Building a cost-effective, flexible sample bank of animal diets

The scope of this protocol

This is the lab's most pared-down protocol for simple, cost-effective banking of animal dietary samples near the point of collection. It is useful for collecting a bank of samples that can be used to identify foods in animal diets and study aspects of animal nutrition. It has been used by multiple governmental agencies, non-profit organizations, and student researchers under a variety of field conditions around the world.

The protocol assumes local access to a reliable freezer but does not require specialized lab supplies or equipment. When the following measures are taken, we have found samples can be stored—or 'banked'—while preserving dietary DNA of sufficient quality for years prior to analysis. The samples may also be useful for paired analyses of various dietary and nutritional metrics as needed (e.g., crude protein, micronutrients, stable isotopes, microbiomes).

Field-sampling considerations for dietary DNA

Material options. When collecting fecal samples in the field, samples should be collected and stored in unused, sealable plastic baggies. We like small 'plastic jewelry bags' for the sake of cost and the ease of labeling and storage, but the size can be matched to the need. Baggies of ~2-3 mm thickness can help prevent brittleness and tearing during storage.

Sample quantity. A fecal sample the size of a pea is all that is required for a typical dietary DNA analysis (~150 mg). It is helpful to avoid bulky and contaminating materials such as soil, twigs, seeds, or hair. Small samples have advantages in that they freeze quickly to preserve quality and that a clean subsample can be obtained from otherwise dirtied material. However, more material or duplicate collections can be made to provide insurance against rare setbacks (e.g., freezer failures, lab mix-ups) or to enable complementary analyses.

Sample number. The number of unique samples to collect inevitably depends on the study objectives and resources as well as animal abundance. Often the objective is to compare the diets of animals based on different groupings—different species, different populations, different seasons—and for this we have some helpful rules of thumb. We have found that 10-30 samples per grouping represents the optimal effort for most groups of consumers. More than 30 is often more than necessary to reveal most of the dietary diversity that exists within a group. Much less than 10 can still provide useful data, and often conditions on the ground may limit collections to this level, but this can

make it difficult to compare groups. We are happy to discuss objectives and develop tailored collecting targets.

Fresh is best. Ultimately, you'll have to make do with what is feasible in the field. Dietary DNA degrades and gets contaminated with time, but that happens faster when it is warm and wet/sunny than under certain other conditions. If you can collect fresh fecal samples (i.e., as soon as an animal defecates), that's obviously ideal. But, within the lab, we often work with samples that fell the night before, or within 24 hours. Consistency and documentation can be important; when collecting samples, we try to note some characteristics of the sample (e.g., how recent, how clean, how certain the source).

Storage considerations

Sample storage depends on what is feasible. If immediate freezing is not possible, it is best to place samples into an insulated box with ice packs until you can access a freezer. Avoid relying on "frost-free" freezers because those can be harmful to DNA—they work by periodically thawing to evaporate frost and then refreezing. For very long-term sample storage, we recommend using a -80^{o}C freezer; we have found that ~20^{o}C freezers are often more affordable and adequate for storage at field sites over periods of months to years.

Step-by-step guide for collection

1. When collecting a dung sample, we suggest turning the baggie inside out with your hand inside (like using a dog poop bag; do not touch the inside).
2. Take a representative sample of the dung that is ~a tablespoon or ~3-5 pellets. If the sample is more like a dung pat, first remove any crust that may have formed on the outside of the dung sample before taking a representative sample.
3. Fold the baggie back around the collected dung sample and seal tightly making sure to remove as much air as possible from the bag.
4. Label the bag with permanent marker-ink to include information such as sample ID number, collector ID, date, and location/GPS. We suggest writing information towards the bottom half of the bag if a specific writing panel is not visible to reduce the risk that it rubs off during handling.
5. Record these collection data in a notebook or spreadsheet for long-term records keeping and sharing.
6. Freeze the sample as soon as possible.

Final considerations

Field conditions vary across sites. Sometimes, access to reliable electricity and freezers is not possible. Sometimes, access to additional lab supplies and/or equipment may be

possible. Although the initial sample collection strategy changes little, we have often used alternative storage methods—including preservation in alcohol, drying, and the use of specialized ‘lysis buffers’ for storing dietary DNA at room temperature. We strive to match the collection and storage strategies to what is practical on the ground.