CHAPTER 6:
VERTEBRATE DIVERSITY

Purpose:

1. To study some vertebrates with emphasis on successful adaptations to aquatic and terrestrial life.
2. To understand the basic characteristics of chordates and the classes of vertebrates.
3. To compare chordates in terms of habitat, mode of feeding, locomotion and body plan.
4. To experience a brief introduction to vertebrate anatomy using a perch, a frog and a rat for dissection.

Materials:

Preserved perch, frog, and rat for dissection. Dissection tools. Live fish in marine and fresh water tanks. Models. Videotape. Film loop.

Classification:

Kingdom Animalia
Phylum Chordata
Subphylum Urochordata
Subphylum Cephalochordata
Subphylum Vertebrata
- Class Osteichthyes
- Class Mammalia
- Class Amphibia

Introduction:

All chordates possess the following three distinguishing characteristics which are present in the embryological or larval stages and may be present in the adult:

1. Dorsal hollow nerve cord
2. Notochord
3. Pharyngeal gill slits

Chordates are divided into three subphyla, the tunicates, the cephalochordates and the vertebrates. The first two groups will be discussed briefly but we will focus our attention on the vertebrates.

Subphylum Urochordata (Tunicates)

The tunicates are the most primitive of the chordates; the adults have lost most of the basic characteristics after the embryonic stage. The larval tunicate resembles a tadpole. In preparation for metamorphosis, the larva attaches itself to a rock, then undergoes rapid changes during which the notochord and nerve cord degenerate but the pharyngeal gill slits are retained for the filtration of water for oxygen and food.

Subphylum Cephalochordata

The representative of this group is the amphioxus, a fish-like animal that retains the chordate characteristics throughout its life. The amphioxus can swim but prefers to bury itself in the sand in shallow coastal waters exposing only its mouth for feeding. In the adult, the notochord , which is flexible and resilient, provides support along the longitudinal axis of the body and is an evolutionary forerunner of the vertebral column in

the vertebrates.
Subphylum Vertebrata

Class Agnatha (Jawless fishes)

The first vertebrates were simple jawless fishes similar to the amphioxus but possessing a true head and vertebrae. These fishes were precursors of the modern living jawless fishes, the hagfish and the lamprey, which are scavengers and often are parasites on other fishes. The lamprey is an elongated, eel-like animal with a long fin on its back (median), a nostril on top of the head between the eyes, seven gill openings behind the eyes and a weird round jawless mouth containing sharp rasping teeth for boring into its fish victims to suck their blood. The agnathans possess the following primitive characteristics:

1. No jaws
2. Scaleless skin
3. Many gill slits
4. Continuous median fin
5. No paired fins
6. Persistent notochord

Class Chondrichthyes (Cartilaginous fishes)

Jawed fishes evolved from jawless ancestors about 350 million years ago. The cartilaginous fishes are a branch of the early jawed bony fishes (class Placodermi). A skeleton of cartilage is the major characteristic of this group which includes the sharks and rays. Other characteristics include:

1. Internal fertilization with male pelvic fins adapted for clasping the female
2. Absence of lungs or air bladders
3. Most sharks have separate gill openings
4. Predominantly marine fishes

The evolution of higher sharks has followed two lines. The first contains the "typical" sharks which are the streamlined, fast, aggressive and predaceous fishes. The other line contains the skates and rays which are adapted for life on the ocean bottom.

Class Osteichthyes (Bony fishes)

Of all the animals that have lived in the water, none has been so successful as the bony fishes. They occur in virtually all aquatic habitats, from the abyssal depths of the ocean to rocky shores and freshwater lakes and ponds. The bony fishes have evolved species showing a remarkably wide range in size and show great diversity of body form and of adaptations, particularly in water balance. Bony fishes are characterized by a skeleton of bone and an air bladder (the swim bladder) which functions in buoyancy control. The swim bladder evolved separately from the lungs of other vertebrates; these two structures are not homologous since they have different embryological origins, and different ultrastructure and different vascularization (pattern of blood vessels). Swim bladders arise from the dorsal surface of the pharynx and are composed of esophageal tissue whereas lungs arise from the ventral surface and are composed of pharyngeal tissue. The perch, a common freshwater fish, is a modern example of the bony fishes and we will examine one in lab.

Procedure:

Perch dissection

External anatomy

1. The body consists of the **head**, which is separated from the **trunk** by the gill openings, and the **tail**. Each of the gill openings is covered by a bony **operculum**. The overall shape of the perch is **fusiform** (torpedo-shaped) reflecting the fact that the head is smaller than the trunk. The **mouth** of the perch is **terminal**, an

indication of the animals' feeding habits of overtaking prey while swimming. Fish which have a ventral mouth are bottom feeders and those which have dorsal mouths are surface feeders.

2. The perch has two sets of paired appendages, the **pectoral** and the **pelvic** fins, as well as the unpaired **dorsal** fin on top of the fish, the **caudal** fin on the tail and the **anal** fin behind the anus and urogenital opening. The outer body covering of the perch consists of **ctenoid** scales, named for the presence of **ctenii** (teeth) on the portions of the surface of the scale. The skin of the perch is also covered by a layer of mucus for reduction of friction as the fish swims through the water.

3. The sense organs are varied and specialized: (1) the **lateral line organs** of the perch are a series of interconnected tubules which are open to the surface of the fish and are sensitive to changes in pressure and temperature in the water; (2) the **ear** consists only of an inner ear that contains **semicircular canals** that enable the fish to maintain the proper position in the water (the swim bladder may serve as an aid to hearing as well); (3) the **eyes**; (4) and the **olfactory sacs**, which have openings to allow water to pass through, detecting odors in the water as the fish swims.

