

LABORATORY EXERCISE 6 **PHYLUM ARTHROPODA**

Arthropods are the most successful of the animal phyla in terms of numbers of individuals, numbers of species and diversity of form. They have adapted successfully to all environments, modifying in a variety of ways the basic body plan of exoskeleton, segmentally repeated appendages, complex internal musculature, and a well-developed nervous system. As with molluscs, our choice of a representative arthropod is dictated by practical considerations. The crayfish *Procambarus* is a good-sized fresh-water crustacean which provides a convenient study animal.

GENUS PROCAMBARUS –*Procambarus clarkii* or *Procambarus rusticus*.

(THE SPECIES USED VARIES FROM YEAR TO YEAR BASED ON AVAILABILITY)

STRUCTURES OF EXTERNAL ANATOMY AND BEHAVIOR. Observe the crayfish in the aquarium for their behavior in a group. Then grasp one animal firmly over the shield-shaped anterior segment, the cephalothorax, just behind the claws. Take it to your table and place it in a shallow container of pond water. The crayfish body is divided into two principal regions, the cephalothorax and the abdomen. It is covered by an exoskeleton composed of a chitinous cuticle impregnated with several inorganic salts. The dorsal surfaces and lateral surfaces of the cephalothorax make up the carapace, and its ventral surface is the sternum. A well-defined cervical groove extending obliquely cephalolaterad from the mid-dorsal line of the carapace divides it into cephalic and thoracic regions. The cephalomedian projection of the carapace is the rostrum, and the paired compound eyes may be seen extending cephalolaterad from beneath it. On the dorsal surface of the thoracic region, locate the branchiocardiac grooves which set off the dorsomedian cardiac region from the lateral branchiostegites. The latter cover the gill chambers. Locate the antennae and antennules.

The abdomen consists of several segments terminating caudad in a flattened structure, the telson, the ventral side of which bears the slit-like anus. Each segment consists of (1) a dorsal arched portion, the tergum; (2) a ventral, somewhat flattened portion, the sternum, and (3) a pair of ventrally projecting portions from the tergum, the pleura (singular, pleuron). Omitting the telson, how many abdominal segments are there? Are any of the segments in any way atypical? Are there obvious advantages in their modifications?

All of the appendages of the crayfish are constructed on the same fundamental plan; i.e., a basal portion consisting of two segments, collectively termed the protopodite, and extending from its distal end are two rami (branches)--the medial one, the endopodite and the lateral one, the exopodite. Touch the antennae lightly and observe the reaction. Repeat on the eyes, claws, walking legs, tail. How does an animal enclosed in rigid exoskeleton perceive stimuli from its environment? Place a small piece of earthworm in front of the animal and watch for feeding reactions. How is the food moved into the mouth? Study the movements of the eyes. Do they relate to

the movement of the animal? Grasp the animal firmly from the dorsal side over the carapace and hold it horizontal, and then tilted in various planes. How do the legs and eyes react to the position of the body and to the direction of gravitational force? From your reading, identify and describe the receptor organ involved in this behavior.

Examine the chela (claws), the walking legs, and the swimmerets. Identify the sex of the animal by examining the first abdominal appendages. They are slender and flexible in the female, and in the male, they are enlarged, rigid, and grooved to form a sperm-transmitting tube. Locate the genital opening in both sexes.

Observe how the animal moves. Does it walk in the same direction as it swims? How do you explain cephalization in this beast? The respiratory organs, or gills, are attached to the base of the legs under the overhanging edge of the carapace. To test the efficacy of the respiratory apparatus drop a few carmine particles into the water just anterior to the brachistegite. Where do the particles emerge? Try putting the carmine posterior to the brachistegite. Is there a rationale for the directionality of flow? After you have tested the behavior of live crayfish as suggested above, you may devise other tests giving your imagination free reign. Upon completing these, place the animal in the bowl of anesthetizing solution (a finger bowl half filled with pond water and 20-30 drops of chloroform mixed in it) in preparation for heart physiology data collection.

HEART PHYSIOLOGY. You may expose the heart as soon as your animal no longer responds to some of the stimuli you tested above. With the tips of your scissors, carefully remove the triangular segment of the exoskeleton outlined by the caudal edge of the cephalothorax and the branchiocardiac grooves. Flush the exposed heart with a pipetteful of physiological saline and take a one-minute "pretreatment" reading. Apply 5 drops of saline to the surface of the heart, wait 15 seconds and take a "post treatment" reading. Are the two readings the same? What could explain differences in heart beat rate even though the heart was flushed with saline in both cases? Why is it important to see whether such differences exist? Can this part of the experiment serve as a "control" against which subsequent treatments can be evaluated? Carefully test and record the effects of solutions of adrenaline/saline, acetylcholine/saline and serotonin/saline by:

1. Carefully flushing the heart with a dropper of saline
2. Taking a 1 minute "pretreatment" reading
3. Applying 5 drops of the test solution drop wise, waiting 15 seconds, and
4. Taking a 1-minute "posttreatment" reading.

