New Light on Phototaxis and Phototropism

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Objectives

This mini-workshop deals with ultra-bright light-emitting diodes (LEDs) and their applications to classroom investigation or student research. Features of ultra-bright LEDs that make them highly useful for general biology applications include: (a) unbreakable bulbs; (b) extremely long bulb-life; (c) very low heat production and low power consumption; (d) intense, highly localized light output; (e) availability in multiple colors with narrow spectral outputs; (f) inexpensive; (g) battery or A.C.-power options; and (h) safe, easy assembly. Potential applications for classrooms and student research include investigations of color sensitivity of invertebrate phototaxis (e.g., responses to blue versus red light), as well as blue-light-mediated phototropism responses in plant seedlings. Technical information is also included about LEDs (Fig. 1), circuit configuration, power supplies, and relevant photobiology research.

Definitions and General References Related to Photobiology

Photopigments are light-absorbing molecules found in certain cells of organisms that function to absorb light. Photopigments in *animals* are often referred to as *visual pigments,* since they provide the animal with capabilities of vision. Visual pigments are abundant in the *photoreceptor (or photosensory) cells* of vertebrates, as well as most invertebrates. In all of these animals, the light-absorbing photopigment is a protein-complex called rhodopsin. Rhodopsin molecules may differ with respect to the peak wavelength of light that they preferentially absorb. The presence of more than one type of rhodopsin in the photoreceptor cells of an animal is required for the capability of color vision - - a capability that some invertebrates have and others do not. Photoreceptor cells of animals may or may not be organized into structures that are recognizable as *eyes*. For example, in some animals (such as earthworms), no eyes are apparent but these organisms still detect light using photoreceptor cells that are diffusely scattered in the skin. Light-absorbing photopigments are also found in *plants* and these serve various functions. Some plant photopigments, called *photosynthetic pigments* (e.g., chlorophyll and carotenoid), function in photosynthesis. Other plant photopigments, called *developmental pigments* (e.g., phytochrome and cryptochrome), function in controlling plant growth and development. As in the photopigments of animals, plant photopigments also differ with respect to the wavelength of light that is preferentially absorbed [see Attridge, 1990; Salisbury and Ross, 1992].

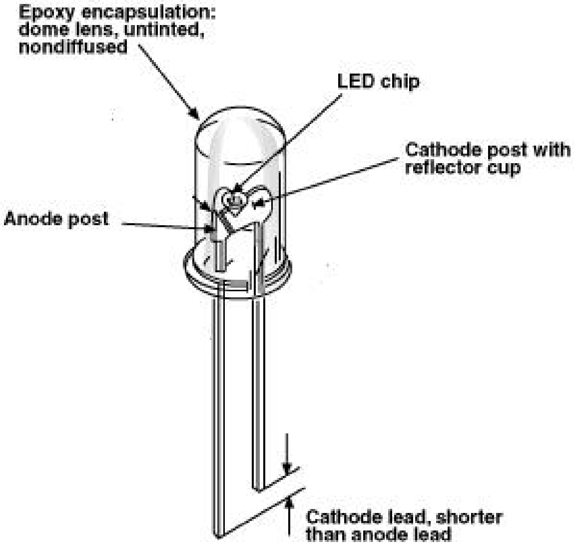


Figure 1. Anatomy of an LED lamp.

Phototaxis is a directed movement of a freely moving organism in relation to the direction of incident light in its environment. Phototaxis is said to be "positive" when an organism moves *toward* the light source or "negative" when an organism moves *away* from the light source. In general, an animal's phototaxic movement serves to bring it into a more favorable relationship with its environment (e.g., improved nutritional status or protection). Examples of positive phototaxis occur in many zooplanktonic crustacea, such as daphnids and copepods. The term, "heliotaxis," is sometimes used to refer to the directed movement of an animal toward or away from *sunlight*.

Photokinesis is the change of an organism's *speed of movement or frequency of movements* in relation to the level of light energy (light intensity) that falls on the organism per unit time. Photokinesis is *independent of the direction of light*. Examples include certain insect larvae (some beetles and fruit flies) that increase their rate of movement in relation to an overall increase in the intensity of ambient light. It is possible for an organism's response to light to involve a combination of both phototaxis and photokinesis.

