Investigation to ascertain the extent to which light intensity is a limiting factor to photosynthesis.

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Abstract

The aim of this investigation is to ascertain the extent to which light intensity is a limiting factor on the rate of photosynthesis of *elodea canadensis*. The hypothesis was that light intensity will have a significant effect on the rate of photosynthesis. As light intensity increases so too will the rate of photosynthesis. The length of gas bubble produced in five minutes was taken at differing light intensities and temperatures. The results showed that light intensity does have a significant effect on the rate of photosynthesis. As light intensity increased so too did rate of photosynthesis until it was limited by another factor such as temperature. When the temperature was raised, the rate of photosynthesis continued to rise.

Introduction

Null hypothesis: Light intensity will not have any effect on the rate of photosynthesis in *elodea canadensis*.

Experimental hypothesis: Light intensity will have a significant effect on the rate of photosynthesis in *elodea canadensis*. As light intensity increases so too will the rate of photosynthesis

Biological knowledge to support hypotheses:

In a freshwater environment PFD (wavelengths of sunlight used for photosynthesis) is low for submerged leaves, because light penetration is reduced when passed through the water. At the surface there is unobstructed full sun for a photosynthetic organ floating. An emergent canopy may intercept high PFD, which may be harmful to the plant. The concentration of carbon dioxide and oxygen dissolved in water is low. Minerals and nutrients are scarce or dilute within the water medium, when compared with drier soil.
Elodea canadensis is a leafy submerged aquatic hydrophyte originating from North America. It is commonly found in still or slow flowing waters in various locations around the state. *Elodea* thrives in temperate climatic zones and grows prolifically during summer once water temperatures exceed 15°C
Elodea canadensis is also known by several other common names such as Canadian waterweed, common elodea, or anacharis. It has been introduced to several countries where it is not native, and is now considered a noxious weed in those regions (parts of Europe, Australia, Africa, Asia, and New Zealand).
Elodea canadensis lives entirely underwater with the exception of small white flowers

which bloom at the surface and are attached to the plant by delicate stalks. It produces winter buds from the stem tips which over winter on the lake bottom. In the autumn leafy stalks will detach from the parent plant, float away, root, and start new plants.[1]
A hydropyhte is a plant that has adapted to live partially or totally submerged in water. They have no osmotic problems in freshwater because of the pressure potential caused by the cell walls. *Elodea canadensis* has only submerged leaves arranged three per whorl, which have many specialised features. There are a large number of small leaves which are long and cylindrical. The leaves are quite delicate so that there is less resistance to movement of water to reduce damage.
Other specialised features of the plant are that there are large intercellular spaces which form air filled cavities extending throughout the plant to provide internal air passages for gas exchange and a carbon dioxide store for photosynthesis. Bubbles are produced from these when the plant is photosynthesising rapidly. Hence when the stem is cut bubbles will come from that point. The cavities provide buoyancy to keep the plant upright in the water so that the leaves can obtain maximum light for photosynthesis.
There is an absence of mechanical and vascular tissue and root systems, this lack of rigidity enables bending with water currents.[2]

Literally, photosynthesis means 'synthesis with the help of light.' This covers a variety of processes. However, the term is usually applied to one reaction only -the synthesis of organic matter by plants in light- a process also called 'carbon assimilation.' This is a fundamental process of life. It creates living material out of inert inorganic materials, replenishes our supply of oxygen in the atmosphere and stores energy provided by the sun to support life activities of organisms.[3]

The equation for this process is:

$$6CO_2 + 6H_2O \Rightarrow C_6H_{12}O_6 + 6O_2$$

Carbon dioxide + water -------- glucose + oxygen

The extent of photosynthesis performed by a plant depends on a number of factors both internal and external. The external ones are: light intensity; ambient temperature; concentration of carbon dioxide. These are the ones this experiment is concerned with. Photosynthesis put simply is this; the capture of the energy of a photon of light by a pigment molecule, in this case chlorophyll, the formation of an electronic 'excited' state, the use of the 'excited' electron to reduce a chemical substance and to form energy rich molecules. These energy rich molecules are used to form other more complex organic molecules[4].
At low light intensities the rate of photosynthesis is low. This is because the electrons are receiving a smaller amount of energy and so fewer of them reach the 'excited' state. When the intensity is increased, the rate of photosynthesis will increase until the saturation point is reached. This is the point when the rate cannot go any higher. A factor other than light intensity is limiting it. A limiting factor is any variable that prevents the reaction from exceeding a particular level.[5]
Temperature affects the rate of photosynthesis in a different way. As temperature increases, more energy is provided. This means that the particles move around faster and so there are more successful collisions between enzymes and substrates. The rate of photosynthesis is slow at cool temperatures rising to a maximum as temperature

