Community Watershed Analysis Final Report

2021 - 2022:

Kezar Lake



Colby-Sawyer College

Community-Based Project Class of 2021-2022

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Executive Summary

Kezar Lake is located in North Sutton, New Hampshire, adjacent to Wadleigh State Park and is approximately 182 acres with a unique history. In the 1960's there were a series of fish kills, caused in large part by algae blooms, that caught the attention of the community and the New Hampshire Department of Environmental Services (NHDES) (NHDES, 2000). In 1971, the Kezar Lake Protective Association (KLPA) formed in response to the declining health of the lake. After many efforts to recover Kezar Lake's health, it was not until 1984 that water quality parameters began showing positive results. The point-source for nutrient loading in Kezar Lake was identified as a sewage treatment plant upstream, adding phosphorus to the ecosystem. To address the elevated phosphorus concentrations, an aluminum sulfate and sodium aluminate injection experiment was approved by the Environmental Protective Association (EPA) (EPA, 2016). For the following five years, the aluminum salt treatment showed that it was effective as different water quality parameters showed positive trends.

Following the aluminum salt treatment, from 1984 to 1999, the NHDES monitored Kezar Lake by measuring certain water quality parameters. Once it was established that the lake improved in many of those qualities, the final phase of their work was finished. From there, the New Hampshire Volunteer Lake Assessment Program (VLAP) has continued to monitor Kezar during the summer months for the past twenty years with varying consistency. Given the data collected by the prior two groups and the capabilities of Colby-Sawyer College, the Community-Based Project class (CBP) has chosen to analyze eight different water quality parameters. Water samples collected during the Fall of 2021 and Spring of 2022 were tested for Acid Neutralizing Capacity (ANC), Chloride, Chlorophyll-a, Conductivity, Escherichia coli (E. coli), pH, Total Phosphorus (TP), and Turbidity. To better understand the influences on Kezar Lake's health, sampling sites consisted of a combination of locations around the watershed, along the tributaries that flow into Kezar Lake, and within the lake itself at the deepest location (Deep Site). The Deep Site specifically focuses on the epilimnetic (surface level) and hypolimnetic (deep level) conditions. Some sample sites are within Wadleigh State Park, at the public beach (also called Horse Beach), the major inlet, and the one major outlet. The remaining locations are along four different streams that flow into Kezar Lake (Lyons Brook, Birch Brook, Rowes Creek, and Clark Pond Outlet).

In the first semester, water samples were taken on a regular basis every two weeks from September 17th until November 12th as well as during three storm events (October 26th, November 1st, and November 12th) in the fall. Similarly, during the Spring semester, bi-weekly sampling was done from January 28th to March 25th. The results for key parameters like Total Phosphorus, Chlorophyll-a, and pH all showed similar historical trends to the summer VLAP data taken in the last 20 years. The CBP's data plays an important role in contributing to the lake's sampling project as samples were taken from not only the summer, but the fall, winter, and spring.

In addition to continued testing from January until April of 2022, the CBP collected sediment core samples to further analyze the success of the aluminum salts injection treatment. Aluminum salts were added to the waterbody to bind to phosphorus, then flocculate and sequester the nutrient in the sediments below, where it would not be consumed by organisms, such as algae. This treatment appears to have been successful because as predicted, a collection of aluminum salts was found in the core samples. The goal was to see if and where the aluminum was within the core that was taken from an undisturbed Deep Site sample location.

Lastly for the Spring semester, the CBP wanted to aid the health of the local water system in a more community focused manner. Two educational outreach opportunities were created, one focusing on landscaping specific to lake health, and the other on local aquatic vegetation. These additions were of high interest to the KLPA and were intended to engage the surrounding community in the protection of Kezar Lake and nearby watersheds. For the 2021-2022 academic year, the CBP worked with the KLPA to monitor Kezar Lake's health.

Introduction

Project Description

The Community-Based Project (CBP) is a class offered at Colby-Sawyer College every year for upper classmen in the Environmental Science and Environmental Studies program. The goal of the CBP is to provide students with both traditional classroom and laboratory experience while also being immersed in a year-long analysis of a local environmental issue with detailed fieldwork and extensive site visits. Each year, the Environmental Science and Studies program collaborates with a new stakeholder and develops a unique project. This year, the CBP class partnered with the Kezar Lake Protective Association (KLPA). The primary goals of the project were to complete an extensive water quality analysis of Kezar Lake and its tributaries, provide Geographic Information System (GIS) mapping and analysis, provide educational outreach projects and community workshops on aquatic vegetation and landscaping, and provide recommendations to the KLPA and other community members on how to maintain and/or improve lake water quality.

The CBP class first started collecting water samples and discharge data every two weeks during the Fall semester, from a total of 13 sites in and around Kezar Lake. During the spring semester the CBP added two sampling sites around the New London Department of Public Works (NLDPW), bringing the total number of sampling sites to 15. These samples were brought to the Lake Sunapee Protective Association (LSPA) approved lab located on the Colby-Sawyer College campus where they were analyzed for eight different water quality parameters. The parameters included Acid Neutralizing Capacity (ANC), Chloride, Chlorophyll-a. Conductivity, *Escherichia coli* (*E. coli*), pH, Total Phosphorus (TP), Turbidity. In addition to biweekly sampling during the Fall semester, the CBP class also collected water samples and discharge data before, during, and after rainstorm events to analyze how rainstorm events affect water quality.

Water quality test results were then incorporated into GIS maps for display. During the Fall semester, the CBP class provided educational materials and updates to the community via online newsletters. At the end of the semester, a mid-term project report and presentation with

the CBP's findings to date were shared with the townspeople along with the next steps for the Spring semester.

By the Spring 2022 semester, the CBP class focused on four main stories that formed from the fall and spring data collection periods. Of the same eight parameters, the data analyses were divided into four main stories, "Eutrophication" (Total Phosphorus, Sediment Coring, Turbidity, Chlorophyll-a), "Under Investigation" (pH, ANC), "Salts" (Conductivity, Chloride), and "Currently No Concern" (*E. coli*). Along with analyzing the parameters, sediment coring was added to the scope of work to further analyze the effectiveness of the 1980's phosphorus inactivation treatment. During the winter, the CBP collected sediment core samples from numerous locations surrounding Kezar Lake's Deep Site.

Additionally, the CBP performed two educational community outreach projects. One focused on aquatic vegetation in the form of a video and the other focused on landscaping practices through a workshop open to the public. Finally, the CBP class provided a final presentation outlining the entirety of the project, shared their findings, and provided recommendations based on those findings. Throughout the process, the CBP continued to work with the KLPA to educate the public on the protection and health of Kezar Lake.

Kezar Lake Protective Association

The Kezar Lake Protective Association (KLPA) was founded by the residents in 1971 to improve the lake quality and to restore the lake to its previous state prior to the negative impacts from nutrient pollution (KLPA, n.d.). Now, the KLPA works with Kezar Lake shoreland owners, NH Lakes Association, and New Hampshire Department of Environmental Services (NHDES) to protect and preserve Kezar Lake. They strive to reach and maintain a high-water quality in both the lake and watersheds surrounding the area. The KLPA works to educate the public on lake biology, ecology, and environmental concerns regarding the lake's health and quality. In addition, the KLPA strives to mitigate or prevent such issues. Currently, the protective association is examining issues such as road salt and sand runoff, erosion, invasive species, changes to the watershed, and other issues that they would like to address. To achieve their mission, the KLPA asked the CBP of 2021-22 to perform water quality tests on the lake, monitor

native and invasive species, educate the public with outreach programs and workshops, build volunteerism opportunities, and maintain public access to the lake. The CBP worked with the KLPA to address and gain a better understanding of these issues and to provide the community with educational outreach projects, a thorough data analysis report of the lake, and suggestions to maintain a healthy and clean watershed for years to come.

History

To understand Kezar Lake, it is important to address the historical context. Kezar Lake is a small lake located in the heart of North Sutton, New Hampshire, a small town within Merrimack County. Despite its small size, Kezar has several tributaries flowing into it and one outlet. Lyon's Brook is the primary inlet of the lake and flows into the northwestern part of the lake from Chadwick Meadows, Clark Pond, and Messer Pond located further upstream. During the early 1900's, Kezar Lake was home to several summer resorts and two youth summer camps that were visited extensively for a variety of recreational uses. Kezar Lake was a popular recreational location until the early 1960's when the lake began to show serious signs of eutrophication. Eutrophication is the process in which excessive nutrients enter a waterbody, leading to an increase in dense, unwanted vegetation growth and aquatic animal fatalities (Connor, J., & Smagula, 2000). One of the primary sources of excess nutrients was determined to be from The New London Sewage Treatment Facility which allowed effluent to enter Lyons Brook beginning in 1931, causing sewage to flow into Kezar Lake (Grimes, C.J., 1979). This source led to high concentrations of phosphorus and nitrogen accumulation and becoming incorporated into the biomass and sediment of Kezar Lake. Historically, a water quality analysis of Kezar Lake conducted in 1938 identified that the lake had an overall water clarity of about eleven feet which steadily declined in the decades to follow (About Kezar Lake, NH). Residents of the watershed reported that the water quality of the lake rapidly declined during the 1960's. The lake underwent alarming physical changes as the water took on a "pea soup" hue, and dense, green slime began to accumulate on the shorelines.

As a result of this nutrient pollution, blooms of blue-green algae and phytoplankton occurred during the summer seasons of 1963, '64, and '65 (Connor, J., & Smagula, 2000;

Grimes, C.J., 1979). The blooms significantly reduced water transparency in the lake. Additionally, the color of the water was altered, the odor became foul, and fish kills increased. The residents of Kezar Lake were concerned about the increased nutrient-laden effluent from the sewage treatment plant flowing into the upper portion of Lyons Brook and pursued legal action against the town of New London.

The case involving homeowners at Kezar Lake would determine whether littoral owners would be able to recover from the intense pollution-caused algal blooms (Grimes, C.J., 1979). The community's primary concern was recovering from the environmental degradation that accumulated over the years due to elevated phosphorus inputs upstream. Residents were outraged by the degradation of the lake as well as the decline in property values surrounding Kezar, prompting residents to create a case against The Town of New London Sewage Treatment Plant (Grimes, C.J., 1979). Evidence from the court case pointed to The New London Wastewater Treatment Plant as the cause of the decreased water quality and reduced enjoyment of Kezar Lake waters. However, the issue was deemed easy to resolve in less than a 10-year timeframe. The court determined that The Town of New London Sewage Treatment Facility was liable for the damages caused by sewage inputs into Kezar Lake from1968 through November of 1977 leading to a decision in favor of inverse condemnation (Sundell v. Town of New London, 1986).

In addition to the court, the State of New Hampshire applied copper sulfate, an algicide, to the lake, in '64 and '65, which resulted in killing off the nuisance algae and increasing the transparency of the water. However, in 1966 there were algae blooms in the lake and this time, the dominant species of algae were *Aphanizomenon holsaticum*. This species of algae proved to be resistant to the copper sulfate treatments as the algae remained despite 3,200 pounds of copper sulfate being added to the lake. In 1967 an additional 400 pounds of copper sulfate was added before the algae bloom stage, however, there was still no success (Connor, J., & Smagula, 2000). In addition to reduced water clarity, the phytoplankton growth in Kezar Lake also led to increased fish kills, offensive odors, and the lake's pea-soup coloration. Due to the decrease in lake quality, lakefront property values and recreational activities at Kezar Lake declined (Connor, J., & Smagula, 2000). After abandoning copper sulfate treatments, mechanical stratification was attempted in 1969 and repeated in 1970. This resulted in some success with

water clarity. In both years, the New London Sewage Treatment Facility made improvements to help limit the amount of phosphorus being released into Lyons Brook. Mechanical stratification of the lake continued until 1981 when the treatment plant was decommissioned (Connor, J., & Smagula, 2000).

After 1974, little was done for Kezar Lake other than routine sampling and it was not until 1978 when Kezar Lake was included in a federal grant that aimed to identify and classify all publicly owned freshwater lakes as a part of the newly enacted Clean Water Act. The Clean Water Act of 1972 was a significant piece of federal legislation that set limits and other criteria on the amounts of pollutants to enter water bodies. The Clean Water Act was a major turning point for the successful restoration of Kezar Lake. In 1979, an assessment of lake qualities across the state of New Hampshire placed Kezar Lake at the highest priority for restoration out of 171 other lakes (Connor, J., & Smagula, 2000; Environmental Protection Agency, 2016). By 1980, Phase I of this restoration program was introduced, leading to the Environmental Protection Agency (EPA) approving a diagnostic/feasibility study grant for Kezar Lake. Samples for the study were collected soon after. As a result of this study, Kezar Lake was classified as eutrophic. This classification indicates that a body of water is rich in nutrients and can support a dense plant population, the decomposition of which kills animal life by depriving it of oxygen. Additionally, in 1981, the town of New London began diverting its sewage to a new treatment facility in Sunapee, NH which eliminated the largest point source of phosphorus from Kezar Lake (Connor, J., & Smagula, 2000). The diagnostic/feasibility study continued until 1983 which led recommendations to conduct hypolimnetic phosphorus inactivation and to conduct phosphorus alteration on Lyons Brook via botanical utilization. Following the recommendations of the report, the Kezar Lake Project went into its second phase which included an EPA clean lakes implementation grant and in 1983 Chadwick Meadows marsh water levels were raised using damming boards to increase water residence time as well as natural phosphorus sequestering and retention within the wetland. Additionally, in 1984 Kezar Lake was treated with aluminum sulfate and sodium aluminate to bind with phosphorus and cause it to become inactive in the sediments (Connor, J., & Smagula, 2000). A 1986 report concluded that because of the aluminum salt injections into the hypolimnetic layer of Kezar Lake, phosphorus levels decreased dramatically, and water clarity increased. Kezar Lake was then reclassified from a eutrophic lake to a mesotrophic lake. In 1988 the Kezar Lake Protective Association (KLPA) joined the New

Hampshire Volunteer Lake Assessment Program (NHVLAP) and in 1989, the final phase two report of the Kezar Lake project was published which reported lasting effects of the aluminum salt injections until the final year of monitoring which showed a decrease in its effectiveness. Finally, in 2000 the Kezar Lake Project Phase III Report was published which evaluates 19 years of pre- and post-treatment monitoring of Kezar Lake (Connor, J., & Smagula, 2000).

Since the year 2000, monitoring at Kezar Lake has continued through the KLPA and the New Hampshire Volunteer Lake Assessment Program (VLAP). VLAP has been continuing the sampling project with the NH Department of Environmental Services (NHDES) to provide annual water quality reports for local watersheds. Kezar Lake has shown itself to be a success story and the years of study and monitoring that have been conducted in the past are a great foundation for the CBP to build upon. This class is in a unique position to continue long-term monitoring on the deep site phosphorus inactivation study as well as the phosphorus budget above and below the Chadwick Meadows wetland. Since the publication of the Phase III Report, development within the Kezar Lake watershed has continued, therefore changes in water quality may have been affected. Additionally, long-term monitoring of aluminum treatments is important as only one other lake in New Hampshire has undergone an aluminum treatment to inactivate phosphorus. In Barrington NH, Nippo Lake underwent an aluminum treatment as well from May to June of 2021. The CBP data collected now from the sampling sites around Interstate 89 and Rt. 11, almost 40 years after the aluminum treatment, may prove to help prepare the Nippo Lake Protective Association and other lakes for what to expect from their own aluminum treatment in the future.

Project Overview and Goals

The CBP made it a mission to work more closely with the community during the second semester. In the end, the CBP produced one informative Aquatic Vegetation video available on the KLPA website and one hands-on, learning experience in the form of a property walk field day around Kezar lake.

The main goal of the Colby-Sawyer Community Based Project (CBP) was to produce a full lake assessment report for Kezar Lake that expands on the existing VLAP data collection.

VLAP data is limited to summers, so the CBP made a goal of more frequent sampling, collecting during the fall, winter, and spring, and analyzing those samples for eight different parameters. Suggestions will be given after data analysis of the parameters and sampling sites in an in-depth discussion.

Geographic Information Systems

Geographic Information Systems (GIS) is a software program that integrates data with geographic coordinates and allows users to create digital maps as a means of displaying various types of data. The depth of knowledge in which a user can engage from using GIS is a valuable tool that can help in understanding and discussing the data analysis. Maps are often a common product developed through using GIS software as certain points displayed on the map contain large amounts of data and can be displayed in relation to its geographic location. For the CBP's purposes, sampling sites are displayed on maps with their associated sampling data. These types of maps allow readers to visualize the correlation of the data collected at each sample site with the physical watershed. For this project, spatial relationships tend to point towards the relationship between water quality parameters and their influences. For example, there is a noticeable difference between chloride levels above and below Interstate-89. Describing this relationship is done easily with the visual assistance of GIS.

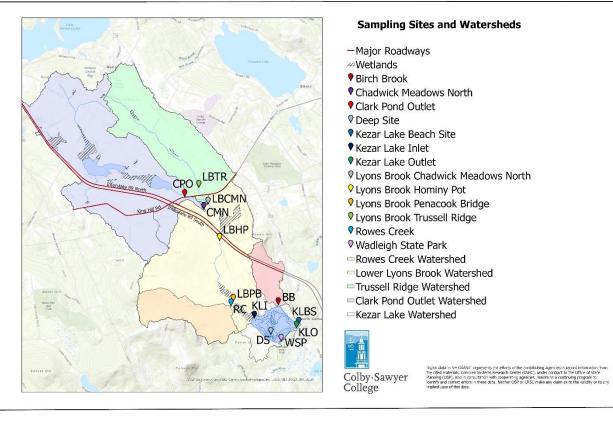
The data collected at all 15 sample sites were recorded and formalized into an interactive timeline made with ArcGIS Pro and shared to the community to view. GIS can store data by month and transform it into the format of an animation much like a timeline. This gives the audience a visualization of potential changes occurring at a spatial point over time and observes the relationship of data patterns with spatial coordinates. The animated timeline for each parameter is available to view <u>here</u> on ArcGIS Map Viewer Classic. To view this timeline, viewers must create an account with Esri, the GIS system software that manages ArcGIS Pro. Creating an account is both easy and free. Once signed up, the link will bring viewers to Map Viewer Classic where each parameter can be viewed. To use this feature, navigate to the Content pane on the left side of the screen and expand the drop-down section to view each selection. Viewers can then toggle each parameter on or off and click the play button centered at the

bottom of the screen. The map will then be animated to show the data for the selected parameter in relation to the date on which it was sampled.

Sampling Site Description

As mentioned before, the CBP sampling sites of the Kezar Lake watershed are scattered around Interstate 89 (I-89) and Route 11 (Rt.11) as seen on Map 1 below. Before reaching Kezar Lake, Lyons Brook flows through a wetland called Chadwick Meadows located north of I-89. Chadwick Meadows was constructed in 1958 specifically for the rehabilitation of Kezar Lake. Messer Pond and Clark Pond outlets flow into Lyons Brook, the primary inlet for Kezar Lake (KLPA, n.d.). The Lane River located on the southeastern part of the lake is the main outlet for Kezar Lake as it flows south into the greater Merrimack River watershed. The Kezar Lake watershed includes two additional public waterbodies (Messer Pond and Clark Pond) and four major wetlands (including Chadwick Meadows) (Connor, J., & Smagula, 2000). There are many seasonal and year-round homes present around the lake as well as a public beach, public boat ramp, and a state park. Kezar Lake has a rich and interesting water quality history making it a perfect lake for this year's Community-Based Project.

Kezar Lake Sampling Sites and Watersheds



Map 1. CBP Sampling Sites of Kezar Lake Watershed.

The Four Stories Overview

The CBP's yearlong analysis was broken down into four different stories based on the Fall and Spring data collection which was then used to educate the KLPA about the lake. The "Eutrophication" story includes Total Phosphorus, Sediment Coring, Transparency, Turbidity, and Chlorophyll-a. The first story is meant to explain why the lake was classified as eutrophic years ago and how the lake is now. The second story is the "Under-Investigation" story which includes pH and ANC, and explains what may be happening in Chadwick Meadows. The third story outlines the "Salts" which includes the parameters, Conductivity and Chloride and explains how the salts added to the roads and properties around the watershed could be affecting the lake. The last story is "Of No Concern" which explains how *E. coli* in the lake is under the statewide standard of 88 colonies per 100ml sample and therefore, currently has no concern.

Story 1: Eutrophication

Total Phosphorus

Total Phosphorus (TP) is a limiting nutrient for algae production which makes it one of the most significant parameters out of the eight used for the assessment of Kezar Lake. It is an essential element for plant life, but when there is an excessive amount in the water, it can increase the eutrophication process (USGS, n.d.; Lee et al., 1978). Eutrophication, also known as accelerated aging and increased production of algae, occurs when phosphorus concentrations reach or exceed 0.04 mg/L and when nitrogen in a waterbody gets high. These can be classified in trophic state levels that are distinguished by its unique lake characteristics (see *Table 1*).

| Trophic State | Description |
|---------------|---|
| Oligotrophic | Young lakes that are low in nutrients and |
| | productivity. |
| Mesotrophic | Middle-aged lakes are moderate in |
| | productivity- the midpoint of their processes |
| Eutrophic | Older lakes have the most buildup of |
| | sediments with an excessive amount of |
| | biological activity. |

Table 1. Trophic levels and their characteristics (Nag, 2017).

| Category | Phosphorus concentrations (mg/L) |
|------------|----------------------------------|
| Low (Good) | .001010 |
| Average | .011020 |
| High | .021040 |
| Excessive | > .040 |

Table 2. Phosphorus categories and concentrations (Citation).

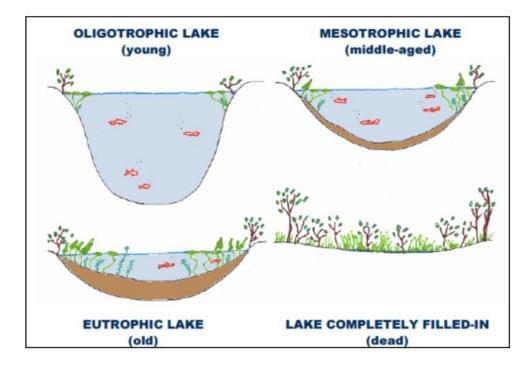


Image 1. A diagram showing the aging of a lake and the classifications used to identify it (The Life of a Lake, NH Lakes).

Eutrophication has become a major issue in water quality all over the world. The most common cause of the decreased water quality and threat to aquatic life is typically excessive nutrient loading through runoff of overused fertilizers and/or discharged human waste (Xiao-e Yang et al., 2009). A eutrophicated lake accumulates large amounts of plant nutrients in a short time span and results in excessive growth of phytoplankton (Xiao-e Yang et al., 2009). Kezar Lake once experienced this in the span of a few decades rather than the natural aging process. The cause of this was the operation of the New London Sewage Treatment Facility that released effluent upstream from Kezar Lake into Lyons's Brook which began in 1931. With this occurrence, the town of New London attempted to mediate the excessive phosphorus issue in the lake. By 1984, the most effective solution was the aluminum sulfate and sodium aluminate treatments which bonded to excess phosphorus, making it inactive in sediments at the bottom of the lake.

