Zoology
Exercise #19: Chordates: Amphibians
Lab Guide

Movement onto Land (Adaptations)

- Oxygen is more abundant in air (20 times more than water) so land animals have evolved lungs and other respiratory structures
- Limbs and skeleton have developed to support more weight since air is 1000 times less dense than water
- Physiological adaptations have allowed land animals to adjust to wider fluctuations in temperature when compared to water
- More opportunities to take advantage of the variety of terrestrial habitats

Terrestrial vertebrates evolved structural characteristics while in the water which made it possible to explore terrestrial habitats

- Swim bladder and paired internal nares have combined to draw in air and fill an air filled cavity (lung)
- Bony elements of paired fins have been modified to support movement on land

Additional adaptations also occurred in the skull, teeth, pectoral girdle and jointed limbs

- Stronger backbone
- Muscles to support body in air
- Muscles to elevate head
- Stronger shoulder and hip girdles
- More protective rib cage
- Modified ear structures to detect airborne sounds
- Forward shortening of the skull
- Longer snout

About 370 million years ago, the first amphibians evolved from lobe-finned bony fish and became the first vertebrates to live on land. There are over 6000 species in modern amphibians separated into 3 orders:

- **Order Gymnophiona** – caecilians
- **Order Caudata** – salamanders
- **Order Anura** – frogs and toads

Metamorphosized adults use redesigned olfactory epithelium to sense airborne odors and ears to detect sounds.

**Order Gymnophiona – caecilians**

- 173 living species
- Elongate, limbless, burrowing animals
- Inhabit tropical forests in South America, Africa, India, and Southeast Asia
- Feed on worms and small invertebrates
- Internal fertilization with protrusible copulatory organ
- Deposit eggs in moist ground with some species guarding them
  - **Viviparity** can be observed in some species where the embryos will eat the wall of the oviduct
- Larva are aquatic

**Order Caudata – salamanders**

- Approximately 533 living species
- Found in temperate regions
- Most are under 15cm with one, the Japanese Giant Salamander, measuring over a meter long
- Limbs are at right angles to the trunk (body)
- Burrowing species and some aquatic forms may have lost their limbs
- They are carnivores as adults and larva, feeding on worms and small arthropods
- They are **ectotherms** (body temperature determined by the environment) with a low metabolic rate
- Internal fertilization with a courtship that has the male depositing a spermatophore and the female picking it up with her cloaca
  - Eggs are laid in stringy masses or clusters, usually in logs or in soft earth
  - Most have aquatic larva and terrestrial adults (but some are aquatic their entire lives)
Terrestrial varieties have direct development and hatch as miniature adults.

- They have extensive vascular networks in their skin to help with gas exchange (cutaneous respiration).
  - Depending on the life stage, some will have gills that may be lost during metamorphosis.

**Order Anura – frogs and toads**

- Approximately 5283 species that must live near a water source.
  - Reproduction requires water and skin must stay moist (in most species).
- They are ectotherms, so this prevents them from inhabiting polar or subarctic regions.
- All have a tailed larva stage and tail-less, jumping adult stage (except for 1 species).
- There are 44 families, with 3 mentioned below:
  - **Family Ranidae** – larger frogs of North America.
  - **Family Hylidae** – tree frogs.
  - **Family Bufonidae** (true toads) – have thicker skin and prominent wart-like structures.
- Anurans are declining worldwide and becoming geographically fragmented.
  - Some are presenting with malformed limbs that has been connected to trematode infections.
  - Humans have had a large impact on anurans due to reducing water depths in native habitats, causing increased exposure to UV radiation resulting in the animal being more susceptible to fungal infections.
  - Other, non-native, amphibian introductions have also caused massive declines.

**INTEGUMENTARY**

The skin of a frog serves two functions: protection and respiration. Breathing through the skin is called cutaneous respiration. Thin, moist skin is very permeable, allowing rapid diffusion of gases. Mucous glands in the skin help keep the skin moist in air and make a frog feel “slimy”. In some amphibians, the skin contains other glands that secrete foul-tasting or poisonous substances that provide protection from predators. However, the same features that allow efficient respiration, make the frog vulnerable to dehydration. So amphibians live in moist, wet places on land and are active at night when loss of water through evaporation is reduced. Coloration in an amphibian’s skin provides camouflage. Notice the webbed feet on the back legs. One of the characteristics of amphibians is feet with no claws.

