Methods for the scientific collection of barred owls (*Strix varia*), including suggested data and specimens to collect to maximize their utility as scientific specimens

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This document has been reviewed and approved by the Barred Owl Science Team.

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Introduction

Large experiments are being done that lethally remove barred owls (*Strix varia*) from Washington, Oregon, and California. Researchers designed these experiments primarily to study the ecological impacts of barred owls on the federally-threatened northern spotted owl (*Strix occidentalis caurina*). However, the lethal removals are producing significant numbers of carcasses that can be used by natural history museums for a variety of other research projects.

Museums typically collect and preserve specimens of living animals in order to document life on earth. These scientific specimens are the most complete, tangible, and permanent record of life at a particular place and time. If done correctly, they can document many aspects of the animal's life (e.g. anatomy, physiology, biochemistry, morphology) as well as aspects of the environment (e.g. environmental contaminants, diet, parasites and diseases, even climate conditions), and potentially many other things that researchers cannot yet imagine.

These large collections of barred owls represent an unprecedented opportunity in ornithology to collect and store large samples of a top predator throughout much of its western range. There are several reasons why this is a unique opportunity: (1) it is unusual to get a series of top predators in collections; (2) it is rare to get such a large series of any bird species today; (3) it is rarer still that these are healthy representatives of a vibrant population (and not salvage – like things that wash up on beaches, or die-off due to environmental poisoning, etc.); (4) these specimens and data are timely and important because there are many ecological and conservation questions being asked about barred owls, and the collections can help answer some (health of birds, diet and niche overlap, genetics and introgression of genes among different species, route to the west, etc.).

However, the collection of barred owls also presents many challenges: (1) there are many specimens to deal with; (2) large birds are expensive to prepare, store, and curate, and require significant space in collections; and (3) we are not sure what materials we really

need or whether collections protocols are capturing those data that will be most important to researchers.

Ornithological collections background

Traditional bird collections consist of a round skin (aka study skin) stuffed with cotton. Preparators typically opened carcasses with an incision on the chest or belly and removed the body, internal organs, and bones (with the exception of a few wing bones, leg bones, and portions of the skull and beak) and replaced the body with an equal volume of cotton wool. These are then dried and stored in cases for researchers and others to use. An excellent description of modern museum specimen collecting and preparation can be found in Winker (2000).

Other types of ornithological specimen (Table 1) include dried cleaned skulls and skeletons, mounts (usually a bird stuffed in a life-life position), eggs, nests, pickles or fluid-preserved specimens (usually formalin-fixed and alcohol stored), and preserved tissues (usually frozen, sometimes buffered). Tissues became standard museum specimens in the early 1990s for the purpose of allozyme and DNA studies, so few museums have well-preserved tissues before that period. Each of these specimen types contains different types of information or data and are useful for a variety of analyses. When embarking on a particular study, it is important to know exactly what information you hope to gather and what preparation type is most likely to yield the needed data. When doing general collecting, it may be important to collect as much material as possible, keeping in mind what future researchers will most likely desire or need for their work. All specimens should carry basic elements of data that describe the specimen. The most important of these data are called "Darwin Core Data" which describe the basic type of specimen, its taxon name, and its origin in time and space, etc. These are important for simply describing what the specimen is, when and where it came from, and other basic attributes.

Table 1. Typical museum specimens collected from birds and their uses.

Specimen type	Tissues preserved	Traditional uses	Modern (new) uses
Study skin	Skin, feathers	Phenotypic ID and variation, color and plumage study	DNA extraction, stable isotopes, environmental contaminants
Pickle	Whole body	Anatomical dissection	CT Scanning, pathology, diet, etc.
Skull and skeleton	Bones only	Functional morphology	Disease, "ancient" DNA
Mounts	Skin and feathers	Decoration, display	Same as study skins, above
Tissues	usually muscle, liver, or blood	Protein or DNA extraction	DNA, RNA, proteins, viruses, other pathogens and disease

Eggs	Eggshell	Species phenotype and ID	DNA, environmental toxins
Bird nests	Nests	Species phenotype and ID	Pollen, plant phenology, avian ectoparasites

