Necropsy and Sampling

IUCN CSG Darwin
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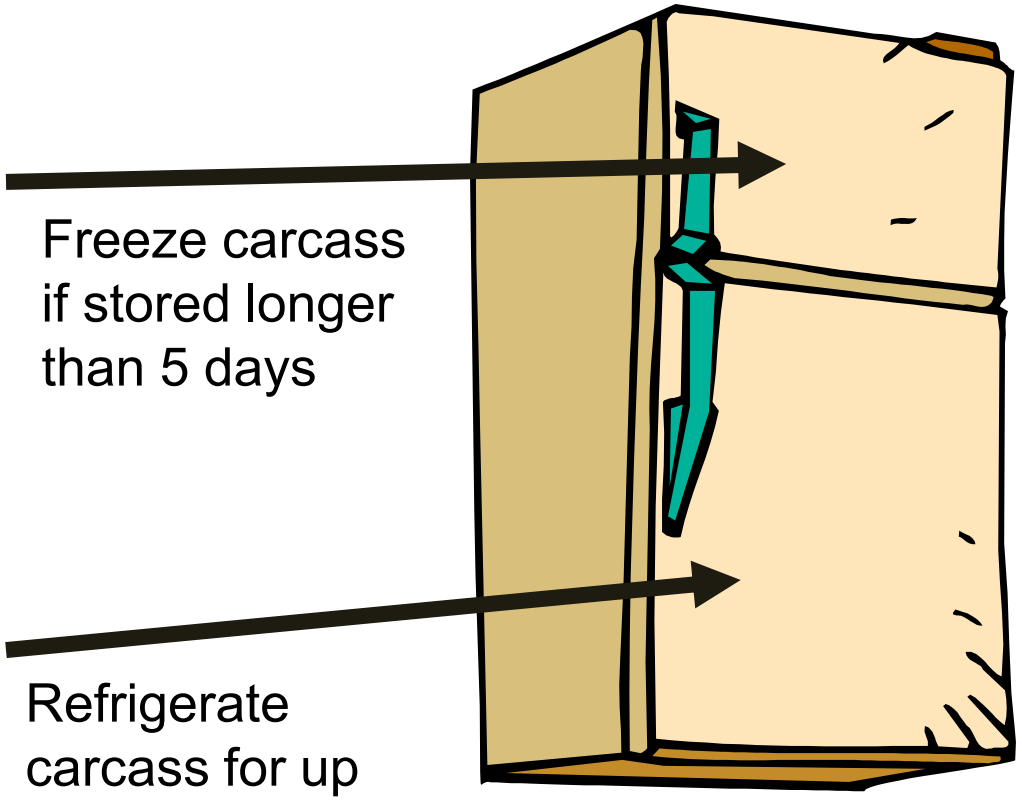
Why necropsy a wild crocodile?

- Determine the cause of illness/death in an animal or group of animals
  - The gross necropsy alone may achieve this but usually additional testing (histology, bacteriology etc.) are also required
- Surveillance: determine which diseases are present in a population, how important and common they are
- Research: take samples aimed at answering a specific question
- Forensics: for wildlife officers, to investigate a possible crime

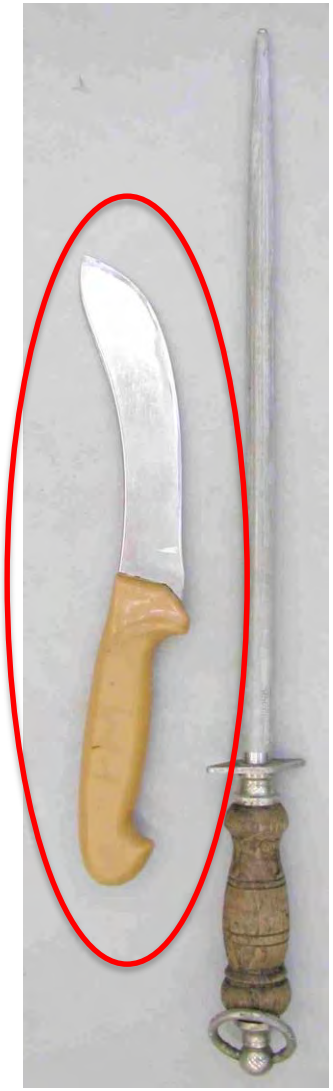
Necropsy tip #1: Maximise carcass quality

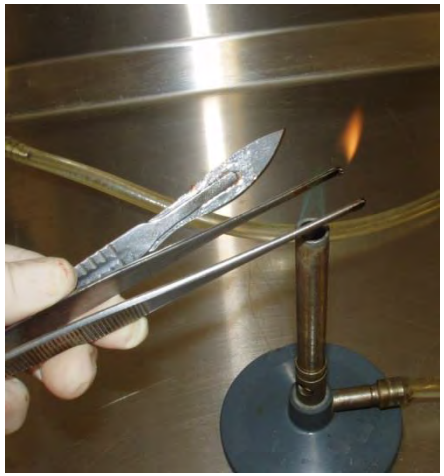
- For diseases, ideally sample several sick and/or <u>recently</u> dead animals
- Rotten (> a few hours old in tropical heat) carcasses are unlikely to be grossly diagnostic for diseases, and are not good sources of samples
  - Tissues look different grossly and real lesions are hard to recognise
  - Bacteriology will yield contaminants
  - Virus isolation and/or molecular tests may be falsely negative
- For wounds and trauma, lesions may still be recognisable in rotten carcasses (or even skeletons)



- Freezing a carcass limits the ability to interpret necropsy findings and histology but samples for bacteriology, toxicology, virology or molecular testing will likely be fine

Necropsy tip #2: be well equipped

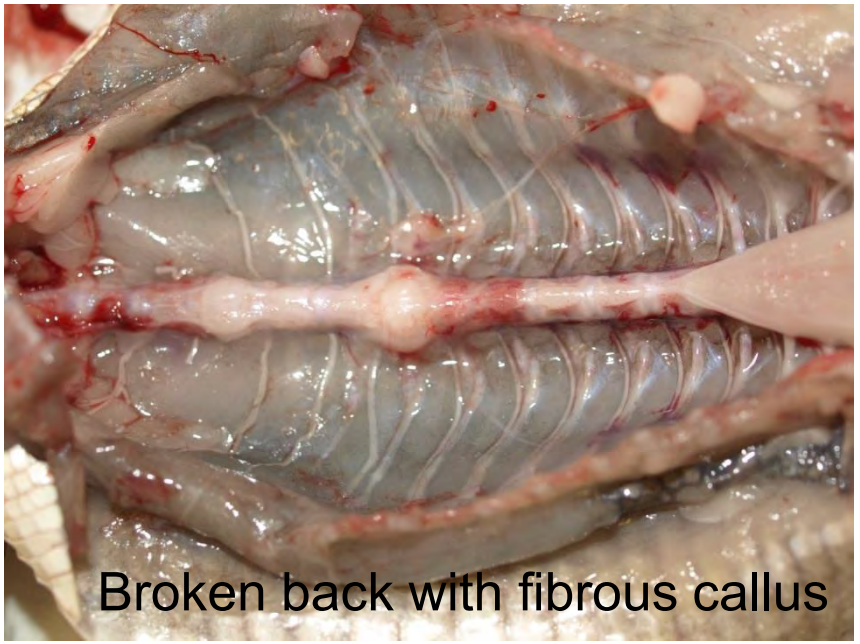




- Eg. Sharp knife, proper tools to do the job, sampling supplies

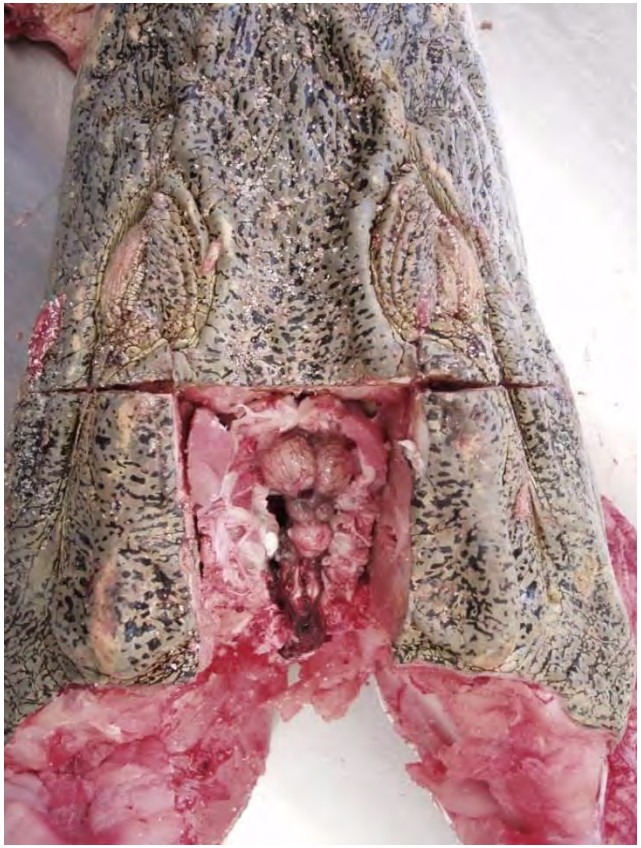
Necropsy tip #3: perform a thorough necropsy every time

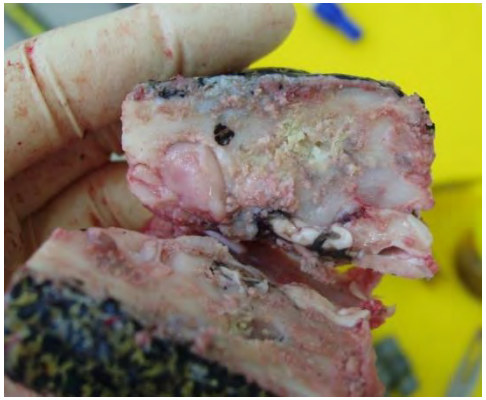

Septic joint infection


Broken back with fibrous callus

- A necropsy isn't just a sampling exercise. Some diseases must be noticed at necropsy and can't be diagnosed by examination of a standard list of samples.

Necropsy tip #4: target a particular organ system if necessary, based on what you need to find out





- Some diseases require special necropsy techniques or samples (eg. brain if neurological signs, stomach and intestines for foreign bodies or parasites if in poor body condition)
- For suspicion of trauma, skin the animal to look for bruising

Necropsy Procedure

- The actual necropsy procedure is simple
- The hard part is recognising abnormal from normal
  - This can be overcome by interest and experience
  - Do it the same way every time and be thorough
- You don't have to know which disease(s) might be causing the abnormalities (lesions), just recognise lesions and describe them
- For general diagnostic purposes, take samples of a standard set of tissues and any lesions for histology and other specific testing

On the CSG website!

Resources

1.1. Step by Step Guide and Reporting Form for the Necropsy of Crocodilians (English) (Dr. Paolo Martelli and Dr. Fritz Huchzermeyer)

1.2. Guia Paso por Paso y Formato de Reporte para la Necropsia de Crocodilianos (Espanol) (Dr. Paolo Martelli and Dr. Fritz Huchzermeyer, translated by Luis Sigler)

1.3. Manuel Operatoire et Formulaire pour la Realisation d'Autposies de Crocodiliens (Francais) (Dr. Paolo Martelli and Dr. Fritz Huchzermeyer, translated by Dr. Samuel Martin)

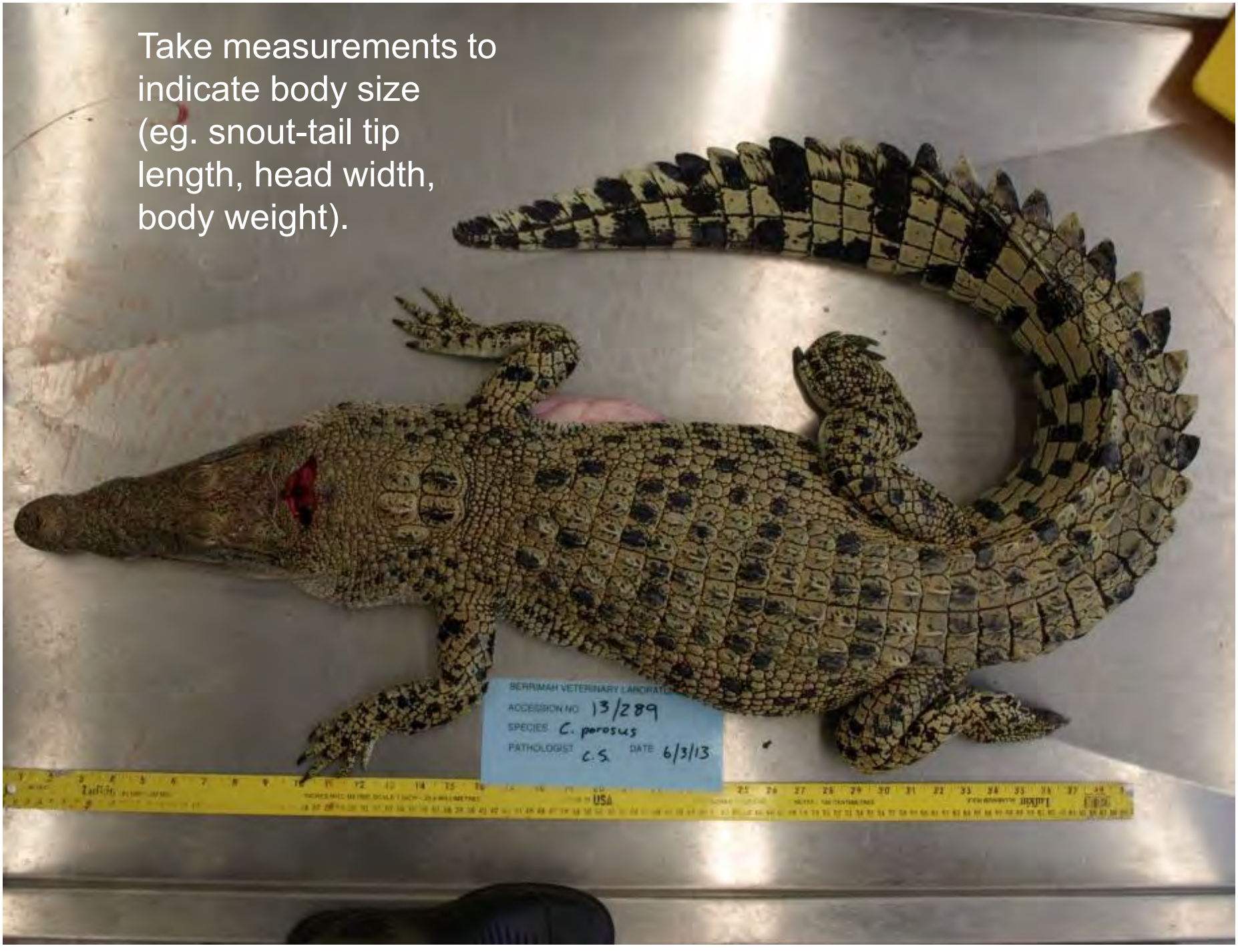
1.4. Langkah-langkah Panduan Dan Formulir Pelaporan Untuk Nekropsi Buaya (Bhasa Indonesian) (Dr. Paolo Martelli and Dr. Fritz Huchzermeyer, translated by Dr. Vidi Saputra)

1.5. Gharial Necropsy Manual (Hindi) (Dr. Gowrie Mallapur and Dhiraj Gopinath; translated by Mrs. Rekha Joshi)

Description of lesions
Compare to normal:

1. Distribution (eg. focal, multifocal, diffuse)
2. Colour (eg. darker, lighter, different colour)
3. Consistency (eg. soft, hard, firm, fluid)
4. Shape (eg. round, linear, elevated, depressed)
5. Size (larger, smaller than normal)
6. Clinical significance (extent, severity)

TAKE PICTURES

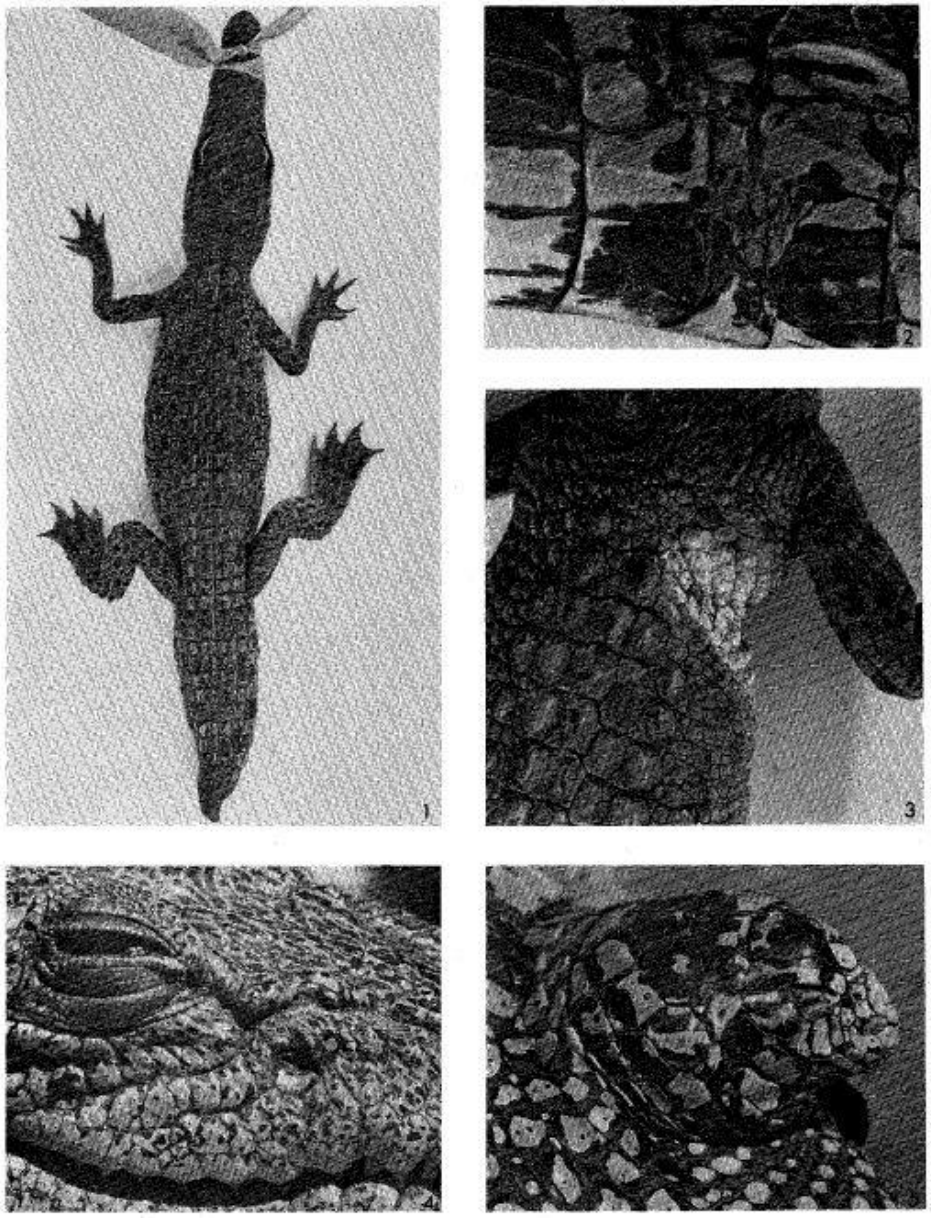

Take measurements to indicate body size (eg. snout-tail tip length, head width, body weight).
ACCESSION NO 13/289
SPECIES C. porosus
PATHOLOGIST C.S. DATE 6/3/13

Examine the outside of the carcass for abnormalities/injuries

Photo: Daily Mail News: Katrina Bridgeford/Rex Features

Photo: Daily Mail News: Adelaide River Cruises

Photo: Paul Simpson, Wetland Cruises



Figs 1–5. *Crocodylus porosus* injuries. *1*, Hatchling with severe tail amputation. *2*, A healed scar on the side of the tail. *3*, Trunk injury. *4*, Puncture mark in the head; presumably caused by another *C. porosus*. *5*, Amputated limb.

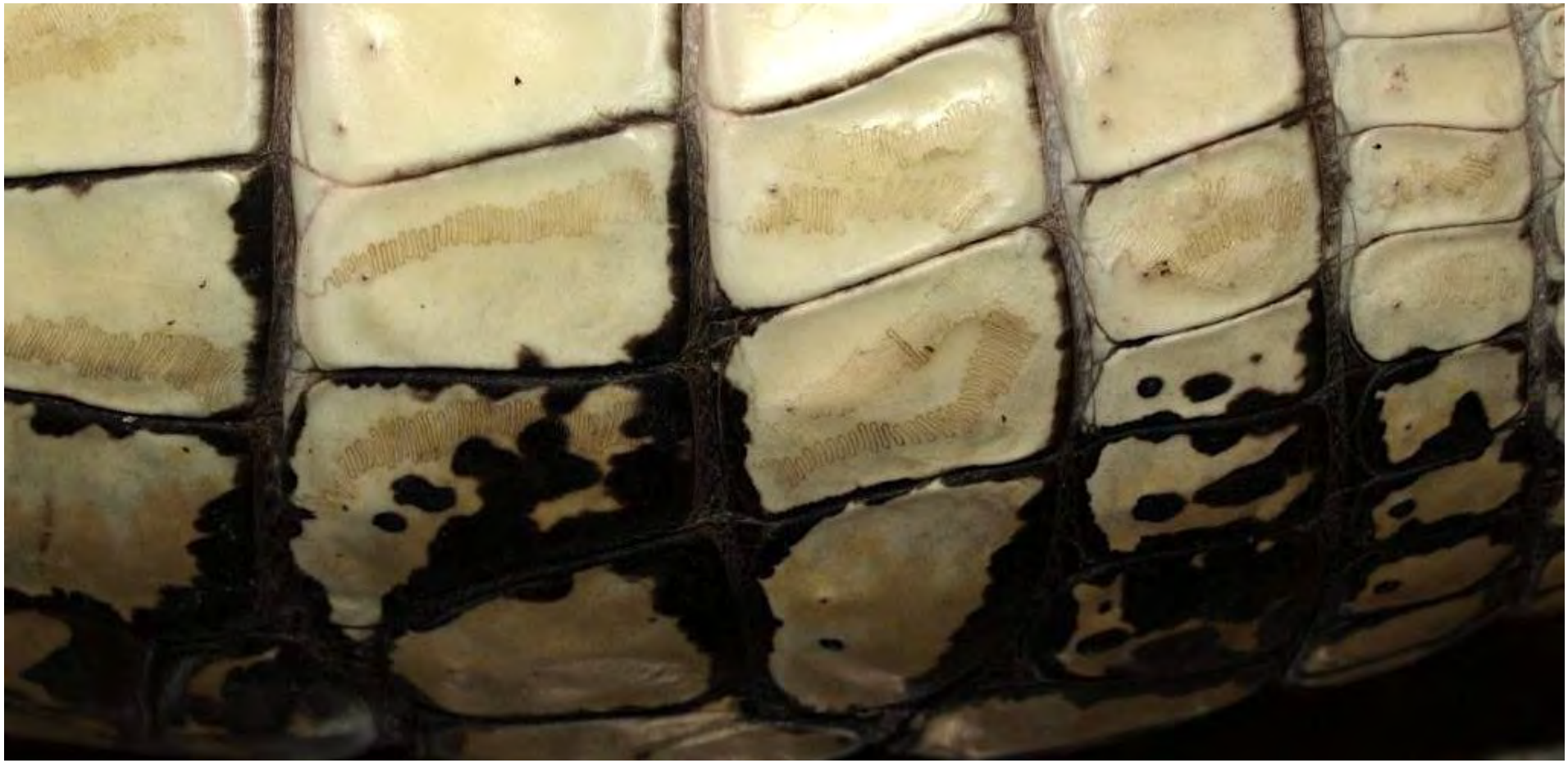
1345 injuries and abnormalities in wild *C. porosus* (SVL 11-210 cm):

- 5 limb amputations (0.4%)
- 24 tail amputations (1.8%)
- 101 scars (7.5%)
- Injuries attributed to failed predation attempts on hatchlings and when older, intraspecific aggression

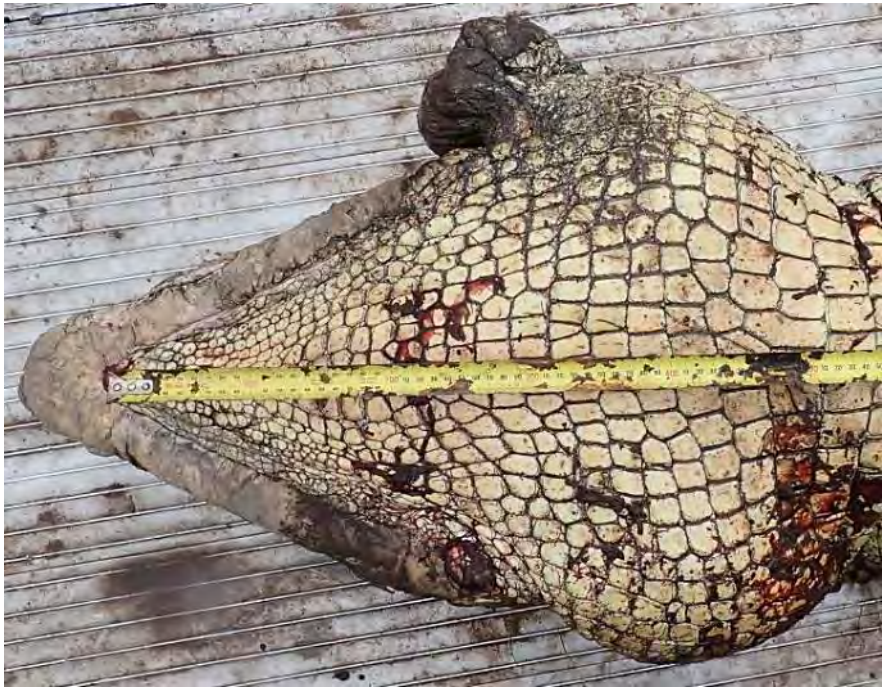
Webb GJW, Messel H. 1977. Aust. Wildl. Res. 4:311-319.

