INVESTIGATIONS AND MANIPULATIONS OF TROPHIC LEVELS IN A POND ENVIRONMENT

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ABSTRACT

Over the course of a four week investigation in Drew University's Long Pond in the Zuck Arboretum, the dynamics of trophic levels were explored through controlled manipulation. Leading concerns dealt with aspects of the "top-down" and "bottom-up" predation theories, testing the validity of each. Exclusion chambers (plastic bags) grouped around a floatation device (PVC framework) provided control of variables, and a simplified experimental setting in a natural habitat. Inquiries dealing with the effect of increased nitrates and phosphates on trophic levels helped investigate the "bottom-up" forces through chemical testing, while manipulation of bullhead catfish as consumer organisms provided a study of the alternative "top-down" forces. Several population counts failed to garner conclusive evidence towards the initial design yet opened doors into alternative investigations. One experiment demonstrated the ability of the pond and nutrient-enriched chambers to sustain *Daphnia* populations. A study designed to test the food preferences of small versus larger catfish revealed an unexplained absence of plankton. Another experiment tested the effectiveness of rye versus white bread for baiting catfish. This final study showed a significant difference in ability of the rye bread to attract bullhead catfish ($p = 0.097$, $d = 0.1$).

INTRODUCTION

Trophic levels in aquatic ecosystems explain the transfer of energy that drives living systems. From the primary producers that derive their energy from the sun, a food chain subsists on the flow of energy through levels (Figure 1). Therefore, the organisms found at the bottom of the food chain, photoautotrophs, are essential in maintaining a balance in the diversity of life. In addition, a specific relationship existing among trophic levels allows the system to be sustained.

Two parameters believed to regulate the balance in an aquatic ecosystem are the "top-down" and "bottom-up" theories. "Top-down" refers to the predation of photoautotrophs being the limiting factor of the trophic level [1]. The "bottom-up" theory, in contrast, asserts that nutrient levels control the growth of the photoautotrophs, thus driving the balance in trophic levels.

In conjecture, the two theories put together serve to stabilize the population of photoautotrophs more than either theory on its own. To further test this idea, ecological factors such as nutrient deposits and predation were manipulated within controlled environments within a pond to observe resulting differences in trophic levels. Studies were conducted relating eutrophication, predation, *Daphnia*, and catfish to the theories on trophic levels.

Eutrophication is a process that through which a surplus of nutrients upsets the balance of trophic levels within an aquatic ecosystem. The agents responsible can be from both naturally-occurring nutrient deposits, or the result of man-made attempts to fertilize land. In the past, recorded incidents of eutrophication in the Delaware Estuary [2] and the Chesapeake Bay [3] have shown to cause significant algal blooms and observable depletion of dissolved oxygen levels [4]. In the Seto Inland Sea of Japan, natural eutrophication resulted in the severe reduction of fish populations [5]. It is believed that an overabundance of algae accumulating on the water's surface will lead to increased turbidity and a depletion of oxygen, thereby choking other photoautotrophs responsible for upholding the food chain of much-needed sunlight and oxygen. In the study at Long Pond, the amount of nutrients placed within an exclusion chamber, the independent variable, was compared to turbidity and dissolved oxygen levels, the dependent variables, to see how this might affect trophic levels.

Daphnia, or water fleas, are freshwater crustaceans named for the jerky motions they use to propel themselves through water. *Daphnia* are capable of asexual and sexual reproduction. Female *Daphnia* reproduce asexually under favorable conditions through a process known as parthenogenesis. However, under less suitable conditions, female *Daphnia* give birth to both males and females, thus fostering sexual reproduction. *Daphnia* are sensitive to changes in salinity, pH, and ammonia and metal ion concentration. However, they are more tolerant to fluctuations in temperature and variations in dissolved oxygen concentration [6].

Besides manipulating the pond environment, experiments were also conducted to determine the effect of nutrient levels on *Daphnia* abundance in a manipulated laboratory setting. It was hypothesized that increased nutrient levels would promote *Daphnia* reproduction, resulting in a notably higher population, while decreased nutrient levels will stunt population growth.

Daphnia play a critical role in pond ecology. They serve as a food source for many fish and feed on other crustaceans, yeast, algae, and bacteria [7]. Population of *Daphnia* and *Cyclopoid copepods* (*Cyclops*) were to serve as dependent variables in the initial experimental design proposed for this study.

The predation of brown bullhead catfish on small organisms is a factor that regulates *Cyclops* and *Daphnia* populations. *Cyclops* are tiny, brown, crab-like creatures; *Daphnia* are larger, clear, and jelly-like. Bullheads dwell in the bottom of freshwater ponds and lakes and prefer to eat small organisms like *Cyclops* and *Daphnia* [8]. In 1999, Darold Batzer, Chris Pusateri, and Richard Vetter conducted a study on bullhead catfish to determine the effect of predation on other small organisms [9]. It was gathered from these individuals' experiment that bullheads prey heavily on freshwater organisms, which make up 87% of their diet. However, the results may have been skewed because other fish were used as well to study the effects of competition in the chambers [9]. These scientists did research from an experiment that concluded that such fish have a minor impact on foodwebs. Based on previous research, it was hypothesized that the size of a bullhead catfish would have no effect on the differences in predation.

