Effects of Biomanipulation with Fishes on Eutrophic Ponds in Andhra Pradesh, India

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Abstract Millions of people throughout the world depend on reservoirs such as ponds as the main source of drinking water. The water in these ponds can sometimes be subject to eutrophication which leads to negative health impacts in humans and livestock. Eutrophication also causes excessive algae growth, which clogs water filtration devices, creates a foul smell and degrades the ecosystem of the water body. A biological method of treating water known as biomanipulation was applied to experimental ponds undergoing eutrophication in Andhra Pradesh, India. The effects of fishes such as rohu, catla, grass carp and silver carp on the concentrations of algal biomass, nitrates and turbidity in ponds were studied over a period of five months from October 2007 to February 2008. Measurements of these water quality parameters were taken weekly in four experimental ponds where three ponds consisted of different combinations of fish and the control pond contained no fish. Results from this study suggest that the introduction of fish in treated ponds did not reduce the concentrations of algal biomass or nitrate. This could be attributable to nutrient loading from fish excrement. The amount of turbidity, however, was lower in the control pond compared to the treated ponds probably because fish were not available to disturb the sediment in this pond. Given the limitations of this study, a longer research of biomanipulation in this region might prove beneficial in terms of understanding the mechanisms involved in treating water in this manner.

Introduction

Many communities throughout the world depend on surface reservoirs such as lakes and ponds for drinking water; however, eutrophication of reservoirs has limited their supply of clean water (Klapper 1991). Eutrophication is caused by the increase in supply of plant nutrients such as nitrogen and phosphorus in natural waters due to human activities and results in excess growth of algae and other aquatic plants (Klapper 1991). Fertilizer application in agriculture can contribute to elevated nutrient levels in lakes, ponds and reservoirs that are linked through canals and groundwater movement (Klapper 1991). The formation of phytoplankton due to excesses in nitrogen and phosphorus in particular proportions poses many problems for the ecosystem including water purification for human and livestock consumption.

The biggest concerns with drinking water are the harmful effects that inorganic forms of nitrogen [nitrate (NO_3^-), nitrite (NO_2^-), ammonia (NH_3)] pose on human health (Reilly *et al.* 1998). The reactions of bacteria like *Nitrosomonas* with the organic matter produced during eutrophication can result in the formation of inorganic nitrogen through the process of nitrification (Shrimali and Singh 2000). High levels of nitrates in drinking water can lead to human health problems, such as the development of methemoglobinemia in infants under the age of six (Mason 1996). Methemoglobinemia occurs when nitrate reduces to the nitrite ion in an infant's stomach and binds to hemoglobin, reducing the transfer of oxygen to the body's cells and resulting in a bluish skin color (Benefield *et al.* 1982).

Other aspects of concern with eutrophication are algal and cyanobacterial toxins, and amenity value. High levels of algae may produce toxins that are lethal to economically important animals (Mason 1996). Grazers such as cattle have been observed to die in hot climates due to the intake of water with high levels of algae (Klapper 1991). Cyanobacteria such as *Microcystis* and *Anabaena* can produce poisons that cause liver damage at low concentrations and can induce paralysis and death (Mason 1996). In eastern England, a number of dogs and sheep have been observed to die rapidly after drinking reservoir water that was going through a bloom of *Microcystis* (Mason 1996). In addition, the excess growth of algae and other aquatic plants can harm aquatic wildlife such as fish (Reilly *et al.* 1998). Eutrophication can also reduce the aesthetic and recreational values of a given body of water. The smell resulting from decaying algae can be highly unpleasant which can be a nuisance to people who use the water or live along the water source (Mason 1996).

Additional effects of eutrophication are reduction of species diversity, increased plant and animal biomass, increased rate of sedimentation, and turbidity (Mason 1996). Many shallow lakes and ponds can shift from a macrophyte-dominated, clearwater state to a phytoplankton-dominated, turbid state with the addition of nutrient loading (Bekiloglu 2003). Consequently, eutrophication can result in difficulty in treatment of potable water due to frequent clogging of filters, unacceptable taste and odor of water supply, and decrease in commercially important species (Mason 1996).

