Protocol for sampling seabirds in breeding colonies – LIFE SeaBiL

European Shag nesting monitoring

The surveys to monitor the effect of marine litter on European Shag occur once a month between January and July each year. The minimum surveys required are four per year (mid February, mid April, mid May and early June).

Requested material:

- Map and photo guide with nest locations;
- Form;
- Pencil and permanent marker;
- Clipboard;
- Binoculars;
- Telescope;
- Camera.

Protocol:

1) Visit all known nesting sites of the area, or the selected nesting sites for monitoring;
2) Select a place with a good view of the nest and its contents;
3) Record the observers' name, date, colony name, subcolony name, nest ID, number of adults presented in the nest, number of eggs, number of alive chicks and number of dead chicks;
4) In the notes, record if the adult was in nesting position or next to the nest;
5) Record the code, colour and position of colour ring in adult birds;
6) In case of an empty nest, add to the "notes" if it has signs of recent activity (e.g. presence of fresh branches);
7) Adults may stay sit on top of the eggs for long times (up to 1 hour) before moving the eggs or being replaced by its mate. This monitoring requires a long time waiting to count the entire clutch;
8) In case of a new nest (not present in the map or photo guide) is noticed, take a picture of the nest location, including the major amount of the cliff where the nest is and the nest itself. Make sure the picture allows the detection and identification of the nest during the following surveys. Right in the "notes" field any detail on the position of the nest, e.g. it is located in the left side of the nest 79. Record also the name of the picture in the form "photo" field;
9) Record the non-natural contents present in the nest. Examples of these items include fishing line, rope, piece of fishing net, hook, wire, piece of metal, cloth, rubber, etc.
10) Record the number of items, colour and size (as 1 - <10cm, 2 – 10 to 25cm, 3 – 25 to 100cm, 4 - >100cm).

Project Life SeaBiL – Saving SeaBirds from marine Litter (LIFE20 GIE/FR/00114)

Form:

Date_____ **Colony**_____ **Observers**_____

Subcolony	Nest	N_adults	N_eggs	N_chicks	Photo	Lixo				Notes
						Item	N	Size	Colour	
Carolinas	1	2	2	0		Fishing line	1	2	blue	One adult is next to the nest
Carolinas	2	1	0	1		No contents				

European Shag diet monitoring

The surveys to monitor the effect of marine litter on European Shag occur once a month along the entire year. The minimum surveys required are 2 per year (October and mid May). These visits intend to sample both the non-breeding and breeding season.

Requested material:

- Map and photo guide with sample locations;
- Form;
- Pencil and permanent marker;
- Clipboard;
- Paper envelop.

Protocol:

1) Visit the known resting sites of the area, or the selected resting sites for monitoring;
2) Search for shag pellets;
3) Store each pellet in a single envelop, properly labelled, including observers' name, date, colony name and site name.

Cory's Shearwater and Band-rumped Storm-petrels breeding success

The surveys to monitor the breeding success of Cory's Shearwater and Band-rumped Storm-petrels should occur three times (minimum) along the breeding season. The first taking place during the egg laying period, the second during the hatching period, and finally a last one just before the chicks desert their nests. This information will be lately be compared with the information gathered through the stomach contents protocol

Surveys are species dependent and should take place during the following periods:

Species	**Phenology**	**Dates**
Cory's Shearwater	Egg laying	First week of June
	Hatching	Last week of July
	Chick rearing	Mid October
Band-rumped Storm-petrel	Egg laying	Early October
	Hatching	Late November
	Chick rearing	Mid January

Requested material:

- Map and photo guide with nest locations;
- Form;
- Pencil;
- Clipboard.

Protocol for nest monitoring:

1) Visit all known nesting sites of the area, or the selected nesting sites and nests for monitoring;
2) Record the observers' name, date, colony name, subcolony name, nest ID, number of adults presented in the nest and the status of the nest (presence of egg or juvenile dead/alive) as O – egg alone, J – chick alone, 1A+O – one adult plus egg, 1A+J, one adult plus chick, etc);
3) Record the ring number of each adult and chick;
4) Collect any dead bird (adult/chick) and proceed as in the **Necropsy Protocol**.

