Invertebrate Experiments

And

Research Projects

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This is an assemblage of exercises, experiments, and research projects that one can do utilizing invertebrates. I developed several of them while working with students; many others have come from a variety of published and unpublished sources. Although some specifically mention species found along the southeastern coast of the United States, similar species can be found elsewhere

Enjoy!

Protistans

1. **Selective vital staining:** Such staining will kill the organisms eventually, but makes detailed analysis of protistans easier. These dyes can be added as 0.01% aqueous solutions. Useful stains and what they color are as follows: Neutral red - food vacuoles, Janus green B - mitochondria, Sudan black B and Sudan IV - lipids, Brilliant cresyl blue - several structures. Although the organisms will be killed rapidly, acidified methyl green can be used to stain nuclei. If appropriate microscopes are available, phase contrast and dark field optics will provide excellent views of unstained specimens.

2. **Digestion in *Paramecium*:** Add equal amounts of *Paramecium* suspension and a congo red-yeast cell mixture to a wet mount. Ring the drop with vaseline to decrease desiccation and add a cover slip. Observe ingestion of the stained yeast cells by the *Paramecium*. Record the color of food vacuole contents after 1, 5, 10, 20, and 30 minutes. What is the significance of the color changes in the food vacuoles (orange above pH of 5; blue below pH of 3)? The congo red-yeast mixture is made by mixing 1-2g of dry yeast with 30 mg congo red and boiling for 10 minutes.

3. **Ciliate Osmoregulation:** Place ciliates in the cavity of a slide and flood them with distilled water, 0.1%, 0.2%, 0.4%, and 0.8% seawater, then distilled water once again. Time the frequency of contractile vacuole contractions at each salinity. What is your initial hypothesis with respect to the likely results of this experiment? What is the basis for your hypothesis? What do the results of the experiment suggest with respect to the comparative importance of contractile vacuoles in marine versus freshwater protistans? As a practical point, attached, stalked ciliates are easier to work with in this experiment. Also, ensure that the slides do not dry out or become too warm.

4. 4. **Protistan Succession:** Put some dry vegetation, such as hay or grass into distilled water in a jar and cover it. Every 2-3 days during the next 2-3 weeks remove drops of water and examine them. Using available references, record the genera present and their relative density (*e.g.*, average number per high power or low power visual field). Does a succession of different taxa occur?

5. ***Euglena* Phototaxis:** Fill a large test tube with a dense culture of *Euglena* and surround it with opaque paper or foil. After thirty minutes remove the paper and note the distribution of organisms. Then cut a 0.5 cm hole in the foil and repeat. What accounts for the difference in distribution?

Porifera

1. **Sponge Symbionts:** Carefully macerate different species of sponge after measuring the volume of each by displacement using a graduated cylinder. Count and identify to the lowest possible taxa all symbionts found within them (if polychaetes are torn up during the maceration process, count polychaete heads). Calculate densities per cubic centimeter of sponge and proportion of the total symbiont fauna for each taxon. Are there differences in the symbiont community or symbiont density among the different species of sponge? One can also do this study sampling one species of sponge during an entire year to see if symbiont species and/or densities change as the seasons change or sampling a species of intertidal sponge from subtidal to its highest occurrence intertidally to see if the degree of exposure influences the symbiont assemblage. Remember to take replicate samples.

2. **Sponge Reaggreation:** You can use a variety of species. The genera *Microciona* and *Cliona* work quite well. Cut a piece of sponge into very small pieces using scissors and place them in the center of a square of silk or similar tightly woven material. Wrap the material around the sponge pieces and dip it into a small dish of filtered seawater or fresh water (depending on the sponge's habitat). Squeeze the sponge material through the fabric until the water is only *faintly* colored by the disaggregated cells; otherwise you could have problems with reduced oxygen and/or a bloom of bacteria. Place the dishes, properly labeled, into a cool area. They will not need aeration or to have the water changed. Before throwing away the pieces of sponge, press them once again into water in a small beaker until the color gets quite dark. Make a wet mount from this beaker and

examine the cells. Disaggregated sponge cells will characteristically send out *very* thin pseudopodia, which will pull other cells in, thereby reaggregating the cells. Do you see any pseudopodia? Do you see any movement of choanocyte flagella? After about 3 days examine the reaggregation experiment dishes using a dissecting microscope. Do you see any clumps of cells? Using a razor blade, remove a mass of sponge from the bottom of the dish, make a wet mount, and examine it using a compound microscope. Do you see any pseudopodia?

3. **Hatching of Gemmules:** Get gemmules of freshwater sponges and keep them in a refrigerator for a few weeks (this mimics winter). Then put each gemmule on a microscope slide in shallow water in a petri dish. Petroleum jelly aids in sticking it to the slide. Check the slides daily. Where does the mass of archeocytes leave the gemmule? How long until you can see spicules in the sponge tissue? How long until oscula are seen? Can you see water canals developing? After hatching, draw the appearance of the sponge each day for a week. Note that, depending on species and environmental conditions, gemmules may take from a couple of days to three weeks to hatch.

4. **Larvae:** Most sponges have parenchymula (= parenchymella) larvae, while calcareous sponges, *e.g. Scypha* and *Grantia*, have amphiblastula larvae. The common East Coast redbeard sponge, *Microciona prolifera*, is a good source for parenchymula larvae during warmer times of the year. Place two to three 3-inch pieces of recently collected *M. prolifera* into a beaker of filtered seawater. From half a dozen to a dozen red, oval larvae should be released within three to four hours. Other species can be treated similarly. Make a wet mount of a larva. Can you see any spicules? Do you see evidence of external flagella (remember to adjust your microscope's diaphragm to make flagella more visible)? Place some larvae in watch glasses, change the water twice daily, and put in cover slips for attachment. Attachment may occur in a day or two. Examine these specimens closely through the next few days. To examine possible changes in larval behavior with age, in a diffused-light room (to avoid phototaxis affecting your results), place recently released larvae into a graduated cylinder, discharging them from a pipette at about mid-depth. Do the larvae consistently swim up or down (*i.e.*, demonstrate geotaxis)? Repeat the experiment with larvae more than a day old. Are the results the same? If they are different, how might this change in larval behavior with age be of adaptive value for the species? Design an experiment to examine larval response to directional light (without results being confounded by gravity). How do the possible phototaxis results compare to results examining possible geotaxis?

