

SUMMARY: SAMPLING AND CONTAMINANT MONITORING PROTOCOL FOR RAPTORS

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INTRODUCTION AND AIM

Research and Monitoring for and with Raptors in Europe (EURAPMON) is a European Science Foundation (ESF) Research Networking Programme (<http://www.eurapmon.net>). One objective of EURAPMON is to spread best practice and build capacity in Europe for harmonised monitoring with raptors. From May 31st to June 2nd 2013, representatives from six countries gathered in Murcia, Spain, to attend the Workshop on “Setting best practices on raptor contaminant monitoring activities in Europe” funded by EURAPMON. The workshop developed a draft protocol that was subsequently completed with the involvement of experts in monitoring contaminants in raptors. The aim of this sampling protocol is to provide guidance on types of best practice that will facilitate harmonisation of procedures between existing and emerging schemes and so maximise the reliability, comparability and interoperability of data. This protocol covers the sampling of blood and feathers from live birds, addled and deserted eggs, internal organs and tissues from dead specimens, and other samples such as faeces, preen oil and pellets. The present document is a summary of this extended protocol that is freely available on the EURAPMON website (<http://www.eurapmon.net>).

1. GENERAL GUIDELINES

- 1.1. Permission.** All necessary licences and permissions must be obtained from the appropriate national agencies before work is undertaken. Samples should be collected by appropriately trained and authorised personnel.
- 1.2. Identification.** Individual sample containers should be labelled, before or immediately after the sample is collected, with a unique code.
- 1.3. Avoid contamination.** Appropriate material for sampling and storage should be used. Equipment has to be pre-cleaned before sampling (take advice from the laboratory undertaking the chemical analysis). Ensure that sampling personnel do not smoke, drink or eat during sampling. Note whether insect repellent was used by the sampling personnel.
- 1.4. Personal health and safety.** Appropriate personal protective equipment should be used and safety requirements for climbing and hiking should be applied.
- 1.5. Animal welfare.** Unnecessary stress to birds during sampling should be avoided (cover the head, avoid unnecessary noise, avoid handling birds or visiting nests when conditions may increase stress, keep the handling time as short as possible). Check with the laboratory to determine the minimum size of sample for the intended analysis, but make sure that the size of sample taken is safe for the bird and compliant with licence conditions.

- 2. BASIC DATA.** The following information should be clearly described in the sampling report, which must always accompany the samples submitted for analysis: contact details of observer or recorder, date and time of sampling, study area, type of samples and number of samples collected, biological data

(species, ringing data, age and gender, morphometric measurements, body condition index, nest information), and other general observations.

3. PROTOCOL FOR EACH SAMPLE TYPE

3.1. BLOOD

- **General requirement:** If possible, prior to and after blood sampling, a clinical exploration should be done by a vet to evaluate the health status of the bird. The collection weight should not exceed 1% of the body weight of the animal at any one time. When considering the combined weight of samples taken during multiple dates from the same individual, a maximum of 2% of its body weight may be collected in any 14-day period. Blood samples can be taken using a hypodermic needle and a syringe. Change needles/syringes between birds. Veins of very small birds can be nicked with a sharp, sterile point (e.g. scalpel or hypodermic needle) and blood collected in a capillary tube. Vacuum systems such as vacutainers should be avoided. The smallest needle possible should be used: 30 to 25-gauge hypodermic needle and a 1 or 2 ml syringe for birds < 500 g body weight, and 23-gauge hypodermic needle and a 5 or 10 ml syringe for birds > 500 g body weight. Anticoagulants for whole blood/plasma are needed. Take advice from the laboratory undertaking the chemical analysis (in general, heparin is recommended).
- **Sampling method:** (1) stimulate the local blood circulation, (2) use antiseptic at the phlebotomy site, (3) take blood samples puncturing vein (brachial vein is the easiest one from which to obtain blood), (4) press the puncture site with sterile cloth before pulling the needle from the vein, and keep pressure for some minutes until no blood is flowing from the wound to avoid bleeding and haematomas.
- **Procedure after blood extraction:** needle has to be removed from the syringe before placing the sample in labelled tubes. Samples should be transported at 4-10 °C. Tubes containing anticoagulants should be adequately filled in order to provide a proper blood-to-anticoagulant ratio. Serum separator tubes are needed for serum and samples should be centrifuged as soon as possible, ideally within 6 hours (maximum 12-24 h) after collection; the longer the elapsed time, the higher the risk of clotting and rupture of red blood cells. Blood has to be centrifuged to separate plasma/serum from erythrocytes (10 minutes, 1600-3000 g). Plasma/serum/red blood cells separation is possible on fresh blood only. All separated fractions should be conserved in different labelled tubes. Different pipette tips should be used for each sample during plasma/serum separation to avoid cross contamination.
- **Storage:** samples should be stored frozen at -20°/-80°C/liquid N₂ (depending on the analyte or the studied biomarker) in darkness until analysis.

