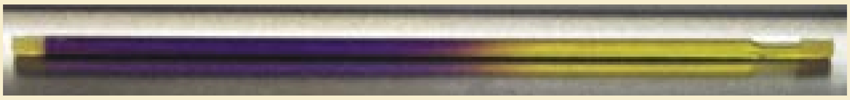

*Figure 2* - *Suspension of Scenedesmus quadricauda in hydrogencarbonate indicator.*

they might observe. Provided that the cylinder is removed carefully and mixing is kept at a minimum then the tube will look similar to the one shown in Figure 2. The algae were originally placed in indicator at pH 7.6 prior to illumination. The left-hand portion of the tube was exposed to the full beam while the right-hand portion was covered with black paper during illumination. Similar effects can be observed with immobilised algae which can be prepared using standard methods [3, 4] and this is shown in Figure 3. In this case approximately 150 immobilised beads, suspended in hydrogencarbonate indicator (pH 7.6), were distributed along the length of the tube and illuminated for some 3 hours under a light bank.

*Figure 3* - *Immobilised Scenedesmus quadricauda in hydrogencarbonate indicator.*

The immobilised algae were originally placed in indicator at pH 7.6 prior to illumination. The left-hand portion of the tube was exposed to the full beam while the right-hand portion was covered with black paper during illumination.

An interesting experiment is to take the tubes in Figures 2 and 3 and illuminate them but in this case cover the purple region with the black paper during the illumination. In both cases a reversal of the colour changes is observed i.e. purple → yellow/orange and yellow/orange → purple.

We believe this to be a simple, effective and above all 'nice' system for demonstrating photosynthesis and respiration in aquatic plants.


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# Nature's Neons

*Figure 1* - *Female glow-worm (Lampyris noctiluca). Image courtesy of David Savory/UK Glow-Worm Survey [9].*

## Background

Now that the Arrangements Documents for the Revised and *CfE* Highers in Biology [1, 2] and Human Biology [3, 4] have been published the Biology Team within SSERC is preparing to publish a series of protocols to support practical work contained therein. Many of these protocols will appear on the SSERC website [5] or in this Bulletin (see for example [6]). The Arrangements Documents [1-4] all include a suggestion that students might explore *'Experiments on ATP dependent reactions, e.g. luciferase, luminescent reactions'*.

The luciferin/luciferase reaction is probably one of the most alluring in nature and is characteristic of bioluminescent organisms; bioluminescence may be conveniently defined [7] as *'the production and emission of light by a living organism'*. Bioluminescence is not an evolutionarily conserved function; in the different groups of organisms capable of undergoing the process the genes, proteins and substrates involved are mostly unrelated and probably originated and evolved independently [8]. It is worth pointing out that although the substrates and enzymes involved from one species to another are often unrelated the terms luciferin and luciferase are still used to describe key reactants in the process. So, firefly luciferin is chemically different from luciferin found in bacteria; possibilities for confusion abound!

An amazing diversity of organisms is capable of emitting light and these include bacteria, fungi, crustaceans, molluscs, fishes and insects. The specific biochemistries of bioluminescence vary from one species to another but all involve an enzyme-mediated reaction between molecular oxygen and an organic substrate leading to the production of light; no external light source is required.

## Functions of Bioluminescence

Bioluminescence is a good example of a function that is not metabolically essential but one which confers an advantage on the individual.

Many organisms utilise their ability to bioluminesce in order to attract a partner. So, for example, a female glow worm of the species *Lampyris noctiluca* (Figure 1) glows in an attempt to attract a sexual partner - males of this particular species do not bioluminesce. Thus, in this

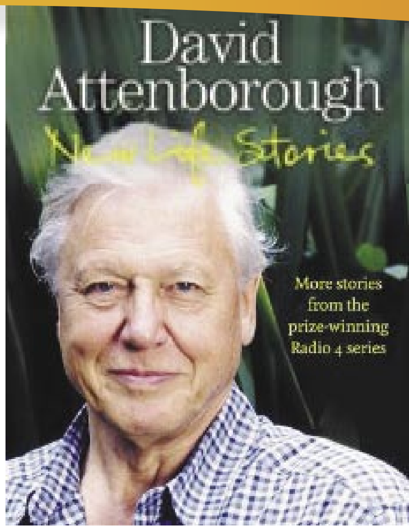



***Figure 3*** *- A recent anthology by David Attenborough.*

case the functional importance of bioluminescence is based largely on the need to be detected by another organism.