increases. At higher temperatures photosynthesis is inhibited because the enzymes are denatured and are unable to catalyse the reactions that form the organic compounds.[6] There have been similar experiments to the one which is to be undertaken which investigate temperature, light intensity and carbon dioxide concentration as limiting factors to photosynthesis. These experiments conclude that the **law of limiting factors** controls the rate of photosynthesis. The law is; when a chemical process depends on more than one essential condition being favourable, its rate is limited by that factor which is nearest its minimum value.[5,6]

Plan

Outline method

A piece of the pondweed is placed in an inverted glass funnel within a beaker containing water and sufficient concentration of carbon dioxide so as not to be a limiting factor. This is achieved by adding sodium hydrogen carbonate to the water. This is not carbon dioxide, however, it dissociates to provide carbon dioxide when needed by the plant. Water is added to the sodium hydrogen carbonate instead of vice versa to ensure that there is exactly 250cm^3 of solution. Adding sodium hydrogen carbonate to water would increase the volume above the required amount due to displacement.
The apparatus is set up so that the oxygen produced by it can after a fixed amount of time be drawn up into a capillary tube so the length of the bubble can be measured against a scale on the capillary tube. This scale can be achieved by simply sticking a section of graph paper on to the capillary tube. The temperature of the solution is to stay at 20°C. A lamp is placed up to a heat screen next to the beaker and left to equilibrate. This ensures that the readings taken are accurate for that particular intensity, because the plant has time to adjust to the new environment. The heat screen is put in place to prevent heat being transferred from the lamp to the solution in the beaker thus raising the temperature above the desired 20°C
The bubble produced is removed from the capillary tube. After this, time for five minutes taking a reading of the length of the bubble. Repeat this method but moving the lamp progressively further away, 0, 8, 14, 18, and 20cm measured using a 30cm ruler for accuracy, any larger size would be impractical in the laboratory. This range of distances was chosen for the benefit of plotting the graph. Light intensity is $1/d^2$ therefore by sequentially deceasing the size of the gap between distances; the graph will show a smaller range and hence be easier to plot.
Two replicates are to be undertaken to provide plenty of results for analysis including a statistical test.
To investigate further, repeat entire method including replicates but raise temperature to 30°C using the same piece of elodea so no extraneous variables are introduced.
The temperature range was chosen as 20°C and 30°C as this represents fluctuations in the environmental temperature that may occur naturally. Also, *elodea canadensis* grows well in temperatures above 15°C
This method of collecting the oxygen given off in five minutes in a single bubble was chosen as it provides a reasonable volume to measure whereas another method of measuring oxygen given off in a gas syringe is impractical as such a small volume of gas is given off. Meaning any readings taken have a very large margin of error.

Variables

- Temperature
- Carbon dioxide levels
- Size of pondweed
- Volume of water
- Disease

Temperature will be controlled with the use of water bath, it will be held at a specific temperature with the thermometer being checked every 2-3 minutes.
The levels of carbon dioxide will be controlled by adding 5g sodium hydrogen carbonate to the water thus ensuring that this is not a limiting factor. Sodium hydrogen carbonate dissociate in water to provide carbon dioxide for the plant when needed.
The pondweed used will be ten centimetres long.
There will be exactly 250cm^3 of water in the beaker measured by the beaker.

Risk assessment

This experiment is relatively low risk. The only risk comes when handling the hydrogen carbonate. Therefore, gloves lab coat and goggles are to be worn to prevent any contact with the chemical.
Care is to be taken when handling the pondweed so not to damage it, as it is a living organism.

Apparatus

Capillary tube	lamp
200 cm^3 glass beaker	light bulbs
Small glass funnel	pondweed (*elodea canadensis*)
Rubber tubing	5g sodium hydrogen carbonate
Stopwatch	thermometer
Heat screen	30 cm ruler
Top pan balance	filter paper

Treatment of results

The raw results are to be placed into a table, then shortened down to show the mean results in another table. From this a graph will be plotted, light intensity against rate. The results from both temperatures will be on this graph. Light intensity will be calculated by $1/d^2$ with d being distance of the lamp from the plant. Rate is calculated by volume of bubble in mm divided by time in seconds. The volume of the bubble is calculated by $l\pi r^2$. L is the length of the bubble in mm and r is the radius of the capillary tube.
Separate graphs will be drawn up for each temperature with error bars, these graphs will be length of bubble produced in three hundred seconds against light intensity using the mean result of bubble length. Error bars are calculated by calculating the standard deviation of the raw results, then creating minimum and maximum deviation bars from the mean by adding and subtracting the standard deviation from the mean respectively.
The results will be analysed for correlation using the Spearman's rank statistical test. This test was chosen because the data is non parametric i.e. not normally distributed and the data is quantitative.

