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Sensitivity of mayfly nymphs to red light: implications for behavioural ecology

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SUMMARY

1. Visual sensitivity of the mayflies *Stenacron interpunctatum* (Say) and *Stenonema vicarium* (Walker) to red (650 nm) and infra-red (950 nm) light was tested using a behavioural assay. Nymphs were placed in a runway and sequentially exposed to green light, red light, infra-red light and no light (control) at one end of the runway. The distance run away from the light, and the number of alarm reactions to the light were recorded.
2. Both species reacted strongly to both red and green light, running significantly greater distances and reacting more frequently to these wavelengths of light than to either infra-red light or the control.
3. These results show that unobtrusive observations of mayfly nocturnal behaviour should be made using infra-red, and not red light, for illumination. Previous studies of aquatic insect nocturnal behaviour may have produced biased results if red light was used.

Introduction

Adult insects are generally insensitive to the red end of the visible spectrum (Menzel, 1979), so red light has often been used as illumination for nocturnal ecological studies (e.g. Madsen, 1968; Corkum, 1978; Kovalak, 1979). Use of red light in studies of aquatic insects has continued despite suspicion of red sensitivity (Allan, Flecker & McClintock, 1986; Rader & Ward, 1990). If aquatic insects can see red, researchers using red light may be getting spurious results.

Insect vision in stream habitats is important because light is the primary exogenous cue affecting behaviour of many species (Wodsedalek, 1911; Lyman, 1945; Harker, 1953; Elliott, 1968; Bishop, 1969; Kovalak, 1979; Bailey, 1981). Many aquatic insects remain on the bottom of stones during the day to reduce risk from visual fish predators (Allan, 1978), moving on to the top of stones only at dusk. The vision of aquatic animals is affected by the differential absorption of wavelengths of light by water (McFarland, 1986; Lythgoe, 1988). Although various fishes are known to have visual pigments that maximize sensitivity to ambient environmental light (Levine & MacNichol, 1979; Muntz & Mouat, 1984; Heinermann & Ali, 1985), little is known of the vision of immature aquatic insects. Although it can be assumed that the vision of aquatic insects is matched to their photic environment, which in organically enriched freshwaters is shifted towards the red (James & Birge, 1938; Schindler, 1971; Spence, Campbell & Chrystal, 1971; Muntz & Mouat, 1984; Howard-Williams & Vincent, 1985; Reimchen, 1989), this idea has not been tested.

This is a report on a behavioural study of the sensitivity of mayfly nymphs to red (650 nm) and infra-red (950 nm) light, the results being discussed in terms of experimental methodology and photic adaptation. Nymphs of two species of heptageniid mayflies were used as heptageniids are found throughout North America, and are often among the more abundant insects in streams in the eastern half of the continent (Bednarik & McCafferty, 1979). They are crepuscular or nocturnal in activity (Casey, 1987), and live on the sides and bottoms of rocks. The species used, *Stenacron interpunctatum* (Say) and *Stenonema vicarium* (Walker), were ideal for a behavioural assay of spectral sensitivity because of their strong negative phototaxis (Wodsedalek, 1911; Lyman, 1945).

Materials and Methods

Nymphs of both species were collected from Reesor Creek (43°56'N, 79°12'W), a fourth-order stream, and the Credit River (43°38'N, 79°45'W), a sixth-order river, both near Toronto in southern Ontario, Canada. Animals were collected on 19 April 1991, 10 days before the start of the experiment, and were held in an environmental room at 10°C, under a 12L:12D light regime. Between trials, nymphs were kept individually in Petri dishes with dechlorinated water conditioned in aquaria containing rocks from the creek. A piece of conditioned leaf from the river provided food and substrate. Experiments were conducted in the same environmental room.

To test the hypothesis that these mayflies see red, but not infra-red light, they were exposed to green, red, infra-red, and no light (control), and their responses measured. Green light was used because most insects are known to be sensitive to this part of the spectrum (Menzel, 1979), and therefore a positive response by the animals would indicate that the experimental apparatus worked properly. A behavioural assay was used because it avoids the controversy over which physiological technique best assesses spectral sensitivity (Bernard, 1979; Paul, Steiner & Gemperlein, 1986).

