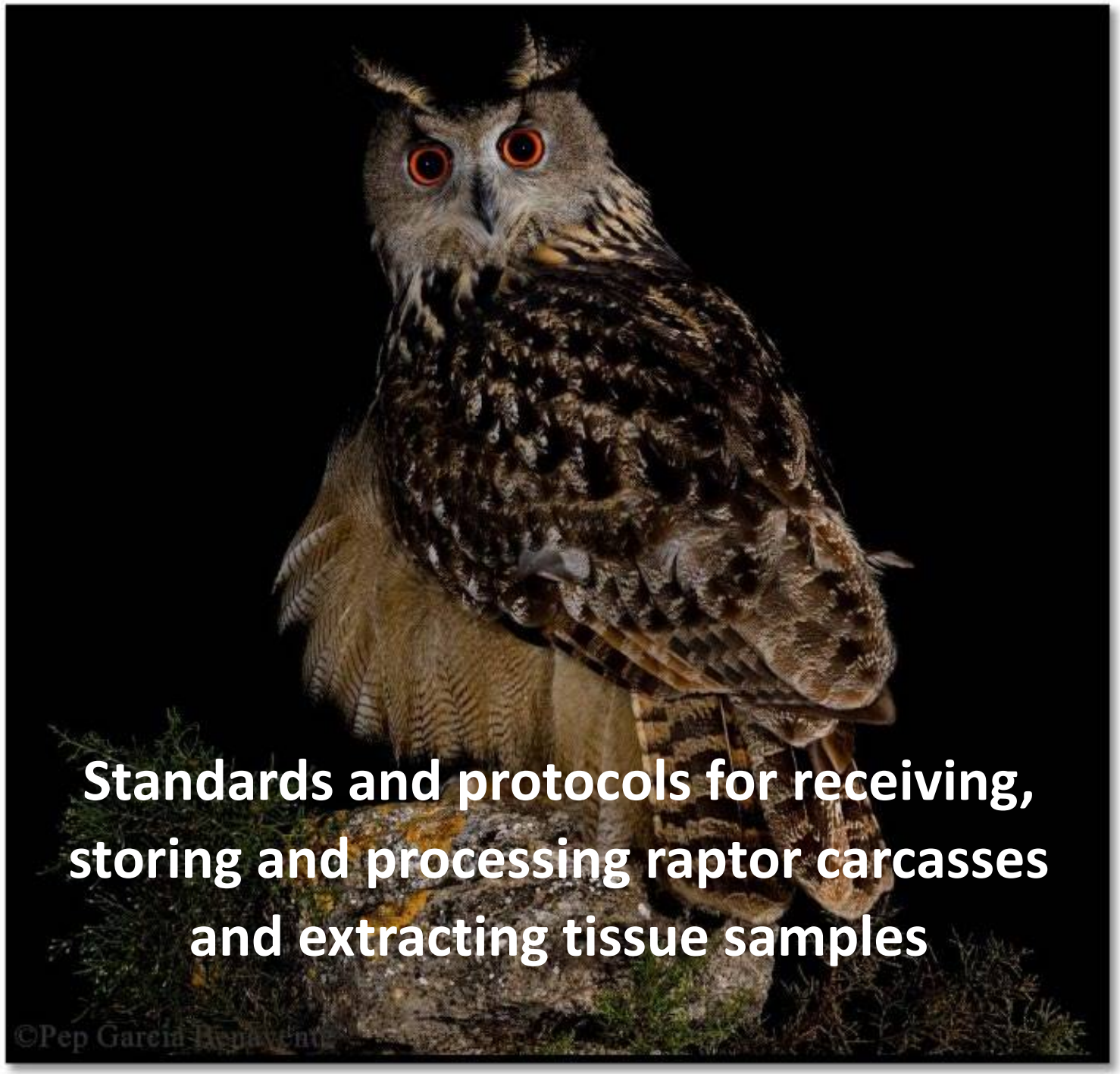


Standards and protocols for receiving, storing and processing raptor carcasses and extracting tissue samples

European Raptor Biomonitoring Facility

ERBFacility WG3 Collections – Virtual Meeting

11-12 February 2021

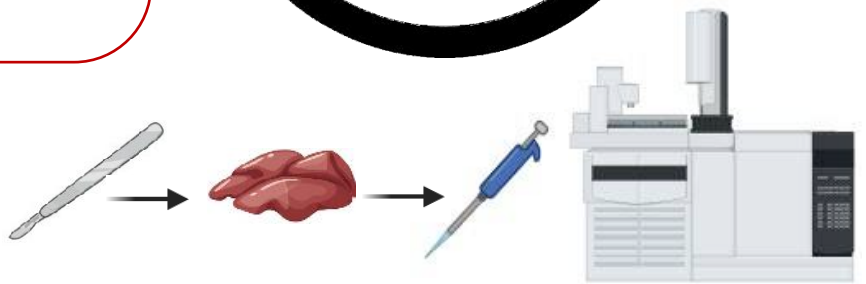
Silvia Espín, Giuseppe Cicero & Paola Movalli

European Raptor Biomonitoring Facility

Context

- Thousands of raptor carcasses arrive annually at Europe's natural history museums (NHMs)
- **NHMs** are very important **collection partners for European contaminant biomonitoring**

