AN INVESTIGATION OF THE ROLE OF AQUATIC PLANTS IN THE CYCLING 
OF PHOSPHORUS AND NITROGEN IN MURPHY’S BOTTOM LAKE, 
ARMSTRONG COUNTY, PA

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By
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AN INVESTIGATION OF THE ROLE OF AQUATIC PLANTS IN THE CYCLING
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ABSTRACT

AN INVESTIGATION OF THE ROLE OF AQUATIC PLANTS IN THE CYCLING OF PHOSPHORUS AND NITROGEN IN MURPHY’S BOTTOM LAKE, ARMSTRONG COUNTY, PA

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Thesis supervised by Dr. Brady Porter

The objectives established for the Murphy’s Bottom Ecological Project are to obtain baseline biological inventories and establish a Master Plan for reclamation of the 100 acre site located in Armstrong County, Pennsylvania. Reclamation plans include connecting the 20+ acre Lake, formed by sand and gravel removal, to the Allegheny River to form a backwater wetlands. This study produced a macrophyte species list, mapped the macrophyte community and determined water and plant phosphorus concentrations and water nitrogen concentrations. Replicable laboratory methods for the analysis of these nutrient concentrations were adapted from established molybdenum blue, Nitrate Electrode and HACH Cadmium Reduction Methods. Geographic coordinates, Global Information System (GIS) software and quadrant sampling was used to estimate the total biomass of various macrophyte species at the site. Total phosphorus
data and total biomass data were combined to determine the total amount of phosphorus in the Murphy’s Bottom Lake.
ACKNOWLEDGEMENTS

This research would not have been possible without the support and encouragement of my advisors, co-workers, family and friends. Dr. Brady Porter eagerly invited me into his lab and provided assistance and advice at all stages of this project. Dr. Alan Seadler helped with formulating the initial research plan and dedicated many hours to lab work and data review. Dr. John Stolz provided suggestions and feedback for the final manuscript. Dr. Beth Dakin’s patience and technological skills helped me to stay sane while working through math problems and data analysis. Field sampling would not have been possible without the assistance of Amanda Muir and Ben Latoche. Porter Lab members including Stephanie Dowell, Maria Wheeler, Katie Boone, and TJ Fireno provided many laughs and a positive work environment. My parents, Tom and Carolyn Mathews, and my fiancé, David Mills, were always patient with my frustration and excited to celebrate with me when methods were successful.

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INTRODUCTION

The Murphy’s Bottom Ecological Project began in 2006 when Duquesne University was offered a parcel of land in Armstrong County, Pennsylvania to be used for student research and a long-term habitat restoration project. In the early 1970s the area was mined for sand and gravel and with succession has transformed into a vegetated parcel with a variety of land cover types. The eastern end of the property is comprised of early successional shrub land and wetland habitat. Centrally located is the 25-acre Lake (Figure 1) and associated two-acre Annex (Figure 2), a smaller body of water attached to the Lake during high water. Upland and lowland forests comprise the western portion of the property. Knapp Run meanders through these forests before emptying into the Allegheny River, which acts as the southern property line. The Turning Basin (Figure 3), a small two-acre inlet on the Allegheny River (Figure 4) is located upriver from the Lake approximately a quarter of a mile. The inlet was previously used for turning barges to offload coal and transport sand and gravel from the mine.

In 2008 Duquesne University hired Lennon, Smith, Souleret (LSSE) Engineering, Inc. in Coraopolis, PA to map the existing land cover types at the site (Figure 5) and to create a habitat enhancement plan (Figure 6). The major component of this agenda is the connection of the Murphy’s Bottom Lake to the Allegheny River. Major chemical and physical changes are likely to occur as a result of this topographical change.

Quantitative and qualitative data has been collected at the Murphy’s Bottom site in the past four years regarding the terrestrial plant community and the planktonic and microbial populations in the water columns of the Lake and Annex. Prior to this study, however, the aquatic plant community had never been investigated. For management and
Figure 1: A photograph of the Murphy’s Bottom Lake in September 2009.

Figure 2: A photograph of the Murphy’s Bottom Annex in August 2009. Photo courtesy of Amanda Muir.
Figure 3: A photograph of the Turning Basin at the Murphy’s Bottom site in May 2010. Photo courtesy of Amanda Muir.

Figure 4: A photograph of the Allegheny River at the Murphy’s Bottom site in May 2006. Photo courtesy of Dr. John Stolz.
Figure 5: A map showing the existing cover types at the Murphy’s Bottom site as of May 2008. Map created by Lennon, Smith, Souleret Engineering, Inc., Coraopolis, PA.
Figure 6: A map showing the proposed cover types at the Murphy’s Bottom site after the implementation of the habitat enhancement plan. Map created by Lennon, Smith, Souleret Engineering, Inc., Coraopolis, PA.
planning purposes it is important to understand the role of the macrophyte community in the cycling of nutrients (Thiebaut and Muller, 2003). With this goal it was imperative to determine the concentrations of phosphorus and nitrogen in the water columns to track trends in nutrient concentrations within the four water bodies. Due to the unnatural formation of the Murphy’s Bottom Lake the underground hydrology of the system is unknown.

The macrophyte community at the Murphy’s Bottom site offers a variety of ecological services to wildlife and acts as a primary energy source for the limnetic system. Macrophytes are excellent biological indicators in aquatic systems due to their response to nutrients, sediment composition, metals, water depth, and pollutants (EPA, 2009). Aquatic plants play a significant role in nutrient retention and sediment stabilization while providing habitat for fish, invertebrates, and waterfowl. Research has shown that large-mouth bass prefer vegetated areas with intermediate stem densities to non-vegetated sites (Savino and Stein, 1982; Killgore et al., 1989 as cited in Pedlow et al., 2006). Habitat heterogeneity provided by a diverse macrophyte community has the potential to support larger populations of macroinvertebrates (Gerking, 1957; McCafferty, 1981; Watkins et al., 1983 as cited in Pedlow, Dibble, and Getsinger, 2006), the major food source for pan fish.

Macrophytes are commonly categorized into four major growth forms: emergent macrophytes (e.g. *Scirpus lacustris*), free-floating macrophytes (e.g. *Lemna minor*), rooted floating-leafed macrophytes (e.g. *Nymphaea odorata*) and rooted submerged macrophytes (e.g. *Potamogeton crispis*).
The most prevalent macrophyte form in both the Lake and the Annex are the submerged macrophytes. These species include *Myriophyllum spicatum*, *Potamogeton crispis*, *Najas guadalupensis*, *Chara spp.*, *Elodea canadensis* and *Zosterella dubia*. With an overshadowing presence in the Annex, *M. spicatum* plays a significant role in nutrient retention and cycling. Both *N. guadalupensis* and *Chara spp.* have formed large beds around the perimeter of the delta (located at the north-central area of the Lake) and in the shallows on the eastern end of the Lake.

The white pond lily, *Nymphaea odorata*, is the only floating-leafed macrophyte documented at the site. This species acts as a sediment stabilizer with expansive rhizome networks (Borman et al., 1997). With its broad leaf structure at the surface *N. odorata* prevents sunlight from penetrating the water column and prevents the proliferation of lower-growing species.

The emergent macrophytes are familiarly known as the sedges and bulrushes and are usually found along moist shorelines and in shallow water less than a meter (Borman et al., 1997). Although they are present at the Murphy’s Bottom site, due to the fluctuations in water levels these plants are not consistently located in the water column and were therefore excluded from this study.

