An investigation into the effects of phosphorus pollution on populations of *Daphnia magna*.

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**Introduction**

**Aim**

The aim of this investigation was to see whether phosphorus pollution has an effect on populations of *Daphnia magna*. The levels of phosphorus pollution to be investigated were above and below the levels recommended for drinking water safety. The effects on the population size of *Daphnia* were investigated. The other areas to be investigated were the effects on the number of *Daphnia* with eggs, the average number of eggs per female, the number of dead *Daphnia*, and the amount of algae in each treatment.

**Hypotheses**

The addition of phosphorus, up to the recommended safe level in drinking water, will cause there to be an increased growth of algae. This increases in algae will lead to an increase in the number of, the number of females with eggs, and number of eggs per female. The addition of phosphorus above the recommended safe level in drinking water, will induce the effects of eutrophication. This will reduce the number of females with eggs, and number of eggs per female.

In 1980, an European Community (EC) Directive on the Quality of Drinking Water Intended for Human Consumption was passed setting a maximum allowable concentration of 50 milligrams (mg)/litre (l) of nitrate in drinking water. (MAFF-SNP). This has meant that agriculture has had to become acutely aware of these issues and the management techniques that can reduce the loss of nitrate from arable soils. Nitrate can b found in natural water in three main forms as combined oxygen nitrogen, ammoniacal nitrogen (ammonia or ammonium) and as oxidised nitrogen (nitrite or nitrate).

The loss of phosphate into aquatic systems is more damaging than the loss of nitrate. Phosphate is applied at the same time as nitrate and is found in fertilisers and manures. Soils, especially agricultural soils are naturally low in phosphate and soil content ranges from 200- 2000 kg (Phosphorus (P))/ ha (Nyle and Weil; 1996). There is a considerable difference in the amount of P* lost from agricultural land under different management techniques. Fields under wheat conventional ploughing have been seen to lose 3.72 kg/P/ha/year, wheat with no-till has been seen to lose 1.42 kg/P/ha/year while a field under heavily grazed grass was seen to lose 0.24 kg/P/ha/year (Nyle *et al*; 1996). When P* enters a water body in sufficient quantities, 0.03 mg/l of dissolved P* and 0.1 mg/l of total P* (Nyle *et al*; 1996), accelerated or cultural eutrophication occurs. The affected water body can lose all ecological, commercial and recreational value.

*Daphnia magna* (Order Cladocera, Family Daphniidae) is frequently referred to as the water flea. They are often used in studies of ecotoxicology because they can withstand unfavourable conditions and are readily available. They occur in nearly all bodies of still water and in some slow-flowing rivers. They can occur with other species of Daphnia, but where conditions prevail, one species will dominate. In the early spring eggs, which have been lying dormant over the winter, hatch. An all-female population is produced and the females can produce more females without the need for fertilisation by males. The eggs hatch into miniature adults within the brood pouch and are released when the female sheds its skin. A few males are produced in the autumn, as they are needed to produce the next stage of dormant eggs. Ephippium are produced that contain to dormant eggs that can resist drought and hard frosts. In the summer the *Daphnia* population feeds on
bacteria and suspended algae and a vital member of the food chain. It is a well-known fact that they feed by using thoracic limbs to filter phytoplankton particles in the water and can also use them to graze algae from stones and plants. Egg production ceases when food, measure as carbon, is < 0.2 mg/Carbon (C)/l and reaches a maximum when food reaches about 0.7 mg/(C)/l. They cannot survive on the blue-green algae produced in eutrophic lakes.

Methods
An experiment had to be designed to investigate the effects of phosphorus pollution on a population of *D. magna*. *D. magna* was chosen because it was readily available from garden centres as fish food. It is a well-known indicator species and experiments to determine the effects of water pollution have been run previously. Potassium dihydrogen orthophosphate was chosen because it is a soluble source of phosphorus. Thus known could be added to attain known concentrations of phosphorus. The experiment tested phosphorus at five different levels (Table 1). There were three replicates of each level and the appropriate amount of chemical was placed in buckets with 20 *Daphnia*. A mixture of lake and tap water was: 1 litre lake water, 4 litres tap water. Lake water was used to provide phytoplankton for the *Daphnia* to feed upon.

