COLLECTING SAMPLES FROM FOXES AND THEIR PREY FOR ISOTOPIC ANALYSES

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PURPOSE

To sample different tissues of fox and their prey to help disentangling the trophic dynamic of tundra ecosystems across the Arctic.

INFORMATION

The isotopic signature of an animal tissue reflects the signatures of the food items eaten during a certain period of time. For instance, the signature of feathers represents the bird's diet during the molting period.

Note that 1- The isotopic signature varies within the same individual as a consequence of different metabolic rates among tissues; 2- The isotopic composition of a predator reflects the isotopic signatures of those prey parts that are digested (i.e. mostly meat); and 3- The isotopic signature of prey changes over the course of the year.

We recommend adoption of the specific guidelines given in this short protocol for collecting samples for isotopic analyses because uniformity and quality of sampling among the different ArcticWOLVES field sites is important.

TIME PERIOD AND LOCATION

Samples can be collected at any time of the year, wherever the opportunity arises.

PROCEDURE

A. General rules

i) *Quantities*

- *Meat:* < 1 cm^3 from the leg.
- *Egg contents:* ~ 1mL of homogenate (white and yellow well mixed).
- *Eggshell membrane:* a few fragments of eggshell (only if freshly hatched/predated).
- *Fur:* ~ 2 cm^2 on dead individual. Pick up fur on several places of the body rather than at a single place.
- *Feather:* 1 large tail or wing feather is sufficient. For small feathers (e.g. cover feathers), 2-3 would be better.
- *Down:* ~ 2 cm^2 on live individuals. Pick up down on several places rather than at a single place.
- *Note:* For each species at each site and each year, we should try to reach 15-20 samples of each tissue (maximum 30 samples).

ii) *Other guidelines*

- *Dry samples:* Dry samples do not need to be cleaned before being placed in paper envelopes.
- *Wet samples:* They should be stored in the 2ml microtubes as soon as possible to prevent degradation by bacteria.
- Prevent contamination of wet samples by using clean tools rinsed with ethanol.

- Whenever possible, clean the wet samples in a small bath of ethanol prior to the final storing in the 2ml microtubes.
- *Freshness:* Meat should not be older than one day in summer, in order to avoid sampling decaying flesh. Older wet samples should be destroyed. For carcasses found during the winter, meat can be much older. The state of the carcass should always be carefully described, with a picture if possible.
- *Choosing what to sample:* Sample what a fox would eat on the animal (On a bird or mammal, this is meat. However, sampling feathers is useful to studying the bird's diet).
- *For meat samples:* The ratio of sample to Ethanol70% in the microtubes should be as close to 1:3 as possible (1 part sample for 3 parts Ethanol70%).
- *For egg contents:* The ratio of sample to ethanol70% in the microtubes should be 1:1 (1 part sample for 1 part Ethanol70%).
- *For eggshell membranes:* With clean tools washed with ethanol, remove the eggshell membrane from the shell fragments, air-dry membranes at ambient temperature, and store in paper envelopes in a dry place. If no proper tools are available, air-dry the membrane with the shell and store both together in the paper envelope, while being careful to avoid contamination.

B. Specific guidelines for arctic fox samples

i) Sample winter fur found around burrows of fox dens in spring (1 sample/den).

ii) If you have access to a carcass with winter fur (field discovery or hunting), sample the following tissues when appropriate: a) meat from the back legs (< 1 cm^3), b) canines and/or the front jaw (for age determination), c) fur, d) nails, e) whiskers.

iii) *Sampling design linked to fox den monitoring:* For samples that are taken from dens, priority should be put on accessible locations that can be sampled from year to year. Furthermore, an ideal setting should be the use of dens that are distributed in a gradient of habitat (e.g. marine to terrestrial; low altitudes to highest ones).

C. Specific guidelines for prey samples

i) *Possible prey in different study sites:* lemmings, voles, muskrat, reindeer carcasses, waterbirds (e.g. geese, eider ducks), shorebirds, ptarmigans, hares, seals, fishes, etc. Consider sampling everything that foxes may eat, even arthropods and berries of plants if they occur in your area. You should capitalize on every opportunity to sample the various potential prey to establish as good a collection of samples as possible.

ii) Sample meat on fresh carcass (< 24h) and on trapped animals (e.g. in the case of lemmings where allowed).

iii) *Timing:* Although sampling should be done at every opportunity, two periods are of particular interest: the spring-beginning of summer and the end of summer. Keep in mind that winter fur of foxes reflects fall diet and feather/down of avian predators reflects summer diet of the previous year. If you are in the field during winter, samples can also be collected in the winter (e.g. reindeer carcasses, hares, ptarmigans).

iv) For eggs, do not forget to homogenize the white and the yellow parts before taking the sample. PRECAUTIONS: priority should be put on eggs rejected by females (i.e. common in waterbirds) or on abandoned nests, in order to limit our impact on nesting birds.

MATERIAL

• Small paper envelopes (for dry tissues; e.g. feather and fur)
• Microtubes (2ml) with screw-top caps (for wet tissues; e.g. meat, egg homogenate)
• Ethanol **70 % (DON'T USE OTHER CONCENTRATIONS!)**
• Permanent markers
• Carbon pens (or pencils)
• Field books
• Gloves (to prevent sample contamination and to secure your health)
• Tweezers (to prevent sample contamination and to secure your health)
• Scalpel and blades
• Reference meter
• GPS (It is critical use the same geo-location system; for robustness reasons, we should use in all the Arctic sites, the universal systems coordinates longitude/latitude hour-min-seconds with the WGS 84. Specify the reference area and your confidence interval in seconds)

DATA MANAGEMENT

i) *On a piece of paper:* Write with a carbon pen the date of sampling, the code for the species (see below), the name of the study site, the GPS location, a unique sample number (see below), and your name. Put the paper in the microtube with the sample and the ethanol. Write also on the microtube, the species code, the sample number and the date. For dry samples, write this same information (described above for piece of paper) directly on the paper envelope with a pencil or carbon pen.

ii) *In the field* book: Starting with the unique sample number corresponding to the appropriate sample, re-write the same information about the sample: date (day, month, year), species code, study site name, GPS coordinates, and your name. In the field book, when appropriate, you can also specify the tissue type and location on the body for the samples. For fox carcasses, you should add information (whenever it is possible) on total mass, sex, left back legs length, age class (young/adult) and the state of the carcass when found (e.g. frozen, newly hunted, deteriorated, etc.). Any other available information about the sample and where it was collected can also be added in the field book (e.g. altitude (use GPS), habitat type, age (or age class) and sex of animals sampled, time elapsed between death and sampling, etc.). Information on freshness of the sample (i.e. confirmation of freshness) can also be noted. Too much information is better than too little.

iii) *Species codes:* Species codes are formed of the first 3 letters of the latin genus name and the first 3 letters of the species name, for example:

Species Code	English name
VULLAG	arctic foxes (note: genus name has recently changed)
BUSCA	snowy owl (note: genus name has recently changed)
LEMSIB	brown lemmings
ONDSIB	collared lemmings
VULVUL	red fox