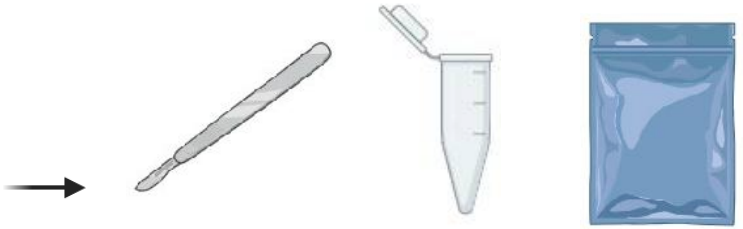
- Many carcasses are stored in freezers, but many are discarded
- Contaminant biomonitoring in raptors is relatively novel for most museums
- Very few NHMs gather, store and process raptor carcasses with contaminant monitoring in mind



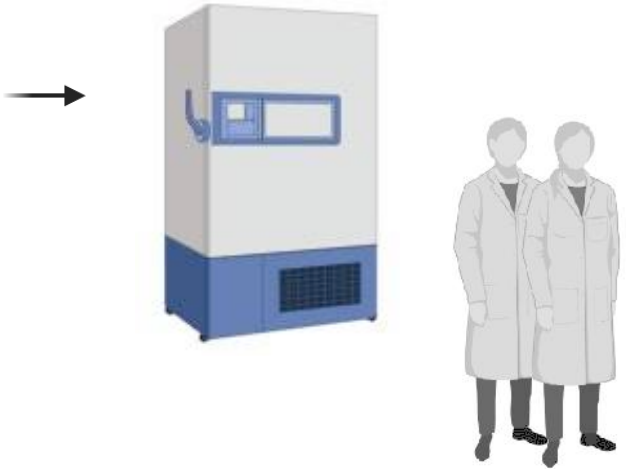
Context

There may be issues regarding:

- Using the correct **processing and storage methods** to conserve samples for contaminant monitoring purposes



- **Constraints** such as freezer capacity to store carcasses or availability of staff to process samples (*Gloria Ramello*)



- **Digitisation** of records to allow for easy access to data on raptor carcasses and tissues (*Konstantinos Vlachopoulos*)

Therefore, a STSM was designed by WG3…

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STSM: Standards and protocols for gathering, processing and storing raptor specimens/samples for contaminant monitoring

STSM objective:

To develop a **detailed protocol**, tailored for NHMs, for the gathering, processing and storage of raptor specimens/samples, with a view to subsequent contaminant analyses.

Host

Group of Toxicology

University of Murcia, Spain

STSM holder

Giuseppe Cicero

University of Palermo, Italy

European Raptor Biomonitoring Facility

The mission has involved a rapid review of **existing standards and protocols**

Example:

Ambio 2021, 50:95–100
https://doi.org/10.1007/s13280-020-01341-9

PERSPECTIVE

A schematic sampling protocol for contaminant monitoring in raptors

Silvia Espín, Jovan Andevski, Guy Duke, Igor Eulaers, Pilar Gómez-Ramírez, Gunnar Thor Hallgrimsson, Björn Helander, Dorte Herzke, Veerle L. B. Jaspers, Oliver Krone, Rui Lourenço, Pedro María-Mojica, Emma Martínez-López, Rafael Mateo, Paola Movalli, Pablo Sánchez-Virosta, Richard F. Shore, Christian Sonne, Nico W. van den Brink, Bert van Hattum, Al Vrezec, Chris Wernham, Antonio J. García-Fernández

Sampling protocol overview

Click on the name of the matrix to see the schematic protocol for each sample type.

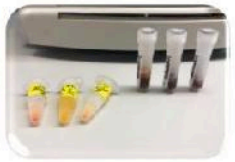
Click here to see important general guidelines related to permits and health and safety issues when sampling.

ACTIVE MONITORING

(Trapped live birds and nests)

Blood

Plasma/Serum



Deserted/Addled eggs

Feathers

Preen oil

Regurgitate pellets/Prey remains

PASSIVE MONITORING

(Dead birds)