In the final experiment, the bait preferences of bullhead catfish were examined. Bullhead catfish (*Ameiurus nebulosus*) are bottom feeding fish tolerant of eutrophic conditions and poor and polluted waters. The catfish usually feed nocturnally and have a keen sense of smell to locate food. Bullhead catfish lack scales and have eight barbells surrounding the mouth [10]. Though mainly scavengers, food in the catfish diet includes insects, plant material, carrion, small fish, snails, crayfish, worms and leeches [11]. However, based on discussions with individuals who have caught catfish, it was conjectured that given a choice between rye and white bread, the catfish would prefer rye bread. Apparently, the rye bread contains certain chemicals and substances such as caraway seeds which affect the olfactory sensors in the catfish more than the chemicals and nutrients in the white bread. In this experiment, traps were baited with either white bread or rye bread and dispersed within a natural pond environment in six locations. The randomly chosen locations included vegetated areas, shaded areas, and sunny, open areas of the pond. The locations of the traps were alternated during trials so that after the six locations were chosen, traps containing white bread and traps containing rye bread received equal exposure to each area. The numbers of catfish caught in traps containing rye bread were compared to the numbers of catfish caught in traps containing white bread to determine catfish bait preference.

METHODS

This study was conducted at Long Pond, a small body of water located in the arboretum on the northeastern campus of Drew University in Madison, New Jersey, primarily because of its convenience and close proximity to the laboratory. Long Pond, approximately 100 meters long and varying from 30 to 40 meters wide, is a relatively isolated environment, which made for an ideal study of pond ecology.

The team first designed and constructed a chamber in order to create an isolated pond environment separate from the pond itself. To do this, eight 67 cm x 67cm squares were built from ½ inch PVC pipes using PVC fittings. Foam pool noodles were placed on each side of the square around the PVC as well as on the 40 cm connector pieces to provide floatation. The squares were grouped into two sets of four holding containers. Large plastic bags with a circumference slightly larger than the perimeter of the square were attached to the PVC using duct tape such that they reached a depth of one meter. These bags were separated from each other by 40 cm so that they would not touch. The two setups were then placed in the middle of the pond with a close proximity to one another (Figure 3). They were attached to anchors to prevent drifting and then filled with pond water. As shown in Figure 2, each holding case was given the specific additive to test the independent variable under investigation.

First tested were the effects of increased levels of nitrates in a pond environment. In order to monitor this, tests on samples of pond water were conducted within exclusion chambers depicted in Figure 1. An exclusion chamber where no additional nitrates were added, chamber 1, served as one control, and the natural pond environment served as another. Exclusion chamber

2, in which nitrogen levels were increased through use of “Espoma,” a 10-10-10 nitrogen-phosphorus-potassium fertilizer, served as the experimental chamber.

Samples of pond water from the chambers were taken from 25 cm below the surface and then chemically analyzed using spectrophotometry to affirm that nutrient levels were higher within the experimental chamber than they were in the two controls. To do this, pond water was tested using a CHEMetrics VVR water analysis system which allowed the spectrophotometer to measure nitrate levels in parts per million.

Data was also collected for dissolved oxygen and phosphates in each chamber and the pond itself using readings taken from CHEMetric vacu-vials. One purpose for this was to estimate the amount of phytoplankton growing in each chamber due to the different parameters. Additives in the water functioned as independent variables. These included the controls of the pond and chamber 1, variables of added nitrates in chamber 2, catfish in chamber in 3, and a combination of the two in chamber 4. Water samples were then taken over a two week period. These were collected by taking boats out to the holding chambers and collecting 250 mL of water from each chamber, and one from the pond itself. Then, amounts of nutrients and dissolved oxygen were measured using testing kits. After the data was collected, it was analyzed to find patterns within the chambers. Data related to turbidity and pH was obtained on site using Vernier probes, a lab-pro interface, and laptop computer.

In the investigation of predation in relationship to predator size, two different environments were created as controls in the exclusion chambers containing different sized catfish. The independent variable was the size of catfish per chamber. In chamber A3, four small fish (14 cm – 16 cm in length) were placed in freshwater only; in chamber A4, four large fish (17 cm - 20 cm in length) were placed with the nutrient mixture. Then, in chamber B, the sizes were switched and four large fish were positioned in the freshwater of chamber 3 while four small fish went into chamber 4 with the same nutrient mixture. From research, it was found that most bullheads prefer bottom water plankton, and that they also eat meat such as beef liver or glass worms (Schmidt, *Minnow Trap Bait*, 1996), although none of the latter was used in conducting the experiment. The purpose was to observe how the bullheads prey on freshwater plankton. After having set up the chambers as stated above, 50mL samples of water (which contained the plankton) was collected from each of the 4 sections, using a plankton net to conduct vertical tows. There was a separate container for each section to store these samples. Ethanol in the containers served to preserve the plankton for future population counts. For each sample, the 50mL were divided up into 10mL increments, which were observed under a microscope. From this, the dependent variable, the number of *Daphnia* and *Cyclops* remaining, was determined, and this was the number that survived predation. This number told how the size of the bullheads affects plankton consumption.

In addition to predation, the foraging preferences of catfish for different breads were also studied. Equal amounts of white and rye bread were obtained and distributed into six bullhead catfish traps (Figure 3), three containing white bread and three containing rye bread. Serving as the independent variable, these traps were then distributed in central locations throughout the pond as indicated in Figure 3. Approximately a half hour following distribution, the traps were collected in the order they were placed. The dependent variable, the number of bullhead catfish in each trap, was enumerated and recorded. This procedure was repeated five more times to accumulate a total of 18 trials. T-tests were used to determine statistical significance in the bread preference.