5. **Examination of Sponge Cells:** Place a living colony of *Leucosolenia* in a dish of seawater and then squirt some carmine particles into the water. Can you see any evidence of sponge-generated water movement? After several minutes, cut one of the members of the colony in half from the osculum to the base. Then cut a very thin slice from around the rim of the sectioned sponge. Make a wet mount of this slice using seawater. Can you see any evidence of flagellar movement by the choanocytes (remember to adjust the diaphragm of your microscope)? Are any of the cells amoeboid in form (teasing the cells apart with a fine needle after lifting the cover slip may make the search for amoeboid cells easier)? Examine your section to see if any cells contain

carmine particles. Which cells would you hypothesize these would be? Another view can be obtained by placing one-half of the longitudinally sectioned sponge in a drop of seawater on a slide with the inner surface facing you. Cover it with a cover slip and then focus down until the action of the choanocyte flagella can be seen.

Cnidaria

1. **Cnidarian Larvae:** Many scyphomedusae will brood embryos for a while, especially on the oral arms. Therefore, any living scyphomedusae should be carefully examined for embryos. If any are present, examine them using a compound microscope. Note the typical planula external ciliation. These larvae will soon attach to the bottom of a dish of clean water and rapidly metamorphose into scyphistomae. If hydromedusae are received in shipment from a supply house, examine the shipping water carefully for larvae; spawning may have occurred in transit. During the summer months hydroid colonies that possess attached, degenerate medusae can be kept in clean water overnight. Depending on the breeding season, many such colonies will release larvae in the morning. Glass slides can be placed in the dishes in the event larval attachment is rapid. If larval release does not occur, embryos may be teased from the gonophores using fine needles (note that usually all the gonophores on one colony will be the same sex). Genera good for such analyses are *Bougainvillia*, *Clava*, *Eudendrium*, *Hydractinia*, and *Tubularia*. If several hydromedusae of a species are available during the summer, keep them in large dishes to see if spawning occurs. Make sure any developmental stages are not crowded.

2. ***Hydra* Regeneration:** Boil some pond water and set it aside for an adequate period to re-aerate. Cut up a *Hydra* into as many pieces as you can using a flamed scalpel or needle. Place the pieces in a small watch glass (so the pieces are in contact with each other) that is sitting in a larger dish of pond water. The pieces should reconstitute in 4-5 days. If you cut up and mix a brown hydra and a green hydra (*Chlorohydra*), you can see if they separate out (indicating species recognition by aggregating cells).

3. **Medusa Equilibrium:** Watch a scyphomedusa swim. Cut away the rhopalia. How is the swimming affected?

Ctenophora

1. **Ctenophore Larvae:** If a living ctenophore has been isolated in a dish or bag of seawater for a night or longer, make sure you examine the water carefully for larvae; they will frequently self-fertilize. If larvae are present, examine them using a compound microscope. How do they compare with a cnidarian planula larva? With an adult ctenophore?

2. **Effects of Prey Chemicals on Ctenophore Swimming Behavior:** To examine whether chemicals released from the bodies of nearby prey affect the swimming speed of a ctenophore, place a large number of *Artemia* larvae in seawater having the same salinity as that your ctenophores are in. After a few hours, pour the *Artemia*-containing water through a filter — this is now "*Artemia*-conditioned" seawater. Draw a large plus sign on a blank sheet of paper. Prepare two large dishes, one of which contains *Artemia*-conditioned seawater and the other unconditioned seawater. Place a ctenophore (lobates such as *Mnemiopsis* work nicely) into one of the two dishes (determined randomly), place the dish on top of the sheet with the plus sign (which needs to be as large as the bottom of the dish), and let it rest for 30 seconds. Then count the number of times the ctenophore crosses one of the lines of the plus sign within a period of 2 or 3 minutes. Repeat for the same ctenophore in the dish containing the other kind of seawater. Do this paired comparison with several ctenophores. Does the presence of prey chemicals change ctenophore swimming speed? If so, do they go slower or faster? Of what adaptive value would this change in swimming speed be for the ctenophore?

Platyhelminthes

1. **Regeneration:** Planarians are well known for their powers of regeneration. Use animals that have been starved for several days. Using a sharp scalpel, make clean, perpendicular cuts through the body of several planarians. Specimens can be cut by putting them on a clean slide with minimal water; putting the slide on an ice cube greatly facilitates the process. Different types of operations can be done: a) removal of the head; b) removal of the region posterior to the pharynx; c) a combination of a and b; d) slicing a long portion of the body in half longitudinally (such an incision must be re-cut in 24 hours). Use several animals and keep them in individual dishes in a cool, dark area for 1-2 weeks. They should not be fed and the water should be changed daily, at which time record the degree of regeneration. How successful was regeneration? Did regeneration of the anterior and posterior ends of the body occur equally rapidly or does there appear to be some anterior-posterior variation in this? Did the proper "end" of the worm regenerate where the excision was made or did you get some two-headed or two-tailed individuals?

2. **Flatworm Movement:** Cilia and muscular activity are both involved in flatworm movement. To determine their relative contributions, animals can be placed in either 1-2% lithium chloride, which reduces ciliary action, or 1-2% magnesium chloride, which reduces muscular action. Is one more important than the other in being responsible for locomotion? Do you see a difference when small animals are compared with large? If living flukes are available, such as lung flukes from frogs, the same analysis can be done. How do the turbellarians and flukes compare?

3. **Feeding Behavior:** Using several starved planarians, examine how rapidly they can locate a small piece of meat in a small dish. Do many repetitions with several

animals, with initial distance between animal and food the same. Then remove the auricles (lateral, grooved lobes) from the sides of the head of most of the animals and time how long it takes them to locate the food, as before. Retest the undamaged specimens as controls. Is there any change in the time needed to find the food in the de-auricled animals? Does this suggest any function of the auricles to you?

