Photosynthesis using seaweed - opportunities for quantitative studies

Background

In a previous article [1] we suggested that seaweeds might offer opportunities for studying a range of parameters in relation to the rate of photosynthesis. We described how one might measure absorbance changes in hydrogencarbonate indicator solutions in the presence of knotted wrack (*Ascophyllum nodosum*) as a measure of photosynthesis activity. We have described other uses of this technique on previous occasions [2-3].

We describe here an updated version of our previous work [1] and offer suggestions for how the technique might be extended to offer opportunities for quantitative studies.

Whilst we anticipate that the methods shown here will be applicable for National 5 Environmental Science, we anticipate that investigations in National 5 Biology using the techniques outlined would also be possible.

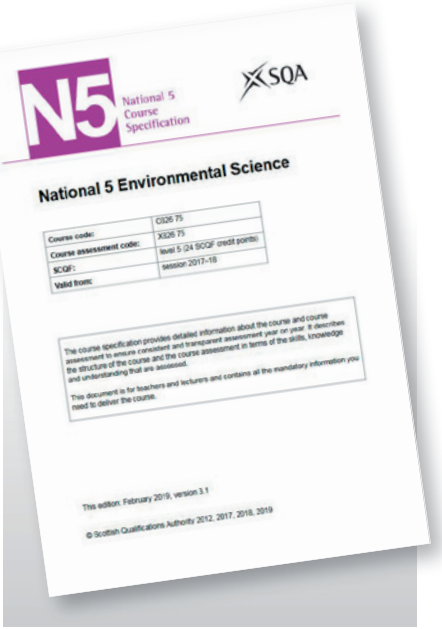

Figure 1 - Image of knotted wrack [5].

Method

1) Knotted wrack (Figure 1) was collected from a local beach and stored in sea water until use; the experiments described here were performed on the day of sample collection although our previous observations [1] suggest that samples, kept moist, can be used for at least a week after collection.

2) Hydrogencarbonate indicator can be prepared [4] or purchased commercially.

3) Twenty-five samples of knotted wrack (approximately rectangular in shape, avoiding the air bladders and fruiting bodies) were cut and the mass of each sample adjusted to be 0.3 ± 0.02 g.

   Because there is a slight time delay (20-30 min) between taking the samples and using them in the experiments which follow they were stored in seawater in the dark until use.

4) A sample of knotted wrack was placed into an empty Bijou bottle and the bottle was filled with hydrogencarbonate indicator (approximately 7 cm^3, pH = 7.2).

5) To test the effect of light intensity on the rate of photosynthesis the Bijou bottles were treated as follows:
   - Five bottles were exposed to the full lamp output.
   - Five bottles were covered in black paper to exclude all light.
   - Neutral density filters that allowed 71% or 50% or 25% of light to be transmitted [7] were used to cover the remaining 15 bottles (5 bottles for each light intensity).

6) All 25 bottles were laid on their side and placed under a standard fluorescent tube (Figure 2) and illuminated for a period of 60 min.

7) After illumination the samples of knotted wrack were removed from the Bijou bottles and the absorbances of the solutions were measured using a Mystrica colorimeter [6] using the green diode (distilled water was used as the blank in these colorimetric readings). Measured absorbance data are shown in Table 1. >>

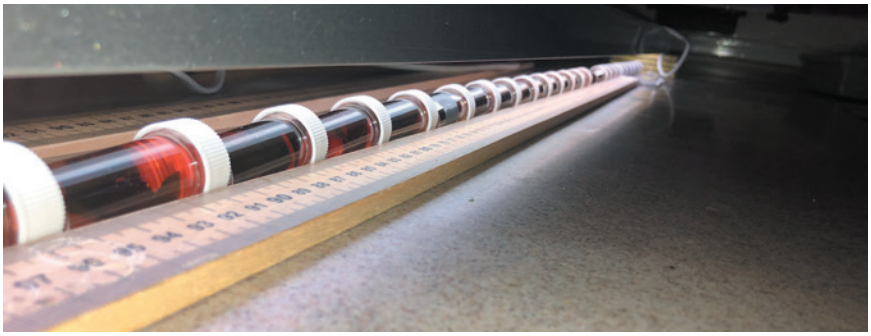

Figure 2 - An experimental set-up allowing for measurement of the effects of light intensity on the rate of photosynthesis. Bijou bottles containing samples of knotted wrack are exposed to light from a fluorescent tube; light intensity is altered by the addition of a suitable filter.

