Biology 3B Laboratory
Muscles of Vertebrate Animals: Shark, Mudpuppy and Rat

Objectives:

- To be able to distinguish between the basic muscles of the rat, mudpuppy and shark
- Identify and learn the origin, insertion and action of selected vertebrate muscles
- Learn the embryological origin for selected vertebrate muscles
- Be able to trace homology of muscles in each of the three species

Introduction

This lab will examine the **external features** and **muscular system** of three animals, the spiny dogfish shark (Chondrichthyes: *Squalus acanthias*), the mud puppy (Amphibia: *Necturus maculosus*) and the domestic rat (Mammalia: *Rattus rattus*). Not every animal will be equally easy to dissect and some animals will require more time than others. You will have to work as a team in order to spread out the work load and make sure that you all have equal time to learn the material.

In the histology lab you learned to recognize **smooth, cardiac,** and **skeletal** (**striated)** muscle. In this lab we only look at skeletal muscles. Most muscles of the body are derived from mesoderm. The process of skeletal muscle fiber development occurs within myotomes (Fig. 1) in the embryonic trunk area, or from loosely organized masses of hypomeric mesoderm in the head and appendage areas. Embryonic formation of skeletal muscle tissue begins as specialized mesodermal cells, called **myoblasts**, which multiply rapidly. New cells continue to form as the myoblast cells fuse into **syncytial myotubes**. A syncytium is a multinucleated protoplasmic mass that forms by the secondary union of originally separate cells. Growth in length continues by addition of myoblasts to the syncytium. Thus, these voluntary muscle fibers are composed of long, unbranched cylinders.

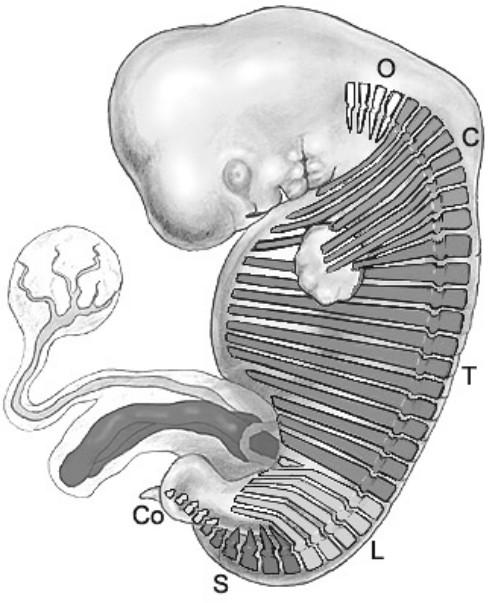

Figure One. Embryological Myotomes. O = occipital, C = cervical, T = thoracic, L = lumbar, S = sacral, Co = coxxygeal

As you already know, muscles work by contraction. As the muscle contracts, it draws various body parts together, creating an **action**. Muscles have points of connection to body parts. These are defined as **origins** (fixed or the most fixed part) and **insertions** (less fixed, or most movement). Muscles often work in pairs

called **agonists** and **antagonists**. Muscle names are often based upon the action.

Table One. Some examples of Agonist/Antagonist muscle pairs and their actions

Agonist/Antagonist Pair	General name	Name of action	Description of action
	Abductor	abduction	move away from midline
	Adductor	adduction	move toward midline
	Flexor	flexion	reduce angle
	Extensor	extension	increase angle
	Protractor	protraction	move limb forward
	Retractor	retraction	move limb backward
	Elevator	elevate	raise the body part
	Depressor	depress	lower the body part

Many muscles insert by means of **fascia** or by **tendons** (dense regular connective tissue) onto bone. The connection of bone to bone is also made of this type of connective tissue, but here it is called **ligament**.

We will be studying the muscles of the vertebrate skeleton. These muscles come from embryonic **myotomes**, which are segmented, in the embryonic body (Fig 1). The first three segments in the head region are located in front of the ear (**pre-otic**). The epimere mesoderm in these segments survives as the muscles of the eyes; the hypomere mesoderm does not develop. Each segment has split to form different eye muscle masses, which have then moved apart. These masses are traced by their innervation (the nerves going to them). We will look in detail at the eye muscles of the shark, but every vertebrate, including man has a similar pattern of eye muscles and innervation.

The hypomeric mesoderm in the head region does not contain a coelom and instead forms a solid sheet called the **branchiomere.** This **branchiomeric muscle** becomes the muscles of the jaw, hyoid arch, gills and spiracle of the dogfish. They are similar in *Necturus*, but in mammals, with the loss of gills, the more posterior muscles are lost or transformed into muscles of the larynx and the trapezius muscle of the shoulder.

The **axial** muscles of the body are from mesodermal somites and form the skeletal muscles of the trunk and tail. In the head region, **hypobranchial muscles** are formed from somites behind the gills that move (as embryonic mesenchyme) forward to the ventral region of the pharynx (between the gills). **Appendicular** muscles are formed in the dogfish from muscle buds in the embryonic myomeres. These operate the pelvic and pectoral fins. In the dogfish the axial muscles do much of the locomotion. In *Necturus* the evolution of limbs makes the musculature more complex. In mammals, with more powerful limbs and increasing speed on land, appendicular muscles overlie many of the axial muscles.

I. The Spiny Dogfish, *Squalus acanthias*

a. External Anatomy and Skin Removal

First, look at the shape of this fish. Observe its adaptations for swimming. Feel the **placoid** scales; they point towards the caudal region of the fish. Identify the fins

- 2 dorsal (with spines),
- caudal (note shape of lobes)
- pectoral (1 pair)
- pelvic (1 pair)
- claspers (present or absent)

Follow the **lateral line** along the side of body. It is used for hearing/pressure sense. On the head find

- pair of lidless eyes
- pair of spiracles
- 5 pairs of gill slits
- pair of nares

On the surface of the head note the continuation of the lateral line into three principal lines: the supra orbital, the sub-orbital and the mandibular. Find the **ampullae of Lorenzini**, fluid filled pits that sense low-level electrical stimulus and sense depth. Examine the **cloaca**, a chamber between the pelvic fins and note the urinary papilla, and anus.

Skin the **left** side of the head and body to about 10 cm behind the pelvic fins. The skin is adherent to the muscles, so be patient and careful. Use a blunt probe to separate the skin from the muscles; the scalpel will cut and damage the muscles. You will have to cut through the dorsal cartilage in the region of the left eye and remove the gelatinous material and blood behind the eye.

b. Dogfish Musculature

The **branchiomeric** muscles of gill slits 4 through 7 are levators (cucullaris, interarcuals) and superficial constrictors innervated by nerves X and XI. Caudal to the gill arches, the myotomes grow ventrally and posteriorly as hypobranchial muscle that fills the ventral area between the gill arches with coracoid muscles. These are innervated by the hypobranchial nerve (XII). Posterior to these muscles we have typical myotomes forming **axial muscles** in the cervical, thoracic and lumbar areas and special **appendicular** muscles for the pectoral and pelvic limbs.

Dogfish Branchiomeric Muscles

The dorsal constrictor muscles lie between the eye and the pectoral fin and are responsible for forcing water our of the gill pouches and closing the mouth. The first dorsal constrictor is made up of two muscles, the **mandibular adductor**, ventral to the spiracle, and the spiracular, anterior to the spiracle. The second dorsal constrictor is a large superficial muscle located posterior to the spiracle. The third to sixth dorsal constrictors lie dorsal to the gill openings.

The ventral constrictor muscles are located on the ventral surface between the lower jaw and the ventral, anterior line of the pectoral fin. The **first ventral constrictor**, **intermandibular**, is located ventral to the mandibular adductor. The **second ventral constrictor** is continuous with the second dorsal constrictor. The third to sixth ventral constrictors aligned with the dorsal constrictors.
The levator muscles elevate the mandible, hyoid and gill arches. The first **levator**, or **palatoquadrate levator**, is located anterior and deep to the spiracular. The **second levator**, or **hyoid levator**, is fuse to the second dorsal constrictor and cannot be easily seen. The **third to sixth levators**, the **cucullaris**, form a single triangle between the dorsal constrictors and the epaxial muscle mass. It results from a fusion of all the levators and serves to elevate the scapula and gill arches.