3.2. FEATHERS

- **Sampling:** the number and type of feathers that need to be sampled, and the age of birds they should be taken from, depends on the contaminant under investigation, the analytical technique, and the objectives of the study. Plucked (or cut at the skin) contour body feathers from nestlings/adults are preferred. Moulded feathers found in the nest or field can also be collected. Down feathers are not recommended for contaminant monitoring. Normally 200-500 mg is required for organic compounds and 10-200 mg for metal analyses. Feathers can be transported at room temperature in aluminium foil and plastic sealed bags for the analysis of organic pollutants and just plastic bags for metals.
- **Storage:** feathers can be kept at room temperature if any soft tissue or blood residue is removed and if they are dry. Feathers can be stored in aluminium foil and/or plastic sealed bags or envelopes, in darkness, and in a dry place (or use silica) if stored at room temperature. Alternatively, feathers can be frozen in sealed plastic bags. The label should not be put directly on the feather, but label the bags with a code.
- **Characterisation of samples:** the type(s) of feather taken should be identified, indicating where applicable which side of the bird they were taken from, and using the conventional numbering system for primary flight feathers (from the inside out to the tip). In the case of contour feathers, the location on the body should be indicated. Date of sampling has to be registered and the time of the last moult should be

estimated. The length of each feather (in mm) should be measured using a caliper, from the base of the vane to the tip. Before taking feather length measurements, calamus or quill (ie, the most proximal part of the shaft lacking vane) should be removed with a pair of sterilised stainless-steel scissors, just at the point where the first barbs of the vane join at the base of the feather. The calamus can be stored for genetic studies (using the blood in the quill). Each feather should be weighed (in mg). Depending on the compound(s) to be analysed, different washing techniques should be employed prior to analysis (see extended protocol).

3.3. UNHATCHED EGGS

- **Sampling:** only deserted eggs or addled eggs should be collected from the nest. A graphite pencil should be used to write information on both the eggshell and the container. Suitable containers for transportation are needed to avoid breaking. Pieces of the eggshells (remains after hatching or crushed eggs) found in the nest can be collected and kept in sealed plastic bags. The contents of the nest, including viable and addled eggs, as well as nestlings, should be recorded; as should the estimated age of the egg (time since laying) when it was collected.
- **Pre-treatment of samples and storage:** eggs should not be frozen because they can crack. They should be kept cool and be processed as quickly as possible. The length and width of the egg should be measured and the weight should be recorded. The egg should be opened at the equator and its contents should be emptied into flasks. The content should be weighed and homogenised and kept frozen at -20°C until analysis. A glass container with Teflon sealed cap may be used if considering determination of industrial pollutants. Plastic containers may be used for inorganic contaminants and PFAS determination. Eggs should be examined for putrefaction, embryo development and deformities. If an embryo is present, it should be separated from the rest of the egg content (prior to homogenisation) and kept frozen.

Eggshell should be rinsed carefully with tap water and dried at room temperature to a constant weight (the constant eggshell weight should be recorded). Eggshell thickness should be measured at equator after drying at room temperature using a caliper. Eggshell index and desiccation index should be calculated according to equations provided in the extended protocol. Contaminant data should be corrected to account for desiccation/moisture loss.

3.4. INTERNAL ORGANS AND TISSUES

- **General considerations:** carcasses should be conserved in sealed plastic bags to avoid desiccation and labels placed both inside the bag (written with pencil or waterproof marker) and on the bag (written with waterproof marker). All the information about the carcass should be recorded, including date, location, circumstances in which it was found, contact information of the finder, species, age, sex, body weight, measurements, decomposition state.
- **Necropsy:** necropsies should be done on fresh carcasses where possible or the carcass should be kept frozen (-20°) until necropsy. If the carcass is frozen, it should be thawed out overnight. External examination of the carcass is necessary to find possible signs of trauma or evidence of clinical symptoms previous to the death. Body condition should be estimated (see extended protocol). During necropsy, organ weight, lesions/alterations, sex and status of the gonads (developmental stage) should be recorded. A standardised necropsy protocol should be followed (see extended protocol).
- **Sampling:** take advice from the laboratory undertaking the chemical analysis as to selection of tissues for analysis. Suitable dissection equipment should be used and the equipment should be cleaned between the organ sampling and between individuals. Sampling of liver, kidney and other internal organs, should be of the whole organ, if possible. When sampling muscle tissue, the pectoral muscle should be preferred. If fat is to be analysed, use abdominal fat in preference to subcutaneous fat. If sampling tissues are dispersed throughout the body, such as abdominal fat or bone, it is recommended that samples of the tissue from multiple individuals are taken consistently from the same part of the body (or, if sampling bone, from the same skeletal location).

- **Storage:** organs should be kept in separate, clearly labelled containers/plastic bags. The use of containers whose materials may interfere with or affect analysis of the contaminants need to be avoided. Aluminium foil (washed with water and methanol) can be used to wrap samples if uncertain about the suitability of plastic containers for analysis of perfluorinated compounds. Aluminium foil should not be used in case of trace metals analyses. Organs should be stored at -20 °C or -80 °C. If uncertain about any aspect of storage, seek advice from the analysing laboratory.
- **Other considerations:** disposal of waste and carcasses should be done according to national regulations relating to biological waste. Where possible, key organs, feathers, and bones should be retained in a long-term archive.

3.5. OTHER SAMPLES

- Fresh faeces may be sampled by inducing individuals to defecate when handling them. Potential for contamination of old faeces falling from nests is large and they are not recommended for contaminant analysis. When sampling preen oil from carcasses the whole preen gland may be removed. In living birds, the preen gland should be pressed softly and the expelled oil collected in a sterile tube. Fresh pellets can be collected from nests, roosts or beneath perches. Samples should be collected in clearly labelled sterile tubes (faeces/preen oil) or plastic bags (faeces/preen gland/pellets), with equivalent information to that recommended for carcasses (see section 3.4). Check with the analysing laboratory to determine the amount required, and acceptable transport/storage conditions. Normally, transport should be under cold conditions and storage at -20° until chemical analysis. In some cases, storage at an alternative temperature (from room temperature to -80°C, depending on the analysis) may be more appropriate.

Acknowledgements. The authors gratefully acknowledge the financial support provided by European Science Foundation (ESF) through the EURAPMON Research Networking Programme. Thanks also to Lee Walker for his valuable comments on the extended protocol, and Mark Wilson, Amy Challis and Chris Wernham for their comments on this summary.

For detailed information, figures and references, see extended protocol available on EURAPMON website (<http://www.eurapmon.net/>).

April 2015