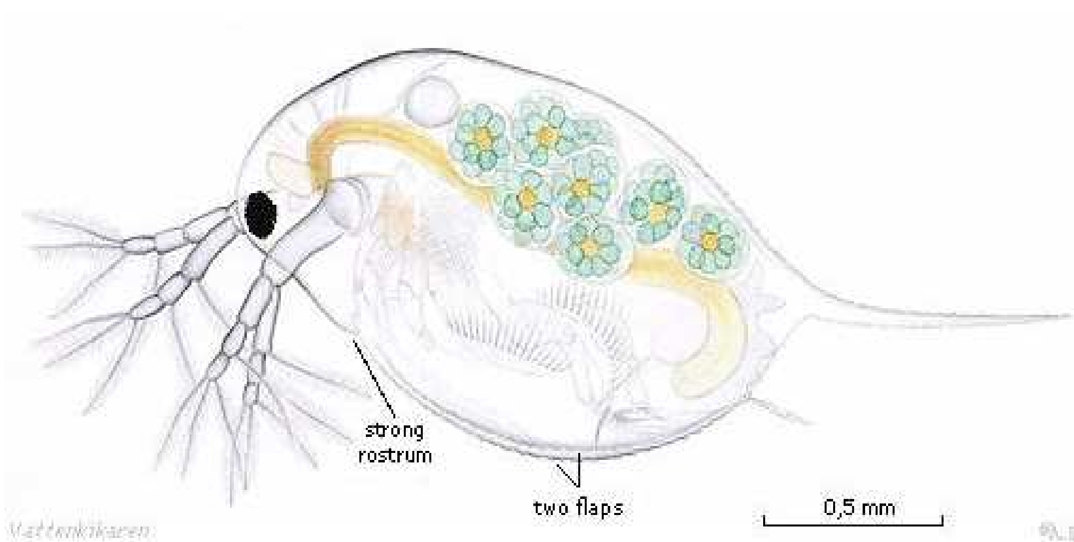
Aim:
To investigate the feeding relationship of invertebrates in ponds of different light levels.

Background Research:

A two-flapped shell that creates an opening on the underside covers the water fleas body. The two quite complex eyes have grown together. The second pair of antennae is large and is used to swim. The head is large, and the rostrum is pointed and powerful. There is also a point on the shells upper side. They are 6mm in length.

Water fleas are mainly freshwater organisms, but a few species tolerate brackish water. Because of this they are found in rock pools close to the sea and in relatively closed seas with a large run-off of freshwater. They are normally found only as deep as a few meters in the water.

Water fleas eat plants and other particles in the water. Therefore they are herbivores (primary consumer). Larger creatures, for example, phantom midge larvae (Carnivores) feed off water fleas. With some females the young water fleas developed in the ovary without being fertilized. Several generations with only females can occur and multiply themselves partenogenetically. When harsher conditions prevail even males and certain females whose eggs were fertilized hatch. The ovaries are released when sloughing takes place or when there is a drought and are spread over large areas by the wind. Water fleas exist globally.



An ecosystem is defined as 'a self-contained interacting community of organisms, considered with the environment in which these organisms live and react'. Freshwater ecosystems include such water bodies as lakes, rivers, ponds and streams. They do not include saltwater bodies such as oceans or seas. A pollutant may be considered as any substance or form of energy, usually an unwanted by-product or waste, which is released into the environment as a result of human activities, that alters the chemical, physical and biological characteristics of the environment. Such substances may be solids, liquids or gases. Freshwater ecosystems can become affected in a number of ways, such as by chemical, organic, and heat pollution.

As the water becomes more polluted, there are more organic nutrients available for the bacteria to break down which leads to an increase in mineral salts. This is known as eutrophication ("good eating" conditions). However, the breakdown of these nutrients leads to depletion in oxygen and an increase in CO_2, which can lead to the death of many of the other organisms such as fish and invertebrates, which depend on the ecosystem remaining balanced. Eutrophication encourages the growth of algal blooms, which cover the surface and prevent light from reaching the submerged plants in the water thus preventing photosynthesis. The problem is greatly enhanced at nighttime, when plants have been unable to produce sufficient levels of oxygen for the invertebrates to respire. Eutrophication has the following effects on the ecosystem:

1. Affects animals and invertebrates, by causing lower oxygen levels, too low for animals to respire effectively
2. Increases turbidity / cloudiness, reducing light levels and thus reducing photosynthesis by plants

This would have a great effect on the freshwater pond, as if there is no light the plants would be able to photosynthesise and would die, resulting in the death of invertebrates as they would have no food to eat, and would probably have a great effect on the freshwater pond food web.