The last experiment was to determine how well manipulated pond environments could sustain *Daphnia* populations. In order to do this, three culture dishes were prepared, each containing five specimens of *Daphnia* from a previously prepared culture kit. Culture dish 1 was prepared with 130 mL of spring water, dish 2 with 130 mL of pond water, and dish 3 with 130 mL of pond water enriched with nutrients from exclusion chamber A4. Each dish received a minute amount of desiccated egg yolk to stimulate bacteria growth. These bacteria served as the food source for the *Daphnia.* Dish 2 served as the control, as it most closely reflected the *Daphnia's* natural environment with no added or withheld nutrients. The extent of the nutrient enrichment served as the independent variable. The dependent variable was the population density of *Daphnia* per 100mL.

To measure the *Daphnia* population growth, three random 10 mL samples were taken from each culture dish using a 1 mL pipette. The number of *Daphnia* per 10 mL sample was determined using visual counts. The three counts from each dish were recorded in terms of population density per 100 mL of liquid. Each set of three counts was then averaged to give one representative population density for each dish. Population measurements were taken every two days over a six-day period.

RESULTS/DISCUSSION

"Top-Down" "Bottom-Up" Theories in Relation to Trophic Levels

When the experiment was first designed the intentions were to use dissolved oxygen and turbidity as indicators of the phytoplankton population. However, after testing and reviewing the result, the levels of dissolved oxygen formed no consistent pattern and were inconclusive. This was possibly due to influences from outside factors such as temperature, sunlight/ shading, weather, etc. Therefore, turbidity was used as the sole indicator of phytoplankton growth.

Several patterns in turbidity readings emerged from two weeks of data collection. To begin, as seen in figure 4, the turbidity levels in control chambers A1 and B1 mimic that of the pond. While the pond had the highest turbidity level, each of the holding containers was not far behind.

The increase is most likely due to natural factors. In fact, an increase in turbidity of the control holding containers may even have been somewhat expected since phytoplankton and other producers were isolated in them without the presence of large consumers like fish. In the nutrient enriched chambers, a high amount of phytoplankton was expected to grow, thus causing a higher level of turbidity. However, testing showed that the nutrient enriched levels actually ended with the lowest turbidity readings. This may seem like this doesn't support what was originally thought. However, when looking at the graph one can see that the phytoplankton population does start to rise. Later in the study period, it falls again. This is due to an increase in herbivores when the phytoplankton increased. The herbivores than ate the phytoplankton, causing a decrease in population. It can also be observed that while equal amounts of phosphate and nitrates were added, only the phosphate readings were off the scale in subsequent testing. Possibly the nitrogen was used via the nitrogen fixation process, while the phosphate could not be used.

Additionally, the chambers containing solely catfish (chamber A3 and B3) ended with the highest turbidity levels although there were no added nutrients. This is due to the fact that the catfish were eating the herbivores, leaving no predators to prey upon the phytoplankton. This would cause an increase in turbidity which indicates an increase in phytoplankton population.

Looking at all chambers as a whole, number four (which contained fish and nutrients) remained the most constant over time. This is because while the nutrients are creating an abundance of phytoplankton, the herbivores are keeping them in check, while the catfish eating the herbivores keeps everything at a balanced level. Thus, to maintain a healthy environment, both top and bottom levels of the trophic system must work together.

Eutrophication

When the phosphate versus nitrate and turbidity graphs are compared, turbidity appeared to follow the same pattern in fluctuations. Phosphate levels exploded into levels beyond measurement by the latter two trials in the nutrient enriched chambers A2, A4, B2, and B4. Nutrient filled containers provoked larger unpredictable disparities from trial to trial than the fairly consistent control groups. In the A-group chambers where data was taken at the experiment's beginning nitrate levels showed a precipitous decline between the first and second trials (Figures 5 & 6).

Phosphates steadily increased to levels beyond the range of the Chemetrics equipment. The pond control group consistently increased in turbidity while three of the four nutrient enriched chambers displayed alternating levels of turbidity. However the chambers with both fish and nutrients varied less than those with isolated nutrients. Turbidity even declined in the case of chamber B4. The chambers containing fish alone accumulated astronomically high turbidities in a rapid and consistent trend.

The gradual balance of nitrates indicates their incorporation into the ecosystem of the enriched chambers (A2, A4, B2, B4). Producers absorb the nitrates and nitrifying bacteria incorporate them back into the ecosystem, as shown by the increase in the third trial. Turbidity follows this pattern as well, initially low while the organisms utilize available nitrates, it only increases once the nitrates begin to re-appear. The turbidity increase corresponds with the reproductive success of those organisms that utilize nitrates and recycle them into the ecosystem. Eutrophication also follows the level of phosphate increase in all the chambers, yet declines while phosphates continue to accumulate, suggesting that organisms fail to reincorporate phosphate while it gathers and pollutes the water. Phosphate pollution explains the decrease of turbidity in exclusion chambers; turbidity rapidly increased where the fish consumed herbivores leaving vegetation to cloud the water. Our hypothesis was supported by correlating nitrogen and turbidity levels yet failed to predict and explain phosphate pollution. Turbidity levels varied along with the expected nitrogen cycle, and appeared self-regulating until disturbed by excess phosphate. One may deduce that excess levels of nitrate and phosphate in a large body of water will disturb natural turbidity levels from their equilibrium states

Daphnia

After six days, population trends were apparent in the three culture dishes. Dish #1 with spring water, after relatively small initial growth, declined in population on days 4 and 6 to virtually no *Daphnia*. Dish #3 with pond and nutrient water also substantially increased, although dish #2 with pond water alone sustained by the highest increase in population.