Internal Anatomy

1. Locate the gill covers and cut away the operculum from the left side, examining the underlying **gills**. With scissors, cut through the angle of the jaws of the left side, separating the cut edges. Examine the **mouth cavity** and the anterior part of the **pharynx**. Can you see the gill slits on the inner side? Probe the gill slits and determine the path of the water through this respiratory system.

2. Remove each of the gills from the left side by cutting through their bony supports above and below. How many gills are there? Place one **gill arch** in water and examine with dissection microscope. Extending outwardly from the gill arch is a double row of **gill filaments**, or **lamellae**. What is their function? Along the inner margin of the gill arch are the **gill rakers**. What is their function?

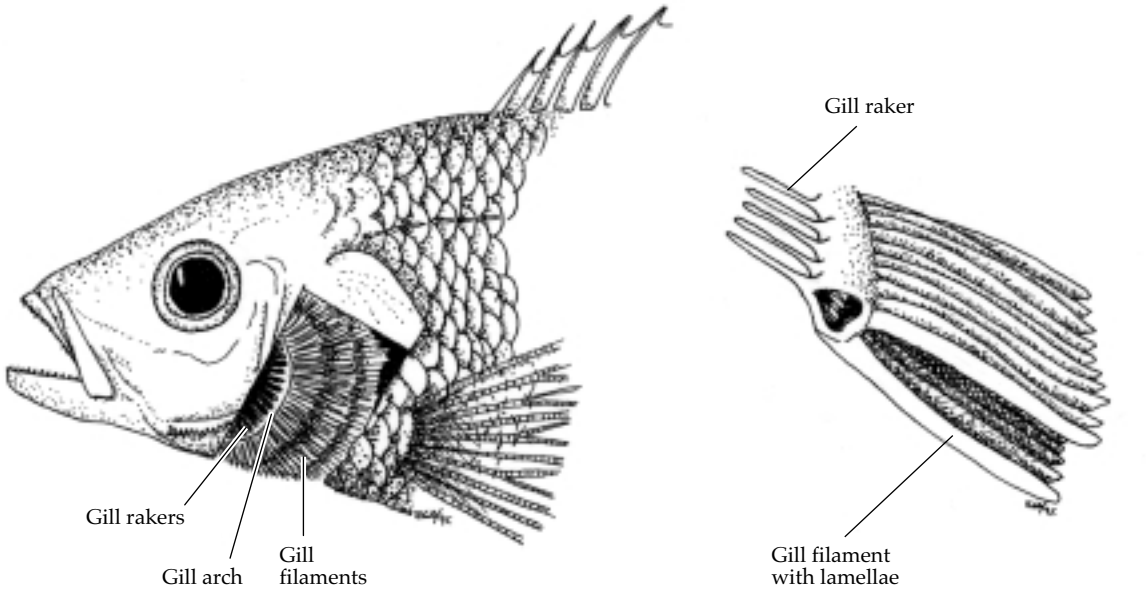

Figure 6.1. Perch gills.

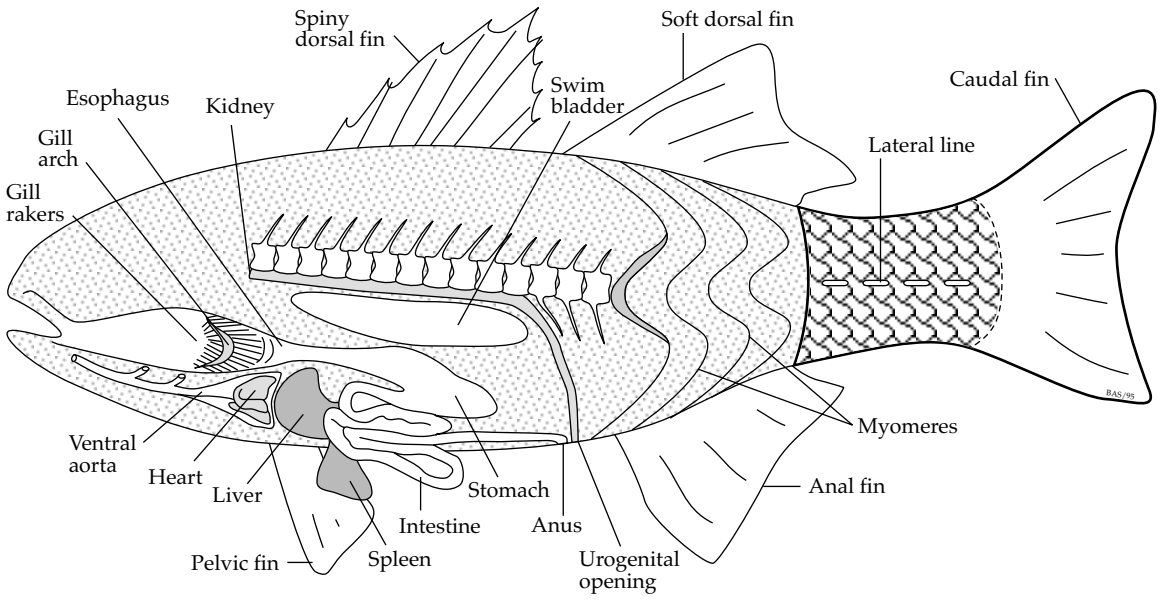

Figure 6.2. Internal anatomy of the perch.