A similar investigation that was carried out by Marianne V. Moore and Susan J. Kohler of Wellesley (Mass.) College had examined how artificial light affects small aquatic invertebrates in New England freshwater ponds. Their data shows that the night time activity of these animals near the surface drops off in proportion to the amount of light reaching them. That could reduce the invertebrates' predation on algae at the surface, potentially leading to algal blooms and poor water quality.

The experiment shall take place in two different ponds, woodland pond and a meadow pond. As the woodland pond has trees surrounding it, the light intensity is a lot less in comparison to the meadow pond where there is no obstruction to light.

Apparatus

Fishing Net – To capture the organisms from the freshwater pond. The fishing net is classed as a 'small' one and has very small fine holes in the net.

Standard Tray – The tray is 30 x 20cm is size and it is going to be used for emptying the contents of the net into it for inspection.

Light meter - To measure the amount of light available to the ponds. There are three different light scales. X10, x100 and x1000, in order to get a high degree of accuracy from our results we shall be measuring light in x1000 mode.

Glass beaker – to measure oxygen-testing liquids.

Goggles – eye protection so that nothing can get into our eyes while we are doing the experiment and wont catch any disease.

Oxygen Kit – This is a kit that is used to measure the amount of oxygen that t there is in the freshwater pond. It contains instructions and 6 reagent bottles.

Spoon – to stir the captured sample and take out pon d plants.

Square container – used to enclose the organisms in a small region of the tray.

Pencil/paper – To use to write down my results/observations.

Rubber gloves – to keep our hands clean.

Hypothesis

I predict that there is a relationship between the abundance of water fleas and the intensity of light.

Null Hypothesis

There is no relationship between water fleas in two ponds of different light intensities.

Site Justification

To test my hypothesis a site had to be chosen where there were two ponds of different light intensity. Background reading revealed that simple invertebrates such as water fleas subsist in freshwater environments. Therefore we went to the nearest field centre where there were two freshwater ponds where our data would be collected.

Method

1. Gather up all required apparatus

2. Put on the rubber gloves and the goggles to ensure we are going to be carrying out the experiment in the safest way possible.

3. Fill half of the tray up with water from the pond.

4. Measure the abiotic factors:

   - Temperature – Use a thermistor and place the needle into the water at the top (don't submerge the whole unit) and record reading.

   - Oxygen levels – This is a very complicated method. First of all, fill up the beaker with the pond water. Then add 10 drops of Manganous Sulphate Solution (reagent 1) and 5 drops of Alkaline Potassium Iodide Azide Solution (reagent 2), then shake the solution (should be a black colour) fill up the syringe with Sodium Thiosulfate (reagent 6) and add drop at a time until the solution has turned clear. Then record your readings.

5. Measure the light levels by standing at the side of the pond and hold the light meter out in front, and then record the reading.

6. Place the net into the freshwater pond only about 10 cm deep, and stir the net in a figure of eight making sure that you are capturing every thing into the net in the same region for 1 minute.

7. Emerge the net from the water, waiting for any excess water to drain from the net and then place the contents of the net into the tray.

8. Use the spoon to mix up the contents of the tray and remove any pond plants weeds or leaf litter that may have accumulated.

9. Use the square container that is 10x10cm (100cm^2) in size and place it in the top right hand corner of the tray.

10. Count the number of water fleas within the confined region.

11. Carefully empty the contents of the tray back into the pond (exactly where you took it out from) taking the up most care, as there are living organisms in there.

Repeat stages 5 – 11 ten times for the meadow pond and the woodland pond.

Sampling Methods

A sample is a collection of numbers (measurements or counts) on the quality characteristic of process variable of units drawn from a process. A sample unit is a part of the process on which the quality characteristic or process variable is measured. The units may be incidents, time (day of week).

There are four main types of sampling:

Systematic: This is a data collection method designed to select at fixed or count intervals. This is mainly used to collect data over extended periods of time. It can also reduce the impact of a variety of conditions to prevent systematic errors from influencing the analysis of the data.

Quota: A data collection method designed to select sample units in a block of a predetermined size. Quota sampling is used to select sample units so as to capture the detailed behaviour of the process. A quota sample is especially useful for situations where the data is time or sequence dependent. This data collection method is likely to capture the detailed behaviour of the process.

Random: The purest form of probability sampling. Each member of the population has an equal and known chance of being selected. When there are very large populations, it is often difficult or impossible to identify every member of the population, so the availability of subjects reduces bias.

Stratified: Stratified sampling is a probability sample selection method in which the population is divided into homogeneous groups (strata) and different sampling methods are applied to the different strata.