Dogfish Hypobranchial Muscles
These somatic (myotomal) muscles are the skeletal muscles of the trunk and tail. The anterior-most myotomes form hypobranchial muscles, which move anteriorly between the gills to form some of the muscles of the throat. Superficially, between the gills and attached to the scapulocoracoid bar can be seen the **common coracoarcual** muscles which open the mouth. Another important muscle that depresses the mandible is the **coracomandibular**, inferior to the intermandibular complex. .

Dogfish Axial Muscles
Remove a 3 cm x 3 cm patch of skin from the tail region centered on the horizontal septum and make a clean section through the tail. The **epaxial** muscles are found dorsal to the horizontal septum and the **hypaxial** muscles are ventral to it. There is a **myomere** for each vertebra. The **horizontal septum** in anchored to the dorsal ribs and the ventral ribs develop in between, in the **myosepta**. The ventral hypaxial muscles meet at the ventral midline forming a connective tissue raphe called the **linea alba**.

Dogfish Appendicular Muscles
Skin the left pectoral and pelvic fins near their bases and look for the muscles that lift the fins: the **pectoral levator** muscles. On the ventral side are the muscles that pull the fins down: the **pectoral depressor** muscles.

II. The Mudpuppy, *Necturus maculosus*

a. External Anatomy and Skin Removal

The flattened head has a mouth with lips, small lidless eyes and external nares. There are three pairs of lateral gills and two pairs of gill slits. The head is separated from the trunk by a **gular fold**. In the head and trunk regions small indents of the **lateral line** system can be seen. The **cloaca** is surrounded by a large **cloacal gland** in males. The skin aids in respiration and has a thin stratum corneum with mucous and poison glands. It is easily removed on the **left** side to the midline by cutting around the gills and turning the skin on the limbs inside out. Make a dorsal incision and do not cut very deeply or you will damage muscles.

b. Necturus Musculature

Necturus *Branchiomeric Muscles*

In the mandibular arch, the adductor mandibulae of the shark splits into the **temporalis (anterior mandibular levator)** and **masseter (external mandibular levator)** muscles. They form a sling for the lower jaw to close the mouth. On the ventral side lies the **intermandibular**, extending from the mandible to the median raphe. It elevates the throat.

In the hyoid arch, the **branchiohyoid** can be found lateral and posterior to the jaw. It waves the gills back and forth in the water for respiration. Ventrally, posterior to the intermandibularius, lays the **interhyoid**, which elevates the throat. The posterior portion of this muscle becomes most of the facial muscles in mammals.

In the gill arches, directly above the gills, lie the **branchial levators** that elevate the gills. The **cucullaris (trapezius)** is posterior to the branchial levators and draws the scapula anteriorly. Most of the other gill constrictor muscles have disappeared or moved to the larynx.

Necturus *Axial Muscles*

Anteriorly, the hypobranchial muscles can be seen in the center of the throat, behind the gular fold. Their segmentation can be seen in the **rectus cervicis,** which retracts the hyoid and gills and depresses the head.

The **epaxial** and **hypaxial** muscles separated by the **horizontal septa** of the myotomes are similar to those of the dogfish. Dorsally the epaxial muscles are fused into a long, strap like **dorsalis trunci**. Ventrally the hypaxial muscles meet at the **linea alba**. Lateral to the linea alba lie the strap-like longitudinal **rectus abdominis** muscles. There are three layers of abdominal muscles, the **external oblique**, **internal oblique** and **transversus abdominis**.

Necturus *Appendicular Muscles*

Muscles of the pectoral girdle

Pectoral muscles developed from the pectoral fin muscles of fish. The dorsal pectoral abductor muscles will be examined first. The **latissimus dorsi**, a large triangular muscle originating on the fascia of the dorsalis trunci and inserting on the humerus, is used to adduct the limb. The **trapezius (cucullaris)** also originates from the dorsalis trunci and inserts on the scapula to pull it anteriorly (this muscle is branchiomeric in origin and is not really an appendicular muscle). The **dorsalis scapulae** originates on the superscapular cartilage and inserts on the humerus to pull it anteriorly. It is related to the deltoid muscles of mammals. Lateral to this is the **procoracohumeralis**, which pulls the humerus anteriorly. The **triceps brachii** lie on the dorsal surface of the upper arm with three heads from the coracoid, scapula and humerus. They insert together on the ulna and serve to extend the forearm.

