Department of Primary Industries
Department of Regional NSW

Cane Toad Sex Identification & Dissection Guide

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Background

This guide has been prepared for use by veterinarians and qualified field staff to assist in the positive identification of the sex of cane toads and to assist in determining the reproductive status of female toads in particular.

Important

Cane toads are highly toxic. Dissection should only be carried out by competent individuals and safety measures should be in place such as the use of PPE (gloves and preferably goggles or glasses) to prevent exposure to toxin. Care should also be taken to avoid placing unnecessary pressure on the parotoid glands during dissection as this may result in the expression of toxin from these glands. N.B. While the toxin is concentrated in the parotoid glands, it is also distributed in other small glands throughout the dorsal surface of the skin. All equipment used for dissection should be washed thoroughly after use.

Figure 1 - A female cane toad exuding toxin from the parotoid gland. Note also toxin exuding from dorsal skin glands and glands on the ridges of the eyes.

Warning: Contains graphic images

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Glossary

Amplexus: The copulatory pairing of anuran (frog or toad) amphibians, where the male clasps the female.

Cloaca: The opening where both waste and gonads are ejected into the environment.

Dorsal: The upper / top, 'back' side.

Metamorph: A toad that has recently transitioned from the aquatic tadpole stage, to the terrestrial 'toad' stage.

Nuptial pads: Dark, slightly rough patches of skin located on the inner surface of the front digits.

Ovarian tubules: Coiled light pink tubes through which ovaries are transported to the cloaca in the female.

Parotoid glands: Large glands located either side of head. These glands are where the majority of toxin in the skin is located.

PPE: Personal Protective Equipment

SUL: Snout-Urostlye Length. The standard measurement to record the size of an anuran (frog or toad) amphibian.

Urostyle: Elongate, modified section of vertebrae. The terminal section of spinal column.

Ventral: The lower / under, 'belly' surface.

Introduction

Cane toads (*Rhinella marina*) were introduced to Australia, being released at various locations in north Queensland, in the late 1930s. They have since become established and widespread (Shine 2010). They now occur across northern Australia where they continue to invade the west Kimberley. On the east coast, they have spread as far south as the Clarence Valley just north of Grafton and on the coast to around Brooms Head (M. Greenlees pers. obs.). Hitch-hiking toads have resulted in the establishment of isolated populations on the Central Coast, in Port Macquarie and even in Sydney - the latter two having been eradicated (White and Shine 2009, Greenlees et al. 2018), with the Central Coast population subject to a current eradication program. It is estimated that approximately 50 toads per year are translocated into Sydney, most likely from the north coast New South Wales and south-east Queensland (White and Shine 2009). Fortunately, most of these would be individual animals posing a lower establishment risk.

Care should be taken to correctly identify toads as they can be confused with native frogs. Additionally, toads can change in general appearance from when they first metamorphose, to when they become mature. Species in New South Wales often confused with toads include Barred frogs (*Mixophyes* species), Banjo frogs (*Limnodynastes dumerilii* and *Limnodynastes terrareginae*), Ornate burrowing frogs (*Platyplectrum ornatum*) and Emerald-spotted, or Peron's, tree frogs (*Litoria peronii*).

Figure 2. Great barred frog (*Mixophyes fasciolatus)* on the left and a similar sized toad on the right.

Figure 3. Giant barred frog (*Mixophyes iteratus*)

Figure 4. Eastern Banjo frog (*Limnodynastes dumerilii*)

Figure 5. Northern or Scarlet-sided Banjo Frog (*Limnodynastes terrareginae*)

Figure 6. Ornate burrowing frogs (*Platyplectrum ornatum*) [on left] are often confused with small toads [on right].

Figure 7. Emerald-spotted or Peron's tree frog (*Litoria peronii*) is a common species in urban areas and often mistaken for a toad.

Figure 8. Cane toads can vary greatly in size, measuring approximately 10mm when they first emerge as metamorphs, to over 10cm as adults. As metamorphs, toads are black in colour, usually changing to mottled grey by the time they are 15mm in length.