3. The **viscera** can now be exposed; they lie in the peritoneal cavity. Remove the left body wall by cutting with scissors. Start the incision from the anus and continue forward along the midventral line to the pelvic fins, diagonally upward to the pectoral fin, then posteriorly along the lateral line of the body to the level of the anus. Do not remove the fins. Locate the **liver** and the **gall bladder** between the lobes of the liver; the **stomach**; **esophagus**; **intestine**; **spleen**; **gonads** (ovaries or testes); **swim bladder**.

4. Remove the swim bladder from the body. Lying in the roof of the swim bladder is a large blood vessel, near the mid-dorsal line, which is the **dorsal aorta**. Parallel to this major vessel is a pair of long, narrow, dark, strap-like **kidneys**.

5. With your scissors make a horizontal cut through the anterior wall of the peritoneal cavity in front of the liver. This cut will expose the **pericardial cavity** in which the 2-chambered **heart** lies. Note that the heart consists of a **ventricle**, an **atrium** and a **sinus venosus**. The large vessel carrying blood forward from the ventricle is the **ventral aorta**. Remove the heart and set it aside for next week in the plastic bag provided.

6. Skin the right side of the body. Notice the thick layer of muscles. This layer is divided into zigzag bands of muscle tissue called **myomeres**. The myomeres are separated from each other by bands of connective tissue, the **myosepta**. This arrangement of muscles as seen in the fish is the primitive vertebral arrangement from which much of the muscular system of all land vertebrates is derived.

7. Remove all of the flesh from the right side so as to expose the vertebral column. Remove one vertebra from the trunk region and another from the tail region. Note the following structures on each of the vertebrae: **centrum**, **neural arch**, **neural spine**, **hemal arch** and **hemal spine**. What structures are contained in each of the arches?

8. Observe the fish in the Biospace aquaria. Your instructor will help you identify the various kinds in the tank. Watch them during normal swimming, feeding and escape behaviors. Notice the fins used under each circumstance. Can you speculate where they would live under natural conditions?

An important factor in determining the speed of fish is the shape of the caudal (tail) fin. The ratio of the height of the fin over the area of the fin (ht/area) is called the **aspect ratio**. A speedy fish has a high aspect ratio, that is, the height is larger than the area of the fin. Sketch the tail fin of a slow fish and a fast fish in the aquarium.

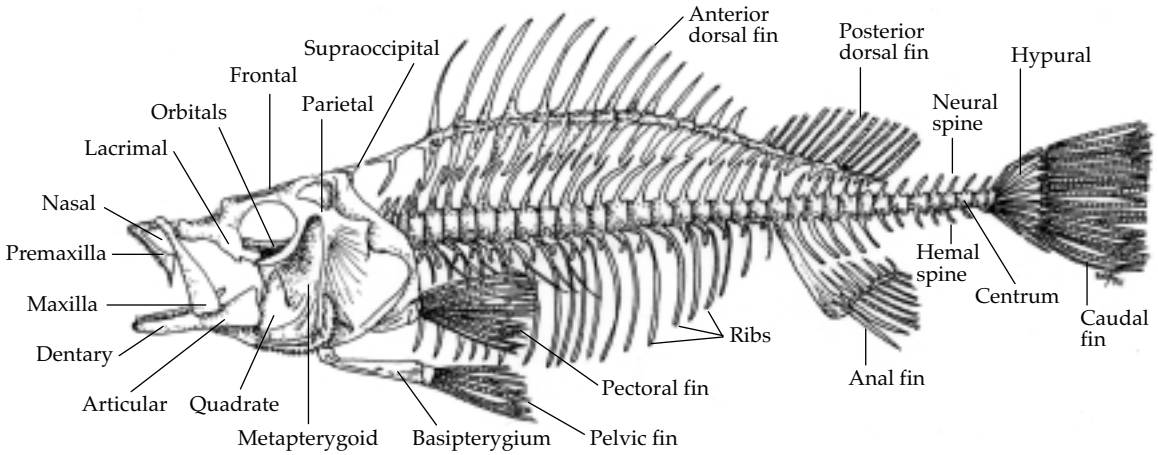

Figure 6.3. Perch skeleton.

Frog dissection

Frogs are members of the class Amphibia. Amphibians are especially interesting because they are the transitory forms between the water and the strictly land vertebrates. Frogs require some moisture for their unprotected skin. They often live near water, in humid environments, under debris, in burrows and are more active at night.

External Anatomy

Head

1. Locate the **external nares**. Insert a probe or bristle into one and observe that it comes out of one of a pair of small openings, the i**nternal nares**, inside the mouth cavity. Find the **eardrum** (**tympanum**), a disc-like structure, just behind the eye. The tympanum is the outer wall of the **middle ear**.

Appendages

2. The forelimbs are divided into three main parts: **upper arm**, **forearm** and **hand**. The hand is divided into a **wrist** (carpus), **palm** (metacarpus) and **fingers** (digits). The hindlimbs consist of a **thigh**, **lower leg** (shank) and **foot** which has 5 webbed toes. The foot is divided into three parts: **ankle** (tarsus), **sole** (metatarsus) and **toes** (digits). Do you see any advantage in the especially long ankle?

Internal Anatomy

1. Open the mouth of your preserved frog as far as possible and cut the angle of the jaws enough to expose the mouth and pharyngeal cavities. The mouth cavity leads into the pharyngeal cavity. On the anterior part of the roof of the mouth cavity locate again the two **internal nares**. At the rear of the mouth cavity, near the angle of the jaw, and on each side of the roof, is a circular opening which leads to the **eustachian tube**. This tube is connected with the tympanic cavity, just beneath the eardrum. What is the function of this tube in both frog and man? Look posteriorly and laterally in the floor of the mouth for the openings to the **vocal sacs** in males.