Data and materials to collect from Barred Owls and hybrids: in the field

Researchers working with barred owls have many questions that can be informed by data recorded during field collections. There are issues about effort and costs of removals, there are questions relating to whether the bird is a territory holder or floater, and, if collected birds turn out to look unusual, there may be questions regarding protocols and evidence that the collectors used to identify the bird as a barred owl or hybrid. Therefore, data about the collecting event, methods, and effort may be useful. Some behaviors of owls (particularly their calls, but also branch crashing, aggressive behavior, or distances that the owls approach) may help provide clues to the bird's species identity, sex, or hybrid origin, and these should be recorded. There are also some key samples that degrade quickly after the bird dies (e.g. RNA) or are more convenient or accurate to take right away (e.g. measurements such as mass and talon spread) before the carcass is frozen or dried. Mass and foot-size can be useful for sexing birds in the field and thus help to confirm that you have collected both the male and female of a territorial pair.

Data sheets can facilitate collecting these measurement data, but, however it is done, the most important data for field workers to collect are any data that pertain specifically to the collecting event, including (1) specimen number (a unique identifier); (2) collection time; (3) collection date; (4) collector's name(s); (5) collection exact locality (usually latitude and longitude or UTM coordinates); (6) locality metadata (items like map datum, projection, UTM grid numbers, etc.). Field workers may want to collect information pertaining to the collecting methods, such as (7) gun type; (8) shot type and size; (9) available daylight; (10) playback recording(s) used; (11) distance and/or height above ground of shot, and (12) number of shots or misses. Others who have been interested in quantifying the effort involved in removal activities have also recorded (13) time of arrival at the locality, (14) timing and series of owl calls played, (15) time and direction of first response and first arrival of owl to the locality, (16) exact time of shot, if one is taken, (17) time that the collecting, measurements, and samples are collected and when the team departs. Valuable specimen measurements that can be taken in the field upon collection of the bird include (18) song or call recordings, especially those used to identify the specimen; (19) mass (in grams); (20) wing cord (typically in mm); (21) culmen length; (22) claw cord; (23) foot-pad length; (24) evidence and pattern of molt; (25) age class (based upon wing and tail feathers); (26) presence of brood patch on females, and (27) photos of the specimen (facial disk, full front with tail, full back with tail, and and spread wing (top and bottom)). There are some specimen types that are best collected in the field when the specimen is fresh, including (28) blood (taken with syringe from the heart for large blood samples, and useful for DNA, parasites, and blood smears); (29) ectoparasites (using pyrethrin powder); (30)

cloacal or oral swabs for virus or metagenomic work (stored in live virus culture media or in RNA*later*® (Invitrogen, Carlsbad, California, United States of America)); (31) tissues for DNA or environmental contaminant screening (typically liver and/or muscle, taken from the breast or abdominal cavity); (32) tissues for RNA analyses (e.g. skeletal muscle, heart, liver, retina, brain, skin, etc., depending upon genes or tissue expression that particular researchers require and typically stored on liquid nitrogen or in RNA buffers such as RNA*later*®). Another option for preserving DNA and RNA from blood and fluids are Whatman FTA filter papers (Santos 2018) that are infused with lysis and storage buffer. Samples can be blotted onto the FTA filter and dried and stored at room temperature and used for a variety of studies involving the host bird DNA or the DNA of parasites and pathogens.

Once field workers sample specimens and take measurements, they then bag specimens for freezing and storage until eventual transfer to a permitted scientific collection facility. It is important to tag or clearly label all carcasses and samples with the specimen's unique identification number. This prevents specimens from becoming mixed up and allows for all parts and samples to be associated with their field data, voucher specimen, and other future data (and this is a legal requirement of most scientific collecting permits). After labeling the carcass, field researchers should insert a small amount of paper towel or ball of cotton into the mouth and cloaca (as well as any other opened wounds) to plug those openings and prevent body fluids from leaking out and staining feathers. Typically, the researcher should then close and tuck in the wings and legs in addition to loosely organizing the feathers to lay flat against the body. The collector can then wrap the body in a piece of paper towel or other paper (newspaper can work for this, provided that ink is dry and cannot rub off onto the specimens), being sure that wings and tail are supported and that feathers are not bent or broken. The researcher can then place the wrapped carcass into a plastic bag that is large enough to enclose the specimen without bending the tail or wings when sealing. The collector should keep the carcass and any samples as cold as possible (in a cooler or ice chest, ideally containing dry ice) until field personnel can freeze the specimen, which should stay frozen until it is prepared as a specimen.

