# Field collection protocols for DNA dietary analysis of seabird scats

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## BACKGROUND

Food DNA present in scats provides a non-invasive tool for studying the diet of seabirds (e.g.Deagle et al. 2007, Bowser et al. 2013, Jarman et al. 2013, McInnes et al. 2016b). Dietary DNA metabarcoding uses high-throughput sequencing of small, highly variable DNA regions that survive digestion to identify the food consumed (Pompanon et al. 2012). This may involve identification of a particular food species using species specific markers (Jarman and Wilson 2004); food within a taxonomic group using group specific markers (Jarman et al. 2004, Murray et al. 2011, Zeale et al. 2011); identification of all food taxa using universal metazoan markers (O'Rorke et al. 2012, Jarman et al. 2013); or a combination of these approaches (Deagle et al. 2009, Bowser et al. 2013). However, characterising the entire diet requires 'universal' markers that are capable of amplifying DNA from any food group (King et al. 2008, Jarman et al. 2013).

Universal metazoan polymerase chain reaction (PCR) primers amplify from all eukaryotic DNA, but will inevitably also amplify unwanted DNA from non-food items (Deagle et al. 2009, O'Rorke et al. 2012). Non-target DNA within the scat may originate from the animal being sampled, its parasites, gut flora; or contamination from external organisms such as insects and vegetation. These sources of DNA can overwhelm the sequences amplified from a sample, making detection of DNA from food items less effective. Sample sizes must consequently be increased to address the underlying questions of a study, increasing processing costs. In some cases, non-target DNA amplification can be reduced by using a blocking primer to suppress amplification of specific DNA types, such as DNA of the defecating animal (O'Rorke et al. 2012). However, development of blocking primers is challenging and food sequences may be inadvertently blocked with this approach. The use of blocking primers becomes more complex when there are multiple non-target DNA groups present.

To improve the quality and quantity of dietary information obtained from scat samples, these optimised scat collection protocols were developed using Shy Albatross (*Thalassarche cauta*) to provide a basis for future experimental designs, enable the collection of high quality diet samples and reduce non-target DNA amplification. Further details can be found in McInnes et al. (2016a).

## CONSIDERATIONS PRIOR TO SAMPLING

Careful planning of DNA dietary metabarcoding studies prior to sample collection is imperative for overall project success. Researchers should consider the dietary question they are targeting and focus

on which scat samples will inform this. This includes marker selection, seasonal changes, fasting and the age of animals.

The ideal marker to choose will be based on the scientific question. Group specific markers can provide high taxonomic resolution of prey within a group (e.g. cephalopods), however will only detect that group of prey (Deagle et al. 2009, Bowser et al. 2013). A universal metazoan primer set will allow the all main prey groups to be identified, but with low taxonomic resolution (Deagle et al. 2009, Jarman et al. 2013, McInnes et al. 2016b). If a universal metazoan marker is chosen, additional care will be needed in the field to reduce the collection of non-food DNA.

DNA dietary analysis is an excellent tool for identifying diet at a population level, however identifying the diet of individuals using this method should be treated with caution. The digestion time is often unknown and depending of foraging duration, the scat may not relate directly to the previous meal.

## FACTORS AFFECTING SAMPLE SUCCESS

### *Sample freshness*

Fresh samples where the bird is observed defecating provide significantly more food DNA than dry samples (McInnes et al. 2016a). Dry scats have had more potential exposure to external contamination, particularly from fungi and also DNA within the scat is likely to degrade with UV exposure (Oehm et al. 2011). Recent scats that were wet, but where time since defecation was unknown, had mixed success. Recent samples had a lower amplification success than fresh samples, but the proportion of food DNA detected in those that did amplify, was not significantly lower than that of fresh scats. Therefore using scats that are still wet may produce dietary information, but larger sample sizes would be required and reliance on small amounts of DNA may reduce data quality (Murray et al. 2015).