The free-floating macrophytes located at Murphy’s Bottom are *Lemna minor* and *Ceratophyllum demersum*. The Lemnaceae family consists of some of the smallest flowering plants on earth; this family is commonly known as the duckweeds. Duckweed is present in the Annex but due to its small size and lack of attached roots was very difficult to successfully collect and categorize. *C. demersum* grows at the western end of
the Main Lake but was not included in any of the sampling quadrants due to its sparse distribution.

Due to their abundance and diversity of species in the Lake and the Annex, it was hypothesized that macrophytes play an active role in the cycling and retention of phosphorus and nitrogen. This research project focuses on this ill-studied community component and provides a baseline data set for the Murphy’s Bottom site prior to the proposed physical connection of the Main Lake and the Allegheny River in coming years. Of particulate interest was the concentration of total phosphorus and total nitrogen in aquatic plant tissues and the corresponding water columns where such plants grow. The importance of data collection in regards to nutrient status, sediment quality and particulate content of the water column has been highlighted in terms of determining the effect of habitat changes on macrophyte populations (Lougheed, Crosbie and Chow-Fisher, 2001).

A number of variables have been said to affect the distribution and community structure of macrophytes in lake systems. Abiotic factors such as temperature, light, water depth, and wind patterns, and biotic factors such as invasive species, predators, and competition are known to be of influence, but the degree of influence is unknown. Research has supported hypotheses suggesting that macrophytes take up nutrients from the sediment (Johnson and Ostrofsky, 2004) and the water column (Granéli and Solander, 1988).

Wetzel (2001) determined total and available nitrogen in a small eutrophic lake in southern Wisconsin. Of the available nitrogen, 20% was held up in the macrophytes while 50% existed in the water column. Organic nitrogen is cycled and retained by
photosynthetic plants and microbes in the water column. Ninety-seven percent of the total nitrogen in the system was trapped in the sediments and did not cycle throughout the system (Wetzel, 2001).

Phosphorus plays a vital role in biological metabolism yet is often a limiting nutrient in lake systems (Wetzel, 2001). It has been argued that the most important measurement of this nutrient is total phosphorus in unfiltered water (Juday, 1927; Ohle, 1938 as cited in Wetzel, 2001) which is a combined measure of soluble and particulate phosphorus. Phosphorus measurement is most often accomplished by reaction with molybdate in an acid to form orthophosphate (Wetzel, 2001). Concentrations of total phosphorus in natural waters are usually between 1-200 ug/L with surface water concentrations normally between 10-50 ug/L; although high variation exists in some areas due to local geography (Wetzel, 2001).

This project quantifies the two primary, and arguably limiting, nutrients in the Lake, Annex, Turning Basin and Allegheny River water columns and determines the concentration of phosphorus in tissues of aquatic plants growing in the Lake and Annex. An aim of this research was to determine which species of native and invasive aquatic plants at the Murphy’s Bottom site are the most prominent sinks and sources of phosphorus and nitrogen in the system.

It is important to note that living macrophytes do not release phosphorus but instead facilitate phosphorus release from the sediments due to changes in pH and oxygen during photosynthesis (Granéli and Solander, 1988; Rörslett et al., 1985; Barko and James, 1997 as cited in Horppila and Nurminen, 2003).
The invasive Eurasian milfoil, *M. spicatum*, removes nutrients primarily from the sediment (Barko and Smart, 1980; Carignan and Kalff, 1980; Carignan, 1982 as cited in Smith and Barko, 1990). But this species also contributes phosphorus to the sediment throughout the growing season as small leaflets on the lower portion of the plant are shaded out, drop off, and fall to the bottom (Smith and Barko, 1990). This particular species is a probable sink for phosphorus in the Annex due to the persistence of shoots overwintering from year to year (Smith and Barko, 1990). The dense canopy growth style of these plants (Smith and Barko, 1990) prohibits the growth of lower-growing species present in the Main Lake such as *Elodea*, *Chara*, and *Najas*. According to Adams and McCracken (1974) *M. spicatum* is prone to two biomass peaks in geographic locations where flowering occurs early (as cited in Smith and Barko, 1990). This dual-peak was noted at the Murphy’s Bottom site in the summer of 2009 with flowering occurring in mid-July and again in mid-September.

*Chara* have the unique capability of utilizing bicarbonate and precipitating calcite. Research by Kufel and Kufel (2002) has shown that phosphorus may become trapped in these crystalline calcite structures. Charophytes were reported to breakdown at slower rates than other aquatic macrophytes and can therefore store nutrients for longer periods of time. Kufel and Kufel (2002) concluded that *Chara* beds act as prominent nutrient sink in shallow lakes where dense beds form and restrict nutrient release from sediments. Light is a critical component of prime charophyte habitat and these macrophytes cannot grow in turbid conditions. In addition to its role in the phosphorus cycle *Chara* grows on rich organic bottom sediments where it participates in nitrogen cycling by distributing oxygen at the sediment/water boundary to create conditions for
nitrification, denitrification and ammonification (Lijklema, 1994 as cited in Kufel and Kufel, 2002).

*E. canadensis* is also known for its high phosphorus storage ability (Thiébaut, 2005). *Elodea* species have the ability to overwinter in a vegetative state and are also adapted to deal with seasonal phosphorus fluctuations (Thiébaut, 2005). These plants have an advantage in the late spring because they have retained some of the phosphorus from the fall season and are capable of fueling an intense growth rate in the spring season when nutrients are limited. Jeschke and Simonis (1965) and Weigl (1967) determined that *Elodea* absorbs phosphorus through its foliage (as cited in Seadler, 1975).

Water quality and sediment composition are two of the greatest contributing factors to macrophyte growth and development (Day et al. 1988; Barko et al. 1991 as cited in Lougheed, Crosbie and Chow-Fraser, 2001). Water quality parameters can include temperature, pH, dissolved oxygen, conductivity, turbidity and nutrient concentration. Water temperature, dissolved oxygen, conductivity, chlorophyll a and turbidity readings were collected electronically via the floating buoy Sonde on the Murphy’s Bottom Lake. The solar-powered probe collects real-time water quality data throughout the year that was incorporated into the results of this project.

Johnson and Ostrofsky (2004) tested the effect of sediment type on the distribution of macrophytes where patterns could not be explained by depth gradients or water chemistry differences. Their data supported the hypothesis that macrophyte community structure responds to sediment nutrient concentrations. Their conclusions explain that the sediment in the littoral zone is a patchwork of varying nutrient concentrations that is not always correlated with depth. Moving water is known to drop
its sediment load when it slows (Cushing and Allan, 2001) which supports the research by Rooney, Kalff and Habel (2003) showing that sediment accumulation adjacent to macrophyte beds can be statistically significant and increases with bed density.

The research outlined in this paper lends evidence to the possibility that macrophyte partitioning at the Murphy’s Bottom site is due to the variety of sediment textures. Sediment texture determination was completed in a rather non-sophisticated manner but determined that differences in sediment texture do occur at the Murphy’s Bottom Lake and Annex.

Chemical analysis and modeling of nitrogen cycling has not been studied extensively, but research by Nichols and Keeney (1976) suggests that nitrogen uptake by *M. spicatum* occurs primarily as ammonium from sediment and also as ammonium and/or nitrate from the water column. Due to supporting evidence from Smith and Barko (1990), a hypothesis for the Murphy’s Bottom site is that phosphorus and nitrogen concentrations in the water column of the Annex will decrease as the *M. spicatum* proliferates throughout the summer growing season, followed by a large spike in phosphorus concentration in late fall after plant die-off. Submerged macrophytes increase the diurnal dissolved oxygen in the water column while decreasing inorganic carbon dioxide. This process results in elevated pH levels perfect for volatilization of ammonia.

