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Biological surface water treatment : replacing copper sulfate with aquatic macrophytes for the control of micro-algae in surface water

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BIOLOGICAL SURFACE WATER TREATMENT:
Replacing Copper Sulfate with Aquatic Macrophytes for the Control of Micro-algae in Surface Water

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ABSTRACT

BIOLOGICAL SURFACE WATER TREATMENT
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This thesis examines the results of a project using aquatic vegetation, specifically *Ceratophyllum demersum* and *Elodea densa*, instead of copper sulfate, to control micro-algae blooms and problem macrophytes that are responsible for the eutrophication of surface waters.

Weekly concentrations of phosphate and nitrate in both the project and control lakes were determined, coupled with observations of water clarity, and a laboratory analyses of phytoplankton abundance throughout the 1993-94 data collection year.

The results indicate that the phosphate levels in the project lake were significantly lower than those in the control lake (t=3.65; df=42; α=0.05). The difference in nitrate concentrations between the two lakes, however, was not significant (t=0.49; df=42; α=0.05). Observations suggest that the aquatic macrophytes, *Ceratophyllum demersum* and *Elodea densa*, were successful in competing with micro-algae in the project lake. Phytoplankton analysis showed a significant increase in species diversity once copper sulfate applications ceased.

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CHAPTER I

INTRODUCTION

There is increasing concern over the state of our environment and how we manipulate it to serve human needs. Water, the common denominator for sustaining life, is one of our most abused resources, and it is frequently necessary to alter its characteristics in order to make it fit for human consumption. This alteration is customarily accomplished with chemicals that are frequently harmful to the aquatic ecosystem. Therefore, it is preferable to discover organic avenues of managing our surroundings and the available resources that are required for survival.

Surface water bodies become eutrophied through the addition of nutrients. These nutrients, such as phosphate and nitrate, cause micro-algae growth which reduces the water quality. Current surface water quality management commonly involves treating these problem areas with algaecides, especially copper sulfate ($CuSO_4$), in order to meet drinking water standards. The use of aquatic herbicides is simply a quick solution to a long-term problem. This treatment method can actually aggravate the problem because micro-algae are known to develop tolerances to chemicals, and concentrations of toxic substances must be increased to control them. Bioaccumulation of these toxic substances should also be taken into consideration because some constituents accumulate in ever-increasing concentrations as they move up the food chain.

Replacing chemical control methods with biological methods is of interest to water management companies for the following reasons. First, as drinking water regulations continue to become more stringent, it is necessary to determine alternate algae control methods that do not impair water quality by introducing potentially toxic substances to water used for human consumption. Second, aquatic life is often more sensitive to foreign chemicals than the human organism and must also be considered in any type of environmental management practice. The application of copper sulfate to fresh water for control of algae at rates ranging from 0.3 to 2.0 mg/l[1] could be fatal not only to algae but also to nontarget organisms like bacteria, plankton, fishes, and other aquatic life (Nor 1987). Using a natural means to treat domestic water supplies will put water companies/managers a step ahead of the agencies and the contemporary movement to protect wildlife.

Biological lake management offers an opportunity to naturally balance the nutrient load within an aquatic

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[1] mg/l = milligrams per liter (equivalent to parts per million (ppm)).

ecosystem in a manner that is both economically favorable and environmentally sound. The success of biological surface water treatment will move the human population away from the trend of jeopardizing the welfare of wildlife in the name of human safety and convenience. We will only benefit in the long run if all organisms are kept as healthy as possible.

Background

Phosphorus is a biologically active element with many natural sources including organic matter decomposition and leaching from sediments (Lind 1979). Other phosphate sources are related to land use practices such as fertilizers used in agriculture, and urbanization. Domestic sources such as sewage and detergents also contribute to the phosphate load. Phosphorus and nitrogen are the elements most likely to encourage stimulation of plant production and are often linked to excessive plant production and problems associated with eutrophication (Goldman and Horne 1983). Phosphorus is the nutrient of greatest concern since it is usually the factor limiting plant production in lakes (Goldman and Horne 1983). For example, for optimum growth, micro-algae requires a ratio of 40 C(carbon):7 N(nitrogen):1 P(phosphorous) (Lind 1979). Therefore, in theory, limiting the phosphorus concentration in surface water should reduce eutrophication, and if managed properly, should create an environment conducive to multi-purpose resource utilization. This theory has been tested and is well-documented in wastewater treatment systems that use aquatic macrophytes as a means for nutrient removal. Middlebrooks and Reed (1981) discuss the effectiveness of water hyacinths for reducing contaminants in wastewater treatment systems to levels equivalent to secondary, advanced secondary and tertiary effluents. Similarly, Sinclair and Forbes (1980) argue in favor of using aquatic plants for nutrient removal because they remove many nutrients capable of stimulating micro-algae growth.

The Nutri-Pod system incorporating *Ceratophyllum demersum* and *Elodea densa* was developed by Limnion Corporation in 1986 to offer a viable alternative to chemical control methods. Previous to 1986, Limnion used similar technology in aquariums and zoological exhibits to improve water quality (Murray, telephone interview, April 1994). The first lake project utilizing the Nutri-Pod method of improving and maintaining aesthetic water quality took place in 1986. This technology has primarily been utilized by housing developments for ornamental lakes. Some of the development agencies that have incorporated this technology are: Grupe Development Company, River West Developments, U.S. Department of Energy, Georgia Pacific

Corporation, and Wilson Construction (Limnion 1990a). The species, *C. demersum* and *E. densa*, utilized in the Nutri-Pod system, have the ability to compete with micro-algae and other aquatic vegetation for nutrients, thus suppressing the eutrophication process.

It is widely accepted that aquatic plants absorb nutrients from both water and sediments more rapidly than micro-algae (Land and Water 1992). Submerged aquatic vegetation provides higher absorption rates per unit area than floating vegetation, which is limited to the volume or surface area they may develop at the water surface (Limnion 1991). Therefore, the Nutri-Pod system of containing submerged aquatic plants, such as *Ceratophyllum demersum*, that are known for high phosphorous uptake could be a successful venture.

The effectiveness of macrophytes in contaminant removal varies according to the treatment system design which depends on the desired contaminant removal goals (Reddy and DeBusk 1987). For the removal of phosphorus, the preferred system designs are those which optimize conditions for plant uptake. The basic function of plants is that of assimilating, concentrating, and storing contaminants (phosphates and nitrates), on a short-term basis (Reddy and DeBusk 1987). Subsequent harvest of plant biomass results in permanent removal of stored contaminants from the treatment system. The Nutri-Pod system offers a design that incorporates the storing and ensuing harvesting or removal of nutrients from the water body.

CHAPTER II

RELATED RESEARCH

Nutrients

The cycle of phosphorus in the biosphere is primarily regulated by biological uptake, precipitation, and one-way flow to sediments and the sea (Vallentyne 1974). The main inputs of phosphorus into lakes are from inflowing rivers and precipitation. However, precipitation is not as important to phosphorus inputs as it is for nitrogen. An additional source of phosphorus is from the excretion and decomposition of aquatic vegetation. Both sediment resuspension and plant decomposition can be thought of as recycling processes that contribute phosphorus to the open water from sediment (Rutherford et al. 1989; Dierberg 1992). Shallow lakes pose the additional problem of having greater percentages of their water mass in direct contact with the bottom sediments, unlike a deep lake. This contact permits the more efficient transfer of nutrients such as phosphorus and nitrogen from the sediments into the lake waters, creating an extra source of nutrient input (Goldman and Horne 1983).

The trophic status of a lake is related to the concentrations of nitrogen and phosphorus; the change from oligotrophy to eutrophy involves a marked increase in the concentrations of these nutrients (Rutherford et al. 1989). Natural phosphorus inputs to lakes are generally insufficient to supply the potential growth of plankton given the amount of available nitrogen. Eutrophication appears to be dependent upon the phosphorus balance (Petts 1988).

Nitrogen is always present in aquatic ecosystems, but is most abundant in the gas phase. Living plant cells contain about 5 percent total nitrogen by dry weight (Goldman and Horne 1983). The availability of various nitrogen compounds influences the variety, abundance, and nutritional value of aquatic animals and plants. Nitrogen is often present in quantities which can limit plant growth. In aquatic systems nitrogen fixation and denitrification are the ultimate sources and sinks of combined nitrogen available to micro-algae (Petts 1988; Rutherford et al. 1989). Nitrogen gas, although abundant in water, is almost inert and is normally utilized for growth in lakes only by some blue-green algae through the process of nitrogen fixation. Nitrogen fixation is a process restricted to a few genera of bacteria and blue-green algae and is defined as the transformation of nitrogen gas to ammonia by an enzyme (Goldman and Horne 1983; Rutherford et al. 1989). Nitrogen fixation is important because it is a major source of new, usable nitrogen. This process can accelerate lake eutrophication. In very eutrophic lakes, nitrogen fixation may be the major source of nitrogen (Rutherford et al. 1989).

Phosphorus is not needed for growth in large quantities like carbon, oxygen, hydrogen, and nitrogen, and is one of the more common limiting elements in freshwater (Goldman and Horne 1983). Most of the phosphate goes into bacteria, micro-algae, or other plants and some is precipitated or adsorbed by physiochemical processes (Goldman and Horne 1983; Dierberg 1992). The N/P ratio for aquatic plant growth is typically 7:1 by weight or 16:1 by element; thus it can be seen that phosphorus depletion is likely in many fresh waters (Lind 1979; Goldman and Horne 1983). There are three forms of phosphorus generally measured in aquatic ecosystems: dissolved orthophosphate (phosphate or PO_4), dissolved total phosphorus, and particulate phosphorus. Only dissolved phosphate can be used directly by micro-algae for growth (Goldman and Horne 1983).