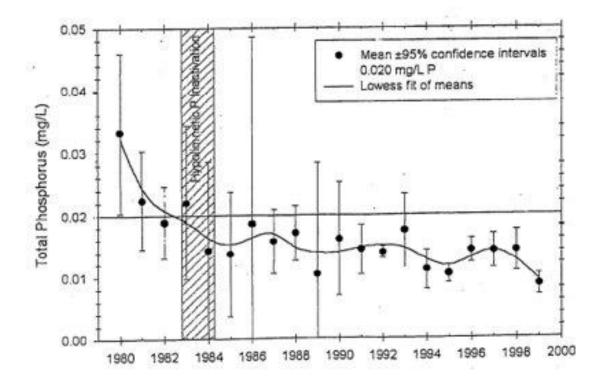


Figure 1. Total Phosphorus (mg/L) mean levels at Kezar Lake from 1980 to 2000. (Connor and Smagula, 2000)

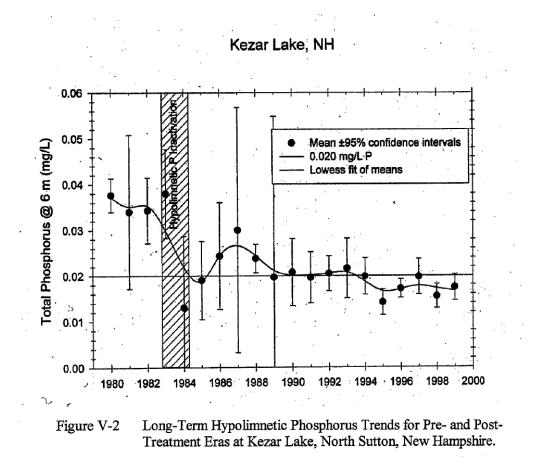


Figure 2. Total Phosphorus (mg/L) mean levels at Kezar Lake Deep Site at 6m from 1980 to 2000. (Connor and Smagula, 2000)

Data from the Phase III Report seen above shows which years the lake received pre- and post-treatments, and the levels of phosphorus present in the water. From 1980 to 1999, three phases of the phosphorus summer concentrations that were collected can be seen. *Figure 1* shows the mean annual epilimnion summer concentrations throughout this time. *Figure 2* shows the mean annual hypolimnetic concentrations. Both figures show a positive trend with decreased levels of phosphorus in Kezar Lake. This suggests success of the phosphorus inactivation treatment. The data represented in the Phase III Report regarding phosphorus levels in Kezar Lake are limited. The number of samples collected at Kezar Lake over the 19-year sampling period varied. This trend seems to be prevalent with all Phase III collected data. Therefore, the CBP recommends that data be collected on a consistent basis to better analyze water quality.

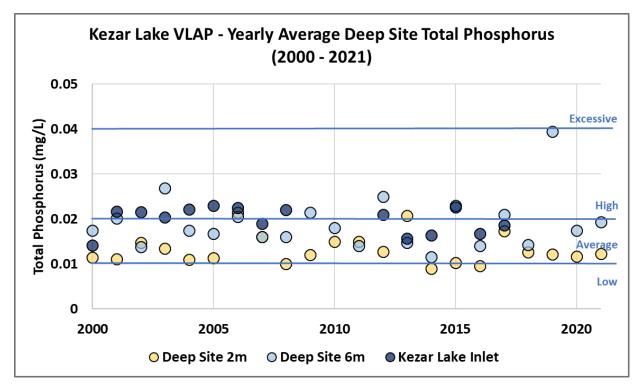


Figure 3. VLAP findings of yearly average Total Phosphorus (mg/L) at different depths of the Deep Site from 2000 to 2021. Satisfactory categories are indicated by the blue lines(s). Each dot has a 30 percent transparency, meaning that dots darker than the rest have more than one dot in the same place.

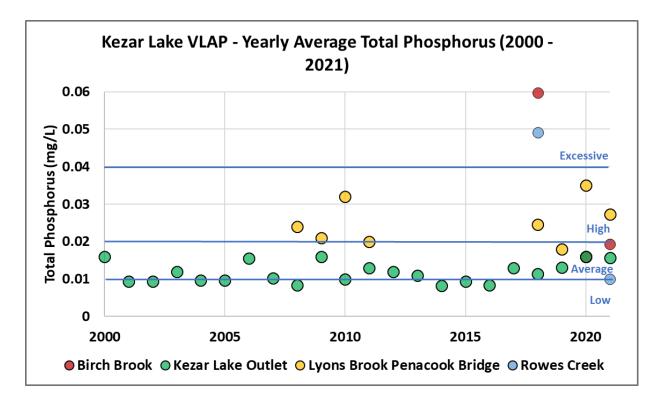


Figure 4. VLAP findings of yearly average Total Phosphorus (mg/L) from 2000 to 2021. Satisfactory categories are indicated by the blue line(s).

Compared to the CBP data, this information from the Volunteer Lake Assessment Program (VLAP) was taken during the summer months. *Figure 3* represents the Deep Site data taken during this time. The data displayed in this graph show high to low levels of phosphorus concentration with a singular data point reaching the excessive range in 2019, which is represented by the light blue dot from the Deep Site at 6 meters. *Figure 4* shows the Kezar lake tributaries with a noticeable even in 2018 from Birch brook and Rowes creek, both in the excessive range.

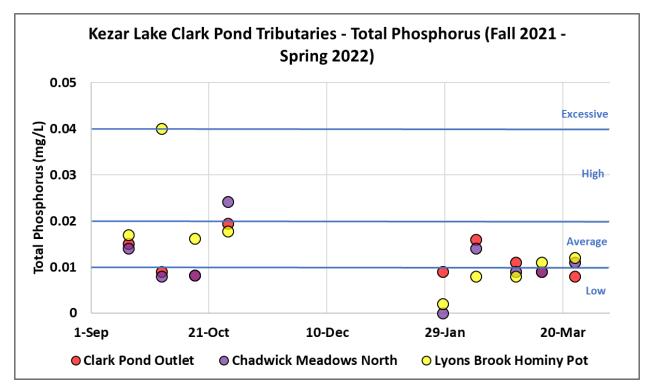


Figure 5. CBP findings of Total Phosphorus (mg/L) at the Clark Pond Tributary during the Fall and Spring 2021-2022. Satisfactory categories are indicated by the blue line(s).

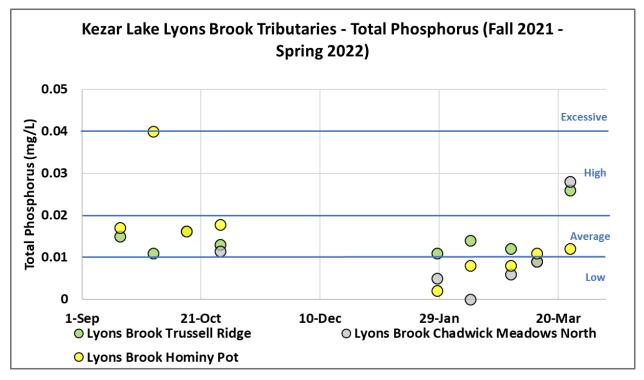


Figure 6. CBP findings of Total Phosphorus (mg/L) at the Lyons Brook Tributary during the Fall and Spring 2021-2022. Satisfactory categories are indicated by the blue line(s).

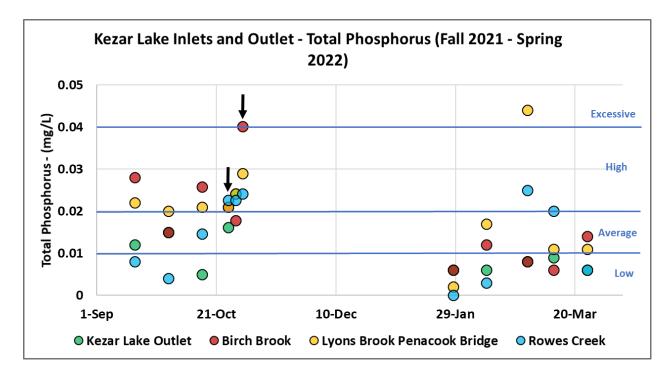


Figure 7. CBP findings of Total Phosphorus (mg/L) at the Kezar Lake Tributaries during the Fall and Spring 2021-2022. Satisfactory categories are indicated by the blue line(s). Storm event(s) are indicated with black arrow(s).

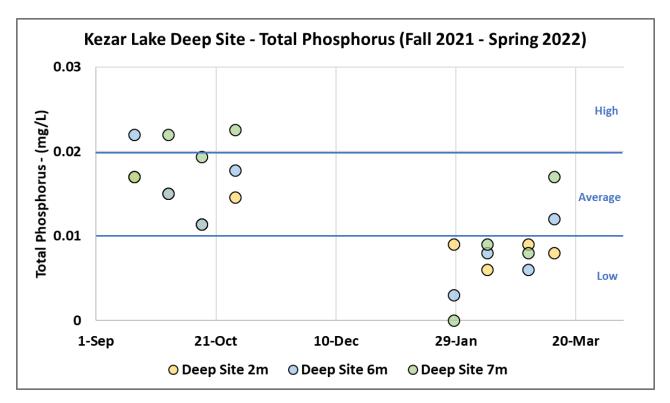
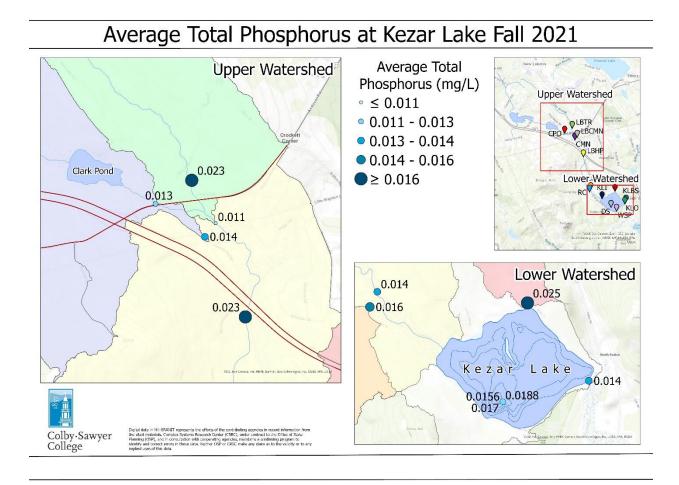


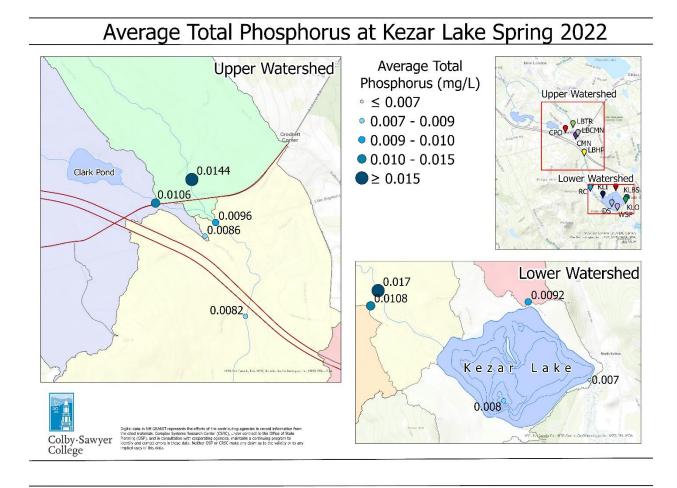
Figure 8. CBP findings of Total Phosphorus (mg/L) at different depths of the Deep Site during the Fall and Spring 2021-2022. Satisfactory categories are indicated by the blue line(s).

Throughout *Figures 6* and 7, October 1st and November 12th experienced an event that reaches beyond the Total Phosphorus excessive threshold of 0.04mg/L at Lyons Brook Hominy pot and Birch Brook. The excessive threshold is not crossed again throughout the time of CBP sampling. In both *Figures 7* and 8, during the spring semester, data falls between average to low concentrations of Total Phosphorus.

Regarding the Kezar Lake Inlets and Outlets, data from *Figure 7* ranges from excessive to low levels of Total Phosphorus. Birch brook in red and Lyons Brook Penacook Bridge in yellow are two sites from *Figure 7* that reach or exceed levels of excessive phosphorus concentrations, one sample in the fall and the other in the spring. Storm events indicated by black arrows on this figure show elevated levels of phosphorus. The Deep Site represented by *Figure 8* shows average to high levels of Total Phosphorus in the fall, while the spring is considerably lower. This could be caused by turnover that occurs during the fall. The CBP GIS map also shows the average Total Phosphorus at each sampling site and its variability.



Map 2. The average total phosphorus in mg/L sampled at sites in and around Kezar Lake for the Fall of 2021.



Map 3. The average Total Phosphorus in mg/L sampled at sites in and around Kezar Lake for the Spring of 2022.

The Phase III Report includes a phosphorus budget which is important in a lake's trophic status because it provides a way of evaluating and ranking phosphorus sources that contribute to the lake productivity (Connor, J., & Smagula, 2000). The phosphorus budget quantifies the maximum amount of available phosphorus (Maine DEP, 2016). To calculate this budget, multiply the phosphorus concentration of the chosen tributary by the corresponding tributary water volume, represented as discharge, a measure of water volume. First convert mg/L to a rational number that corresponds with the discharge being used, in this case it will convert liters to meters cubed (m³). This will make the unit mg/sec, since there are 60 seconds in a minute; multiply the number by 60, this will make the unit mg/min. This will make the unit mg/min.

There are 60 minutes in an hour; once again multiply the last number by 60, this will give you the amount mg/hour. Finally, there are 24 hours in a day therefore you must multiply the last number received by 24, this results in the unit mg/day (Meyer, 1979).

This will give a measure of the relative contribution of each inlet to the total phosphorus entering the lake. From here, the Total Phosphorus inputs and outputs of Kezar Lake can be modeled to create a phosphorus budget. This will help to assess the net changes in the lake. For example, On September 17th, Lyons Brook Penacook Bridge was sampled, the phosphorus concentration was 0.022 mg/L and discharge was .2 m³/Sec. This converts to a daily rate of 380,160 milligrams of phosphorus entering the lake from Lyons Brook Penacook bridge. On September 17th, Kezar lake outlet was also sampled: the phosphorus concentration was 0.012 mg/L and discharge was 0.25 m³/Sec. This converts to a daily rate of 259, 200 milligrams of phosphorus exiting the lake from Kezar Lake outlet.

The CBP can make recommendations about maintaining low levels of phosphorus, which would decrease the likelihood of algal blooms. Continuous septic system care can help limit phosphorus as well as other actions individual property owners can make. It is important to educate the community about proper landscaping management around a watershed and understand how different practices can impact phosphorus levels. Consistent sampling will allow for better data analysis. In addition, more sampling will indicate detailed trends over time that can be used to understand Total Phosphorus levels in depth. Based on the data that the CBP class collected, there is a trend of Birch Brook being high in Total Phosphorus concentration. This trend should be investigated further. However, Birch Brook is a small volume of water entering Kezar. Further analysis of Total Phosphorus concentrations in Kezar Lake could be better understood by examining the sediment cores collected by the CBP class.

Sediment Coring

At the beginning of Spring 2022, the CBP added a sediment core analysis of the Deep Site to their scope of work. This additional analysis allowed students to analyze the presence of the phosphorus inactivation treatment that occurred at Kezar Lake in the 1980's. If the phosphorus inactivation treatment has been successful throughout the past 40 years, then the phosphorus is expected to be bonded with the aluminum in the sediment of Kezar Lake.

During the winter, the CBP collected samples in plastic sediment core tubes at numerous locations surrounding the Deep Site for a total of eight samples- one being a testing sample. Using a series of connected pipes, the CBP was able to lower the sediment core to the bottom of the lake and take a core sample to store and freeze in plastic tubing at a later date. In the image below, the CBP had just pulled the core from the bottom and were capping the plastic tubing for storage.



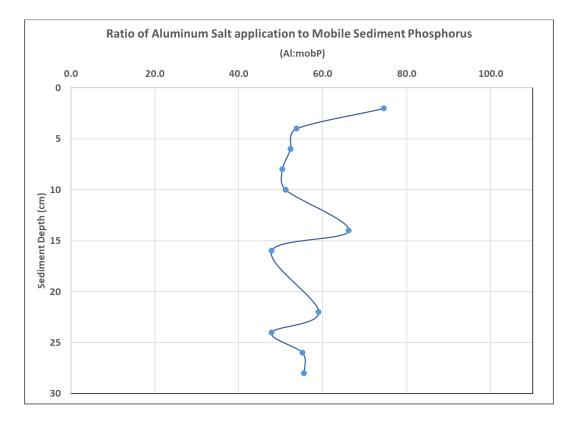
Image 2. The CBP is pictured here taking sediment core samples from Kezar Lake using their new sediment corer.

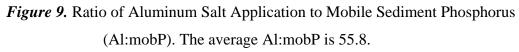


Image 3. The CBP is attempting to cap the plastic tubing which stores the core sample.

To analyze the sediment core samples, the CBP cut the cores into 2cm discs. Each disc was labelled with the site name and its depth. The discs were then processed in the LSPA approved lab on the Colby-Sawyer College campus to get the concentrations of Water-Soluble Phosphorus, Loosely Sorbed Phosphorus, and Aluminum and Iron Bound Phosphorus. For details, refer to the Sediment Coring SOP in the appendix.

The individual sediment coring samples collected and tested may help better understand the story of the lake's history (Van Metre, n.d). As sediment builds on the bottom, through the natural aging process of a lake, some of the conditions of that time were captured in the sediment through layers. This process of sediment coring was a great extra tool for the CBP to use in their analysis of Kezar Lake's water quality. A study by (J. Huser, et al., 2016) developed a model to predict the longevity of aluminum treatments based on factors such as watershed area to lake area, surface area of the lake to depth, as well as the original aluminum treatment dose. They found that there was a relationship between the longevity of aluminum treatments and the ratio of the aluminum dose rate to mobile phase phosphorus (Al:mobP), with larger ratios being correlated with greater longevity of aluminum treatments. As seen in *Figure 9* below, the CBP Deep Site average ratio of Salt application to Mobile Sediment Phosphorus was 55.8 with a high variability throughout depth.





During the spring semester, the CBP was restricted to time with each core taking almost 72 hours in total to analyze, this reason is why only one sediment core was analyzed. In the future, to investigate these cores, the CBP would recommend looking at the remaining 6 sediment cores for their iron to aluminum rations to further this study at Kezar Lake. This could be done for an internship, as a senior project at Colby-Sawyer College or even as another Community-Based Project.

Transparency

Another indicator of how clean a waterbody is transparency which is confused with turbidity. However, with transparency, if there are more solids suspended in the water, it becomes murkier, thus decreasing the transparency. In the Fall of 2021, the CBP class measured transparency at the Deep Site of Kezar Lake using a Secchi disk. The Secchi Depth measures the transparency by how far light can penetrate the water column (Leszek Błędzki, 2009). Lower Secchi Depths indicate a low transparency which is often associated with a high turbidity (Leszek Błędzki, 2009). Below, VLAP shows data collection for both transparency and turbidity of Kezar Lake.

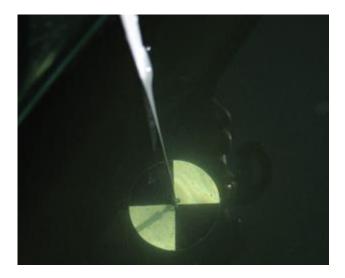


Image 4. Secchi Disk is being used to measure the lake's transparency.

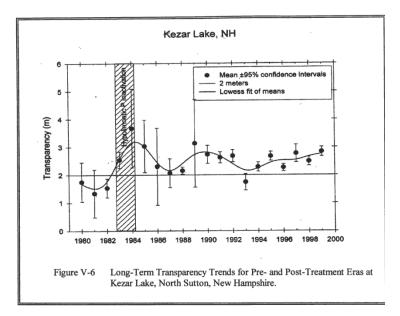


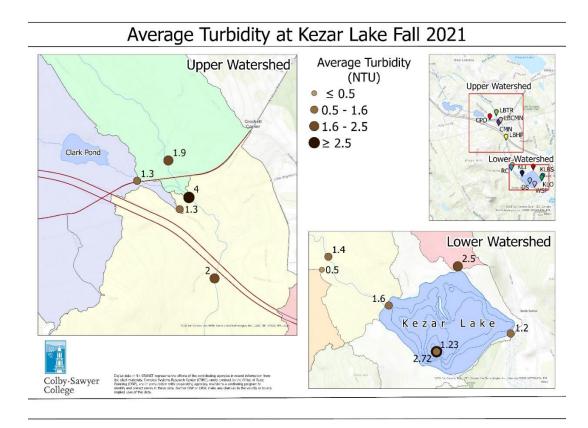
Figure 10. Average Transparency at Kezar Lake from 1980 to 2000 (Phase III Report).

Records of Kezar Lake's transparency began in 1938 when a study of the lake recorded the transparency of eleven feet, however, regular transparency data collection began in 1980 (Grimes, 1979). *Figure* 10 above shows the average transparency in meters, throughout the course of the Phase III Report. The shaded portion represents the implementation of the aluminum treatment from start to end. Around 1983, transparency increased gradually but decreased for roughly three years, and eventually increased again. The transparency after 1938 diminished due to the algal blooms in the 1960's. Overall, since the aluminum salt treatment, the long-term transparency has a positive trend for both the health and aesthetic value. It has rebounded close to the value that was measured in 1938. The results suggest that the aluminum treatment has so far been effective. An effective aluminum treatment would reduce or eliminate nutrient particles in the water therefore increasing transparency, because of the considerably good trend seen by the CBP. The measurement of transparency stopped during the Spring of 2022.

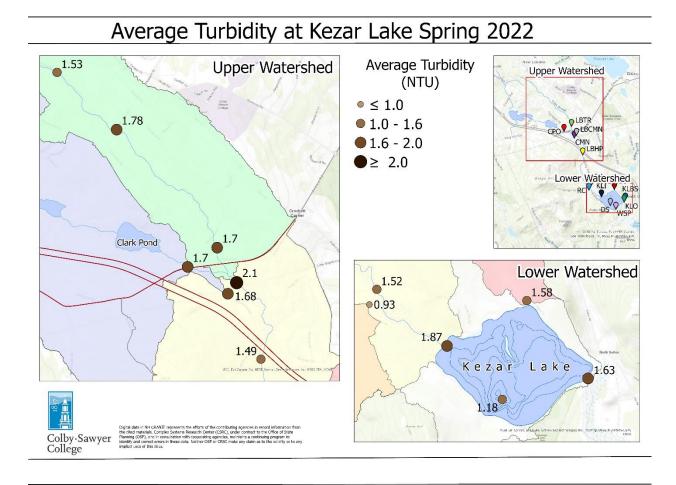
Turbidity

Turbidity, often confused with transparency, refers to the overall clarity of a water sample and can be affected by suspended matter such as clay, silt, algae, or other organic materials and is measured in nephelometric turbidity units (NTUs). Common influences of turbidity include soil erosion, water discharge, storm runoff, bottom feeding aquatic animals, and algal growth. As the water becomes cloudier, the amount of light that can penetrate through the water column decreases. This can impact the aesthetic of a lake and have ecological consequences (e.g., loss of aquatic vegetation productivity due to obstructed sunlight).

Generally, a waterbody with a turbidity of 5.0 NTU or above is considered undesirable for water clarity, as it has more suspended matter within it, causing a decrease in its transparency and an increased cloudiness. Anything below the 5.0 NTU threshold is considered normal (QA Manual, 2020).



Map 4. The average turbidity in NTU was sampled at sites in and around Kezar Lake for the Fall of 2021.



Map 5. The average turbidity in NTU was sampled at sites in and around Kezar Lake for the Spring of 2022.

The CBP samples were in a variety of places within the Kezar Lake watershed, aiming to collect a broad representation of different conditions throughout the area. Each sample site is part of a sub watershed that may have different characteristics than the next. By having a broad range of sampling sites, the CBP had hoped to monitor turbidity levels and identify possible sources contributing to the changes of those turbidity levels. At the Deep Site, two samples were taken at different depths, 2 meters and 6 meters, to examine both the epilimnion (surface level) and hypolimnion (deep level).

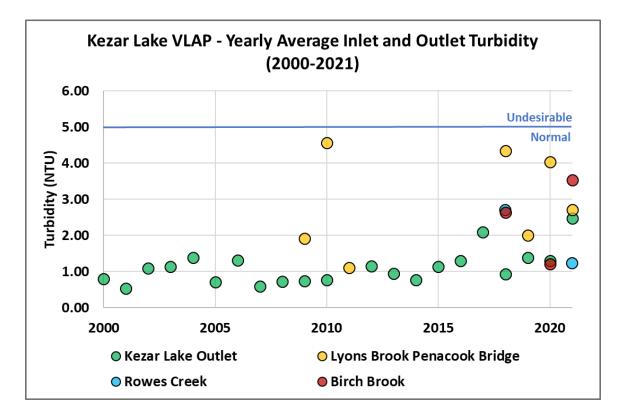


Figure 11. VLAP findings of yearly average Turbidity at the inlets and outlets from 2000 to 2021. Satisfactory threshold of 5.0 NTU indicated by the blue line.

VLAP has continued to track turbidity although data collection has been inconsistent over the years. *Figure 11* displays the average turbidity of the inlet and outlet sample sites from 2000 to 2021, sampled by VLAP. The average of all sample sites per year show to have a steady incline and all fall under the desirable threshold (blue line) of 5.0 NTU. This Figure shows no data above that threshold, and all considered normal, while gaining a slight increase towards the undesirable range as the years progress. Turbidity in smaller tributaries is likely to show greater variability in values due to its smaller water volume ratio to sediment runoff. Typically, they are more affected by sediment runoff because there is less water that can dilute potential suspended sediments in comparison to the larger waterbodies thus is why turbidity is expected to have a higher value as water volume decreases to an extent.