Photophobic response is a change in an animal's behavior (such as rapid retreat or reversal in direction of movement) in response to a sudden increase or decrease in the overall light intensity. Photophobic responses are *independent of the direction of light*. Examples of photophobic responses include: (a) change in the direction of locomotion in bacteria as a result of a sudden change in light intensity, (b) rapid withdrawal of the exposed head or tail end in certain tube-dwelling polychaete worms or oligochaete worms (*Lumbriculus*) in response to abrupt onset of a shadow (Drewes and Fourtner, 1989), or (c) rapid withdrawal of an earthworm into its burrow in response to sudden flash of bright light.

Optokinetic response is a change in the orientation of an organism's eye(s) in relation to the *changes in the direction of incident light*. Such responses are the result of contraction in specific muscles (oculomotor muscles) associated with the eyes. An example of an optokinetic response is the eye rotation in certain crustaceans (e.g., daphnids and crabs) in response to a change in the direction of light (Drewes, 2003).

Phototropism is the change in position or orientation of some part of an organism in relation to the direction of light. The term "positive" phototropism is used when some part of an organism shifts position *toward* the light, and "negative" phototropism when part of the organism shifts position *away* from the light. The bending of the stem of a plant seedling toward blue light is an example of a "positive phototropic" response. In general, phototropic movement of an organism tends to bring it into a more favorable relationship with its environment (e.g., enhanced nutrition). Another term, "heliotropism," is sometimes used to specifically refer to the directed growth of plants toward or away from *sunlight*. The term phototropism may also be used to describe bending in the body of *sessile animals* toward or away from a light source (Note: sessile refers to animals that are attached to a substrate). Examples of sessile animals that bend toward the sun include some marine, tube-dwelling polychaete worms and some colonial hydrozoa.

Photonastic response is a response in a plant caused by a *change in light intensity*. Such responses are *independent of the direction of light*. Examples include the closing of a flower and closing of leaf stomata in response to an overall decrease in light intensity.

Possible Applications of Ultra-Bright LEDs to Phototaxis and Phototropism

- Phototaxic orientation in zooplankton, such as daphnids (Fig. 2) and copepods
- Phototaxic orientation in nauplius larvae and later stages of brine shrimp (crustaceans)
- Phototaxic, photokinetic, and photophobic responses in oligochaetes and planarians
- Phototaxic orientation in protozoa and algae (e.g. ciliates; flagellates, motile algae)
- Phototropism in plant seedlings (Figs. 3, 4)
- Light-induced stomatal opening
- Photonastic responses in plant leaves

Practical Suggestions for Investigating Invertebrate Phototaxis

(1) Before testing animal responses to a photo stimulus, first try to reduce the overall intensity of overhead or window lighting on the organism by turning off some or all room lights, or by placing the test container and animal under an opaque canopy. Reducing background lighting will generally accentuate the animal's responses to stimulation with the LED lights. A canopy should still permit

easy access for delivering the light stimulus and for viewing by the experimenter. A small, inverted cardboard box, with appropriate cut-outs in the sides, often suffices as a canopy. [NOTE: It may take several minutes of exposure to darkness for an animal to become dark-adapted and for its responses to light to become accentuated. Patience and time are often needed to obtain clear-cut responses.]

(2) In some cases, such as newly hatched brine shrimp or daphnids, it works well to study phototaxic responses to numerous organisms at once rather than individual organisms. A dozen or more of these animals may be placed in a relatively confined space, such as a shallow petri dish of water, with the LED light source placed somewhat horizontally at the side of the container, rather than shining down from above. To enhance contrast, it may be helpful to place a piece of paper (light-colored for daphnids or dark-colored for brine shrimp) under the transparent bottom of a container, such as a petri dish. Then, studies and comparisons of the direction of swimming and overall location of animals may be made at two different times: (a) immediately *before* the LED is turned on at side of the dish, and (b) about 15 seconds *after* the light has been turned on (Fig 2).

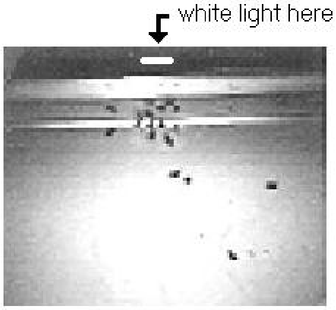
(3) Some aquatic organisms, especially daphnids, tend to periodically cease swimming and momentarily cling to the sides or bottom of their container. Under these circumstances, locomotor movements toward or away from a light source may not occur. In such cases, it may be necessary to gently vibrate the container, thus stimulating the organisms to release their attachment and begin swimming movements that are oriented toward or away from the LED source (Fig. 2).