Although phosphorus is often deficient in lake water, micro-algae have evolved devices for overcoming these deficits. One device is termed *luxury consumption*. This process entails the uptake of more phosphate than is required for growth and its consequent storage with the cell (Vallentyne 1974; Goldman and Horne 1983). Dissolved phosphate is often low during the appearance of micro-algae blooms because the phosphate is contained in the algae. Another evolutionary adaptation by which algal species compensate for low phosphate levels is the enzyme, *alkaline phosphatase*. This enzyme breaks the bond between phosphate and the organic molecule to which it is attached in organic phosphate complexes. The result is free phosphate available for plant growth (Goldman and Horne 1983).

The removal of both nitrogen and phosphorus from eutrophic lakes is recommended for several reasons. The removal of phosphorus alone may produce no measurable improvement in lake conditions unless phosphorus is the limiting nutrient. The removal of nitrogen alone may not be sufficient because nitrogen-fixing organisms could increase the nitrogen content of the lake, and nitrogen control would reduce the N:P ratio in the lake, thus favoring those blue-green algae which can fix atmospheric nitrogen (Rutherford et al. 1989).

Aquatic Plants and Water Treatment

Aquatic plants have been used for small-scale wastewater treatment for the past several years. Studies have shown that aquatic macrophyte-based treatment systems offer a promising, low-cost method for removing contaminants from wastewaters and polluted natural waters (Reddy and DeBusk 1987). Aquatic plants possess a remarkable ability for assimilating nutrients and creating favorable conditions for microbial decomposition of organic matter. This ability can be exploited in the restoration process of natural streams, lakes, and wetlands, and in wastewater treatment systems (Middlebrooks and Reed 1981; Brix and Schierup 1989). The potential for resource recovery by harvesting and utilizing the plant material produced can be regarded as a step in the direction of a holistic solution where waste products will be regarded and utilized as a resource (Brix and Schierup 1989).

Shallow, eutrophic, aquatic ecosystems stocked with macrophytes are among the most productive in the world (Brix and Schierup 1989). Much of the attention focused on vascular aquatic plant has been directed toward their elimination from water bodies, since dense stands of aquatic vegetation can impede navigation and threaten the balance of biota in aquatic systems. Despite their nuisance characteristics, the high productivity and nutrient removal capability of many aquatic plants has created substantial interest in their photosynthetic and physiological characteristics and in their potential use for beneficial purposes (Reddy and DeBusk 1987). The significance of macrophytes in nutrient removal varies according to the treatment system design which depends on the desired contaminant removal goals. For instance, Sinclair and Forbes (1980) discuss the use of vascular aquatic plants for nutrient removal because they remove many nutrients capable of stimulating micro-algae growth. When listing considerations for selecting possible plants for nutrient removal, Sinclair and Forbes (1980) suggest that preferred plants should have large standing crops, rapid growth rates, be easy to harvest, have high nutritive value, and have an economical use after harvest. To this list, Reddy and DeBusk (1987) add having the ability to adapt to local climate, resistance to pests and disease, and tolerance to adverse concentrations of pollutants.

The water hyacinth (*Eichhornia crassipes*) is one of the most popular aquatic plants used in wastewater treatment systems. The water hyacinth is one of the most prolific and productive plants in the world, and is often considered a serious weed in the tropical and subtropical regions (Reddy and DeBusk 1987). However, its high productivity can and has been exploited in wastewater treatment facilities (Middlebrooks and Reed 1981; Reddy and DeBusk 1987; Brix and Schierup 1989). Water hyacinths control micro-algae in wastewater effluent by limiting the light available to the algae (Gupta 1982). Nutrient removal is a result of hyacinth growth, physiochemical reactions, and accumulation by other organisms growing in the ecosystem (Middlebrooks and Reed 1981). Documented performance of the water-hyacinth-based system indicate that more than 0.8 g N and 0.15 g P $\cdot$ m^{-2} $\cdot$ d^{-1} can be removed by harvesting (Sinclair and Forbes 1980; Middlebrooks and Reed 1981; Gupta 1982; Reddy and DeBusk 1987; Brix and Schierup 1989). Brix and Schierup (1989) suggest growing water hyacinths in tertiary treatment effluent, rich in nutrients. Nitrogen and phosphorus are removed as they are incorporated into water hyacinth biomass. The biomass is then harvested frequently to sustain maximum productivity and to remove incorporated nutrients.

Submerged aquatic plants have also been documented as having a high affinity for nutrient uptake. However, since submerged plants only grow well in oxygenated water, they cannot be used in wastewater with a high content of readily biodegradable organic matter because the microbial decomposition of the organic matter will create anoxic conditions (Brix and Schierup 1989). The prime potential use of submerged macrophyte systems is for the "polishing" of secondarily treated wastewaters or as a final step in integrated systems (Brix and Schierup 1989). In order to achieve effective nutrient removal, the biomass must be harvested; otherwise the amount of nutrients assimilated by the plants would be released to the water upon decay of the dead plant material. *Ceratophyllum demersum* and *Elodea* species are often considered ideal macrophytes for water treatment systems(Reddy and DeBusk 1987; Brix and Schierup 1989).

Macrophyte-based wastewater treatments systems have several potential advantages and disadvantages compared to conventional treatment systems. The advantages as described by Brix and Schierup (1989) include low operating costs, low energy requirements, the ability to establish plants at the site where the wastewater is produced, flexibility, and lower susceptibility to shock loading. The disadvantages include the increased land area required compared to conventional systems, decreased performance during winter in temperate regions, and the fact that macrophyte based systems are more suitable for smaller contributors (small villages, amusement parks, camping sites, small industries) than for larger cities or industries (Brix and Schierup 1989).

Nutri-Pod Technology

Limnion Corporation investigated the feasibility of controlling pollutants and nutrients that cause eutrophication through biological means. Limnion's Nutri-

Pod technology is an evolution of the technology they developed for aquatic life support systems design and manufacturing, and industrial water filtration (Murray, telephone interview, April 1994). A Nutri-Pod is a fiberglass mesh sphere used to hold submersible plants that can consume large quantities of nutrients. The spheres range in diameter from three to 12 feet (0.914 to 3.66 meters), and remain below the water surface (Murray 1992). They have had several successful projects using the Nutri-Pod method in man-made lakes in which both humans and wildlife benefited. For example, the Groupe Development Company enlisted Limnion's expertise to control a problem lake in one of their housing developments in Sacramento, California. Limnion introduced the Nutri-Pod technology into the lake, along with annual harvesting of rooted aquatic vegetation. Approximately 75% of the aquatic plants within the Nutri-Pods are removed every few months and are then used as seed for other Nutri-Pods. The introduction of this system has eliminated chemical control of micro-algae since 1988. The water is reportedly clear, with 20 to 30 feet of visibility, and the lack of chemicals has allowed the development of a healthy aquatic ecosystem. Limnion has had similar successful tests of the Nutri-Pod system. Recreational value and wildlife habitat were restored to different developments using the Nutri-Pod method of establishing environments limited in both nitrogen and phosphorous, effectively preventing micro-algae growth from occurring in nuisance quantities. Experiments in ornamental lakes found in housing developments demonstrated that within a ten day period, total N within the experimental lake was reduced by 87% and total P was reduced by 88% (Limnion 1990b). Limnion's successful projects have involved ornamental lakes where high nutrient concentrations are problematic to aesthetic and recreational uses. Limnion projects are based upon the fact that aquatic vegetation has an impressive ability to accumulate nutrients and trace metals in concentrations thousands of times higher than the surrounding water (Limnion 1990b).

The Lake Kittridge project, initiated by the San Jose Water Company, has expanded upon the Nutri-Pod system. This experiment tests Nutri-Pod effectiveness and limitations in nutrient removal in a natural lake environment that is surrounded by a moderately-sized watershed (200 acre). The parameters which were monitored were chosen to determine if this method of surface water management is sufficient for precluding the use of algaecides (copper sulfate) to prevent algal blooms in multi-purpose (fisheries, drinking water, aesthetic, recreational) reservoirs. Although Nutri-Pod technology has been incorporated into the management of new man-made lakes in such areas as housing developments, whether or not this type of lake management could be successful in a natural eutrophic lake is not known.

One difference between an ornamental lake and one that is to be utitized as a domestic water source, is that the water quality of the latter must be maintained under federal, state, and local regulations. It is desirable to prevent algal blooms from occuring in an ornamental lake largely due to the odor and unsightly qualities that are thus produced. A surface water body that will be used for drinking water has the additional burden of combating the unpleasant tastes and odors that are associated with micro-algae. Besides controlling the nutrient levels in surface water in order to prevent micro-algae blooms, there is also the need to meet drinking water regulations which state that nitrate nitrogen (NO_3-N) must be kept to 10 mg/l in drinking water. This is due to the fact that excess nitrate can cause a blood disorder called methemoglobinemia or blue baby syndrome, which can be fatal to infants under four months old (USGS 1981; USEPA 1990). Such high levels of nitrate are not likely to be found in surface waters utilitzed for domestic water supply because this much nitrate would be accompanied by unacceptable algal growth and related problems.

Summary

There are numerous cases of the successful use of macrophytes to control nutrients levels. However, whether this method is appropriate for Bay Area drinking water supplies and whether *Ceratophyllum demersum* and *Elodea densa* are effective locally is not known. This research builds on the documented macrophyte water treatment systems, and reveals that this surface water treatment technology can meet standards for aquatic health and safe drinking water.