2. Examine the **tongue**; how is it attached? What is its function? Behind the tongue on the floor of the mouth cavity is a round elevation with a closed slit in its midline, the **glottis**, which opens into the larynx (voice box), then into a very short tube, the **bronchus**, and thence into the lungs. The posterior portion of the pharynx leads into the **esophagus**.

3. If an incision is not found on the right midline, on the ventral surface of the specimen, then make one. With

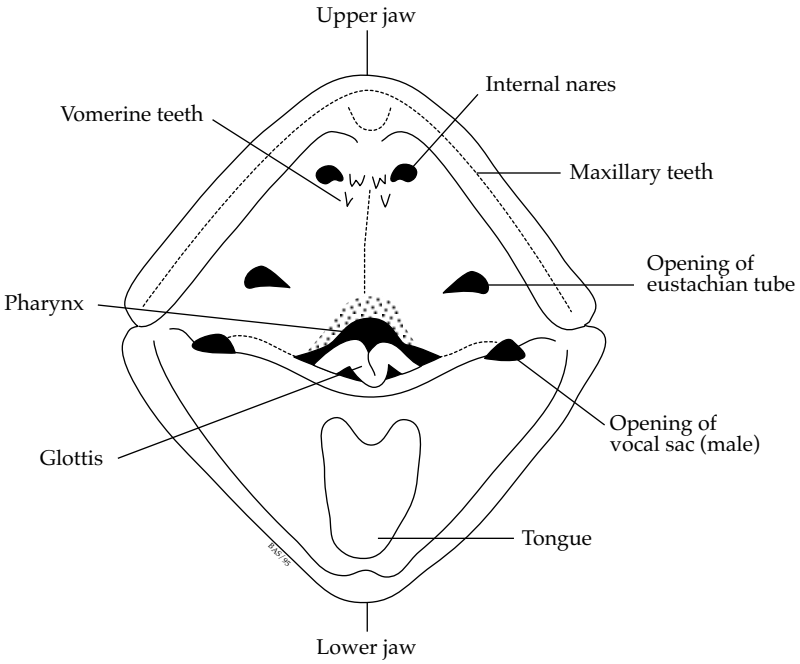

Figure 6.4. Frog mouth.

scissors extend the incision posteriorly to the end of the body, and anteriorly to the angle of the jaw. Make transverse cuts just anterior to the hindlimb and just anterior to the forelimbs each way from the first incision, and pin back the flaps. A heavy pair of scissors may be used to cut through the breastbone (sternum) but under no circumstances should you use a scalpel. Do not disturb the organs underneath.

4. Follow the esophagus as it joins the **stomach**; identify the **cardiac** (upper) and **pyloric** (lower) sections of the stomach. Locate the long, coiled **intestine** that consists of the **small intestine** which is attached to the pyloric region of the stomach, and the thick, short **large intestine** leading to the **anus**. Look at the large, reddish-brown **liver**; between two of the lobes of the liver on the right side of the animal is a round, greenish sac, the **gall bladder**.

5. In the loop formed by the stomach and the proximal portion of the small intestine (**duodenum**) a lobe of the **pancreas** may be found. It is a long, ribbon-like gland, yellowish white in color, and held in place by connective tissue (mesentery). What is the function of this organ?

6. The reddish-brown **kidneys** lie deep in the posterior cavity, one on each side of the midline. The location of the kidneys is termed as **retroperitoneal**, meaning that they lie between the body wall and the lining of the body cavity, the peritoneum. Lying on the ventral surface of the kidneys are thin yellowish strips, the **adrenal** glands. The ureters are the ducts running from the kidneys to the **urinary bladder**, a flimsy, often collapsed structure lying posterior, and opening out into the **cloaca**.

7. The **gonads** are anterior to the kidneys. If you have a mature female, the **ovary** containing ripe eggs will fill much of the body cavity. The **testes** in the male are oval yellow structures. Finger-like yellow **fat bodies**, attached to the anterior region of the gonads, are the primary site of fat storage and are important in the development of mature sex cells.

A film loop will show mating (**amplexus**) and egg laying in the frog. Some kinds of frogs escape the necessity of returning to ponds to breed by laying terrestrial eggs. These eggs hatch into miniature frogs instead of tadpoles, and as such provide a rare example of incomplete metamorphosis in a vertebrate. Moist habitats for these animals are still required. Other frogs brood eggs or young in pouches on the back, on the side, in the vocal sacs and even in the stomach! Frogs which have some kind of parental care lay few eggs (1 - 30). Frogs that lay their eggs in water typically lay many thousand eggs. Why?