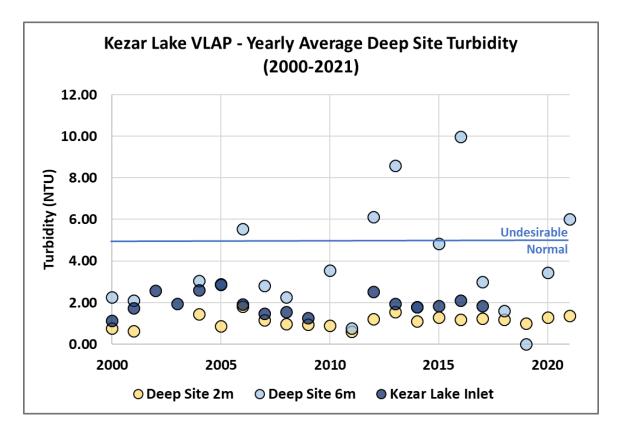


Figure 12. VLAP findings of yearly average Turbidity at different depths of the Deep Site from 2000 to 2021. Satisfactory threshold of 5.0 NTU indicated by the blue line.

Figure 12 shows the average turbidity of the Deep Site at depths of 2 and 6 meters and the Kezar Lake Inlet from the VLAP data. All sample site average measurements are within the satisfactory line of 5.0 NTU except for the Deep Site at 6 meters. Samples at the deepest depth, in the hypolimnion level are closer to the fine sediments settled below which may have had an influence on the accuracy of the collected samples. Sampling at the bottom of the lake could have disturbed the settled sediment either from the anchor or by collecting samples too close to the lake bottom which would have captured a large amount of sediment. Due to the location and depth of the Deep Site at 6 meters, a higher turbidity is to be expected. The VLAP data from *Figure 12* indicates a pattern where turbidity will be higher at samples that are closer to the bottom of the lake than samples that are further from the lake floor. The Inlet also supports this idea as turbidity levels range in between those of the Deep Site 2m and 6m.

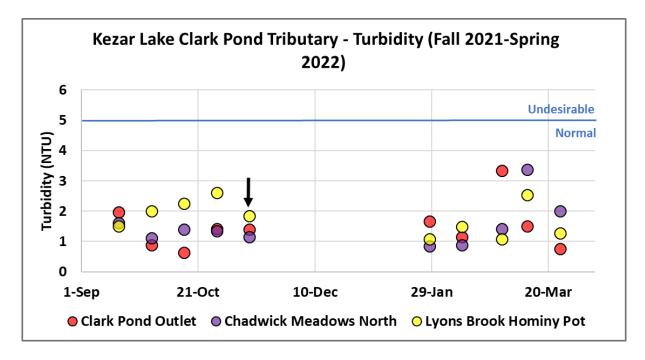


Figure 13. CBP findings of Turbidity at Clark Pond Tributary from the Fall 2021 to Spring 2022. Storm event(s) are indicated by the black arrow(s).

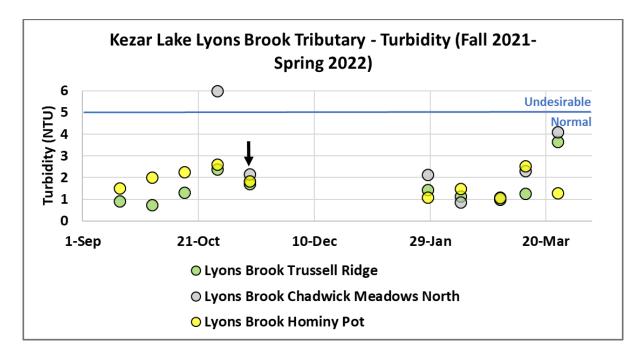


Figure 14. CBP findings of Turbidity at Lyons Brook Tributary from the Fall 2021 to Spring 2022. Storm event(s) are indicated by black arrow(s).

Data collected from the CBP class all stays in the satisfactory range apart except for two samples from different sites that surpassed the 5.0 threshold (LBCMN and Deep Site 6m). More will be discussed later in the paper.

During the fall 2021, storm events (indicated in black arrow(s) were recorded to observe if there was any relationship between rain fall and turbidity. However, this year's CBP findings show little to no correlation between the two and so, rainstorm events were no longer recorded by the beginning of the spring 2022 semester.

In *Figure 13*, Chadwick Meadows North has consistent values below 2 NTU while in *Figure 14*, Lyons Brook Chadwick Meadows North has one high reading of 6 NTU in late October. The CBP cannot determine the cause of this but suggests more research and sampling specific to Chadwick Meadows to understand the cause for the increased turbidity levels from Chadwick Meadows North to Lyons Brook Chadwick Meadows North.

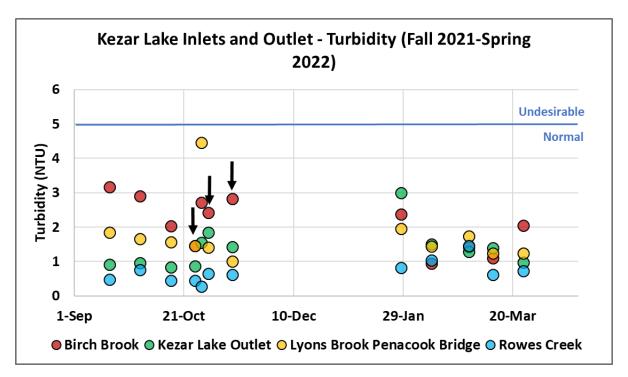


Figure 15. CBP findings of Turbidity at Inlets and Outlets from the Fall of 2021 to Spring 2022. Storm event(s) are indicated by black arrow(s).

In *Figure 15*, every sampled turbidity level fall below the threshold of satisfactory level. Storm events that are represented by black arrows display little to no change compared to other sampling dates without rain events throughout the Fall semester.

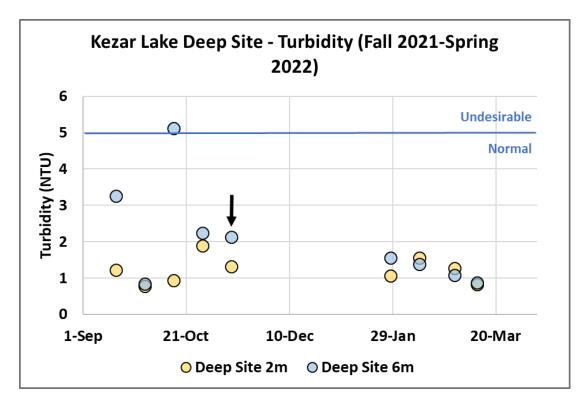


Figure 16. CBP findings of Turbidity at different depths of the Deep Site from the Fall of 2021 to Spring 2022. Storm event(s) are indicated by black arrow(s).

Figure 16 solely focuses on the Deep Site at 2 meters and 6 meters. Data points vary during the months and has one event around mid-October with Deep Site at 6m having one high recording where turbidity went over 5.0 NTU. The storm event represented as black arrows, displays relatively similar data compared to the previous samples taken during the year and so, was not recorded in the Spring semester.

The CBP observed from the two sample sites, CMN and LBCMN, that there is some sort of relationship causing turbidity to rise. Many factors could increase the levels that with more tested samples, can reveal such causes. Most sampled data from CBP 2021-2022 meet the satisfactory line of 5.0 NTU and has formed a steady turbidity trend. The sample site with the most concern regarding turbidity is the Deep Site at 6m. However, as mentioned previously, variability in the recordings is somewhat expected as the CBP sampling method may have caused disturbance to the Deep Site which would have made the samples less accurate.

Turbidity and transparency can be used to indicate the quality and health of a lake. To increase transparency and reduce turbidity, lakeshore and watershed properties can educate themselves on environmentally friendly landscaping practices. Encouraging property owners to take part in lake assessments and proper landscaping management can help to improve the overall clarity of a water sample.

Chlorophyll-a

Chlorophyll-a is a green pigment that gives photosynthetic organisms their green color. Chlorophyll-a absorbs light and converts it to chemical energy as part of the photosynthetic process. Photosynthesis produces sugars for plant growth and development (Urry et al., 2016). While chlorophyll-a is a key indicator of lake health and quality, it is also a direct measure of the photosynthetic activity that occurs within the lake. High levels of this parameter in a waterbody can indicate an overabundance of nutrients such as nitrogen and phosphorus (Bennett, 2017). Sources of excess nutrients include fertilizers and wastewater containing untreated sewage (Nutrients and Eutrophication, United States Geological Survey). The accumulation of excess nutrients within the water column leads to a process known as eutrophication. Eutrophication is the natural aging process of a lake; however, it is often accelerated by humans from farm fertilizer runoff and private residences. Algal blooms are a result of eutrophication and often produce high amounts of cyanotoxins, which are toxic to local organisms within the ecosystem, including humans (Merel et al., 2013). Algal blooms are both aesthetically unappealing for the appearance and recreational use of a lake as well as extremely detrimental to a lake's health. When left unchecked, algal blooms quickly take over a waterbody, causing a rapid decline in the amount of dissolved oxygen present in the water column. This decline of dissolved oxygen can be identified by fish kills, increased levels of nutrients, and toxins in the waterbody (Merel et al., 2013). Algal blooms have proved to be a prominent issue for many fresh and saltwater systems across the United States in recent years, including Kezar Lake from the mid 1960's to 1984 when the Kezar Lake Restoration Program was put into effect. The CBP is examining the chlorophylla content of the Kezar Lake Deep Site, located above its deepest point of approximately 25 feet. Below are *Image* 5 and *Image* 6 showing the before and after appearance of Kezar Lake.



Image 5. Algal blooms in Kezar Lake from 1963 as a result of intense phosphorus loading prior to the alum treatment (Marcoux & Beck, 2017).



Image 6. Kezar Lake years after the alum treatment (Marcoux & Beck, 2017).

The New Hampshire Department of Environmental Services (NHDES) composed an indepth analysis of the trends seen throughout the Kezar Lake Restoration Project. The Phase III Report observes trends seen in chlorophyll-a data taken both before and after the initial phosphorus inactivation of 1984 (Connor, J., & Smagula, A., *New Hampshire Department of Environmental Services*).

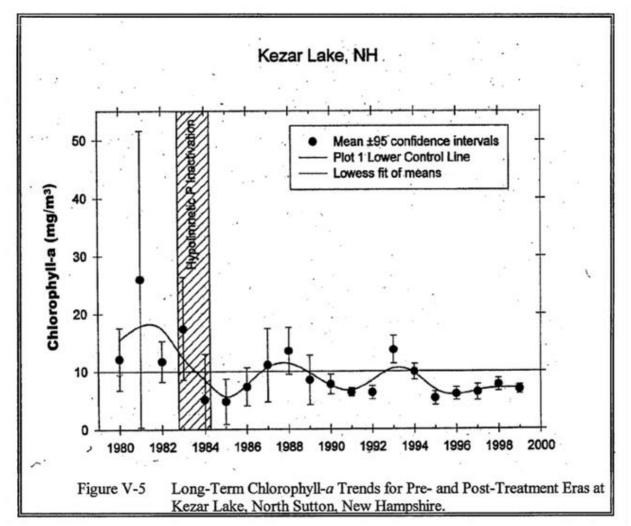


Figure 17. Average Chlorophyll-a levels at Kezar Lake from 1980 to 2000 (Phase III Report).

Figure 17 shows the yearly average chlorophyll-a content at the Kezar Lake Deep Site from the years 1980 to 2000. The state of New Hampshire has outlined three distinct thresholds to classify lakes based on their average chlorophyll-a content. These classifications are listed in table 3 below.

| Oligotrophic | $0-4 \text{ mg/m}^3$ |
|--------------|-------------------------|
| Mesotrophic | $4 - 15 \text{ mg/m}^3$ |
| Eutrophic | >15 mg/m ³ |

Table 3. The three lake classifications given by the state of New Hampshire (Connor, J., & Smagula, A., New Hampshire Department of Environmental Services).

The trends seen in *Figure 17* can be viewed as a good indicator that the health of the lake is improving, because of the phosphorus inactivation treatment. After 1984 as outlined in a black, crosshatch design on the figure, chlorophyll-a levels for most years were below 10 mg/m³, indicating that quality of Kezar Lake has improved post treatment (Connor, J., & Smagula, A., New Hampshire Department of Environmental Services). It is important to note that after the aluminum injection, chlorophyll-a content peaked above 10 mg/m³ in the year 1987 and again six years later in the year 1993, but then returned to below 10 mg/m³ again in subsequent years, however, concentration levels remained within thresholds regarded as eutrophic (Connor, J., & Smagula, A., New Hampshire Department of Environmental Services).

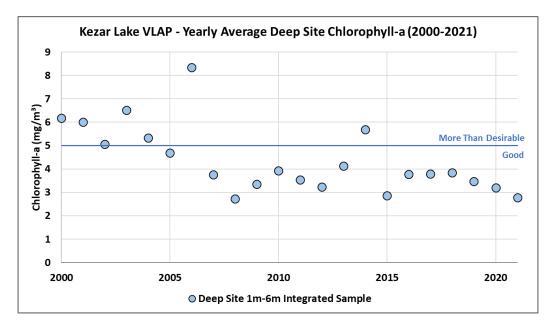


Figure 18. VLAP findings of the yearly average chlorophyll-a levels at the Deep Site from 2000 to 2021. Satisfactory threshold of 5 mg/ m³ indicated by the blue line.

Data from the Volunteer Lake Assessment Program pertaining to historical summer averages for chlorophyll-a from the years 2000 to 2021 are represented above. Some of the latter years have as little as only one sampling date for chlorophyll-a, making it difficult to confidently assess trends. *Figure 18* shows the trend of chlorophyll-a averages collected by VLAP over the span of 21 years. The figure displays an overall decreasing trend for chlorophyll-a content of Kezar Lake as the years progress. Earlier years show averages in the 5-7 mg/m³ range which is considered higher than optimal for lakes in the state of New Hampshire. After the year 2006, chlorophyll-a averages begin to steadily decrease to acceptable amounts in the 2-4 mg/m³ range. This is a great indicator that Kezar Lake has had relatively low productivity compared to levels from 21 years ago.

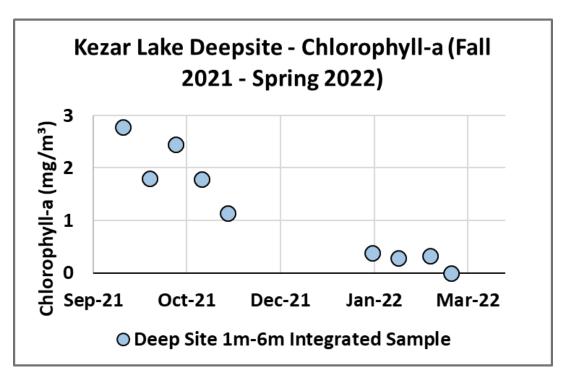
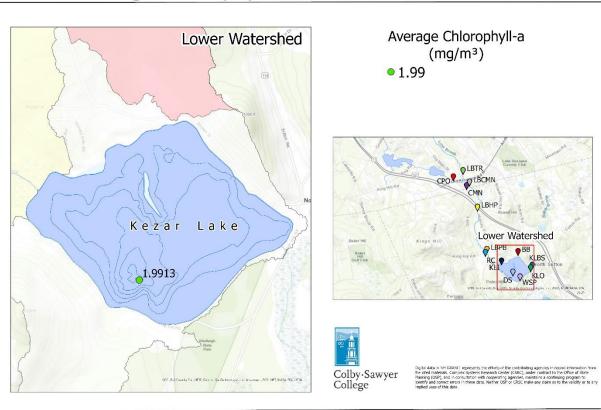


Figure 19. CBP findings on Chlorophyll-a sampled at the Deep Site during both Fall 2021 and Spring 2022.

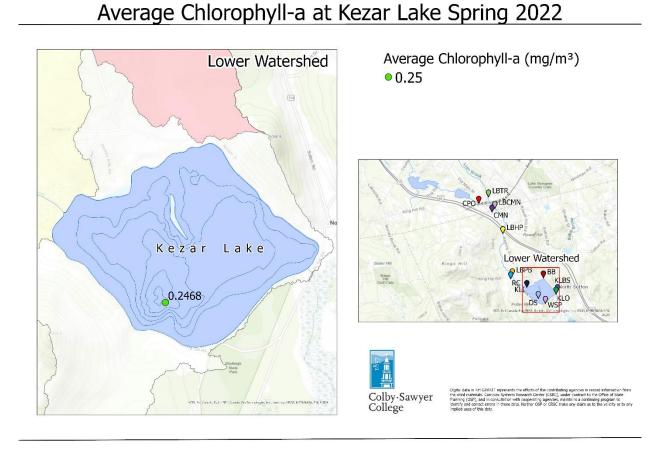
The CBP data seen in *Figure 19* above shows a similar trend to that seen in the VLAP data. Chlorophyll-a was sampled on five dates throughout the Fall 2021 semester and shows declining chlorophyll-a at the Kezar Lake Deep Site, as data collected during spring continued this trend. All data points collected thus far fall within the 1-3 mg/m³ range, indicating that Kezar

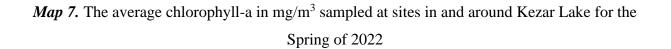
Lake is maintaining good levels of chlorophyll-a. This is expected to be true as photosynthetic processes decrease through the fall and winter seasons. The average chlorophyll-a content of Kezar Lake Deep Site sampled by the CBP during the fall of 2021 was 1.99 mg/m³ and can be seen in *Map 6* below.



Average Chlorophyll-a at Kezar Lake Fall 2021

Map 6. The average chlorophyll-a in mg/m^3 sampled at sites in and around Kezar Lake for the Fall of 2021.





Chlorophyll-a was sampled again on four separate dates throughout the Spring 2022 spanning from late January to late March. When compared to the results received from the previous semester, chlorophyll-a levels were much lower with the highest value only reaching to around 0.25 mg/m³. This is an expected trend to see as Kezar Lake was completely frozen over on each day in which the samples were collected, causing plant productivity to be much lower in cold conditions. The overall data pertaining to chlorophyll-a collected by the CBP over the course of the duration of the project indicates that Kezar Lake is maintaining healthy levels of plant productivity. The highest recorded chlorophyll-a content recorded throughout this project was 2.78 mg/m³ which is well below the acceptance threshold shown in the previous graphs. Given the lake's history of extensive algal blooms, the lake continues to thrive at healthy levels.

Some recommendations that the CBP can provide to the KLPA for maintaining healthy chlorophyll-a levels is to acknowledge and understand property water runoff. A common driving factor that leads to detrimental algal blooms in lake systems often stems from fertilizer runoff. To lower the risk of nutrient runoff from properties surrounding Kezar Lake, the CBP asks homeowners to be mindful of the number of fertilizers that homeowners apply to their lawns. Another recommendation from the CBP is for the KLPA homeowners to monitor and check their septic systems. Nutrient accumulation from untreated sewage was the main factor that caused the original algal blooms in Kezar Lake in 1963 before the application of the alum treatment.

Story 2: Under Investigation

pН

The pH indicates the logarithmic measure of hydrogen ion concentrations in a solution, such as water (Wurts, 1992). Normal waters will contain both acids and bases as well as biological processes that influence the pH. The water ranges for New Hampshire consist of an average between 5.8 and 6.5 for eutrophic waterbodies, 6.1 and 6.9 for mesotrophic waterbodies, and 6.15 and 7 for oligotrophic waterbodies (Nelson & Neils, 2020). For example, when carbon dioxide dissolves in water, a reaction occurs where a small portion of carbon dioxide becomes carbonic acid and reduces pH (Wurts, 1992). Higher carbon dioxide concentrations result in the production of carbonic acids, and acidic inputs lower the pH of a waterbody. These changes happen more quickly depending on the qualities of the waterbody being affected, like it's historical pH value and associated buffering capacities. Limestone, calcium, and silicate which are seen in geologic formations, can dissolve through carbon dioxide and increase the bicarbonates in water (Boyd, 2015). While many places have these geologic formations, New Hampshire does not. These bicarbonates, as well as acids like sulfur dioxide and nitrogen dioxide interact with the waterbody to increase the water's acidity. To know more about buffering capacity, refer to the ANC section of this report. See the table below for the pH ranges and their meanings.

| PH range (0 – 14) | Representation of logarithmic scale | |
|-------------------|-------------------------------------|--|
| Below 7 | Acidic | |
| 7 | Neutral | |
| Above 7 | Basic/Alkaline | |

Table 4. The pH range on a 0-14 scale (USGS Logarithmic Scale of pH and water, n.d.).

| Category | pH (units) |
|--------------|------------|
| Acidified | <5 |
| Critical | 5.0 5.4 |
| Endangered | 5.5 6.0 |
| Satisfactory | 6.0 - 8.0 |

Table 5. Satisfactory Category levels for pH (QA manual, 2020).

Historically, NH lakes have had a pH range of 6.5 to 6.9, meaning they are just below the neutral range. pH naturally fluctuates during daily and seasonal changes. pH levels below 6 or above 9 can have detrimental effects on aquatic life. Extreme levels outside the tolerable range limits fish growth, reproduction, and causes death in aquatic biota. (Banrie, 2013).

The last twenty years of data collection from Kezar Lake show a positive trend of increasing pH values from lower 6's to higher 6's, and this can be seen in *figure 20* and *figure 21* below. While local efforts are an important part of any ecosystem's long-term health, for pH and ANC it is important to look at largescale, outside factors, like the United States Clean Air Act (CAA), for example. The CAA is a piece of legislation that has regulated national air pollution since 1970. Two of the chemicals targeted directly by the CAA are sulfur dioxide (SOXs) and nitrogen dioxide (NOXs) because of their known adverse human health effects (EPA, December 2021). Their production is mainly from energy production and the introduction of SOXs and NOXs causes an environmental phenomenon called acid rain (EPA, June 2021). Their involvement with national pH levels, from rains to lakes, is most of what has made SOXs and

NOXs reductions a long-lasting goal of the CAA. Since the 1970's, SOXs and NOXs reductions have been recorded every decade, alongside data collection that shows improving freshwater pH levels (EPA, June 2021; EPA December, 2021). This supports a case that the CAA is working. Results to the contrary are interesting and have been found during our study, localized to the Chadwick Meadows wetland.

Chadwick Meadows has been observed for pH levels. Lyons Brook Chadwick Meadows North (LBCMN) and Chadwick Meadows North (CMN) are located just north of interstate 89. To look at the implications of the wetland affecting the sample sites, LBCMN and CMN were compared to the sample site, Lyons Brook Hominy Pot (LBHP) which is farther south of Chadwick Meadows and below Route 11. The CBP data suggests that the pH levels from LBHP are impacted by the Chadwick Meadows wetland, the site shows lower pH levels compared to the pH levels from above the wetland. This could be influenced by natural occurrences in the Chadwick Meadows wetland like organic acids, known as humic and fulvic acids. These acids are dead organic compounds that have been produced by aquatic or terrestrial plants and then broken down. In conditions of low oxygen conditions these acids can build up in the water body and create a brown or tan color. As organic compounds are acidic inputs, they ultimately lower the pH of the wetland and surrounding watershed (Dodds & Whiles, 2010).

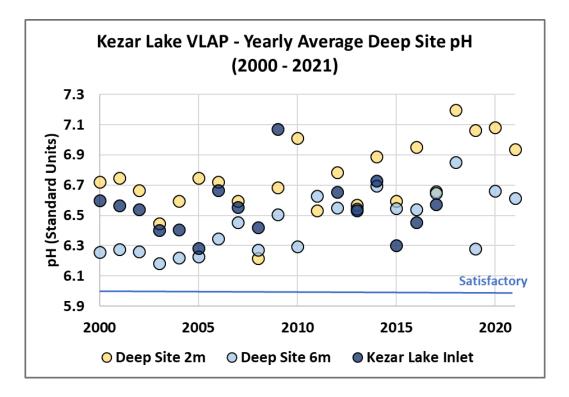


Figure 20. VLAP findings of yearly average pH levels at the Deep Site and Kezar lake inlet from 2000 to 2021. Satisfactory threshold is 6.0 or above, indicated by the blue line.