The sampling method that I am going to exercise in my experiment is stratified. This is because as it is a large pond, the best chance of getting the most accurate results is to sample different areas of the whole pond.
We are also going to take 10 samples of each pond due to my statistical test analysis.

Risk Assessment and ethics

As we will be working outside in the wilderness, it is extremely important that we do not disturb any of the wildlife. So once we have finished with a sample of the pond, we must make sure that it is placed carefully back into the pond. There is a risk element that we could fall into the pond and drown, so we must take extreme precautions. We should always make sure that we are with someone, and perhaps wear a life jacket.

Another danger is that we must wash our hands and keep them clean, as there is a possibility of catching all sorts of diseases. For example Weil's disease, this is when animals excrete the organism Leptospira ictero-haemorrhagiae in their urine. Infection in humans can cause illness and in 10% of cases death. For this reason I will be wearing rubber gloves and goggles.

We will be using some dangerous chemicals to measure the oxygen levels (I have listed them in my method) of the ponds. It is crucial that we dispose of these chemicals safely and not to pour them into the pond, as it toxic to the pond creatures and could cause some ecological havoc. Precautions will also have to be taken, as we will be working close to an electric power station. Which has a great potential of danger in itself.

Glass apparatus must be handled with care so that they do not break. If they do, that is also another hazard, and should be cleared up and disposed of in an appropriate fashion.

This is a table that shows the severity of an incident happening and the likelihood. They are scaled from 1 to 5, 5 being the most severe. They are then multiplied together to give the outcome.

Risk	Likelihood	Severity	Chance
Disturbing wildlife	4	1	4
Diseases	4	3	12
Chemicals	3	5	15
Glass	2	3	6

<u>Variables</u>
The independent variable of this experiment is the intensity of light.
This will be changed and should cause changes in my dependant variables. Light intensity should be a lot greater in the meadow pond than in the woodland pond this is because in the woodland pond the amount of light is blocked by the vast number of trees that are there. In the meadow pond there are no trees blocking the light and therefore there shall be a greater light intensity in the meadow pond. The intensity of the light shall be measured by using a light meter. The dependant variables of this experiment are the changes in the population of invertebrates, which should occur as a result of changing the independent variabl e.

In order to make this a fair test I am going to have to try and keep all other variables constant. This is going to be very hard for me to do as the pond is outdoors and controlling variables is not possible. To overcome this problem we are going to have to carry out the experiment quickly and during the same time of the day so that pH and temperature will not have enough time to change drastically in order to affect the validity of my results.

Table of results (Raw data)

Table 1: Meadow pond

Obs. #	Number of water fleas	Light intensity x 1000 (Lux)
1	4	19
2	1	15.4
3	8	18.24
4	6	13.71
5	2	**25.5**
6	**10**	13.52
7	5	12.96
8	3	15.65
9	5	16.35
10	6	11.2