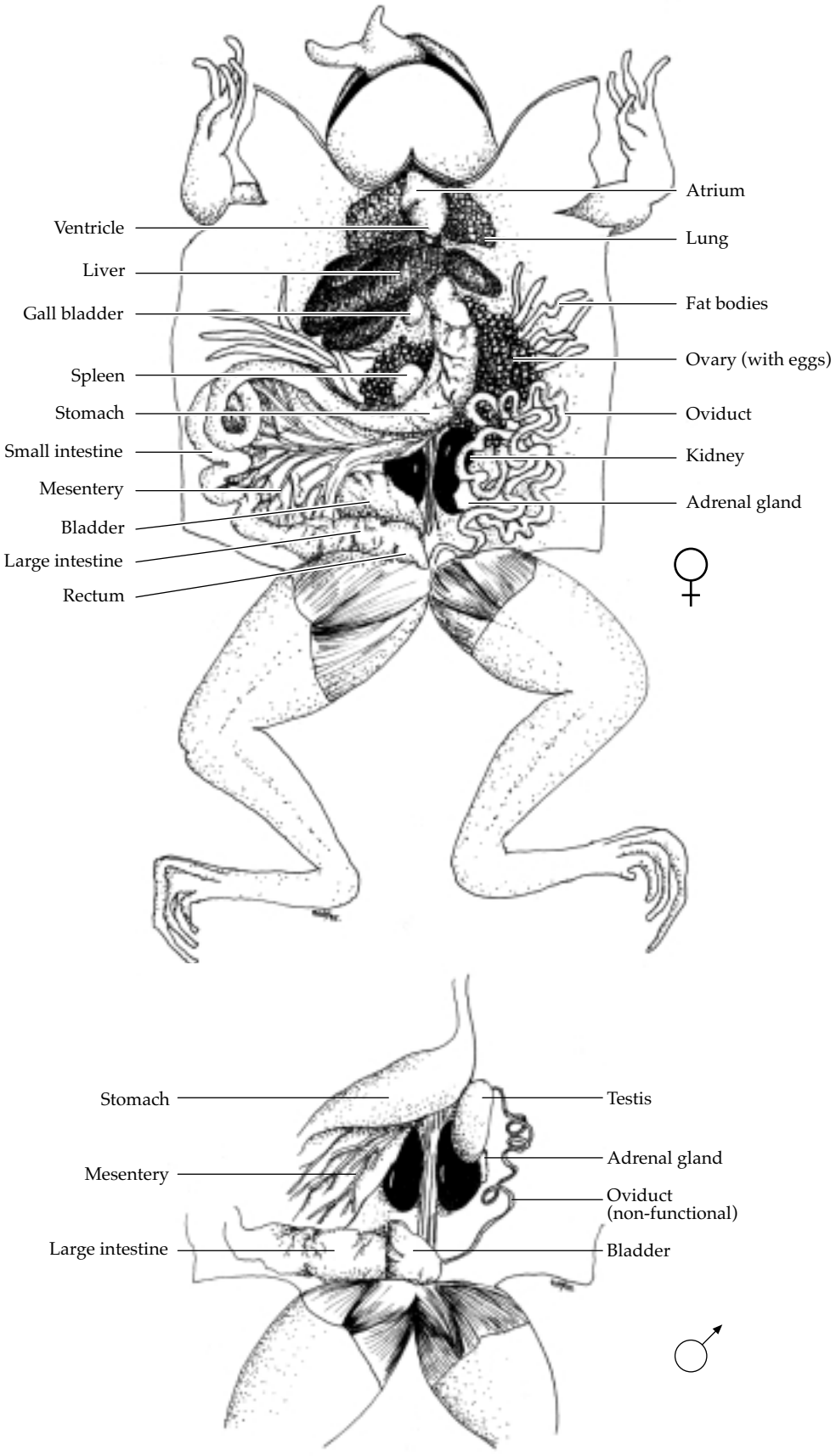

Figure 6.5. Internal anatomy of the frog.

8. Above the liver, locate the **heart** which is surrounded by a thin membrane, the **pericardium**. Remove the pericardium to study the heart structure. There are three main divisions of the heart: the **right atrium**, **left atrium**, and the **ventricle**. The ventricle is the thick-walled conical portion pointing posteriorly. Note the two dark-colored, thin-walled sacs, the right and left atria, which are anterior and dorsal to the ventricle. The **sinus venosus** is the thin-walled, dark-colored, triangular shaped sac on the dorsal surface of the heart, opening into the right atrium. Entering the left atrium , at the anterior dorsal edge, are the two **pulmonary veins**. Remove the heart and set it aside for next week in the plastic bag provided.

9. The general functions of the respiratory system are to deliver oxygen to the tissues and to eliminate carbon dioxide formed in the body. Frogs have lungs like other land vertebrates but considerable exchange of gases takes place through the **membrane lining the mouth** (buccopharyngeal respiration) and through the **moist skin** (integumentary respiration).

Lungs are not an efficient respiratory structure in the frog but have another important function. Air is forced back and forth between the lungs and vocal sacs passing over the vocal cords, producing the distinctive calls that characterize many species of frogs. The openings to the vocal sacs are located in the floor of the mouth (males only). The leopard frog has 2 separate vocal sacs located behind the corners of the mouth. Perhaps you can see this in a live frog. Most tree frogs have a single median vocal sac which distends the entire throat. Many frogs return to the water to lay their eggs, and therefore calling has an important function in locating mates. Evolutionarily speaking, the call of the frog may have been the first voice heard across the land.

10. Continue your dissection by finding the **glottis**. Observe that it opens downward into the cavity of the **larynx** (voice box). Remove the digestive organs, being careful not to damage the lungs. Using scissors, cut backward from the angle of the jaw (not too deeply or the lungs will be cut) and around the glottis, in a circle, to free the larynx, then approach your dissection from the body cavity side. Carefully dissect the lungs free from their attachments to the body, but be careful not to cut the lungs away from the larynx. Now remove the entire respiratory system and place it in water for observation. Is there a distinct **trachea** (windpipe)?

11. Muscles constitute the "flesh" of the body. Practically all movements of animals result from the contraction of muscles. A large proportion of the body musculature is composed of skeletal muscles, which in most cases, are attached to some part of the skeleton. These muscles are chiefly involved with locomotion and with the movement of bones. Skeletal muscles are also called **striated** and **voluntary** muscles. Why?