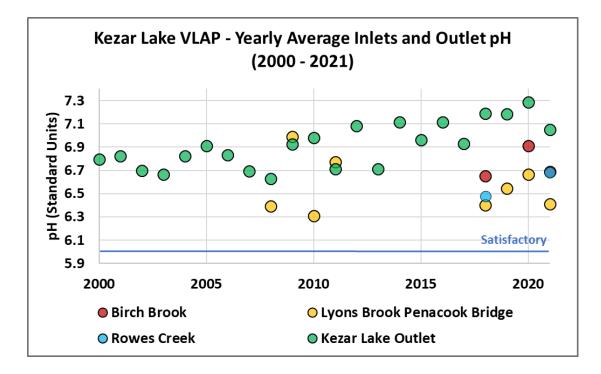


Figure 21. VLAP findings of yearly average pH levels at the Inlets and Outlets from 2000 to 2021. Satisfactory threshold is 6.0 or above, indicated by the blue line.

Figure 20 illustrates the average pH from the VLAP data from 2000-2021 for the Deep Site and Inlet. All pH values are within a satisfactory range of 6.0, and all values are showing a positive trend as they move further away from the endangered range. *Figure 21* shows the yearly average pH for the inlet and outlet from the VLAP data. This also shows a positive trend as it is leading away from endangered and towards a neutral level while staying in the satisfactory line. These trends indicate that Kezar Lake is improving away from acidification and there are lower concentrations of hydrogen ions seen in the waterbody.

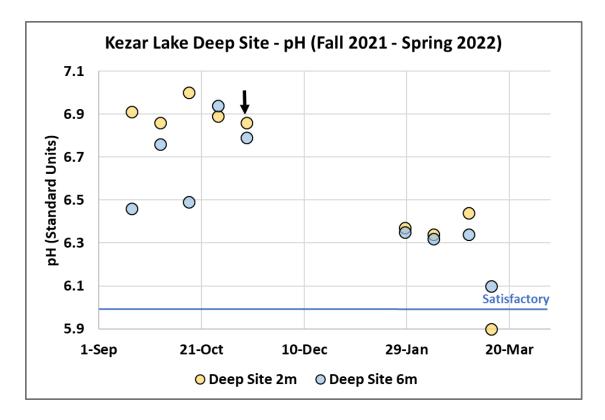


Figure 22. CBP findings of pH levels at different depths of the Kezar Lake Deep Site in the Fall and Spring 2021-2022. Storm event(s) are indicated with black arrow(s).

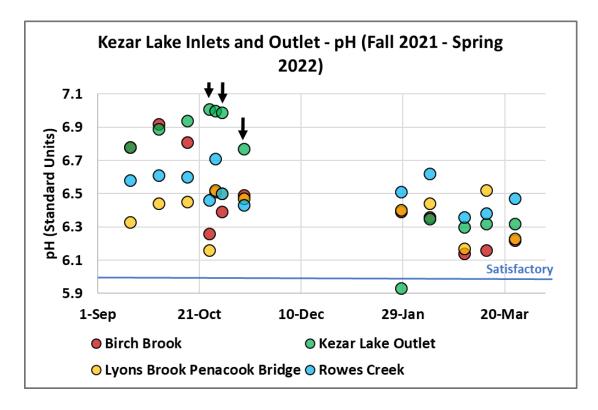


Figure 23. CBP findings of pH levels at the Inlets and Outlets in the Fall and Spring 2022. Storm event(s) are indicated with black arrow(s).

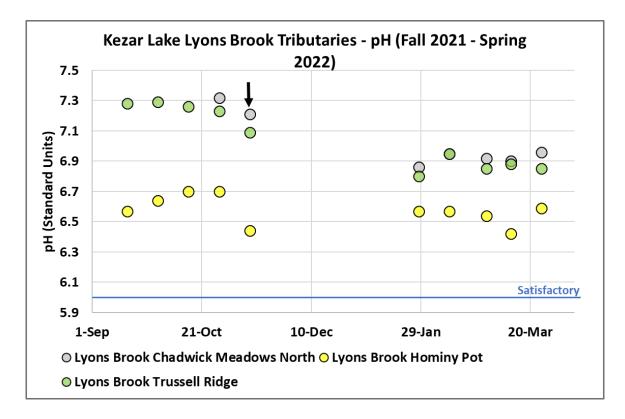
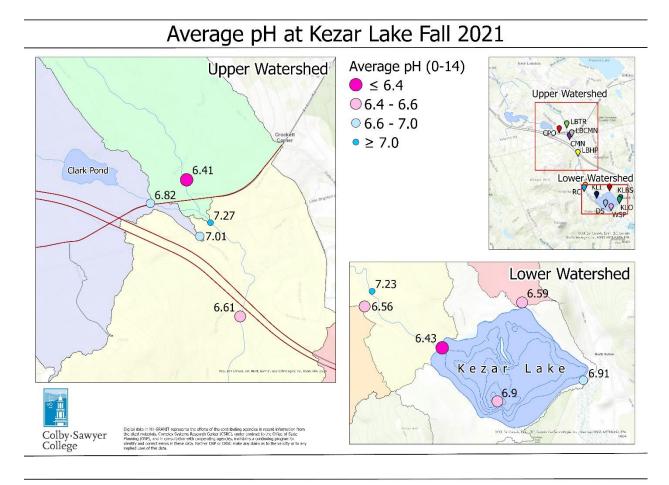
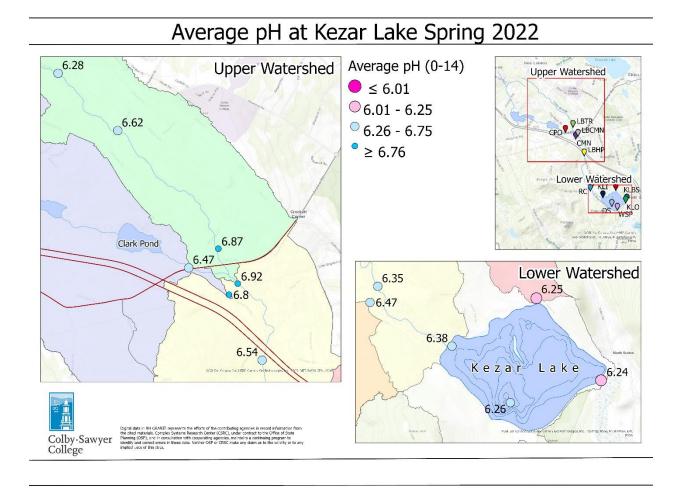


Figure 24. CBP findings of pH levels at Lyons Brook Tributary in the Fall and Spring 2021-2022. Storm event(s) are indicated with black arrow(s).

Figures 22, 23, and *24* all show the findings of pH levels during the Fall semester and spring semester of the Community-Based Project class. They all stay in satisfactory ranges while varying between pH ranges of 6.1 to 7.4. It can also be seen in the past mentioned figures, that Lyons Brook Chadwick Meadows North is typically highest in pH and Lyons Brook Hominy Pot ranges in typically lower levels. This could potentially be caused by the decomposition aspects that allow for natural changes in a wetland. Compared to the Fall semester the spring semester is relatively lower in value until it begins to bump at the end of sampling time because of the spring thaw that occurred.



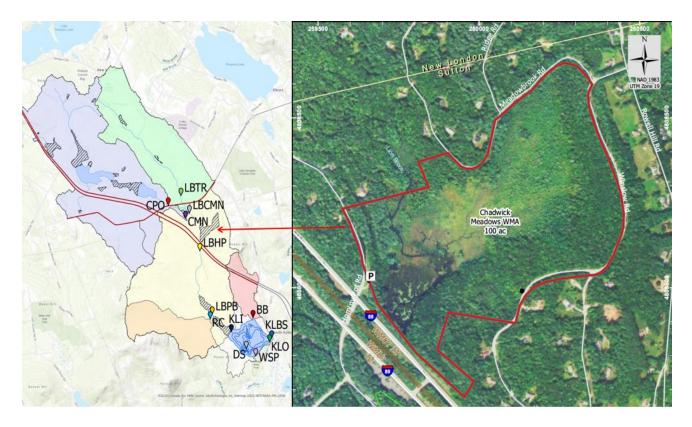
Map 8. The average pH on a 0-14 pH scale sampled at sites in and around Kezar Lake for the Fall of 2021.



Map 9. The average pH on a 0-14 pH scale sampled at sites in and around Kezar Lake for the Spring of 2022.

CBP findings indicate Lyons Brook Chadwick Meadows North tends to have higher pH ranges of 7.0 to 7.5 compared to below Chadwick meadows where Lyons Brook Hominy Pot is within pH ranges of 6.5 or less. In addition, tributaries closer to the Lake such as Birch brook and the Inlet are in range from 6.0 to 7.0 as the trends after Chadwick Meadows stay the same throughout the rest of the tributaries and lake. This data can conclude that all tributaries and pieces of the lakes that are connected are in a good range of pH even though some are higher than others. No pH goes over the values of 7.5 which shows relatively good information regarding the lake and its tributaries.

The CBP recommends further study on what is happening to the water's pH values within Lyons Brook as it passes through Chadwick Meadows. Further study is needed to be able to tell what exactly is causing this effect. It could be from a number of factors, both human and natural, and nothing specific enough to be sure has been studied by the CBP this year.



Map 10. Aerial view of Chadwick Meadows and where it is in reference to the greater watershed and our sampling sites.

Acid Neutralizing Capacity (ANC)

Acid Neutralizing Capacity (ANC) is measured as the concentration of calcium carbonate (CaCO₃) dissolved in water. This is utilized as a measurement of the ability of water to act as a buffer for acidic inputs such as acid rain (Boyd, 2015; QA Manual, 2020; Dyer, 2007). ANC for natural waters ranges anywhere from 0 mg/L to more than 500 mg/L (Boyd, 2015) and the New Hampshire Department of Environmental Services outlines six sensitivity categories that they use to classify their lakes.

| Sensitivity Category | ANC (mg/L) |
|----------------------|------------|
| Acidified | <0 |
| Critical | >0 - 2 |
| Endangered | >2 - 5 |
| Highly Sensitive | >5 - 10 |
| Sensitive | >10 - 20 |
| Not Sensitive | >20 |

Table 6. Satisfactory Category Levels for Acid Neutralizing Capacity (QA Manual, 2020;NHDES, 2019).

The amount of CaCO₃ is partially determined by the underlying composition of bedrock that a lake or stream sits on in addition to surficial deposits. Lakes positioned over hard rocks can be particularly sensitive to acidic inputs. These hard rocks, such as granite, quartzites, and quartz sandstone are resistant to weathering and produce waters that contain very low concentrations of neutralizing compounds (Novotny, 2003; Dyer, 2007). Moderate to high levels of ANC are often associated with limestone deposits in watershed soils (Boyd, 2015). Lakes with limestone deposits are far more common in New Hampshire's neighboring state, Vermont. Due to its geology, New Hampshire has far more granite bedrock than it does limestone, which does not have the same buffering capacity associated with limestone, resulting in relatively low ANC levels throughout the state. The median for NH lakes ANC is 4.9 mg/L which would be in the Department of Environmental Services endangered sensitivity category (QA Manual, 2020). When acidic inputs, such as acid rain, fall on watersheds with low acid neutralizing capacity, the acids are not neutralized during the overland flow and streams and lakes that the water flows into can become acidified (Novotny, 2003).

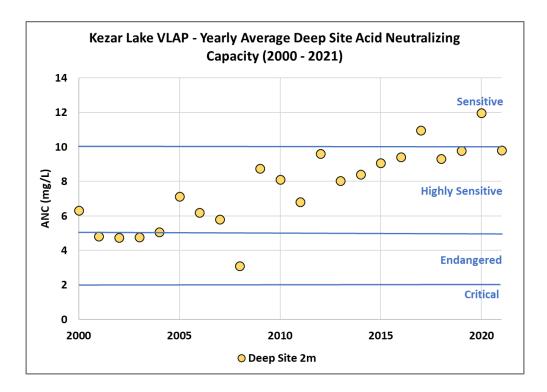


Figure 25. VLAP findings of annual average ANC at the Deep Site of 2m from years 2000 to 2021. The blue lines indicate the sensitivity thresholds for ANC.

Figure 25 shows the VLAP yearly average ANC for the Kezar Lake Deep Site at 2m from 2000-2021. The data indicate a positive trend as ANC levels increased from an endangered to a sensitive classification over the twenty-one-year period. Initially, ANC levels were near the endangered sensitivity category and were similar to mean NH Lake ANC values. In the late 2000's, the positive trend begins with the ANC of Kezar Lake increasing into the highly sensitive category and eventually reaching the sensitive category in the years 2017 and 2020. Given that the mean NH lake ANC is 4.9 mg/L, this trend is a healthy change for Kezar Lake. This positive trend in ANC is very similar to the positive trend in pH over the same time period. This change occurred both from local and national input. National policies such as the Clean Air Act are, in part, responsible for these positive changes. Regulating harmful emissions like sulfur dioxides and nitrogen oxides in the air caused by burning fossil fuels, and reducing their national output, has opened the way for lakes like Kezar to rebound in ANC, pH, and other parameters.

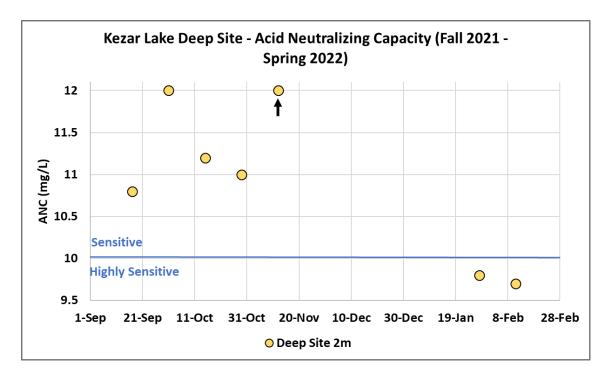
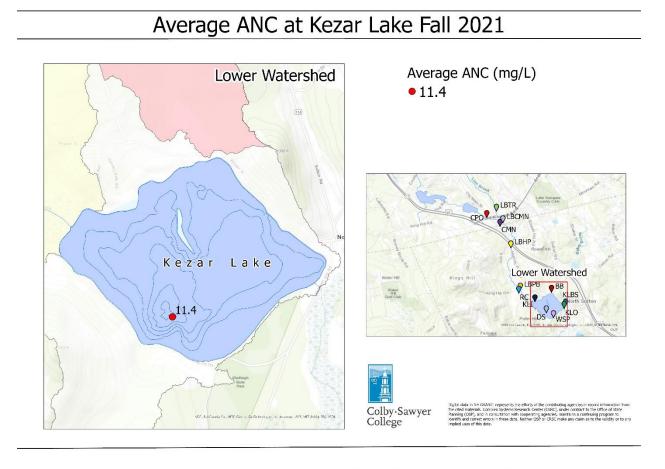
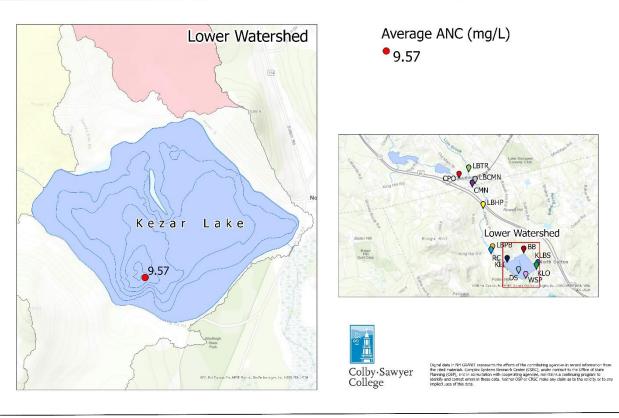


Figure 26. CBP findings of ANC levels at the Deep Site of 2m for Fall 2021 and Spring 2022 semesters. Storm event(s) are indicated by black arrow(s).



Map 11. The average ANC in mg/L sampled at sites in and around Kezar Lake for the Fall of 2021.



Average ANC at Kezar Lake Spring 2022

Map 12. The average ANC in mg/L by the CBP at sample sites in and around Kezar Lake for the Spring of 2022.

The fall 2021 and spring 2022 data demonstrated that the trend identified in the VLAP data is continuing. Regarding the fall semester, all five samplings at the Deep Site had ANC values averaging 11.6 mg/L, which is within the sensitive sensitivity category of >10-20 mg/L. Unfortunately, due to equipment failures in the lab coupled with melting ice during sampling periods, the CBP were only able to obtain two additional ANC values during the spring semester. However, these two additional samples continue to support the general trend established by the VLAP data with some results remaining in the sensitive category of >10-20 mg/L and some results dropping down into the highly sensitive category of >5-10 mg/L. The two spring ANC samples had an average of 9.75 mg/L which does fall within the highly sensitive category. However, this decrease in ANC can most likely be contributed to seasonal factors. Overall, like the more recent VLAP data, all the CBP ANC values were well above the mean NH lake ANC

value of 4.9 mg/L meaning that acid neutralizing capacity is not of immediate concern. Despite this, Kezar lake is still hovering around the sensitive / highly sensitive categories outlined by the New Hampshire Department of Environmental Services and because of this, the CBP recommends more frequent testing for acid neutralizing capacity. This would allow the KLPA to better understand the seasonal differences in ANC and would provide more opportunities to detect any drastic changes in ANC. Additionally, the CBP class recommends supporting local, state, and federal legislation aimed at maintaining and strengthening regulations such as the Clean Air Act.

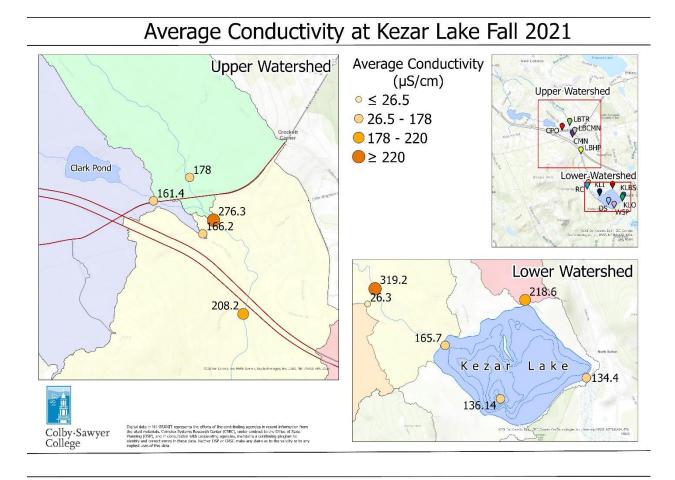
Story 3: Salts

Conductivity

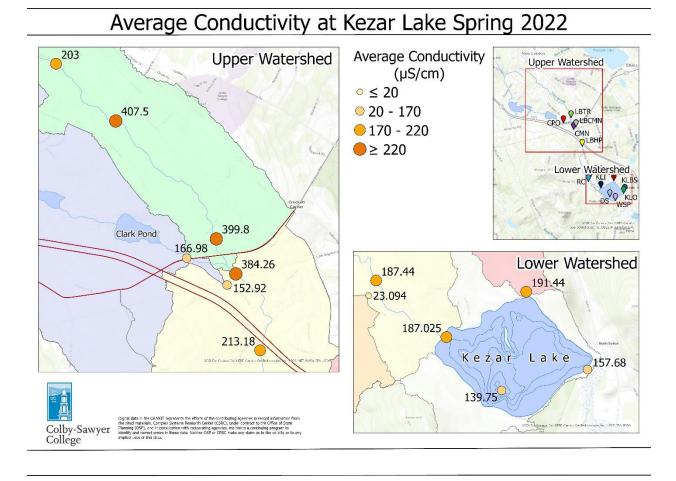
The Community Based Project measured the parameter, conductivity, in micro siemens per centimeter (μ S/cm), to look at the water's ability to conduct an electrical current (Kegley & Andrews, 1997). This is a key indicator for the overall health of a waterbody as high ion concentrations indicate the presence of human sourced contaminants in a waterbody. If the conductivity of an observed waterbody increases significantly, this indicates a source of pollution entering the waterbody (Mathur, 2014, EPA, n.d), however, conductivity alone does not make a sufficiently negative impact. Conductivity is closely related to chloride, as they both tell a similar story. Both chloride and conductivity can indicate human impacts such as salts from de-icing techniques in the winter.

New Hampshire's waters typically have low conductivity, in part because of the substantial amounts of granite in the state's bedrock (VLAP, n.d.). The state contains a large amount of granite, which does not contain minerals that easily dissolve or ionize. While granite bedrock has no contribution to conductivity, anthropogenic pollution such as sewage releasing into inland waters does the opposite due to the presence of chloride, phosphate, and nitrate (Mathur, 2014). These two influences will have a major impact on the water's ability to conduct electrical currents. Historically, during the VLAP data collection of Kezar Lake, the DES established values below 100 μ S/cm as the baseline for healthy waterbodies. Readings above this baseline can indicate human activity affecting the watershed. Road salting, agricultural runoff,

lawn care practices, and septic systems are often the source of raised water conductivity. Measuring the water's ability to conduct electrical currents continuously is important to understanding the effect of Kezar Lake's aluminum salt injection treatment from the 1980's. If the aluminum bound phosphorus in the soil of Kezar Lake's floor were to begin to reenter the water, then the conductivity would rise significantly (Kegley & Andrews, 1997). For information on how conductivity is collected and analyzed please refer to the appendix.



Map 13. The average Conductivity µS/cm sampled at sites in and around Kezar Lake for the Fall of 2021.



Map 14. The average Conductivity µS/cm sampled at sites in and around Kezar Lake for the Spring of 2022.

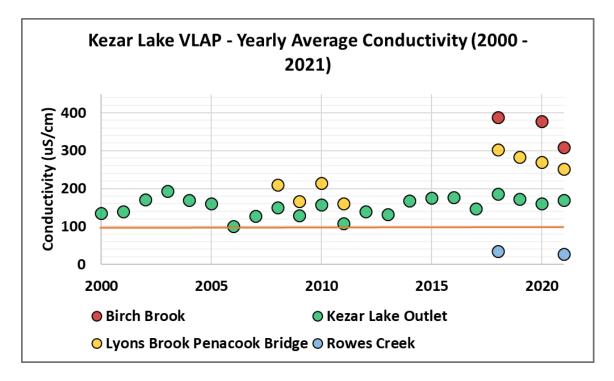


Figure 27. VLAP findings of annual average conductivity from 2000 to 2021. A satisfactory level of $100 \,\mu$ S/cm is indicated by the orange line.

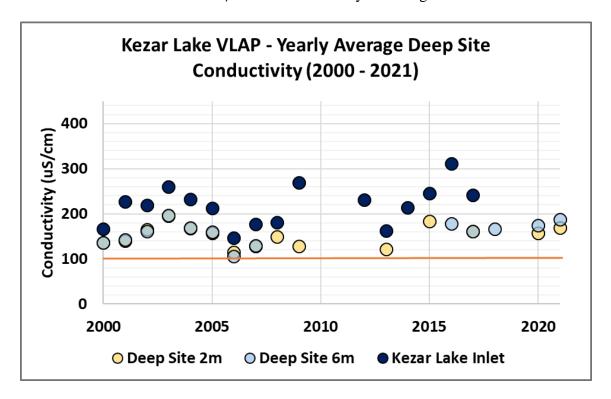


Figure 28. VLAP findings of annual average conductivity at different depths from 2000 to 2021. A satisfactory level of $100 \,\mu$ S/cm is indicated by the orange line.

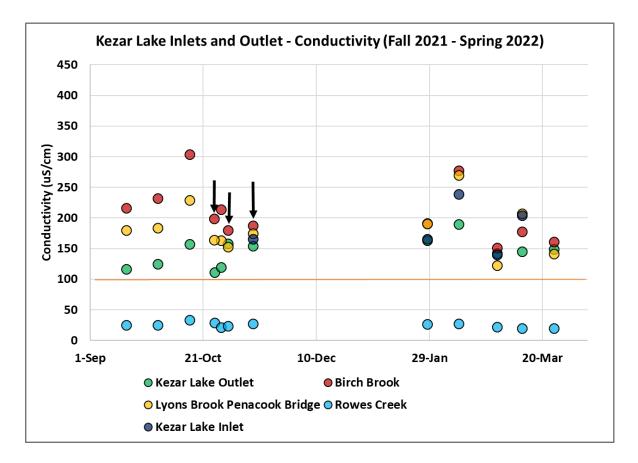


Figure 29. Community Based Project findings of conductivity levels at Kezar Lake's Tributaries during the Fall Semester of 2021 and Spring Semester of 2022. Storm event(s) indicated by the black arrow(s).