Internal tissues

Gastric content

Feathers

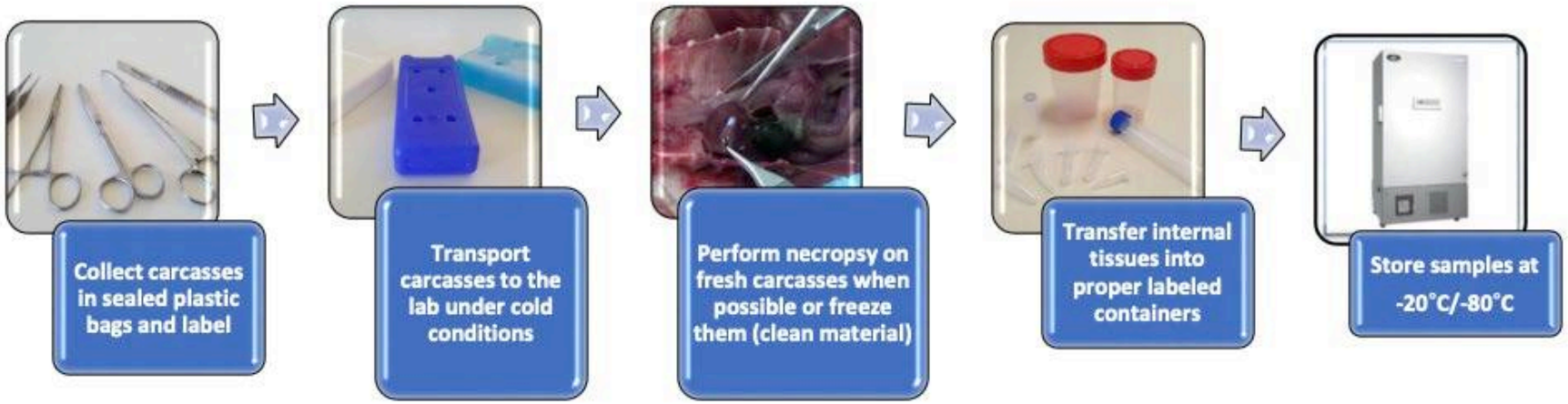
Preen oil/Gland

Click here to see Table 1. Volume/Mass of sample, type of container and transport conditions required for contaminant monitoring in different matrices

Click here to see Figure 1. What can we measure in each sample type? (a. Active monitoring / b. Passive monitoring)

Schematic protocol for internal tissues/gastric content

Click here to get additional information



Click here to see video

Click here to see Table 1. Volume/Mass of sample, type of container and transport conditions required for contaminant monitoring in different matrices

Click here to see Figure 1. What can we measure in each sample type? (a. Active monitoring / b. Passive monitoring)

Go back to the main menu here

Espín *et al*. 2020. A schematic sampling protocol for contaminant monitoring in raptors. *Ambio (Electronic Supplementary Material)*

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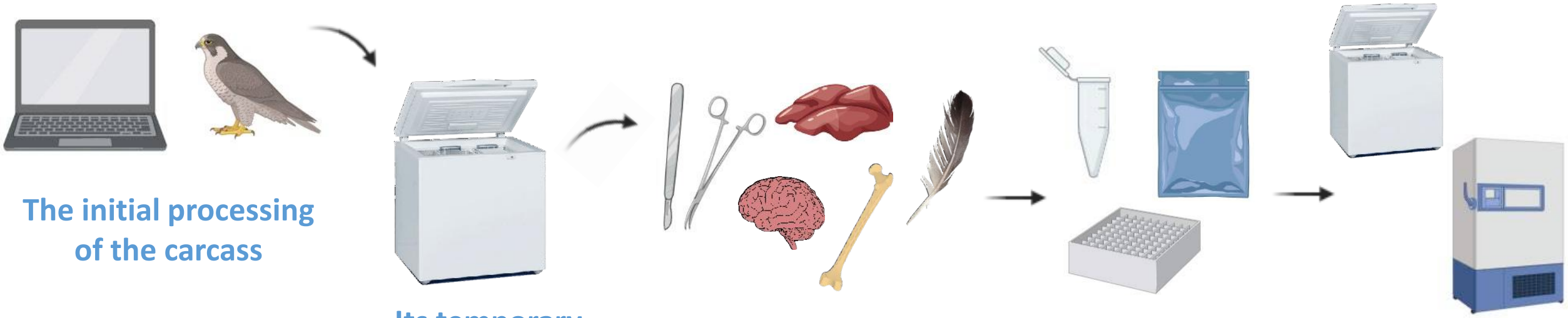
The new protocol for NHMs

- Protocol focused on **fresh carcasses and tissue samples** (for contemporary contaminant monitoring)
- The protocol is **not** intended to cover larger historical archive collections of **dry tissues** (e.g. skins and bones)

The protocol will start from the receipt of a raptor carcass at a collection, and covers:



The initial processing of the carcass

Its temporary storage in freezer

Subsequent preparation (necropsy) and storage of tissue samples for contaminant analyses