Critically examine the distribution of skeletal muscles in your frog and compare it with that of the perch. What does the striking difference between the two cases reflect?

12. The skeleton is an essential part of the body machine of every vertebrate animal. It has at least three important functions: (1) serves as a support or framework for the softer parts of the body; (2) provides a firm surface for the attachment of muscles used in movement and locomotion; (3) protects soft and delicate parts. The skeletal system is subdivided into (1) the **axial** and (2) the **appendicular** skeleton. The axial skeleton is composed of the **skull**, **vertebral column** and **sternum**. The appendicular skeleton is composed of the **pectoral girdle**, **forelimbs**, **pelvic girdle** and **hindlimbs**.

Carefully examine the mounted frog skeletons on demonstration and compare with that of the perch. There are many striking anatomical differences that reflect functional differences. Try to explain as many as you can.

From what you have seen and learned of the internal and external gross anatomy of the perch and frog, you should be able to summarize the great structural and functional modifications that reflect the transition from life in water to life on land. Refer back to lab #2 on Bryophytes and the plant invasion of land. Have amphibians solved the problems of life on land in similar or dissimilar ways?

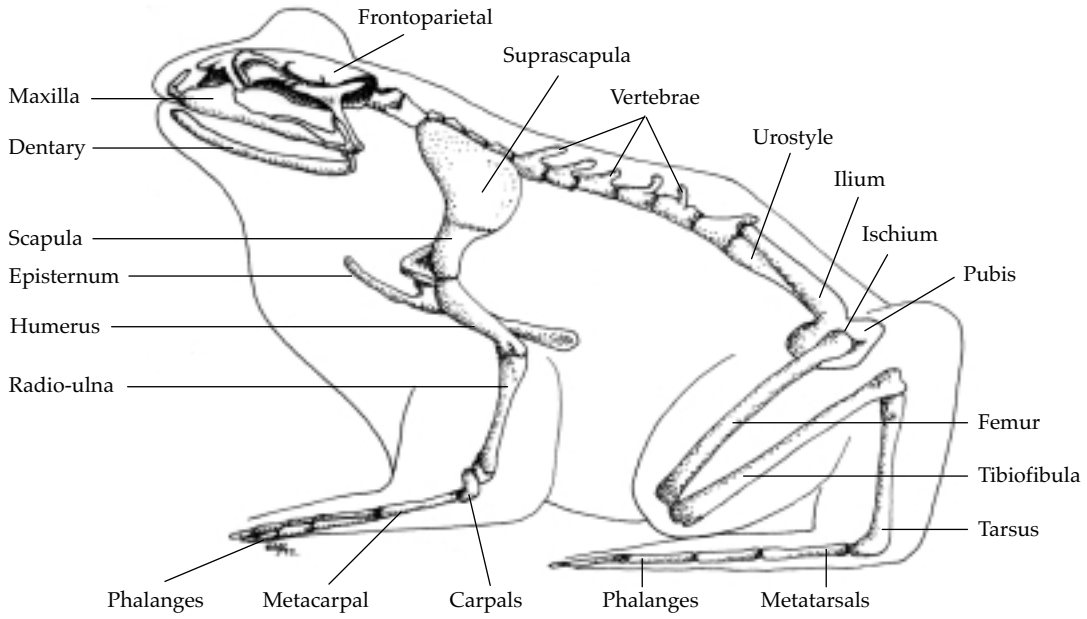

Figure 6.6. Frog skeleton.

Rat dissection

Mammals are tetrapods ("four-legs") that use metabolic processes to set their body temperature, which requires a relatively high metabolism. This characteristic is correlated with the four-chambered heart found in mammals in which the oxygenated arterial blood is kept entirely separate from the deoxygenated, carbon dioxide-rich venous blood, increasing the efficiency of oxygen uptake. In addition, mammals have two distinguishing characteristics that separate them from other vertebrates: (1) all mammals have a protective and insulating outer layer of hair at some phase during their development; (2) all female mammals possess mammary glands which produce milk for nourishing the young. Also of note is that the young are usually born live except in the most primitive mammals, the monotremes, which lay eggs. Finally, one of the most important features of mammals is their relatively large brain case, indicative of greatly increased intelligence.

External Anatomy

1. Examine the external features of your rat noting especially the **incisor teeth** which protrude from the mouth between the well defined lips. Find the **ears** which have a long, flexible external fold called the **pinna** that directs sound waves into the **external auditory meatus** (ear). Note the **eyes** with upper and lower lids, the **external nares** on the end of the nose, and the long, sensory hairs on the face called the **vibrissae** (whiskers).

2. The **trunk** is divided into an anterior **thorax** and a posterior **abdomen**. The **teats** (nipples; there are normally 12 in the rat) are the external openings of the mammary glands and are located on the ventral surface of the trunk. The tail of mammals is usually well developed as it is in the rats but is reduced in rabbits and absent in humans. The **anus** is at the base of the tail. If your rat is a male the **testes** may be descended into the **scrotum**, a sac ventral to the anus. In immature or non-breeding males the testes are within the body cavity. Why are the testes in the scrotum (outside the body) during the breeding season? The **penis** is within a sheath and is anterior to the anus. In the female the genital papilla or **clitoris** is more posterior and closer to the anus. The urinary opening is on the papilla, and the **vagina** is between the clitoris and anus. What sex is your rat?