Treatment of water undergoing eutrophication can be problematic and costly. One method of purifying water is through a water treatment plant that uses sand filters to remove phytoplankton (Mason 1996). A treatment plant in an area with a large quantity of phytoplankton can be subject to blocked filters, which tremendously reduce the throughput of water and bring the filtration process at a halt. In order to remedy this problem, the treatment plant has to be shut down temporarily to be cleaned (Mason 1996). If this area is the only source of potable water for a given community, then a water treatment plant shutdown can pose serious problems for inhabitants. Furthermore, water treatment plants can incur tremendous costs involved with construction and operation. According to Kovacic *et al.* (2006), the construction of a 20 mgd (million gallons per day) treatment plant in the United States was estimated to cost $8 million in 2003 with an additional cost of up to $1 million per year for maintenance.

Many communities throughout the world do not have adequate access to potable water from treatment plants but rather depend on natural reservoirs such as lakes and ponds. As many as 300 ponds in the state of Andhra Pradesh, India, which serve as the primary source of drinking water for villagers, may be undergoing eutrophication (Gadgil 2007, pers. comm.). These man-made ponds in the catchment area are going through algal blooms which create a foul odor and affect the taste (Gadgil 2007, pers. comm.). Currently, WaterHealth International, a company that provides potable water to underserved people, is treating the water from ponds in localized water centers using filtration in order to improve the availability of drinking water. However, the algae have created problems for filters installed by WaterHealth and has affected the final filtered product. In order to remedy this problem, a cost-effective solution as well as one that is ecologically sound is being explored.

Biomanipulation of eutrophic water sources is a procedure that changes the food web in order to favor grazing on algae by zooplankton or reduces algae by introducing planktivorous fish.

This method is fairly new and does not require machinery or toxic chemicals (Klapper 1991). A study performed by Starling (1993) showed that the introduction of silver carp, a phytoplanktivorous fish, in a reservoir led to a reduction in blue-green algae. It is important to note that biomanipulation is not always as simple as a fish/zooplankton-algae food chain. It can achieve success when fish removal triggers other processes like the increase in herbivorous zooplankton such as *Daphnia*, which are effective grazers of phytoplankton (Beklioglu *et al.* 2003). Reduced internal loading, a state which occurs when a lower amount of phosphorus is available for phytoplankton, is another effective process for reducing phytoplankton after fish removal (Persson 1997). It has also been found that the introduction of silver carp can stimulate phytoplankton growth (Gophen 1990). While biomanipulation has had successes in controlling algae growth and improving water quality it has also been shown to have negative impacts.

The goal of this study is to examine whether biomanipulation is a useful method in treating eutrophic man-made ponds in southern India. The effectiveness of biomanipulation will be studied by looking at the impacts of top-down control with different fish species over time on algal biomass, nitrate levels, and water clarity or turbidity. I hypothesize that the introduction of fish in the ponds will result in a reduction of algal biomass through direct consumption of algae, reduce nitrate levels and decrease the turbidity of the water. Furthermore, the results of this study could be used to inform local villages about the types of fish that could potentially improve the water quality of their ponds.

Methods

Study Site This study took place in an area around the city of Vijayawada in Andhra Pradesh, India. The Department of Fisheries in the nearby town of Badampudi provided the site in order to conduct the experiment. Four small man-made ponds measuring approximately 15m by 8m by 1m were used to mimic the conditions of actual ponds used for drinking water. The first pond served as the control pond which did not have any fish and the other three ponds consisted of different combinations of fish species (Table 1). The combinations of fishes in each pond were decided based on the type of algae or vegetation that was present. For instance, a pond that had vegetation on the surface was stocked with grass carp, whereas one that consisted of unicellular algae was stocked with silver carp. Because the number of ponds available for manipulation were limited and with different eutrophication states it was not possible to replicate treatments. Stocking rates of the fishes were on a species by species basis and were established based on previous studies (Table 2).