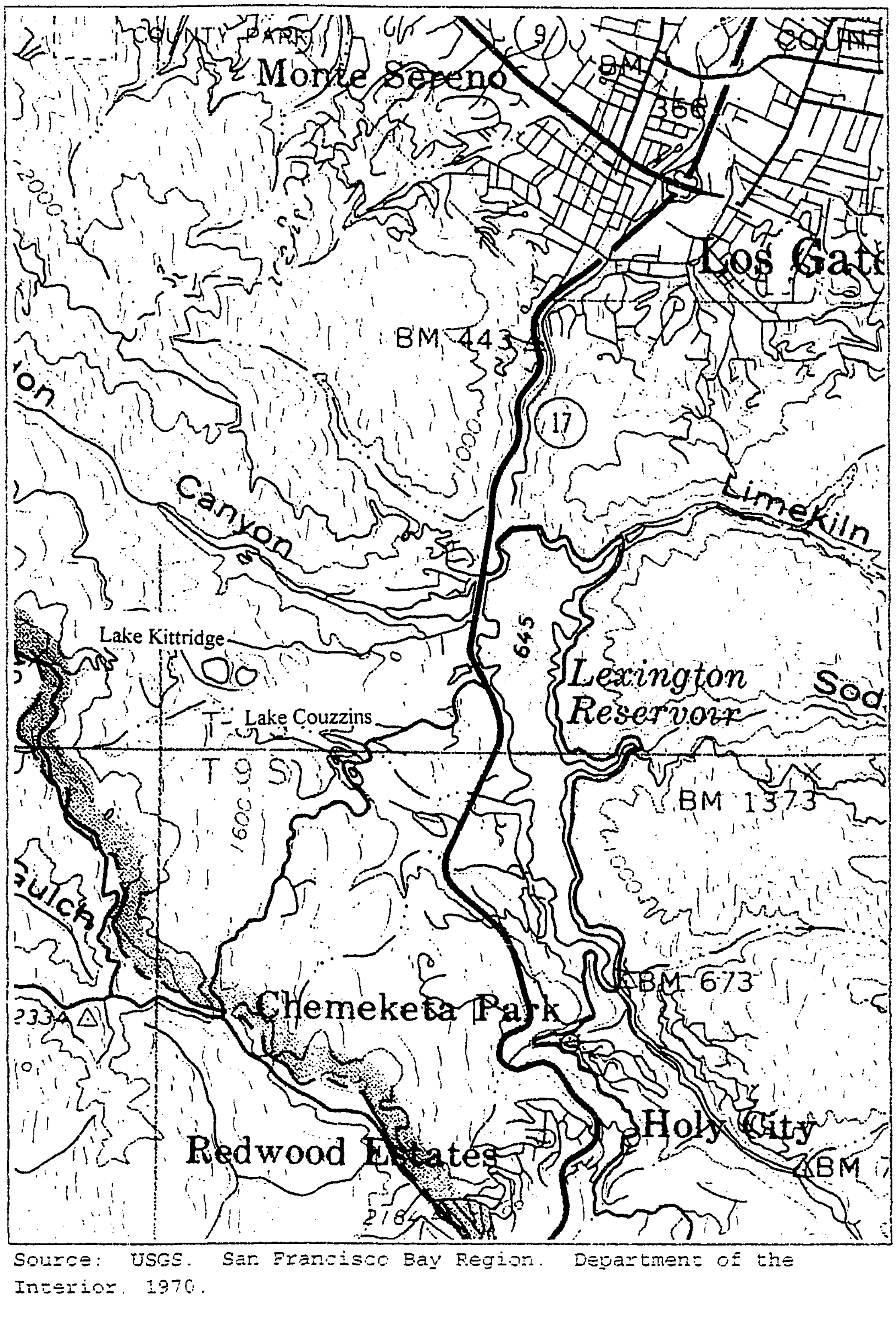
CHAPTER III

STUDY AREA

The study site is Lake Kittridge, a 12.5+ acre lake found on the northeastern slopes of the Santa Cruz Mountains off of Highway 17 near the town of Los Gatos (Figure 1). The lake is owned and managed by San Jose Water Company, a local water supplier, and is not open to the public. Lake Kittridge is surrounded by a 200-acre watershed, has a depth of 15 feet, and has the potential for eutrophication. It is a secondary domestic water supply for a private picnic area and at least four households in the area with additional residences pending (Yoo, personal communication, January 1993). As a potential water supply, the lake must be managed according to drinking water quality regulations. Part of this management has included using copper sulfate to control undesirable micro-algae.

The possible nutrient inputs into Lake Kittridge include Briggs Creek, which is partially diverted from its normal route to Lexington Reservoir into Lake Kittridge, an artesian source which is seasonal, nutrient cycling within the water column from the sediments at the lake bottom, and

Figure 1: Site Location Map



Source: USGS. San Francisco Bay Region. Department of the Interior, 1970.

general runoff from the surrounding watershed during the rainy season.

Immediately adjacent to Lake Kittridge, on its eastern side, is Lake Couzzins. This Lake is also approximately 12+ acres in surface area, and was created as a spill-over from Lake Kittridge. The possible nutrient inputs into Lake Couzzins include nutrient cycling within the water column from the sediments at the lake bottom, general runoff from the surrounding watershed during the rainy season, and from Lake Kittridge when the water level exceeds the spill-over.

The Nutri-Pod project was started in January of 1992 by San Jose Water Company and Limnion Corporation. Nutri-Pods were placed in Lake Kittridge, while Lake Couzzins was used as the control lake. Each Nutri-Pod is 6 feet in diameter and contains *Ceratophyllum demersum*. The concentration of pods is approximately one per acre for a total of 13, submersed six to eight feet below the surface. They are anchored to prevent them from floating to the surface. This depth allows for the most efficient nutrient uptake and permits adequate light to the plants, while maintaining the aesthetic and recreational values of the lake. The pods are inspected for growth, damage, and movement or drifting, and they are harvested, if necessary, to remove biomass and assimilated nutrients from the lake.

In January of 1992, five acres of *Elodea densa* were planted in the bottom sediments of Lake Kittridge. This planting was designed to work in conjunction with the *Ceratophyllum demersum* to absorb excess nutrients and to compete with the undesired aquatic plants: watermilfoil (*Myriophyllim spp.*), pondweed (*Potamogeton spp.*), and tules (*Scirpus actus*).

History of Copper Sulfate Applications

Historically, copper sulfate was applied to Lakes Kittridge and Couzzins in order to control algal blooms, thus delaying the symptoms of eutrophication. The control of micro-algae is futher desired due to taste and odor problems associated with mass quantities of micro-algae present in drinking water sources. Prior to 1992, copper concentrations in Lake Kittridge following treatments were as high as 0.33 mg/l in 1989. In October 1991, San Jose Water Company discontinued the copper sulfate treatment program and, afterward, copper levels in both lakes decreased to <0.05 mg/l and remained so (Yoo, personal communication, January 1993).

CHAPTER IV

METHODOLOGY

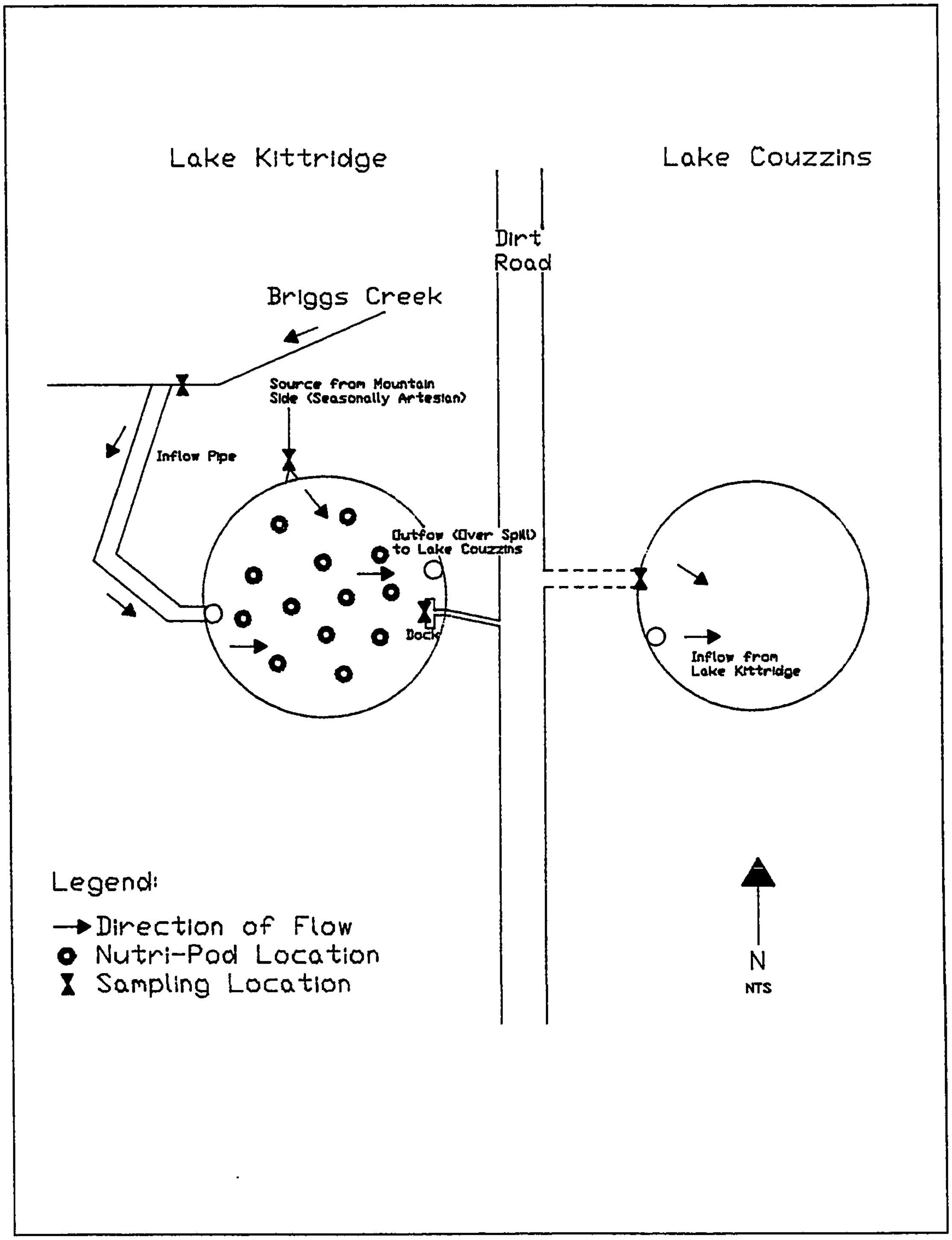
Nutrient Samples

From March 1993 to February 1994 Lakes Kittridge and Couzzins were tested weekly for nitrates and phosphates with a Hach[2] water test kit. The testing methods included simple grab samples at designated locations on shore, at various input sources, and from the control lake (Figure 2). Four samples were collected at each sampling location, two for each nutrient test, nitrate and phosphate. The two samples for each test were averaged then recorded for analysis. Observations of the conditions surrounding the lakes, weather, input sources, and Nutri-Pod placement were recorded. Samples were collected and analyzed on a monthly basis by Limnion Corporation, using the same Hach water test kit for analysis.