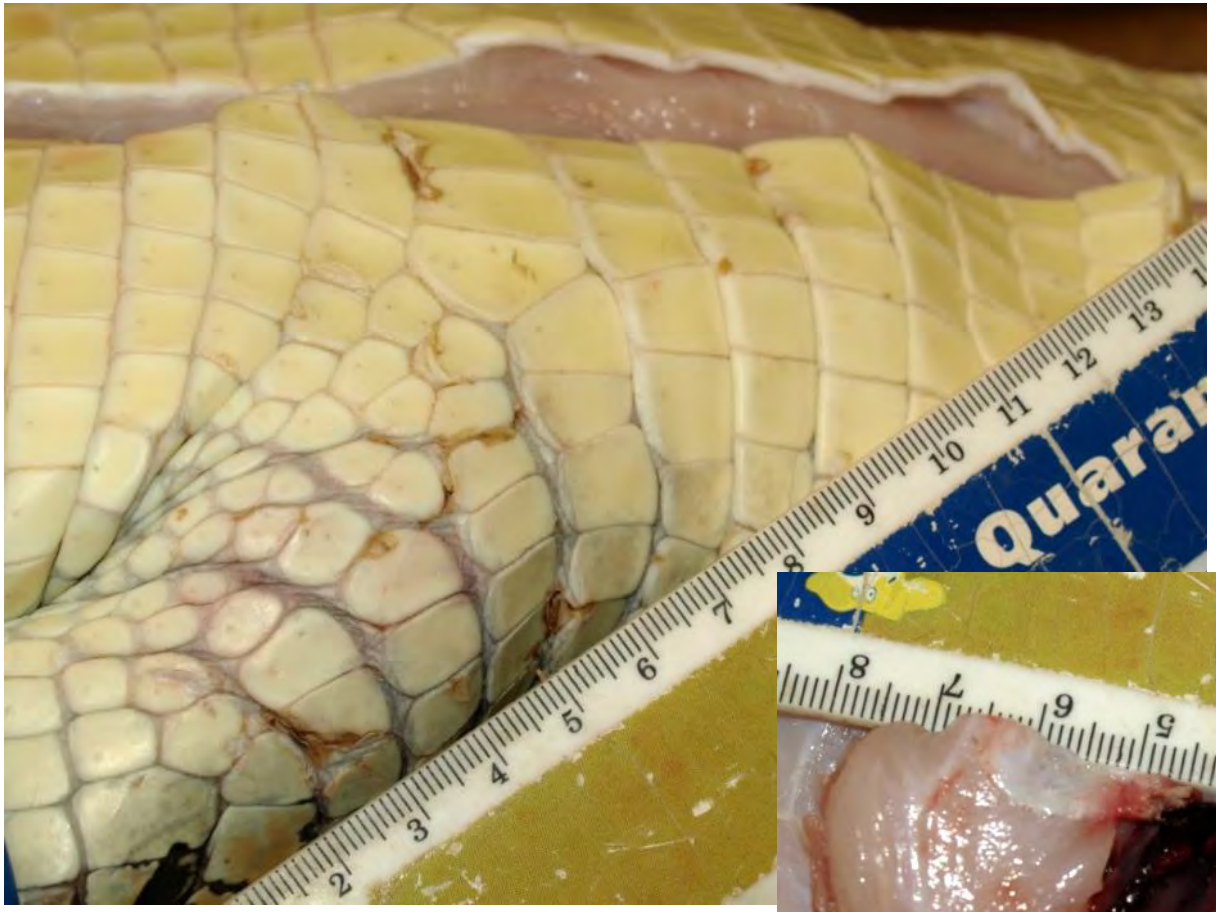
Paratrichosoma nematode worm tracks

- Present in 0.9% wild salties

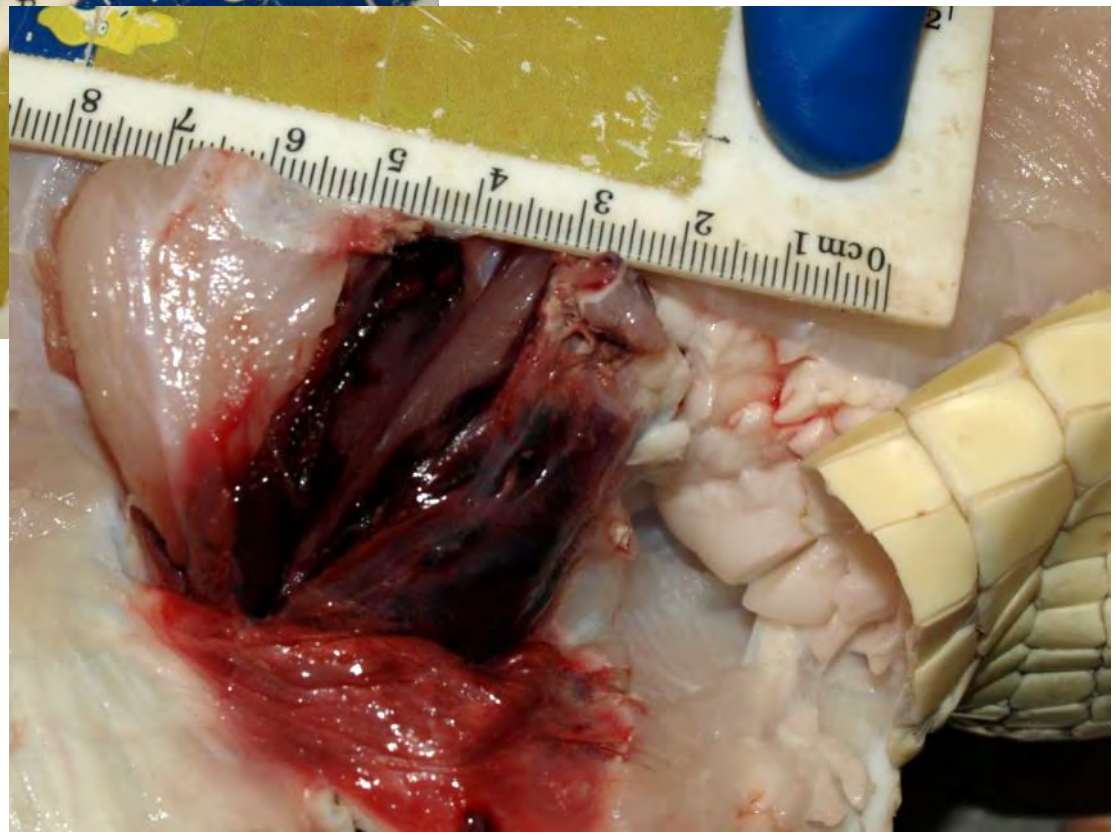


- 3.5 m male crocodile, long-term captive in naturalistic pond
- Was inactive, losing weight, with large protruding mass at left angle of jaw
- Due to size of mass, difficulty of surgical removal, and other signs of illness, was humanely euthanised



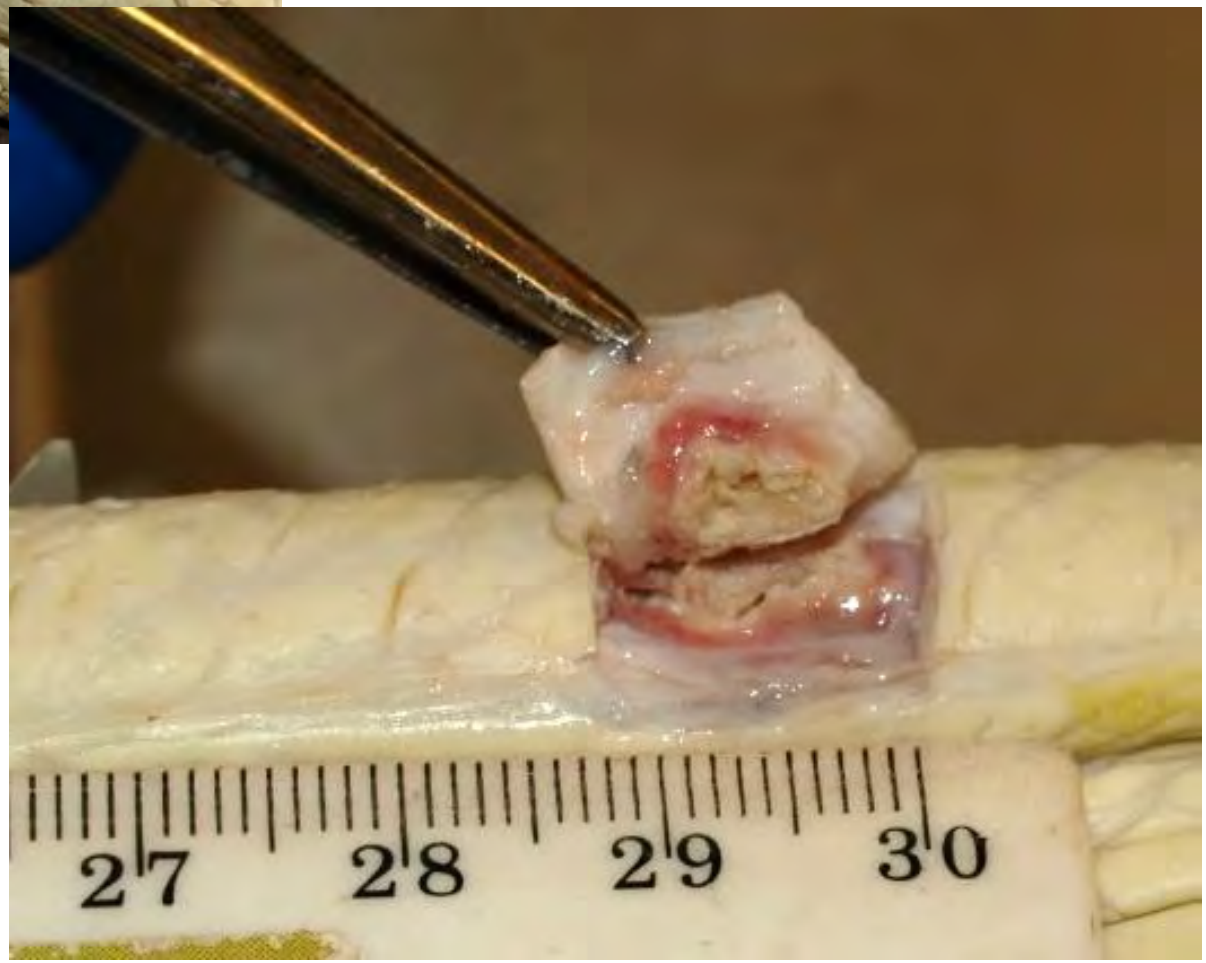


Check over the exterior of the carcass for abnormal discoloration or swellings

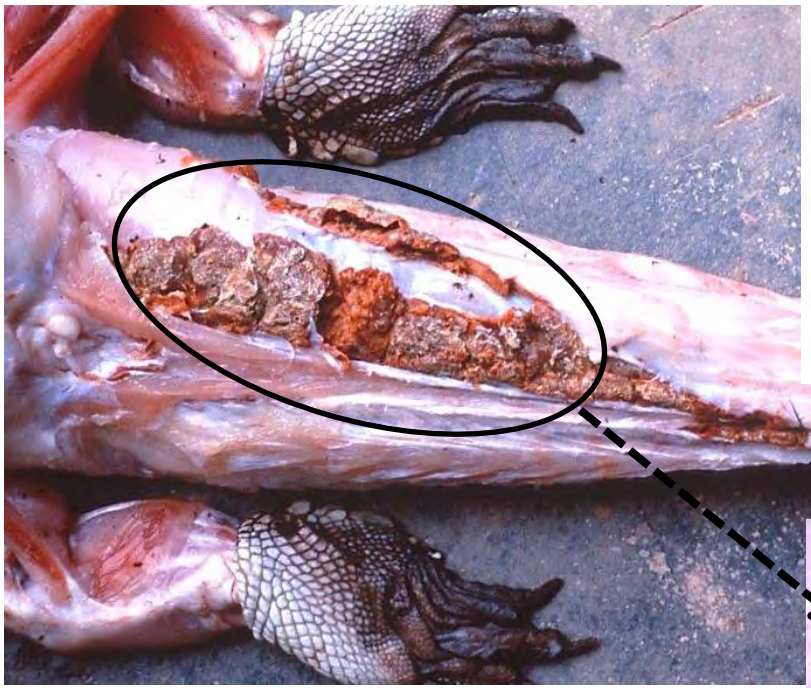


The reddening of the skin of the upper arm is a clue to dissect this area further, and reveals haemorrhage deep in the muscle

Focus of mixed bacterial infection with caseous core of necrotic exudate surrounded by red inflamed rim



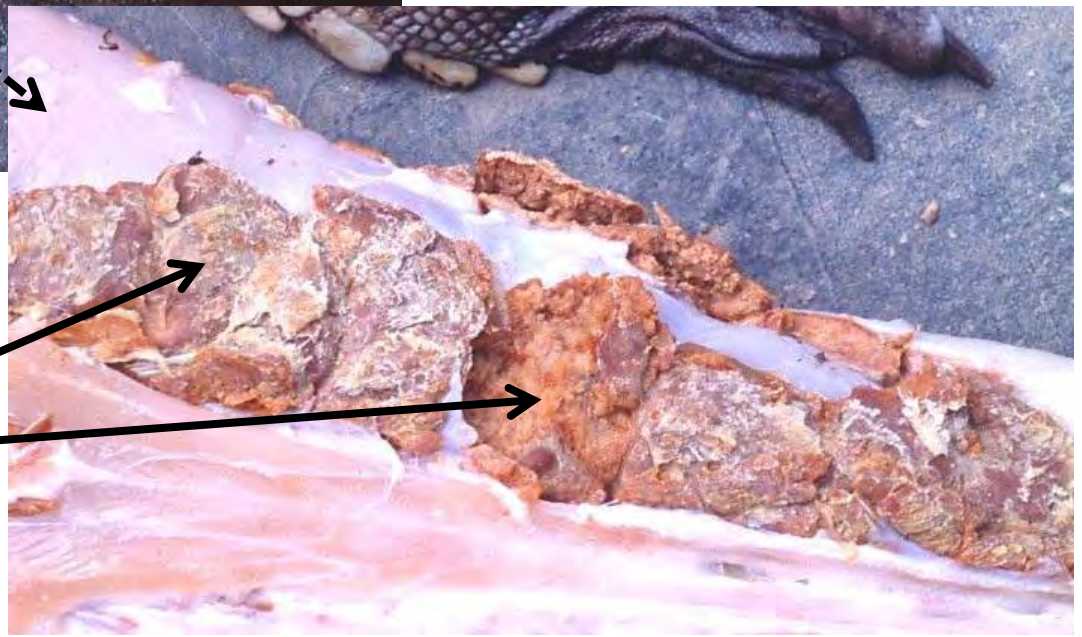
Crocodile skin can hide lesions underneath
Eg. fat necrosis



Journal of Zoo and Wildlife Medicine 44(4): 899–910, 2013

PANSTEATITIS OF UNKNOWN ETIOLOGY ASSOCIATED WITH LARGE-SCALE NILE CROCODILE (*CROCODYLUS NILOTICUS*) MORTALITY IN KRUGER NATIONAL PARK, SOUTH AFRICA: PATHOLOGIC FINDINGS

Emily P. Lane, B.V.Sc., M. Phil., Dipl. A.C.V.P., Fritz W. Huchzermeyer, Dr. med. vet., Ph.D., Danny Govender, B.V.Sc., M.Sc., Roy G. Bengis, B.V.Sc. M.Sc., Ph.D., Peter E. Buss, B.V.Sc., M. med. vet., Markus Hofmeyr, B.V.Sc., Jan G. Myburgh, B.V.Sc., M. med. vet., Johan C. A. Steyl, B.V.Sc., M.Sc., Daniel J. Pienaar, B.Sc. Honours, M.Sc., and Antoinette Kotze, B.Sc. Honours, Ph.D.

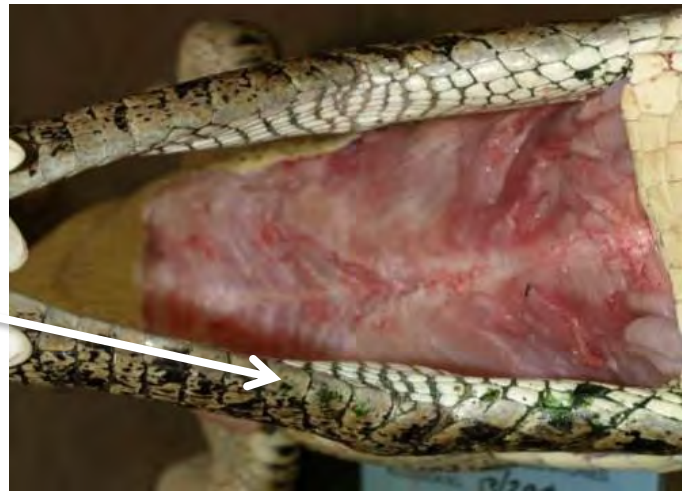


Fat: Hard and brown instead of soft and white

(Photos courtesy of Dr. Phil Ladds)

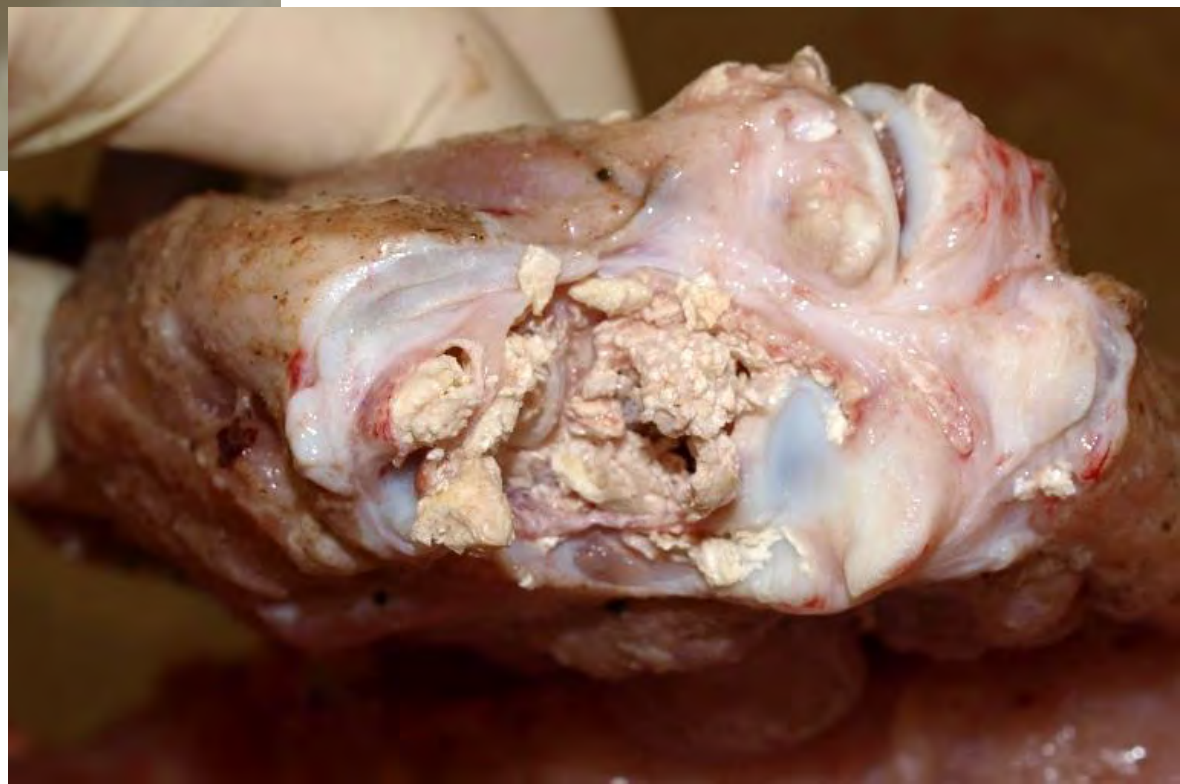
Compare left and right sides to judge possible asymmetries

This croc had a misshapen elbow on the left side compared to the right.



Dissection revealed irregular bone surfaces in the joint (chronic arthritis), possibly due to a prior injury (left side compared to normal right side)

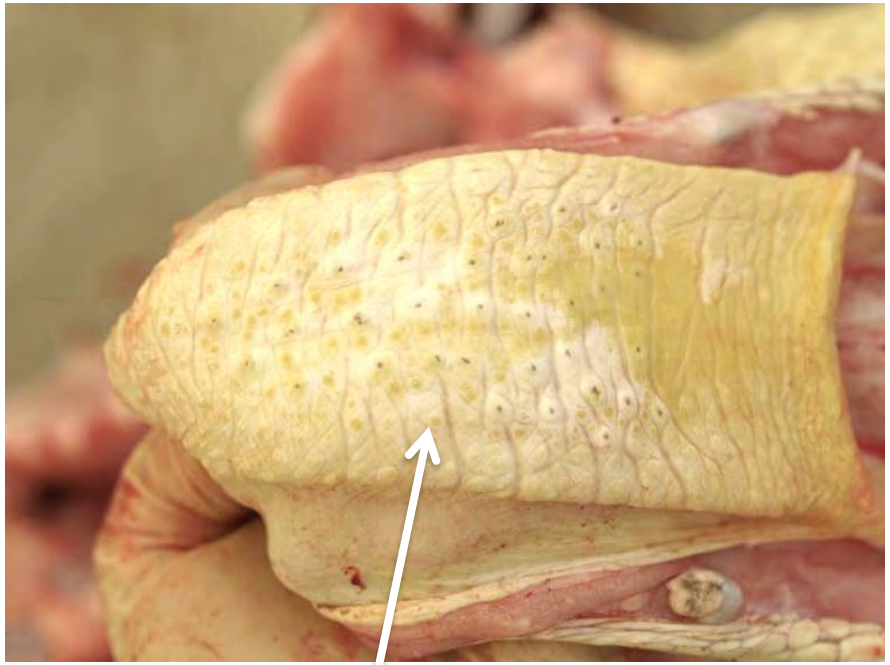
Gout caused by problem with uric acid excretion by the kidneys



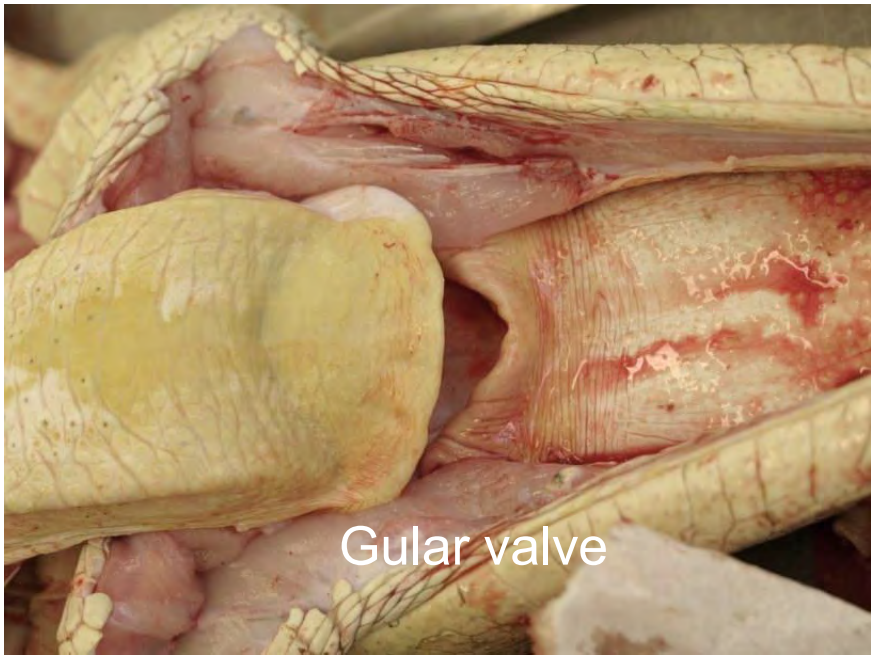
Examine the eyes

"Red" eye and "white" eye due to Chlamydia, herpesvirus etc.