Methods

Method

Using a ten cm piece of elodea, the apparatus was set up as in the diagram so that the oxygen given off by the plant accumulated in the tubing to create a single bubble that was measured against a scale when it was drawn up through a capillary tube. This meant placing the pondweed in an inverted glass funnel in a glass beaker to collect all the oxygen produced and funnel it into the rubber tubing connecting to the capillary tube. A syringe was connected to the capillary tube by rubber tubing to enable the bubble to be drawn up through the apparatus into the capillary tube for measurement. The water level was higher than that of the funnel to keep the apparatus airtight. This was then placed into a water bath held at 20°C with a 5% concentration of sodium hydrogen carbonate. These measures controlled the temperature and concentration of carbon dioxide variables. This concentration was achieved by adding 6.25g of sodium hydrogen carbonate to 250cm^3 of water measured in a beaker. The sodium hydrogen carbonate was weighed on a top pan balance correct to two decimal places. A filter paper was placed on the scales, the weight zeroed, and then 6.25g were carefully measured out ensuring that gloves, lab coat and goggles were worn to ensure there was no contact with the chemical. The sodium hydrogen carbonate was then put into a beaker adding water slowly to it until 250cm^3 was reached.

A lamp was then placed up to the water bath with a heat screen put in place between them. The apparatus was then left for five minutes so that the plant could equilibrate. The temperature was taken of the water bath and adjusted if needed so that it remained at the given temperature. The bubble was removed from the capillary tube. The experiment was then timed for five minutes, a reading of the bubble length was taken after the time had elapsed putting the result into a suitable table. During the timed five minutes the temperature of the water bath was closely monitored with any adjustments needed being made.

This method was repeated moving the lamp further away from the plant, 0, 8, 14, 18, and 20 cm

To investigate further, this was all repeated but raising the temperature of the water bath to 30°C, using the same apparatus ensuring exactly the same volume of water was used. The same piece of pondweed was used which was thus the same size and state of health.

Results

Results from experiment to ascertain the extent to which light intensity is a limiting factor on photosynthesis

		Length of bubble produced in 300 seconds (mm)		
	Distance of lamp from plant (cm)	First reading	First repeat	Second repeat
Temperature 20°C	0	23	32	32
	8	27	28	28
	14	23	12	17
	18	20	17	21
	20	12	11	16
Temperature 30°C	0	40	45	39
	8	2	40	46
	14	9	26	20
	18	6	23	10
	20	0	0	2

Analysis

At 20°C the trend shown was one of positive correlation. The exact correlation was 0.86 as obtained from the Spearman's rank statistical test, indicating a strong positive correlation. Meaning that as light intensity was increased the rate of photosynthesis also increased. (Appendix 3)
The graph demonstrates this relationship, (Appendix 5) showing a steady increase from the starting point at 2.5 light intensity through to the final result at 100. This is excluding the anomalous result, the second point on the graph at 3.08 light intensity. The trend shows a sharp increase in rate between 2.5 and 15.6 light intensity. A very large overall increase of 0.038mm^3 per s as compared with the change in rate from 15.6 to 100 light intensity of 0.003mm^3 per s. This much smaller increase in rate although over a very large increase in light intensity indicates that rate of photosynthesis is limited by another factor other than light intensity. It may be that there were insufficient minerals present. The anomalous result at 3.08 light intensity shows a higher rate of photosynthesis than the previous point which, given the correlation does not fit in with the overall pattern of a decreasing rate with decreasing light intensity. There is a significant error bar for this result showing that there is a large margin of error. The error bar overlaps with the error bars of the previous and following results casting doubt over the reliability of the result.
At 30°C the trend is again one of positive correlation. The result from the statistical test is 0.80, a strong correlation. (Appendix 4)
The graphs demonstrate this relationship, (Appendix 6) showing a huge change in rate as light intensity was increased from 1.5mm^3 per s to 108mm^3 per s. The increase is very sharp from the first to the second point the line is nearly vertical. After that point the line continues to increase though not as sharply through the third and fourth points. The increase in rate from the fourth to the final result is 0.032 mm^3 per s which is substantial until is it recognised that this is spread over a change of 15.6 to 100 light intensity. This means that the increase in rate of photosynthesis has slowed down, possibly indicating that another limiting factor has come into play. It may be that there is an insufficient supply of minerals in the water. However, the error bar on the fourth point shows a massive span of 47.72, therefore casting doubt over the reliability of the results for that point and those of the third and fourth as they are contained within it. The reason for the massive span of this error bar is that there is one particular point that is much lower than the others, therefore bringing the mean result down and increasing the standard deviation.