Great disparities existed among the appearances of the three culture dishes by day number six. On day zero, the culture dish containing only spring water appeared clear, with the visible particles of yellow desiccated egg yolk floating in the water. By day six, the egg yolks developed a red region surrounding them, which appeared to be a fungus. All Daphnia life had virtually ceased in this dish. On day zero, the pond water in dish 2 had a light green tint. By the sixth day, scattered greenish-brown algae had grown throughout the culture dish. Virtually all the Daphnia were relatively smaller than their counterparts in the dish with pond water and nutrients. The pond water in dish 3 also appeared green on day zero, but by day six concentrated dark green algal masses had grown throughout the dish. Dish 3 contained more algae on day six than dish 2. Also, the Daphnia in dish 3 were relatively larger than those in dish 2.

Dish 1 containing spring water could not maintain a stable Daphnia population, possibly because it lacked the natural pond algae upon which the Daphnia feed. This suggests that the bacteria serving as sole food source for Daphnia in dish 1 were insufficient to support a population.

All dishes experienced Daphnia growth with respect to population density by day two, with Dish 3 having the highest density. However, the population density in Dish 2 far exceeded the others' on days four and six, meaning that the pond and nutrient solution did not support Daphnia reproduction as well as the pond water alone. This unexpected result did not support the hypothesis. Perhaps this occurred because the nutrient enrichment in culture Dish 3 was too drastic, prohibiting optimal Daphnia reproduction.

Another observation was that the culture dish with pond water and nutrients had a smaller population when compared to the culture dish of only pond water. However, the Daphnia in the dish of pond water and nutrients appeared considerably larger than those in the dish with only pond water. Possibly, the added nutrients in Dish 3 stimulated greater algal growth. This larger food source may have contributed to the greater size of the individual Daphnia. An excess of nutrients, while increasing the size of the individual Daphnia, may also increased the mortality rate of the population as a whole. Moreover, it is possible that the Daphnia in the culture dish with only pond water adapted to the high population of organisms by reducing their size, whereas the Daphnia in the culture dish with pond water and nutrients were able to increase in size due to the smaller population.

Predation

Population counts of *Daphnia* and *Cyclops* were low to non-existent in all of the chambers. On day 1 of data collection, populations were relatively higher than counts on following days, but population comparisons between the chambers per trial showed no significant differences, as in most cases no organisms were found in the samples.

It should also be noted that three of the bullheads in chamber A4 died, possibly due to starvation, although all other bullheads survived the experiment. What is also interesting is that in the chambers not including the catfish, population counts were also down; this may have resulted due to the unusual precipitation occurrences in New Jersey this summer. The water that was placed in the chambers came primarily from the surface of the pond; deeper pond samples may have yielded different results.

Table 1 Plankton Population Counts

		Daphnia			*Cyclopod*	
Date	*29-Jul*	*2-Aug*	*5-Aug*	*29-Jul*	*2-Aug*	*5-Aug*
A1	1	0	0	80	0	0
A2	0	0	0	0	0	0
A3	0	0	1	1	0	0
A4	0	0	0	1	0	0
B1		0	0		0	0
B2		0	1		0	0
B3		2	0		7	0
B4		0	6		0	2

Bait Preference of Catfish

The catfish preferred rye bread significantly more to white bread ($p<0.10$, $\alpha = 0.10$).

Other than the catfish, other species of fish were collected and considered as well. See Table 2. A t-test showed that fish, in general, do not show preference of one type of bread to another.

Figure 3 shows a sketch of the pond habitat and the location of the traps. These locations could have affected the number of fish obtained. For example the location with the most fish was location 1. This could have resulted from the depth at that area of the pond, vegetation, a possible breeding area, or high levels of nutrients. In other locations, such as location 3, few fish if any were found. This may have occurred from a lack of food in the area. In addition, all other fish besides catfish were caught at locations 4-6. These areas were typically shallow and contained many branches, silt, and other predators such as turtles. This sort of environment seems more suitable for goldfish and carp. Also, some areas were more shaded or exposed to more sunlight than other. Locations were purposely varied as much as possible to expose each type of trap to the varying conditions.

Many factors could have affected this experiment. For example, on a given day, there may have been less fish present in a location as a result of weather changes and the behavior of the fish. In addition, some samples were obtained on the same day immediately following prior ones. In this case, there were usually less fish on the second and third set of trials. This could be due to the fact that the fish were disturbed by the unusual activity in the pond. Besides location and environmental factors, human error could have occurred as well. For example, some fish may have fallen out of the trap upon retrieval, or the entrance to the trap may have been blocked by a log, branch, silt, etc., during deployment.