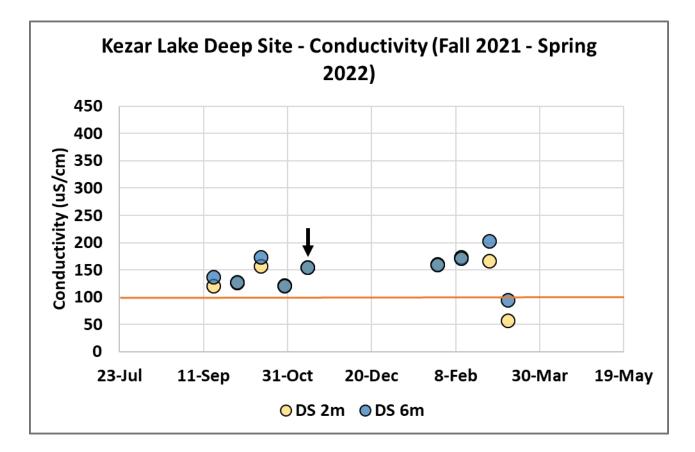
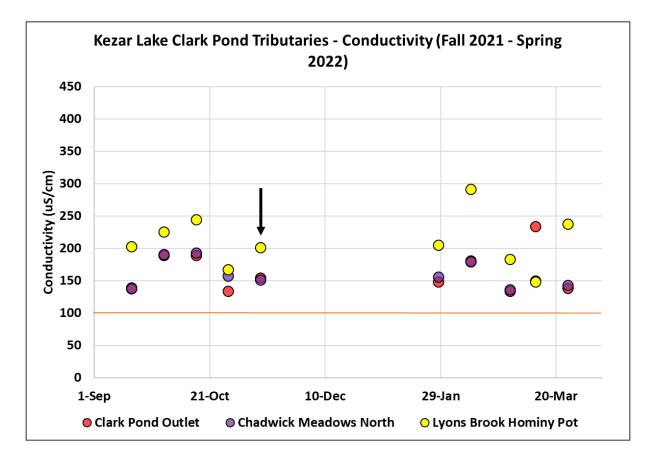


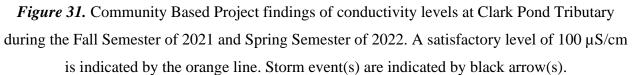
Figure 30. Community Based Project findings of conductivity levels at different depths of the Deep Site during the Fall Semester of 2021 and Spring Semester of 2022. Storm event(s) indicated by the black arrow(s).

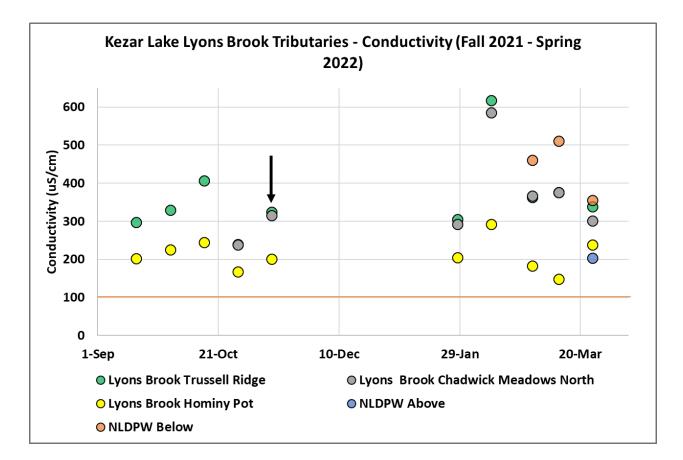
The results of the CBP's class analysis show a healthy trend of stable conductivity readings for Kezar Lake and its tributaries. The orange line on each figure represents the established 100 μ S/cm baseline conductance for the sampled lake. While there is a wide range of results, each location has an established history of results by the VLAP data from the prior twenty years, as shown above in Figures 27 and 28. The CBP data shows that these locations are following those trends up to late November 2021. Figures 29 and 30 show the CBP data, which includes collection days during storm events, represented with black arrows. These results suggest that weather has an insignificant effect on the conductivity of the waterbody.

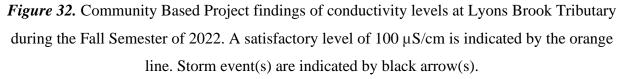
Sampling sites around Kezar Lake display levels of conductivity that suggest strong human influences from actions like deicing practices, except at Rowes Creek which is the only site that stays under the NHDES established value for conductivity throughout the entire sampling period. Birch Brook displayed constant high levels of conductivity in the Fall semester of 2021 and the Spring semester of 2022. However, Birch Brook contains a very low volume of water entering Kezar Lake, it is unlikely that the conductivity coming from Birch Brook has a significant impact on lake conductivity. What is likely influencing the conductivity of Kezar Lake is the input from Lyons Brook. Seen above in *maps 13 and 14* are the results from the CBP's water samples broken down by location. It's seen on the maps that the conductivity in Lyons Brook is twice that of its neighboring Clark Pond tributary. Noticing this in the fall, the CBP added two new sampling sites along Lyons Brook to investigate further human influence.

The results in the spring show a similar pattern. The values in the Lyons Brook tributary are routinely higher than the values from the Clark Pond tributary. The additional locations are upstream and downstream from a location likely influencing the quality of water in Kezar Lake, which is the New London Department of Public Works (NLDPW). The results from these samples can be seen below in *Figure 32*, with points assigned to "NLDPW Above" and "NLDPW Below". It can be seen driving through town, and from an aerial view on maps online, that the NLDPW stores their road salts very close to the banks of Lyons Brook. This is likely influencing the conductivity, and the related parameter chlorides, of the waters tested by the CBP, because of the limited number of samples collected by the CBP. It's recommended that further testing be used to confirm the NLDPW's influence on the Lyons Brook tributary.









Figures 31 and 32 both look at conductivity related to sampling site location, where the two sub watersheds for Lyons Brook and Clark Pond are compared. The Clark Pond outlet has generally lower values than the Lyons Brook watershed, and importantly, the Trussell Ridge location in the Lyons Brook Watershed has the highest values among those locations. During the Spring sampling period the CBP took a sample of over 600 μ S/cm at Trussell Ridge. Trussell Ridge is the most developed area of these sites, with a noticeable concentration of large homes. Due to the higher human activity of this area, this could be one cause of higher conductivity (Mathur, 2014). The CBP class monitored the relation between conductivity and chloride, as the two have a positive correlation. This was observed more closely from the CBP's spring 2022

data as road salting is more frequent and thus, has substantial chloride and conductivity levels in water samples of every sampling site.

As a recommendation for conductivity, continued and consistent monitoring of the lake and its tributaries is necessary. In addition to consistent monitoring, to reduce chlorides and conductivity, the CBP recommends that the KLPA investigates the Green Snow Pro certification. This certification is for commercial plowing and salt applicators, who through the program will learn best management practices to reduce salt applications during winter. Further sampling at Lyons Brook close to the Department of Public Works Salt Shed in New London is also recommended.

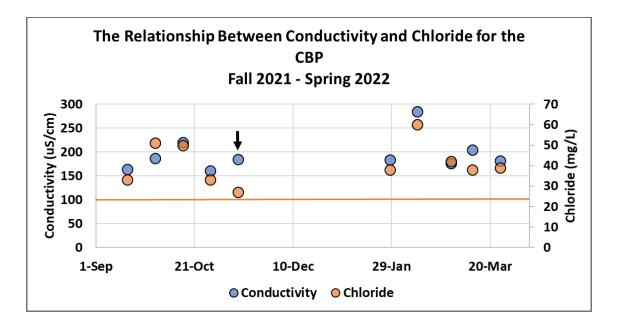


Figure 33. Average Conductivity and Chloride CBP data from the Fall and Spring semesters. Made to represent the relationship between the two parameters. Storm event(s) are indicated by black arrow(s).

Figure 33 seen above is representative of the correlation between conductivity and chloride. This shows signs that as either conductivity or chloride rises so does the other, while the correlative R value does not show significant data, there can be seen that some points are either closely related or right next to one another. The data seen here can be a representation that the conductivity or the chloride of Kezar lake have relied on the other.

Chloride

Chloride content is directly related to deicing and dust mitigation treatments on roads. Salts, such as sodium chloride and calcium chloride, are commonly used as road salts that will eventually flow into a waterbody in runoff. These salts are considered highly corrosive as they can be detrimental to both infrastructure and wildlife. Chlorides in higher concentrations can degrade inorganic materials such as concrete and steel (Lindsay, n.d.). Waterbodies with chloride contents considered high, which is generally an amount over 100 mg/L, will consequently see inhibited plant growth and that will eventually have a negative effect on other aquatic life in that system (Hunt et al., 2012).

The chloride content for fresh water in New Hampshire is historically low, with normal levels ranging from 1 and 100 mg/L (Hunt et al., 2012). However, higher chloride values are usually seen in areas with a lot of human influence, like septic input and salted roads (Medalie, 2013). Kezar Lake has a partially paved road that runs the perimeter of the lake, is influenced by nearby major roadways NH Route 11 and Interstate 89 and has many homes in its watershed likely contributing to the chloride levels measured by the CBP.

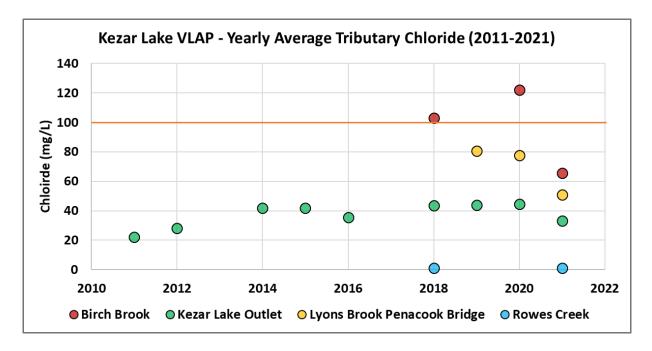


Figure 34. VLAP data for the yearly average chloride levels from 2011 to 2021. Normal threshold indicated by the orange line at 100 mg/L.

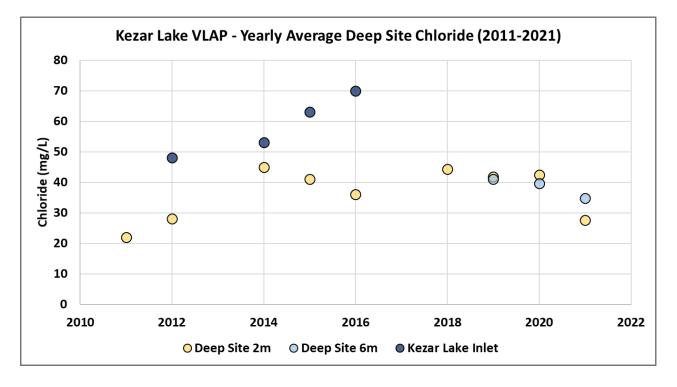


Figure 35. VLAP data for the yearly average chloride levels at different depths of the Deep Site from 2011 to 2021.

Compared to the other parameters VLAP recorded for Kezar Lake, chloride data collection began much later in 2011. There are gaps in the data that occur in 2013 and 2017. *Figure 34* shows the VLAP average chloride levels at Kezar Lakes tributaries from 2011 to 2021. Rowe's Creek, displayed in blue, shows the lowest levels of chloride while Birch Brook, in red, contributed the highest chloride levels to Kezar Lake in 2018 and 2020. These two locations are the smallest tributaries to Kezar Lake and likely have a minimal impact of the water chemistry of the lake. *Figure 35* shows the Deep Site data, which did not return any values of the normal threshold for chloride.

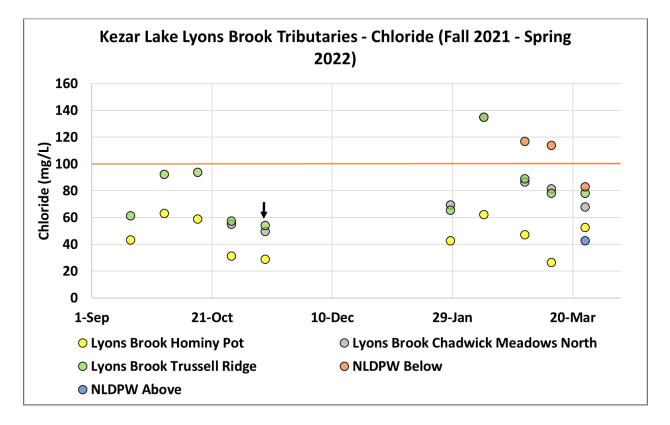


Figure 36. CBP findings of chloride levels at Lyons Brook tributaries during the Fall of 2021 and Spring of 2022. Storm event(s) are indicated by black arrow(s). Normal threshold indicated by the orange line at 100 mg/L.

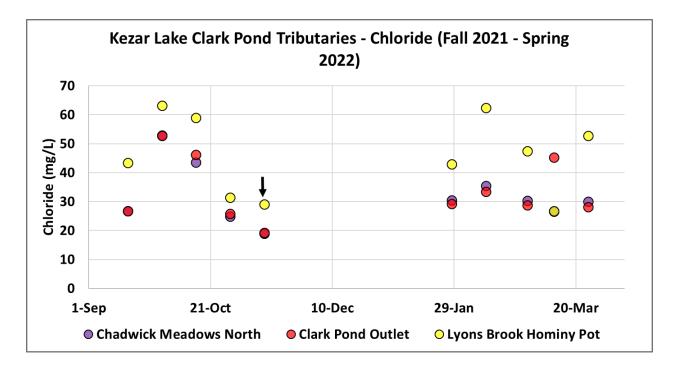


Figure 37. CBP findings of chloride levels at Clark Pond tributaries during the Fall of 2021 and Spring of 2022. Storm event(s) are indicated by black arrow(s).

The CBP sampling data illustrates a trend between two tributaries during the fall semester, the input from Clark Pond and Lyons Brook. The Chadwick Meadows North and Clark Pond outlet sampling sites appear to be diluting the chloride content where the two tributaries meet at the Lyons Brook Hominy Pot sampling site. In *Figure 36*, a new sampling site was added on October 29th, 2021, called Lyons Brook Chadwick Meadows North, represented by grey dots to collect more data on that dilution effect. A similar dilution effect from the rainfall can be seen after storm events, which are represented as black arrows on the figures above.

Lyons Brook Trussell Ridge, represented in green, is the sampling site with the CBP's highest chloride reading of 135 mg/L. Lyons Brook Trussell Ridge is on the perimeter of a housing development and is near NH Route 11, which may contribute to why this result exceeded the normal chloride threshold of 100 mg/L. It is important to note that this happened on February 11th, 2022, during winter when it is reasonable to higher chloride readings from road salts.

The CBP noticed a trend of raised chlorides in Lyons Brook compared to Clark Pond's outlet. In response, two sampling sites were added along upstream Lyons Brook for chloride and conductivity only. These locations were above and below the New London Department of Public Works (NLDPW) operational facility because of the large amount of road salt stored less than 1000ft from the banks of Lyons Brook. Of the three results from below the NLDPW, the first two were above the normal threshold, with a decreasing trend as time went on. The one result from above the NLDPW was 42.9 mg/L which is much lower than the results from downstream. Further testing is recommended because of the concerns that these results raise to the CBP.

To give context for the CBP results from Kezar Lake in 2021 and 2022, two nearby waterbodies recent water chemistry tests were looked at. In the same town of Sutton, NH, is a waterbody of similar size at approximately 160 acres, called Blaisdell Lake. In a much less developed part of the town, Blaisdell's differences from Kezar Lake in terms of the CBP's parameters shows much less human influence. In 2019, Blaisdell's chlorides were on average, one-third to one-half of what Kezar's results were (Steiner, et. Al., 2020). Upstream from Kezar Lake, along the Clark Pond tributary, is the smaller Messer Pond. Messer Pond is in the town directly north of Sutton, New London, and was studied by the prior year's community-based research project class. The results from that study show that the much smaller, 45-acre pond, has similar chloride levels to Kezar Lake. Many of Messer Pond's average water sample chloride results were close to the 100mg/L human influence threshold that Kezar's results crossed nearly every time (Page et. Al., 2021).

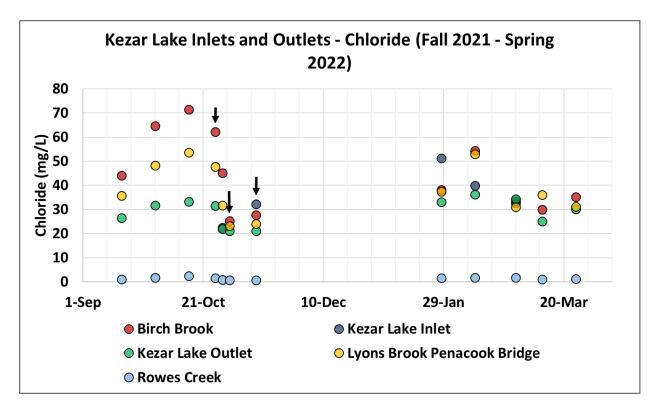
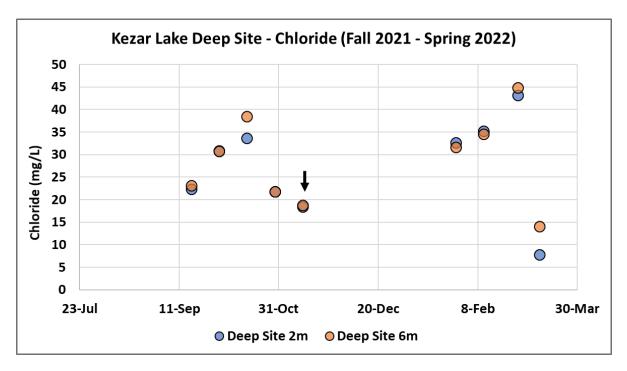


Figure 38. CBP findings of chloride levels at Kezar Lake Tributaries during the Fall of 2021.

Storm event(s) are indicated by black arrow(s).



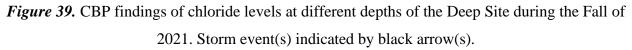
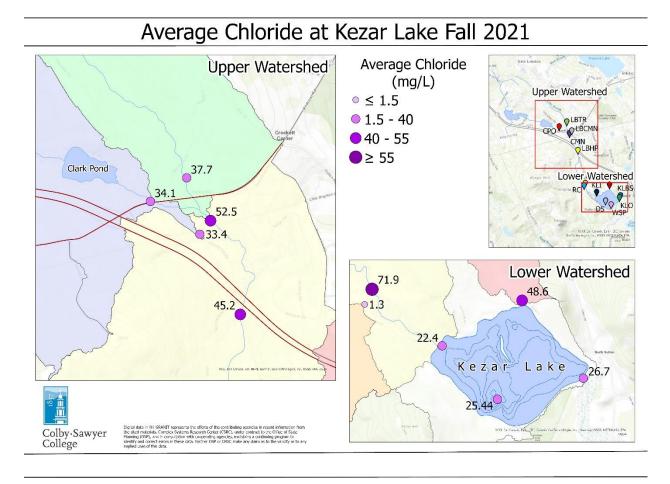
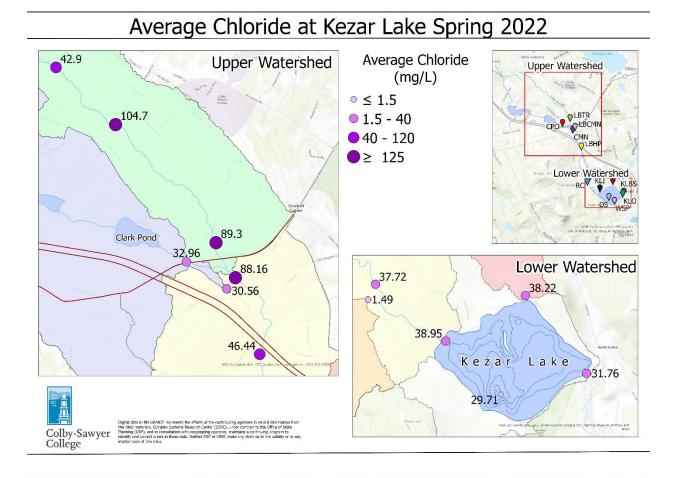


Figure 38 displays Kezar Lake's four tributaries and one outlet. Birch Brook, shown in dark red consistently displays higher levels of chloride while Rowe's Creek, shown in light blue, displays consistently low levels of chloride which matches the trend seen in the VLAP data. The CBP data collected in the fall of 2021 had higher individual readings but lower average readings than the CBP data collected in the spring of 2022. All readings throughout the year for Kezar Lake's Deep Site seen in *Figure 39* and most of the tributaries were below the normal threshold of 100 mg/L.



Map 15. The average Chloride in mg/L sampled at sites in and around Kezar Lake for the Fall of 2021.



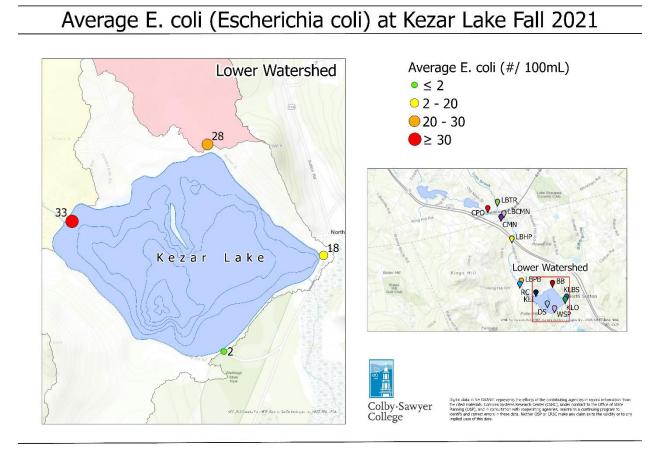
Map 16. The average Chloride in mg/L sampled at sites in and around Kezar Lake for the Spring of 2022.

Story 4: No Concern Currently

E. coli

Escherichia coli, more commonly known as *E. coli*, is one of the most common species of fecal coliform bacteria. *E. coli* is naturally found in the large intestines of warm-blooded animals and is often found in high quantities in animal waste, including sewage. *E. coli* is used as an indicator species for the presence of waste in waterbodies (DOH 2017; NHDES, 2019). While most *E. coli* bacteria are harmless, the presence of *E coli* indicates recent fecal contamination that may contain pathogenetic organisms (NHDES, 2019; WSDOH, 2016).

E. coli thresholds are established for both drinking water and water used for recreation. Drinking water should contain no *E. coli* after treatment while designated beaches should not exceed 88 colonies per 100 mL in a single sample or exceed a three-sample average of 47 colonies per 100 mL over a 60-day period (NHDES,2019; QA Manual, 2020). It is not unusual for higher numbers of *E. coli* to occur, specifically after storm events. Increased rainfall can lead to agricultural or urban runoff which often leads to increased levels of *E. coli*. These spikes in *E. coli* are generally not considered unsafe, however they are still being investigated to determine the source of fecal contamination. Other possible sources of fecal contamination could include wastewater treatment plants, failing septic systems, and domestic and wild animal waste. (NHDES, 2019; QA Manual, 2020).



Map 17. The average E. coli colonies sampled at sites in and around Kezar Lake for the Fall of 2021.

Map 17 above shows the four sampling sites where *E. coli* samples were collected during the Fall 2021 semester along with average colony counts per 100 mL of samples. Sensitive areas around the lake, including Wadleigh State Park and the Kezar Lake Beach Site, which both draw large numbers of people for recreational use, include a swimming area qualifying the water as recreational, demanding regular *E. coli* testing. If levels were to exceed the established thresholds, the beach site would need to be closed and an investigation would need to take place to determine the cause of the source. *E. coli* sample analysis can be found under the *E. coli* standard operating procedures in the appendix.



Image 7. Petri dish and filter which has been incubated for a 24-hour period after having 100 mL of water sample filtered through it. The blue dots are *E. coli* colonies.

