Investigating the growth of Lemna (Duckweed)

Aim: investigate one factor which affects the growth of Lemna.

Introduction:
Lemna is a floating aquatic plant that is generally known as "duckweed". There are several common species of duckweed including the large-leaved *Spirodela (Lemna) polyrhiza*, the three-lobed duckweed, *L. triscula*, and the common duckweed, *L. minor*. These duckweeds thrive on lakes and multiply until a layer of it covers the entire surface of the water, but are not usually a pest as the layer is thin and light and oxygen can still pass into the water beneath their leaves.

There has been a case in Britain where a particular species of duckweed has been so successful that it blanketed the entire lake in the nature reserve of Canterbury with a mat that was five to twelve centimeters thick, thus isolating the lake from light and oxygen and killed the rich growth of underwater vegetation which then decomposed. The result of the decomposition was high concentrations of hydrogen sulphide which proved toxic to most organisms living in the water. Once rid of its competitors, the duckweed proceeded to use the decomposition products, particularly ammonia, as their source of nitrogen.

The success of *L. minuta* in the Canterbury lake can be credited to a number of human-generated factors. Firstly, the water in the lake is enriched by fertilizers and fully treated sewage thus providing a rich source of nutrients for the duckweed to thrive upon. Secondly, humans accidentally move organisms around the planet. Examples of the latter include the introduction of sycamores to Britain, rabbits to Australia and probably *L. minuta* to England. Thirdly, there may have been increased amounts of sunlight (insolation) warming the lake over the past three years.

Lastly, one crucial factor appears to be that the Canterbury lake is sheltered from the wind by trees, and water disturbance is known to reduce the success of *L. minuta*.

From this example, I can deduct from it, the factors which will affect the growth of Lemna.

Variables to Investigate:

- Temperature – Lemna grow particularly well in warm water and is deterred by colder climates.
- Area – when the leaves of the Lemna touch, they stop reproducing, as there is no space left.
- Water Disturbance – wind and waves are known to slow the growth of Lemna
- Light Intensity – as Lemna is a plant, it photosynthesizes which requires light thus the importance of light intensity as a factor.
- Nutrient concentration – as Lemna is a life form, it needs nutrients to survive thus the importance of nutrient concentration as a factor.
- Nutrient deficiency – see "nutrient concentration".
- Light wavelength – As chlorophyll is green, it has different levels of receptivity to various wavelengths and reflect some wavelengths thus the importance of light wavelength as a factor.

Scales of Growth to consider:

- Counting
- Area Covered
- Biomass (dry weight)
- Number of Leaves
- Health

My choice of Variable to investigate is: "Nutrients and the effects of their deficiency".

The Nutrients involved:

Phosphorus / Phosphates:

Phosphates promote root establishment and formation as well as flowering. Phosphorus is also used in production of DNA and RNA during cell mitosis. Therefore if a plant is phosphorus deficient, it may have a purplish cast on the underside of the leaves (corn) or purplish/red leaves and stems (tomatoes). Flowering and fruiting will be limited. Plants will be slow to form new roots and establish. Plants are slow growing. Premature fruit drop may occur. (Include diagram of cell mitosis and "before a cell can divide.doc"). Mention RNA and its roles and illustrate the importance of phosphorus in creating DNA and RNA. Phosphorus is also involved in the energy dynamics of plants. Without it, plants could not convert solar energy into the chemical energy needed for the synthesis of sugars, starches, and proteins.

Potassium:

Potassium is involved in photosynthesis, sugar transport, water and nutrient movement, protein synthesis, and starch formation. Potassium helps to improve disease resistance, tolerance to water stress, winter hardiness, tolerance to plant pests, and uptake efficiency of other nutrients. Strong, stiff stalks are also an effect of potassium. Additionally, potassium promotes productions of sugar, starches and oils, increases size of grains and fruits and improves the quality of the crop's yield. This is because potassium plays a vital role in activating enzymes throughout the plant. Potassium deficiency includes reduced vigour, increased disease problems, thin fruit or vegetable skins as well as small fruit.

Calcium:

Calcium is an element which contributes towards the strengthening and building of cell walls. Calcium is a constituent of the cell wall and keeps the cell membranes stable. Visual evidence of calcium deficiencies generally occurs in growing points of the plant at the fruit, stem, leaf, and root tips. Calcium deficiency will show up as weak stems or trunks as well as a reduction of any new growth from the plant's growing points.