The nitrate test involved collecting a 5-mililiter (ml) water sample and adding a potassium phosphate based NitriVer3 Nitrite Reagent powder pillow. The sample is

[2] Hach Company facilities are certified to meet ISO 9001 and ISO 9002 international quality standards. ISO standards are a series of guidelines established by the International Organization for Standardization to provide industry with a baseline for achieving consistent levels of quality (Cooper 1994).

Figure 2: Sampling Location Map



shaken vigorously for one minute then stands for 10 to 20 minutes for color development. A red color will develop if nitrite is present in the sample. After color development, the sample is placed in a colorimeter and compared to a 5-ml sample of original sample water. This procedure is repeated and the average of the two nitrite readings are taken and recorded in units of mg/l. A conversion factor is used to convert the nitrite reading into nitrate by multiplying the nitrite result by 4.4.

Sample collection for phosphate is similar to the nitrate. A 5-ml water sample is mixed with a potassium chloride based bromphenol blue indicator powder pillow. The sample is gently swirled until the solution is well mixed then allowed to stand for 1 to 5 minutes for color development. A blue color will develop if orthophosphate is present. After color development, the sample is placed in a colorimeter and compared to a 5-ml sample of original sample water. This procedure is repeated and the average of the two orthophosphate readings are taken and recorded in units of mg/l. A conversion factor is used to convert the orthophosphate reading into phosphate by dividing the orthophosphate result by 3.0.

Monthly samples are taken by a San Jose Water Company from both lakes and sent to a lab for phosphate, nitrate, pH, alkalinity, turbidity, and Total Dissolved Solids analysis. There are no samples taken from the input sources. Limnion Corporation was also involved in taking monthly grab samples from both Lake Kittridge and Lake Couzzins. Using the Hach test kit, the samples were analyzed for pH, alkalinity, phosphate, nitrate, and dissolved oxygen. This masters thesis study, however, concentrated on the levels of nitrate and phosphate.

Plankton Analyses

Additionally, monthly samples were taken from Lake Kittridge by the San Jose Water Company and sent to California Water Service Company for plankton analysis. The abundance and diversity of plankton is a good indicator of the health of an aquatic ecosystem. High numbers of blue-green algae may render the water unuseable for domestic water supply due to the development of obnoxious tastes or odors. The plankton samples were collected in one liter bottles and analyzed within 24 hours of collection by California Water Service Company. The method used is the Sedgwick-Rafter Method as given in The Microscopy of Drinking Water (Whipple 1914). An interview with Lori Zylker (personal communication, April 1994), a Lab Technician II with California Water Service Company, provided the following information on how the plankton analysis was conducted. Five-hundred ml of the sample are filtered through a cylindrical filtration funnel. The filter is cheese cloth and approximately one-half inch of sand. The sand retains the organisms, which are separated by washing with 10-ml of deionized water. A known amount of the sample is placed on a slide and put under a microscope that is equipped with an ocular micrometer that defines the area of the field. The micrometer is necessary in order to obtain quantitative estimates of the number and varieties of organisms present in the sample. The Whipple micrometer is ordinarily used in the Sedgwick-Rafter method and consists of a grid where one square is approximately 1 millimeter (mm) on each side. The number of organisms in each grid is counted in 10 different fields, then the number in each field is counted to get a total. To calculate the standard size of the organism, you multiply how many squares the micro-algae or protozoa take up by the size of the square (1 mm). The next step is to do a cell survey. The organisms are killed with chloroform so that each cell can be counted. The cell count is a result of the average size multiplied by the survey of cells multiplied by a standard number of 0.02. The total units per one cell count (cc) is equal to the average size multiplied by the total count of fields multiplied by a factor of 2.

While the water sampling method may show trends in nitrates and phosphates, it may not be adequate to determine the full significance of nutrient loading within the lake. One problem may be the determination of rates of nutrient cycling between the water, lake sediments, and decaying vegetation. This is further exacerbated by the fact that the area was plagued by drought from 1986 to 1992. Therefore, the rains of 1992-94 may have washed an unusually large reservoir of nutrient rich biomass from the surrounding watershed into the lakes. Additionally, there may be limitations to the use of the Hach field test kit methods. Even though Limnion used the same kit, the test methods are subjective and the mixing methods are sensitive enough to give different results if each test is not treated in exactly the same manner. However, the results should show trends in phosphate and nitrate concentrations that may be valid for drawing conclusions about the success of the project.

The objective of the Nutri-Pod project was to determine if using aquatic vegetation in surface water is as effective as the traditionally used copper sulfate in controlling nuisance micro-algae. The success or failure of this project was based on comparative phosphate and nitrate concentrations between the project lake, Lake Kittridge, and the control lake, Lake Couzzins. Further, there was an analysis of phytoplankton abundance pre- and post-introduction of the Nutri-Pods that correlates to the use and subsequent suspension of copper sulfate applications in Lake Kittridge.

PART II. THE DATA

CHAPTER V

RESULTS

Phosphate and Nitrate

Phosphate levels were compared between the project lake, Lake Kittridge, and the control lake, Lake Couzzins, using the data that was collected from March 1993 through February 1994. The null hypothesis (H_o) is that the phosphate and nitrate levels in Lake Kittridge (with the addition of the Nutri-Pods as a method of water treatment) would not be significantly lower than the levels in Lake Couzzins (without a method of water treatment). The alternative hypothesis (H_A) is that there will be a significant difference in phosphate and nitrate levels between Lakes Kittridge and Couzzins with the Nutri-Pod water treatment system in Lake Kittridge.

The mean for the 43 data points for phosphate in Lake Kittridge was 1.66 mg/l (s^2=0.47) which is significantly lower than the mean for Lake Couzzins at 2.32 mg/l (s^2=1.05). A t-test comparison of these means shows a significant difference in phosphate levels between the project lake and the control lake with the project lake having the lower values throughout the year ($t=3.65$; $df=42$; $\alpha=0.05$).

The mean for the 43 data points for nitrate was 0.117 mg/l ($s^2=0.022$) in Lake Kittridge compared to the similar mean of 0.127 mg/l ($s^2=0.024$) in Lake Couzzins. A t-test comparison of these means shows that the difference in nitrate levels between the project and control lakes were not found to be significant ($t=0.49$; $df=42$; $\alpha=0.05$).

Phytoplankton

Phytoplankton species diversity in Lake Kittridge was compared during $CuSO_4$ applications and after $CuSO_4$ cessation. The H_o is that phytoplankton species diversity during $CuSO_4$ applications will not be significantly different after its suspension. The H_A is that phytoplankton species diversity will be significantly different after the suspension of $CuSO_4$.

The diversity of phytoplankton species during $CuSO_4$ treatment was different from species diversity after the suspension of $CuSO_4$ applications (See Table 1). Plankton data from 1989 to 1991 show that the highest total cell count was 3,288 with only five species total. After the addition of the Nutri-Pods, and the termination of copper sulfate applications in 1992 and 1993, the maximum cell count increased to a high of 8,781 in 1992 with a total number of species elevated to 17 in 1993. While this difference has not been tested statisically, there is an indication that the null hypothesis should be rejected.

Table 1: Lake Kittridge Plankton Data

Year	Cell Count (cc)	Number of Species	Average $CuSO_4$ Concentrations (mg/l)
1989	3,288	5	0.121
1990	1,616	4	0.089
1991	2,768	3	0.020
1992	8,781	11	0.000
1993	8,280	17	0.000

Summary of Lake Kittridge plankton data from 1989 through 1993 (Source: California Water Service Company).

CHAPTER VI

DISCUSSION

Nutrient Levels and Micro-algae Blooms

Monitoring nutrient levels within a lake is a way to determine its eutrophic state. Shortly after a nutrient "spike" there will be a significant reduction in nutrient concentrations caused by an increase in micro-algae within a lake. This occurs after a nutrient spike because the micro-algae stores the nutrients, accounting for the decline in concentrations. Then, after a short delay time, the micro-algae will grow and blooms will appear. Visual observations made during data collection in 1993-94 supports this hypothesis. Increasingly high concentrations of phosphate in Lake Couzzins throughout June 1993, followed by a significant decline, appeared to correspond to the observation of micro-algae blooms in mid-July 1993, covering approximately 20% of the Lake's surface. In the future, a good test would be to take chlorophyll A data and correlate it to the quantity of nutrients present.

Phosphate

In most lakes soluble phosphate concentrations fall from relatively high winter levels at the start of the spring bloom and continue to be low until early winter (Goldman and Horne 1983). Luxury uptake by phytoplankton may use up many times the requirement for one cell division and may account for the rapid decline in spring levels. This cycle of phosphate as described by Goldman and Horne (1983) is reflected in the phosphate data collected from Lake Couzzins from March 1993 to February 1994 (Figure 3). Sources of phosphate include excretion by animals feeding on phytoplankton and direct sediment resupply in shallow areas.

The phosphate levels in Lake Kittridge remained relatively low throughout the year (Figure 4), with the highest level reaching only 3.33 mg/l in January 1994. This was expected because when healthy, the *C. demersum* within the Nutri-Pods have the ability to uptake phosphate at relatively high rates. The high concentration of phosphate in January 1994 may be the result of phosphate being released from decomposing vegetation or lake sediment stirred up during the heavy rains that occurred during that month.

