FWS Form 3-200-43 (Rev. 01/2020)
U.S. Department of the Interior

OMB Control No. 1018-0093
Expires 08/31/2023

TAKE/IMPORT/EXPORT OF MARINE MAMMALS FOR PUBLIC DISPLAY, SCIENTIFIC RESEARCH, ENHANCEMENT, OR RESCUE/REHABILITATION/RELEASE ACTIVITIES OR RENEWAL/AMENDMENT OF EXISTING PERMIT (MMPA and/or ESA)

■New ☐Reissue/Renew ☐Amendment

Complete Sections **A or B**, and C, D, and E of this application. U.S. address may be required in Section C.**

A. Complete if applying as an individual

1.a. Last name	1.b. First name	1.c. Middle name or initial	1.d. Suffix

2 Date of birth (mm/dd/yyyy)	5.a. Telephone number	5.b. Alternate telephone number	6. E-mail address

B. Complete if applying on behalf of a business, corporation, public agency, Tribe, or institution

1.a. Name of business, agency, Tribe, or institution	1.b. Doing business as (dba)
University of Memphis	

2. Tax identification no.	3.a. Description of business, agency, Tribe, or institution	3.b. Website URL (if applicable)
	University	https://www.memphis.edu/

4.a. Principal officer (P.O.) last name	4.b. P.O. first name	4.c. P.O. middle initial	4.b. P.O. Title
Dhaliwal	Jasbir		Executive Vice President

5. Primary contact name	6. Primary e-mail address
Emily Puckett	puckett3@memphis.edu

7.a. Business telephone number	7.b. Alternate phone no.	8.a. Primary contact telephone no.
901-678-1618		901-678-3005

C. All applicants complete address information

1.a. Physical address (Street address; Apartment #, Suite #, or Room #; no P.O. Boxes)
239 Ellington Hall, 3770 Walker Ave

1.b. City	1.c. State	1.d. Zip code/Postal code	1.e. County/Province	1.f. Country
Memphis	TN	38152	Shelby	USA

2.a. Mailing Address (include if different than physical address; include name of contact person if applicable)

2.b. City	2.c. State	2.d. Zip code/Postal code	2.e. County/Province	2.f. Country

D. All applicants MUST complete

1. Include a check or money order, payable to the U.S. FISH AND WILDLIFE SERVICE, **a nonrefundable processing fee** [50 CFR 13.11(d)(4)]. Federal, Tribal, State, and local government agencies, and those acting on behalf of such agencies, are exempt from the processing fee – ***attach documentation of fee exempt status as outlined in instructions.*** (50 CFR 13.11(d))

2. If you are requesting a reissue/renew/amendment, what is your permit/file number?

3. Certification: I hereby certify that I have read and am familiar with the regulations contained in Title 50, Part 13 of the Code of Federal Regulations and the other applicable parts in subchapter B of Chapter I of Title 50, and I certify that the information submitted in this application for a permit is complete and accurate to the best of my knowledge and belief. I understand that any false statement herein may subject me to the criminal penalties of 18 U.S.C. 1001.

JASBIR DHALIWAL — 11/13/24

The individual/principal officer of the business must print and sign the application. (No photocopied or stamped signatures) — Date (mm/dd/yyyy)

** Further instructions for the above application may be found on our ePermits website. See the last page for information on the Privacy Act, Paperwork Reduction Act, Estimated Burden, and Freedom of Information Act aspects of this application form.

Mail your application(s) to Division of Management Authority, Branch of Permits, MS:IA 5275 Leesburg Pike, Falls Church, VA 22041-3803.

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E. TAKE/IMPORT/EXPORT OF MARINE MAMMALS FOR PUBLIC DISPLAY, SCIENTIFIC RESEARCH, ENHANCEMENT, OR RESCUE/REHABILITATION/RELEASE ACTIVITIES OR RENEWAL/AMENDMENT OF EXISTING PERMIT (MMPA and/or ESA)

Allow at least 90 days for the application to be processed. Applications for marine mammal permits must be published in the Federal Register for a 30-day public comment period.

Use this application for the take[1], import, export, or re-export of marine mammal species (or their parts) under the jurisdiction of the U.S. Fish & Wildlife Service (sea otters, marine otter, polar bears, walrus, manatees, and dugong; see our marine mammal webpage) for purposes of public display of live animals, scientific research, or enhancement under the U.S. Marine Mammal Protection Act (MMPA) and/or U.S. Endangered Species Act (ESA). This application may also be used to apply for a letter of authorization (LOA) under MMPA Sections 109(h)/112(c) and/or an ESA permit for enhancement of propagation or survival of the species, which would provide authorization to work as a "cooperator" for the purpose(s) of **rescue, rehabilitation, and/or release of stranded marine mammals.** Finally, this application may be used for the **renewal and/or amendment** of an existing permit for these activities.

Note: Renewal and amendment requests require responses to all questions pertaining to your requested activity.

This form should NOT be used:

- For activities involving marine mammals under jurisdiction of the National Marine Fisheries Service (NMFS) (i.e., whales, dolphins, porpoises, seals, and sea lions); please contact NMFS.
- For activities involving photography in the wild for educational or commercial purposes; use Form 3-200-86.
- For transport/transfer of live captive-held animals within the United States; use Form 3-200-87.
- For transfer within the United States of dead marine mammal specimens for the purpose of public display or scientific research; use Form 3-200-87.

If you already have MMPA/ESA authorization and need a CITES permit:

- For CITES export/re-export of captive-held **LIVE** animals, use Form 3-200-53.
- For export, or re-export of parts or biological samples, use Form 3-200-29; for import of parts of Appendix-I animals, use Form 3-200-37; and for introduction from the sea, use Form 3-200-31.
- Provide a copy of your FWS or NOAA Fisheries permit or authorization with your CITES permit application.

All international shipment(s) must be through a designated port. A list of designated ports (where an inspector is posted) is available from the list of designated ports. If you wish to use a port not listed, please contact the Office of Law Enforcement for a Designated Port Exemption Permit (form 3-200-2).

[1] The term, "take," as defined by the MMPA means to harass, hunt, capture, or kill, or attempt to harass, hunt, capture, or kill any marine mammal. As defined by the ESA, "take" means to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect, or to attempt to engage in any such conduct.

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Permit Types and Processing Fees

Please review the complete application carefully before beginning. Provide complete answers to all the questions in the sections relevant to the activity for which you are requesting authorization. If a question is not applicable, answer with "N/A." You will need to use additional sheets of paper. On all attachments or separate sheets you submit, indicate the application question number you are addressing. If you are applying for multiple species and/or activities, be sure to indicate which species/activity(ies) you are addressing in each response.

