PROTOCOL FOR TRANSPORTATION & NECROPSIES OF STRANDED SEABIRDS' CARCASSES AND TISSUES

LIFE SEABIL "SAVING SEABIRDS FROM MARINE LITTER"
LIFE20 GIE/FR/000114

Coordinating beneficiary

Associated beneficiaries

PROTOCOL TRANSPORTATION/NECROPSIES FOR STRANDED SEABIRDS

LIFE SEABIL "SAVING SEABIRDS FROM MARINE LITTER"
LIFE20 GIE/FR/000114

CONTEXT :

According to estimates, **almost 90% of seabirds** have plastic in their stomachs. When they are not killed by ingestion, plastic threatens their survival and affects their habitats and their reproduction.

Launched in October 2021 and until September 2024, **the LIFE SeaBiL Project "Saving SeaBirds from marine Litter"** intends to evaluate and reduce the impact of plastic pollution on seabirds. The project involves 5 pilot sites in 3 countries:

- **France :** Gironde estuary and Pertuis sea Marine Natural Park (PNMEGMP);
- **Portugal :** Berlengas Natural Reserve;
- **Spain :** Ebro Delta Natural Park, Urdaibai Biosphere Reserve / Santoña Marshes Natural Park; Cabo de Gata Natural Park.

One of the main actions of the project is to set up **a transnational monitoring network** for stranded birds' collection and storage in care centers. Carcasses and tissues are then transmitted to La Rochelle University and University of Cadix to **proceed to necropsies and store the tissues**. The establishment of a tissue bank will provide analysis of collected stranded birds to identify **indicator species of good ecological status** for seabirds and plastic ingestion on our coastlines, as per MSFD indicator D10.

The following protocol is for the necropsy and transportation of the tissues. It has been written within LIFE SeaBiL project framework with the participation of all the partnership and after the consultation of the relevant scientific community.

Infos, contacts et documents

https://lifeseabil.fr
life.seabil@lpo.fr

PROTOCOL FOR THE TRANSPORT OF BIOLOGICAL SAMPLES:

Before transportation

1. Authorization and documentation: before sending the samples, make sure to obtain all the necessary authorizations and permits in both Spain and France. This may include export/import permits and customs documents. Also make sure you have the proper documentation about the research and the purpose of the transport.

2. Use a courier service experienced in handling biological samples.

Preparing samples for transport

1. It is important that samples are in leak- and breakage-resistant containers. Tupperware or large zipper bags can be used for this purpose.

2. Samples should be well identified with labels that include at least the sample identification, number or code to identify the sample belonging to the individual, sender of the sample, date of sample collection.

3. Adequate temperature preservation during transport. It is important not to break the cold chain. The samples have been stored at -20ºC. During transport, it will be necessary as far as possible to maintain their frozen state and to keep them as short as possible on route. There are several options for this: prepare the samples in coolers with dry ice for their transport, in case this is not provided by the transport company; see the cold transport options offered by the transport companies.

4. Plan the shipment so that the samples are in route as little time as possible, use courier options that ensure delivery within 24 hours.

PROTOCOL FOR THE NECROPSIES:

Material required for dissections – make sure to avoid any plastic tool. The manipulators must wear a clean cotton gown (and mask) and pay attention to the cleanliness of the material. **All the dissection tools must be made of metal or glass, and should be cleaned and rinsed 2 times with filtered alcohol.**

Requested material:

- Dissection tools
- Cotton gown
- Glass vials and jars of different sizes to put the organs in (with aluminum or PTFE caps; if you use a different cap, put some aluminum foil in between the vial and the cap)
- 5 clamps (metal clips) to separate the different parts of the digestive tract
- Filtered alcohol
- Glass disk for microplastics control (control disk)
- 0.001g precision scale
- Morphometric tools (caliper, wing ruler, scale)
- Paper envelops
- Eppendorf with ethanol
- Parafilm to avoid losses of ethanol