Table 2 Catfish Trials

Trial #	# of Catfish in White Bread Trap	# of Catfish in Rye Bread Trap
1	1	2
2	0	1
3	0	0
4	0	6
5	1	0
6	1	0
7	0	1
8	0	0
9	0	0
10	0	3
11	0	0
12	1	2
13	0	0
14	0	0
15	0	1
16	0	0
17	0	0
18	0	0
Total	4	16

SUGGESTIONS FOR FURTHER STUDY

For Further Study on Eutrophication

Further analysis on the effect of nutrient-enrichment on the ecosystem could be carried out in a number of ways. Instead of using a combined nutrient solution of nitrates, phosphates, and potassium, studies on each individual nutrient's effect on the ecosystem could be accomplished by adding only one type of nutrient to each experimental chamber. Results relating turbidity to the levels of the nutrients could then be compared and analyzed.

For Further Study on *Daphnia*

Future research may focus on the connection between asexual and sexual Daphnia reproduction and nutrient levels. Daphnia may reproduce both asexually and sexually, depending on their environment. Optimal nutrient levels would stimulate asexual reproduction in Daphnia. One possible hypothesis is that excess nutrient levels are adverse, and therefore cause Daphnia to revert from asexual to sexual reproduction, in order to adapt to unfavorable conditions. This research could provide valuable insight into the survival of Daphnia, and thus the ecology of the entire pond.

For Further Study on Predators through *Chaoborus* populations

Should populations be discovered during sampling, future studies might consider examining the vertical migration patterns of *Chaoborus*. During the daytime, the larvae of this species, usually common to Long Pond, remain buried in the mud to avoid predation. However, when darkness falls, the *Chaoborus* rise up from the mud to feed and take advantage of the dissolved oxygen at the lake's surface [12]. Consequently, the goal of this study would be to examine effects of decreasing light exposure on vertical migration in controlled circumstances. In order to test this hypothesis in a laboratory setting, organisms obtained from the pond could be housed in clear plastic core sampling tubes oriented in a vertical fashion and exposed to varying intensities of light. After a period of time, the number of Chaoborids at each depth would be recorded. A large population of Chaoborids at the surface under lower light levels would tend to support the hypothesis, while an unchanged or random distribution would nullify it. Due to the unexpected scarcity of the larvae in Long Pond, the experiment could not be conducted this year. Changes in pond nutrient levels or species populations in Long Pond, however, could result in an increase in the *Chaoborus* population, making the study possible in future years.

For Further Study on Catfish Predation

For further study, it will be best to take pond samples from the bottom of the pond instead of the top, for better counts of plankton populations. In addition, the bullheads must be kept alive throughout the experiment. Possibly, if they are kept in a tank instead of a plastic bag, they will not be as crowded; the bigger space could allow for more plankton as well, which would help keep the bullheads alive by providing nutrition. Competition of bullheads with other species of fish may also be studied, to see if the bullheads actually do prey on plankton as much as the results in this experiment seemed to have shown, or if there are other species that prey on plankton more.

Suggestions for Further Study on Fish Bait Preferences

Further study of fish bait preferences could be expanded by employing the use of different variables. The white bread/rye bread test could be conducted on carp or goldfish alone. To further test the hypothesis that fish prefer rye bread to white bread, certain chemicals from the rye bread could be isolated and decoyed to attract fish. In addition, an investigation could correlate the number of fish caught and the depth the respective baits were decoyed, or the number of fish caught in relation to how sunny or shaded the area of the pond was.