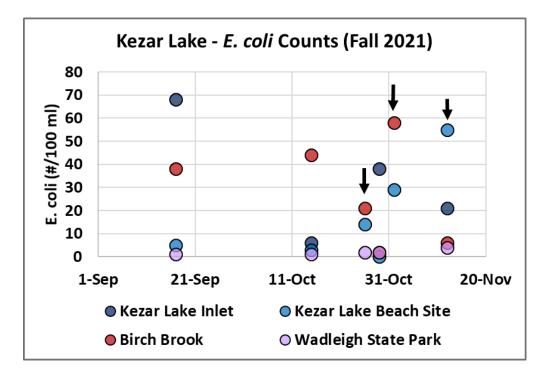


Figure 40. CBP findings of E. coli levels of Kezar Lake Tributaries during the Fall of 2021.

E. coli levels were sporadic during the Fall 2021 semester. Birch Brook and Kezar Lake Inlet consistently had higher *E. coli* levels while Wadleigh State Park consistently had the lower *E. coli* levels, below 10 colonies per 100 mL for each sampling date. Excluding Wadleigh State Park, each of the other sampling locations all had events over 50 colonies per 100 mL per sample. However, two of these occurred during storm sampling events that are represented with black arrows in figure 40. This is likely due to excessive runoff caused by the extra water that a rainstorm produces. Despite those spikes, all *E. coli* values collected during the Fall 2021 semester remain below the 88 colonies per 100 mL per sample limit outlined for waterbodies with designated swimming sites. However, low *E. coli* levels during this time of year are to be expected due to decreasing temperatures. The timing of this sampling period is one explanation for why *E. coli* levels have been dropping over time.

E. coli samples were not collected during the Spring 2022 semester. Cold temperatures, coupled with the fact that all samples from the Fall 2021 semester were well under the 88 colonies per 100 mL per sample limit outlined by the NHDES for waterbodies with designated swimming sites led to the decision that sampling for *E. coli* was not necessary during the Spring semester. However, more frequent sampling should take place during the warmer months along sensitive sites such as Wadleigh State Park and the beach site, as well as the major inlets into the lake.

More frequent sampling during warmer months, and sampling at all the sites outlined above, could potentially act as an early detection system for any future *E. coli* issue if they were to occur. Additionally, community encouragement and education on the importance of regularly maintaining septic systems is a fantastic way to be proactive about potential *E. coli* issues. Properly designed and functioning septic systems help treat wastewater that is generated in homes from toilets, showers, sinks, and dish and clothes washers. If septic systems are not functioning properly, or if they have not been regularly pumped, the effluent containing harmful bacteria can travel to streams, rivers, and lakes potentially contaminating drinking water and prevent the recreational use of lakes such as Kezar.

Community Outreach

Data collection took place from September to November and resumed in January to continue until March for a total of six months of data collected by the CBP for the KLPA. Those months of work amounted to a final presentation and a written paper, accompanied by a handful of smaller projects chosen by the KLPA based on their members' interests. Two smaller groups formed within the CBP to create an educational workshop on healthier landscaping practices for a lake's watershed, and a video explaining the effects of aquatic plants in a local ecosystem. These smaller deliverables were first planned in the Fall semester and executed during the spring semester, to increase the CBP's community outreach efforts for the KLPA.

These goals were made possible through regular communications with the CBP's chosen stakeholders. Three very active members of the KLPA regularly met with the CBP during class meetings and scheduled lab time. In those weekly meetings, both the CBP and the KLPA were given opportunities to discuss their respective progress and goals for the project. The KLPA connected the CBP to the larger Kezar Lake community through their personal lives and the clubs they attend outside of the project. With these connections, the CBP meetings thought to create volunteer opportunities for the Kezar Lake community and provide them with newsletters explaining each semester of work. Regular communications helped make the CBP's regular activities like lab work, field work, and meetings, make sense to the larger community and interested members.

Of the two smaller crews within the CBP, the landscaping crew worked with the program, NH LakeSmart, and created an opportunity for residents of Kezar Lake to walk through volunteer properties to teach them about lake-friendly landscaping practices.

Aquatic Vegetation applies to more than just the landscaping practices, as well. The second group of students focused on aquatic vegetation. More specifically, the work agreed upon with the KLPA was to investigate their growing concern about the native aquatic plant elodea and deliver the information in the form of a video.

Finally, projects happening within the community relating to the work being done by the CBP for the KLPA have easily been connected to the Kezar Lake community. An example of this is a regional water testing program called All About Arsenic (AAA) that Professor Nick Baer at Colby-Sawyer College is involved in, where a water chemistry test is conducted on local lakes. AAA provides at-home water tests which the CBP encouraged the community to use within the Kezar Lake watershed and beyond. Having more research and water tests done on Kezar Lake will keep the community informed and bring people together in a series of different water-testing parameters which can be used for educational opportunities such as the CBP's final report paper

In the end the CBP recommends that the KLPA invests in setting up an internship program between themselves and Colby-Sawyer College and or the larger New Hampshire area. An internship opportunity is a great way to continue the work done by the CBP during this last academic year and has potential to continue adding to the decades of study on Kezar Lake.

Aquatic Vegetation

The Community Based Research project has agreed to create an educational video for the Kezar Lake Protective Association to inform the members and the community of the effect of the growing elodea population in the lake. *Elodea canadensis* and *Elodea nuttallii*, commonly known as elodea, is a group of submerged aquatic waterweeds native to shallow New England lakes (Connor and Smagula, 2007). Both species of elodea are classified as submergent vegetation, indicating that the plants grow within the sediment at the bottom of a lake or pond and that all of the plant's parts including its roots, leaves, and stems are submerged entirely underwater. Elodea is a relatively thick plant that propagates easily, and quickly, which has made its population growth over the last few years noticeable to recreators on Kezar Lake. Activities like kayaking and canoeing may become more difficult with an elodea population left unchecked in a smaller lake like Kezar making it a KLPA's main concern. The slow-moving waters that elodea thrives in allows for seeds to easily grow in the sediment of the lake (Ozimek et. Al., 1993). The KLPA's desire for this outcome was to be a management plan for reducing the

amount of elodea present in the lake. Instead, the CBP used the community outreach project to inform the community about the importance of elodea.



Images 8 & 9. A closeup photo of E. canadensis (left) and E. nuttallii (right).

Though elodea can become a nuisance when it grows in large, dense quantities, clusters of these waterweeds have been shown to provide vital ecosystem services to plants, fish, birds, other wildlife, and even humans. Elodea tends to grow in dense clusters along the bottom of a lake or pond creating ideal habitat for fish nurseries (Aquatic Biologists Inc., 2022; Drociak, et. Al., 2008). The two species of elodea native to the state of New Hampshire look similar in appearance at first glance, however, it is easy to identify which is which. E. canadensis tends to grow broad leaves with rounded tips while *E. nuttallii* will grow thin leaves with sharp tips (Connor & Smagula, 2007). Elodea clusters also act as a food source for many birds within the surrounding lake ecosystem such as swans, ducks, geese, and occasionally loons (Aquatic Biologists Inc., 2022). Elodea is also excellent at nutrient cycling, taking in large amounts of phosphorus, nitrogen, and other elements directly from the water and lake sediment (Ozimek, 1993). In depth studies on the nutrient sequestration rate of elodea have shown that both species of elodea can sequester significant amounts of inorganic nutrients such as phosphorus and nitrogen, as well as toxic heavy metals such as cadmium, lead, mercury, arsenic and much more (Dogan, et Al., 2009; Picco, et Al., 2019). Many of these nutrients and metals are harmful to the health of humans and local biota, even when present in low quantities. Given the lake's history, phosphorus is of particular interest to the KLPA. Elodea is a valuable part of nutrient cycling in

shallow lakes (Ozimek et. Al., 1993). Against similar submergent plants species, elodea takes in up to twice as much phosphorus relative to its size (Bishop & Eighmy, 1989). Noticing a population increase in elodea is worth paying attention to, because that could be an indication of nutrient loading (Penn State, 2022). Algal blooms thrive on excess nutrients and elodea is a much healthier presence in Kezar Lake's ecosystem than an abundance of algae.

Research shows that removing elodea is only truly possible through harsh chemical intervention (Penn State, 2008). Hand pulling elodea shoots from the lake is not a recommended method of removal by the CBP. This is due to the fact that seeds rapidly disperse into the water column if the plant becomes damaged, either by animals or from human recreation. In addition, the usage of aquatic herbicides as an alternative method to controlling the population of elodea is also not recommended by the CBP as it will cause more harm than good by killing off other species of aquatic vegetation present in Kezar Lake. The CBP recommends that residents of Kezar Lake leave any elodea in the lake as is. Though it may be a nuisance, the bloom of elodea seen in the lake is likely just a temporary event and will return to normal levels in the years to come (Connor & Smagula, 2007). The CBP recommends continuing to monitor elodea in Kezar Lake. As weather patterns continue to warm our climate it is likely that elodea with outcompete other submergent vegetation (Zhang et. Al., 2015). It is likely that the elodea population will continue to grow over the years but addressing nutrient sources to the lake like encouraging reduced fertilizer use and maintaining septic systems will be the most effective way for the KLPA to manage elodea in Kezar Lake (Penn State, 2022; Zhang et. Al., 2015).

The CBP has provided the KLPA and residents of the Kezar Lake community with a brief educational video detailing the physical attributes of elodea as well as its importance to the health of Kezar Lake and the surrounding ecosystem. This video can be found on the Kezar Lake Protective Association's website at https://www.kezarlakenh.org.

Landscaping

An additional community outreach portion of the CBP was a focus on lake-friendly landscaping practices. The KLPA expressed high interest in protecting Kezar Lake and the surrounding watershed through proper landscaping management practices and taking preventative measures to avoid issues of concern such as erosion. To best assist the KLPA with education on proper landscaping practices, the CBP partnered with the New Hampshire Lake Smart Program and to encourage community members to participate in the NH LakeSmart online self-assessment. The NH LakeSmart Program is a part of the charitable non-profit organization NH LAKES whose mission has been to keep New Hampshire's lakes clean and healthy since 1992. The NH LakeSmart program is a free, voluntary, educational opportunity for property owners to be evaluated on their lake-friendly living practices. The first step of the program is for homeowners within the watershed to take the confidential online self-assessment. The assessment only takes about 15 minutes to complete. It includes questions regarding driveways, parking areas, structures, wastewater treatment systems, yard and play areas, and for properties along the water it asks about the shoreline and shallow water areas near the property. Upon completion of the online self-assessment, homeowners who do not initially achieve LakeSmart status will be provided with a personalized follow-up report with tips and recommendations on how they can improve their property to be more lake friendly. The next step, after implementing improvements provided in the follow-up report, is to request a free and confidential follow-up site visit from a LakeSmart evaluator. These visits take about an hour and will determine if a property is ready for the LakeSmart Award. Owners of properties that have achieved LakeSmart status will be awarded two LakeSmart yard signs and a dock sign (if applicable). More information on the NH LakeSmart program can be found here at https://nhlakes.org/lakesmart/



Image 10. The logo for the LakeSmart Program that helped conduct property assessments during the landscaping field-day on April 9th, 2022.

In addition to encouraging community members to participate in the online selfassessment, the CBP also hosted a community landscaping field-day on April 9th, 2022, to educate residents of the Kezar Lake watershed about proper landscaping practices. To accomplish this, the CBP again partnered with NH LakeSmart who provided evaluators to conduct a more in-depth site assessment of two different properties within the Kezar Lake watershed. These site assessments were open to the community and were treated as a learning opportunity in which community members were encouraged to ask members of the CBP and the evaluators from NH LakeSmart questions while the site visits were being conducted. Additionally, a Q&A session was held after the site visits to ensure that community members could ask any additional questions that they may have had.

Turnout for the landscaping field day was more than initially expected with approximately 20 community members, including members from the KLPA, attending the event. Krystal Costa Balanoff, and Gloria Norcross from NH LakeSmart facilitated the two property assessments hosted by the Follansbee Inn, and a private three season residential camp. Each assessment focused on several areas including driveways / parking areas, structures / wastewater, pesticide / fertilizer use, and lastly lakefront / shoreline. As a group, Krystal and Gloria facilitated the assessments by leading the group around each property. While doing so, they explained what exactly they were looking for in each area of the property, how they would rank each area, and what sort of recommendations they would make to the property owner to make their property more lake friendly.

The CBP, LakeSmart Evaluators, and community first met at the Kezar Lake Beach Site (also known as Horse Beach) on Saturday, April 9th, 2022. The two volunteered properties showed great contrast as one exemplified almost achieving the criteria of the NH LakeSmart Award and the other gave insight on some possible constructive criticism one may receive. The private three season camp was a high-quality lake-friendly property that had very few needed improvements. If a property does not initially meet the criteria of the LakeSmart Award, personalized suggestions and tips are then given during the property walkthrough as well as in a follow-up written report.

For this private property, Jonathan (or Jon) was given just a few suggestions. As a lakefront property owner, he understands the impact his decisions will have on the environment and so Jon is constantly doing his best to be environmentally friendly. His property is used ³/₄ of the year and is shut down during winter. This puts less stress on the land as it is allowed a season for nature to take its natural course and recover. During the summers, when the property is in use most, Jon has established one pathway throughout the land that emulates the "Stay on The Trail" mindset. By having one consistent pathway, Jon is protecting the healthy undergrowth of the forest, thus preventing erosion, and overall protecting the shorelines. The private property also has rich soil that acts like a sponge due to the forest roots and natural organic layer. Less water is likely to run off as the roots on the slope absorb a large amount of water and hold the soil together. Another method to limit the amount of erosion is implementing a vegetation buffer. A few shorelines friendly plants include, Alder, Sheep Laurel, Clethra, Buttonbush, and Nanny Berry. They provide a buffer between the lakefront and sloped property to reduce substantial portions of nutrient runoff as well as provide natural habitat for birds and other organisms.

To preserve the healthy and natural land that exists currently, Jon does not remove trees from his property unless it is necessary. However, most lakefront property owners desire a view which is typically achieved by removal of trees. Instead, evaluators of the NH LakeSmart Program suggest a process of trimming branches to attain the lake view and to limit the damage done to the forest. Jon's property had many lake-friendly practices incorporated into the land and only received a few suggestions that were confidential to the NH LakeSmart evaluator and property owner. In this case, both property owners gave permission for their property evaluations to be discussed in this paper. The most impactful issue seen on Jon's property is one that is common among property walk-throughs including the Follansbee Inn.

Often, septic systems, specifically the septic drain fields, or leach fields, in New Hampshire are outdated, or have not been pumped on a regular basis which has environmental consequences. The leach field on the private three-seasoned property has been overgrown with young hemlock that has grown densely despite Jon's efforts of cutting them back. Woody plants grown over the leach field are highly likely to cause damage to the septic pipes as their roots can puncture through causing leakage problems. Water-loving plants are also not recommended as they can negatively impact the leach field. Instead, the NH LakeSmart Program suggests planting wildflowers over the leach field. Wildflower gardens planted over a leach field have many benefits in addition to it looking aesthetically pleasing. Wildflowers are a great source of food and habitat for pollinators such as bees, butterflies, hummingbirds, and other species. The garden would also provide a strong root system that would not affect the pipes but would prevent erosion and keep the soil together. Both properties shared this issue of managing their septic systems but received unique and personalized recommendations by an NH LakeSmart evaluator.

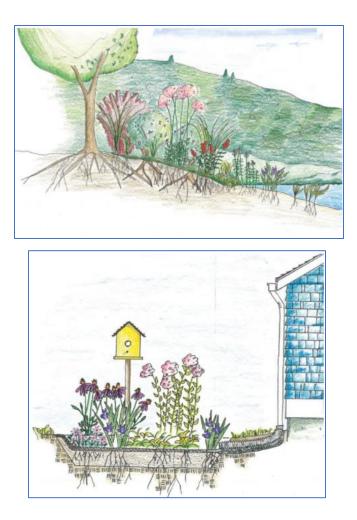
Both property evaluations contrasted from each other and displayed different concerns but shared similar themes of recommendations. The Follansbee Inn is used all year round and has up to 17 guest rooms as opposed to Jon's one-family summer residence camp house. The land is thus under a greater amount of stress and requires more attention. Firstly, the LakeSmart evaluator could not identify precisely where the septic system leach field was located. Wherever the leach field was, the LakeSmart evaluator recommended removing any woody species nearby as they can cause harm to the system. The evaluator also expressed concern for the septic system care as it has not been inspected in 10 years. NH LakeSmart evaluators suggest that septic systems be inspected every 3 years. The more that the tank is being utilized, the more sludge accumulates and builds up (NHDES, 2011). To function properly, the tanks must be inspected continuously and when necessary, pumped out. By doing so, the property owner prevents the system from failing, saves money, and protects the health of the environment. A functional septic system should effectively remove most of the wastewater pollutants such as nitrogen and phosphorus before seeping into the Earth's soil (NHDES, 2011).

It is also important to consider the management of roadways around a watershed. They should never be built over a septic system leach field and should only accommodate for the required number of people- never exceeding that number-to limit the amount or erosion and runoff. As mentioned before, vegetative buffers are always recommended to reduce erosion and sediment runoff. In addition, they provide an aesthetically appealing component and set clear boundaries to a property. The LakeSmart evaluator recommends vegetative plants like Osier Dogwood, Blueberries, and other plants that are salt tolerant and unyielding to the damage caused by snowplows. During the property walk, there was discussion about the appropriate types of road applicants and salt alternatives to reduce the amount of ice in the winters. The Follansbee Inn property sat on a slope meaning any salt applications or alternatives will have a direct effect on the lake as it sits just below the property and road. Substantial amounts of sodium chloride can have multiple environmental consequences, so efforts have been made to limit the amount of road salt used that could enter waterways. Beet juice, sand, and programs like the Green SnowPro Program are all great alternatives to salt application. Sand, in particular, is not the solution to the salt issue but is a better alternative as it contains only about 10-4% salt content depending on the source and has a lot of sediment that could lower the transparency and increase turbidity of the water if not used in proper amounts. These are just a few suggestions that the NH LakeSmart program provided to these locations specifically, but each property has a unique situation and will vary in suggestions and tips.

One of the main concerns is erosion or the process by which soil and rock are moved by natural elements such as wind or water (Novotny, 2003; Fernandez, 2003). The chance of erosion can increase with the steepness of a property, changes in water movement, storm events, and soil structure (UNH, n.d.). Additionally, the development of impervious surfaces upstream in a watershed inhibits large consequences of stream bank erosion over a greater area than impervious surfaces downstream (Booth, 1991).

Methods to control erosion include installing a rain garden in the depression of ground close to a building, planting vegetation to stabilize the soil, and constructing a terrace system on sloped property. A rain garden uses the soil and plants to absorb and filter stormwater coming from buildings or driveways (Soak Up the Rain NH). More specific guidelines and tips about planting a rain garden can be found in the New Hampshire Department of Environmental Services (NHDES), Soak Up the Rain website. Rain Gardens use soil and plants to absorb and treat stormwater and runoff (Soak Up the Rain NH). More specific guidelines and tips about planting a rain garden can be found at the NHDES, Soak Up the Rain website.

Managing stormwater is a significant aspect of proper landscaping that can protect and restore clean water in New England's lakes and rivers. It is important to prevent harmful stormwater pollutants such as: road salts, heavy metals, fertilizers, pesticides, and petroleum products from reaching those bodies of water. (UNH, n.d.; McCarthy & Loosigan, 2019). Stormwater runoff contributes to over 90% of water quality issues in New Hampshire (McCarthy & Loosigan, 2019). The contribution from these pollutants can add excessive nutrients to the watershed tributaries that can allow more plant and algae growth (McCarthy & Loosigan, 2019). In addition to carrying pollutants, stormwater can carry sediments into waterways making it easier for plants and invasive species to grow (Novotny, 2003). One way to manage stormwater on a waterfront property is to construct a vegetative buffer between the land and the lake. The vegetative buffer is used to prevent pollutants, harmful to biotic species, from reaching open waters. A vegetative buffer can also help reduce flooding at the bottom of a sloped property, which allows water to infiltrate the soil and enter the groundwater.



Images 11 & 12. Vegetative Buffer & Rain Garden illustrations by Jillian McCarthy

Nitrogen rich fertilizers cause dissolved oxygen deficiencies in lakes and pollute groundwater systems when used in excess amounts (Bijay-Singh, 1999). Plants, such as grass, can take up limited amounts of fertilizer. When plants reach the threshold of their ability to absorb fertilizer, fertilizer becomes an additive that can enter nearby waterways (Stigliani et al., 1991). The first step of applying fertilizer to a lawn is testing the soil to see the nutrient status and pH value (UNH, n.d.). After testing the status of the soil, fertilizer use can be determined and applied at appropriate rates. An important note is that fertilizer is prohibited within 25 feet of the reference line of a waterbody, however it is also important to check local rules which may be stricter (McCarthy & Loosigan, 2019; NHDES, 2020).

Maintaining septic systems within a watershed, specifically close to a waterbody, is crucial to protecting the environment as previously mentioned. It is the responsibility of the property owner to maintain the septic system properly. Consistent and regular maintenance can prevent a failure in the system leading to unwanted costs and untreated wastewater leaking into a waterbody. It is recommended that septic systems be pumped and inspected every three years. Untreated wastewater can become a health risk to those who live in the area as well as those who use the waterbody recreationally. Nutrients such as phosphorus can enter the waterbody and influence bacteria and algae growth within the water (DES, 2019). Additionally, when a septic system is not properly and regularly maintained, *E. Coli* counts can rise. *E. Coli* is a bacterium found in warm blooded species and can be harmful when found in large amounts. If *E. Coli* counts surpass 88 colonies per 100 mL, the lake will be closed off for recreational use like mentioned above.

Recommendations

Of the CBP's many repeated recommendations, the most frequent is to continue monitoring the water quality of Kezar Lake. This kind of work takes a lot of time and money. Thanks to organizations like Colby-Sawyer College and the Kezar Lake Protective Association, this work is accessible to students, community members, and anyone else interested in water chemistry. The relationship between an educational facility and local community volunteer group is valuable. Creating an internship for other students to continue this project would be a great opportunity.

Next, the LakeSmart and Green Snow Pro programs discussed in the Landscaping community outreach section of the CBP report are both very valuable. The CBP recommends incorporating these programs with Kezar Lake's management strategy because they are equipped with state workers who are passionate about supporting the well-being of New Hampshire's lakes.

Supporting continued interest in water chemistry reports is the CBP's final recommendation. Reading this report, remaining involved in watershed health with at home practices like limited fertilizer use, proper plumbing maintenance like regular septic system inspections, and awareness of the effect personal landscaping has on local water is vital for the long-term health of waterbodies. Please continue to support local lake protective associations.

Acknowledgements

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Appendix

Project Scope Statement

Community Watershed Analysis: Kezar Lake

Colby-Sawyer College Community-Based Project 2021

Key Stakeholders Kezar Lake Protective Association

Kezar Lake Ecosystem

Stakeholders Sutton Citizens

Colby-Sawyer College

Property and Homeowners of Kezar Lake

Town of Sutton, NH

- Sutton Department of Public Works
- Sutton Conservation Commission

NH Department of Environmental Services

NH Fish and Game

Environmental Protection Agency

NH Department of Transportation

Project Purpose

The Colby-Sawyer College Community-Based Project class of 2021 aims to produce a professional and in-depth water quality analysis of Kezar Lake and the surrounding watershed. An analysis of both biotic and abiotic factors that influence the overall health and quality of the lake will be conducted over the course of several months. The analysis will closely examine pH, conductivity, chloride, turbidity, chlorophyll-a, total phosphorus (TP), acid neutralizing capacity (ANC), dissolved oxygen, and *E. coli* for Kezar Lake and its surrounding tributaries. The results will be shared with the Kezar Lake Protective Association (KLPA), NH Department of Environmental Services, and the surrounding community through project reports and public presentations.

The results of this project will provide the KLPA with a series of recommendations to further improve the water quality in Kezar Lake to encourage recreational use and allow the native biota to thrive. The data collected for this project will be analyzed and shared as a series of interactive maps on Arc GIS Online, allowing the community to observe nutrient and contaminant levels over the progression of several months. As a secondary goal, we will provide community outreach / educational materials such as fact sheets, blog posts, bi-weekly newsletters, or short videos that are specific to the interests of the KLPA that are available online for other potential stakeholders. The final analysis is intended to help the KLPA have a comprehensive understanding of the Kezar Lake watershed and water quality. With this information, they will have the opportunity to make informed decisions regarding the improvement of Kezar Lake.

