



A Non-invasive Method to Collect Fecal Samples from Wild Birds for Microbiome Studies

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Received: 29 January 2018 / Accepted: 22 March 2018
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Abstract

Over the past few decades, studies have demonstrated that the gut microbiota strongly influences the physiology, behavior, and fitness of its host. Such studies have been conducted primarily in humans and model organisms under controlled laboratory conditions. More recently, researchers have realized the importance of placing host-associated microbiota studies into a more ecological context; however, few non-destructive methods have been established to collect fecal samples from wild birds. Here, we present an inexpensive and easy-to-use kit for the non-invasive collection of feces from small birds. The portability of the collection kit makes this method amenable to field studies, especially those in remote areas. The main components of the collection kit include a flat-bottomed paper bag, a large modified weigh boat (tray), vinyl-coated hardware cloth fencing (grate), a clothespin, and a 10% bleach solution (to sterilize the tray and grate). In the paper bag, a sterile tray is placed under a small grate, which prevents the birds from contacting the feces and reduces the risk of contamination. After capture, the bird is placed in the bag for 3–5 min until it defecates. After the bird is removed from the bag, the tray is extracted and the fecal sample is moved to a collection tube and frozen or preserved. We believe that our method is an affordable and easy option for researchers studying the gut microbiota of wild birds.

Keywords Avian · Feces · Fecal collection · Method · Microbiome

Introduction

The gut microbiota can play an important role in regulating the behavior, physiology, and fitness of the host. Most of these studies focus on humans or model host systems (e.g., lab rodents) but more recently, a growing number of wild bird gut microbiota studies have been published (reviewed in [1–3]). Avian studies have used several different methods to collect gut bacterial community samples from the hosts, with no clear consensus on the best methodology [4]. For example, destructive sampling (by euthanizing live birds including, in some cases, sampling from birds that are prepared as museum samples) is likely the best method to characterize the microbiota of

the digestive system [5–11]. However, researchers cannot always destructively sample their study organisms, especially when attempting to link the gut microbiota to proxies of fitness or when working with federally protected species [9].

When euthanizing focal individuals is not an option, studies have collected fecal samples as a proxy for the gut microbiome. Although studies show that the bacterial community in bird feces does not always reflect the gut community of the host (e.g., in the cecum; [12–14]), fecal samples are generally representative of the bacterial community in the large intestines [13, 14]. To collect fecal samples, a variety of non-invasive methods have been employed, which have potential drawbacks. For one method, researchers follow individuals until they defecate and then collect the fecal sample from the environmental substrate [5, 15–18]. If the study requires sterile sampling or is focused on a microorganism that is not found in the environment, collecting samples from a non-sterile substrate can potentially contaminate the sample [4]. Another option is to place “catching” materials, such as paper, wax paper, or plastic, in a container (e.g., bag or cage) under a captive or recently captured bird [11, 17, 19]. Alternatively, researchers have placed catching materials under objects where birds are likely to perch in the wild (e.g., bird bath, feeding station) to

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collect the fecal samples [5, 17, 20]. Two issues can arise from collecting feces directly from these substrates: (1) the substrate is typically not sterile, and/or (2) the birds are not restrained and could then potentially contaminate the fecal samples by contacting it (e.g., stepping in the feces) (Fig. 1a, b). Indeed, a study found that contamination of feces was low in traps with desert woodrats (*Neotoma lepida*) [21]. However, it is unclear the extent to which the rodents contacted the feces in the traps whereas birds often contact the entirety of the feces in the bags (Fig. 1a, b); rodents' feces are also relatively solid compared to the liquid-based feces of birds so the rodents likely contact only the surface of their feces as opposed to the entire sample. Another option is to collect cloacal swabs instead of fecal samples [22, 23], but a recent study suggests that the fecal microbial community reflects the true gut community better than that of cloacal samples [14].

