and the dashed dark blue line is the lower limit of the 95% confidence interval for Below Lower Lake.

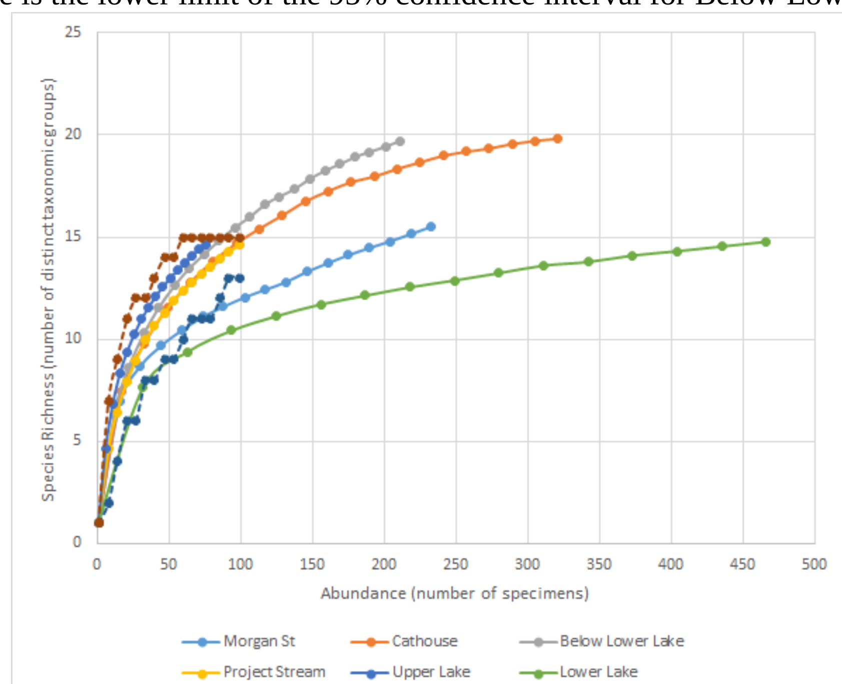

Figure C-4. Species richness at each site in relation to the number of specimens collected (abundance). The dashed red line is the upper limit and the dashed dark blue line is the lower limit of the 95% confidence interval for Project Stream.

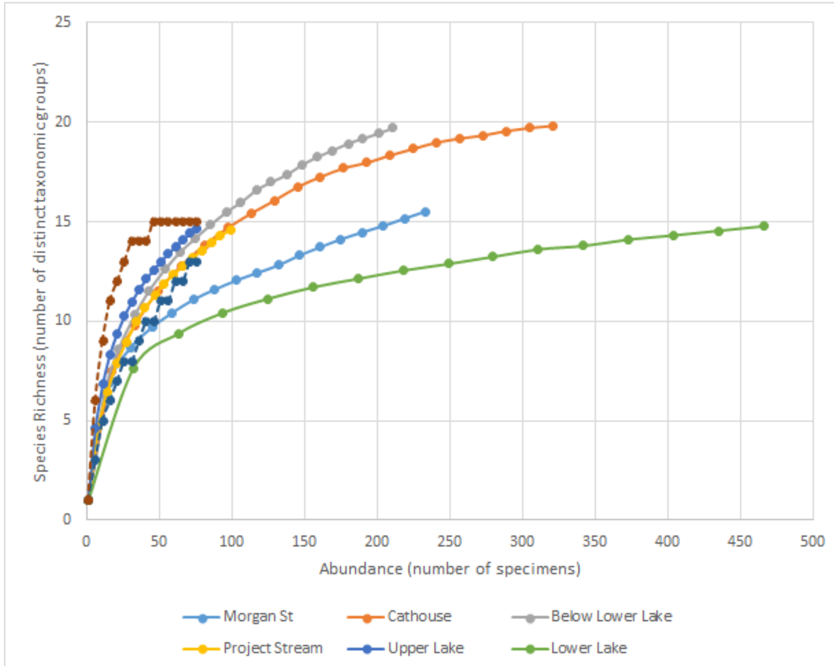

Figure C-5. Species richness at each site in relation to the number of specimens collected (abundance). The dashed red line is the upper limit and the dashed dark blue line is the lower limit of the 95% confidence interval for Upper Lake.

Figure C-6. Species richness at each site in relation to the number of specimens collected (abundance). The dashed red line is the upper limit and the dashed dark blue line is the lower limit of the 95% confidence interval for Lower Lake.

APPENDIX D

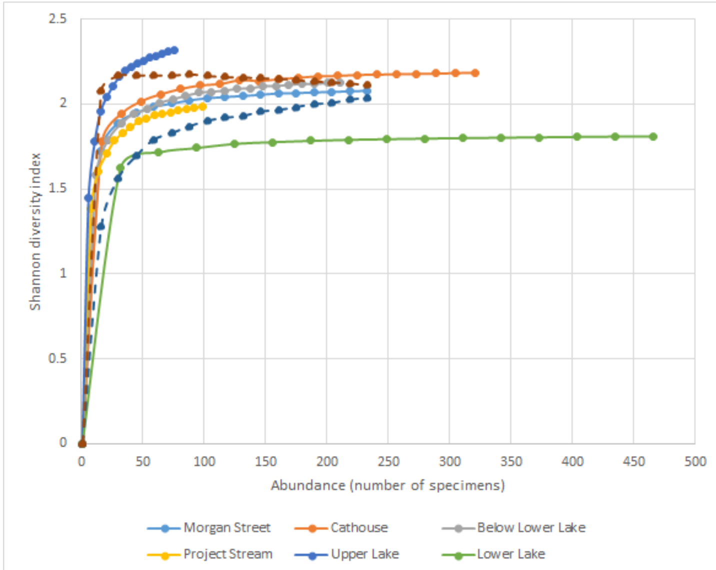

Figure D-1. Shannon’s diversity indices from each site in relation to the number of specimens collected (abundance). The dashed red line is the upper limit and the dashed dark blue line is the lower limit of the 95% confidence interval for Morgan Street.

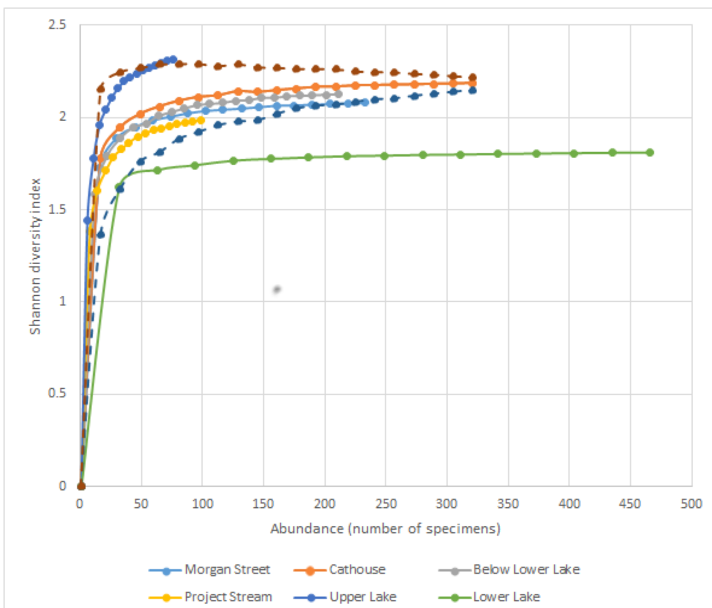

Figure D-2. Shannon’s diversity indices from each site in relation to the number of specimens collected (abundance). The dashed red line is the upper limit and the dashed dark blue line is the lower limit of the 95% confidence interval for the Cathouse.

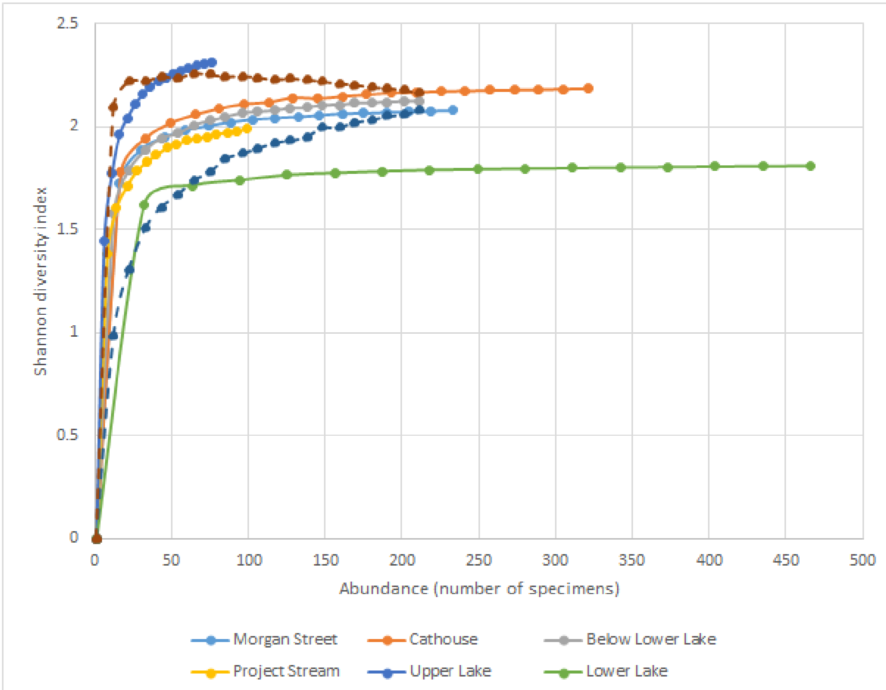

Figure D-3. Shannon's diversity indices from each site in relation to the number of specimens collected (abundance). The dashed red line is the upper limit and the dashed dark blue line is the lower limit of the 95% confidence interval for Below Lower Lake.

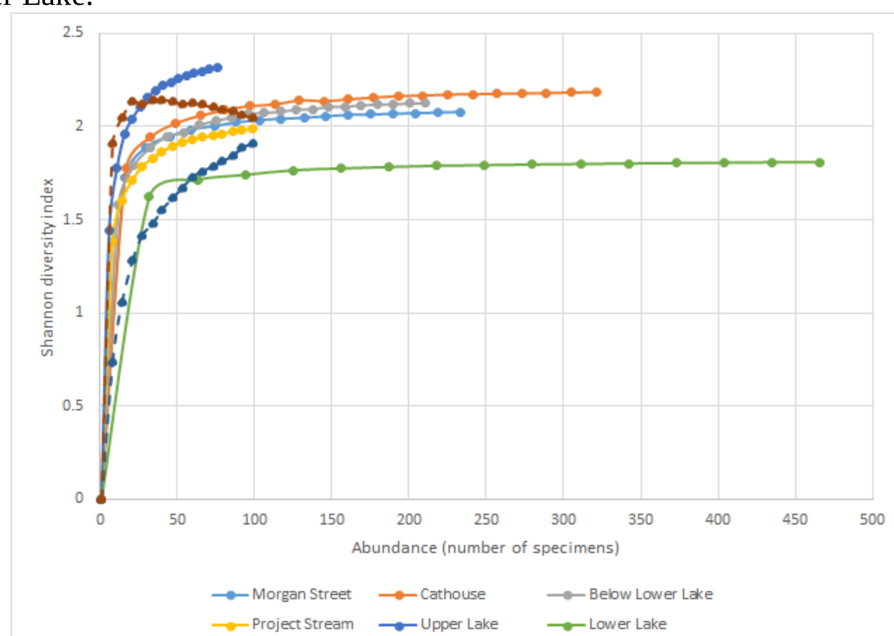

Figure D-4. Shannon's diversity indices from each site in relation to the number of specimens collected (abundance). The dashed red line is the upper limit and the dashed dark blue line is the lower limit of the 95% confidence interval for Project Stream.

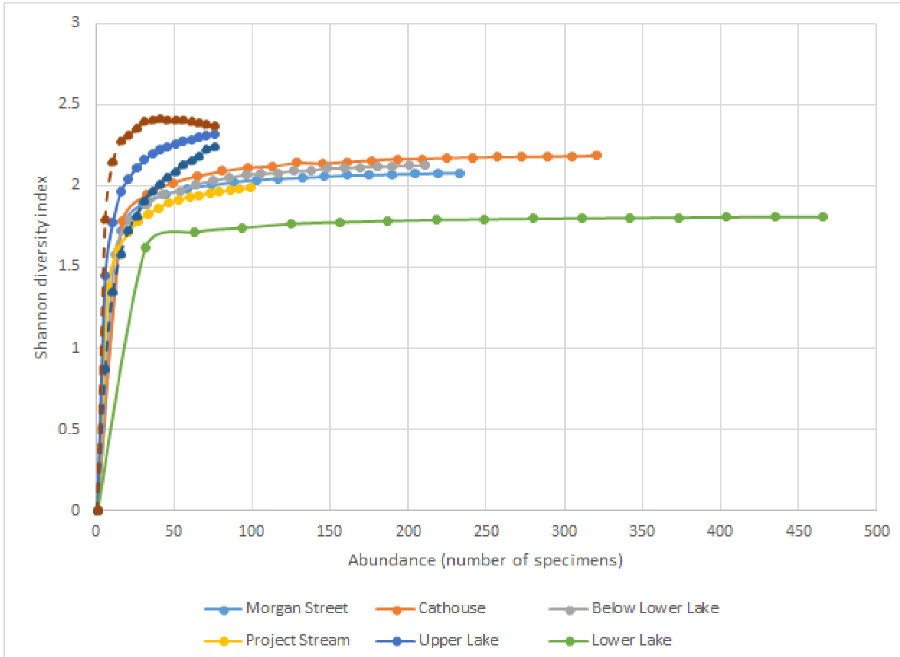

Figure D-5. Shannon's diversity indices from each site in relation to the number of specimens collected (abundance). The dashed red line is the upper limit and the dashed dark blue line is the lower limit of the 95% confidence interval for Upper Lake.

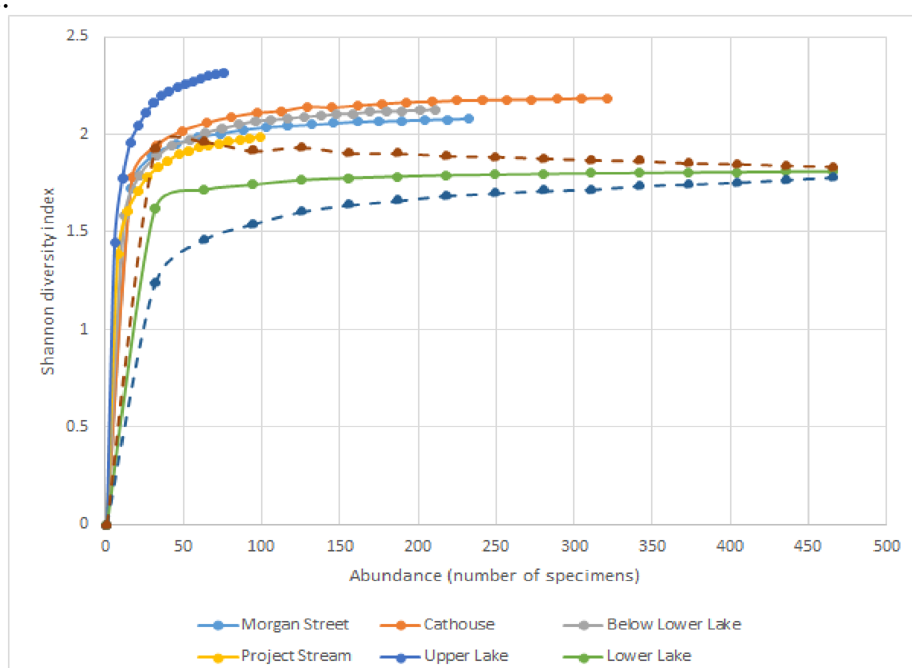

Figure D-6. Shannon's diversity indices from each site in relation to the number of specimens collected (abundance). The dashed red line is the upper limit and the dashed dark blue line is the lower limit of the 95% confidence interval for Lower Lake.

APPENDIX E

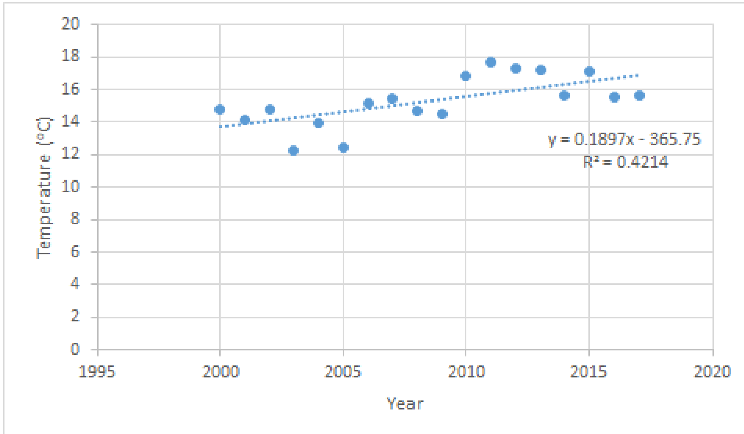

Figure E-1. Average annual temperature over time at the Morgan Street site.

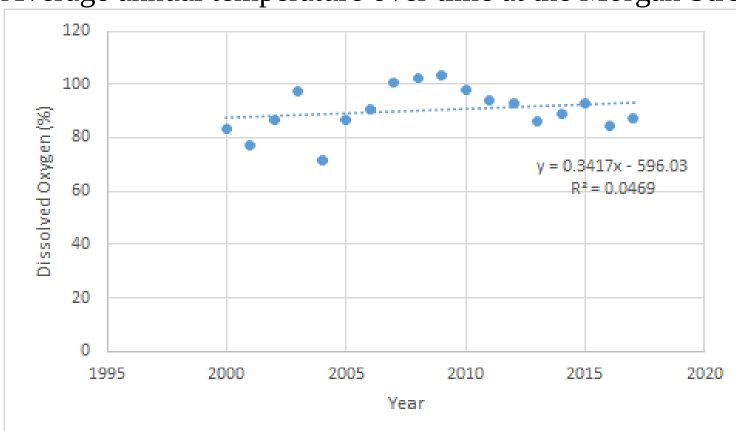

Figure E-2. Average annual percent dissolved oxygen over time at the Morgan Street site.

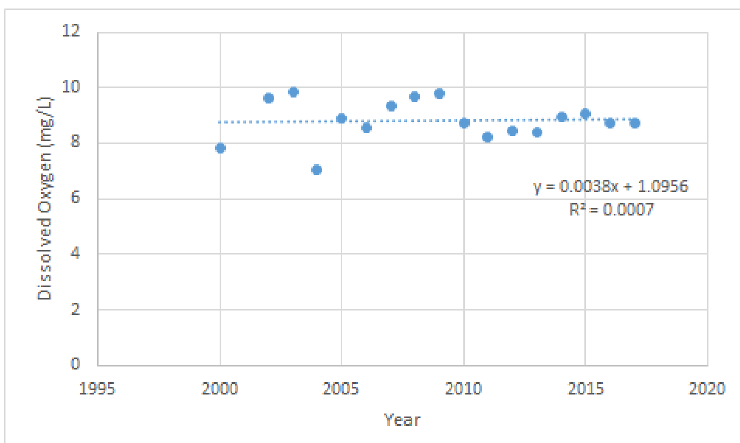

Figure E-3. Average annual dissolved oxygen concentration over time at the Morgan Street site.

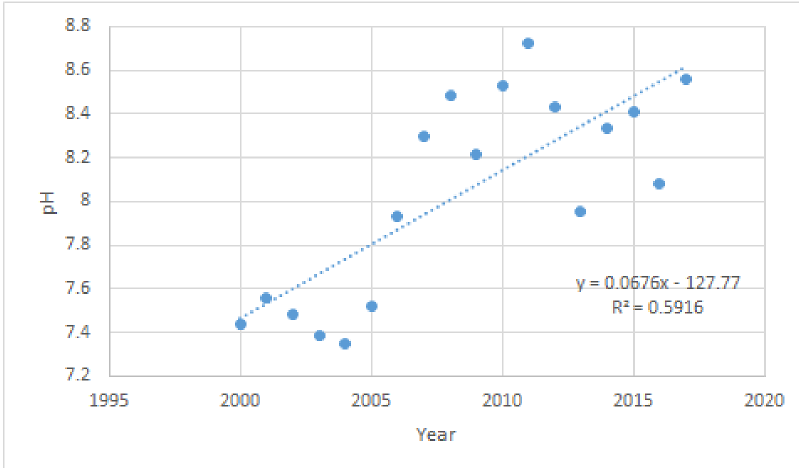

Figure E-4. Average annual pH over time at the Morgan Street site.

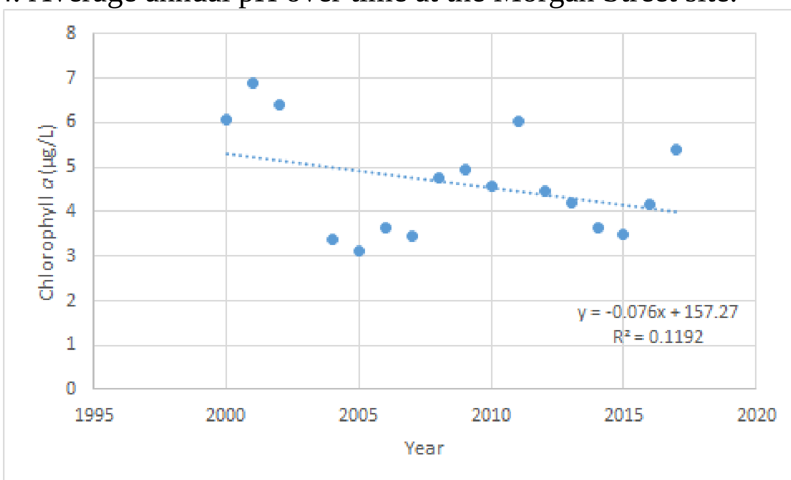

Figure E-5. Average annual chlorophyll *a* concentration over time at the Morgan Street site.