4. **Influence of Fluke Parasites on Host Behavior:** *Ilyanassa obsoleta*, the "mud snail," is a common estuarine species found along the eastern coast of North America. It is commonly infected by larvae of a fluke species in which the next intermediate host includes several intertidal crustaceans, such as fiddler crabs and amphipods. It would certainly appear to be advantageous to the fluke if infected snails were close to the intertidal zone, enabling the released cercariae to swim only a short distance to their next host. One can examine whether infection of *I. obsoleta* by fluke larvae causes the snail to migrate into shallower water. Collect many specimens of *I. obsoleta* from each of several depths, break open their shells, and tease apart gonadal and digestive gland tissue in small dishes of seawater to search for fluke larvae. Calculate percent of hosts infected for each depth. Is there convincing evidence for increased infestation in snails found in shallower water? If so, how might the fluke larvae induce such a behavioral change in their host?

Mollusca

1. **Scavenging Gastropod Feeding Behavior:** Cover the bottom of a small aquarium with 1-2 cm of muddy sand. Then put in several specimens of gastropods such as *Ilyanassa* or *Nassarius*. Permit them to disperse throughout the aquarium and move beneath the sediment surface. Then place a small piece of fresh meat into the aquarium. What is the response? Do the animals seem to be using their osphradia for locating food? What is the basis for your conclusion?

2. **Trail Following in Gastropods:** Using snails crawling over a relatively flat, hard substrate, examine what behavior takes place when an individual encounters a trail left by another individual. If encountering the trail alters the movement of the snail, in which direction does the individual then move? Are there differences between different species of gastropod with respect to trail-following? Do individuals only follow trails of conspecifics or only certain sexes or sizes? What effect does tentacle removal have on this behavior?

3. **Nematocyst Storage in Nudibranchs:** Collect some nudibranchs with cerata (aeolid nudibranchs) from their cnidarian prey and make a compressed wet-mount of the nematocyst storage sacs (cnidosacs) at the tips of their cerata. Examine the contents of these sacs using a compound microscope. Can you recognize nematocysts? Add some acetic acid/methylene blue solution (which stimulates nematocysts to "fire"). Does anything happen?

4. **Prey Selectivity and Predator-Prey Interactions in Nudibranchs:** Do hydroid-feeding nudibranchs show evidence of prey species specificity? There is evidence that some species avoid *Tubularia* (which has large polyps with powerful nematocysts) when young, but feed on it when older. Is there evidence for this prey selectivity in your species? Does the nudibranch orient into low-velocity current when the water source includes a prey item, but move randomly in the absence of prey? Does this reaction differ for different prey species? What is the behavior of the nudibranch when it encounters a polyp? Does it vary among different hydroid species (*e.g.*, holding the rhinophores away from *Tubularia* tentacles)? Expose nudibranchs that have fed on *Tubularia* and those having fed on *Eudendrium* (with smaller polyps) to potential predators, *e.g.* fish or crabs. What is the response of the predator to nudibranch contact? Consumption? Mouthing and rejection? Complete rejection? Does it depend on the size (age) of the nudibranch? Is there any evidence that the source of the nudibranch's nematocysts (prey species) affects the response by the predator to nudibranch contact? What is the reaction of the polyp to nudibranch contact? On what does the nudibranch feed — does it just engulf polyps; suck tissue; eat everything, including branches; prefer gonozooids or gonophores in those colonies having them; or feed on any associates of the hydroid colonies (*e.g.*, stalked ciliates, bryozoans, entoprocts)? Do the nudibranchs completely destroy a colony or is there only partial destruction, allowing for prey recovery? What is the reaction of nudibranchs to stimulation, *e.g.* touching it with a fish or crustacean or mimicing predation by squeezing with forceps? Are there secretions, behavioral responses, pH changes of the body surface? Do predators still avoid nudibranchs that have had their cerata and/or brightly colored portions removed? Does it depend on whether you have previously exposed these predators to non-modified nudibranchs?

5. **Nudibranch Reproduction:** Examine the behavior associated with egg mass deposition in a species of nudibranch. In coiled egg masses, is there consistency with repect to whether they are laid clockwise or counter-clockwise? How many eggs are found in each mass? Does this vary depending on nudibranch size? How frequently does an individual nudibranch deposit an egg mass? Are egg masses laid at certain locations of the host colony, *e.g.* where polyps have been removed or not removed, where there is branching, toward the inside or outside of the colony, on zooids having brown bodies (& lacking lophophores) in bryozoan feeders? What is the reaction of a potential predator when it encounters an egg mass? Is there any evidence that a chemical in the egg mass discourages predators? If so, what is the reaction of a predator to being placed in a small dish having an extract of egg mass added? What animals, if any, eat the egg masses? When they hatch, do you find planktonic veligers, crawling veligers, or does it vary? Will the veligers settle and metamorphose on any solid surface? Must the substrate be a specific hydroid colony or bryozoan for them to settle early or at all? Is it species specific with respect to the potential prey substrate?

6. **Substrate Selection in Gastropods:** Many gastropod species, like many other invertebrates, are quite selective as to the substrate on which they are found. This is particularly true for species that are very specialized in their diet, such as many nudibranchs. For the following experiment, construct a Y-tube apparatus in which water

flows down each arm of the Y from a different container. Several gastropods can be used, including nudibranchs removed from their cnidarian, bryozoan, or sponge hosts; other nudibranch-like opisthobranchs removed from their algal food; or *Boonea*, a small pyramidellid opisthobranch that parasitizes oysters. Place the actual substrate of the test animal, such as a hydroid colony, in one container of the Y-tube and another possible substrate, such as a sponge, in the other. Place the test animal in the neck of the Y-tube as water passes by it from the two containers. Record which neck of the Y the animal enters. Repeat this several times with different animals for each of several substrate choices (clean the inside of the Y-tube between experimental runs to remove the mucus film of the previous animal). Also do it several times with controls (clean water in both sides). Do any of the test species show evidence of substrate selection? Does the degree of selection vary between species?