Data and materials to collect: in the museum and lab

Deciding on a preparation type and materials to preserve

Specimen preparation should be completed by experienced and permitted preparators in museums or labs. At the museum or lab, researchers need to determine what materials to preserve for scientific research and as a long-term voucher specimen. Freezing specimens long-term is one option, but keeping a continued chain of freezing is expensive and requires a lot of space. Frozen specimens are not easily accessible for work and, over time, portions of the carcass may become freezer-burned or freeze-dried making working with them difficult. For these reasons, it is usually preferable to determine what material to keep and to prepare the necessary material and specimens as soon as possible (generally within a year or so of initial freezing and storage). The basic choices are (1) to prepare the main

voucher specimen as a research skin, (2) to prepare as an ethanol or formalin-fixed complete carcass, or (3) to prepare as a skeleton. These are not entirely mutually exclusive as there are some options that combine vouchers of multiple types of material, such as a skin with partial skeleton (schmoo) and skins along with partial fluid-preserved material (Dickerman 1989; Spaw 1989; Johnson et al. 1984; Longmore and Boles 1990). Due to the large gun and shot size used to collect barred owls, the bones of the specimens are often shattered to the point that entire skeletal specimens are only marginally useful. It is possible, however, to palpate the major bones of the wing, legs, neck, and skull before deciding on a preparation type to determine how severely the bird has been shot and whether the skeleton will be useful. Traditional skins are likely to be very valuable in determining the range of phenotypic variation within western barred owls, and skins are easily measured to obtain a range of often-used ornithological measurements. *Thus, skins are among the most useful specimen types, and skins should be prepared whenever resources permit*. Limiting resources often include the time for preparation and curation of skins as well as the available storage space. In the event that time and/or resources are limited, museums and labs can preserve whole bodies in formalin or ethanol after preserving tissues and other necessary genetic samples. Such fluid preparations will preserve the full carcass, which is useful for a variety of studies, including diet, morphology (including CT-scanning), plumage (although plumage color may be damaged or affected), and genetics. The drawbacks to this type of preparation are that the preserved fluid materials are messy and difficult to retrieve, measure, or otherwise use, and direct comparison of many side-by-side specimens is impossible. Regardless of whether preparators decide to preserve skins, skeletons, or pickles as the primary voucher specimens, there are many other measurements and samples that should be taken to compliment the voucher specimen.

Data collected during preparation

Museum personnel preserving barred owl specimens at the California Academy of Sciences (CAS) have typically taken a specific set of measurements and saved several preparation types from each individual. Measurements should include a date to differentiate them from measurements taken in the field. These measurements should include (1) mass; (2) total length; (3) wing length; (4) tail length; (5) tarsus length; (6) exposed culmen; (7) foot pad; (8) bill measurements (usually bill depth, exposed culmen, or nares to tip); (9) gonad measurements (testes length, width, and color; ovary length and width, diameter of largest developing ova); (10) fat class and location; (11) molt; and (12) stomach contents. Whether it is necessary to take some of these measurements will depend on the preparation type. For instance, researchers can use preserved study skins to take external measurements involving hard parts, such as wing length, tail length, tarsus length, exposed culmen, and bill measurements. For barred owls, preparators should preserve the whole stomach so that researchers can analyze stomach contents at a later time. Measurements and description of soft parts must be taken as these measurements and colors change with drying; these include measurements of gonads, foot pad, and fat, and include colors of all soft parts such as iris, leg, and bill.

In addition to data, the materials that preparators typically preserve at CAS (and what we recommend) include (1) full study skin; (2) tissues (usually two muscle and two liver samples, each containing 1-2ml tissue each in 2ml cryovials); (3) liver (all remaining liver (usually several grams) frozen in whirl-pack airtight bags for rodenticide screening); (4) cloacal and oral swabs (for microbiome / virome studies, either frozen in liquid nitrogen or in RNA*later*® buffer and frozen); (5) stomach/intestines with contents preserved in ethanol (for diet and parasite analyses); (6) any dissociated feathers placed in ziplock bags and kept with skin; and (7) all remaining materials (bones, muscle, internal organs, etc.) preserved in 95% ethanol as "post-cranial material". The above preparations are what is typically done at CAS, but museum preparators have preserved a small number of individuals as flat half skins (in order to explore the potential benefits of storing smaller skin specimens), full skeletons, or as full body fluid specimens for the purposes of CT scanning. A few have also been prepared as full mounted specimens for public outreach and for our exhibit floor (at least two barred owls are in our current "California Giants" exhibit) and a few more are in our Naturalist Center for interpretation and public programs.

If specimens are properly prepared and preserved, researchers can gather many measurements and types of data from the specimens at a later date. Feather color and pattern should be well preserved in study skins provided that the specimens are well cared for and kept out of light and high temperatures. Although researchers can obtain DNA from dried museum skins, museum skin DNA is poorly preserved and highly fragmented; it is not a substitute for the high quantities of long-strand DNA provided by tissue samples. It is not possible to imagine everything that researchers might want to study in 50 or 100 years or more, but we must try our best to anticipate some of those needs and prepare as much material as possible.

Finally, as time passes, researchers, managers, and policymakers have additional questions and need new information. We will need to periodically ensure that collecting efforts are serving the needs of these and other groups. Thus, we appreciate feedback on what should be collected and how. For example, after collecting for multiple years, we received a request for one gram of liver tissue for anticoagulant rodenticide screening. For many of our specimens, the total mass of liver collected did not equal one gram, so we were not able to fulfill that request to the extent that we would have liked. In subsequent preparations, we preserved much more than one gram (in many cases preserving the entire liver, frozen without buffer) for use in such analyses. It is important to obtain feedback when needs change and research objectives expand.

Costs

Cost of specimen preparation and storage

There are several factors limiting what specimens are taken, prepared, and stored for long term usage. These include field time and staff, preparation time and staff, storage space, and long-term storage resources. Often this comes down to funding to pay for these

different resources, especially personnel. Museums typically have some resources for basic staffing and storage, but most projects, especially large ones, require extra resources. The museums that have been involved to date with the barred owl collections have mostly done so using existing resources. Additional money, staff time, and storage space will likely be required if museums are to continue to preserve additional barred owl specimens. To some extent, we have a good sample of western barred owls that might not need augmentation if its primary purpose is to simply document the invasion and study basic population genetics and morphological diversity. On the other hand, the number of owls becoming available could potentially allow for new and innovative studies that would require and use large series of additional specimens. Whenever the lives of wild animals are being sacrificed for research, we want to be sure that those specimens will be used in some way, and preferably will have maximum scientific utility. But collectors must be deliberate and thoughtful in order to ensure that the cost of collections do not exceed the present and future benefits.

To help evaluate the cost-benefit equation, we have outlined some of the basic costs of collecting and curating barred owl specimens. The total cost of creating barred owl specimens includes both the costs involved in performing field work to collect the specimens as well as the costs in the museum of preparing the specimen, curating it and the associated data, and long-term storage and maintenance of the collections. Some of these estimates may be more accurate than others, but we attempt to tally the various types of costs and estimate some error around each to give an idea of the potential costs. Field costs include (1) time; (2) field staff; (3) transportation; (4) supplies (collecting supplies and sample preservation buffers and containers); and (5) the cost of shipping to specimen repository. Preparation costs include (1) materials to preserve and store specimen preparations and samples; (2) preparator staff time; (3) cataloging staff time; and (4) the cost of serving data online. Long-term storage and maintenance depends tremendously on the types of specimens taken, but these include costs of (1) storage for specimens, including the costs of ethanol changes, freezers and their power requirements and/or liquid nitrogen resources, and (2) packaging, loaning, and shipping specimens to users.

Field costs

Field costs of barred owl removals have been estimated elsewhere (Diller et al. 2014), and are mostly covered by existing projects that are currently conducting removals. The most important costs for removals are staff field time and transportation to the field, which are significant; however, for most projects underway producing specimens, these costs are already covered by other funds. Depending upon the field techniques used for removals or scientific needs for the specimens, there may be additional field costs involved for converting the carcass to a useful museum specimen. These costs may include supplies required to package, store, and transport the carcass (such as bags, tags, specimen vials, coolers, ice, liquid nitrogen), time for training field personnel and collecting data and samples that may pertain primarily to the museum collection (such as taking additional fresh viral samples in RNA buffers or live virus buffers). Any field protocols will need to be agreed upon before costs can be estimated.

Museum costs

Materials to preserve

These are minimal, but include gloves (worn by preparators), printed tags, cotton, paper, sterile swabs for viral samples, cryovials and labels for tissues and samples, glass jars and alcohol for storing stomachs and contents as well as post-cranial material, and disposal of any animals parts (incinerated as hazardous waste), and other miscellaneous items. These costs amount to about $6 per specimen.

Preparator staff time

At CAS as well as at UC Berkeley, we sometimes have full-time staff preparing specimens and sometimes we are able to hire students to do such tasks. The hourly cost of students is cheaper; however, students require training, oversight, and they generally take longer. Thus, the cost of students ends up being similar to hiring full-time staff, but the ability to hire students can greatly increase the number of specimens that can be prepared.

- Professional – 2.5 hrs/bird (at CAS our costs are approximately $30/hr for professional staff salary+benefits). Total cost approximately $75 per bird.

- Student – 4-5 hours/bird (at CAS our costs are about $19/hr). Total cost at 4 hrs per bird is $76.

Cataloging staff time

This is usually done in batches to save time. When done in this way, data can be entered, double checked, databases updated, and final museum tags printed in about 10-minutes/bird, or ~6 birds/hr. This is done by professional staff, and would be charged at the $30/hr rate, total approximately $5/bird.

In addition to creating the catalog entry, each entry needs to be updated with additional information as other studies are conducted on the specimen or materials are loaned. For example, when genetic work is done, the catalog should contain links to GenBank records and publications. If tissue is loaned, then the catalog should be updated to reflect the remaining material.

Cost of storing specimens (to be covered by the museum)

This is very difficult to define, since museum cases are rarely purchased, and most museums have limited space, so it is unlikely that new cases can be easily purchased and installed. However, the cost of a museum case is several thousands of dollars, and at CAS, each case holds approximately 100 barred owl skins. Ethanol specimens (including stomachs and post-cranial specimens) require additional open shelf storage and periodical maintenance to change or top-up ethanol. In addition, staff must inventory and store materials in their final cases, which also takes staff time. If museum space were to become filled or if museums with space for extra cabinets were to be involved, we would expect that costs could easily exceed ($5000/case)/(100 specimens/case) plus shelving for

ethanol specimens (totally $50-$60/specimen), plus annual rental or purchase of storage space to house the collection. Although museums may not request or be able to use these funds, these costs are real and should be considered as part of the museums' contribution to the project.

Serving data online and in loans (to be covered by the museum)

- All data from the collections are currently uploaded and maintained in collaborative databases. Our cost simply includes maintaining our own databases, which is minimal on a per-specimen basis, but can be considerable for large projects contributing much new data. The cost of creating the data is captured above under "cataloguing staff time."

- Museums also make specimens available to researchers who need them. In some cases, researchers visit the museum to access, measure, and sample specimens. In other cases, materials are shipped as loans to researchers elsewhere. The costs of hosting researchers and processing loans (reviewing, approving, packing, shipping, unpacking, reshelving, etc.) can be considerable, but are part of our annual budget and part of our contribution to science and to this project.

- Museum specimen data can be queried online and full data can be downloaded from sites such as vertnet.org (http://portal.vertnet.org/search) and gbif.org (https://www.gbif.org/species/search). These data aggregators typically serve data from any specimens catalogued and lodged in major museum collections, as well as sighting from some citizen science databases such as eBird and iNaturalist.

