Carolina™ Arthropods

CAROLINA

World-Class Support for Science & Math

Carolina™ Athropods Manual

Contents

For your convenience, we have listed throughout this manual the catalog item numbers of some of the products available from Carolina Biological Supply Company. For more information, please refer to the most recent Carolina™ Science catalog, call toll free 800-334-5551, or visit our Web site at **www.carolina.com**.

Introduction

Carolina Biological Supply Company maintains a large variety of living arthropods for use in teaching and research. Animals leaving our laboratories are healthy, but we have no control over shipping conditions. Occasionally animals may be traumatized during shipment, so it is critical that you give them your full attention immediately upon receipt.

We hope you find this information useful for maintaining and using living arthropods in the classroom and laboratory. We have developed this manual in an attempt to provide relevant background information for each arthropod, including its nutritional requirements and proper habitat setup. For species-specific information, we recommend consulting textbooks, species-specific publications, journal articles, the Internet, or an arthropod expert.

USDA Safeguards for Insect Pests

If you ordered bean beetles, cabbage white butterflies, flour beetles, greater wax moths, harvester ants, hornworms, subterranean termites, or superworms (*Zophobas morio*), you must adhere to the U.S. Department of Agriculture Standard Safeguards listed below. Your state regulatory agency has required that these safeguards be followed for keeping and using these insects. Other insects also may be regulated in your state. Because state laws regulating live insects can change without notice, we strongly recommend following these guidelines for all pest insect species.

USDA Standard Safeguards

1. Upon receiving living pests, all packing material media, substrate, soil and shipping containers shall be destroyed immediately upon removing insects.
2. Pests shall be kept only within the laboratory, classroom or designated area at the purchaser's address.
3. No living pests kept under this permit shall be removed from confined area except by prior approval from State and Federal regulatory officials.
4. Without prior notice and during reasonable hours, authorized Plant Pest Quarantine and State regulatory officials shall be allowed to inspect the conditions under which the pests are kept.
5. All pests kept under this permit shall be destroyed at the completion of the intended use.
6. All necessary precautions must be taken to prevent escape of pests.

Advisory on Releasing Organisms

Carolina provides living organisms for educational purposes only. As a general policy, we do not advocate the release of organisms into the environment. In most states, it is illegal to release organisms, even indigenous species, without a permit. The intention of these laws is to protect native wildlife and the environment.

After completing classroom activities, we suggest that organisms be

- maintained in the classroom.
- donated to another classroom or science department.
- donated to a nature center or zoo.
- humanely disposed of, as a last resort.

Amphipods

Amphipods are commonly referred to as "sideswimmers," because they often swim on their sides. Carolina supplies freshwater amphipods. They are primarily scavengers of plant and animal material; however, they have been known to attack injured or stressed animals.

Upon receipt, remove the lid from the shipping jar and allow it to just sit on the jar, but do not aerate the culture with a pipet or any other device. Keep the jar in a cool area (21°C, 69°F) out of direct sunlight. The optimal habitat for amphipods is a glass or plastic container (e.g., a 20-liter or larger aquarium) filled with springwater or bottled water. **Note:** Do not use distilled water or municipal tap water. The organisms are sensitive to metal ions, which are usually present in at least trace amounts.

Amphipods feed on algae as well as bacteria and yeast. Place a few small pieces of algae in the habitat. To prepare a yeast suspension as food for amphipods, mix baker's yeast and springwater in a clean 2-liter soft drink bottle. Add baker's yeast until the water becomes milky, then store in a refrigerator. Always agitate the water before use to resuspend the yeast, and feed amphipods a few drops each day. An alternative is to crush three or four grains of dry baker's yeast on clean paper and dust this on the surface of the culture. Avoid overfeeding. Do not allow the water in the culture to become clouded. Draw off and discard about one-fourth of the culture water and replace it with new springwater on a weekly basis.

Ants

Ants supplied by Carolina can be stored for up to 2 weeks in the refrigerator. Until ready for use, check the ants daily with special attention to the moisture content of the culture. If the culture is too damp, use a fine mesh screen as a temporary cover to allow moisture to escape. Remove any mold; however, if there is excessive mold, move the ants to a fresh container with a very small piece of potato or apple for food and moisture. If the culture seems too dry, add damp paper, cotton, or sponge to the container.

Typically, the inhabitants of ant farms are worker harvester ants. Before introducing the ants into the ant farm or habitat, place them in a refrigerator for 5–10 minutes. Do not place them in a freezer, as extremely cold temperatures will kill them. Roll a piece of paper into the shape of a funnel. Remove ants from the refrigerator, and pour them through the funnel into the ant farm. **Caution:** *Do not touch the ants, as they are able to deliver a painful, stinging bite.* It may take 12–24 hours for the ants to become acclimated to their new home. Although their activity level is high, their nutritional demands are low. Feed your ants a small piece of fruit or vegetable every 2 or 3 days. Remove any uneaten food after 2 days to prevent mold from accumulating. Provide your ants with a few drops of water every day, and maintain them in a cool room at 16–21°C (60–70°F). With proper care, harvester ants will live 2–4 weeks.

Carpenter ants are active at night. It is then that they leave the nest to forage for food. Carpenter ants feed on sources of protein and sugar. Their typical foods include aphid honeydew, other insects, plant juices, and food products containing fat, meat, or sugar. Other foods eaten by carpenter ants include syrup, honey, jelly, pieces of sweetened cereal, and a sugar-water solution on a sponge. Remove uneaten food before it molds. Carpenter ants do not eat wood. The carpenter ants we ship are normally used as a food source for other animals. They should be kept in cool areas (such as a refrigerator) until needed, so that they will survive longer and be easier to handle due to their sluggish movement.

The life cycle of carpenter ants is an example of complete metamorphosis, with egg, larval, pupal, and adult stages of development. Depending on temperature, the life cycle can take from 3 months to a year to complete. Worker ants have an average lifespan of 7 years, while queen ants may live as long as 25 years.

Note: Harvester ants are potential agricultural pests. Do not release them into the environment. It is illegal to ship queen ants, due to the threat of infestation.

Beetles

Bean Beetles

Bean Beetles, *Callosobruchus maculates*, are pests of stored legumes (Fabaceae). The larvae require the seeds for nutrition, while the adult beetles do not require food or water. Culturing bean beetles is simple, as the only materials needed are dry beans and a container that will prevent the beetles from escaping. We recommend using organic mung beans and black-eyed peas (cowpeas). It is best to maintain a ratio of one or two adult beetles per bean, and temperatures between 22°C and 30°C (72°F and 86°F). Keep all cultures out of direct sunlight and away from heat sources.

To begin a culture, cover the bottom of a container with a single layer of beans equivalent to a volume of 50 mL. Add 10 adult males and 10 adult females to the container. Adult females are black in color with an enlarged abdomen that has dark stripes on each side of the abdominal plate. Males are a brownish color with a smaller abdomen that lacks stripes. Females lay their eggs on the surface of host beans. The eggs will hatch 4–5 days later. The larva hatches from the egg, burrows into the bean, and feeds on it. While still inside the bean, the larva goes through a series of molts and then pupates directly under the skin of the bean. The adult will chew through the skin of the bean when it is ready to emerge. Adults live 1–2 weeks, and spend that time mating and laying eggs. The entire life cycle of the bean beetle, from egg to adult, takes from 3–7 weeks. The length of metamorphosis is dependent upon temperature and humidity.

Note: Bean beetles are potential agricultural pests. Do not release them into the environment. Prior to disposal, place the live cultures in a freezer for at least 3 days to ensure that all the beetles are dead.

Bessbugs

Bessbugs (*Passalus cornutus*), or shorthorned stag beetles, can be kept for a considerable length of time if provided with a supply of damp, slightly decaying hardwood (oak is preferred). The beetles are shipped with a generous amount of wood, which should last several weeks.

Place 4–5 cm of moist potting soil or humus in a terrarium as a substrate. Add some leaf litter and the beetles, along with the rotten wood that was shipped with the animals. Do not place terraria in direct sunlight, as this may overheat and kill the beetles. Maintain the beetles at room temperature (20–22°C, 68–72°F). Bessbugs require humidity, so keep the soil damp and mist the habitat daily. However, if mold becomes a problem, increase the ventilation or reduce the amount of misting. Bessbugs eat microorganisms found in wood, chewing through damp, rotting wood to

create tunnels inside the log where they live. If they eat all the rotten wood shipped with them, collect additional rotten hardwood locally or order it from Carolina (14-4153).

Bessbugs can live as adults for up to 1½ years, which is unusually long for a beetle. They also undergo complete metamorphosis, so their entire life cycle (egg, larva, pupa, and adult) can extend beyond 2 years. The beetles you receive are of unknown age, but they will probably live several weeks or months. They will not reproduce in a terrarium.

Convergent Lady Beetles

The life cycle of the lady beetle (*Hippodamia convergens*) spans 1 year. Eggs that are laid in the spring develop into darkish gray larvae with orange spots. These soft-bodied, carrot-shaped, spiny larvae appear quite unlike the round, hard-bodied adults, yet both have a voracious appetite for aphids. It has been claimed that a convergent lady beetle is capable of eating more than 50 aphids a day.

The pupa does not spin a cocoon, but cements the tip of its abdomen to a leaf. When disturbed, the pupa lifts its body to a vertical position, then drops back again.

Adult beetles emerge during the first week in June. They are active crawlers and fliers, and can disperse nearly 5 miles in as little as 3 days. Throughout the spring and autumn, adults are abundant in fields. As winter approaches they migrate to mountainous regions, where they congregate in large numbers before winter hibernation.

For about 9 months the adults do not feed but live off stored fat. The adult beetle's ability to tolerate long periods of fasting under cool temperatures allows *H. convergens* conveniently to be stored under refrigeration (5–8°C, 41– 46°F) for months.

Carolina supplies adult beetles that can be satisfactorily held in the refrigerator until spring, when they should be released in the garden. Providing a vial of water and a vial of sugar water, each with a cotton wick, may help the survival of the beetles during refrigeration. Beetles received after the last frost, but before June, should be released immediately and not stored in the refrigerator. At this time they have used up their stores of fat and need to begin actively feeding.

If one wishes to culture the convergent lady beetle, aphids should be provided as a food source or our artificial diet (14-4230).

Darkling Beetles (Mealworms, Superworms)

There are over 20,000 species of darkling beetles. The larval stages of various species are used as a food source for animals like fish, reptiles, and birds. Carolina provides two species of larvae: mealworms (*Tenebrio molitor*) and superworms (*Zophobas morio*). The species are similar in appearance; however, superworms are larger (1–2 inches) than mealworms and require different care. Additionally, superworms do not always pupate easily and may remain in the larval stage for 3–5 months.

Note: Superworms (*Zophobas morio*) are potential agricultural pests. Do not release them into the environment.

Culturing Mealworms

To culture mealworms put 5–10 cm of bran meal in the bottom of a plastic pan. Add 25–50 beetles and place a small slice of potato on top of the meal for moisture. A thin layer of shredded paper can be added for the beetles to crawl on. Mealworms may also be cultured in chicken mash, which has been spread on layers of moistened burlap. After 3–5 months, check the culture regularly for adults and pupae. These can be removed and used to set up new cultures.

The beetles start laying eggs 7–10 days after emergence and the eggs hatch about 14 days later. The time spent in the larval stage varies considerably with temperature and food availability. The larvae grow rapidly under ideal conditions until they are about 25 mm long, then they pupate. The adults emerge 2–3 weeks after pupation.

Culturing Superworms

To prevent escape, keep the superworms in a container with closed sides, such as a bucket or pan lined with 2–3 inches of substrate material (such as peat moss, oat bran, bran cereal, etc). Small glass aquariums, plastic storage boxes, and plastic food containers are also great options for a habitat. The key to choosing a habitat is to select one that has smooth sides, because this will prevent the worms from crawling out. It is very important that you maintain proper levels of humidity by misting the substrate every other day. (Too much moisture will kill the superworms, while too little moisture may cause them to cannibalize one another.) Because these are tropical insects, it is important to maintain them at a temperature between 21°C and 27°C (70°F and 80°F). Be sure not to let the temperature drop below 16°C (60°F) or rise above 29°C (84°F). Unlike mealworms, superworms should not be refrigerated.

Good sources of both nutrition and moisture are fruits and vegetables, such as sweet potatoes, apples, and carrots. Superworms should also be

fed a diet of oats, wheat bran, or chicken meal (sold commercially by Purina Mills® as Layena®). An added benefit of Layena is that it is fortified with vitamins and minerals, which add to the superworms' later effectiveness as a food source.