Electronic submission of inventories, photographs, and receipts/invoices: For hard copy submissions, if you wish to provide information electronically, please include a flash drive containing your information with your physical application.

PURPOSE for which you are applying (check below):

☐ PUBLIC DISPLAY of live animals: **Complete All of Part I and Part II**.

Note: A public display permit is not available for marine mammal species listed as depleted under the MMPA or listed under the ESA; a public display permit may be valid for the life of an animal and is not renewable; a public display permit may be available for a facility that would hold multiple animals of a particular species and would be renewable every 5 years.

☑ SCIENTIFIC RESEARCH: **Complete All of Part I and Part III**.

☐ RESCUE, REHABILITATION, and/or RELEASE of stranded marine mammals: **Complete questions 1-7 of Part I and Part IV**.

☐ MMPA ENHANCEMENT of survival or recovery of the species or stock: **Complete Part I and Part V**.

Request is for (check below):

☑ A NEW PERMIT

☐ A RENEWAL of Permit # _____ (Complete all questions for your requested activity, as described above).

☐ AN AMENDMENT of Permit # _____.

If requesting renewal or amendment of your current permit, provide an update of any activity that has occurred under the permit since your last report.

Part I.

1. Name and address where you wish the permit to be mailed, **if different from physical address**. If you would like expedited shipping, please enclose a self-addressed, pre-paid, computer-generated, courier service airway bill. If unspecified, all documents will be mailed via the U.S. Postal Service.

   239 Ellington Hall- Biology
   3770 Walker Ave, University of Memphis
   Memphis, TN 38152

2. Who should we contact if we have questions about the application (name, phone number, and e-mail)?

   Emily Puckett, 901-678-3005, puckett3@memphis.edu

3. Have you or any of the owners of the business (if applying as a business, corporation, or institution), been assessed a civil penalty or convicted of any criminal provision of any statute or regulation relating to the activity for which the application is filed; been convicted, or entered a plea of guilty or nolo contendere, for a felony violation of the Lacey Act, the Migratory Bird Treaty Act, or the Bald and Golden Eagle Protection Act; forfeited collateral; OR are currently under charges for any violation of the laws mentioned above?

   ☑ No ☐ Yes

   If you answered "Yes" to Question 3, provide: a) the individual's name; b) date of charge; c) charge(s); d) location of incident; e) court, and f) action taken for each violation. Please be aware that a "Yes" response does not automatically disqualify you from getting a permit.

4. List the scientific name (genus, species, and, if applicable, subspecies) and common name of each species for which you are applying.

   Ursus maritimus, polar bear

5. Provide a copy of any other applicable Federal, local, or state permissions (e.g., National Wildlife Refuge Special Use Permit, NOAA National Marine Sanctuary permit, etc.) required to conduct your proposed work, OR indicate whether you have applied for, secured, or will apply for such permissions (please provide contact information).

6. Is/are the species or population stock(s) for which you applying listed under the U.S. Endangered Species Act (ESA), a species proposed for listing, or a candidate species?

   ☐ **No** ☑ **Yes;** complete a-d, below.

a. Attach a justification for taking an ESA-listed species, and explain why your proposed activities are not appropriate for a similar non-ESA-listed species;

b. Describe both the short- and long-term anticipated effects of each of your activities alone or cumulatively on the behavior and physiology of the target animals and critical habitat or proposed critical habitat for the species.

Please see Appendix-3-200-43.docx for a full explanation

c. Describe how the animals will react to your actions and the consequences of those reactions.

Polar bears will be supervised by veterinarian or researchers while under anesthesia during the collection of hair and skin biopsy samples; thus, they will not be under stress during sampling. They will likely minimally notice the wound. We have heard of no instances of rubbing behaviors from the black and brown bears samples thus far.

d. Identify how you would mitigate any potential negative effects.

Please see Appendix

7. Do you plan to conduct activities with **MARINE MAMMALS IN THEIR NATURAL ENVIRONMENT** (i.e., in the wild) where "non-target" marine mammal and ESA-listed species occur in the United States? ("Non-target" species are species that are not the subject of your activities.)

☑ **No** ☐ **Yes**; We will need to assess impacts to marine mammal and ESA-listed species that are not the subject of your activities; therefore, provide responses to a-c, below:

a. A list of all non-target marine mammals and ESA-listed species that might occur in your project area or might be affected by your activities;

b. The maximum number of animals of each non-target marine mammal and ESA-listed species (# per species) that might be harassed by your activities, the precautions that you will take to minimize the likelihood that harassment will occur, the actions that you will take should harassment occur; and

c. The maximum number of animals of each non-target marine mammal and ESA-listed species (# per species) that might be taken (e.g., killed, injured, feeding activities disrupted, etc.) by your activities, your precautions to minimize the likelihood that take will occur, and your actions should take occur.

(Note: The following link provides access to resources that might be useful for gathering the required information to answer this question, including links to FWS and NMFS offices responsible for managing marine mammals stocks, and Stock Assessment Reports, which provide population status information on marine mammal stocks.

8. Do you plan to conduct your public display, research, or MMPA enhancement activities with **MARINE MAMMALS that are CURRENTLY HELD IN A CAPTIVE ENVIRONMENT (including, but not limited to import into the U.S. of captive-held live animals/specimens)**

☐ **No** ☑ **Yes**;

If yes, specify the number of captive individuals for each species of interest: 9 _____; and for each individual animal of each species of interest, respond to a-i, below.

Note: You may provide the information in tabular form, as in the example below:

a. Species	b. Sex	c. Birth date	d. Description (e.g., ID #, ISIS #, transponder #, tattoo #)	e. Country of origin	f. Source (i.e., wild, captive-born, or captive-bred)	g. Current location of animal
Example: *Enhydra lutris kenyoni*	Female	Approx. 04/09/2010	House # XXX123 Transponder # 45678	USA	Wild	ABC Aquarium, Anchorage Alaska

h. For **captive-born or captive-bred animal(s)**, provide a breeder's statement, ARKS/ZIMS specimen report, or other information that documents the animal was born in captivity, location of birth, and information on the source of the parental stock (e.g., captive-born, wild).

i. For **captive-held animal(s) already taken from the wild**, provide:

i. Information (e.g., ARKS/ZIMS specimen report(s)) on the source of the animal, including when the animal was removed from the wild, by whom, and the location.
ii. A copy of the MMPA permit or LOA under which the animal is currently being held in captivity or a copy of the MMPA permit or authorization for removal of the animal from the wild.
iii. Has the U.S Fish and Wildlife Service deemed the animal(s) non-releasable to the wild?