Examine the mouth

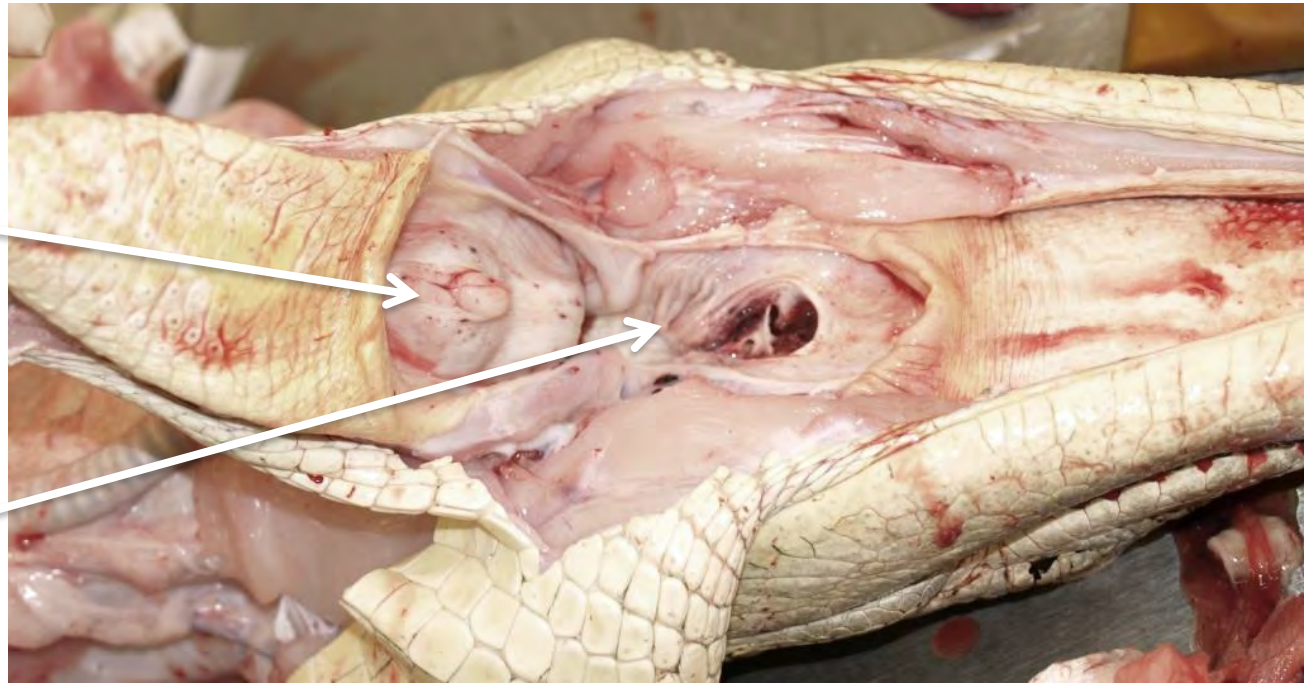


Tongue salt glands

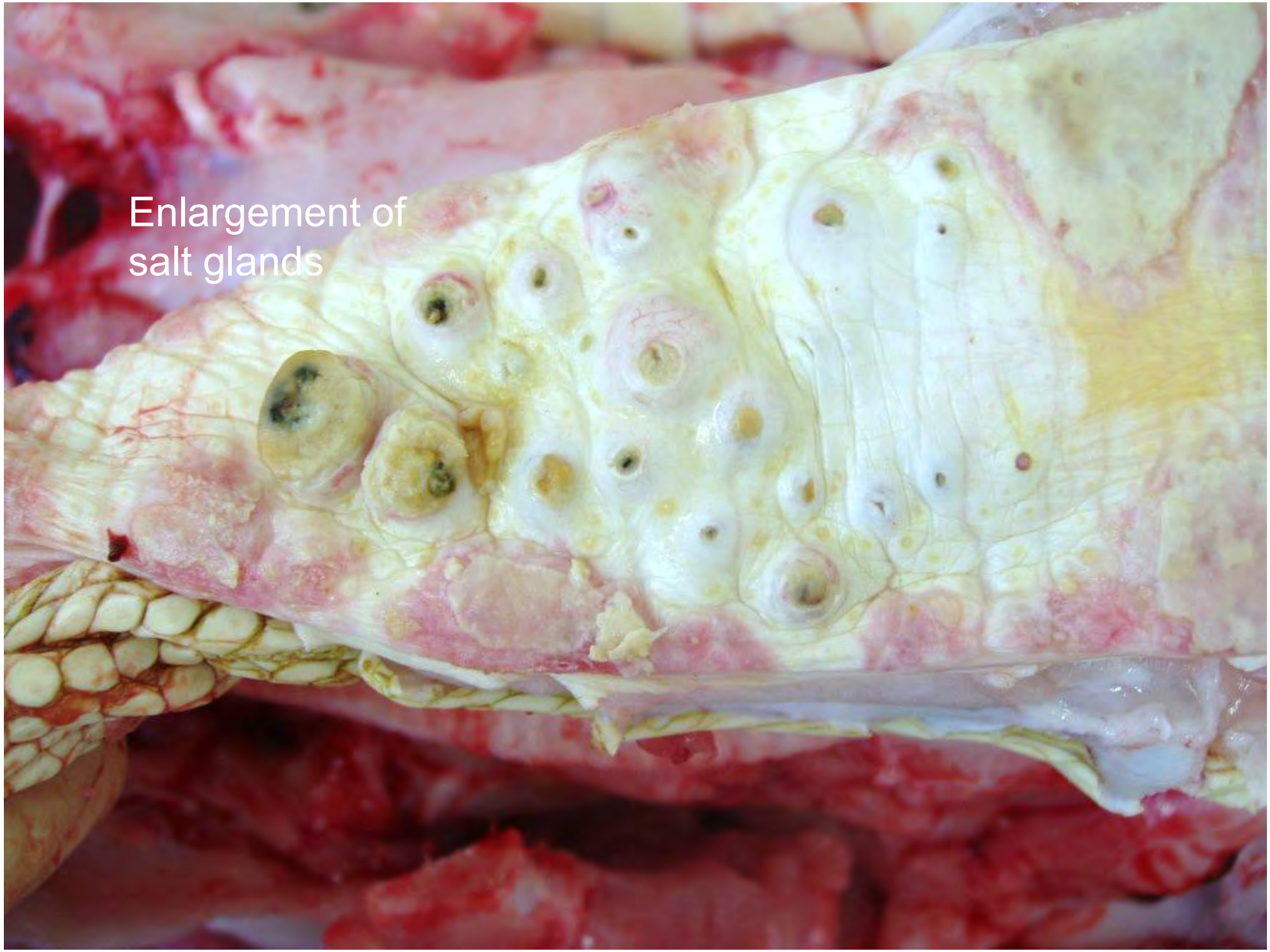


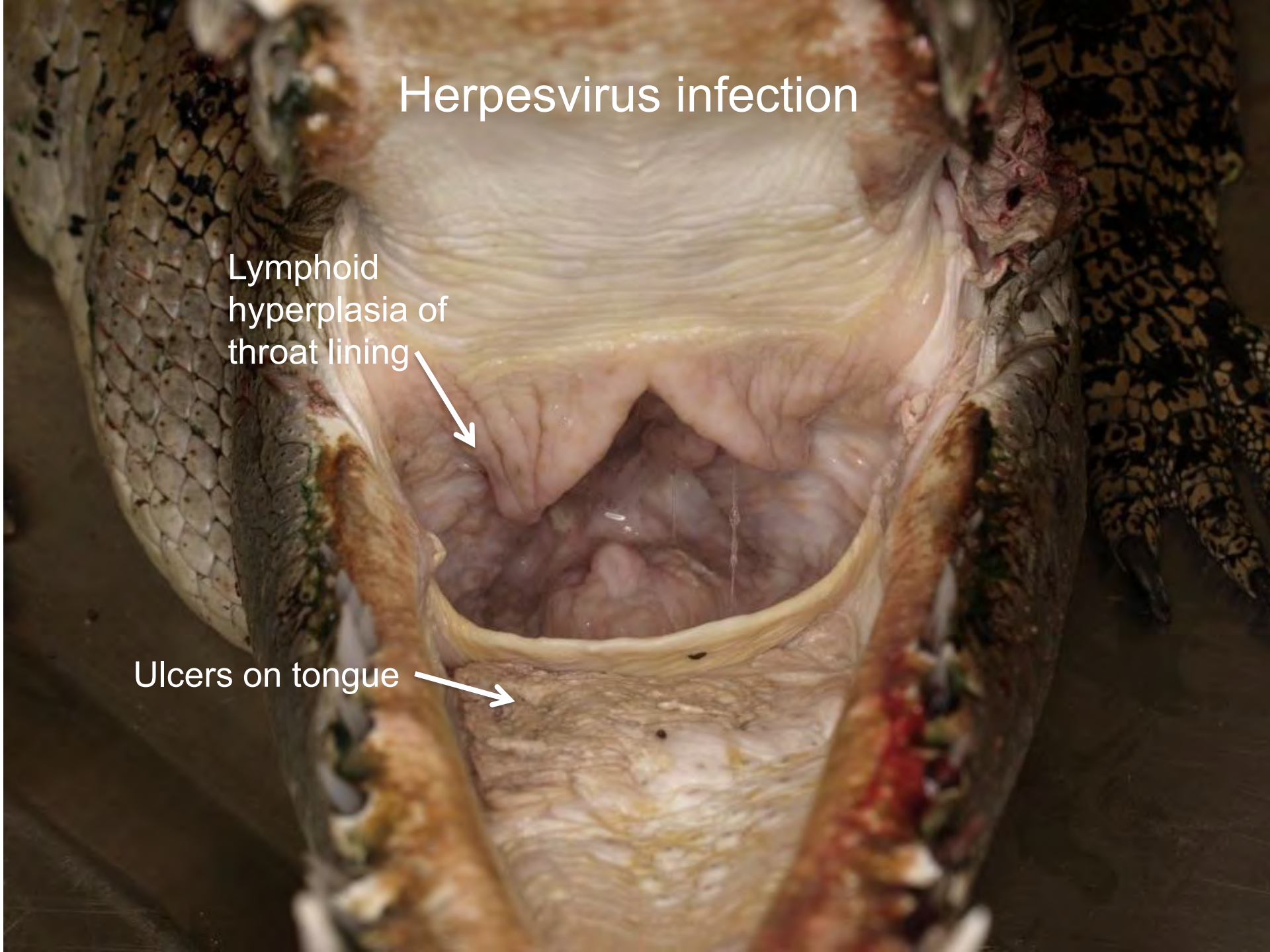
Opening of windpipe

Tonsil



Vitamin A deficiency




Herpesvirus infection
Lymphoid hyperplasia of throat lining
Ulcers on tongue

High lead exposure and clinical signs of toxicosis in wild Nile crocodiles (*Crocodylus niloticus*) from a World Heritage site: Lake St Lucia estuarine system, South Africa

Marc Humphries [a, *], Jan Myburgh [b], Robert Campbell [c], Xander Combrink [d]

[a] School of Chemistry, University of the Witwatersrand, Johannesburg, South Africa
[b] Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, South Africa
[c] National Zoological Garden, South African National Biodiversity Institute, Pretoria, South Africa
[d] Department of Nature Conservation, Tshwane University of Technology, South Africa

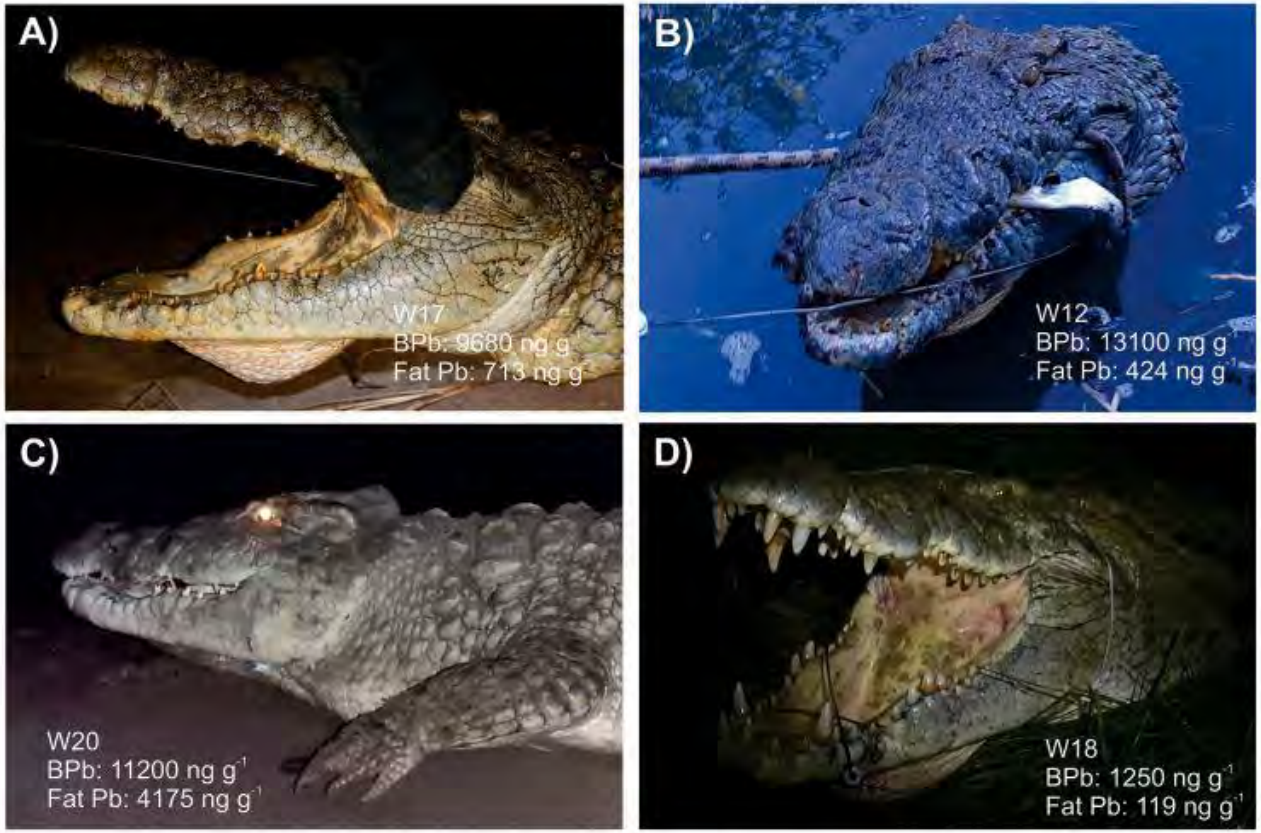
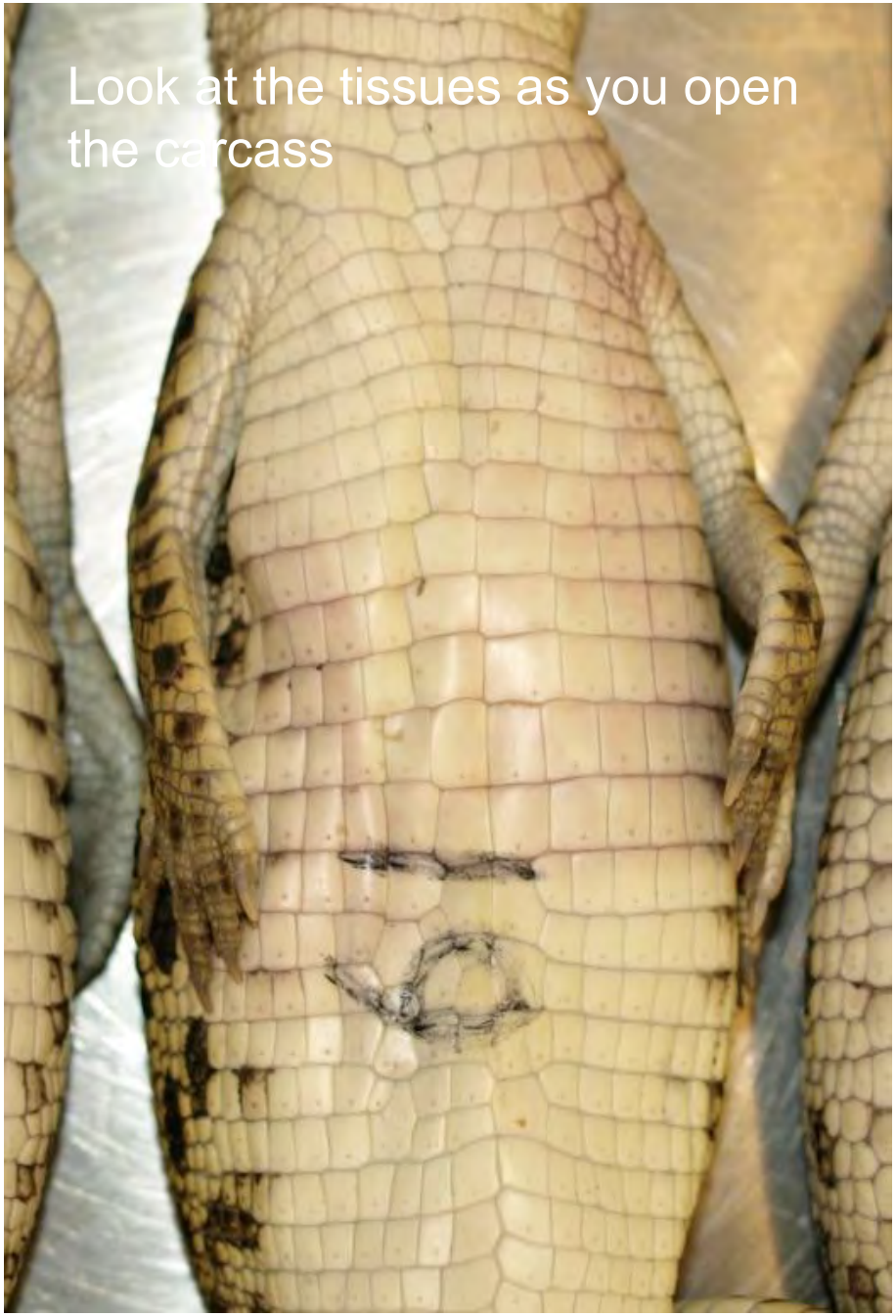
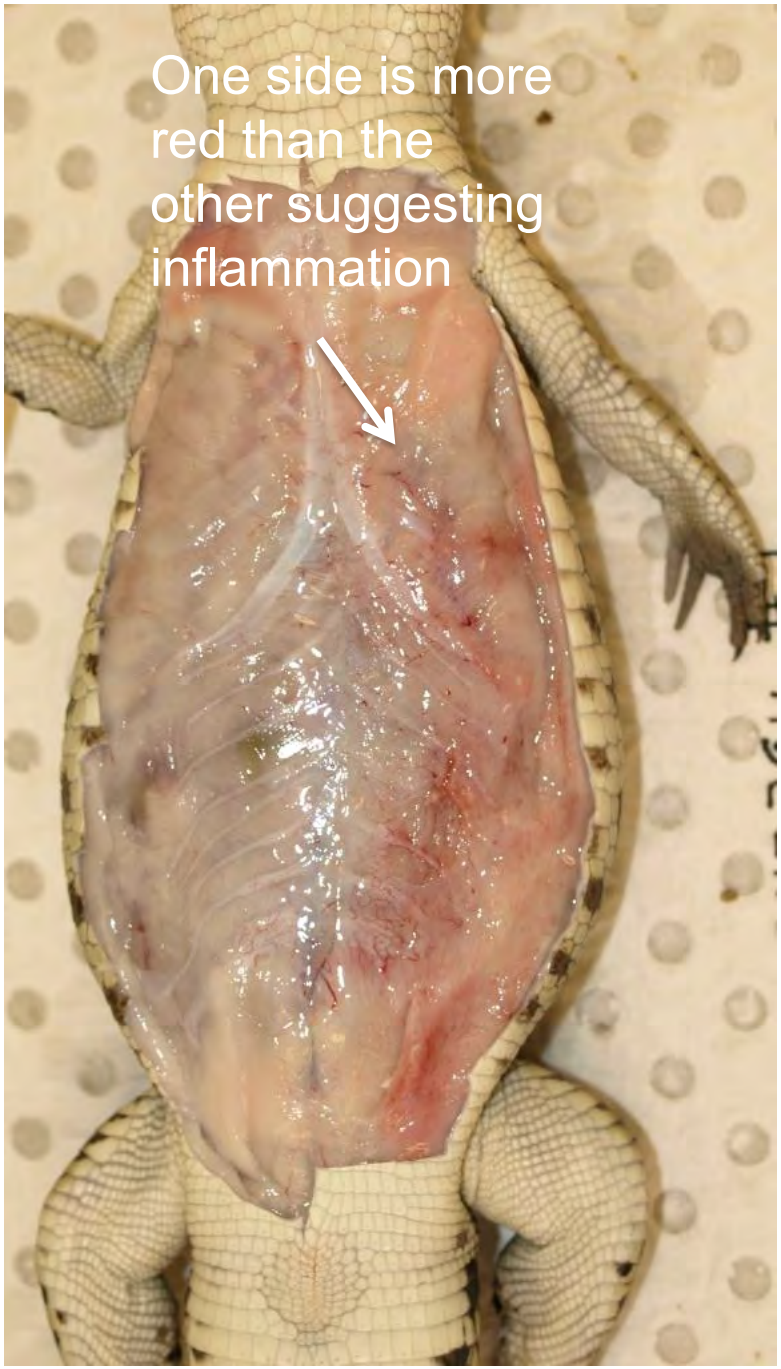
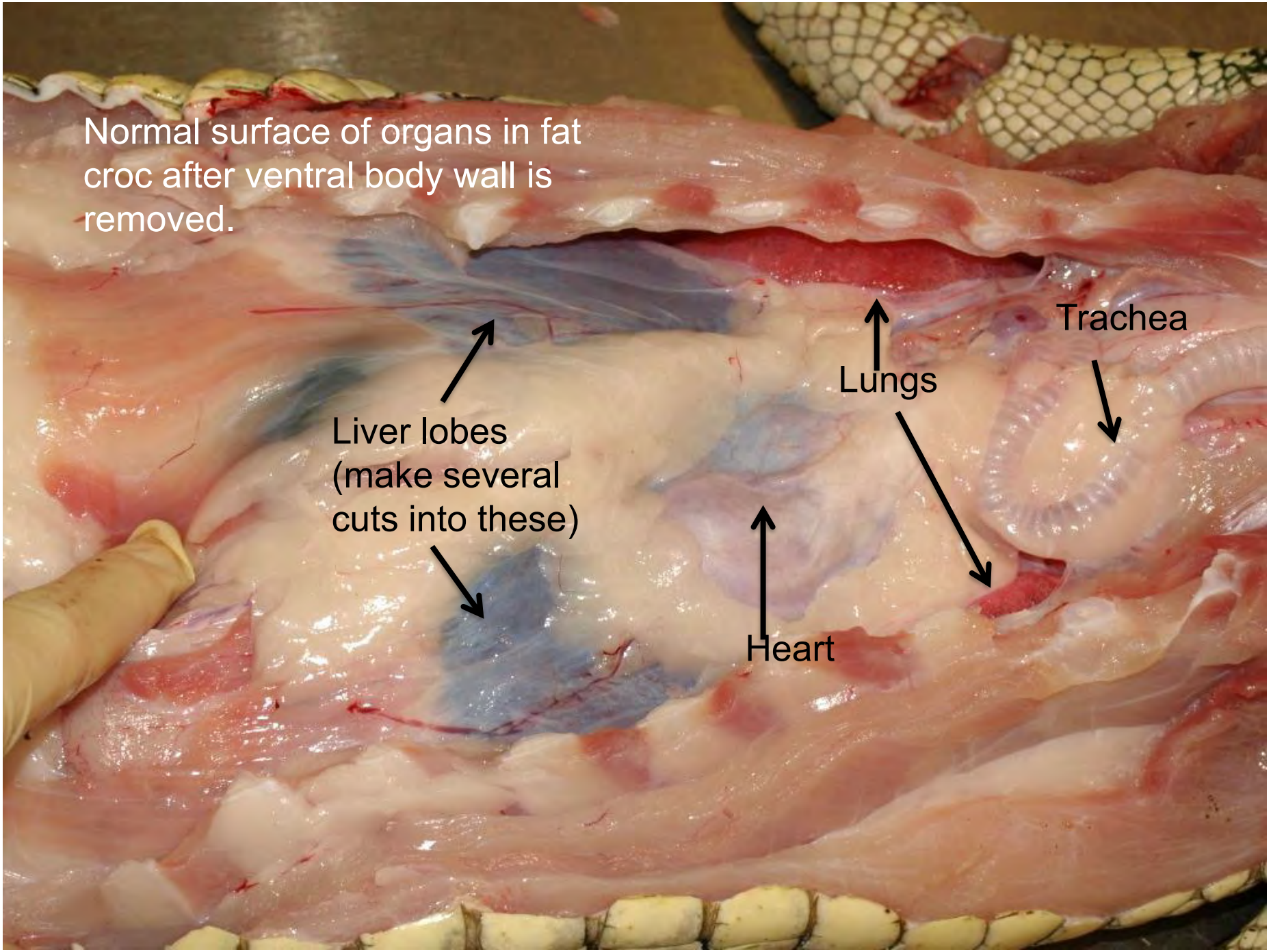


Fig. 5. Selected photographs of sampled male crocodiles highlighting the variation in physical tooth condition among individuals. Deterioration in tooth condition, discoloration, and tooth loss (examples A, B and C) was observed in individuals with highly elevated blood Pb concentrations. A photograph of the teeth associated with a typical healthy male (D) is provided for comparative purposes. Photos taken by Marc Humphries, Xander Combrink and Philip Jordaan.