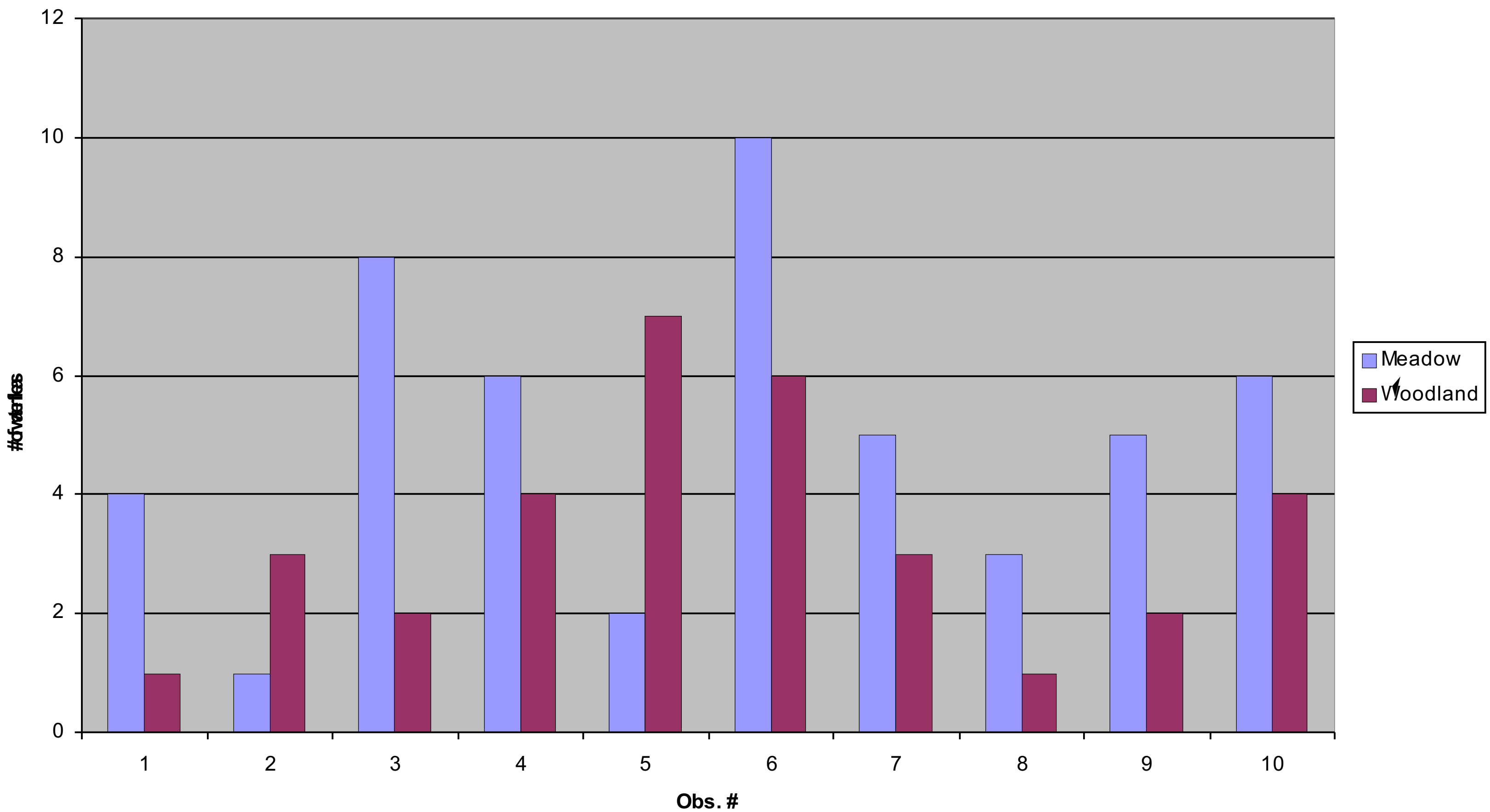
Table 2: Woodland pond

Obs. #	Number of water fleas	Light intensity x 1000 (Lux)
1	1	1.25
2	3	1.53
3	2	1.97
4	4	1.74
5	7	1.9
6	6	1.85
7	3	2.08
8	1	2.06
9	2	2.21
10	4	2.05