☐ **Yes**; provide a copy of the official letter confirming the animal's non-releasable status.

☐ **No**; if you are requesting to have the animal(s) deemed non-releasable at this time, provide an explanation of the following: a) why release of the animal to the wild will not likely be successful given its physical condition; b) why release of the animal to the wild will not likely be successful given its behavior, including adverse interactions with humans or marine mammals; or c) why release of the animal to the wild may jeopardize the wild population of the species.

9. For **animal(s) to be taken from the wild and brought into a captive environment for public display, research, or MMPA enhancement activities**, provide for each species:
   a. Information on the actual or proposed date(s) and location(s) of collection;
   b. The numbers of animals of each age class and sex to be taken from the wild (include a definition of each of these age classes by range of months and/or years).
   c. An estimate of the species' population stock in the wild; Note: stock assessment reports might assist you with this information and are available at the following FWS field offices, depending on the species involved:

Southern sea otter: Ventura Fish and Wildlife Office

Northern sea otter: Washington Fish and Wildlife Office

Northern sea otter, walrus, polar bear: Marine Mammals Management, AK

Manatee: North Florida Ecological Service Office

d. A description of the efforts made to acquire captive-held animals in lieu of taking animals from the wild.

Note: for holding and maintaining animals you must also provide the information requested in question 14.

10. Are you requesting to **CAPTURE LIVE** marine mammals in the wild? (i.e., for research, public display, or MMPA enhancement)

☑ **No** ☐ **Yes**

If yes, specify the number of individuals to be captured for each species of interest: _____ and provide responses to a – i, below:

a. A description of the manner in which the animal will be captured, type of gear used, and deployment method (e.g., from shore or boat approach and net deployment).
b. Methods of restraint and holding, including dimensions/type of holding container, if used;
c. The holding time required prior to transport or release of the animal;
d. Number and roles of personnel participating in the captures;
e. Duration of restraint/holding from capture to release; and
f. The number of non-target individual animals of the target species that will be incidentally harassed during capture activities, and precautions you will take to minimize incidental harassment of non-target animals;
g. If capturing females with calves/pups/cubs, describe:
    i. How calves/pup/cubs will be held;
    ii. Which procedures will be conducted on them;
    iii. The duration of time the pair will be separated; and
    iv. Procedures used to reunite the pair, and if they do not reunite, explain the disposition of the calf/pup/cub.
h. A description of the use of drugs during capture, including:
    i. Name of each drug/chemical used, its dosage rate (ml/kg), method of administration (IV, IM, SQ, topical and whether remotely-deployed IM), and purpose of the drug;
    ii. Duration of drug and required holding time;
    iii. The names of the personnel who would administer the drugs;
    iv. Provisions to minimize adverse reaction(s), including the use of appropriate drug reversals;
    v. Procedures to be used to minimize the chance that drugged animals will escape or enter the water prior to complete immobilization; and
    vi. Measures to be taken to ensure that the animal is fully recovered prior to release.
i. What emergency procedures would be employed (e.g., drugs, bagging, CPR, etc.) in the event that an animal's condition starts deteriorating during capture activities?

11. Are you requesting to **IMPORT LIVE** marine mammals?

☑ **No** ☐ **Yes**

If yes, specify the number of individuals to be imported for each species of interest: _____; and provide responses to a – m, below:

a. The proposed date of import;
b. The name and address of the foreign exporter, including the country of export;
c. For wild-sourced animal(s), a description of the manner in which it was taken from the wild and a copy of the foreign collecting/capture authorization(s);
d. The age (approximate or known) of the animal at the time of removal from wild or from its mother;
e. The age (approximate or known) of the animal at time of weaning; and

f. For females, respond to i & ii, below:
   i. At the time of removal from the wild, was the female pregnant? ☐ **No** ☐ **Yes**
   ii. At the time of the proposed import, will the female be pregnant? ☐ **No** ☐ **Yes**

g. A description of the means and duration of the transportation used to move and import the animals;
h. A description of the type, size, and construction of all shipping containers used to transport the animals;
i. A description of the arrangements for watering or otherwise caring for the animals during transport;
j. A description of the qualifications of each person accompanying the animal that demonstrates their ability to address the animal's needs during transport;
k. A copy of the transport plan;
l. Quarantine plans, including location and time-frame; and
m. Any additional documentation showing compliance with U.S. Department of Agriculture (USDA) regulations for transport and care of live marine mammals (7 U.S.C. 2131-2159; 9 CFR 3, Part E).

NOTE: A separate CITES permit will be required from our office prior to the import of live CITES Appendix I species.

12. Are you requesting to **IMPORT PARTS/SPECIMENS** of/from marine mammals?

☐ **No** ☑ **Yes**; provide a – m, below:

a. The proposed date of import;
b. The name and address of the foreign exporter, including the country of export;
c. The current location of the specimens;
d. The country of origin of the animals from which the specimens were/will be collected;
e. List the number of animals by species, age class/life stage, and sex from which parts/samples are sought. If you are requesting opportunistic sample import, you may request an unlimited number of samples from a specified number of animals, by taxa (e.g., unlimited samples from up to 100 polar bears annually).
f. The types of specimens to be imported (e.g., blood, skin biopsy, carcasses, etc.) and number of each type from each animal;
g. The source of the specimens to be imported (wild, captive-bred, or captive born);
h. Were the animals/will the animals be alive or dead at the time of sample collection?
   ☐ DEAD ☑ LIVE
i. Provide a detailed description of the source of the specimens to be imported and the manner in which the sample was/will be taken or collected. For example, this might include the following sources:
   i. Animals in captivity (samples taken during routine husbandry procedures or under separate authorization; distinguish between permanently captive in public display or research facility and temporarily captive in rehabilitation facility);
   ii. Animals in foreign countries stranded alive or dead or that died during rehabilitation;
   iii. Animals killed during legal subsistence harvests;
   iv. Animals killed incidental to legal commercial fishing operations;
   v. Samples from other authorized researchers or collections;
   vi. Soft or hard parts that are sloughed, excreted, or discharged naturally.
j. Provide a copy of the foreign collecting/capture authorization(s) (if not required, indicate "not required");
k. If importing samples from subsistence-hunted marine mammals in foreign countries, describe the subsistence method. Include documentation, if available, that verifies that the taking was/will be conducted in a humane manner (i.e., using the method that involves the least possible degree of pain and suffering);
l. If importing samples from live animals, describe how the samples were/will be collected, including animal handling and sample collection protocols. This should include a description of how the take was humane; and
m. Describe how the specimens will be preserved, shipped, and stored/curated.