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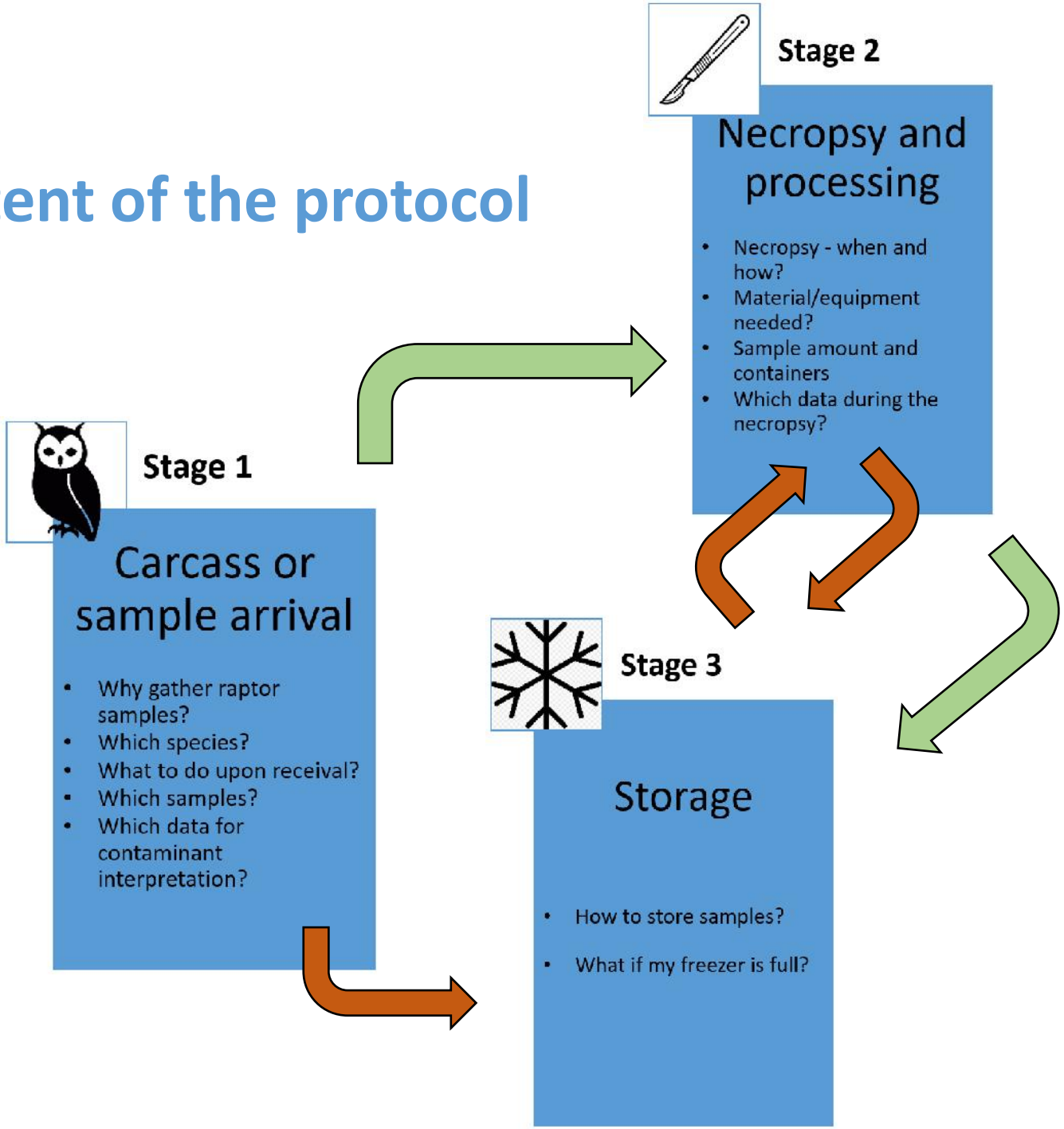
Structure & content of the protocol

Easy-to-follow format: Q&A



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Stage 1: Carcass or sample arrival

Why should I gather raptor samples?

Europe-wide biomonitoring of contaminants in raptors

Evaluate and reduce chemical risks to raptors themselves, to the wider environment and to human health

Cooperation between different arenas (field, collections & analysis)

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Stage 1: Carcass or sample arrival

Which species may be most suitable for biomonitoring studies?

Science of the Total Environment 731 (2020) 139198

Contents lists available at ScienceDirect

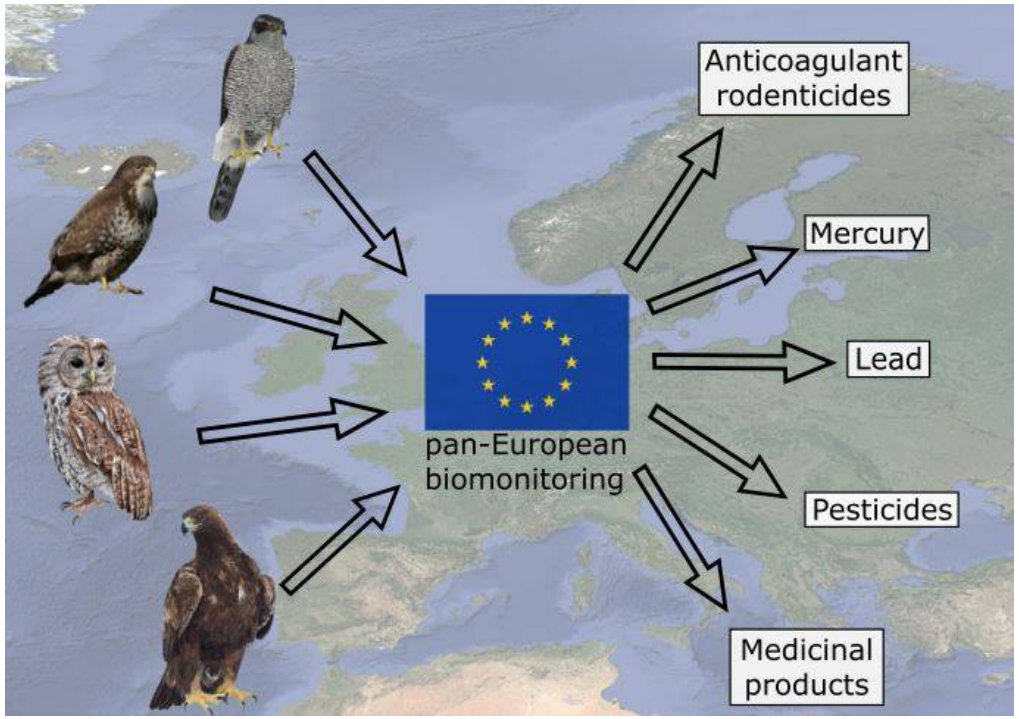
Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Review

Towards harmonisation of chemical monitoring using avian apex predators: Identification of key species for pan-European biomonitoring

Alexander Badry [a,*], Oliver Krone [a], Veerle L.B. Jaspers [b], Rafael Mateo [c], Antonio García-Fernández [d], Madis Leivits [e], Richard F. Shore [f]



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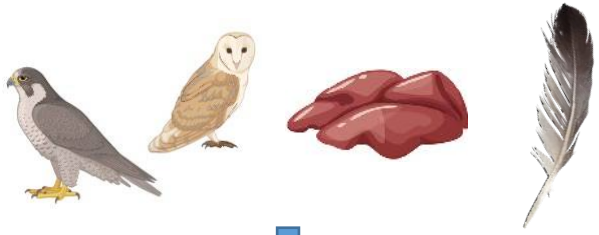
Stage 1: Carcass or sample arrival

What should I do when I receive a raptor carcass/sample?

Raptor Carcass / tissue



Unique Identifier
(track specimens)

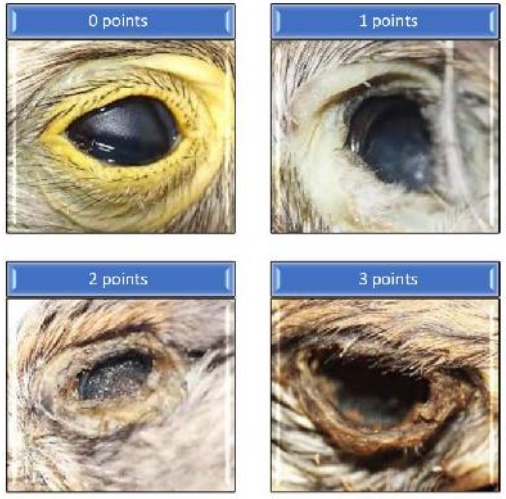
Initial inspection and examination
(state of the carcass/sample)

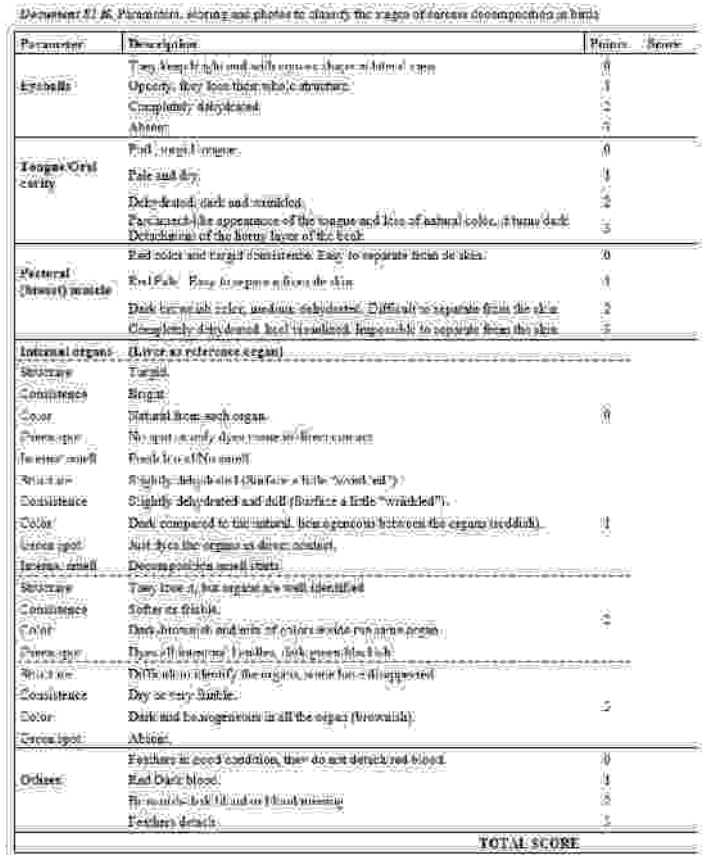
European Journal of Wildlife Research (2020) 66: 93
https://doi.org/10.1007/s10344-020-01429-3

ORIGINAL ARTICLE

Protocol to classify the stages of carcass decomposition and estimate the time of death in small-size raptors

Irene Valverde[1] · Silvia Espín[1] · Pedro María-Mojica[1,2] · Antonio J. García-Fernández[1]





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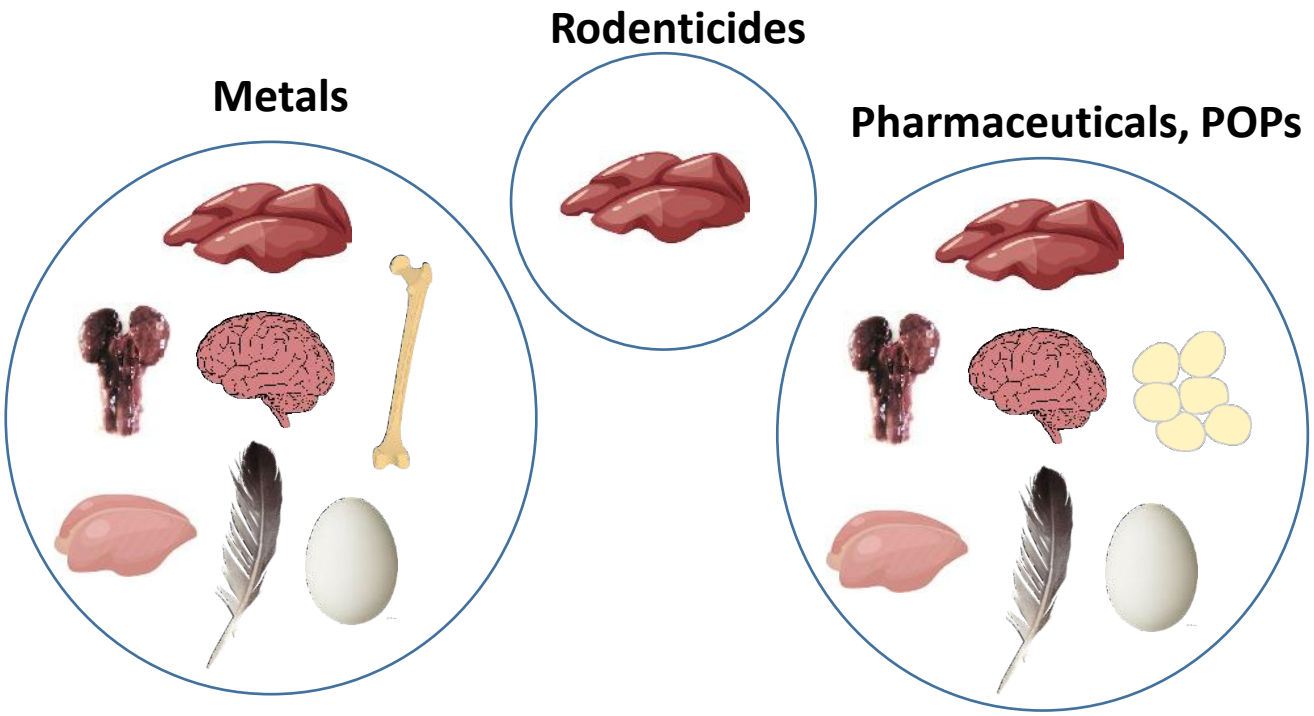
Stage 1: Carcass or sample arrival

Which tissue types are useful for contaminant biomonitoring?

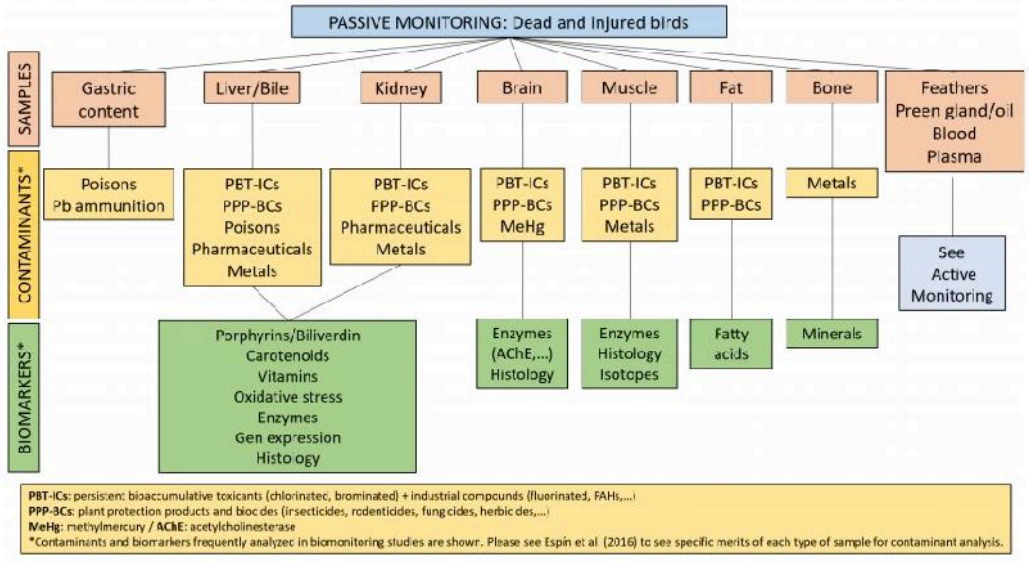


Ecotoxicology (2016) 25:777–801
DOI 10.1007/s10646-016-1636-8

Tracking pan-continental trends in environmental contamination using sentinel raptors—what types of samples should we use?

S. Espín[1,2] · A. J. García-Fernández[1] · D. Herzke[3] · R. F. Shore[4] · B. van Hattum[5,16] · E. Martínez-López[1] · M. Coeurdassier[6] · I. Eulaers[7,14] · C. Fritsch[6] · P. Gómez-Ramírez[1] · V. L. B. Jaspers[7,8] · O. Krone[9] · G. Duke[10] · B. Helander[11] · R. Mateo[12] · P. Movalli[13] · C. Sonne[14] · N. W. van den Brink[15]

Figure 1b. What can we measure in each sample type? -**Passive monitoring**



See References here
Go back to the main menu here

Espín et al. 2021. A schematic sampling protocol for contaminant monitoring in raptors. Ambio 50: 95-100.

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Stage 1: Carcass or sample arrival

Which data should I record for each carcass or tissue sample, to support subsequent contaminant interpretation?

	A	B	C	D	E	F	G	H	I	J	K
1		UID	Species	Sex	Age	Country	Region/Province	Date of death	Cause of death	Body mass (g)	Wing length (mm)
2	1										
3	2										
4	3										
5	4										
6	5										
7	6										
8	7										
9	8										
10	9										
11	10										

Maintain near real-time digitised raptor specimen database (*Konstantinos Vlachopoulos*)

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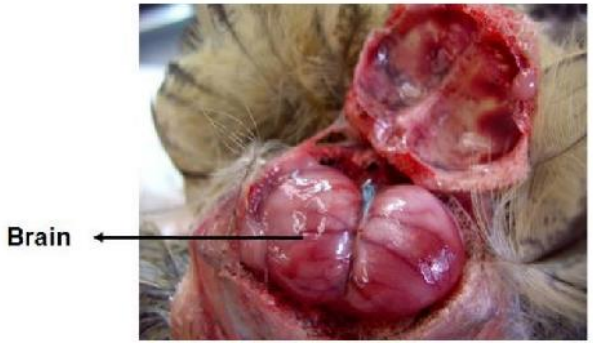
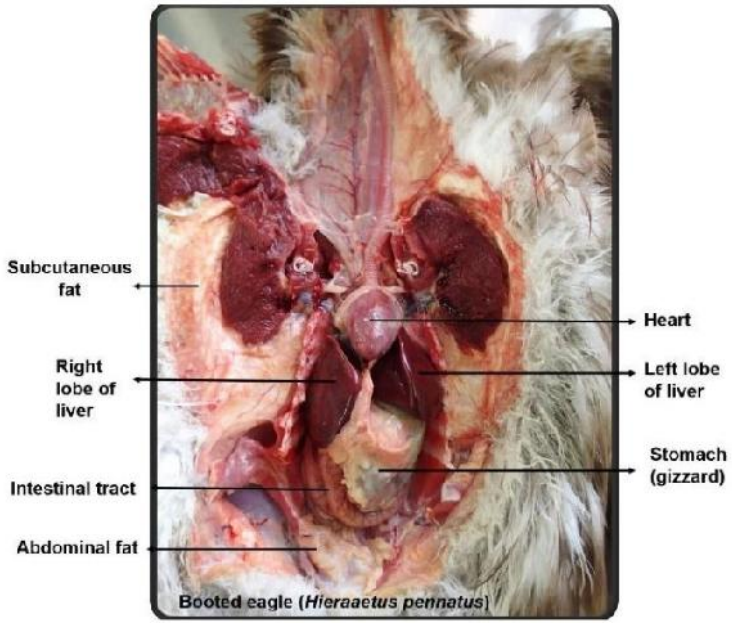
Stage 2: Necropsy and processing

When and how should I perform the necropsy?

Video: Necropsy and sample collection

Eagle owl (*Bubo bubo*)
Photo: Pedro Maria-Mojica





Which material and equipment are needed to perform a necropsy?

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Stage 2: Necropsy and processing

What is the amount of sample and containers needed for contaminant analysis?

Depends on the contaminant to be analysed...

- Volume/Mass sample
- Type of container
- Storage conditions

Table 1. Volume/Mass of sample, type of container and transport conditions required for contaminant monitoring in different matrices

			Matrix[a]	Blood[b]	Plasma/serum[b]	Feathers[c]	Eggs[d]	Liver[e]	Kidney[e]	Brain[e]	Bone[e]	Muscle[e]	Fat[e]	Preen oil	Regurgitated pellets / Prey remains
Pharmaceuticals	Volume/Mass (range)			P-NC	0.1-0.25 ml	1 g	2 g	2 g	2 g	2 g	NA	2 g	2 g	MI	For prey remains different tissues could be analysed (see other columns)
	Type of container			P-NC	PP tubes[2]	Sealed plastic bag / Envelope[2]	PP jar[2]	PP jar[2]	PP jar[2]	PP jar[2]	NA	PP jar[2]	PP jar[2]	MI	
	Transport conditions	Temperature		P-NC	Cold blocks	Cold blocks	Cold blocks	Cold blocks	Cold blocks	Cold blocks	NA	Cold blocks	Cold blocks	MI	
		Time		P-NC	< 24 h	< 24 h	< 24 h	< 24 h	< 24 h	< 24 h	NA	< 24 h	< 24 h	MI	
	Storage conditions	Temperature		P-NC	-20°C/-80°C[3]	20°C/ 80°C[2] (preferrably in darkness)	-20°C	-20°C	-20°C	-20°C	NA	-20°C	-20°C	MI	
		Time		P-NC	See note[4]	See note[4]	See note[4]	See note[4]	See note[4]	See note[4]	NA	See note[4]	See note[4]	MI	
Rodenticides	Volume/Mass (range)			1 ml	1 ml	NA	0.5-2 g	0.5-2 g	MI	NA	NA	MI	NA	NA	Plastic sealed bag for pellets. For prey remains different tissues could be analysed (see other columns)
	Type of container			PP tubes	PP tubes	NA	PP jar	PP jar	MI	NA	NA	MI	NA	NA	
	Transport conditions	Temperature		Cold blocks	Cold blocks	NA	Cold blocks	Cold blocks	MI	NA	NA	MI	NA	NA	
		Time		< 24 h	< 24 h	NA	< 24 h	< 24 h	MI	NA	NA	MI	NA	NA	
	Storage conditions	Temperature		-20°C	-20°C	NA	-20°C	-20°C	MI	NA	NA	MI	NA	NA	
		Time		See note[5]	See note[5]	NA	See note[5]	See note[5]	MI	NA	NA	MI	NA	NA	
Perfluorinated compounds	Volume/Mass (range)			0.2 1 ml	min. 0.2 ml	ca. 0.1 1 g	0.5 1 g	ca. 1 g	ca. 1 g	ca. 1 g	MI	ca. 1 g	0.5 1 g	0.01 0.1 g	ca. 1 g
	Type of container			PP tubes	PP tubes	Sealed plastic bag / Envelope	PP jar	PP jar	PP jar	PP jar	MI	PP jar	PP jar	PP jar	PP jar
	Transport conditions	Temperature		Cold blocks (<4°C)	Cold blocks	Ambient temperature/Cold blocks	Cold blocks	Cold blocks	Cold blocks	Cold blocks	MI	Cold blocks	Cold blocks	Cold blocks	Cold blocks
		Time		ca. 24 h	ca. 24 h	ca. 24 h	ca. 24 h	ca. 24 h	ca. 24 h	ca. 24 h	MI	ca. 24 h	ca. 24 h	ca. 24 h	ca. 24 h
	Storage conditions	Temperature		-20°C	-20°C	Ambient temperature/ -20°C[1] (preferrably in darkness)	-20°C	-20°C	-20°C	-20°C	MI	-20°C	-20°C	-20°C	-20°C
		Time		Indef	Indef	Indef	Indef	Indef	Incef	Indef	MI	Indef	Incef	Indef	Indef