Important defensive strategies include the ability to frighten, serve as a decoy, to provide camouflage and to aid in vision.

## Firefly Bioluminescence

Probably the best know example of an organism which can bioluminesce is the firefly (Figure 2). The variety of fireflies with their different habitats and behaviours is impressive. The major function of light emission in fireflies is for communication during courtship, in which one sex emits a flash as a signal to which the other responds usually in a species-specific pattern. Typically one sex (often the female) is stationary and emits light or a flashing signal and the other sex is attracted to it. The time delay between signals is an important characteristic of a species. For example, at 24° C the female of the eastern US firefly species, *Photinus ignitus*, waits for 3 s before answering the male signal; the time delay increases to about 9 s at 13° C. Timing is crucial - a firefly with a poor sense of timing will be doomed to a life of celibacy!

In a recent publication (Figure 3 and [11]) David Attenborough describes one of the most fascinating displays of cooperation amongst fireflies. In the mangrove swamps of southeast Asia [12] just after dusk male fireflies sitting on the leaves of so-called display trees will start to flash in an attempt to attract a female for sex. After several minutes the males coordinate their activities in such a way that they all flash at the same moment in time followed by a pause before the process is repeated. The overall effect is of a tree which 'lights up' at regular intervals. Because of the large numbers of fireflies present at any one time the flashes of light become so strong that the trees are used by local inhabitants as navigational aids. Written observations of the firefly tree phenomenon are not new - indeed in 1949 Somerset Maugham wrote [13] *'The fireflies give the shrubs the look of a Christmas tree all lit up with tiny candles. They sparkle softly; the radiance of a soul at peace.'*

***Figure 2*** *- Photinus pyralis (taken from [10]).*

*Figure 4A - mating fireflies (Photuris hebes) hang in the shelter of a leaf.*
*Figure 4B - a female (Photuris versicolor) dismembering an alien male firefly. Images taken from [16].*

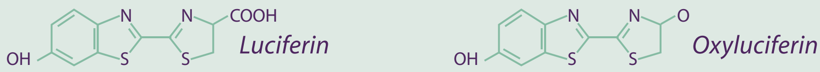
Video clips of the phenomenon are available (see for example [14]) but few are as impressive as that first shown in the Wildlife on One TV programme entitled *'Nature's Neons'* [15]. Sadly copies of the programme are difficult to locate.

The images in Figure 4 demonstrate potential outcomes arising from the light displays [16]. In the first case (Figure 4A), male and female fireflies from the species *Photuris hebes* have exchanged signals and are mating. Figure 4B shows the results when food rather than sex is the goal. A female firefly *(Photuris versicolor)* has cracked the code of a male firefly from an alien species and captured him. Despite his imminent demise, the male firefly in Figure 4B is still signaling in the hope that he might mate with the female.

## Biochemistry

The firefly system was the first to be well-characterised. The series of reactions (catalysed by the enzyme luciferase) can be summarised in the reaction sequence shown.

$$\text{Luciferin} + \text{ATP} + O_2 \longrightarrow \text{Oxyluciferin} + \text{AMP} + PP_1 + CO_2 + \text{Light}$$




The mechanism is not yet fully understood but it appears that luciferase amino acid residues promote the addition of molecular oxygen to luciferin, which is then transformed to an electronic excited state oxyluciferin molecule and carbon dioxide. Visible light emission results from the rapid loss of energy of the excited state oxyluciferin molecule via a fluorescence pathway. The very high quantum yield for this process (in alkaline solution, nearly each reacted luciferin molecule emits a photon) reflects not only efficient catalytic machinery, but also a highly favorable environment for the radiative decay of an electronic excited state.

It should be noted that ATP is not a reactant in the light-generating reactions in all organisms (see for example [17-19]).