Open the carcass with sharp side of knife upwards to avoid cutting organs underneath
BERRIMAH VETERINARY LABORATORIES
ACCESSION NO 13/289

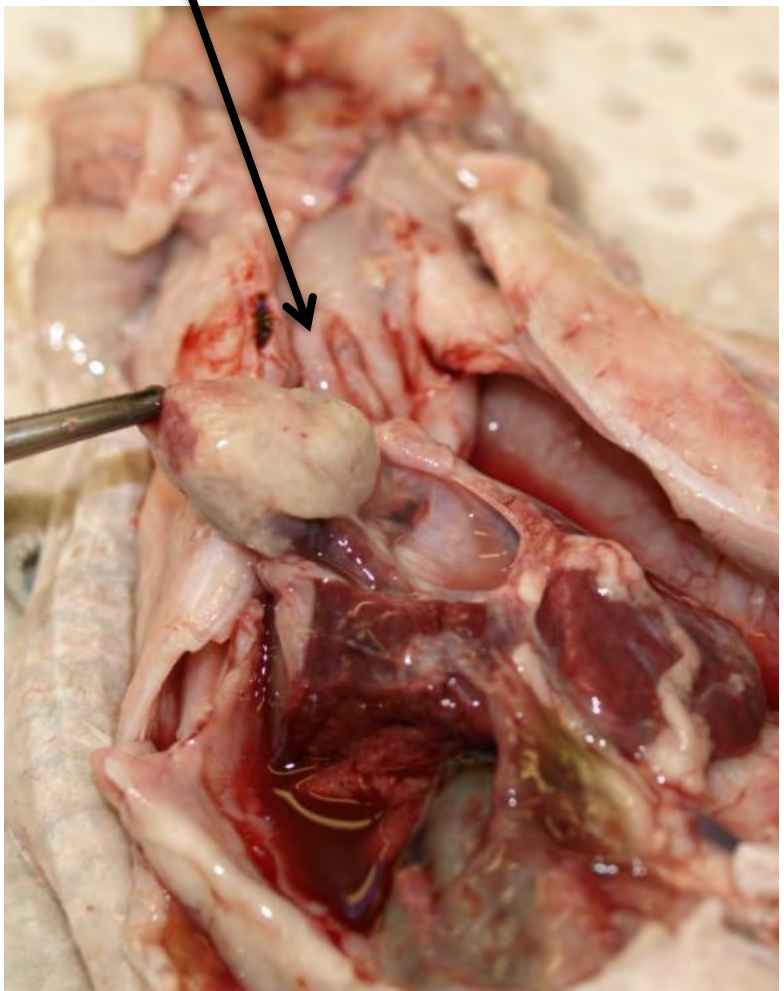

Look at the tissues as you open the carcass

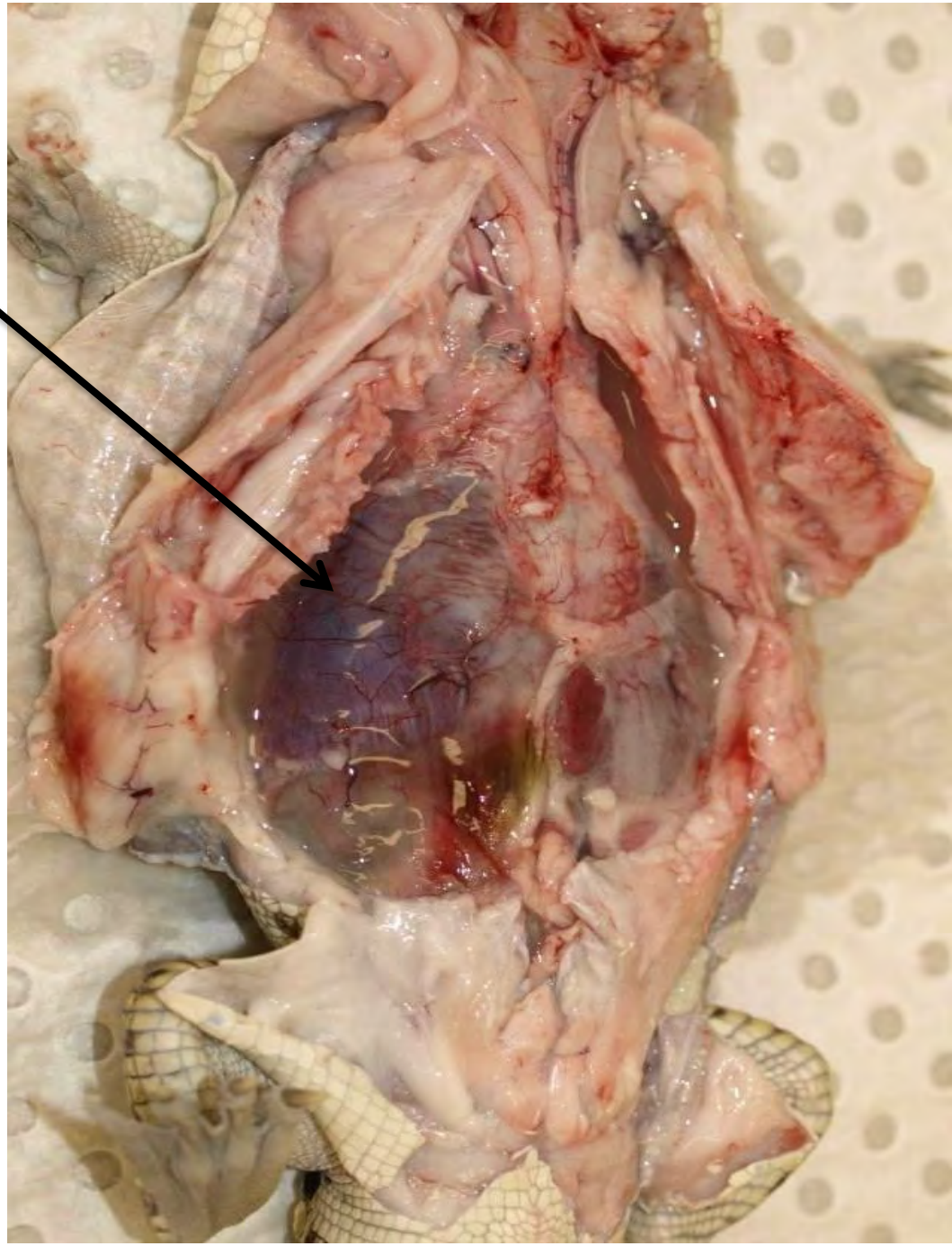

One side is more red than the other suggesting inflammation

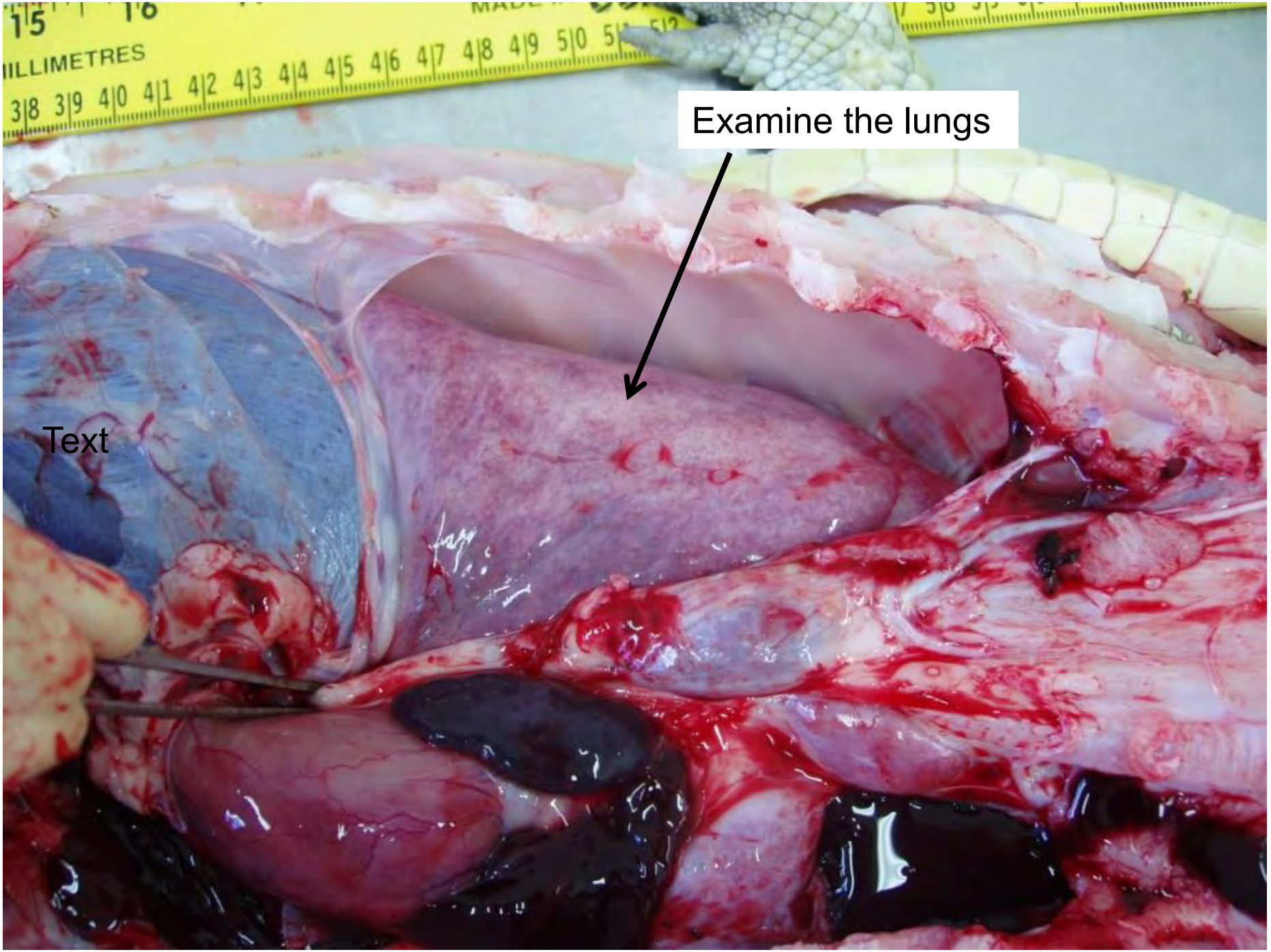

Normal surface of organs in fat croc after ventral body wall is removed.
Liver lobes (make several cuts into these)
Lungs
Trachea
Heart

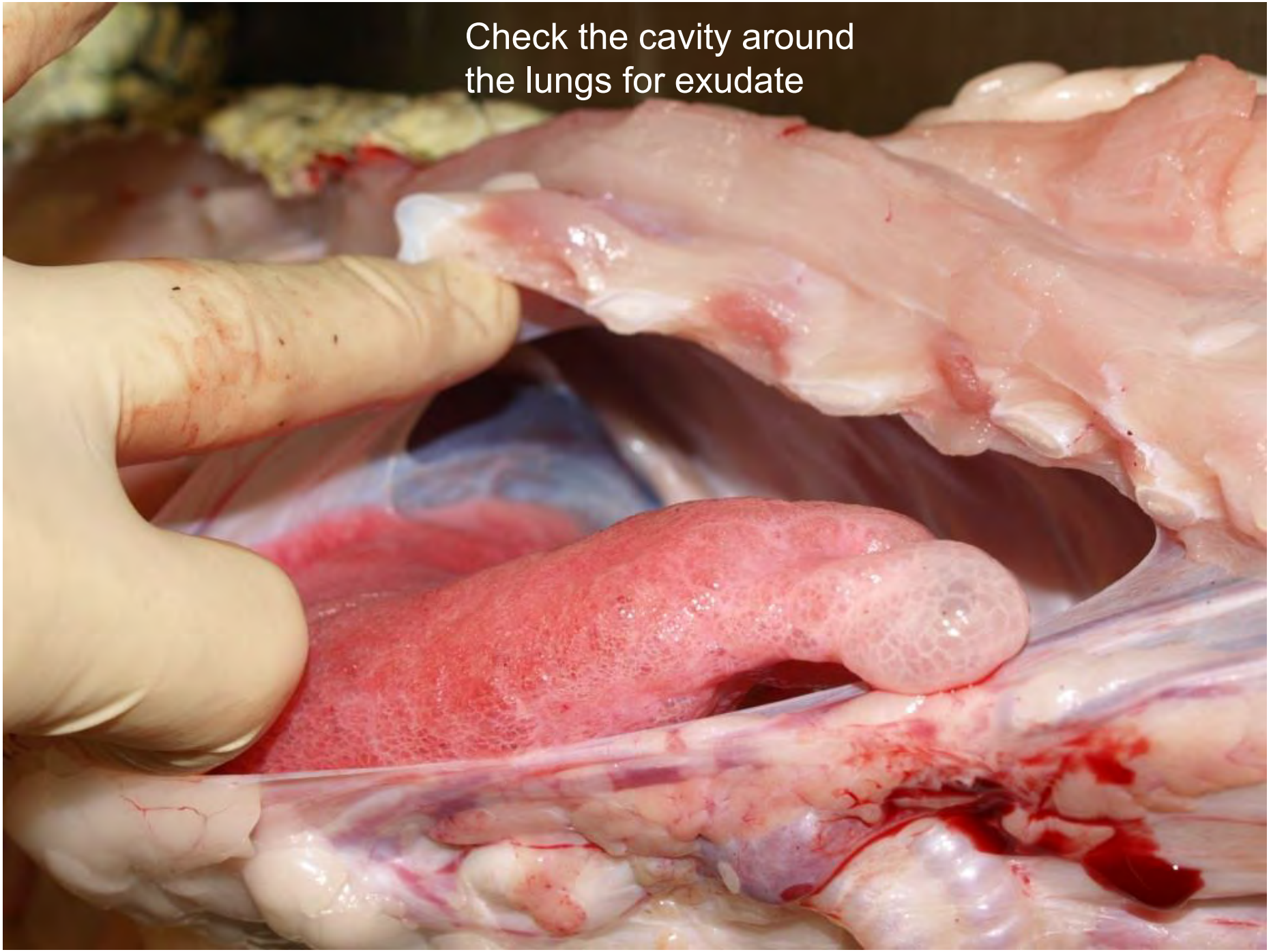
Bacterial infection. Surface of liver is very wet with prominent blood vessels