NOTE: A separate CITES permit will be required from our office prior to the import of specimens of CITES Appendix I species.

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13. Are you requesting to **EXPORT or RE-EXPORT PARTS/SPECIMENS** of/from marine mammals?

☐ **No** ☑ **Yes**; provide a – e, below:

a. The types of specimens and quantity of each to be exported/re-exported;
b. The complete name and address of person/facility receiving the specimen(s);
c. A description of the origin of the specimens to be exported/re-exported;
d. The name(s) of the facility/institution that currently holds the specimens; and
e. Whether a portion of the specimen will need to be re-imported following export/re-export.

NOTE: A separate CITES permit will be required from our office prior to the export/re-export

14. Are you a facility requesting **MAINTENANCE of LIVE ANIMALS** (i.e., holding and caring for animals) for public display, research, or MMPA enhancement activities?

☑ **No** ☐ **Yes**

If yes, specify the number of individuals to be held for each species of interest: _____; provide responses to a – h, below:

a. A complete description, including photographs and/or diagrams (no blueprints), of the area and facilities where the animals will be held (including the dimensions of pools and haul-out areas);
b. The number of animals of the same species (include age and sex) presently maintained at the facilities and information indicating whether there is space for additional animals without exceeding USDA/Animal and Plant Health Inspection Service (APHIS) limits (i.e., provide the maximum # of animals of each species that could be held).
c. A list of all animal caretakers and a description of their specific duties/responsibilities;
d. A description of the animal caretakers' experience in the care, handling, and maintenance of the marine mammal species that is/are the subject of this application and copies of curriculum vitae (CVs) that demonstrate such experience for each caretaker;
e. A description of specific State requirements regarding who (e.g., attending veterinarians, vet technicians, researchers) may handle and administer certain drugs;
f. A list of all marine mammals under the jurisdiction of FWS maintained at the facility (specify whether they are held in the same exhibit/holding area as the target animals will be held and maintained);
g. A description of all deaths of FWS-jurisdiction marine mammal species at the facility within the past five years and the steps taken to prevent or decrease similar mortality;
h. A copy of the facility's USDA/APHIS, Animal Welfare Act (AWA) license and the most recent APHIS inspection report.

15. If you are a facility requesting maintenance of live animals for which the primary purpose is scientific research, or enhancement of survival or recovery of the species, are you seeking approval to publicly display the subject animals?

☑ **No** ☐ **Yes**; in a-c, below, provide information to show that:

a. The facility is open to the general public without limitations or restrictions (other than by the charging of an admission fee);

b. The facility offers a program for education or conservation purposes that is based on professionally recognized standards of the public display community; and

c. Such display will not interfere with attainment of the objectives of the permitted/authorized activity.

Part II.
For Public Display

16. For U.S. facilities, provide information to show that the facility:
    a. Is open to the general public without limitations or restrictions (other than by the charging of an admission fee);
    b. Offers a program for education or conservation purposes that is based on professionally recognized standards of the public display community (include copies of outreach/educational materials and photos of signage); and
    c. Is registered or holds a license issued by the USDA Animal and Plant Health Inspection Service (APHIS) under the Animal Welfare Act (AWA).

Part III.
For Scientific Research

17. Explain how the proposed research meets the MMPA definition of "bona fide research," i.e., scientific research on marine mammals, the results of which: (A) are likely to be accepted for publication in a referenced scientific journal; (B) are likely to contribute to the basic knowledge of marine mammal biology or ecology; or (C) are likely to identify, evaluate, or resolve conservation problems.

    See appendix

18. Provide a detailed description of the proposed project. You may attach a formal research proposal, provided it includes all the requested information, including:

    a. Objectives and hypotheses and associated methodology;

    b. Background information discussing relevant published literature on the subject of your proposal, with citations;

    c. An explanation of how this study is different from, builds upon, or duplicates past research;

    d. An explanation of how you determined your sample size/take numbers (e.g., based on previous encounter rates or abundance estimates for the study area). If appropriate for your study, include a power analysis or other sample size estimation to show whether the sample size is sufficient to provide statistically significant or otherwise robust results appropriate for your study;

    e. If proposing novel procedures, include a discussion on results from pilot studies or studies on other species, if available; and

    f. Disposition of animals or remaining specimen material once your project is complete.

19. Provide the expected research schedule (clearly specify the proposed start date and end date of your research or field season(s) and overall duration of the project). Include the months of the year and frequency of fieldwork/sampling (e.g., number of times per year). If your research extends beyond five years, or is a continuation of previously authorized research, give information about when the research began and when you expect it to end.

20. Indicate which research procedures/activities you will be conducting that will or might result in **TAKE or HARASSMENT of TARGET species**, and describe each activity in detail, including the information indicated in a-i, below.

Level A harassment means any act of pursuit, torment, or annoyance, which has the potential to injure a marine mammal or marine mammal stock in the wild.

Level B harassment means any act of pursuit, torment, or annoyance, which has the potential to disturb a marine mammal or marine mammal stock in the wild by causing disruption of behavioral patterns, including, but not limited to, migration, breathing, nursing, breeding, feeding, or sheltering.

Take, as defined by the MMPA means to harass, hunt, capture, or kill, or attempt to harass, hunt, capture, or kill any marine mammal.

☐ a. Administration of drugs (including emergency drugs and prophylactic antibiotic use) or other substances (e.g., stable isotopes); include i-vii, below, in your activity description:

i. Name of each drug/chemical used, its dosage rate (ml/kg), method of administration (IV, IM, SQ, topical and whether remotely-deployed IM), and purpose of the drug;
ii. Duration of drug and required holding time;
iii. The names of the personnel who would administer the drugs;
iv. A description of specific State requirements regarding who (e.g., attending veterinarians, vet technicians, researchers) may handle and administer certain drugs;
v. Provisions to minimize adverse reaction(s), including the use of appropriate drug reversals;
vi. Procedures to be used to minimize the chance that drugged animals will escape prior to complete immobilization; and
vii. Measures to be taken to ensure that the animal is fully recovered prior to release.

☐ b. Aerial and vessel surveys (manned); include i-v, below, in your activity description:

i. Type of survey craft and vessel;
ii. Type of survey (e.g., line transect, photogrammetry);
iii. Number of surveys per year;
iv. Minimum and maximum altitude/approach distance; and
v. Duration spent with group or individual per day.