Form:

Date_____ **Colony**_____ **Observers**_____

Subcolony	Nest	Status	Ring Adult 1	Ring Adult 2	Ring chick	Notes
Melreu	1	1A+O	LV26309	LV26512		
Melreu	2	2A	LV26513			
Melreu	3	J			LV26515	

Cory's Shearwater and Band-rumped Storm-petrels stomach contents

The surveys to monitor the effect of marine litter on Cory's Shearwater and Band-rumped Storm-petrels should occur twice or one time (minimum) along the breeding season, respectively. Regarding Cory's Shearwater, the first survey should take place early during the breeding season, before female exodus (namely from March to early May). A second survey might take place during chick rearing (between September and October). Both surveys will target live adult birds presented in the nesting areas. In the case of Band-rumped Storm-petrels, adults birds are intended to be caught using mist-netting during a night of early October. Stomach content of 30 birds of each species should be collected each year using water off-loading technique.

Requested material:

- Map and photo guide with nest locations;
- Form;
- Pencil and permanent marker;
- Clipboard;
- Cloth bag;
- Metal rings and ringing material;
- 5-mm (for Cory's Shearwater) or 2-mm (for storm-petrels) stomach pump;
- 1.5 lt bottle filled with seawater;
- Tray or bucket;
- 0.5 (for Cory's Shearwater) or 0.25 lt (for storm-petrels) vials.

Protocol for sampling stomach contents:

1) Adult birds are caught when resting or arriving at the nesting area (in the case of Cory's Shearwater) or using mist-nets (in storm-petrels);
2) Put the bird inside of a cloth bag;
3) Read the ring code or mark with a new ring as convenient;
4) The stomach pump plastic catheter is pushed down the bird's esophagus until it reaches the base of the stomach (Fig. 1 a);
5) Seawater at ambient temperature is then pumped in using an the pump until it begins to flow back out round the sides of the catheter;
6) The catheter is removed and the bird is restrained by both legs with one hand while the other holds the head, the first and second fingers holding the beak open;
7) The bird is then inverted over a bucket or tray and gentle pressure applied below the bird's rib cage with the knees until regurgitation occur (Fig. 1 b);
8) When regurgitation appears complete the neck should be massaged gently to remove any items remaining in the esophagus;
9) Occasionally the food mass gets stuck in the buccal cavity. In this case steady pressure on

the stomach will stop reswallowing and eventually force the bird to shake out the remaining contents since these block the epiglottis and prevent breathing;

10) Drain the bucket/tray contents to a vial, properly labelled, including sampling ID, observers' name, date, colony name and species;
11) Record the observers' name, date, colony name, subcolony name, sample ID, species, ring code, nest and breeding status (non-breeding, egg, chick or unknown).

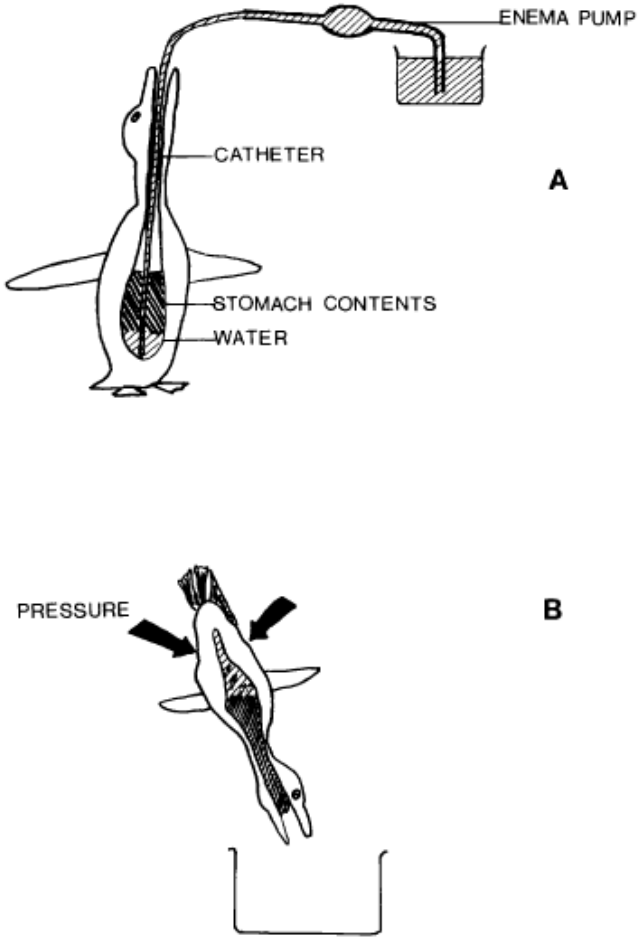

FIGURE 1. Mode of operation of a stomach pump for seabirds. Figure from Wilson, R. P. 1984. An improved pump for penguins and otherseabirds . Journal of Field Ornithology 55: 109-112