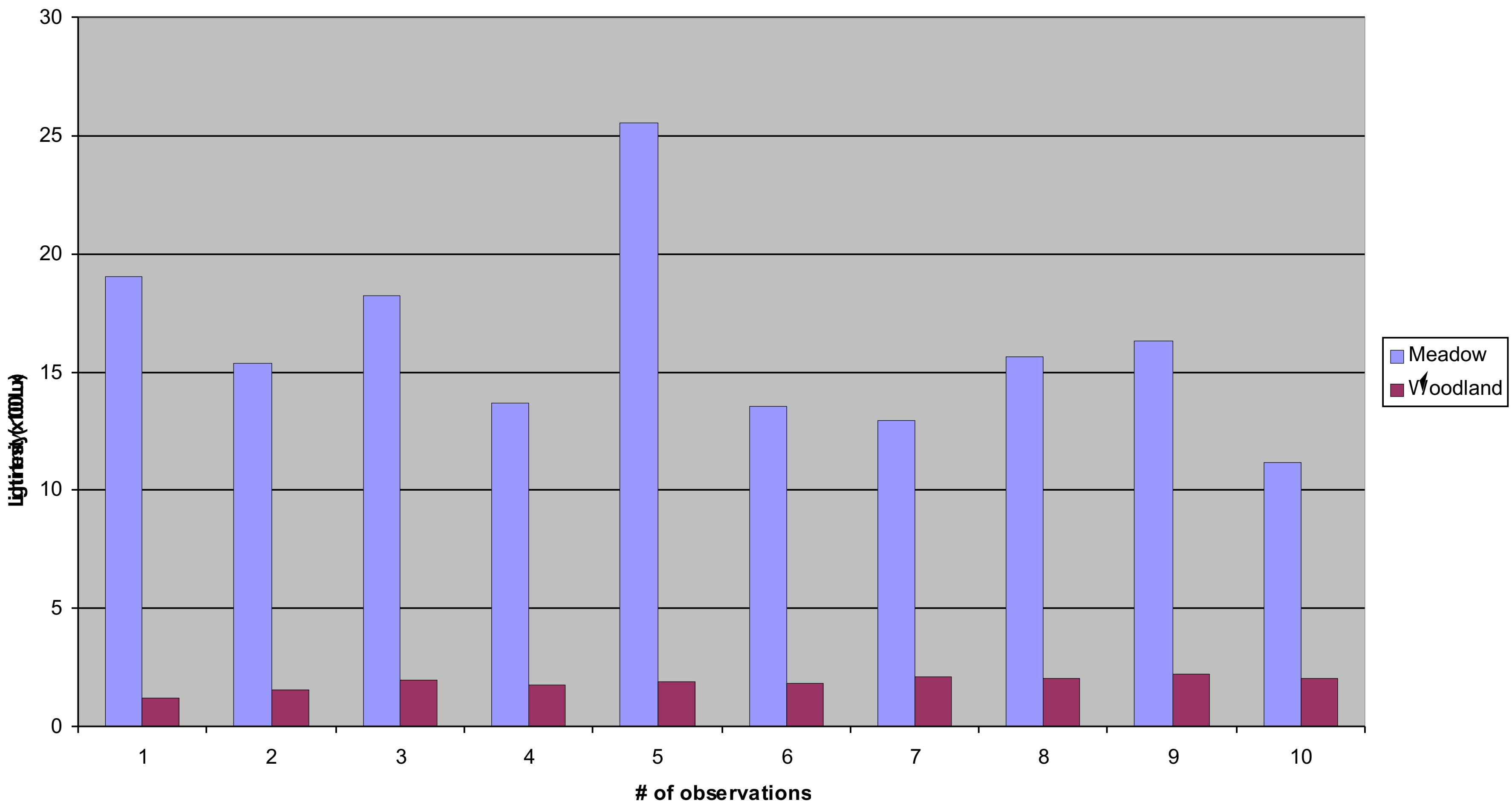
Table 3: Comparison

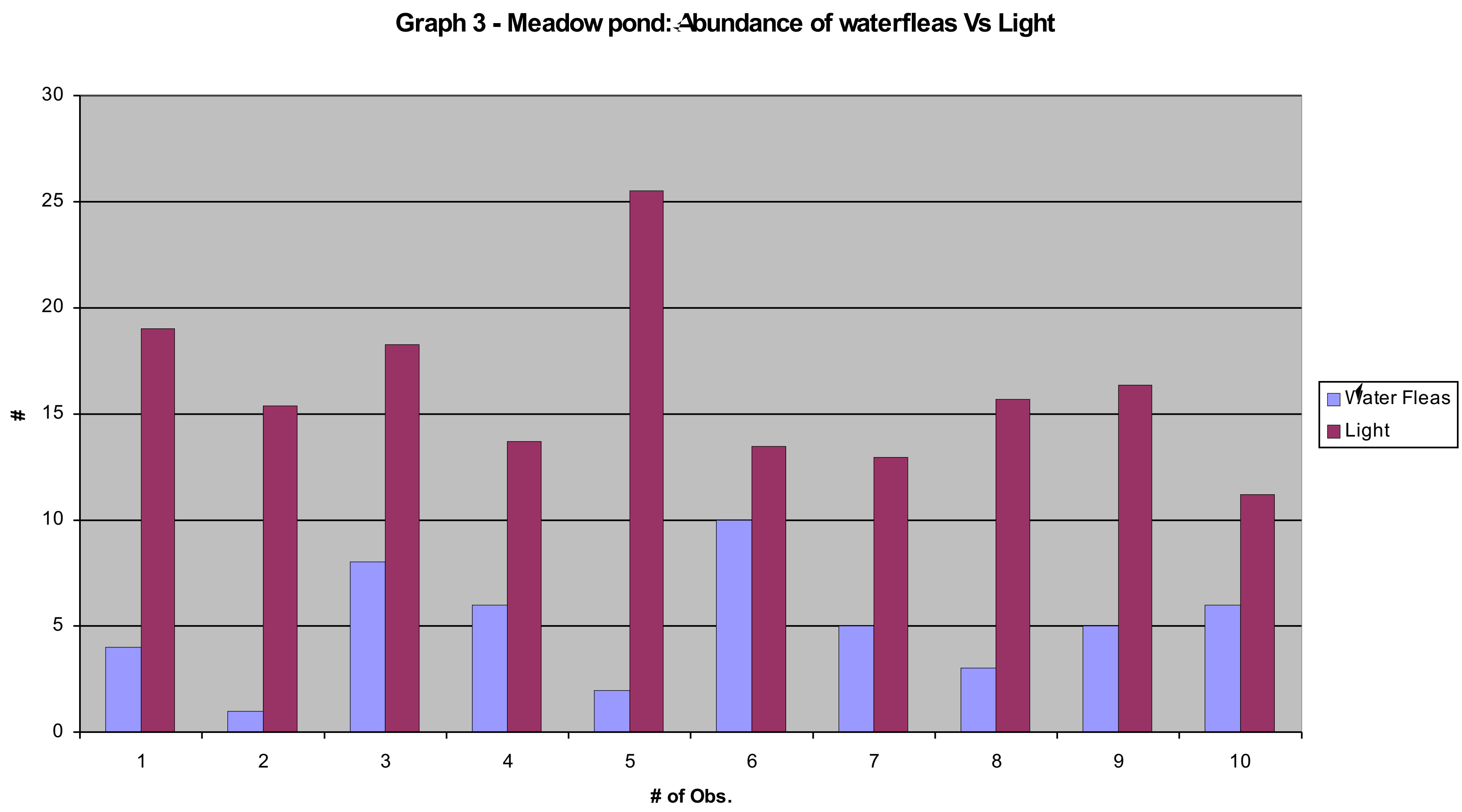
	Meadow Pond	Woodland pond
Total Water fleas	50	33
Average # of water fleas	10	3.3
Total light intensity	161.53	18.64
Average # of light	16.153	1.864