Discussion

With respect to the results obtained from the experiment and the subsequent analysis of those results it is possible to reject the null hypothesis and accept the experimental hypothesis. The results from the Spearman's rank statistical test for both temperature conditions were higher than the critical value at the five per cent level showing that that light intensity does have a significant effect on the rate of photosynthesis which is not due to chance. As light intensity increases so too does the rate of photosynthesis. The actual results obtained from the statistical test were for 20C 0.86>0.514 at the 5% level and for 30C 0.80>0.514 at the 5% level.

Conclusions

The results from the experiment have shown that there is a positive correlation between light intensity and the rate of photosynthesis in *elodea canadensis*. This is in agreement with the experimental hypothesis put forward. As light intensity increases the electrons within the chlorophyll in the plant are receiving more energy and more of them are able to reach the 'excited' state where this energy is used to reduce chemical substances which in turn form energy rich molecules used to form complex organic molecules needed by the plant for survival. The rate of photosynthesis showed an increase as the intensity increased, when investigated at the higher temperature of 30°C the rate increased beyond that reached at 20°C. This demonstrated that raising the temperature of the water increased the rate of photosynthesis as predicted in the experimental hypothesis. Raising the temperature increases the kinetic energy of the particles and thus increases the number of successful collisions between enzyme and substrate molecules enabling the energy rich molecules and complex organic molecules to be formed.

Evaluation

The results from the experiments vary greatly in their range. The average standard deviation reflects this with 3.204 at 20°C and 9.146 at 30°C. (Appendix 2) The average standard deviation for 30°C is nearly three times that of 20°C throwing doubt over the reliability of the readings taken in that experiment. The huge variability present makes the results very unreliable and hence makes it very hard for solid conclusions to be drawn from them. A possible explanation for this variability is that at the higher temperature, the rate was much higher and therefore a larger amount of gas was evolved. Thus it made it harder for accurate readings of the bubble length to be taken. A slight mistake in the measuring of the bubble length would have had a detrimental effect on the rest of the experiment leading to subsequent calculations of volume and rate being incorrect. A Possible solution to this problem would be to use a smaller scale on the capillary tube

against which the bubble length was measured. This would have made is easier to accurately take the length of the bubble evolved during the given time limit. However, this solution does not combat another problem that was found. This was that on occasion the gas evolved was not a single measurable mass but a stream of tiny bubbles. This lead to estimations being made by the experimenter as to the possible volume of these tiny bubbles. A way of overcoming this problem would have been to add a little detergent to the solution. This would have made it easier for the bubbles to escape into the capillary tube and merge into a single bubble.

For the experiment at 20°C, the results obtained showed much lower variability making conclusions drawn much more reliable. The lower variability was due to the fact that the rate of photosynthesis was much lower and hence a smaller volume of gas was evolved making it easier to measure the length of the bubble in the capillary tube. The average standard deviation was much lower compared with at 30°C. Individual standard deviation for each light intensity showed quite low variability showing reliability in the method. The method used for both experiments was the same however; the results obtained from the two conditions vary greatly in their reliability throwing doubt over the design. It would seem that this method was a very good way of obtaining reliable results when the rate of photosynthesis was lower, as the rate increased it became much more difficult to obtain the same standard of reliability and validity of the results. A solution to this problem would be to take more readings of the bubble length
at the higher temperature. Instead of taking one single reading at three hundred seconds, take one reading at one hundred and fifty seconds and another at three hundred seconds adding them together to gain the final result. This method would provide smaller volumes of gas to measure which would be more manageable than one very large bubble.

A limitation of this experiment was that the range of light intensities was not large enough. A range of five is very small compared with for example fifteen. A range of that size would show more accurately the relationship between light intensity and rate of photosynthesis.

To obtain much more reliable and valid results from this experiment it would be necessary to undertake many more replicates therefore making the mean result much more reliable and possibly ironing out any anomalous results which would greatly affect the mean. Also to validate the conclusions drawn from this experiment it would have to be repeated following the same design and the same results obtained

Bibliography

1. www.ecy.wa.gov/programs/wq/plants/aqua003.