A primary desire of this project was the compilation of an aquatic macrophyte species list for the Murphy’s Bottom site. ArcView GIS software has been used in the ecologic realm in the past decade to reflect the presence of plants and to highlight the significant plants beds within a water body.
Various methods for mapping macrophytes have been employed and cited throughout the literature. Airplanes and satellite imagery have been used on expansive and diverse lakes (Lehmann and Lachavanne, 1997) while some data has been collected visually with SCUBA gear (Rooney, Kalff, and Habel, 2003). Random sampling has included data collection with sampling quadrants (Johnson and Ostrofsky, 2004) and transects. Horppila and Nurminen (2003) estimated macrophyte density as percent volume infested (PVI) using plant height, percent coverage and water depth. Due to the small size of the Murphy’s Bottom Lake and relatively shallow depth a sampling method was developed using a metal quadrant. Sampling plots were chosen in a manner that targeted areas of high plant density and a variety of species per quadrant. The goal was to collect as many species as possible in an attempt to catalog the average phosphorus content per plant species to eventually determine the total phosphorus concentration of the entire Lake system.

Studies have documented the difficulty in obtaining intact macrophyte samples from the field (Forsberg, 1959). Due to the turbidity in the water column upon disturbance, not all macrophytes within a demarcated area can be accurately removed (Forsberg, 1959). A number of shoots typically remain within the quadrant after sampling. Various systems have been used for the function of macrophyte sampling, but for the purposes of this project a simple four-sided metal frame was used and macrophyte shoots were removed by hand.

Global Information System (GIS) technology has become popular in mapping landscapes and plotting data. A combination of GIS and handheld Global Positioning System (GPS) units has been used to map macrophytes in lake systems. Within the
ArcView GIS software, layers can be established to compare depth, sediment type, light penetration and water chemistry to species presence and abundance (Remillard and Welch, 1992). This software is especially useful for mapping changes over time, such as those that are likely to occur after Duquesne University implements the proposed Murphy’s Bottom habitat restoration plan.

The management and extirpation of invasive species is an objective of the Murphy’s Bottom Ecological Project’s habitat restoration plan. In an effort to maximize biodiversity it is essential to acknowledge the presence of alien species and to contain or destroy such species before they out-compete the native species. The presence of a number of non-native aquatic plant species was noted at the Murphy’s Bottom site during the preliminary survey but a comprehensive study outlining the potential threats of these species to the health of the Main Lake or the Annex has yet to be completed. By completing a comprehensive survey of the aquatic plant community, a more diligent and thought-out plan can be constructed, and a timeline of removal or containment can be implemented.

The purpose of this research is to analyze the macrophyte community at the Murphy’s Bottom site and determine its role in the cycling of phosphorus and nitrogen in the limnetic system. This was accomplished by compiling a macrophyte species list for the Murphy’s Bottom site, formulating specific and replicable methods for analyzing phosphorus and nitrogen in the water columns and investigating which macrophytes are having the most impact on the phosphorous cycling in the Lake and Annex systems.
METHODS

2.1 Macrophyte mapping

Due to the relatively small size of the Murphy’s Bottom Lake and the accuracy of visual monitoring, macrophyte presence and abundance was visually determined by boat during the prime growth period of late July and early August. Macrophyte mapping methods were adapted from those published on the Pennsylvania Department of Environmental Protection’s website (PA DEP, 2008).

A map of the site including the Main Lake and Annex was transferred from a Google Earth image found online onto waterproof paper prior to field data collection. Snorkeling equipment was used to compile a general layout of the underwater macrophyte community before more specific methods were employed.

Beginning at the southwest corner of the Main Lake, the rowboat was maneuvered along the Lake’s shoreline at approximately four meters out from shore. This is the zone of prominent macrophyte growth due to lack of light penetration and turbidity at greater depths. Points were marked with a handheld Garmin GPS unit at 15-meter intervals around the Lake shore. The latitude and longitude of each point, as well as a Secchi disk reading was recorded. The depth and bottom type was determined with a calibrated copper pole. Sediment would become lodged in the end of the pipe as it was tamped on the bottom. The pole was pulled to the surface and tapped in the boat to release the enclosed sediment. The sediment core was visually and texturally assessed. Color and texture of the core was recorded for each site.

Macrophyte species’ presence and abundance was noted at each point and unknown plants were collected for later expert identification. Plants were placed in
plastic Ziploc bags marked with the sampling location, date and species. Significant macrophyte beds and landmarks were noted on the map for purposes of comparison of the present macrophyte community to that of future years. The large *N. odorata* bed on the east end of the lake was circumvented by the rowboat and marked with multiple GPS points; all of these points were transferred to the paper map. After the mapping of the Main Lake, a similar method was used to plot macrophytes in the Annex. Due to the narrow width of the Annex a zigzag pattern was employed rather than following the shoreline. Plant data, water depth and bottom type were used to create a GIS map of the Murphy’s Bottom site with ESRI ArcGIS version 9.3.1 computer software.

Representative specimens of all macrophyte species were collected from the field and taken back to the lab where they were dried and pressed. Plant samples were placed between sheets of newspaper and stacked between cardboard and herbarium sheets in a plant press for approximately one week, or until dry. Dried samples were removed from the press and glued to herbarium sheets labeled with the collection date, location, and collector’s name. Bonnie Issac of the Herbarium at the Carnegie Museum of Natural History verified plant samples. Through this process a comprehensive Murphy’s Bottom macrophyte species list was compiled.

2.2 Water sample collection

Beginning in May of 2009 weekly water samples were collected from the Murphy’s Bottom Lake, Annex, Turning Basin, and Allegheny River. In an attempt to track changes over time, sampling occurred on a weekly basis until cold temperatures and freezing prevented sampling in late October 2009. The sampling site for the Main Lake was located mid-lake, approximately 25 meters east of the floating buoy Sonde. The
sampling site for the Annex was located in the center of the water body in alignment with
the large deadfall tree closest to the Turning Basin on the Allegheny River side.
Allegheny River water was collected from mid-channel slightly downstream from the
Turning Basin inlet, and the sample in the Turning Basin was collected in the center of
the pool in alignment with the ladder steps on the old barge. GIS coordinates for each of
these sites in listed in Table 1.

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<tr>
<td>Turning Basin</td>
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<tr>
<td>Allegheny River</td>
<td>40.691751°, -79.629378°</td>
</tr>
<tr>
<td>Lake</td>
<td>40.693012°, -79.637157°</td>
</tr>
<tr>
<td>Annex</td>
<td>40.692666°, -79.63493°</td>
</tr>
</tbody>
</table>

Table 1: GIS coordinates for water sampling locations at the Murphy’s Bottom site.

A rowboat was used to facilitate collection of water samples. Air temperature,
water temperature and a Secchi disk reading was collected and recorded at each site. The
bucket, the grab sample jars, the plastic sample bottle, and the grab sampler were rinsed
three times in the water body prior to collection to prevent contamination. The glass
subsample jar was connected to the grab subsampler and three water samples were
obtained from the epilimnion 0.5 meters below the water surface. All water samples were
poured into the bucket as they were collected. The water sample in the bucket was poured
into an acid washed 100 mL polypropylene sample bottle, capped, and placed in a cooler
with ice for transport back to the lab. Upon return to the lab, each 100 mL water sample
was divided into two sterile 50 mL labeled plastic polypropylene tubes and stored in a
4°C dark refrigerator for a maximum of ten months.
All 50 mL water samples were transferred into clean, labeled glass test tubes. Labeling tape was used to mark a 50 mL water line on the tubes for future reference and to note potential loss of water during autoclaving. Approximately 0.4 g +/- 0.1 g of potassium persulfate was added to each 50 mL water sample. The glass tubes were capped tightly and autoclaved for 30 minutes (M. Ostrofsky, personal communication, September 8, 2009).