Desired Results

- Water analysis of 12 sites around Kezar Lake and the surrounding watershed (pH, conductivity, acid neutralizing capacity (ANC), chloride, chlorophyll-a, E. coli, chloride, total phosphorus (TP), dissolved oxygen)
- An analysis of discharge from Kezar lake tributaries that will help us understand the loading and mass balance coming into the lake.

- This discharge collection will include most rain events in the beginning, middle, and end of the storm when possible.
- Detailed interactive maps of the Kezar Lake watershed
- Provide recommendations for the Kezar Lake Protective Association to improve water quality
- Design potential community outreach / educational material projects for the Kezar Lake community (please choose whichever method you'd prefer)
 - Blog post on the KLPA website
 - Bi-weekly newsletter
 - o Informational fact sheets
 - Short videos
- Water analysis report, collected data displayed through the usage of GIS maps, and a final presentation to share with the community and stakeholders

Deliverables

- Aquatic vegetation assessment
- Landscaping

Communication Needs

Regular communication with the KLPA

Follow up emails if needed

Presence at weekly meetings

Educational Outreach

Maximum of 2 educational outreach projects outside of the water analysis and GIS components

Constraints

- Time Limit
 - 2 Semester Limit (September December & January April)

- Resources
 - Limited to CSC materials
- Implementation action(s)
 - We are only able to make recommendations, we are not able to implement any plan to address the water quality of Kezar Lake.

Approval(s)

Kezar Lake Protective Association

Semester One Outcomes

- Final GIS map(s) of the Kezar Lake watershed displaying elevation and tributaries
- Completion of sample data analysis to date
- Mid semester project paper and presentation containing a comprehensive analysis of all parameters listed in a previous section displayed through graphs and GIS maps to be shown to the KLPA and surrounding community
- Communication outreach / educational materials to be sent out to the KLPA
- Recommendations

Semester Two Outcomes

- Completion of sample data analysis to date
- Final project paper and presentation containing a comprehensive analysis of all parameters listed in a previous section displayed through graphs and GIS maps to be shown to the KLPA and surrounding community
- Updated GIS map(s) of the Kezar Lake watershed displaying elevation and tributaries.
- Communication outreach / educational materials to be sent out to the KLPA
- Recommendations

Standard Operating Procedures

Acid Neutralizing Capacity (ANC) ANC by Gran Plot Titration using VWR SympHony B10P Meter

A. Sampling Methodology

| 1. | Preservation: | Refrigerate |
|----|-------------------|--|
| 2. | Hold Time: | 24 Hours |
| 3. | Required Volume: | 50 ml |
| 4. | Required Titrant: | .02 N HCL |
| 5. | Container Type: | Polyethylene or glass |
| 6. | Reference Number: | 2320B (Standard Methods 20th Ed. 1998) |

B. Equipment Calibration and Set Up

(once per day, or if CCV fails) - 2 point calibration with standard buffers.

1. Follow calibration procedures 1 - 8 from pH calibration section.

C. ANC Sample Analysis Procedures

ANC analysis is required for the top (Epilimnion) layer of Lake Sunapee Station

numbers 200-230, and for the top layers of all area lakes!

- 1. Rinse probe and sample vessel with DI water. Gently tip sample bottle to disperse solids from the bottom of the bottle and rinse probe and sample vessel with a small volume of sample.
- 2. Place probe into exactly 50 ml of sample, swirl once, and press the **READ** button. <u>Turn</u> off the Auto-Eye feature on the pH meter.

- 3. Place a small stir bar into the sample cup and the magnetic stirring unit under sample cup. Turn the stirring unit on slowly and adjust the speed until the stir bar mixes the sample evenly.
- 4. Add 0.05 ml increments of .020N HCL to the sample with pipettor **UNTIL pH of the** sample STABILIZES AT APPROXIMATELY 4.60

Use a hand tally counter if necessary, to keep track of the number of increments of acid added!

- 5. When the pH of the sample reaches approximately 4.60, enter the <u>exact</u> pH value and the corresponding titrant volume on line one of the ANC benchbook data sheet. The titrant volume is calculated by multiplying the number of 0.05 ml increments of acid added (from step #5) by the 0.05ml volume of each increment. (For example: 6 initial hits of acid times 0.05ml for each hit, equals a titrant volume of 0.30ml).
- 6. Continue to add 0.05 ml increments of acid, allowing the stirring unit to mix the sample and the pH of the sample to stabilize after each hit of acid. Record the pH reading after each 0.05 ml hit of acid on the subsequent pH lines of the ANC benchbook data sheet.
- 7. Stop adding 0.05 ml increments of acid and recording the stabilized pH values when a pH of approximately 3.60 is reached. As in step #6, record the <u>exact</u> final pH.
- 8. Remove probe from the sample, rinse probe and sample vessel with DI water and prepare next sample by following steps 1 through 8 or continue with shut-down procedures.

Duplicate analysis is performed on every tenth sample.

D. Shut-down Procedures

- 1. Rinse probe and sample vessel with DI water.
- 2. Replace probe cover.
- 3. Press power button to shut off instrument.

Acid Neutralizing Capacity Titration (ANC)

Acid Neutralizing Capacity Titration

For tributaries:

- 1. Add 50 mL of the sample to a beaker.
- 2. Fill the burette with 0.02 normal H_2SO_4 (sulfuric acid). Make sure to let a bit run out into a waste beaker to get any air bubbles out of the tip.
- 3. Place the sample on top of a stir plate with a stir rod in the sample. Turn it on low.
- 4. Record the initial pH of the sample with the pH meter and the initial volume of the burette. Remember, the burette can be read to 2 decimal places! Detail on reading burettes below.
- 5. Add small quantities of the H_2SO_4 while monitoring the pH of the sample.
- 6. Once the sample pH is near 5, record the burette volume and sample pH.
- 7. Continue adding the titrant drop by drop while recording the burette volume and sample pH. Be careful, the pH will begin to make large, sudden drops.
- 8. The titration is complete once the sample pH is at 4.5. Make sure you have recorded: Initial Volume, Initial pH, Final Volume, and Final pH.

-If you go past 4.5, it's okay as long as recordings were taken along the way (preferably in the 4.5-4.7 range).

9. When recording values in bench book, record volume of titrant used, *not* volume recorded on burette.

Standardization Process:

Prepare a standard solution by combining 5 mL Na_2CO_3 and 95 mL DI water. Run this titration exactly the same way as you did with the samples.

Use this standard to calculate the actual normality of the H₂SO₄ using this formula:

$$(M_1)(V_1) = (M_2)(V_2)$$

Where M1 and V1 are the molarity and volume of the Na_2CO_3 . V_2 will be the volume of the H_2SO_4 used. Solve for M_2 , the molarity of the H_2SO_4 .

Normality is equivalents/liters, and H_2SO_4 has 2 equivalents per mole. Use the molarity of the H_2SO_4 to find its normality like this:

Example: H_2SO_4 molarity = 0.001M

How to read the burette:

Burettes start at 0 mL and typically go to 100. You read where the bottom of the meniscus is: This burette would read 15.45 mL

Tips to remember:

- · The initial and final volumes are essential
- · Use two decimal places
- Putting your hand behind the burette helps read the lines
- Be on eye level with the meniscus

ANC formula:

ANC is standardized into mg CaCO₃/L.

Sample calculation: This procedure calls for 0.02 normal H_2SO_4 and 50 mL of sample. Let's say the volume of H_2SO_4 used is 0.5 mL:

So, the ANC in mg CaCO₃ /L is 10.

Chloride

STANDARD METHOD FOR DETERMINATION OF CHLORIDE USING ORION VersaStar Pro Meter and Orion 9617BNWP probe

A. Sampling Methodology

- **1. Preservation:** Refrigerate
- **2. Holding Time:** 28 days
- **3. Required Volume:** 100 ml
- 4. **Container Type:** polyethylene or glass
- 5. **Reference Number:** ASTM D512(C)

B. Startup and Calibration

- 1. IMPORTANT: Remove Probe cap and clip covering filling hole from probe and set these aside to be replaced on probe when finished. <u>Failure to do so will result in inaccurate</u> <u>data</u>. When removing cap do not apply pressure to the top of the probe.
- 1. Turn unit on and wait for initial screen. Once that has passed, there should be a mg/L displayed in the middle of the screen
- 2. Wait 5 minutes for meter to warm up. 'AR Stabilizing' will change to 'AR Ready'.
- 3. Go to 'Calibration' section in bench book to record the calibration process.
- 4. Rinse the probe in DI.
- 5. Place the probe in the 100 standard.
- 6. Press the f1 button under 'Cal'.
- 7. Press f3. Wait until 'Stabilizing' changes to 'Ready'.
- 8. If not reading 100 mg/L, press f3 to Edit. Type in correct concentration. Press f2 to Accept.
- 9. Press f2 again for Next.
- 10. Note the temperature of the calibration standard in the temperature column in the calibration log.
- 11. Rinse probe with DI.
- 12. Place probe in 1000 mg/L Standard. Press f3 for Next.

- 13. Wait for 'Stabilizing' to stop flashing. 'Ready' will appear.
- 14. Repeat steps 9-11 with the 1000 mg/L standard, only pressing f2 once to Accept.
- 15. Once the meter has been calibrated to both standards, the slope should be near 50mV/Dec.
- 16. Press f3 for Done.
- 17. Rinse probe in DI.
- 18. Run 100mg/L QC and press Measure. Write value in calibration log under initial CCV value. Make sure it is within 10% of the 100 mg/L value.
 - a. If sample is not within 10% press Measure again. If still not within 10% start calibration process from beginning.

C. Sample Measurement

- 1. Rinse probe and sample beaker with DI water. A sample rinse of beaker is recommended if there is sufficient sample.
- 2. Place probe into sample, press 'Measure' button (#1 on number pad)
- 3. Wait for probe to stabilize (AR will stop flashing).
- 4. Complete a logbook entry to record the sample.
- 5. When recording temperature make sure sample is within 4°C of standard temperatures (noted during calibration in the calibration log). This must be between 20°C and 25°C.
- 6. Repeat steps 1-5 for subsequent samples.
- 7. Every 10th analysis should be a replicate of a previously analyzed sample. Randomly select one of the previous 9 samples with a value of 3 mg/L or higher.

D. Shut Down

- 1. Run 100mg/l standard QC write value in calibration log under CCV.
- 2. Rinse probe with DI.
- 3. Turn unit off.
- 4. Replace probe cap and plug covering filling hole.

E. QA/QC Review

During QA/QC review the QA officer or his/her designee shall review all temperatures recorded during analysis and confirm that they are within 4°C of ambient. The QA officer

or designee shall flag all results that do not meet temperature requirement with a result comment in the Limnology Database that the data is suspect due to improper temperature during analysis.

Chlorophyll-a

Chlorophyll-a using the Hach Genesys 30

A. Sampling Methodology

- 1. Preservation: in dark, refrigerate
- 2. Hold Time: 24 hours
- 3. Required Volume: 500 mL
- 4. Container Type: polyethylene, opaque/dark
- 5. Reference Number: 10200H (Standard Methods 20th Ed. 1998), EPA 446.0

B. Preparation of Special Reagents

- 1. Saturated Magnesium Carbonate Solution: Dissolve approximately 2-3g of finely powdered magnesium carbonate in 200 mL of DI water. Shake to suspend the powder and allow to settle at least 24 hours before use.
- 2. Reagent Grade Acetone: 100% acetone should be shaken with approximately 3g of granular anhydrous sodium carbonate and decanted before being used to make aqueous acetone solution.
- 3. Aqueous (90%) Acetone Solution: Prepare a 90% acetone solution by filtering 100 mL of saturated magnesium carbonate solution in to 900 mL of reagent grade acetone, using volumetric pipettes (the final volume will be less than 1 liter after completely mixing).
 - a. Note: 90% acetone solution must be filtered through a Grade 1 filter before being used!

C. Processing Samples

1. Place chlorophyll-a sample bottles on lab bench next to vacuum rack.

- 2. Copy sample information from bottles into bench book. Be sure to note the TIME as well as the date filtered. ****Label all tubes with sample date****
- 3. Attach plastic filter collars securely to vacuum rack and dampen with a small volume of DI water.
- **4.** Using forceps, place .45μm (47 mm), 45 micron pore size membrane filter onto filter collar. Avoid contact with filter.
 - a. NEVER TOUCH WITH FINGERS AND BE SURE THAT FILTER HAS BEEN SEPARATED FROM BLUE DIVIDER PAPER BEFORE PLACING ON HOLDER.
- 5. Thread plastic 250 mL Millipore funnel onto filter collar, ensuring it has a rubber gasket. Avoid any tearing or wrinkling of filter paper.
- 6. Be sure vacuum rack is attached to a flask 'trap' before entering the Welch-ILMVAC vacuum pump (model 2546B-01). Caution: Watch that flask does not overflow. Empty flask between sample runs to prevent moisture entering the pump.
- 7. Pour 250 ml of sample into funnel. Turn vacuum pump power on (power switch is on the back side of the motor) and open valves on vacuum rack. To avoid potential contamination, open one valve at a time.
- 8. Drain the filter thoroughly under suction. Rinse sides of filter funnel with DI water during last 10 mL of sample. Note: Do not let vacuum run when no water is in the funnel!
- 9. Remove filter with *forceps*, fold, and place in a 15 mL screw top centrifuge tube. Never touch filter with fingers. Add sufficient 90% acetone to bring the final volume up to 8 mL. Remember that the 90% acetone must be filtered before being used!
- 10. Put screw top on tube, wrap tube in aluminum foil, and shake vigorously until the filter is completely dissolved. Place in freezer in complete darkness for 24 hours. ***make sure the cap properly fits before freezing***
- **11. Update bench book to reflect samples tested**

Duplicate analysis should be performed on every tenth sample.

ALL ACETONE WASTE SHOULD BE DISPOSED OF VIA EVAPORATION UNDER THE HOOD-NOT DUMPED DOWN THE DRAIN.

IMPORTANT NOTES:

One method blank should be run once a week.

To prepare a method blank, use DI instead of sample water in steps 1 and 2.

D. Centrifuge

- 1. Centrifuge the samples for 10 minutes
- 2. After the 10 minutes, remove the tubes, tapping them **Three times** to clean the sides
- 3. Replace the tubes and centrifuge for another 10 minutes

E. Sample Analysis, Genesys-30

To read Chlorophyll-a tests on the Genesys 30:

Follow the sample handling and preparation steps on the Section titled S.O.P Chlorophyll-a, Sections A through C, of the latest edition of the <u>Colby-Sawyer/Lake Sunapee Protective Assoc.</u> <u>Satellite Laboratory QA Manual.</u>

1. Connect laptop with the program.

Make sure it is reading the wave lengths of 750, 664, 647, and 630

2. Click the "Measure" button at bottom of the list, to measure each sample, beginning with the water blank, (which should still be in the instrument). The instrument will automatically read the samples at each of the 4 wavelengths. As it finishes each sample, it will request the next sample. *label the samples in the computer corresponding with the sample being tested*

Do not open the instrument to load another sample until the sample request appears!!

*rinse the cuvette between each sample with a small amount of 100% acetone.

- 3. Wait for the absorbance reading for each sample to stabilize before clicking the "Measure" button each time.
- 4. Continue to load/measure samples until all have been read. At the end of the sample run, re-insert the cuvette containing the water blank. Click on "Measure". This serves as a CCV measure.

Replicate samples must be within 3 ug/L of original sample.

At the end of the run, close the window, go to File > Export Results as a .csv

NOTE: At the end of each test session, or if something has spilled or spattered, clean the windows of the sample compartment with distilled water, then alcohol. Use the liquids and a soft, lint-free cloth to clean the windows. Do not apply too much pressure or the surface of the windows may be damaged. Be sure to remove all fingerprints.

DO NOT USE ACETONE TO CLEAN THE WINDOWS!

F. Cleaning Procedures

- 1. Chlorophyll-A Centrifuge Tubes
- 2. Allow material in tube to thoroughly dry before washing.
- Scrub the inside of the tube with a centrifuge tube brush until dried material has been removed from the bottom of the tube. BE SURE TO REMOVE ALL SEDIMENT FROM TUBES
- 4. Rinse tubes three times in DI water.
- 5. Hang on rack for drying.
- 6. Remove any remaining materials from tubes with the centrifuge tube brush

Conductivity

Specific Conductance using Orion Meter, 3 Star Model

A. Sampling Methodology

- 1. Preservation: Refrigerate
- 2. Holding Time: 28 days
- 3. Required Volume: 100 ml
- 4. Container Type: polyethylene or glass
- 5. Reference Number: 2510B (Standard Methods 20th Ed. 1998)

B. Equipment Set-Up

- 1. Place the instrument probe (Conductivity Cell) in a cup of DI water. Plug the instrument in (if necessary) and press the On/Off switch, located on the upper middle area of the key pad.
- Confirm that the instrument is in the Conductivity Mode by locating the reporting units for Conductivity, "µS/cm", on the right, middle section of the display screen. (* If it is necessary to change to the Conductivity Mode, refer to page EN-35, in the instrument manual which is located on the labeled shelf, directly under the instrument.)
- 3. Confirm that the instrument is in the Auto-Read Mode by locating the letters "AR" in the upper right corner of the display screen.(* If it is necessary to change the Reading Mode, refer to page EN-20, in the instrument manual which is located on the labeled shelf described above.)
- Confirm that the instrument is in the Measurement Mode screen display shows: Temperature, in degrees Celsius in the upper left corner;

Measurement Icon (Probe) and letters "AR" in upper right corner; Triangular arrow, numerical read-out, and "µS/cm" in middle portion of screen.

C. Instrument Calibration

- Place the probe into a cup of freshly Deionized water and press the "Measure, Save/Print" key. Wait for the "AR" to stabilize. Reading should be 2.0 μS/cm or less. Record reading in the appropriate section of the Triple Bench-Book.
- 2. Place probe in cup of Traceable Conductivity Standard, which has been freshly poured weekly, as necessary.
- 3. Press the "calibrate" key on the upper left area of the key pad. "COND/CAL", "CELL", and then "CAL.1" will be displayed on screen.
- 4. When the digital readout for the calibration standard is stable, push the "Measurement, Save/Print" key to store the value.
- Instrument will automatically advance to Measurement Mode and when "AR" stops flashing, check the reading for the Calibration Standard. This reading should fall within 10% of the μS/cm_value which is printed on the label of the Conductivity Standard bottle.
- 6. (*N.B.: This <u>value</u> changes slightly with each new batch of standard purchased/opened and must be noted and changed accordingly, in the standards section of the Triple Bench-Book)
- 7. Lift probe out of the Calibration Std. solution. Rinse it thoroughly and repeatedly with DI water.

D. Conductivity Sample Analysis Procedures

- 1. Rinse probe and sample vessel with DI water.
- 2. Warm all samples (in a warm water bath in the sink) to 20-25 degrees, Celsius before analysis. Invert sample bottle repeatedly, in order to thoroughly disperse solids from the bottom of the bottle. Rinse probe and sample vessel with a small volume of the sample, then discard. Fill vessel with sample.
- 3. Immerse probe into sample and press "Measure, Save/Print" key. When "AR" stops flashing, the Conductivity and Temp. reading may be recorded in the appropriate section of the Triple Bench-Book.

Duplicate analysis is performed on every ninth sample.

CCV is required after every duplicate analysis, at the end of each sample run, and/or at the end of the day's analyses, as appropriate.

E. Shut Down Procedures

- 1. Rinse probe and sample vessel with DI water.
- 2. Store probe dry in Kim-wipe "nest" in sample vessel.
- 3. Turn instrument off.

Note: For natural water (eg., drinking water, surface water, ground water) the meter uses a special built-in non-linear function for temperature compensation (as described in DIN 38404). To accurately measure solutions that have a Temperature Coefficient other than natural water (eg., acids, leaches, salt solutions, and seawater), the solution must be brought to reference temperature before being measured (20 - 25 degrees Celsius).

Escherichia coli in Surface Water using m-Coli Blue

A. Sampling Methodology

| 1. Preservation: | 4°C |
|----------------------|--|
| 2. Holding Time: | 8 hours (24 hours maximum) |
| 3. Required Volume: | 250 mL |
| 4. Container Type: | Sterilized polypropylene |
| 5. Reference Number: | 9222B (Standard Methods 20 th Ed. 1998) Hach Method 10029 (40 CFR 141) |

B. Equipment Calibration and Set-Up

- 1. Fill stainless steel pan with DI water and heat to a boil on hot plate.
- 2. Immerse glass filter funnels (250 mL Millipore), and glass support bases into boiling water to sterilize.
- 3. If the filter funnels are stored on the blue foam in the lower cupboard shelving it means that they are sterile and you do not need to sterilize them. However, you will need to sterilize them after you have finished the procedure. It is a good idea to boil water as soon as you enter the lab so it will be boiled by the time you are finished.
- 4. While *E. coli* apparatus is in water bath, place a petri dish with absorbent pad next to each sample bottle on laboratory bench.
- 5. Mark sample stations on agar plate covers with permanent marker. Write the sample site on the side of the petri dish that has the manufacturers name (PALL) imprinted on it. This is the bottom of the dish.
- 6. Place the dish top side up (PALL on the bottom) on a flat surface and take the lid off. Use a sterile pipet and fill it with m-ColiBlue24[®]. There will be a pipet on the cart that has a line marking the 2mL point. Compare your sterile pipet to this pipet when measuring out the m-ColiBlue24[®]. Pour 2 mL of m-ColiBlue24[®] broth evenly over the absorbent pad. Replace petri dish lid.
- 7. Check the data section of the *E*. *coli* bench book to see if the replicate sample is required and copy information from the bottles into bench book.

- 8. Retrieve support bases from water bath (Or from the lower shelf if they are already sterile) with beaker tongs and insert into vacuum rack. Ensure that you use beaker tongs because you want the bases to remain sterile.
- 9. Sterilize forceps by immersing into boiling water bath (Or wiping them down carefully with disinfectant spray and a paper towel).
- 10. Using sterilized forceps place .45µm sterile membrane filter grid side up on support base pad. Then using the DI squirt bottle squirt a few circles of buffered DI solution onto the filter that sits on the support base pad.
- 11. Retrieve glass filter funnel from water bath with beaker tongs and place on support base.
- 12. Secure funnel to base with blue aluminum clamp.
- 13. Allow funnels to cool to room temperature before proceeding to Section C.

C. E. coli Preparation Procedures

- Be sure vacuum rack is attached to a flask 'trap' before entering the Welch-ILMVAC vacuum pump (model 2546B-01). Caution: Watch that flask does not overflow. Empty flask between sample runs to prevent moisture entering the pump. Set the pressure to 5PSI = 10 in. mercury = 250 mm mercury (refer to manual to adjust pressure). Vacuum pump does not require lubrication for maintenance.
- 2. Pour 100 mL of shaken sample into glass funnel.
- 3. Turn vacuum pump power on (power switch is on the back side of the motor) and open valves on vacuum rack. To avoid potential contamination, open one valve at a time.
- 4. Rinse sides of glass funnel with sterile buffered DI water once volume has been almost completely filtered.
 - Note: If 100 mL will not pass through membrane, re-sterilize funnel and support base, and repeat set up procedure with new membrane filter. Filter 50 mL of sample and record sample volume filtered in bench book. Be sure to multiply final results by 2, since we report the number of E.coli colonies per 100 mL.
- 5. When sample volumes have been filtered and rinsed, turn vacuum pump power off. *Do not let vacuum run when there is no sample remaining in the funnel.*
- 6. Remove aluminum clamp and glass funnel.
- 7. Remove membrane filter from support base with sterilized forceps and transfer the filter to the previously prepared petri dish in the following manner: Place the Petri dish bottom (the side with "PALL" and the side that you labeled) down on a flat surface. Remove the lid from the Petri dish. With a slight rolling motion, place the filter, grid side up, on the absorbent pad. Check for trapped air under the filter and make sure the filter touches the entire pad. DO NOT WRINKLE FILTER PAPER! Replace the petri dish lid.
- 8. Invert petri dish and incubate at 35 ± 0.5 °C for 22 ± 2 hours.