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Figures

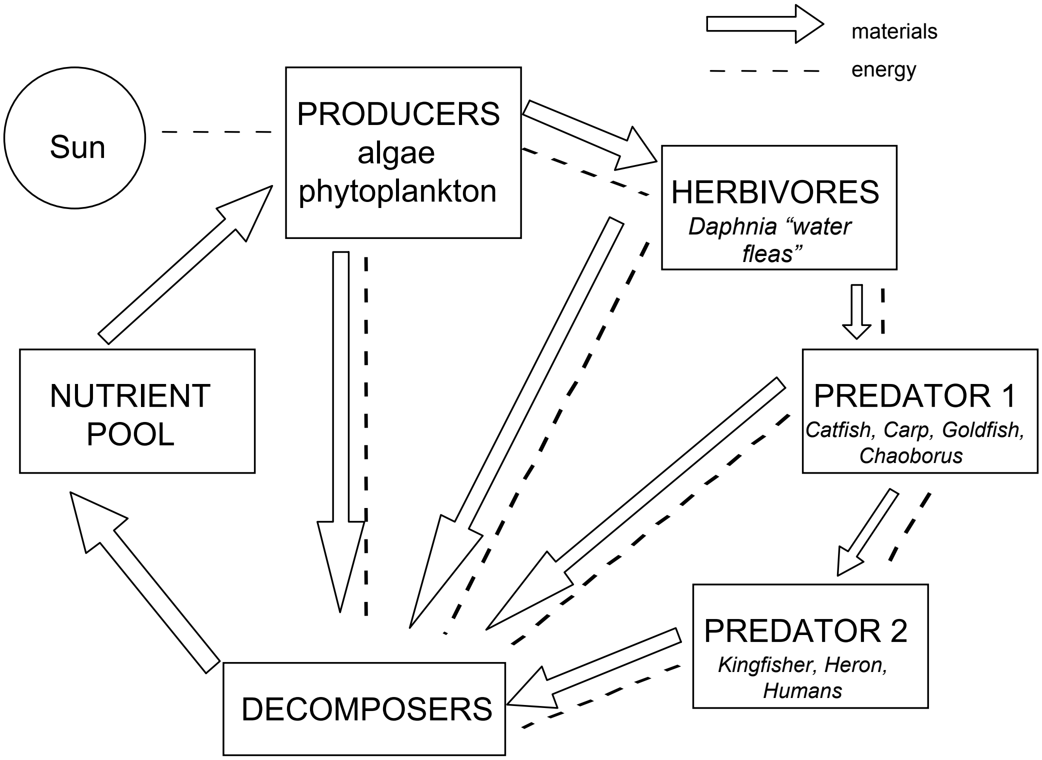

Figure 1 Trophic Levels of an Ecosystem

Diagram depicts flow of energy and cycling of matter through Long Pond

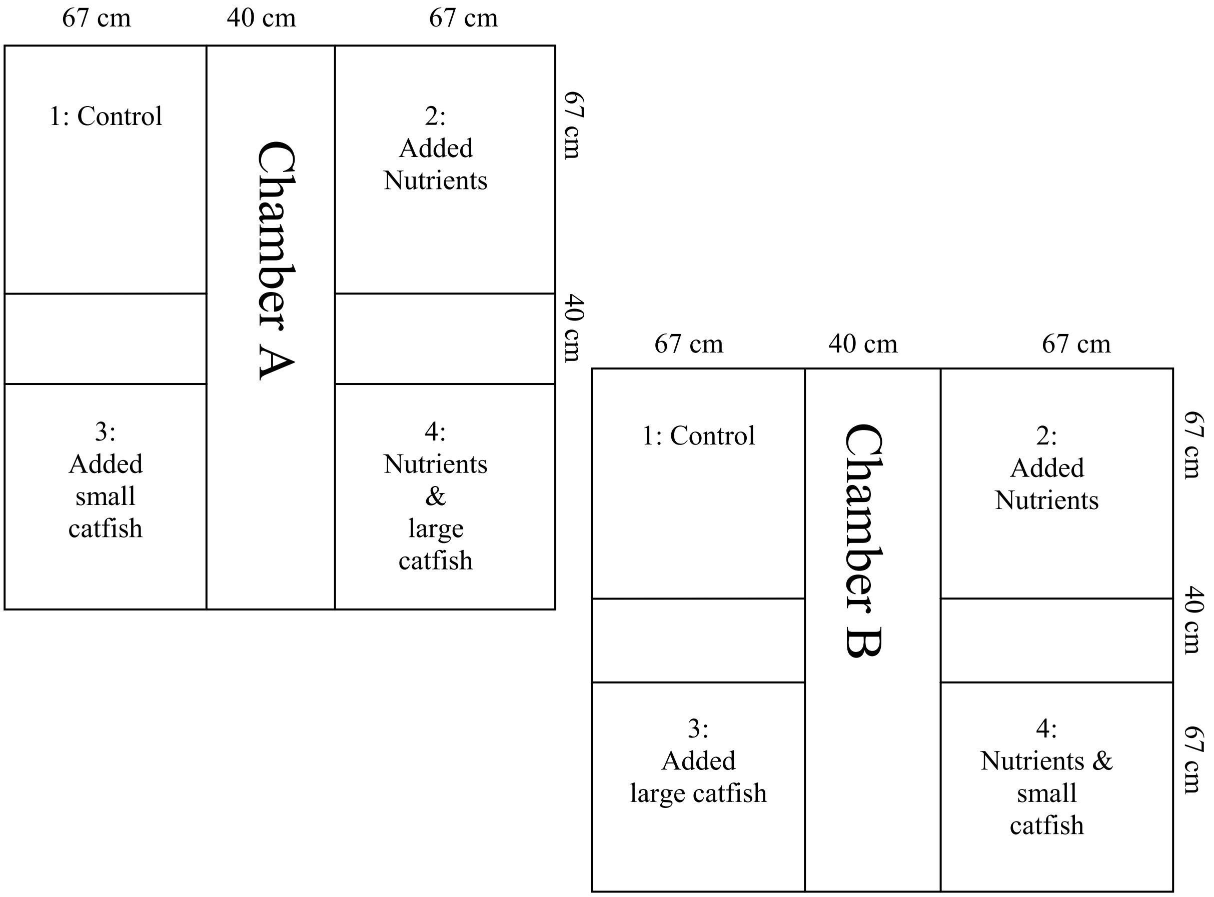