☐ c. Aerial surveys using unmanned aircraft systems (UAS); include i-xii, below, in your activity description:

i. Dimensions, mass, and battery life of UAS;
ii. Will the UAS ever be beyond the line of sight?
iii. Does the device have an auto-return feature should the device fail?
iv. Ground control station description (what it is, where it will be located, e.g., on shore or on vessel, number of stations, and how close the station will be to animals);
v. Spotter roles (e.g., one spotter monitoring the UAS, another for monitoring the ground control station);
vi. Do you have the appropriate FAA permits/authorizations (including pilot licenses)?
vii. Type of survey (e.g., line transect, photogrammetry);
viii. Number of surveys per year;
ix. Minimum and maximum altitude/approach distance;
x. Duration spent with group or individual per day;
xi. The names of the personnel who will pilot the aircraft, and
xii. Mitigation measures you will use to minimize disturbance including specific measures you will use to avoid separating female-calf/pup/cub pairs, and measures to ensure the UAS will not collide or crash into any of the animals.

☐ d. Capture and restraint; if you will be capturing animals, ensure that you have completed question 10, above.

☐ e. Instrumentation, Marking, and Tagging (MTI); include i-x, below, in your activity description:

i. The type of MTI (including dimensions and mass);

ii. The maximum number and total mass of MTIs to be attached to/implanted in an animal at a given time;
iii. The maximum dart penetration depth if MTI is attached via darts;
iv. Methods and location of attachment, including minimum approach distance for remote MTI attachment;
v. If surgeries for implantable tags are being conducted, specify who will be conducting them, where (in the field or in a facility), and if antibiotic prophylactics will be administered;
vi. The maximum number of times an animal would be fitted with MTIs in a given year;
vii. Will recapture be necessary (if so, how many times will animals be captured annually), would the instrument/tag have a release mechanism, or would the instrument/tag fall off?
viii. Have the proposed MTIs been used previously on this species?
ix. What are the potential adverse effects and the means of monitoring new MTIs for adverse effects?
x. What actions will be taken in the event that the MTI has a significant adverse impact on the animal(s), and what is the method of animal release from the MTI?

☐ f. Intrusive sampling (e.g., blood, blubber, muscle, skin); include i-xiii below, in your activity description:

i. Will sampling be remote or under restraint?
ii. Will local anesthetics be administered?
iii. Type of tissues sampled;
iv. Size or volume of sample (diameter and depth or total volume);
v. Target sampling location on body;
vi. Maximum number of samples per animal per day and per year;
vii. Sampling intervals (e.g., for serial blood or biopsy samples);
viii. Collection method and equipment/materials used (e.g., dart fired from rifle, dart depth, sterilization/disinfection);
ix. If remote, what is the minimum approach distance?
x. If restrained, describe treatment of site of sample collection (e.g., cleansing, wound left open or closed);
xi. Number of attempts per animal per day (include total number of attempts needed for all work if requesting multiple procedures (e.g., remote tagging and biopsy) on same animal on the same day);
xii. The names of the personnel who will conduct the sampling; and
xiii. Sample preservation and analysis.

☐ g. Non-intrusive sampling (e.g., behavioral observations via focal follows and ground surveys, scat collection, passive acoustic monitoring and recording, photo-ID, photogrammetry, remote video monitoring, underwater photography); include i-vi, below, in your activity description:

i. Approach, sampling methods, and platform type;
ii. Minimum and maximum approach distance (specify different distances for each deployment method);
iii. Are researchers within sight of animals or not (e.g., from a blind)?
iv. Frequency of observations/sampling;
v. Duration of observations/sampling per day; and
vi. If conducting underwater photography/videography, specify the method (e.g., snorkeling, underwater pole cam, or divers using typical gear or rebreathers) and number of people in the water at a given time, including the safety diver/snorkeler.

☐ h. Testing methodologies on captive-held animals; include i-iii, below, in your activity description:

i. A description of the methodologies and equipment to be used;
ii. Duration and times of testing and data analyses; and
iii. Methods used to decondition the animals that will be released to the wild after testing.

☐ i. Other procedures/activities; list each additional procedure/activity and provide a detailed description of each, including all appropriate mitigation measures (note, we might contact you with follow-up clarification of methodologies), novel procedures, and any procedures involving active acoustic or hearing studies).

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21. For each procedure/activity, provide the information in a-j, below, including the maximum number of animals of each species expected to be taken by the procedure annually, broken down by sex and age class; the number of takes per animal per year; and an estimate of the number of animals of the study species that might be incidentally harassed (i.e.,# of non-target animals of your study species that might be harassed by your activities). Also, include the time periods and specific locations of the takes. This information may be provided in table format such as:

a. Species	b. Procedure/Activity	c. Level A or Level B Harassment*or other Take**	d. Age Class(see question 23, below)	e. Sex	f. Max. # Animals Per Year	g. Max. # Takes Per Animal,Per Year	h. Max. # non-target conspecifics incidentally harassed	i. Time-period	j. Location

*** Level A harassment** means any act of pursuit, torment, or annoyance which has the potential to injure a marine mammal or marine mammal stock in the wild. **Level B harassment** means any act of pursuit, torment, or annoyance which has the potential to disturb a marine mammal or marine mammal stock in the wild by causing disruption of behavioral patterns, including, but not limited to, migration, breathing, nursing, breeding, feeding, or sheltering.

****Take**, as defined by the MMPA means to harass, hunt, capture, or kill, or attempt to harass, hunt, capture, or kill any marine mammal.

22. Will any female-pup/calf/cub pairs be targeted for any of the proposed research activities? If so, describe how you would minimize impacts on pups/calves/cubs and associated females during each of those activities.

    No

23. Define each age class listed in your response to question 21(d), above, for each species (i.e., list the range of months or years (or mass for otters) constituting each age class); provide the minimum age (or mass) that animals will be targeted for take activities; and indicate whether females with calves/pups/cubs less than that minimum age will be targeted for take activities?

24. Describe the precautions that will be taken to minimize the likelihood that harassment of non-target individuals of the study species will occur and the actions that will be taken should harassment occur.

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25. Explain how you determined that your methods involve the least possible degree of pain and suffering and why there are no feasible alternative methods to obtain the desired data or results.

26. Provide: a) an estimate of the possible number of unintentional deaths or serious injuries that might result from your research activities; b) the number of unintentional and intentional (via euthanasia for humane purposes if an animal is seriously injured) deaths or serious injuries you seek approval for annually; c) the steps you will take to reduce the likelihood of deaths or injuries; and d) if euthanasia might occur, provide the method of euthanasia (e.g., gunshot, drug, etc.) and who would conduct the euthanasia procedure.