Results and discussion

The data from Table 1 are shown graphically in Figure 3. It is important to recognise that an increase in absorbance represents a decrease in the concentration of CO_2 present in solution; the converse is also true. So, as light intensity is increased, we see that photosynthesis is the dominant process. One of the samples that had been irradiated with no filter (i.e. 100% value in Table 1) was returned to a Bijou bottle together with the hydrogencarbonate indicator in which it had been irradiated and left in the dark at room temperature. After a period of 1.5 hours the absorbance of the indicator had returned to a value of 0.40 (from a starting value of 1.08) indicating that respiration had taken place (we might assume that absorbance would continue to fall if samples were left for longer periods although we have not tested this hypothesis). The sample was once again illuminated and an increase in absorbance from 0.40 to 0.90 noted after 45 min. Whilst the observation that respiration in the dark dominates is not surprising, the fact that photosynthesis can be 're-started' makes a useful demonstration.

The observed relationship as light intensity increases is interesting and offers scope for further investigative work for example a detailed analysis of the compensation point, and the effect of temperature on photosynthetic rates could both be studied.

With one exception (Run 1, 100% light transmission, A = 0.8), the consistency of results, as shown in Table 1, implies that the system should allow for quantitative studies to be undertaken. To firm up our thoughts on this we repeated the experiment but used fresh samples of knotted wrack collected some two weeks later. The corrected mean absorbance data for this second batch of knotted wrack are shown in Table 2. For comparison we have copied the corrected mean absorbance data from Table 1. >>

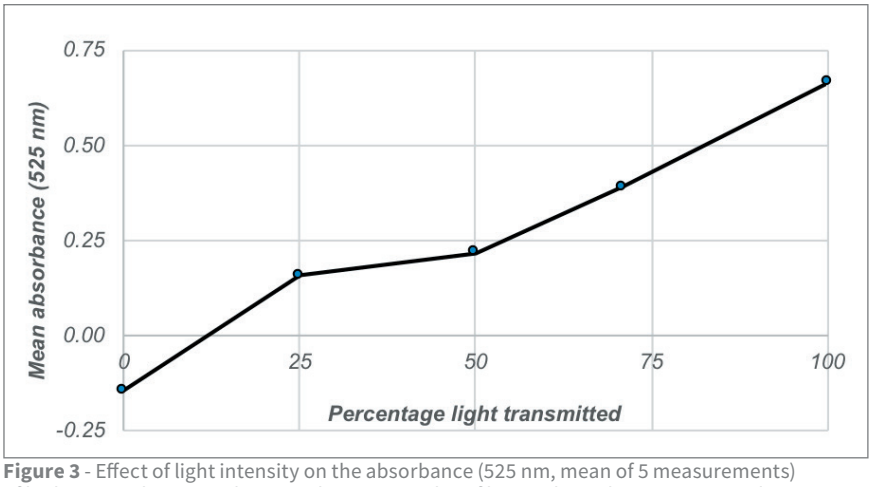

Figure 3 - Effect of light intensity on the absorbance (525 nm, mean of 5 measurements) of hydrogencarbonate indicator solution. Samples of knotted wrack were immersed in hydrogencarbonate indicator solution and neutral density filters used to reduce the intensity of light incident on the sample. Further experimental details are in the text and the legend to Table 1.

	Absorbance (525 nm) of hydrogencarbonate indicator						
Light Intensity (%)	Run 1	Run 2	Run 3	Run 4	Run 5	Mean of runs 1-5	Corrected mean absorbance
0.0	0.28	0.28	0.29	0.29	0.29	0.29	-0.14
25	0.59	0.57	0.58	0.60	0.59	0.59	0.16
50	0.63	0.65	0.64	0.65	0.66	0.65	0.22
71	0.82	0.85	0.85	0.83	0.87	0.84	0.39
100	0.8*	1.13	1.06	1.08	1.11	1.10	0.67

Table 1 - The effect of light intensity on the rate of photosynthesis in samples of knotted wrack. Data shown are the absorbance (wavelength of observation ~ 525 nm) values of hydrogencarbonate indicator solutions after illumination (standard fluorescent tube) for a period of 60 min. Other experimental details are in the text. The absorbance of a sample of hydrogencarbonate indicator in the absence of added knotted wrack was measured to be 0.43 and this value has been subtracted from the mean values to yield the absorbance value in the column named 'Corrected mean absorbance'. The data point marked*, deemed to be an 'outlier,' has been excluded from the calculation of mean absorbance.

The mean absorbance values for the data in Table 2 are shown in Figure 4. We conclude that comparisons of photosynthetic rates between samples collected on separate days are entirely feasible.