There were little to no observable micro-algae blooms during the 1993-94 data collection year in Lake Kittridge. The absence of micro-algae blooms in the project lake during

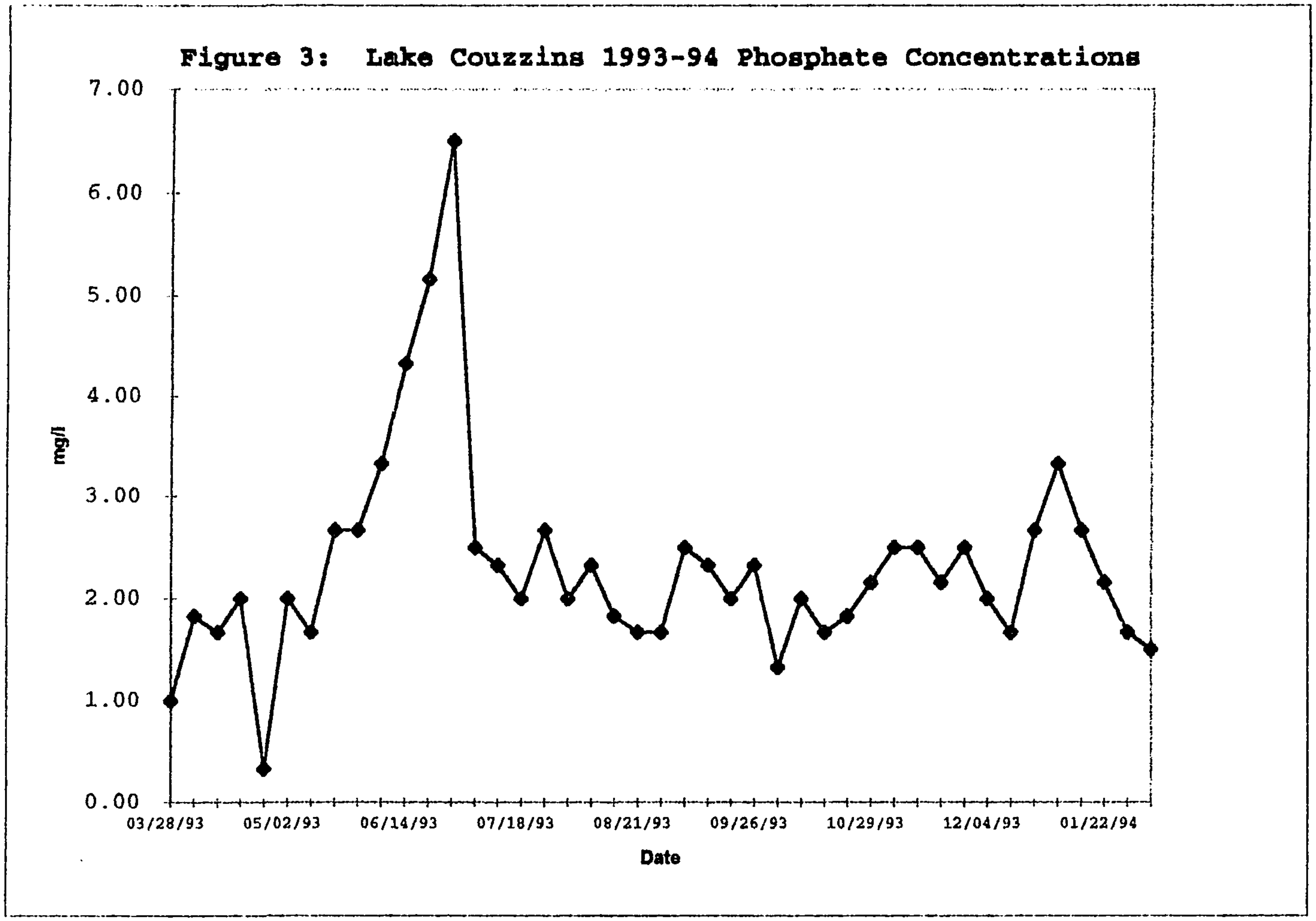


Figure 3: Phosphate concentrations from March 1993 to February 1994 in Lake Couzzins. Algae blooms appeared shortly after the decline in phosphate concentrations in July, 1993.

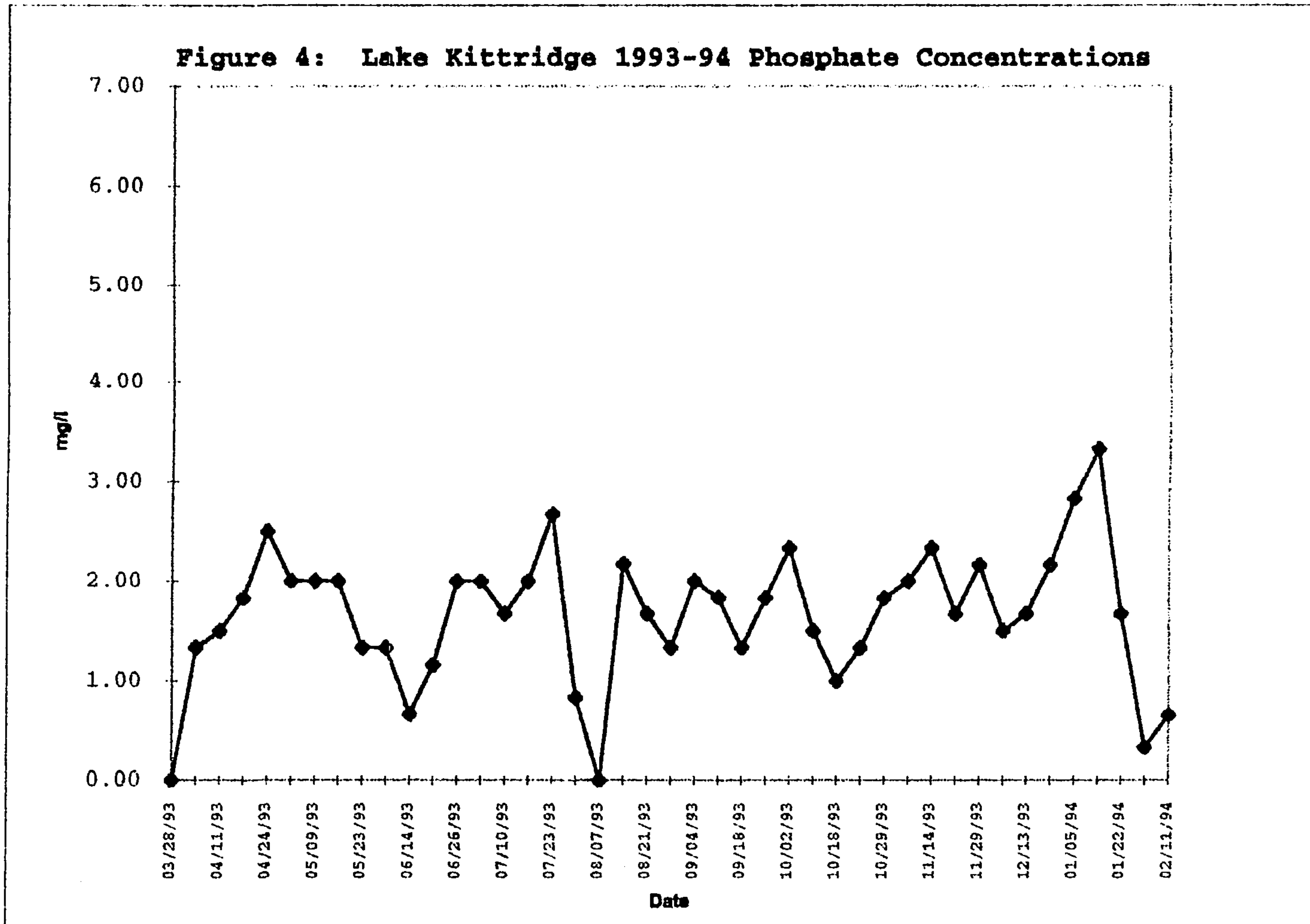


Figure 4: Phosphate concentrations for Lake Kittridge from March 1993 to February 1994. The highest concentration occurred in January 1994 during severe rain storms which probably stirred-up sediment that released phosphate into the lake water.

the 1993-94 data collection year suggests that the Nutri-Pod system using *C. demersum* and *E. densa* was suppressing the growth of micro-algae.

The levels of phosphate in Lakes Kittridge and Couzzins differ noticeably in the 1993-94 data collection year (Figure 5). Lake Couzzins has an obvious significant increase in phosphate concentrations throughout June 1993, that is followed by an extreme decline from 6.50 mg/l to 2.50 mg/l. Micro-algae blooms were evident in Lake Couzzins shortly after the decline in phosphate levels.

Nitrate

The seasonal cycle of nitrate tends to be similar in most lakes. In winter nitrate inflow exceeds uptake by micro-algae and is further supplemented by nitrate released from the sediments. In summer, nitrate uptake by plants is faster than inflow, and recycling from the hypolimnion is limited by the thermocline (Goldman and Horne 1983). In eutrophic lakes, high demand will often completely remove nitrate from the lake water (Goldman and Horne 1983). Figure 6 illustrates various points throughout the 1993-94 data collection year where nitrate levels were recorded at 0.0 mg/l in both the project and control lakes. However, the 1993-94 seasonal cycle of nitrates in each lake does not