Internal Anatomy

As an example of gross dissection we will do a small group of muscles. Skeletal or striated muscles are attached to other muscles or to bones either directly or by means of tendons. Limb movements are controlled by 2 or more pairs of opposing muscles. The muscle that extends a limb is an **extensor** and the muscle which bends it is a **flexor**. **Abductors** move a part away from the median line and **adductors** move it toward the midline.

1. Cut through the midline of the skin of the chest and continue to skin the rat over the arm, shoulder and neck up to the base of the skull. Look for the muscles listed in the chart below. Place the animal on its back and make a cut from the anus anterior to the **diaphragm** (base of the ribcage). Make lateral cuts in the body wall both anteriorly and posteriorly so flaps can be laid back.

Note the fat deposits under the skin. How does this arrangement compare with that of the frog? Examine the viscera starting with the **liver** which may be removed. Is there a gall bladder? Refer back to the situation in the frog. Can you explain any differences? The **stomach** is found below the liver on the left side of the animal. Find the **esophagus** following it up to where it penetrates the diaphragm.

2. Pull out the intestinal tract. Note the **mesentery** that attaches the intestine to the body wall. Are there blood vessels in the mesentery? The posterior part of the stomach that is constricted is the **pyloric** region that connects to the **duodenum**, the upper portion of the **small intestine**. Continuing posteriorly trace the **jejunum**, **ileum**, **caecum**, ascending and descending **colon** and finally the **rectum** that ends in the anus. Note the size of the caecum in the rat. Does this structure occur in humans? The **pancreas** is buried in the mesenteries just below the stomach. How does the length of the intestinal tract in relation to the size of the animal compare to the situation in the frog? Look for the **spleen** which is located below and to the right of the stomach. What color do you expect the spleen to be?

3. Now move posteriorly and look for the **kidneys**, dark red structures lying dorsally on each side of the midline (retroperitoneal). The **adrenal** glands are small oval structures at the anterior end of each kidney. Follow the **ureters** from the kidneys to the bladder. From the bladder the **urethra** continues to the external urinary opening.

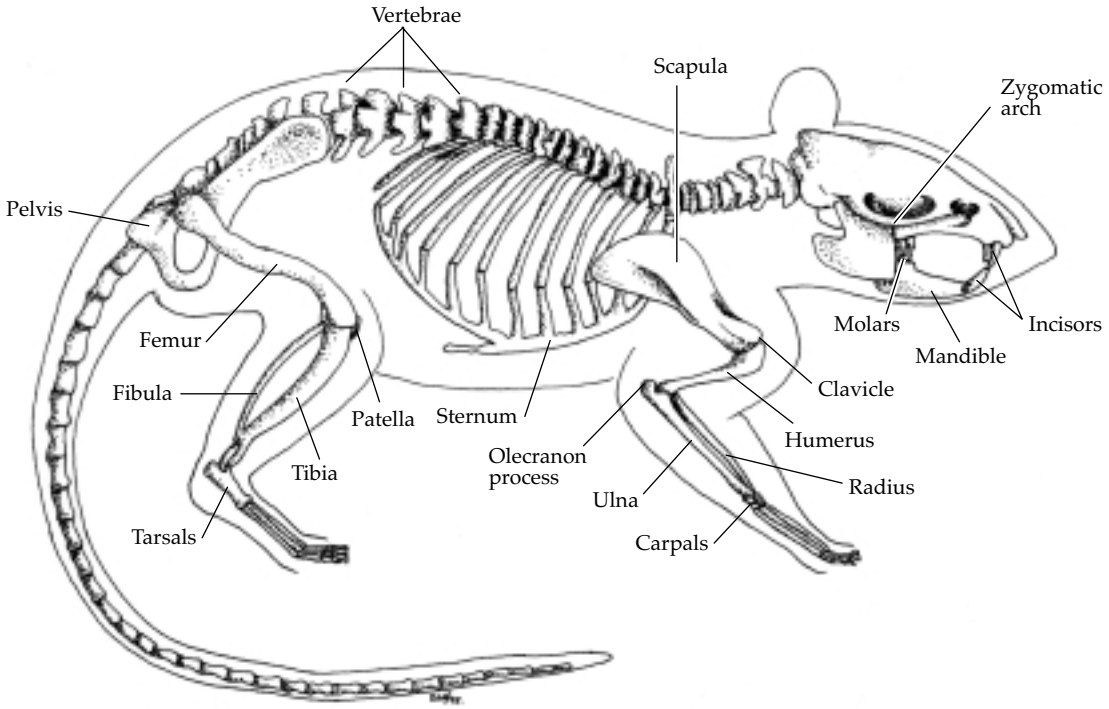

Figure 6.7. Rat skeleton.