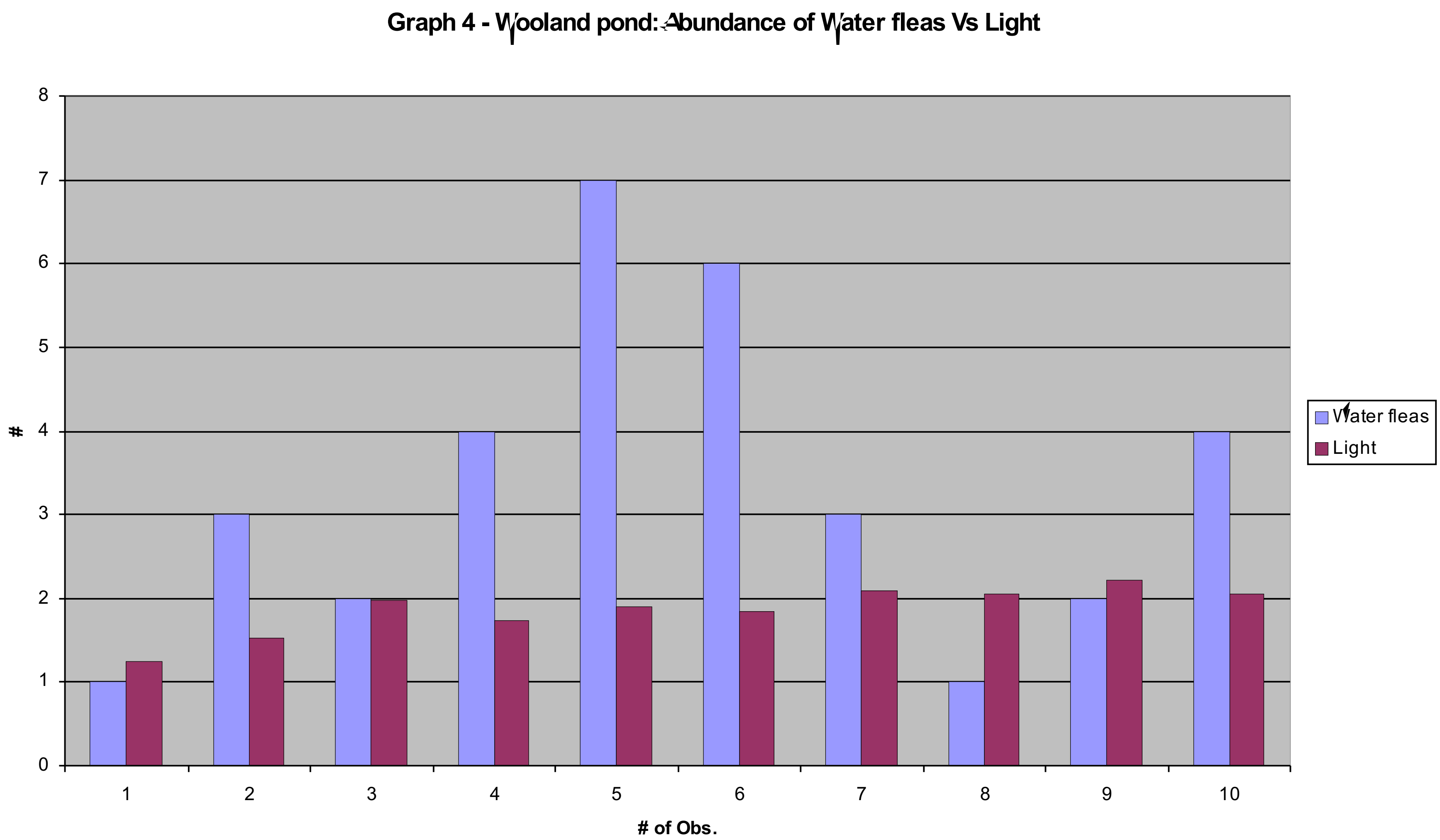
Graph 1 - Comparison of the abundance of water fleas



