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ABSTRACT

*Using fecal samples to analyze trophic levels, glucocorticoids, and diet of birds is common in avian research. However, several methods of fecal sample collection often do not provide a complete sample and can induce unnecessary stress in birds. Parrish et al. (1994) described a new fecal sampling apparatus to opportunistically collect feces of mist-netted birds. However, based on the paucity of citations for this method, it appears that it has not been widely accepted or implemented in field studies. In this paper we describe a modification of the fecal sampling apparatus outlined in Parrish et al. (1994), and suggest that this technique eliminates many of the shortcomings associated with other common methods of fecal sample collection. Our compact fecal sampling apparatus is constructed using inexpensive materials and can be implemented in many banding studies, including remote operations. In the summer of 2016 we tested the efficacy of this fecal sampling apparatus on 99 passerines of three species (44 Worm-eating Warblers (*Helmitheros vermivorum*), 29 Ovenbirds (*Seiurus aurocapillus*), and 26 Scarlet Tanagers (*Piranga olivacea*). Eighty birds successfully defecated in the apparatus (31 Worm-eating Warblers, 26 Ovenbirds, and 23 Scarlet Tanagers), resulting in an 81% success rate for this method. We deem this technique highly effective and recommend its implementation in future banding studies that incorporate a fecal sampling research component.*

INTRODUCTION

Given the increasing number of research applications involving avian feces (Fair et al. 2010), there is a recognizable need for an efficient field sampling method to collect quality fecal samples from birds. Fecal material can be used to perform trophic analyses using stable isotopes, measure stress response using glucocorticoid metabolites, and determine specific components of avian diet using next generation sequencing (Podlesak et al. 2005, Sheriff et al. 2011, Pompanon et al. 2012). However, it can be difficult to collect quality fecal samples from wild birds on a consistent basis.

In many passerine studies, birds are extracted from mist nets and placed in cotton handling bags before they are banded and released (Bocetti 1994). During this holding period some birds defecate in the bags and feces are opportunistically scraped off the bags to be used in subsequent analyses (Ralph et al. 1985, Podlesak et al. 2005). There are two main problems intrinsic to collection via this sampling method. First, there is a possibility of sample cross-contamination if bags are re-used. Second, it is often difficult to attain a complete sample because feces can rub off on feathers, and cloth bags quickly soak up the liquid component of feces, which prevents collection of a complete sample. This can be especially problematic if the liquid component makes up a majority of the fecal sample, as is common for many frugivorous birds and small passerines.

In some instances, birds may predictably defecate during handling, in which case a vial can be held under the cloaca to catch the feces as they are released (Poulsen and Aebischer 1995). However, this method is most often used with nestlings and is not as effective with older birds. Additionally, some birds defecate as soon as they become entangled in the mist-net and feces can be collected on plastic sheets placed underneath nets (Hernández-Dávila et al. 2015), although there can

be challenges using this method. Transporting large plastic sheets may be cumbersome in remote banding operations. In addition, depending on the method of fecal analysis there may be potential for sample contamination if feces come in contact with a soiled net. Finally, if many birds are simultaneously caught in the same net, correctly assigning fecal samples to individual birds may become difficult.

Another common method for collecting fecal samples involves placing birds in an enclosed box or paper bag for a pre-determined period of time and collecting any deposited feces after this period (Poulin et al. 2002, Lindström et al. 2009). This "box method" is also employed in diet studies in which birds are given emetics to induce a regurgitation response (Poulin et al. 2002, Carlisle and Holberton 2006). Using this method, a bird may defecate (or regurgitate) shortly after being placed in the container, but must remain inside until the predetermined time has elapsed. Not only can this method cause unnecessary stress for the bird, but it may also compromise the quality of the fecal sample. Similar to cloth handling bags, the bird can rub the fecal sample into its feathers and onto the holding container walls as it flaps and moves around, effectively decreasing the amount of the remaining available fecal sample.

Parrish et al. (1994) described an elegant solution to these problems. By attaching wire mesh and a Ziploc® (SC Johnson, Racine, WI) bag to the bottom of a polypropylene sock, Parrish et al. (1994) collected feces from 347 migratory birds in Rhode Island with a 72% success rate. This method also minimizes stress in birds by opportunistically collecting feces during the holding period prior to banding. Although the Parrish et al. (1994) method yields improved results compared to many other common methods of fecal sample collection, it appears that it has not been widely accepted or implemented in avian field studies. After completing a thorough literature search using several online databases (e.g., Agricola, Web of Science, Wildlife and Ecology Studies Worldwide, etc.) we only identified three references to the Parrish et al. (1994) technique over the past 20 years. Based on the paucity of citations for the Parrish et al. (1994) method, and the continuation of less desirable fecal sampling collection techniques in the literature, we feel the need to reiterate this technique and advocate for its use in future avian research.

In the present manuscript we describe a modification of the Parrish et al. (1994) technique for collecting fecal samples from small passerines. We implemented this modified fecal sampling apparatus in a field study in southern Indiana (Brown and Monroe counties) in the summer of 2016. Here we report the efficiency of this fecal sampling apparatus and provide a detailed description of the pros and cons of our modification with the original Parrish et al. (1994) design.

METHODS

The modified fecal sampling apparatus was constructed using inexpensive materials: a white paper lunch bag, 1.27-cm hexagonal plastic-coated wire mesh (Menards®, Eau Claire, WI), and duct tape. To begin assembly, the bottom of the lunch bag was cut or torn away, leaving an open-ended, 27 x 13 x 8 cm chamber. Wire cutters were used to cut an 18 x 13 cm piece of wire mesh that was larger than the base of the paper bag, allowing for approximately 2.5-cm clearance on all sides. The excess 2.5-cm of mesh material was bent upward on all sides to create a rectangular base of equal dimension to the base of the bag. The newly formed wire base was inserted into the paper bag, and duct tape was used to secure the folded ends of the mesh to the inside of the bag, thoroughly covering any exposed wire points (Fig. 1). Replacing the bottom of the paper bag with a wire mesh platform provided a "pseudo-perch" for birds and allowed fecal samples to pass through unimpeded.

To collect feces from birds, we positioned the fecal sampling apparatus approximately 0.25-m off the ground with a small Ziploc® bag placed directly underneath. We closed the top of the paper bag by folding it over, and attached the apparatus to a young sapling or supple woody stem (*Smilax* spp. worked best in our field sites). Although we attached the apparatus to natural structures in our study, it could also be attached to a low-hanging clothesline in more permanent banding operations. In addition, we used Ziploc® freezer bags placed flat on the ground below the apparatus to catch fecal samples, but any reusable plastic sheet would suffice (Fig. 2).

During the summer of 2016 we tested this fecal sampling apparatus on 99 wild-caught passerines representing three different species in southern Indiana: Worm-eating Warbler (*Helmitheros vermivorum*), Ovenbird (*Seiurus aurocapillus*), and Scarlet Tanager (*Piranga olivacea*). Birds were captured with 12-m long, 30-mm mesh, four-tier, black, tethered, nylon mist nets. After we extracted birds from the nets, we placed them in cotton handling bags and carried them back to a central banding station. We banded each bird and recorded morphometric data (i.e., wing chord length, tail length, culmen length, and mass) before placing it in the fecal sampling apparatus. Once a bird was placed in the apparatus it was checked every minute for a total of 10 min. If a bird provided a fecal sample before the maximum time, it was immediately released.

We used a 27-gauge insulin syringe (BD Micro-Fine IV, Becton, Dickinson and Co., Franklin Lakes, NJ) to effectively collect both the liquid and solid components of feces. We collected the liquid component of feces first, and then used the fine tip of the syringe to scrape the remaining solid component into a 1.5-ml microcentrifuge tube. Complete fecal samples were then frozen for future analyses. To prevent sample cross-contamination, the wire mesh and plastic bags were cleaned with alcohol swabs between uses. As a result of constant folding and unfolding, the paper bag component of the fecal-sampling apparatus generally needed to be replaced after approximately 20 uses, but the wire mesh base could be saved and reused.

RESULTS

We tested the efficacy of the fecal sampling apparatus on 99 small passerines in southern Indiana (44 Worm-eating Warblers, 29 Ovenbirds, and 26 Scarlet Tanagers). Eighty birds successfully defecated in the apparatus (31 Worm-eating Warblers, 26 Ovenbirds, and 23 Scarlet Tanagers) resulting in an 81% success rate for this method. Average masses for Worm-eating Warblers, Ovenbirds, and Scarlet Tanagers was 13.2 g ± 1 g, 19.0 g ± 1.1 g, and 28.7 g ± 1.8 g, respectively (mean ± SD). Although birds were given 10 min to defecate, most defecated within 2 min of being placed in the apparatus.

DISCUSSION

The fecal sampling apparatus was highly effective, producing complete fecal samples 81% of the time. The scale of this apparatus also proved to be adequate for passerines ranging in size from 11.0 – 32.0 grams (the size of the smallest Worm-eating Warbler to the largest Scarlet Tanager, respectively). This efficient design produced complete fecal samples with inexpensive materials and eliminated many of the pitfalls associated with pre-existing fecal sampling strategies.

Unlike most pre-existing methodologies (excluding Parrish et al. 1994) in which the moment of defecation may be unclear, this apparatus allows feces to fall through holes in the wire mesh and onto a plastic bag, enabling researchers to easily observe the exact moment of defecation. Birds can then be released immediately after defecating, preventing them from being detained for an unnecessary period of time. In addition, defecation through the wire mesh prevents birds from ruining the fecal sample by flapping or rubbing against it as is commonly the case when birds are enclosed in a box or bag (P. Ruhl, personal observation). This becomes even more crucial as the size of the fecal sample decreases. In our study, fecal samples from Worm-eating Warblers and Ovenbirds could be as small as 0.1 mL. Thus, our fecal sampling apparatus allowed us to collect complete samples from birds whose feces may have, otherwise, been difficult to salvage using other methodologies.

Our design differs from the apparatus described in Parrish et al. (1994) in three main aspects, namely composition, sample collection, and timing. Our apparatus is composed of a white paper lunch bag instead of a polypropylene sock. This paper composition is advantageous in two ways: First, it provides a more rigid structure (similar to a cage) providing a larger platform, giving the bird more space to move, and minimizing the chance of defecation (i.e., sample loss) on the apparatus wall. Second, the white material allows for quick inspection

of potential contaminants after defecation. Although paper is not as durable as polypropylene (which can affect longevity), the material is inexpensive and recyclable, and one lunch bag can easily withstand 20 uses.

Our modification does not involve the attachment of a sampling bag to the bottom of the apparatus (although this could easily be added if desired). By placing Ziploc® bags on the ground below the apparatus and collecting feces directly from these bags we were able to concentrate feces in a small (1.5 ml) tube. This allows for easier processing for certain lab applications (e.g., stable isotope studies) that require freeze-drying and powdering. In addition, by specifically collecting feces with a syringe immediately after defecation, we ensured that the fecal samples were not contaminated with feathers or other foreign objects that might drop into an attached sampling bag.

Unlike Parrish et al. (1994), we did not place birds in the fecal sampling apparatus immediately after removing them from the mist nets. Instead, we placed birds in the apparatus for a maximum of 10 minutes after they had already been banded and processed. Because our modification of the apparatus is composed of a paper lunch bag rather than a polypropylene sock, it is not as durable and cannot be easily tied off. Thus, it cannot serve the same function as a cloth handling bag. Rather, our paper lunch bag iteration of the Parrish et al. (1994) method replaces other "box methodologies" commonly used for fecal sample collection, but with the added benefit of immediate recognition of defecation. Although birds in our study were not placed in the fecal sampling apparatus immediately after they were removed from mist nets, our success rate was higher than that reported in Parrish et al. (1994), suggesting that the timing (i.e., how soon birds are placed in the fecal sampling apparatus) may not be critical. It is possible that the 19 birds that did not provide a fecal sample in our study may have defecated while they were entangled in mist nets or being carried in cloth handling bags. However, we observed several birds (~ 10) defecate prior to placement in the apparatus, yet still provide an adequate fecal sample after being placed in the apparatus. We posit that the most important factor is the birds' ability to stand or perch on the wire platform. Because birds often defecate just prior to flight (Van der Veen and Sivars 2000), we suggest that placing them in the container with a wire bottom provides the impetus for defecation. Thus, the timing is less important than the physical placement in the apparatus itself.

Our described modification of the Parrish et al. (1994) fecal sampling apparatus is lightweight and compact (17-g), allowing for easy transport to remote banding stations. In contrast to other methods (e.g., placing plastic sheets under every mist net) our apparatus is much more efficient. This design also allows for a high level of adaptability in set-up, without compromising productivity. Composed of inexpensive materials, this fecal sampling apparatus can be implemented in many field studies, regardless of budget.

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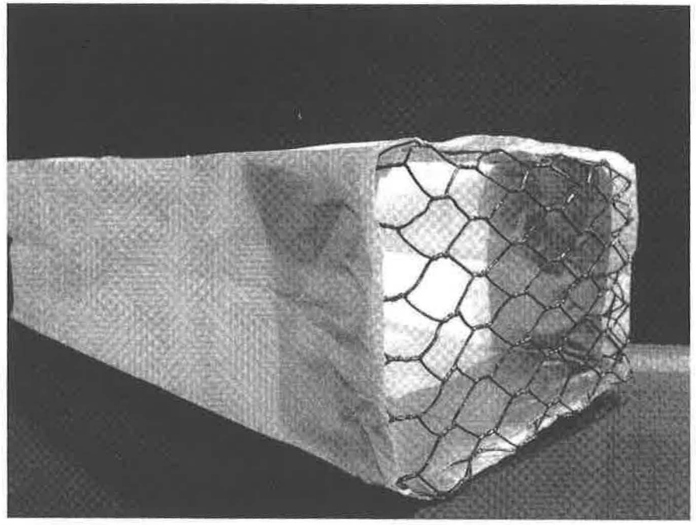

Fig. 1. Photograph of a fully assembled fecal sampling apparatus.

Fig. 2. Photograph of a typical field set-up: fecal sampling apparatus with Ziploc® bags and fecal sample underneath.

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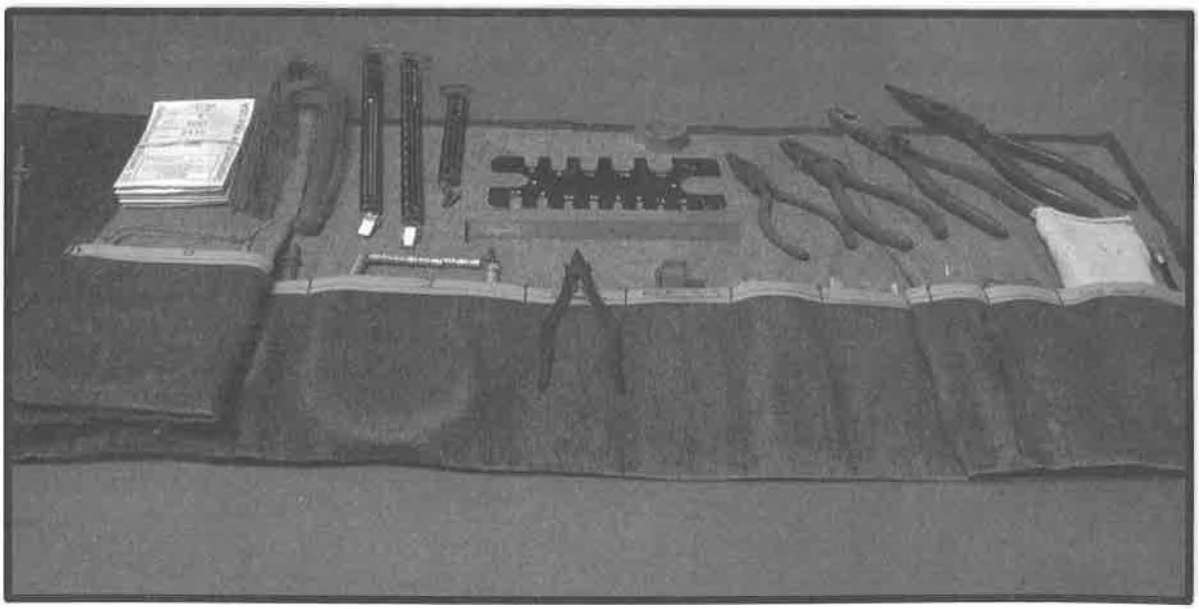

Banding Equipment (tools) Photo by R. Pantle