Dissection of a Squid

Note: At the end of class, you will turn in your annotated drawings. Please bring either a hard (#3) or a #2 pencil and an eraser. Colored pencil may be used for details; bring a set, if you have one. Do not use ink.

When drawing, use clean, simple, continuous outlines. Think about the details you choose to show—do not try to show everything. The relationships of parts, especially their connections (which sometimes require an “exploded” diagram) are your primary goals. Microscopic views can be shown with an arrow to the place you are examining on the whole squid. You will be graded on understanding, not technical facility in drawing. Shading is not usually helpful.

There are several questions in the lab. Do not turn in answers to these questions, but do not be surprised if some reappear on the final examination.

A. Preliminary matters:
This dissection concentrates on parts of the squid’s anatomy that can be related to topics covered in lecture. If in doubt, probe, look at references, and ask questions. The least useful approach is to snip the confusing bit into tiny pieces!

1. **Anatomical orientation** on a squid is tricky if you try to be logical. The rule in animals is that we draw a line between the head and the anus to set up the long axis. The back side is up (dorsal), and the bottom side is down (ventral). Since the internal anatomy of a squid is bent like a hair pin, the line between mouth and anus is very short, and the obvious functional up and down of the squid, at least from the way it leads its life, are not the standard anatomical dorsal and ventral. To simplify things, we will orient according to how the animal lives its life: head is **anterior**; the arrow shaped end of the trunk is **posterior**; the siphon (= funnel) is **ventral**, and the pen is under the **dorsal** surface. Some other useful words: **proximal** means toward the body, **distal** means away from the body (for instance, on the arms, distal suckers are smaller than proximal suckers); **medial** means toward the midline (e.g., position of the siphon), **lateral** means on or toward the side of the body (the fins are lateral).

2. The **species** we will be examining depend on what was available in the Boston fish market back in September. If we can get fresh-frozen East Coast squid from Massachusetts waters, they will be **Loligo pealei**. Frozen west coast squid are **Loligo opalescens**. Both species are fundamentally similar, but it is possible that details of one (say, the color of an organ) will not turn out to be true for another. All are near shore squid, often found in great schools. People, sea birds, predatory fish and marine mammals feed heavily on these schools.
B. Superficial matters—the outside parts of the animal

1. **Form:** All squid are streamlined for swimming in open water. The triangular fins aid in hovering, maneuvering and slow swimming. The posterior end of the body is notably stiff and arrow-shaped—when squid are in a particular hurry, this is the most likely part to be cutting into the water. Rapid swimming is effected by jetting—water that had been pulled into a muscular sheath (the mantle) that forms a cavity under the body (mantle cavity) is forcibly ejected. We will look at the details of this system later.

2. **Skin:** Use scissors to remove a small piece of the very thin skin. Put it on a slide, cover with a couple of drops of seawater, and then add a coverslip. Examine the skin under a compound microscope and draw the chromatophores. Often you will see the sac surrounding the pigment granules pulled by some of its radial muscles (but not all of them) – the result resembles a partial star. In life, the pigments are wine red, black, and golden yellow.

3. **Arms and tentacles:** the pair of long tentacles capture prey and the eight shorter arms manipulate prey and other objects of squidish interest. The arms and tentacles differ in functions, sucker distribution, internal structure, subcellular organization, and biomechanical properties. The two tentacles can be retracted into sheaths when not in use. Although death (and freezing) will alter some features, others survive. Before lab, you might take a look at a brief high speed video of how tentacles are used, made by William Kier at UNC, Chapel Hill. (His URL is at the end of this hand out.)

   Examine the suckers under the dissecting microscope. Note that they are borne on stalks (pedicles); their edges are supported by horny rings. The circumference of the sucker is pressed to a surface, then muscles in the pedicle contract, lifting the center of the sucker away from the surface. This creates a vacuum, and the sucker sticks. It is logical that the horny ring acts to resist the deformation of the muscular contraction; octopi, however, manage even greater feats of suction though they lack the horny ring on their suckers.