2.3 Total phosphorus analysis of water

Water samples were analyzed for total phosphorus using an adaptation of Strickland and Parsons’ molybdenum blue method following potassium persulfate oxidation (Strickland and Parsons, 1972). The persulfate oxidation technique oxidizes all forms of phosphorus in the sample into orthophosphate (Seadler, 1975). Following oxidation, the sample is reacted with ammonium molybdate and potassium antimonyl tartrate to form phosphomolybdic acid, which is then reduced by ascorbic acid to form a deep blue color (Standards Method for the Examination of Water and Wastewater, 2005). The maximum absorbance occurs at a wavelength of 880 nm and was read with 1 cm² cuvettes in a Cary 100 1E UV Visable spectrophotometer. Phosphorus concentration is linearly related to absorbance for the approximate range between 150 and 1300 ug/L (Standard Method from the Examination of Water and Wastewater, 2005).

Phosphorus standards were prepared prior to analyzing the field samples. Standards of 0, 25, 50, 100, 200, 400 and 800 ug/L were prepared using a three-step dilution process described in the Ascorbic Acid Method 4500 P.C.3e and 4500 P.E. 3g in Standard Methods for the Examination of Water and Wastewater (2005). Careful
measurement was imperative to produce an accurate standard phosphate curve to which all samples were compared.

All glassware used for these water samples was washed with H₂SO₄ followed by three rinses with deionized water and used solely for phosphorus samples. Between uses, glassware was sealed with parafilm to prevent contamination.

The modified stock reagent solutions from the Strickland and Parsons method were prepared at concentrations outlined in Table 2, then labeled and stored for later use.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium Molybdate</td>
<td>30 g/L</td>
</tr>
<tr>
<td>5 N Sulfuric Acid</td>
<td>254 g/L</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>54 g/L</td>
</tr>
<tr>
<td>Potassium Antimonyl Tartrate</td>
<td>1.36 g/L</td>
</tr>
</tbody>
</table>

Table 2: Stock reagents used in the preparation of Strickland and Parsons’ mixed reagent (Seadler, 1975).

The ammonium molybdate and sulfuric acid solutions are stable for many months when stored at room temperature in capped glass bottles. The ascorbic acid solution was frozen and thawed to room temperature when needed (Strickland and Parsons, 1972). The potassium antimonyl tartrate was initially stored at room temperature but harbored fungal growth. New reagent was prepared and stored in the 4°C refrigerator.

The Strickland and Parsons reagent was created by mixing together the stock reagent solutions in volumes outlined in Table 3.

<table>
<thead>
<tr>
<th>Stock</th>
<th>mL</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium Molybdate</td>
<td>100</td>
<td>6.0 g/L</td>
</tr>
<tr>
<td>5 N Sulfuric Acid</td>
<td>250</td>
<td>127.4 g/L</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>100</td>
<td>10.8 g/L</td>
</tr>
<tr>
<td>Potassium Antimonyl Tartrate</td>
<td>50</td>
<td>0.14 g/L</td>
</tr>
</tbody>
</table>

Table 3: Quantities of stock reagents needed to prepare Strickland and Parsons’ mixed reagent (Seadler, 1975).
The mixed reagent was prepared for immediate use and excess discarded (Strickland and Parsons, 1972). The quantities of stock reagents in Table 3 are suitable for 50, 100 mL samples or 100, 50 mL samples. To analyze samples or standards, a 10 mL water sample was removed via hand pipette from each 50 mL sample and placed into a labeled 50 mL polypropylene tube. An automatic pipette was used to place 1 mL of mixed reagent into each 10 mL water sample. A 10 mL subsample of each of the seven standards was also placed in 50 mL polypropylene tube with 1 mL of reagent.

After ten minutes, and within the first two hours, the standards and samples were read at 880 nm (Seadler, 1975) in the Cary 100 1E UV-Visible spectrophotometer. The seven standards were read in increasing concentration to create a calibration curve with the CaryWin UV computer software version 3.00(182). The samples were read and the software determined their total phosphorus concentrations based on the standards.

2.4 Macrophyte sampling

Following the mapping of the macrophyte community at Murphy’s Bottom in late June and early August plant samples were collected from the Lake and Annex in August, September and October and used for phosphorus analysis. Sampling areas were determined based on the presence and abundance of individual plant species. The goal was to collect and analyze representative individuals for each species growing in the Main Lake and the Annex. All plants were represented in the sampling with the exception of *C. demersum*, *L. palustris* and Lemnaceae. Sampling quadrants were chosen depending on abundance of varying macrophytes and density of plants.

A sampling quadrant was constructed with four metal plated slotted angles at lengths of one meter purchased at a local home improvement store. Each corner was
secured with hardware. Braided polyester ropes at lengths of approximately eight feet were attached to each corner with metal snap hooks for lowering and retrieval purposes. The rowboat was used to maneuver around the lake to gather samples. At each site the anchor was dropped and the Garmin handheld GPS unit was used to note the latitude and longitude of the sample point (Table 4). The metal quadrant was lowered into the water and positioned so that it lay flush to the bottom. The four ends of the corner ropes remained inside the boat for easy retrieval of the quadrant after plant removal.

<table>
<thead>
<tr>
<th>Quadrant No.</th>
<th>Coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>40.693618°, -79.63419°</td>
</tr>
<tr>
<td>3</td>
<td>40.693569°, -79.634496°</td>
</tr>
<tr>
<td>4</td>
<td>40.693793°, -79.635955°</td>
</tr>
<tr>
<td>5</td>
<td>40.693205°, -79.634528°</td>
</tr>
<tr>
<td>6</td>
<td>40.693734°, -79.637658°</td>
</tr>
<tr>
<td>7</td>
<td>40.692715°, -79.634861°</td>
</tr>
<tr>
<td>8</td>
<td>40.692520°, -79.635252°</td>
</tr>
</tbody>
</table>

Table 4: GIS coordinates for macrophyte sampling quadrants at the Murphy’s Bottom site.

Plants were snapped off at the base and roots were left in the sediment. Tall macrophytes like *M. spicatum* and *P. crispis* were removed first, followed by smaller, shorter plants such as *Elodea* and *Chara* spp. Plants were rinsed in the water body to remove epiphytes and then placed in a large plastic Tupperware container in the boat. Following collection the quadrant was pulled from the water and the Tupperware container of plants was taken back to the lab for sorting.
Plants were sorted by species in the lab and placed in labeled Ziploc bags. Due to
the small size of the available drying oven some of the specimen needed to be
temporarily stored in a 4°C refrigerator. Plants were removed from the Ziploc bags,
placed in a clean, dry, metal tray and dried at 60°C for 48 hours (Seadler, 1975). Plants
were removed from the oven and dry weights were recorded. After careful wrapping in
aluminum foil, plant packages were labeled and stored in a clean, dry Tupperware
container lined with powdered silica and aluminum foil.