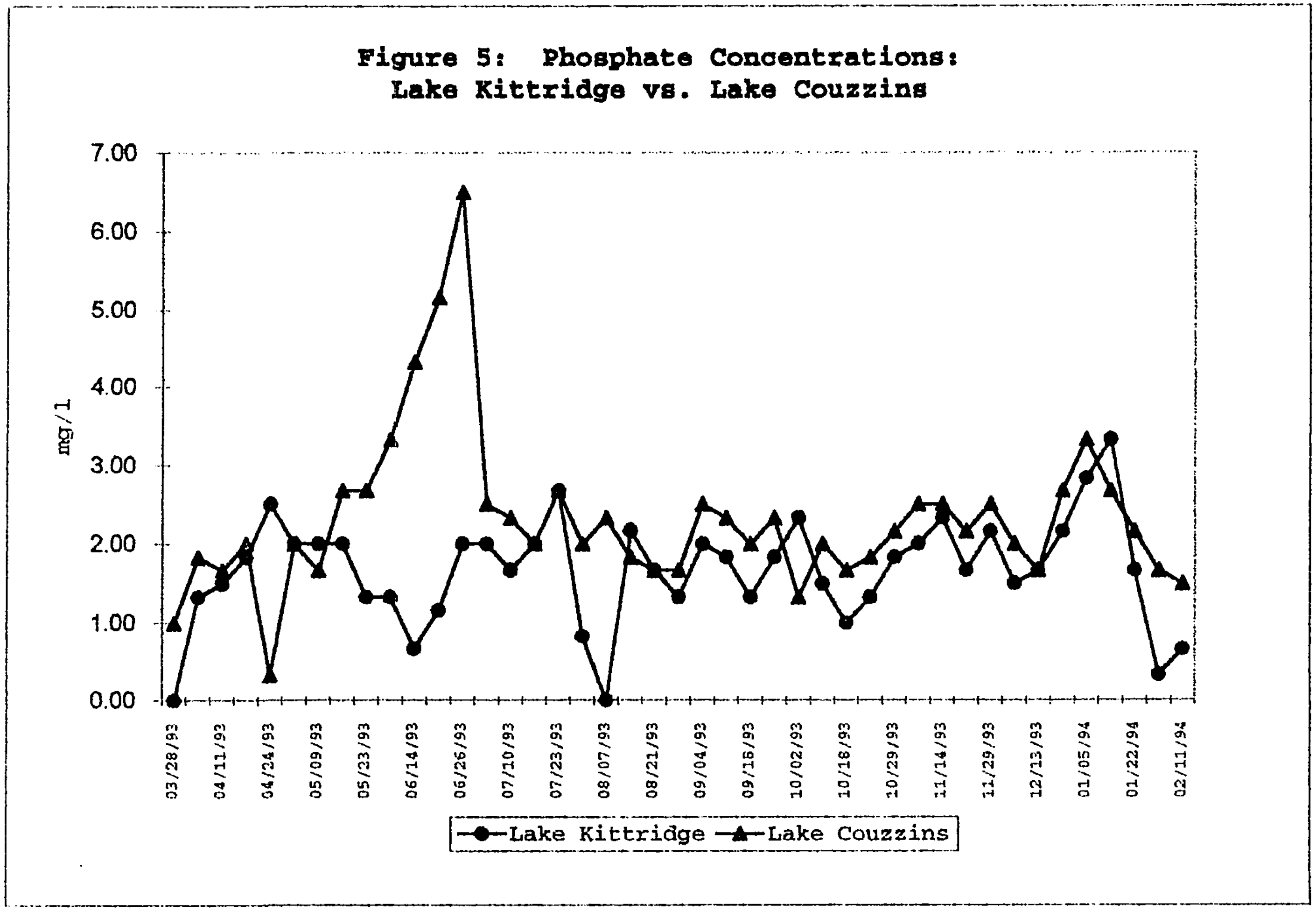


Figure 5: Phosphate concentrations in Lakes Kittridge and Couzzins. Note that micro-algae blooms began to appear shortly after the phosphate spike in June 1993, covering approximately 20% of Lake Couzzin's surface area.

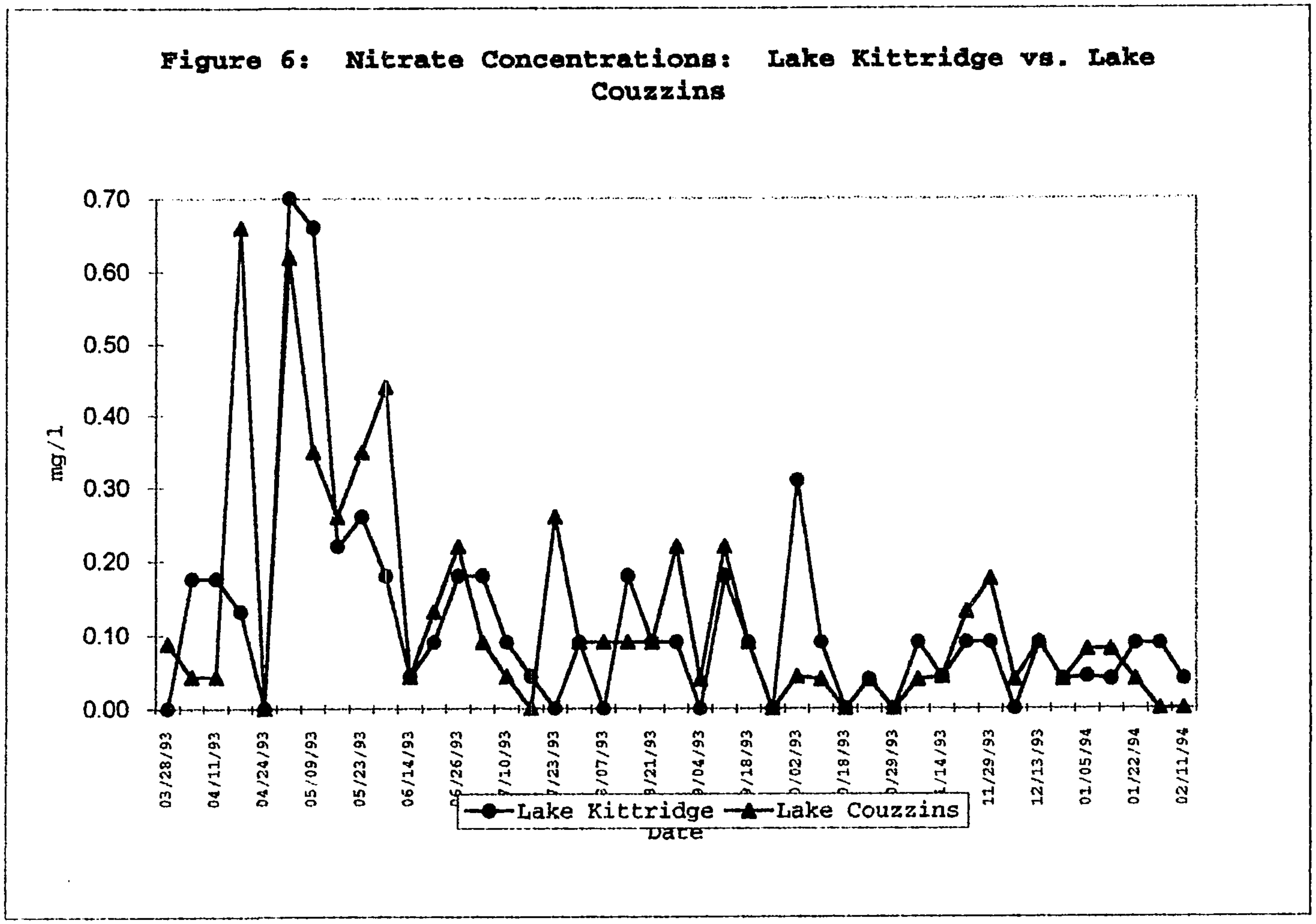


Figure 6: Nitrate concentrations in Lakes Kittridge and Cozzins. There appeared to be no correlation between micro-algae blooms and nitrate spikes. Lake Couzzins was covered with algae blooms over approximately 20% of its surface area in July through October 1993.

seem to follow the norm of higher levels in the winter and lower concentrations in throughout the rest of the year. Instead, low concentrations of nitrate occurred in the winter months and higher levels occurred sporadically during the rest of the year. Sources of nitrate include inputs from rainfall, streams, the sediment at the lake bottom, and biomass from the surrounding watershed.

Significant nitrate spikes were found in Lake Kittridge during the month of May 1993, while the rest of the 1993-94 data collection year had relatively consistently low levels of nitrate present (Figure 7). There was no observable difference in the water clarity of Lake Kittridge during the nitrate spikes or subsequent decline. The nitrate spike may have occurred because it represented an excess that was not needed by the *C. demersum* or *E.* densa, and therefore was not readily taken up by the plants. In addition, the low concentrations of phosphate may indicate that it is a limiting nutrient within the lake system.

In Lake Couzzins there were various spikes in the nitrate concentrations followed by a remarkable decrease in April and May 1994, respectively, but there was no noticeable increase in micro-algae blooms during or after this period (Figure 8).

