PRACTICAL 3

Dissection of the toad

Purpose

To examine the structure and organisation of the Queensland cane toad, *Rhinella marina* (formerly known as *Bufo marinus*).

Pre-practical quiz on Moodle

A quiz will be posted on the SLE132 page of Moodle, in the Resources and Assessments section, ONE WEEK before the first practical, and will close the day before the practical class. This quiz will test your knowledge of practical 3 and ensure you have prepared yourself for the class. You will have two opportunities to complete this quiz so please ensure you have thoroughly read the practical notes before you attempt the quiz. Your result from this quiz will contribute 1% of your overall SLE132 mark.

Introduction

Cane toads were introduced to Queensland last century from Hawaii (to where they had originally been introduced from South America) as a biological control mechanism to deal with the cane beetle which damaged the sugarcane crop. Since its introduction, the cane toad has successfully invaded and occupied many ecological niches, displacing native species, and its geographical distribution has increased enormously, now extending to New South Wales, the Northern Territory and Western Australia.

In addition to the ecological damage caused by the competitive displacement of native species and the modification of microhabitats, the cane toad also poses a threat to predator species not previously exposed to it because of the poisons it releases from its skin. The toads that you will dissect today have been collected on farms in Queensland, and then humanely euthanized before being frozen and air-freighted to Deakin. For quarantine purposes, **all of the tissue that you remove during the dissection and the remaining carcass must be placed in the special waste containers in the laboratory at the end of this practical class.**

The complete classification of the cane toad is:

Phylum:	Chordata
Sub-phylum:	Vertebrata
Class:	Amphibia
Super order:	Salientia
Order:	Anura

Family: Bufonidae
Genus: *Rhinella*
Species: *Rhinella marina* (*marina* is the specific epithet).

Note that when asked to provide a species name, you must always include the genus, since a specific epithet on its own is meaningless. That is, though the genus that the cane toad sits in is *Rhinella*, the species is *Rhinella marina*.

There are three orders of the vertebrate class Amphibia still living today: the Anura (frogs and toads); the Caudata or Urodela (newts and salamanders); and the Gymnophiona or Apoda (limbless, 'wormlike' burrowers). These modern Amphibia, like living reptiles, birds and mammals, apparently evolved from a group of primitive amphibians known as *labyrinthodonts*, which first appeared in the Devonian period of the Palaeozoic era (about 400 million years ago). It is important to remember that present-day amphibians, and especially anurans, are highly specialised animals that bear little resemblance to the 'stem amphibians'—the common ancestors from which the modern amphibians as well as the modern reptiles, birds and mammals all evolved.

As their name implies (*amphi* = both; *bios* = life), the life cycle of amphibians has two distinct components. Larval amphibians (tadpoles) are wholly aquatic and rely in part on gills for respiration and ion exchange; in this respect, they resemble fish. Some amphibians remain and even reproduce in the aquatic larval form. This condition is known as neoteny, and is commonly seen in the axolotls (Caudata), which retain their gills throughout their entirely aquatic life. Most amphibians undergo a radical metamorphosis at the end of the larval stage to become a more-or-less terrestrial tetrapod (i.e. an adult frog or salamander).

Today's dissection will cover the important features of the anatomy of the internal organ system of the toad, as well as aspects of the external features of the animal and its skeletal structure. Before you start cutting anything, read all of the information and instructions provided. Take your time and be careful.

WARNING: The skin of the cane toad contains many glandular structures that secrete a variety of noxious and poisonous substances onto the surface of the skin. Some of these substances, in particular those which are secreted from the parotoid glands located on each side of the head near the shoulders of the toad, can cause extreme irritation of the eyes. For these reasons, you should wear safety glasses during this dissection, so that you do not inadvertently touch your eyes with your hands, and so that your eyes are protected from any poison which might be ejected into them if you squeeze the toad tightly. You should also wear a lab coat and rubber gloves, especially if you have any cuts or abrasions on your hands. (RISK: Low.)