If the study uses group specific dietary markers, then food DNA may be detected for longer. For example, in carrion crows (*Corvus corone*), food DNA could be detected for up to five days when protected from UV and rain exposure (68% success), however, this was dramatically reduced when scats were left in exposed areas (17.5% success) (Oehm et al. 2011). Similarly, Steller sea lion (*Eumetopias jubatus*) scats also produced detectable prey DNA for up to five days in some samples using group specific markers (Deagle et al. 2005).

### *Collection substrate*

Seabird colonies are often surrounded by tussock grass and exposed dirt, both of these provide contamination from other DNA sources through vegetation or unicellular organisms in the dirt. Consequently, the substrate the sample lands on can affect the amount of food DNA obtained. Scats that land on substrates that contain other sources of DNA, such as plant or dirt, will increase the risk of contamination (McInnes et al. 2016a). Samples collected from rock or ice enable more food DNA to be detected. If samples are obtained from dirt or vegetation, ensure that the collection of soil or plant is minimised. Take care in colonies with multiple bird species, especially when they might be a possible prey item. For example if the target animal is known to feed on carrion and breeds around penguins or seals, ensure the DNA obtained is not contamination.

### *Breeding stage*

The detection of food DNA in scat samples throughout the season is strongly linked to fasting (McInnes et al. 2016a). Longer periods of fasting during incubation cause a low detection of food DNA in scats, whereas food DNA detection can be much higher for breeding birds during brood. This is likely to be linked to more frequent and shorter feeding trips during this stage. During periods of fasting, non-target DNA was dominated by endoparasites and avian DNA. Depending on the aim of the study, try to collect samples from birds with the minimum time since feeding. During incubation, target birds that are back at the colony for less than a day. This may involve observing birds newly returned to the colony or marking one bird on the nest to monitor incubation length and know when they change over. Samples from non-breeding birds in the colony during brood also had a lower detection of food DNA, which was attributed to increased time at the colony and fasting.

### *Developmental stage*

Collections from young animals are likely to pose problems for DNA dietary analysis with reduced detection of food in scats (McInnes et al. 2016a). As food is delivered by regurgitation, food items are likely to be partially digested before they are fed to the chick. Consequently, digestive processes may excessively degrade food DNA in chick scat samples. Additionally, there is presumably cross-over of parental DNA to the chick during regurgitation, which may cause the amount of bird DNA to be inflated, thereby reducing the food DNA proportionately. If a blocking primer is used that supresses bird DNA, the amount of food DNA detected in chick scats can be increased, but care should be taken that the blocking primer doesn't block other vertebrates such as fish.

Scat samples from older chicks enabled a higher detection rate of food DNA than small chicks (McInnes et al. 2016a), which may reflect larger meals or a reduction in stomach oil. This oily liquid can contribute up to 80% of the sample mass in some albatross stomachs (Thompson 1992). In shy albatross, there is a greater mass of oily liquid in younger chicks than older chicks (Hedd & Gales 2001), which may dilute the food DNA.

## SUMMARY

DNA metabarcoding provides a valuable dietary tool to identify the prey in predator scats. This method can provide broad diet information across multiple taxa or species specific resolution of targeted prey groups. There are some benefits over hard-part analysis with the detection of soft-bodied prey and hard-bodied prey items that are usually retained in stomachs aren't overestimated. Samples can be collected during all breeding and life-history stages when scats are accessible (Bowser et al. 2013) however care should be taken during some breeding stages and ages as discussed above. As no handling of birds is required, it provides an ideal method of assessing the diet of sensitive species. The limitations are that prey age, size class and mass cannot be assessed. Scats must also be available, so the approach is not feasible for determining diet during the non-breeding period for species that remain far from land. The method is not currently commercially available (2016), however there are numerous research institutes that are using this method and as demand increases it will hopefully become commercially available in the future.