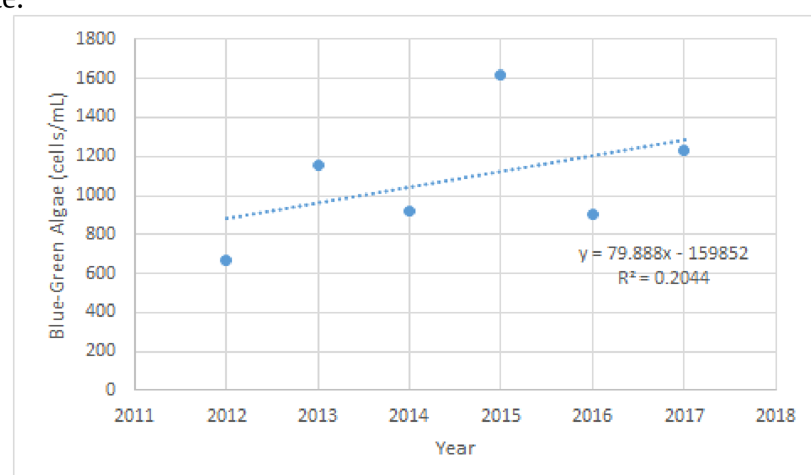

Figure E-6. Average annual blue-green algae concentration over time at the Morgan Street site.

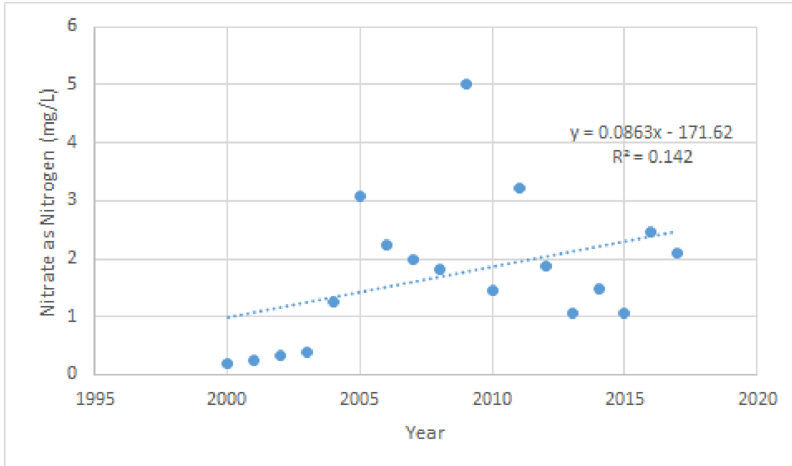

Figure E-7. Average annual nitrate nitrogen concentration over time at the Morgan Street site.

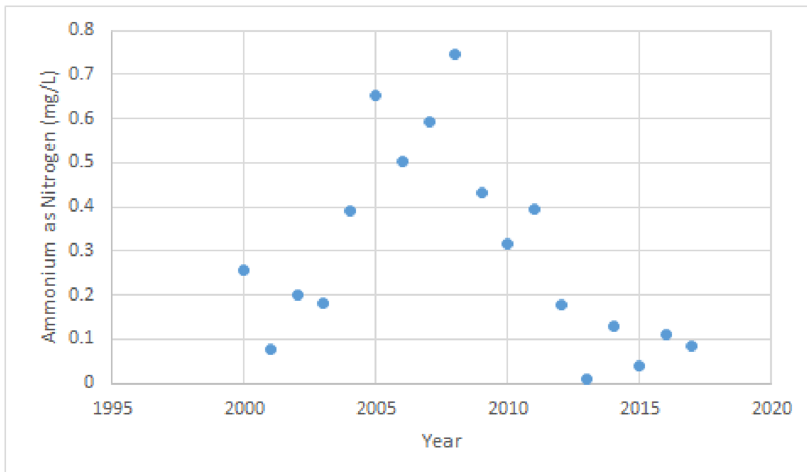

Figure E-8. Average annual ammonium nitrogen concentration over time at the Morgan Street site.

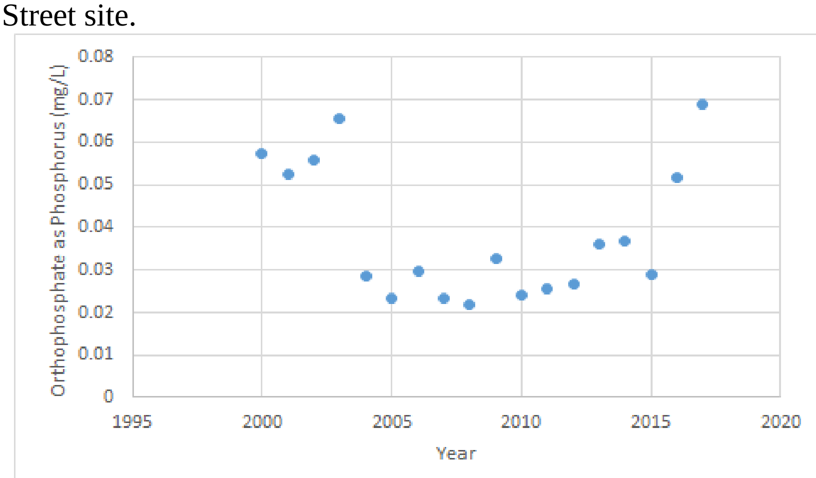

Figure E-9. Average annual phosphate phosphorus concentration over time at the Morgan Street site.

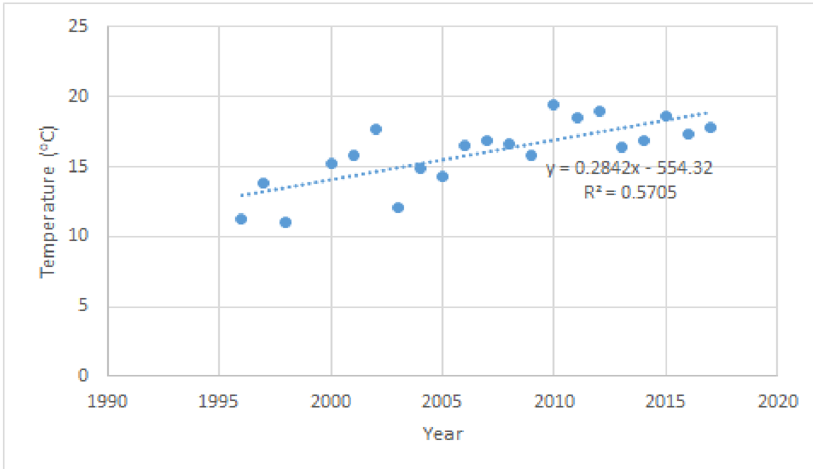

Figure E-10. Average annual temperature over time at the Cathouse site.

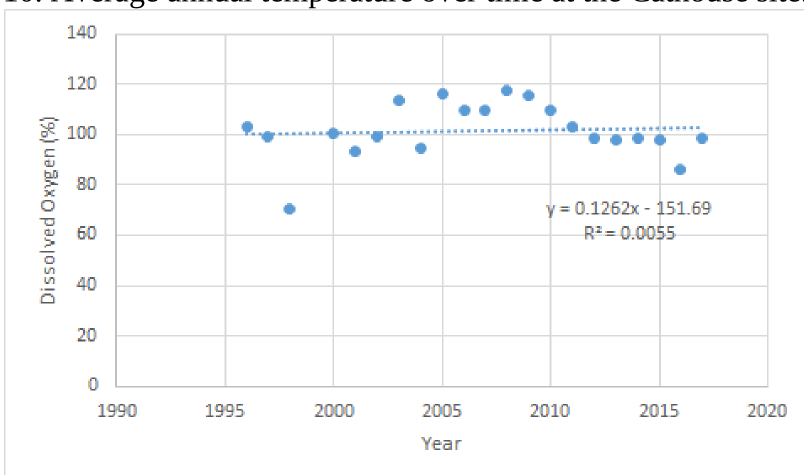

Figure E-11. Average annual percent dissolved oxygen over time at the Cathouse site.

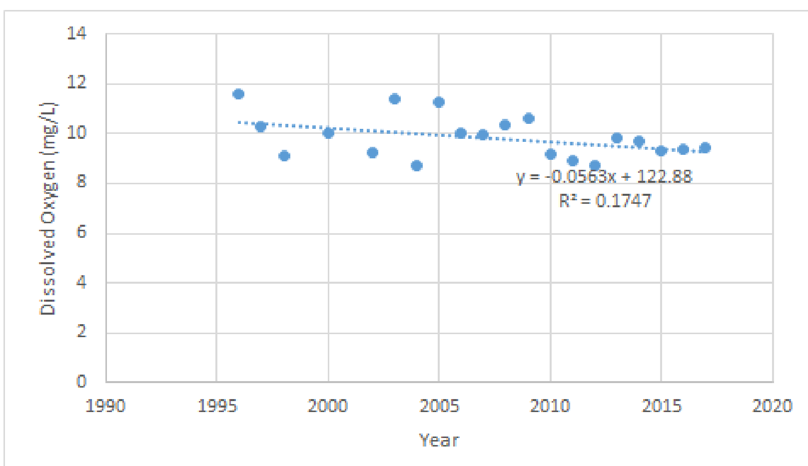

Figure E-12. Average annual dissolved oxygen concentration over time at the Cathouse site.

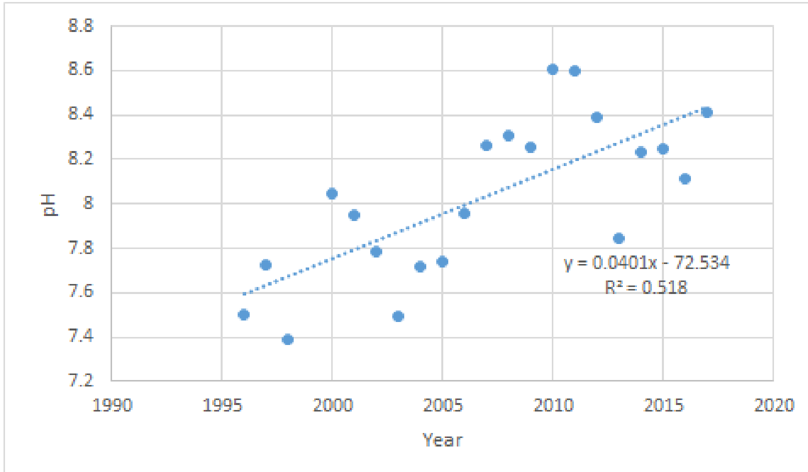

Figure E-13. Average annual pH over time at the Cathouse site.