Espín et al. 2021. A schematic sampling protocol for contaminant monitoring in raptors. Ambio 50: 95-100.

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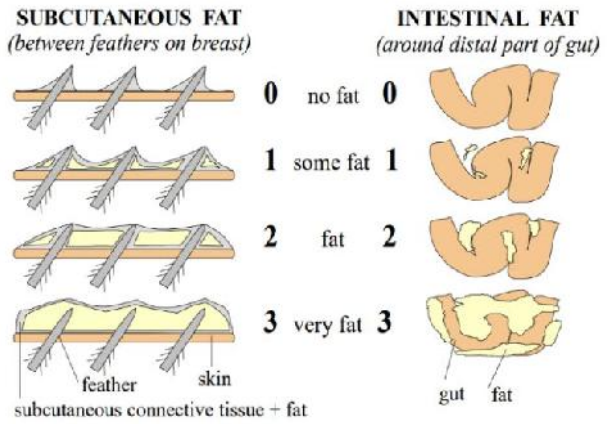
Stage 2: Necropsy and processing

Which data should I record during the necropsy?

NECROPSY FORM					
Carcass information					
Species			Identification code		
Age			Sex		
Wing length		Tarsus length		Tarsus width	
Head length		Bill length		Body weight	
Location of the carcass					
Date collected					
Circumstances in which the carcass was found					
Collector/Finder (contact information)					
Necropsy information					
Thawing	Date and time				
Necropsy	Date and time		Person in charge		
DESCRIPTION OF ALTERATIONS					
Status of decomposition					
External examination					
Cause of death					
Body condition					
Head and neck	Eyes		Ears		
	Oral cavity		Nares		
	Brain (weight)				
	Observations (lesions/alterations)				
Thoracic cavity	Pectoral muscle		Subcutaneous fat		
	Heart (weight)				
	Lungs (weight)		Trachea		
	Observations (lesions/alterations)				
Abdominal cavity	Liver (weight)			Abdominal fat	
	Kidney (weight)				
	Esophagus			Crop	
	Intestine			Cloaca	
	Observations (lesions/alterations)				
Gonads	Developmental stage				
	Observations (lesions/alterations)				
SAMPLES COLLECTED FOR CONTAMINANT MONITORING					
Liver	Kidney	Bone	Feathers	Subcutaneous fat	
Abdominal fat	Brain	Pectoral muscle	Preen oil	Crop content	
Other					

Body condition index



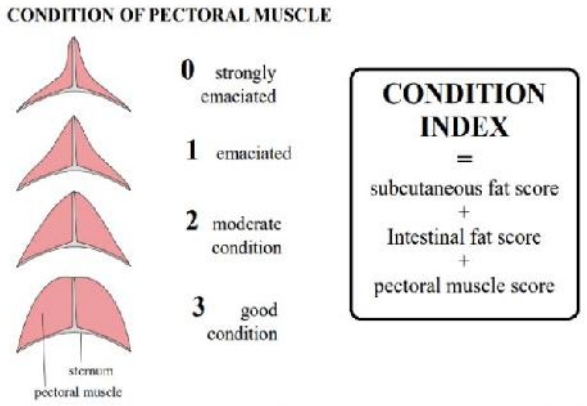


Figure: Van Franeker (2004)

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Stage 3: Storage

How should I store the samples?

Storage

-20°C

-80°C

Room temperature

What if my freezer is full?

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What happens after this protocol has been put into practice?

Shipping tissues to lab

Packaging and legal considerations

List of labs in Europe

WG4 (*led by Chris Wernham*)

ERBFacility Raptor Advice Hub

Click on the picture (CTRL + CLICK) to get additional information.

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Standards and protocols for receiving, storing and processing raptor carcasses and extracting tissue samples

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