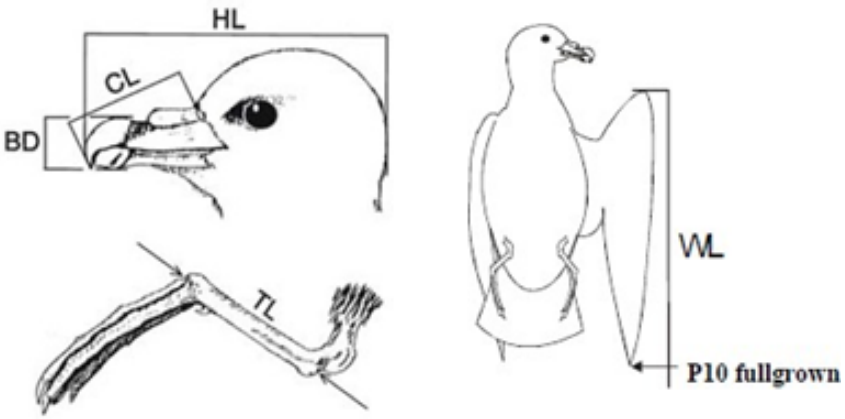
Protocol:

1. Take the carcass out of the freezer about 12-24h before the necropsies and let it thaw covered with a cloth. The collection of the different organs is easier if the bird (and organs) is still slightly frozen.

2. Weigh the empty vials and jars that will be used to store the different organs

3. Weigh the carcass

4. Take morphometrics (WL - flattened wing length, TL - tarsus length, CL - culmen length, DB - bill depth and HL - headbill)



5. Rank the freshness and completeness of the carcass

Table 2 Categories of freshness (CIRCLE ONE of the codes on the form)

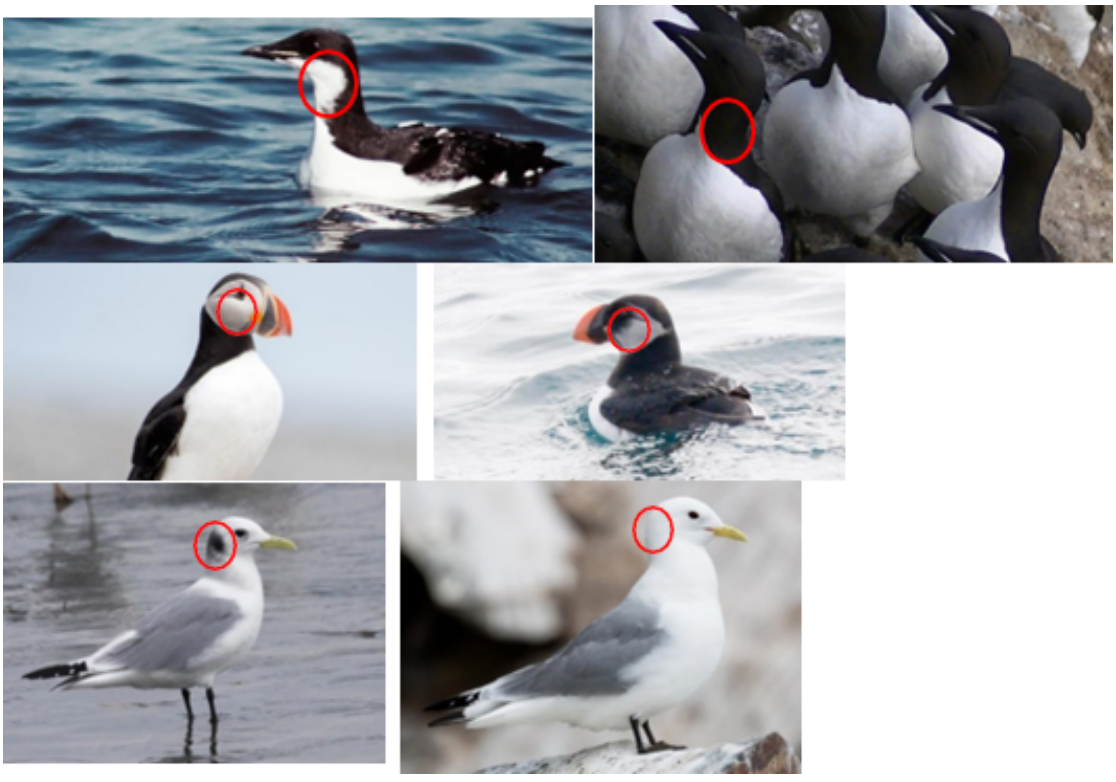
very fresh	**FFF**	eyes bright and shiny
fresh	**FF**	eyes dull and bit shrunken, but tissue eg in mouth looks fresh
rather fresh	**F**	shrunken eyes, tissue in mouth starts discolouring
rather old	**O**	shrunken eyes, discoloured tissue, feathers becoming loose
old	**OO**	feathers easily pulled out
very old	**OOO**	mummified or strongly decaying eg bill-cornea easily falls off