7. **Phototaxis in Gastropod Veligers:** Prepare a deep dish containing large numbers of swimming veliger larvae, such as those recently hatched from *Ilyanassa* or *Polinices* egg capsules. In dim, diffuse room light (or red light), shine a light on the dish from one direction. After several minutes examine the distribution of larvae. Do they show evidence of positive or negative phototaxis? Maintain the larvae for a while and repeat the experiment. Does the response to light vary with larval age? How might positive or negative phototaxis be of adaptive value to a swimming larva?

8. **Burrowing in Bivalves:** Using a burrowing bivalve such as *Donax*, time how long it takes to burrow into sand. Then make up various mixtures of sand and mud from all sand to all mud. Determine burrowing time in all substrate types. Does type of substrate have an influence on how rapidly the bivalve can burrow? Does drying out the substrate have any effect (note that *Donax* is intertidal)?

9. **Escape Behavior in Scallops:** Place a living starfish in an aquarium that contains a living scallop or *Lima* (closely related "file shell"). Note the swimming escape behavior by the bivalve. Since scallops and *Lima* have eyes, it is possible that sight caused the reaction. To see if sight (*e.g.*, detection of movement) is all that is involved, add some fluid from the macerated arm of a starfish (or water from an aquarium that has contained starfish) into the aquarium with the bivalve. Is there any reaction? What is your conclusion about the senses that may be involved in scallop escape behavior?

Annelida

1. ***Chaetopterus* Feeding:** Carefully remove a *Chaetopterus* from its parchment-like tube and insert it into a similarly sized, transparent U-tube. *Chaetopterus* will live well in the tube once it is placed in an aquarium. Watch feeding by adding carmine suspension or congo red-yeast suspension near the mouth of the tube closest to the anterior end of the worm. Watch the action of the worm's pumping "fans," movement of water through the tube, and capture of particles by the secreted mucous bag. Periodic ingestion and re-secretion of this bag will occur. After you are through with the worm, remove it from its

tube and leave it undisturbed in the dark for at least an hour. While still in the dark, stimulate it with a sharp probe in different body locations. You may notice that it secretes small quantities of a luminescent chemical.

2. **Burrowing Polychaete Observation:** Burrowing polychaetes, such as *Arenicola*, can be observed by sticking two glass plates together so that the space between them is only slightly greater than the diameter of the worm. Add sediment to the observation chamber and place it in an aquarium. The polychaete can be added and its burrowing observed. You can modify the nature of the sediment to see how this affects the worm's activities or success at burrowing.

3. **Polychaete Regeneration:** Many species of polychaetes will regenerate lost portions of their bodies. Good genera to use for examination are *Sabella*, *Chaetopterus*, *Clymenella*, and *Autolytus*. For example, cut *Chaetopterus* in three or four locations, separate the body parts, and allow them to regenerate over successive weeks. Change the water daily. Does regeneration occur more rapidly at one end of the body than at the other? Do regenerated portions resemble the parts of the body that are missing? Sabellid fanworms will autotomize their crown of radioles if they are grabbed by a predator, such as a grazing fish. Induce a fanworm to lose its crown and follow its regeneration over successive weeks.

4. **Tube Building in Polychaetes:** Several groups of polychaetes build tubes from sand or miscellaneous debris. Carefully remove such a worm from its tube, scatter a variety of possible tube-constructing material around it, and watch how particle selection and tube-building occurs. The bamboo worm *(Clymenella*) and *Diopatra* are particularly good subjects. *Diopatra* will have a partially completed tube in less than a day.

5. **Epitokous Spawning:** Occasionally, invertebrate zoologists will be made aware of an unusual abundance of polychaetes swimming at the surface of the water or washing in with the ocean surf. These are usually episodes of surface swarming by reproducing epitokes. An example along the southeastern coast of the United States is the spring epitokous spawning of *Nereis succinea*. If such an opportunity arises, collect some worms, place them in clean seawater in dishes, and watch their behavior. Note how effectively they swim (their parapodia are larger than the atokes from which they metamorphosed). Watch for gamete release. If both eggs and sperm have not been spontaneously released, squeeze the soft bodies of the animals to release them. Expose the eggs to a very dilute sperm suspension, make a wet mount, and watch fertilization occur using a compound microscope. Maintain a culture of the embryos so that the living trochophores can be seen in one to three days

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6. **Respiratory Behavior in *Tubifex*:** *Tubifex* oligochaetes are very common in freshwater areas where the bottom is rich in organic material. They live in tubes and wave the posterior ends of their bodies from the mouth of the tube to increase movement of water past their body and, thereby, oxygen uptake. The lower the oxygen content of the water, the more active is this waving behavior. Place some *Tubifex* (frequently sold in aquarium stores) in a dish of water and note the waving activity. Now place them in

water having reduced oxygen content and see if you notice a change. You can reduce the oxygen content by boiling the water and letting it stand for varying periods of time.

7. **Metanephridium Functional Morphology:** The earthworm *Lumbricus* can be used to examine metanephridia. Because the water earthworms are exposed to is fresh, their metanephridia are large, making it possible to eliminate large volumes of water from the body during osmoregulation, such as following a rain event. Anaesthetize an earthworm by placing it in 0.2% chlorotone for 5 minutes. Immersing your worm in a saline solution, carefully open the animal and remove a metanephridium. Place it in a small dish of saline under the dissecting microscope and examine its morphology. Then place it in a saline-filled well of a depression slide, cover the fluid with a cover slip, and examine it using a compound microscope. Can you locate the nephrostome? Is there evidence of ciliary activity around this opening? Are there remnants of blood vessels attached to the metanephridial tubule? If so, what does the presence of both a nephrostome and blood vessels suggest possible sources of waste that the metanephridium collects and eliminates from the body? On occasion, organisms, *e.g.* nematodes, may be seen living in the bladder of this organ. Are any present?

