# Diversity and abundance of plankton in an artificial wetland, Tottino Ponds

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Capstone
5-5-01

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## Big Picture, Little Picture

Wetlands play an important role in our ecosystem. A haven for wildlife, they also regulate the hydrology by moderating the effects of a heavy rain and continuing the flow of groundwater in a dry period (Odum, 1978). Water is a scarce commodity here due to population growth and agriculture (Lyons et al, 1996). Managing our wetlands in an efficient way will produce the greatest amount of habitat for the many animals that need wetlands while conserving the greatest amount of water. The wetland being studied is located near Castroville, in central California, in the middle of agriculture fields. This site was chosen because it is public land located in an area that would naturally be a wetland extension of the Moro Cojo Slough if it were not for the diking and draining of the surrounding agricultural areas (Lyons et al, 1996).

Wetlands are valuable for a variety of reasons. They are effective at removing nitrates and fertilizers from the water, which causes the outflow from a wetland to be cleaner than the inflow (Moss, 1988). This would be valuable in our coastal environment where algal blooms in the ocean can potentially cause eutrophication. In the United States, 20% of endangered or threatened plants or animals are associated with wetlands (Moss, 1988).

Before the appearance of Europeans in California, the many rivers that emptied into the Monterey Bay, such as the Carmel, Salinas, and Pajaro, sprawled out and covered much of the coastal lands with wide marshy valleys (Margolin, 1978). There was such an abundance of life here that explorer, la Perouse, was quoted as saying "There is not any country in the world that which abounds more fish and game of every description." (Margolin, 1978). The water table was so close to the surface that one would only have to dig a few feet to strike clear, fresh water. Within a few generations of European colonization, some species of birds and animals had been completely exterminated. Within a century, the land was changed permanently from its natural, productive state by destroying marshes and wetlands for conversion into farming parcels (Margolin, 1978).

Local wetlands are currently seriously depleted as a result of management policies that have been conducted for the last 200 years. Most of Elkhorn Slough has been diked and drained at some time in history. This valuable ecosystem was at one time a fresh water system that made up the estuaries of the Salinas and Pajaro Rivers (Lyons et al, 1998). Currently Elkhorn Slough is comprised mainly of a tidal inlet that has salinities close to the ocean's salinities.

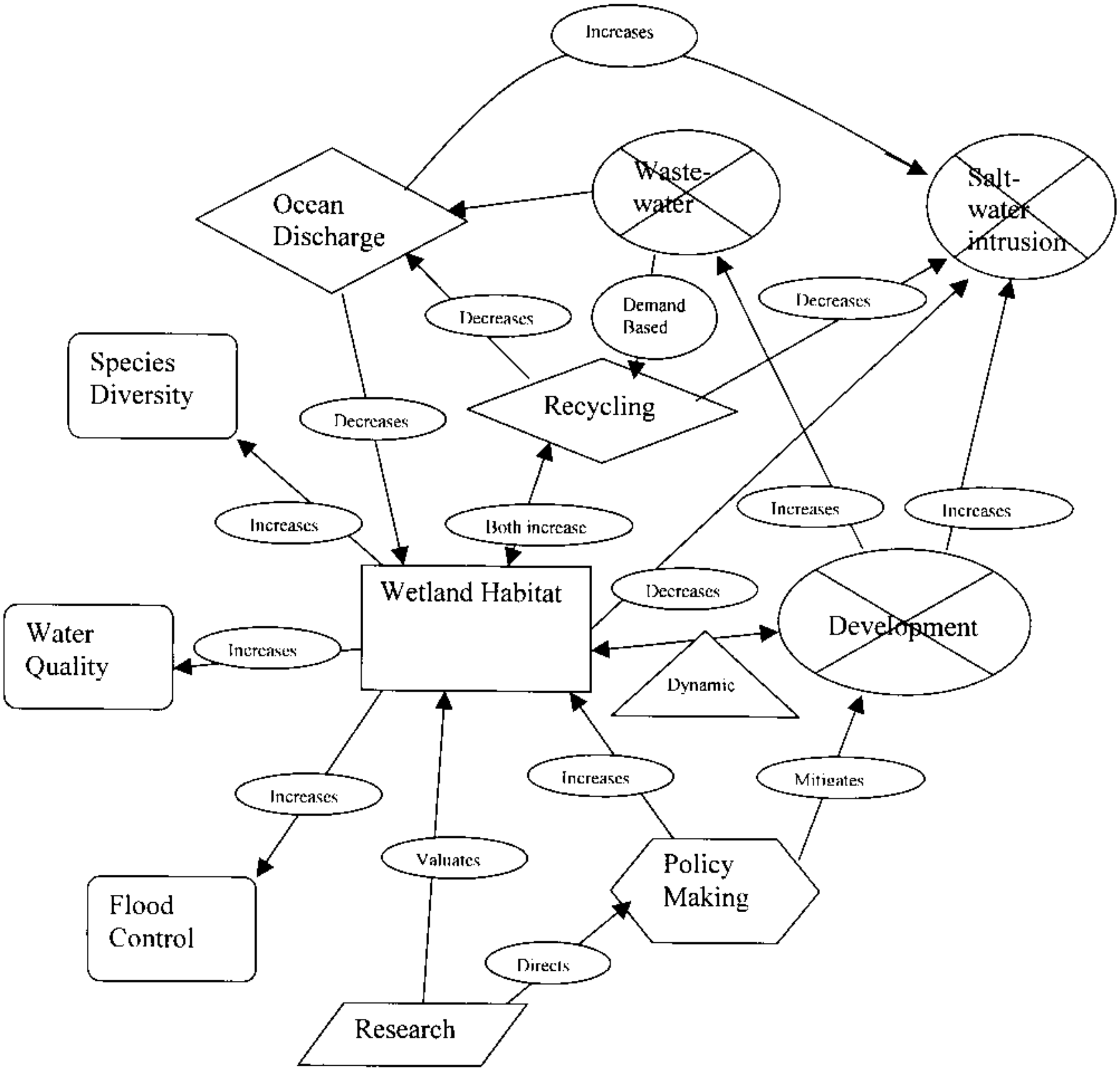
In the surrounding area, many wetlands have been drained and used for agricultural or residential purposes. The policies regarding fresh water have long been to remove it from the land as fast and efficiently as possible and channel it into the Monterey Bay. Little effort has been made in the past to protect this commodity or preserve the wetlands in which fresh water accumulates. Few people wish to surrender the land that could be economically valuable if used for a farm or a housing development in order to create a habitat for wildlife that helps to recharge the groundwater supplies. The result has been a massive loss of wetland habitat and the organisms that live in them as well as saltwater intrusion into the water table.

In an effort to reduce the amount of saltwater intrusion, a 70million dollar wastewater treatment facility was constructed in the city of Marina. They claim that by recycling the wastewater collected from 200,000 homes, they could reduce salt-water intrusion into the Salinas Valley Aquifer by 30% (MRWPCA). At this time, they are recycling up to 70% of the wastewater, but this number is dictated by demand and if there is no demand for the water, they discharge the partially treated sewage into the Monterey Bay. Farmers in the North Salinas Valley use the recycled water during the growing season, but in the winter months, much of the waste water is diverted into the bay after secondary treatment (See appendix I. For more information on the treatment of wastewater).

In order to increase demand for recycled wastewater and restore some of the depleted wetlands, Dr. John Oliver obtained permission to convert a strip of public land into a wetland. The land was to be used as a research base into the new field of using recycled water for wetland restoration. In 1999, a series of small ponds were constructed using a tractor and were filled up with recycled wastewater. The water level was maintained by periodically adding recycled wastewater to the system.

Dr. Oliver began to recruit researchers to conduct studies on the site in the areas of the benthos, fishes, birds, water quality, mammals and amphibians, native plants, and plankton. With this team of researchers, he hopes to discover the value of artificial wetlands and the effects of recycled wastewater on biological communities. Using the information gathered by the researchers and him, Dr. John Oliver hopes to write grants to fund more wetland restoration projects. He hopes to learn techniques on how to create and manage future artificial wetlands that can be developed into policies regarding their construction and maintenance. These policies may come into play in mitigation cases where developers wish to build on wetland areas.

## Diagram of Wetland System




From a systems perspective, wetland restoration plays a key role in helping to reduce salt-water intrusion both by increasing the amount of water recycled and absorbing more surface water into the water table. Development plays a key role in wetland habitat as can be seen in the past draining of many wetlands. This is a dynamic relationship, however, as mitigated development can add to the amount of wetland habitat. This is where research-based policies can be helpful to increase the amount of wetland habitat and the resulting benefits.

I was contracted by Dr. Oliver to conduct a study on the zooplankton and periodically measure the quantity of chlorophyll in the water column. My study began in October of 2000 and is still ongoing. The following report is a summary of the results and a discussion of contrasts and interactions that have occurred up to this date. I have been working at Moss Landing Marine Labs, a graduate school, since January of 2000. I began my internship there as a benthic macrofauna sorter which is how I became proficient in microscopy and species surveying. I spent much of my time there working on coastal dune restoration where I learned the interaction of species and successions under the guidance of the researchers and students there. In the summer of 2000, I became the sedimentologist for the benthic lab and conduct grain size analysis for the benthic lab and the Department of Fish and Game.

I have been attending CSUMB since the fall of 1999 studying in the ESSP program with an emphasis in marine and coastal systems. I have personal interests in mathematics, physics, computer programming, ecology, and the ocean. I hope to continue my studies after completion of my studies at CSUMB. I wish to study at Moss Landing Marine Labs where I can continue my survey of Tottino Ponds and see the system develop and even more systems if Dr. Oliver can write enough reports and I give him sufficient data. So what does one do with 69,000 copepods, 52,000 rotifers and a mayfly? Observe them.

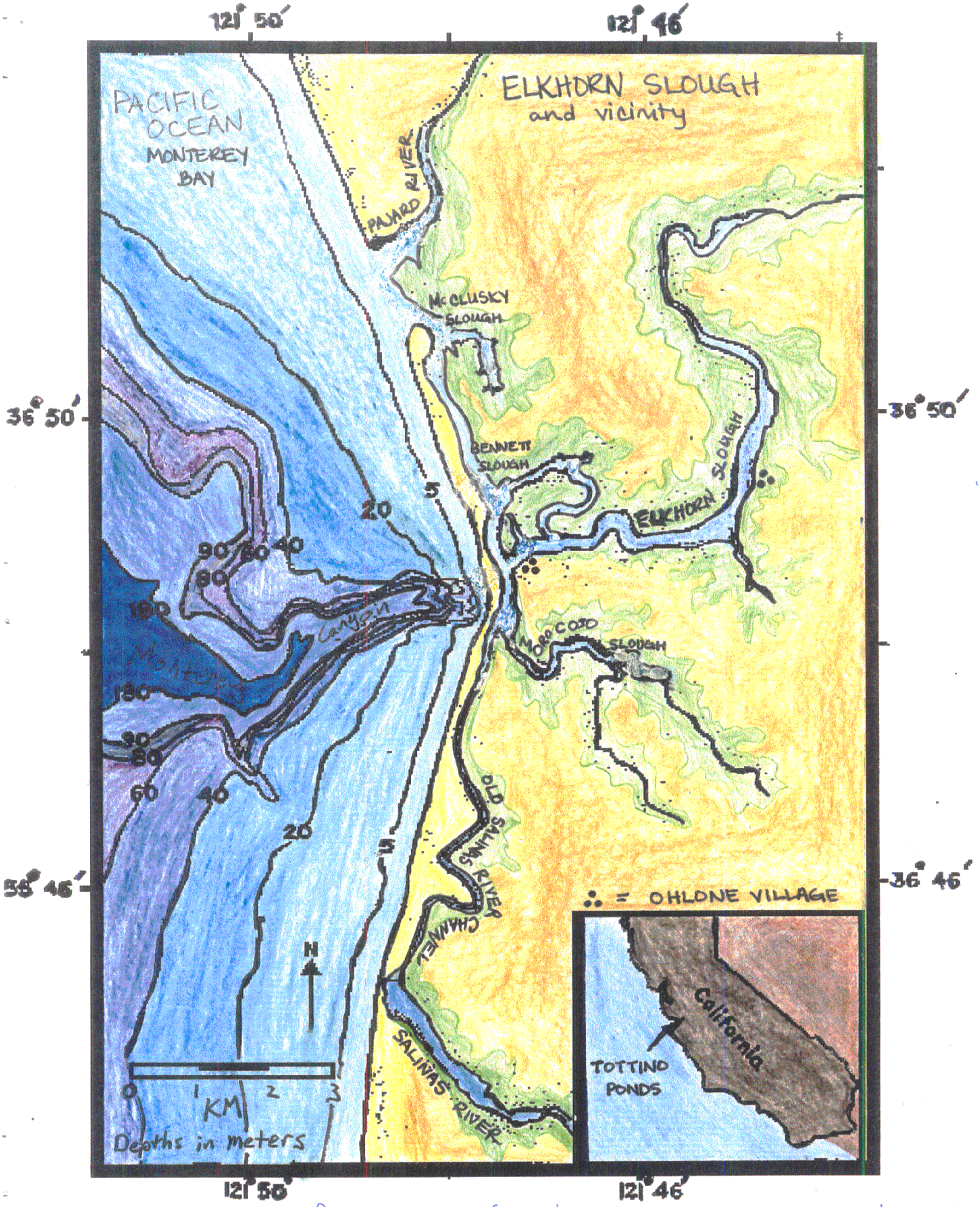



Fig 1. The Pristine Slough (Silberstein, 1991) p. vi

## I. Abstract:

**Tottino Ponds is an artificial wetland created in the middle of agricultural fields, near Monterey Bay, and filled with recycled waste water. The site consists of four 25-meter square ponds. A study was conducted between October 2000 and April 2001 on the planktonic organisms living in the ponds. A series of one-liter samples were taken on four different dates. The samples were preserved and sorted. The samples contained a total of 121,526 organisms ranging in size from rotifers to a tadpole (*Pseudacris*). A measure of chlorophyll concentration was taken at the beginning and end of the study. The results showed that copepod, rotifer, and cladoceran abundance varied with time and remained constant from pond to pond. There was a negative correlation between cladoceran and chlorophyll abundance. The diversity of Tottino ponds throughout the survey, using Simpson's diversity index (1-l), was 0.51.**

## II. Introduction:

This study combines the issues of wetland and wastewater management. What happens when a hole is dug with a tractor and filled with tertiary treated sewage? This happened in 1999, one year after the Monterey Regional Water Pollution Control Agency began recycling waste water in an effort to curb saltwater intrusion by 30-40% in the north Salinas Valley (MRWPCA, 2001). Since that time, a biological community has developed from migrating birds to red-legged frogs (*Rana aurora*). The field of wetland management is not new. A study has shown that by managing a wetland's hydroperiod, one can increase species diversity and abundance (Anderson & Smith, 2000). The part of this study that is unique involves the variables of a completely artificial setting and recycled wastewater.