Heart is covered in tan exudate

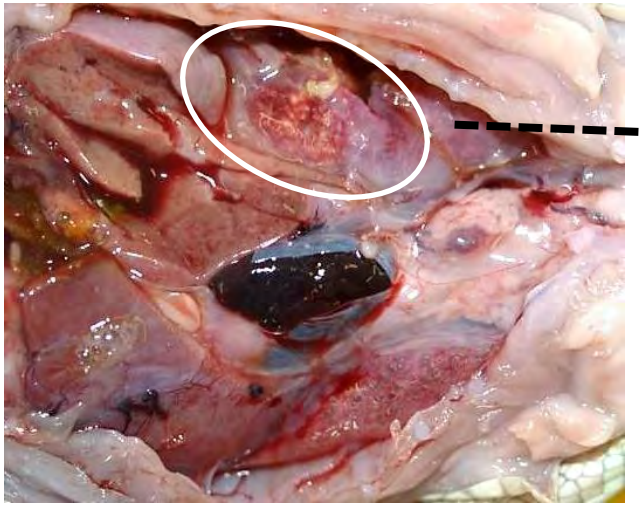


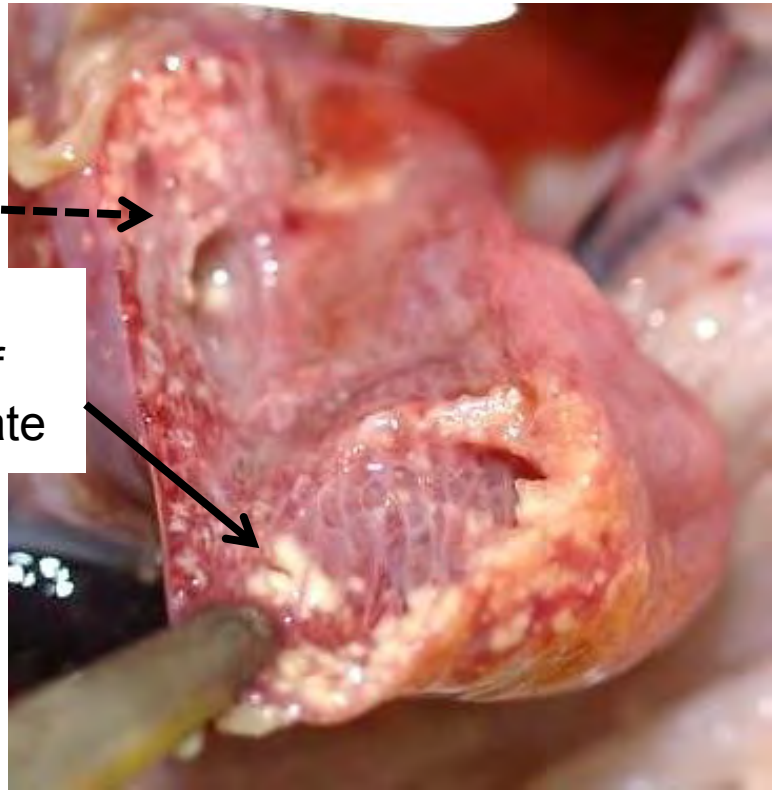



Examine the lungs
Text

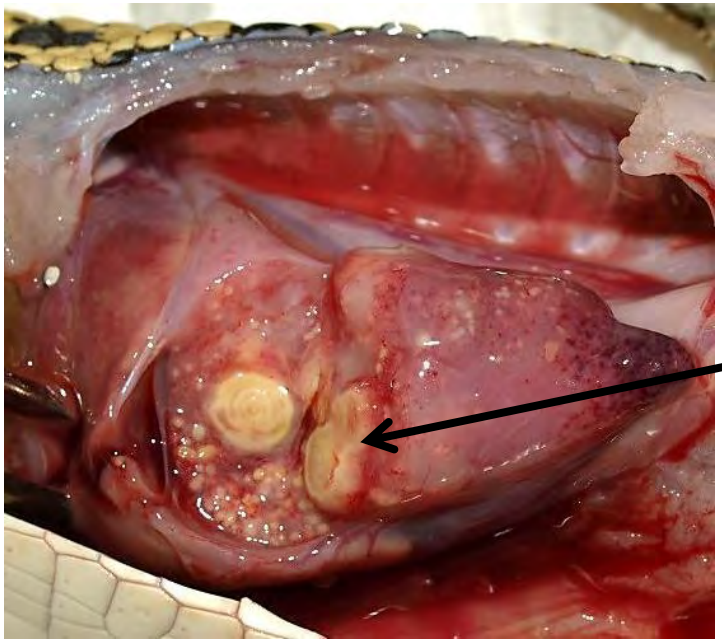

Check the cavity around
the lungs for exudate

Cut open the lung and look for exudate or parasites in the lumen

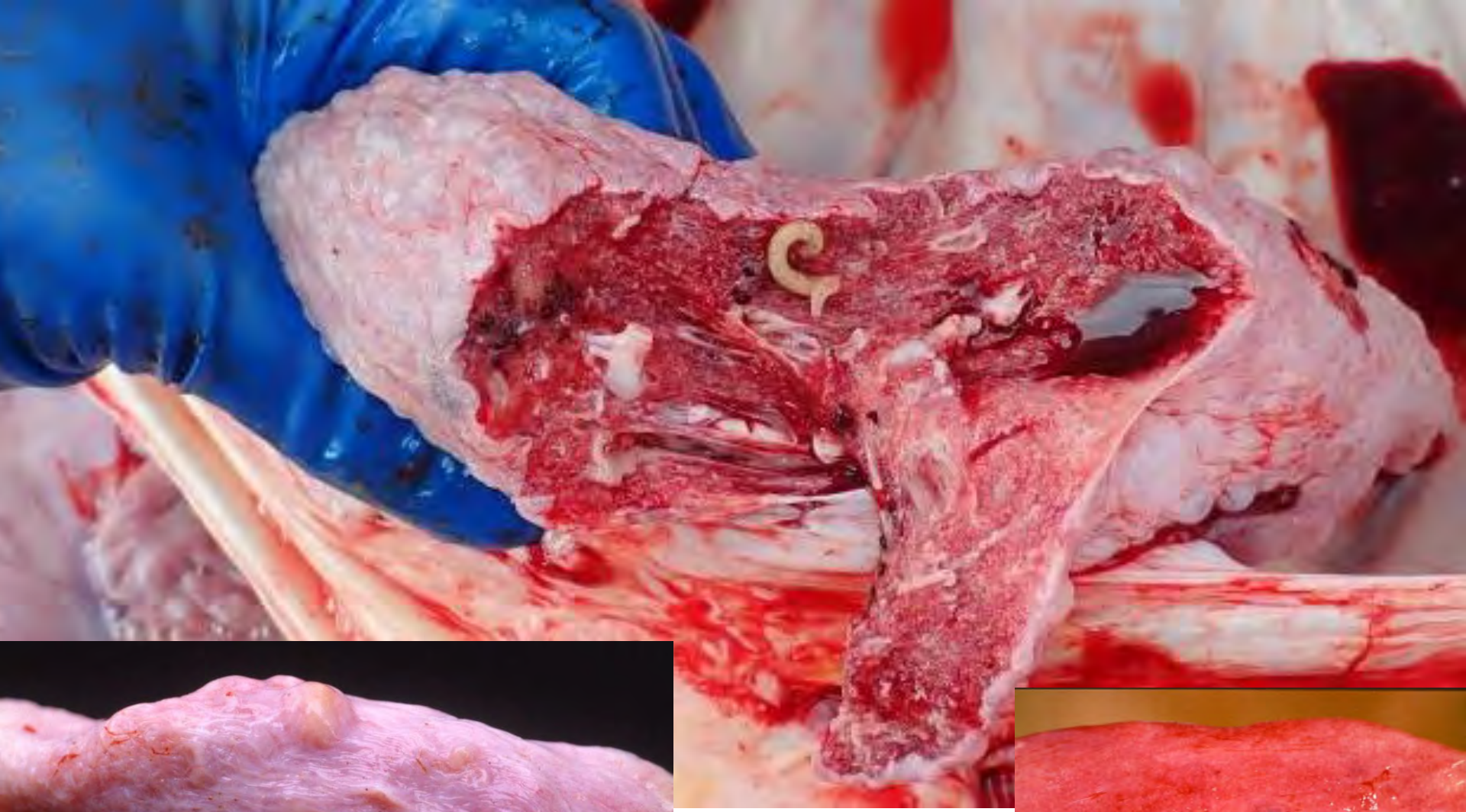




Many small yellow areas of caseous exudate



Larger laminated foci of caseous exudate



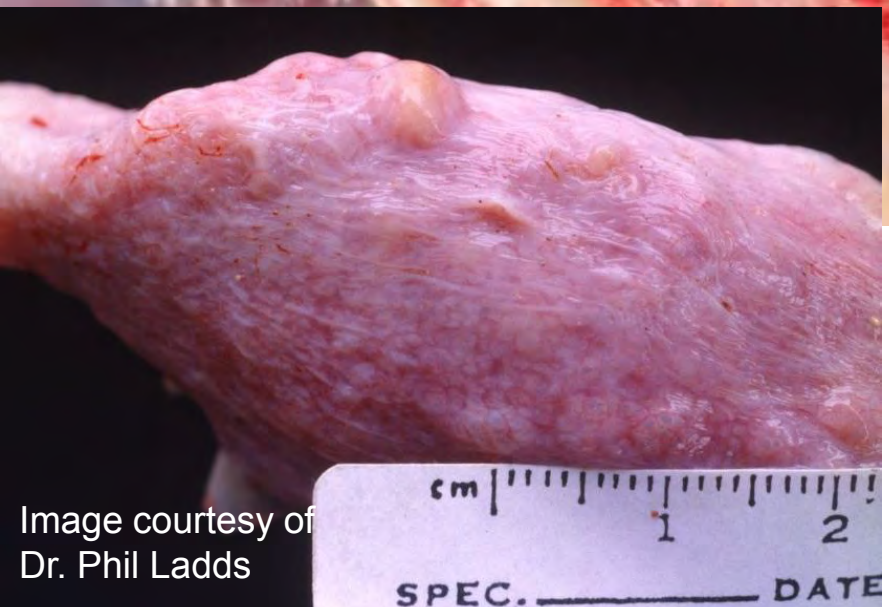


Image courtesy of Dr. Phil Ladds

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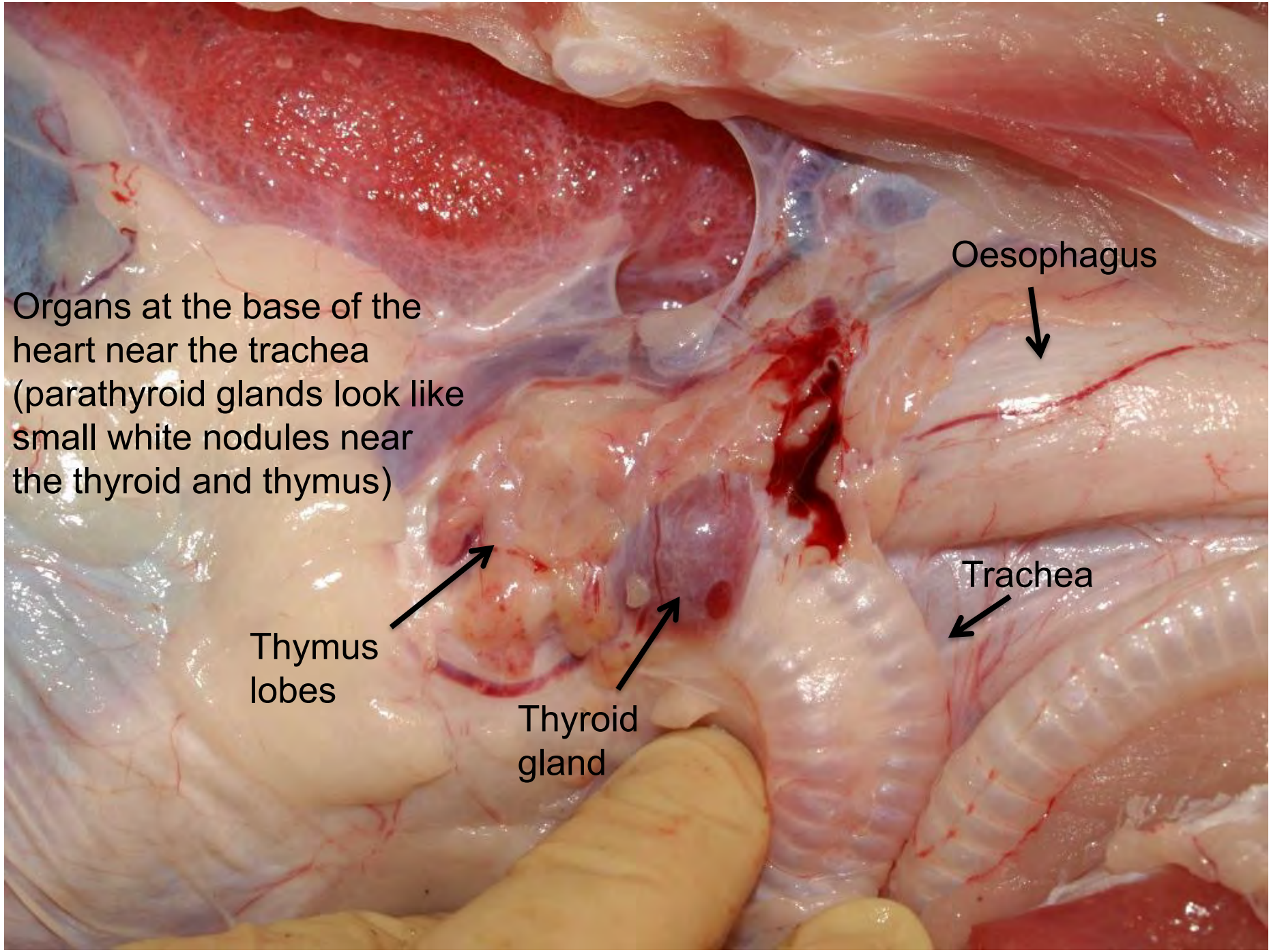
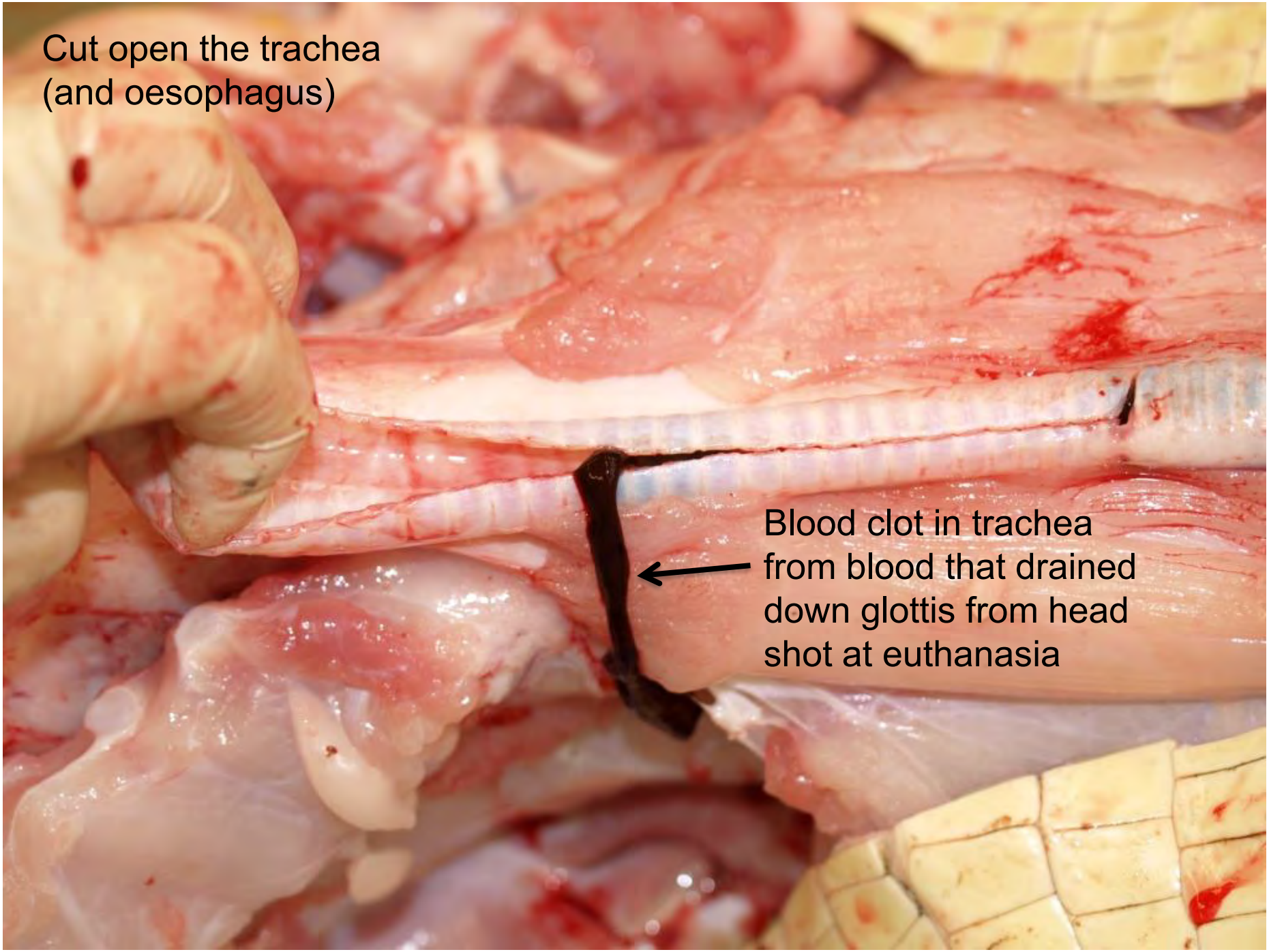
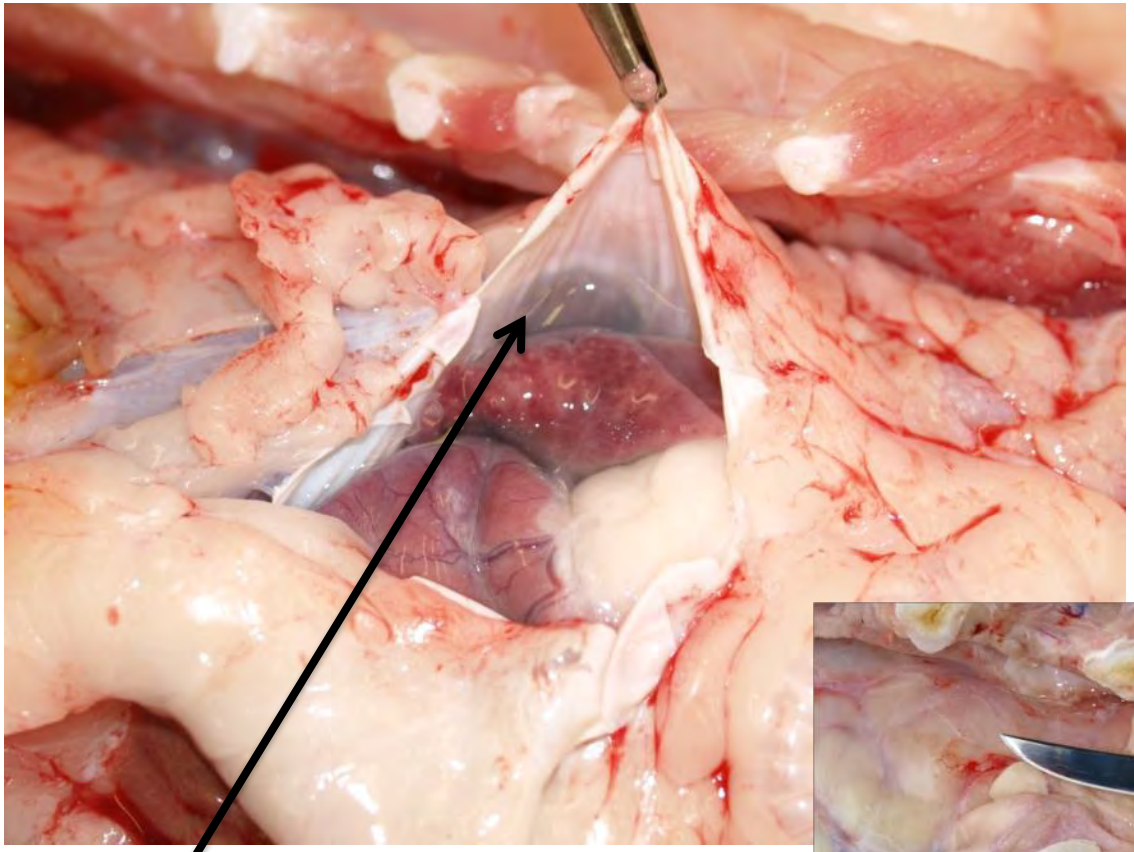
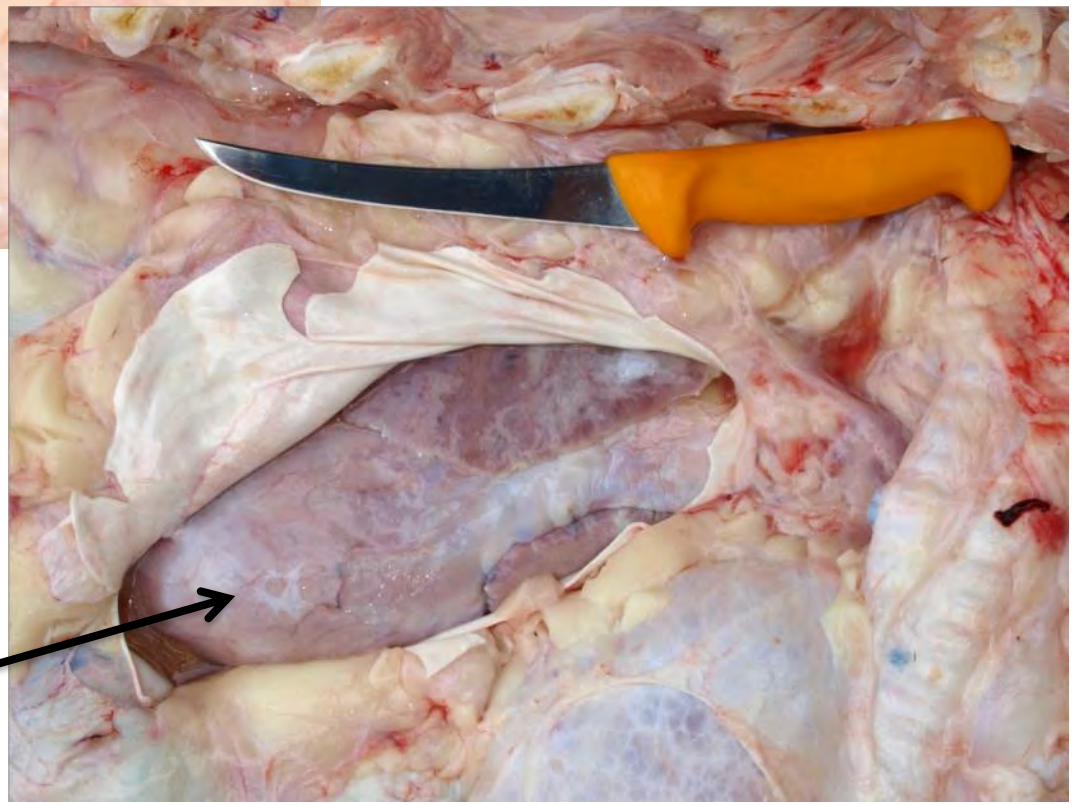

Organs at the base of the heart near the trachea (parathyroid glands look like small white nodules near the thyroid and thymus)
Oesophagus
Trachea
Thymus lobes
Thyroid gland


Cut open the trachea
(and oesophagus)
Blood clot in trachea
from blood that drained
down glottis from head
shot at euthanasia



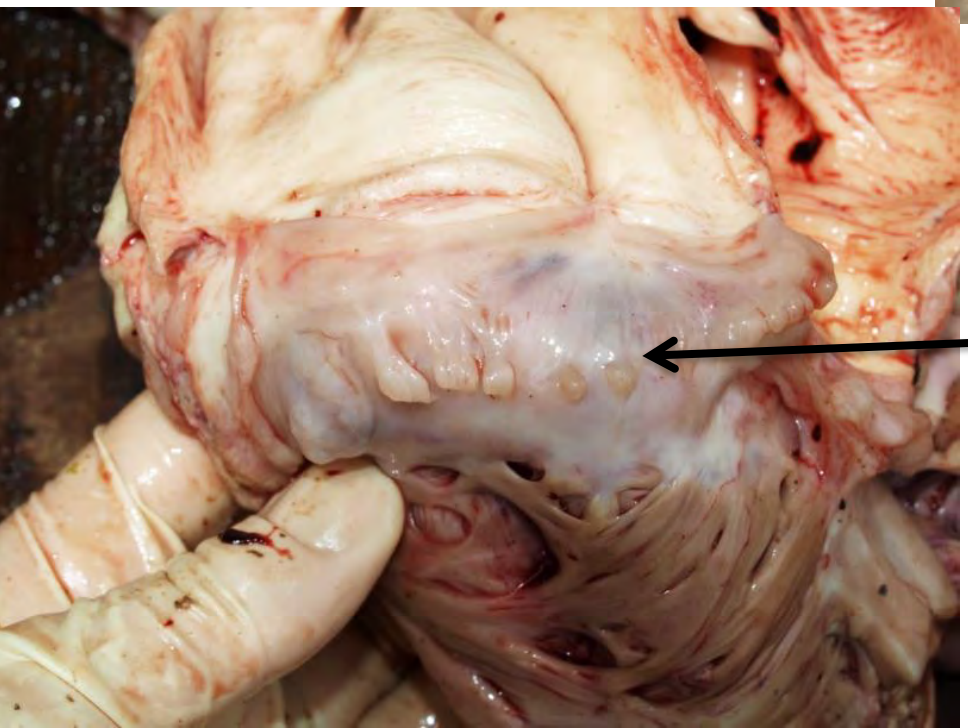
A small amount of clear fluid in the heart sac is normal

Open the sac that encloses the heart and examine the surface of the heart

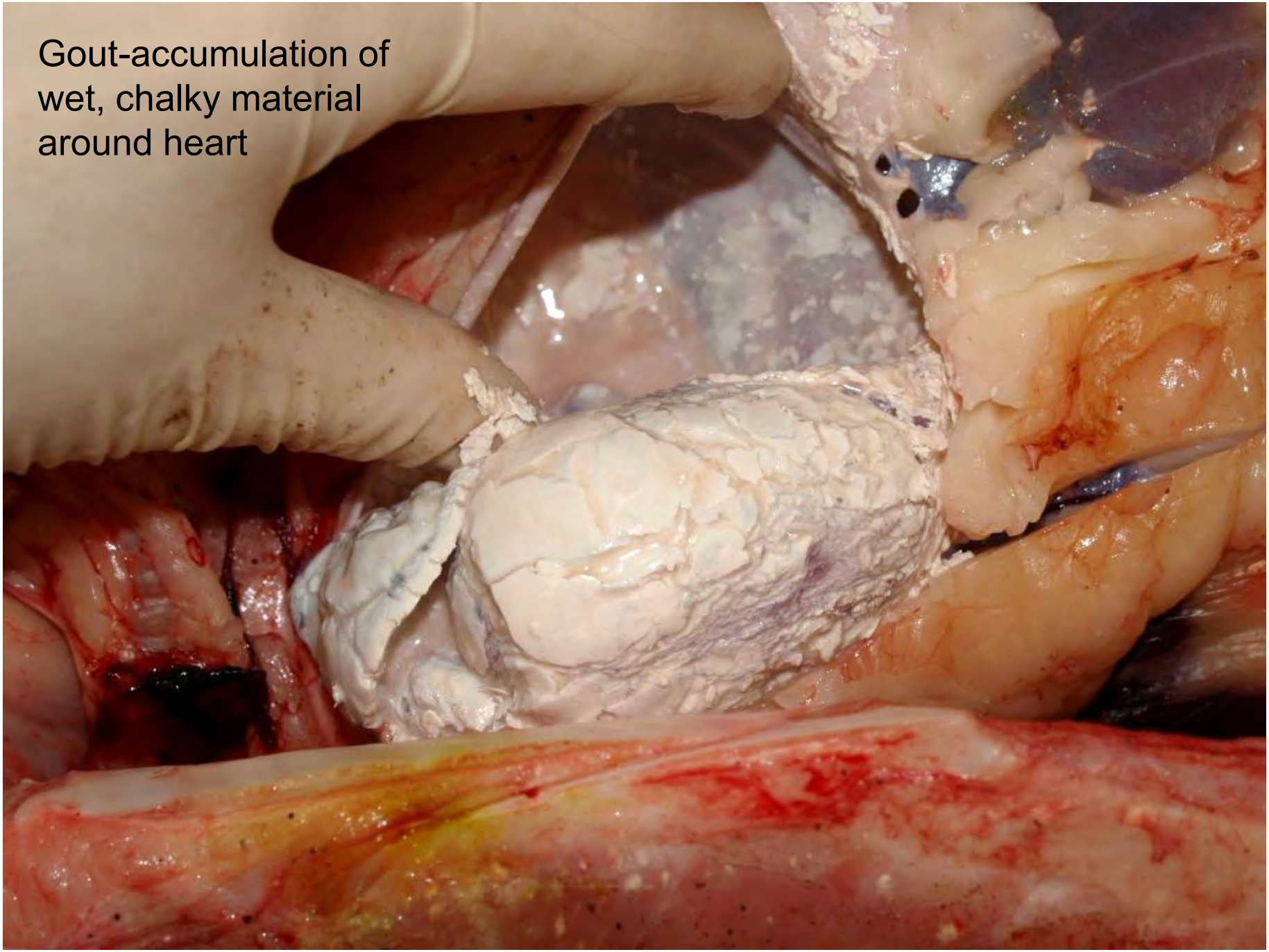


Thickened, slightly opaque covering of heart in old, large croc

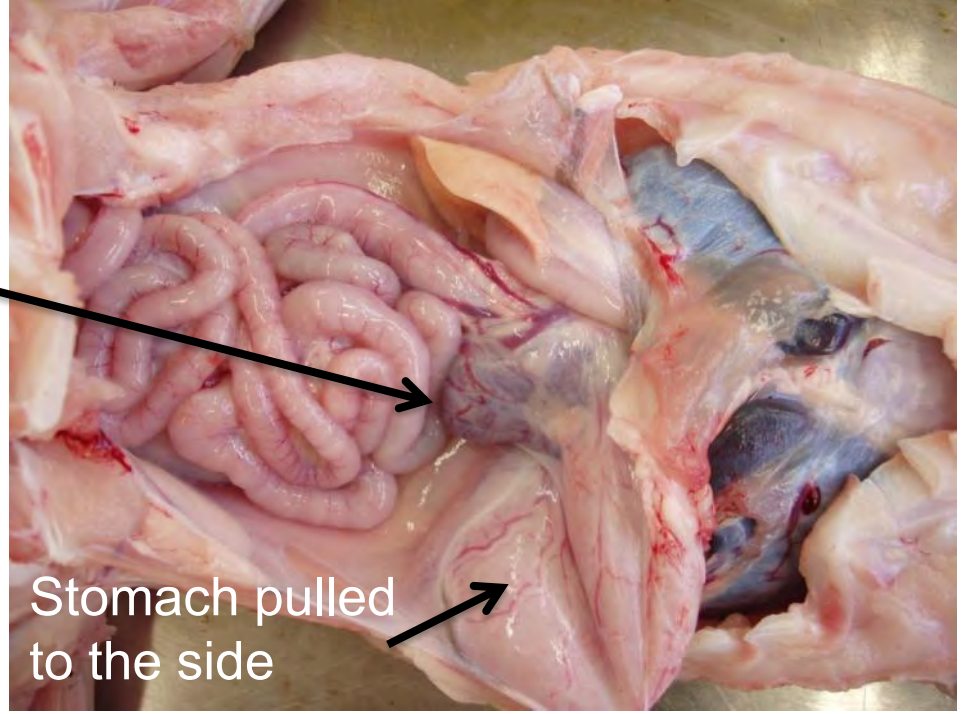
Remove the heart and open it to look at the inside surface and the valves.