Nitrogen:

Nitrogen is combined with sugar molecules or smaller carbohydrate molecules to create amino acids that are then combined to form the proteins that make up the enzymes and cytoplasm of the cell. Deficiency symptoms include a yellowing of foliage or a light green-yellow overall colour. Plant growth is reduced and/or stunted.

Magnesium:

Magnesium is a key element in chlorophyll, thus is essential in the photosynthesis process. Chlorophylls is any member of one of the most important classes of pigments

involved in photosynthesis, the process by which light energy is converted to chemical energy through the synthesis of organic compounds. Chlorophyll is found in virtually all photosynthetic organisms, including green plants, cyanophytes (blue-green algae), and certain protists and bacteria. It absorbs energy from light; this energy is then used to convert carbon dioxide to carbohydrates. In green plants chlorophyll occurs in disk-like units in organelles called chloroplasts. The chlorophyll molecule consists of a central magnesium atom surrounded by a nitrogen-containing structure called a porphyrin ring; attached to the ring is a long carbon-hydrogen side chain, known as a phytol chain. Variations are due to minor modifications of certain side groups. Magnesium is also involved in energy metabolism in the plant and is required for protein formation. Stem strength is also influenced by magnesium. Leaves become chlorotic and yield is reduced when leaves are deficient. Rose flowers may droop at the neck.

Sulphur:

Sulphur helps build the plant proteins by forming the cross-links in the proteins called sulphur-bridges. Therefore a deficiency would result in similar conditions as a nitrogen deficiency.

Iron is a catalyst throughout the plant; important in chlorophyll and protein formation, enzyme systems, respiration, photosynthesis, and energy transfer. It is also applied to form cytochrome, which is an enzyme required for cell respiration.

Cytochrome is a group of hemoprotein cell components that, by readily undergoing reduction and oxidation (gain and loss of electrons) with the aid of enzymes, serve a vital function in the transfer of energy within cells. Hemoproteins are proteins linked to a non-protein, iron-bearing component. It is the iron (heme) group attached to the protein that can undergo reversible oxidation and reduction reactions, thereby functioning as electron carriers within the mitochondria (the organelles that produce energy for the cell through cellular respiration).

THE PLAN:

I will have a choice of eight different solutions in my investigation, seven of which contain a deficiency of one of the nutrients explained in the introduction. The eighth solution is a complete solution which includes: Calcium, Nitrogen, Potassium, Phosphate, Magnesium, Sulphur / Sulphates, Iron, Sodium and micronutrients. The chemical makeup of the solutions can be illustrated in more detail in the table:

Solution:	Phosphorus	Calcium	Nitrogen	Magnesium	Sulphur	Iron	Potassium	Micronutrients
Complete Solution	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Phosphorus Deficient	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Calcium Deficient	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Nitrogen Deficient	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
Magnesium Deficient	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Sulphur Deficient	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Iron Deficient	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Potassium Deficient	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes

I will, choose from the eight solutions, a selection of five which will be used in the investigation. This is because there is a limited supply of equipment, therefore I should only choose the variables which will yield the best and most significant results. For example, nitrogen and sulphur are both important in the creation of proteins in the plant therefore a deficiency of any one of them will result in the same effects therefore I can eliminate sulphur from the list. From here, I assess the nutrients, comparing them against one another. Calcium is the next element to be removed from the list because, firstly, the interaction of Calcium and the cell walls are not entirely understood and therefore no detailed explanation can be made of the effects. Secondly, there is little external effects to be noted, thus making it hard to record.

The final list of solutions to be used in the investigation is as follows:

- Phosphorus / Phosphates
- Potassium
- Nitrogen
- Magnesium
- Iron
- Complete Solution

I will use twelve petri dishes and fill every two petri dishes with 15 millilitres of one different nutrient of those mentioned above. I will then use a small paintbrush to transport three Lemna plants from the container provided to each petri dish, discarding those that are currently in the stage reproduction, as that will mean that when placing one individual Lemna into the petri dish I am, in fact, getting two. I will also reject those that are unhealthy so that if the test subjects die, it will be due to the variable I chose to investigate and not because they were unhealthy before the investigation even began. The petri dishes will be name 1A, 1B, 2A, 2B, 3A, 3B up until 6A and 6B. The numbers stand for the nutrient deficiency. 1 stands for complete solution, 2 for phosphorus deficiency, 3

for potassium, 4 for nitrogen, 5 for magnesium, and 6 for iron deficiency. The measures I am taking are to ensure that the investigation and comparison will be a fair one.