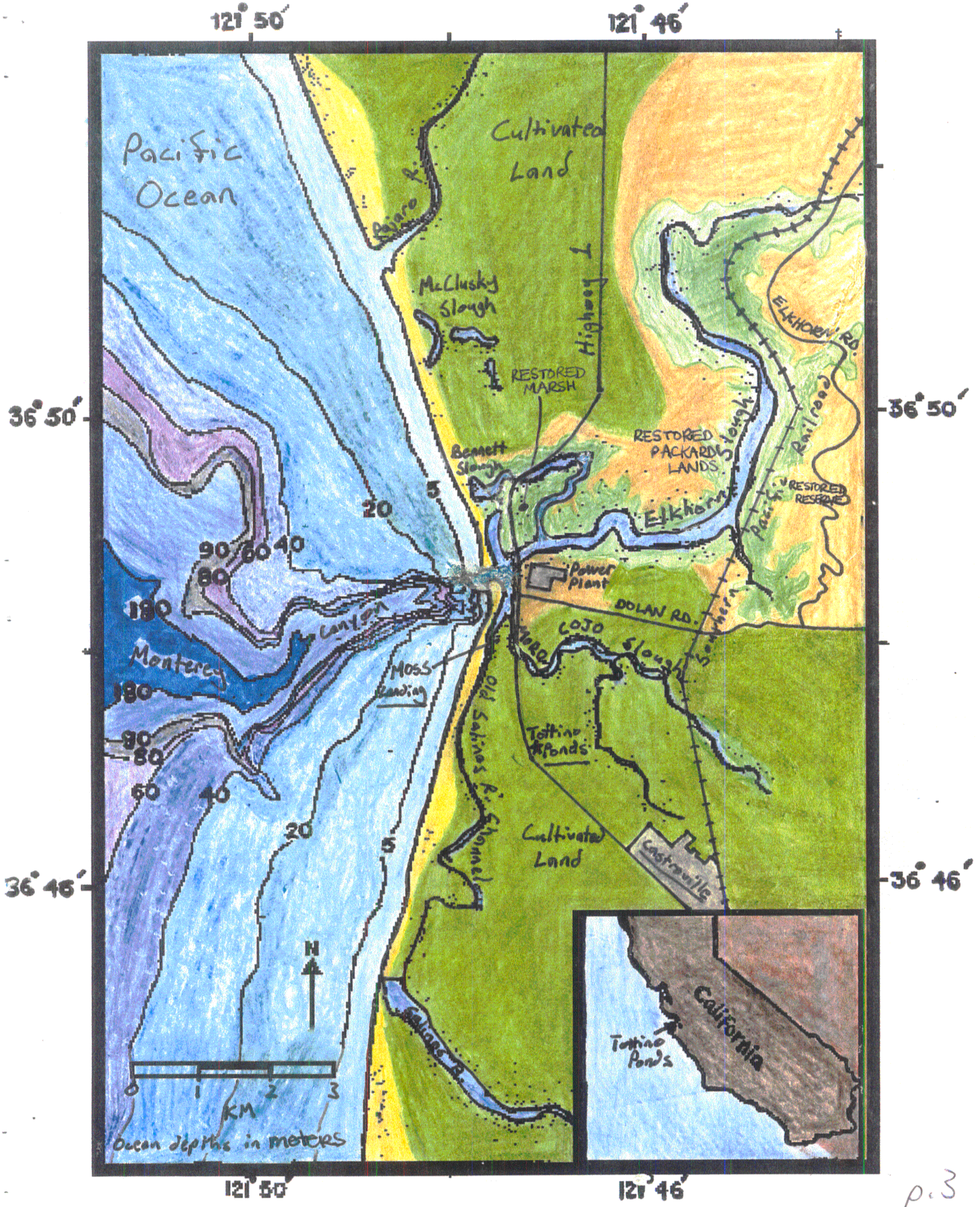
This study looks into the type of biological community that will develop given these surroundings. Planktonic organisms were sampled for a period of six months. A measurement of the chlorophyll content was done at the beginning and end of the survey. The diversity of the system is assessed in order to determine its complexity and stability (Bower, 1990). Does the abundance of organisms vary with location and time throughout the system? Is there a correlation between the abundance of organisms and the level of chlorophyll present? What are the underlying causes for these relationships?

## III. Site Description:

The Tottino Ponds are located on the western boundary of the Moro Cojo Slough watershed 2.2-Km north of the town of Castroville. The ponds are adjacent to Highway 1 on the East Side of the highway. Elkhorn Slough and the Moss landing power plant are located 2.8 Km to the north of Tottino Ponds. The Pacific Ocean is 1.5 Km to the west of Tottino Ponds. The ponds are surrounded on all sides by agricultural lands and are located in a natural depression between the fields. (Fig 3.a)

The site consists of four square ponds constructed with a tractor in 1999 that are approximately 25m on all sides. The contours of the ponds were constructed so that there is a 1.5m deep hole in the NE corner of each pond. The diameter of the holes is about 10m and they slope gradually down from a depth of 0.5m. The water level is controlled by periodically adding recycled wastewater from the Monterey Regional Water Pollution Control Agency (see appendix 1.). The water is also used to irrigate farmland in the North Salinas Valley. The water source is located between Highway 1 and the ponds. Water is fed into a 0.5m wide ditch that runs along the north side of the ponds. A short ditch fills each pond from the main ditch.

There are four ponds and they are staggered eastward from the highway at 20m intervals. Pond one is closest to the highway and the pond numbers increase with distance from the highway. The elevation of the ponds decreases with distance from the highway. There is a dominant west wind at the site. The total width of the wetland created from these ponds is 75m and the last pond is located 175m from the highway. Any excess water flows into a pre-existing drainage ditch located along the south side of Tottino Ponds via 0.5m wide ditch.

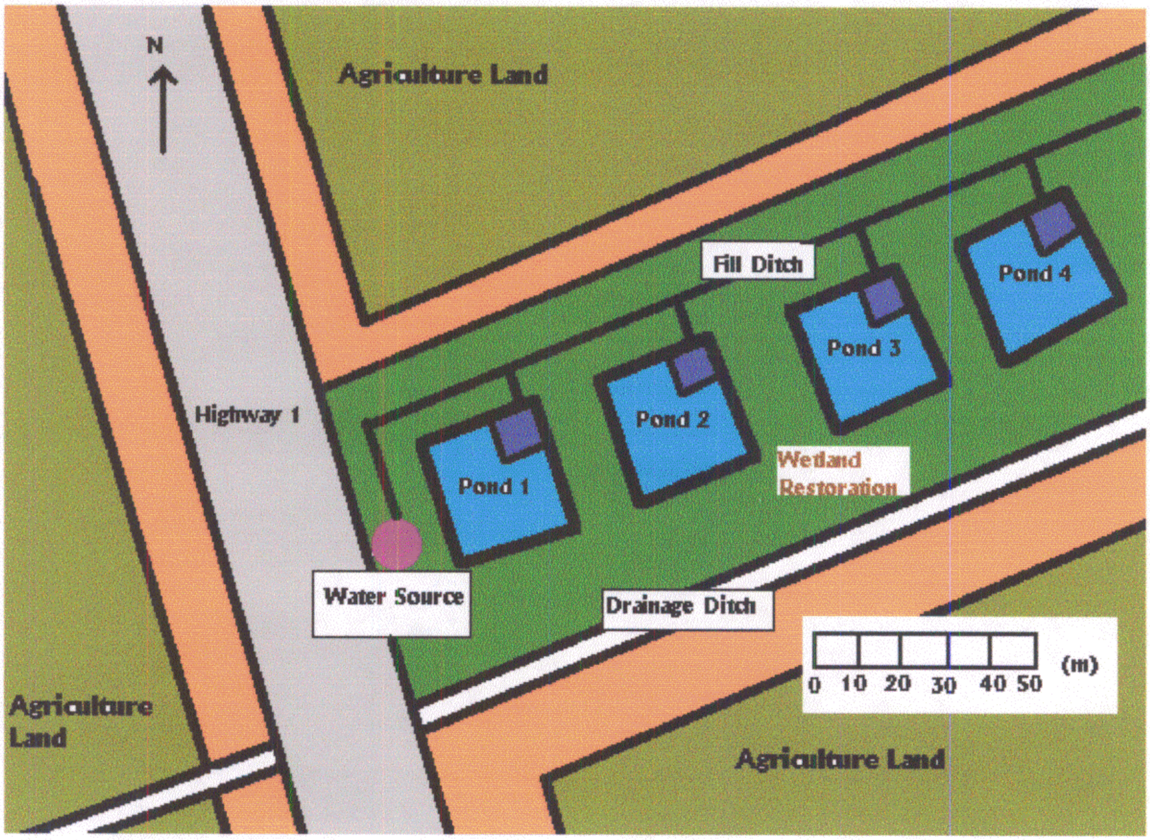



– Location of Tottino Ponds –

Fig. 3.a

p. 3

Fig. 3.b




**Detailed schematic of Tottino Ponds**

p. 4

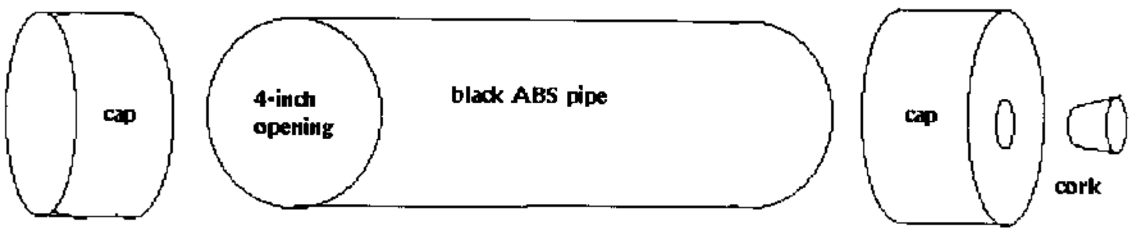
## IV. Materials and Methods:

As there were two components to the study of the water column biota of Tottino Ponds, the method of each component will be listed separately. The two components were **macro-zooplankton**, and **chlorophyll content**.

A. Macro-zooplankton Survey:

Materials:

- For sampling the water column, a sample device was constructed from a section of 4-inch ABS pipe with a 1L volume. The sampler had removable caps on each end that were sealed by applying a coat of petroleum jelly to exterior ends of the sampler. One of the caps had a small hole with a cork in it to allow water to pass out while sealing and opening the sampler.




**1 L volume water column sampler**

- A 63 micron sieve was used to concentrate the sample.
- 100% formalin for fixing the zooplankton.
- 70% isopropyl alcohol for preservation.
- Squirt bottle.
- Petroleum jelly.
- Sample jars.
- Dissection microscope.
- Petri dishes.
- Forceps.
- Small specimen vials.
- Plankton splitter.

- Multiple variable counter.

Methods:

- Note.

Samples were collected on four dates between 10/19/00 and 4/2/01. Samples were taken between 8am and 12 noon. Three replicates were taken from each pond on each sample date. There was one exception to the sampling procedures that occurred on 1/3/01. At this time, the ponds were being drained with a mechanical pump and three samples could not be obtained in time from ponds 2-4. Only one sample was able to be obtained from these ponds on 1/3/01. These samples were obtained by scooping 1L of water in a jar instead of using the standard sampling device. The three samples from Pond 1 were taken using the standard sampling device on the following afternoon.

Throughout the study, the condition of the ponds varied dramatically. At the beginning, the ponds had been full for a year and submerged aquatic vegetation had grown in ponds 3 and 4. Ponds 1& 2 had no aquatic vegetation in them. 1 month before the study, mosquito fish (*Gambusia affinis*) were introduced by Mosquito Abatement into the fill ditch system. The ponds were re-filled after waiting for the mosquito fish to be exterminated. The ponds were re-filled naturally by rains in early February of 2001. The water level was maintained in the ponds by the addition of recycled waste-water. For the February samples, no submerged aquatic vegetation was present in any of the ponds. Trace amounts of submerged aquatic vegetation began to develop in Pond 4 at the final collection date.

- Collection.

Samples were taken at random along the perimeter of eastern half of each pond using the above sampling apparatus. This was done to limit the number of samples because the dominant winds concentrated the plankton along the eastern edge. This was also done to ensure that some samples would be taken near the basin located in the north-east corner of each pond.

The samples were taken from the top 30cm of the water column by inserting the tube vertically into the water column in a rapid motion so that no animals avoided the sampler. Once submerged, the caps were applied, beginning with the bottom cap and ending with the upper uncorked cap. The cork was then inserted in the top to seal the sample. The sample was then sieved through a 63 micron mesh. The contents of the sieve were flushed into a container by means of a squirt bottle. 100% formalin was added to the contents of the container until a concentration of 5-10% was achieved. After fixation, the samples were transferred to 70% isopropyl alcohol for preservation.

- Processing.

The preserved samples were transferred to water and sorted under a dissection microscope. All planktonic species were removed and placed in specimen vials with the exception of the Copepods and Rotifers. Due to their small size, delicacy, and high densities, a few specimens of these were sub-sampled for identification purposes. The collected specimens were identified and counted using a multiple variable push-button counter. In the case of densities exceeding 500 individuals for the Copepods and Rotifers, a plankton splitter was used to sub-sample until a manageable level of individuals was obtained while still preserving a minimum number of individuals of a species above 100. The sub-samples were counted using a counter and a gridded petri dish.

B. Chlorophyll Content:

Materials:

- 20mL vials
- 90% acetone
- 7 micron Glass Fiber Filter (GFF)
- Vacuum filtration system
- Freezer
- Centrifuge
- Test tube vials with caps
- Flurospectrometer
- Graduated pipet

Methods:

- Note.

  Chlorophyll content was only studied on two dates. The first date was at the beginning of the study and the second was at the last sample date of the study.