2. Abbey College Manchester, AS and A2 Biology notes, Units 3,5.

3. E. Rabinowitch and Govindjee (1969). *Photosynthesis*. pp1-3. John Wiley and Sons Inc.

4. David W. Lawlor. (1987). *Photosynthesis*. pp1-4, 20-27, 260-276. Second edition, Longman Scientific and Technical.

5. Hall and Rao. (1994). *Photosynthesis*. pp24-26. Fifth edition, Cambridge University Press.

6. MBV Roberts. (1983). *Biology, a functional approach*. pp144-148. Third edition, Thomas Nelson and Sons.

Appendix 1

Conversion of data to calculate rate of reaction

	Distance of lamp away from plant (cm)	Mean bubble length produced in 300 seconds (mm)	Mean volume of bubble produced in 300 seconds (mm^3)	Rate of photosynthesis x 10^3 (mm^3 per s)
Temperature 20°C	0	29.0	22.76	75.92
	8	27.6	21.68	72.26
	14	17.3	13.59	45.29
	18	19.3	15.16	50.53
	20	13.0	10.21	34.03
Temperature 30°C	0	41.3	32.44	108.12
	8	29.3	23.01	76.71
	14	18.3	14.37	49.91
	18	13.0	10.21	34.03
	20	0.6	0.47	1.57

Appendix 2

Table containing standard deviation to produce error bars on corresponding graphs

		Length of bubble produced in 300 seconds (mm)				
	Distance of lamp from plant (cm)	First reading	First repeat	Second repeat	Mean reading	Standard deviation
Temperature 20°C	0	23	32	32	29.00	5.20
	8	27	28	28	27.60	0.58
	14	23	12	17	17.30	5.51
	18	20	17	21	19.30	2.08
	20	12	11	16	13.00	2.65
Temperature 30°C	0	40	45	39	41.30	3.21
	8	2	40	46	29.30	23.86
	14	9	26	20	18.30	8.62
	18	6	23	10	13.00	8.88
	20	0	0	2	0.60	1.16

Appendix 3

Analysis of data for Spearman's rank statistical test

Temperature 20°C

Distance of lamp from plant (cm)	Rank	Length of bubble produced in 300 seconds (mm)	Rank	D	D^2
0	3	32	1.5	1.5	2.25
0	3	32	1.5	1.5	2.25
0	3	23	6.5	-3.5	12.25
8	5	28	3.5	1.5	2.25
8	5	28	3.5	1.5	2.25
8	5	27	5	0.0	0.00
14	8	23	6.5	1.5	2.25
14	8	17	10.5	-2.5	6.25
14	8	12	13.5	-5.5	30.25
18	11	21	8	3.0	9.00
18	11	20	9	2.0	4.00
18	11	17	10.5	0.5	0.25
20	14	16	12	2.0	4.00
20	14	12	13.5	0.5	0.25
20	14	11	15	-1.0	1.00

$\sum d^2 = 78.5$ $\qquad r_s = 1 - \frac{6\sum d^2}{n(n^2-1)}$

$r_s = 1 - \frac{6(178.5)}{15(224)}$

$r_s = 0.859821429$ $\qquad 0.86 > 0.514$

0.514 is the critical value at the five per cent level for thirteen degrees of freedom

Appendix 4

Analysis of data for Spearman's rank statistical test

Temperature 30°C

Distance of lamp from plant (cm)	Rank	Length of bubble produced in 300 seconds (mm)	Rank	D	D^2
0	3	45	2.0	1.0	1.00
0	3	40	3.5	-0.5	0.25
0	3	39	5.0	-2.0	4.00

8	5	46	1.0	-4.0	16.00
8	5	40	3.5	-1.5	2.25
8	5	2	12.5	-7.5	56.25
14	8	26	6.0	-2.0	4.00
14	8	20	8.0	0.0	0.00
14	8	9	10.0	-2.0	4.00
18	11	23	7.0	4.0	16.00
18	11	10	0.0	2.0	4.00
18	11	6	11.0	0.0	0.00
20	14	2	12.5	1.5	2.25
20	14	0	14.5	0.5	0.25
20	14	0	14.5	0.5	0.25

$\sum d^2 = 110.5$ $\qquad r_s = 1 - \frac{6\sum d^2}{n(n^2-1)}$

$r_s = 1 - \frac{6(110.5)}{15(224)}$

$r_s = 0.803678571$ $\qquad 0.80 > 0.514$

0.514 is the critical value at the five per cent level for thirteen degrees of freedom