**WHAT SEX IS IT?**

Male frogs are usually smaller than females and have thick “THUMB PADS” which enable the male to hold onto the female so that sperm and eggs are released at the same time and in the same place (AMPLEXUS). This increases the chances for external fertilization. Locate the exit opening between the frog’s hind legs. This is the opening to the CLOACA, a multipurpose cavity shared by the digestive, reproductive, and excretory systems. In animals with a cloaca, the exit opening is called a VENT.

Locate the structures shown in the diagram at the left. The NICTITATING MEMBRANE, is a transparent third eyelid, which covers and protects the eye while swimming under water. The TYPANUM (eardrum) are located directly behind the eyes. A bone called the COLUMELLA transmits sound from the eardrum to the inner ear. EUSTACHIAN TUBES connect the inner ears to the mouth cavity. The EXTERNAL NARES (nostrils) also connect inside to the mouth so frogs can breathe with their mouths closed while swimming.
Cut the hinges to the mouth and look inside to find the following:

A flexible **TONGUE** is attached at the front rather than in the rear like ours. Two **INTERNAL NARES (connect to EXTERNAL NARES outside)** which allow the frog to breathe with its mouth closed.

Two **VOMERINE TEETH** in the middle of the roof of the mouth and the **MAXILLARY TEETH** along the jaw, which grab and hold prey to keep it from escaping. Frogs don’t chew, but swallow their food whole.

The **GLOTTIS**, a small round structure with a vertical slit just behind the **TONGUE**, is the opening to the respiratory system. Posterior to the glottis is the opening to the esophagus and the digestive system. The muscular back of the throat where food is pulled into the digestive system is the **PHARYNX**. The opening where food enters the digestive system is the **GULLET**.

Mouth Sketch
Follow the dashed lines on diagram at the left and cut through the skin only.

Notice the numerous blood vessels in the skin for gas exchange.

If your frog is female, the abdominal cavity may be filled with black and white eggs. Amphibian eggs are surrounded by a single cellular membrane and are coated with a jelly-like material as they are laid for protection.

The yellowish fingerlike structures are **FAT BODIES**. Frogs do not store fat in layers under the skin like humans do. The size of the fat bodies varies depending on the season. These are reservoirs for food used during hibernation, estivation, and breeding.

Amphibians, as well as the other organisms we have dissected so far, are **ECTOTHERMIC**; commonly called “cold blooded”. They don’t make their own body heat. Their body temperature is dependent on the temperature of their environment. Animals that are ectothermic have evolved ways to survive in environments with seasonal extremes in temperature. Many animals hibernate in order to stay alive in cold times (winter season) and many amphibians (like frogs and toads) **ESTIVATE** [or aestivate] in hot, dry conditions. When hot and dry times come, estivators will find themselves a safe place to sleep—usually underground. This is the only way some animals can live through conditions with high heat and no water. The metabolism, breathing and heartbeat slow down. The animal doesn’t need as much food and water to live. Animals don’t move, grow or eat during this time. Fat stored in the fat bodies provides energy for the animal during estivation, hibernation, and breeding seasons.

**DIGESTIVE SYSTEM**  Use the diagram to locate the following:

- **ESOPHAGUS**- connects the mouth and stomach. The elastic esophagus and STOMACH (found under the lobes of the liver) allow the frog to swallow large amounts of food. Gastric juices secreted by the walls of the stomach and the muscles work to break down food. The circular **PYLORIC SPHINCTER** muscle at the end of the stomach controls the passing of digested into the **SMALL INTESTINE**.

The upper portion of the small intestine closest to the stomach is the **DUODENUM**. The coiled middle section is the **ILEUM**. A fan-like membrane called the **MESENTERY** holds the folds of the small intestine together. The small intestine receives bile from the **LIVER** and pancreatic enzymes (including trypsin) from the **PANCREAS**. Digestion is completed here and nutrients are absorbed through the surface of the small intestine lined with **VILLI**, the finger-like extensions which increase surface area. The lower end of the small intestine leads into the **LARGE INTESTINE**, where indigestible wastes are collected and passed into the **CLOACA**, a multipurpose cavity. Waste from the kidneys (urine), as well as eggs OR sperm also pass through the cloaca on its way out of the body. Waste materials exit through the **VENT**.