- Collection.

  A 20mL vial was numbered and dipped into a pond. This was done on the first and last sample dates. One sample was taken from each pond.

- Processing.

Between 5 and 20mL of the sample was filtered through the GFF using a vacuum filtration system. The GFF containing the filtrate was placed in 5mL 90% acetone solvent in a capped test tube and placed in a freezer. After waiting a minimum of 24 hours, the extract was taken out of the freezer. The filter was pressed down to the bottom of the test tube to avoid interference with the flurospectrometer. The extract was centrifuged to remove solid particles from suspension.

The outside of the vials were cleaned and dried. A blank of 5mL acetone was placed in the flurospectrometer to calibrate the device. The extracts were then placed in the flurospectrometer and the RFU was recorded. For the October 2000 samples, it was necessary to dilute the extracts to be within the bounds of the flurospectrometer. Dilutions were made by removing a fraction of the extract with a graduated pipet and adding the fraction to 5mL of 90% acetone.

## V. Results:

### A. Macro-zooplankton Survey:

### 1. Classification of total diversity of Tottino Ponds using Simpson's Index:

The Simpson's dominance index was calculated for the species using the formula

D(s) = 1 – Sum(number in group)*(number in group-1) / ((total number individuals)*(total number individuals-1))

Although the species were not identified to the same taxonomic level, each group contains only one type of organism so it was possible measure the diversity of the system.

D(s) = 0.50935

**Total Individuals Counted at Tottino Ponds**

| Common Name | Taxonomic Group | Total Abundance |
|---|---|---|
| (Copepod) | Cyclopoidae | 6691 |
| (Copepod) | Nauplii | 60290 |
| (Rotifer) | Brachionidae | 52494 |
| (Cladocera) | Daphniidae | 1891 |
| (Boatmen) | Corixidae | 20 |
| (Backswimmer) | Notonectidae | 1 |
| (Beetle) | Dytiscidae | 17 |
| (Midge larva) | Chironomidae | 33 |
| (Damselfly) | Coenadrionidae | 2 |
| (Mayfly) | Baetidae | 1 |
| (Tadpole) | *Pseudacris* | 1 |
| (Ostracod) | Podocopida | 85 |

**Table V.A.1**

## 2. Variation in group density

The data collected were analyzed using the statistical program SPSS. The data were analyzed in two ways, sample date vs. abundance and location (Pond#) vs. abundance.

### Copepods

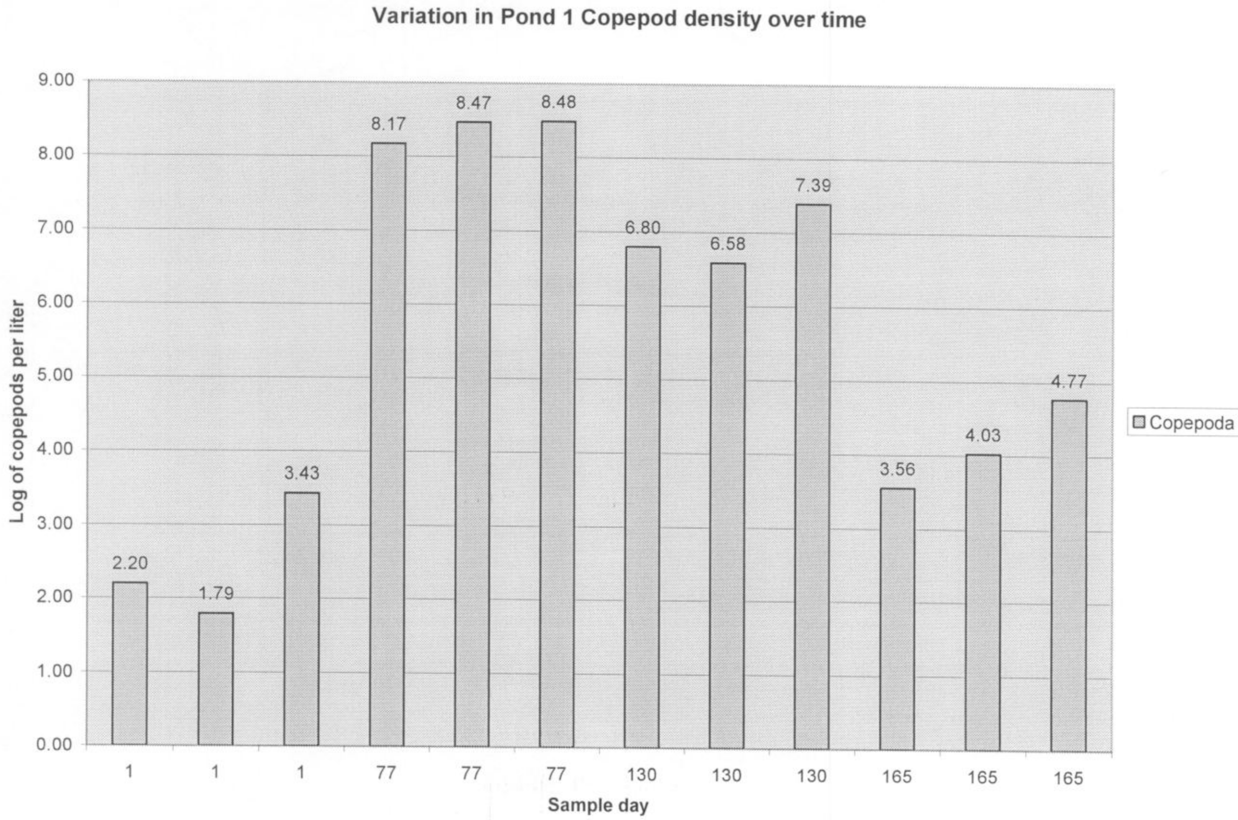
For analysis, due to the extreme abundance of copepods, the data were transformed using the natural log of the density per liter.

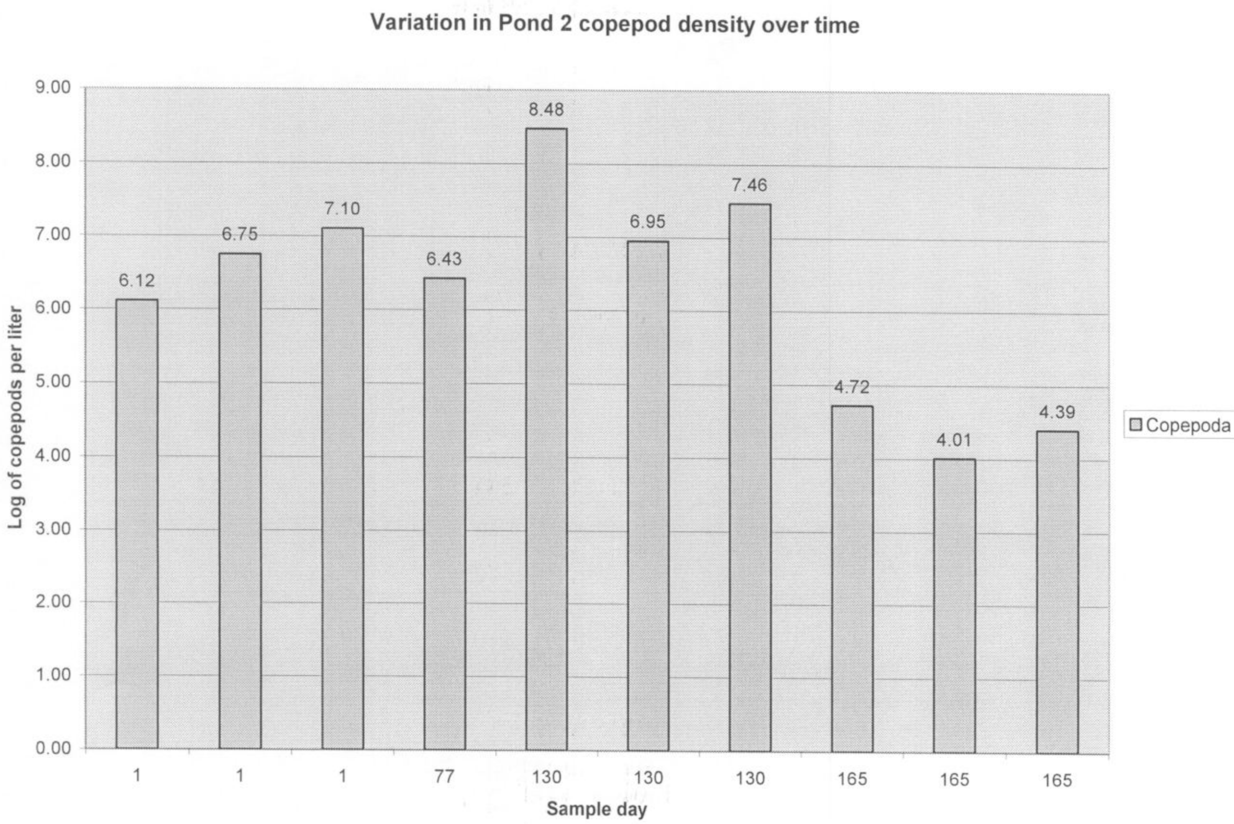
- Location vs. abundance

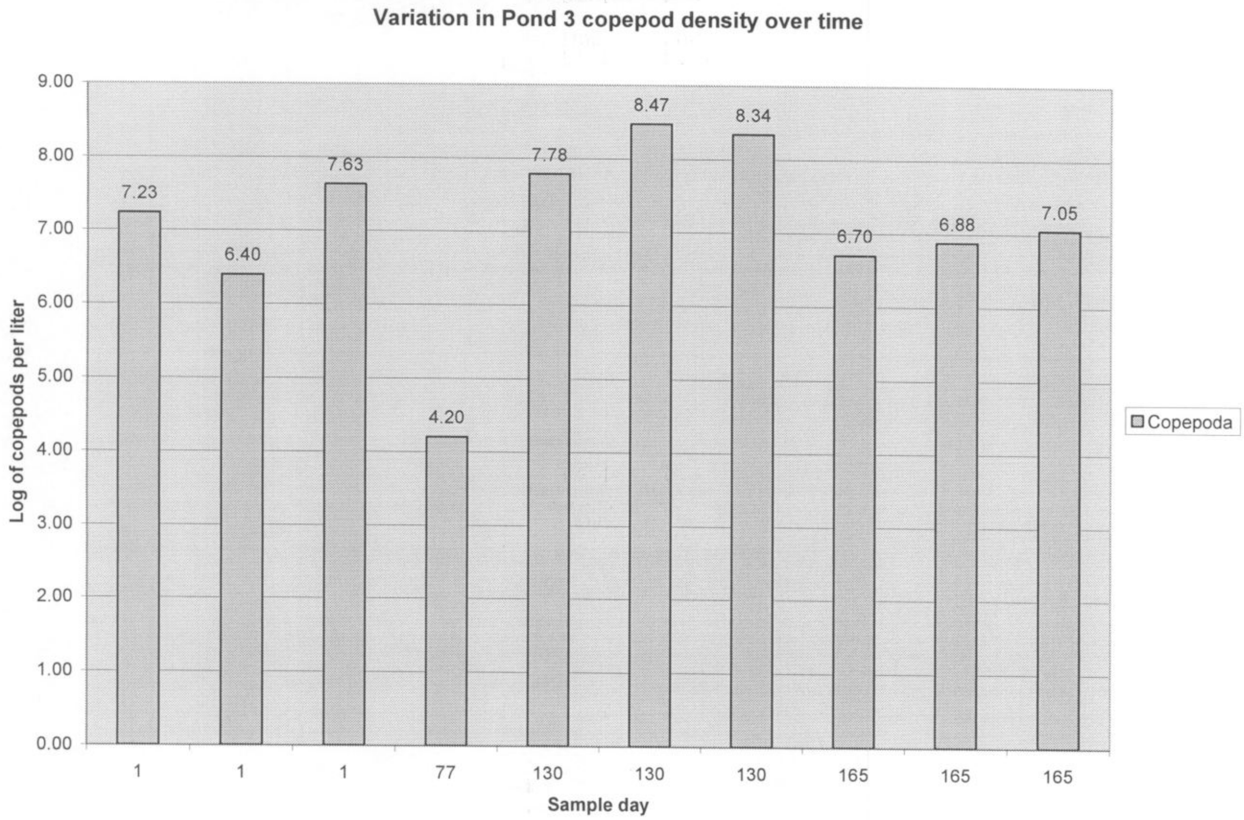
The Levene statistic rejected the hypothesis that the variances of each pond were equal. ($p < 0.01$) Due to unequal variances, a Games-Howell multiple comparison was made. The results of the comparisons failed to reject the null hypothesis that there was any variation in copepod density between ponds. ($p > 0.2$)

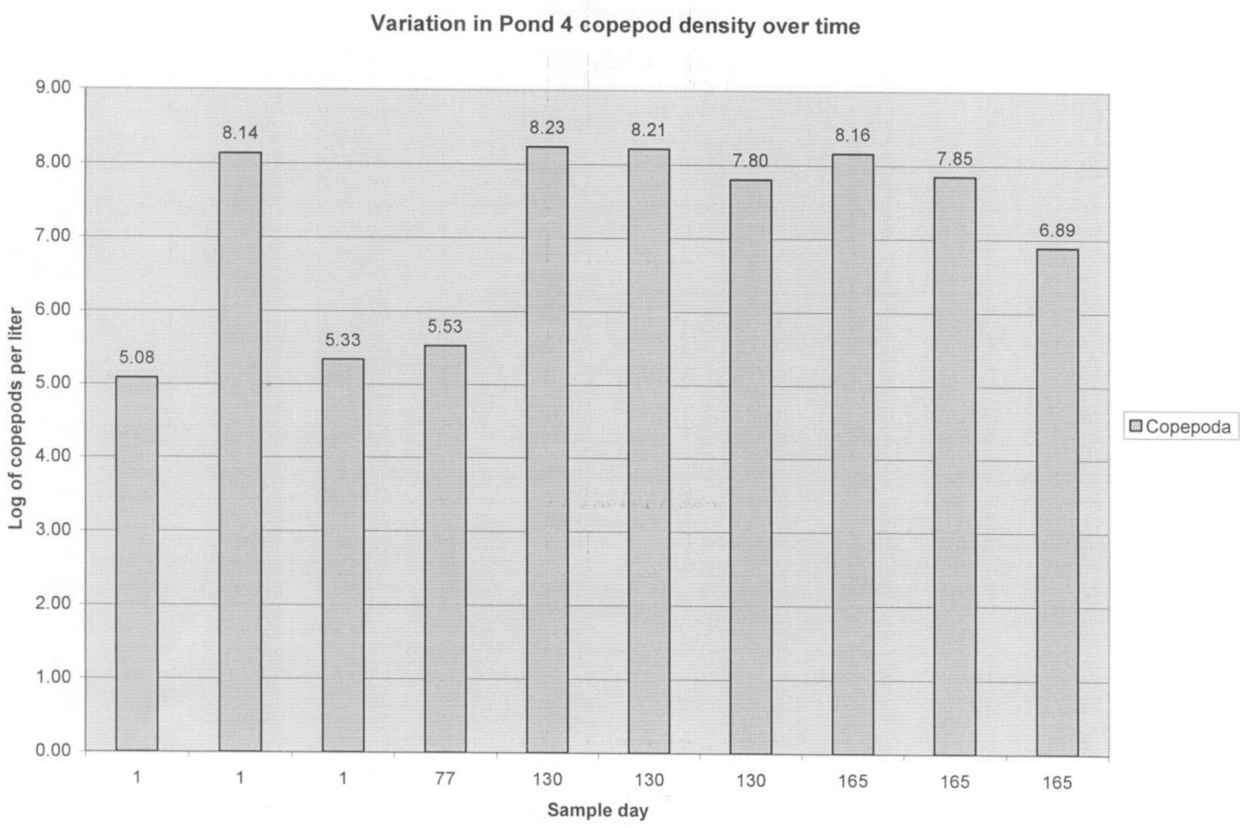
- Sample date vs. abundance

The Levene statistic rejected the hypothesis that the variances of each pond were equal. ($p < 0.01$) Due to unequal variances, a Games-Howell multiple comparison was made. The results of the test rejected the hypothesis that there was no variation in copepod density between sample dates for 10/19/00 vs. 2/26/01 ($p < 0.05$) and 2/26/01 vs. 4/2/01 ($p < 0.01$).