Fish that were used for this study were: rohu [*Labeo rohita* (Hamilton, 1822)], catla [*Catla catla* (Hamilton, 1822)], grass carp [*Ctenopharyngdon idella* (Valenciennes, 1844)] and silver carp [*Hypothalmichthys molitrix* (Richardson, 1845)]. Rohu is a water column feeder that mainly feeds on phytoplankton and zooplankton, while catla is mostly a surface feeder that eats plankton but can also feed in other layers of a pond (Jhingran 1968, Jhingran 1975). Rohu and catla were selected for this study since they are readily available and because they are commercially important fishes. Additionally, grass carp is an effective grazer of vegetation along with filamentous algae and silver carp feeds on phytoplankton or unicellular algae (Chilton and Muoneke 1992, Starling 1993). Grass carp and silver carp were chosen for the study because they are typically used to treat water with vegetation and phytoplankton, respectively.

Table 1. Types of fish used in each treatment.

Treatment	**Control**	**A**	**B**	**C**
Fish	No Fish	Rohu Catla	Rohu Catla Grass Carp	Silver Carp Grass Carp

Table 2. Stocking rates and feed for each fish along with references.

Fish	**Rohu**	**Catla**	**Grass Carp**	**Silver Carp**
Stocking Rate	100 fish/acre	100 fish/acre	50 fish/acre	30 fish/acre
Primary Feed	Phytoplankton Zooplankton	Phytoplankton Zooplankton	Vegetation Filamentous Algae	Phytoplankton
Study	Jhingran 1975	Jhingran 1968	Opuszynksi and Shireman 1995	Starling 1993

Initially, the test ponds at the study site were not undergoing eutrophication or did not have algal biomass which was typically observed in actual village ponds. In order to replicate situations going on in real village ponds, nutrients in the form of urea at 20 kg and diammonium phosphate at 15 kg were added to each of the ponds on July 20 2007. At the beginning of the experiment, small fingerling fish, weighing about 5-7 grams each, were added to a small pond close to the test ponds. In order to acclimate the fish to the surroundings, fish feed was supplied and after a week the fish were transferred to the test ponds.

Parameters of concern To determine the remediation potential of introduced fish on eutrophication and water clarity, nitrate, algal biomass (chlorophyll-*a*), and turbidity were observed in the test ponds (Greenfeld et al. 2005). Nitrate, which acts as a fertilizer promoting the growth of algae and vegetation, was measured using an azo dye method (Bodine and Janzen 1973). The amount of algal biomass was analyzed by measuring chlorophyll-*a* content using a Hach DR/2500 spectrophotometer. Turbidity, a measure of water clarity and particulate matter that may clog downstream filtration efforts, was analyzed using a LaMotte turbidity meter. One liter samples were collected by Department of Fisheries staff from the top layer within 1 m of the shoreline of each pond without disturbing the water. Samples were taken every week for six months which were analyzed in a laboratory located near the ponds. Data from sample analysis by local Indian technicians were emailed to California for subsequent statistical analysis.

Statistical Analysis The data collected for this study were not substantial enough to constitute any statistically significant findings. But, a repeated measures multivariate analysis of variance (MANOVA) was used to display and compare the trends of nitrate, chlorophyll-*a*, and turbidity from the experimental ponds to the control pond over time. The statistical software used for these analyses was JMP 7.0.1.

Nitrate and algal biomass (chlorophyll-*a*) levels were measured from October 2007 to February 2008 and turbidity levels were measured from November 2007 to February 2008 in the test ponds. The information gathered below discusses the trends observed of mean values over time for chlorophyll-*a*, nitrate and turbidity.

