Chapter 1

Investigations in Orientation Behavior

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Introduction

This is an investigative laboratory in which students observe the orientation behavior of a variety of organisms, formulate hypotheses based on their observations, and use simple equipment to collect data and test their hypotheses. These laboratory systems have been used at both the introductory college and advance placement high school levels. One 3-hour period is adequate for this laboratory, although a second period would allow students to conduct follow-up studies and arrive at more meaningful generalizations. Students work in groups and report on their studies either orally at the end of the laboratory, by writing individual reports, or in both ways. This laboratory can be scheduled either during the portion of the course treating behavior or as a general introduction to scientific methodology.

Notes for the Instructor

Student Preparation

To prepare for this laboratory, students should read the Student Outline and whatever additional information may be in their textbook on orientation behavior. In your introduction to the laboratory, you may wish to discuss the definition of orientation behavior given in the Student Outline. Typically, students associate orientation behavior with migration and homing, but it should be emphasized that it need not involve movement. General body orientation to light and gravity, as in the dorsal light reaction in fish, is orientation behavior without movement. You can demonstrate the dorsal light reaction by shining an intense light to the side of an aquarium containing a good-size fish (angelfish work well). The fish will dip its head (if facing the light) or its side (if lateral to it) toward the light. Because the fish also uses information on gravity from the labyrinth organ of the inner ear, it will not show a complete dorsal light reaction to a lateral light.

Terminology has been kept to a minimum in the Student Outline; kinesis is contrasted with taxis and several types of taxes are discussed. You may wish to present a more complete introduction to the types of orientation responses shown by animals. Consult Fraenkel and Gunn (1961) for a comprehensive treatment of their classification scheme and terminology.

The two types of taxes mentioned in the Student Outline can be demonstrated in the laboratory with a blindfolded student. Asked to orient to a sound, such as a buzzer in the corner of the laboratory, the student will turn so that the sound is equally intense in both ears (a tropotaxis). When trying to orient to an odor in the laboratory, the student will typically turn his or her head first to one side, then to the other side, comparing the stimulus intensity on both sides, and then turn in the direction in which the smell is strongest (a klinotaxis, our nostrils are so close together that we can't usually compare one with the other!).

A moth flying into a candle is showing a menotaxis. As with many insects, it tends to fly at a fixed angle relative to a light source (usually the sun or moon). However, with a nearby light source, as the moth moves it must constantly change its flight path in order to maintain a fixed angle relative to the light. This results in the characteristic spiralling flight into the flame.

Laboratory Logistics and Timing

This laboratory can be managed in a number of different ways depending on how structured you wish it to be. Students could be allowed to form their own groups and design a project based on their interests. Unfortunately, this approach sometimes leads to competition for the more popular organisms (usually fish and sowbugs) and the neglect of less desirable ones (fleshfly larvae). You could assign specific groups to work on different projects so that material is shared more equitably and a greater diversity of orientation types are studied. You may also wish to eliminate certain organisms/studies based on your own interests, availability of materials, or budgetary constraints, or have the whole class study different aspects of orientation behavior in a single species of organism. The number of different studies that can be done on fish, *Daphnia*, or sowbugs is sufficiently great to keep a class of 18+ students profitably occupied for 2–3 hours.

For each organism, students first make some preliminary observations, designed in conjunction with the background information provided in the Student Outline to help them ask questions and formulate hypotheses. The following general guidelines indicate the time requirements for this laboratory:

Activity	*Time required*
Initial observations	10–20 minutes
Design study	15–30 minutes
Conduct study	90–120 minutes
Discuss results	45–60 minutes

Isopods

Basic Set-up: Sowbugs should be made available in covered plastic (sandwich-size) dishes with moist paper towel covering the bottom one-half of the container. Generally, most of the sowbugs will be under the towelling. If students remove the towel, randomize the sowbugs, replace the towel, and the sowbugs will quickly resume their positions under the towel. Light and moisture gradients direct this response.

Apparatus: The basic apparatus consists of two petri dishes and some flexible screen (see Figure 1.4 in the Student Outline). The lower dishes hold the desiccant and wet paper towelling. The upper dishes hold the sowbugs and are separated from the lower dishes by the screening. Arrange the upper dishes so their "doorways" are joined. Animals are inserted through the top central holes.

Two basic approaches are to study the response of a group of animals by counting the number in the two dishes (also the number moving and motionless) or follow in detail the path taken by one

individual in the two areas, using a marking pen and a map measurer to determine distance travelled. This approach will show that the response to humidity involves both the rate of movement and the turning rate; that is, the response is both an orthokinesis and klinokinesis.

Suggested Studies: In addition to examining the effect of humidity, the apparatus can be set-up to study the sowbug's response to light. Cover one upper dish with black construction paper or have some tops that have been spray-painted black; leave the other top exposed to light. Under damp conditions (with wet towelling in the dish bottoms), the sowbugs will move directly into the dark dish (a phototaxis). However, under dry conditions (with desiccant in both dishes or empty dishes), they will usually ignore the light-dark stimulus and continue to search for a more humid environment. Therefore, in order to examine their response to light, both chambers should be kept humid. In pitting one environmental variable against the other, students will see that humidity is a more important stimulus than light; that is, if offered light-humid verses dark-dry, the animals will prefer light-humid. If two or more species are available, students can do comparative studies on orientation to humidity and light or investigate possible orientation to conspecifics (do individuals show a preference for the paper towelling from their own species container?). See Sutton (1972) for more information on sowbug orientation behavior.

Fleshfly Larvae

Basic Observations: As with the sowbugs, the fleshfly larvae will be under the paper towelling within the plastic container and will return there fairly quickly if moved into the open.

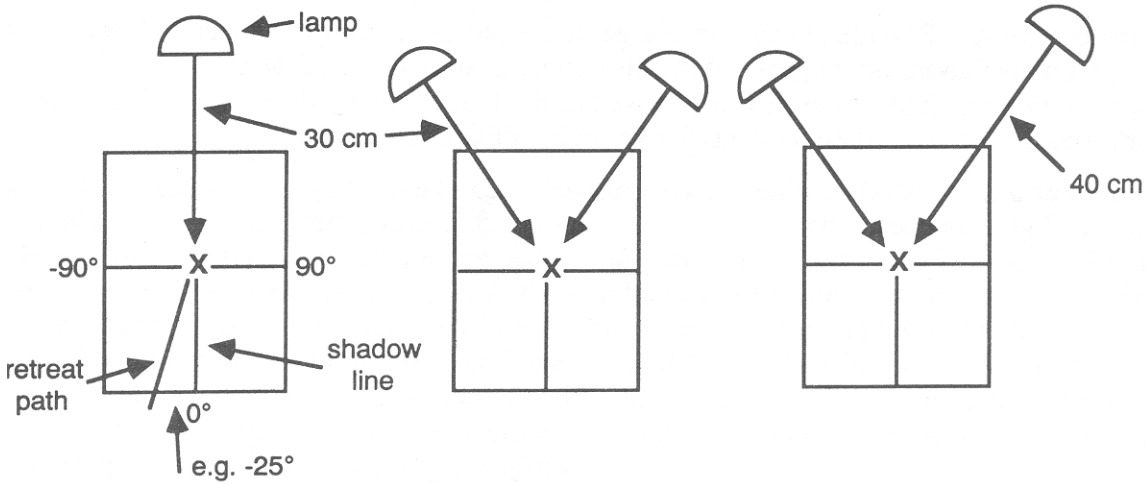
Suggested Studies: These organisms show a well-developed negative klinotaxis to light, most consistently seen in the younger larvae; the swinging, side-to-side head motion is characteristic of a klinotaxis. Locate students working on fleshfly larvae in a darkened portion of the laboratory, since these animals are very sensitive to stray light.

Fleshfly larvae will move directly away from a single light, applied as suggested in the Student Outline. Two lights cause the larva to describe a path of retreat an equal distance from the two lights. If one of the two lights is moved away, the larva's path will be angled toward the light that is now less intense. If two lights are used, but one is switched off whenever the larva swings its head toward that light, then the larva will ignore that light and move directly away from the light that is not turned off. Goose-neck lamps equipped with 60-watt bulbs are best for these studies. Jennings (1975) provides additional information on methods to use with this species.

Students have difficulty deciding how to quantify the response of the larvae in these studies. A good method is to measure the angle of retreat relative to the shadow line created by holding a pencil vertically at the intended release point with the light(s) on. Protractors can be used to measure the angle between the retreat path (a straight line connecting the release point and the point where the larva left the paper) and the shadow line. Figure 1.1 shows typical data collected by students testing larvae subjected to three different lighting conditions.

Fish Schooling

Basic Observations: Initially students should make observations on a variety of species in a community tank, in order to rank them according to their school-forming tendencies. Try to provide species differing widely in this regard.



Degrees (°)

1	-6	20
11	6	11
-9	-4	12
0	-2	18
2	3	14
5	-3	19
-10	3	-1

Figure 1.1. Investigative set-ups and typical student data for retreat paths (degrees) of fleshflies exposed to a single lamp at 30 cm (left), two lamps at 30 cm (center), and two lamps, with the left lamp at 30 cm and the right lamp at 40 cm (right).

Test Tank: At least one test tank is needed by each group of three to four students (see Figure 1.5 in the Student Outline). A clear plastic cylinder is used to release the test fish in a controlled manner when each trial begins. The two cylinders containing the artificial schools should be switched during each trial to control for any preference the test fish may have for a particular end of the tank.

Suggested Studies: In collecting data on the degree of orientation to conspecifics or larger versus smaller groups, students should compare several species differing in school-forming tendencies. This will allow them to evaluate the importance of these factors in the school-forming process. Model fish schools can be drawn on plastic sheets rolled up to fit into the cylinders. Each model school should have fish that all differ in a single way from other schools. The fish in some schools might be simply outlines; in other schools, outlines plus a species typical color patch; in others, just the color patch, etc. The importance of school movement can be tested by taping the plastic sheet to a long glass rod and using it to move the "school." See Keenleyside (1975) for more information on this approach to the study of fish schooling.

Planaria

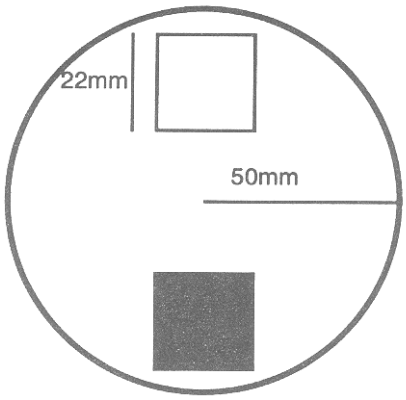
Basic observations: Provide planaria in a large, white pan covered with a piece of black construction paper. Before the laboratory begins, set the dish under a lamp with a 60-watt bulb at 30 cm and adjust the paper so that one half of the dish is exposed to the light. Most individuals will have moved into the dark area of the pan when students make their initial observations.

Apparatus and Suggested Studies: Two approaches can be used to study planaria. To study their response to light, use petri dishes that have been divided into a light and a dark side with black construction paper. Or to examine possible photo- and/or thigmotaxis, students should have access to petri dishes, each with a pair of clear and black coverslips. These coverslips have four short legs on the underside to allow planaria to move under the coverslips. The basic approach is to monitor the numbers of planaria under clear and black coverslips in the light and in the dark (covered by a box). If movement of planaria is random then the number under coverslips should be proportional to the surface area of the coverslips to the total surface area of the dish. Students measure the dimensions of coverslips and dishes, determine surface areas, and calculate the expected numbers in different areas if neither photo- or thigmotaxis is shown (Figure 1.2). Table 1.1 shows four possible outcomes and predictions.

Using either the dishes divided into light/dark areas or the dishes with the special coverslips, initially place 15–20 planaria into each dish using suction with a pasteur pipet to transfer them from the pan to the dish. Four to five replicate dishes can be set up and monitored at the same time. Allow the organisms at least 5 minutes to respond and scrub the dishes between trials to remove the mucus trails. Counting animals is tricky because of the mirror images created along the edges of the petri dishes.

Table 1.1. Expected results in studies in which planaria have access to clear and black coverslips in the light and in the dark, assuming that planaria show only phototaxis, only thigmotaxis, both photo- and thigmotaxis, and no orientation at all.

Orientation behavior	Light	Dark
Phototaxis only	Significantly more under black than expected	No more under coverslips than expected
Thigmotaxis only	Equal number under clear and black and more than expected	Equal number under clear and black and more than expected
Both photo- and thigmotaxis	More under black than clear and more under both than expected	Equal number under clear and black and more under both than expected
No orientation at all	No more under coverslips than expected	No more under coverslips than expected



Calculations: Area of circle= (π)r2
Petri dish area = 3.14 × (50 mm)2 = 7850 mm2
Coverslip area = (22 mm)2 = 484 mm2

Expected planaria under coverslip if moving randomly (no orientation):

484 mm2/7850 mm2 × (total planaria in petri dish)

Figure 1.2. Measurements and calculations for determining the expected number of planaria under coverslips in the absence of any orientation.

Water Fleas *(Daphnia magna)*

Basic Observations: Students should first observe *Daphnia* within a 100-ml graduated cylinder suspended by a clamp from a ring stand. Have them use a goose-neck lamp to present light, first from above, and then from below, noting the response in each case. Generally, *Daphnia* will be at the bottom of the cylinder under diffuse light (fluorescent room lights) or darkness. Moderate light from above will attract the *Daphnia*. Intense light from above or below (150-watt bulb at 10 cm from the water surface) may cause the *Daphnia* to move away from the light source.

Suggested Studies: In order to eliminate side light in these studies, students should place sleeves of black plastic around the graduated cylinders and additional pieces of black plastic can be applied to the top, bottom, or both ends, depending on the treatment. To protect the *Daphnia* from excess heat, use a heat shield consisting of a petri dish filled with water and supported by a beaker holder. After 5 minutes exposure to the treatment, the sleeve is quickly removed and the number of *Daphnia* in each of the 10 10-ml volumes is counted. If three or four students work as a group, they can each be responsible for counting *Daphnia* in specified volumes. Several cylinders can be set up at the same time to make data collection more efficient. Light direction (from above or below), intensity (adjusted by varying the distance from the light source), or no light can be tested in this manner. Students can use a self-paired investigative design (i.e., each cylinder subjected to all treatments, and comparisons made between data collected from the same cylinder), in order to reduce the effect of cylinder-specific variation.

To examine the effect of light quality on *Daphnia*, we recommend using colored filters sold by Edmund Scientific (101 E. Gloucester Pike, Barrington, NJ 08007-1380): Dark Urban Blue (#866), Medium Green (#874), and Medium Red (#823). If you use a combination of incandescent and

fluorescent light sources, the transmission spectra of these filters are fairly distinct and non-overlapping (see Figure 1.7 in the Student Outline). Students can obtain these transmittance spectra by using a Spectronic 20 spectrophotometer equipped with a wide-range bulb and a "blank" consisting of an empty cuvette. A radiometer can be used to obtain a light intensity-distance curve (see Figure 1.6 in the Student Outline).

Our results show that *Daphnia* are relatively higher in the water column under blue light than green or red light, although the response is somewhat variable. See Baylor and Smith (1957) for background information on vertical movement of *Daphnia* in response to important environmental variables.

Notes for the Preparator

Keep room lights low during this laboratory; stray light can cause unexpected results in many of these studies.

Isopods

Although isopods can be purchased from numerous biological supply companies, they are easy to collect. Look under logs, rocks, etc., in woodland areas, mulching material in gardens, around woodpiles, and under flower pots in greenhouses. An afternoon of collecting usually yields more than enough for these studies. Maintain your cultures in plastic containers with loose fitting lids and a soil bottom covered with several pieces of moist paper towelling. Feed them fresh lettuce, spinach, and cut potatoes. Keep the cultures in a cool location.

The test apparatus is constructed from two 150 mm × 25 mm petri dishes and a piece of fiberglass window screen (200 × 350 mm). The introduction hole should be large enough to accommodate a #2 solid stopper. Use a hot pipe to melt through the plastic lid to create the hole. A hot knife is used to make the passageways (10 mm high, 15 mm wide) in the two lids. Melting plastic produces fumes so you should do this in a hood or well-ventilated area.

Fleshfly Larvae

Although you can raise your own larvae it is easier to order them from a biological supply company, get them from a local colleague with a culture, or locate a pet store or bait supplier offering them. As you get the larvae, place them in covered plastic dishes and keep in an incubator at about 15°C. Depending on their age, they can be used for several days before they begin pupation. You will need two or three shipments for 1 week of laboratories. See Galtstoff et al. (1959:414–427) for methods to culture your own fleshfly larvae.

Fish Schooling

Try to provide at least 10 individuals of 5–6 different species selected from the following:

Tiger barb (*Barbus tetrazona*)
Tetras (*Hyphessobrycon sp.)*
Swordtails (*Xiphophorus maculatus*)
Juvenile convict cichlids (*Cichlasoma nigrofasciatum*)
Juvenile Jack Dempseys (*Cichlasoma octofasciatum*)
Scissor tail (*Rasbora trilineata*)
Harlequin fish (*Rasbora heteromorpha*)

Pristella (*Pristella riddler*)
Zebra danios (*Brachydanio sp*.)

If possible get danios, barbs, tetras, a cichlid species, swordtails, and pristellas. Divide the species up among the two community tanks. You may need to isolate the more aggressive cichlids and the swordtails. All tanks will need to be filled with aged tap water. Install a bubbler in the community tanks. Plexiglass cylinders for holding the artificial schools work especially well because they need not be filled with water, as with a jar, but can be simply immersed into the tank.

Planaria

Keep planaria in spring water or aged tap water. We collect our own planaria from a local pond, but several species are available from biological supply companies. Feed them every several days—a small amount of canned cat food is good—but change the water immediately after feeding.

Very small dabs of silicone aquarium sealant can be applied to the corners of coverslips to create a 1 mm space beneath the coverslip. Spray the tops of some of the coverslips with black enamel paint. We made permanent coverslips by milling 2 mm plexiglass squares (clear and black) to provide the 1 mm legs.

Water Fleas (*Daphnia magna*)

Use spring water or aged tap water to culture *Daphnia*. (You will need to try the water sources available to you to discover which are suitable). When the *Daphnia* arrive, divide them into several gallon jars filled two-thirds with water. Change the water every 5–7 days by pouring the culture through a fine mesh fish net and returning the organisms to a jar with fresh water.

Maintain an algal culture to feed *Daphnia*. We scrape algae from an overgrown aquarium and add it to fish-tank water in a gallon jar. Install a bubbler and keep the culture in a lighted area. Periodically add more fish-tank water as a nitrogen source for the algae. When a "good" algal culture is evident, add about 10 ml to the *Daphnia* jars every 2–3 days. Feeding a unialgal culture of *Chlorella* or *Scenedesmus* also works well.

A medium-size mesh fish net can be used to scoop up *Daphnia* to the graduated cylinders. The mesh size should allow the small, immature *Daphnia* to pass through, but retain the adults. Float the net in a beaker of water and use a large-bore dropper to pick up adults for transfer to the cylinder. Fire polish a glass tube or cut off a portion of the tip of a plastic pipet to make this dropper; add a rubber bulb. Be sure to gently release the *Daphnia* below the water surface without bubbling. If *Daphnia* get air under their carapace, they float to the surface and die. Each cylinder should have at least 20 individuals. Replace the *Daphnia* in the cylinders every day.

Materials

General Supplies (Class size of 24; six groups of four students)

Gooseneck lamps with 150- and 60-watt bulbs (15)
Fluorescent lamps (two 15-inch bulbs per lamp) (4)
Stopwatches (6)
Scissors (6)
Masking tape (6 rolls)
Soluble marking pens or wax pencils (assorted colors) (12)
Meter sticks (6)

Isopods

Isopods of any available species (100)
Drawing compasses (needed to draw circles on black construction paper to be used as cover to darken sowbug apparatus) (3)
Dririte® desiccant or equivalent (2 kg)
Soft (insect) forceps (for handling sowbugs and fleshfly larvae; available from Wards as "featherweight" forceps) (6)
Map measurers (Forestry Suppliers, Inc., 205 West Rankin St., P.O. Box 8397, Jackson, MS 39204-0397) (2)

Fleshfly Larvae

Larvae (20+)
Black construction paper (20 sheets)
Protractors for measuring retreat angles (2)
Soft (insect) forceps (6)

Fish Schooling

Fish of several species (60)
10-gallon test tanks per lab (4–6)
Black plastic (4 mil garden mulching material works well, used to make side curtains for fish test tanks) (1 roll)
Clear plexiglass cylinders (10–12 cm O.D., 20–25 cm high) per test tank (3)
20-gallon community tanks per lab (2)
Fish nets (6)
Clear acetate sheets (used to make model fish schools) (12)

Planaria

Planaria of any available species (40+)
50-mm petri dishes (12)
Coverslips with legs, some clear some black (12)
Boxes to cover some petri dishes (3)

Water Fleas

Daphnia magna (200+; one MagnaCulture 100 per lab section is sufficient; obtained from obtained from Wards Natural Science, 5100 W. Henrietta Rd., P.O. Box 92912, Rochester, NY 14692-9012)
100-ml graduated cylinders (6)
Ring stands with clamps to hold the cylinders and beaker plates to hold heat shields (6)
Petri dishes (medium-size; used as heat shields) (6)

Student Outline

(Read this entire outline before coming to the laboratory)

Laboratory Synopsis

During this investigative laboratory, you will design and conduct a study concerned with some facet of the orientation behavior of a selected animal. You will begin by making preliminary observations on one of the animal species available in the laboratory, and then design an investigative study to test a hypothesis inductively derived from these observations. In this study, you will direct your efforts at providing answers to several basic questions of interest to biologists: What stimuli elicit orientation behavior? What is the nature of the orientation response? How might the observed response be adaptive? At the conclusion of this laboratory session you will report your results to the class.

Laboratory Objectives

At the end of the laboratory, you should have:

1. Secured a basic understanding of the types of stimuli used by organisms in orientation, the types of behavioral responses leading to orientation, and terms useful in describing these phenomena.
2. Acquired experience in designing and conducting investigations in the area of animal behavior.
3. Gained experience in using descriptive statistics and graphing techniques to better understand data collected in your study.
4. Developed experience in the oral communication of results and conclusions from a scientific study.

Questions to Prepare You for this Laboratory

1. What are three types of orientation stimuli that can be sensed by some organisms but not humans?
2. What characterizes a taxis orientation response?
3. What characterizes a kinesis orientation response?
4. Is the manner in which you would locate the source of an odor in a dark room a taxis, a kinesis, both, or neither?
5. Is the manner in which you would locate the source of a sound in a dark room a taxis, a kinesis, both, or neither?

Introduction

For many, the orientation behavior of animals is one of the most fascinating phenomena in all of biology. In a general sense, humans have been aware of animal orientation, migration, and navigation for thousands of years. Whole civilizations have thrived or perished based on their understanding of the movements made by principal animal food sources. Yet, it was not until early in the 20th century that a rigorous analysis of orientation mechanisms began, when our knowledge of sensory systems and how other animals detect the world improved considerably.

Orientation refers to the spatial organization of movements. Since movements are elements of behavior, orientation and behavior are intimately associated. For simplicity, we will define behavior as any overt manifestation of life by an animal, especially one that takes the form of movements. A behavior pattern is the fundamental unit of behavior, and is defined as a sequence of movements characterized by a specific configuration in time and space. This underscores the special significance that spatial organization has for behavior. Every behavior is spatially oriented in some way. Whether an animal walks, grooms, catches prey, or interacts with a social partner, "where" and "in which direction" are indispensable features of its behavior pattern. Thus, we can define orientation as the process that animals use to organize their behavior with respect to spatial features.

The specific orientation systems used by an animal correspond to the features of its environment. Many terrestrial organisms are sensitive to humidity levels, and are therefore capable of orienting with respect to moisture gradients. But humidity is an environmental feature that is not relevant in a totally aquatic habitat, and as a result animals that live in water must use physical gradients based on other parameters (e.g., temperature or salinity) to help direct their movements. Some orientation stimuli are available to both terrestrial and aquatic organisms; these include gravity, light and magnetism.

This laboratory exercise will allow you to investigate orientation behavior in a variety of animals. It will also help you understand the scientific method by giving you first-hand experience with designing, conducting, and interpreting a study involving animal orientation.