Variation in Pond 1 Copepod density over time
Log of copepods per liter
Sample day
Copepoda
2.20
1.79
3.43
8.17
8.47
8.48
6.80
6.58
7.39
3.56
4.03
4.77
1
1
1
77
77
77
130
130
130
165
165
165


Variation in Pond 2 copepod density over time
Log of copepods per liter
Sample day
Copepoda
6.12
6.75
7.10
6.43
8.48
6.95
7.46
4.72
4.01
4.39
1
1
1
77
130
130
130
165
165
165


Variation in Pond 3 copepod density over time
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Log of copepods per liter
7.23
6.40
7.63
4.20
7.78
8.47
8.34
6.70
6.88
7.05
1
1
1
77
130
130
130
165
165
165
Sample day
Copepoda


Variation in Pond 4 copepod density over time
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Log of copepods per liter
5.08
8.14
5.33
5.53
8.23
8.21
7.80
8.16
7.85
6.89
1
1
1
77
130
130
130
165
165
165
Sample day
Copepoda

p.16

Rotifers:

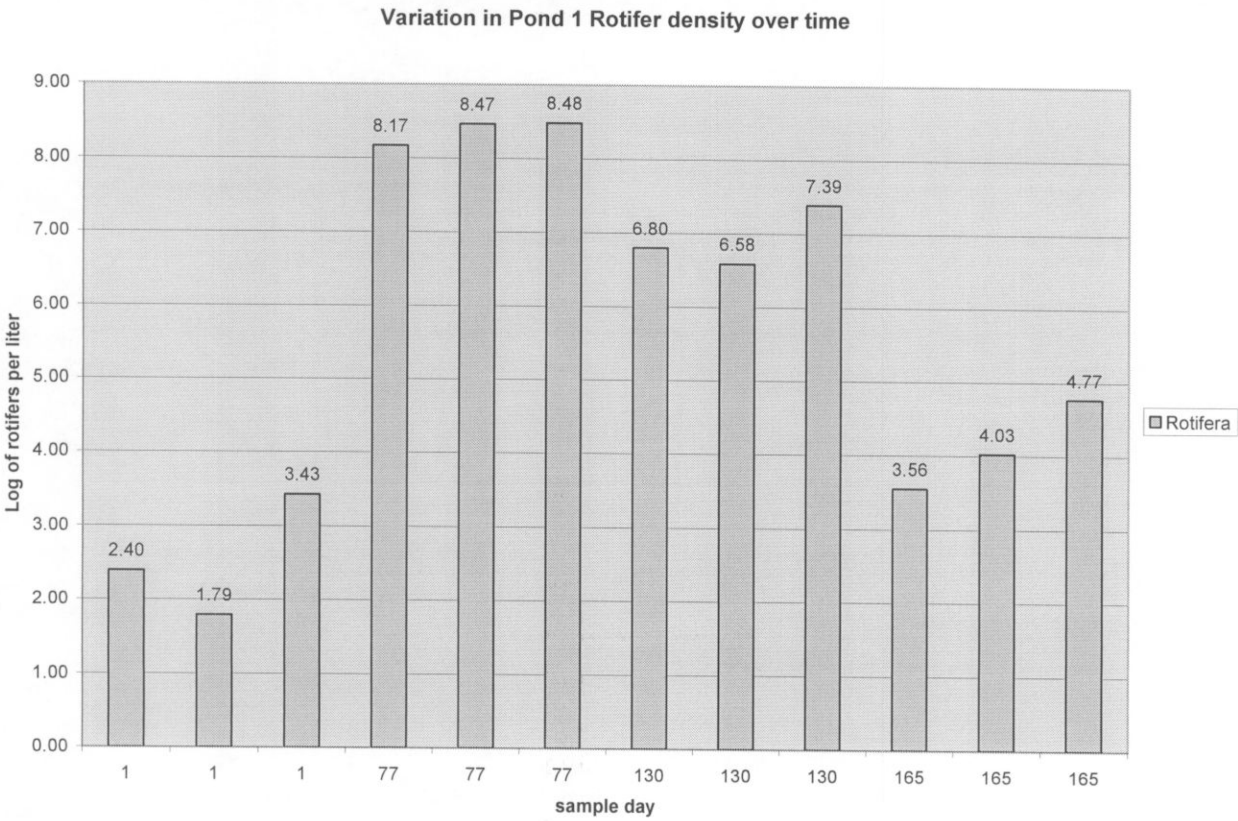
For analysis, due to the extreme abundance of rotifers, the data were transformed using the natural log of the density per liter.

- Location vs. abundance

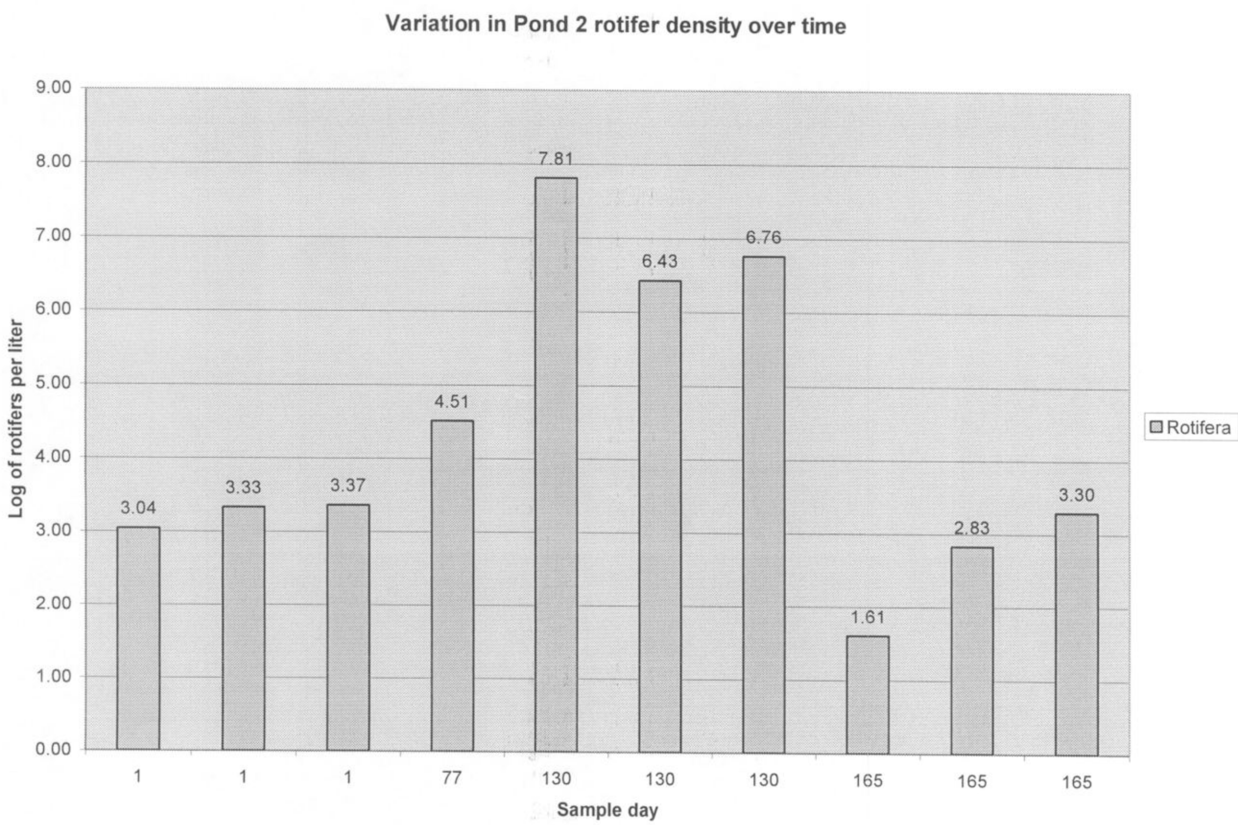
The test of equal variance using the Levene statistic failed to reject the null hypothesis that the variances were equal. ($p > .05$) Analysis of variance failed to reject the null hypothesis that the rotifer density varied with location. ($p > 0.25$)

- Sample date vs. abundance

The Levene statistic rejected the hypothesis that the variances of each pond were equal. ($p < 0.05$) Due to unequal variances, a Games-Howell multiple comparison was made. The results of the test rejected the hypothesis that there was no variation in copepod density between sample dates for 10/19/00 vs. 1/4/01, ($p < 0.05$) 1/4/01 vs. 4/2/01 ($p < 0.01$) and 2/26/01 vs. 4/2/01. ($p < 0.001$)

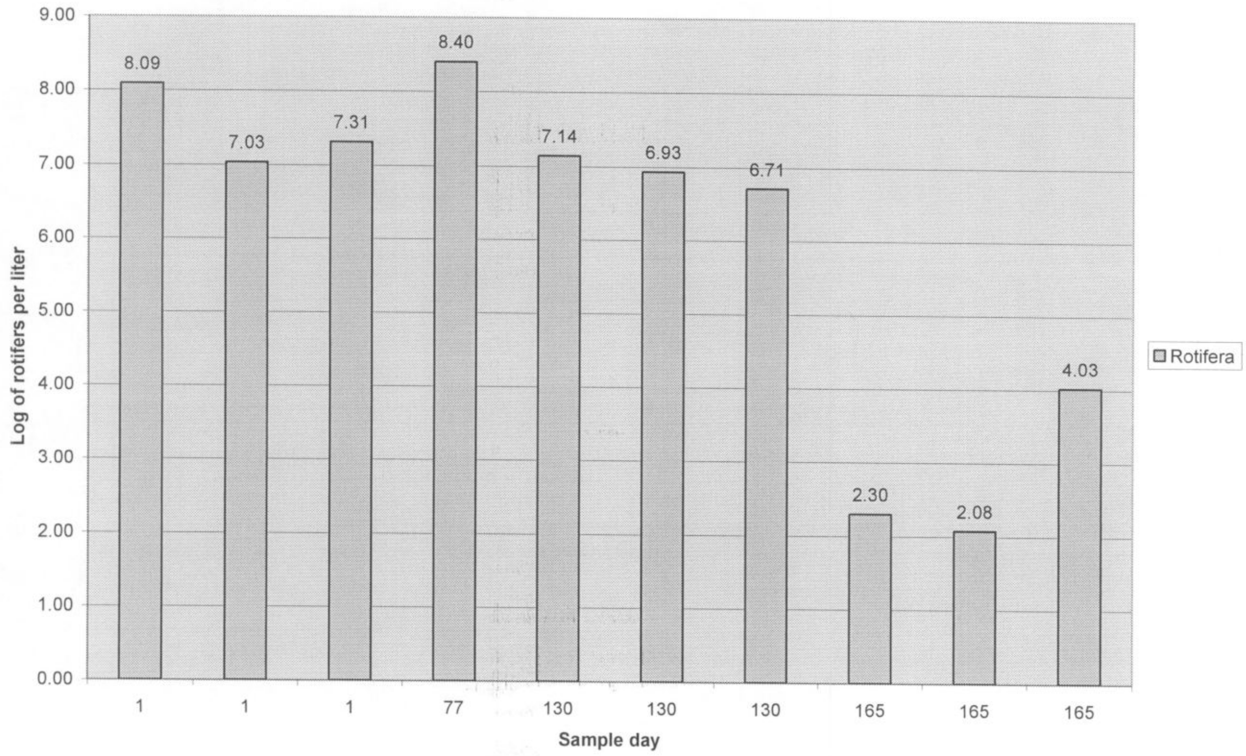


Variation in Pond 1 Rotifer density over time
Log of rotifers per liter
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
2.40
1.79
3.43
8.17
8.47
8.48
6.80
6.58
7.39
3.56
4.03
4.77
1
1
1
77
77
77
130
130
130
165
165
165
sample day
Rotifera