Graph 2 - Comparison of light levels




Graph 3 - Meadow pond: Abundance of waterfleas Vs Light
30
25
20
15
10
5
0
#
1
2
3
4
5
6
7
8
9
10
# of Obs.
Water Fleas
Light


Graph 4 - Wooland pond: Abundance of Water fleas Vs Light
#
0
1
2
3
4
5
6
7
8
1
2
3
4
5
6
7
8
9
10
# of Obs.
Water fleas
Light

Statistical Analysis – Spearman's rank

Spearman's Rank is used to analyse the data, which is related back to the hypotheses. The sampling techniques can be evaluated.
Spearman's rank order correlation coefficient is computed as the ordinary (Pearson) correlation coefficient between two groups of rankings. In other words, it is the correlation coefficient between two permutations of the integers. The reason why I had chosen to use Spearman's rank statistical analysis was because we have two sets of data and we comparing them together and therefore Spearman's would be the most appropriate to use in this scenario in comparison to Mann-Whitney and Chi squared.

Obs.	Woodland (x)	Rank (Rx)	Meadow (y)	Rank (Ry)	D (Rx - Ry)	D^2
1	1	1.5	4	2	-0.5	0.25
2	3	5.5	6	5.5	0	0
3	2	3.5	8	8.5	-5	25
4	4	7.5	6	5.5	2	4
5	7	10	8	8.5	1.5	2.25
6	6	9	10	10	-1	1
7	3	5.5	5	3.5	2	4
8	1	1.5	3	1	0.5	0.25
9	2	3.5	5	3.5	0	0
10	4	7.5	7	7	0.5	0.25
				Σ	0	37

The formula for Spearman's

$$r_s = 1 - \frac{6\sum d^2}{n(n^2-1)}$$

Calculations

$$Rs = 6 \times \Sigma\,(37) = 222$$

$$n = 10 \rightarrow n^3 = 1000 \rightarrow n^3 - n = 990$$

$$Rs = 1 - \frac{(6\Sigma D^2)}{n^3 - n} \rightarrow 1 - \left[\frac{222}{990}\right] \rightarrow Rs = 1 - (0.2242)$$

Rs = 0.775757575

Below is part of a table concerning tests of the hypothesis that a population correlation coefficient, Spearman's Coefficient, r, is 0. The values in the table are the minimum values, which need to be reached by a sample correlation coefficient in order to be significant at the level shown, on a one-tailed test.

To assess the validity of my result I will need to compare the test value (Rs) with the table of critical values. If my test value is equal or greater than the critical value of 95%, we can reject our null hypothesis.

Sample Level	Spearman's Coefficient Level		
	0.05	0.025	0.01
4	1.0000	-	-
5	0.9000	1.0000	1.0000
6	0.8286	0.8857	0.9429
7	0.7143	0.7857	0.8929
8	0.6429	0.7381	0.8333
9	0.6000	0.7000	0.7833
10	0.5636	0.6485	0.7455
11	0.5364	0.6182	0.7091
12	0.5035	0.5874	0.6783
13	0.4835	0.5604	0.6484
14	0.4637	0.5385	0.6264
15	0.4464	0.5214	0.6036
	0.4294	0.5029	0.5824

<u>Hypothesis</u>
I predict that there is a relationship between the abundance of water fleas and the intensity of light.

<u>Null Hypothesis</u>
There is no relationship between water fleas in two ponds of different light intensities.

Rs is greater than the 98% confidence level, which was greater than I had expected it to be. Therefore I can reject our null hypothesis.

Analysis of results and Evaluation of evidence

Overview of results

From my background information and my experiment results I can therefore say that the intensity of light in the area of a freshwater pond does affect the number of herbivore freshwater invertebrates.

As you can tell from the results table there was a significant difference in the abundance of water fleas in each ponds. A total of 50 were identified in the meadow pond with a greater light intensity with an average of 10, and a total of 33 in the woodland pond with a lower light intensity with an average number of 3.

Explanation of results

We can therefore say that the abundance of water fleas in a pond of greater light intensity is greater. This could relate back to my background information as to the fact that water fleas are herbivores and that if there is a greater amount of light in the pond, there shall be a greater amount of plants, which is more food for the water fleas, and this affects the food chain/webs and the pyramids of number in the ponds.