Dissection technique

To perform a dissection well, you must proceed slowly and with considerable care.
DO NOT USE A SCALPEL FOR ROUTINE CUTTING. In this dissection, there is no need for your scalpel. You should always make major cuts with blunt-ended scissors.

(**WARNING**: Sharps; risk of cuts; use great care. **RISK**: Low.)

Whenever you insert one blade of the scissors into an opening where you can't see the other side (which is most of the time) insert the blade with the most rounded end into the cut. When cutting, point the tips of the scissors up and away from the underlying tissue. Be conservative.

Most of the tissues separate best by gentle pulling with your fingers, or, for smaller parts, forceps. The blunt, rounded handles of forceps make useful tools for gently displacing organs. When you are trying to separate tissues, tease the specimen apart rather than cutting or pulling vigorously. If you have to separate two robust structures that are held together by tough connective tissue, it is sometimes useful to use a technique known as **blunt dissection**. This involves gently placing the closed tips of a pair of blunt-ended scissors between the two layers to be separated and then opening the scissors—you will find this technique useful for separating large areas of skin from the underlying body wall musculature.

Be particularly careful when dissecting blood vessels. Although the heart is no longer beating, blood will still leak from vessels if you damage them, and will obscure the structures you are trying to display. The blood vessels will be reasonably elastic but you will still have to be careful not to break them. The veins will be large and dark blue-black because the vessels are thin-walled and blood tends to pool in them when the animal dies. The arteries actually have very little colour since they do not retain the blood. This sometimes makes them harder to find, but they are more muscular than veins and therefore stronger and easier to dissect.

Remove connective tissue and fat to display structures clearly when necessary, but remember to use your finest pointed forceps and always make notes and diagrams as you go because it is all too easy to destroy some structures as you attempt to display others. You may find that the blood vessels of your toad differ slightly from the description in the practical notes—draw them as you find them, not as you think they should be.

Terminology

The naming and directions of the organs of an animal with regards to right or left are from the perspective of the animal's right or left.
Anterior is towards the animal's head,
Posterior is towards the animal's tail or hind end.
The **dorsal** surface is the back or upper surface.
The **ventral** surface is the abdominal or under surface.
Medial means towards the animal's midline,
Lateral means away from the midline.

Procedure

Work in groups of three.

Examination of the external features of the toad. The body of the toad is divided into a head, trunk and limbs. There is no neck and no tail. The forelimbs are held at right angles to the body and the pectoral muscles are strongly developed to support the body's weight. The hind limbs are long and specialised for hopping. The feet are only slightly webbed since *Rhinella* is more terrestrial than aquatic.

Reptiles, including the toad, have a single posterior orifice, the **cloaca**, through which faecal material, excretory products and gametes pass to the outside. The cloaca is present in all vertebrates, except placental mammals, which have separate openings for the digestive (anus), excretory (urethra) and reproductive systems (vagina/penis), though the latter two are combined in the male penis.

The skin is cornified (i.e. the outermost epidermal cells accumulate large amounts of the waterproof protein keratin) but does not possess scales (distinct from reptiles) and is loosely attached to the body. In *Rhinella*, there are many glands in the skin. An aggregation of glands, the parotoid glands - which are on the dorsal side of the pectoral region behind the head, produce a creamy secretion which is poisonous.

Male toads generally have a patch of reddish skin on the anterior ventral surface of the body.

Examination of the head, buccal cavity and pharynx

Food capture

Adult frogs and toads are carnivorous while the majority of larvae are herbivorous. In adults, the mouth is broad allowing a wide gap. The tongue is attached at the anterior part of the buccal cavity and is used to capture food. In *Rhinella*, there are no teeth and prey is swallowed whole. In other amphibia, teeth are used for holding prey rather than for mastication. The opening to the oesophagus is a wide slit across the pharynx (the posterior part of the buccal cavity).