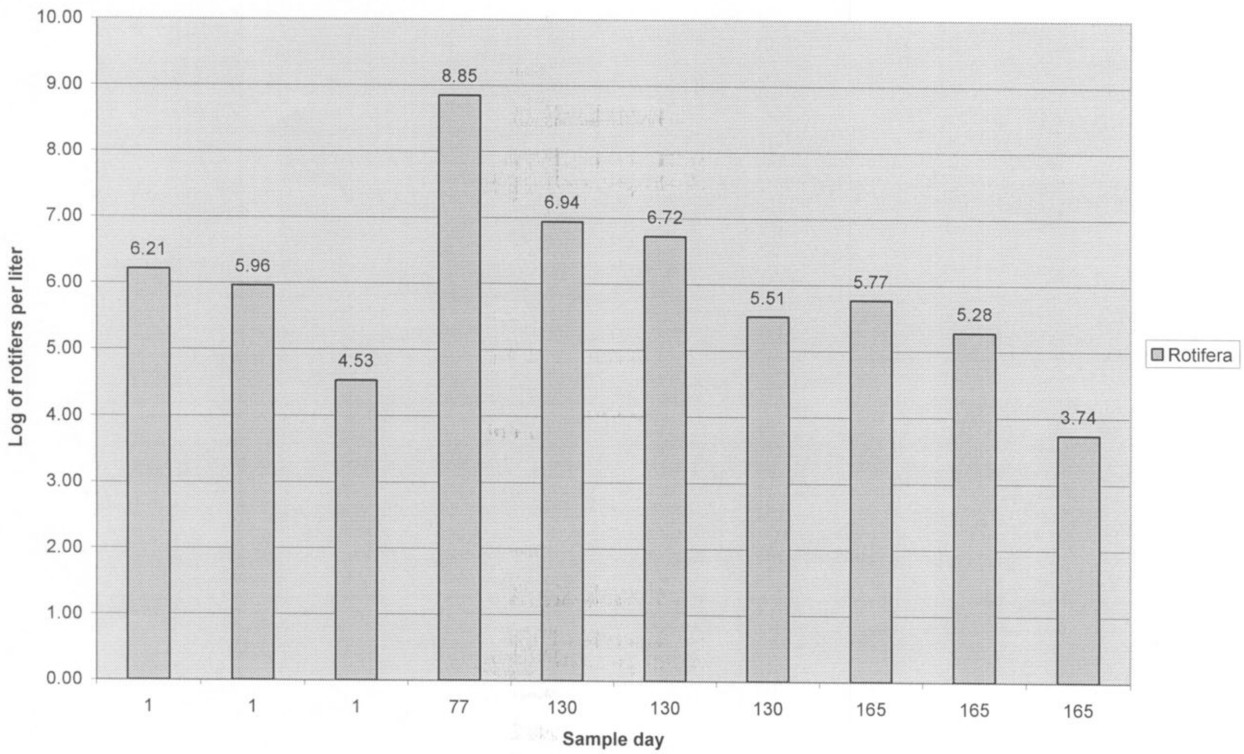



Variation in Pond 2 rotifer density over time
Log of rotifers per liter
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
3.04
3.33
3.37
4.51
7.81
6.43
6.76
1.61
2.83
3.30
1
1
1
77
130
130
130
165
165
165
Sample day
Rotifera

Variation in Pond 3 rotifer density over time




Variation in Pond 4 rotifer density over time



## Cladocera

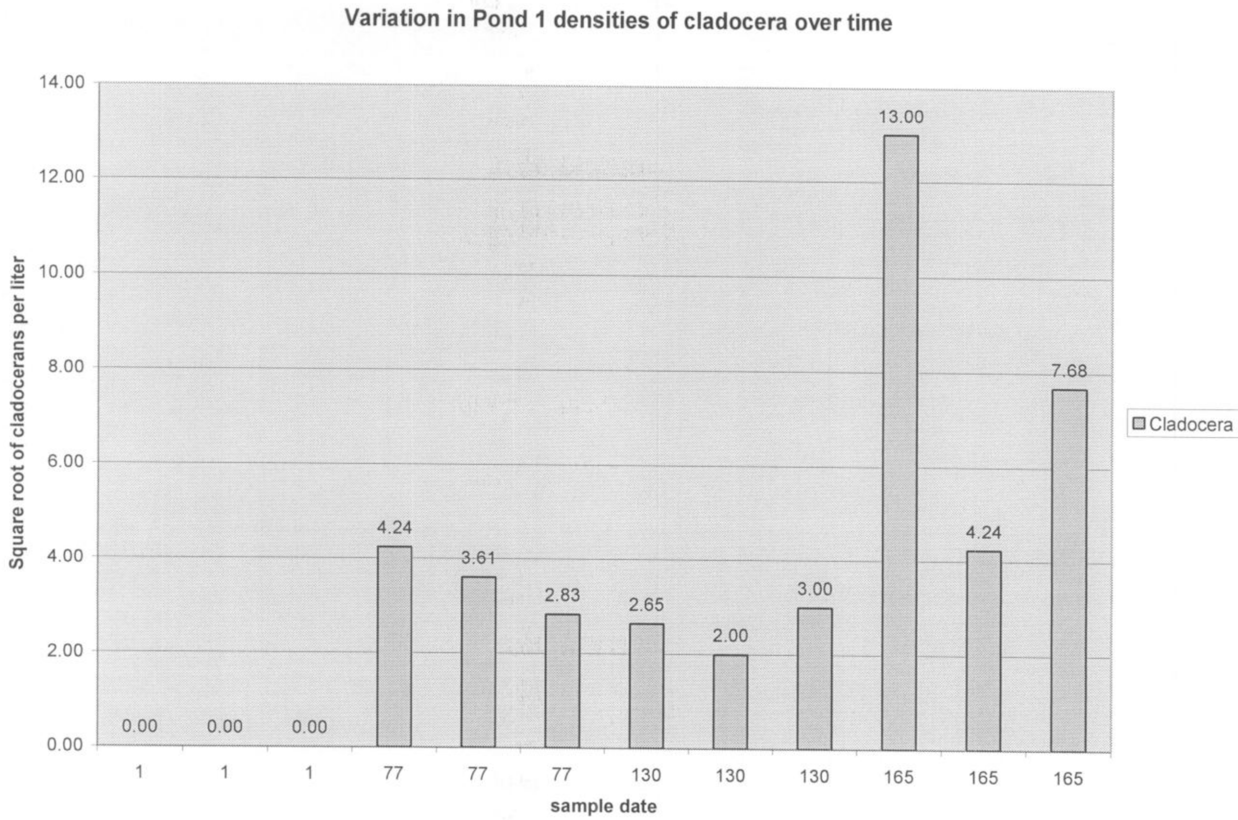
For analysis, due to the variation in densities, the data were transformed using the square root of the density per liter.

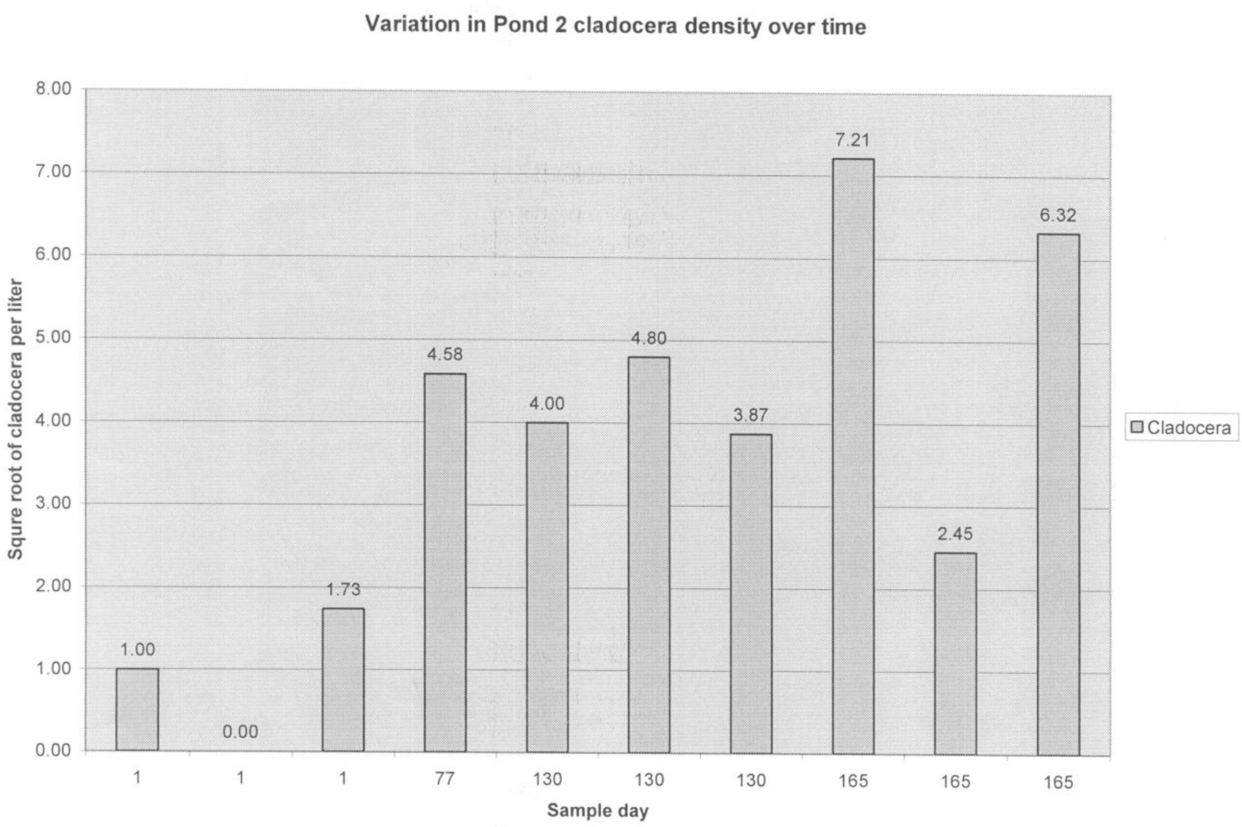
- Location vs. abundance

The test of equal variance using the Levene rejected the hypothesis that the variances of each pond were equal. ($p < 0.05$) Due to unequal variances, a Games-Howell multiple comparison was made. The result of the tests failed to reject the hypothesis that there was no variation in cladocera density between ponds. ($p > 0.40$)

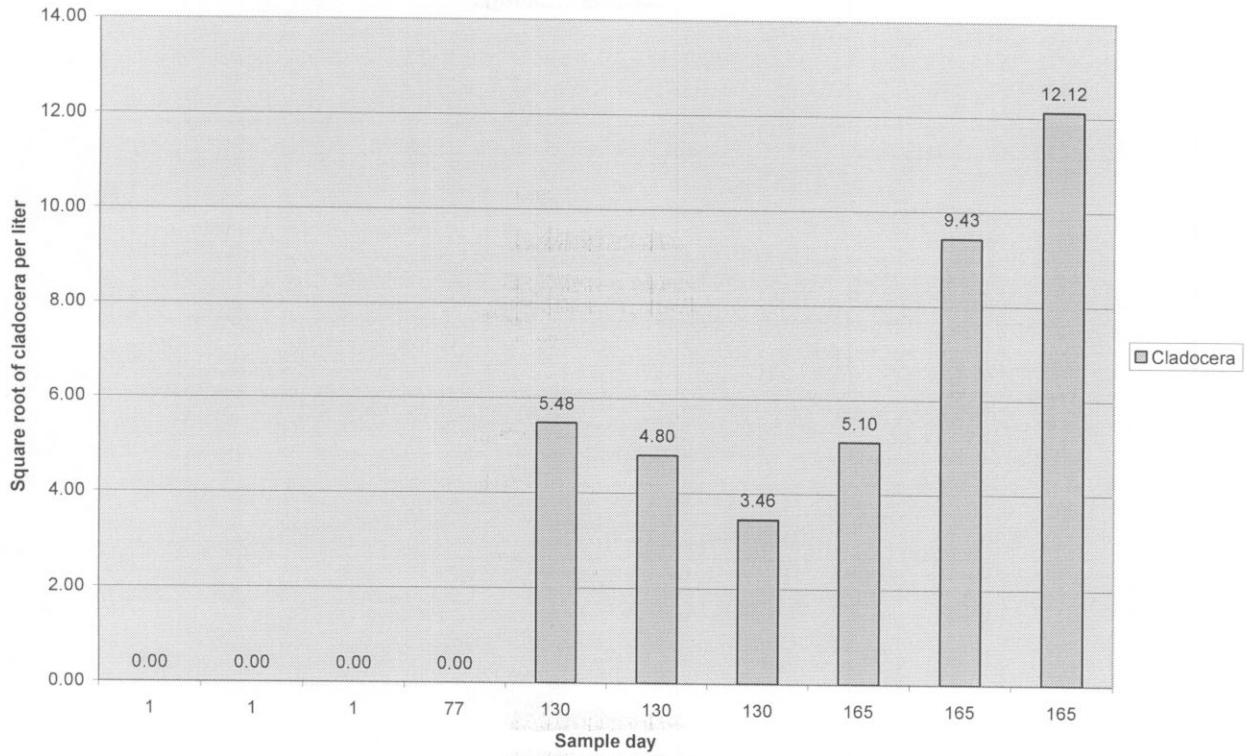
- Sample date vs. abundance

The Levene statistic rejected the hypothesis that the variances of each pond were equal. ($p < 0.001$) Due to unequal variances, a Games-Howell multiple comparison was made. The results of the test rejected the hypothesis that there was no variation in cladocera density between sample dates for 10/19/00 vs. 2/26/01, ($p < 0.01$) 10/19/00 vs. 4/2/01, ($p < 0.01$) 1/4/01 vs. 4/2/01, ($p < 0.01$) and 2/26/01 vs. 4/2/01. ($p < 0.05$)