Arthropoda

1. ***Limulus* Blood:** Certain common characteristics of arthropod blood, such as phagocytic properties of special cells, clotting, and the presence of a respiratory pigment (in some arthropods), can be examined using the horseshoe crab. Insert the needle of a syringe into the heart of a large *Limulus* by bending the animal at the prosoma-opisthosomal junction and pointing the needle toward the anterior end as it is slipped in the dorsal midline. Extract 10-20 ml of blood and put it in a vial. Also put a drop on a slide. Now shake the vial. As is also true of most crustaceans (but not insects), *Limulus* blood contains hemocyanin. As you shake the vial, the color of the blood changes from clear to faint blue. What accounts for this color change? After a short time you will also notice a blood clot forming in the vial. Examination of the drop of blood on the slide will show the fibers developing that form the clot. After removal of blood from your specimen, inject an equal volume of carmine suspension. After an hour, take another blood sample and examine it using a compound microscope. Is there any evidence of phagocytosis of the carmine particles by cells?

2. **Spider Behavior:** Choose a grassy habitat, such as an old field, for an analysis of spider diversity and behavior. Carefully walk through the habitat looking for the aerial webs of orb weavers as well as other, less conspicuous spiders, such as the cryptically colored jumping spiders on blades of grass. When a spider is found, stop and observe it for several minutes. If it has a web, does touching the web elicit a response? How about touching the web so as to mimic movement of a prey organism? Place a live insect in the web. Does the spider's response seem greater than with your attempts to mimic prey? Watch how the spider approaches, kills, and wraps the trapped insect. Also observe its feeding. If you have a tuning fork, how does touching it to the web affect the spider? If

tuning forks of different frequencies are available, try each of them. Is there a difference among frequencies or among different species of spiders with respect to how great a capture response the tuning forks elicit? When watching non-web-building species, such as jumping spiders, place an incapacitated insect in front of the spider to watch how prey-capture takes place. By moving a stick at various locations around the spider's body, examine how wide its field of view is. How might spider eye placement and size be related to prey-capture behavior in spiders? Using sweep nets, pit-fall traps, and examining debris and logs, thoroughly sample the habitat. Note the large numbers of species found. Return some of the more interesting species to the laboratory to examine their behavior using a dissecting microscope.

3. **Statocyst Function in Decapod Crustaceans:** Use small shrimp (*e.g., Palaemonetes*) or crayfish in this exercise. Place a specimen in a dish and remove a first antenna at its base, thus removing a statocyst. Has the orientation of the animal been affected? Turn the animal over on its dorsal surface and compare its righting behavior with that of an undamaged specimen. Now do the same, but remove both eyes as well. Is there evidence that vision may also be involved in a maintaining proper balance and spatial orientation? If the animals survive, see if they become more able to compensate for the statocyst loss over time. If many *Palaemonetes* are collected, keep them over-night in individual clean dishes; some may molt. Place iron filings in the dishes of molted individuals and watch them place the particles into the statocyst chambers. If you place a *very* strong magnet above the shrimp, is its orientation affected? What would you expect to occur? Why?

4. **Decapod Chromatophore Analysis:** Put some *Palaemonetes* on a white back-ground and some on a dark background. Using a dissecting microscope, compare their chromatophores after one hour. Of what adaptive value are the differences you observe? In *Palaemonetes*, a hormone secreted by the x-organ-sinus gland complex in each eye stalk causes lightening of the animal through dispersion of lighter pigments and concentration of darker pigments in the chromatophores. When this hormone is not present, the animal darkens. What do you suppose would happen if the eye stalks were removed? Remove the eye stalks from a specimen after carefully examining its chroma-tophores. Are the changes, if any, as you expected?

5. **Antennal Gland Function in Crayfish:** One function of the antennal ("green") glands in decapods is removal of toxic chemicals from the blood. To analyze this, inject a 0.25% aqueous solution of methyl green, safranin, or acid fuchsin into crayfish. The next day dissect the animals and examine the antennal glands. Is there any evidence that these chemicals have been "picked up" by these excretory structures? Have any other tissues, such as hepatopancreas, retained any dye?

6. **Localization of Salt Exchange Tissues in Crustaceans:** Crustaceans in low salinities or extremely high salinities (such as a hypersaline lake) have specialized "salt pump" tissues that actively move salt ions into the body (in reduced salinity habitats) or out of the body (*e.g.*, in *Artemia salina* in a hypersaline lake). Collect some crustaceans from estuarine and/or freshwater habitats. Shrimp, small crayfish and crabs, amphipods,

and isopods work well. Wash the animals thoroughly in several changes of distilled water to remove salt from their outer surfaces. Then put them into 1% silver nitrate solution for 5 minutes. After carefully washing them once more in several changes of distilled water, place them into photographic developer. Any silver ions that entered the ion exchange ("salt pump") areas will bond to chlorine in the blood to form silver chloride precipitate. The photo developer then turns the silver black. Where does uptake occur in these animals? Are salt-pump tissues localized or are they present over the entire body surface? Are such areas more prominent in animals found in less saline or fresh water (which would require more osmoregulation than animals in higher salinities)?

7. **Decapod Gill and Limb Symbionts:** Remove gills and selected limbs from small decapods, such as crayfish, shrimp, or crabs. Using both dissecting and compound microscopes, examine them carefully in dishes containing fresh or saline water (as appropriate) as well as on microscope slides. Identify to the lowest possible taxa those organisms found on these appendages. Some may be attached to the exoskeleton while others may move actively over its surface. Are there differences with respect to these symbionts among different decapod species? How about between different appendages from the same animal? How about different locations on a gill or limb? If you collected your animals from different habitats, does this habitat difference result in different symbiont faunas? One can also compare symbionts of hosts of different ages or different stages in the molt cycle.