- **i.** Note: If no colonies are visible after 22 hours, incubate the filters an additional 2 hours. If no colonies are visible at that time, the sample is free of total coliforms and *E. coli*.
- 9. Remove the petri dish from the incubator and count the colonies using a 10 to 15X stereoscopic microscope (optional). Red and blue colonies indicate coliforms and blue colonies indicate *E. coli*.
 - i. Note: Red colonies may vary in color intensity. Count all the red and blue colonies as total coliforms. Blue colonies may appear blue to purple. Count all blue to purple colonies as *E. coli*.
 - ii. One reading will be taken by one person and then another will be taken by another person. If the counts are very different contact Teriko. If the results are only slightly different go with the first one. This is for quality assurance purposes.
- 10. You may keep the lid of the Petri dish on when you are counting the colonies as long as you are clearly able to see the e-coli, or you may remove the lid. You may count the colonies without the light platform and use a flashlight instead as long as you are clearly able to see all of the colonies.
- 11. Take a photograph of each of the petri dishes. The lid should be off and the label should be clearly visible within the photograph. Upload each of these photographs on to Teams under the sampling and analysis channel, in the sampling-data folder, in the eColi_photos folder. This is for quality control purposes.
- 12. Record number of colonies in data section of *E. coli* bench book. Make appropriate calculations for samples that had less than 100 mL filtered.
- 13. To dispose of the petri dishes (as listed below) tape them together and place them in the bio hazard waste disposal in middle prep.

D. Instrument Setup and Sample Reading Procedures

- 1. Remove Scienceware® Mini Light Box platform from container. Plug in Mini Light Box platform to power source. On right side of platform, flip switch from 'Off' to 'On'. The top of the Light Box should now be lit.
- 2. Remove the Petri dish from the incubator/ Place the Petri dish onto the lit platform. Red and blue colonies together, indicate the total number of coliforms: Blue colonies indicate E. coli specifically. Note: Red colonies may vary in color intensity. Any red colonies should be considered other coliforms, not *E. coli*. Blue colonies may appear blue to purple. Count all blue to purple colonies as *E. coli*.
- 3. Record E. Coli count in E. Coli book. Record only the number of E. coli (blue) colonies in data section of E. coli bench book. (Make appropriate calculations for samples that had less than 100 ml filtered).

4. When E. Coli counts have been completed, turn the Mini Light Box platform off by flipping the switch on the right side of the platform from 'On' to 'Off''. Unplug the Mini Light Box platform from the power source and place it back into the appropriate storage area.

E. QC Procedures

- 1. Plate check Inspect petri dishes before using. Discard any with cracks, chips or visible indications of possible contamination.
- 2. Negative control Once each day of testing: Perform entire test/incubation protocol using 100 mL of buffered DI water.
 - i. After 24 hours, plate should show no growth. Put check mark in the bench column labeled "Neg. Control OK." **NOTE: If there is growth on the control notify lab manager immediately!**
- 3. Positive control Before putting each new bottle of m-ColiBlue24® broth into use, and whenever you suspect contamination of broth currently in use: Add the 2 ml of m-Coli Blue24 into the petri dish, add a BioBall from the supply in the freezer. Allow to dissolve. Add a drop of buffered DI to aid with dissolving if necessary. Incubate and read results as you would any sample.
 - i. <u>Keep in mind the known presence of E.coli; guard against cross-</u> <u>contamination!</u>
- 4. Duplicate count On every tenth sample, count is done twice.
 - a. Two different people perform count, or,
 - b. Same person performs count twice.

Results of all duplicate counts should be within 10% of each other. Enter results of duplicate count in appropriate space in bench book.

F. Shut Down Procedures

- 1. Resterilize all appropriate pieces of equipment after each use.
- 2. Dispose of plates by placing them in an autoclave bag and autoclaving them for 30 minutes. After autoclaving, bag may be discarded in a standard trash bin. OR, if there is room in the red bio hazard bucket outside of room 202, the bag of contaminated plates may be sealed and placed in the bucket for disposal via the contracted waste disposal company.
- 3. TURN OFF HOT PLATE!!!

G. Preparation/Handling of Positive Control and Buffered DI

- 1. Measure 1000ml of DI water and place into a flask. Add one pillow of magnesium chloride and one pillow of potassium dihydrogen phosphate to the water. Separate water into two flasks to prevent overflow in autoclave. Autoclave both flasks for 15 minutes at 121oC. Pour into squirt bottles for use in E.coli procedures.
- 2. Place 2 ml of M.coli blue into petri dish. Place filter paper on top of absorbent pad. Place BioBall onto filter paper (pour directly from container onto petri dish). Use a drop of buffered DI to dissolve bioball. Counts should be 13 +/- 3.

pH using Beckman pH Meter, Model pHi 510

A. Sampling Methodology

- 1. Preservation: Immediate Analysis is optimal
- 2. Holding Time: 24 hours
- 3. Required Volume: 50 ml
- 4. Container Type: Polyethylene or glass
- 5. Reference Number: 4500H*B (Standard Methods 20th Ed. 1998)

B. Equipment Calibration and Setup

(once per day, or if CCV fails) - 2 point calibration with standard buffers. Calibration and sample procedures are posted at the workbench.

- 1. Remove storage container and black clip from probe. Put somewhere it won't get lost.
- 2. Place electrode probe in cup of fresh DI water and press the Power Key (**O**).
- 3. Press **pH**, to put into pH mode.

4. Immerse probe in the pH 4.0 buffer solution. Check to make sure buffers have been changed within last week.

5. Gently stir buffer around probe.

6. Press the up arrow under **READ**. The instrument will show "stable".

7. When calibration is complete for first calibrator, meter display will show a flashing CAL 2 and the next pH calibrator value (7.00).

8. Rinse probe as above.

9. Immerse the probe into the pH 7.0 buffer solution and press the **ENTER** key as before, "stable" will show when calibration is complete.

10. Check calibration by placing probe in 6.0 buffer. Value should read between 5.9-6.1.

C. pH Sample Analysis Procedure

1. Rinse probe and sample vessel with DI water. Gently tip sample bottle to disperse solids from the bottom of the bottle and then rinse probe and sample vessel with a small volume of sample.

- 2. Place probe into 50-100 ml of sample and swirl once.
- 3. Press the **READ** key.
- 4. Record pH in benchbook when the reading stabilizes.
- 5. Follow Steps 1 through 4 for next sample.

Replicate analysis is performed on every tenth sample.

CCV is required after every duplicate analysis, or at the end of the day's analyses, whichever comes first.

D. Shut-Down Procedures

- 1. Rinse probe and sample vessel with DI.
- 2. Replace probe cover
- 3. Press power key (O) to turn off the instrument.

Sediment Coring

Sediment Coring - Community Based Project Class Spring 2022

Equipment:

- o Jet Sled
- 7, 5ft lengths of ³/₄ inch galvanized pipe
- Couplings for pipe
- Handle for metal poles
- Rope with clip
- Sediment core sample tubes with oranges caps
- Sediment sample plugs (white half oval plugs)
- Sediment corer
- Sediment core cutting tip
- o Ice auger
- Ice scoop
- Wrenches and vice grips
- Microspikes
- GPS w/ spare batteries
- Spare socks
- o 25 lb weight
- Bungee cords
- 5 gallon bucket
- o 1 liter bottle
- Silicon Spray
- Sharpie

Procedure:

Preparation:

1. Wear proper clothing for the weather

- 2. Wear insulated waterproof gloves for protection and proper grip
- 3. Label all tubes with date and sampling location

Retrieving the sample:

- 1. Use auger to cut hole in the ice
- 2. Place plastic sediment core tubes uncapped into sediment corer
- 3. Use ropes to clip the bottom pole
- 4. Connect metal poles one at a time as it drops in the water, with the T bar on top for every bar connection to prevent losing the gear.
- 5. Move very slowly and gently the whole way, no quick movements. The sediment is easily disrupted and suspended.
- 6. Continue to push the corer past the point of initial resistance until the sediment corer stops
- 7. Slowly retrieve the sediment corer and remove pipes as they emerge.

Securing the sample:

- 1. Keep sediment core upright and avoid jarring movements
- 2. Prepare orange caps
- 3. Remove the cutting tip and quickly cap the bottom
- 4. Remove the sample tube and quickly cap the top.
- 5. Rinse off any excess sediments
- 6. Carefully place sample into the Jon Brown Sediment Sample Caddy (JBSSC)

Preparing the sample for analysis:

- 1. Let the sample sit for some time (on 04MAR22 it was from 12:30pm-3:30pm) to allow the sediment to settle
- 2. In a sink and with two people, drill a small hole (a 5/16in bit was used) just above the top of the sediment to allow excess water to drain
- 3. Scoop out approximately 1 tablespoon of diaper polymer and spread that over the top of the drained sediment sample. Let it sit for ten minutes
- 4. Move sediment sample into freezer to chill for at least 24 hours

Total Phosphorus (TP)

Total Phosphorus using the Hach Genesys 30

Technique Used: Ammonium Persulfate Digestion, Single Reagent/Ascorbic Acid.

A. Sampling Methodology

- 1. Preservative: 4oC, H2SO4 to pH 2 (.5ml of 9N H2SO4/L)
- 2. Holding Time: 28 Days
- 3. Required Volume: 25 mL
- 4. Container Type: polyethylene (preferred) or glass

B. TP Sample Preparation Procedure

- 1. Triple rinse all flasks with DI water.
- 2. Arrange numbered TP flasks (50mL Erlenmeyers) on bench with appropriate number of replicate and spike flasks. (See #s 7 & 8). Make sure to label with site name and date sampled.
- 3. Triple rinse 25 mL graduated cylinder with DI water.
- 4. Season flasks and graduated cylinder by rinsing with small volume of sample after DI.
- 5. Prepare potassium persulfate while preparing flasks in following steps by dissolving 5 grams K₂S₂O₈ per 95 mL of DI water. This solution should be prepared in acid washed glassware warmed on a stirring hot plate (low setting) to be sure that the solution is completely dissolved. This solution is prepared immediately prior to use.

- 6. Measure and pour 25 mL of standard/QC/sample into the flask. Season graduated cylinder between each sample.
- 7. Prepare two blanks of 25 mL of DI.
- 8. Do a repeat analysis on every 10th sample, by simply pouring the sample twice, and handling each sample exactly alike. Label with site name, date, and REP.
- 9. Prepare two spikes. One is a spiked blank as part of the QCs, one is a spiked random sample by pouring the sample twice. For the second pouring, add 0.5 ml of the 5 mg P/L QC intermediate substock #2 (blue label in Teriko's fridge) to 25 mL of sample, for an added concentration of 0.100 mg/ml.
- 10. Add 4 mL of freshly-made potassium persulfate to the flasks.
- 11. Cover each flask with new aluminum foil, crimped tightly around the top. Add autoclave tape to the top of each.
- 12. TP samples are then digested to release cellular phosphorus. This is done by placing them in an autoclave at 121°C / 250°F for 45 minutes. Autoclave instructions at end of procedure.
- 13. Cool samples to room temperature before adding the color reagent.
- 14. Make the color reagent immediately prior to use. Mix reagents in the following order, each should have a corresponding graduated cylinder to use. They should <u>all</u> be at room temperature. The color will be yellow if mixed correctly.

Approximate #

| TP flasks (25ml) | 20 | 40 | 60 | 80 | 100 | 120 | 140 |
|--------------------|------|------|------|-------|-------|-------|-------|
| Sulfuric Acid 4.8N | 25ml | 50ml | 75ml | 100ml | 125ml | 150ml | 175ml |
| Ammonium Molybdate | 10ml | 20ml | 30ml | 40ml | 50ml | 60ml | 70ml |

| Ascorbic Acid | 10ml | 20ml | 30ml | 40ml | 50ml | 60ml | 70ml |
|---------------------|------|-------|-------|-------|-------|-------|-------|
| Potassium Tartrate | 5ml | 10ml | 15ml | 20ml | 25ml | 30ml | 35ml |
| | | | | | | | |
| Final Vol. Of Mixed | 50ml | 100ml | 150ml | 200ml | 250ml | 300ml | 350ml |

- 15. Add 2.5ml of **Freshly** mixed color reagent to each flask. The color will become blue if phosphorous is present, and the standards and QCs should develop a gradient.
- 16. After 10 minutes, but within 6 hours, measure absorbance of each sample using Genesys 30 spectrometer.
- 17. Select fixed display, set wavelength (λ) to 880 nm.
- 18. Season cuvette with DI, then fill with DI and run as blank.
- 19. Wipe sides of cuvette with kim wipe and place into spectrometer with arrow facing left.
- 20. After running blank, start running the standards from lowest to highest concentration, seasoning the cuvette between each.
- 21. Once the standards have been run, it is a good idea to check the standard curve in excel to ensure the procedure has been successful thus far.
- 22. Next, the QCs can be run from lowest to highest concentrations.
- 23. Finally, the samples can be run in the order that makes sense to you.

24. To clean up, the spectrometer should be shut off, chemicals correctly disposed of (the majority in this procedure can go down the drain, check with Teriko or Harvey if unsure), and glassware should be acid-washed and hung back up on our rack above the sink in middle prep. If unsure about acid washing, rinse well with DI and label rack as non acid-washed.

OPERATION OF THE AUTOCLAVE:

- 1. Turn the black and silver knob which is located <u>directly under the door of the</u> <u>autoclave chamber</u> to the closed setting.
- 2. Do not touch any other knobs and levers. (If something is not draining correctly, check that yellow lever #1 is vertical, yellow levers #2 are both horizontal, red knob #2 is closed, red lever #2 is perpendicular to the pipe and the yellow valve closest to the wall way in back, hard to see, must be in the open (horizontal) position at all times.
- 3. Turn red knob #2 to open ONLY if the temperature or pressure becomes too high.
- 4. Fill the chamber with approximately 1900 mls of DI and 1900 mls of tap water.
- 5. (The half and half mixture of tap/deionized water is recommended by the manufacturer.)
- 6. Ensure that both the handle on the breaker box and the white switch on the smaller box next to it are in the ON positions. (These are located on the wall to the left of the autoclave.
- 7. Replace the removable stainless steel tray with holes in it. It sits on the bottom of the autoclave chamber.
- 8. Replace the bevel plate. It leans against the back of the chamber (the wear spots on chamber mark the spot!). Its "feet" sit on the two side mounted racks. Make sure there is some space between the bevel plate and the back wall of the chamber this allows the steam to be circulated evenly during sterilization.
- 9. Load the autoclave. Never overload or block the free circulation of steam. Apply autoclave tape to the most difficult place for the steam to penetrate.
- 10. Close the door by pulling it down by the handle. Engage the curved portion of the handle under the rod that acts as its catch. Push the handle down and back to lock door securely.
- 11. Set the exhaust selection to liquids. Fast exhaust is not much quicker and may cause lids to blow off or break the items being sterilized.
- 12. Set the temperature to 121 degrees Celsius, or 250 Fahrenheit.
- 13. Set the time the autoclave should run to 45 minutes. The autoclave only starts timing when it has reached the proper temperature and pressure. It is normal for the autoclave to drain during the cycle.

AFTER THE RUN

- 1. Before the door is opened the orange running light must be off and the pressure gage must read zero. Caution, the temperature should be below 40 degrees.
- 2. Open the door cautiously about an inch. Let the steam escape and the chamber cool down before opening it all the way. Do not leave the door closed after a run for long periods of time or the gasket will warp.
- 3. Open the black and silver know to drain the water from the autoclave chamber.
- 4. Remove the stainless steel tray and the bevel plate. Wash both in the sink, drain and dry them.
- 5. When the chamber is cool it must be rinsed and dried. If this procedure is not followed the metal will corrode and may cause a hole in the lining or interfere with the temperature sensors.

QC Acceptability Ranges

Duplicate tests:

Duplicate readings should be within .004 (concentration, not absorbance) of the original sample readings. If readings exceed this range, report immediately to the Lab Manager for troubleshooting and corrective action procedures.

Spikes:

Perform the following calculation:

% Recovery = Absorbance values of: <u>Spiked Sample</u>

Spiked + Original X 100

blank sample

Recovery rate should be 82%-114%.

Preparation of Special Reagents

NOTE: <u>When preparing the following reagents use only pure, fresh deionized water (from lab sill) and glassware which has been acid-washed and thoroughly rinsed with DI water.</u>

- **1. Ammonium Molybdate:** Dissolve 15g of analytical reagent quality ammonium molybdate (NH₄)₆Mo₇O₂₄**?**4H₂O in 500 mls of deionized water. Store in a plastic bottle in a refrigerator. This solution is stable for 30 days.
- 2. Sulfuric Acid:
 - **a. 4.8N FOR COLOR REAGENT** Add <u>140mls</u> of concentrated sulfuric acid to <u>900 mls</u> of deionized H₂O. Allow to cool and store in a glass bottle.
 - **b. 9N H2SO4** Used to periodically acid wash glassware and as a preservative in sample bottles. 250mL H2SO4 Q.S. to 1000mL DI water.
 - c. Sulfuric acid is stored in a glass bottle in the cabinet labeled for acids.
- **3.** Ascorbic Acid: Dissolve 27g of good quality ascorbic acid in 500 mls of distilledH₂O. Store this solution in a plastic bottle <u>frozen</u>. Best Policy is to make large batches occasionally and freeze 30 and 60 mL portions for one time thawing and immediate use. (In freezer, in centrifuge tubes)
- **4.** Antimony potassium tartrate dissolve 1.3715g APT in a 500 ml volumetric with 400 ml of water. QS to 500 ml once dissolved, warming if necessary. Store in a dark plastic bottle in refrigerator. Stable for 30 days.

Preparation of Calibration Curve Standard Solutions

(Labeled in red)

NOTE: <u>When preparing the following solutions use only pure, fresh deionized water (from lab</u> still) and glassware which has been acid-washed and thoroughly rinsed with DI water.

A concentrated form of the standard solution used in the TP method is purchased each spring. (See inventory sheets for order information). The stock solution is stored at room temperature, and is good until the expiration date on the label.

The concentrated standard contains the equivalent of 1000 ppm (mg P/L) of Phosphorus. A working sub-stock of 5 mg P/L must be made first, and used to make further dilutions.

To make the 5 mg P/L sub stock: Volumetrically measure 5 ml. of concentrated standard (1000 mg P/L), into a 1000 ml volumetric flask. Add fresh, pure deionized water to bring the final volume up to the fill line on the neck of the flask.

Then use the 5 mg P/L sub stock to make the following concentrations:

Concentrations needed for the calibration curve are:

- 0.300 mg Phosphorus/Liter
- 0.100 mg P/L
- 0.050 mg P/L
- 0.025 mg P/L
- 0.010 mg P/L
- 0.005 mg P/L

All dilutions are made using the formula $C \times V = C \times V$ where:

- C= concentration of the working sub-stock standard solution (5 mg P/L)
- V = amt. of stock standard solution needed to make the desired dilutions (ml)
- C = final concentration needed (0.300 mg P/L, 0.100 mg P/L, 0.050 mg P/L...etc.)
- V = final amount desired 1000 (ml)

FOR EXAMPLE:

To make the .300 mg P/L calibrator from the 5 mg P/L sub-stock standard solution then:

C= 5 V = x (unknown) C = 0.300 V = 1000

Then, 5x = 300, and x = 60

Therefore, diluting 60 ml of the working sub-stock solution, (5mg P/L), to one Liter using deionized water, will result in 1000 ml of a 0.300 mg P/L solution for the calibration curve. The rest of the concentrations for the curve are made as follows:

20 ml of sub-stock to one Liter of water = 0.100 mg P/L,

10 ml of sub-stock to one Liter of water = 0.050 mg P/L,

5 ml of sub-stock to one Liter of water = 0.025 " "

2 ml of sub-stock to one Liter of water = 0.010 " "

1 ml of sub-stock to one Liter of water = 0.005 " "

Preparation of QC Solutions

(Labeled in blue)

NOTE: <u>When preparing the following solutions use only pure, fresh deionized water (from the lab still) and glassware which has been acid-washed and thoroughly rinsed with DI water.</u>

A concentrated form of the QC solution (stock) used in the TP method is made by DES personnel and provided to the CSC/LSPA Satellite Laboratory each spring. The stock solution is kept refrigerated, and is good for one year. *The concentration of this stock solution may vary from year to year, and must always be diluted for use.*

Concentrations of working QC solutions needed:

0.300 mg Phosphorus/Liter

0.050 mg P/L

0.025 mg P/L

0.005 mg P/L

All dilutions are made using the formula $C \times V = C \times V$

<u>Please refer to Section C of this procedure for a complete explanation of the use of this</u> <u>formula.</u>

<u>The concentrated QC solution, (1000 mg P/L) is also bought and diluted to a 5 mg P/L</u> working sub-stock. The .300, .050, .025, and .005 dilutions are made in the same way as the dilutions of the stock standard solution, as previously discussed in section C.

Turbidity

Turbidity using the HF Scientific Micro 100

A. Sampling Methodology

- 1. Preservation: in dark, refrigerate
- 2. Holding Time: 24 hours
- 3. Required Volume: 100 ml
- 4. Container Type: polyethylene or glass
- 5. Reference Number: 2130B (Standard Methods 20th Ed. 1998)

B. Equipment Calibration and Set-Up

- 1. Turn meter on and allow it to warm up for 30 minutes.
- Press the CAL key. Once this key is pushed, the "Ident" block and the "Cal" block will illuminate on the display. The instrument is not calibrated if "Ident" block and the "Cal" block are flashing.
- 3. The turbidity value in the lower row of the display should read 1000 NTU.
- 4. With a KIMWIPE, wipe clean and dry the 1000 NTU calibration standard. Insert the calibration standard into the sample well and align the arrow on the standard cap with the white indexing pin on the meter. Wait for turbidity reading to stabilize.
- 5. When reading has stabilized, Press the Enter Key. The instrument will calibrate on the 1000 NTU level (the "Store" block will flash) and the upper row of the display should read 1000 NTU. The lower row of the display should now read 10.0 NTU. *Remove the 1000 NTU Standard*.
- 6. With a KIMWIPE, wipe clean and dry the 10.0 NTU calibration standard. Insert the calibration standard into the sample well and align the arrow on standard cap with the white indexing pin on the meter. Wait for reading to stabilize. Note: the 10 NTU standard needs to be freshly poured on the first lab day of each month.
- 7. When reading has stabilized, Press the Enter Key. The instrument will calibrate on the 10.0 NTU level (the "Store" block will flash) and the upper row of the display should read 10.0 NTU. The lower row of the display should now read 0.02 NTU. Remove the 10 NTU Standard.

- 8. With a KIMWIPE, wipe clean and dry the 0.02 NTU calibration standard. Insert the calibration standard into the sample well and align the arrow in the standard cap with the white indexing pin on the meter. Wait for turbidity reading to stabilize.
- 9. When reading has stabilized, Press the Enter Key. The instrument will calibrate on the 0.02 NTU level (the "Store" block will flash). The display should read 0.02 NTU since this is the turbidity level of the standard still in the sample well. *Remove the 2 NTU Standard*.
- Calibration is complete. Record the date of calibration, "ok" in each of the calibration standard columns, and your initials in the bench book's calibration data sheets. Instrument will automatically exit the calibration mode and return to automatic sampling mode.
- 11. Re-test 10.0 NTU standard and record (just insert, do not press enter).
- **Note:** The meter has been calibrated for all ranges. The meter automatically shifts to required NTU range for high or low value samples. However, samples with NTU values greater than 1000, must be diluted until the sample NTU value is less than 1000.

C. Turbidity Sample Analysis Procedures

- 1. Warm samples to 25°C.
- 2. Check calibration record and verify that meter has been calibrated.
- 3. Gently agitate all samples before examination to ensure a representative measurement.
- 4. Rinse sample cell with DI water and small volume of sample and pour sample into sample cell. Wipe clean and dry with KIMWIPE. The meter reads through the cell wall and must be free of water and fingerprints.
- 5. Insert sample cell into sampling well by aligning the white mark on the cuvette with the white indexing pin on the meter. (The meter should be in the normal mode and the "**Auto**" block should be illuminated).
- 6. Wait for the reading to stabilize and record turbidity value in bench book.
- 7. If further sample analysis is needed, repeat steps 3-6. If sample analysis is complete, continue with shut down procedures.

Duplicate analysis is performed on every tenth sample.

CCV is required after every duplicate analysis, or at the end of the day's analyses, whichever comes first.

*Note: Be sure no bubbles are present in sample cell and that the cell is free from fingerprints and water on the outside surface.

D. Shut Down Procedures

- 1. Turn the meter off by pressing the on/off button.
- 2. Remove sample cell.
- 3. Insert storage cell into the sample well.
- 4. Ensure that all information from sample bottles has been entered correctly into bench book and that the appropriate number of replicates has been run.