

HOW TO



PREPARE BIRD SPECIMENS

Part 8a – Tissue sampling for DNA, Toxicology
and other studies

Part 8b – Gut Analysis

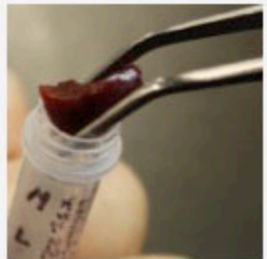




Photo taken at the Field Museum

The Migratory Bird Conventions Act regulates the take and possession of birds in Canada. The Migratory Bird Treaty Act regulates the take and possession of birds in the United States. In addition, the provinces (in Canada) and the states (in the United States) also require permits. For some species SARA, ESA, or CITES permits may be required.

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When handling dead birds, it is probably impossible to tell if a bird is infected with a pathogen that may cause human illness even if you know the cause of death to be a wound or an injury. Take reasonable precautions to protect yourself. The Ornithological Council offers a peer-reviewed fact sheet on avian zoonotic disease and safety precautions for those who handle birds in the field and in the lab.

<http://www.nmnh.si.edu/BIRDNET/documents/WNV&H5N1-FactSheet.pdf>



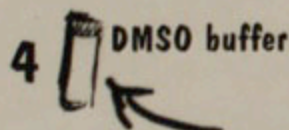
Tissue samples are preserved in a buffer solution or by freezing. Sometimes it is wise to use both methods simultaneously.

Specimen preparation in the field— tissue samples

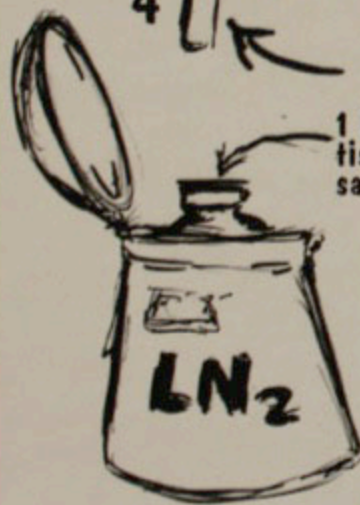
Four nunc tubes (pre-labeled)



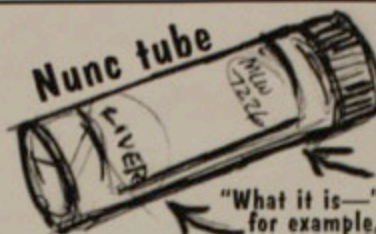
- 2 breast muscle
- 2 liver and spleen



1 breast
tissue
sample



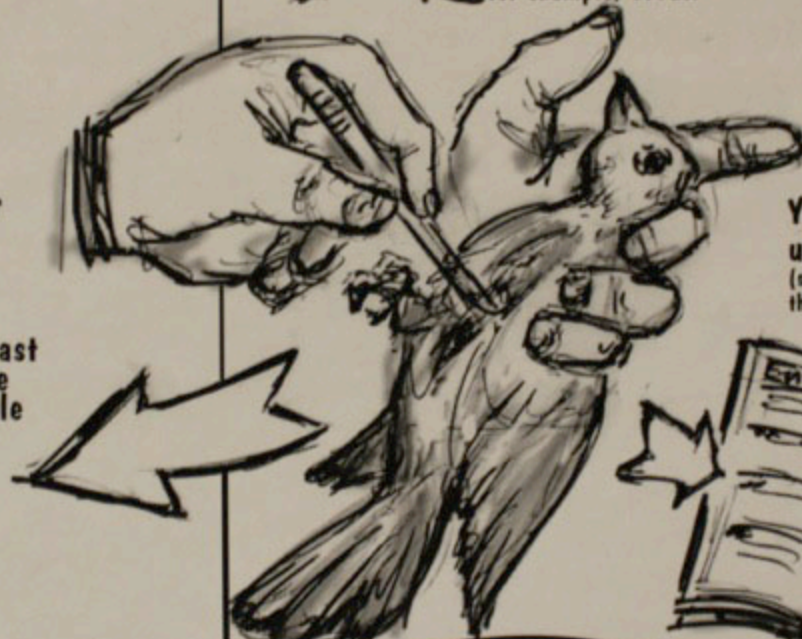
Liquid
Nitrogen



Proper labeling of
tubes is important!

Project location and
specimen number

"What it is—" for example, LIVER



Yellow sheet
used in field
(stays with specimen
throughout processing)



As you do this,
everything is recorded
in your notebook!

Field notebook





The possibility of a complete loss is one of the arguments in favour of storing tissue samples in a buffer solution or ethanol. The purpose of the buffer is to solubilize DNA or RNA while protecting it from degradation.

Ethanol dehydrates minced tissue.



For field collection, these methods eliminate the need for electricity (freezers), dry ice, or liquid nitrogen flasks.

Buffer use and formulations are beyond the scope of this presentation. Contact your local university or museum for up-to-date information.



These buffer filled vials are numbered inside and out - plus on the lid.

In order for the buffer to penetrate the tissue, each piece is minute.



For long term storage, tissues prepared with or without buffer are best stored at -80°C or colder. If budgets permit, using liquid nitrogen in cryogenic freezers (-196°C) is optimum.



Photo taken at the Field Museum

More commonly,
-80 °C chest or
upright freezers
are used.

Back up power
systems are key.
Be sure to have
some kind of
monitoring system.

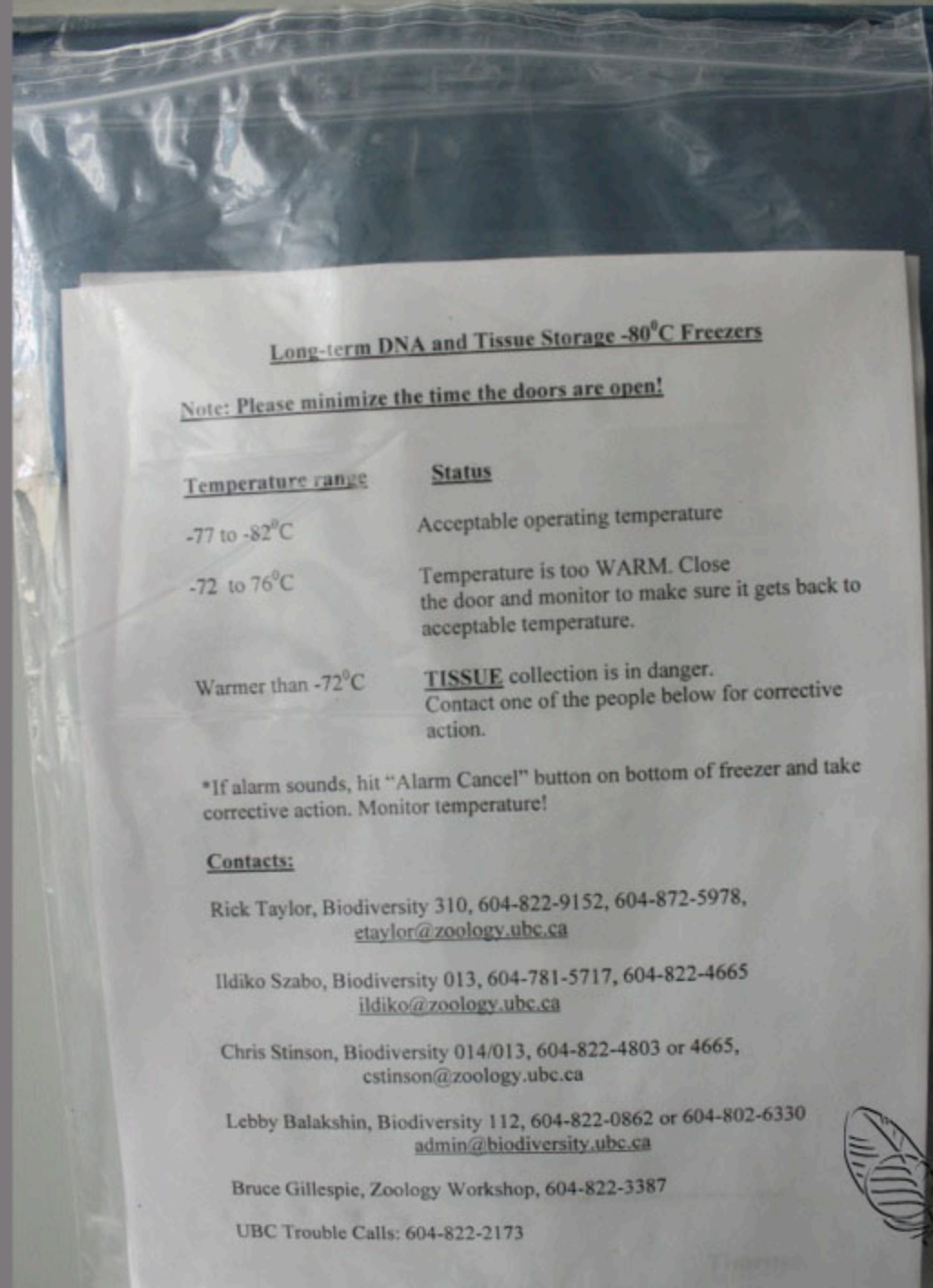
24 hours without
electricity, a flood,
or a fire could
destroy tens of
thousands of hours
of collecting effort.