The ventral pectoral muscles have become large masses that adduct the humerus. The anterior muscles, the **supracoracoid** and the **pectoralis** originate on the linea alba and insert on the humerus. Flexion of the forearm is achieved by the **humero-antebrachialis,** which originates on the humerus and inserts on the radius and the **coracobranchialis**, which originates on the coracoid process and inserts on the humerus.

Muscles of the pelvic girdle
The pelvic dorsal abductors have also been highly modified. They have given rise to small muscles on the anterior of the limb. The **iliotibialis** and the **iliofibularis**, which form the sartorius and gluteus muscles of mammals, originate on the ilium and insert on the tibia and fibia to abduct the hind limb. The pelvic adductor muscle from the ventral surface of the shark fin becomes highly diversified in *Necturus*. On the ventral side, between the limbs, is the large **puboischiotibialis,** which along with the **pubotibialis** adducts the hind limb. Medial to these and projecting anteriorly is the puboischiofemoralis externus, which also adducts the thigh (with some anterior movement) The more posterior **ischioflexorius** flexes the shank and foot.

On the dorsal surface can be seen the **iliotibialis** and **ilioextensorius**, which both originate on the ilium and insert on the proximal tibia, casing extension of the lower leg. The **iliofibularis** originates on the ilium and inserts on the fibula. It causes flexion of the lower leg.

III. The Rat, *Rattus rattus*
a. External Anatomy and Skin Removal
Note the skin with **hair** and the **mammary glands**, characteristic of mammals. The head has a mouth with lips. The upper lip is split by a groove, the **philtrum**. The nose has **nares** and the eyes bear upper and lower eyelids and a reduced nictitating membrane. The external ears or **pinna** direct sound into the auditory canal. The whiskers or **vibrissae** are sensory. The trunk has an anterior **thorax** and posterior **abdomen**. The anus is at the base of the tail. The urinary and genital openings are separate in females, but in males they run as one duct to the tip of the penis. Males have a double pouch, the **scrotum** containing the **testes**. In the female, between the skin and muscles lies granular, mammary gland tissue in a band under the lines of nipples.

Cut through the skin of the rat from the pelvic region to the throat and the upper limbs on the **left** hand side. Remove the skin from the left side of the head. Skin the animal by inserting the handle of the scalpel or a blunt probe between the skin and the muscles. The muscles are connected to the skin by fascia.

b. Rat Musclature
Mammal Branchiomeric Muscles
In the mandibular arch, the **temporalis muscles** lie from behind the eye to the ear, occupying the temporal fossa of the skull. It elevates the mandible (lower

jaw). Behind and below the eye is the large **masseter** muscle, which elevates the jaw and allows for complex chewing. Ventrally and medially lies the **digastric** muscle, which depresses the mandible.

In the hyoid arch, the most important muscle in mammals is the spincter coli, which is the posterior portion of the interhyoideus muscle. This muscle spreads onto the neck and becomes the **platysma** in mammals. It then spreads over the head and face to form the **facial muscles** of higher mammals.

The gills are now gone and the muscles of the gill arches have for the most part disappeared. Some muscles remain on the larynx and for swallowing. The cucullaris muscle has survived as the **trapezius** muscles of the pectoral girdle. The trapezius complex consists of three muscles, two of which originate on the spine and insert on the scapula to adduct and move it dorsally and forward. The third muscle inserts on the clavicle and protracts the humerus. The **sternomastoid** muscle is also derived from the cucullaris muscle and serves to turn or flex the head.

Mammalian Axial Muscles
In mammals the hypobranchial muscles form the deep muscles of the throat and tongue.

The hypaxial muscles are three sheets (layers) joined ventrally at the **linea alba**. These formed by a muscle-to-muscle attachment of fascia called an aponeurosis. Cut out a rectangle of abdominal muscle about 1 cm x 1 cm, which includes the mid-line. Pull apart the layers of muscle from the lateral side towards the mid-line. On either side of the linea alba is a band of longitudinal muscle, the **rectus abdominis**, where the three muscle layers sit in a common sheath. The three muscle layers from the outside to the inside are: **external oblique, internal oblique,** and **transversus abdominis.** These muscles support the abdominal viscera in a muscular sling. Other, deeper hypaxial muscles will not be not seen. These are the intercostals and supracostals of the rib cage and the **diaphragm**, which acts as a suction pump in respiration. The muscles of the tail are continuations of the epaxial and hypaxial muscles bundles.