Breathing

The skin, buccal cavity and lungs all contribute to aerial respiration. Amphibia do not have a diaphragm and air must be forced into the lungs rather than sucked in as in mammals. The driving force is provided by the floor of the buccal cavity. Air is drawn into the buccal cavity by depressing the floor and opening the nares. The external nares are located on the tip of the snout and can be opened and closed by movement of the tip of the upper jaw. The **internal nares** open into the anterior of the buccal cavity. Air can be passed in and out of the buccal cavity without entering the lungs. To deflate the lungs, the floor of the mouth is depressed, the nares opened and the **glottis** opened. The elastic recoil of the lungs expels stale air through the open nares. To inflate the lungs, the nares close and the buccal floor is raised to force air into the lungs through the open glottis. It may take several cycles to fully inflate the lungs. The glottis is a slit-like opening bounded by cartilage and is located on the ventral surface of the **pharynx** anterior to the **oesophageal opening**.

Hearing and vocalisation

The tympanic membrane (eardrum) is located on the external surface of the head posterior to the eye. There is no outer ear structure (pinna, auditory canal) of the form typically seen in mammals. The tympanic membrane transmits acoustic pressure waves via the columells (stapes) across the middle ear cavity to the inner ear. The middle ear communicates with the buccal cavity by the **eustachian tubes**. The openings of the eustachian tubes are on the cranial surface of the pharynx near the articulation of the upper and lower jaws.

Most male frogs and toads use acoustic signals to define their territories and attract females. The vocal cords are situated longitudinally across the larynx which is immediately posterior to the glottis. Air is passed between the vocal sacs and lungs to set up vibration in the vocal cords. To amplify sound, the vocal sacs beside the pharynx act as a resonator. The openings to the vocal sacs are lateral to the glottis near the outer margin of the pharynx. Females do not have these structures.

Dissection of the buccal cavity and pharynx

- Open the mouth and cut through the angle of the jaw with coarse scissors.
  (**WARNING**: Sharps; risk of cuts; use great care. **RISK**: Low.)

- Extend the cut about 5 mm and pull the lower jaw to the right side to expose the pharynx. **DO NOT** cut across the floor of the buccal cavity.

- If necessary, wash out the buccal cavity to remove excess mucus.

- Locate the **internal nares** and demonstrate their continuity with the external nares using a blunt probe.
- Examine the **pharynx** and locate the opening to the **oesophagus**, the **glottis** and **eustachian tubes**. By piercing the tympanic membrane, show continuity of the middle ear and buccal cavity.

- In male toads, attempt to find the opening to the vocal sacs. This may be difficult.
- Examine the attachment of the **tongue**. Why is it attached in this manner?

Biological drawing 1: Make a fully labelled drawing of the head including the features of the buccal cavity.
Your drawing should occupy at least half a page and have labels for at least the six features that are in bold in the preceding dot points.

Dissection to reveal the internal organs of the body cavity

- Pin out the toad with ventral surface uppermost (Figure 1).
- Using large forceps and coarse scissors, lift the skin in the mid-ventral line and make a mid-ventral incision to the tip of the lower jaw. Make lateral cuts along each limb.
  WARNING: Sharps; risk of cuts; use great care. **RISK**: Low.)
- Using blunt dissection techniques, free the skin from the body wall. **TAKE CARE** in the auxiliary (armpit) regions to avoid cutting superficial blood vessels. Pin back or cut off the separated skin.