I will then cover the petri dishes with the lids and place them side-by-side. I will choose an area which has sufficient light intensity, but not too much as the sunlight may bleach the Lemna and kill them, to place the petri dishes. The area must not have excessive temperature fluctuations, for example, the windowsill where the temperature changes with sunrise and sunset, as that will slow the growth of the plant. Nor should there be too much air disturbance i.e. wind which, although the petri dishes have lids, may still affect the growth of the Lemna. Lastly, is to consider the amount of gas diffusion that the lid of the petri dish allows, but according to previous experience with similar experiments, the petri dish allows adequate amounts of carbon dioxide to diffuse in for plants to thrive. This is all to provide the optimum conditions for the growth of Lemna, even though they are subject to all the same conditions, whether it is extreme temperature fluctuations, ultraviolet bombardment, high winds or CO_2 starvation, but these conditions will result, in the end, of the death of the test subjects and thus ruining the investigation.

I will also keep a light constantly switched on above the petri dishes so they may have light throughout the entire day. But from here, arises question of the heat generated by the light which may go to the Lemna and may be different in each petri dish thus making the investigation unfair, also, the heat may make the solutions evaporate and kill the Lemna. The amount of light each petri dish gets may also be different. I will approach the problem of different amounts of heat and light affecting the petri dishes, by assuming that light intensity is directly related to the heat the petri dish receives. It is an assumption because the wavelength of visible light and heat radiation are different but I am assuming this because I believe that in such short distances, the differences do not matter. If that is so, then I can ensure that every petri dish has the equal amount of light intensity just by measuring the light with the light intensity metre at each position of the petri dishes and changing their positions to create equal amounts of light and heat intensity thus solving the problem. The second problem is that the excess heat generated by the light causes undue evaporation of the solution vital to the survival of the Lemna. This can be remedied by placing a water barrier between the light and the Lemna. A second petri dish can be placed above the first, which contains water. This water will let the light penetrate but will absorb the heat that comes from the light. But this requires me to use 12 extra petri dishes which may produce a problem due to lack of equipment. Other changes may be necessary to accommodate the shortage of equipment later on in the investigation.

On account of the problem of equipment, I may need to conduct the experiment without the lamp or without the extra petri dishes. The reason which I repeat the nutrients in another set of petri dishes is so that I am conducting a repeat of the experiment simultaneously with the current experiment so that I have a second set of results to obtain averages from which can reduce the impact of errors in one or both sets of results.

If I conduct the experiment without the lamp, I will gain benefits from the extra set of results and I must find an area of optimum light intensity for the Lemna, but if I do use a lamp, I may decrease the amount of errors caused by light deficiency, but I will lose the extra results. Another alternative is instead of using petri dishes with water, I use a transparent tray or several beakers filled with water and place that on top of the test subjects. Out of all of these methods, I find the method replacing petri dishes with a tray

of water, the most appealing as it helps to keep the temperature in the petri dishes constant as well as providing an abundance of light therefore providing the optimum conditions as well as controlling them.

My range of variables is 6, as stated above under the heading:

"The final list of solutions to be used in the investigation"

I will take recordings of the progress of the growth of the Lemna. This can be done by:

- Counting
- Area covered
- Biomass
- Health

In the early stages of the experiment, where the number of Lemna is small, counting is seems the best method. But when the investigation progresses, the count of the Lemna will increase exponentially, i.e. very fast and so counting become inaccurate as it is hard to keep track of which individuals have been counted and which have not. At this stage, the logical method to use is the area covered which also has its errors, but is much more time efficient that counting. Biomass is not really an option that should be considered as it is stupid to dry your test subjects and weigh them as it will kill them and after the first reading, there is no way to continue the experiment. Health is not a quantitive measure of growth but it does show the progress of the plant and probably the effects of the nutrient deficiency in the solution. Therefore I shall record results in three ways.