To produce light of the correct wavelength, visible light from a 30-W tungsten incandescent source was made into a parallel beam using apertures and lenses and focused on to a 1-cm diameter area at the front of the experimental chamber (Fig. 1). Band pass colour filters (Melles Griot, Irvine, CA, U.S.A.) provided light at 550 nm (half power bandwidth = 40 nm) for green and 650 nm (half power bandwidth = 34 nm) for red light. Intensity at the front of the chamber was adjusted to 0.95 mW using a variable attenuator and power meter (Newport Corp., Fountain Valley, CA, U.S.A.). Intensity decreased over the length of the experimental chamber because of absorbance of light by the water. Infra-red radiation was produced using an array of diodes emitting at 950 nm (full bandwidth = 50 nm), situated in place of the second lens. Baffles were placed between lenses, and a cardboard box enclosed the entire set of lenses to prevent any stray light from reaching the chamber. The experimental chamber (20 × 2 × 2 cm) was divided into an anterior 'waiting cell' and a posterior 'runway' by a sliding door. The chamber was made of black Plexiglas, except for the inside bottom, which was white, and the anterior end, which was clear to receive light. A 1-cm increment scale was drawn on flanges which extended perpendicularly along the outside of the chamber to enable distances run by nymphs to be recorded with a video camera.

Freshly conditioned water was placed in the chamber to a depth of about 6 mm for each trial. Before each trial a nymph was placed in the 'waiting cell' for 1 min to acclimate. The light source was then turned on and the sliding door raised simultaneously. Nymphs could reduce the light intensity they experienced by moving down the length of the runway. Nymphs were allowed to react for 1 min, then the door was closed and the runway illuminated with infra-red light. The distance travelled by the animal and any behaviours were recorded by the video camera, which was sensitive to green, red and infra-red light. For the control, the power source was turned on and the door of the chamber was raised, but no light source was connected.

Each animal ($n = 24$ for each species) was exposed to all light conditions. Twelve nymphs of each species were randomly assigned to a testing sequence. The species to be tested first was assigned randomly, and thereafter the species alternated. One wavelength was tested per night, in the sequence 550, 650, 950 nm and no light (control). This process was repeated with a second set of twelve nymphs of each species. All trials were run with the room in darkness at night to coincide with endogenous behavioural rhythms.

Two responses to light were analysed. Distance run was the net distance travelled by the animal away from the light source at the end of the 1-min trial, regardless of mode of locomotion (crawling or swimming) or of doubling back towards the light source. Because each animal was tested at each wavelength, repeated measures analysis of variance were used to test the hypothesis that wavelength of light had no effect on the distance run by each animal. To test for differences between distance run in red light *v* control and in infra-red *v* control, pre-planned orthogonal contrasts (Sokal & Rohlf, 1981) were used. The three contrasts used were (green and red) *v* (infra-red and control), green *v* red, and infra-red *v* control. Because it was known a priori that animals would run further in the (green and red) treatments than the (infra-red and control) treatments, a 1-tailed test was used for this comparison. A 2-tailed test was used for the other two comparisons.

The second response was the action of pulling the

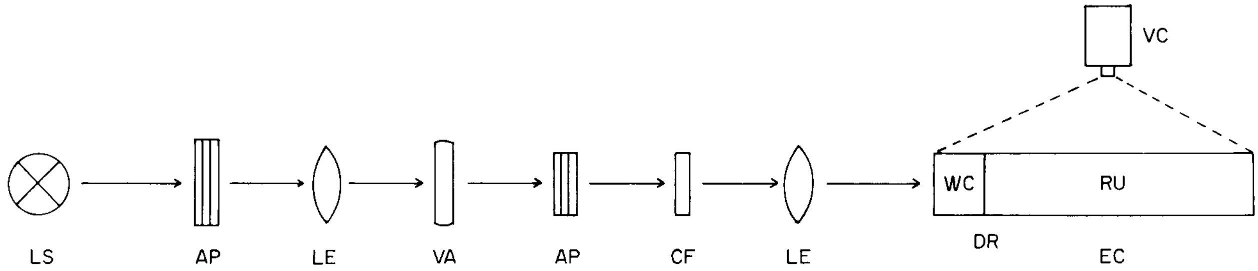

Fig. 1 Diagrammatic representation of the apparatus showing the light path through the light source (LS), apertures (AP), lenses (LE), variable attenuator (VA), and colour filter (CF) before it reached the experimental chamber (EC). The chamber contained a door (DR) separating the waiting cell (WC) from the runway (RU). Dotted lines indicate the field of view of the video camera (VC).