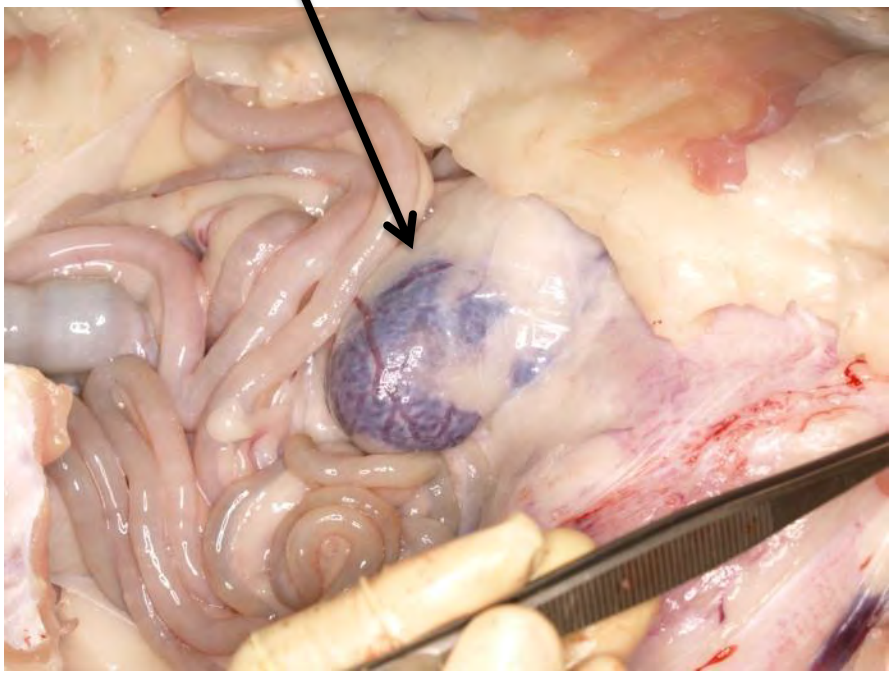


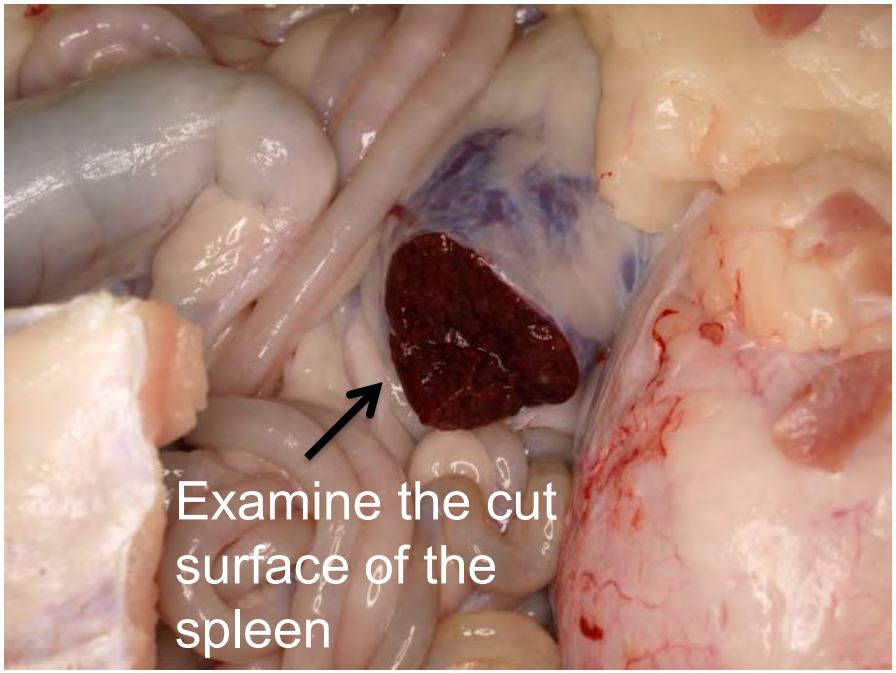
Note normal nodular appearance of valves on right side of heart


Gout-accumulation of
wet, chalky material
around heart

Examine the spleen. It is located under the stomach.

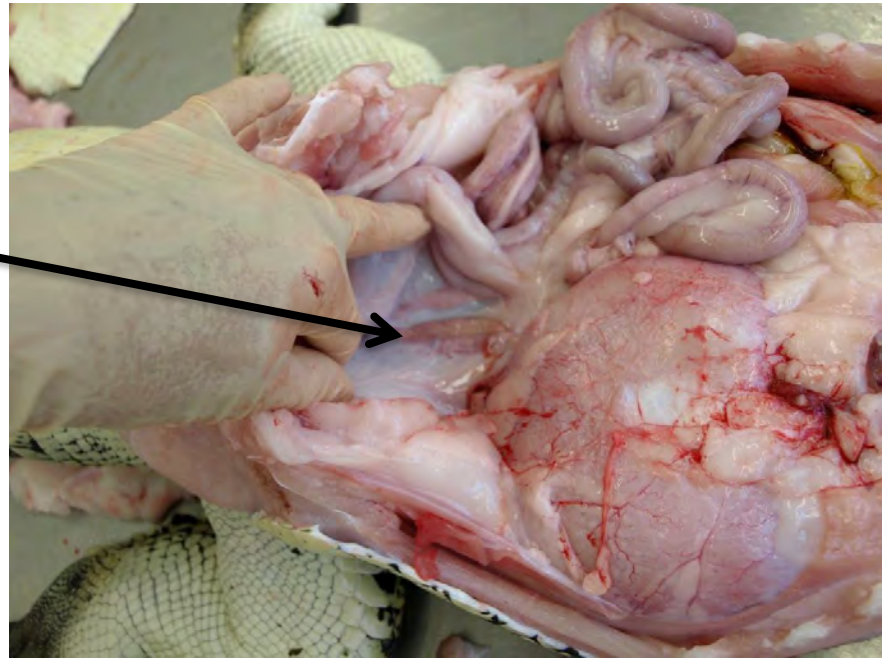







Assess the size of the
fat body

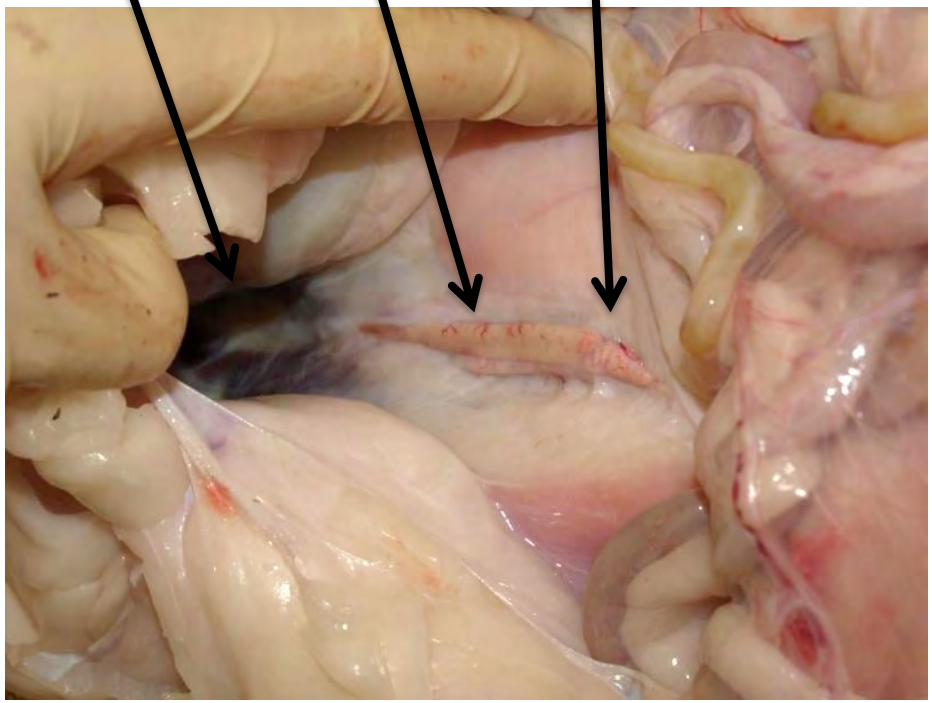
Examine the kidneys located level with the hind legs between the hind legs

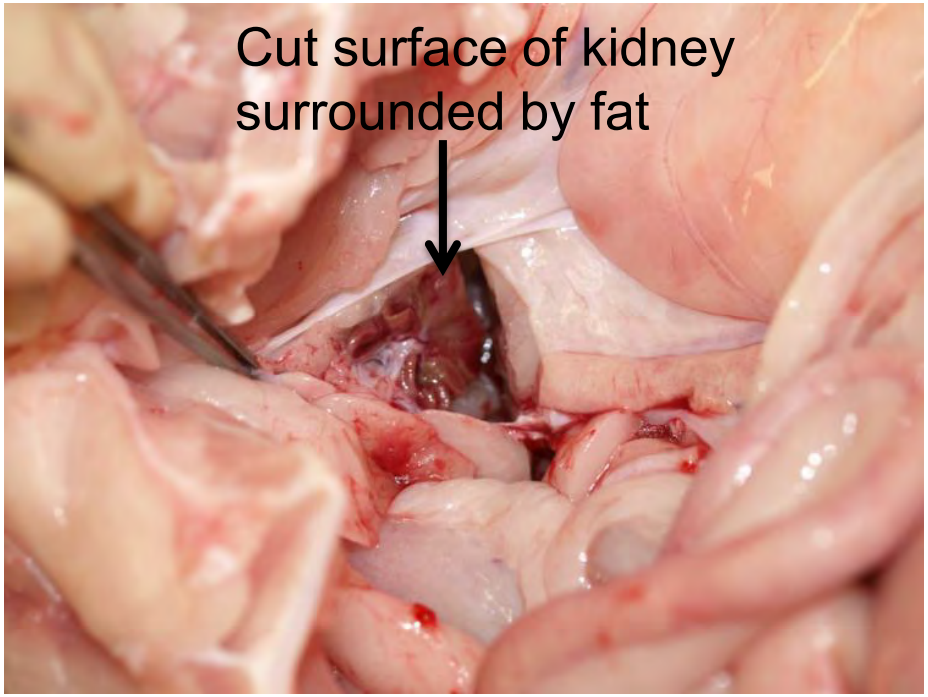


Testis (small in hatchling)

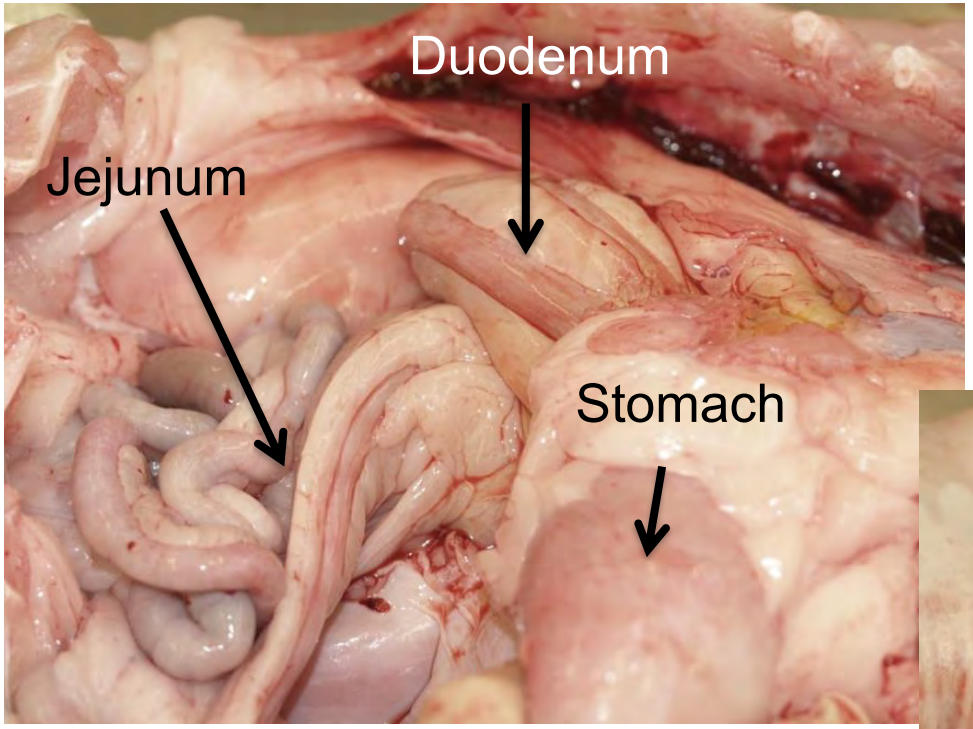
Kidney

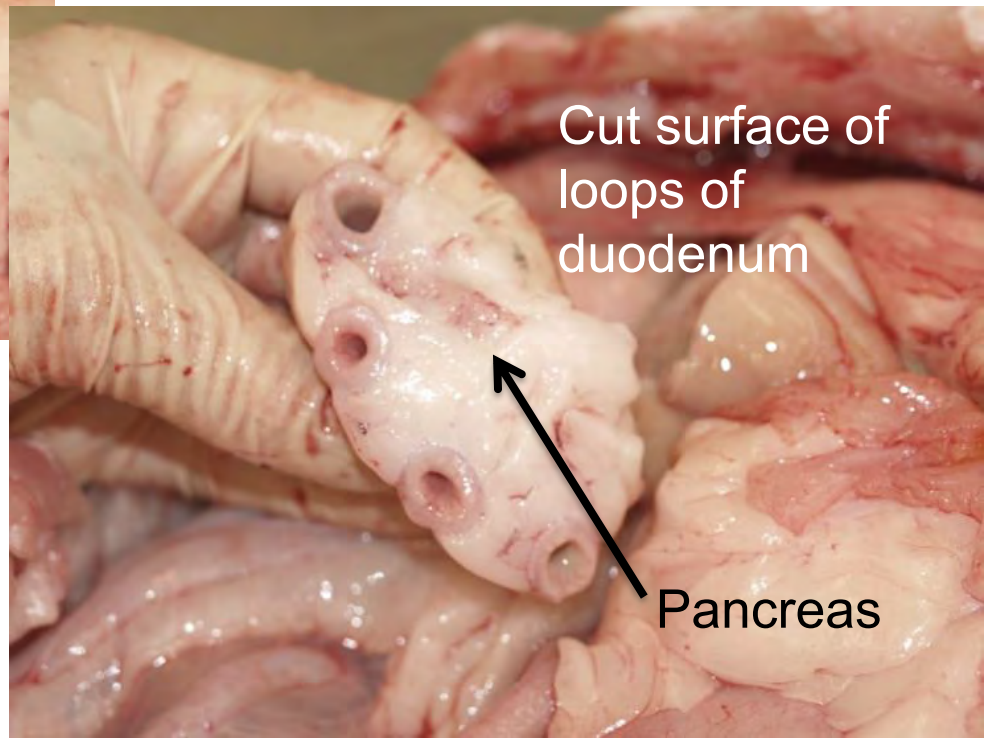
Adrenal gland

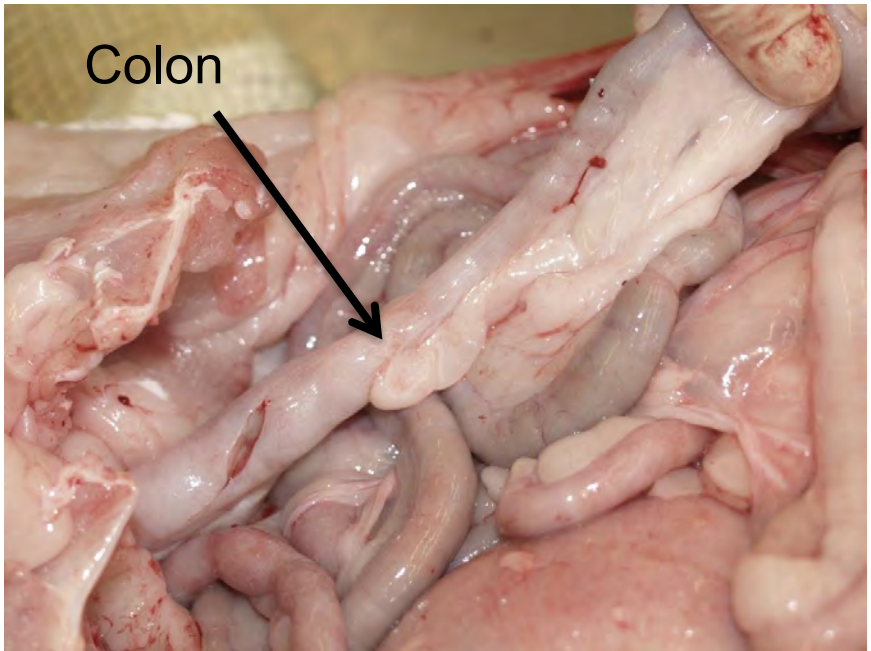


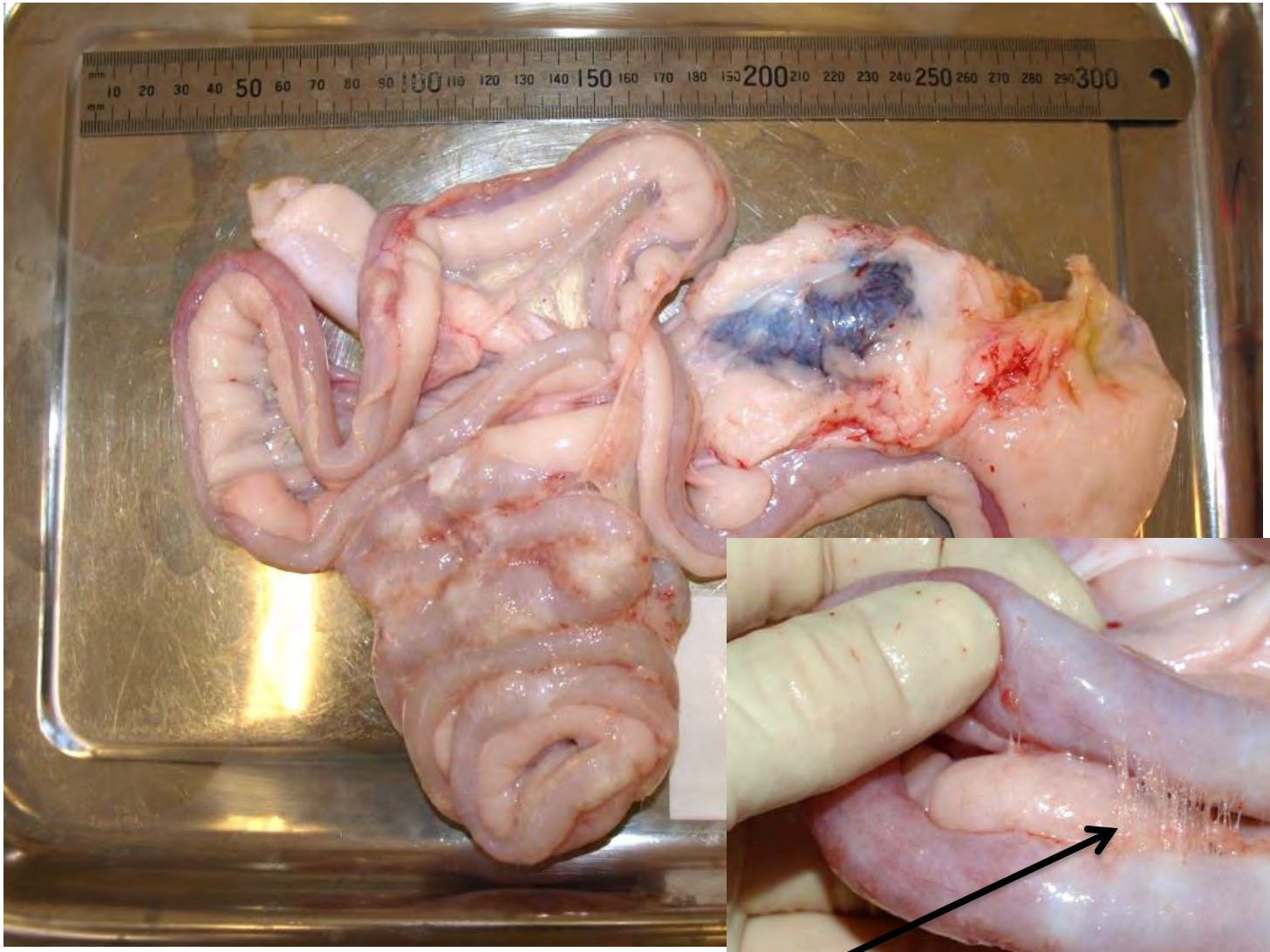


Examine the gastrointestinal tract last (outer surfaces and inner lumens at several levels along intestine)

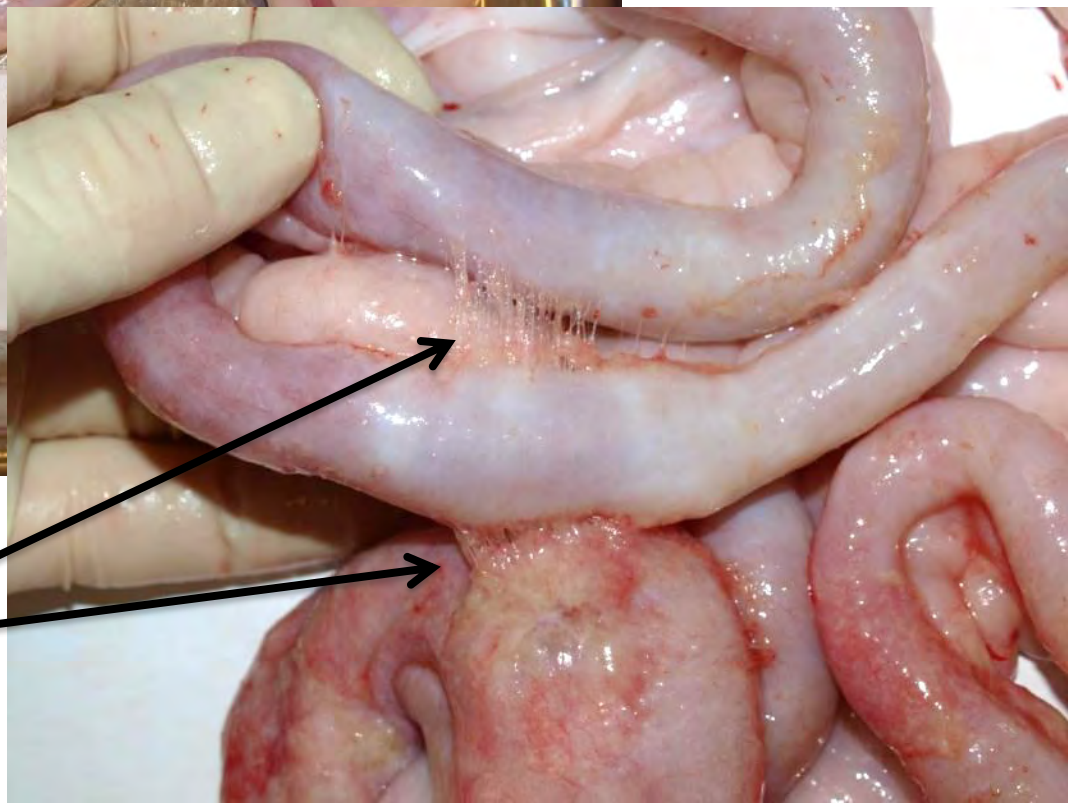








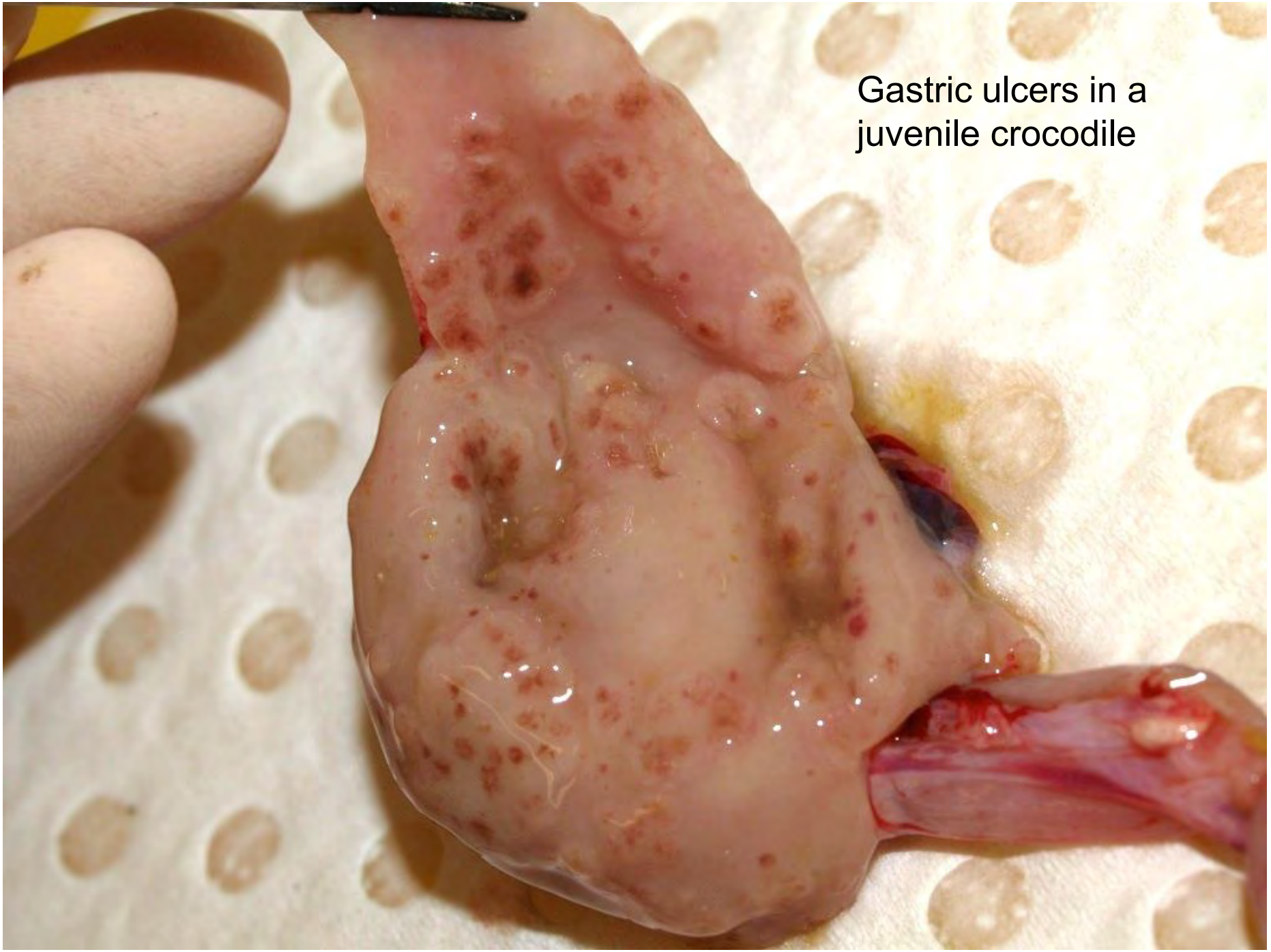
Intestinal mass stuck toegether due to inflammation from chronic, severe coccidiosis



Intestinal adhesions

Have a look inside the stomach and intestines

Open the stomach and
examine the content


Gastric ulcers in a
juvenile crocodile

Wild croc with cancer!