(4) Responses to LED-stimulation in creeping and crawling organisms such as flatworms, earthworms, or aquatic oligochaetes (e.g., *Lumbriculus variegatus*) are likely to involve a combination of both negative phototaxis and photokinesis responses. Such responses may be studied by placing one worm into a narrow, linear-shaped container that has straight sides, covered transparent top, and transparent bottom. "Flexible foam-well slides" made from foam tape are especially useful for this purpose [see**:** *http://www.eeob.iastate.edu/faculty/DrewesC/htdocs/*]. Alternatively, worms may be placed in culture tubes with flat tips <*http://www.eeob.iastate.edu/faculty/DrewesC/htdocs/FLATTUBE.PDF*> or glass capillary tubes <*http://www.eeob.iastate.edu/faculty/DrewesC/htdocs/looking2.JPG*>. With a worm confined to this narrow, linear space, study its reactions and locomotor movements toward or away from a bright, constant light positioned at the worm's head or tail end.

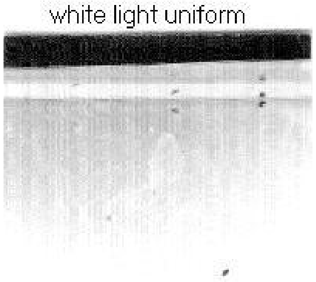
(5) Keep in mind that in order for an animal to produce a phototaxic response in relation to a gradient of light, *the animal must somehow be able to sense, and respond to, small differences in light intensity that exist within its immediate environment*. To do this, the animal needs one or more photoreceptor cells in its body. In theory, if an animal has only a single photoreceptor cell, then in order for it to produce a phototaxic response within a light gradient, it would be necessary for the animal to be moving. Such movement is needed to allow the animal to "detect and compare the light intensity reaching its photoreceptor" at two different times – initially, when its body is in one specific position and then, a short time later, when its body is in a somewhat different position within the light gradient. Given sensory information about the comparative intensity of illumination at these two different locations, the animal may then appropriately respond by turning and moving toward (or away from) the direction in which the light was most intense. If the animal has more than one photoreceptor cell then, in theory, it is possible for it to sense the direction of the light gradient even if it is not moving. This is possible if the animal is able to simultaneously detect and compare the intensity of light striking two or more photoreceptors that are necessarily localized in different parts of its body and receiving different levels of light intensity. In invertebrates with two or more photoreceptors, it is likely that a combination of both of these detection and comparison strategies is probably used. That is, the organism may make sequential comparisons of intensity using the same

photoreceptor, as well as simultaneous comparisons of intensity using spatially separated photoreceptors.

(6) For studying phototropic responses of plants, the plants may be placed under an inverted cardboard box with one or more LED lights inserted into holes in the box (Fig. 3). Different colored lights, placed at opposite ends of the box, may be used to study the color-sensitivity of phototropism (Fig. 4). Responses of plants seedlings could also be studied in relation to a combination of both light and gravity by placing lights at differing positions in relation to the direction of gravity.

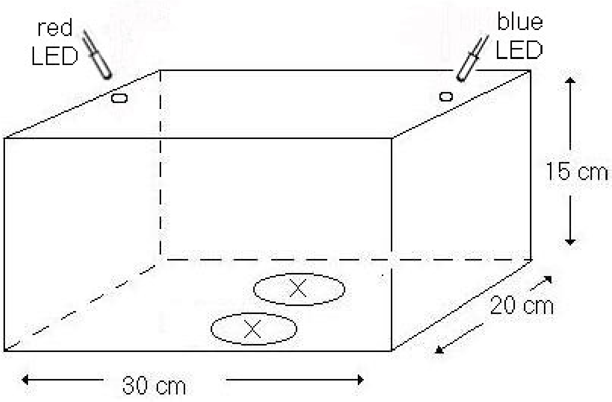


A) Daphnids aggregate where the white LED lamp is directed at the side of the container.



B) Daphnids show no aggregation when container is uniformly and diffusely illuminated with white light.

Figure 2. Phototaxis in daphnids in response to white light.



NOTE: LEDs should be anchored in holes at the top of the box. Both LEDs should be angled so that lights are directed at the emergent plant seedlings, which are placed in small pots at points marked "X" that are equidistant from the LEDs.