   Arms are used to subdue and orient prey to the mouth, but they are also used to taste, maneuver, and hold objects, such as the squid’s egg masses and potential mates. Mating itself is consummated using a specially modified arm of the male that carries sperm packages (spermatophores) to the female’s mantle. This arm is called the hectocotylus. Later, she can decide whether to transfer the sperm package to her genital opening, which is inside the mantle cavity.

   The hectocotylus in some cephalopods (though not our squid) breaks free of the male and takes up residence in the female’s mantle cavity. In some octopi, it is so highly modified that its derivation from a standard cephalopod arm is not at all obvious. Cuvier, the greatest anatomist of the early 19th century, encountering one in a female octopus, made one of his few mistakes: he described it as a new genus (and class) of parasitic worm, Hectocotylus. The name stuck.
Examine the left ventral arm of your squid. If the pedicels (stalks) of the proximal suckers are notably longer and thinner than those on the right ventral arm, it’s a hectocotylus. If, however, we have relatively small squid, many will be immature. Some years, we see a predominance of immature males, and difference in sucker development are best seen through the eye of faith. The large squid used in next week’s lab might be better to examine for sexual dimorphism.

The existence of fundamental asymmetry in a streamlined, bilaterally symmetrical animal might well occasion some surprise. The male uses left and right ventral arms very differently, in a precise order, during courtship and mating (actually, handing over the sperm packages). Internal sex organs also show some notable asymmetries.

We often ascribe a function to a structure: the heart pumps blood. To what physiological function(s) should the arms and tentacles of squid be assigned?

C. Deeply superficial matters: The mantle cavity is to molluscs what the hole is to the donut—everything is organized around it, and, although it is inside, it is really outside. The mantle cavity is a crescent shaped space, open to the environment and normally filled with sea water, below the torpedo shaped visceral mass. The wall of the mantle cavity is a sheet of muscle. Water enters the mantle cavity though the dorsal slit behind the head. A squid can put about half its weight in water into the cavity. The water is then squeezed out through the ventral siphon (also called the funnel), a muscular tube with valves that allow the water to move in one direction -- out. The siphon can be aimed forward, in which case the squid scoots backward, or turned into a U, in which case the squid jets forward.

Determine the path of water through the mantle cavity, including its relation to the organs of the mantle cavity (we will establish these points through dissection. You can use a syringe and tubing to put water into openings, but dead squid are too flaccid and leaky to get good information about the path the water would take through the squid. If enough hands are available, you may be able to figure out how much water a squid mantle holds--note that in nature, they have water on the outside, pushing in, and do not inflate into spheres! With some ingenuity, a graduated cylinder, and a big beaker, you should be able to establish the volume of your squid. Think about the biological significance of x mls of mantle and y ml of squid for jetting and respiration.

Place the squid on its back, (so the siphon is facing you), and open the mantle cavity by making a medial longitudinal cut. Carefully section the siphon. Note the cartilaginous resisting apparatus along the edge of the mantle--these ridges and sockets effectively seal the mantle cavity, so that the only way water can exit is through the siphon.
On each side of the mantle is a feather-like gill (a molluscan gill is officially a *ctenidium*, an allusion to its comb-like structure). Gas exchange requires movement of two fluids past each other—an external source relatively high in oxygen, and deoxygenated blood. Squid have a heart at the base of the gill that pumps blood through the gills. (It takes a tremendous amount of pressure to pump large volumes of blood through very fine spaces—it is no accident that fish hearts are adjacent to their gills, or our heart sits between our lungs, and two of our four heart chambers deal with the lungs alone.) The gill/heart arrangement is standard molluscan issue. But when we turn to the other fluid, cephalopods have done something very unmolluscan: (almost) all other molluscs use sheets of synchronized cilia on the gills to pull water through the gills. Cephalopods have replaced this system in favor of pumping water though the mantle cavity using muscles. In squid, thick sheets of muscle supply the pumping. (In most molluscs, the mantle has notably little musculature.)

If you closely examine the gill, you will see a white blood vessel running along one edge. When you fold back the gill to look at the other side, you will see another white vessel. One of these vessels runs to the gill from its branchial (gill) heart, and the other carries oxygenated blood to the systemic heart for distribution to the rest of the body. Which vessel would you predict to be thicker (because it deals with blood under higher pressure)? Test your hypothesis by injecting water colored with food coloring into these vessels, using a very fine hypodermic needle.