- Case detail and photos courtesy of Matt Brien, Wildlife Operations, QLD Dept. Environment and Science
- 4.8 m wild croc captured as part of management program
- Croc age estimated at 70 yrs +
  - Very poor body condition
  - Floating askew in water, large bulge right side of abdomen


Keystone Omni
LEVELS
ZOOM
PAN
ROLL
LENGTH
RECT
CIRCLE
ELLIPSE
ANGLE
ANNOT
ERASER
CALIBRATE
VHS
TPLO
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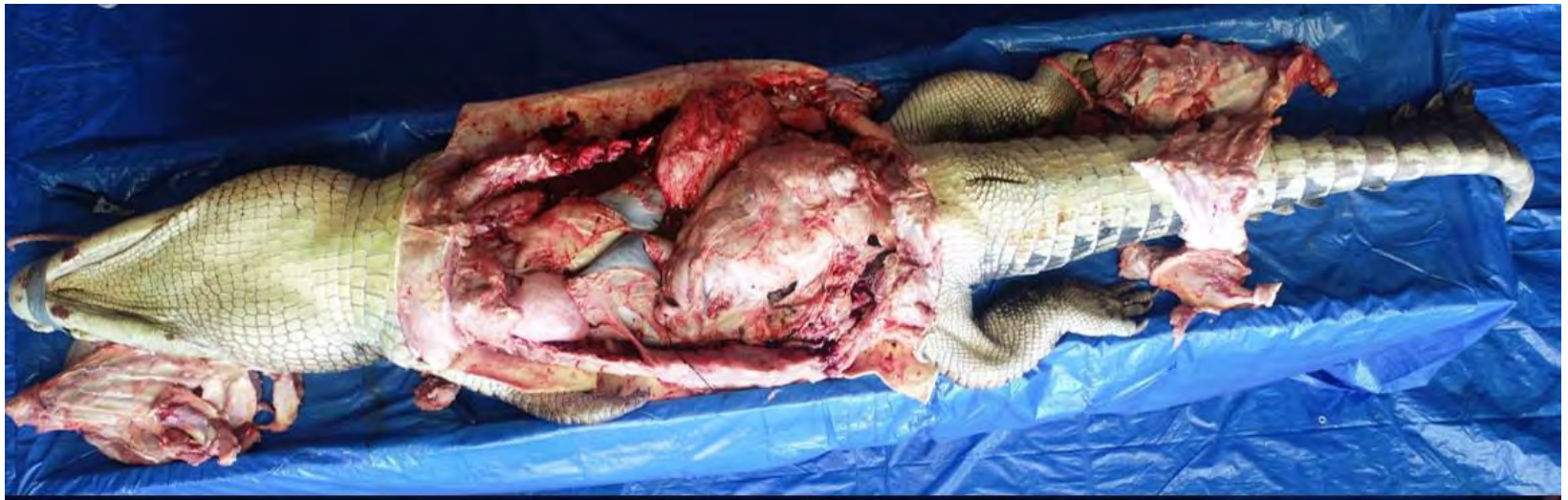
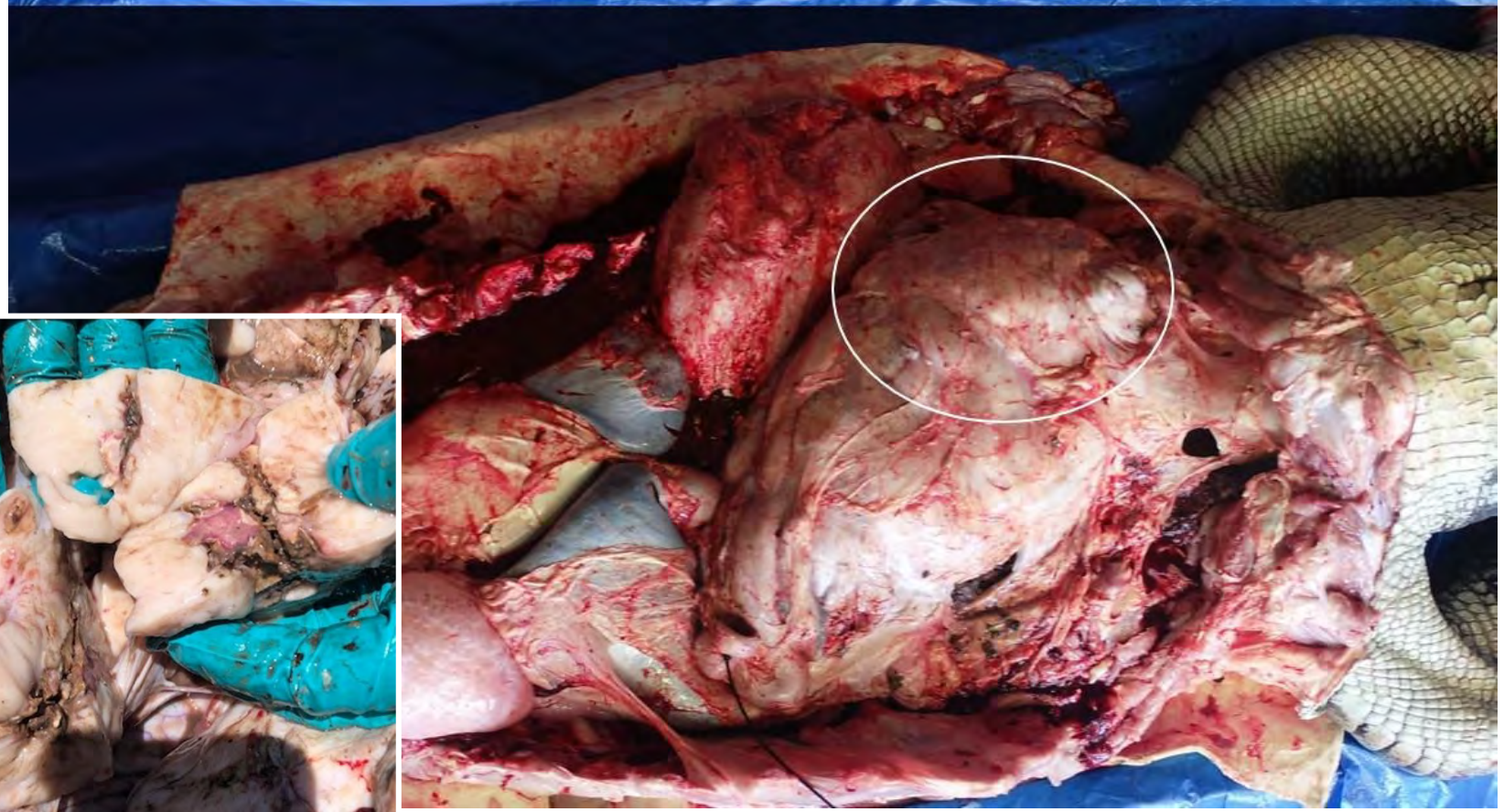
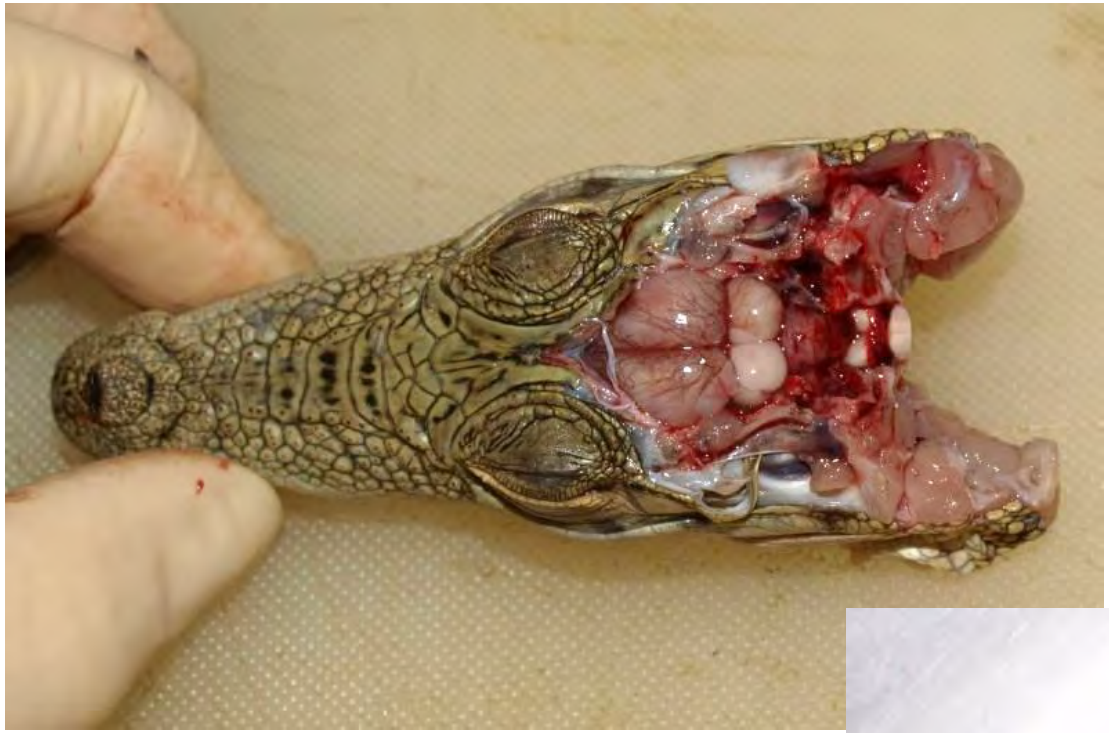
SYNC
ROTATE
RESET
DICOM
ACTIONS
ABDOMEN_LAT RIGHT OBL S:1
ABDOMEN_LAT RIGHT OBL S:2
ABDOMEN_LAT RIGHT OBL S:3
ABDOMEN_LAT RIGHT OBL S:4
Zoom: 29.14%
Series: 4 / 20
Image: 1 / 1
View: ABDOMEN_LAT RIGHT OBL
Name: Croc
Id: 123
Owner: 123
Institution: Tableland Veterinary Service - Malanda
Study Date: 15/10/2020, 9:54 am
Acq Time: 15/10/2020, 10:27 am
W/L: 4096/2048
Rotation Angle: 0°



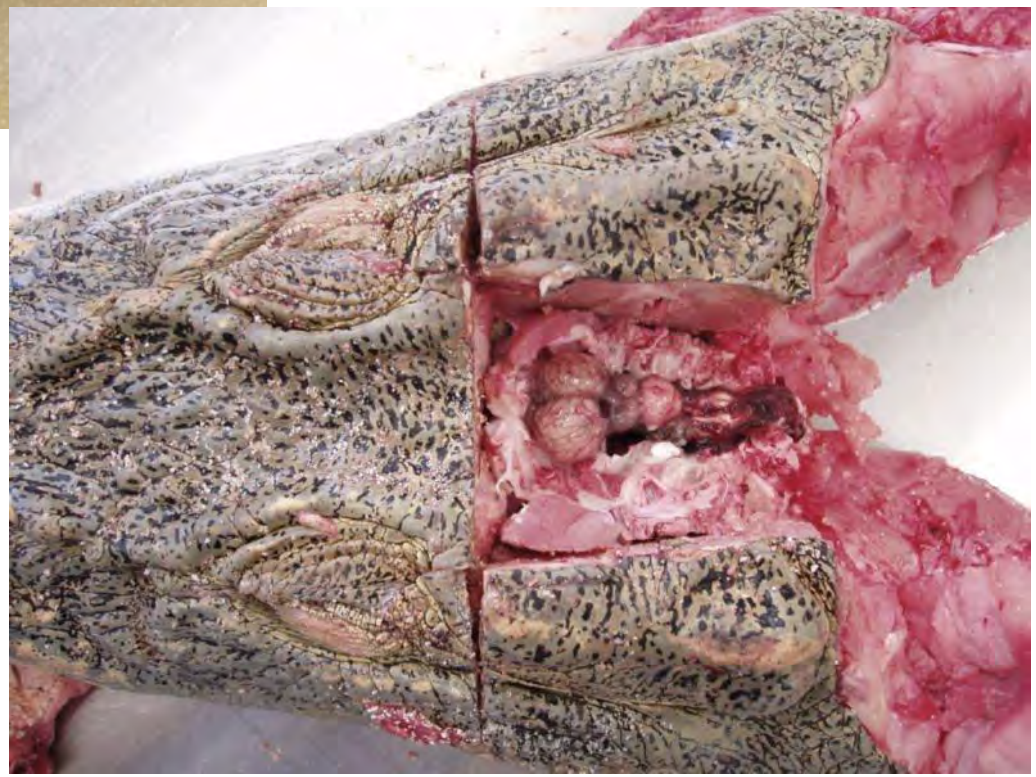




Consider examining and removing the brain, particularly if there is no diagnosis by the end of the necropsy

For a small croc, bone cutting pliars (rongeurs) can be used.

For a large croc, a hacksaw is used.

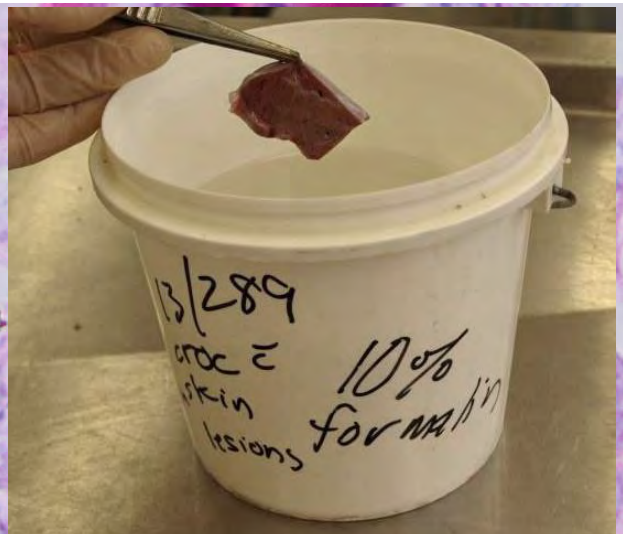


Necropsy tip #5: sample thoroughly and thoughtfully

- Quality and value of lab results depends on quality and types of samples submitted
- Take a variety of samples even if you don't think they'll be needed
- Collection technique, storage & transport of samples may determine whether a diagnosis can be made
- If you have a specific test in mind, get advice from the lab before you sample

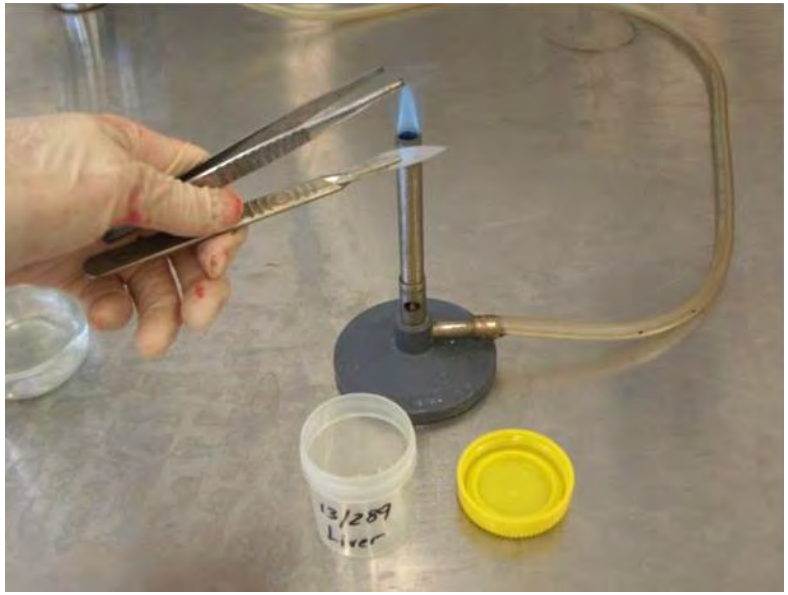
Histology

- Looking at tissues under the microscope
- Is the standard first test to investigate disease
- Severely hindered by tissue decomposition and freezing
- 1-2 cm tissue sections into 10% neutral buffered formalin (1 part tissue:10 parts formalin)
- A general range of tissues can be placed in one pot (only separate if notable lesion or tissue)



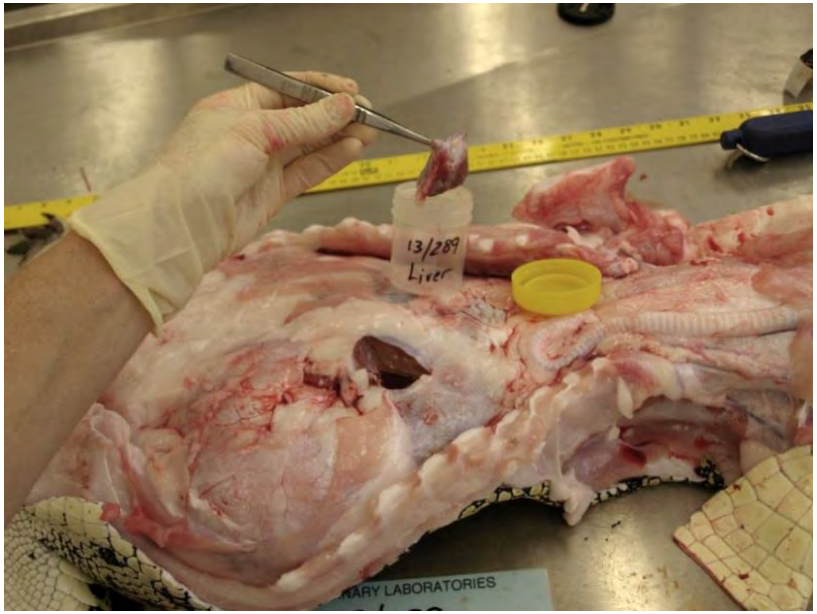
Sending formalin-fixed tissue by mail or courier:

- Make sure tissue is well-fixed in 10% formalin
- Wrap formalin-fixed tissue in paper towel moistened with 10% formalin
- Put into a zip-lock plastic bag
- Then put bag into a solid plastic crush-proof container to make sure no leakage occurs



Bacteriology

- Used to look for diseases due to bacteria
- Aseptically (or at least cleanly) taken samples into sterile jars or swabs in transport medium, store @ 4^{o}C
- During PM, collect tissues for bacteriology 1st (3 filtering organs, 2-4 cm diameter sections)
- Range of specific culture techniques (let lab know which organism(s) you suspect)



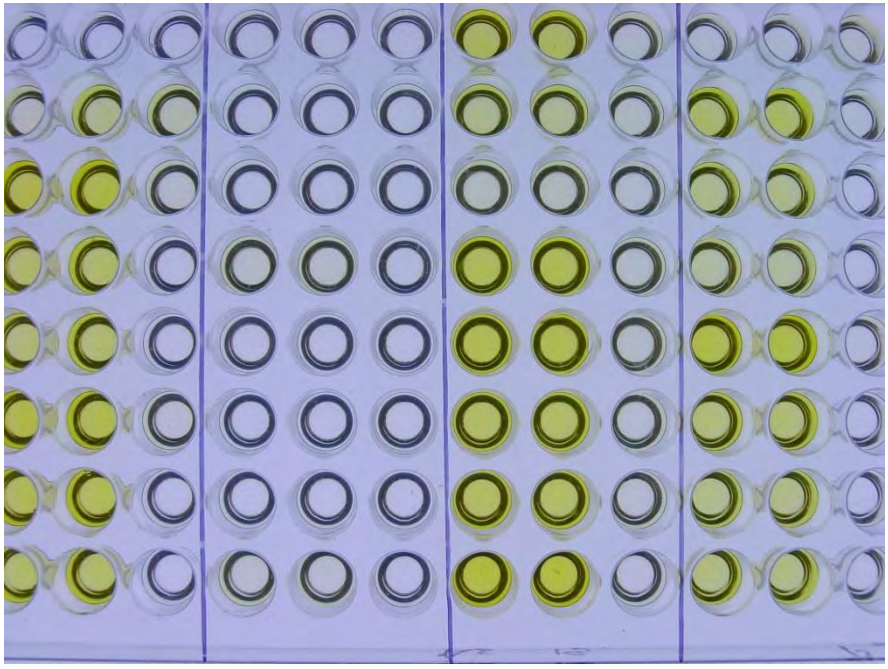
Crocodile cell line infected with Crocodyline herpesvirus

Virology & Molecular

- Definitive diagnosis for viral diseases in crocodiles is problematic
  - VI takes a long time, is costly, and generally requires specialist crocodile cell lines
  - Molecular tests (PCR, next generation sequencing) are used increasingly but may be expensive and availability limited to specialised labs, interested researchers
- Common samples are oral/cloacal swabs, blood in EDTA anticoagulant (live croc) or frozen filtering organs
- Store @ 4^{o}C short-term, freeze if several days to lab
- Specialised nucleic acid preservative may be required

Serology

- Testing for antibodies to specific disease agent to show prior exposure
- Reptiles generally don't make antibodies well
- Tests are usually not validated for crocs
- Available for a limited number of diseases at research labs



(An ELISA plate used for serological testing)

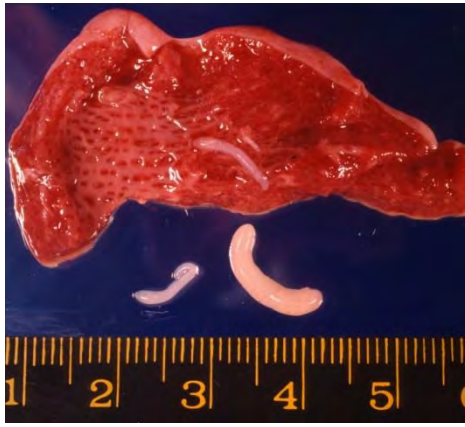
Parasitology

- An increasingly rare specialty
- Can use morphology or molecular techniques.
- For worms, preservation technique is crucial:

Crocodilian Parasite Preservation

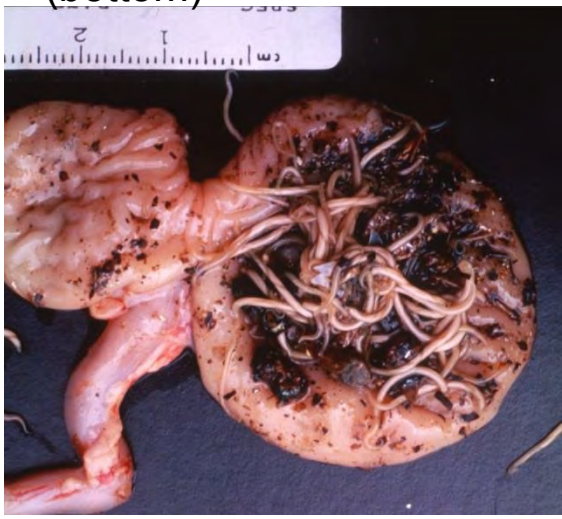
Prepared for the CSG by Dr. Marisa Tellez

The following guide is designed to provide basic information on the killing, fixation and preservation of crocodilian parasites.