Figure 9. Toads can also vary substantially in colour and pattern, particularly as sub-adults.

Euthanasia

There are various methods available for the euthanasia of cane toads, such as using registered products like Hopstop® spray. Another simple and effective method is to place a toad in a suitable container and hold it in a refrigerator (at 4°C) for 8-12 hours (i.e., usually overnight) and then transfer the container to a freezer (at -20°C) and hold for a further 24 hours as a minimum (ANZCART 2016).

When using this method, it is important that there is sufficient cool air around each individual toad to allow for uniform cooling and freezing. It is not recommended that large numbers of toads are placed in a bag together as it will take much longer for those in the centre of the bag to be cooled. As such, it is recommended that toads are placed in containers and stacked to allow appropriate circulation of air and consistent cooling.

Where it is not possible to refrigerate toads before freezing, or where toads are likely to be captive for some time before they can be placed in a fridge/freezer, they should be placed into a bucket (or similar container with lid) and euthanised immediately with Hopstop®. These animals can then later be placed into a freezer to ensure death and/or if specimens need to be kept for further analysis.

Dead toads must not be left in the field, they need to be carefully disposed of to ensure they do not pose a risk to domestic animals or native fauna that are susceptible to cane toad toxin.

Sexual maturity in toads

In populations from tropical regions, toads have been recorded as reaching maturity at approximately 1 year of age and/or approximately 70mm (Zug & Zug 1979). It is difficult to determine the age of a cane toad from visual appearance thereafter as male and female growth and growth rates may differ (Zug & Zug 1979). As ectotherms, growth rate is likely to be dependent on temperature and food availability which can vary in time and space. Traits such as growth and morphology may also be affected by environmental conditions experienced by the toad as a tadpole (e.g. Relyea 2001). Maturity in toads is essentially size dependant and generally, maturity for both sexes is reached and is easily discernible by the time they have reached a length of 70-80mm SUL. By this size, males will usually be demonstrating secondary sexual characteristics and females capable of producing eggs. Male testes will be apparent upon dissection by the time they reach 50mm SUL; if they are not apparent by this stage it can safely be assumed that the animal is female, even if ovaries are not yet developed (M. Greenlees pers obs.).