On each side of the central, supporting rod are a series of filaments—the teeth of the comb. Examine a small piece in water under the dissecting microscope. Why are there so many filaments? Why are they so finely divided?

The next obvious landmark in the mantle cavity is the silvery black, oval *ink sac*. It opens by way of a duct into the rectum, just before the anus. The rectum is on a short median tube, sensibly aimed into the excurrent that leads out the funnel. Although squid ink has many attractive properties (especially in Spanish and Italian cuisine) please defer artistic endeavors until the end of the dissection. An open ink sac will make the rest of this dissection very difficult.

In female squid, the ink sac and rectum may be partially covered by the *nidamental glands* (a pair of whitish elongated sacs) and the smaller *accessory nidamental glands* (oval, and reddish in mature females). These glands produce the jelly in which the squid eggs are embedded. The paired, red, accessory glands are visible through the translucent mantle, and may serve to announce sex and maturity to other members of the school.

The openings of the *renal sacs* (one on either side of the rectum, nearer its base than the anus) and the genital openings are small and very difficult to find in squid that have been frozen. The *penis* or *oviduct* (depending on the sex of your animal) should be to the right of the midline (assuming the head of your squid is oriented up), very near the anus. Those who fail to find these organs are encouraged to take their existence on faith.
Finally, the stellate ganglion. These huge ganglia, are found one on each side of the visceral mass, near the collar, on the inside of the mantle. They are giant nerve cells, and they control the muscular contractions that produce jetting. The nerve axons emerging from them are some of the largest neurons in the animal world, and they have been the leading experimental system for the chemistry, electrical properties, and cell biology of axons and synapses. Anyone interested in neuroscience should attempt to trace some of the neurons. Why they are so large is a very sensible question—the answer is intimately connected to the nature of neuromuscular coordination in squid. J.Z. Young discovered the potential of this model system in 1937; he was a leading worker on the cephalopod nervous system for the next 70 years—see his 1984 paper for his reflections on this experimental system.

D. The Visceral Mass

Probe before you cut, and cut only when you know you should. Cut into the clear, thin, body wall. If you have a female, carefully remove the nidamental and accessory nidamental organs. Free the ink sac from its connections to the body wall, tie off its duct with some silk, and cautiously push it to the side.

With luck (regarding the quality of preservation of your squid) and/or skill, you will now be faced with the thin, bilobed renal sac. If the sac has broken down or been cut, you won’t see anything. The sac connects a complex of organs of the coelom (true body cavity)—the circulatory system, the excretory system, and the reproductive system.

The venous system (notably, the vena cava—paired posteriorly, single anteriorly) can be followed by injecting the branchial heart with colored water. The arterial system (aorta, one anterior, two posterior) can similarly be traced from an injected systemic heart.

Beneath the venous system is the wall of the pericardium, the (coelomic) body wall that surrounds and mechanically buffers the heart. (Our body cavity has an equivalent water balloon surrounding our heart, with the same name.) When the ventricle takes in blood, it puts pressure on the fluid in the pericardium. This pressure pushes the fluid through the thin walls of the cauliflower-textured kidneys (actually, a proliferation of vessels off the vena cava). The membrane between the pericardial fluid and the kidneys are lined with special cells called podocytes, so named because under very high magnification (electron microscope, not light microscope) they stand on little feet. The spaces between the feet are lined with extracellular matrix which serves as a molecular sieve, through which waste products are preferentially pushed. Two sets of fluid, at different pressure levels, separated by a membrane supporting podocytes, is the same system we use in our kidneys. Molluscs have merely taken advantage of the alternating high and low pressures in the pericardium—a point our bodies have missed. (When the squid heart contracts, pressure on the pericardium is lowered, which pulls in more fluid to for the next round of filtration.)
To study the digestive system, continue the cut into the body cavity forward, past the siphon, and past the eyes to the mouth. The large, smooth organ in the anterior of the body cavity is the digestive gland, often called the liver. In most molluscs, it receives very small particles of food from the stomach, which are taken in (phagocytosed) by digestive gland cells and digested intracellularly. Squid, however, pour enzymes into their stomach, as we do, and then take the dissolved products of digestion into the blood. Their digestive gland has become functionally equivalent to our liver; both perform chemical manipulations on the dissolved products of digestion. Why have squid departed from typical molluscan digestion, by phagocytosis in the digestive gland?