If you want pupae or adult beetles, you will have to force the superworms to pupate. First, separate the larvae into artificial pupation chambers, such as film canisters. Each container should contain only one larva. Next, add some wheat bran or oatmeal to serve as a substrate and a source of nutrition. Then, add a carrot slice to provide the beetles with some moisture. Finally, punch a few holes in the lids of the containers for ventilation.

Adult Darkling Beetles

Adult darkling beetles are scavengers, eating both fresh and decaying vegetation. In nature, they feed on dried or rotting plant matter. In captivity, they feed on bran meal, apples, oranges, potatoes, cucumber, romaine lettuce, and pears. Remove uneaten food before it molds. Providing them with water on a regular basis is not necessary, as they extract water from their food. Additionally, their hard carapace is covered with a wax-like substance that prevents water from evaporating from their bodies. Placing a moistened sponge or cotton wick into the habitat will provide the beetles with enough water.

Darkling beetles are active both during the day and at night. Their habitat should provide them with enough space to move around. Set up the terrarium by covering the surface with paper towels, leaf litter, oats, or bran meal. They prefer dark, cool, dry places. With proper care, darkling beetles can live from three months to over a year.

Dermestid Beetles

Dermestid beetles (*Dermestes* sp.) are scavengers that perform a valuable service in nature by consuming the soft inner remains of dead animals (muscle and connective tissue and the soft tissue surrounding bones). Museums and taxidermists commonly use these flesh-eating beetles for skeleton preparation. A female beetle will lay her eggs, sometimes hundreds of them, on a carcass, where they hatch 3 days later. As the tiny, brown larvae consume the remains, they leave behind the toughest meat, hair, and skin. They do not eat rock-hard flesh.

Proper care and a suitable habitat are essential to maintaining a dermestid colony. Adult dermestids tend to fly from the colony in search of food, thus it is important to have a good cover on their holding container at all times. Glass aquariums are great options for setting up a dermestid habitat.

Caution: *We advise against using habitat made of wood or plastic, as beetles are able to chew through these materials.* The enclosure should include some bedding where the bugs can shed their larval skins, pupate, and lay eggs. The deeper the substrate, the bigger the colony will become. Add wood shavings (not cedar) in the bottom of the container. Dermestid beetles prefer a dark environment with 50–60% humidity and a temperature of about 27°C (80°F). Keeping the culture in a detached building where portable heaters and humidifiers can help control these conditions is ideal. Additionally, the colony should be kept dry. Too much moisture may result in the emergence of mites, which could potentially destroy your entire colony. Lightly mist any material in the colony about once every day. You may also provide the dermestids with water by adding a moist paper towel to the habitat each day. The habitat should be checked daily to ensure that all conditions are satisfactory.

Dermestid beetles can consume enough protein from dried dog or cat food to survive, but to cultivate a thriving colony for skeletal preparations, supplement the dried food with bits of meat, such as beef, pork, or hotdogs. Cooked chicken that has not completely dried out is a good supplement. Dermestids do not feed well on rotting meat; therefore, it is important to thoroughly dry any food material.

A dermestid beetle can live 60–70 days, depending on its environment. The larval stage lasts about 30 days, toward the end of which larvae bore into wood and other hard materials to pupate for about 7 days before emerging as adults. Larvae are light brown and covered with tiny hairs. Usually, dermestid larvae are reclusive and like to hide in dark places. Fully developed larvae search for wooden materials to pupate. After adult beetles emerge, they will mate and lay their eggs near a food source. Adults are small, hairy, dark-colored beetles.

It takes about 90 days to cultivate a "hot" culture containing a large percentage of larvae that can clean a skeleton quickly. Large skulls will require a large container, such as an aquarium. Paint the sides of the aquarium black or cover it with a dark cloth to promote the colony's activity. Put a wire mesh screen on top of the aquarium to keep the beetles from escaping.

Remove the skin and organs of the specimen before putting it into the aquarium with the beetles. The larvae will attack the cartilage between the bones last, so be sure to remove the skeleton before the cartilage is damaged. Then, remove as many larvae and adults as possible from the specimen with forceps or a brush. Dip the bones in a 50% alcohol solution to kill any remaining dermestids.

Diving Beetles

Predaceous diving beetles make up a large group of aquatic beetles that are common in ponds and quiet streams. There are about 500 different kinds of diving beetles in North America, and about 5000 worldwide. These beetles get air at the surface but can remain submerged for long periods because they carry air in a chamber under the elytra (the wing covers). They often hang head downward from the surface of the water. These insects may leave the water at night and fly to lights. **Caution:** *Diving beetles can deliver a painful bite. Do not handle them.*

Both adults and larvae are highly predaceous, feeding on a variety of small aquatic animals, including small fish. The larvae, often called water tigers, have long, sickle-shaped, hollow jaws, with which they grab prey and suck out the body fluids. These larvae are very active and will not hesitate to attack animals much larger than themselves. Larvae can eat up to 20 tadpoles a day. Diving beetles have a life span of one year or longer.

Diving beetles may be held in a holding container with aged tap water (at least 2 inches deep). Change the water when it becomes fouled. You may also put a few aquatic plant sprigs in the holding container for the insects to float on and some living amphipods for them to eat. The holding container must have a secure lid; otherwise, the beetles will escape.

Flour Beetles (*Tribolium*)

With proper care, the flour beetle (*Tribolium*) is one of the easiest insects to culture. Although *Tribolium* can survive on a number of finely ground grains, we recommend a medium consisting of 4 parts white flour, 4 parts whole-wheat flour, and 1 part brewer's yeast. (Prepared *Tribolium* Medium is available from Carolina upon special request.)

A 1-gallon jar with a cloth top makes a suitable rearing container. Twenty to 50 adults placed in the jar one-fourth to one-half full of medium will produce a large culture. Humidity should be maintained at 70%. If conditions are dry, a small slice of potato or lettuce can be added to the culture. The potato or lettuce should be replaced when it becomes dry or moldy.

A female flour beetle may live for a year or more and lay 400–500 eggs, and a culture can quickly become overpopulated. Check the population in the culture each month and set up new cultures if overcrowding occurs. Flour beetles are cannibalistic; adults will eat eggs while larvae will feed on eggs, pupae, and other larvae. Overcrowding in a culture naturally increases cannibalism. Additionally, overcrowding in the culture aids in the transmission of a protozoan parasite, *Adelina tribolii*. If present, *A. tribolii* will reduce colony vigor and can kill the colony. Beetles under stress often

produce a gas containing certain quinones that can cause the appearance of aberrant forms of *Tribolium* and can even kill the beetle colony.

Note: Flour beetles are potential agricultural pests. Do not release them into the environment.

Butterflies

While all butterflies undergo complete metamorphosis (egg, larva, pupa, adult), not all feed on the same plants. This section provides descriptions and proper care instructions for the butterfly species supplied by Carolina. The last section focuses on the care of adult butterflies, which is similar for most species.

Note: Cabbage white butterflies are crop pests. Do not release them into the environment.

Brassica Butterflies

The cabbage white butterfly (*Pieris rapae*) is found throughout much of North America. This butterfly feeds on plants from the brassica family (e.g., radish, turnip, cabbage, kale, mustard, collards, broccoli, and cauliflower).

The eggs should hatch within 48–72 hours after receipt. Prior to the eggs' arrival, sow at least 10 radish or brassica plants (per unit of eggs) on which to start your larvae. If you choose not to prepare radish or brassica seedlings on which to hatch the eggs, then get some cabbage leaves right away. When using cabbage as a substitute, discard the outer leaves and then tear off a few leaves and place them in three or four plastic containers, such as 12-ounce deli cups. Cut the egg strip into smaller pieces and place each piece, eggs facing down, on one or more cabbage leaves. The larvae develop more slowly and are more difficult to see on the cabbage leaves than on potted plants. Expect to see the larvae in 5–6 days. When placing the eggs on the leaves of potted plants, cut the egg strip into four to six smaller sections and place each section, eggs facing down, on a separate leaf.

Growing on Plants

Once the larvae have eaten down the potted plants, you can gently remove the larvae and either place them on more plants you have prepared, or place them on any available brassica, such as cabbage, Brussels sprouts, or broccoli. These should be set up in plastic containers.

Growing on Leaves

Immediately after a larva chews its way out of the egg, it may begin to feed on adjacent eggs on the strip. Newly hatched larvae cannot chew through

tough plant material as well as older larvae can. Therefore, it is best to feed them young, tender leaves. Due to their small size, the larvae can dehydrate. Mist the larvae daily with dechlorinated, room-temperature water.

After hatching, the larvae grow very quickly, as they molt two to three times within the first week. The excrement of the larvae, called frass, dries to form fine, dark granules that can be used as plant fertilizer. When using cabbage leaves, you will need to carefully remove the cabbage and larvae from the dish occasionally to clean out the frass and prevent the growth of mold on the leaves. Remove any old, dry leaves and replace with fresh ones whenever needed. *Do not discard the larvae with the leaves.*

After 18–20 days, the larvae will begin to pupate. When preparing for pupal formation, a larva will weave a strong carpet of silk on the plant or container. Allow the exoskeleton to harden for several hours. Six or 7 days after pupa formation, a butterfly should emerge. Wings usually take 15 minutes to expand, and another several hours to harden. Adult butterflies live about 2 weeks.

Painted Lady Butterflies

The painted lady butterfly (*Vanessa cardui*) is one of the most common butterflies in the world. It is a migratory species. Because of its wide range, it is sometimes called the "Cosmopolitan."

To rear the painted lady butterfly, set the culture cup containing three to five larvae in a well-lighted area where the temperature remains between 22°C and 25°C (72°F and 77°F). Do not set the cup in direct sunlight. In 5–10 days, the larvae will crawl to the top of the cup and hang from the tissue paper. Each larva is preparing to form a chrysalis, so the cup should not be disturbed. Occasionally, just before the larvae prepare to form their chrysalis, they will begin to chew through the tissue paper. If it becomes a problem, remove the old paper and replace it with a fresh piece of tissue paper. Avoid papers that contain perfumes or lotions. After the chrysalises have formed and darkened (a 1- or 2-day process), place the tissue paper with the insects in a cage and attach it to the side or top so that the chrysalises are hanging in a natural position. The cage can be an empty covered terrarium or similar container 1–2 cubic feet in volume. At room temperature, adult butterflies should emerge 7–10 days later.

To prepare individual cultures, place one-quarter inch of artificial food in a 1-ounce cup. Use a fine-tip paintbrush or cotton swab to transfer one caterpillar into the cup. Cover the opening of the cup with unscented tissue paper, and secure with a ventilated lid. Once a chrysalis has formed, allow 1 or 2 days before transferring the tissue paper with the pupa to the adult habitat.

From 5–7 days after emergence, female butterflies should begin to deposit eggs on the leaves of host plants. The eggs are small, pale green spheres with 12–14 longitudinal ridges. Place the leaves with eggs attached to them into a ventilated container. They should begin to hatch in 3–5 days. However, if the leaves begin to dry out, wrap the stem in a wet paper towel. Seal the paper towel with either plastic wrap or aluminum foil. Keep a supply of mallow leaves in the refrigerator to feed to the larvae once they hatch. If you choose to provide your larvae with plant leaves, replace them with fresh host plant leaves each day. This protocol may also be followed (i.e., replacing leaves daily) to prevent the eggs from dehydrating and becoming non-viable.

Care of Adult Butterflies

Flower nectar is the natural food source of adult butterflies. A sugar-water solution is a convenient alternative. Make a feeding solution by dissolving 2 teaspoons of sugar in a cup of water. Fill the feeder with the sugar water. Keep this feeding solution refrigerated when not in use. You can make a feeder from any small, clean, empty plastic container that has a lid (e.g., a sauce cup or 35-mm film canister). Clean the container with a mild solution of household bleach in water (1 part bleach to 9 parts water), and then rinse it thoroughly. Fill it with warm water. Punch a hole in the lid of the container, large enough to easily insert two white felt wicks. The wicks should be approximately ¼ inch wide by 2 inches long. Dip the felt pieces into the sugar-water solution, and then squeeze out the excess water. Insert the felt into the hole in the lid, leaving approximately ¾ inch extending out the top of the lid. Place the lid on the container. Fold the protruding wicks over toward the opposite sides of the feeder, so the wicks resemble the petals of a flower. The sugar-water solution should be replaced every other day.

Butterflies need 18 hours of light per day to mate and lay eggs. Otherwise, normal room lighting is sufficient. With proper care, adult butterflies can live up to 14 days.