Measuring Toads

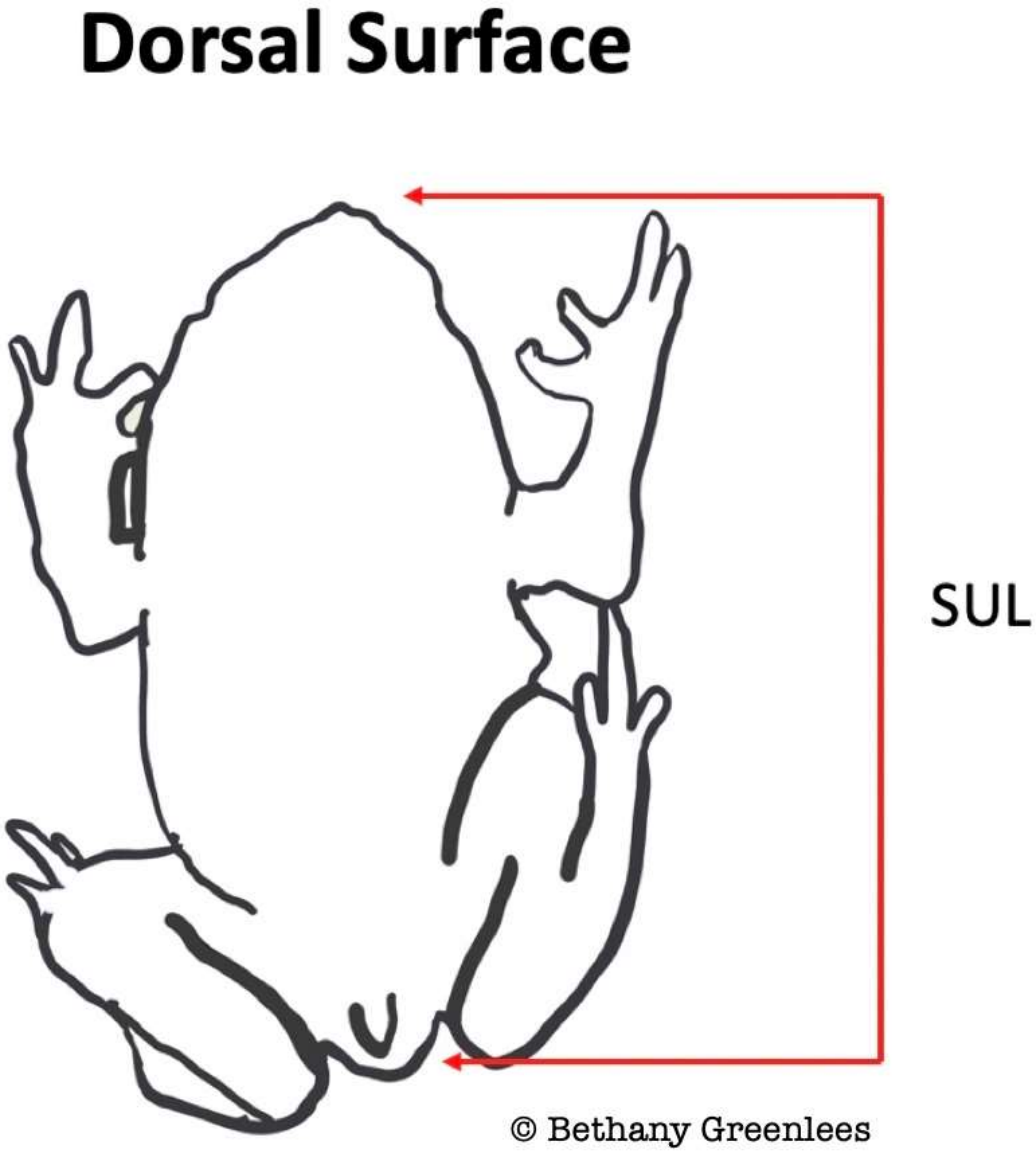


Figure 10. To measure a toad, a ruler should be placed along the dorsal midline and the length from the snout to the end of the urostyle recorded. The end of the urostyle can be located by gently running a finger from between the back legs toward the head.

Auditory sexing of mature toads

Once mature, live male cane toads will emit an audible ‘release’ call while simultaneously ‘vibrating’, usually while being held around the mid-body, simulating amplexus. This call is emitted by male toads to signal to other male toads which may have ‘amplexed’ them that they are not female. Female toads may also vibrate, but do not emit any sound. The ability of male toads to emit the call appears to be temperature dependant, so this indicator should not be the only one used to determine sex.

Figure 11. Male toads will emit an audible release call when gently gripped around the mid-body. Females may vibrate but will not emit any sound.

Figure 12. Toads in amplexus.

Sexing using external morphology

During the breeding season, typically spring and summer months, male cane toads develop a number of secondary sexual characteristics that can make them readily identifiable as male. Care however should be taken as these characters can become far less prominent, or even absent during non-breeding season (generally over winter). Not all males will necessarily be displaying these characteristics at any given time during the breeding season (generally spring and summer) and finally, the degree to which each character is expressed can also vary (Kelehear & Shine 2019).

Nuptial pads

Nuptial pads are probably the most consistently useful and accurate morphological character that will allow for positive identification of sex (M Greenlees pers. obs.). Male toads develop dark, rough patches on the inner surface of their front digits. This provides grip when in amplexus with female toads. When males are in peak condition for breeding, these pads may cover two or three toes and may connect at the base of the fingers. During non-reproductive periods, the pads are almost always still apparent, but may only cover a small part of the inner most frontal digit (Kelehear & Shine 2019). By 70mm SUL, it is almost certain male toads will be displaying some evidence of nuptial pads (M Greenlees pers. obs).