Results

Chlorophyll-*a* As observed over time, average cholorphyll-*a* values were highest in treatment pond B containing rohu, catla, and grass carp (Fig. 1). All of the ponds behaved the same way displaying similar trends regardless of treatment. While there was a rise in average chlorophyll-*a* values for treated ponds from October to November, the control pond experienced a decline in algal biomass from 6 μg/L to 5.75 μg/L. There was a decline in chlorophyll-*a* levels from November (5-20 μg/L) to January (0.5-1.5 μg/L) in every single pond and all of the ponds had the same average algal biomass levels in February (2.5 μg/L).

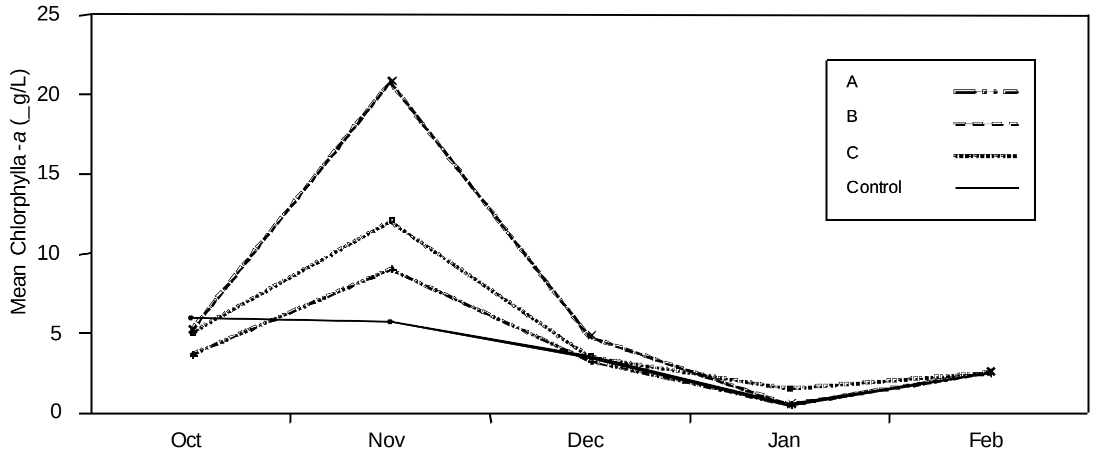

Figure 1. Monthly mean Chlorophyll-a by treatment. Measurements were taken about once a week during each month

Nitrate Treatment A (rohu, catla) and the control ponds experienced similar average nitrate values over time, whereas treatment B (rohu, catla, grass carp) and C (silver carp, grass carp) had higher average nitrate concentrations during the month of December at 0.0325 ppm compared to 0.01 ppm for the control and treatment A ponds (Fig. 2). Even though all of the treated ponds had lower average nitrate levels compared to the control pond between January and February, over the course of the experiment, a statistical difference between treatments could not be quantified due to the lack of replication. Additionally, all of the ponds experienced higher levels of nitrate in the month of February (0.02-0.03 ppm) compared to October (0.007-0.012 ppm).