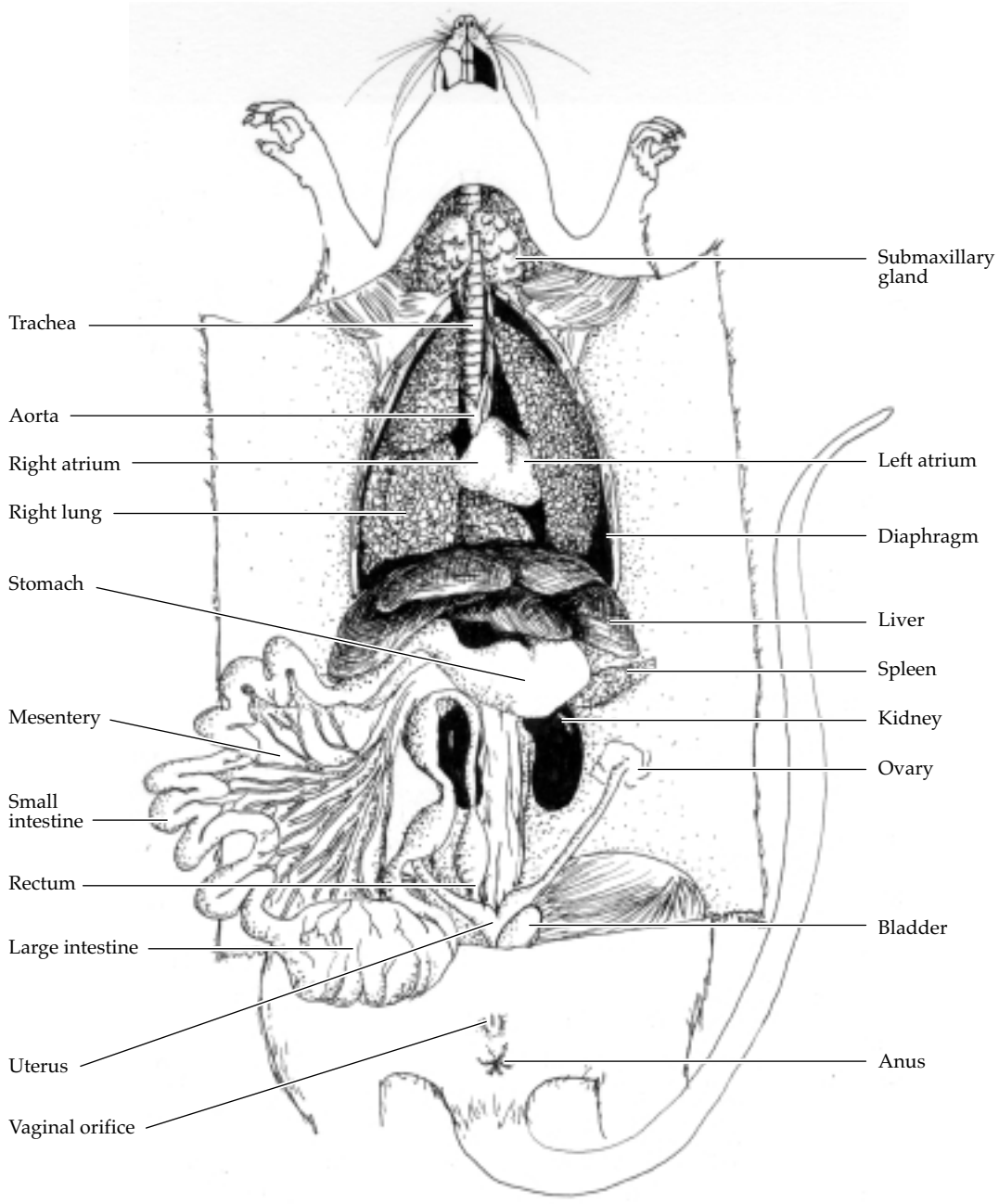

Figure 6.8. Internal anatomy of the rat.

4. In the male, locate the **testes**. The vas deferens leads from the testes to the **prostate** glands which are located on each side of the bladder. In the female, look for **ovaries** which are small and posterior to the kidneys. The **uterus** has 2 separate horns (bifurcated) which lie near each **ovary**. The **oviducts** or Fallopian tubes form a tight coil near the anterior horns of the uterus. Posteriorly the horns of the uterus unite and join the **vagina**.

5. Cut through the sternum from the diaphragm anteriorly to the neck. Pull the ribs back on both sides. Dissect away neck muscles to expose the **larynx** and **trachea**. Follow the trachea posteriorly as it divides into 2 **bronchi** which lead into each **lung**. The **thyroid** gland may be found as a small brownish bilobed gland on the anterior ventral surface of the trachea near the larynx.

6. Examine the heart and major blood vessels. Review mammalian circulation in your textbook. Try to locate the **dorsal aorta** and **vena cava**. A demonstration model of an injected rat is available with some major vessels labeled. Compare the model with your rat. Cut out the heart and identify the **ventricles** and **atria**. Slice open the heart and examine the valves. Set the heart aside for next week in the plastic bag provided.

Questions:

1. A frog is one of the few vertebrates (humans and apes are others) without tails. Is this an advantage or disadvantage to these animals? Why?

2. What structural features adapt the perch to life in water?

3. About how many young does a female rat have in each litter? How does this compare with the number of eggs in the frog and fish? What significance do these figures have in terms of survival to adulthood?

Table of Comparison of Vertebrate Systems and Characteristics (Adaptations)

Animal	Digestion/ Waste Disposal	Gas Exchange	Musculo/ Skeletal	Nervous system	Circulation	Reproduction	Fat Distribution
Frog							
Perch							
Rat							

Preparation for next lab:

Treatment of bush beans

1. Of the twenty bush beans you planted last week, choose ten of the best plants that germinated.

2. Measure the height of each plant from the cotyledons to the shoot apex and record the data.

3. Pair the plants according to size for a maximum of five pairs.

4. Apply one drop of gibberellin to the shoot apex of one of the plants in each pair and one drop of deionized water to the other of the pair as the control. Label each plant accordingly.

5. Choose a member of your group to take the beans home to care for them. Remember, **the beans must be brought back to lab next week**! You are responsible for making sure the vermiculite is kept moist. **DO NOT SOAK!** Put the plants in the light and make sure they are kept moist until next week for data analysis.