The **LIVER** consists of three dark lobes. Its main functions are to make bile, store vitamins, store glycogen, and process toxins (including nitrogen waste) which the **KIDNEYS** remove. The **GALL BLADDER** stores bile made by the liver. It is a greenish colored sac found between the left and right lobes of the liver. **PANCREAS** is an elongated yellow organ located in the first loop of intestine between the small intestine and stomach. It secretes trypsin (used in the small intestine to break down proteins) and enzymes to regulate blood glucose levels.
RESPIRATION:
Adult frogs breathe in two ways. The respiratory organ in the adult frog is **LUNGS** (two large air sacs below the liver and heart). Breathing with lungs is called **PULMONARY RESPIRATION**. Adult frogs also breathe through the skin on their bodies (**CUTANEOUS RESPIRATION**) and through the skin in their mouths. Raising and lowering the floor of mouth, opening and closing nostrils pushes air into the lungs **(called POSITIVE PRESSURE breathing)**.

The respiratory organ in tadpoles is the **GILL**. (3 external pairs upon hatching)
These gills will transform into internal gills with a flap of skin covering them. A spiracle on the left side will develop to pass water over the internal gills. As tadpoles undergo **METAMORPHOSIS** they grow lungs and must change from breathing with gills to breathing with lungs.

Digestive/Respiratory System Sketch

CIRCULATORY SYSTEM

While **TADPOLES** have a **CLOSED** circulatory system similar to fish (**two chambers/1 loop**) adult amphibians have a **CLOSED 3 chamber/2 loop** circulatory system. The **HEART** is surrounded by a **PERICARDIAL MEMBRANE**. Locate the **RIGHT ATRIUM, LEFT ATRIUM, and VENTRICLE**. The **PULMONARY CIRCULATION** carries deoxygenated (**LOW oxygen**) blood from the heart to the lungs and returns oxygenated (**HIGH oxygen**) blood to the heart. **ARTERIES** carry blood leaving the heart and veins carry blood returning to the heart from the body. **Capillaries** connect arteries and veins and allow gas exchange in the lungs and body tissues. Adding a second loop has the advantage of “faster blood flow to the body organs”. Low oxygen blood enters the **SINUS VENOSUS** from the large vein bringing blood back to the heart (**VENA CAVA**) from the body. From there it enters the **RIGHT ATRIUM**. At the same time high oxygen blood returning from the lungs via the **PULMONARY VEINS** enters the **LEFT ATRIUM**. When the atria contract both kinds of blood are sent to the **VENTRICLE**. Although the ventricle is not divided, a spongy irregular surface inside, the coordinated contractions of the 2 atria, and a valve in the **CONUS ARTERIOSUS** keep the **HIGH and LOW OXYGEN** blood from mixing, even though both kinds of blood share this single pumping chamber. When the ventricle contracts, high oxygen blood is sent into the **CONUS ARTERIOSUS**, that has a valve to prevent mixing of the high and low oxygen blood. High oxygen blood is carried via a large artery (**AORTA**) out to the body organs and muscles and low oxygen blood is sent to the lungs to pick up oxygen via the **PULMONARY ARTERIES**.
**SYSTEMIC CIRCULATORY SYSTEM**

The **SYSTEMIC CIRCULATION** carries oxygenated blood from the heart to the muscles and body organs and brings deoxygenated blood back to the heart. Parts of the systemic system are named for the organs they service. **RENAL** circulation carries blood to the kidneys. The **CORONARY** circulation supplies blood to the heart itself. The **HEPATIC** circulation carries blood to the liver.

**Circulatory System Sketch**

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**EXCRETORY**

Like fish, most amphibian larvae excrete nitrogen waste as ammonia through their gills. Ammonia is highly toxic and must be excreted quickly (through their gills) or diluted with large amounts of water to make urine. *in order to conserve water* terrestrial adult amphibians transform their ammonia into **UREA**, which is less toxic and does not require as much water to dilute.

The **KIDNEYS**, which lie on either side of the spine against the dorsal body wall, are the primary excretory organs. The kidneys filter nitrogen wastes (urea) from the blood which is diluted with water to make **URINE**. Urine flows from the kidneys through urinary ducts to the cloaca. The **URINARY BLADDER**, which branches from the ventral wall of the cloaca, stores urine until it is released through the vent. During dry periods, water can be reabsorbed from urine in the bladder.