Photo taken at the Louisiana State University
Museum of Natural Science



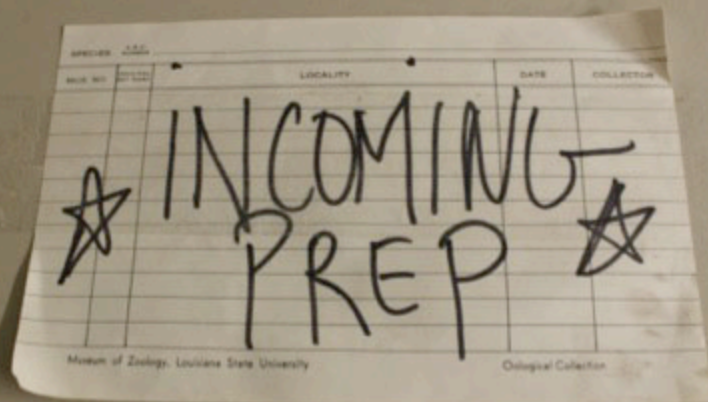
Post emergency instructions on or near the -80 °C Freezers.



Inside, 5 individual Styrofoam doors help keep the temperature constant in the sections not being accessed.

These metal racks, each holding 16 boxes facilitate quick access.





To minimize the time the freezer door is opened, many institutions have a Ziploc bag or a slot to drop tissue samples into.

Depending on volume, sequence vials and distribute to final storage location every 2-6 months.



Photo taken at the Louisiana State University
Museum of Natural Science

Collecting tissue is the easy part.

The real challenge is finding a specific vial in a large collection without melting or degrading tissue.

A good retrieval system is key.



Left photo taken at the Louisiana State University
Museum of Natural Science

Right photo taken at the Field Museum

FileMaker Pro - [birdtissues.FP5]

File Edit View Insert Format Records Scripts Window Help

Database: [1]

Records: 131198
Found: 42990
Unsorted

BIRDTISSUES

Collector No. **SML1167**

Location Tissue Remarks

10	6	23	10-6-23	frozen, muscle
10	6	28	10-6-28	frozen, liver
10	6	29	10-6-29	frozen, muscle
10	6	30	10-6-30	frozen, heart, testes

New

Catalog No. **320358** Bar Code No. **"320358"**

Order **Tinamiformes** Family **Tinamidae**

Genus **Crypturellus** Species **undulatus** Subspecies **undulatus**

Country **Peru** State/Province **Madre de Dios** District/County

Specific Locality **Hacienda Amazonia**

Latitude **0** Longitude **0** Altitude **500m**

Collector **S M Lanyon**

Sex **f** Preparation **k** Tissue **y** Tissue calc **Yes**

To find tissue with no location

Location Print

Database systems for internal collection use and sharing on the web are beyond the scope of this presentation.



Before collecting tissue samples contact the facility where you would like your vouchers and tissue samples stored at the end of your study.

Use their protocols and labelling system from the start.

It is practically impossible to adhere a new label to a frozen vial. A little homework makes a big difference in the long run.



Opinions differ as to which manufacturer produces the best tissue vials.

Look for a vial that has an O-ring or other sealing mechanism.

1.8ml is the most common size used.



Photo taken at the Louisiana State University Museum
of Natural Science

Post detailed vial labelling instructions.

Once the vials have been frozen, even for just 5 minutes, the vial surface is slippery. Neither ink nor pre-glued labels will adhere.

After the fact labelling or correcting labels usually involves thawing and transferring the tissue to a new vial.

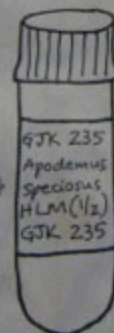
This is NOT acceptable.

INFORMATION TO PUT ON TISSUE TUBES:

TISSUE NUMBER
GENUS SPECIES
TISSUE TYPE (# OF TUBES)
TISSUE NUMBER

E.G.:

Writing "sideways" is also fine.



Write with a Sharpie, or better yet, a VWR lab pen. Never use a technical pen - the ink smears right off tube labels.

Procedures for filling and labeling tissue tubes

Top line: specimen's prep # on single line,

(RT) if applicable *Tamiasciurus only*

Next line: Genus species (if necessary, write only first 3 letters of species name)

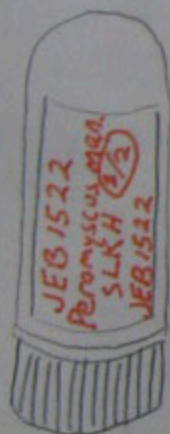
Next line: organs taken (tissues should be placed into & written onto tubes in this order: **S,L,K,H,M**),

tube # if applicable (ie: 1/2 or 2/2)

Bottom line: repeat specimen's prep #

Always use VWR lab pen

Always fill tubes to (but not over) 1.8ml





Note the M, L, H on the side of the vial.
This is a quick reference that the heart tissue
is at the bottom, the liver is in the middle,
and the muscle is on the top.
Placing the liver in first is common.

Decide on a protocol and stick to it.
Neither the heart or the liver should take up
more than $\frac{1}{3}$ of the vial.