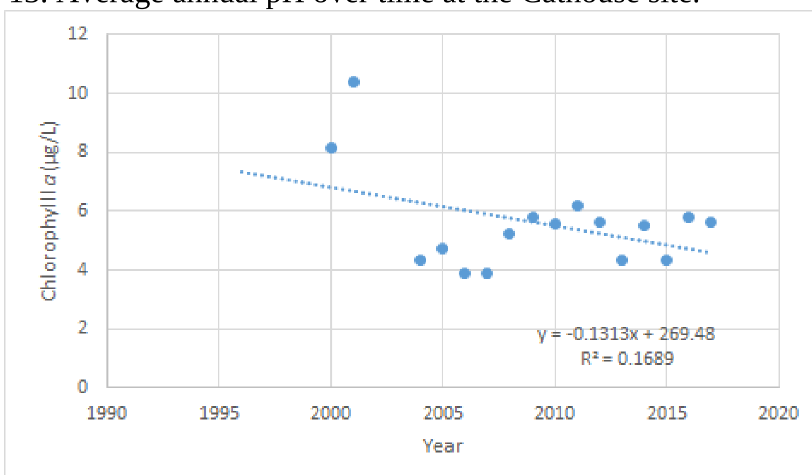

Figure E-14. Average annual chlorophyll *a* concentration over time at the Cathouse site.

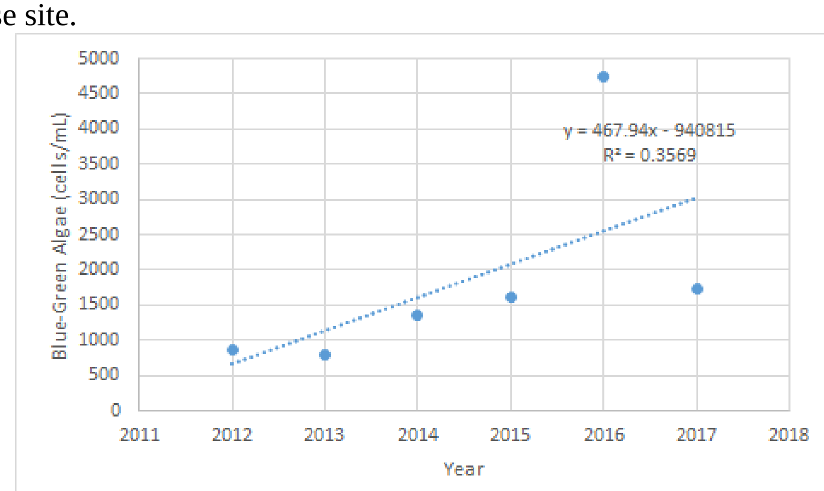

Figure E-15. Average annual blue-green algae concentration over time at the Cathouse site.

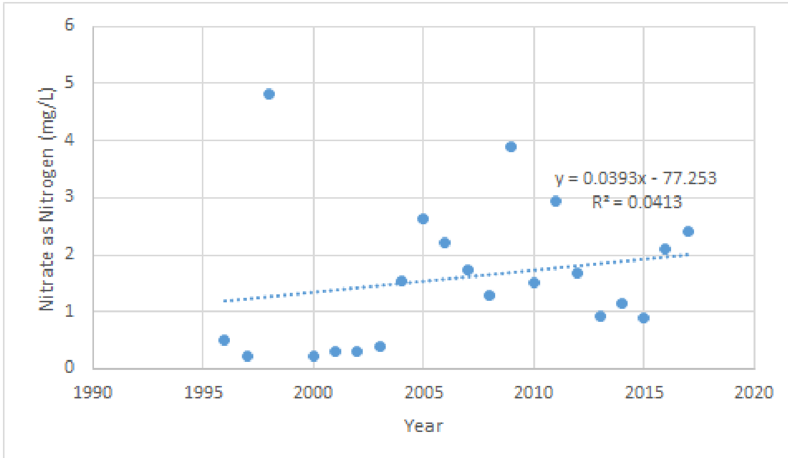

Figure E-16. Average annual nitrate nitrogen concentration over time at the Cathouse site.

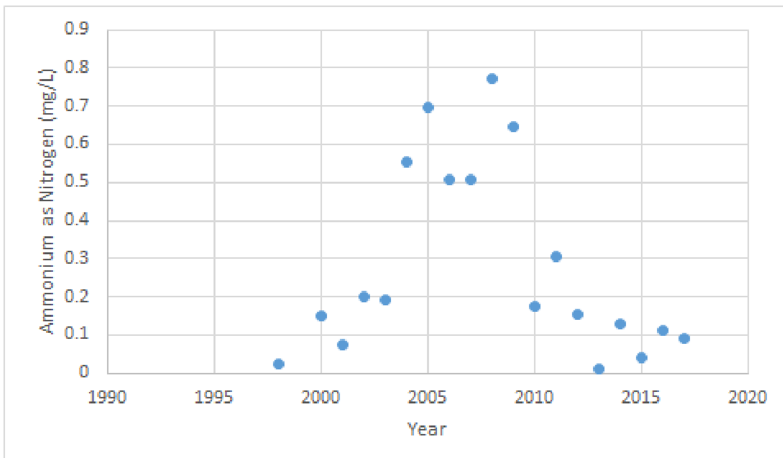

Figure E-17. Average annual ammonium nitrogen concentration over time at the Cathouse site.

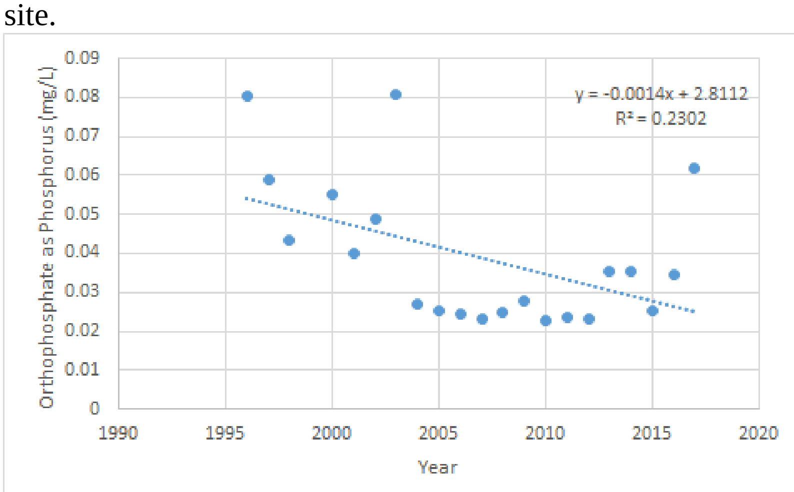

Figure E-18. Average annual phosphate phosphorus concentration over time at the Cathouse site.

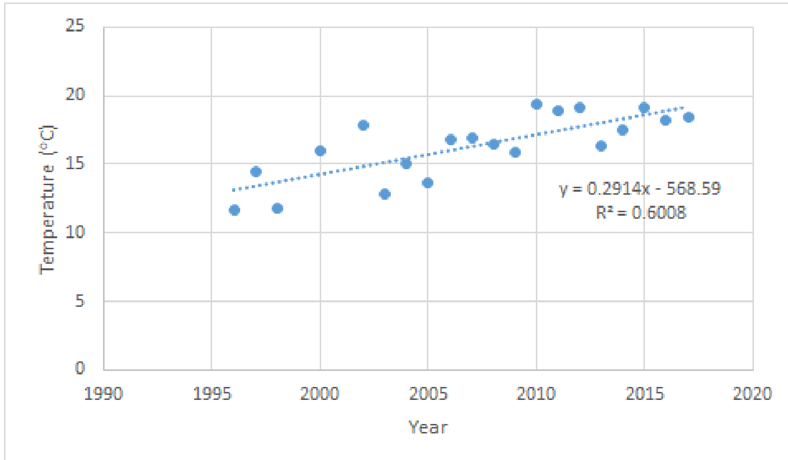

Figure E-19. Average annual temperature over time at the Below Lower Lake site.

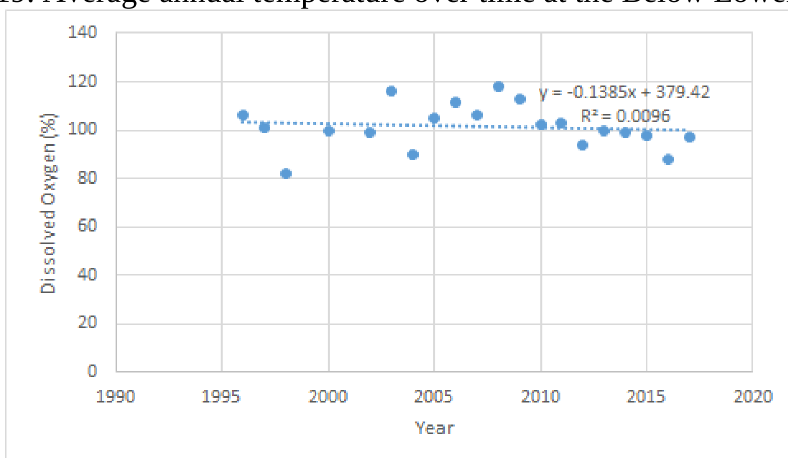

Figure E-20. Average annual percent dissolved oxygen over time at the Below Lower Lake site.

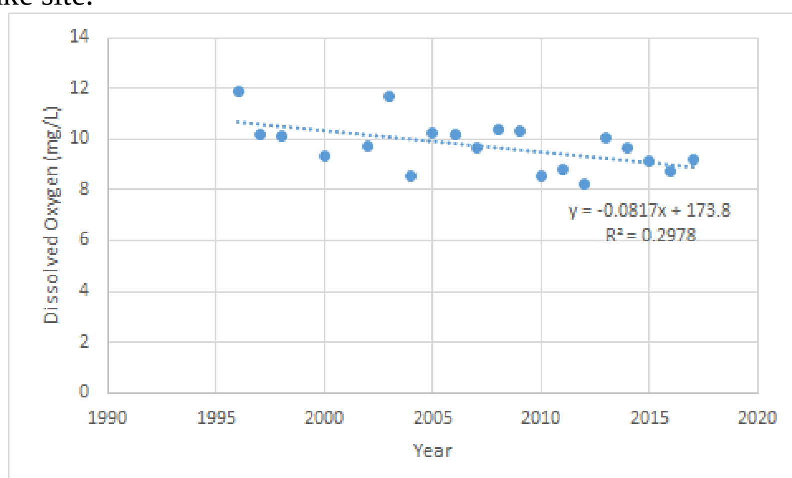

Figure E-21. Average annual dissolved oxygen concentration over time at the Below Lower Lake site.