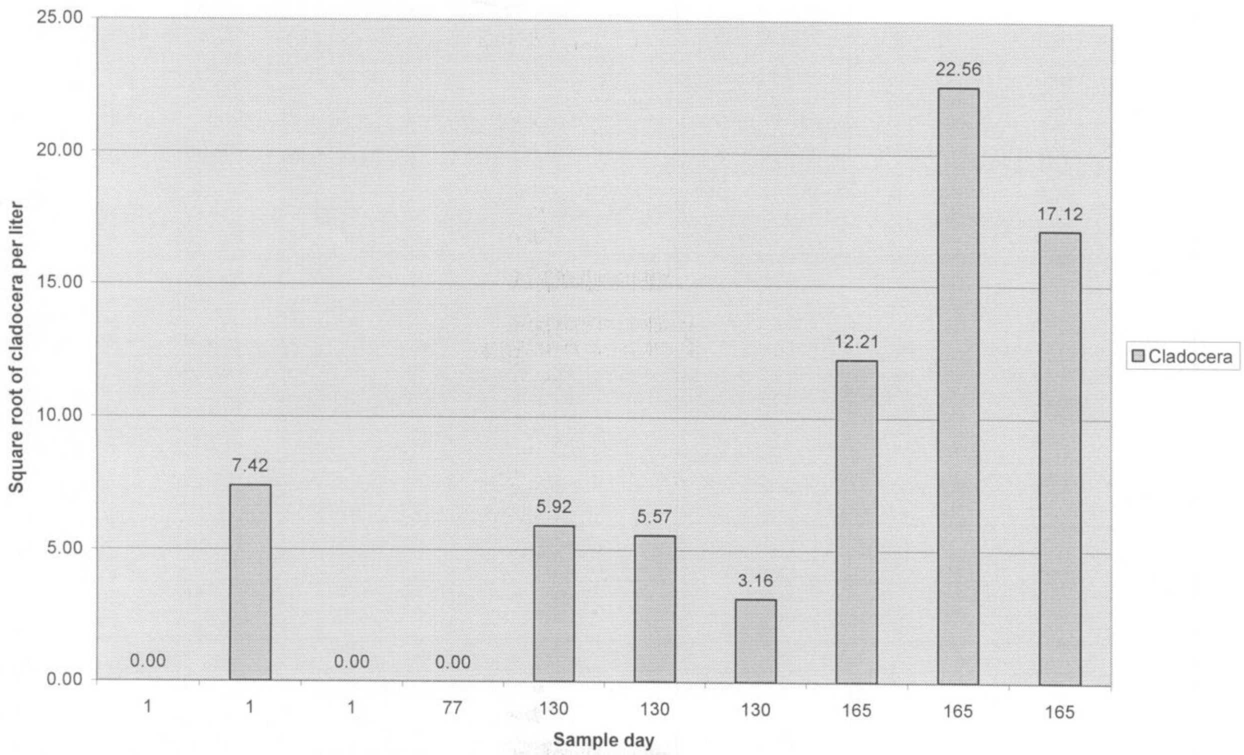

Variation in Pond 1 densities of cladocera over time
Square root of cladocerans per liter
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
0.00
0.00
0.00
4.24
3.61
2.83
2.65
2.00
3.00
13.00
4.24
7.68
1
1
1
77
77
77
130
130
130
165
165
165
sample date
Cladocera


Variation in Pond 2 cladocera density over time
Squre root of cladocera per liter
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
1.00
0.00
1.73
4.58
4.00
4.80
3.87
7.21
2.45
6.32
1
1
1
77
130
130
130
165
165
165
Sample day
Cladocera

## Variation in Pond 3 cladocera density over time




## Variation in Pond 4 cladocera density over time



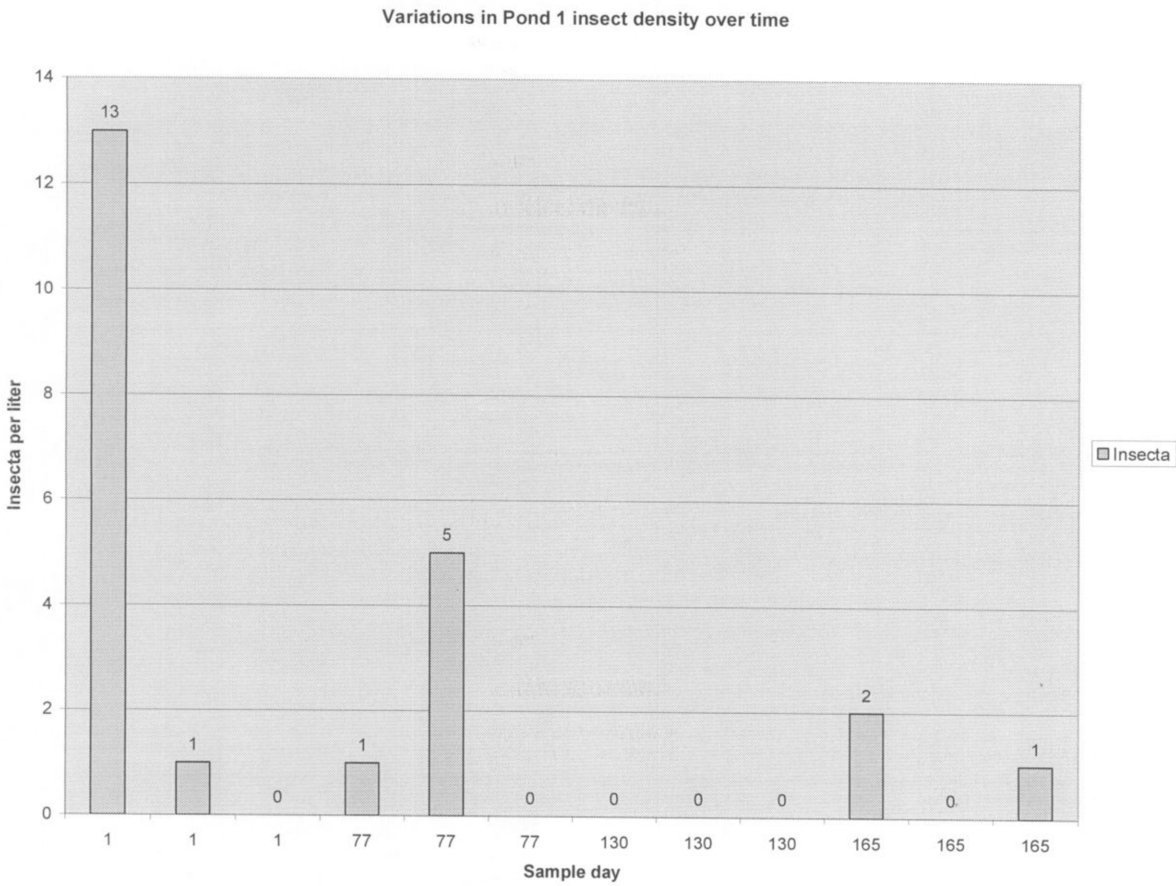
Insects

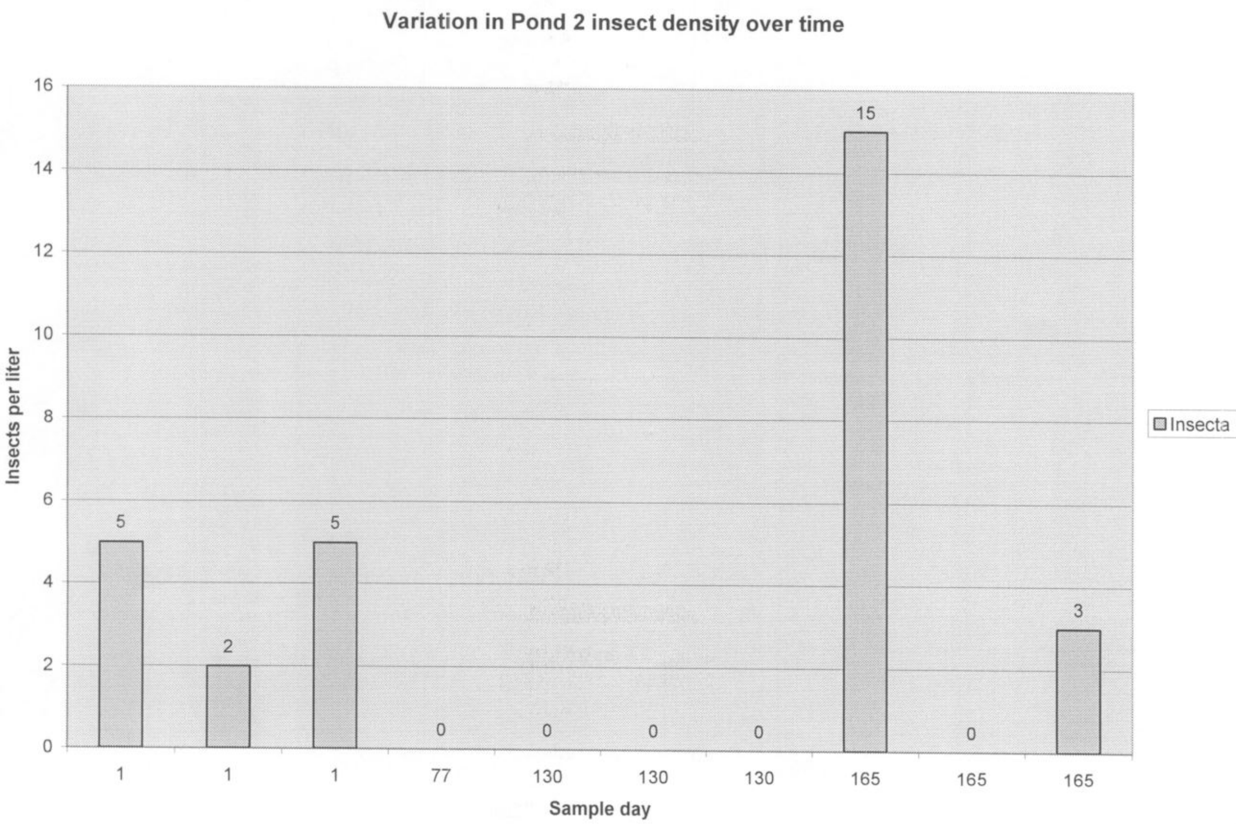
- Location vs. abundance

The test of equal variance using the Levene accepted the hypothesis that the variances of each pond were equal. ($p > 0.05$) Analysis of variance failed to reject the null hypothesis that there was no variation in insect density between ponds. ($p > 0.40$)

- Sample date vs. abundance

The Levene statistic accepted the hypothesis that the variances of each date were equal. ($p > 0.05$) The significance of equal variances was questionable (0.053) so both a standard analysis of variance and a Games-Howell comparison were made. The standard analysis of variance failed to reject the null hypothesis that there was no variation in insect density between sample dates. ($p > 0.1$). A Games-Howell multiple comparison agreed with the results of the standard analysis of variance. ($p > 0.05$)


Variations in Pond 1 insect density over time
Insecta per liter
Sample day
Insecta
14
12
10
8
6
4
2
0
13
1
0
1
5
0
0
0
0
2
0
1
1
1
1
77
77
77
130
130
130
165
165
165


Variation in Pond 2 insect density over time
Insects per liter
Sample day
Insecta
16
14
12
10
8
6
4
2
0
5
2
5
0
0
0
0
15
0
3
1
1
1
77
130
130
130
165
165
165

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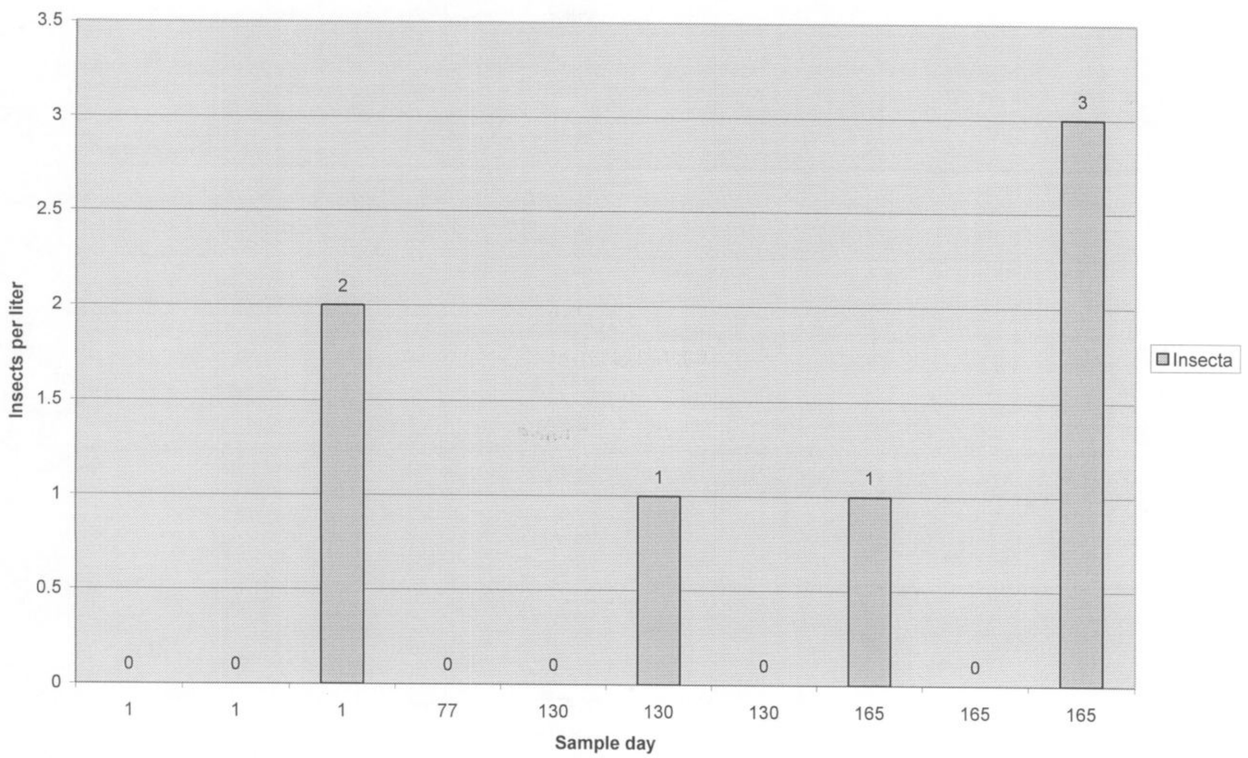
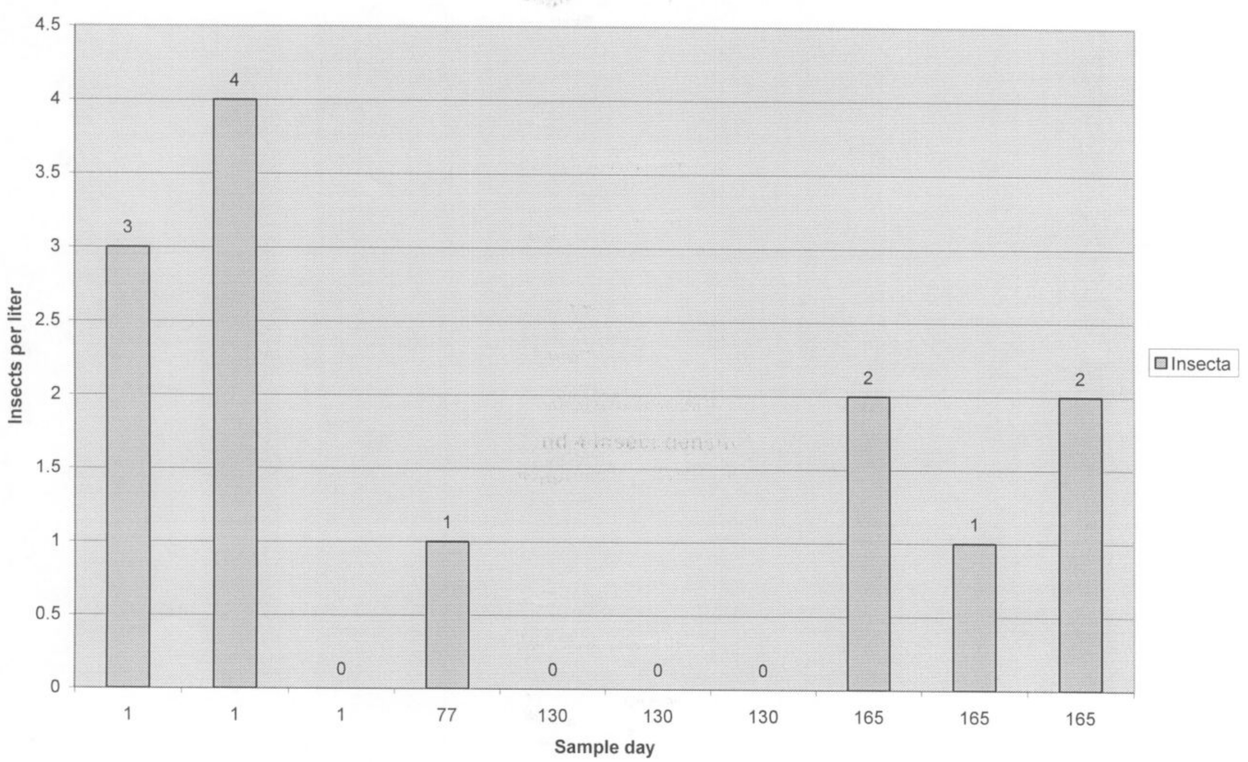
## Variation in Pond 3 insect density over time