8. **Cuticle Permeability in Crustaceans and Insects:** Because arachnids and insects have a waxy covering on their cuticle, moisture is less able to pass through it than in the crustaceans, which lack such a covering. This makes the crustaceans more susceptible to desiccation and less adapted to terrestrial habitats. Weigh a few terrestrial isopods and a few meal worm larvae. Keep them in a warm, dry room for 4 hours. Reweigh them and determine average percent weight loss for each species. What does this weight loss represent? If you abrade some meal worm larvae lightly with sandpaper, much of the waxy covering will be removed. See how such removal affects weight loss after 4 hours. You can see how much of the weight loss is due to loss of moisture through the spiracles by covering the spiracles with fingernail polish and see how much this reduces the loss of weight (moisture). As you might expect, crustaceans show variation in their cuticle permeability, with it being low in terrestrial species and higher in aquatic species. We can examine these differences by placing specimens in 70% ethanol. The more permeable the cuticle, the faster the ethanol will pass through it, and the sooner the animal will die. Drop specimens of aquatic, intertidal, and terrestrial crustaceans (*e.g.*, isopods and amphipods of different species) into 70% ethanol and measure how long it takes for each to stop moving (if they will not move, touch them with a probe). Determine the mean survival time for each crustacean species. Is there a relationship between survival time and degree of usual exposure to air?

9. **Function of Malpighian Tubules:** Malpighian tubules are the major excretory structures of most terrestrial arthropods. In a similar experiment to that described above for antennal gland function in crayfish, inject meal worm larvae with indigo carmine. Dissect the larvae after thirty minutes. Did any of the dye accumulate in the Malpighian

tubules? If you examine these tubules as well as some from uninjected specimens using a compound microscope, you may be able to see uric acid crystals inside. Of what adaptive significance is this to terrestrial arthropods?

10. **Learning Behavior in Cockroaches:** Construct a floating maze by cutting wooden strips about 2 cm wide and attaching them together in a simple, branching maze pattern, which is then floated in a pan of water. Place about ten cockroaches one at a time at the origin of the maze and a box shelter at the end. Record the time it takes for each animal to reach the shelter as well as the number of incorrect choices (at maze branches) that are made. Then calculate the mean completion time and mean number of errors for all animals for the first trial. Repeat this for each animal about twenty times and calculate mean completion time and mean numbers of errors for each trial for all animals. Make two graphs. In the first plot mean time to run the maze versus trial number. In the second plot mean number of errors versus trial number. Is there any evidence that learning has occurred?

11. **Predatory Feeding Mechanisms of Aquatic Insects:** Establish a freshwater aquarium using pond water and introduce into it a variety of aquatic predatory insects collected from local habitats. Excellent predators to use are odonate (dragonfly and damselfly) nymphs; a variety of predatory beetle larvae (their large jaws will be clearly evident); dytiscid diving beetles; and such hemipterans as gerrids (water striders), notonectids (back swimmers), naucorids (creeping water bugs), nepids (water scorpions), and belostomatids (giant water bugs). Note how the adults all have some mechanism for breathing air. Some hang from the water surface while scanning below for prey, while others carry a bubble of air with them as they swim beneath the surface. Introduce a variety of possible prey organisms into the aquarium; these may include other insects, *Daphnia*, copepods, amphipods, isopods, freshwater shrimp, and small tadpoles. Watch carefully as prey-capture occurs. Odonates will use a lightening-fast extension and retraction of their very long, hinged labium to capture their prey, while an hemipteran will use its grasping forelegs for prey-capture and a piercing beak to kill it and suck in its bodily fluids. Do any of the predatory taxa show a preference for certain types of prey? Is there any evidence for cannibalism? Do any of the predators stalk their prey or use other types of distinctive prey-capture behavior? If any vegetation is present in the tank, do any of the predators use it for hiding or the prey for shelter?

12. **Sampling of Soil Arthropods:** Several methods can be used to separate minute arthropods from the soil and vegetation debris in which they live. One of these techniques is the use of a Tullgren funnel, which uses heat and desiccation to drive these animals through a funnel into a container of preservative. One can make an inexpensive Tullgren funnel using a 2 lb. coffee can, 25-watt light bulb, and a polyethylene funnel having a mouth diameter equal to or greater than the diameter of the can. Remove the top and bottom of the can and secure a disk of one-fourth-inch hardware cloth to one end of the can. Hang the can with the hardware cloth end down and put the wide end of the funnel against the lower opening. Place a vial of preservative (70% ethanol is usually fine, although some organisms may be repelled by it) below the small end of the funnel. Wrap the soil or leaf litter sample in one to two layers of cheesecloth (to help retain soil

particles) and place it onto the hardware cloth within the can. Hang the light bulb just above the sample. Extraction of virtually all arthropods should be complete after 48 hours for a soil or leaf litter sample taken from an area of approximately 25 cm^2. Collect soil and leaf litter samples from a variety of habitats, including grassy field, coniferous forest, deciduous forest, and, if desired, a homeowner's lawn or agricultural field. Samples may also vary by season or soil depth. Compare the major groups of arthropods and their densities from each of these samples. How do they compare? What environmental factors might explain these differences? Were any animals in addition to arthropods extracted from your samples? Can you think of other sampling techniques that may be more effective for some of these other soil-inhabiting taxa?

Tardigrada

1. **Collection of Tardigrades from Moss:** Collect healthy moss from a living tree and soak it in a beaker of aged tap water for at least three hours (overnight is fine). Remove the moss and squeeze remaining water into the beaker. Let it settle, then decant the top layer of water. Pour the bottom layer of water and debris into a dish and search for movement using a dissecting microscope. Present in addition to tardigrades should be nematodes, rotifers, mites, and larval insects. Also try this using dried moss to collect animals that are in a cryptobiotic state.