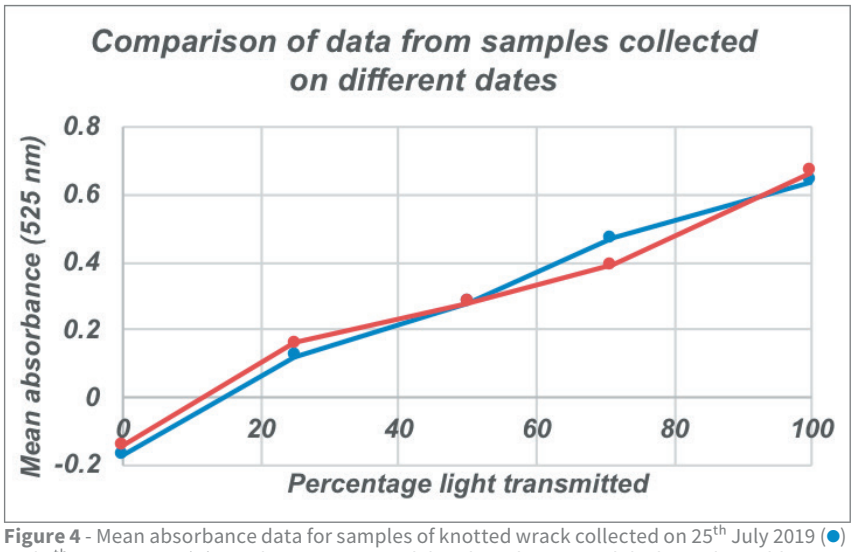

Figure 4 - Mean absorbance data for samples of knotted wrack collected on 25th July 2019 (●) and 7th August 2019 (●). Further experimental details in the text and the legend to Table 2.

Acknowledgements

Several years ago, Liz McMillan (formerly Head of Science at Stromness Academy, Orkney) flagged up the possibility of using seaweeds for experiments with the 'hydrogencarbonate indicator system'. It has taken us some time to act on Liz's suggestion…

Much of the experimental data reported here was generated by Thomas Beaumont from Linlithgow Academy during a period of work experience in SSERC in July/August 2019.

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	Absorbance (525 nm) of hydrogencarbonate indicator							
Light Intensity (%)	Run 1	Run 2	Run 3	Run 4	Run 5	Mean of runs 1-5	Corrected mean absorbance	Corrected mean absorbance (from Table 1)
0.0	0.23	0.25	0.21	0.21	0.28	0.24	-0.17	-0.14
25	0.55	0.53	0.51	0.54	0.52	0.53	0.12	0.16
50	0.72	0.69	0.68	0.67	n.d.	0.69	0.28	0.28
71	0.91	0.87	0.93	0.80	0.90	0.88	0.47	0.39
100	1.04	1.06	n.d.	1.08	1.03	1.05	0.64	0.67

Table 2 - The effect of light intensity on the rate of photosynthesis in samples of knotted wrack. Data shown are the absorbance (wavelength of observation ~ 525 nm) values of hydrogencarbonate indicator solutions after illumination (standard fluorescent tube) for a period of 60 min. Other experimental details in the text. The absorbance of a sample of hydrogencarbonate indicator in the absence of added knotted wrack was measured to be 0.41 and this value has been subtracted from the mean values to yield the absorbance value in the column named 'Corrected mean absorbance'.

References

[1] SSERC (2018), Photosynthesis using seaweed - opportunities for investigative work in Environmental Science? *SSERC Bulletin*, **263**, 4-5.

[2] Andrews, K., Beaumont, P.C. and Crawford, K. (2015), Measurement of limiting factors in photosynthesis. *School Science Review*, **96** (356) 31-35.

[3] Andrews, K. and Beaumont P.C. (2017) National 5 Biology assignment packs. *SSERC Bulletin*, **261**, 10-12.

[4] To make a concentrated stock of indicator (10 times the concentration required for the experiments) use the following protocol:
- dissolve cresol red (0.1 g) and thymol blue (0.2 g) in ethanol (IDA, 20 cm^3).
- dissolve sodium hydrogencarbonate (0.85 g) in freshly boiled distilled water (200 cm^3).
- add the solution of cresol red and thymol to the hydrogencarbonate solution and make to 1 dm^3 with freshly boiled distilled water.
- for use in experiments dilute the stock indicator prepared above and adjust the pH to approximately 7.4.
- aerate this diluted indicator prior to us.

[5] Field Studies Council (2008), Knotted or Egg Wrack (Ascophylum nodosum). Image taken from https://www.theseashore.org.uk/theseashore/SpeciesPages/Knotted%20or%20Egg%20Wrack.jpg.html (accessed 29th July 2019).

[6] Lee Filters (2019), Neutral density (filters). Information available at http://www.leefilters.com/lighting/technical-list.html (accessed 21st July 2019).

[7] Mystrica: Practical solutions (2019). Information available at http://www.mystrica.com/Colorimeter (accessed 21st July 2019).