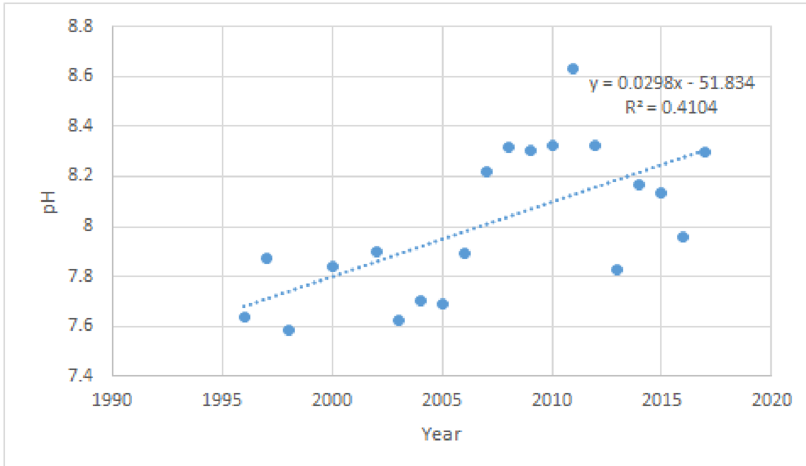

Figure E-22. Average annual pH over time at the Below Lower Lake site.

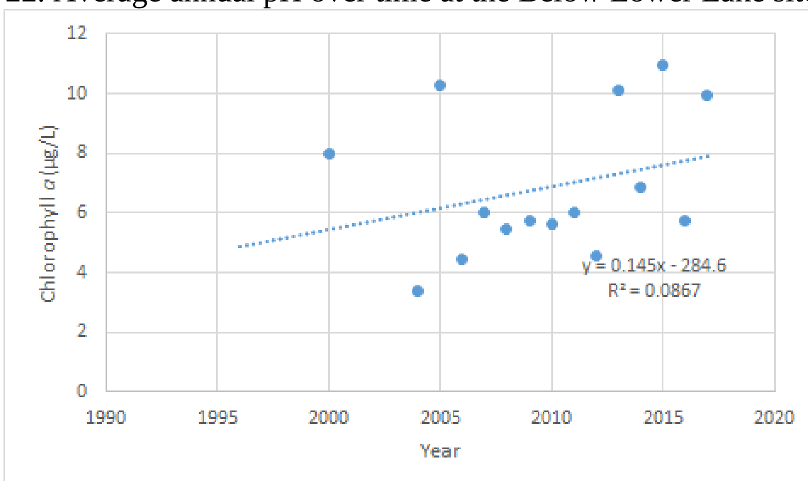

Figure E-23. Average annual chlorophyll *a* concentration over time at the Below Lower Lake site.

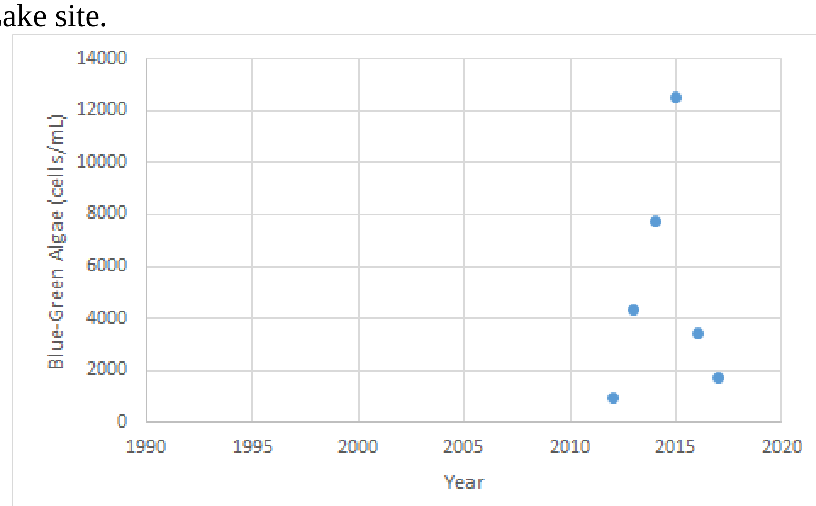

Figure E-24. Average annual blue-green algae concentration over time at the Below Lower Lake site.

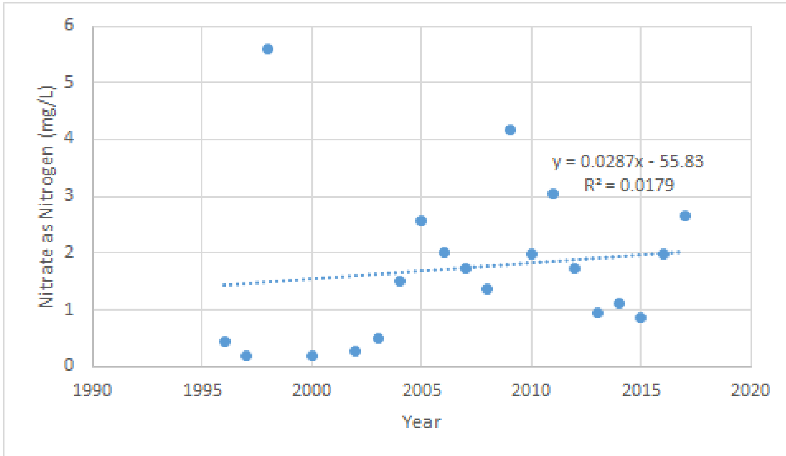

Figure E-25. Average annual nitrate nitrogen concentration over time at the Below Lower Lake site.

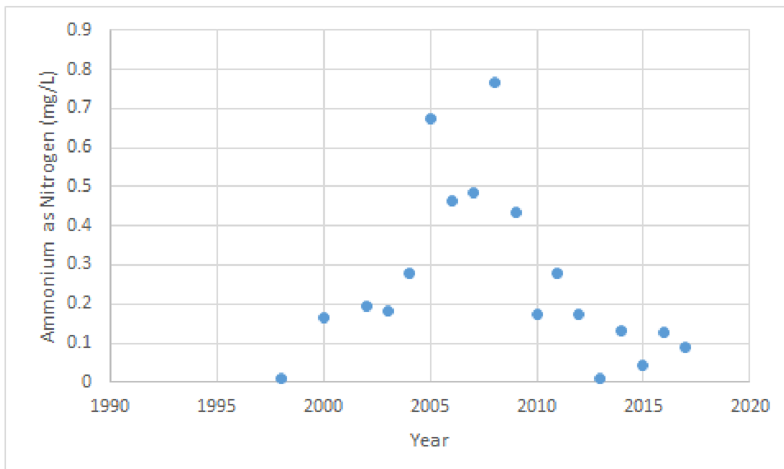

Figure E-26. Average annual ammonium nitrogen concentration over time at the Below Lower Lake site.

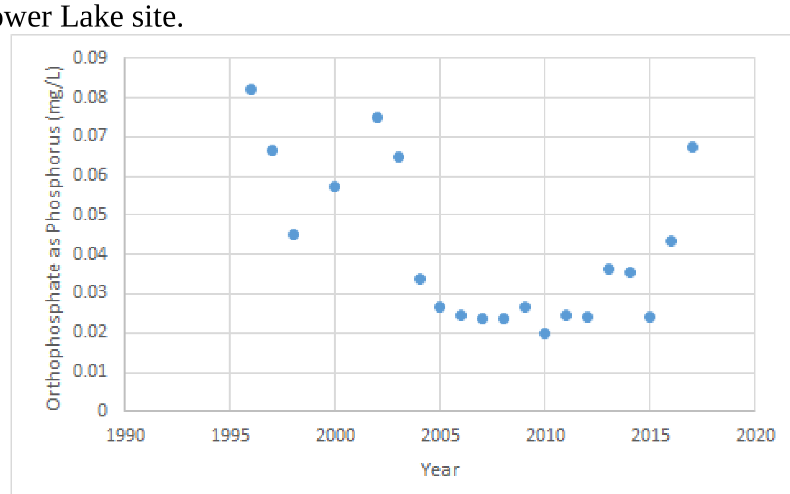

Figure E-27. Average annual phosphate phosphorus concentration over time at the Below Lower Lake site.

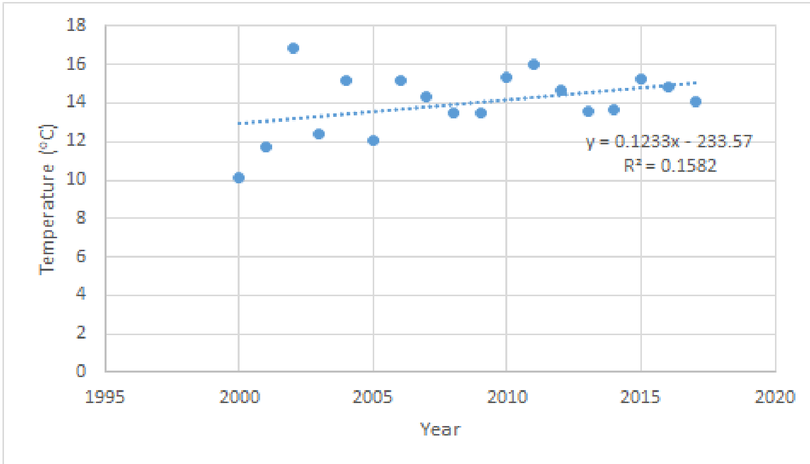

Figure E-28. Average annual temperature over time at the Project Stream site.

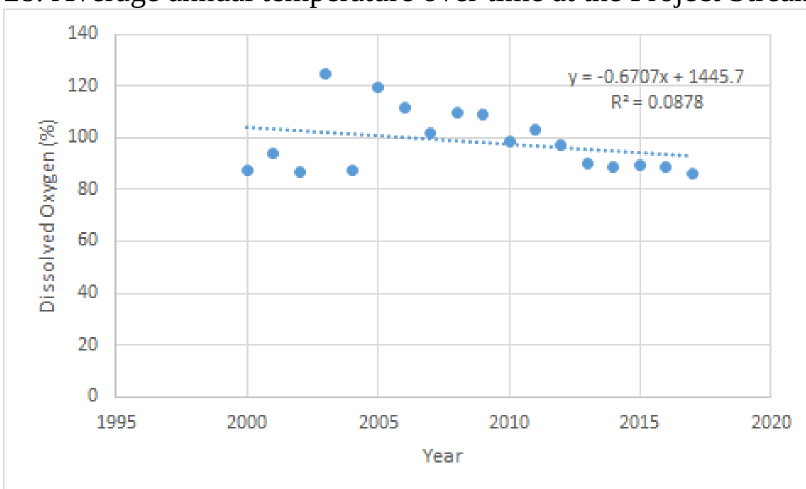

Figure E-29. Average annual percent dissolved oxygen over time at the Project Stream site.