## Variation in Pond 4 insect density over time

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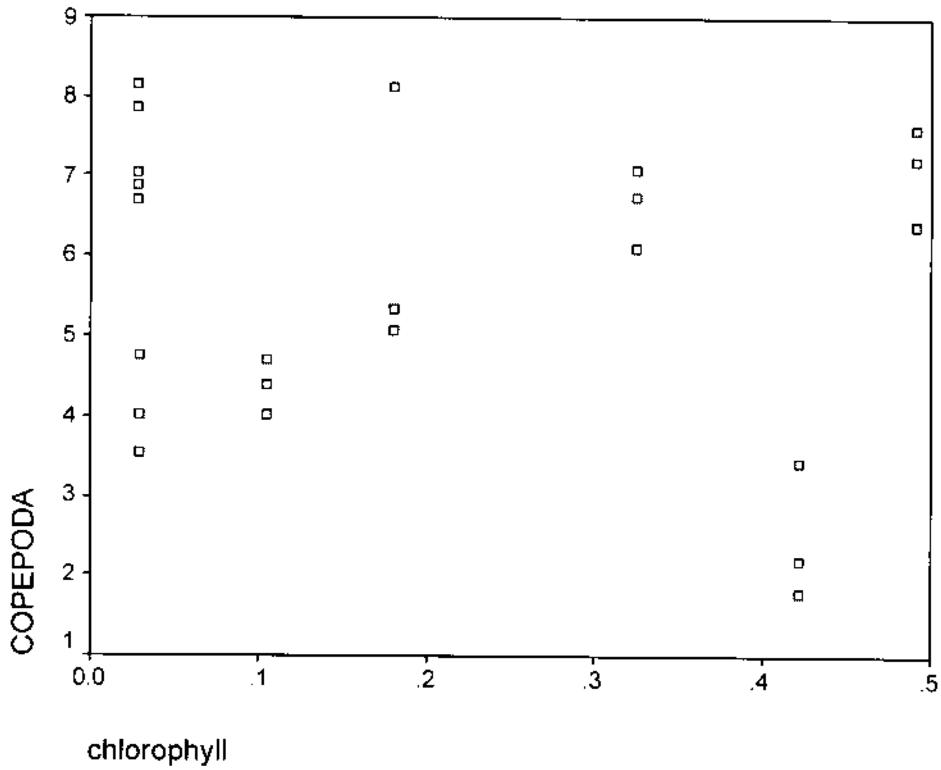
B. Chlorophyll Content:

The readings from the flurospectrometer were converted to milligrams per liter pond water.

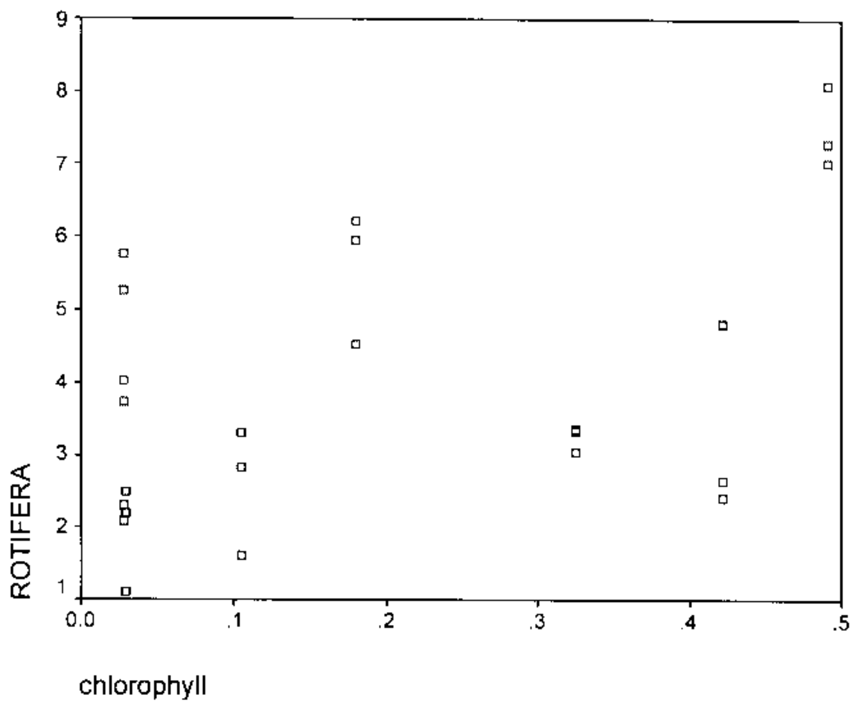
**Chlorophyll A**

| day | pond # | mg/L |
|---|---|---|
| 1 | 1 | 0.178241 |
| 1 | 2 | 0.105949 |
| 1 | 3 | 0.241738 |
| 1 | 4 | 0.032319 |
| 165 | 1 | 0.000817 |
| 165 | 2 | 0.010904 |
| 165 | 3 | 0.000755 |
| 165 | 4 | 0.00078 |

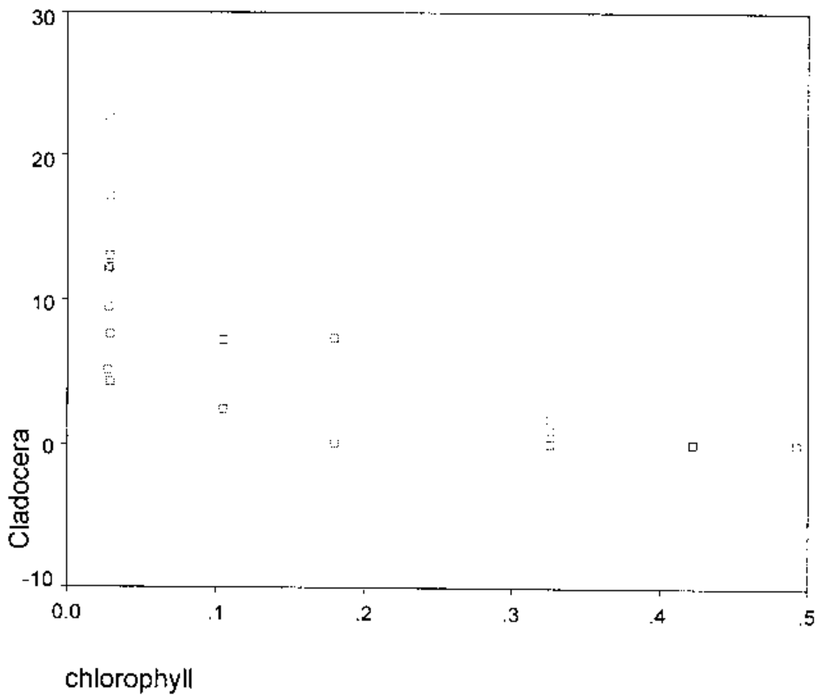
Tests were then conducted to determine if there was a correlation between the density of zooplankton and chlorophyll content. The square root of the chlorophyll values was taken to linearize the data. The converted data were first plotted against the density of each group to see which groups may be correlated to chlorophyll content.



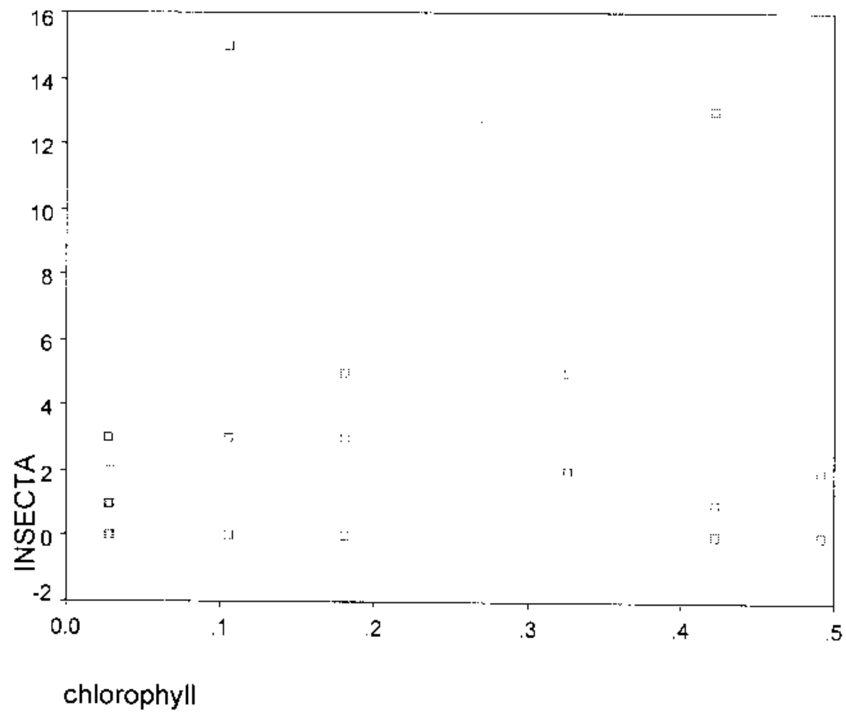

**Square root of mg chlorophyll vs. log copepod abundance**




**Square root of mg chlorophyll vs. log rotifer abundance**




**Square root of mg chlorophyll vs. square root cladocera abundance**




**Square root of mg chlorophyll vs. insect abundance**

The only data that appeared correlated to chlorophyll content were the density of cladocera. The data still did not appear linear so the fourth root of the density of cladocera was used. These data were plotted against the square root of the mg per liter pond water. The result of this transformation produced a linear trend in the data and these transformed data were used to construct a linear model.

The linear model constructed had a correlation coefficient of 0.870. The significance of the variables in the model was below 0.001. The equation for the model was

**Cladocera*-4 = -6.461(chlorophyll*-4) + 4.287**

Results of linear regression using SPSS.

Model Summary

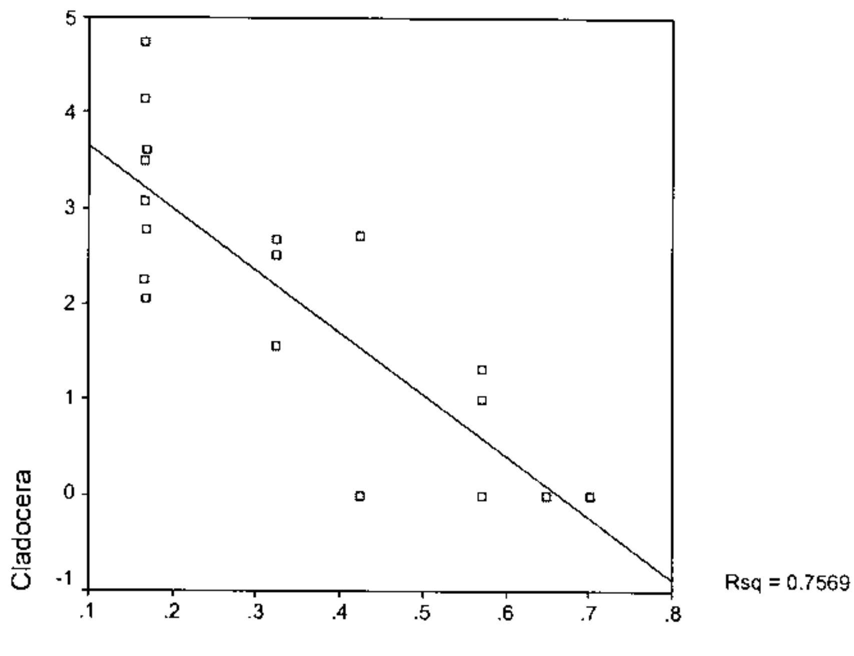
| Model | R | R Square | Adjusted R Square | Std. Error of the Estimate |
|---|---|---|---|---|
| 1 | .870 | .757 | .746 | .1079 |

a Predictors: (Constant), Cladocera
b Dependent Variable: CHLORO4

Coefficients

| Model | Unstandardized Coefficients B | Std. Error | Standardized Coefficients Beta | t | Sig. |
|---|---|---|---|---|---|
| 1 (Constant) | 4.287 | .350 | | 12.251 | .000 |
| CHLORO4 | -6.461 | .781 | -.870 | -8.277 | .000 |

a Dependent Variable: Cladocera




Fourth root of mg chlorophyll vs. fourth root cladocera abundance with linear regression line.