Figure 3. LED light-box configuration for demonstration of phototropism in plant seedlings. The inside surface of the box should be black.

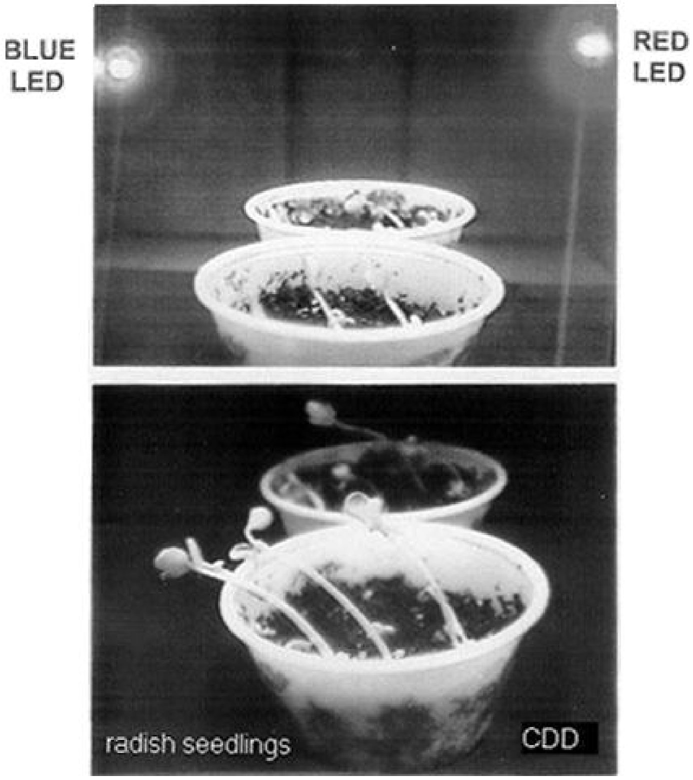


Figure 4. Phototropic growth in radish seedlings. Seedlings were grown in potting soil for several days while covered with a small cardboard box (Fig. 2). The plants were continuously illuminated by red and blue light from ultrabright light-emitting diodes that were inserted into holes in the top of the box, at opposite ends. The lights were about 30 cm apart. [for color photo, see: *http://www.eeob.iastate.edu/faculty/DrewesC/htdocs/phototrop.jpg*]

Sources and Specifications for Ultra-bright LED Kits

A commercial source for ultrabright LEDs is Allied Electronics (Table 1), which has distributors in many states (ph: 800-433-5700). For general information about LEDs, see:

(1) *http://www.theledlight.com/technical.html*
(2) *http://www.lrc.rpi.edu/Futures/LF-LEDs/index.html*

Table 1. Specifications for ultra bright LED lamps.

Allied #	Mfg Type	mm	peak λ	COLOR	lv@20 mA	view<	~cost	$V_{forward}$
505-9877	AND157HRP	5	644 nm	RED	1800	20	1.25	2.2 V
505-9887	AND157HAP	5	612	AMBER	2000	20	1.25	2.2 V
505-9698	AND157HYP	5	590	YELLOW	2500	20	1.25	2.2 V
505-9705	AND520HG	5	540	GREEN	5000	20	2.45	3.75 V
505-9706	AND520HB	5	466	BLUE	1400	20	2.45	3.75 V
505-9707	AND520HW	5	420-700	WHITE	5000	20	2.31	3.75 V

Examples of LED circuitry, general assembly, battery power pack, and AC power adapter

(6V DC, 200 mA) are shown in Fig. 5. Contact author (C. Drewes) for additional information about wiring details, component specifications, and calculation of appropriate current-limiting resistor (R).