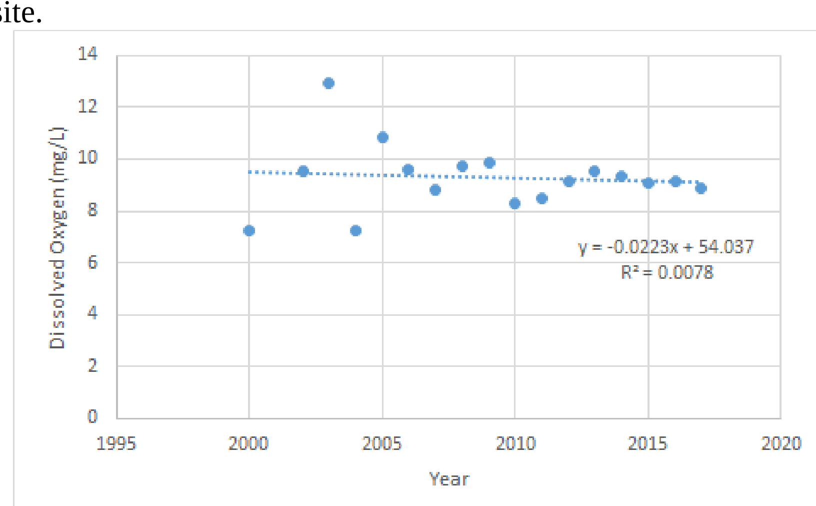

Figure E-30. Average annual dissolved oxygen concentration over time at the Project Stream site.

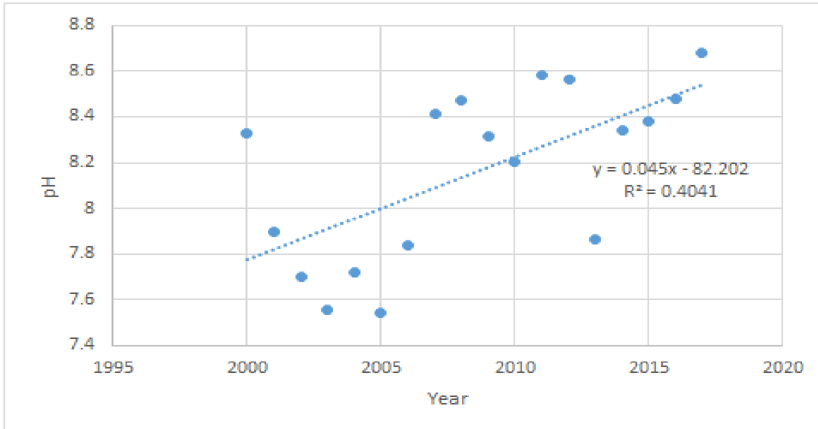

Figure E-31. Average annual pH over time at the Project Stream site.

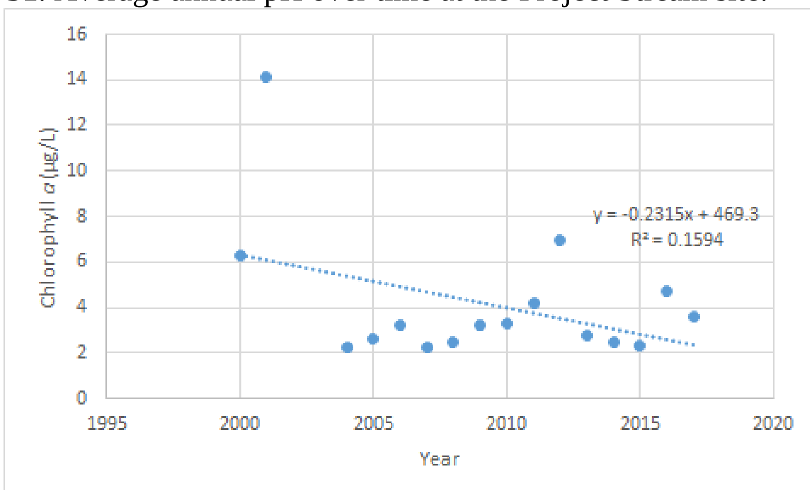

Figure E-32. Average annual chlorophyll *a* concentration over time at the Project Stream site.

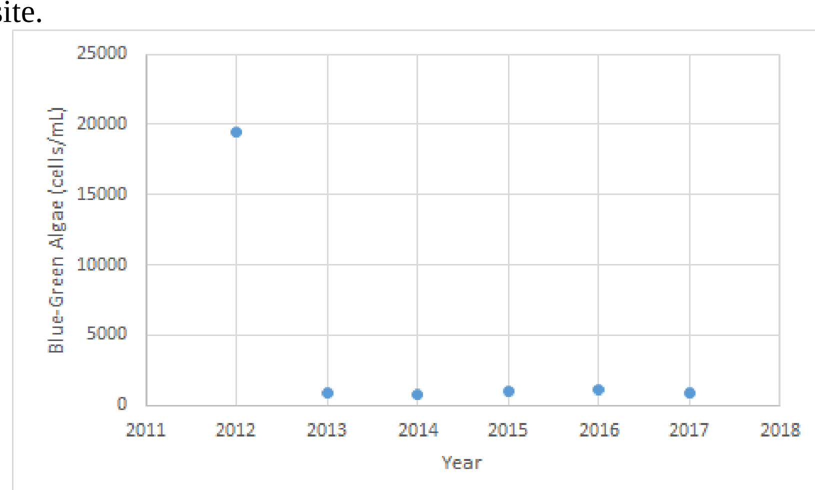

Figure E-33. Average annual blue-green algae concentration over time at the Project Stream site.

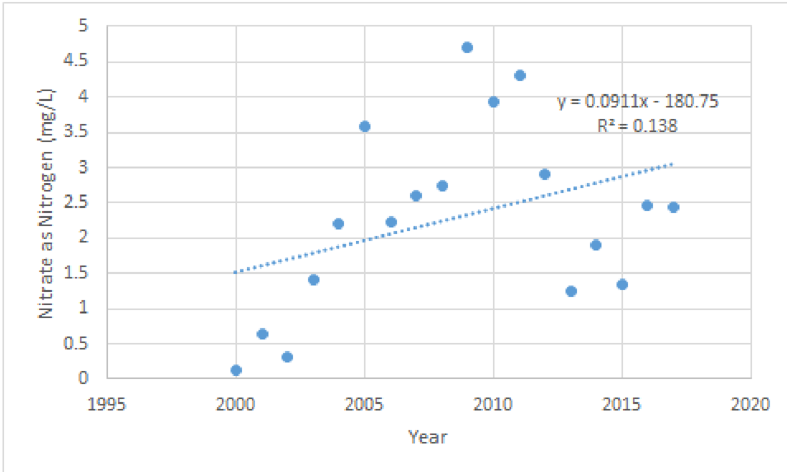

Figure E-34. Average annual nitrate nitrogen concentration over time at the Project Stream site.

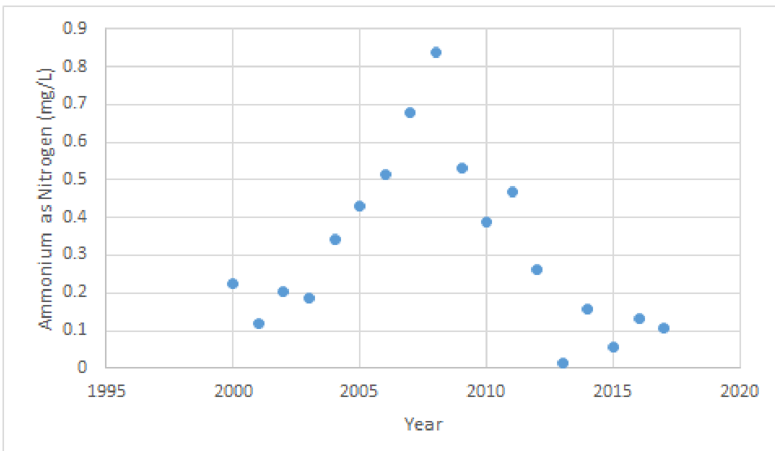

Figure E-35. Average annual ammonium nitrogen concentration over time at the Project Stream site.

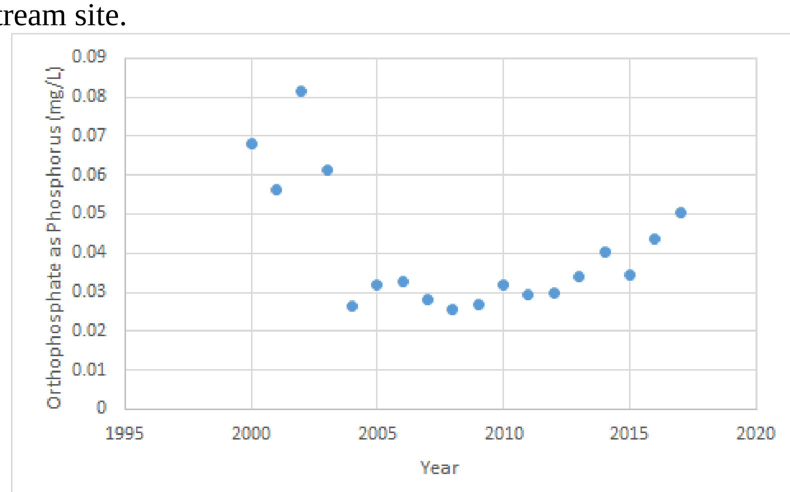

Figure E-36. Average annual phosphate phosphorus concentration over time at the Project Stream site.

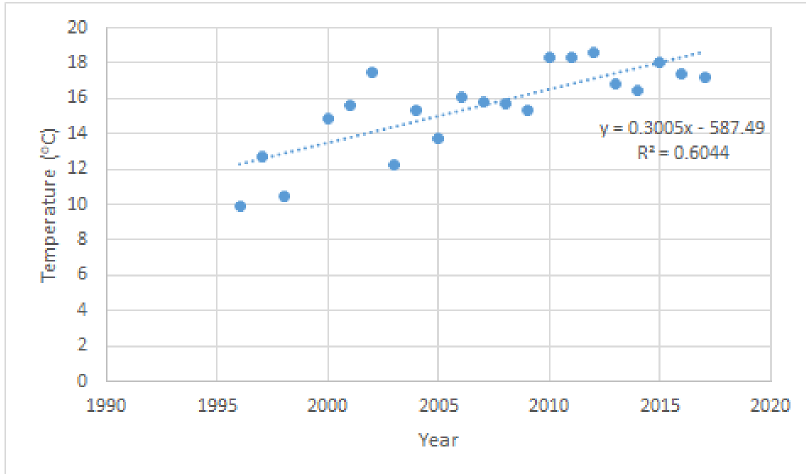

Figure E-37. Average annual temperature over time at the Upper Lake site.

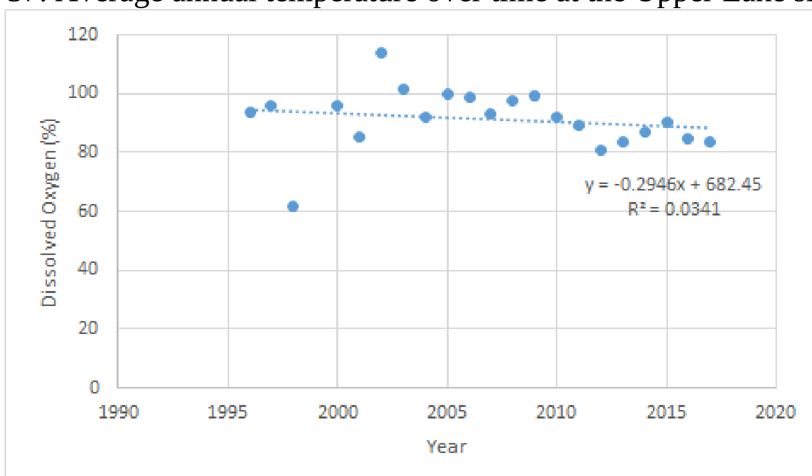

Figure E-38. Average annual percent dissolved oxygen over time at the Upper Lake site.