Appendices:

Appendix 1: Field data sheets from Hoopa Tribal Forestry removal projects

Appendix 2: Field data sheets from USFWS / USGS removal study

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Hoopa Study Area Experimental Barred Owl Removal Form

StudyArea Hoopa
Date
Territory_No
BO_ID 20400
Time
Status MB14305B-6
SEX
Collector
Est_Age
Species Barred Owl
Observers
Effort_Code

UTM_x_cs
Terr_UTMx
UTM_y_cs
Terr_UTMy
Condition
Method Shotgun
NumShots
Euthanasia
StorageSite Hoopa Office

Gauge 12 2 3/4
Shot_type steel
ShotSize 6
Factory_H Factory

Blood
EctoParasites
Stomach
Cloacal
Feathers
Liver
Measurements
Photos
Recording

Weight
WingCord
Culmen
ClawCord
Foot Pad
TalonSha
WingMolt
TailMolt
WingNew

Notes

Entered_____By_____

Ver 6.05.2018 Circle one: Non-breeding season Breeding season Proofed ☐ Entered ☐ Copied ☐ Additional information on back ☐

BARRED OWL SPECIMEN COLLECTION FORM

(complete for each individual barred owl collected)

OWL ID#: _____; SEX:_____; AGE_____; DATE: _____ / _____ / _____ ;
STUDY AREA: _____ ; HEXAGON #: _____; OBSERVER(S) INITIALS: _____;
(CLE = Cle Elum, COA = Coast, KLA = Klamath)

TIME COLLECTED: _____; UTM_X: _____; UTM_Y: _____;
METHOD: 12-gauge shotgun ; NUMBER OF SHOTS:_____; SHOT TYPE (S=steel; QG=quiet gun):_____;
DISTANCE (M):_____; BROOD PATCH? (1=Yes, 0=No):_____; CARCASS RECOVERED (1=Yes, 0=No)_____
EUTHANASIA REQUIRED? (1=Yes, 0=No):_____; PAIR or SINGLE (circle one);
Pair Owl ID: #_____OR Single OR Not Collected OR SPOW OR SPAR
(record the OWL ID# of other member of pair OR circle one of the options)

MEASUREMENTS	VALUE	COMMENT
Specimen condition	Good Moderate Poor Excellent*	
Net mass (g)		
Wing chord (mm)		
Tail length (mm)		
Halix (mm)		
Footpad length (mm)		
Beak length (mm)		
Beak depth (mm)		
Primary1 Tip (mm)**		
Secondary 1 Tip (mm)**		
Foot/claw condition		

*Reserved for specimens that are near-perfect: no broken bones, no blood, feathers perfect, beak undamaged. Taxidermy quality.

**Measure the white feather tip along the vein. Right wing only

PLUMAGE DESCRIPTION Enter one of the following into circles: 1 = checked, none obvious 2 = not checked 3 = entered in boxes

Enter one of the following into each box: J= juvenile feather; O=adult old; N=adult new if fully grown, or proportion of full growth (e. g., .1, .4, .9 etc); A=adult unknown; M = missing; U = unknown

RIGHT WING ◯

10	9	8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8	9	10	11	12

LEFT WING ◯

10	9	8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8	9	10	11	12

Comments

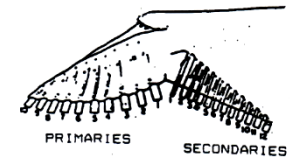
RETRICES ◯

6	5	4	3	2	1	1	2	3	4	5	6

L R

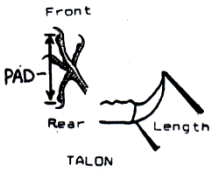
BLOOD & TISSUE	Circle appropriate value
Contaminants tube	Percent full: ZERO ¼ ½ ¾ FULL
DNA/Parasite vials	Number of vials: 0 1 2 3
Blood smear/parasites	YES=1 NO=0

NOTES:

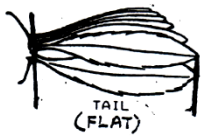

PRIMARIES
SECONDARIES


6
5
4
3
2
1
RECTRICES

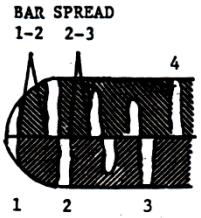

WING
CHORD
(RELAXED)


Front
PAD
Rear
Length
TALON


Length
BEAK


TAIL
(FLAT)


Adult
2yr
1yr
AGING FROM TIP


BAR SPREAD
1-2 2-3
4
1
2
3
COMPLETE
TAIL BARS = 4