Mammalian Appendicular Muscles
Muscles of the pectoral girdle
In the pectoral region, the pectoral abductor muscles of the dogfish now are represented by a number of forelimb muscles. Note the triangular **latissimus dorsi**, which originates from the neural spines of most of the thoracic vertebrae and inserts on the humerus, to pull the forelimb dorsally. Anteriorly it is overlain by the trapezius complex, which is branchiomeric in origin. From the scapula there are five deltoid muscles, which insert on the humerus to adduct and rotate it. Locate the **spinodeltoid**. The **triceps brachii** consists of three muscles originating on the scapula or humerus and inserting together on a process of the ulna, to extend the forearm.

The ventral pectoral adductor muscles of the dogfish have also become complex in mammals. The **biceps brachii** is located on the anterior of the forearm. They originate on a tendon inserting on the scapula and insert by a tendon on the tuberosity of the radius, to flex the forearm. Turn the animal on its back to see the large **pectoralis** muscles. There are several muscles in this group in mammals, some of which adduct or pull the forelimbs towards the midline, others retract the forelimb backwards. These muscles originate on the sternum and insert on different parts of the humerus. The most obvious of these muscles will be the anterior **pectoralis major** and the more posterior **pectoralis minor**.

Muscles of the pelvic girdle
The pelvic abductor muscles of the dogfish have become specialized for locomotion in the mammal. In the thigh is found the large **sartorius** muscle, which runs from the ilium to the knee to adduct and rotate the femur and extend the lower leg. More posteriorly lay the thin, wide, **gluteus** muscles of the hip, originating on the last sacral and first caudal vertebrae and inserting on the femur to abduct the thigh.

The pelvic adductor muscles now occupy both surfaces of the hind limb. Anterior on the limb is the **tensor fascia latae**, a triangular muscle originating on the ilium and inserting on the knee by a **fascia lata**. It extends the lower leg. Posterior to it is the **caudofemoralis**, partly hidden by the **biceps femoralis**. It originates on the tail (caudal) vertebrae and inserts on the lower leg. The posterior thigh has the large, thick **biceps femoralis**, which runs from the ischium to the tibia and knee to abduct the thigh and flex the lower leg. The posterior-most muscle is the **semitendinosus**, which is stuck to the biceps femoralis and runs from the ischium to the tibia to flex the lower leg. Ventrally you will see the **gracilis** muscle which occupies the posterior half of the thigh. It runs from the pubic symphysis to the tibia and adducts and retracts the leg.

Table Two. Vertebrate Muscle Homologies

	SHARK	**MUD PUPPY**	**MAMMAL**
Branchiomeric muscles	mandibular adductor	masseter temporalis	masseter temporalis
	intermandibular	intermandibular	digastric
	palatoquadrate levator	branchiohyoid	platysma
	hyoid levator	interhyoid	digastric
	cucullaris	brachial levators cucullaris	trapezius sternomastoid
Hypobranchial muscles	coracorarcuals coracomandibular	rectus cervicus	tongue & throat muscles
Axial muscles	epaxials	dorsalis trunci epaxials	deep longitudinal muscles
	hypaxials	rectus abdominus external oblique internal oblique transversus abdominis	rectus abdominus external oblique internal oblique transversus abdominis
Appendicular muscles	pectoral abductors	latissimus dorsi dorsalis scapulae triceps brachii procoracohumeralis	latissimus dorsi deltoids triceps brachii
	pectoral adductors	supracoracoid pectoralis humero-antebrachialis coracobranchialis	pectoralis biceps brachii coracobranchialis
	pelvic abductors	iliotibialis iliofibularis	sartoris gluteus
	pelvic adductors	puboischiotibialis pubotibialis iliofemoralis ischioflexorius	gracilis caudofemoralis tensor fascia latae biceps femoralis semitendinosus