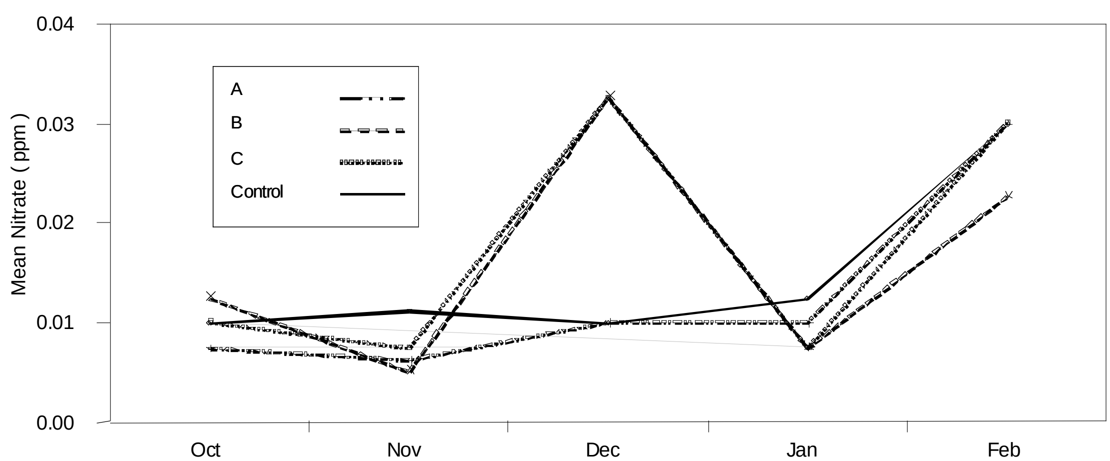

Figure 2. Mean Nitrate (ppm) concentrations over time by treatment.

Turbidity The control pond had the lowest amount of turbidity compared to the rest of the treatments throughout most of the study (Fig. 3). The turbidity levels for each treatment declined over time at different rates. All of the treatments had different levels of turbidity at the beginning of the study but converged to about similar values in the range of 2.9-5.4 NTU in February. Treatment C (silver carp, grass carp) experienced the greatest decline over time with an average of 61.9 NTU in November to 5.4 NTU in February.

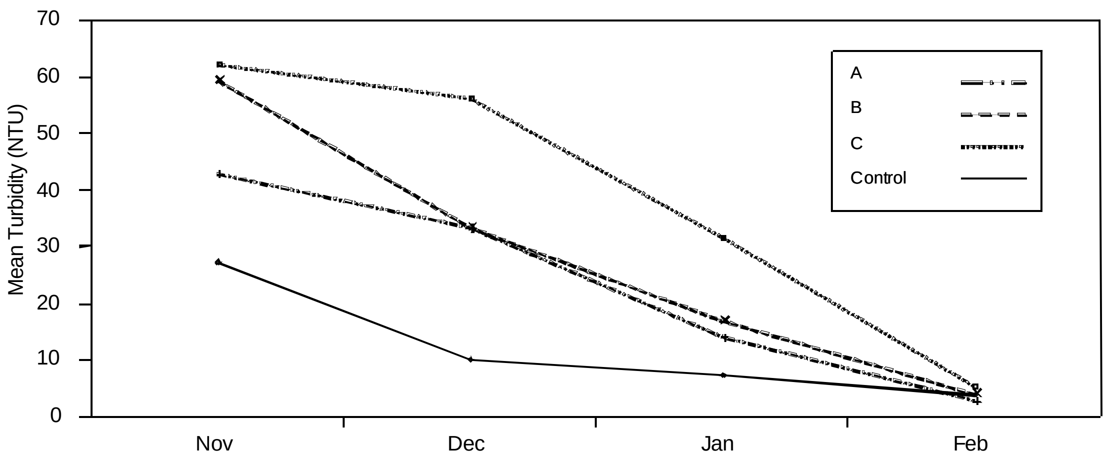

Figure 3. Mean Turbidty (NTU) levels over time by treatment. Measurements for turbidity did not begin until November 2007.

Discussion

The results gathered over a period of five months for chlorophyll-*a*, nitrate, and turbidity levels in the experimental ponds did not provide a clear distinction as to which treatment is the most effective at reducing the parameters of concern. There were, however, some discernible trends that may provide some insight as to what was occurring in the treatment ponds. Most of the parameters being measured in each pond were slightly different from each other at the beginning of the study, however, all of them seem to have similar averages at the end of the study.