Here, we present a new protocol to collect fecal samples from live wild birds, which is particularly amenable to field conditions. The purpose of this protocol is to provide a relatively sterile method in which birds do not directly encounter the feces after defecation (Fig. 1a–d); this proposed technique is particularly useful in remote field locations, such as the Galapagos Islands where we designed and used the method. All materials are relatively inexpensive and the collection kits are easy to construct. The collection kit described in this paper are ideal for small to medium passerines (< 30 g), but can be easily scaled based on the size of the focal bird.

Materials to construct the collection bag include paper lunch bags with a flat bottom, hardware cloth fencing, plastic weigh boats, scissors, and clothespins (or any type of clip) (Table 1, Fig. 2a–e). We advise that the researcher wears laboratory gloves while constructing the collection bags so that they do not transfer human-associated microbes to the bags. First, cut off two sides of the weigh boat so that it is 13.97 cm × 7.62 cm × 2.54 cm and thus will fit in the paper bag (Fig. 2b, c). Then, cut the fencing material into smaller pieces that are 15 squares (19.5 cm) by 11 squares (14 cm); remove 4 squares from each corner (Fig. 2d, e). Fold the outer two rows of each side of the fencing to make a raised grate and place it in the paper bag over the modified weigh boat (from here on: tray) (Fig. 2f). Use a clothespin to secure the bag (Fig. 2g).

Collection materials should be sterilized between uses to reduce potential cross-contamination. We recommend sterilizing the tray and grate by soaking them in a 10% bleach solution for at least 10 min [24]. If this method is not possible, then the bleach solution can be placed in a travel-sized spray bottle and used to treat the kit prior to use. In both cases, ensure the collection materials are dried before use to prevent the bleach from degrading the DNA in the sample. The collection bags can be constructed prior to traveling to the field site, entering the field, and/or while in the field. Similar materials should also be readily available in any town that has a hardware and/or grocery store near the field site. If the researcher is constructing the bags while in the field, we suggest that they

Fig. 1 Pictorial comparison of fecal collection methods after individual birds were held in their respective bag for 3 min with and without a grate above a tray. Panels **a** and **b** represent two different attempts to collect feces from birds with only a tray and no grate. In both examples, most of the fecal matter splattered on the bag, and in panel **a**, the fallen feathers are saturated with fecal matter from the bird. Panels **c** and **d** show an attempt to collect feces using a tray and grate. Fecal matter remained on the tray and although a feather had fallen off the bird, the feather remained attached to the grate

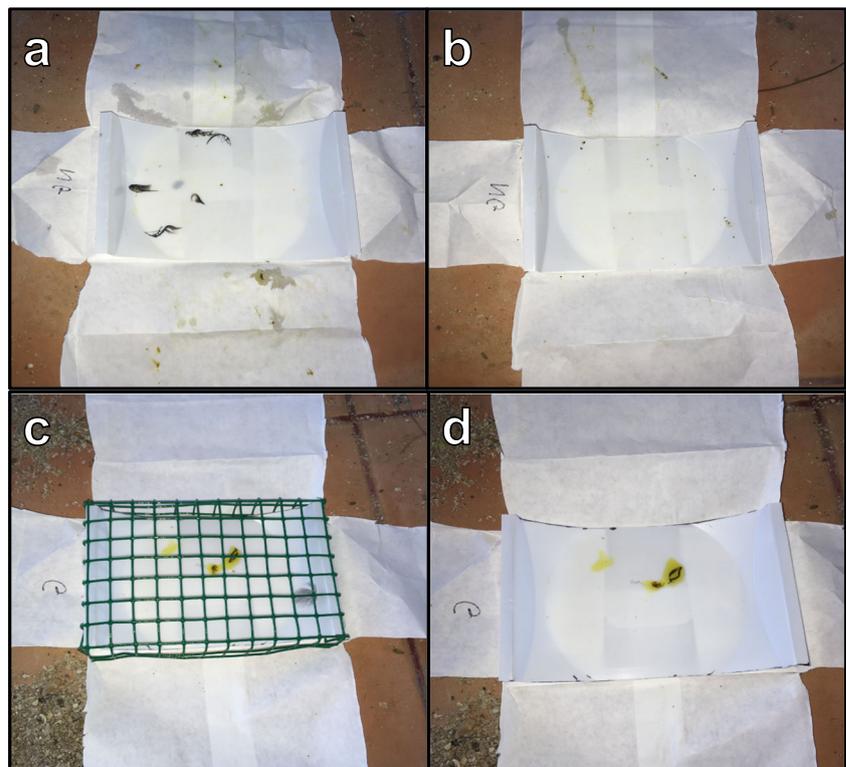


Table 1 Materials and examples of brands/vendors for proposed fecal collection protocol

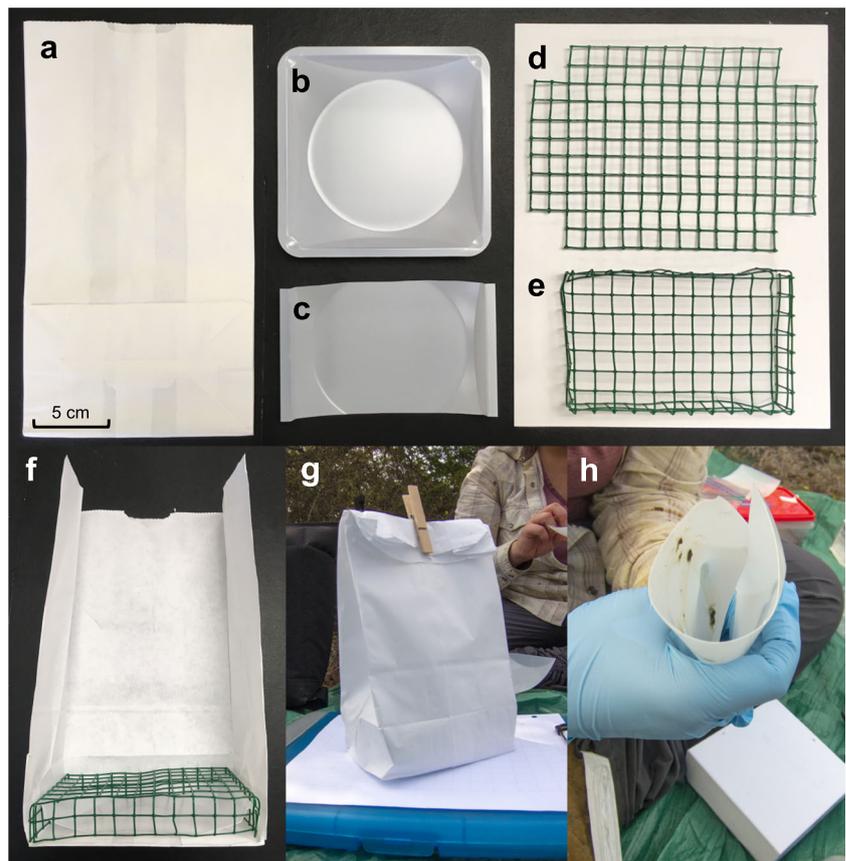
Supplies	Dimensions	Suggested brand (vendor)
Paper lunch bags	15.24 cm × 7.62 cm × 27.94 cm	School Smart (Amazon.com)
Large weigh boats	13.97 cm × 13.97 cm × 2.54 cm	Scientific Equipment of Houston (Amazon.com)
Galvanized hardware cloth (vinyl coated)	1.27 cm, 19-gauge	Everbuilt (Home Depot)
Sterile swabs	15.24 cm	Fisherbrand (Fisher Scientific)
Sterile collection tubes (snap or screw top)	2 mL	Thermoscientific (Fisher Scientific)
Bleach	473 mL	Clorox (Amazon.com)
Plastic container	700 mL	Koax Biokips Food Storage Containers (Amazon.com)
Kimwipes	11 cm × 21 cm	Kimtech (Fisher Scientific)
Travel spray bottle	74 mL	Fantasea Fine Mist Spray Bottle (Amazon.com)
Nitrile gloves	NA	Fisherbrand (Fisher Scientific)
Clothespins	NA	Whitmor Natural Wood Clothespins (Amazon.com)
Scissors	NA	Fiskars Softgrip Scissors (Amazon.com)

transport all sterilized material in a sealed container (e.g., plastic container or bag) to prevent contamination.

Immediately after capture, individual birds should be placed directly in the collection bag; the bag is then sealed by folding the top edge over and secured with a clip. We recommend that you place and brace the bag on the ground, as opposed to hanging it on a laundry line, to minimize movement of the bag. The amount of time the birds spend in the bag

depends on the species. In our experience, passerines typically defecate immediately after capture and therefore 3–5 min in the bag is sufficient. Once the bird has defecated, the bird can be removed and processed according to the researcher’s protocol (e.g., banding, measurements, blood samples). We do not reuse bags for multiple birds because the bags are often destroyed to obtain the tray and grate; however, the bags could possibly be used for multiple birds, if needed.

Fig. 2 Examples of the **a** paper bag, **b** original and **c** modified weigh boat tray, and **d** flattened and **e** folded hardware cloth grate. Once constructed, panel **f** represents a cut away view of the inside of the collection bag, **g** a sealed collection bag with bird, and **h** a demonstration of creating a funnel with the tray to collect feces



While collecting fecal samples from the tray, researcher should wear gloves, if possible. Sterile swabs and microcentrifuge tubes (e.g., 2 mL for small- to medium-sized birds) are also needed for the collection of the fecal samples. Carefully remove the grate from the bag and then remove the tray. Create a funnel with the tray and place the smallest end into the collection tube (Fig. 2h). Use the sterile swab to move the feces into the tube, place the swab in the tube, and snap off or cut the end of the swab. We recommend that the swabs are included in the tube and DNA extraction since the swab will contain fecal bacteria from the transfer. Any debris, such as feathers, that falls off the birds should be removed from the tray before the feces are transferred. Once feces and swab are collected, the tube should remain on wet ice in the field and then placed in a freezer (-20 or -80 °C) as soon as possible for long-term storage and/or until DNA extractions can be performed. However, if a freezer is not available, the fecal samples can be stored in DNA/RNA shield (Zymo Research, CA, USA) at room temperature for over 2 years for DNA and 1 month for RNA.

As the interest in gut microbiota of wild birds increases, fecal collection methodology that minimizes potential contamination and allows for among-study comparisons is needed. Here, we described a novel method for collecting fecal samples from wild birds that we believe fills this need. Our protocol combines two previously published methods: the bird bag for wild birds (e.g., [5, 11]) and grated cage for captive birds (e.g., [18, 20]). Although we did not compare collection methods empirically, our observations suggest that the method without a grate results in a smaller fecal sample and potentially has a higher risk of contamination from the focal bird compared to the method with a grate (Fig. 1a–d). Our specific design was intended for our small budget and system (Darwin’s finches) but can be easily modified to suit the researcher’s needs (e.g., larger birds). Our fecal collection protocol represents an affordable and easy option for researchers who are interested in wild bird gut microbiomes.

Acknowledgements We thank Johanna Harvey for field assistance and Brian Trevelline and three anonymous reviewers for comments on this manuscript.

Funding Information The work was supported by funding through Instrumentl Crowdfunding to KMG and SAK; a Le Fonds Québécois de la Recherche sur la Nature et les Technologies Postdoctoral Fellowship, Natural Sciences and Engineering Council of Canada Banting Postdoctoral Fellowship, and British Ornithologists’ Union Research Grant to KMG; and a British Ecological Society Large Research Grant (5599-6643) and the University of Connecticut to SAK.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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