legs towards the body in the 'drift position', a typical alarm response by nymphs to disturbance, such as being dislodged from the substrate. The total number of reactions was considered to be the number of animals that *either* ran from the light *or* assumed the 'drift position'. To test for overall differences across all wavelengths Cochran's Q-test was used (Sokal & Rohlf, 1981). To test for differences in reaction between two wavelengths of light the McNemar test was used for significance of change, with Williams' correction (Sokal & Rohlf, 1981). For all statistical tests the level used to accept significant differences was $P \leq 0.05$.

Results

Both *S. interpunctatum* and *S. vicarium* reacted strongly to both green and red light, running significantly greater distances from these light sources than from either infra-red, or the control (Fig. 2, Table 1). The mean distance run in red light was slightly but not significantly greater than that run in green for both species (Fig. 2). There was also a significant effect of red light for both *S. interpunctatum* ($P = 0.0005$) and *S. vicarium* ($P = 0.025$) when the total number of reactions ('drift position' or run) was considered (Table 2). Reactions to infra-red light were not significantly different from the control. These results, together with those on distance run, indicated that nymphs of both mayfly species could detect and react to red light, but not infra-red light.

Discussion

The use of red light as unobtrusive illumination in studies of aquatic insect nocturnal behaviour has been done without documented justification (Madsen, 1968; Corkum, 1978; Allan & Feifarek, 1989; Culp, Glozier & Scrimgeour, 1991), or with reference to previous work reporting red insensitivity (Kovalak, 1979; Glozier & Culp, 1989). Red light has also been used despite qualitative knowledge of red sensitivity (Allan *et al.*, 1986; Rader & Ward, 1990). A few studies have measured red sensitivity indirectly (Elliott, 1968; Bishop, 1969; Bailey, 1981; Casey, 1987), with ambiguous results. Infra-red illumination has been used to study aquatic insect nocturnal behaviour (e.g. Sjöström, 1985), as the only insects documented to

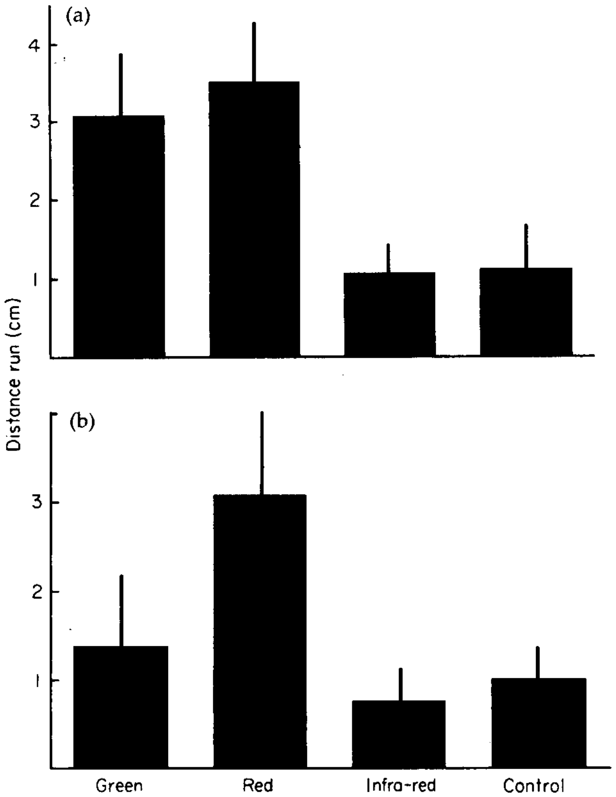

Fig. 2 Mean (+1 SE) distance run by (a) *Stenacron interpunctatum* and (b) *Stenonema vicarium* in response to light of various wavelengths. Each individual nymph was used once at each wavelength, with at least 24 h between trials.