Chlorophyll-*a* The average amount of biomass in the treatment ponds was higher over time compared to the control pond (Fig. 1). Treatment B (rohu, catla, grass carp) and C (silver carp, grass carp) had higher chlorophyll-*a* values throughout the duration of the study, but treatment A (rohu, catla) experienced levels that were below the control pond. The amount of algal biomass increased significantly from October to November in all of the ponds containing fish but not for the control pond. It was hypothesized that through direct consumption, fish would reduce the biomass of algae, but this increase in chlorophyll-*a* was unexpected. Biomanipulation with the introduction of certain fishes has been shown to increase the amount of biomass within a water body. According to Opuszynksi (1979) and Voros (1997), introduction of silver carp suppressed herbivorous zooplankton populations which consequently reduced grazing pressure on algae. A similar pattern may have occurred in pond C due to the presence of silver carp. Pond A, with rohu and catla, in comparison to pond B and C had the least amount of algal biomass. This combination of fish may be reducing algal biomass because it had a fewer number of fish than pond B (rohu, catla and grass carp). The presence of grass carp may have increased the amount of nutrient loading through excretion which could further enhance algal biomass growth (Persson 1997).

Nitrate There was an overall increase in average nitrate levels in all ponds from 0.01 ppm in October 2007 to about 0.03 ppm in February 2008. This rise in nitrate concentration might be due to the amount of fish excrement that is added in the form of ammonia which is later converted to nitrate. Nitrate levels for all of the treated ponds were below the control pond throughout the study except for the month of December 2007 where ponds B and C had higher average values. The reason for the rise in concentration during this month was not identified. Nitrate levels for the treated ponds were well below the World Health Organization's standard for safe drinking water of 10 ppm (Townsend *et al.* 2003). Throughout the experiment, nitrate levels were on an increasing path, however, it was not possible to see their implications in the warmer months due to the time limit of the study.

Turbidity Average turbidity declined steadily in all of the ponds (Fig. 3). The control pond had the lowest amount of turbidity compared to the treated ponds. Studies done by Bekiloglu *et al.* (2003) and Zombrano *et al.* (2001) suggest that turbidity levels in ponds and lakes can increase with carp-induced feeding. According to these studies, a lake can go from a clear-water state to a turbid state due to high suspended solid concentration from the presence of carp. This analysis probably holds true for the beginning of the turbidity measurements which were taken a few weeks after the fish had been introduced to the ponds. However, the cause of drop in turbidity among all ponds over time was unknown.

With the trends observed from the data at hand, it seems that treatment A (rohu and catla) was most effective in terms of reducing the levels of chlorophyll-*a* and turbidity, however, this has not been statistically proven. It had the lowest levels compared to other treatments, but the control pond maintained the lowest levels among all treatments. In terms of reducing nitrate levels, treatment A might again be best since it had average values that were similar or lower compared to the control pond throughout the study. Note that this may not hold true in future months due to the fluctuations of the nitrate levels as seen by ponds B and C.

It is important to keep in mind that there were some limitations to this study. The lack of replication of treatments over time did not provide robust results for the parameters being measured. The pre-treatment effects, which serve as an important baseline for comparison, were not recorded. The water in the treated ponds was in a closed system and did not have an exit path as was the case in ponds used by the villages. This could have negatively influenced the treated ponds by making them more likely to retain higher concentrations of algae, nitrate and turbidity. Also, the time span of the study did not overlap with warmer temperatures in India during the months of April through June when more algal blooms occur (Gadgil 2007, pers. comm.). According to Suzuki (2000), warmer temperatures along with the amount of light and nutrients stimulate more algal blooms. Measurements through this time period could have provided information on how the fishes influence the different treatments under warmer conditions.

The results from this study show that the introduction of certain fish into ponds may be useful in some cases but may also result in unexpected behaviors and trends. The goal of this study was to provide more insight into the effects of biomanipulation on eutrophication control within this region of India. While in doing so, it also brought about questions such as what would the results look like if this study were to take place in real-size ponds with replication of treatments? To address this issue, more applications of similar studies along with longer monitoring are needed in order to better quantify the effects of fish on eutrophication in this region of the world.

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