2. **Collection of Marine Tardigrades:** Greatest abundance and diversity of marine tardigrades probably occur in coarse sandy sediments, ideally with small pieces of shell or coral mixed in. However, specimens can also be found in moist intertidal sand close to the low tide mark. Place a few handfuls of sediment in a bucket containing about 10 liters of freshwater. The freshwater induces an osmotic shock, which causes tardigrades and other interstitial organisms to release their strong hold on the sand grains. [As an alternative technique, small quantities of sand (about 10 cm^3) can be placed into 10 times its volume of 3.5% ethanol to anaesthetize the organisms.] Gently agitate the mixture for about 10 seconds, let the sediment settle, and then decant the liquid through a 62-µm mesh net. Repeat this several times for each sample of sediment. Dip the net into seawater so that the captured animals can be restored to osmotic equilibrium. Animals retained on the net can be washed with seawater into a small dish to be examined alive or preserved in buffered 5-7% formalin or 70% ethanol. What other animals were captured along with the tardigrades?

Nematoda

Collection of Terrestrial Nematodes: Use a Baermann funnel to collect nematodes from soil, roots, or organic debris. Take a 100-mm diameter glass funnel, attach a short piece of rubber tubing to the end and close off the rubber tubing by means of a metal clamp. Place a circular piece of wire screen inside the funnel so that it rests

about 2-3 cm below the top rim. Place a wet-strength facial tissue on the screen to retain soil; allow it to slope up the sides of the funnel, but not over the rim. Fill the funnel with tap water to just above the screen. Gently place a small amount of nematode-containing material in the water on top of the facial tissue. Ideal sources of nematode-rich material are top soil or decaying organic debris from a compost pile. Living nematodes in the material will migrate downward and settle to the bottom of the funnel tubing. After one or two days, remove 5-10 ml of water from the tubing and examine it for nematodes. Microbe-feeding nematodes can be readily distinguished from those that parasitize plants due to the rapid movement of the former. Make a wet mount of a plant-parasite nematode and, using a compound microscope, locate the oral stylet. Why might you expect to see such a structure in a plant-eating nematode rather than in a species that feeds on microbes or animal tissues? Several Baermann funnels can be set up, each containing soils from different sites. How do nematode densities differ between these locations/soils? What hypothesis(es) can you propose that could account for these differences? What other animals were collected in addition to nematodes?

Bryozoa/Ectoprocta

1. **Examination of Bryozoan Larvae and Larval Metamorphosis:** Collect living *Bugula* sp. colonies the day before initiating your study and aerate them overnight in a lidded container (it doesn't have to be opaque). In the morning remove some of the colonies and place them into dishes of clean seawater. Within an hour, small, dark larvae will start being released from ovicells and swim around the dish. Examine colonies using a dissecting microscope. Can you see any ovicells releasing larvae (one larva per ovicell)? Different types of larvae are found within the bryozoans; this kind is termed a coronate larva. Is there any evidence that these larvae are photopositive? What change in photo-response might you expect to take place as the larva ages? Make a wet mount of one of these larvae and note the cilia covering its external surface. If you look carefully, you may notice a site on the larva's body where there is a tuft of longer cilia. This is the location where attachment to the substrate occurs. Transfer several larvae to a dish of seawater. Within a few hours the larvae will attach to the bottom (as well as to the surface film). Follow metamorphosis by periodically removing one of the metamorphosing individuals and examining it using a compound microscope. Within a day or two the first individual of a new colony, the ancestrula, will be formed.

2. **Colony Morphology in *Bugula*:** Examine different *Bugula* colonies using a dissecting microscope. Examine the distribution of avicularia (not all *Bugula* species have them), ovicells, and spines. Is there some predictability with respect to their locations, such as near or away from branching points, or at a predictable periodicity as one goes along a branch? Are there differences with respect to ovicell, avicularium, or spine positioning between old (large) and young (small) colonies? How about any differences between young individuals and second generation individuals on a large colony? (As one goes from the top of a colony to its base, one can recognize different generations by looking for zoecia containing brown bodies, which occur in groups; these

separate one generation from the next as one goes from the tip to the base of a branch.) One can also examine the influence of differing current velocities or sediment loads on colony morphology by collecting colonies from habitats differing with respect to these factors. What symbionts are found on the colonies? Are sessile symbionts found at particular locations on the colony, such as near or away from branching points? Do they appear to influence colony form or polypide feeding behavior in any way? One could compare the proportion of zoecia that consist of second generation feeding individuals, first generation feeding individuals, and individuals with brown bodies between colonies that are subtidal and those that are intertidal (intertidal colonies can't feed as much). One could also determine how many ovicells there are per number of zoecia for intertidal vs. subtidal colonies.

Echinodermata

1. **Echinoderm Development:** The major echinoderm taxon utilized for the study of embryology has been Echinoidea. Inject specimens of *Arbacia* (or another genus) with 0.5 M potassium chloride. Gametes will begin to seep out of the aboral gonopores; eggs will be purple, sperm white. Invert females over a 250 ml beaker of clean seawater and permit the eggs to drop into it. Invert males over small, empty dishes, in which the sperm will collect. Make a wet mount of unfertilized eggs; note the uniformly distributed, pale yolk granules and red pigment particles. Dip a toothpick in some sperm and touch it to the edge of your wet mount (do not add too much sperm). Watch the swimming sperm contact the eggs and the subsequent lifting of a fertilization membrane from the egg, thus blocking the entrance of other sperm. This membrane can be best seen by the presence of sperm collecting outside of it. Now, using the same method, fertilize some eggs in a depression slide and, adding a small amount of sperm, fertilize the eggs in the beaker. Keep the depression slide wet and examine it periodically. The first cleavage should occur in 50-70 minutes. The next cleavage will occur 1½ hours after fertilization. Periodically examine the embryos in the beaker by making wet mounts. Echinopluteus larvae will be formed in 24-48 hours, depending on temperature. Examine these larvae and note the use of the ciliated larval arms for feeding and movement.

2. **Circulation of Coelomic Fluid and Phagocytosis in Echinoderms:** Movement of fluid within the coelom, caused by a ciliary lining, is important for internal transport in echinoderms. To examine this circulation, inject 1-2 ml of carmine suspension into the distal portion of a starfish arm. Look at the papulae under a dissecting microscope and note the rapid circulation of carmine (and possibly injected air bubbles) within. How long does it take for carmine to be seen inside papulae of other arms? These carmine particles will be taken up by coelomocytes and removed from the body. After about fifteen minutes remove some coelomic fluid from the tip of the injected arm (just cut the very tip off — do not cut the pyloric cecae) and make a wet mount. Are carmine-containing coelomocytes present?