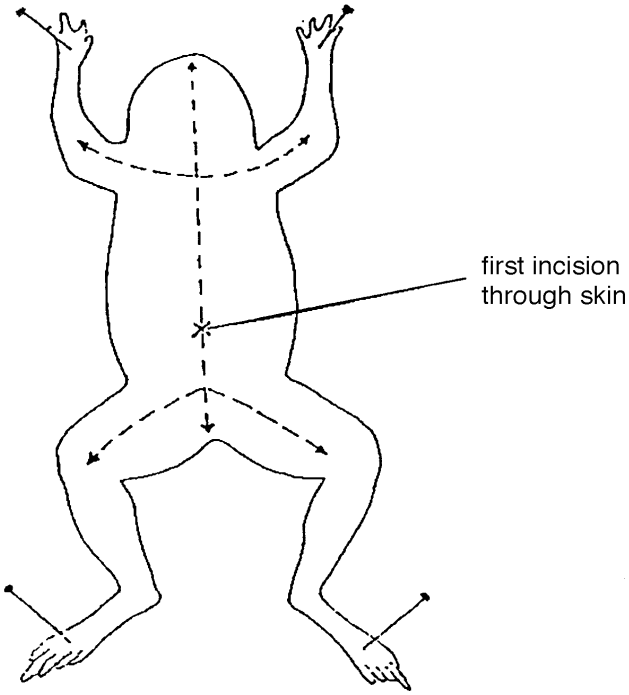

Figure 1

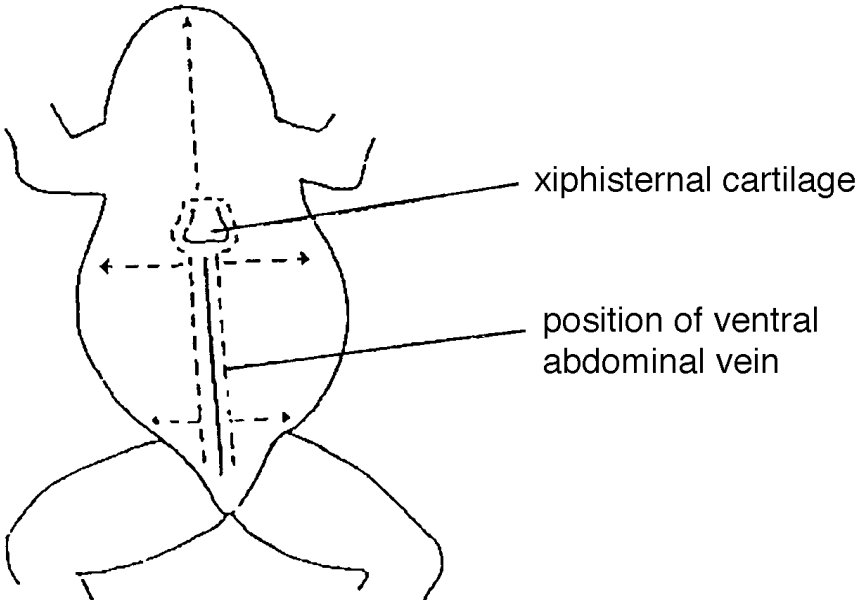

Figure 2

- The ventral abdominal vein lies adjacent to the abdominal muscles in the midline (Figure 2). To avoid cutting this vessel, make two incisions on each side of the midline

and leave the vessel attached to the body wall (Figure 2). Continue the incisions forward and around each side of the xiphisternal cartilage (part of the sternum or breastbone). Keep lifting up on the scissors while making these cuts to avoid damaging the underlying organs.

- Make lateral cuts from each end of the two median incisions so that a flap of muscle can be reflexed to expose the underlying organs. **AVOID** auxiliary blood vessels.

- Lift the strip of muscle with the attached ventral abdominal vein near the xiphisternal cartilage and locate the point where the ventral abdominal vein diverges from the body wall to join the hepatic portal vein. Make a transverse cut through the strip of muscle, leaving the ventral abdominal vein intact.

- Carefully continue the midline incision through the pectoral girdle and forward to lower jaw. Be very careful to avoid the heart and the anterior blood vessels that lie underneath the pectoral girdle.

- Cover the animal with water. This causes the organs to float apart from one another. You should change the water whenever it becomes cloudy.