In the woodland pond there are a vast number of trees, the leaves fall off the tree and fall into the pond. As the water becomes polluted with the 'leaf litter' there are more organic nutrients available for bacteria to break down. This is called Eutrophication, and this causes algae to grow on the top on the pond preventing light, therefore killing many plants and invertebrates. This could be a reason for the diminutive amount of water fleas in the woodland pond.

The greater intensity of light shall also prevent low levels of oxygen in the freshwater pond preventing invertebrates to respire.

Trends

Graph 1 shows us the difference between the abundance of water fleas in the two different freshwater ponds. There was a significant difference here, as you can see there is a greater population in the meadow pond in comparison to the woodland pond. However, there were anomalous results obtained, the graph shows on two occasions where the number of water fleas in the woodland pond was greater than the meadow. These were on observation number 2 where there were 2 water fleas in the woodland and 1 in the meadow, and observation number 5 where there were 7 in the woodland and 2 in the meadow. Data found from both ponds proved that there was at least one water flea in each of my samples.

Graph 2 shows us the difference between the intensity of light in the two freshwater ponds. The difference of the light intensity of the meadow pond compared to the woodland is almost 10 – 20 times difference. Therefore the trees in the woodland pond do obstruct the light intensity available to the pond. There was one anomalous result that I had found; this was on observation number 5, where the light intensity was 25.5 x 1000 lux. This was rather odd because the experiment was carried out in one morning and consequently the light levels did not have a chance to change. Although a reason for this could have been as it was in the meadow pond, there could have been a quick burst of sunshine that could have increased the level of light.

My results may have been slightly inaccurate; this could have been for a number of reasons, for example:

- The method of counting the number of water fleas is extremely inaccurate, as I could make many mistakes. We could count the same water flea twice or not count some that we may not see at all. For this reason there was a great chance of human error and would lower the accuracy of results.
- I measured the abiotic factors at the beginning of the experiment (check method), and I found it very hard to control all these variables, this included pH, temperature and oxygen levels. This was because the temperature varied throughout the day, as in the morning it was very sunny and later on in the afternoon it was raining and cloudy. This might not have made it completely a fair test, as for the pH levels; the rain could have altered them slightly throughout the course of the experiment.

The apparatus used was not adequate to give conclusive results; this was because we did not have enough money to buy better equipment and enough time in order to carry out the experiment properly. Hence giving a moderately accurate conclusion that our hypothesis was correct. There were however some anomalies in our investigation, this could have been for a number of reasons; the method of counting the water fleas was a very imprecise and could have lead to abnormal results. The number of anomalies could also contradict the validity of my results.

<u>Improvements</u>
I believe the method used was generally the best possible, considering the equipment available. If I were to do the experiment again, I would change my method, as it is a very conventional, and as we were outside and counted the organisms manually this has increased the chance of human error.
If I was to do the investigation again, and I had more time and resources, I would first of all take my sample of the pond back to a laboratory and then and place it under a microscope and manually count the number of water fleas. This would be very time consuming but would give more accurate results.
Another method that we could undertake would be to use a binocular microscope that has a eyepiece quadrat, and to count the number of water

fleas using this method. It is still has a good chance of human error, but there will be an increase in the accuracy of my results.
Another idea would be to create an artificial pond under controlled conditions. This would be because then we would be able to control the other variables, giving us more reliable results. Although this would probably take years of research and would be an idea for future research.

Anomalous Results
By referring to the table of results you can see two separate results that are in bold. These indicate anomalous results. They are anomalous because they are very different from the other readings that I had taken. This should not occur because there should be readings for each pond.
Another anomaly that I had noticed in my results was the fact that in two ponds we had still found the same number of water fleas. Although in general there were more in the meadow pond, there were occasions where there were 1 or 2 observations that were the same, this can contradict the reliability of my results.

Limitations
Another limitation in my method could be the time of the year. First of all it was March and as it was very cold, the experiment was rushed and most probably not done to a high degree of accuracy. Another factor could have been that some of the freshwater invertebrates could have either died or be hibernating due to the cold weather. I had taken the temperature of the water during the experiment and it turned out to be 5.1`c.
We also had a great problem with controlling the other variables in outdoor conditions.

Conclusion Validity
Taking into account the inaccuracies and problems mentioned above it can be concluded that although the results are not accurate enough to give a reliable conclusion, we can say that the results obtained were 'fairly' reliable with about a 20% of human error. My hypothesis was correct and we can say there is a relationship between the number of water fleas in freshwater ponds and the intensity of light.

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www.waterfleas.com