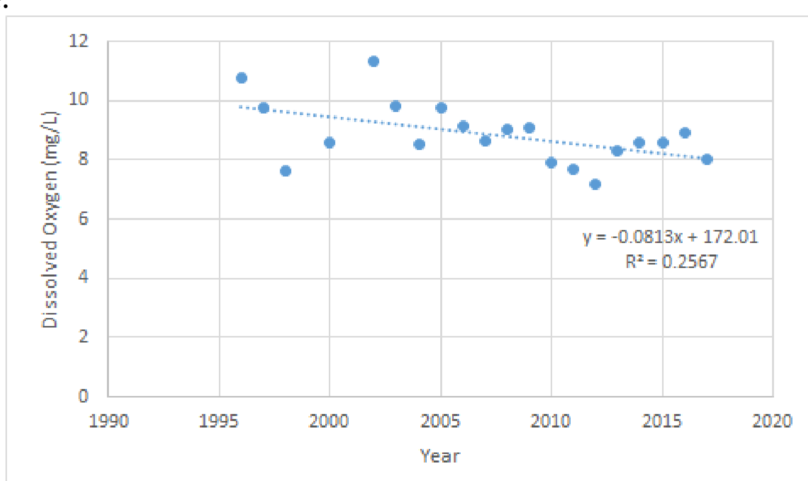

Figure E-39. Average annual dissolved oxygen concentration over time at the Upper Lake site.

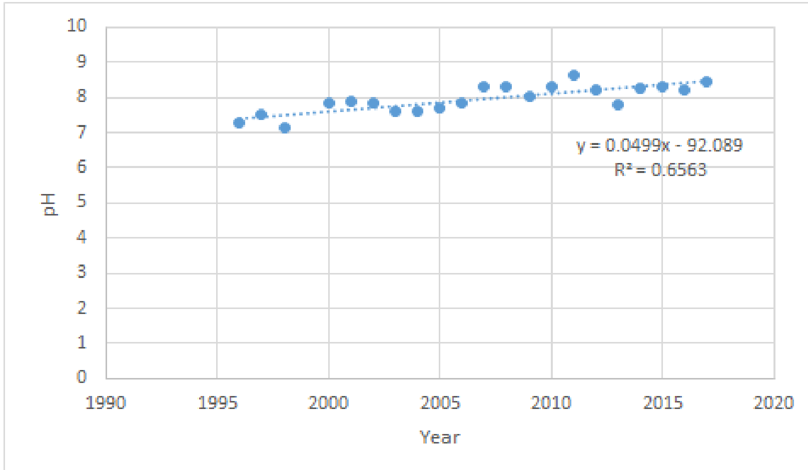

Figure E-40. Average annual pH over time at the Upper Lake site.

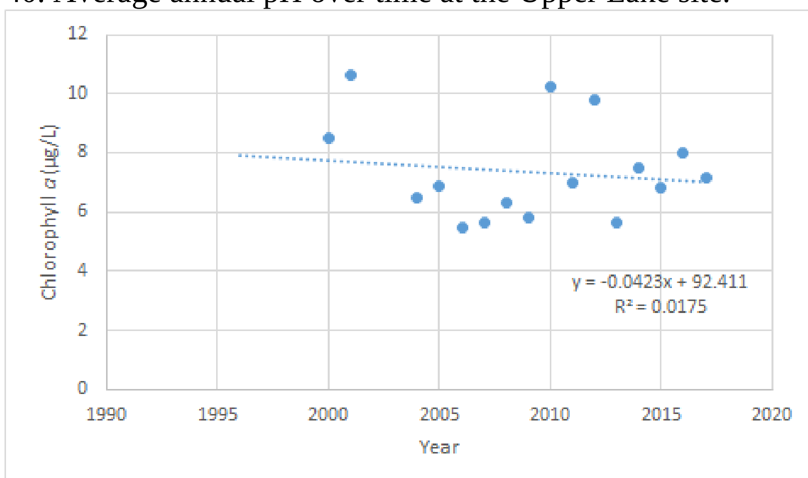

Figure E-41. Average annual chlorophyll *a* concentration over time at the Upper Lake site.

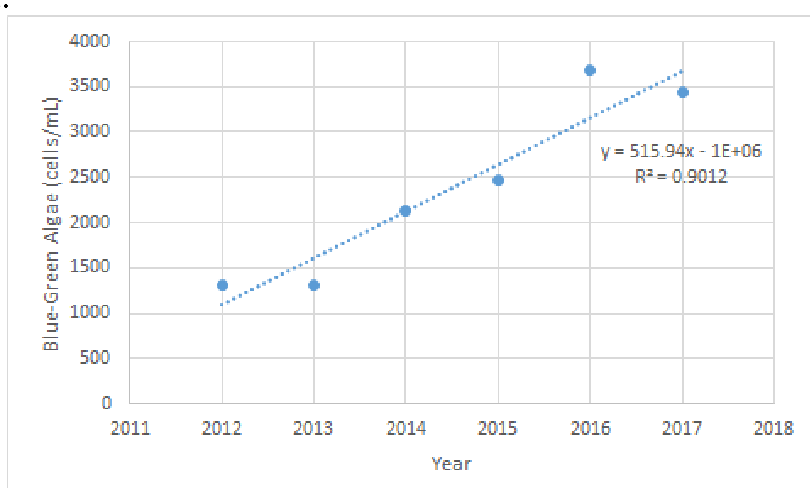

Figure E-42. Average annual blue-green algae concentration over time at the Upper Lake site.

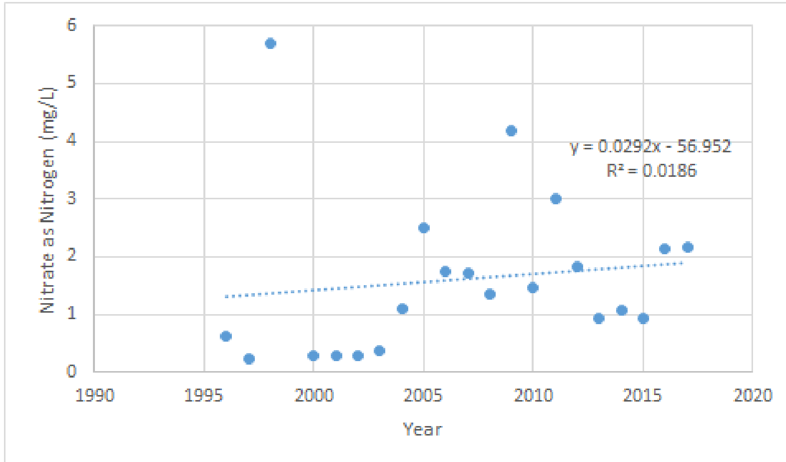

Figure E-43. Average annual nitrate nitrogen concentration over time at the Upper Lake site.

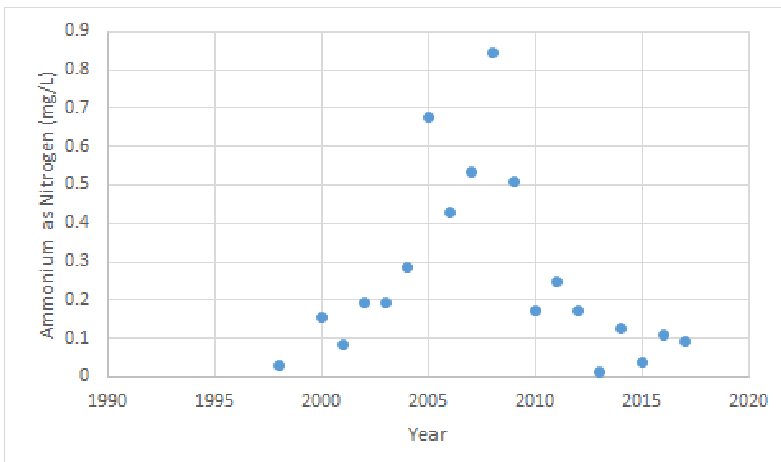

Figure E-44. Average annual ammonium nitrogen concentration over time at the Upper Lake site.

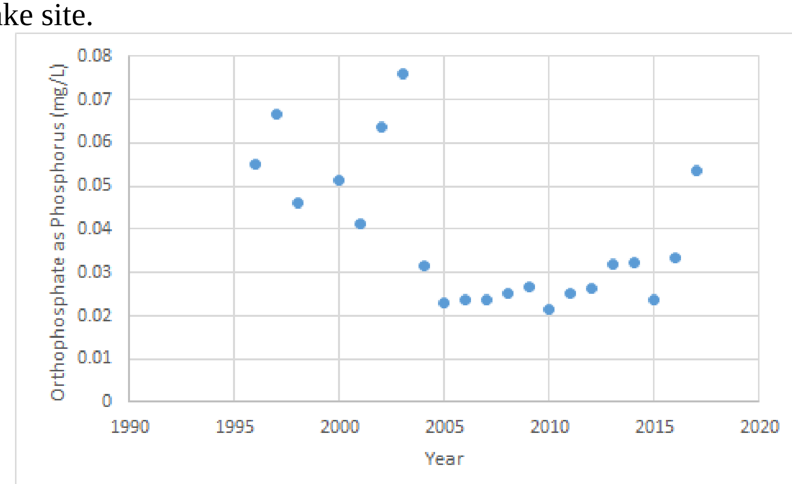

Figure E-45. Average annual phosphate phosphorus concentration over time at the Upper Lake site.

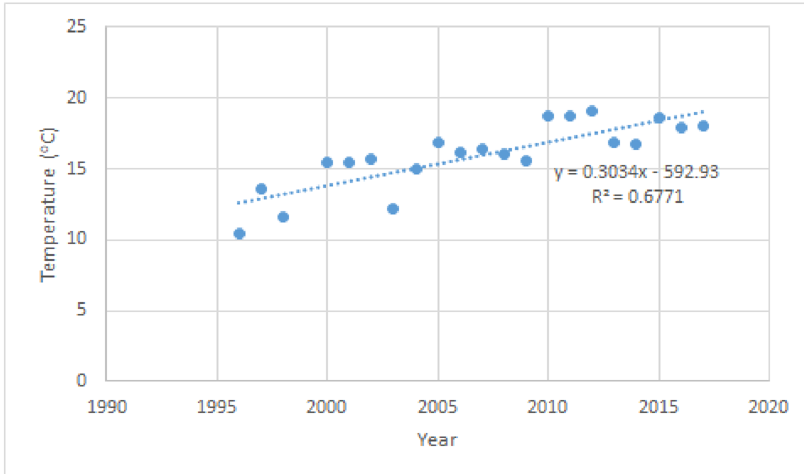

Figure E-46. Average annual temperature over time at the Lower Lake site.