## COLLECTION METHODS

### *Field equipment*

- 2ml vial half filled with ethanol (70-80%)
- Straw, small spatula or tweezers
- Permanent marker pen
- Kimwipes™/tissues
- Plastic bag to collect dirty tissues
- Notebook and pencil

### *Sample Size*

When using universal metazoan markers, the average success for of samples collected randomly can be as low as 15%, even when fresh. Whereas when following these guidelines, sample success was between 50-60%. This success rate is a guide only and may be useful to determine the sample sizes required to achieve the desired number of data points. In large colonies, it is usually possible to get 10 fresh scats per person per hour. Some stages such as incubation, may be lower.

### *Collecting the sample*

Sit on the colony edge and wait for a bird to defecate. This can be time consuming, but will be faster for people that know the behavioural cues. There are distinct behaviours prior to defecation. These include the bird standing, shuffling around usually to face into the wind, before they lean slightly forward to defecate. This is usually followed by a tail shake. Sometimes you will hear the defecation, so quickly look for the bird shaking their tail and the direction their bottom is facing to be able to find the scat. For burrowing petrels where observations aren't possible, laying a sheet outside the burrow may enable fresh scats to be collected.

Once a sample is located, collect a small fragment of the non-uric acid portion of the scat (dark part) was using tweezers or a plastic straw. Only a small portion is required (30-50µl or equivalent to half a baked bean). Seabird scats are well mixed, but if the scat is large, take a small amount from multiple parts to ensure the selection is representative of the sample. Avoid the white liquid as this is primarily urea and does not contain dietary DNA. Place the sample in the labelled vial containing 70-80% ethanol. Screw the lid shut and shake the sample to ensure the DNA is well mixed and preserved. Clean the spatula or tweezers between scats by wiping with Kimwipes™ or a tissue, or use clean tweezers between samples and bleach at the end of a day.

### *Storage*

Once samples have been collected, keep them out of direct sunlight and try to keep them cool to reduce any degradation. Although they should keep well in the ethanol in the short term, for long periods store them in the freezer at -20°C or if possible -80°C.

***Watch video at [https://www.youtube.com/watch?v=CQ_6bUX91Is](https://www.youtube.com/watch?v=CQ_6bUX91Is)***

**Target the dark part of the scat avoiding the runny white urea**

## GUIDELINES

- Collect fresh samples where the animal is seen defecating. If this is not possible, try to collect only samples that still have moisture (recent samples).
- Using a straw, spatula or tweezers collect a small amount of the dark part of the scat (not the white liquid, which is primarily urea and does not contain dietary DNA);
- Place the sample in a 2ml vial containing 70-80% ethanol.
- Tightly close the lid and mix the scat with the ethanol by shaking the tube.
- Clean the spatula or tweezers between scats.
- Take into consideration the seasonal behaviour and feeding ecology of the study animal prior to sample collection.
- Consider the scat substrate type, as contamination from substrate can overwhelm the food DNA signal. Ideally, collect scats from surfaces with minimal sources of DNA contamination (e.g. rocks or ice). If collecting from dirt or vegetation, try to minimise the collection of foreign material and record the substrate (and species where applicable) to cross-check and validate results.
- Collect from animals that are likely to have fed within a short time frame, i.e. not from fasting animals during incubation or when defending nests/territories.
- During incubation, target birds that have returned to the colony within the last 24 hours.
- Samples collected from young chicks may be problematic due to degraded DNA passed on by parents or large amounts of bird DNA. Target scats from adults or older chicks.
- If only a single collection is available and the timing in the season or cohort is not the focus of the dietary question, target the time period with the shortest foraging trip duration and focus on breeding birds.
- Scat collections in the morning may reduce DNA degradation from UV.
- If multiple study sites are used, keep collection protocols and timing consistent between sites.

## RECOMMENDED CITATION

McInnes, J. 2016. *Field collection protocols for DNA dietary analysis of seabird scats.* Agreement on the Conservation of Albatrosses and Petrels. http://www.acap.aq/en/resources/acap-conservation-guidelines Date viewed.

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