27. In the event of a death, will a necropsy be conducted on the animal?
☑ No ☐ Yes

28. If a female animal accompanied by calf/pup/cub(s) dies during research activities, specify the disposition of the associated calf/pup/cub(s).

n/a

29. If biological samples are to be collected or received domestically, provide responses to a through j, below, for each individual animal per species. This information, or part of the information, may be provided in table format such as the table below. (Note: if your **only** proposed activity is to transfer dead marine mammal specimens for purposes of public display or scientific research, complete application # 3-200-87).

a. Species	b. ID #	c. Sex	d. Source (Wild or Captive/ Live or Dead)	e. Birth Date or age class	f. Type of Samples (blood, tissue, DNA)	g. Number of animals sampled annually	h. Number of times each animal will be sampled annually	i. Packaging and Preservation of samples	j. Use/ Disposition of Samples

a. Provide a detailed description of the source of the specimens, including the circumstances under which the animals were/will be taken. For example, this might include the following sources:
   i. Animals stranded alive or dead;
   ii. Animals killed during legal subsistence harvests;
   iii. Animals killed incidental to legal commercial fishing operations;
   iv. Samples from other authorized researchers or collections;
   v. Soft or hard parts that are sloughed, excreted, or discharged naturally;
   vi. Samples that will be/were intrusively collected from captive-held animals;

vii. Samples that will /were collected from wild animals.

b. If collecting samples from live animals, describe how the samples were/will be collected, including animal handling and sample collection protocols.

c. For samples received domestically from U.S. permitted researchers, include the researcher's name, affiliation, and permit number under which samples will be/were collected.

(Note: if samples are to be imported, you must answer question 12, above).

30. Provide a list of all personnel that will be involved in the project, identifying each as either a principal investigator or co-investigator, their project duties/responsibilities, and a brief description or CV that demonstrates their experience and expertise to perform their designated duties, including knowledge of the marine mammal species that is/are the subject of this application.

31. Describe how you will collaborate or coordinate with other researchers in your study area. Who are they? Explain how this will occur and how it will minimize negative impacts on the species. For example, will it involve sharing resources, samples or data; timing surveys to minimize disturbance, etc.?

32. If you intend to conduct research on animals in a captive-holding facility such as a zoo or aquarium, provide documentation showing that the facility(ies) has authorized you to conduct your proposed activities.

33. Animal Welfare Act (AWA) Compliance (for research on live animals only): AWA requirements apply to all research facilities, which include institutions, organizations, or people that use or intend to use LIVE animals in research, tests, or experiments; AND, that receive funds under a grant, award, loan, or contract from a department, agency, or instrumentality of the U.S. for the purpose of carrying out research, tests, or experiments, or acquires or transports the animals in commerce. **Provide the following documentation:**

    a. Registration under the AWA as a research facility:
        i. Attach a copy of your APHIS certificate of registration as a research facility, or for Federal facilities, a letter from your Institutional Officer that you are compliant with applicable requirements for scientific research under the AWA; OR
        ii. If your facility does/will not conduct activities requiring registration under the AWA, attach a letter from APHIS confirming that registration is not required.

    b. Institutional Animal Care and Use Committee (IACUC) documentation: If your facility is registered as a research facility under the AWA or is a Federal research facility (see a.i), attach the applicable IACUC documentation from the list in i-iii, below. Please note that all activities that involve an invasive procedure, harm, or materially alter the behavior of an animal under study, even if the activities are carried out in the field, are subject to IACUC review and approval. See (AWA regulations and standards for definition/explanation of covered research activities.):
        i. Attach a copy of your final protocols with the IACUC signed approval; **OR**
        ii. Attach a copy of your proposed protocols to be reviewed by your IACUC along with an explanation as to how and when the protocols will be reviewed (Note: A copy of your final signed protocols and certification will be required prior to permit issuance.); **OR**
        iii. Attach the IACUC determination that your research activities are not subject to IACUC review and approval.

    c. If your facility is not registered as a research facility under the AWA, please provide an explanation of how your take activities are reviewed and monitored to assure that the proposed takes are humane (i.e., using the method that involves the least possible degree of pain and suffering).

Part IV.

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For Rescue, Rehabilitation, and/or release of stranded[2] Marine Mammals

Marine mammals may be captured from the wild by duly authorized U.S. Fish and Wildlife Service personnel or **authorized cooperators** for the protection or welfare of the marine mammal or for the protection of public health and welfare and held at cooperating authorized facilities. This section of the application is for those parties interested in applying for a letter of authorization (LOA) under MMPA Sections 109(h)/112(c). Parties interested in rescue, rehabilitation, and release activities involving ESA-listed marine mammals would also use this section of the application to apply for an accompanying ESA permit for enhancement of propagation or survival of the species OR to apply as a "sub-permittee" working under the authority of an ESA permit held by different organization or agency. Authorized "sub-permittees" would be responsible for coordinating their activities with the designated ESA permit-holder (i.e., "Permittee") and would be required to comply with the conditions of that permit. Each authorized party's MMPA LOA will document the ESA permit number associated with that LOA, whether the party is a sub-permittee or the Permittee on the ESA permit.

The MMPA LOA or, for ESA-listed species, the combined MMPA LOA and ESA permit would provide authorization for individuals or institutions to work as "cooperators" for the purpose(s) of rescue, rehabilitation, and/or release of stranded marine mammals. Marine mammal rescues are dangerous activities that require trained staff, specialized equipment, and clear communication among stranding partners. The U.S. Fish and Wildlife Service provides opportunities for different levels of involvement for approved cooperators: verifiers, rescuers, transporters, critical care facilities, and rehabilitation/holding facilities. These roles are defined in question 37, below.

34. Are you/your organization currently conducting research activities with marine mammals?

☐ **No** ☐ **Yes**

If yes, provide the permit number under which you are conducting research_____.

35. What type of authorization are you requesting (check all that apply)?

☐ LOA under MMPA Sections 109(h)/112(c)

☐ ESA permit for enhancement of propagation or survival of the species

☐ Sub-permittee under ESA permit #_____.

36. What type of stranding event are you requesting to respond as a cooperator for a U.S. Fish and Wildlife Service marine mammal rescue, rehabilitation, and release program?

☐ Oil spill events

☐ Other contaminant spill events; Specify types_____.

☐ Other stranding events

[2] The term, "stranding," as defined by the MMPA means an event in the wild in which: (A) a marine mammal is dead and is on a beach or shore of the United States or in the waters under the jurisdiction of the United States (including any navigable waters); OR (B) a marine mammal is alive and is on a beach or shore of the United States and unable to return to the water, on a beach or shore of the United States and, although able to return to the water, is in need of apparent medical attention, or in the waters under the jurisdiction of the United States (including any navigable waters), but is unable to return to its natural habitat under its own power or without assistance.