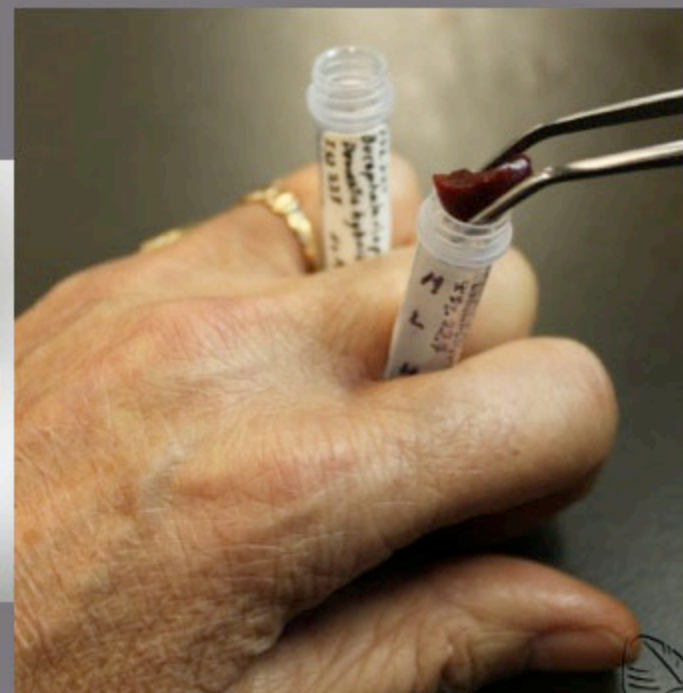
Passerine hearts are so small
that the entire heart is placed
in the vial.





To access the heart, tear the air sacks.
Do not remove medium or large hearts.
Subsample in place.

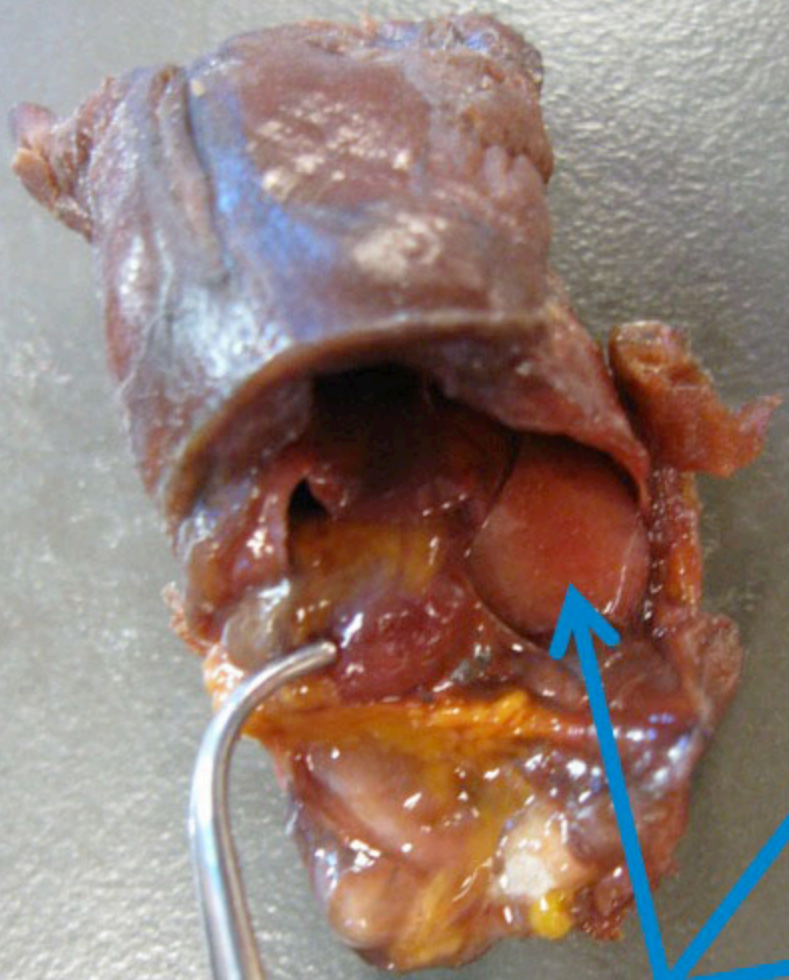
Note how the vials are braced between the fingers. This keeps the vials upright and allows you to use both hands.



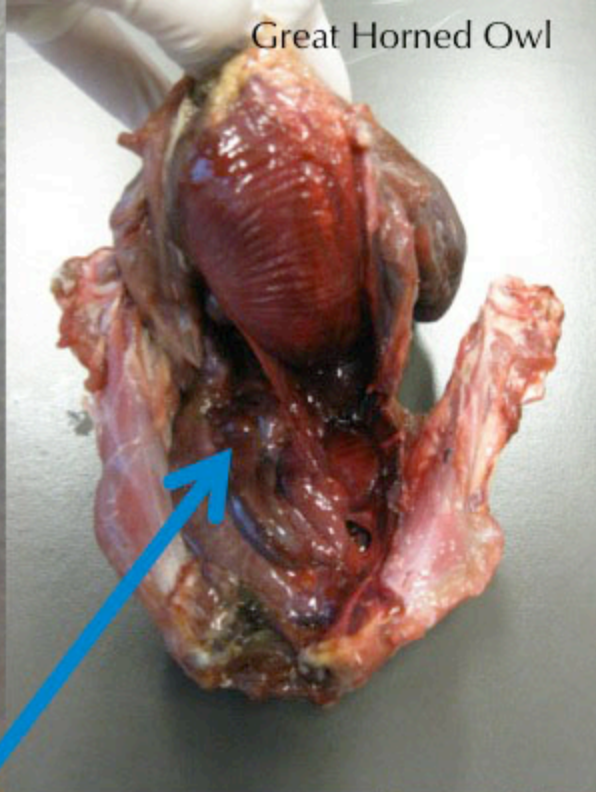
Use the same tools to skin the bird and take the tissue samples.
If the tools have touched another bird or are suspect, sterilise them first.



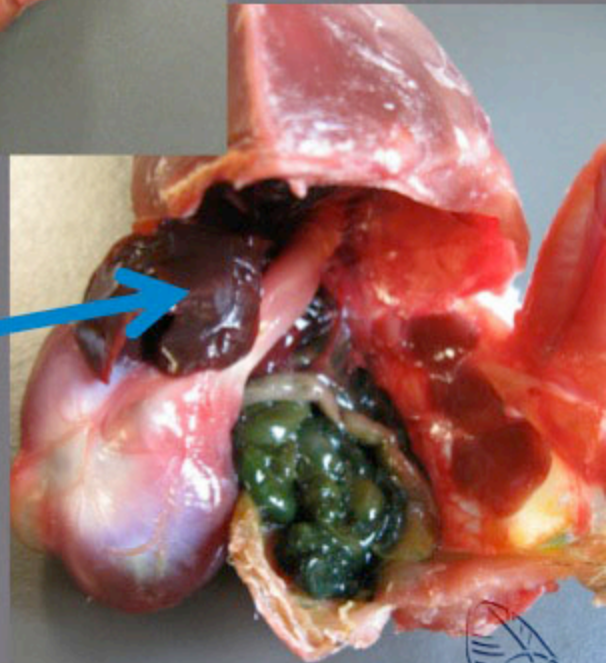
All these birds have healthy livers.