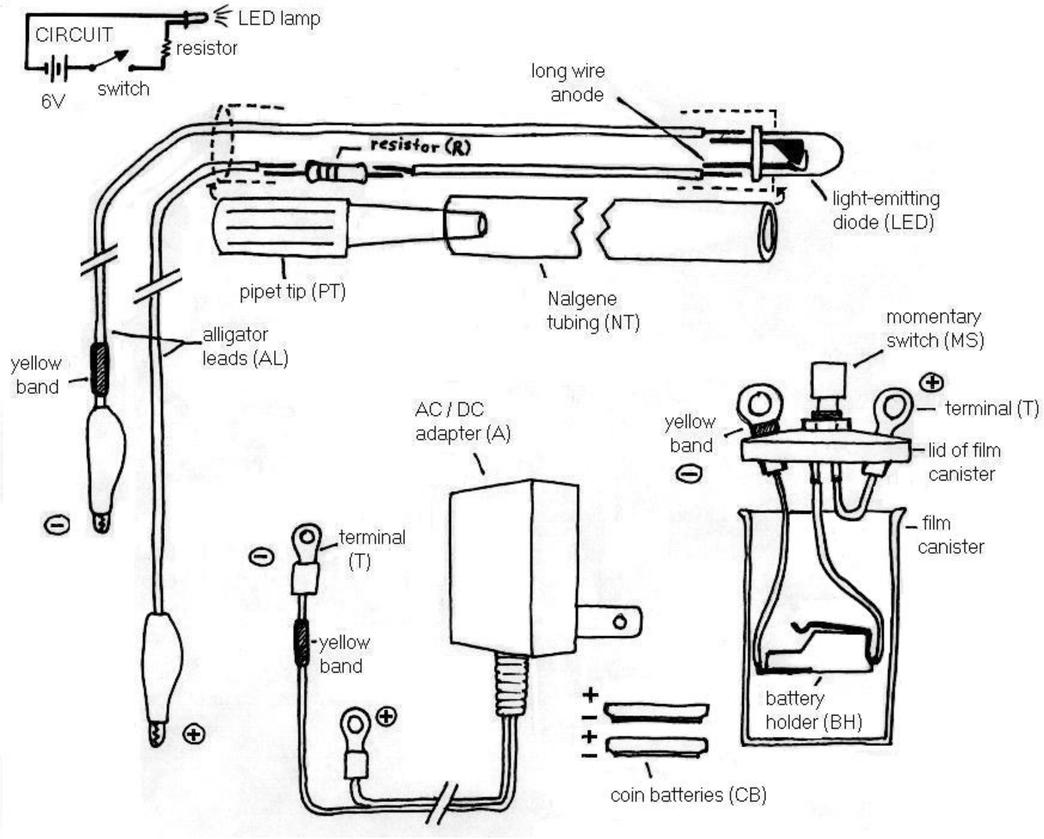


Figure 5. Wiring schematics, power supplies, and assembly of LED lights.

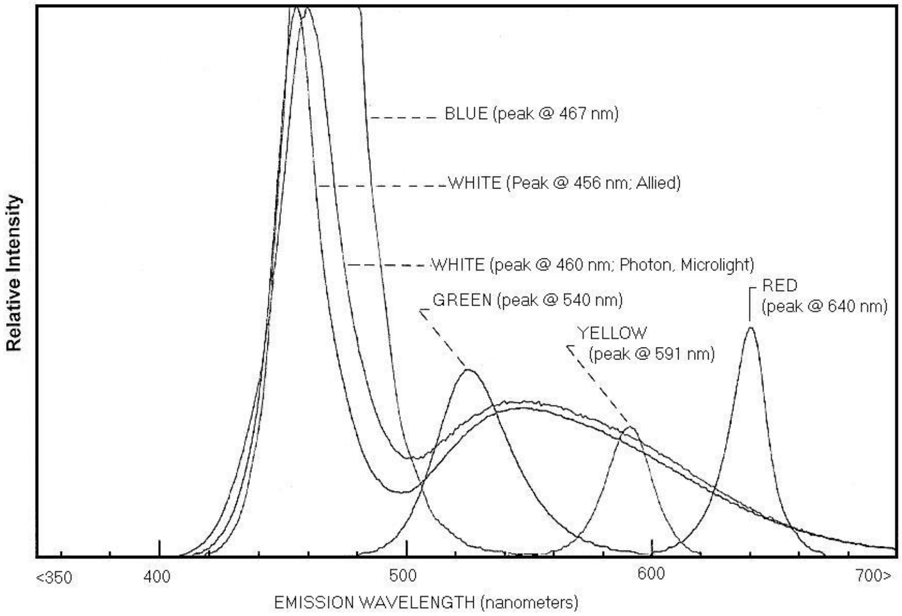


Fig. 6. Ultrabright LED Emission Spectra

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DISCLAIMER: The user assumes all responsibilities for the safe and proper assembly and use of electrical components described herein. The author (CDD) assumes no liability relating to the unsafe handling and use of these materials.