Table 3 Categories of completeness (CIRCLE ONE of the codes on the form)

complete	CC	body & plumage fully intact; no scavenger marks
near complete	C	lightly damaged or scavenged, but all major feather areas present
incomplete	I	seriously damaged or scavenged, with feather areas incomplete
parts only	I I	whole body parts missing, e.g. only wings + breastbone

6. Collect feather samples.
In alcids: 5 belly feathers, 5 back feathers, 5 head/throat feathers, P1, P10
In larids: 5 belly feathers, 5 back feathers, 5 neck feathers, P1, P10
In other species: 5 belly feathers, 5 backfeathers, P1, P10

Place feathers in paper envelops – use one envelop per individual and feather type and makesure to write on the envelop the bird ID and the feather type.



7. Put a control disk next to the bird during the necropsy to measure aerial microplastic contamination.
8. Sample the brain (take the sample with a small spoon if liquid)
9. Put the bird in dorsal position and soak the feathers with alcohol to clear the belly (it will avoid the feathers fly everywhere). Make a longitudinal incision through the skin from the cloaca to the sterum. Be careful not to cut too deeply so as not to perforate the proventriculus and the intestinal cavity, which must remain intact.

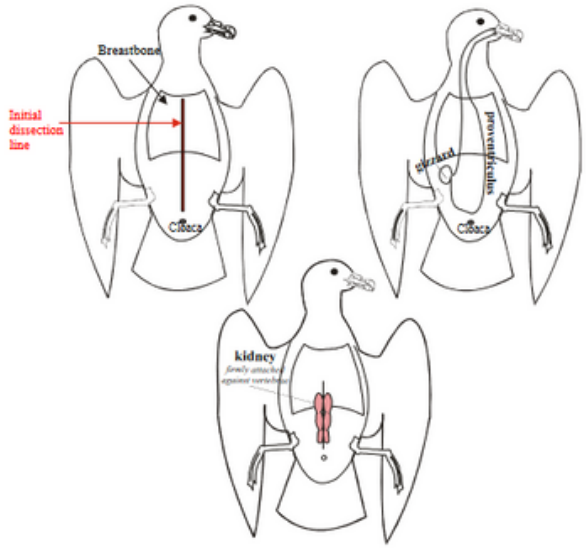


Figure 10 Dissection: first skin-incision, position of stomach, and position of kidneys

10. Peel off the skin on each side, keeping the fat layer attached to the skin. Note the condition of the pectoral muscles and the quantity of subcutaneous fat.