3. **Coordination of Tube Feet in Asteroids:** Watch a starfish move and note which arm is the leading arm. Then cut the ambulacral ossicles at the base of this arm, which severs the radial nerve. How does this affect movement? Do the same thing in another specimen on a non-leading arm. How do the results compare? Now sever the nerve ring between two undamaged arms on opposite sides of the body (this requires severing the nerve ring at two locations). How is walking affected? Do the stepping movements of the tube feet become uncoordinated? What are your conclusions about the role of these nerve cords in movement?

4. **Defensive Response in Echinoids**: It has been reported that sometimes when a sea urchin is touched with an object, the moveable spines swing toward the spot, providing a spiny defense. Others have reported that when a sea urchin is touched, the spines bend away from the point of contact, giving the pedicellaria ample room to pinch the intruder. Perhaps these different responses depend on the nature of the object. Touch the surface of a sea urchin with a pointed instrument. How does it respond? After a several minutes, touch the animal with a blunt instrument. Is the response different?

5. **Pedicellaria Use in Asteroids**: Drop crushed (but not powdered) chalk onto the surface of a pedicellaria-bearing asteroid (use forcipulate genera such as *Asterias, Pisaster,* or *Leptasterias*). Within a very short period of time the small pieces of chalk are reduced to a fine powder that is then removed from the surface of the animal by cilia. One can also note the pinching nature of these structures by resting the back of your hand or arm briefly against the aboral surface of a pedicellaria-bearing sea star. When you then pull your hand or arm away, you can feel the tugging caused by pedicellaria that have pinched hairs that have fallen between their jaws.

6. **Evisceration in Holothuroids:** One strange type of behavior seen in some holothuroids is evisceration of most of the internal organs. The true adaptive value of this bizarre phenomenon is unclear. After a few weeks, the lost organs are regenerated. Place a living *Thyone* or *Stichopus* in 0.1% ammonium hydroxide for a couple of minutes, then hold it in the air by its posterior end. It may then eviscerate most of its internal organs by rupture of the body wall behind the tentacles. What organs have been lost? Keep the specimen in an aquarium for 2-3 weeks and then dissect it. Can you see any evidence of regeneration?

Chordata

1. **Procurement of Tadpole Larvae from *Amaroucium* or *Perophora*:** These colonial tunicates brood developing embryos in the atria of colony members. Squeezing a colony of *Amaroucium* over a dish of fresh seawater will provide, in addition to individual members of the colony, brooded embryos of a variety of developmental stages. Alternatively, you can collect larvae released from undisturbed colonies of *Amaroucium* or *Perophora*. Larvae will usually be released at dawn. Freshly collected colonies can be kept in well-aerated seawater and kept in a dark room or covered with opaque material

until larvae are desired. At that time, expose the colony to light. Actively swimming larvae should be released in less than one hour and for the next few hours. Are the larvae responding to the direction of the light? If so, are they positively or negatively phototactic? How might this behavioral response aid their dispersal? If they appear to be showing a phototactic response, how might you experimentally distinguish between positive phototaxis and negative geotaxis or negative phototaxis and positive geotaxis? Make a wet mount of a living larva and, using a compound microscope, see what structures you can identify. Larvae will swim for only a short period of time (typically a matter of minutes for colonial ascidians), at which time they will attach to a solid surface using anterior adhesive papillae and begin to metamorphose (this may even occur if attachment in a dish is unsuccessful). Do older, attaching larvae have the same phototactic response as young larvae? Why might a change in phototaxis with larval age be advantageous? Do larvae temporarily attach, changing locations until they find a suitable site for permanent attachment? Examine the attached, metamorphosing individuals periodically during the next few days until metamorphosis appears to be complete.

2. **Procurement of Tadpole Larvae from Solitary Ascidians:** Most solitary ascidians (*e.g., Molgula, Styela*, or *Ciona*) do not brood their embryos. Macerate gonads (ovaries are usually yellow-orange and testes white) from 2-3 individuals of a species in a dish of clean seawater (many species will not self-fertilize). In order to reduce the possibility of polyspermy (multiple fertilization of an egg), don't make your suspension of gametes too dense. Initial cleavage should occur within one hour after fertilization. Isolate developing embryos and follow their development. Formation of a tadpole larva may be complete after as little as 12 hours or as long as several days, depending on the species. The tadpole larvae of solitary ascidians usually stay in the plankton longer than those found in colonial species, probably on the order of 12 hours or so. If you are successful in rearing the embryos to the tadpole larval stage, examine them periodically so that you can watch their attachment and metamorphosis.

3. **Ascidian Endosymbionts:** The enlarged ascidian pharynx, constantly flushed with well oxygenated water, lined with nutritious mucus and captured particles, and sheltered from potential predators, would appear to be an ideal dwelling for an invertebrate. In fact, there are several invertebrates that can be found living there. Among the more common symbionts one might find in the pharynx of an ascidian along the eastern coast of North America are the amphipod *Leucothoe* and notodelphyoid copepods. Although *Leucothoe* can be found living outside of a host, it is most commonly encountered as an internal symbiont of sponges and ascidians. It can be recognized by its unusual first gnathopods (the first pair of thoracic limbs behind the smaller maxillipeds); these are complexly chelate, with article 5 extending as a slender, pointed projection along the entire inner margin of article 6. Notodelphyoid copepods, which are obligatory symbionts, have a noticeably enlarged midsection. Collect solitary ascidians from benthic habitats, open them up and look for symbionts. Determine the size of the host ascidian using volume displacement of water within a graduated cylinder. Is there a relationship between ascidian size (age) and the presence and absence or abundance of symbionts? Are there differences between different species of solitary ascidians? Do

occurrences of amphipods and copepods differ from each other? Do amphipods and copepods cohabitate the same host? Were any other symbionts found?

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