Orienting Stimuli

In orientation studies, one first attempts to identify the nature of the stimuli to which the animal is orienting. Light, gravity, sound, and mechanical stimuli, as well as temperature, chemical, and moisture gradients are all likely candidates. As with a moth flying into a candle, the nature of the orienting stimulus may be clearly apparent. However, if the animal is orienting to a stimulus for which humans have no receptor organs, identification of that stimulus will be much more difficult. Orientation to ultraviolet and polarized light, magnetism, electrical fields, and some acoustic stimuli are of this sort. Frequently, organisms respond simultaneously to several stimuli while orienting. Thus, one must be cautious in interpreting observations of orientation behavior since the stimulus most obvious to human senses may not be the most important factor determining the animal's behavior. Frequently an orienting stimulus also elicits a behavioral response. For example, in prey-catching and courtship behavior, the animal often first orients toward the prey or mate, then performs the appropriate behavior to capture the prey or attract the mate. The presence of the prey or potential mate in the environment causes the animal to orient appropriately as well as to perform other behaviors. Of course, at the same time that an animal is stalking prey or courting, it is also using gravity as a stimulus for body orientation relative to the earth. As a tuna pursues a mackerel in the open ocean, the mackerel elicits and orients the predatory behavior of the tuna, but gravity and light stimuli are also used by the tuna for general body orientation.

In addition to species differences for a given orientation behavior, the nature of the orienting stimulus itself may vary as a function of the animal's age. Many nestling birds, for example, show a gaping response which elicits parental feeding. When the nestlings first hatch they are blind, and the

gaping response is released by mechanical or auditory stimuli provided by the parent birds. The nestlings gape vertically, with gravity being the main orienting stimulus. Later, after a nestling can see, the sight of the parent bird not only elicits the gaping response, but also orients it.

Classification of Orientation Responses

The ways that animals orient to their environment are diverse, and certain schemes have been developed to classify these responses in reference to underlying similarities. The classification system presented in this laboratory was first suggested by Fraenkel and Gunn (1961) in their classic book, The Orientation of Animals.

Kinesis

One important distinction that Fraenkel and Gunn make depends on whether the animal's body is oriented with respect to the stimulus source. A movement that does not involve orientation with reference to a stimulus source is known as a kinesis. In a kinetic response, the stimulus produces either a change in the speed of the animal's movement (orthokinesis) or in the animal's turning rate (klinokinesis). These two responses effectively change the position of the animal with respect to the stimulus source. Several examples should clarify this point.

Isopods (terrestrial crustaceans) prefer moist habitats. In some species, as the relative humidity of the environment increases, the amount of time the animal is stationary also increases. This response tends to keep an isopod in damper areas. As another example, some insects can not detect the direction of an odor gradient, but their rate of locomotion varies with the strength of the odor. Thus, if an insect moves rapidly at low concentrations of a chemical and slowly at high concentrations, it should eventually arrive at the source of the odor. The human body louse (Pediculus corporis) finds its host by a kinetic response to a number of stimuli including temperature, humidity, and odor. When in a favorable environment with respect to these stimuli, the louse travels in straight lines. However, if it encounters an unfavorable environment, it turns until a favorable environmental zone is once again encountered. In summary, a kinesis involves quantitative variations in an animal's speed or turning rate with no fixed orientation of the body relative to the stimulus source.

Taxis

In a taxis, the animal's body is oriented in some linear manner relative to a stimulus; either directly toward it, directly away from it, or at a fixed angle to it. Locomotion may or may not be involved in a taxis. This kind of response may be shown for light, heat, moisture, gravity, sound, chemicals, or other stimuli.

Procedures: Investigations of Orientation Behavior

You will be involved in studying some aspect of the orientation behavior shown by one of the organisms discussed below. After making some initial observations, organize yourselves into small, common interest groups and, while consulting with your instructor, design your study. After your project has been approved, your group can begin collecting data to test your research hypothesis. After your data collection are complete, analyze data by calculating descriptive statistics, such as means and standard deviations, and graphing the data in appropriate ways. Your graphs and data tables should be transferred to overhead transparencies in preparation for reporting the results and conclusions to the class at the end of laboratory. In interpreting your results, try to relate what you have learned to the animal's natural environment, and consider how the behavior might be adaptive for the animal's way of life. In the study you do, be sure to collect adequate evidence *before* forming your conclusions!

Isopods

Background Information

These terrestrial crustaceans, sometimes called sowbugs or pillbugs, are common inhabitants of leaf litter and soil. They feed on decaying organic material as well as algae, moss, and bark. Isopods have a pair of compound eyes, two pairs of antennae (although only the second pair is prominent), and seven pairs of legs. When disturbed or desiccated they will roll up into a ball, looking rather pill-like. You will have access to two genera for study: *Porcellio* sp. (woodlice) and *Armadillidium* sp. (pillbugs). Figure 1.3 shows the gross morphological differences between these genera.

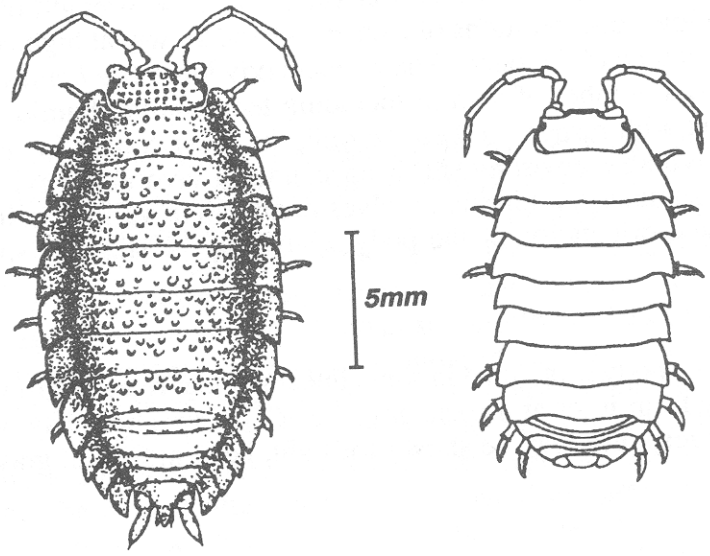


Figure 1.3. Two genera of isopods: *Porcellio scaber* (left) and *Armadillidium vulgare* (right). Modified from Edney (1954).

☐ Obtain a plastic container with individuals of either genus. Carefully remove the lid and observe the location of the animals relative to the moist paper towelling on the dish's bottom. How many are under the towelling? How many are in the open? Remove the towelling and randomize the distribution of isopods within the dish. Replace the towelling and watch how the sowbugs respond. List the environmental stimuli that might be important to the animal in eliciting any observed orientation behavior.

Suggested Studies

A pair of modified petri dishes will be available for studies in which animals are given a choice between two different environments (Figure 1.4). This apparatus can be used to collect quantitative data on the response of individuals to light, humidity, or a combination of these stimuli. For example, to examine the possible effect of humidity on the distribution of individuals within the two dishes, set up the apparatus with anhydrous CaCl2 (a desiccant) in one of the lower dishes and a wet paper towel in the other dish. Expose the apparatus to low, uniform illumination. After about 5 minutes place 10 individuals in each upper dish via the central stoppered holes. Every minute count the number of moving and motionless individuals in each dish. Continue this until your data indicate that the isopods are no longer changing position or moving between dishes.

The same approach can be used to determine if a preference is shown for light or dark environments. One dish can be left exposed to light and the other can be masked with black construction paper on top and sides. After you have gained some insight into the sowbug's response to humidity and illumination (and have collected enough data to support your views!), you can examine the interplay between these two stimulus types. Is the response to humidity changed under conditions of high illumination? What if the individuals are offered a brightly illuminated, humid environment versus a dark, dry environment?

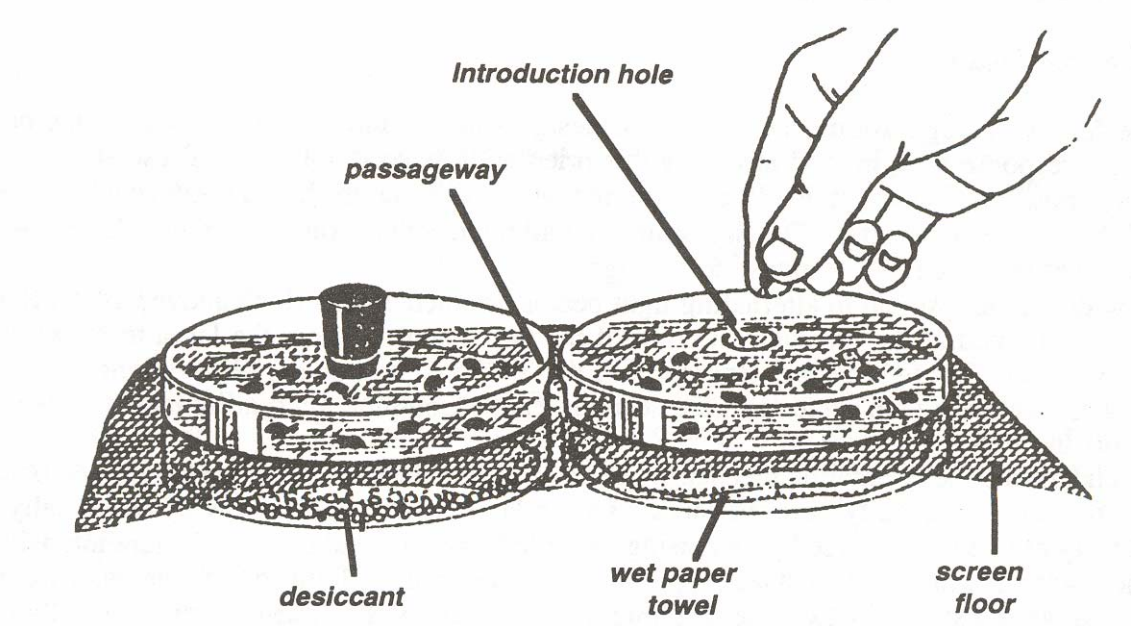


Figure 1.4. Apparatus for studying the orientation of isopods, set up to study humidity preference in constant light.

To better understand the orientation response of isopods, examine the response of individuals within the apparatus. Place one animal in the apparatus and track its progress by marking its path with a pen. Continue this for 4–5 minutes. Use a map measurer to find (1) the total distance travelled in both environments, and (2) the total distances in the two halves of "direct" travel (i.e., maintaining a straight line or following the dish's curve). Also, count the number of sharp curves of more than 90°. Do your data indicate whether the response shown is a kinesis or a taxis? If a taxis is involved, what would you do to determine the taxis type? (The various kinds of taxes are described at the end of this outline.)

Fleshfly Larvae

Background Information

Fly larvae (maggots) feed on decaying plant and animal tissue. As they reach full size and are about to undergo pupation, they exhibit very distinctive reactions to light that presumably help them locate a suitable pupation site. Anatomically, fly larvae (including the fleshfly *Sarcophaga sp*., which will be available to you) possess a single photoreceptor located on the larva's anterior end.

☐ Obtain a plastic dish containing fleshfly larvae. Open the dish and carefully note the location of the animals. Based on these observations can you hypothesize what response the larva might exhibit to light? *Note* the locomotory behavior of the larva. How might an organism with a single photoreceptor be able to detect a light gradient? Place a single large larva in the center of a piece of black construction paper. Position a gooseneck lamp at a distance of about 30–40 cm from the larva. Turn on the lamp and describe the larva's response. Note: If the larva does not respond it may have already begun the first stages of pupation. Return it to the container and obtain another individual.

Suggested Studies

The following suggestions can be used to design studies aimed at understanding the nature of the larva's response to light, and also how this orientation is accomplished. These studies will use two lamps positioned at about 30–40 cm from the larva. Each lamp should produce a beam oriented about 30° from the horizontal. The two beams should intersect at about 90°. You will need to decide how to quantify the larva's response to the light.

How do larvae respond to alternating light beams oriented as described above? Note: Start with the larva positioned at the intersection point of the two beams. Allow the larva to move 5–10 cm in response to the first light beam. Now turn off that light and turn on the second light. Again, allow the larva to assume a path relative to the second beam. Continue alternating lamps until the larva moves off the paper.

To understand the orientation mechanism better, position the larva as above but turn on both lamps at the same time. Carefully note the larva's path relative to the light. Now systematically reduce the intensity of one light source by increasing the bulb-to-larva distance. *Note*: Light intensity varies with the inverse square of the distance separating the larva and the light source; that is, if we increase the bulb-to-larva distance by two, the resulting light intensity will be one-quarter. Carefully measure the initial bulb-to-larva distance and estimate relative light intensity at different distances. Record directional change in the larva's path as the light intensity of one lamp is decreased.

Are there any differences in the response to light shown by larvae of different ages (sizes)? What differences might you expect based on the needs of young, actively growing larvae and older larvae nearing pupation. In studies involving larvae of different sizes, be sure to account for body-size differences in how you measure the behavioral response to the stimulus.

Fish Schooling

Background Information

A fish school can be defined as a group of individuals maintained through time because its members are showing positive orientation towards each other, and not because they are responding similarly to an external factor such as food, light, or shelter. A simple feeding aggregation disperses after the food is consumed, but a true school is a long-term phenomenon. Investigators (see Burgess and Shaw, 1979) have determined that in many fish, vision is the major factor involved in the orientation of schooling fish toward each other; olfaction, sound, and changes in the pattern of water pressure seem to help maintain the cohesion of an established school. Equipment available in the laboratory will allow you to develop studies for testing the role of the visual component in schooling behavior. In most of your investigative set-ups, fish will be separated by barriers which prevent communication by sound or chemical signals.

☐ Examine the large community tank containing five to six fish species, and with the help of your instructor identify the species that are present. Species available may include the following: zebra danios (*Brachydanio sp.*), tiger barbs (*Barbus tetrazona*), swordtails (*Xiphophorus maculatus*), tetras (*Hyphessobrycon sp.*), and a cichlid species in the genus Cichlasoma. Make careful observations so that you can rank the species from weakest to strongest with respect to their school-forming tendencies. What cues did you use in making these decisions? What type of taxis response (described at the end of this outline) do you think is most likely involved in school maintenance?

Suggested Studies

A test tank (Figure 1.5) is available so that you can do studies directed at answering the following three types of questions: (1) How will species differ in their degree of orientation to conspecifics (members of the same species), and is this influenced by the species' school-forming tendencies? (2) What is the effect of group size on orientation to conspecifics, and will this vary for species exhibiting different school-forming tendencies? (3) What visual stimuli are most important in orientation to conspecifics?

Using a marking pen to mark the glass sides of the tank, divide the test tank into sections of equal volume. Place black plastic curtains around the outside of the tank to minimize the disturbing effects of outside movement. Two cylinders are placed in the peripheral sections of the tank. In order to address the first question mentioned above, you can place 4–5 individuals of two different species into the two cylinders. Using a third cylinder, carefully release a test fish in the central section of the test tank and, for the next 10 minutes, record the amount of time that the test fish is in each section. Now test a fish of the other species. Repeat this procedure until all fish of the two species have been tested. Note: What could you do in your use of the test tank to control for any possible preference that the test fish is in each section. Now test a fish of the other species. Repeat this procedure until all fish of the two species have been tested. *Note*: What could you do in your use of the test tank to control for any possible preference that the fish may show for a particular end of the tank?

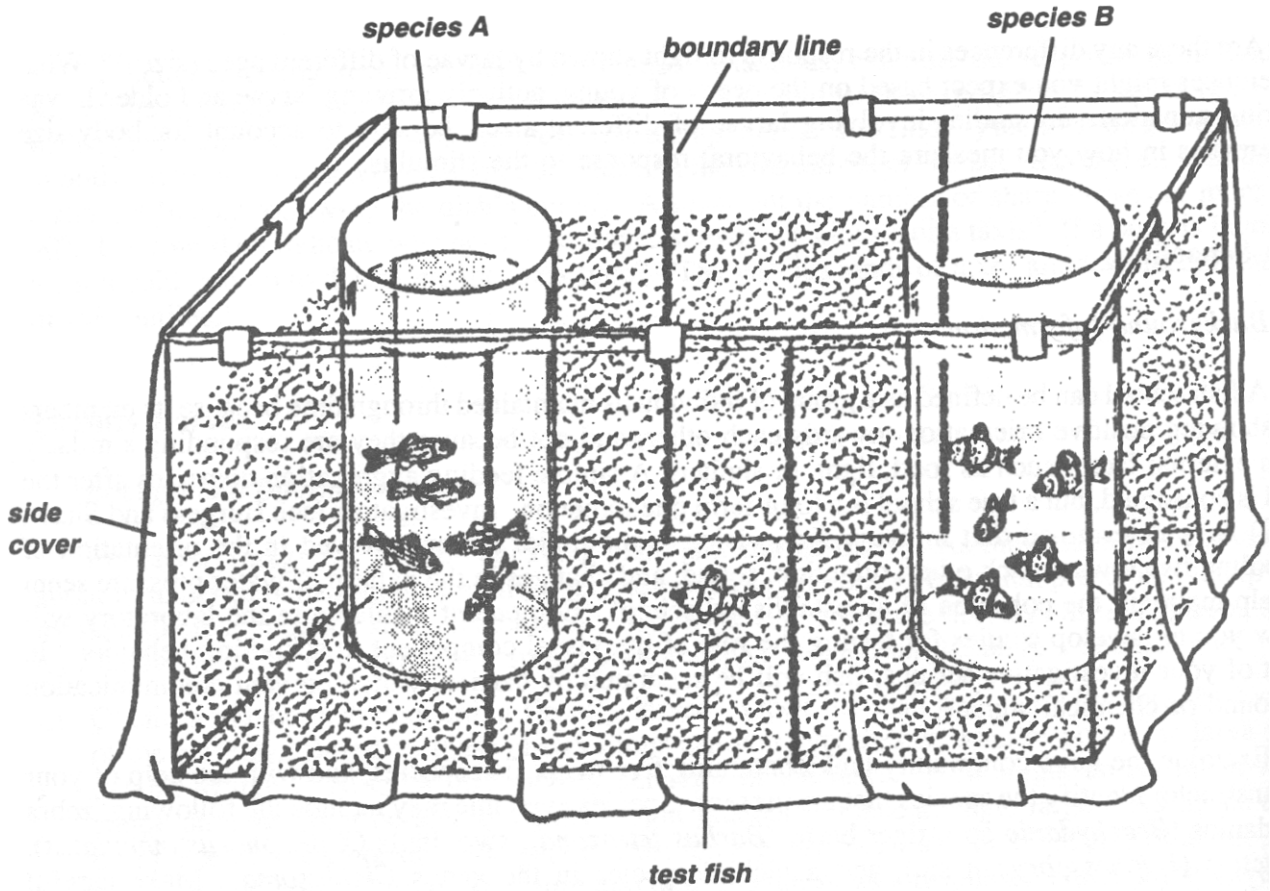


Figure 1.5. Test tank used to study factors affecting fish school formation.

A similar procedure can be used for examining the effect of group size on orientation to conspecifics. In this case, place 2–3 individuals of one species in a cylinder at one end of the tank and 7–8 of the same species in a second cylinder at the other end. Release the conspecific test fish into the central section and record your data as described above.

Behaviorists frequently construct simple physical models to help them better understand which visual stimuli are most important in eliciting a behavioral response. If schooling results because individuals show positive orientation towards conspecifics, then we can ask which physical features of a species are most important in eliciting attraction. After you have selected a species showing strong schooling tendencies, you can construct two-dimensional models of the species with transparent sheets of plastic and marking pens. Test a school of "complete" model fish against schools in which the fish lack certain potential visual stimuli. Use the same procedures to collect data as described in the previous two studies. The plastic sheets of model fish schools should be constructed so they will fit into the cylinders. Perhaps school movement per se is also an important stimulus. How could this hypothesis be tested?

Planaria

Background Information

Planaria are common freshwater flatworms usually found under rocks in streams and ponds. They glide about using cilia located on their lower surface. The two "eye" spots near the front end are groups of pigmented photosensitive cells that allow the planarian to sense light levels in its environment.

☐ Observe the white enamel pan with a collection of the Brown Planaria (*Dugesia tigrina*). Describe the location of the planaria relative to the piece of black paper covering half of the container. What environmental variables might be eliciting any orientation you observe?

Certain animal species orient themselves in the environment so as to maximize contact with solid surfaces. This type of orientation behavior is called thigmotaxis. Animals showing thigmotaxis tend to aggregate under solid objects and their orientation is sometimes incorrectly assumed to be a response only to light.

Suggested Studies

Petri dishes and both blackened and clear glass coverslips can be used to observe the response of planaria to light and solid objects. Place one clear and one blackened coverslip in a petri dish filled with pond water and carefully add 15–20 planaria. Illuminate the petri dish from above with a lamp placed 20 cm from the dish. Every several minutes, determine the numbers of planaria under each coverslip and in the open. Repeat this until no significant change in the distribution of planaria is observed. What are your expected results if planaria are only reacting to light? What are your expected results if planaria are only showing a thigmotaxis? (*Hint*: In order to analyze your results quantitatively, you may want to measure the surface area of the petri dish and the coverslips). Perhaps planaria respond to light and *also* show thigmotaxis. How might petri dishes and coverslips be used to test such a hypothesis?

Water Fleas (*Daphnia magna*)

Background Information

This freshwater crustacean is part of the primary consumer, zooplankton populations present in most ponds and lakes. *Daphnia* have a single median compound eye and flattened, leaf-like legs that are their chief respiratory organs. Several pairs of legs have distinct comb-like setae used for filtering phytoplankton from the water. A pair of branched antennae are used in locomotion. Many zooplankton are known to make diurnal vertical migrations within lakes, presumably in reference to changes in light intensity levels, moving up within the water column after sundown and returning to greater depths as day approaches. These vertical migrations are thought to place the zooplankton within the upper, more productive parts of the aquatic ecosystem at a time when predation and the damaging effects of excessive illumination would be at a minimum. Based on these observations, one would expect that *Daphnia* should respond to changes in light intensity. Other than light cues what additional stimuli might be important in these vertical migrations?

In addition to light intensity, the proportion of different wavelengths within the light (light quality) may also serve as an orientation stimulus for *Daphnia*. In developing expectations, two ecologically important light-related phenomena should be considered. First, water tends to differentially absorb more long- than short-wavelength light. So at a greater depth, the proportion of blue light increases, as red light decreases. Thus, the proportion of light wavelengths striking

Daphnia could potentially provide it with information on its location in the water column (its depth). Second, phytoplankton contain the green pigment chlorophyll that also differentially filters out certain light wavelengths more than others, potentially giving *Daphnia* information on whether phytoplankton populations are present in the water column above it or not (useful information for a herbivore). Both types of information are only available to *Daphnia* if it has evolved the sensory equipment to see colors. What are your expectation if it has not?

☐ Observe the suspended 100-ml graduated cylinder containing a *Daphnia* population. Using the cylinder's 10 10-ml divisions, characterize the vertical distribution of the population. Position a light below the cylinder and look for changes in the population's distribution.

Suggested Studies

Additional *Daphnia* populations in cylinders are available for studies on light intensity and quality. Try comparing the distribution of populations exposed to the following conditions: no light, low-intensity light from above, and low-intensity light from below. Each cylinder with its population of *Daphnia* should be tested under all conditions. A black plastic sleeve can be made so that light can be applied from only one direction. After 5 minutes, the sleeve can be quickly removed and the distribution characterized. How will these results allow you to relate *Daphnia*'s response to both light and gravity? Does a higher light intensity produce predictable results based on your initial studies? Be sure to collect adequate data before making conclusions!

To look at the effect of light quality, you will have access to three colored filters and additional lamps. One concern in using filters to examine an organism's response to different light wavelengths is that the filters may not transmit the same light intensity. Light intensity must be the same under all conditions or the study would have two confounding treatments. Figure 1.6 shows the relationship between the distance from the light source and the light intensity measured by the photometer for the three filter types we have available. As you can see, a lamp-sensor distance of about 10 cm for blue, 25 cm for green, and 30 cm for red produces an equal light intensity for all three filters of about 17 μE m-2 sec-1 (microeinsteins per meter square per second). The other concern with filters is that you determine exactly what light wavelengths are transmitted, since filters may not transmit a single or narrow range of wavelengths. Figure 1.7 shows the transmission spectrum for each filter; these spectra were obtained with a spectrophotometer, using techniques that some of you may have used in the photosynthesis study earlier in the semester.

Classification of Taxis Types

Taxes (singular taxis) have been classified by Fraenkel and Gunn (1961) according to (1) the type of stimulus eliciting a response and (2) how the organism accomplishes the orientation. For example, the response would be termed phototaxis if the stimulus were light, geotaxis if the stimulus were gravity, and chemotaxis if the response involved a chemical stimulus. To be even more precise, since the organism is orienting toward the light, its response may be described as a positive phototaxis. Use the data you have collected to characterize the behavior of your organisms. Do they show a kinesis, a taxis, or both? Try to be as precise as possible when describing the orientation response of your study organism.

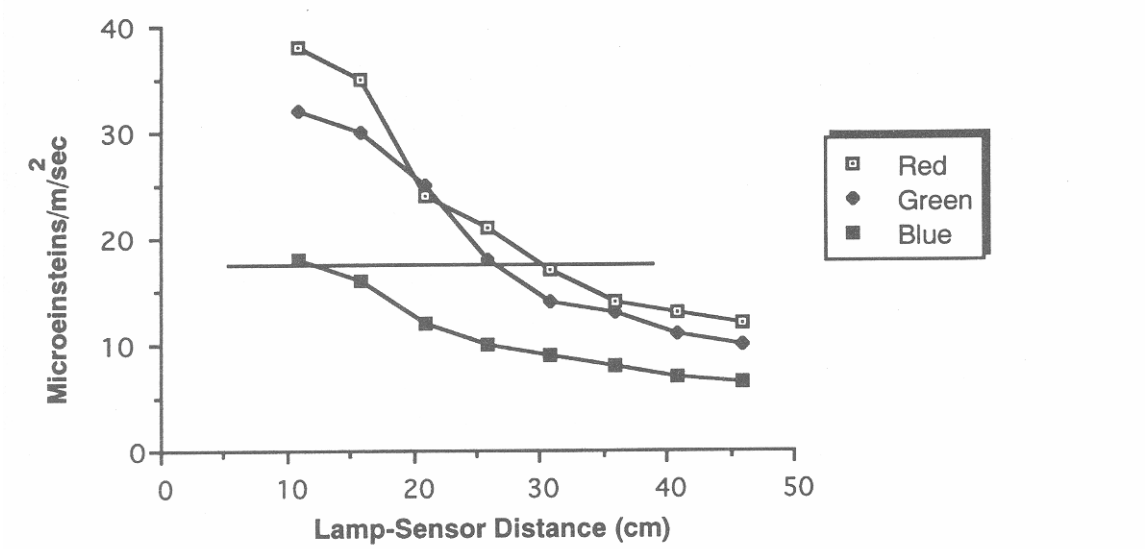


Figure 1.6. Relationship between light intensity (μE m-2 sec-1) and the lamp-bulb distance (cm), using 150-watt incandescent and two 25-watt fluorescent bulbs as a light source, for three filters (one sheet Dark Urban Blue, #866; one sheet Medium Green, #874; and three sheets Medium Red, #823). Light intensity was measured with a Li-Cor model LI-185A quantum/radiometer/photometer.

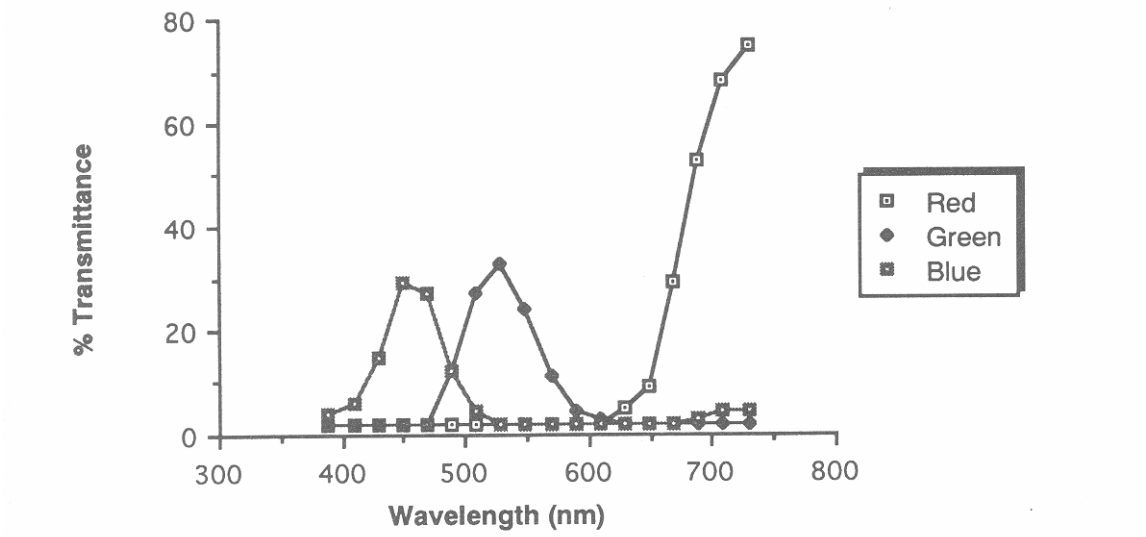


Figure 1.7. Transmission spectra for three filters (one sheet Dark Urban Blue, #866; one sheet Medium Green, #874; and two sheets Medium Red, #823) obtained with a Spectronic 20 spectrophotometer.

One type of taxis is dependent on bilaterally symmetrical receptor organs. The animal orients either directly toward or directly away from the stimulus source by positioning itself with reference to the stimulus such that both organs are receiving equal stimulation. If the animal is locomoting, it moves directly toward or away from the stimulus source. This type of orientation can be recognized in two ways, as exemplified by the isopod *Armadillidium* (see Figure 1.8). Under certain conditions, this animal is photopositive and will move toward a light source by orienting so that its bilateral photoreceptors are equally stimulated. If blinded in one eye and then exposed to diffuse light, it will continuously attempt to equalize stimulation. The circling movements of unilaterally-blinded animals are characteristic of a taxis dependent on bilateral photoreceptors. Also, if *Armadillidium* is offered two light sources of equal intensity, it will advance to a point equally distant between them before turning toward either the right or the left. It should be noted that pillbugs are not always positively phototactic. The positive response appears particularly after a sudden rise in temperature, or a period of starvation or desiccation. What adaptive significance might this variability in response to stimuli have for a terrestrial animal?

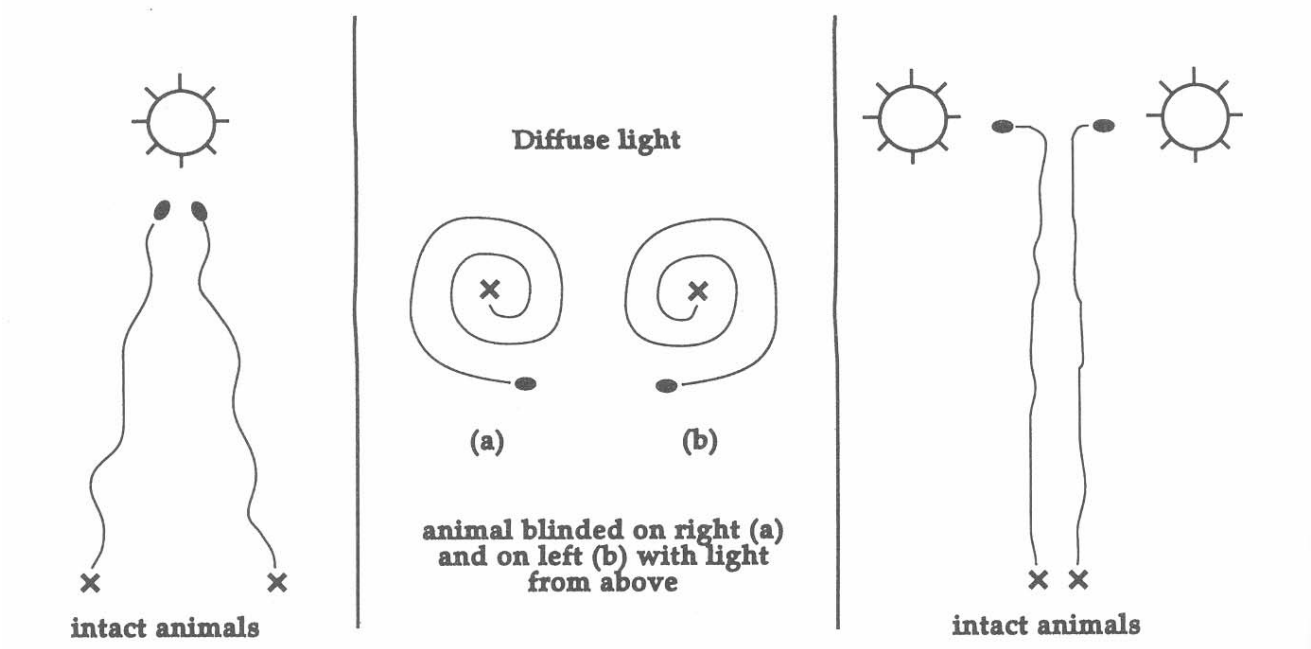


Figure 1.8. Taxis response based on bilateral photoreceptors of the pillbug, *Armadillidium* sp., under different conditions. X = starting point; • = location of pillbug.

The dorsal light reaction is another important form of a taxis dependent on bilateral receptor organs that is exhibited by many aerial and aquatic organisms. This reaction functions to keep the animal's dorsal surface uppermost and oriented toward the natural light source. Again, this is accomplished by maintaining equal stimulation of bilateral photoreceptor organs. Argulus, a marine crustacean, normally swims with its dorsal surface toward light. In a natural situation, the light always comes from above. However, if an artificial light is presented from below, the animal will swim about upside-down. If one eye is removed, it makes the characteristic continuous circling movements.

Many fish show the dorsal light reaction, but in most it is modified by information on gravity detected by the labyrinth organ of the inner ear. Thus, the orientation of labyrinthectomized fish is determined only by the position of the light, whereas normal intact fish orient using both the direction of light and the pull of gravity (Figure 1.9). See the demonstration of the dorsal light reaction in angel fish.

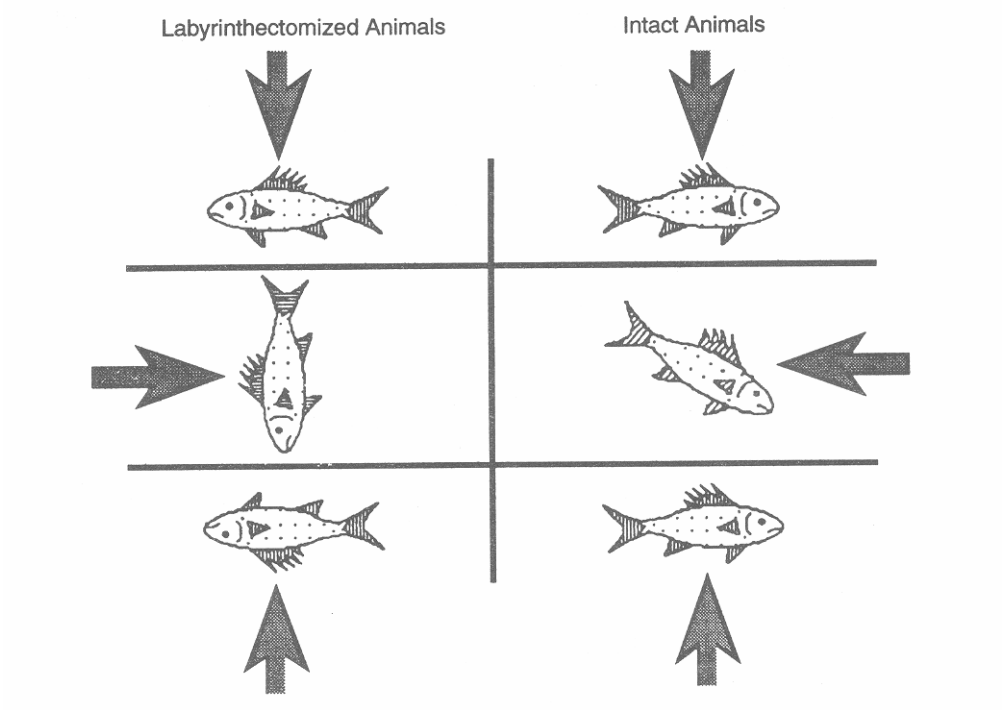


Figure 1.9. Dorsal light reaction of fish. Arrows indicate direction of light beams.

Some animals with but a single receptor organ can still show a taxis movement either directly toward or away from a stimulus source. In this case, the animal first turns its single receptor one way, then the other way, determining in this manner the direction in which the stimulus source is strongest. Certain fly larvae show this form of orientation during the period preceding pupation. Larvae are photonegative and thus seek out dark areas. They do this by turning their head (where the light receptor is located) first in one direction, then the other. Based on this information, the animal advances in the direction of least light. This type of taxis is most common in orientation to chemicals, as in the food finding behavior of planaria, and is characterized by the side-to-side movement of the animal's forebody during locomotion.

In another type of taxis, known as goal-directed orientation, the animal orients directly toward or away from the stimulus source even if one receptor organ of a pair is removed (the animals do not show circling movements). Moreover, if presented with two equally intense stimuli the animal does not pursue an intermediate path, but rather aims directly at either source, in some way disregarding the other. This suggests that the animal is not orienting by equalizing the stimulation intensity on the two sides of the body, but rather perceives the stimulus as a discrete goal.

Examples of goal-directed orientation are many and include the orientation of the nestling gaping response toward the parent bird's head, the positioning of toads towards their prey prior to striking, and the photopositive response of hermit crabs (see Figure 1.10).

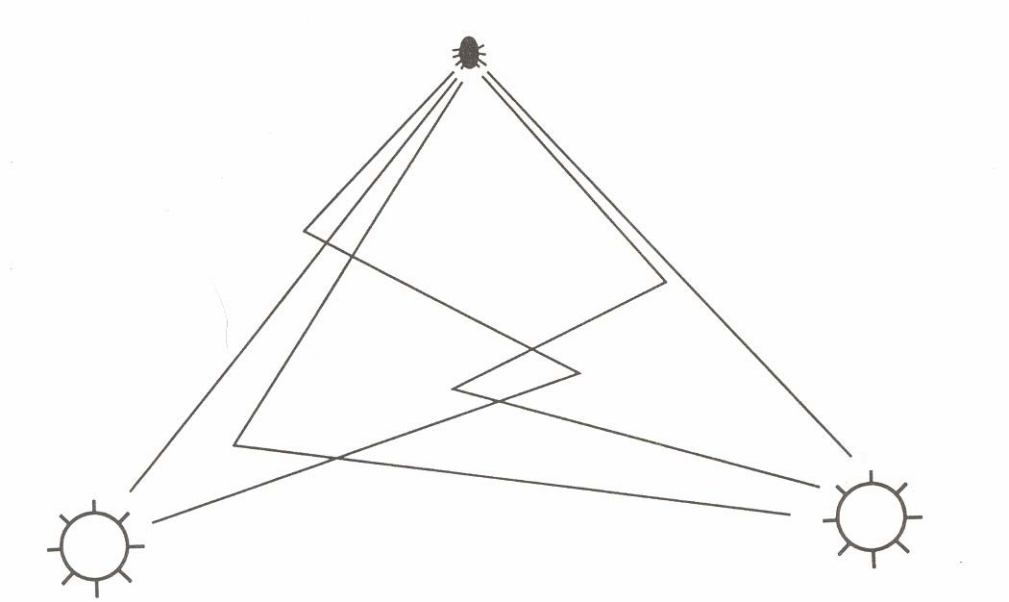


Figure 1.10. Photopositive response of hermit crabs presented with two light sources.

Considered by some to be a special type of goal-directed orientation, light-compass orientation is found in many insects and vertebrates. Light-compass orientation involves orientation at a constant angle with respect to a light source, such as the sun or moon, and enables the animal to obtain compass information while moving through unfamiliar territory. Changing the apparent position of the celestial body with a mirror results in a predictable change in the animal's orientation. A time sense is necessary in order to compensate for the sun's apparent movement across the sky.

A moth flying into a candle is showing light-compass orientation that has gone awry. As with many nocturnal insects, the moth tends to fly at a fixed angle relative to the moon. However, with a nearby light source, such as a candle, as the moth moves it must constantly change its flight path in order to maintain a fixed angle relative to the light. The closer it gets to the light source, the more curved its flight path becomes. This results in the characteristic spiralling flight into the flame.

Orientation can also be based on memory (sometimes called landmark orientation), where the animal learns the prominent features of its environment and uses this "stored" information to guide its future movement. This type of taxis is certainly common in vertebrates, many of which learn the "lay of the land" in the areas where they spend much of their time. Some invertebrates, such as the solitary sand wasps, use landmarks to find their nests (Tinbergen, 1958).

The mechanisms underlying taxes are often complex and depend on the morphological and physiological characteristics of the organism. Furthermore, these forms of behavior are not as rigid as is sometimes assumed. For example, the response of an animal to light may be changed or strengthened according to whether it is hungry or satiated, desiccated or has had access to water, or whether it has most recently been in the dark or not. In addition, it is biologically important for animals to be released occasionally from behavioral responses which would otherwise trap them. Woodlice (*Porcellio sp.*) clustered under bark in darkness and relatively high humidity must emerge at some time to feed. It is not surprising to find, therefore, that as the temperature drops in the evening their positive-humidity response weakens, and they emerge to run about and feed.

You should also recognize that a species may possess several different orientation responses, even those involving the same sensory receptors. Grayling butterflies (*Eumenis semele*), for example, will fly into the sun to escape predators. This response is dependent on paired photoreceptors because, if blinded in one eye, the butterfly will perform circling movements. However, during courtship males fly after females and are also guided primarily by optical stimuli; yet, because they can still do this if unilaterally blinded, the mechanisms controlling the two responses must be different.

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