Brine Shrimp

Live brine shrimp (*Artemia salina*), also called "Sea Monkeys," are found worldwide in saltwater, though not in oceans. Under ideal conditions, adult brine shrimp live up to 3 months.

To hatch brine shrimp, use a container with a large surface area, such as a plastic pan, an enameled bucket, or an 8-inch culture dish. Fill the container to within 3 cm of the top with a 1% non-iodized salt solution. Sprinkle the shrimp eggs evenly over the surface of the solution. For a liter of solution,

add about 1 gram of eggs; for 4 liters of solution add up to 3 grams. Maintain the culture at room temperature (21°C, 70°F). Constant light is needed for hatching, so you need a lamp. Add a coarse bubbling air stone or other bubbler to provide circulation and oxygen. Eggs hatch in 24–36 hours. Prepare a new bottle of saltwater and add unhatched eggs to it for a second, delayed hatch.

Strain the shrimp from the salt solution with a fine mesh aquarium net or handkerchief. Before feeding them to a freshwater predator, pour fresh springwater over the shrimp three times to remove all traces of salt. This saltwater arthropod can survive in freshwater 3–5 hours before dying.

Carolina Biological Supply also provides adult brine shrimp. To maintain adult brine shrimp, add synthetic sea salt, lobster salt, or a mixture of rock salt (9 oz) and Epsom salts (3 oz) at the rate of 12 ounces of salt per gallon of springwater or conditioned tap water. Carefully pour the brine shrimp into a net and allow the shipping water to go down the drain. Rinse the brine shrimp quickly under the cold-water tap, and then release them into the saltwater at the rate of 2 ounces of shrimp to 2 quarts of solution. Do not cover the container unless the water level is less than 4 inches below the top. Aerate the entire length of the container using a bubble-wand aerating device. Any temperature between 25°C and 30°C (77°F and 86°F) is acceptable. **Note:** Adults may be maintained at cooler temperatures (7–16°C, 45–60°F) for short-term storage. Continuous aeration will keep the brine shrimp from dying.

If brine shrimp are kept for more than 2 days, it is necessary to feed them. Brine shrimp are nondiscriminating, particulate filter feeders. One food option is a yeast suspension (baker's or brewer's yeast). To prepare a yeast suspension, make a salt solution as before. Stir in enough baker's yeast to make the water milky, and store in a refrigerator. Agitate the water mixture before use to resuspend the yeast. Feed shrimp a few drops of the yeast suspension each day. An alternative is to crush three or four grains of dry baker's yeast on clean paper and dust this onto the surface of the brine shrimp culture. Avoid overfeeding. Water quality deteriorates quickly when feeding. Therefore, monitoring of water quality is essential to the survival of the adult brine shrimp.

To prevent buildup of waste products, draw off one-fourth of the water weekly and replace it with fresh saltwater.

Centipedes and Millipedes

Centipedes and millipedes are land dwellers; however, they prefer habitats that are moist or humid. They are easily maintained in terraria at room temperature. They need humidity, thus the terrarium soil should be kept damp by misting with a water bottle. Other than humidity, they require little attention. Centipedes can be kept in crushed leaf mold; however, each one must be kept in a separate container. Millipedes can be housed together in leaf mold and rotting hardwood.

Centipedes are long-lived arthropods, as some have been known to live up to 6 years. They have a pair of poison claws under their head, which enables them to successfully capture their prey. Centipedes are very active predators and may be fed crickets, roaches, moths, worms, or spiders. Some centipedes are able to deliver a painful bite, so do not handle them.

The lifespan of a millipede is 2–5 years when maintained in relatively humid environments. As herbivores, they mostly eat decaying vegetable matter and plant material. They may also be fed fish food flakes, pieces of raw potatoes, apples, lettuce, mushrooms, or carrots. Chitin is essential for molting and growth of a new exoskeleton. Oak leaves are a great source of this nutrient. If mold begins to develop, removing any uneaten food from the habitat or increasing the ventilation may resolve the problem. Unlike centipedes, millipedes do not bite. However, they can secrete a liquid that emits a repugnant odor. The millipedes provided by Carolina have a very mild secretion, but be sure to avoid contact with your eyes or mouth, and wash your hands after touching the organisms.

Cockroaches

Remove cockroaches from the shipping carton as soon as possible. If the roaches are not to be used immediately, they should be placed in a container and given food and water. They will chew through cloth and cardboard, so provide the roaches with housing made of metal or plastic, such as a waste can covered with a screen top. As a substrate, cover the bottom with a few layers of paper towel or cardboard. A 30- $\times$ 30-cm cage is sufficient for up to 24 American (*Periplaneta americana*) roaches or 24 German (*Blatella germanica*) roaches. It is sufficient for up to 10 Madagascar hissing roaches (*Gromphadorhina laevigata*) or 10 *Blaberus* cockroaches. Due to differences in native environment, a viable habitat for the Madagascar hissing roach has different requirements. Wood chips or bark are good substrates for the bottom of the Madagascar hissing roach cage. You may provide half an egg crate or the paper tube from a roll of paper towels or toilet tissue to increase

hiding space. You may also want to add a branch for climbing. Carolina Biological Supply's Madagascar Hissing Roach Habitat set comes complete with a lidded cage, two Madagascar hissing roaches, wood chips, food, a water dish, a tree branch, and instructions (14-3660).

Cockroaches can be fed dog food, rolled oats, wheat germ, bread, apple, and potato. Water should be given by the inverted vial method. Fill a culture vial half-full of water. Place the bottom half of a petri dish face-down over the mouth of the vial, and then quickly invert the dish and vial so that the vial is upside-down and water trickles into the (now upright) petri dish bottom.

Alternatively, use a small plastic container with a hole punched in the lid. Insert a cotton wick through the hole down into the water. The wick should extend at least 1 inch through the lid. *Periplaneta*, *Blatella*, and Madagascar hissing roaches can be maintained between 21°C and 26°C (70°F and 79°F). However, *Blaberus* cockroaches should be maintained between 27°C and 30°C (81°F and 86°F).

Madagascar Hissing Roaches

Madagascar hissing roaches hiss by pushing air out of their spiracles, tiny holes in the sides of the abdomen that are also used for breathing. After the roaches become accustomed to being handled, they seldom hiss except when courting.

Madagascar roaches sometimes have mites. These mites will remain only on the roaches and do not harm them; the mites do not live on humans. If desired, the mites can be removed by gently shaking the roach in a plastic bag with a small amount of flour (i.e., the "shake and bake" method). The mites fall off the roach into the flour. Tie off and discard the bag, and gently spray the excess flour off the roach with plain water from a mist bottle.

Mature male Madagascar roaches have bumps on the top of the thorax (the section behind the head) that resemble horns. The males use these protrusions to nudge other males out of their territory. The male's antennae are thicker than those of the female, and the male's last abdominal segment is narrower than the others, having a C-shaped flap at the end. In females, the last abdominal segment is wider than any of the others.

Madagascar roaches are livebearers. Females carry the ootheca (egg case) internally, and release young nymphs only after the eggs have hatched. Sometimes, females release undeveloped eggs that are in an egg case; these are not viable. Babies are born in batches of 30–40. The young roaches reach adult size on the seventh molt, which usually occurs in fewer than 6 months. If you have nymphs, be sure your habitat does not

have openings large enough for them to escape. If your habitat has large ventilation holes, place a thin layer of cloth under the lid to prevent the nymphs from escaping.

Copepods and Ostracods

Copepods, ostracods, and small cladocerans such as *Alonella* can be cultured on wheat germ medium. Pasteurize springwater by heating it to 65°C (149°F) or bringing to a momentary boil. While the water is still hot, pour 200 mL into each culture dish. Add four grains of previously boiled wheat to each dish. When the water cools to room temperature, inoculate with the copepod, ostracod, or other organism.

Crayfish

Crayfish can be kept in an aquarium tank in a cool location out of direct sunlight. Do not refrigerate them. The water should be 1–2 cm deep for small crayfish, and 3–4 cm deep for large crayfish. Change the water daily. The ideal temperature range of the water is 18–25°C (65–77°F); however, crayfish are able to withstand much cooler or warmer temperatures. Upon arrival, acclimate the crayfish to their new environment by floating the shipping bag in the prepared habitat water for 45–60 minutes. The water should have a pH range of 7.5–8.5, because water with extremely low pH promotes a risk of a calcium deficiency that could interfere with molting. Place rocks at one end of the tank so the animals can get out of the water. Crayfish will endure some crowding, but they should not be piled one upon another. Add a few healthy sprigs of *Elodea* or other aquatic plants for food.

Some crayfish are shipped "dry" in moist sphagnum moss, so the acclimation process is different. Remove the box containing the crayfish from its shipping container. Inspect the crayfish and mist with room-temperature dechlorinated water. If the box and contents are at room temperature, you can proceed. If the box is not at room temperature, close the lid to conserve moisture and wait 15–20 minutes before proceeding. Once you are certain that everything is at room temperature, transfer the crayfish into holding containers or habitats.

Crayfish are omnivores. They will consume many types of plants and animals, living or dead. Feed crayfish sparingly. Remove uneaten food after a few minutes to avoid contaminating the water. Crayfish will eat small pieces of raw meat such as ham, beef, fish, and frog. They may also be fed pieces of dried cat food. Place the food conspicuously in front of the crayfish.

The average lifespan of crayfish is 2–3 years; however, some species live longer. During their lifetime, they grow through the process of molting. Immediately after molting, they are soft and not well protected from predators. Typically, it takes several days for their new exoskeleton to completely harden. Young crayfish may molt every week, while adults may molt only a few times a year.

Damselfly and Dragonfly Nymphs

Damselfly and dragonfly nymphs are cannibalistic and should be maintained separately. Keep them in aquaria, small jars, or glasses of water. Nymphs keep satisfactorily at a temperatures ranging from 10–31°C (50–88°F), but do not place them in direct sunlight, as overheating may occur. They eat *Daphnia* and the larvae of various Diptera (e.g., mosquitoes and house flies).

Daphnia

When the *Daphnia* arrive, remove the lid from the shipping jar but do not aerate the culture. Keep the jar in a cool area (21°C, 70°F) out of direct sunlight. This is all that is needed for short-term care (3–5 days). If you plan to maintain the culture for a longer period, provide *Daphnia* with a glass or plastic habitat (e.g., a 2-liter or larger aquarium) filled with springwater. If springwater is not available, collect water from a local unpolluted stream, lake, or farm pond, and then run it through rapid filtering paper or close-mesh, undyed white cotton cloth. Do not use city tap water or distilled water, as these sources may contain contaminants or chlorine levels that are too stressful.

Prepare the culture container in the following way. Hard boil an egg and remove the yolk. Cut a portion equivalent to one-fourth of the total egg yolk, mash finely, and put into the aquarium. This will start vigorous bacterial growth upon which *Daphnia* will feed. **Caution:** *Do not add too much egg yolk.* Allow 5 days for the container to age. Remove any scum that appears on the surface, and do not aerate the culture.

To introduce *Daphnia* into the culture container, hold the shipping jar at a 45° angle and gently submerge it. Then, "pour" underwater and remove the empty jar. **Important:** Do not pour the *Daphnia* through the air into the culture container. If you do, air will become trapped beneath the carapace of the animals, and they will rise to the water's surface and die.

As the tank water begins to clear, add an additional small amount of freshly boiled egg yolk once weekly. Do not clean debris that builds up on the tank bottom as it may contain *Daphnia* eggs that will hatch later. The water in

the culture should not become clouded. Each week draw off and discard about one-fourth of the culture water and replace it with springwater. If the *Daphnia* do not survive, the difficulty is most likely from the type and age of the water used or from an excessive amount of added egg yolk.

Drosophila

The common fruit fly, *Drosophila melanogaster*, is the animal most widely used in genetic studies. The fruit fly is easily cultured and its generation time is only 2 weeks at 21°C (70°F).

When cultures arrive, remove the caps but leave the plugs in place. Keep cultures out of direct sunlight, and maintain at 20–25°C (68–77°F). The development of the flies is normally slowed when the cultures are kept at lower temperatures, while higher temperatures may promote male sterility, growth of bacteria and fungi, and mite infestation.

Use transparent vials or glass or plastic bottles as culture vessels for *Drosophila*. Fruit flies can be raised on a variety of fermenting plant materials. However, to minimize problems with excess moisture and mold, use Carolina's Formula 4-24® Instant *Drosophila* Medium. It does not require cooking or sterilizing, and is available with a blue coloring agent to facilitate observation of larvae, which are white (17-3200, plain; 17-3210 blue). Food medium should be moist at all times, as dry food will inhibit larval growth and result in few flies eclosing. Transfer flies to new culture vials every 10–14 days.