Three controls did not have any chemical added, so there was a total of 18 buckets used. Buckets were set up in the arrangement indicated in Fig. 1. The buckets were left for three days to settle. This allowed algal growth to begin so that, when *Daphnia* were added, there would be food available.

The experiment was conducted for two weeks in a glasshouse, which had an average temperature of 22 °C. At the end of this period, the percentage of algal growth covering the bucket was estimated by eye. The percentage estimate was estimated by the same person, so the results were consistent. For each replicate, the following population parameters were measured: total number of *Daphnia*, number of females with eggs, and number of eggs in each female.

Results

*Phosphorus and number of Daphnia magna*

The highest average number of *Daphnia* was 273.67, found in phosphorus treatment 2, this accounted for 46% of the total number of *Daphnia* found in the phosphorus treatments. The lowest number of *Daphnia* found was 0, in treatment 5. The lowest number of *Daphnia* in a single treatment was 313- in treatment 2: replicate 5 (Fig. 3). The *Daphnia* in treatment 1, replicates 1 and 2, covered the full range of body sizes.

The highest average number of *Daphnia* with eggs was 64.34, found in phosphorus treatment 2. The lowest average number of *Daphnia* with eggs was 0 in treatment 5 (Fig. 3). The highest number of *Daphnia* with eggs in a single treatment was 70 in treatment 2: replicate 2.

<table>
<thead>
<tr>
<th>Treatment strength</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorus mg/l</td>
<td>0.55</td>
<td>1.1</td>
<td>2.2</td>
<td>4.4</td>
<td>8.8</td>
</tr>
</tbody>
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*Table 1.* A table showing the amount of pollutant needed for each treatment strength.
The highest average number of eggs per female was 4.42, found in phosphorus treatment 2. This accounts for 32% of the total average number of eggs found in the phosphorus treatments. The lowest average number of eggs per female was 0, found in nitrate treatment 5. The highest average number of eggs per female in a single replicate was 5.15 in treatment 1: replicate 3.

Control replicates
The water in all the control and experimental buckets had a pH of 7. This meant that pH was not a variable. There were an anomalous number of Daphnia in replicate 1. This was 621. The average number was 282.67. The average number of Daphnia with eggs was 7.67, and the average number of eggs per female was 3.17.

Discussion
The Daphnia in the phosphorus treatments were expected to have favourable conditions, as P is usually the limiting nutrient in the growth of algae. The algae would have taken up the phosphorus added to the water and growth would have increased. This abundance of food would have allowed the Daphnia to graze all the time. The Daphnia would not be food stressed so breeding and numbers increase. The highest population size was in phosphorus treatment 2. The treatments after this had sufficient phosphorus to start eutrophication and high algal growth. This is what was expected. Phosphorus treatment 5 had no Daphnia. This may have been due to a rapid growth of algae at the start of the experiment. This would rapidly use the oxygen present in the water resulting in the death of the Daphnia and causing conditions to be unfavourable for algal growth.

Fertility
Fertility rates were highest in phosphorus treatment 2, where population number was the highest. The treatments above and including the recommended safe level of phosphorus showed a decrease in the number of Daphnia with eggs. This was predicted in the hypotheses. The presence of phosphorus would have allowed for growth. In order for a Daphnia to release its young, it has to be able to shed its skin. Increased growth would have meant that the Daphnia shed their skins more often, releasing more young and increasing the size of the population.

The average number of eggs per female did not differ very much. This may have been because food quantity and quality had no effect on the number of eggs a Daphnia produces, just how many times they produce them.

References
Fig. 1. Physical layout of the buckets to which phosphorus was added. A, B and C refer to the three replicate blocks; P1- P5 refers to the five levels of phosphorus addition.
Fig. 2 Graphs showing which phosphorus treatment resulted in a) the highest number of Daphnia b) the highest number of female Daphnia with eggs c) the highest average number of eggs per female.
**Assignment**

1: In your own words, write down the objectives of this study. Were the objectives adequately addressed? What assumptions underpin the study? Are these assumptions clearly discussed/justified in the manuscript?

2: Having read the manuscript, write a report on this paper to the author. The report should be about one and a half pages of single spaced, size 12 font. Although the criticism is most important, remember to not just make criticisms, but also indicate good aspects (if any), and suggest how to improve the paper.

3: Make an appropriate form for the collection of data from this experiment.