The levels of nitrate in both lakes (Figure 6) were

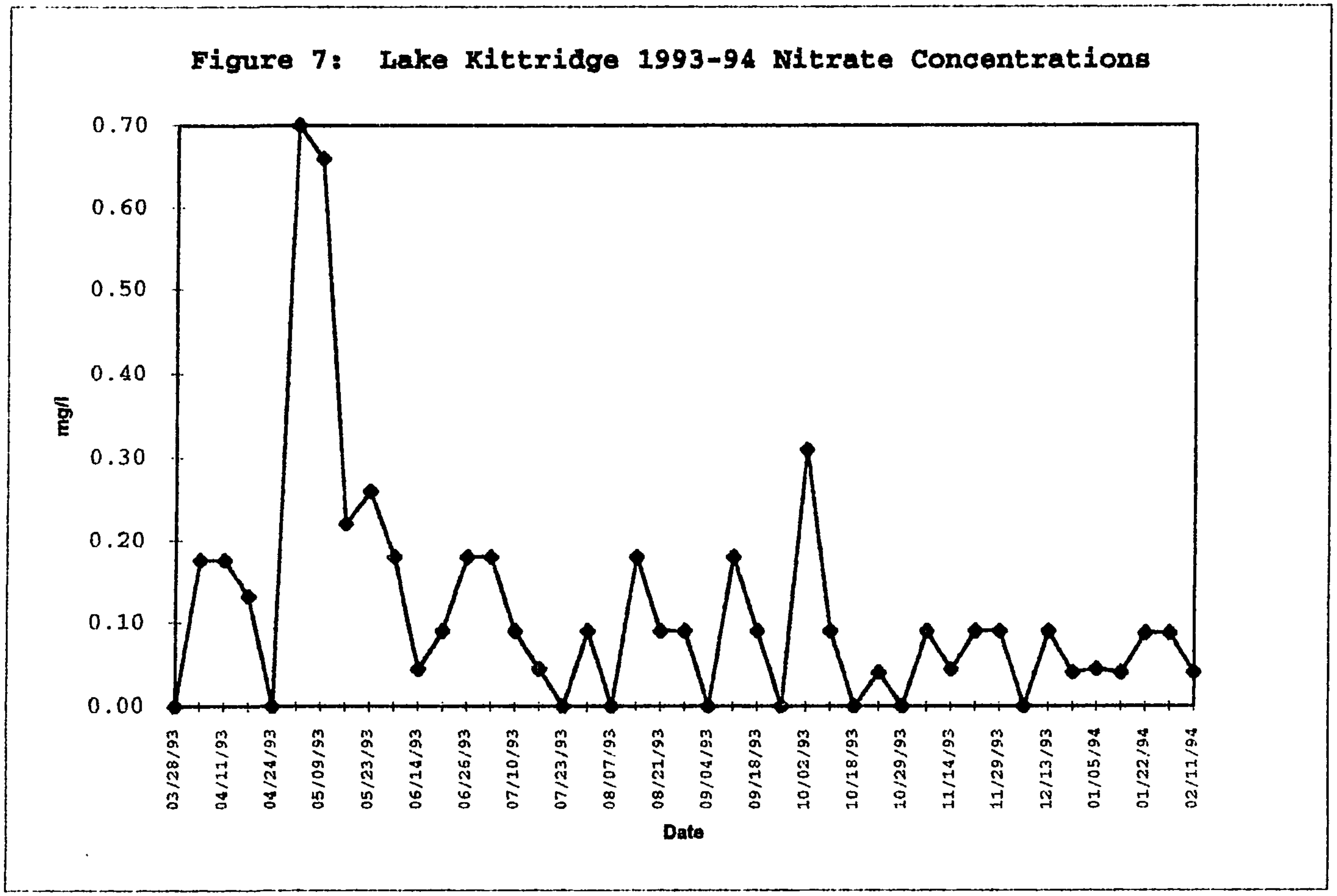


Figure 7: Nitrate concentrations in Lake Kittridge from March 1993 through February 1994.

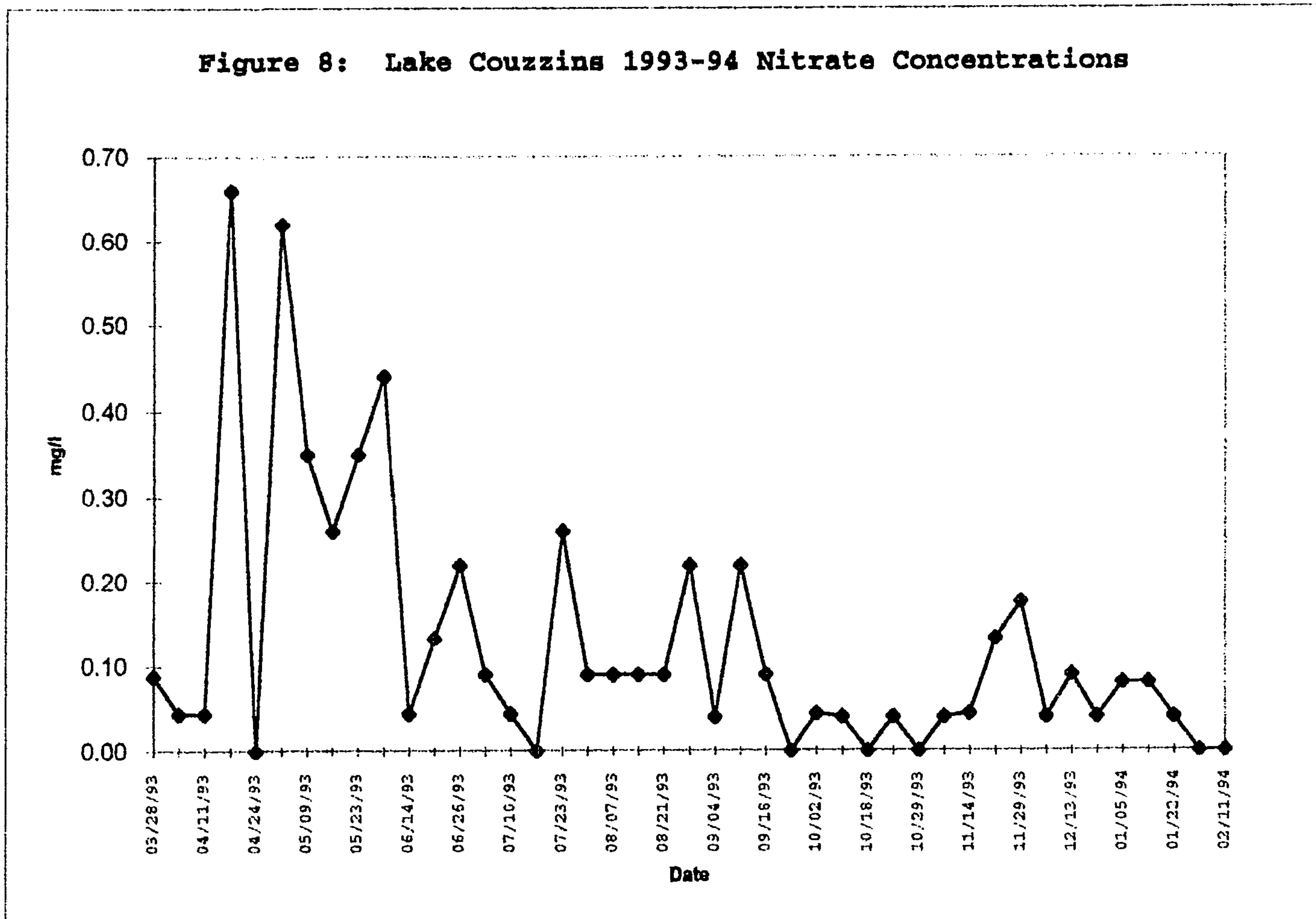


Figure 8: Nitrate concentrations in Lake Couzzins from March 1993 through February 1994.

relatively sporadic throughout the 1993-94 data collection year. The highest nitrate level was found in Lake Kittridge in May at 0.70 mg/l while the highest level in Lake Couzzins was 0.66 mg/l in April. The spikes of nitrate in either of the lakes did not seem to correspond with any observable event such as rain washing biomass into the lakes or a stirring-up of the sediment at the lake bottom. Additionally, the declines in the nitrate concentrations did not seem to correlate with any observable events except for the decrease of nitrate in Lake Couzzins in July 1994 when micro-algae blooms were present.

Plankton Analyses

The abundance and diversity of aquatic organisms within a lake is a good indication of the health of the ecosystem. As indicated in Table 1, there was a significant increase in the number and diversity of algal species present in Lake Kittridge from 1989 to 1993. Since copper sulfate applications ceased in January 1992, it can be inferred that the jump from three species in 1991 to 11 species present in 1992, to 17 in 1993, is a result of no longer treating the Lake with copper sulfate. Furthermore, there appears to be a notable difference between the 1991 total cell count of 2,768 and the 1992 total cell count of 8,781, also illustrating an increase in organisms once copper sulfate was no longer utilized as an aquatic herbicide. This trend is consistent with Goldman and Horne's (1983) observation that the toxic effects of copper are much more pronounced at lower trophic levels, especially in phytoplankton and zooplankton, than at higher trophic levels. As little as 0.1 μ/l[3] (0.0001 mg/l) ionic copper can kill some micro-algae in water with low-chelation[4] potential. Copper sulfate applications prior to 1992 were reportedly as high as 0.33 mg/l (330.0 μ/l) in 1989, and only as low as 0.05 mg/l in 1991.

Blue-green algae were more abundant prior to the cessation of copper sulfate applications (Table 2). This was probably caused in part by their resistivity to the copper sulfate due to tolerance developed during repeated applications. It is also likely that the regular copper treatments upset the natural balance of the ecosystem causing an over abundance of blue-green algae relative to other species. However, once *C. demersum* and *E. densa* were introduced, the number of species and the cell count of blue-green algae dramatically decreased from eight species and 3,060 cc in 1989 when copper sulfate was used, to two species and 360 cc in 1993. The reduction in blue-green algae species and cell counts after the introduction of the

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[3] μg/l = micrograms per liter (equivalent to parts per billion (ppb)).
[4] Chelation is the ability to combine with a metal ion.

Nutri-Pod system (including the free-standing *E. densa*) was probably a result of competition between the blue-green algae, other algal species, and the introduced macrophytes. It has been well documented that aquatic macrophytes have an outstanding ability to absorb and store large amounts of nutrients. Blue-green algae usually float near the surface of a water body and restrict light from the depths of the water column and outcompete other micro-algae species (Fogg 1975; Golterman 1975). However, with the Nutri-Pod system in place, the appropriate spacing between pods throughout the lake, and the addition of *E. densa*, it appears that the introduced aquatic vegetation were in a better position than the blue-green algae to take up the nutrients. Thus, the Nutri-Pod system kept the prevalence of undesirable blue-green algae low and enhanced general algae biodiversity.

Table 2: Comparative Micro-Algae Cell Counts (cc)

Year	Average $CuSO_4$ (mg/l)	[a]Blue-Green algae cc	[b]*Ceratium spp.* cc	All Other Plankton cc
1989	0.121	3,060	0	228
1990	0.089	216	176	1,224
1991	0.020	400	2,272	96
1992	0.000	2,368	1,705	4,708
1993	0.000	360	4,850	3,070

Table 2 illustrates the difference in cell counts from 1989 to 1993. [a]Blue-green algae decreased in 1993. [b]Ceratium increased from 1989 to 1993, totaling more than half the total cell count of 8,280.

Plankton Succession

There are many environmental variables to which micro-algae growth rates might respond, such as temperature, average light intensity, and nutrient availability, in natural waters (Fogg 1975). Phytoplankton usually grow in a series of pulses or blooms depending on the environmental conditions. For example, temperature and/or light may be limiting in the winter months, while nutrients are more likely to be critical during the summer (Reynolds 1984). These seasonal shifts influence the sequence in which various species will each be able to grow and, possibly, to dominate the plankton assemblage (Fogg 1975).