Table 1 ANOVA table for distances run by *S. interpunctatum* and *S. vicarium* when exposed to green, red, infra-red and no light (control). Treatment variance is partitioned into the three orthogonal contrasts

Source of variation	df	SS	*F*	*P*
S. interpunctatum				
Light	3	117.0	4.600	<0.005
(Green + red) *v* (infra-red + control)	1	114.8	13.55	<0.001
Green *v* red	1	2.167	0.256	NS
Infra-red *v* control	1	0.035	0.004	NS
Error	92	779.5		
Total	95	896.5		
S. vicarium				
Light	3	79.67	2.850	<0.05
(Green + red) *v* (infra-red + control)	1	44.08	4.726	<0.05
Green *v* red	1	34.85	3.736	NS
Infra-red *v* control	1	0.738	0.079	NS
Error	92	858.1		
Total	95	937.8		

Table 2 Total number of reactions ('drift position' or run) of *Stenacron interpunctatum* and *Stenonema vicarium* to various wavelengths of light. Total number of trials was twenty-four for each species. Overall differences between treatments were significant for both *S. interpunctatum* ($P < 0.05$) and for *S. vicarium* ($P < 0.005$)

	Green (550 nm)	Red (650 nm)	Infra-red (950 nm)	Control (dark)
S. interpunctatum	18	21	7	5
S. vicarium	13	19	5	9

detect infra-red light are beetles that use extraocular receptors (Meyer, 1977; Evans & Kuster, 1980).

Why then, were there discrepancies between the results of this study and those of previous studies? Earlier studies of the effect of red light on aquatic insects have used indirect responses, such as suppression of insect activity and changes in the density of animals on the bottom of a stream (Elliott, 1968; Bailey, 1981; Casey, 1987). Responses were observed under conditions of dim white light, with and without the addition of red light. Lack of a red-light effect could have been due to the confounding effects of the white background light. In another study often cited as showing that insects cannot detect red (Bishop, 1969) the conclusions are questionable, as drift was suppressed at high intensities of red light.

Individual variation among insects may also account for differences between studies. Both intra- and interspecific differences occur in the sensitivity of mayfly nymphs to visible light (Elliott, 1968). For most aquatic insects the age of the animal may also be important. The visual receptor peaks in dragonflies change from nymph to adult (Ruck, 1965), as the photic environment changes with the emergence from the aquatic to the terrestrial environment. Hemimetabolous insects such as mayflies do not pupate, but undergo a rapid transition from aquatic nymph to terrestrial adult. Any change in photic pigments must therefore take place in the developing nymph prior to emergence. It is logical to assume that mayfly nymphs just about to emerge may have spectral sensitivities very different from younger ones. Age may account for some of the variability in the results of this study, as nymphs of various ages were used in this experiment (though none were in the dark wing pad stage directly preceding emergence). Nevertheless, these results do show (based on responses by mayflies) that future studies of nocturnal behaviour should use infra-red illumination, or at least test quantitatively the insensitivity of the study animal to red light.

In no studies where red light has been used have both the exact wavelength and intensity of red light used been reported. Thus, the 'red' lenses placed on the front of flashlights in various studies may have let through a small number of green photons in addition to red ones. In my study the red band pass filter cut off all light at 600 nm (=orange); therefore no green photons would have been present. Researchers examining light responses in aquatic animals should ideally express their light intensity in photons, as it is the number of photons, not the power of each photon, which is important for vision.

Visual sensitivity in the red end of the spectrum seems to be rare in insects, and has been substantiated only for some Lepidoptera (Bernard, 1979), Diptera (Goldsmith & Fernandez, 1968), Odonata (Meinertzhagen, Menzel & Kahle, 1983) and Hemiptera (Schwind, Schlecht & Langer, 1984). However, all insects shown to have such sensitivity have been adults and none are benthic in streams. The sensitivity of mayfly nymphs to the red end of the spectrum may represent adaptation to ambient light conditions. Water in many freshwater habitats contains short-wavelength-absorbing organic compounds such as tannins and lignins resulting from organic decomposition (Levine & MacNichol, 1979; McFarland, 1986). As a result, fresh waters can have transmission maxima beyond 600 nm (James & Birge, 1938; Schindler, 1971; Spence *et al.*, 1971; Howard-Williams & Vincent, 1985; Muntz & Mouat, 1984). According to the sensitivity hypothesis (Muntz & Mouat, 1984), visual pigments of an animal should be adapted to the spectral characteristics of ambient light in order to maximize photon capture. To test this hypothesis spectral transmission data from a wide range of aquatic habitats are needed, as well as spectral sensitivity data for a phylogenetically diverse assemblage of aquatic insects. Red vision is probably far more widespread in aquatic insects than is presently thought.

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