I will record the count, area covered and health of the Lemna while it is still possible. When it becomes hard to count the individuals, I will change mainly to area and from that, calculate the approximate number of individuals, assuming that they are of equal sizes and that not spaces exist between each separate plant. When there small numbers, I will comment generally on the health of each plant but when numbers increase I will comment on the percentage of individuals which are unhealthy. To calculate the area, I will use a flexible ruler which I can then form in the shape of a circle, which I will then place into the petri dish and gather the Lemna together in a circle. The circumference may then be measured and subsequently, the area to be calculated from the formula:

$$C \div 2\pi = r$$
$$(C \div 2\pi)^2 = r^2$$
$$\therefore A = \pi(C \div 2\pi)^2$$

$$\therefore \mathbf{C^2 \div 4\pi = A}$$

(**C** = Circumference **A** = Area)

I chose to use a circle instead of any other geometrical shape because the leaves of the Lemna are rounded and these fit the best with a circle. Also, the petri dish is round and so it is more convenient to work with a circle. The results will be recorded over a period of weeks where the long-term effects of nutrient deficiency can be effectively illustrated. I will collect results every Tuesday and Friday so that there are equal times in between each recording. I can then process the information to create a growth rate graph where the growth of Lemna each day can be approximately calculated and can be used in later analysis of the results. The results will be recorded in a table similar in layout to this:

Date	Method	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
12/12	Area												
12/12	Count												
“”	Area												
“”	Count												
“”	….												

I will repeat my experiment by conducting two of the same experiments simultaneously; therefore I have two of the same variable and thus, two sets of results. This will enable me to combine the results and form an average which will reduce the impact of errors in one or both sets of results and increasing the reliability of the results.

List of Equipment Required:

- 12 petri dishes
- Paintbrush (small)
- Nutrient solutions
- Measuring cylinder (10 ml) or syringes (5 ml)
- Transparent tray
- Flexible ruler (paper substitute will do fine)
- Lamp or Light
- Light intensity metre

Prediction:

I predict that the Lemna in the complete solution will be the most successful as it does not contain any deficiencies and they are exposed to only the optimum conditions. As illustrated in the introduction analysis of the nutrients, every deficiency will result, whether early or late, in poor health and stunted growth of the Lemna and probably even death. Therefore a complete solution will provide all the essential nutrients and so the plants will remain healthy. To predict the effects of the various nutritional deficiencies is notably difficult as more information is required at this stage, but I will venture to make a few proposals in the direction of which I believe there to be significant developments.

Phosphorus is a key element in the creation of DNA and RNA and so is a vital part of the processes of a cell. Without DNA, the cells cannot reproduce, as the DNA cannot replicate completely, mutations may also occur, or if the DNA is badly damaged or altered, it will trigger a self-destruct sequence within the cell and the cell will die. The production of proteins is virtually all brought to a halt and when the current proteins break down, all cell activity will cease. The period required for these proteins to break down vary between different types. Short ones may breakdown faster while long, complex ones take longer. I believe that the population will grow for a while, using the nutrients present in the solution until the store of phosphorus is depleted, then the population will drop as metabolism and cell processes stop.

Potassium is an activator of enzymes throughout the plant. I believe that without potassium, the plant’s processes will slow down, if not stop altogether. Therefore killing the plant slowly. I think that there will probably be an increase in population for a while

as the solution will contain more nutrients than in the original tank, but then stop reproducing slowly due to potassium starvation.

Nitrogen deficiency affects the production of protein not to mention that the four bases for DNA construction are also nitrogenous. Nitrogen is a part of the base amino acid which makes it so important for producing protein. Thus it's deficiency affects the cell processes even greater than phosphorus as it stops both DNA and protein creation. The population of Lemna will grow, thriving on the extra nutrients and the original store of nitrogen, then; stop reproducing quickly due to nitrogen deficiency. I predict that the Lemna with nitrogen deficiency will stop reproducing earlier than the Lemna with a phosphorus deficiency.

Magnesium is essential in chlorophyll. Without magnesium, no new chloroplasts can be manufactured and so the Lemna cannot photosynthesize. I predict that the Lemna with magnesium deficiency will have a population increase for a longer time than any of the others. That is because the plants have their current chloroplasts that can last them for some time, and then they have a store of sugars, starches and proteins that they can use.

Iron is mainly applied to produce an enzyme, cytochrome, which acts in cell respiration. Without iron, the cytochrome cannot undergo reversible oxidation and reduction reactions and thus stunting cell respiration. I predict that Lemna with iron deficiency will have a long period of population increase, but just under that of magnesium, and then stop reproduction due to difficulties in cell respiration.

All the Lemna will have a period, whether short or long, of population growth due to the nutrients already taken up and stored in the plant from the original tank in which they were bred. When this store of nutrients is depleted and in combination with other factors, the Lemna will stop reproducing and begin dying out. It is only a matter of which nutrient deficiency will result in what rate of population growth and decay.