Examination of the viscera

Viscera is the collective term given to the organs in the body cavity. Each of these organs is covered by a thin membrane called the peritoneum, as is the internal surface of the body cavity. The peritoneum also extends between individual organs in the form of thin sheets of connective called mesenteries. The mesenteries hold the different organs in relatively fixed positions with respect to one another and to the body cavity, while allowing some freedom of movement.

The alimentary system comprises the digestive tract (= the alimentary canal) through which ingested food passes from the mouth to the cloaca (or anus), together with the accessory organs that supply enzymes and other substances that are involved in various stages of the digestive process.

Four major regions of the alimentary canal can be recognised:

i. the receiving region comprising the buccal cavity and pharynx, where food is captured, tasted and its palatability assessed, lubricated and broken down mechanically.
ii. the conducting region, comprising the oesophagus.

iii. the digestion and absorption region, comprising the stomach and the small intestine, where ingested food is broken down enzymatically and the breakdown products absorbed into the bloodstream.
iv. the storage and water absorption region of the large intestine.

Food is propelled along the alimentary tract by peristaltic contractions of the circular and longitudinal muscle layers in the wall of the tract. Movement of food between i. and ii. and between ii. and iii. is regulated by sphincters.

Locate the organs of the alimentary system without breaking the mesentery. If necessary, move the organs gently with your fingers to reveal the relationship of one to another.

The **liver** is a large, brownish, three-lobed organ which lies dorsal and posterior to the xiphisternal cartilage.

The **gall bladder** is located between the lobes of the liver. It is spherical and usually filled with green bile.

The **stomach** is a thick-walled, tubular organ just posterior to the liver. Anteriorly, its continuity with the oesophagus may be seen by turning the stomach to the right side. The posterior end of the stomach can be closed by the pyloric sphincter.

The pylorus leads to the duodenum or first part of the **small intestine**. The duodenum loops back to lie parallel to the stomach and between these two organs lies the pancreas.

The pancreas is pink and multi-lobed. Ducts from the pancreas join the bile duct, giving rise to the common bile and pancreatic duct. This duct runs to the duodenum.

The second part of the small intestine, the ileum, is a narrow, convoluted tube slung in mesentery.

The **large intestine** is a short, wide tube which merges into the cloaca.

Other organs of the body cavity

Ventral to the large intestine is the large, bi-lobed, thin-walled urinary bladder. It is attached to the large intestine and also to the body wall.

The **kidneys** are elongated, red-brown organs on the mid-dorsal body wall. Move the viscera to locate the kidneys.

The adrenal tissue is an orange streak on the ventral surface of the kidneys.

In males, the **testes** are closely associated with the anterior part of the kidneys. The testes are ventral to the kidneys and are cream coloured.

In females, the **ovaries** may be very large in mature animals. The ova are many and each ovum is half black and half white giving a mottled effect to the ovary. In mature females, the oviducts are easily located on the dorsal body wall. They are opaque, coiled tubes.

The **fat bodies** are yellow, branched structures anterior to the testes or ovaries.

By turning the viscera to the animal's right side, the small red **spleen** can be found in the mesentery at about the level of the duodenum.

Anteriorly, the **lungs** are situated dorsal and anterior to the liver and stomach. If they are inflated, they have the appearance of reticulated balloons.

Beneath the pectoral girdle, the **heart** is contained within the pericardium. The heart lies within the **pericardium** at the anterior end of the body cavity where it is protected ventrally by the pectoral girdle and xiphisternum. There are two separate atria. The left atrium receives oxygenated blood exclusively from the pulmonary veins. The right atrium receives systemic venous blood from the sinus venosus. Both atria deliver blood to a common, single ventricle. This pumps blood through the truncus arteriosus which is internally divided by a 'spiral valve'. The truncus arteriosus divides into a left and a right arterial trunk.

Biological drawing 2

Arrange the viscera so that the organisational relationships between the different components are clear to an observer. Ensure the stomach and intestines are pinned to the right-hand side.

Make a labelled drawing of the viscera from a ventral view.