Figure 13. Male toad nuptial pads showing varying degrees of darkness and coverage of fingers.

Figure 14. Female toad hands lack any evidence of nuptial pads

Skin colour and texture

At maturity (approx. 70mm SUL), male toads will generally be a uniform brown to yellow colour. During the breeding season, males become increasingly yellow in colour, with the first indication of this usually along the flanks. Females are generally more mottled and consistently brown in colour. Female toads will also usually have a lighter coloured dorsal stripe. This however is the least consistent character and is often present in both sexes before they reach sexual maturity. At maturity, and especially during the breeding season, male toads develop small granular glands all over the dorsal surface and upper sides of the hind limbs giving their skin a rough, 'sandpapery' texture. When males are mostly yellow in colour, these granular glands will be dark in colour. Even

when males are not strongly yellow-coloured, the upper surface of the legs is almost always noticeably rough. Mature female toads do not develop these glands so are always smooth in texture.

Figure 15. Male toads are uniformly brown to yellow, the latter especially during breeding season and have dark, granular glands on the skin giving it a rough, 'sandpapery' texture.

Figure 16. Female toads are more mottled in appearance with large warty bumps on their skin. Their skin is however smooth in texture. Female toads will often also have a lighter-coloured dorsal stripe.

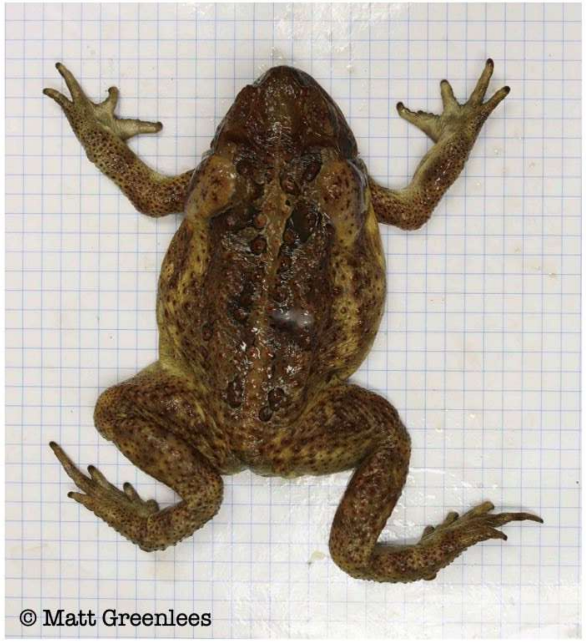
Quick guide to sexing toads using external morphology

Quick guide to sexing toads by morphological characters

Male

Nuptial pads present	Nuptial pads absent
Skin uniform (yellow in breeding) colour	Skin mottled and never yellow
Skin texture rough	Skin texture smooth

Female



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Toad Dissection

Adult cane toads have surprisingly thick skin so scissors may be preferable to a scalpel when performing dissection. To assess the sex and reproductive condition of a toad, the ventral skin should be removed.

General

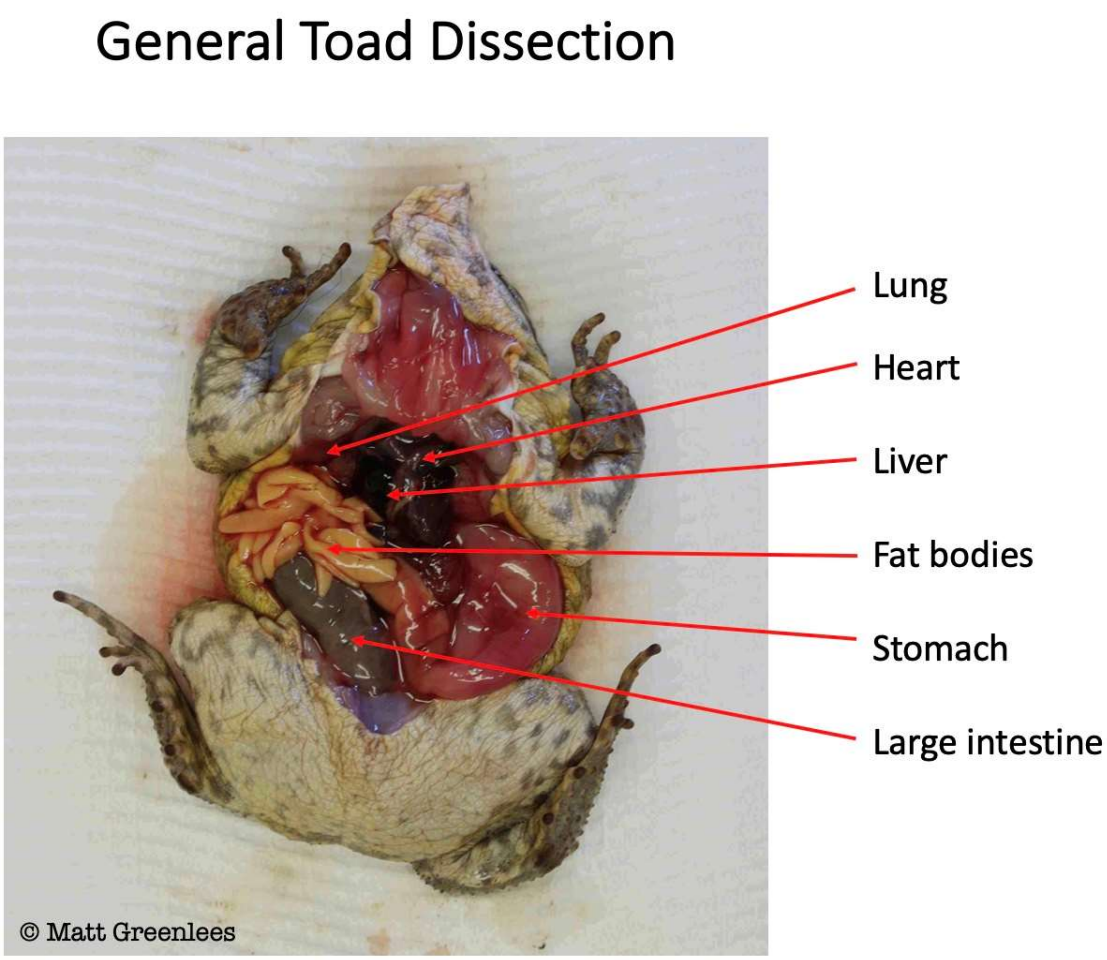

Figure 17. General Dissection

Males and non-reproductive females will generally accumulate fat bodies that appear as yellow to almost white strands or 'fingers' below the liver. If females have accumulated sufficient fat stores, they will convert these fat bodies into ovaries. Note that if inflated, lungs will be easily apparent when the toad is first opened, appearing as light pink 'balloons'. However, when deflated, the lungs will retract towards the toads shoulders and may not be apparent if these are punctured during initial incision.

Male

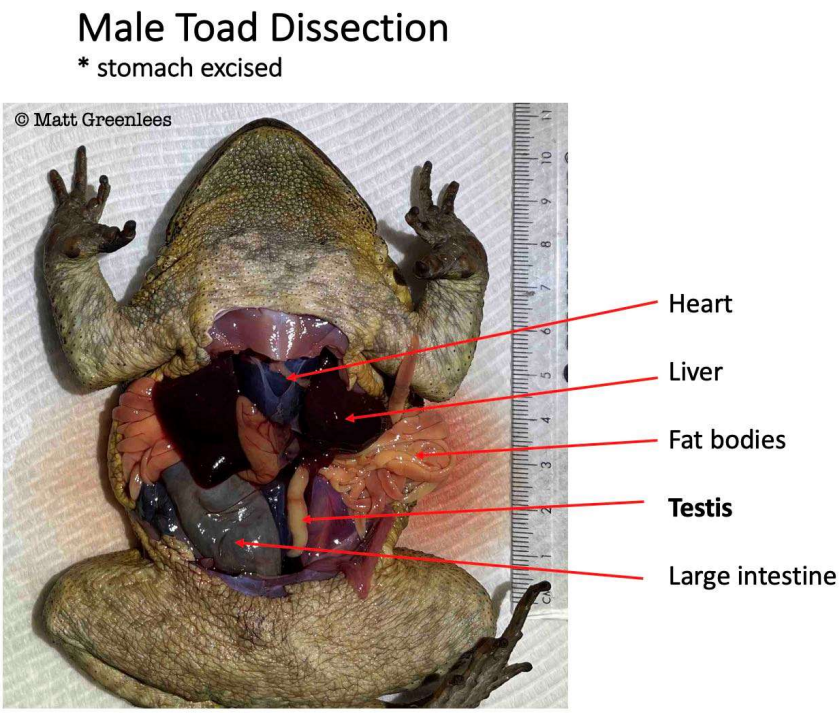

Figure 18. Male dissection – stomach excised

Testes should be apparent in male toads greater than 50mm SUL, even if they are not yet displaying morphological secondary sexual characteristics such as nuptial pads or yellow skin (M Greenlees, pers. obs.).

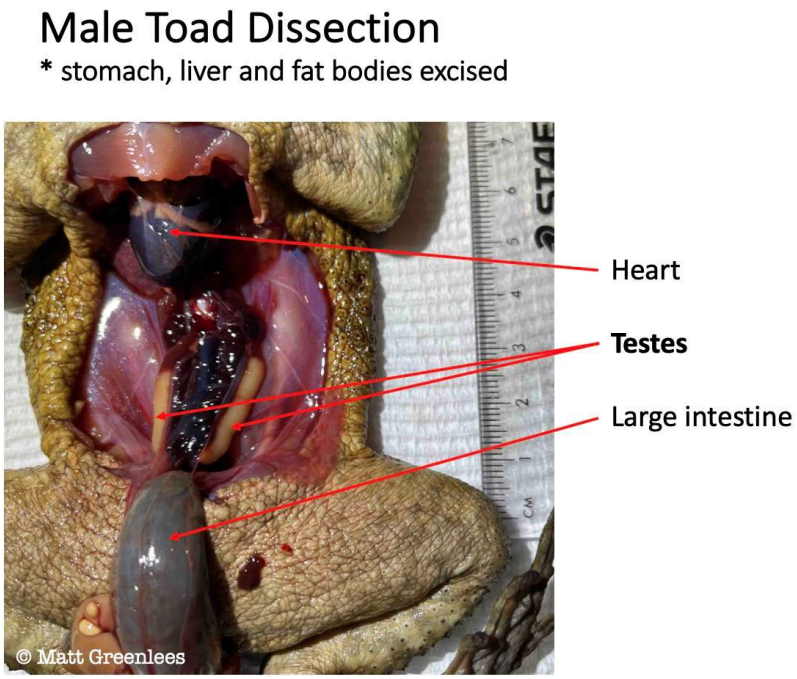

Figure 19. Male dissection – stomach, liver and fat bodies excised, large intestine displaced

Testes should be apparent with the excision or displacement of the stomach and large intestine as they (the testes) are located toward the dorsal surface of the toad. The testes can be white to light yellow in colour.

Female

Toads greater than ~50mm where testes are not discoverable are almost certainly female. Ovarian tubules will also be present. In non-breeding females, these are coiled, thin light-pink tubes that will be detectable adjacent to and dorsal to the stomach and intestine. When females are ready to breed, these tubes will swell and become darker pink and more loosely coiled until the eggs are laid. Tubules will remain swollen for up to a month post-laying. Fat bodies can be apparent in non-gravid and non-breeding females that will eventually be utilised for the development of ovaries. Like in males, these fat bodies are pale to yellow in colour and appear as finger-like structures below the liver. During early development, ovaries appear as a granular, grey to yellow mass, developing darker spots of pigment. These masses increase in volume during development and eventually turn black (see images).

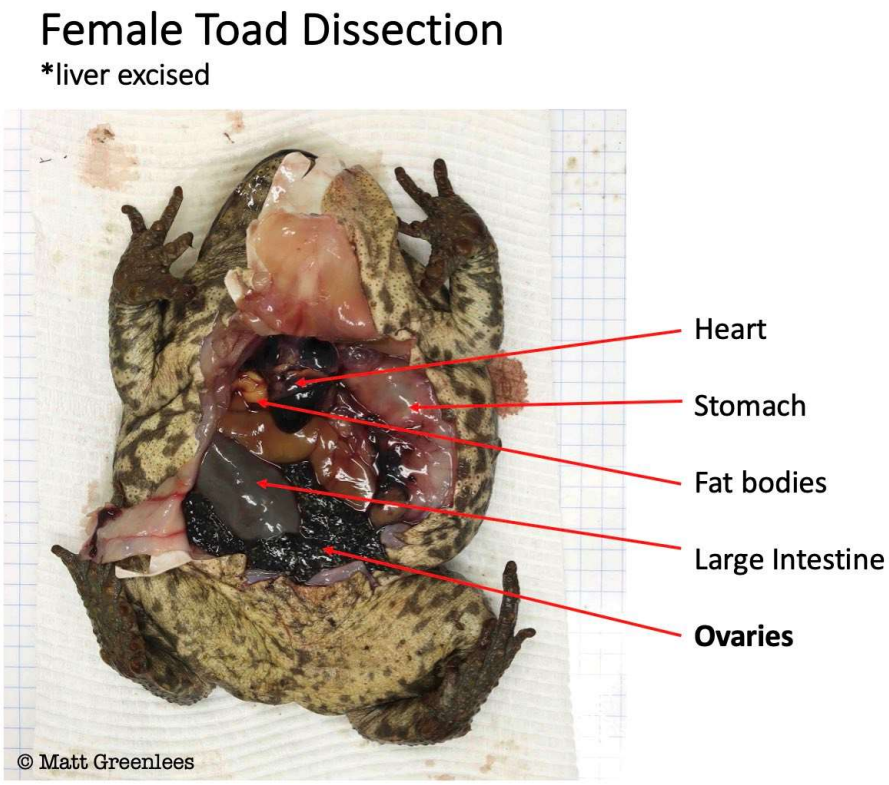

Figure 20. Female dissection. Liver excised, ovaries almost fully developed.

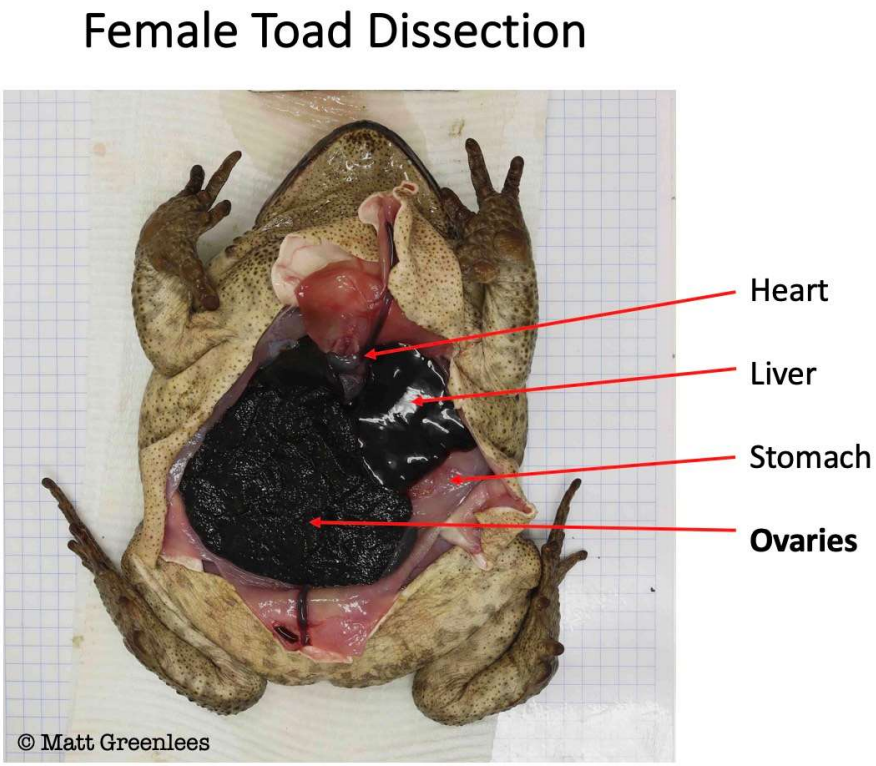

Figure 21. Female dissection. Ovaries fully developed.

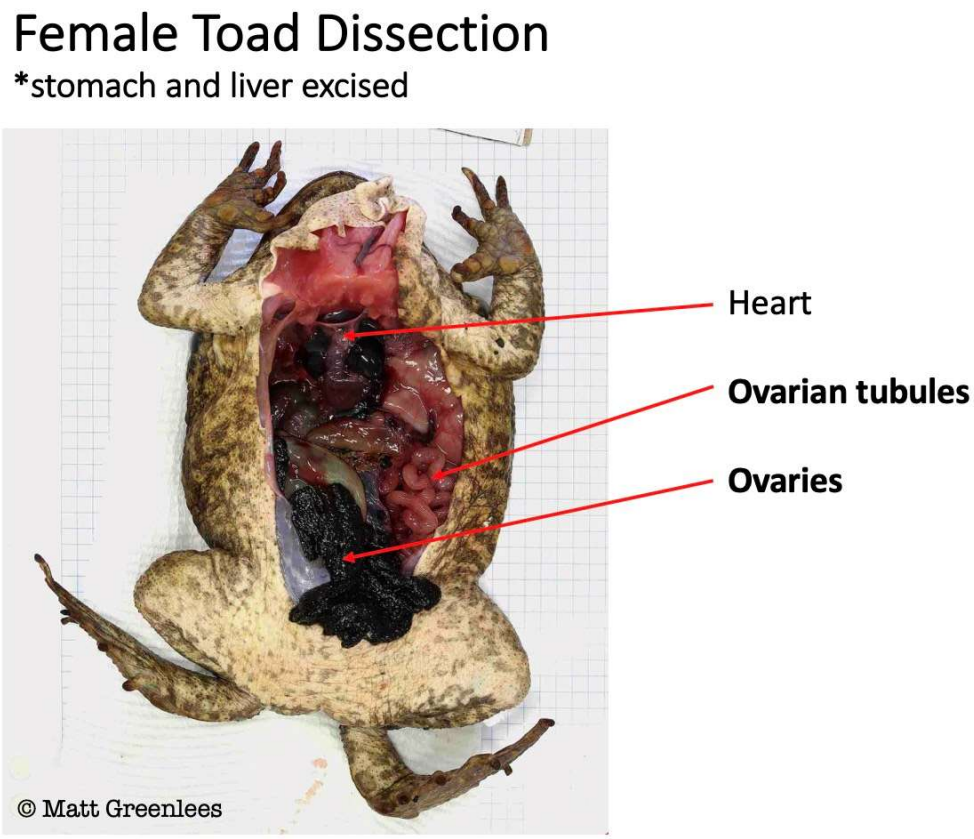

Figure 22. Female dissection. Liver, stomach and portion of ovaries excised revealing ovarian tubules.

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