## School Practical Work

As noted previously the Arrangements Documents [1-4] for the revised and *CfE* Highers in Biology and Human Biology suggest that students might explore luminescent reactions. In our experience the costs associated with such experiments will be prohibitive for most school budgets. We have recently used the Firefly Bioluminescence BioKit (see Figure 5) from Carolina Biological Supply www.carolina.com) and sold in the UK by Blades Biological

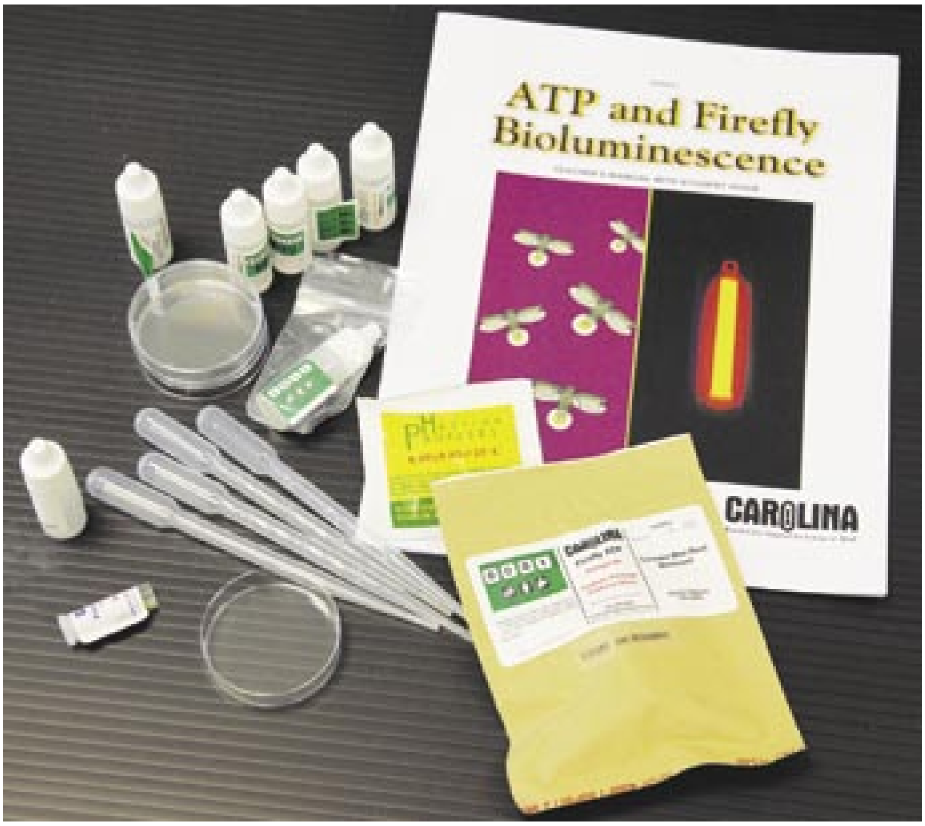



*Figure 5 - Contents of the Firefly Bioluminescence BioKit from Carolina Biological Supply.*

*Figure 6* - *Firefly tails glowing in Petri dishes after addition of ATP and buffer. (Image supplied by Ian Birrell).*

www.blades-bio.co.uk, telephone 01342 850242) although the kit is not listed on the latter's website. The current cost via Blades is about £130 including VAT and carriage.

In our opinion some aspects of the kit worked really well. The manufacturers claim that the kit contains sufficient materials for 30 students. Detailed and easy-to-follow instructions are included with the kit and we have adapted those to produce 3 protocols which will shortly be available on the SSERC website [5]. In terms of laboratory facilities we would emphasise 2 things:

- to make observations effectively requires a room which can be efficiently blacked out
- ensure that students' eyes are well-adapted to dark conditions at the start of any given experiment since changes in light intensity can be subtle.

We trialled the protocols at a recent Summer School for Biology teachers (Figure 6) and overwhelmingly the participants thought the experiments were 'very useful' although the cost of kits is an issue for most schools. In due course we will place additional support materials on the SSERC website although we recognise that video materials are a poor substitute for the 'wow' factor which students will experience when dried firefly tails light up after addition of ATP.

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[5] The SSERC website is available at www.sserc.org.uk. Please note that to access all resources on the website you will need to register and be provided with a log-on ID and password.

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## Additional Resources

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