Figure 2 Diagram of the Exclusion Chambers Used in the Pond

Top view of contents in chambers for experiments

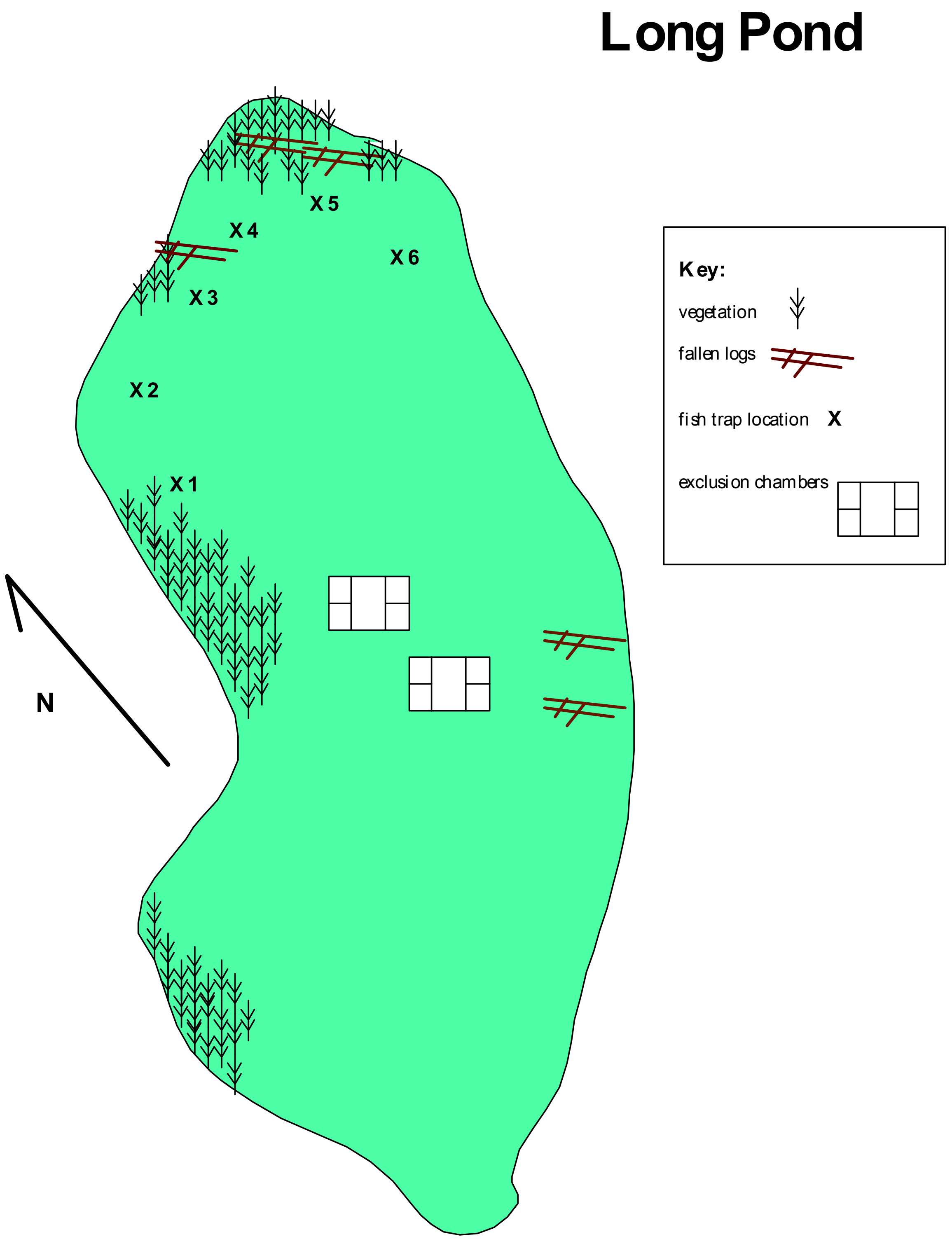

Figure 3 Diagram of Long Pond

Aerial depiction of Long Pond showing relative locations of two exclusion chamber set-ups and six catfish trapping sites