2.5 Total phosphorus analysis of macrophytes

The method for determining total phosphorus in plant samples was similar to the
method outlined above for water samples. All of the glassware used for this method was
first acid washed with either 5 N H₂SO₄ or the Strickland and Parsons Mixed Reagent to
react and remove any residual phosphorus. A 0.25 g subsample of each dried plant
species was crushed with a mortar and pestle and placed in individual labeled glass test
tubes. The glass tubes were then filled with 50 mL of deionized water. Using a clean
balance boat and plastic scooper, 0.4 g +/- 0.1 g of potassium persulfate was added to
each glass tube with the crushed plant samples. The glass tubes were capped tightly with
autoclaveable lids, swirled to mix and autoclaved for 30 minutes. The persulfate
oxidation technique oxidizes all forms of phosphorus in the sample into orthophosphate
(Seadler, 1975).

Standards of 0, 25, 50, 100, 200, 400 and 800 ug/L were created using a three-step
dilution process described in the Ascorbic Acid Method in Standard Methods for the
Examination of Water and Wastewater (2005) as described previously. Care was taken to
treat standards in the same manner as samples in an attempt to prevent inconsistencies.
Standards were allowed to cool after autoclaving. From each 50 mL standard, 10 mL was removed by pipette and placed in a clean, labeled 50 mL polypropylene tube.

After samples had cooled the plant material was allowed to settle to the bottom. One mL of each water sample was removed from the top portion of each sample by pipette and placed in a clean, labeled 50 mL polypropylene tube. Nine mL of deionized water was added to each 1 mL sample.

The Strickland and Parsons Mixed Reagent, outlined in Table 3 was prepared and 1 mL of Mixed Reagent was added to each 10 mL water sample and standard (Strickland and Parsons, 1972). All samples and standards were gently swirled and allowed to sit for ten minutes. The seven standards were read at 880 nm in increasing concentration in a Cary 100 1E UV Visible spectrophotometer to create a calibration curve with the CaryWin UV computer software version 3.00(182). Then the samples were read and the software determined their total phosphorus concentrations based on the standards.

The dilution of the plant sample outlined above was determined via trial and error. Previous trials using 0.5 g of dry plant or more produced a very dark blue color beyond the readable range for the Strickland and Parsons’ molybdenum blue method. A 0.25 g dry plant sample with the addition of 0.4 g +/- 0.1 g of potassium persulfate was determined as the optimum. This amount of plant provided a concentration within the readable range. A formula was developed to determine the amount of total phosphorus incorporated into each 0.25 g dry plant sample. All sample concentrations were multiplied by ten to obtain the undiluted concentration of phosphorus per liter. This determination was multiplied by 0.05 to determine mg total phosphorus per 50 mL and then multiplied by four to calculate mg phosphorus per gram dry weight. Total
phosphorus as percent of dry weight was then calculated based on the total dry weight of each plant sample collected from the field.

2.6 Total nitrogen analysis of water samples

Two methods were investigated for the determination of total nitrogen in water samples. The Nitrate Electrode Probe method (Standards Methods for the Examination of Water and Wastewater, 2005) and HACH Cadmium Reduction Method were employed and the readings were compared.

2.6a Nitrate electrode method

The Nitrate Electrode Probe method published in Standard Methods for the Examination of Water and Wastewater (2005) uses an ion selective electrode to determine NO₃ by measuring the changes in potential (mV) with increasing concentration. Nitrogen standards were prepared using the Nitrate Ion Standard (1000ug/L) that was included with the Fisher Scientific Nitrate Combination Electrode. Standards of 0.1, 1, 10 and 100 mg/L were created using serial dilution and deionized water.

The Fisher Nitrate Combination Electrode was attached to the Fisher Scientific accumet Basic AB15 pH meter and set to read millivolts (mV). The probe was gently lowered into the standard samples and the mV recorded. After each reading the probe was rinsed with deionized water to prevent contamination. Readings were recorded for each standard and a logarithmic calibration curve was created.

Five mL of water was removed from each autoclaved water sample tube and placed in a clean, labeled 50 mL polypropylene tube. One drop of the Ionic Strength Adjuster (2 M(NH₄)₂SO₄) was added to each 5 mL water sample (a recommendation on
the instruction sheet that accompanied the probe). The nitrate probe was lowered into the sample and the millivolt reading was recorded. For greater accuracy an average of two readings was determined. The probe was rinsed with deionized water between samples. Total nitrogen in each sample was determined using a logarithmic equation to fit a curve to the standards. The data from this method was skewed by the addition of persulfate and will be reviewed in subsequent discussion.

2.6b Cadmium reduction method

The cadmium in the NitraVer 6 pillow packet (catalog number 14120-99) is used to reduce all nitrates to nitrites. The nitrite ions react with sulfanilic acid to form diazonium salt that forms the red color complex with chromotropic acid (HACH). The intensity of red color is read in a spectrophotometer at 540 nm.

The nitrogen standards were prepared using the Nitrate Ion Electrode Standard (1000ug/L) that was included with the Fisher Scientific Nitrate Combination Electrode, part number 302578.0/300743.1. Standards of 0.1, 1, 10 and 100 mg/L were mixed in glass tubes using serial dilution and deionized water. Potassium persulfate in the amount of 0.4 g +/- 0.1 g was added to each tube and autoclaved for 30 minutes.

The autoclaved standards and 50 mL water samples collected between June and October 2009 were used for the HACH pillow packet (catalog number 2460800) cadmium reduction method to determine total nitrogen in the Lake, Annex, Turning Basin and Allegheny River. Both standards and samples contained persulfate. After the autoclaved samples had cooled, 5 mL of water was removed from each of the samples by pipette and placed in clean, labeled, 50 mL polypropylene tubes.
The two-stage Low Range Nitrate Test Kit instructions from HACH were followed. One NitraVer 6 Nitrate Reagent Pillow Packet (catalog number 14120-99) was emptied into each 5 mL water sample and standard. The glass tubes with reagent were shaken for three minutes. The samples and standards were allowed to sit for 30 seconds as the cadmium metal particles settled to the bottom of the tube. Each sample and standard was carefully poured into a second labeled 50 mL polypropylene tube leaving all cadmium particles in the first tube. Cadmium particles were disposed of in the hazardous waste container.

After standards and samples were transferred to the second tubes a NitraVer 3 pillow packet (catalog number 14078-99) was added to each. All tubes were capped, shaken for 30 seconds and allowed to sit undisturbed for ten minutes while a red color formed in the tubes. Each of the standards was poured into a 1 cm glass cuvette and read at 540 nm against a blank of deionized water. The CaryWin UV version 3.00(182) computer software was used to read the absorbance of the samples and standards at 540 nm in the Cary 100 1E UV-Visible spectrophotometer. Standards were read in increasing concentration as the software program plotted the absorbances on a linear graph. After the standard curve was created each of the samples was poured into the cuvette and read against a deionized water blank at a wavelength of 540 nm. The CaryWin software determined the concentration of total nitrogen in each sample based on the standard curve.

To determine any effect of persulfate on the determination of total nitrogen in the water samples a comparison of data was completed. A set of standards containing persulfate was compared to a set of standards without persulfate. The HACH cadmium
reduction method was identically carried out with both sets of standards. The results of this mini experiment determined that there is no significant difference in the determination of total nitrogen in samples with and without persulfate.

Figure 7: A graph reflecting the combined data of a total nitrogen standard curve with persulfate and a total nitrogen standard curve without persulfate. The $R^2$ value is 0.99842 and reflects that the absorbance readings from the two sets of standards are indeed similar.
RESULTS

3.1 Macrophyte and sediment mapping and collection

An ArcView GIS map was created using the results of the macrophyte mapping technique (see Figure 10). Eight aquatic plant species were located in the Lake during late July and early August. These included *N. odorata*, *M. spicatum*, *Z. dubia*, *P. crispis*, *N. guadalupensis* and *C. demersum*. The species located in the Annex were *M. spicatum*, *L. palustris* and individuals of the Lemnaceae family (duckweed).

When macrophyte sampling occurred at the Lake in August, September and October two additional species were observed (*Chara spp.* and *E. canadensis*). Due to their small size and low profile these species may have been overlooked during the August mapping procedure or they could have proliferated after the mapping dates.

From the surface perspective in early August the Lake looked to be comprised primarily of *N. odorata* with a concentration of plants at the eastern sector. However, the results of the quadrant samples, shown as a graph in Figure 8, reflect that *N. guadalupensis* and *M. spicatum* produce the greatest biomass in the Lake. *M. spicatum* was the sole species growing in the two quadrants collected in the Annex (see Figure 9).

*N. guadalupensis*, the second largest biomass producer in the Lake, surrounded the delta at mid-Lake and formed a bottom community below the *N. odorata* stands. *Chara spp.* also formed dense mats at mid-Lake in shallow shoreline waters and its presence was noted around and within the *N. odorata* beds that surrounded the small island in the northeastern corner of the Lake.
Figure 8: Total dry weights (g) of macrophyte species collected from the Murphy’s Bottom Lake as a result of quadrant sampling. Error bars represent the standard error of the mean.

Figure 9: Total dry weights (g) of macrophyte species collected from the Murphy’s Bottom Annex as a result of quadrant sampling. A dominant species, and the sole species collected in the Annex, is *M. spicatum*. Error bars represent the standard error of the mean.
Figure 10: Map depicting the macrophyte distribution in late July/early August 2009. Quadrant sampling sites are labeled with black squares. Gray lines represent changes in water depth. Contour data provided by Lennon, Smith, Souleret Engineering, Inc. Coraopolis, PA.
*M. spicatum* and Lemnaceae (duckweed) covered the majority of the Annex. *M. spicatum* grew at extraordinary densities throughout the Annex. *L. palustris* was located in the very shallow water (less than one foot) at the far eastern end of the Annex. During a few weeks of the late summer months during low water this area of the Annex became very shallow, less than three inches, and the *L. palustris* population declined.

Using the sediment texture data collected with the copper pole method a layer was added to the ArcView GIS map to reflect the textures of bottom sediment in the Lake and Annex. There was distinct variation in sediment textures throughout the Lake whereas the Annex was more homogeneously stony with finer silt. Figure 11 shows the macrophyte beds with an additional layer depicting the varying sediment textures in the Lake and the Annex.
Figure 11: Map depicting the various sediment textures throughout the Lake and Annex at the Murphy's Bottom site. Quadrant sampling sites are labeled with black squares. Gray lines represent changes in water depth. Contour data provided by Lennon, Smith, Souerel Engineering, Inc., Coraopolis, PA.
Surrounding the delta and in the northwestern portion of the Lake the sediment texture was muddy. The eastern end of the Lake had a silty texture and the *N. odorata* created a dense macrophyte root mat. In the southeastern portion of the Lake where the *Z. dubia* had propagated where the sediment was very dark and fine. The small inlet in the south portion of the Lake contained *M. spicatum* and small patches of *N. odorata* but was otherwise free of macrophytes. The sediment in the inlet had a dense clay-like texture. The Annex had a homogeneous bottom type consisting of stones and silt. Of particular interest is the correlation of depth with macrophyte presence and proliferation. A visually noticeable correlation in Figure 11 exists between the macrophyte beds and the contour lines of the Lake.

### 3.2 Total phosphorus analysis of macrophytes

Macrophyte quadrant sampling uncovered the presence of two overlooked species (*Chara spp.* and *E. canadensis*) and supported the findings of the other eight species noted during the macrophyte mapping. Figures 11 through 17 reflect the varying macrophyte community compositions of each of the five sampling quadrants and the percentages on the graphs represent the species’ contribution of total phosphorus to the quadrant. The Strickland and Parsons’ (1972) method was used to determine total phosphorus concentration of the plants.
Figure 12: Breakdown of phosphorus contributed by each macrophyte species collected from Quadrant 2. Quadrant 2 was located at the eastern end of the Lake at GPS coordinates: 40.693618°, -79.63419°. Chart colors for each macrophyte species are the same as those in Figure 10.

Figure 13: Breakdown of phosphorus contributed by each macrophyte species collected from Quadrant 3 located east of the delta at the center of the Lake at GPS coordinates: 40.693569°, -79.634496°. Chart colors for each macrophyte species are the same as those in Figure 10.
Figure 14: Breakdown of phosphorus contributed by each macrophyte species collected from Quadrant 4 located to the west of the delta at coordinates: 40.696793°, -79.635955°. Chart colors for each macrophyte species are the same as those in Figure 10.

Figure 15: Breakdown of phosphorus contributed by each macrophyte species collected from Quadrant 5 located to the west of the delta at coordinates: 40.693205°, -79.634528°. Chart colors for each macrophyte species are the same as those in Figure 10.
Figure 16: Breakdown of phosphorus contributed by each macrophyte species collected from Quadrant 6 located to the east of the beaver dam in the southeastern corner of the Lake at coordinates: 40.693734°, -79.637658°. Chart colors for each macrophyte species are the same as those in Figure 10.

Figure 17: Breakdown of phosphorus contributed by each macrophyte species collected from Quadrant 7 located in the Annex at coordinates: 40.692715°, -79.634861°. Chart color for the species is the same as that in Figure 10.
Figure 18: Breakdown of phosphorus contributed by each macrophyte species collected from Quadrant 8 located in the Annex at coordinates: 40.692520°, -79.635252°. Chart color for the species is the same as that in Figure 10.

*N. guadalupensis* surprisingly contributed the greatest quantity of phosphorus in Quadrant 2, which was located in an area with dense floating-leafed *N. odorata*. *Chara* spp. and *E. canadensis* contributed a very small percentage of the nutrient. Quadrant 3 was located to the east of the delta and was composed of primarily *N. odorata* and *N. guadalupensis*. *E. canadensis* and *M. spicatum* grew sparsely. *N. odorata* was the primary species in Quadrant 4 and contributed 76% of the total phosphorus. *N. guadalupensis* and *Chara* spp. were present but were not strong nutrient contributors. *M. spicatum* was the dominant species in Quadrant 5 located to the west of the delta. *Z. dubia* and *Chara* spp. were also present at the site but in meager quantities. Quadrant 6 was the most evenly partitioned with no species overtaking the site. *M. spicatum, E. canadensis* and *N. guadalupensis* contained the greatest amounts of phosphorus.

The phosphorus concentration data from the collected quadrant species was compiled to produce Table 5.
Table 5: Concentration of total phosphorus (mg P/g DW) in macrophyte species at the Murphy’s Bottom Lake and Annex. Total phosphorus determined with an adapted method from Strickland and Parsons (1972).

Table 5 shows that *N. odorata* contains the greatest amount of phosphorus per gram of dry weight. Although *M. spicatum* is one of the most abundant species it is not necessarily the largest sink for phosphorus. On average *N. odorata* contained the largest concentration of total phosphorus per gram of dry weight followed by *M. spicatum* and *E. canadensis*.

![Graph reflecting the average concentration of total phosphorus in macrophytes collected during quadrant sampling. Error bars represent the standard error of the mean.](image)

<table>
<thead>
<tr>
<th>Quadrant</th>
<th><em>Nymphaea odorata</em></th>
<th><em>Najas guadalupensis</em></th>
<th><em>Myriophyllum spicatum</em></th>
<th><em>Elodea canadensis</em></th>
<th><em>Chara</em></th>
<th><em>Zosterella dubia</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration of Total Phosphorus (mg P/g DW)</td>
<td>2.78</td>
<td>1.58</td>
<td>0.94</td>
<td>1.63</td>
<td>0.87</td>
</tr>
<tr>
<td>2</td>
<td>1.91</td>
<td>1.30</td>
<td>1.00</td>
<td>1.96</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.30</td>
<td>0.75</td>
<td>1.02</td>
<td>0.80</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>4</td>
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<td>1.87</td>
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<tr>
<td>5</td>
<td>0.94</td>
<td>0.66</td>
<td>0.71</td>
<td>0.66</td>
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<td>6</td>
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<td></td>
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<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>1.99</td>
<td>1.13</td>
<td>1.22</td>
<td>1.27</td>
<td>0.74</td>
<td>1.50</td>
</tr>
</tbody>
</table>
After the macrophyte community was mapped at the Murphy’s Bottom site (Figure 10) and quadrant samples were collected, zones of similar community structure were outlined on an ArcView GIS map, shown as Figure 20. Zones were outlined based on areas of similar macrophyte community structure. The area (square meters) of each zone was determined with the ArcView GIS software program.

Next the dry weights of all similar species collected from the quadrants were averaged and a percentage was created to reflect the average representation of each species in each zone. This value was calculated based on the total dry weights of each species per quadrant. See Table 6 for calculations.
Figure 20: Map depicting macrophyte beds and various zones used to calculate biomass and total phosphorus of the Lake system. Zones were determined based on macrophyte species composition in the area. Quadrant sampling sites are labeled with black squares. Contour data provided by Lennon, Smith, Souleret Engineering, Inc., Coraopolis, PA.
Table 6: Extrapolation of data to determine the amount of total phosphorus in the Murphy’s Bottom Lake and Annex. Percent representation of each macrophyte species per zone was calculated based on the average representation of species in the sampling quadrants. Total area (m²) of the species in each zone was determined by multiplying the total area of the zone by the average percent representation in the quadrants. Average mg TP/m² was determined by averaging the TP of the species collected in the quadrant samples. The average mg TP/m² was determined by multiplying the average TP for each species by the species area of the zone. The average TP concentration of each zone was added to determine the amount of TP in the system.
3.3 Total phosphorus concentration of water

Total phosphorus concentration in the four water bodies at the Murphy’s Bottom site fluctuated dramatically within the five-month study period. Figure 18 reflects the changes in concentration.

![Graph showing concentration of total phosphorus](image)

Figure 18: Concentration of total phosphorus from water samples in the Lake, Annex, Allegheny River and Turning Basin between 20 June 2009 and 28 October 2009. Water samples collected on 26 June, 2 July, 10 July and 31 July were analyzed by M. Ostrofsky at Allegheny College, Meadville, PA.

The phosphorus concentration in the Lake and Annex produced a similar trend as both were around 100 ug/L in mid-June, spiked in late July and remained around 150 ug/L for the remainder of the summer season. The Allegheny River usually contained the least amount of phosphorus although in very similar concentrations to the Allegheny River. A spike in phosphorus concentration was noticeable in mid-June and mid-July and in the Lake during the first week of October. The peak in concentration in late July in the Annex could have been due to the die-off of *M. spicatum* while the small increase in the
Lake total phosphorus concentration in early October may have been influenced by the *N. odorata* decay.

### 3.4 Total nitrogen concentration of water

The cadmium reduction method used by HACH to determine nitrates in the water column was slightly modified with the addition of persulfate to determine the concentrations of total nitrogen in the Allegheny River, Turning Basin, Annex and Lake during the sampling period of 20 June 2009 to 28 October 2009 as shown in Figure 13.

![Figure 19: Concentration of total nitrogen in the Lake, Annex, Allegheny River and Turning Basin between 20 June 2009 and 28 October 2009 as determined by the HACH cadmium reduction method.](image_url)

Figure 19 shows a number of interesting trends in total nitrogen concentrations within the four Murphy’s Bottom water bodies. Between 20 June 2009 and 24 July 2009 nitrogen concentrations increased dramatically and then plummeted in early August. Nitrogen in the Annex spiked in late August followed by another drop in concentration at all sample locations. From mid September until the end of October concentrations slowly increased. Both nitrogen and phosphorus increased in late July, which could signal the
die off of most macrophytes and the availability of phosphorus in the water column for a second wave of growth.

3.5 Turbidity of the Lake

The solar powered floating Sonde located on the Murphy’s Bottom Lake collects a number of water quality parameters and sends the data to an online database by satellite. The turbidity readings from each sampling date were compiled to produce Figure 20.

![Figure 20: Comparison of data collected with two different methods to determine the turbidity of the Lake between May and October 2009. The Sonde probe buoy located on the Lake transmitted real-time data to an online database by way of satellite. Secchi disk measurements were collected each week on site near the buoy at coordinates 40.693012°, -79.637157°.](image)

The turbidity of the Lake was relatively constant throughout the sampling period. Due to the low values of the original Secchi disk readings the data points were multiplied by ten to produce the red points in Figure 20. The average turbidity reading with the Secchi disk was 0.26 NTUs throughout the sampling season. Turbidity readings spiked
on the Sonde probe on 3 September 2009 and 9 October 2009 but this was most likely
due to the low water levels in the Lake and the occasional contact of the probe with the
sediment due to wave action.
Numerous laboratory and field studies have been conducted in an attempt to determine the role of macrophytes in the cycling of nutrients within limnetic systems. Due to the proposed habitat restoration project and potential connection of the Murphy’s Bottom Lake with the Allegheny River it was imperative to determine a baseline species list for aquatic macrophytes and quantify the concentrations of phosphorus and nitrogen that are incorporated into the hydrologic systems of the Allegheny River, Turning Basin, Lake and Annex.

The macrophyte mapping method in late July and early August 2009 determined that the Annex supports only three aquatic plant species (*M. spicatum*, *L. palustris*, and Lemnaceae). Biodiversity is a key component of a healthy ecosystem and a monotype such as *M. spicatum* in the Annex should be noted and potentially addressed. It is not likely that the Lake will become inundated with *M. spicatum* at the same scale as the Annex due to the differences in sediment and water volume but there should be concern that this exotic species is out-competing the native species for resources. Newroth (1985) found that *M. spicatum* invasions have the potential to alter the aquatic macroinvertebrate community and deter fish species from spawning (as cited in Madsen et al., 1991). A possible addition to the current proposed habitat restoration plan at the Murphy’s Bottom site could be the eradication of *M. spicatum* in the Annex.

The general biodiversity of the Lake is encouraging. A specific observation is the similar distribution of *N. odorata* and *N. guadalupensis* (Figure 10). In most areas of the Lake where floating-leafed *N. odorata* beds existed, *N. guadalupensis* grew amongst the shoots in great densities. These areas of the Lake may be richer in nutrients and capable
of supporting a larger biomass of macrophytes. *N. guadalupensis* may be able to tolerate the lower light conditions. Differences in sediment texture and sediment nutrient concentrations could also be contributing factors. Figure 11 reflects the various sediment textures in the Lake and the Annex. Macrophytes grow in a distinct pattern around the Lake shore and only grow in high densities out to approximately 15 feet from shore, or four feet in water depth.

*M. spicatum, N. guadalupensis* and *N. odorata* were the three greatest biomass contributors to the Lake and Annex. The result was surprising due to the noticeably large beds of *N. odorata* that are spread around the Lake. Interestingly, *N. odorata* is the most involved with phosphorus retention (Table 5) whereas *M. spicatum* may be contributing the most phosphorus to the water column during summer season die-offs. *M. spicatum* died off in mid-July and mid-September in the Annex. The earliest die-off was reflected vividly in the total phosphorus concentration of the Annex water column at that time (Figure 18).