37. Indicate at which level(s) of responsibility the cooperator will participate (Check all that apply, and respond to the questions below).

☐ **VERIFIER:** The role of verifiers is limited to answering requests to provide physical verification of the condition of reported live, distressed animals and communicating the location and status of an animal to the appropriate person(s), including the rescue program coordinator and, if so directed, the nearest approved rescue facility. In most cases verifiers are required to stay with the animal until an approved rescue and transport team arrives. No physical interaction with animals are authorized under this designation. Verifiers may handle animals only under the guidance of an onsite designated rescue team(s).

a. Describe your organization's experience in verifying the condition of reported live, distressed or injured animals of each species requested (e.g., years of experience, number of responses, etc.).

b. Describe the qualifications of each of your staff who would be serving as a verifier in your organization that demonstrates their ability to verify the condition of reported, live, distressed animals of each species requested (including any work and/or volunteer experience that describes where, with what authorized organization, approximate number of hours, approximate number of verifications, and other relevant experience). Resumes, curriculum vitae (CV), and other supporting documents may be used to describe qualifications, including experience with the marine mammal species (or another similar marine mammal species) that is/are the subject of this application.

c. List and describe any specialized training that your staff have completed to perform this duty, including where and when the training occurred, which organization provided the training, types of training, and other relevant information.

d. Describe numbers and types of: a) vehicles (cars, trucks, boats, etc.) that will be used to travel to/from locations of reported, live, distressed animals; b) communications devices that will be used to communicate with rescue responders (phones, radios, etc.); and c) any other related equipment.

e. Provide a statement that you will be available to respond to reports of live, distressed animals of the subject species when needed.

☐ **RESCUER**: Rescuers respond to reports of injured and/or distressed animals and can initiate hands-on rescue and transport efforts as needed. This level of involvement requires substantial expertise and training in species-specific rescue techniques. Rescuers must meet U.S Department of Agriculture (USDA) standards for Humane Handling, Care, Treatment, and Transportation of Marine Mammals when rescuing live animals.

a. Describe your organization's experience in rescuing distressed or injured animals of each species requested (e.g., years of experience, number of rescues, etc.).

b. Describe the qualifications of each of your staff who would be serving as a rescuer in your organization that demonstrates their ability to rescue distressed animals of the subject species (including any work and/or volunteer experience that describes where, with what authorized organization, approximate number of hours, approximate number of rescues, and other relevant experience). Resumes, CVs, and other supporting documents may be used to describe qualifications, including experience with the marine mammal species (or another similar marine mammal species) that is/are the subject of this application.

c. List and describe any specialized training that your staff have completed to perform this duty, including where and when the training occurred, which organization provided the training, types of training, and other relevant information.

d. Describe how you meet or exceed USDA standards. Include a description of the number and types of:
a) vehicles (cars, trucks, boats, etc.) that will be used to support the rescue of distressed animals;
b) rescue equipment (nets, stretchers, etc.) that will be used for rescues;
c) communications devices that will be used during rescues (phones, radios, etc.); and
d) any other related equipment.

e. Describe your methods of capture of the species of interest, including:
   i. Methods of restraint and holding, including dimensions/type of holding container, if used;
   ii. Minimum number of personnel participating in captures at any given time;
   iii. Precautions you will take to avoid separating female-calf/pup/cub pairs, and protocol in the event they are separated, including disposition of the separated calf/pup/cub; and
   iv. Precautions you will take to minimize incidental harassment of non-target animals of the target species.

f. Provide a statement that you will be available to respond to reports of live, distressed animals when needed.

☐ **TRANSPORTER:** Transporters respond to reports of injured and/or distressed animals and initiate transport efforts as directed. This level of involvement requires substantial expertise and training in the species-specific transport methodology, as well as the necessary equipment and trained staff to accompany and move the animals to or between approved critical care and/or rehabilitation/holding facilities. Transporters must meet U.S Department of Agriculture (USDA) standards for Humane Handling, Care, Treatment, and Transportation of Marine Mammals when transporting live animals. Transports must also be consistent with Animal Welfare Act requirements for transportation and USFWS transport regulations.

a. Describe your organization's experience in transporting animals of each species requested (e.g., years of experience, number of transports, etc.).

b. Describe the qualifications of each of your staff in your organization who would be accompanying animals during transport, demonstrating their ability to transport, accompany, and support animals of the subject species (including any work and/or volunteer experience that describes where, with what authorized organization, approximate number of hours, approximate number of transports, and other relevant experience). Resumes, CVs, and other supporting documents may be used to describe qualifications, including experience with the marine mammal species (or another similar marine mammal species) that is/are the subject of this application.

c. List and describe any specialized training that your staff have completed to perform this duty, including where and when the training occurred, which organization provided the training, types of training, and other relevant information.

d. Describe how you meet or exceed USDA standards:

   i. Include a description of the number and types of: a) vehicles (trucks, boats, airplanes, etc.) that you will use to transport animals of the subject species; shipping containers that will be used to transport the animals (including type, construction, dimensions, and weight); other equipment that will be used in the transport of the animals (foam pads, water sprayers, stretchers, etc.); communications devices that will be used during transports (phones, radios, etc.); and any other related equipment.
   ii. Describe how the subject animals will be cared for during transport, including the number of attending staff and a description of the arrangements for watering or otherwise caring for the animals during transport.

e. Provide a statement that you will be available to transport animals of the requested species when needed.

☐ **CRITICAL CARE FACILITY**: These facilities hold and medically treat sick and/or injured animals whose lives would be jeopardized if care were not provided. These facilities have the species-specific equipment, experience, and credentials necessary to rescue, stabilize, rehabilitate and release animals. These facilities may also provide long-term care, as needed, for generally healthy animals awaiting release, or they may provide long-term care for those individuals designated as "non-releasable". Critical care facilities must meet or exceed USDA standards for Humane Handling, Care, Treatment, and Transportation of Marine Mammals when maintaining, treating, and holding live animals.

a. Describe your organization's experience in maintaining, holding, and caring for distressed or injured animals of each species requested (e.g., years of experience, number of animals held, etc.).

b. Describe the qualifications of each of the staff in your organization who would be caring for, handling, and maintaining animals of the subject species (including any work and/or volunteer experience that describes where, with what authorized organization, approximate number of hours, approximate number of animals, and other relevant experience). Resumes, CVs, and other supporting documents may be used to describe qualifications, including experience with the marine mammal species (or another similar marine mammal species) that is/are the subject of this application.