Although there was a significant increase in the cell counts of the species overall, the most significant difference occurred in *Ceratium spp.*, a dinoflagellate, from none found in 1989 to a total of 4,840 cc in 1993 (Table 2). *Ceratium spp.* generally grow best in summer and fall and succeed because they can actively swim to positions of favorable light and nutrients. Their populations may decline due to heavy zooplankton grazing, competition from other micro-algae, and possibly nutrient depletion (Goldman and Horne 1983). It is apparent that *Ceratium spp.* did not have much competition from other plankton, especially in 1993 when the total cell count of *Ceratium spp.* is greater than one-half the total cell count of all plankton species combined. Zooplankton grazing on *Ceratium spp.* or a lack of nutrients can cause its cell count to decrease, with phosphate being the limiting factor. There did not appear to be any zooplankton in the 1993 plankton data; therefore, predation was not a threat to the *Ceratium spp.* Also, the relatively consistent levels of phosphate in Lake Kittridge probably allowed the *Ceratium spp.* to thrive.

The abundance of *Ceratium spp.* could be due to the re-structuring and succession of species within Lake Kittridge without copper sulfate present. Several more years of data collection is necessary to determine the significance of the abundance of *Ceratium spp.* within the Lake's system.

PART III. THE ENVIRONMENT

CHAPTER VII

ENVIRONMENTAL IMPLICATIONS

The practical implications of biological surface water management can be profound in terms of public water policy issues. These issues include the ever stricter requirements as mandated by federal, state, and local water quality regulations, storm water runoff, and the public desire to eliminate manipulating our environment through artificial means.

Copper in the Bay

Copper is a naturally occurring constituent in the San Francisco Bay. Many species are dependent upon copper for their survival. Therefore, too much or too little copper within the Bay's aquatic ecosystem can be detrimental to its constitution.

The increased concentrations of copper into aquatic ecosystems are of great concern, not only in the San Francisco Bay Area, but nationwide as well. We use copper, or its various derivatives for a variety of purposes, including in water pipes, in copper-based root control chemicals, in copper containing cooling water additives, and, of course, as an algae control in aquatic herbicides, among others. Unfortunately, these copper containing materials often end up in surface waters as a result of non-point runoff. Copper in the San Francisco Bay, or any other aquatic system, causes a great deal of harm to the species that are dependent upon the aquatic ecosystem. Copper can be toxic to many organisms in low concentrations, and is extremely bioavailable[5] in the Bay (San Francisco Estuary Project 1991a).

High concentrations of copper have been found in the South San Francisco Bay (San Francisco Estuary Project 1991b), particularly in organisms in the middle of the food web, signifying bioaccumulation up through the food chain. Regardless of the small quantities of copper used as an algaecide in surface water systems, it still has the capability of bioaccumulation through the food chain. Bioaccumulation occurs when a constituent moves up through the food chain in increasing concentrations.

Water Policy

Due to the close proximity of the San Francisco Bay and the issue of increased concentrations of copper within the Bay ecosystem, Assembly Bill, AB 3394, was proposed by

[5] Bioavailability refers to the extent to which a compound is obtainable for biological use by organisms.

California Assembly Member Sher, to eliminate the sale, use, and discharge of copper-based root control chemicals and copper-containing cooling water additives. When used, root killers and cooling water additives are discharged into sanitary sewer systems or storm drains, contributing to pollutant loading in local creeks and in San Francisco Bay. A representative of Palo Alto's Regional Water Quality Control Plant (RWQCP) stated in a May 1994 meeting that studies have shown that copper from copper-based root killers comprises about three to ten percent of the total influent copper loading to Bay Area wastewater treatment plants. Alternatives to copper-based root-killers and cooling tower additives are readily available and in common use, according to Palo Alto's RWQCP representative, eliminating some of the strain of changing products on the consumer.

Copper is a problem pollutant for Bay area publicly-owned treatment works like the Palo Alto RWQCP. Because water quality limits are very low for copper, discharges of relatively small quantities of copper is significant. According to the RWQCP, in order to achieve compliance with stringent effluent limitations, the RWQCP must decrease the copper levels in its influent by approximately 75 percent[6], from 1.0 mg/l to 0.25 mg/l per month. This reduction of

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[6] Meeting with the RWQCP in May 1994, the speaker, Kelly D. Moran, Ph.D., was a representative of the RWQCP.

influent levels is therefore the burden of industry who discharges into the sanitary sewer system that is connected to a RWQCP.

Local regulatory agencies have increased industrial wastewater discharge requirements in order to comply with the federal Clean Water Act (CWA) and the California Porter-Cologne Act with respect to discharges to San Francisco Bay and its tributaries. Public sewage systems, referred to commonly as publicly owned treatment works (POTWs), who receive wastewater discharge from the sanitary sewer system, are subject to federal pretreatment requirements under Section 307(b) and 307(c) of the Clean Water Act.

The Porter-Cologne Water Quality Control Act[7] (1969) regulates the discharges of wastes that may affect the quality of the state's waters. The State Water Resources Control Board and nine regional water quality control boards are responsible for exercising the powers of the state in the field of water quality (Cal/EPA 1993). The state board formulates state policies for water quality control. The nine regional boards are to formulate regional water quality control plans that included "water quality objectives." The objectives must be designed to ensure the "reasonable protection of beneficial uses and the prevention of nuisance" (Cal/EPA 1993). The concept of "beneficial uses"

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[7] Water code sections 13000-13999.18, and California Code of Regulations, Title 23, Chapter 23.

includes, among others, the preservation and enhancement of fish, wildlife, and other water resources, including surface waters. It can therefore be argued that this provision could be extended to include phytoplankton and other microorganisms that are important to the health and survival of an ecosystem. The abundance of aquatic organisms within a lake is a good indication of the health of the ecosystem. Unless organisms at the bottom of the food chain are healthy and abundant, fish and other wildlife, defined as a "beneficial use", and who are dependent upon the affected organisms, will not remain healthy. Decreases in aquatic microorganisms makes food scarce for the fish and wildlife dependent upon them. Even though fish may have a higher tolerance to copper, bioaccumulation could create high concentrations in fish that are detrimental. The provisions of the Porter-Cologne Act should be an incentive for water quality managers to substitute chemical treatment methods with a more natural viable means of surface water treatment.

However, the effort to reduce sources of copper is not generally related to drinking water because copper containing algaecides is not considered large enough of a point or non-point source, comprising of less than one percent of the total (Moran, RWQCP meeting, June 1994). Furthermore, it is often the opinion of water supply and government agencies (i.e. RWQCB[8]) that there is no adequate alternative to copper for controlling algae (Moran, RWQCP meeting, June 1994). Nutri-Pod technology may be the answer for an alternative to copper sulfate when controlling nuisance algae.

[8] RWQCB is the Regional Water Quality Control Board of California who is one of the principle state agencies with primary responsibility for the control of water quality. The other agency is the State Water Resources Control Board.

CHAPTER VII

SUMMARY AND CONCLUSION

There is a growing desire by the public to find natural, rather than artificial, means to manipulate the environment. This is evident in our use of plants instead of chemicals to increase the quality of our water. Water hyacinths have been commonly used to treat waste waters due to their incredible ability to absorb and store various constituents. The Nutri-Pod system using the aquatic plants, *Ceratophyllum demersum* and *Elodea densa,* is an alternative to using copper sulfate to control nuisance micro-algae in domestic surface waters. *C. demersum* and *E.* densa have an exceptional ability to absorb and store nutrients, thus out competing micro-algae for nutrients and preventing the potential problems (e.g., eutrophication) associated with micro-algae blooms within a lake system.

Although the difference in phosphate and nitrate levels between the project lake, Lake Kittridge, and the control lake, Lake Couzzins, was significant only for phosphate, the lack of micro-algae blooms in the project lake indicate that the Nutri-Pod system is a viable alternative to copper sulfate when treating surface water for nuisance micro-algae growth. On the contrary, the control lake, without any treatment method, experienced micro-algae blooms during the 1993-94 data collection year. Data collected during copper sulfate applications and after its cessation, indicates that the Nutri-Pod system is better at controlling unwanted blue-green algae than the copper sulfate.

Further, using biological instead of chemical means to treat surface water can restore the balance of the aquatic ecosystem. Plankton data from Lake Kittridge, from 1989 through 1993, showed a significant increase in the number and diversity of plankton species found within the project lake once the copper sulfate applications ceased and the Nutri-Pods were in place. Before the cessation of the copper sulfate applications, there was an abundance of blue-green micro-algae compared to the number of other plankton species, with as few as three plankton species present. Once the copper sulfate applications were suspended and biological water treatment was introduced, the number of species increased to seventeen and the total blue-green algae cell count decreased from 3,060 in 1989 to 360 in 1993, indicating a restructuring of the aquatic ecosystem in the project lake.

Water policy is designed to protect the environment as well as humans. Eliminating potential point and non-point sources of copper can only help those aquatic environments, such as the San Francisco Bay, that are negatively affected by an unnatural influx of copper products. Nutri-Pod technology supports the beneficial use objectives of the nine regional water quality control boards under the federal Clean Water Act and the California Porter-Cologne Water Quality Control Act, where beneficial use includes the preservation and enhancement of fish and wildlife.

In conclusion, the Nutri-Pod system of surface water treatment is a viable alternative to copper sulfate as a means to control nuisance micro-algae in surface waters. Additionally, Nutri-Pod technology has the advantage of restoring the balance of species within an aquatic ecosystem by promoting natural competition between species and demoting chemical control methods. Finally, the Nutri-Pod water treatment system conforms to the basic function of water policy which is to protect the quality of water for both humans and wildlife.

Further research should be conducted to determine if the Nutri-Pods containing *Ceratophyllum demersum* can achieve similar results without the aid of the free-standing *Elodea densa*. Additionally, the effectiveness of the *E. densa* in competing with micro-algae for nutrients without the Nutri-Pods needs to be assessed.

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