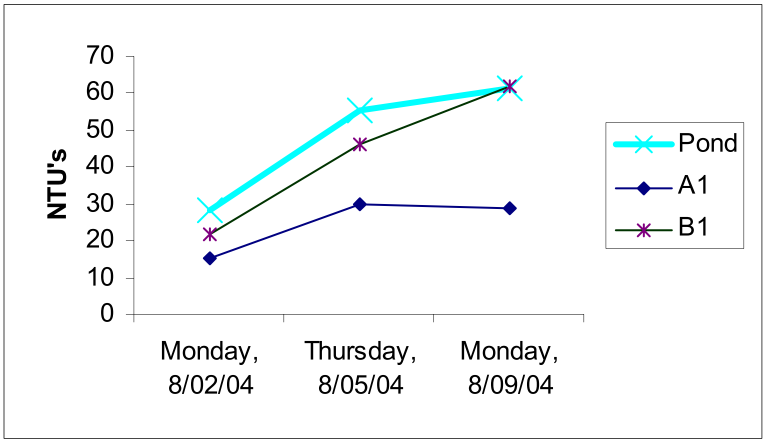

Figure 3 Turbidity Levels of Controls

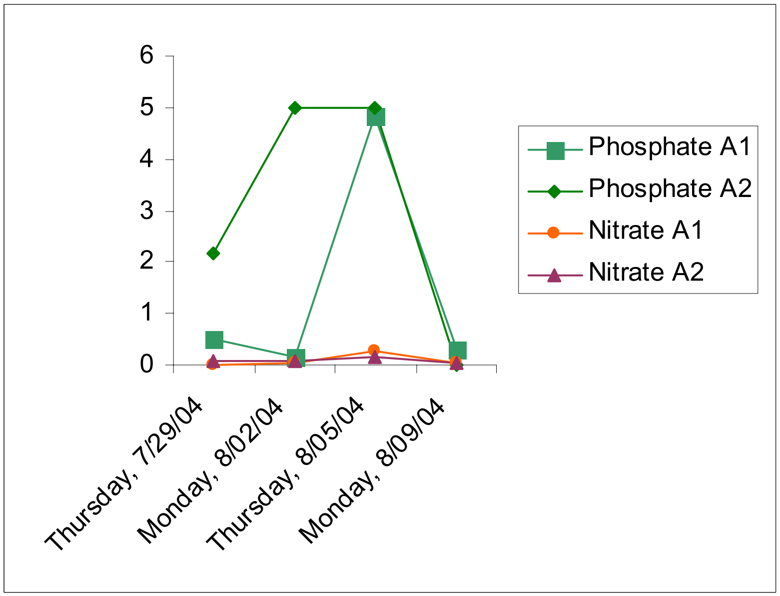

Figure 4 Phosphate vs. Nitrate Levels in Chambers A1 and A2

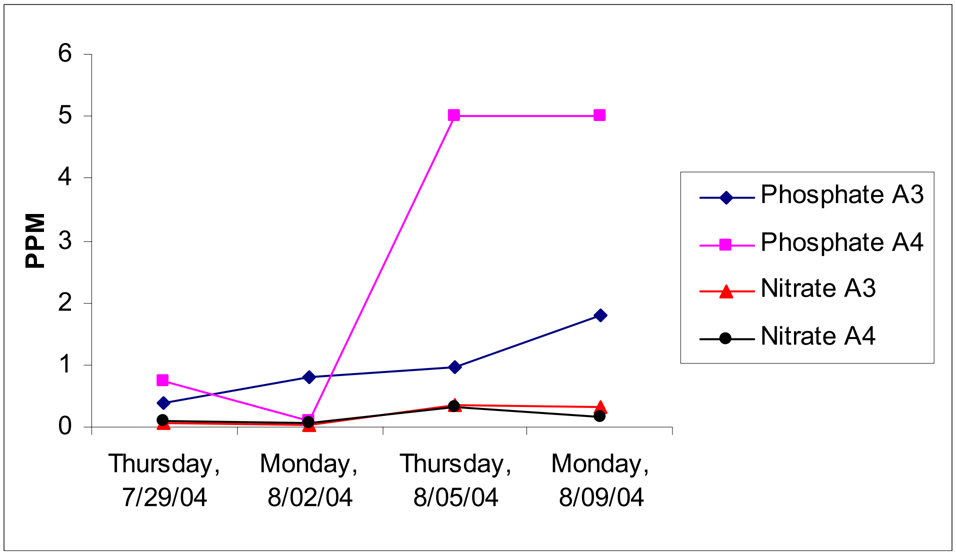

Figure 5 Phosphate vs. Nitrate Levels in Chambers A3 and A4

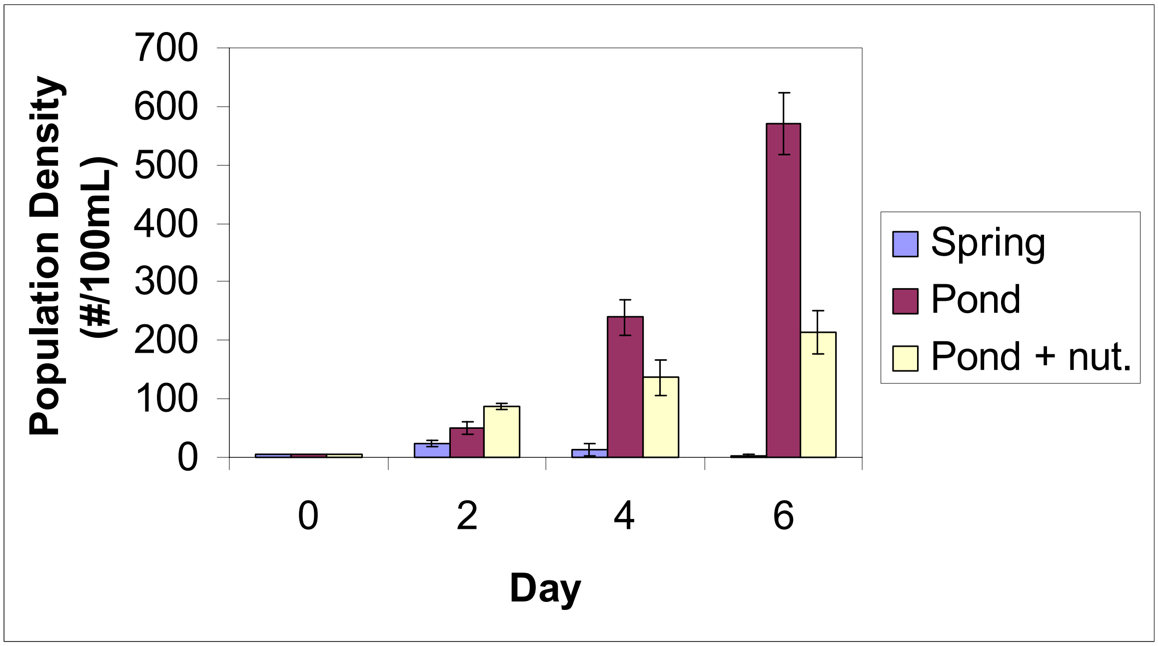

Figure 6 Daphnia Population

Appendix A: Chart used to synthesize experimental designs

	Top Down Manipulation →					← Bottom Up Manipulation
	Carnivores		Herbivores		Autotrophs	
	Upper level predator	Primary predator	Cladocera	Copepods	Phytoplankton	Nutrients
Exclude	Simulated	None	🟩	🟩	🟥	None added
Catfish	None	4 added	🟥	🟥	🟩	None added
Exclude & Nutrients	Simulated	None	Up+	Up+	🟩	Added
Nutrients & Catfish		4 added	?	?	Stim x2 dir	Added
Open Pond	Used as control					None added

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