Trace the digestive system from the end (anus, rectum, intestine) back to the relatively small stomach. Inside the stomach, you might find evidence of a last meal, which should be examined under the dissecting microscope. We have found fish scales, bones, and otoliths (fish ear bones) in the stomach. The cuticle lined gizzard is the part of the stomach that continues the physical breakdown of food. The large, thin walled, blind sac, or caecum, is where the chemical digestion of food occurs. (A duct from the “liver,” passing through the “pancreas,” brings digestive enzymes to the caecum.)

The esophagus passes through a valley in the liver to the stomach. Anterior to the esophagus is the muscular buccal bulb. It powers the parrot-like jaws (unlike parrots, the lower beak goes over the upper beak). Behind the beak is the radula, a rasp with hundreds of teeth on a tough ribbon that shred the prey. It is an intriguing object under the microscope, but do not attempt to get it (or the beak) out until after you have attended to the brain, below.

There is either a single, medial testis (thin, long, white) or an ovary. In season females may be largely filled with developing eggs, parked in the coelom. The reproductive system is closely associated with the coelom, the rule in all animals that have coeloms.

Finally, the nervous system: There are two ways to do this.

1) If your squid is large, and you like careful dissection, turn the squid over, and make a dorsal cut into the head musculature, between the eyes. The brain is surrounded by a skull of cartilage. Cut through the skull to reveal the cerebral ganglion--this is the seat of most of the remarkable behavior and sensory integration. The amazing visual prowess of cephalopods is controlled by the optic ganglia--large, bean shaped structures close to the eyeballs, and connected to the brain by narrow stalks. Dissect from the midline laterally toward the eye to expose the optic ganglion and its connective to the brain. Then look for a large ganglion beneath the esophagus, the pedal ganglion, responsible for connecting the body to the sensory and integrative center above. Beneath it is another cartilage. Commissures connect it to the cerebral ganglion above, and to a more anterior subesophageal ganglion that controls the radula, beak, arms, and tentacles, and a posterior subesophageal ganglion, (sitting on another cartilage), which connects to the stellate ganglion and nerves of the viscera.
2) If you have a small squid, limited time, or less than stellar preservation, use a razor blade to slice through the head, between the eyes, and examine the brain in cross-section.

Either way, note that the brain surrounds the gut, and is encased in a skull of cartilage. Any large piece of a meal that cannot be broken down by the jaws must be swallowed whole. The pen of a cannibalized squid nearly as long as that of the cannibal has been found ingested. In order to swallow it, the meal would have squeezed the brain flat against the skull. As Anna Bidder, the leading student of squid visceral anatomy, noted, this would have been “almost as inconvenient for the eater as the eaten” (1950).

Dissect the eyes. Note the wonderful, hard, clear lens and reflective pigment layer behind the retina. The ring around the lens is the attachment for the ciliary body, by which the lens is moved. Very small cartilages help hold the eye’s shape. Even in dead squid, the eyes are often still under higher pressure than the rest of the body--they deflate when nicked. Why are the interior of the eyes kept under greater pressure than the rest of the body?

Finally, dissect out the beaks and radula; examine the latter under the microscope. Also dissect out the pen--it is a highly modified shell. Shells are, anatomically, outside the body. If the pen is a shell, how can you interpret its topological relation to be “actually” outside the mantle, when it so obviously seems to be inside it? Note that several of the materials we have just removed--skull cartilages, pen, ribbon of radula, layers of lens--may reveal unexpected details under darkfield or polarized light under the dissecting microscope, or with phase microscopy.

**Next time:** You will be designing an experiment on the mechanical properties of squid mantle. It would be very useful for you to think about how the squid can pull water into its mantle after it jets. A thoughtful examination of longitudinal and transverse microscopic sections of young, whole squid might be a very useful way to prepare. You should also read the lab instructions well before you come in to lab, and plan your experiment in advance.

**Literature Cited:**


Kier, W., on WWW: http://www.unc.edu/depts/biology/kier.html