Indicate whether your specimen is a male or a female. Your drawing should occupy a full page and include at least 10 labels.

The nervous system

The central nervous system consists of the brain and the spinal cord. The brain is made up of the fore, mid and hindbrain. The brain is surrounded by the bone of the skull, and the spinal cord is located inside the vertebral column.

In the toad, there are ten pairs of spinal nerves. The first pair of nerves pass to the tongue, and the others are distributed to the muscles of the limbs and the body wall. Each pair of nerves emerges from the **vertebral column** below the corresponding vertebrae (i.e. the first nerves emerge between the first and second vertebrae, the second pair from between the second and third vertebrae and so on). The tenth pair arises from the anterior part of the **urostyle**, a long spine-like bone at the posterior part of the vertebral column.

Associated with the spinal nerves is the sympathetic nervous system. The sympathetic nervous system lies more or less parallel to and on either side of the vertebral column. It consists of two chains of ganglia (masses of nerve cell bodies) joined by longitudinally running nerve strands. These nerves pass ventrally to the spinal nerves and each ganglion is connected to its corresponding spinal nerve by fine fibres. From some of the ganglia, visceral nerves pass to the various organs of the viscera.

Dissection of the spinal nerves and the sympathetic system
Expose the nerves on one side of the body.

Trace the **hypoglossal nerve (first spinal)** from its origin between the first and second vertebrae into the head region towards the tongue.

Find the origin of the **brachial nerve (second spinal)** between the second and third vertebrae and follow it into the arm.
Note the **third spinal nerve** which at first runs alongside the brachial and then passes into the armpit.

The **fourth, fifth** and **sixth spinal nerves** pass to the muscles of the trunk.
The **seventh, eighth** and **ninth spinal nerves** are larger than the immediately preceding ones. They run close together and at first lie near the vertebral column.

The seventh divides into two branches. One passes to the muscles of the trunk and the other joins the sciatic plexus. This is formed by the fusion of the eighth and ninth spinal nerves with the branch of the seventh.

The **tenth spinal nerve** is thin and often pigmented. It may also join the sciatic plexus or pass to the muscles near the cloaca.

Trace the sciatic nerve from the sciatic plexus into the leg.

Look for the sympathetic trunk along the side of the vertebral column. Note the ganglia associated with the spinal nerves. The first four are close to the point where the spinal nerves emerge from the vertebral column. The posterior ganglia are larger and are further from the vertebral column.

Look for the visceral branches to the aorta, kidneys and other organs of the viscera.

Biological drawing 3

Make a labelled drawing of the spinal nerves.
Your drawing should occupy a full page and include at least 11 labels.

Question 1: List the organs of the toad digestive tract - from buccal cavity to cloaca (don't include accessory organs). If the analogous organ is different in mammals, state this organ in brackets after the amphibian organ (3 marks)

Question 2: With respect to the number and types of chambers within the heart, what are the differences between fish, toad and human hearts? (3 marks)

Summary:

Biological drawing 1: Toad buccal cavity

Label at least 6 structures including:

- internal nares
- pharynx
- oesophagus
- glottis
- eustachian tubes
- toung

Biological drawing 2: Toad viscera

Label at least 10 structures including:

- liver
- gall bladder
- stomach
- small intestine
- large intestine
- kidneys
- testes or ovaries and oviducts
- fat bodies
- spleen
- lungs
- heart
- pericardium

Biological drawing 3: Toad spinal column and nerves

Label at least 11 structures including:

- vertebral column
- urostyle
- hypoglossal nerve
- brachial nerve
- third, fourth, fifth, sixth, seventh, eighth, ninth and tenth spinal nerves

Answer questions 1 and 2.

Assessment (7%)

- Pre-prac quiz on Moodle: Resources and Assessment section: 1% of final assessment.
- Three fully labeled, biological drawings with complete legends, and answers to two questions (36 marks for 6% of final assessment).