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## VI. Discussion

A. Macro-zooplankton Survey:

1. Total Diversity of Tottino Ponds:

Throughout the six-month study, a total of 121,526 organisms of 11 species were sampled. By using these total numbers found, it was possible to come up with a way of categorizing the overall diversity of the organisms living in the water column. I used Simpson's diversity index which gives the probability that, if two species are taken randomly from the Tottino Ponds water column, they will not be the same species (Brower, 1990). The index from the data collected was 0.51 which is a moderate one. It is not expected that a newly created ecosystem would have a high diversity. Tottino Ponds is still in an early succession and it is not until a mature community develops that one would see a high diversity.

Having a baseline diversity index will be helpful in tracking the development of Tottino Ponds. If the community develops, as it should, one would expect the diversity to increase. Diversity is also related to a number of other factors such as complexity and stability (Brower, 1990). In order to make these conclusions; one must take into account the ecosystem being studied. The system at Tottino Ponds is new, but it may have reached its maximum potential due to the physical setting and the water quality. This is why a look at the level of the diversity index is important towards understanding the potential for using recycled water to create artificial wetlands.

For a diversity index to be accurate, it is important to classify all organisms to the same taxonomic level. While it was not possible to identify all the species sampled, all groups were identified to at least family with the exclusion of the copepods and ostracods, which were identified to the level of orders. The juvenile and adult copepods were combined for this, as all juveniles of all other species were included in their respective populations. Only female rotifers were counted in the survey, as it was not feasible to distinguish the much smaller males from copepod eggs. All of the groups contained only one species so it was possible to create a diversity index without knowing the identity of each species. In the case of future comparisons, it is also possible to compare indices that are based on family and still preserve this diversity index. Assessments based on higher taxonomic levels are often carried out in cases of high invertebrate diversity where "total taxonomic interpretation of large numbers of accumulated samples is impossible" (New, 1998, p. 28).

2. Variations in Zooplankton Density.

In studying how the animals living in Tottino Ponds reacted to environmental disturbance and change, it was first necessary to see if there was any significant variation in abundance. In order to differentiate between spatial and temporal factors, I looked at how a group of organisms changed from one pond to the next, and then studied how the abundance varied over the time of the survey. It was necessary to convert the densities of some of the groups in order to display the data graphically and reduce the amount of variance. Once the changes (or lack of change) were assessed, it was possible to draw conclusions about the behavior of the populations.

The four groups studied were copepods, rotifers, cladocerans, and insects. None of the groups varied in density from one pond to the next. This is contrary to what initially was expected because Ponds 3& 4 appear quite different from Ponds 1 & 2 as they more readily develop vegetation in them. It is possible that due to the early colonization stage of Tottino Ponds, they have not yet had time to develop unique communities, and that with time, communities will develop that are more suited for each habitat.

Where the groups did show some variation was on the temporal scale. Only the insects did not show any variation over time. The mean number of copepods per liter went from 270 in October 2000, to 2,230 per liter in February of 2001 before dropping back down to 314 per liter in April. The density of rotifers rose from a mean of 133 per liter in October to a level of 3,204 per liter in January and declined until a sharp decline to 21 per liter in April. Cladoceran density rose exponentially throughout the survey from a density of 0.72 per liter in October to a density of 99 per liter in April. Insect density remained at a constant mean of 1.76 per liter throughout all the ponds and for all sample dates.

During the course of this study, the ponds were altered in two dramatic ways. The first was the introduction of mosquito fish, _Gambusia affinis,_ which occurred in the month preceding the survey. In an effort to remove the unwanted fish from the ponds, the ponds were drained in January, after sampling the water column, and then filled again a few weeks later. This shift in the ecosystem is responsible for the temporal change in invertebrate abundance. Mosquito fish are voracious predators of invertebrates and would be responsible for the low abundance of copepods and cladocerans. As the number of copepods dropped, the numbers of rotifers increased as rotifers are a main prey of copepods (Thorp & Covich, 1991). When the ponds were once again filled, an initial boom in copepods occurred due to the abundance of prey and lack of predators. This steadily drove down the number of rotifers in the system until they were scarce enough to cause a decrease in copepod abundance. Without competition from the rotifers, the cladocerans had more food available in the form of algae, which caused them to grow exponentially. The initial low numbers of cladocerans also can be related to the presence of mosquito fish.

A longer study should be done to determine the carrying capacity of the system. With the immigration of new species, more trends may appear and the levels of planktonic invertebrates could once again fluctuate. It is still uncertain as to weather the species surveyed have reached equilibrium. Once equilibrium is established (if ever) it will be possible to determine the carrying capacity of Tottino Ponds.

B. Chlorophyll Content:

The chlorophyll content varied dramatically from the first sample day to the last. The mean level of chlorophyll in Tottino Ponds was 0.140mg/L on October of 2000. When the chlorophyll was measured in April of 2001, it had decreased to a mean level of 0.003314mg/L. The pond with the highest amount of chlorophyll in October was Pond 3 with 0.242mg/L. The pond with the highest amount in April was Pond 2 at 0.0109mg/l. The pond with the lowest amount of chlorophyll for both dates was Pond 4 with 0.0323mg/L in October & 0.00078mg/L in April.

Only one group or invertebrates, the cladocerans, showed any correlation to chlorophyll concentration. This is unusual as all four groups contained species that grazed on algae. Rotifers are known to respond to algal concentrations either when excess is present or when there is a shortage (Thorp & Covich, 1991). The abundance of rotifers, although it varied throughout the study, did not vary between the October and April samples. The chlorophyll concentration varied by a factor of almost 100 between these two sample dates. It can be concluded that the influence of predation by copepods is a much more determining factor than algal abundance for the rotifers in Tottino Ponds.

The cladocerans, however, varied exponentially between these two sample dates and are also known to negatively respond to algal abundance (Thorp & Covich, 1991). A linear regression of the chlorophyll and cladoceran densities had a correlation coefficient (R) of 0.87 and an R-squared value of 0.75. This means that the linear model can account for 75% of cladoceran variation. The coefficients of the model were significant with an alpha level below 0.001. It can not be determined from the model if chlorophyll concentrations are controlled by cladoceran abundance or vice-versa. It can be deduced, however, that since the algal levels are so much less in April, when the cladocerans are the only significantly different population in the water column, that they are responsible for the reduction in chlorophyll. Cladocerans, specifically daphnids, have been reported to deplete algal levels in other similar situations (Thorp & Covich, 1991).

The ability for Cladocerans to significantly reduce algal density can be taken into consideration when wishing to suppress algal blooms and eutrophication of a system. When introducing fish into the system, caution should be used to select a species that will not adversely affect cladoceran abundance. To further explore the connection between cladocerans and algal density, a growth and grazing study could be conducted. Further monitoring of Tottino Ponds may also discover more of the complexity of the food web.

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## VIII. Acknowledgements:

All my teachers:

Dr. Suzanne Worcester, California State University of the Monterey Bay

Dr. Sharon Anderson, California State University of the Monterey Bay

Dr. Rikk Kvitek, California State University of the Monterey Bay

Dr. John Oliver, Moss Landing Marine Labs

Peter Slattery, Moss Landing marine Labs

Dr. Daniel Shapiro, California State University of the Monterey Bay

Dr. Daniel Fernandez, California State University of the Monterey Bay

Dr. Rene Perry, California State University of the Monterey Bay

Dr. Steve Moore, California State University of the Monterey Bay

Dr. Susan Alexander, California State University of the Monterey Bay

Dr. James Harvey, Moss Landing Marine Labs

Dr. Mike Dalton, California State University of the Monterey Bay

Dr. Daniel Orange, California State University of the Monterey Bay

Dr David Takacs, California State University of the Monterey Bay

Dr. Robert Curry, California State University of the Monterey Bay

Special thanks to:

Bill Chevalier- Phytoplankton survey

Lisa Gilbane- Benthic study at Tottino Ponds

Terra Eddy- Illustrations

Sue Shaw- Draining the ponds and water testing

**Appendix I.**

Monterey Regional Water Pollution Control Agency (MRWPCA) Water Recycling Plant:

A.

Description:

The plant is located 2miles north of Marina, CA off Highway 1. They treat up to 21 million gallons per day (MGD) with a capacity to treat 29.6 MGD. They recycle up to 20 MGD during the growing season that is used for irrigation in the north Salinas Valley.

Any water not recycled is discharged into the bay 2 miles off the coast at a depth of 100 ft. after the secondary treatment process.

B.

Recycling Process:

Sewage enters the treatment plant at the **headworks** and large items are removed along with any sand and grit. The water then enters the **primary clarifiers** where small particles that settle to the bottom or float to the surface are skimmed and removed and sent to the **anaerobic digesters**. Methane is produced and collected from the **anaerobic digesters** and powers a **cogeneration plant** that generates enough electricity to operate the secondary treatment facilities.

After primary treatment, the water is passed through the **trickling filters** by spraying it onto plastic sheets where algae found in the water grows and cleans the water. The algae falls off the plastic sheets and is sent to the **anaerobic digesters**. From here, the water is sent to **biflocculation basins** where aerobic bacteria found in the water remove dissolved material as they feed. The **secondary clarifiers** settle the biflocculation organisms and send them either back to the **bifocculation basins** or to the **anaerobic digesters**. At this point the secondary treatment process is completed and the water is either discharged into the bay or sent through the tertiary treatment process depending on demand.

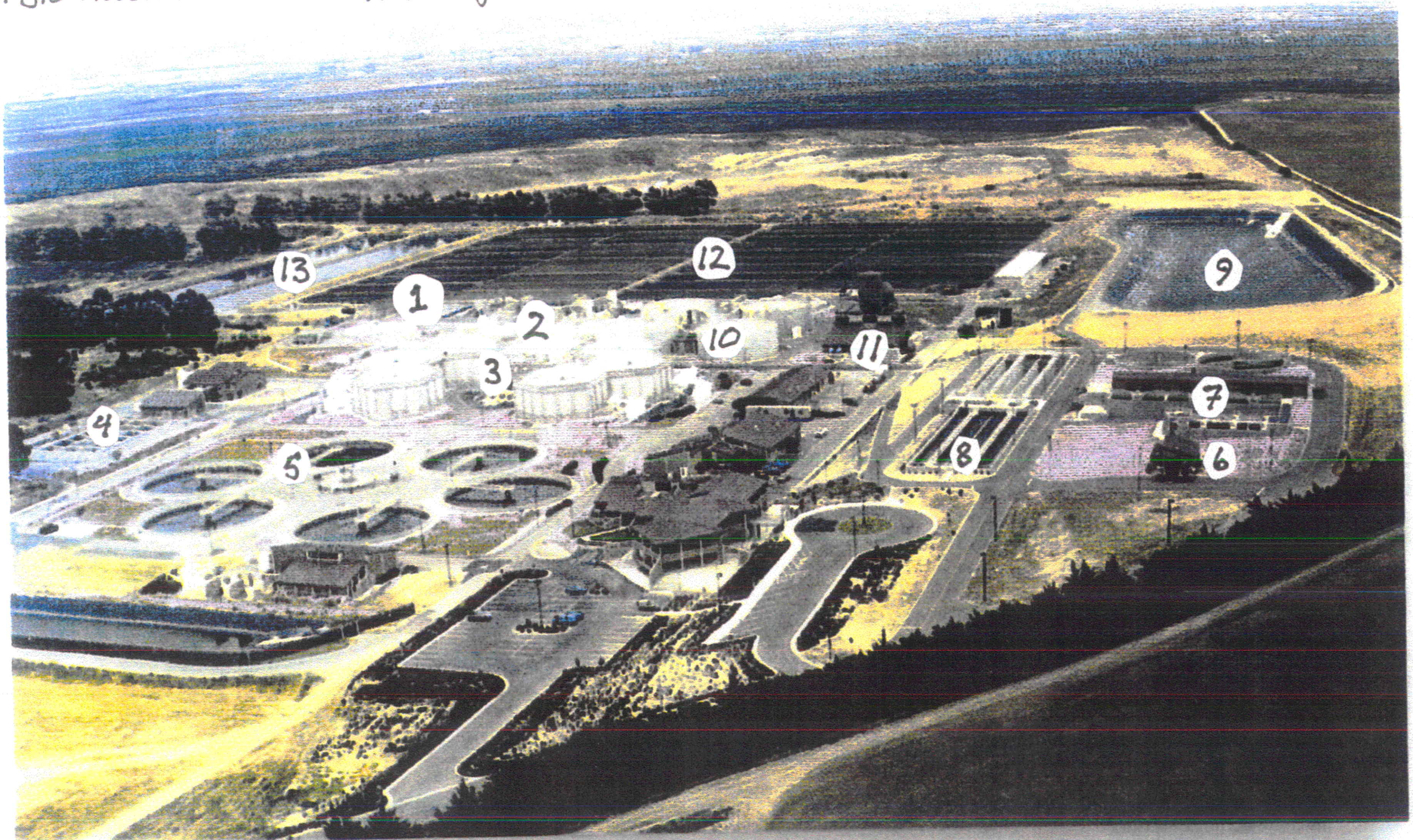
The tertiary treatment process begins by adding a chemical flocculant to the water in **flocculation basins**. The flocculated trace particles are filtered through **filters** of pea gravel, sand and anthracite coal. The water is then disinfected in **chlorine contact tanks** for at least 2 hours. Sulfur dioxide can be added to remove excess chlorine. This completes the tertiary treatment process.

The recycled water is held in a 5-acre **storage pond** before piping it to food crop fields. The **storage pond** is the highest point in the distribution system. Biosolids produced in the **anaerobic digesters** are spread across **solar drying beds**. If the **solar drying beds** are full, which occurs during the rainy season, the biosolids are temporarily held in a **winter storage lagoon** while awaiting application to the beds.

Appendix I.c



1. Headworks
2. Primary Clarifiers
3. Trickling Filters
4. Bioflocculation Basins
5. Secondary Clarifiers
6. Flocculation Basins
7. Filters
8. Chlorine Contact Tanks
9. Storage Pond
10. Anaerobic Digesters
11. Cogeneration Plant
12. Solar Drying Beds
13. Winter Storage Lagoon




Monterey Regional Water Pollution Control Agency (MRWPCA) Water Recycling Plant