Can you investigate the effects of changing climatological conditions on pond microflora using flow cytometry?

Moulton School and Science College

University of Cambridge and Cambridge Institute for Medical Research

Overview

Our project is investigating a pond on our school site which has been confirmed to be home to a population of great crested newts. We are seeking to discover what life lies in our pond and why these conditions are favourable to great crested newts.

Fig 1 – A great crested newt an ecologist caught using a bottle trap.

Aims

- Map out the ecosystem of the pond to establish the links between the flora and fauna.
- Use techniques such as microscopy and flow cytometry to identify microscopic life and microorganisms.
- Use the data to support the great crested newt population and encourage conditions which will help them thrive.

Background information

To identify microorganisms in our pond we have been learning to use flow cytometry. This shines a laser through cells and reflects, or scatters, the light based upon what is inside the cell. If the cell is complex the laser will scatter into a wide beam, the less complex the more narrow the beam – this is known as side scatter. Similarly, large cells will cause the beam to widen vertically whilst small cells will not cause this – this is known as forward scatter. Side scatter can give an idea about the complexity of the cell and forward scatter indicates size.

Fig 2 – Dr Reiner Schulte demonstrating how to run flow cytometer samples.

Methodology

1) Desk research has been undertaken to see what research has been published in journals such as The Royal Society for Cytology. This has demonstrated that flow cytometry is a viable and suitable way to analyse microorganisms and informed some of our procedures.
2) We worked with ecologists to identify animal and plant life around the pond. Following this, we then conducted microscopy of water samples from the pond to begin to identify smaller organisms.
3) Flow cytometry of pond water has been conducted, and then centrifuging samples to isolate biological matter. We have also run control samples through the machine to give us a point of comparison (grown chlorella vs pond chlorella).

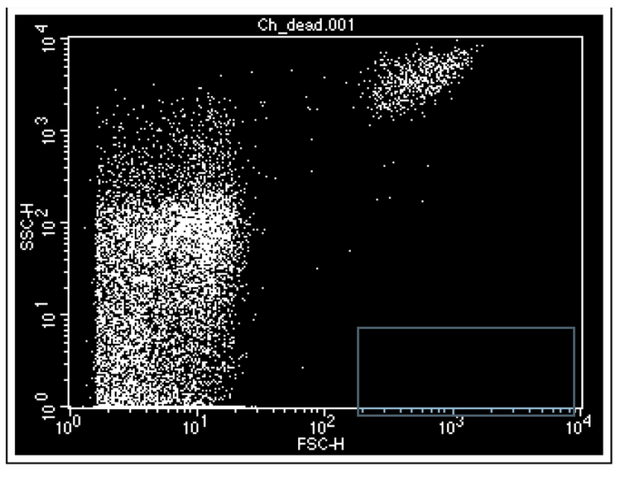

Fig 3/4 – Flow cytometer results showing size, complexity and response to a DNA dye.

Results or Predicted results

We have had success in mapping out a significant part of the ecosystem for the pond. As our knowledge of flow cytometry grows, we will be able to interpret the data more effectively and stain some of the samples to begin to identify what DNA is present. We will also implement PCR testing techniques to multiply any DNA present in the pond so it is easier for us to identify.

Conclusion

We have already confirmed that great crested newts are present in the pond, and we have found some interesting ways in which they are using litter that is present as protection for their eggs. The conditions of our pond so far do agree with what our literature review stated, and as a result we suspect that the newts may also be present in the grounds of the nearby college.

Evaluation or next steps

Our plan is to monitor changes throughout the seasons and on an annual basis. We are preparing to use PCR to get a more informed idea about the DNA present in the pond. We are also hoping to obtain a basic weather station to place next to the pond so our data can include temperature, humidity, light intensity, air quality etc to increase our data about the conditions in, and around the pond.

FUNDED BY A PARTNERSHIP GRANT FROM THE ROYAL SOCIETY

MOULTON SCHOOL & SCIENCE COLLEGE

UNIVERSITY OF CAMBRIDGE

CIMR Molecules Mechanisms Medicine