Why is it necessary to continually take "pretreatment" data? How can you "normalize" the data so that the results from test treatments can be compared with the initial results from physiological saline treatment?

Carefully excise the heart and place it in a vial of clean saline. Does it still beat? Explain! Where does the heartbeat stimulus for an arthropod originate? How does this compare with the mechanism in a mollusc? Enter your data on the master sheet which will be in lab. This sheet, or the information on this sheet, will be made available to the class. Before next week, use the Microsoft Excel (see separate instruction sheet on use of Excel for data analysis) to test the class' results for statistical significance and save the data for a formal laboratory report on comparative heart physiology which you will prepare and submit later this term.

STRUCTURES OF INTERNAL ANATOMY. With your scissors, make two longitudinal dorso-lateral incisions from the triangular incision previously made to points just behind each eye. Cut laterally across the head, joining the two incisions and then carefully lift off the central portion of the carapace, gently teasing away any adhering tissue. The thoracic organs in dorsal view, beginning anteriorly are the thin-walled stomach, the mandibular muscles, the gonads, the digestive glands or "liver" and the heart.

Locate the gonads again, immediately anterior to the heart in both sexes. In the female they appear as a pair of tubular structures, which continue around each side of the heart and coalesce behind it as a single large mass. In the male, the testes are highly coiled white tubular structures. Locate the mouth. A short tubular esophagus leads from the mouth to the large sac-like stomach. The stomach consists of the large anterior cardiac portion in which food is stored, and the small posterior pyloric section in which most of the digestion occurs. Carefully remove the stomach, open it and locate the hard tooth-like gastric mill. In which portion of the stomach did you find the mill? What function does it perform? Locate the remaining digestive organs, the greenish "liver" and the straight intestine, which terminates at the anus. Between the stomach and the base of the antennae are the excretory organs commonly called the "green glands" (which are cream-colored when uncooked). Can you locate their external openings at the bases of the antennae?

Locate the supraesophageal ganglia just ventral to the eye stalks and carefully expose the nerves extending to the eyes, antennae and antennules. Follow the circumesophageal connectives to the subesophageal ganglia, carefully removing the remaining viscera as it becomes necessary for their exposure. Note the paired nerves emanating from the subesophageal ganglia.

From this point, the solid, paired nerve cord lies in a ventral compartment (sternal blood sinus) below a series of chitinous plates. These serve as points of attachment for the leg muscles and the powerful flexor muscles of the abdomen. Cut these plates, or "false floor", away with scissors and forceps. The whitish nerve cord lies along the mid-ventral line. It is delicate and easily destroyed. This cord is really a series of ganglia giving off pairs of nerves to the appendages and internal organs. The nerve cord continues into the abdomen. To trace it you must remove the muscles of the abdomen.

To remove the muscles, use the tips of your scissors cut through the abdominal exoskeleton along each lateral border to the line of articulation of the telson. Cut across this articulation *very shallowly*. Slowly lift off the great mass of abdominal muscles by grasping

the anterior edge between two fingers and gently pulling upward and back. As you do so, the ventral nerve cord will become visible. Many branch nerves connect the nerve cord with the segmental muscles. As you lift the muscles away be certain to cut these nerves; otherwise the cord will be torn into pieces.

Observe now the series of ganglia (evident as slight swellings) and the branch nerves. Terminally the cord divides into three parts. Trace these out. What anatomical parts do they innervate? If you have trouble distinguishing the nerve cord from muscles or connective tissue drop some methylene blue on the abdomen, wash it off, and the nerve cord should be stained blue.

With a sharp razor blade remove an eye and its stalk, slice it in median section, place it in water and with the binocular microscope distinguish: the cornea, the thin transparent external covering; the ommatidia, the small radial components of the compound eye; and the nerve ganglia in the stalk. Slice the cornea from the other eye, scrape away any pigmental ommatidial material and mount flat on a microscope slide. Add a coverslip. What shape is the outline (facet) of each ommatidium? How does this shape compare with that of an insect eye?

Carefully dissect away all of the appendages on one side of the body and arrange them in a labeled array on a piece of paper. Which of these bear gills? What are the functions of each type? Carefully remove the exoskeleton from the dorsal surface of the claw and study the action of the muscles which open and close the claw. Locate the pivot upon which the movable part of the claw rotates. Which of the muscles has the greater mechanical advantage? Why? Which muscle is larger?

At the completion of the exercise carefully wrap the remains of your dear departed specimen.