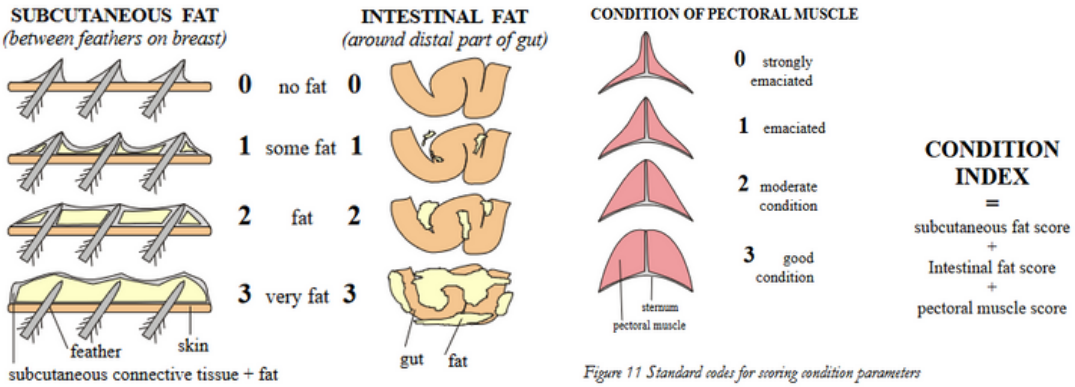
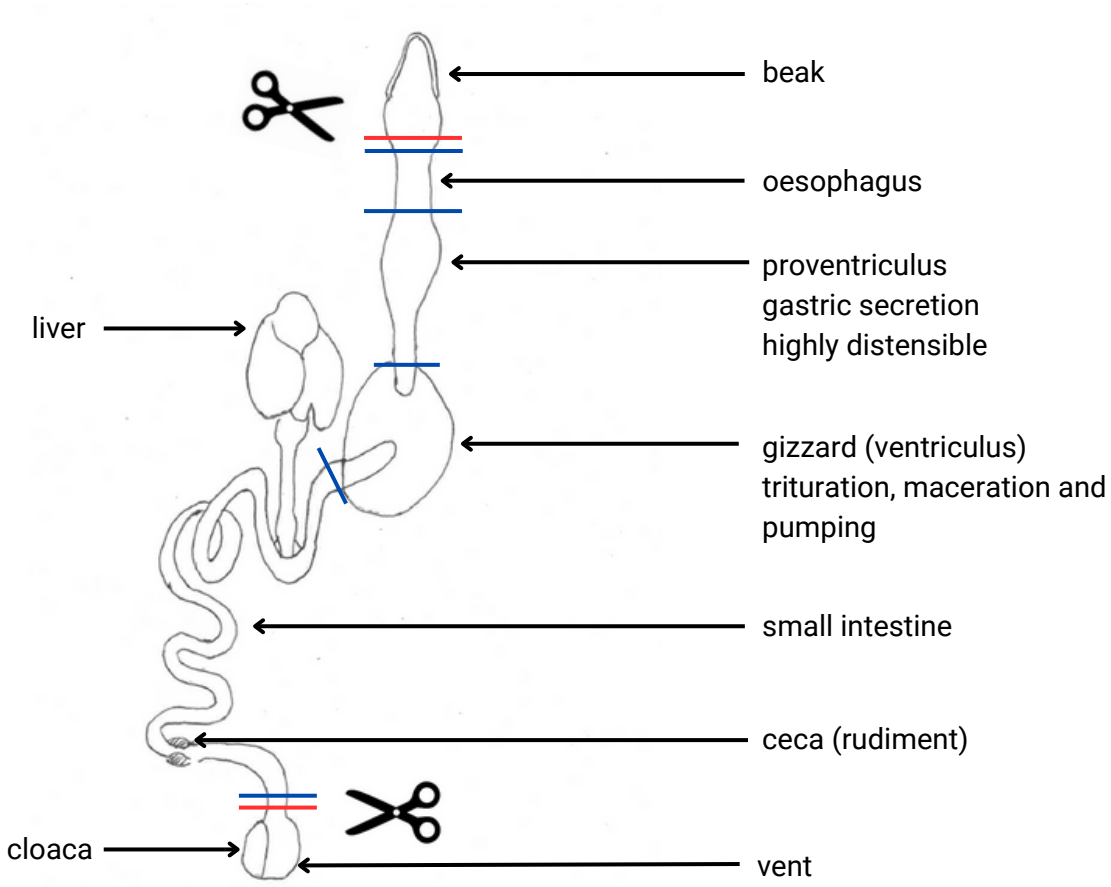


Figure 11 Standard codes for scoring condition parameters

10. Peel off the skin on each side, keeping the fat layer attached to the skin. Note the condition of the pectoral muscles and the quantity of subcutaneous fat.
11. Sample two pieces of pectoral muscle (one complete muscle in a dry vial and one small piece of the second musclein an Eppendorf with ethanol for DNA)
12. Sample some adipose tissue (if not too degraded) in a dry vial.
13. Cut the ribs on both sides to “open” the keel.

14. Collect the liver in a dry vial
15. Remove the heart and open it to recover the clot (coagulated blood mostly in the atria and to some extent in the ventricles). Put the blood in a dry vial.
16. Assess the fat in the mesenteries (see figure above)
17. Determine the sex of the individual by moving the digestive tract and looking at the reproductive organs (located behind the intestine).
18. Clamp the digestive tract in order to separate the 4 different parts: esophagus, proventriculus, ventricle, intestine (blue lines on the figure below). Then cut the extremities of the digestive track (red lines on the figure below) and put it in a jar with the clamps. (For small birds, the digestive track can be sampled without separating the different parts.)



19. Collect the 1-2 kidneys and store in a dry vial.
20. Weigh the different vials together with the collected organs.

Protocol modified from van Franeker (2004) within the framework of the LIFE project SeaBiL (LIFE/20 GIR/FR/00114) and following EcoDIS (ANR-20-CE34-0002) and CIPPE (MITI CNRS 2020-2022) research projects.

Franeker, J. A. van. « Save the North Sea Fulmar-Litter-EcoQO Manual Part 1: Collection and Dissection Procedures ». Wageningen: Alterra, 2004. https://library.wur.nl/WebQuery/wurpubs/334786.

Coordinating beneficiary

Associated beneficiaries

Financed by: