Standard Operating Procedure for Opportunistic House Mouse (*Mus musculus*) Hair Sample Collection

Compiled by Wieteke A. Holthuijzen, Co-PI (3/22/2022)

Project Overview

The house mouse (*Mus musculus*), after humans, is the most ubiquitous mammal on the globe, and has adapted to a wide range of environments, colonizing every continent except Antarctica. Plasticity in diet is one of the key adaptations that allows house mice to survive and thrive across various regions and climates. House mice are considered a generalist omnivore and consumes a diverse array of arthropods and vegetative material. However, on islands, mice have exhibited surprising prey-switching behavior, in some cases attacking and depredating breeding seabirds that are magnitudes larger than the mice themselves. Despite increasing observations of seabird depredations by house mice, it remains unknown if seabird consumption constitutes an incidental or foundational component of mouse diet on islands—which represents a critical knowledge gap of house mice behavior and ecology. Specifically, this gap underscores the need to understand adaptive capacity of house mice in insular ecosystems and the resulting ecological consequences of that adaptability.

The novel behavior of seasonal prey-switching to island avifauna by house mice often occurs when other preferred food sources, such as arthropods and seeds, are limited. In particular, this prey-switching behavior has only been documented when mice are the sole (introduced) predatory mammal in island ecosystems. Under these circumstances, the species' effects on insular food webs may be amplified as house mice shift to a predatory behavior more commonly associated with *Rattus* spp. However, when house mice co-occur with *Rattus* spp., evidence suggests that these rodent species collectively participate in niche partitioning. Thus, prey-switching by mice could be very important in top-down control of island ecosystems, given that invasive rodents are typically the only vertebrate predators in these ecosystems. Moreover, with the increasing use of rodent eradications to restore island ecosystems, predicting both the nature and magnitude of direct and indirect ecosystem effects that follow eradications is critical. For example, if house mice effectively operate as a top predator on islands, their eradication could result in a sudden and unanticipated release of prey species, which could be challenging if mice were also controlling other problematic species (e.g., invasive plants or agricultural pests). Few studies have examined the diet and trophic niche of house mice on island ecosystems; consequently, the broader island ecosystem effects of this species are largely unknown.

Our Objective

Conduct a global study of islands to investigate the trophic position and diet of invasive house mice and understand the broader effects of house mice on insular food webs.

Equipment Needed

- Small plastic bags/plastic vials (clean)
- Tweezers (preferably)
- Alcohol and cotton swabs/alcohol wipes/hand sanitizer/paper towels
- Sharpies/permanent markers
- Gloves (and other relevant PPE)

Field Operations

For this project, we will use mouse hair for both compound-specific isotope analysis and bulk tissue isotope analysis to understand the trophic position of mice on islands. Only 0.8-1.0 mg of hair per specimen is required for analysis; we typically collect at least 3.0 mg of hair per specimen as a precaution and to ensure that we have an adequate amount of sample material to work with.

Given that existing house mouse monitoring efforts are in place (which may involve live traps, snap traps, or other approved methods for capturing mice), we have developed this SOP to outline a protocol for opportunistic house mouse hair sampling. Opportunistically, for each mouse captured, pluck a small patch of hair (<0.25 cm^2) from the VM3 region (Figure 1), using a clean pair of tweezers (cleaned with 70% ethyl alcohol or isopropyl alcohol or hand sanitizer and a clean paper towel). If it is not possible to use alcohol in the field, we ask that you clean or wipe tweezers using a paper towel or cloth to ensure that hair samples from one specimen do not contaminate hair samples of the following specimen. Once an adequate amount of hair is plucked (approximately 3.0 mg, see Figure 2), place the hair sample in an individual, clean, sealed, and pressed (air pressed out) plastic bag or an individual, sealed tube. Please write an ID on the bag or tube with a permanent marker, and in an additional data sheet (which should be shared electronically and in hard copy), please provide the following metadata, as possible:

- sample ID
- GPS location/approximate location of mouse sample, island name/country
- date of sample
- sex/age of mouse sampled
- habitat (general description, % cover, plant species present, etc.)
- any other additional/relevant information normally collected (e.g., mouse body condition)
- names and affiliations of the sample collector(s)

Hair samples should be kept in a cool, dry location prior to shipment.

Preferred Mouse Attributes

Equal mix of males/females, preferably **adult** mice sampled from various habitats/seasons (if possible), *n* = 20 would be ideal, but at least 10 per island.

Figure 1. VM3 region indicated on mice for hair removal.

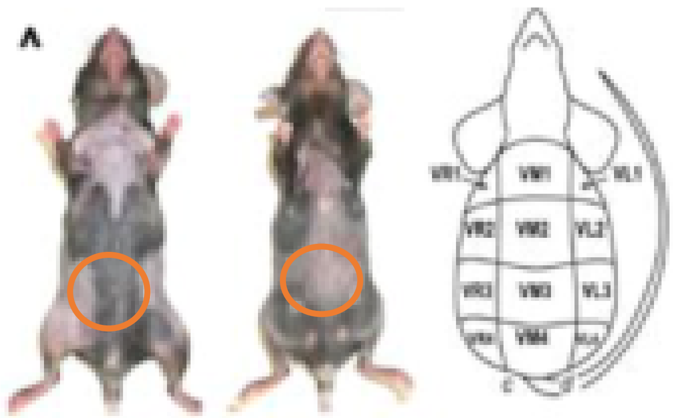
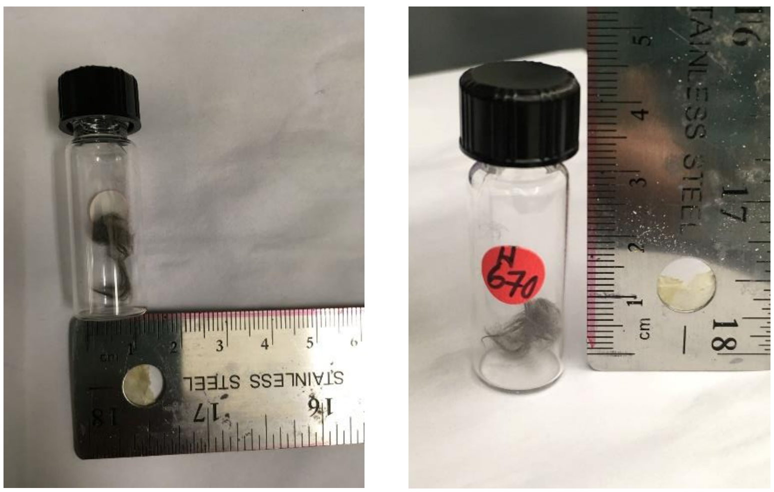


Figure 2. Average amount of harvested (and cleaned) mouse hair used for stable isotope analysis.



Example of cleaned, dried mouse hair. Typically, only 3 mg (maximum) is harvested from each specimen; only 0.8-1.0 mg is needed for bulk tissue or compound-specific stable isotope analysis.

Shipment

Contact Wieteke Holthuijzen (wholthuijzen@gmail.com) to arrange shipment and necessary permits and paperwork. To import the samples, we require a letter/documentation from you (or another supervisor/scientist involved with the project) stating the circumstances/details of this project (i.e., mice collected from XXX island as part of a collaborative research project). In particular, in this documentation, it should be noted that the species collected are *Mus musculus* and have been collected from wild populations for scientific purposes. A template of this letter which can be updated is provided in Appendix A, along with information highlighted in yellow that will need to be supplied. Additionally, for all imports, it is required to fill out the US import permit (USFWS 3-177). I need the following information for USFWS 3-177:

1) Mail carrier used for shipping the specimens
2) The number of boxes used to ship the specimens
3) Markings on boxes containing wildlife (such as the name/abbreviation of your institution or "wildlife parts")

4) Collaborator information (Business/Institution name, first/middle/last name, address, city, country, phone number, email address, country code)
5) Quantity of individual samples in the box(es)

This is a list of paperwork that will need to be mailed with the samples:

1) Copies of any permits that are used to collect samples, and any relevant ethics permits or otherwise;
2) First page and second page of US declaration form (with collaborator signature in box 22)
3) Two copies of the above must be printed; one to go into the courier label and one that goes inside the box with the samples

Information required for shipment:

1. To / Address information is: Wieteke Holthuijzen & Dan Simberloff
The University of Tennessee
Ecology & Evolutionary Biology Department
1416 Circle Drive/569 Dabney Hall
Knoxville, TN 37996-1610
USA
1 208 871 4321
Email: wholthuijzen@gmail.com

2. From / Sender: You

3. Customs declaration
Other: Scientific material
Mouse hairs: value $1 / No. XX
Mouse fecal samples: value $1 / No. XX
Comments: Permits included
Export number (if needed): XXXXXXX
Reason for export: Scientific research

Shipping Details

For shipping samples, you can use the University of Tennessee – Knoxville Department of Ecology & Evolutionary Biology FedEx account. Simply fill out the necessary information and forms (using the information above) and use this FedEx account number: 3055-9109-2. On the airbill, select for payment, "Recipient", and use this account number.

Analysis

Although herbivores have well-understood trophic tendencies, the difficulties associated with characterizing the trophic positions of higher-order consumers has remained a major problem in food web ecology. To better understand trophic linkages in food webs in relation to invasive house mice, we plan on analyzing the stable nitrogen isotopic composition of amino acids to estimate trophic position (TP) of mice. Knowledge of the trophic position (TP) of organisms in food webs allows ecologists to track biomass flow, apportionment among trophic groups, and the trophic compositions of communities (Chikaraishi et al. 2014)—and, in the case of invasive house mice, to assess their trophic position and relative impact in a given ecosystem. This approach is based on contrasting isotopic fractionation during metabolic processes between “trophic” and “source” amino acids (TrAAs and SrcAAs, respectively). Most food chains start with primary producers (TP = 1) such as algae and plants, which are eaten by herbivores (strict plant-feeders: TP = 2) and omnivores (both plant-and animal-feeders: TP > 2). Herbivores and omnivores are eaten by carnivores (animal-feeders: TP > 3) and finally by tertiary predators (carnivores at the top of the food chain). Trophic omnivory among carnivorous species can be measured as the degree to which consumers’ trophic positions depart from an integer-based trophic position (i.e., trophic level 3.0, 4.0).

Prior to analysis, all hair samples will be cleaned by soaking them in a 2:1 mixture of methanol and chloroform for about 2 h to remove any lipids or residue and then rinse twice in water. Hair samples will then be carefully packed into aluminum capsules and sent off to the University of Rhode Island for analysis by the Stable Isotope Lab (under Dr. Kelton McMahon) since the University of Tennessee does not have the equipment available to complete these analyses in-house.

Import/Export Cover Letter

1 January, 2020 (add institutional letterhead, if possible)

YOUR NAME
YOUR INSTITUTION NAME
INSITUTION ADDRESS
CITY, COUNTRY
PHONE NUMBER
EMAIL ADDRESS

U.S. Fish and Wildlife Service
Office of Law Enforcement
3150 Tchulahoma Rd., Suite #6
Memphis, Tennessee 38118

To whom it may concern,

This letter accompanies the Declaration for the Export/Import of scientific specimens (USFWS Form 3-177) pertaining to a shipment of specimen parts shipped to the Port of Memphis on 1 January, 2020 from XXXX ISLAND via MAIL CARRIER NAME for Wieteke Holthuijzen and Dr. Daniel Simberloff at the University of Tennessee, Knoxville, Tennessee. Specimen parts in this shipment are of mouse hairs, removed from a wild population of the house mouse (*Mus musculus*) from XXXX ISLAND; these specimen parts are exported by myself, an accredited scientist at XXXX RESEARCH INSTITUTION, for scientific research purposes only. I am sending XX mouse hair samples in XX boxes to Ms. Holthuijzen and Dr. Simberloff as part of an international, collaborative research program to study mouse trophic position on islands. Mice are invasive on XXXX ISLAND and will be eradicated in 2021. ADD 1-2 SENTENCES ABOUT XXXX ISLAND RESEARCH AND MOUSE ERADICATION, IF RELEVANT. None of the specimens imported are threatened, endangered, CITES listed, or require permits pursuant to part 16, 17, 18, 21, 22, or 23 of CFR50. The specimens have no domestic or commercial value. Included for your records are copies of supplemental documents that authorize collecting activities on XXXX Island. (If you have documentation/permits that allow for collection of mouse parts per your institution, please provide copies of those as well.)

Sincerely,

YOUR NAME
YOUR TITE, DEPARTMENT
YOUR INSTITUTION NAME
YOUR EMAIL
YOUR PHONE NUMBER

Literature Cited

Chikaraishi, Y., Steffan, S. A., Ogawa, N. O., Ishikawa, N. F., Sasaki, Y., Tsuchiya, M., & Ohkouchi, N. (2014). High-resolution food webs based on nitrogen isotopic composition of amino acids. *Ecology and Evolution*, 4(12): 2423-2449.

O'Connell, T. C., and Hedges, R. E. M. (1999). Investigations into the effect of diet on modern human hair isotopic values. *American Journal of Physical Anthropology*, 108(4):409-425.

Quillfeldt, P., & Masello, J. F. (2020). Compound-specific stable isotope analyses in Falkland Islands seabirds reveal seasonal changes in trophic positions. *BMC Ecology*, 20:21.

Whiteman, J. P., Elliott Smith, E. A., Besser, A. C., & Newsome, S. D. (2019). A guide to using compound-specific stable isotope analysis to study the fates of molecules in organisms and ecosystems. *Diversity*, 11(8):d11010008.