Lapland Longspur



Great Horned Owl

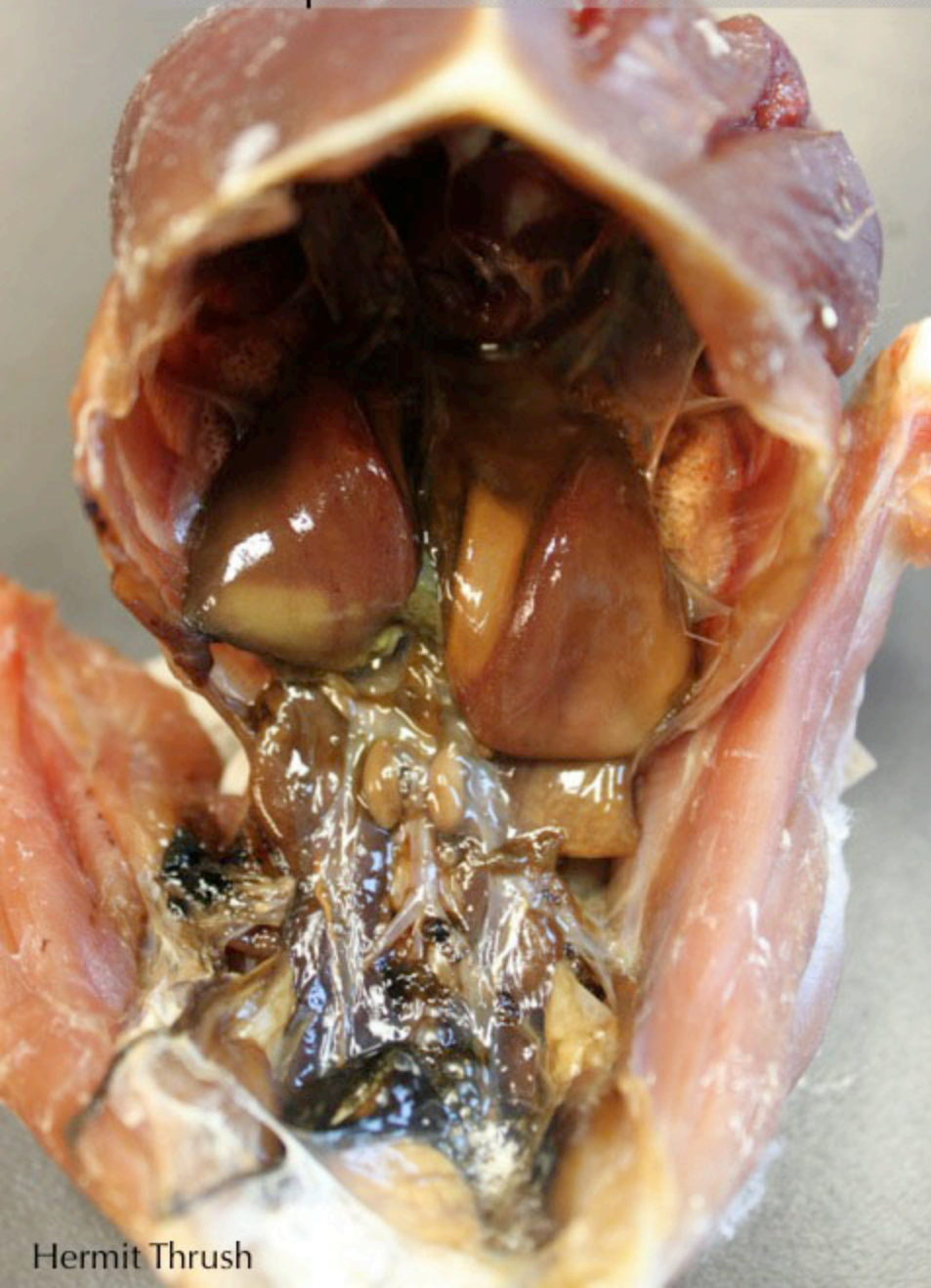


Band-tailed Pigeon

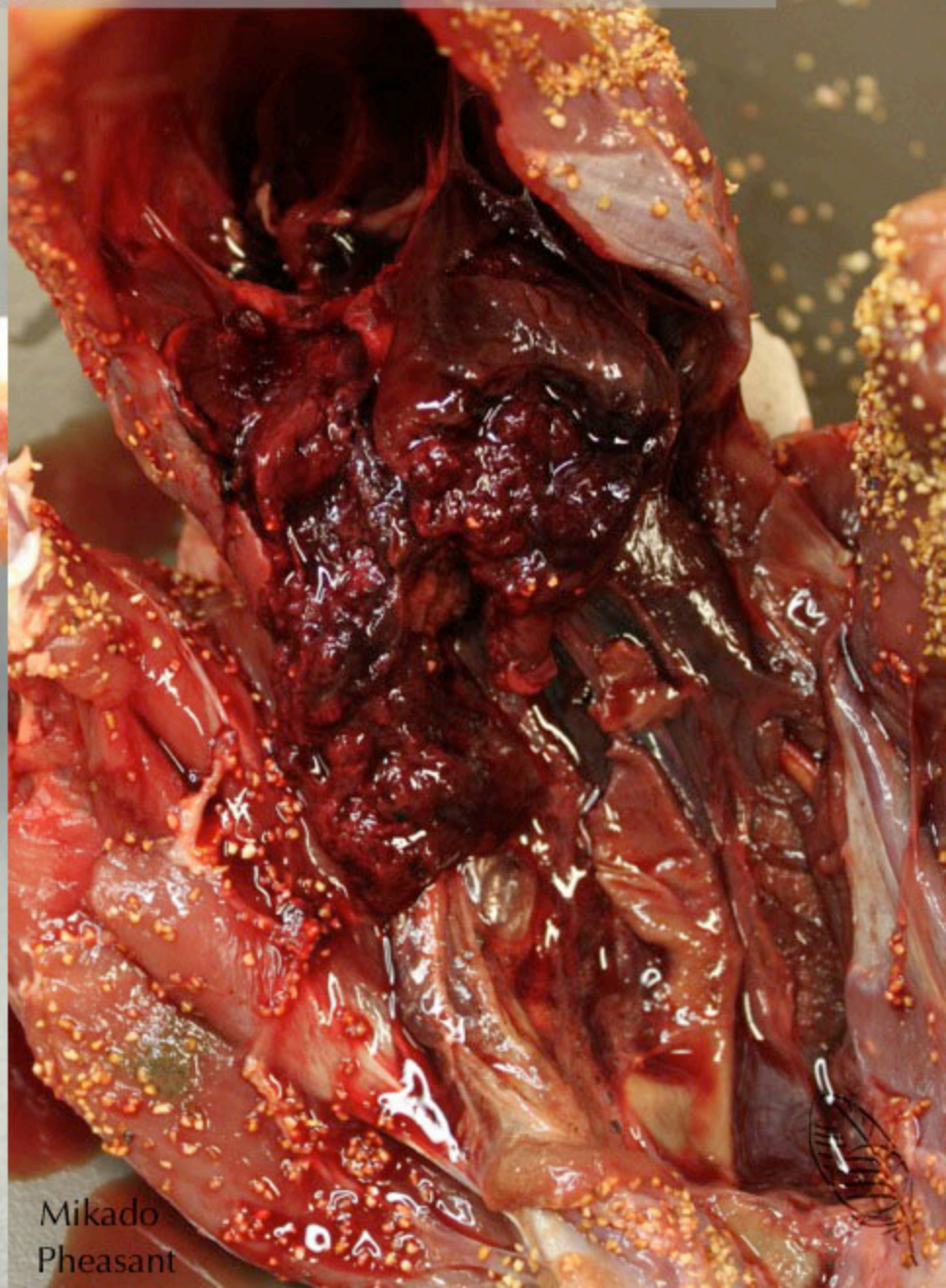
The liver is the first organ to decay.



When in doubt either do not take a sample or put the suspect liver tissue in a separate vial to avoid contamination.



Hermit Thrush



Mikado
Pheasant



Unclotted blood in the body cavity and inside the intestines is indicative of Warfarin-type rodenticide poisoning.

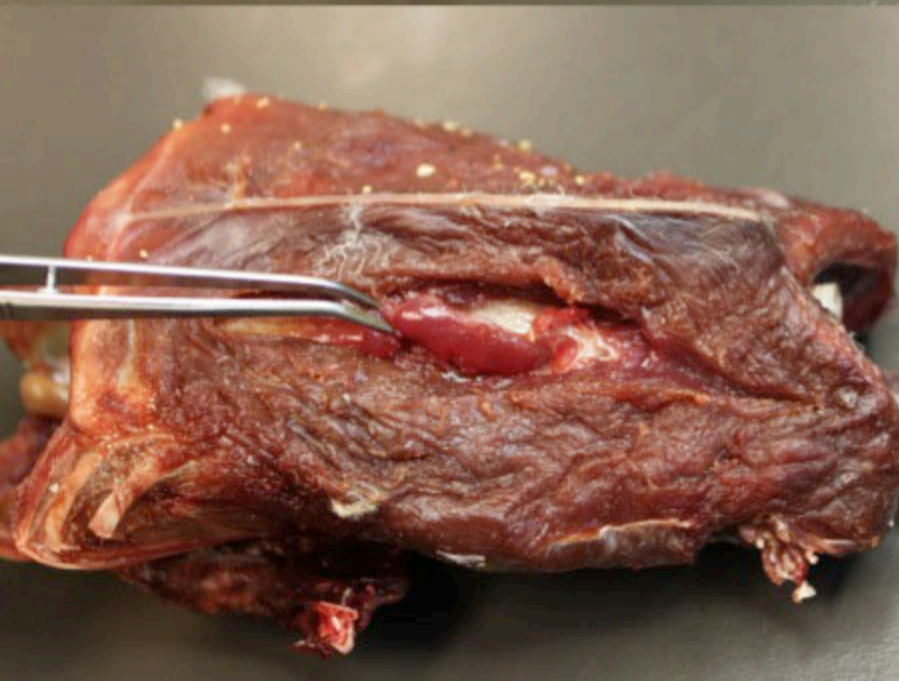


A green liver is indicative of lead poisoning.

If the organs look unhealthy, download Part 13a – Determining cause of death to see if you can find a match.



In medium and large birds, slice the first layer of breast muscle. Take the muscle tissue sample from the ultra-clean inner layer.



Consider putting a small muscle sample in the lid for easy extraction.



How the breast muscle is sampled depends on the bird.

In small birds, most of the breast muscle is required.



Lids of overfilled vials split when frozen. Dehydration is a problem when large air spaces are present.

Fill to the 1.8ml mark or leave only 2-3 mm of air space at the top. Visually check the vial when finished

Morphometric data must be collected before tissue sampling begins. Body weight and wingspan cannot be measured after tissue extraction (see Part 2).

Unless otherwise indicated, it is assumed that muscle tissue is breast muscle. Muscle quality is extremely important.

- The breast muscle near the furcula is fine
- The freezer burnt (dehydrated) muscle tissue is too degraded, do not sample
- Record on the datasheet if wing or leg muscle is substituted
- For hummingbirds and other extremely small birds, consider inserting the entire torso into the vial (breast muscle, bones, organs, but NOT the digestive track).



DO NOT COMPLETELY THAW BIRDS

Test the bird from time to time. Start skinning as soon as the skin will slide on the breast muscle and the legs wiggle.

There should still be ice crystals in the body cavity indicating that the liver, etc. are still partially frozen.



Collect tissue samples **IMMEDIATELY** !

Half thawed birds bleed less making them easier to skin.



Note the 2nd to last column of this tissue catalogue.
Each time a specimen is frozen or thawed is recorded.

B-Tissue Number	Preparator Number	Genus species	sex	material dry ice, LN2, etc.	LOCALITY	collection date	prep date	collector	remarks: specimen handling	tissue types
73307	JKI 21	<i>Corcyzus americanus</i>	♂	freezer	Texas: Jeff Davis County; Limpia Canyon/Ft. Davis; Davis Mountains	11 Jul 2008	12 April 2011	Bill Wright	Re-thawed → freezer	M, H, L
73308	BMP 06	<i>Zonotrichia leucophrys</i>	♀	dry ice	Texas: Hutchinson Co. Burgess-Herring Ranch, Short Creek, Ca 8 mi WSW Stinnett	3 Feb 2006	13 April 2011	John P. O'Neill	netted → freezer free prep dry ice	M, H, L
73309	DLD 10216	<i>Melospiza melodia</i>		dry ice	"	2 Feb 2006	"	"	shot → dry ice → freezer → prep	"
73310	DLD 10217	<i>Zonotrichia leucophrys</i>		"	"	3 Feb 2006	"	"	"	"
73311	DLD 10215	<i>Spinus tristis</i>		freezer	LOUISIANA: Iberville Par.: 4 mi. N St. Gabriel, 435 Pecan Drive	13 Jan 2003	"	SWC	shot → freezer → prep	"
73312	CEB 32	<i>Passerculus sandwichensis</i>	♂	"	LOUISIANA: Jefferson Davis Par.; 1 mi. SW Thruwell	20 November 2010	14 April 2011	Clare E. Brown	"	M, H, L
73313	BMP 07	<i>Bombacilla cedrorum</i>		"	Texas: Brewster Co.; Christmas Mountains Oasis	28 May 2010	14 April 2011	Britt M. Perry	found dead → freezer → prep	"
73314	DLD 10218	<i>Pheucticus melanocephalus</i>		"	TEXAS: Jeff Davis Co.; Davis Mountains Resort, 414 Limpia Canyon Trail	5 May 2009	"	"	"	"
73315	" 10219	<i>Rallus longirostris</i>		"	LOUISIANA: East Baton Rouge Par.; Baton Rouge, 6765 Corporate Blvd.	21 March 2011	"	N Bryce Hill	"	"
73316	JKI 22	<i>Tonostoma curvirostre</i>	♂	"	TEXAS: Jeff Davis Co.; Hwy 166 x Blue Mountain Vineyard	5 September 2009	"	Kelly B. Bryan	"	"
73317	CEB 33	<i>Passerculus sandwichensis</i>	♀	"	LOUISIANA: Jefferson Davis Par.; 1 mi SW Thruwell	20 November 2010	"	Clare E. Brown	shot → freezer → prep	M, H, L
73318	CEB 34	<i>Poecetes gramineus</i>	♀	"	TEXAS: Grimes Co.; 3 mi. SE Anderson, 5893 FM 1794 Road	17 May 2006	"	Leticia A. Alania	found dead → freezer → prep	M, H, L
73319	JKI 23	<i>Zonotrichia leucophrys</i>	♀	"	TEXAS: Hutchinson Co.; Burgess-Herring Ranch, Short Creek, Ca 8 mi WSW Stinnett	3 Feb 2006	26 April 2011	John P. O'Neill	netted → freezer fridge → prep	M, H, L
73320	BMP 08	<i>Melospiza melodia</i>	♂	"	"	"	"	"	"	M, H, L
73321	DLD 10220	<i>Catharus guttatus</i>			TEXAS: Culberson Co.; Sierra Diablo WMA	24 Oct 2007	"	"	"	"
73322	DLD 10221	<i>Melospiza melodia</i>			TEXAS: Hutchinson Co.; Burgess-Herring Ranch, Short Creek, Ca 8 mi WSW Stinnett	3 Feb 2006	"	"	shot →	"
73323	JKI 24	<i>Cardinalis</i>			LOUISIANA: Iberville Par., 4 mi N St. Gabriel, 435 Pecan Drive	3 May 2011	3 May 2011	SWC	found (mortimer) → fridge → prep	"

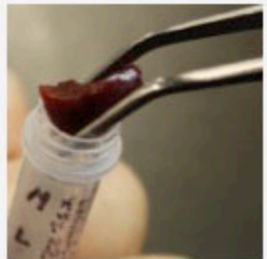
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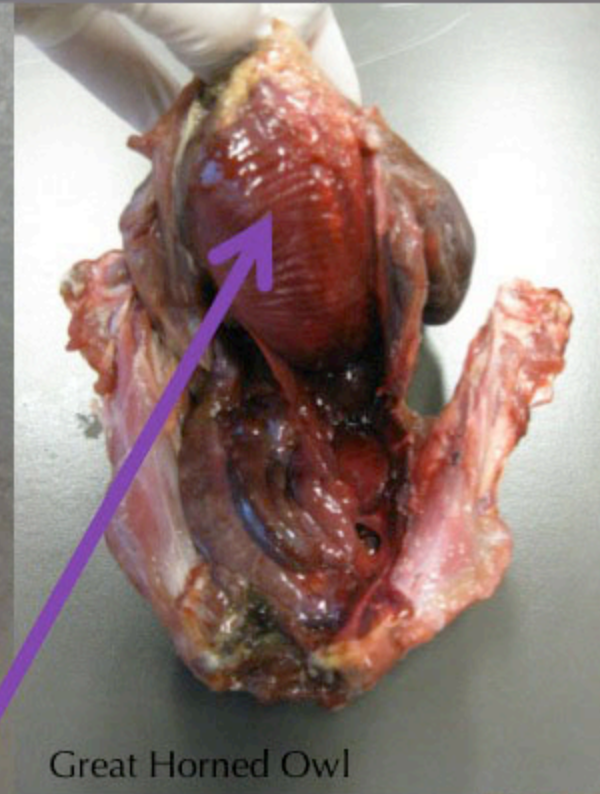
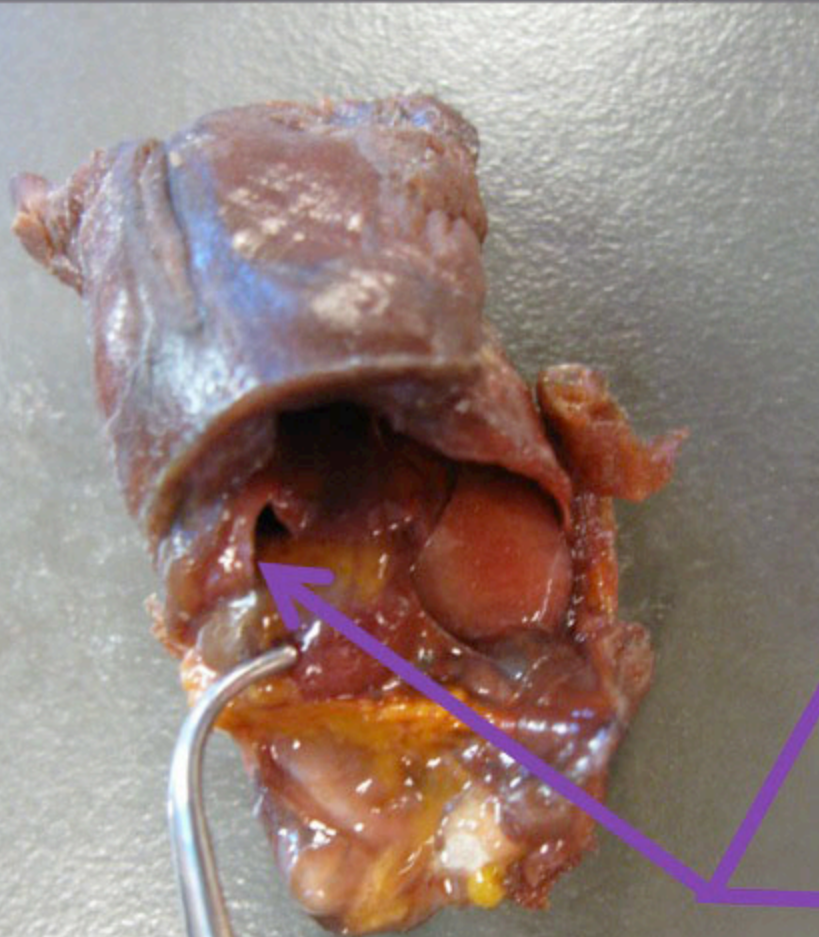
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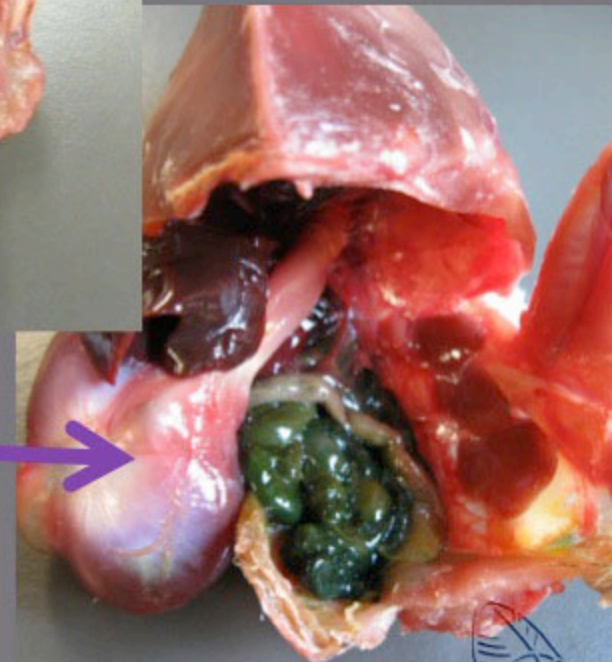
Juvenile California Quail



★ Always take tissue samples and measure the gonads **before** starting gut contents analysis.



Great Horned Owl



Band-tailed Pigeon

The gizzard is usually called the stomach. The gizzard is technically the second part of a bird's stomach. It is surrounded by muscular walls.

Find the stomach (gizzard) and cut it open.

Lapland Longspur

On the specimen label,
rank the different items from
highest to lowest frequency.
This bird's label would read:

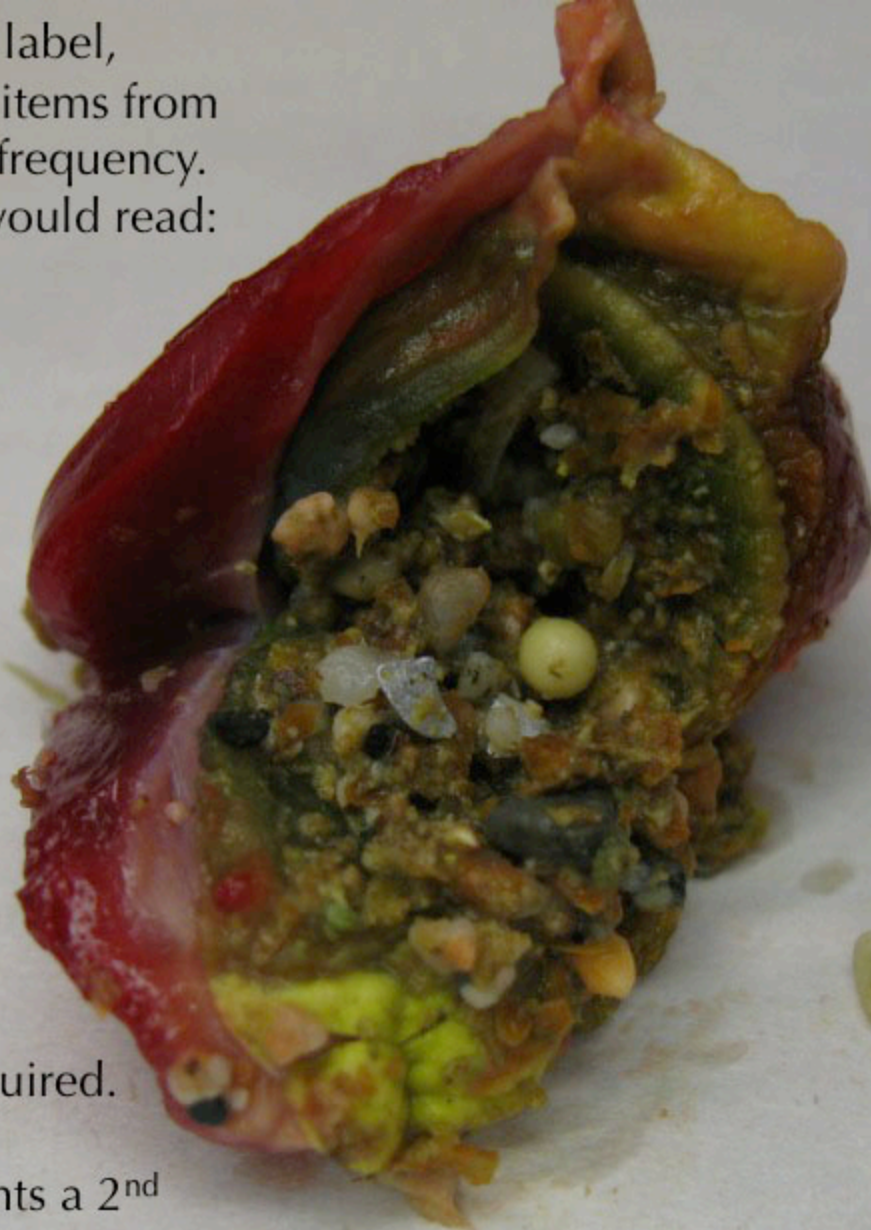
*Stomach: grit,
digested brown
matter, seed*

If the stomach is
empty, record as:

Stomach: empty

If the crop is
empty,
no notation is required.

Record gut contents a 2nd
time on the data sheet.



Many types of birds swallow
grit and small rocks to aid
digestion.

If you are studying foraging
behaviour or food intake,
detailed descriptions and
measurement of the grit may
be important.



Rock Pigeon

Exceptionally fine or large grit should be noted.



Gray Peacock-pheasant

Always note unusual items.



This domestic Sandhill Crane chick has a piece of clear glass and zipper tab fragments.

Finding plastic in seabirds, landfill, or urban garbage specialist is becoming more and more common.

Emaciation is not unusual in such birds. A full stomach reduces the urge to eat.

This Northern Fulmar has eaten air gun bullets and plastic fragments. Count and measure items from anthropogenic sources.



Photo courtesy of Stephanie Avery-Gomm



These stick-like fragments are arthropod appendages.

How plant, arthropod, avian, or mammal remains are labelled depends on your expertise.

Ideally identify all items to species. If, for instance, you recognize that these arthropod appendages are crustacean appendages, record this on the data sheet.

.....
Depending on lab protocol, all or only interesting gut contents are preserved in 70%-90% ethanol.

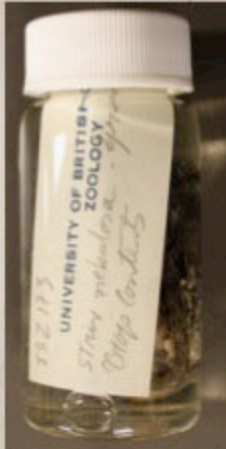
Use a permanent ink that does not dissolve in alcohol for the internal vial label. If you do not have one, use a pencil. Writing the prep number or specimen number in addition to the bird species name on the top of the lid speeds up specimen retrieval.



Not only can these prey items be identified, one of them is in good enough condition to be prepared as a specimen. If a prey item is accessioned and given it's own specimen number, make sure there are cross-references.



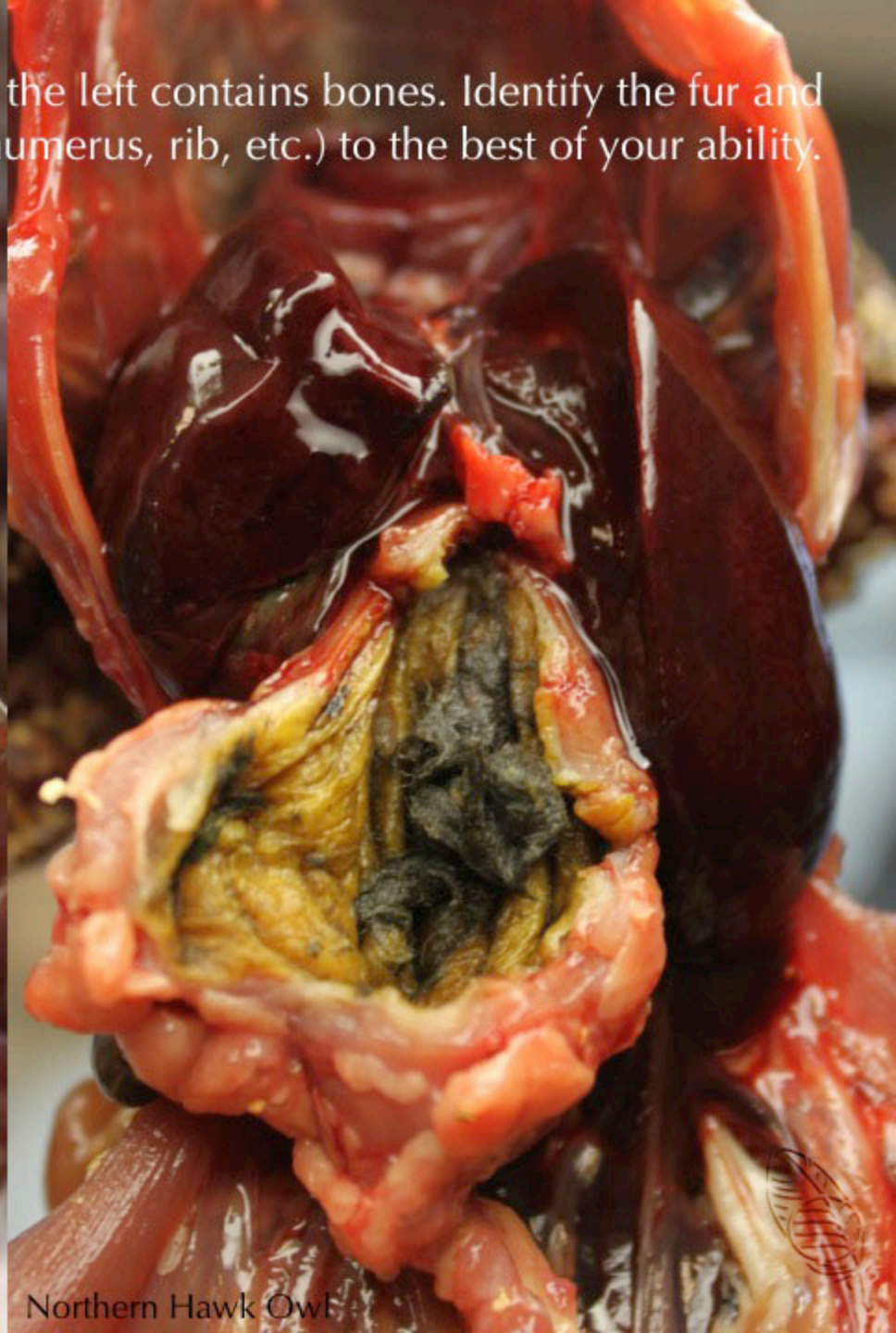
Short-eared Owl



Record that the fur ball on the left contains bones. Identify the fur and the type of bones (femur, humerus, rib, etc.) to the best of your ability.



Great Horned Owl



Northern Hawk Owl

The crop is a widening of the oesophagus. If present, it is a food filled sac on the ventral side of the neck and often covers the furculum.

Identify the crop and stomach contents at the same time. Food items in the crop are usually less digested than those in the stomach making them easier to identify.

It is imperative to removing these blackberries before they stain the feathers.





The stomach has digested green plant matter (clover & unidentified), yellow seeds, and grit.

The crop of this Ruffed Grouse contains liquorice fern and clover.

Common name: Osprey
Scientific name: *Pandion haliaetus*
Date collected: 1/1/2018
Collector: Cow

For your own comfort and those working around you,
remove the crop contents of fish eating birds immediately!
In these images, the crop contents is a frozen block.
This Osprey was not skinnable for another two hours.

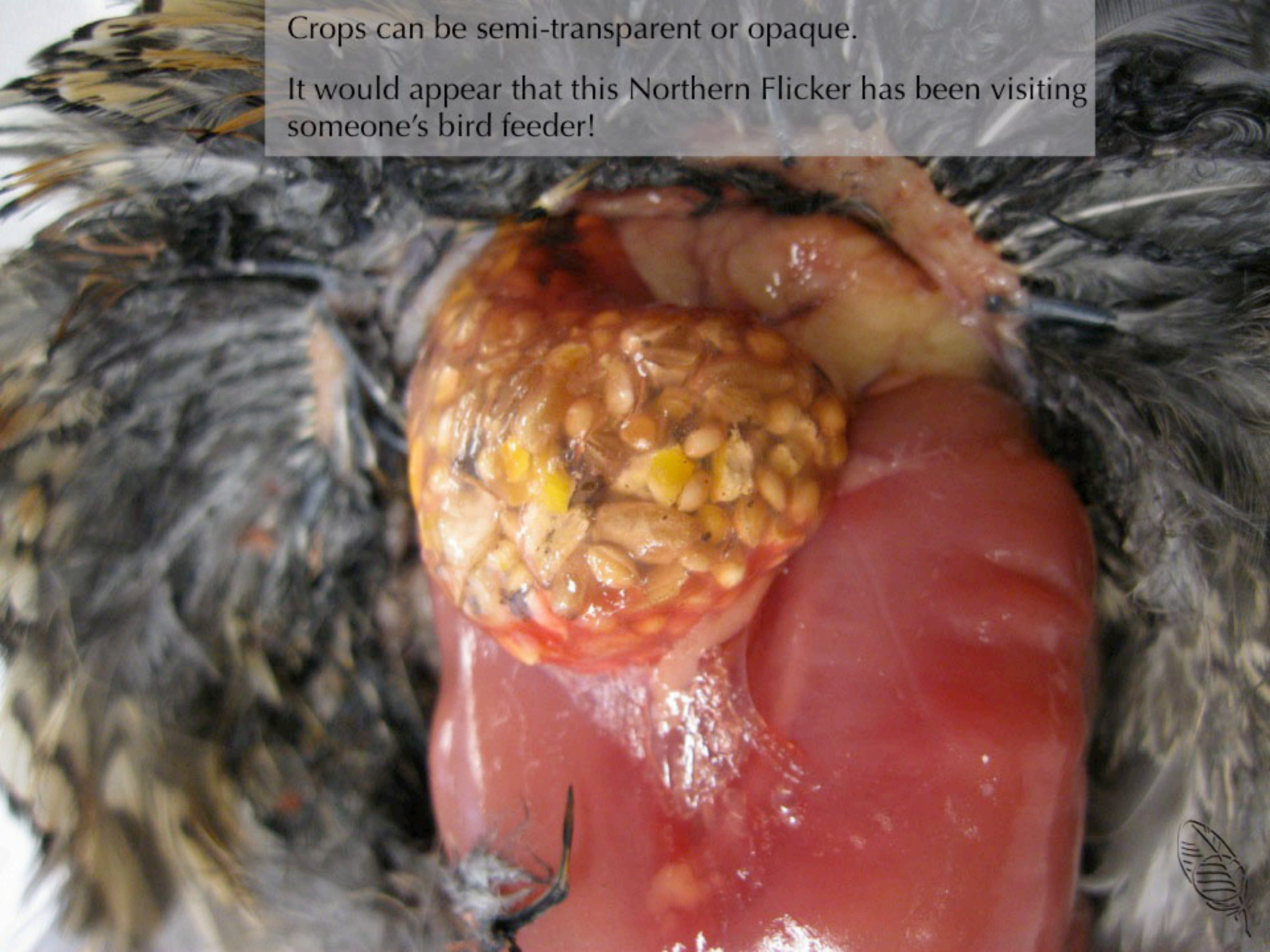
Freezing is another way to preserve crop/stomach
contents for future analysis.

Record the weight of sizable crop contents.



Crops can be semi-transparent or opaque.

It would appear that this Northern Flicker has been visiting someone's bird feeder!



IN MEMORIAM



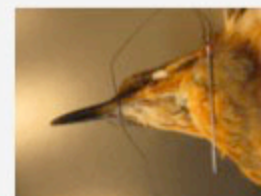
DR. REX KENNER

Former Curator of the Cowan Tetrapod Collection who encouraged me to begin this project.

Special thanks to David Willard, Donna L Dittmann, Steven W Cardiff, Darren Irwin, Eve Szabo, Peter Mortensen, Hans van Brandwijk, Victoria Bowes, Christopher M Stinson, Chris Wood, Ellen Paul, and all the museum curators and collection managers who has helped and encouraged me to complete this project. I take full responsibility for any remaining mistakes.

Without the technical assistance of Derek Tan, this project would never have gotten off the drawing board. Dr. Darren Irwin kindly suggested and made the arrangements for this series to be posted on the Beaty Biodiversity Museum website. A huge thank you to the staff and volunteers at the Cowan Tetrapod Collection for providing space and creating a terrific work environment.

Unless otherwise indicted, all pictures were taken by the author at the Cowan Tetrapod Collection, University of British Columbia Beaty Biodiversity Museum.



OTHER



PRESENTATIONS IN THIS SERIES

Introduction: The look of the bird & A few things to look for

Part 1 - Spread wings, a good way to start

Part 2 - Skinning your first bird

Part 3 - Other skinning methods

Part 4 - Stuffing your first bird

Part 5 - Other stuffing and pinning methods & Bird parts

Part 6 - Sexing birds using gonads (includes 2 quizzes with answer sheets)

Part 7 - Determining skull pneumatization & Skeleton preparation

Part 8 - DNA tissue sampling & Gut analysis

Part 9 - Washing skins for ectoparasites & Drying washed skins

Part 10 - Recording fat levels & Cleaning fatty or stinky skins

Part 11 - Flat skins, shmoos, and other types of study skins

Part 12 - Preserving eggs and shell fragments (in prep)

Part 13 - Determining cause of death

Part 14 - Labelling: the most important step

To download another PowerPoint presentation in this series go to:

<http://www.beatymuseum.ubc.ca/research/birds>