Differences in same-species nutrient concentrations (Table 5) were noted and may be related to sediment nutrient concentrations or natural variation within plant tissues. *M. spicatum*’s primary pathway for phosphorus uptake is from sediment by roots (Barko and Smart, 1980; Carignan and Kalff, 1980; Carignan, 1982; as cited in Smith and Barko, 1990). The macrophyte mapping procedure and sediment texture quantification determined that bottom-type variation does exist in the Lake and could possibly contribute to macrophyte distribution. A future project at the Murphy’s Bottom site should be to collect sediment core samples from each macrophyte quadrant and analyze for phosphorus and nitrogen.
According to Lougheed, Crosbie and Chow-Fisher (2001) the most important predictors of macrophyte distribution are total suspended solids (turbidity), total phosphorus and total nitrogen. But phosphorus does not seem to be the limiting nutrient in macrophyte population at the Murphy’s Bottom site. The total phosphorus concentrations of the Lake and the Annex were quite similar throughout August, September and October with the Lake containing slightly greater concentrations (Figure 18). If phosphorus in the water column was the limiting nutrient for macrophyte growth then the Annex would have less biomass, which is not the case. However, most macrophytes acquire their nutrients from the sediment.

Although the graph of the concentration of total nitrogen in the water samples from the Allegheny River, Turning Basin, Annex and Lake shows vivid peaks, the scale is rather small. Overall the total nitrogen concentration did not fluctuate more than 1.4 mg/L the entire summer. The concentrations of nitrogen in the Lake and the Annex mirrored one another. This factor reduces the importance of nitrogen as a limiting nutrient reagent for native macrophyte growth in the Annex because such species grow in the Lake with similar nitrogen availability (Figure 10).

Results from a study by Lougheed, Crosbie and Chow-Fisher (2001) in the Great Lakes region indicated that *P. crispis* and *N. odorata* can tolerate a turbid, nutrient rich water column. These species as well as *E. canadensis* and *C. demersum* are categorized as somewhat tolerant to turbidity and eutrophication. *M. spicatum* (a dominant species in the Annex) was categorized as a very tolerant species to turbidity due to its ability to survive on the surface in light limiting situations (Lougheed, Crosbie and Chow-Fisher, 2001). The Annex Secchi disk measurements throughout the summer revealed that the
water column is relatively free of suspended solids. On most sampling dates light reached the bottom in areas absent of *M. spicatum*. Light penetration is not the limiting factor of macrophyte biodiversity within the Annex in the few areas free of the exotic species.

A possible concern is that the proposed connection of the Allegheny River to the Annex will cause an influx of nutrients from the Allegheny River into the Annex and result in eutrophication and algal blooms. However, the results of the water sampling show that the Allegheny River actually contains lower concentrations of nitrogen and phosphorus than both the Murphy’s Bottom Lake and Annex (Figure 18).

According to Thiébaut (2005) plant tissue nutrient concentration is lowest in the spring and highest in the fall. Because the plant specimens at Murphy’s Bottom were collected in late August, September and early October the concentration of phosphorus in the tissues should be high.

Thiebaut (2005) determined total phosphorus in *E. canadensis* to be between 5.13 and 9.25 mg P/g DW using a perchloric and nitric acid digestion followed by a molybdenum blue technique. Samples were taken from northeastern France. *E. canadensis* from the Murphy’s Bottom Lake contained between 0.71 and 1.96 mg P/g DW.

Kufel and Kufel (2002) cited three authors who determined nitrogen and phosphorus concentrations in *M. spicatum* to be between 16.7 and 41.4 mg N/g DW for nitrogen and 0.3 and 5.0 mg P/g DW for phosphorus (Boyd, 1967; Riemer and Toth, 1968; and Bernatowicz, 1969). The values for total phosphorus in *Myriophyllum spicatum* tissues at the Murphy’s Bottom site were between 0.64 and 2.41 mg P/g DW.
and compare well with the published boundaries. Concentration of nitrogen in plant tissues was not determined.

Pereyra-Ramos (1981) noted that Chara biomass in late fall was half of that of the maximum stand in July implying that half of the nutrients remain stored in the plants from year to year (as cited in Kufel and Kufel, 2002). Due to the minimal root structures of Chara it is likely that their nutrients originate in the water column. Compared to most vascular plants, Chara species are more likely to act as nitrogen and phosphorus sinks due to their overwintering capabilities (Kufel and Kufel, 2002). It will be interesting to note if Chara bed location and density remains constant from year to year in the Lake if water nutrient concentrations change due to the proposed connection with the Allegheny River.

A primary objective of this research was to produce methods that would facilitate the determination of the total amount of phosphorus in the Murphy’s Bottom Lake and Annex systems as a whole and to establish which macrophytes are the largest sinks for the nutrient. The delineation of the five similar-macrophyte-community zones (Figure 20) in combination with the average biomass of species per sampling quadrant (Figure 19) was able to produce the data in Table 6. Even though N. odorata contained more phosphorus per gram of biomass it did not contribute as much phosphorus to the system as the M. spicatum. Z. dubia contained the second highest phosphorus concentration per gram but due to its low density was not a major contributor. Because Chara spp. and E. canadensis were not visible at the time of macrophyte mapping it was impossible to incorporate their phosphorus content into the final tabulation. By combining the total
phosphorus amounts of all five zones in Table 6 it was determined that the macrophytes growing in the Lake and Annex contain a total of 3,326 grams of total phosphorus.

The greatest hurdle in the completion of this project was the construction of replicable methods for determining total phosphorus in water and plant samples and total nitrogen concentrations of water samples. The original aim was to also determine total nitrogen concentrations in plant samples but the method could not be finalized within the available timeframe.

The Strickland and Parsons (1972) molybdenum blue method is most accurate with larger cuvettes (>3cm) but these were not available. Future studies should use 2.5 cm cuvettes or longer to produce more accurate total phosphorus results (Standards Methods for the Examination of Water and Wastewater, 2005).

The spectrophotometer was originally producing extremely high absorbances for total phosphorus and it was difficult to determine why the standards were not corresponding to those in the literature at the correct 880 nm wavelength. After testing a number of possible problems it was determined that the spectrophotometer had not been cleaned or calibrated in many years. After calibration the readings were accurate.

Two additional macrophyte species were found in the Lake during quadrant samplings that were overlooked with the rowboat mapping method. In the future it may be beneficial to use both the rowboat method and snorkeling in tandem to ensure that all species are assessed. The use of snorkeling equipment ensures that the smaller profile plants such as *E. canadensis*, *N. guadalupensis* and *Chara* are not overlooked.

A byproduct of this project is a set of replicable methods for the determination of total nitrogen and total phosphorus in water and plant tissues. Many weeks were spent
tweaking the phosphorus standards and determining how much plant material was usable to produce results within the readable range of the Strickland and Parsons (1972) method. Future studies should be completed to produce more easily replicable methods for the determination of total nitrogen in the lab.

The purpose of this research was to analyze the macrophyte community at the Murphy’s Bottom site and determine its role in the cycling of phosphorus and nitrogen in the limnetic system. This was accomplished by compiling a macrophyte species list for the Murphy’s Bottom site, formulating specific and replicable methods for analyzing phosphorus and nitrogen in the water columns and investigating which macrophytes have greatest influence on the phosphorous cycling in the Lake and Annex systems.
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