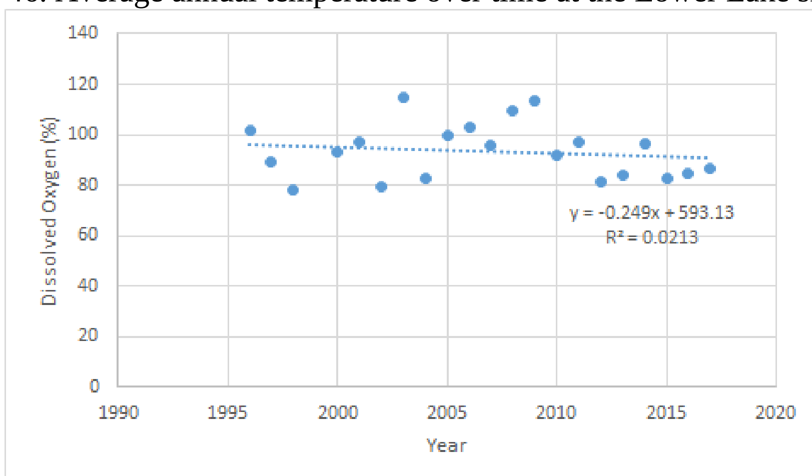

Figure E-47. Average annual percent dissolved oxygen over time at the Lower Lake site.

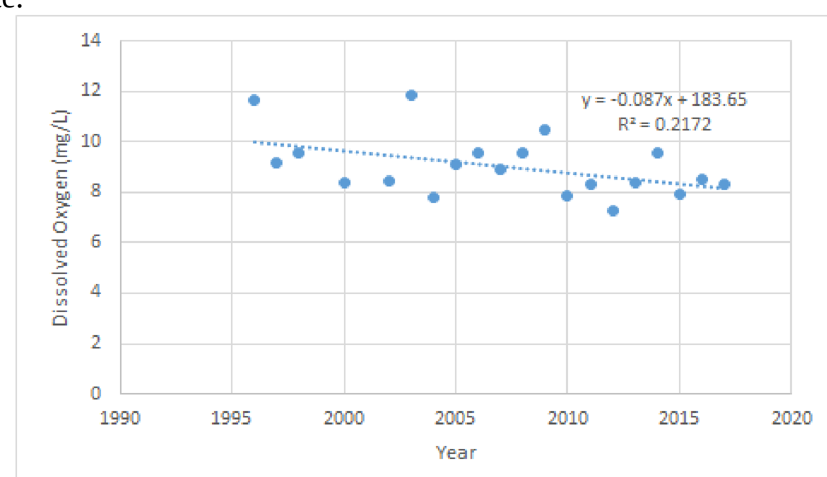

Figure E-48. Average annual dissolved oxygen concentration over time at the Lower Lake site.

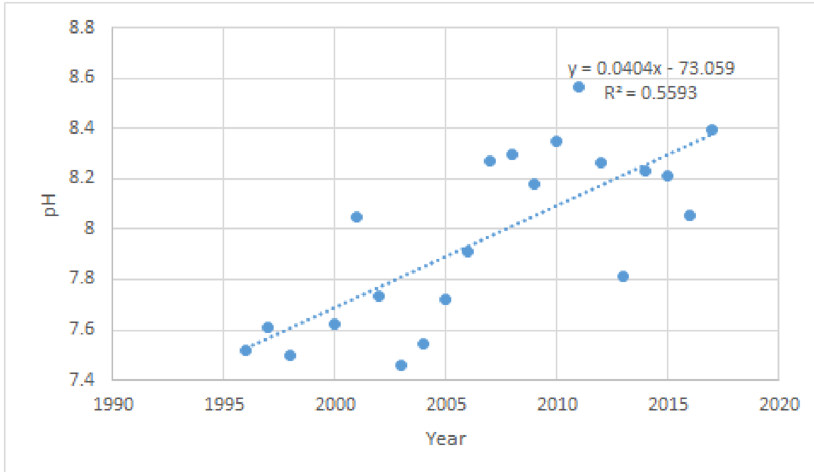

Figure E-49. Average annual pH over time at the Lower Lake site.

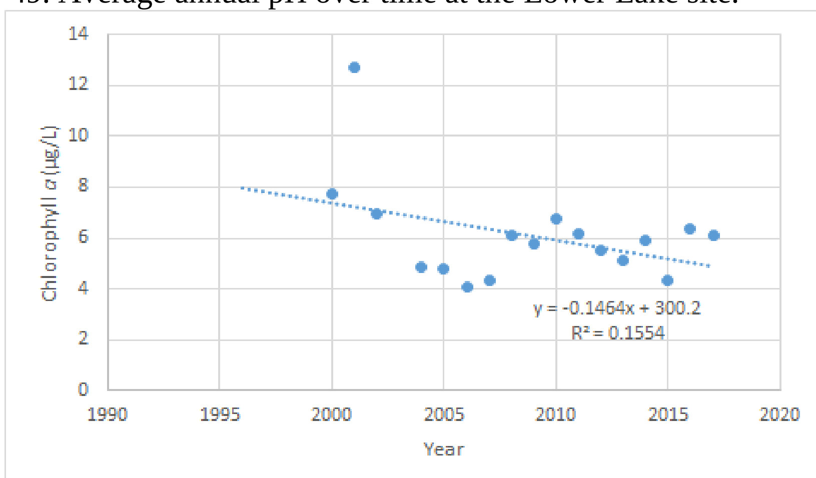

Figure E-50. Average annual chlorophyll *a* concentration over time at the Lower Lake site.

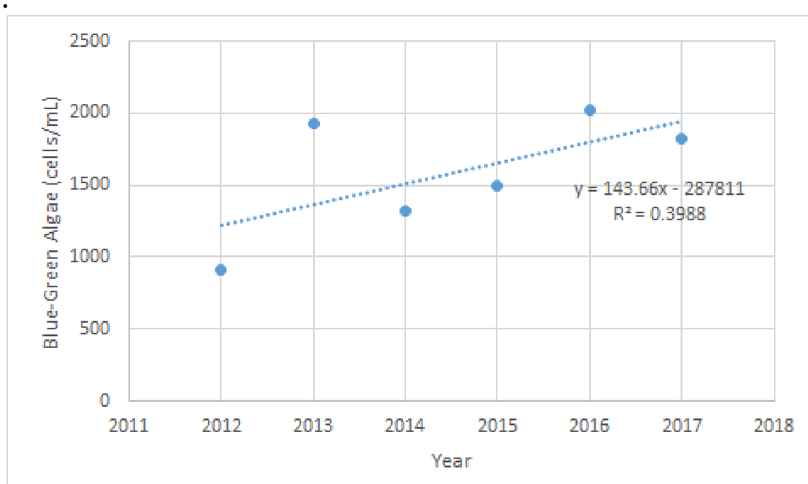

Figure E-51. Average annual blue-green algae concentration over time at the Lower Lake site.

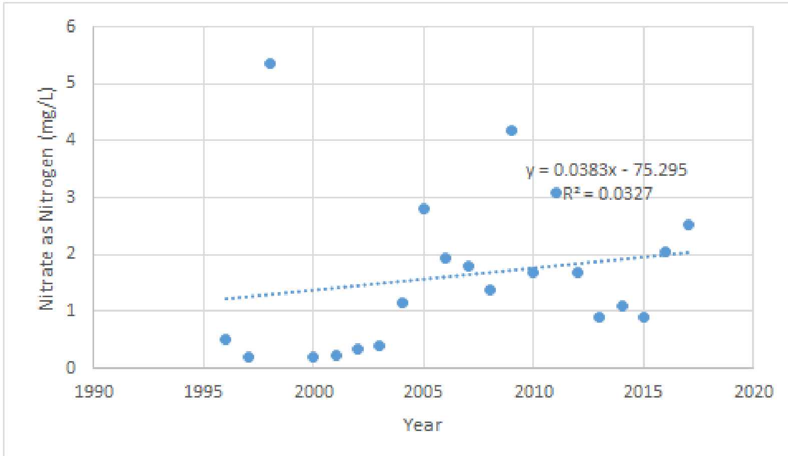

Figure E-52. Average annual nitrate nitrogen concentration over time at the Lower Lake site.

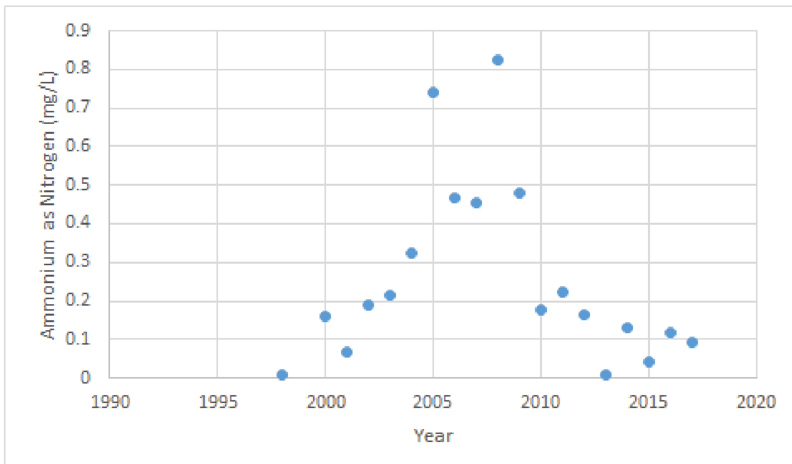

Figure E-53. Average annual ammonium nitrogen concentration over time at the Lower Lake site.

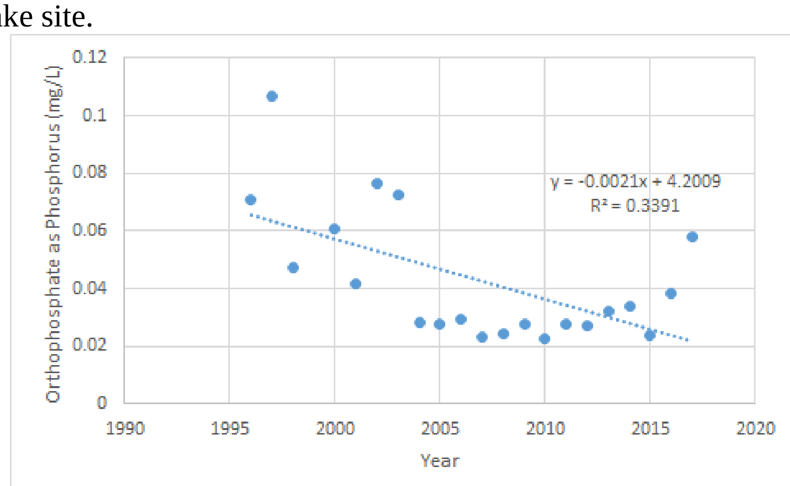

Figure E-54. Average annual phosphate phosphorus concentration over time at the Lower Lake site.