Pentastomes in lung (top) and ascarids in stomach (bottom)

- Histology is fairly good for small parasites (protozoa) but poor for large parasites (worms)
- High quality blood smears are required for haemoparasites



(Photos courtesy Phil Ladds)

Toxicology

- Toxins may accumulate in crocodilians since they are long-lived apex predators
- Specialised testing done by various labs
  - You must know which toxin you are looking for and find a lab that tests for that
  - Eg. Heavy metal testing may require a different sample than pesticide testing
- The most common samples saved for toxicology at necropsy, to cover the possibility of a toxin are liver and kidney (at least 5 g of each into separate containers)
- *If you are serious about toxin testing, contact the lab beforehand to discuss sampling*

Haematology and clinical chemistry

- Only used in live crocs or freshly dead (<10 minutes)
- There are problems with interpretation in crocs ("normal" not known)
- Haematology
  - Provides information on blood cells (inflammation, anaemia)
  - Ensure sufficient EDTA or lihep anticoagulant for sample size, make smear if >12 hrs. to lab, protect smears from formalin vapours
- Clinical chemistry
  - Provides information on organ function
  - Serum or lihep plasma from live or immediately post-euthanasia
  - Artifacts if serum not separated-do before transport if possible

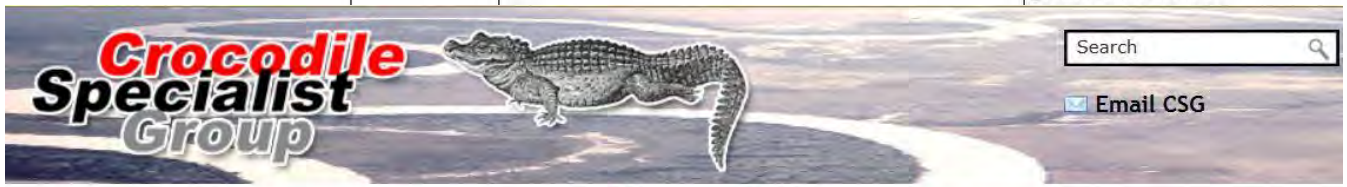
- Anticoagulated blood for haematology
  - Edta: best cell preservation is best but plasma not useful for biochemistry
  - Lihep: cell preservation adequate, plasma useful for biochemistry

- Clinical biochemistry
  - Plain or gel tube: provides most versatile sample, since no added chemicals
  - Lihep: plasma can be used for most clinical biochemistry and sometimes other types of analyses
  - Other special tubes for less common analyses (eg. lactate)

Sampling Summary (CSG Website)

Sampling tips for various types of ancillary necropsy testing (CSG Veterinary Workshop, 2016)

Test	General comments	Specific sampling notes
Histology	-Primary routine ancillary testing -Submit a standard broad range of tissues, even if they appear grossly normal -Also sample any lesions and describe them to the pathologist -Sample any margins of lesions with normal tissue	-Preserve tissues in formalin (10% phosphate buffered), at 1:10 tissue:formalin ratio. -Tissues may be mixed in one jar -Tissues should generally not exceed 1 cm diameter -For small crocs, entire "pluck" of tissues in body cavity can be removed as one piece and placed in formalin -Store formalin-fixed samples at room temperature
Cytology	-Not routinely performed when necropsy tissues for histological evaluation are available -Organ impressions may be useful in conjunction with histology to provide cellular detail or detect organisms -In some cases, cytology performed by the clinician may provide sufficient diagnostic information	-Slides are fixed in methanol and usually stained with Wright's type rapid stain -Additional diagnostic potential can be obtained by use of Gram's stain for bacteria and periodic acid-Schiff stain for fungi -Smears are destroyed by formalin fumes (keep separate)
Bacterial and fungal culture	-Minimise contamination as it will influence culture results and/or confound interpretation -Some bacteria (eg. *Mycoplasma* spp.) require specialized storage media and culture conditions (contact lab) -Accurate anaerobic culture requires use of specialised swabs or immediate transport of sample to lab -For fungal culture, tissue samples are generally better than swabs -If you have sampled a heavily contaminated site (mouth, intestine), advise the lab of the bacterial species of interest -If *Salmonella* spp. is suspected, request specific culture	-Aseptically sample filtering organs as soon as the body cavity is opened (liver, kidney, lung and spleen) -Sterilise instruments with flame prior to sampling -Sample 1 cm or larger pieces of tissue into individual sterile vials. -Moisten small (<5 mm) pieces of tissue with normal saline -Use swabs in bacterial transport medium for exudates -Store refrigerated (a few days) or frozen
Molecular testing	-Polymerase chain reaction and/or genetic sequencing -Commonly used for detecting reptile viruses -May also be used for fastidious bacteria and fungi -Tests are specialised and may only be available at research labs	-Generally, fresh tissues or plain swabs can be used (contact lab) -Moisten small (<5 mm) pieces of tissue and swabs with normal saline -Store refrigerated (a few days) or frozen -Testing of formalin-fixed, paraffin embedded tissues may be possible but is not ideal



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Resources provided by the CSG Veterinary Science Group

The Mission of the CSG Veterinary Science Group is to:

- provide a platform for the exchange of and access to specific veterinary knowledge and advise the CSG on veterinary matters related to crocodilian conservation;
- contribute to advancing crocodile veterinary medicine and science; and,
- provide support to animals under human care: farms and zoological or educational institutions, biologists and researchers that require veterinary support in their work such as sampling, anesthesia, surgery, etc., conservation, research, NGO and Government organizations investigating *in-situ* mortalities and population health status.

As part of this overall mission, the Veterinary Science group aims to compile information on selected topics, which will be posted on the CSG website as it becomes available (see Resources below).

Brief Introduction to Forensic Crocodilian Necropsy and Sampling Considerations

- "Forensic" refers to investigation in relation to a crime
  - What constitutes a crime may vary with jurisdiction
    - May refer to crocodile being the "victim" of a crime, for example:
      - Illegally interfering with wild crocodiles (trapping, hunting, poisoning), possessing crocodiles without a permit, illegal trade in live crocodiles or their products (eg. CITES), animal welfare issues (usually captive crocodiles), crocodiles found dead in unexpected or suspicious circumstances
    - Or, may refer to crocodile as "perpetrator", for example:
      - Human-crocodile conflict

Forensic Investigations

- What is admissible as evidence and who is considered sufficiently expert to investigate may depend on jurisdictional legislation
- Consider whether you need to call in someone else to the scene (an authority or an expert)?
- Store the carcass for necropsy by an specialist?

Forensic Investigations

- A forensic necropsy is characterised by thoroughness and inclusive documentation (sentiment expressed by both Frederic L. Frye and John E. Cooper, 2008, Applied Herpetology Special Issue: Forensic Science and Herpetology)
- Rigorous requirements for:
  - Demonstration of **chain of custody** of samples
  - Detailed labelling, appropriate storage and testing of samples (eg. accredited lab containing suitable testing experts)
  - Detailed necropsy records, including details of body weight, measurements, condition, sex, and tags or markings

- Digital images taken at necropsy should have ID marker in image with animal/case ID, date, investigator, scale.
- Special requirements may apply for download and storage (eg. as “raw” format, original file unopened)

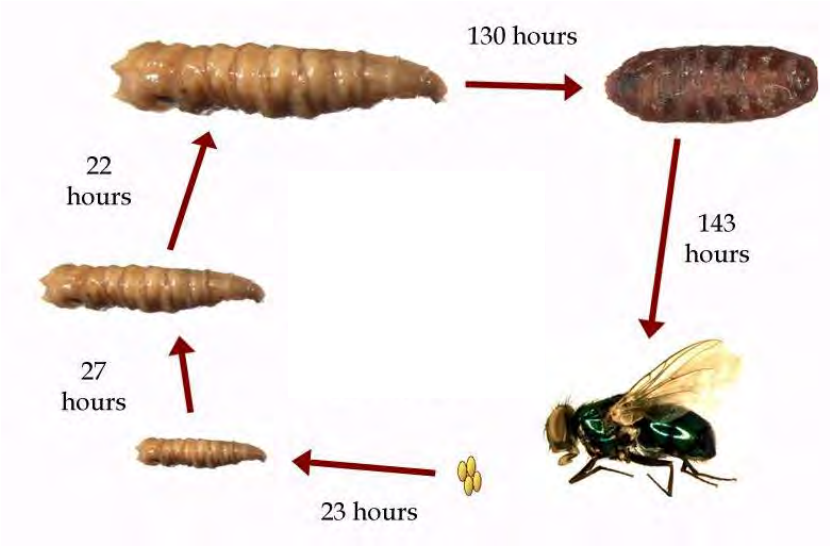
Forensic Necropsy Considerations: Time of Death?

- Because crocodilians are ectotherms, is very imprecise since indicators vary with temperature:
- Rigor mortis (stiffening of the body after death)
  - Occurs when muscle no longer has enough oxygen to pump calcium out of cells, usually takes a few hours
  - Occurs more rapidly (can be within minutes) with high temperature and muscle hypoxia prior to death
  - Rigor mortis ceases when tissue starts to break down
  - A relaxed carcass is more often than not *past* rigor
- Suffusion of pigments (haem and bile) generally take hours (eg. bile staining of belly wall usually takes >12 hours)
- Decomposition varies with temperature, so croc dying in the sun (or on a heated surface) rots much faster than if in shade or cool water
- Scales hide signs of decomposition

(See further discussion and ref list: Cooper, J.E. 2012. The estimation of post-mortem interval (PMI) in reptiles and amphibians: Current knowledge and needs. Herpetol. J. 22: 91-96).

Forensic Necropsy Considerations: Age of Carcass Beyond 24 hours?

- Eg. Forensic entomology: rates of development of fly larvae and other insects
- Exact rates vary depending on temperature and species:
- For sheep blowfly (in hours):

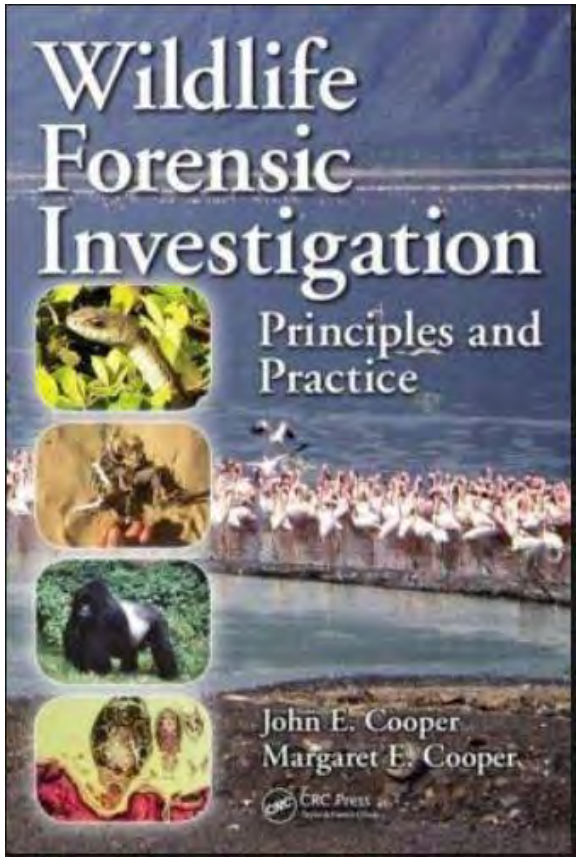


Temp ° C	Egg	1st instar	2nd instar	3rd instar	Pre-pupa	Pupa	Total days
16	41	53	42	98	148	393	32
20	21	31	26	50	118	240	20
27	18	12	40	40	90	168	14

- Other fly species at 20°C range from 17-28 total days

(Anderson GS, 2000. Minimum and maximum development rates of some forensically important calliphoridae (Diptera). J. Forensic Sci. 45: 824-832).

Forensic Investigation References



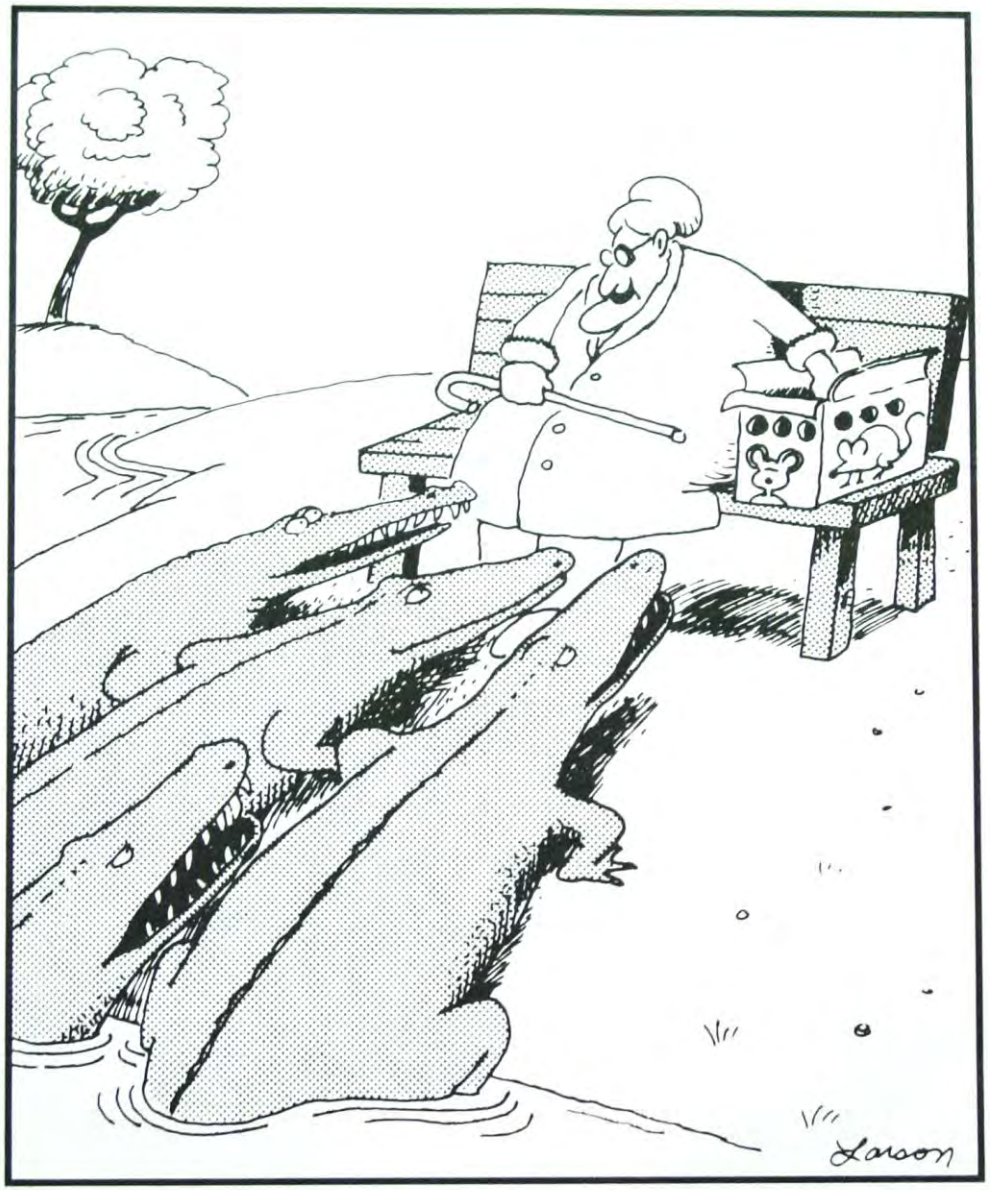
Forensic Science and Herpetology

Authors: John E. Cooper; Barry W. Baker and Margaret E. Cooper

Source: Applied Herpetology, Volume 5, Issue 4, **pages** 305 – 306

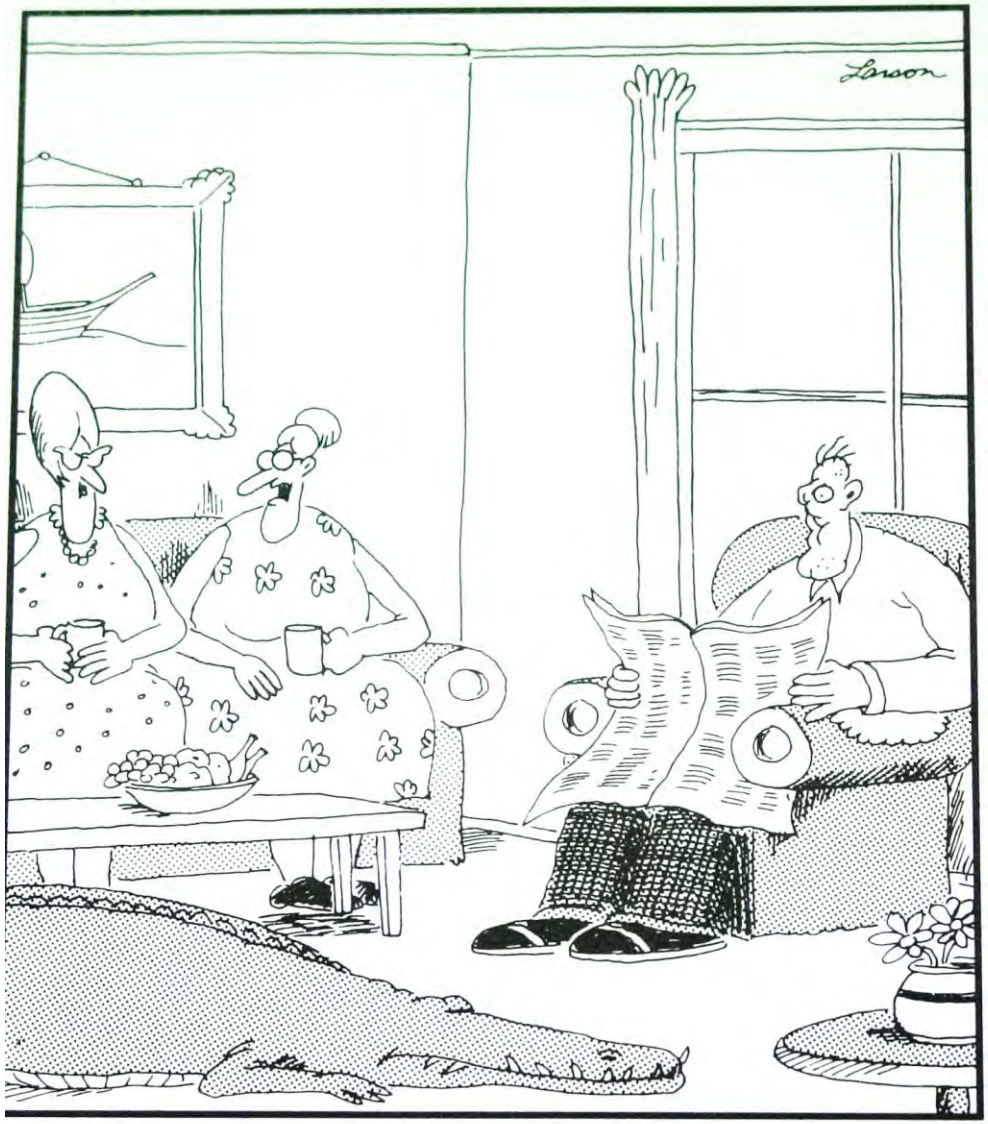
Publication Year : 2008

Two volumes: Includes chapters on legal aspects, methods (sampling, lab, post-mortem), DNA technology, record-keeping, crime scene visits etc.)



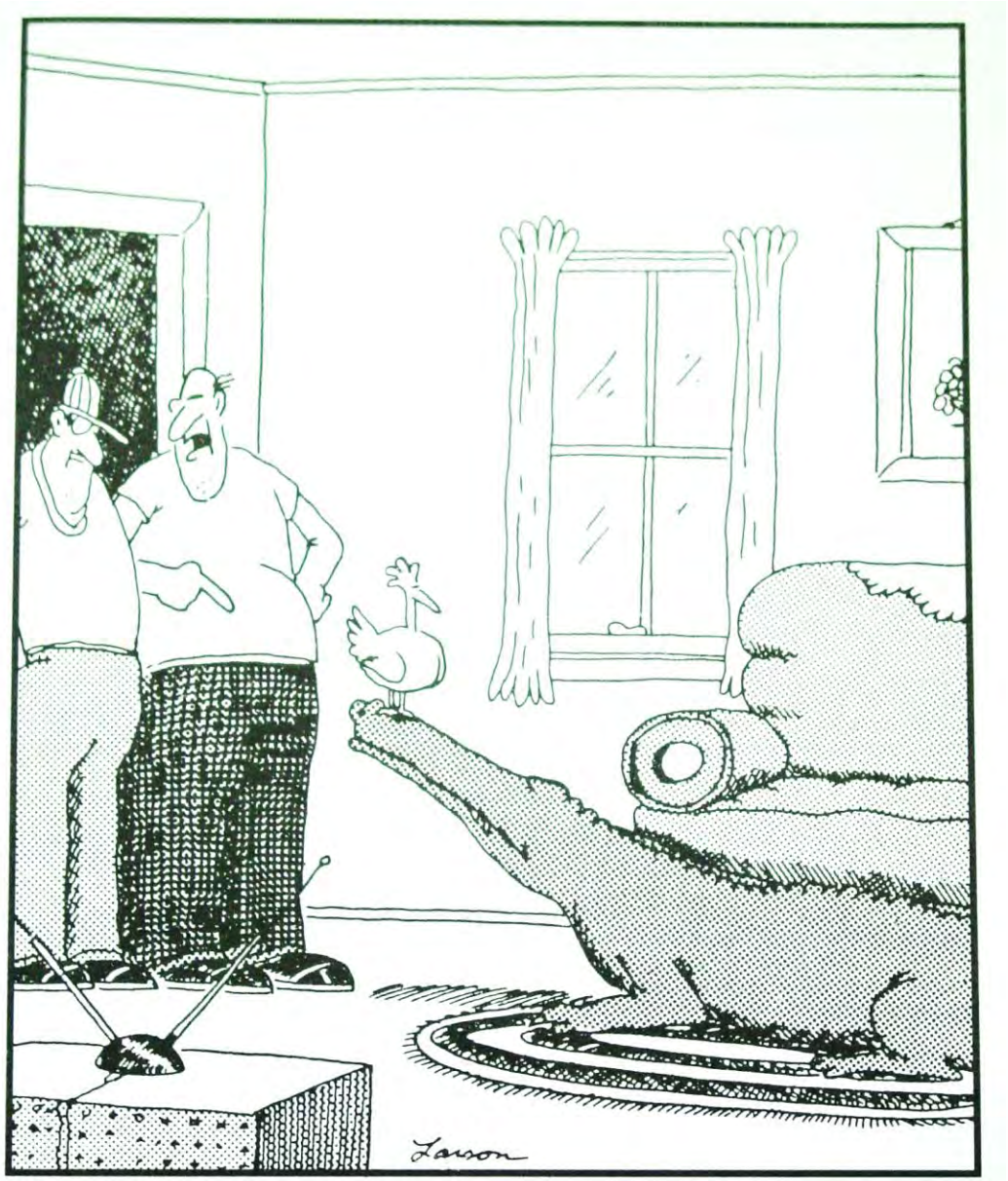
"Yes, yes ... now don't fuss. ... I have something for you all."

"I just don't like it, Al. ... Whenever Billy goes outside, the new neighbors seem compelled to watch every little thing he does."



"No, they're not real exciting pets—mostly they just lie around and wait to be fed—although a couple years ago Charles tried teachin' him to take a cookie from his mouth."

"Get, you rascal! Get! ... Heaven knows how he keeps getting in here, Betty, but you better count 'em."



"Now watch this. He'll keep that chicken right there until I say OK. ... You wanna say OK, Ernie?"