**ENDOCRINE**

As we have seen, the endocrine system controls a variety of body functions including heart rate, metabolism (blood sugar levels), and sexual development. **METAMORPHOSIS** (changing form from a tadpole to an adult frog) is controlled by **THYROXIN**, a hormone produced by the **THYROID GLAND**. The **PANCREAS** makes insulin which causes cells to take up glucose from the blood stream and store it as glycogen and glucagon which causes cells to release their glucose into the blood stream.

**Excretory/Endocrine Sketch**
REPRODUCTION

In amphibians, the excretory, reproductive, and digestive systems all share a multi-purpose exit space called a CLOACA. This space collects urine, eggs/sperm, and digestive waste before it exits the body. The exit opening in animals with a cloaca is called a VENT.

The female frog has paired OVARIES located near the kidneys, containing thousands of tiny eggs. During breeding season, the eggs enlarge, mature, and burst through the ovarian walls into the coelom. Cilia move the eggs into OVIDUCTS, where they are coated with their protective jelly-like covering, and passed out of the body via the CLOACA through the VENT.

The male reproductive system includes 2 bean shaped TESTES located near the kidneys. Sperm cells develop in the testes and pass through tubules to the KIDNEYS, down the urinary ducts to the CLOACA, and out through the VENT.

Reproductive System Sketch

As amphibians, frogs may live some of their adult lives on land, but must return to water to reproduce. In the first warm days of spring, frogs emerge from hibernation. Males call to attract females and to warn off other males. During mating the male grabs onto the female (AMPLEXUS) and holds on until eggs are released. Millions of sperm are released over the eggs to fertilize them. This firm grasp increases the chances that sperm will find egg and fertilization will occur. Eggs (2000-3000 at a time) are laid with a jelly-like coating, but have no shell or multicellular membranes. Some tropical anurans will deposit eggs in foam masses that float on the surface of the water. Some deposit the eggs on leaves overhanging ponds so the tadpole will drop in once it hatches. Other use bromeliad plants to form a reservoir to hold water and the eggs. A few poison dart frogs will tend to their eggs on their backs, even carrying the tadpoles for varying lengths of time. The marsupial frogs even carry eggs in a pouch on their back, hatching perfect little froglets (bypassing the tadpole stage).

Most amphibians have INDIRECT DEVELOPMENT. The larval form is a tadpole. A newly hatched tadpole lives off yolk stored in its body until its mouth opens and it can feed using horny jaws. They are herbivores at this stage. The aquatic larvae breathe with gills and must undergo METAMORPHOSIS to become terrestrial, adult air breathers with lungs. Many land dwelling amphibians lay eggs in moist places on land such as under rocks, inside a rotting log, or in a tree.

An endocrine hormone called thyroxin made by the thyroid gland stimulates these body changes. Legs grow, tail and gills disappear, lungs develop, the circulatory system changes from a fish’s one loop-two chambered heart to an adult’s two loop-three chambered heart.
Analysis

1. Why are amphibians considered a "transition" group? How is this seen in the way they reproduce. Be clear in your explanation.

2. What is the advantage of having a difference in color between the dorsal and ventral sides in some amphibians?

3. Frogs capture prey with its protrusable tongue. How is the position of the eyes advantageous in this situation?

4. What is the purpose of the nictitating membrane and how is it being used by the frog? (Be specific)

5. There are a multitude of blood vessels in the mesenteries supporting the digestive system. Why must it be so well supplied with blood?

6. Amphibians use two methods to breathe. Explain each.

7. The frog has no diaphragm. Explain how air is drawn into its mouth cavity. Be specific in your explanation.

8. Why is there a difference in the amount of fat bodies seen in frogs that are captured in the spring compared to the fall?

9. What is “amplexus”? 
10. Some weed killers used in the United States (ex. Atrazine) have been shown to have a detrimental effect on male frogs. Explain.

11. Amphibians have a 3 chambered heart. How is this different from other groups that have a 4 chambered heart?

12. This lab describe the flow of blood in amphibians. Summarize this pathway below. Make sure to use proper terminology as you create your summary.

13. Frogs are considered “ectothermic”. What does this mean? How would this condition affect the heart? What would be the advantage of being “ectothermic”?

14. The nervous system was not addressed in this lab, but it is made up of two distinct parts: central (cerebrospinal) nervous system and the peripheral nervous system. Explain what makes up each and their primary function.