Form:

Date_____ **Colony**_____ **Observers**_____
Species_____

Subcolony	Sample	Ring	Nest	Breeding status	Notes
Melreu	1	LV26309	1	chick	
Melreu	2	LV26310		unknown	
Melreu	3	LV12241	3	non-breeding	

Processing European Shag's regurgitated pellets

Requested material:

- Regurgitated pellets;
- Stereomicroscope or binocular microscope;
- Fine-tipped tweezers;
- Petri dishes;
- Scale or precision scale (when applicable);
- Water (when applicable);
- Millimeter paper (5x5mm square);
- Needle and a source of heat (e.g. lighter);
- Datasheet;

Protocol for processing the regurgitated pellets:

This methodology follows the recommended practices by Provencher et al. 2019, and the minimum size considered for found items is 1mm.

1) Before beginning the analysis, evaluate the state of the regurgitated pellet (complete/partially complete/very fragmented) and register it in the datasheet.
2) Proceed to put the millimeter paper on the stereoscope base and on top of it, put the petri dish with the regurgitated pellet to begin the analysis.
3) Slowly pull the regurgitate pellet apart with the help of tweezers. If this process proves to be challenging, you can put the pellets under running (warm) water for a few minutes/hours to soften it.
4) If the sample is cluttered with biological matter, a digestive treatment may be necessary to separate the plastics.
5) Put aside any material that resembles plastic or are of non-plastic origin. Proceed to identify each piece as Industrial Plastic (pellets), Domestic Plastic (nylon, plastic bag, rope, cigarette filter, etc…) or Other (paper, fabric, aluminum foil, metal, glass, etc…).
6) Register the color of the observed item, as well as its length, width, volume and weight.
7) When possible try to identify each item to its origin (nylon, fabric etc…). If there is any uncertainties of its classification proceed to do the hot needle test based on Witte et al. 2014, where you press the tip of a hot needle on the plastic and register what happens visually. A useful reference guide is the Spotter's Guide to Plastic Pollution developed by Civic Laboratory for Environmental Action Research.

Processing Cory's Shearwater and Band-rumped Storm-petrels regurgitations for marine litter analysis

This methodology follows the recommended practices by Provencher et al. 2019.

Requested material:

- Regurgitation samples;
- Stainless steel or brass sieves;
- Datasheet.

Protocol for prepping regurgitation samples:

1) In order to separate solids from liquids a series of stainless steel or brass sieves can be used. Stacked sieves (e.g., 5, 1, and 0.3 mm) can be used to separate different fractions.
2) Rince the sample with water using the sieves and discard the any remaining liquid.
3) All the remains and debris should be air dried for at least 24h at room temperature. Alternatively, the debris can be put in a drying oven at 40 °C for a minimum of 12h on a covered stainless steel or glass plate to prevent any cross-contamination.
4) After the drying process, the diet items of interest can be removed for analysis, while the plastic sample can be further processed to remove any biological matter residue (e.g. digestive treatment).
5) Process samples using the guidelines previously described for the European Shag.

References:

- Provencher, J.F., Borrelle, S.B., Bond, A.L., Lavers, J.L., Franeker, J.A., Kühn, S., Hammer, S., Avery-Gomm, S., Mallory, M.L. (2019). Recommended best practices for plastic and litter ingestion studies in marine birds: Collection, processing, and reporting. FACETS.

Form:

Colony_____ **Laboratory workers:**_____

Species_____

Date	Nest	Pellet ID	Integrity	Item	Cassification	Color	Length (mm)	Width (mm)	Volume (mm^3)	Weight (mg)	Notes
11.2.2021	1		Complete	Cigarette	Domestic plastic	White	20	10	10	100	
11.2.2022	8		Very fragm.	Pellet	Industrial plastic	Blue	1	1			
11.2.2023	117		Partially frag.								