For more detailed information on collecting virgins, sex determination, anesthetizing, and mutants, purchase the Carolina™ *Drosophila* Manual (45-2620).

Fiddler Crabs

The fiddler crab is a small burrowing crustacean that lives along the shores of beaches, salt marshes, and estuaries in tropical and temperate zones. The crabs supplied by Carolina are easy to maintain in the classroom.

Fiddler crabs require access to brackish water deep enough for them to crawl around in, and sand deep enough for them to burrow into. A small plastic aquarium is ideal for housing the fiddlers. They are scavengers in nature and can be fed dry or canned dog or cat food, fruits, and vegetables. Room temperature is sufficient although the crabs may be more active at higher temperatures.

Fiddler crabs are easily sexed. The male has one large claw and one small claw; the female has two small claws. The small claws are used for feeding. The male uses his large claw to threaten other fiddlers and to attract

females during the mating season. The waving motion of the large claw has been compared to the movements a musician makes while playing the fiddle; hence, the name "fiddler crab."

Occasionally, the male will lose his large claw during a fight. The missing claw will be replaced during the next molt; however, the large claw will be on the opposite side. This explains why some fiddlers have the large claw on the right while others have it on the left.

Preparing the Cage

Place a small square bowl in one end of the cage. The bowl should fit snugly against three sides of the habitat. Prepare a brackish water solution by adding 1 gram (¼ tsp) of marine salts to 1.5 L of dechlorinated water or springwater. These amounts do not have to be exact. Keep the bowl half full of water at all times and change when it becomes dirty. Pour sand into the opposite end of the cage. The sand should not be deeper than the height of the water bowl. Place the food dish on the sand and add a small amount of food. Put the lid on the habitat after the crabs are placed inside.

Ghost Shrimp

These freshwater shrimp are excellent for studies of crustacean behavior and anatomy. They are simple to maintain in a freshwater aquarium, and are an excellent food source for both freshwater and saltwater fish.

Ghost shrimp are excellent scavengers to keep in a freshwater aquarium. They are non-aggressive toward fish, even the day-old fry of livebearers. Some fish will attempt to eat the shrimp or nip at them. Therefore, it is important to not mix them with large fish. On the other hand, many types of fish, especially smaller types, ignore ghost shrimp.

Allow 60 minutes to acclimate your ghost shrimp. Avoid the three deadly stresses: temperature shock, pH stress, and toxin buildup. Open the bag containing the ghost shrimp and fold down the top to form a sleeve. Place the open bag in the tank but do not allow any water from the tank to enter the bag. Every 15 minutes, discard about one-fourth of the water in the shipping bag and replace with an equal amount of water from the tank. After an hour, transfer the ghost shrimp into the tank. Ghost shrimp do jump, so place the cover on the tank.

Do not feed the ghost shrimp the first day they are in the newly established tank. They need time to recover from the stress of shipping and to adjust to the new habitat. Thereafter, feed them a few flakes of food every 2–3 days. Ghost shrimp are omnivorous scavengers. They will eat just about any food that you offer. If you are keeping them in a tank with fish, they will eat the food left over from feeding the fish.

Dead plants or animals will need to be removed daily, and conditioned water will need to be added to the aquarium periodically to replace water lost through evaporation. Each month, siphon about one-fourth of the water in the tank and replace it with aged or conditioned water. This prevents the buildup of toxic wastes yet helps maintain pH and mineral levels. With proper maintenance, ghost shrimp can provide a long-term tool for studying biological processes in the classroom. When provided with proper nutrition and a suitable environment, ghost shrimp can live up to 2 years and grow up to 1½ inches (4 cm) in length.

Giant Water Bugs

Giant water bugs are found at the bottom of shallow ponds and lakes, often in the vegetation there. By devouring organisms as large as tadpoles, frogs, salamanders, crayfish, and fish, these bugs can be a nuisance to pond owners and fish hatcheries. Their saliva contains an enzyme that helps them immobilize, kill, and digest prey. Their bite is painful, so they should be handled with caution. Larvae meet their nutritional requirements by consuming aquatic invertebrates. It is important to make sure that they are fed live food, as motion triggers their attack. If you are feeding the adults larger animals, then it is only necessary to feed them every 4–5 days. However, if you are providing small animals (i.e., insects or other aquatic invertebrates), then feed them more frequently.

Giant water bugs are easy to maintain. For a habitat, set up a small aquarium with an air pump to keep the water oxygenated. Place some sticks in the aquarium to provide the bugs with something to grab or crawl along. Be sure to secure the lid, as the bugs as very strong fliers.

Some female giant water bugs lay their eggs on water plants, while others will lay their eggs on the back of the males. The eggs cemented on the backs of the male giant water bugs are carried until they hatch. This kind of back-brooding is a reproductive advantage, as it ensures hatching and provides protection from predators. In giant water bugs, metamorphosis is incomplete (i.e., they transition from egg to nymph to adult); therefore, the nymphs closely resemble their parents. The nymphs usually hatch in 6 days. Giant water bugs go through about eight instars over the course of a month to 10 weeks.

House Crickets

Because the house cricket, *Acheta domesticus*, can be raised year-round, it is a useful classroom insect for demonstrating incomplete metamorphosis without diapause (arrested development). A female house cricket is easily

distinguished from a male by her long, needle-like ovipositor, which extends posteriorly from the body.

Any clean container with about 3 centimeters of sand covering the bottom makes a suitable cage. If the container is at least 20 centimeters deep, no cover is needed unless the crickets can crawl up on something and jump out. Cardboard containers, egg cartons, and peat cups make good hiding places. Keep the sand in the bottom of the habitat dry unless egg-laying is desired. If the sand is dry, a water supply (e.g., fresh fruits or vegetables) must be provided.

House cricket eggs are small, banana-shaped, yellowish or white objects. They are always laid singly, not in clusters, and an adult female can lay as many as 2600 eggs during her lifetime. At room temperature, the eggs usually hatch in 2 or 3 weeks.

Adult crickets may cannibalize the eggs. If this is a problem, a dish of moist sand 3 cm deep with a wire screen should solve it. The screen mesh should be large enough for the ovipositors to pass through but small enough to prevent the adults from eating the eggs. When hatched, the nymph is the same size as the egg and is difficult to see with the unaided eye. It reaches maturity through a series of molts. The rate of development of the nymph to the adult depends on a number of factors: food, temperature, moisture, disease, and population. Nymphs held at 32°C (89°F), usually require only 30–35 days to mature, while those held at 27°C (80°F) may take twice as long. The adult usually lives about 90 days when reared at 27°C (80°F). Crickets can be fed a variety of foods. Fresh apples, pears, and lettuce are foods that provide moisture, while dry dog food provides high protein and cuts down on cannibalism.

House Flies

House flies (*Musca domestica*) are easily cultured on nothing more than sugar, powdered milk, and wood shavings. Although in nature the house fly is associated with the spread of certain pathogens, a clean culture is easily maintained under laboratory or classroom conditions.

House fly larvae can be reared in a gallon plastic container with a cloth top. Fill it 8–10 cm full with wood chips or wood shavings. Do not use cedar, redwood, or pine chips, because these are toxic to the larvae. Use shredded paper if wood chips are unavailable. Mix 250 mL of powdered milk with 500 mL of water, and pour this over the wood chips. The wood chips should be thoroughly wet, but should extend about 1.5 cm above the milk surface. Place a clump of 100–200 fly eggs on top of the chips. Try to place the eggs near the milk but not in it. Otherwise, the eggs will be drowned.

Maintain the larvae between 27°C and 32°C (80°F and 89°F), and check the culture daily. When you see the larvae crawling on the sides of the container (in 5–6 days), they are ready to pupate.

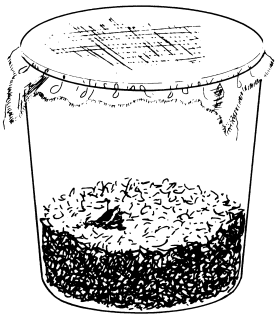

Figure 1. Larval culture vessel.

To obtain pupae, you will need two pans, one larger than the other. Line the bottom of the larger with paper towel and place two petri dishes or objects of similar thickness on the toweling. Spread the medium containing the larvae in the smaller pan until it is within 2.5 cm of the top of the pan. Set the smaller pan on the petri dishes in the larger pan and add water to wet the medium, but not enough to flood the surface or the larvae will drown. The larvae will crawl out of the wet medium and fall into the larger pan, where they will crawl under the toweling to pupate. Once the larvae have left the smaller pan, remove it and discard the wet medium. Once pupation has occurred, collect the pupae and place them in a cage for emergence of adults.

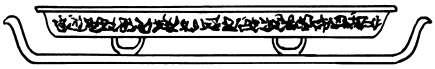

Figure 2. Apparatus for collecting pupae.

Adult flies are fed a 1:1 mixture of granulated sugar and powdered milk. A bowl filled with wood chips and water (up to the top of the chips) serves as a source of water. A small piece of meat placed on the top of the wood chips serves as a site for egg-laying. The small, white eggs are laid on or near the meat, where they can be easily collected with the point of a knife or similar instrument.

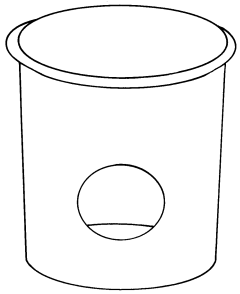

Figure 3. Wastebasket with hole.

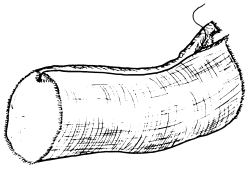

Figure 4. Cloth sleeve.

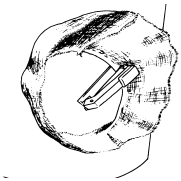

Figure 5. Attaching sleeve.

Cage Construction

We recommend a sleeve cage for holding adult house flies (e.g., Carolina's Insect Sleeve Cage, item 67-4285). This type of cage prevents the flies from escaping when the cage is opened. Another option is to make a sleeve cage from a plastic wastebasket, cloth, and staples. To construct a

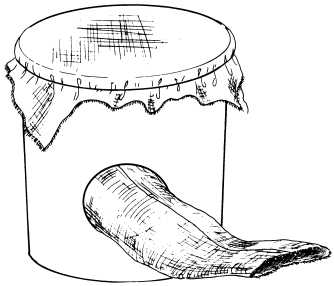

Figure 6. Completed fly cage.

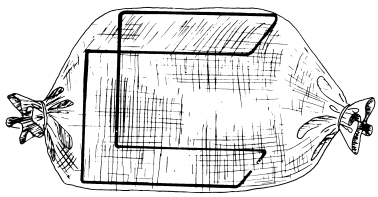

Figure 7. Simple fly cage.

sleeve cage, cut a hole in the side of the wastebasket 15–20 cm in diameter, depending on the size of the wastebasket (Fig. 3). Make a muslin sleeve, open at both ends and slightly larger in diameter than the hole in the wastebasket (Fig. 4). Fold the cloth sleeve over the edge of the hole, and then staple it (Fig. 5) or glue it using a hot-melt glue gun. Secure a piece of nylon organdy around the top with a large rubber band (Fig. 6).

A simpler sleeve cage can be made from a piece of nylon organdy sewn into a tube and supported by a simple frame (Fig. 7). The frame can be made of heavy gauge wire (e.g., a wire coathanger). After the frame is inserted midway into the cloth tube, tightly tie one end. The other end is tied loosely and serves as the opening.

Isopods

Aquatic Isopods

Aquatic isopods reside at the bottom of shallow freshwater environments such as streams, marshes, springs, and ponds. The family of aquatic isopods commonly collected in North America is Asellidae, which contains about 100 species. The cultures supplied by Carolina contain *Asellus* or a similar genera. *Asellus* thrive in muddy water and are usually active at night. They remain incognito, as they usually spend most of their time under the substrate (i.e., rocks and leaf litter). They can be used as food for salamanders and fish.

Upon receipt, remove the lid from the shipping jar and allow it to just sit on the jar, but do not aerate the culture with a pipet or any other device. Keep the jar in a cool area (21°C, 69°F) out of direct sunlight. The optimal habitat for aquatic isopods is a glass or plastic container (e.g., 20-liter or larger aquarium) filled with springwater or bottled water. **Note:** Do not use distilled water or municipal tap water. The organisms are sensitive to copper and other metal ions, which are usually present in at least trace amounts.

Cover the bottom of the habitat with dead leaves and mud from a pond to provide both food and shelter for the isopods. Aquatic isopods are scavengers, omnivores, and detritus feeders. They eat leaf matter and dead and injured aquatic organisms (i.e., small fish and tadpoles). Periodically, supplement their diet with fish flake food.

Terrestrial Isopods

Terrestrial isopods are land-dwelling crustaceans commonly known as sow bugs (*Porcellio laevis*) or pill bugs (*Armadillidium vulgare*). These two creatures look very similar, as their bodies consist of a head, thorax, and abdomen. Although similar in size, color, and life cycle, pill bugs and sow bugs can be distinguished from one another when threatened by a predator. When disturbed, pill bugs curl up into a protective ball. Sow bugs will either run or play dead when touched.

Sow bugs and pill bugs thrive in areas of high moisture, and hide under objects during the day to avoid light. Pill bugs may be kept in a plastic container with a slightly vented lid. Layer their habitat with 4–5 centimeters of damp soil and decaying organic material (wood, leaves, compost, etc.) and mist the habitat to keep it humid. To maintain humidity, place moist paper towels in the container; however, this step may not be necessary if a lid is used. Additionally, if mold becomes a problem, increase ventilation or reduce the amount of water used to mist the habitat. Place the container in a dimly lit area or dark room at room temperature, as too much heat will kill them. If a large number of pill bugs are kept in the container for long periods of time, change the soil occasionally to keep them healthy. They can be fed fish food flakes, leaf litter, and pieces of potatoes, apples, or carrots.

After mating, female isopods lay several dozen eggs, which they carry in a brood pouch located under their thorax. The young hatch in the pouch and stay there about 3 weeks. Young isopods closely resemble the adults, except for their smaller size and paler color. Small pill bugs and sow bugs molt within a day after leaving their mother, and they are sexually mature 6–7 weeks later. As with all crustaceans, they shed their shells in order to grow. During the molting process, the shell is released, and a soft, underlying shell expands before it hardens. Young isopods will molt 4 or 5 times as they grow.

Jewel Wasps (*Nasonia*)

The small jewel wasp (*Nasonia* [*Mormoniella*] *vitripennis*) is parasitic on pupae of several fly species. The adult female wasp oviposits (lays eggs) within a fly puparium, the hardened larval skin that protects the fly pupa.

The small, white, pear-shaped egg is deposited on the surface of the fly pupa in the space between the pupa and puparial wall. Usually, a number of eggs are deposited on each fly pupa.

At 25°C (77°F), the average developmental time is about 2 weeks: 2 days as an egg; 6 days as a white, grub-like larva; and 6 days as a pupa. At the end of this period, the adult wasp chews a small exit hole in the puparial wall and emerges. Adults mate immediately after emerging, and females begin to oviposit in new fly puparia if they are available.

Females that have not mated produce only male offspring, while mated females can produce both females and males. Approximately 80–85% of the progeny of mated females are female, and the remaining 15–20% are male. In the mated female, the sex of the offspring is determined by whether or not sperm (stored in a special organ, the spermatheca) are released for fertilization. Males develop from unfertilized eggs and are haploid; females develop from fertilized eggs and are diploid. This type of reproduction, common in Hymenoptera, is called arrhenotokous parthenogenesis.

Occasionally one encounters mature (no longer feeding or growing) jewel wasp larvae that do not pupate. These larvae have entered a dormant or resting stage called diapause. Diapause can be prevented by culturing the wasps at 26–30°C (78–86°F) and by keeping the culture vials in total darkness except for times of transfer.

To obtain wasp pupae, break open a parasitized fly puparium that is about 10 days old at 26°C (78°F). Use a probe or thumbnail to crack through the wall of the puparium. By carefully separating the puparial halves, you should observe the wasp pupae attached to the outside of the fly pupa. If wasp pupation has not occurred, simply place the halves of the fly puparium back together, replace the puparium in the vial, and wait for a day or two.

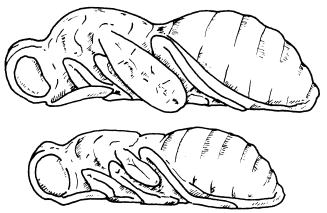

Figure 8. Female (top) and male jewel wasp pupae.

Newly formed jewel wasp pupae are white, gradually changing to a golden color within a day. As white pupae are quite delicate, do not transfer them until they have begun to darken. As a pupa ages, the eye color begins to show, the thoracic region becomes a dark gray, and finally the entire body becomes uniformly dark. Eclosion, adult emergence, occurs approximately 2 days after the complete darkening of the pupa.

Put 10 or 11 female and 6 or 7 male jewel wasp pupae in a 2-dram vial. Because adult wasps are active, it is much easier to sex the wasps as

pupae. The female pupa can be recognized by the longer wings (Fig. 8) and the ovipositor, which is seen as a pale ventral streak. Males of this species have vestigial wings; therefore, as adults they cannot fly.

Maintain the culture vial containing sexed wasp pupae at 26°C (78°F). Add fresh host pupae when the adult wasps become active. To add host pupae, tap the bottom of the culture vial against the palm of the hand. This will jar the wasps to the bottom of the vial and allow the experimenter to uncap the vial, drop in the fresh fly pupae, and recap the vial without wasps escaping. Adult female *Nasonia* are negatively geotactic. Therefore, when setting up a new culture, you can dump a few adult females on a surface and place the test tubes or vial over them and they will crawl up into the culture container.

If *Sarcophaga* pupae are being used as the host, place about the same number of fly pupae in the vial as female wasps. If house fly pupae are used, about twice as many pupae should be supplied. To obtain a maximum number of progeny, fly pupae can be replaced every day or two. To test whether *Sarcophaga* pupae are young enough to be parasitized, crack open a puparium. The fly pupa inside should be white-eyed or only slightly pink-eyed. A fly pupa that has begun to turn dark gray is no longer suitable.

Land Hermit Crabs

Land hermit crabs are best kept in a large, dry container such as a terrarium or an aquarium with 10–15 cm of sand or clean wood shavings in the bottom. A 10-gallon tank is appropriate for housing two small to medium hermit crabs for an extended period of time. Keep only crabs of similar size together, as larger crabs may dominate smaller crabs. A small branch or piece of driftwood can be added for the crabs to climb on, but the container should be large enough so they cannot climb out. A small dish of fresh, conditioned water should be available at all times. Dry moss or crumpled paper towel can be added to one side of the terrarium for the crabs to burrow under, especially during times of molt when the crabs must leave their shells.

It is critical to keep land hermit crabs warm. They are tropical and will die if exposed to temperatures below 10°C (50°F). The crabs are most active at temperatures between 22°C and 27°C (72°F and 81°F). At temperatures below 18°C (65°F), all activity stops. If your hermit crab seems lethargic or stops moving, check the temperature. If you are using a container with a cover, a 25-watt light bulb will usually give off sufficient heat to keep the crabs warms. Additionally, maintaining a humidity level between 70–90% is important. Lightly misting the sand will provide the humidity that the crabs require; however, sand should not be dripping wet.

Hermit crabs are omnivores. Dry food, pieces of apple or other fruit, lettuce, toast, and cookies all are acceptable. A vegetable diet is preferable because uneaten meats spoil or mold. Hermit crabs in captivity do not eat large quantities of food; feeding once or twice a week should be adequate. Do not be concerned if food is not immediately eaten.

At the time of molting, a hermit crab needs a source of calcium to assist in the hardening of the new exoskeleton. A few pieces of eggshell placed in the terrarium will usually meet this need. As the crab grows, it will need to move into a larger shell. Several shells of different sizes should be kept in the terrarium so the crab can change homes as necessary. Any of the spiral gastropod shells are acceptable, but keep in mind some types of shells have more interior room than others.

Mealworms

See Darkling Beetles.

Milkweed Bugs

The large milkweed bug (*Oncopeltus fasciatus*) is a member of the order Hemiptera (true bugs). Because it is a fairly typical member of the order, it is ideal for studying hemipteran morphology. The development of the milkweed bug from egg to adult is an example of incomplete metamorphosis. The young nymphs closely resemble the adults, but do not have wings or reproductive organs. Nymphs begin to develop wing stubs during the fourth and fifth instars.

Culturing

In nature, the milkweed bug feeds on several milkweeds (*Asclepias* sp.). However, in the laboratory *O. fasciatus* has been reared on various plant materials, including cracked seeds of sunflower, watermelon, squash, cashew, and almond. Initially, survival and reproduction on these unnatural foods were poor for the first seven generations, but gradually the strains became adapted to the new foods. The sunflower-seed strain is the most successful.

The obvious advantages of rearing milkweed bugs on food such as cracked sunflower seeds are that the food is relatively inexpensive and readily available. (Unsalted, cracked, sunflower seeds are available from Carolina upon request.)

Gallon jars with cloth tops make suitable culture vessels. Water can be provided from a paper towel or cotton wick protruding through a hole in the lid of a small jar. Cracked sunflower seeds serve as food for adults and nymphs. Change the water and seeds when they become dirty; seeds are not completely consumed and should be replaced when they become shrunken. Crumpled paper or paper towel provides a surface for the bugs to get to the water. A cotton ball or cheesecloth stuffed in a small jar or cup will provide a substrate for egg deposition. To reduce cannibalism, collect eggs daily and place them in a new culture vessel. Cultures do well at room temperature.

A female lays about 30 eggs a day, and 2000 during her lifetime. Egg-laying begins 10–15 days after mating and peaks at about 20 days. At 29°C (84°F) the egg stage lasts 4 days. The color of the egg gradually changes from yellow to deep orange as it nears hatching. The newly emerged nymph is about the size of a pinhead and is bright orange. The nymph grows by a series of molts. The stages between molts are called instars. There are five nymphal instars, each lasting about 6 days at 29°C (84°F). The adult lives for about 1 month. Mating takes place 5–12 days after the last molt for females and 2–3 days for males. Actual copulation may last for up to 10 hours. A high frequency of mating is necessary for maximum egg production and fertility.

In the fifth instar, the sexes can be easily distinguished by examining the posterior abdominal segments. The female has median black spots on the ventral side of the two posterior segments; the male has only one black spot on the last segment. The adult bugs are also easily sexed. The ventral side of the fourth abdominal segment (counting from the thorax) bears a black band in the male and two prominent black spots in the female.

Moina

Moina, smaller relatives of *Daphnia*, are ideal for feeding fry of freshwater fish. Although they are half the length of *Daphnia*, adult *Moina* are longer than newly hatched brine shrimp and 2 to 3 times the length of adult rotifers. They are an excellent substitute for newly hatched brine shrimp, as brine shrimp die fairly quickly in fresh water.

There are several advantages to culturing *Moina*. These freshwater crustaceans are tolerant of poor water quality; however, we recommend using conditioned tap water for optimal growth. A temperature range between 16°C and 24°C (60°F and 75°F) will yield the best growth results. Temperatures below this range, however, will not dramatically reduce production. High population density does not adversely affect *Moina* as it does *Daphnia*. Additionally, *Moina* has a higher protein content than *Daphnia*.

Culturing *Moina* can be accomplished successfully by even the most inexperienced individual, as the cultures usually reset themselves when an error is made. For instance, if an individual does something to destroy the culture, by allowing the culture to sit for a few days to a few weeks, it will adjust and start over. Most populations consist of females that undergo asexual reproduction. Under adverse conditions, males are produced and sexual reproduction takes place between the males and females.

Immediately upon receiving the culture, examine the jar of *Moina* and look for individuals that are actively swimming. Loosen the lid to permit air exchange. Place the culture in an undisturbed location that is shielded from direct sunlight. Maintain the culture at or below 22°C (72°F). Fill a 1.5-gallon plastic aquarium with a quart of algae food. Allow the aquarium content to match the ambient room temperature (waiting 30–60 minutes should be sufficient). Once the water temperature in the aquarium is close to the ambient room temperature, remove the lid from the jar of *Moina* and slowly submerge it in the aquarium. After the jar is completely full and underwater, gently turn the culture of *Moina* into the aquarium. Introducing the *Moina* into their new home this way prevents air bubbles from becoming trapped under their carapaces, which would lift them to the surface where they would die.

Two weeks after the initial setup of the culture, you will need to set up a new culture tank. You should already have a source of the algae food growing that can be used. On the same day that you initially set up the *Moina* culture tank, you should begin a new culture of the algae food source. In a transparent glass or plastic container, add the tube of Alga-Gro® concentrate to 1 liter of springwater, and mix it well. Add freshwater cultured *Nannochloropsis* to the algae medium. Place this under a cool white fluorescent light for 7–10 days or until the culture achieves a deep green coloration. Aerate or mix the algae culture once or twice a day if possible. **Option:** An alternative food source is to create a bacterial food chain. Add 1/16 teaspoon (0.2 g) of *Daphnia* Food (14-2316) to the aquarium per gallon of water.

Mosquitoes

Mosquitoes undergo complete metamorphosis, with four stages to their life cycle: egg, larva, pupa, and adult. Carolina supplies mosquito egg rafts, larvae, and pupae.

Egg Rafts

Mosquito eggs must be laid in or very near water. Some mosquitoes lay their eggs in masses known as rafts. These egg rafts float on the top of the

water and contain as many as several hundred eggs each. Our egg rafts contain about 50 eggs. The egg rafts you receive will come shipped in a petri dish with wet paper toweling. Fill the culture container with conditioned or aged tap water, which is at room temperature. Transfer the egg rafts into the culturing container. (You may soak the toweling in the culturing container until the egg rafts float off. At this time, remove the paper toweling from the culturing container). Due to fluctuations in temperature, occasionally larvae emerge during shipping and are present on the wet paper towel. Allow the eggs to sit until they hatch. Under optimum conditions, eggs usually hatch in 1–5 days.

Larvae

The larval stage is aquatic, feeding on bacteria, algae, and other tiny organisms found in the water. Larvae undergo four growth periods known as instars. At the end of each of three instars, they shed their skins and become larger. Once larvae hatch, provide them with food every third day. Each time you add food, add a small amount of fresh (aged or dechlorinated) water to the culturing container. Mosquito larvae feed well on powdered milk dissolved in water. The growth of the larvae will slow if not enough milk is provided. However, using too much milk will cause a film to develop on the surface, which will suffocate the larvae. The film must be broken up for the larvae to remain alive. If film is a continual problem, the solution should be changed. The amount of powdered milk to use depends on the volume of water and number of larvae to be reared. Allow the larvae to grow in the culturing container until they are approximately 10 days old. At this time, you will need to change the water once per week. Follow directions for changing water. For most species, the entire larval stage lasts between 1 and 3 weeks.

Changing Water

Select a clean container for continuing the culturing process. Fill this container with aged or dechlorinated water. You will now transfer the mosquito larvae from the old container into this newly established one. Using a fine net (65-1345), pour the water (from the old container) containing the larvae through the net. Once you have netted all the larvae, transfer them to the newly established container.

Pupae

After the fourth instar in the larval stage, the larva rises to the surface of the water and undergoes a process known as pupation. This process lasts 3–5 minutes. The mosquito sheds the larval body covering and is now shaped like a comma, with a large head region and a tiny tail region. This

pupa must remain in water. The pupa can be very active, wiggling the tail to swim in rapid jerks. The pupal stage lasts from 1–3 days, depending primarily on the water temperature (warm temperatures speed development). Pupae in water should be placed inside a mosquito cage before adults emerge.

Adults

When it is time for the adult to emerge, the pupa is at the surface of the water, where the outer covering of its body splits open. Emergence takes about 5 minutes to complete. The outer covering of the newly emerged mosquito must harden and the wings must expand before it can fly. The mosquito floats on the surface of the water until this happens. Adults may survive from several days to several weeks.

Moths

Greater Wax Moths

The greater wax moth (*Galleria mellonella*), while providing a ready food source of live food for other organisms, is an interesting insect for classroom study. It is easier and less expensive to rear than many species of Lepidoptera, yet demonstrates lepidopteran morphology and life cycle. In addition, specific nutritional and behavior experiments can be performed using this species.

The wax moth undergoes complete metamorphosis: egg, larva, pupa, adult. In nature all stages of the wax moth may be present at the same time and except during cold weather, development is continuous. The mated female moth deposits small, white, slightly oblong eggs in masses in cracks away from the light. Eggs hatch in 5–8 days at temperatures between 24°C and 27°C (75°F and 81°F), but may require up to 35 days of incubation at temperatures between 10°C and 15°C (50°F and 59°F).

Depending on the temperature, larval development ranges from 1–5 months. The warmer the temperature, the faster the larvae will develop. The most favorable temperature range for development is 29–34°C (84–93°F). Young larvae are white, about 1 mm long, and active. Mature larvae are about 25 mm long, golden-gray or brown in color, and move slower. When ready to pupate, the larvae spin cocoons. Pupation takes from 8 days to 2 months depending on temperature. The wax moth is about 2 cm long with a wingspread of 2.5–3 cm. The size of an adult depends on the quality of food consumed while a larva. The male is slightly smaller than the female and has scalloped wing edges.

Culturing

Up to 50 wax moths can be kept in a gallon jar if there is adequate ventilation. Adults do not need food and water, and will live for 1–3 weeks under these conditions. For continuous culturing, you will need to transfer your culture into a wide-mouthed jar (1–4 liters) with a fine mesh lid for ventilation. Transfer your complete wax moth culture into this. If necessary, add Greater Wax Moth Medium or used bee comb (impurities in used wax provide nutrition for the larvae) to the jar before making the transfer, so that the jar will be about two-thirds full after the addition of the culture. Greater Wax Moth Medium is available from Carolina upon special request. It is best to keep the culture jar in the dark.

To collect eggs, place a folded piece of wax paper (accordion-folded and held together with a paper clip) into the jar. The eggs masses will be laid between the folds of the paper and can be easily collected by flicking them off with the thumbnail or sharp blade.

Once collected, the eggs can be counted to set up new cultures. Put about 75 eggs in a quart jar two-thirds full of medium. Because the newly hatched larvae are so small, it is advisable to place a piece of cloth under the screen lid (do not use cloth alone as larger larvae can chew through it). When the larvae are so large they cannot pass through the screen, remove the cloth. Development from egg to adult at 29–34°C (84–93°F) takes 4–6 weeks. When the larvae are ready to pupate, they will leave the medium and pupate on the top and sides of the jar. The white cocoons can be easily removed and placed into a new container to await adult emergence.

Note: Greater Wax Moths are potential agricultural pests. Do not release them into the environment.

Hornworms

Hornworms (*Manduca sexta*) have been the focus of many behavioral, developmental, and ecological studies. The name hornworm comes from the thick extension located on top of the last abdominal segment of the larva. The hornworm is the larval (caterpillar) stage, while the adult stage is commonly referred to as the hawkmoth, sphinx moth, or hummingbird moth. In nature, this large moth first emerges in early summer. The large size of the larvae enables this organism to defoliate valuable crops quickly. It is categorized as a major agricultural pest.

Note: Do not release hornworms into the environment.

The host plants for *M. sexta* larvae are solanaceous plants. Larvae pass through five instars (stages between molts) before the pupal instar. The

greatest amount of larval growth occurs during the fourth and fifth larval instars, especially the fifth.

When the larva is approaching maturity, the dorsal aorta can be seen pulsating through the dorsal body wall. At this time, the larva stops eating, leaves the host plant, and tunnels into the soil. There it forms a chamber or cell in which to pupate. Before the actual larva-to-pupa molt, the larva enters a stage called the prepupa. It becomes inactive, shrinks in size, and its color fades somewhat. On about the sixth day after entering the ground, pupation occurs.

Initially the pupal cuticle is transparent, revealing the bright green of the inner tissue. During this stage the pupa is soft and vulnerable to being damaged. Gradually the cuticle acquires a reddish-brown color, becomes opaque, and hardens.

Culturing Hornworms

The hornworm is shipped in either the egg, larval, or pupal stage. For a shipment of eggs, begin with the instructions under "Eggs." For a shipment of larvae, begin with the instructions under "Larvae." Hornworm pupae are shipped in a roll of packing material. Gently unwrap the pupae and handle them carefully. Do not break the tongue cases or the pupae will die. Adults should begin to emerge from the pupae within 1–3 weeks. For a shipment pf pupae, begin with the instructions under "Moths."

Eggs.

Our hornworm eggs are shipped in a vial with a foam plug. Preparation of a hatching chamber (Fig. 9) for the eggs is the first step toward observing the hornworm's life cycle. Building the hatching chamber requires an 8-ounce plastic cup with a lid, an artificial hornworm diet, a plastic netting (that allows the hornworms to climb to the food and also keeps the food from falling and crushing newborns), and a plastic lid. Invert the hatching chamber so that the lid is on the bottom and the food is on the top. Remove the lid from the hatching chamber and punch four holes through it with a hole punch. This will allow air to circulate into the chamber and help prevent mold from developing on the food. Place the lid, inside facing up, on a table and put two sheets of tissue on top of it. Put the eggs on the tissue and reposition the chamber (containing the food) over the lid. Gently work the lid back onto the chamber without inverting the cup and spilling the eggs onto the food. Place the hatching chamber on a wire rack (or elevate using small props), this will help facilitate air circulation through the holes in the lid. Additionally, the lid should face down, in a warm location (approximately 27°C, 81°F) with a relative humidity of 40–50%. The eggs should hatch in about 3 days.

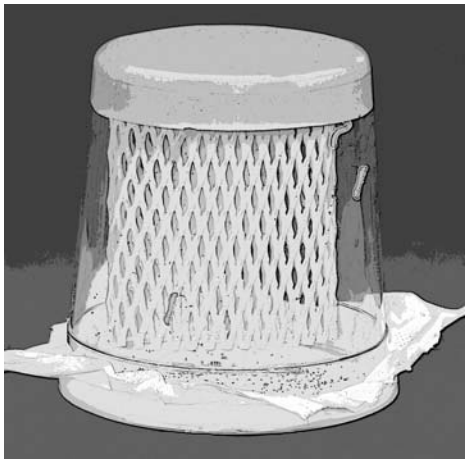

Figure 9. Hornworm egg hatching chamber.

Larvae.

It is possible to carry the hornworm through its complete life cycle in about 30 days. The critical factors are light and temperature. The larva must receive constant light; otherwise, the pupal stage may be extended for several months (diapause). A temperature of about 27°C (81°F) should be maintained throughout the larval stage. Lower temperatures can prolong the life cycle, while temperatures over 32°C (90°F) can kill the organism.

The young larvae are easily damaged by handling; therefore, it is best not to remove them from the shipping cup until they are at least 2 cm long. When the larvae are at least 2 cm long, remove them from the shipping cup and put one larva into each vial of medium you have received. (Do not put more than one larva in each vial.) Lay the vials on their sides and place a lamp with a 100-watt bulb about 25 cm above them. Regulate the temperature by moving the lamp toward or away from vials. Check the temperature several times until it has stabilized around 27°C (81°F); thereafter, check it at least twice a day.

If mold appears in the culture medium, remove the larva, wipe excess moisture from the vial, and remove the mold with the point of a sharp knife or other instrument. Replace the larva.

Pupae.

The larva should be ready to pupate 4–8 days after arriving. Every day, examine each larva for the appearance of a dark, pulsating line (aorta)

along the dorsal surface. This line usually first appears just anterior to the horn. When the line first becomes visible, prepare the larva for pupation. **Note:** Delay at this point often results in the death of the larva.

Remove the larva from its vial and wash any remaining culture medium from the vial with a jet of water. Do not use soap or detergent. Replace the larva and cover it with a dry inert material (e.g., shredded paper, sawdust, potting soil) until the vial is about half full, then recap. Do not overfill or pack the vial; the larva needs space in which to pupate. The larva also needs darkness, so wrap the vial in newspaper and secure with rubber bands. Continue to maintain the larva at 27°C (81°F). If necessary, use a lamp or an incubator. A dish of water in an incubator will help maintain the necessary humidity.

After 7 days, the pupae can be examined. Be careful when handling them, as they are easily damaged. No later than 14 days after the larvae have been prepared to pupate, the pupae should be readied for adult emergence.

Moths.

In preparation for the emergence of the moth, pupae should be transferred to a cage or other container (e.g., a terrarium). While in the cage they should be maintained at room temperature and exposed to a normal day-night cycle. The cage must contain a vertical surface (cardboard, newspaper) that the moths can climb and cling to, or their wings will crumple as they unfold and the moth will be unable to fly.

Note: Hornworms are crop pests. Do not release them into the environment.

Luna Moth and Saturniid Moth Cocoons

The moth cocoons supplied by Carolina belong to the family Saturniidae. The saturniids are some of the largest and most spectacular moths in the world. Known for its long tail and beautiful green wings, the Luna moth (*Actias luna*) is one of America's most popular silkmoths. The Luna moth has a distinctive appearance; each wing bears a small eyespot. Each moth cocoon contains a living animal, a pupa. The pupa is delicate; take special care in handling the very thin-layered cocoon. Hold the cocoon up to a bright light, and you should be able to see the pupal outline.

If the cocoons you receive do not produce moths within 3 weeks, they are probably in diapause (hibernation). To break diapause, refrigerate the cocoons for at least 2 weeks at 7°C (45°F), and then allow another 2 weeks at room temperature for emergence. During this time, the cocoons can be kept in the shipping container or in a paper bag. If the adults still do not

emerge, refrigerate them again for 4–8 weeks before bringing them out to room temperature. To prevent the cocoons from drying out, either place a bowl of water near them, or lightly mist the cocoons daily with room-temperature water.

When the moth is ready to emerge, it will attach itself to one end of the cocoon and rip the silk using hornlike projections. It also will secrete a substance that breaks down the binding of the silk. Usually, the moth will make a lot of noise during the process, which is an indication that the grand event is about to take place. Adults usually emerge from their cocoons in mid-morning. Adult moths expand and stretch their wings as soon as they emerge. Therefore, you should make sure that you provide them with adequate room, or the moth's wings will be deformed. Provide a piece of screen, cheesecloth, or some other object for the newly emerged moth to climb onto. The moth will stay on this surface until its wings have dried and hardened. After the moth's wings are dry, it will rest until nighttime, as moths are nocturnal. At night, the moths will fly around to find a mate.

Saturniid moths have a short lifespan, as they typically die within a week of finding a mate and reproducing. Moths do not feed during the adult stage. Adults survive on fat stored from the larval stage; therefore, it is not necessary to provide them with food.

Silkworms

Silkworm (*Bombyx mori*) production dates back 5000 years to China. Each cocoon a silkworm spins is composed of a single thread. It takes roughly 3000 cocoons to make one pound of silk.

Silkworm larvae must be fed fresh mulberry leaves or Silkworm Artificial Dry Diet (14-3966). The following instructions assume the use of mulberry leaves. If using Silkworm Artificial Diet, mix it according to the instructions included with the diet, and use it in place of leaves.

Hatching Eggs

When you receive your silkworm eggs, place them in a petri dish. Usually, hatching will begin in 2–3 weeks. Monitor the dish periodically for hatchlings. The eggs will turn dark just before hatching. Remove the larvae as they hatch, and transfer them to the mulberry leaves.

Larvae

Petri dishes (9 cm) are recommended as culture containers. Disinfect glass petri dishes with boiling water or use sterile, disposable petri dishes. Place a piece of filter paper in the bottom of the petri dish and moisten it (use a spray mister if possible) so that it is damp but not soggy. Wrap the end of the stem of a young, tender mulberry leaf with a piece of moist paper towel

and place in the petri dish. Using a small brush, transfer the larvae onto the leaf. Maintain the culture at an optimal temperature of 29°C (84°F). The temperature should not drop below 20°C (68°F).

During the first week, remove the leaf each day and replace it with a fresh one. After the first week, add several leaves bunched together to keep up with the increasing appetites of the larvae. Approximately 4 days after hatching, the larvae undergo their first molt. They become slightly larger and stop eating. When they have completed the molt, remove everything from the dish and disinfect it with boiling water, or use a new, sterile petri dish. Place a dry filter paper on the bottom of the petri dish, and then add new leaves. Repeat this procedure for the second molt. Remember, as the larvae grow, so does their appetite and their need for more room. The third instar larvae should be distributed into two petri dishes.

Maintain the fourth and fifth instars at temperatures between 25°C and 27°C (77°F and 81°F). It will be important to set up additional petri dishes to prevent overcrowding. Toward the end of the fifth instar, their bodies become slightly yellow and transparent; this indicates that they are ready to spin their cocoons.

Construct cocoon nests from paper towels or newspaper. Make a roll of the paper, twist one end, put two or more larvae in it, and twist the other end closed (like a Tootsie Roll® wrapper). Store the rolls in a dark room at 25°C (77°F). A mature larva needs about 3 days to spin its cocoon. Once the cocoon is completed, it takes another 2–3 days for the larva to pupate. When you are sure the pupae are fully formed, you can remove some pupae from their cocoons for observation. To do this, cut open the end of the cocoon with a razor blade and gently extract the pupa. After observation is complete, return the pupae to their cocoons. If they are handled with care, they should emerge as adults.

If you are interested in collecting silk, following a few simple procedures will allow you to produce a very fine sample. Start by boiling some intact cocoons in water for 5 minutes while turning them over continuously with a dissecting needle or similar instrument. The cocoons will begin to loosen, and you will see some tangled strands around each. Remove the cocoons from the heat. Use the dissecting needle to pick up strands until you find a single strand of silk that pulls away easily. Tie the end of the strand on a pencil and wind the silk by turning the pencil. **Note:** Boiling kills the pupa, so the moth will not emerge.

Adults

Adults will begin emerging about 2 weeks after cocoon formation. They tend to emerge in the early morning. The females are larger and less active

than the males. The males flutter their wings and crawl about in search of females. Neither sex can fly. Adults do not feed and live only a few days.

Praying Mantids (Mantises)

Praying mantids or praying mantises, are arthropods belonging to the family Mantidae. Minimal care is required for your praying mantis egg cases to hatch successfully. The most important requirement is that the egg cases (ootheca) be kept at room temperature, between 22°C and 24°C (72°F and 74°F). At this temperature range, the nymphs will emerge in 4–8 weeks. Lower temperatures will delay hatching. Temperature can be maintained by placing a desk lamp near (but not touching) the cups.

A single egg case may contain up to 200 eggs, but typically only 10–150 nymphs emerge. If the egg case is to be attached to a stick or in a bush for hatching, a thread can be passed through the outer layer without damaging the eggs. Spray mist the inside of the container weekly. It is important that you do not spray the egg case directly. Doing so might promote the growth of mold, which could be fatal. It is also important that you do not create puddles by spraying excessively. Proper humidity is essential to the survival of the mantids. To maintain proper humidity, either place a small dish of water close to the container, or wet a chemical-free white cotton ball with springwater and place it inside the container.

When the mantids hatch, separate them into individual containers such as small cups with lids. This is important because mantids are cannibalistic. For proper ventilation, punch small pin-sized holes in the lids. As the mantids grow, they will shed their exoskeleton several times, becoming increasingly larger after each molt, and they should be provided with a habitat that will accommodate their change in size. Their home should also contain a moist stick or a piece of cardboard for the insect to hang from when it sheds. A moist wick/stick may also serve as a water source. We suggest a mesh container, such as Carolina's Amazing Bugs™ Habitat (14-4092), because it will provide the mantids with something to cling to. Carolina's Plastic 1.5-gallon Aquarium/Terrarium and Cover (67-0388 and 67-0389) is another excellent habitat option.

The praying mantis eats only live prey, and must be fed soon after hatching to avoid cannibalism. Fruit flies are good food source for young mantids. Carolina Biological Supply Company offers flightless fruit flies (14-4455) for this purpose. When your package arrives, the culture vial containing fruit flies will have larvae embedded in the diet. Prepare one of the starter vials by adding 8.5 mL of water to the dry diet contained within it. This diet may be either white or blue.

Once the diet absorbs all the water (which should take 1–2 minutes), transfer the adult flies from the stock culture vial to the starter vial. To do this, gently tap the stock culture against a hard surface to knock the flies from the opening of the vial. Place the opening of the stock culture vial flush against the opening of the starter culture vial and gently shake the flies into the starter vial. (Do not use too much force; the diet could fall into the new starter vial.) Quickly replug both vials after the transfer.

The larvae in the stock culture vial will begin to emerge as adults over the next several days. The flies in the starter vial will mate and lay eggs, which will undergo complete metamorphosis and emerge as a new generation of adult flies in about 2 weeks (if stored at room temperature). Once the food in the first starter vial begins to break down, repeat the transfer process to begin the second starter vial. For best results, we recommend waiting 7–10 days before starting each subsequent culture. Discard each vial after about 4 weeks. The supply of flies and diet in the package will feed the young praying mantids for about 2 months. After this time, your mantids should be big enough to eat larger prey, such as pinhead crickets, house flies, blow flies, and young mealworms. However, crickets should not be introduced into the mantids diet until the mantids have completed their fourth molt. An alternative to fruit flies for the young mantids are aphids, which can be found in gardens.

Sarcophaga

If *Sarcophaga* pupae are to be used as hosts for Jewel Wasps (*Nasonia*) or WOWBugs™ (*Melittobia*), they should be placed in a refrigerator immediately upon receipt and left there until they are to be put with the wasps.

If the pupae are to be hatched, place them in a warm room (27°C, 80°F). *Sarcophaga* normally pupate in the ground where the moisture content is high. Therefore, it is necessary to prevent excessive drying while they are maintained in the laboratory. Usually, flies emerge about 7 days after pupae are removed from the refrigerator. Check development periodically by breaking open one or two pupae. If the flies are developing normally, their eyes will begin to darken (red) within 1 or 2 days. Then, the setae on the thorax turn black. Finally, the body darkens. Adults emerge 2–3 days after turning black.

If moisture is insufficient, the pupae will shrink and become angular in appearance. These pupae will not hatch unless moisture is provided. Often such pupae develop normally up to the time when they should hatch. Then, they are unable to break through the puparium and soon die.

A screen wire cage 60 × 60 × 60 cm with an access door is a suitable home for an adult colony of *Sarcophaga*. Dry sugar should be provided in a shallow dish. To provide a constant water source, add a shallow tray (about 20 × 30 × 5 cm) containing a bed of wood shavings 1–2 cm deep with water. When the flies are ready to lay eggs (about a week after emergence), put about 200 g of scored liver on the shavings in the water tray. A new tray of shavings and liver are required daily when the flies are laying well.

Place 1.5 kg of thinly sliced fresh liver in a tray (about 45 × 25 × 8 cm) as a rearing platform for larvae. Distribute the existing water, the scored liver on which the flies have laid eggs, and the wood shavings across the surface of the fresh liver. Place the tray in a warm, dark, humid room. Maintain high humidity in the room so the development tray does not become dry.

To collect the mature larvae, place the larval development tray in a large wooden box with openings at the bottom corners, with jars placed underneath the openings. The larvae drop from the development tray into the wooden box and migrate to the corners, where they will fall into the jars beneath.

Scorpions

Scorpions are in the arthropod class Arachnida, in the order Scorpionida. There are between 1000 and 1500 species of scorpions, all of them venomous. Although the scorpions sold by Carolina Biological Supply are relatively harmless and seldom sting, there is no reason to risk receiving a painful welt. Even the mildest sting will swell and hurt. Scorpions are more agile than they sometimes appear, and they are quick with their stinging tails. If a scorpion must be transported, nudge or herd it into a transparent cup or some other container that can be covered with a lid until the scorpion can be released into a secure enclosure. Provide air holes if the scorpion will be in the cup for longer than a few minutes. Always keep the scorpion in sight while it is being moved.

Maintain scorpions in a secure container such as a glass aquarium or plastic terrarium with a tight-fitting, ventilated lid. Scorpions can climb, so it is imperative to use a high-sided terrarium. A 5-gallon terrarium generally provides adequate space for one or two animals. However, four or five scorpions may be kept together in a community tank that is at least 20 gallons in size. This will prevent overcrowding, which is important because scorpions are cannibalistic and will eat one another when placed in close quarters. Scorpions kept together should be of similar size. When keeping multiple scorpions in a single container, provide them with numerous hiding places.

The scorpions sold by Carolina originate in the tropics and subtropics. They require a warm, humid terrarium with a substrate of bark or mulch 2–3 inches deep. Saturate the bottom ½ inch (no more) of bark or mulch with water. Spray the substrate every few days so that it stays moist. Layering the substrate with sphagnum moss will help to seal in moisture. If mold begins to grow on the substrate or if condensation appears on the walls of the aquarium, then the environment is too humid.

Scorpions are nocturnal and reclusive. They are inactive during the day and generally feed at night. They tend to burrow beneath the substrate in their cage. Because scorpions are nocturnal, they do not need to bask and do not require supplemental lighting. The best heat source is an under-tank heater (67-4411). A constant temperature of about 27°C (80°F) is ideal. Place the heater on no more than one-third of the tank bottom. This provides a heat gradient in the tank, allowing the animal to regulate its own body temperature. If a scorpion does not feed, it may be too cold. If it remains on the surface (not burrowing), acts aggressive, and appears uncoordinated or "angry," the temperature may be too high. Maintaining proper heat and humidity is vital to maintaining healthy scorpions.

Scorpions' food of choice is live crickets. Feed scorpions 1–3 live crickets once each week, occasionally supplementing their diet with mealworms, moths, or other insects. The crickets should also be provided with hearty meals, as what they consume will be passed on to the scorpions. Food should be provided at night, simulating the scorpion's normal feeding cycle. Scorpions are not equipped to swallow their prey. They secrete digestive enzymes to dissolve their prey into a liquid that can be sucked in through the mouthparts. Scorpions have a very low energy requirement, and they can survive without eating much. When they are full or when they are not hungry, they simply do not eat.

Scorpions have the potential to live a long life in captivity if they receive proper care. Scorpion life spans typically range from 3–5 years, although some species are believed to live up to 15 years. Scorpions do not undergo metamorphosis. The growth rate of scorpions is very slow; they typically go through five or six molts before they reach maturity.

Spiders

There are more than 39,000 identified species of spiders in the world, varying in both shape and color. As many as 50,000 spider species are believed to exist. Carolina Biological Supply Company offers the following spiders: cellar spiders, orb weavers, and wolf spiders. Spiders of the family Pholcidae are commonly known as daddy long-legs (not the same as the

phalangids by that name) and long-bodied cellar spiders. They weave loose, irregular webs that are primarily used as a living space; however, they also release web material over their prey to immobilize it. Orb weavers belong to the family Araneidae (ca. 2500 species). A distinguishing characteristic of this family is the orb web, which they construct to capture prey. The intricate webs of these spiders have caused them to become the favorite subjects of research into distinctive behavior and the effects of drugs on web construction. Orb weavers have poor vision and locate prey by feeling the vibration of the web. Wolf spiders, members of the family Lycosidae, are known for their keen vision and great hunting ability. Unlike cellar spiders and orb weavers, wolf spiders do not use webs to capture prey.

These spiders have similar requirements, but differ in some aspects of feeding. Flying insects are best for orb weavers, and non-flying prey is best for wolf spiders. Cellar spiders require small prey. Offer orb weavers and wolf spiders prey that is no larger than the body of the spider. Good live foods include *Drosophila*, crickets, house flies, blow flies, wax moth larvae, and roaches. Offer food two or three times weekly; spiders can survive long periods without feeding.

Spiders are cannibalistic and therefore must be kept in separate containers. Immediately upon receipt of your spiders, transfer them to habitats or holding containers. Vials, cups, or small jars with lids make good holding containers. Wolf spiders and cellar spiders, depending on their size, can be kept in 1-gallon to 5-gallon tanks. Desiccation can quickly kill small spiders, so moisten a bit of cotton and add it to each container. Make a simple water system from a small cup with lid and a cotton wick. Punch a hole in the lid and insert the wick. Fill the cup with water and snap on the lid. Alternately, offer wet cotton or bits of wet sponge in a lid. Mist the habitat daily (or at least two or three times weekly) with room-temperature dechlorinated water. Check the cover of the habitat regularly to be certain the spider cannot escape.

Use dechlorinated or deionized water. If the spiders are to be kept for only a short period, no further care is needed. Long-term maintenance requires a suitable habitat and live food. Cover the bottom of the tank with several centimeters of moist potting soil or woodland soil mix. Never use cedar chips, other wood chips, or products made for animal beddings. These can harm your spider. Add a rock or a few sticks for variety. **Caution:** *The fangs of most spiders cannot penetrate human skin; however, all spiders are venomous. Therefore, we advise against handling them.*

Superworms

See Darkling Beetles.

Tarantulas

The common name "tarantula" is actually a misnomer derived from the scientific name of a European wolf spider, *Lycosa tarantula*. Neither the species *L. tarantula* nor the tarantula are significantly dangerous to man. The tarantulas supplied by Carolina are members of the family Theraphosidae. These spiders are ground dwellers and hunt their food rather than using a web to capture prey. Both females and males, however, spin silk. The female wraps her eggs in a silk pouch (usually in spring or summer). Their bite is not significantly venomous to humans. However, a tarantula's bite is painful and there is always the chance of an allergic reaction or infection. Do not handle a tarantula until you have been instructed in proper handling techniques and you feel comfortable doing so. One of the biggest risks in handling tarantulas is that they may accidentally fall from a height and split their abdomens upon impact. Another factor to consider is the urticating hairs flicked off by some tarantulas when handled. These easy-to-remove hairs are part of a defense mechanism utilized by tarantulas when under attack. Their location on the dorsal and posterior surface of the tarantula's abdomen makes it possible for them to come in contact with sensitive areas of the human body and cause irritation. If you wish to hold the spider, place a hand in front of the spider with the palm upward. Gently prod the spider from the back with a pencil or your finger. It will crawl up on the hand. Do not try to pick up a spider that is showing a threatening pose. It might bite.

Females kept in captivity usually live between 6 and 14 years and molt after maturity. Some tarantulas have been known to live for 30 years. Males may live up to 6 years, and do not molt after maturity. The male lives only a few months to a year after reaching sexual maturity. Sexually mature males are identified by the enlarged and hooked palps they use to transfer sperm to the female. A palp resembles a leg and is located near the mouth.

Because tarantulas are cannibalistic, each spider must be housed separately in a screen-covered container. In nature, most tarantulas come together only at mating and even then the male is in danger. Habitats can range from store-bought tanks, jars, plastic storage boxes, to custom-built tanks. The only requirement is that it has proper ventilation via a screen, cheesecloth, or small holes (never larger that the carapace of the spider). Furnishing can consist of a shelter such as a cork hollow or tube, ceramic molded products such as those used in fish tanks, plants, or floral foam. It is recommended that real cork be used for furnishing, due to its superior rot resistance, and plastic for greenery, as live plants are often damaged. Add 5 cm of soil as the substrate. A mixture of peat and vermiculite holds moisture well and allows easy burrowing. Sterile soil, paper toweling, or any other moisture retaining material may be used.

Providing a moist environment is essential to maintaining healthy tarantulas. Maintain the humidity at 50–80% depending on the species. Keep the soil moist, minimizing evaporative loss of moisture from the animals and use water dishes that are just deep enough to drink from but shallow enough to escape from. Most tarantulas fare well at temperature ranges between 24°C and 29°C (75°F and 85°F). Tarantulas are susceptible to overheating, and may crawl into the water dish to cool off. An under-tank heating pad covering one-third to one-half of the tank bottom is excellent for establishing a heat gradient. Lights are not recommended as a heat source. They are stressful to animals that are nocturnal, they will dry out the cage, and they could dangerously overheat the spider.

Although tarantulas eat almost any insect they can capture (e.g., beetles, mealworms, grasshoppers, crickets), initially feed it a diet of crickets. Food should be offered twice weekly. Introduce two or three prey each time. The spider may feel threatened if too much prey is introduced at a single feeding. Lightly mist the container every 2–3 days in order to maintain humidity. Spiders obtain a significant amount of water they need from the food they eat. Remove any uneaten food after 3 hours.

If the tarantula becomes sluggish or stops feeding, it may be getting ready to molt. This normally occurs every few weeks with young tarantulas but less frequently as the spider reaches maturity. Lightly mist the tarantula once or twice daily when you suspect it is molting. Never move a molting tarantula as damage to the new, soft exoskeleton may occur, causing the spider to bleed and die. Withhold food for 2 days after the spider molts to allow the exoskeleton time to harden. The molted exoskeleton will look like a dead spider in the cage. Remove the molted exoskeleton to prevent growth of fungus and bacteria in the cage.

Termites

Termite castes include large soldiers with big mandibles; medium-sized workers with smaller jaws; and tiny, white immature forms. The queen is not included in the units Carolina ships. On average, soldiers and workers have a lifespan of 1–2 years. However, the age of your termites at the time of shipping is unknown.

Place the termites in an 8-inch culture dish or in a bucket and add layers of moist cardboard or paper towels between layers of termites. Pieces of untreated, rotting wood may also be added. Cover the container and keep it in a dark, cool place at 10–15°C (50–59°F).

Control of moisture is critical. A dry culture will soon die. A saturated culture will mold, producing foul conditions that are unsuitable for

termites. It is best to sprinkle a little water daily, or as necessary, onto the top paper to maintain a slightly moist condition. Check the moisture frequently.

Note: Termites are potential agricultural pests. Do not release them into the environment.

Tribolium

See Flour Beetles.

WOWBugs™

Melittobia digitata (WOWBugs) are tiny, stingless, parasitic wasps. WOWBugs are harmless to humans. In nature, female WOWBugs must find host organisms, usually mud dauber pupae. WOWBugs raise their young on the larvae and pupae of over 20 other insect species including, bees, wasps, beetles, and flies. Due to its small size and weak flight, the chance of an individual *Melittobia* successfully dispersing beyond its immediate area is quite small. However, if a female finds a suitable host organism, the payoff could be tremendous. Depending on the size of the host, up to 1000 young can be produced by a single female WOWBug.

This prolific reproduction rate makes WOWBugs ideal for classroom use, as does the rapid and easy to observe life cycle. With hundreds of young emerging from a single rearing container, it takes only a few cultures to produce enough WOWBugs for several classes.

At temperatures between 22°C and 30°C (72°F and 86°F), a WOWBug matures from egg to new adult in only 18–21 days. Eggs are laid first on the host organism (we use *Sarcophaga* pupae). In 3–4 days, the eggs hatch and the emerging larvae begin to consume the host organism. Larvae usually feed on the host for 7–10 days. As the host organism is consumed and the WOWBug larvae reach a certain size, they begin to pupate. The WOWBug larva does not spin a cocoon. This means that students can observe pupal development and determine the sex of the new adults before they emerge. The skin will harden into a tough outer casing. Inside this casing, the insect's body is broken down and reorganized. While in the pupal stage, the WOWBug does not move around or eat. The pupae of the WOWBugs are cream colored.

During the later part of the pupal stage, differences between males and females become evident. Females develop prominent red eyespots and four normal wings. Conversely, males do not develop eyes, and their

wings are reduced in size. The adult sexes also differ in color, as males are either the color of honey or amber, while females are black. Males spend most of their lifetime inside the host. Just before the adults emerge, the pupae become dark in color.

During the adult stage, individuals court and mate. After mating, females lay up to 800 eggs on a new host, and the life cycle continues. WOWBugs have never been observed feeding during the adult stage, although some evidence suggests that males cannibalize one another, and it is possible that females may feed on the juices of their host organism.

Your culture of WOWBugs was shipped in a rearing chamber (clear plastic box) while the insects were in the pupal stage. What you receive should resemble dark, pill-like structures. These are the puparia, the cases that enclose the fly pupae. Normally, adult flies will not emerge from the puparia because they have been so heavily parasitized by the WOWBug larvae. However, some adult WOWBugs may have emerged while in transit. If the majority of the WOWBugs are still in the pupal stage, simply wait several days and adults will emerge. There is no need to feed the adult WOWBugs. It is possible that you will see some dead adults, which were the parents of the WOWBugs that will emerge.

Culturing

To establish your own culture, you will need a supply of *Sarcophaga* pupae (17-3480) and vials with foam or cotton plugs for culture chambers. Our WOWBug Culture Kit (14-4574) contains all needed materials. When not in use, store the blow fly pupae in a refrigerator to retard their development or they will quickly develop, and adult blow flies will emerge. The blow fly pupae will remain viable several months if stored in this manner.

When you are ready to begin, place several vials on a clean sheet of white paper. Remove the lids and place them beside the vials. Pick up and examine an individual *Sarcophaga* puparium. One end should have an opening that allows for air circulation. The puparium surrounding this opening is usually thicker than at the opposite end. Damage to this end of the puparium will kill the pupa. Using your fingernails or a small knife, carefully remove the tip from the other end of the puparium to expose the pupa inside. Visually inspect the pupa. It should appear whitish and smooth-textured. If it shows signs of advanced development, or if any fluid is leaking from it, discard the puparium and try another. Place three opened puparia in each vial.

Once your vials are ready to receive the adult WOWBugs, firmly tap the culture of WOWBugs on the work surface to cause the insects to drop to the bottom of the container. Quickly remove the lid from the culture of

WOWBugs and use the sorting brush to carefully transfer 8–10 adults to each vial. Plug the vials and replace the lid on the original culture.

Note: WOWBugs are negatively geotactic and will quickly crawl up the sides of the rearing chamber and escape. Escapees can easily be seen on the sheet of white paper and can be returned to the container using the sorting brush. New cultures should be set up approximately every 3 weeks, or as soon as new adults begin to emerge in your established cultures.

Carolina Biological Supply Company
2700 York Road, Burlington, North Carolina 27215
Phone: 800.334.5551 • Fax: 800.222.7112
Technical Support: 800.227.1150 • www.carolina.com
CB130560908