c. For authorization as a critical care facility, you must have a qualified, critical care veterinarian. Provide the name of the person assigned this role and describe his/her qualifications, including a CV or resume that demonstrates his/her ability to perform this role.

d. Describe how you meet or exceed USDA standards. Include a description of:
    i. critical care and holding areas, including descriptions of holding tanks and haul-out areas. The description should include photographs, drawings, and/or diagrams illustrating the area(s) and facility (or facilities) where animals of the subject species will be held. When describing holding tanks, include dimensions (tank length, width, depth, water volume); describe pumps and filtration systems in tanks (including type and capacity and other relevant information); describe lifting apparatus; describe water heaters (including degree to which tanks can be heated); describe water source and type (and ability to use freshwater, saltwater and/or both); and any other relevant features.
    ii. The maximum number of animals of the subject species that can be housed at your facility.
    iii. The current distribution and number of animals of the subject species by holding tank at your facility (include sex, age (if known), time in captivity, age/size class, calves/pups/cubs, etc.).
    iv. All deaths of the subject species at your facility within the past five years and the steps taken to prevent them.

e. Describe quarantine plans, including location and time-frame.

f. Provide a copy of i) your USDA Animal and Plant Health Inspection Service (APHIS) Animal Welfare Act (AWA) license; and ii) your most recent APHIS inspection report.

g. Provide a statement that you will be available to maintain, care for, and house animals of the subject species when needed, including round the clock veterinary care.

☐ **REHABILITATION/HOLDING FACILITY:** These facilities provide routine husbandry for generally healthy animals that require a minimum of specialized treatments. These facilities may provide long-term care, as needed, for generally healthy animals awaiting release, or they may provide long-term care for those individuals designated as non-releasable. Holding facilities must meet USDA standards for Humane Handling, Care, Treatment, and Transportation of Marine Mammals when holding live animals.

a. Describe your organization’s experience in maintaining and holding animals of each species requested (e.g., years of experience, number of animals held, etc.).

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b. Describe the qualifications of each of the staff in your organization who would be caring for, handling, and maintaining animals of the subject species (including any work and/or volunteer experience that describes where, with what authorized organization, approximate number of hours, approximate number of animals, and other relevant experience). Resumes, CVs, and other supporting documents may be used to describe qualifications, including experience with the marine mammal species (or another similar marine mammal species) that is/are the subject of this application.

c. For authorization as a holding facility, you must have a qualified veterinarian. Provide the name of the person assigned this role and describe his/her qualifications, including a CV or resume that demonstrates his/her ability to perform this role.

d. Describe how you meet or exceed USDA standards. Include a description of:
   i. holding areas, including descriptions of holding tanks and haul-out areas. The description should include photographs, drawings, and/or diagrams illustrating the area(s) and facility (or facilities) where animals of the subject species will be held. When describing holding tanks, include dimensions (tank length, width, depth, water volume); describe pumps and filtration systems in tanks (including type and capacity and other relevant information); describe lifting apparatus; describe water heaters (including degree to which tanks can be heated); describe water source and type (and ability to use freshwater, saltwater and/or both); and any other relevant features.
   ii. The maximum number of animals of the subject species that can be housed at your facility.
   iii. The current distribution and number of animals of the subject species by holding tank at your facility (include sex, age (if known), time in captivity, age/size class, calves/pups/cubs, etc.).
   iv. All deaths of the subject species at your facility within the past five years and the steps taken to prevent them.

e. Describe your facility's quarantine plans, including location and time-frame;

f. Provide a copy of i) your USDA Animal and Plant Health Inspection Service (APHIS) Animal Welfare Act (AWA) license; and ii) your most recent APHIS inspection report.

g. Provide a statement that you will be available to maintain and house animals of the subject species when needed.

h. Are you seeking approval to display the animals while holding and maintaining them for rehabilitation purposes?

☐ **No** ☐ **Yes**

If yes, in i-iii, below, provide information to show that:

i. The facility is open to the general public without limitations or restrictions (other than by the charging of an admission fee);
ii. The facility offers a program for education or conservation purposes that is based on professionally recognized standards of the public display community; and
iii. Such display will not interfere with attainment of the objectives of the permitted/authorized activity.

Part V.
For MMPA Enhancement of Survival or Recovery of a Species or Stock

Note: This section of the application should not be completed unless you are specifically requesting MMPA Enhancement activities (e.g., this section is not intended for those parties requesting to conduct rescue, rehabilitation, and release activities for marine mammals).

38. Provide information to show that your proposed activities are likely to contribute significantly to maintaining or increasing the distribution or population numbers necessary to ensure the survival or recovery of the species or stock in the wild.

39. Provide information to show that your proposed activities are consistent with any conservation or recovery plan for the species or stock, or, if no plans are available, that the activity is consistent with the actions required to enhance the survival or recovery of the species or stock and that would be addressed in a conservation or recovery plan. For activities that involve captive maintenance of live animals:

a. Provide an explanation on the benefit of removing animals from the wild into captivity; and
b. Include a description of plans in place for returning animals and any offspring to the wild.

(Note: You must also provide the information requested in question 14, above.)

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NOTICES

PRIVACY ACT STATEMENT

Authority: The information requested is authorized by the following: the Bald and Golden Eagle Protection Act (16 U.S.C. 668), 50 CFR 22; the Endangered Species Act (16 U.S.C. 1531-1544), 50 CFR 17; the Migratory Bird Treaty Act (16 U.S.C. 703-712), 50 CFR 21; the Marine Mammal Protection Act (16 U.S.C. 1361, et seq.), 50 CFR 18; the Wild Bird Conservation Act (16 U.S.C. 4901-4916), 50 CFR 15; the Lacey Act: Injurious Wildlife (18 U.S.C. 42), 50 CFR 16; Convention on International Trade in Endangered Species of Wild Fauna and Flora (TIAS 8249), 50 CFR 23; General Provisions, 50 CFR 10; General Permit Procedures, 50 CFR 13; and Wildlife Provisions (Import/export/transport), 50 CFR 14.

Purpose: The collection of contact information is to verify the individual has an eligible permit to conduct activities which affect protected species. This helps FWS monitor and report on protected species and assess the impact of permitted activities on the conservation and management of species and their habitats.

Routine Uses: The collected information may be used to verify an applicant's eligibility for a permit to conduct activities with protected wildlife; to provide the public and the permittees with permit related information; to monitor activities under a permit; to analyze data and produce reports to monitor the use of protected wildlife; to assess the impact of permitted activities on the conservation and management of protected species and their habitats; and to evaluate the effectiveness of the permit programs. More information about routine uses can be found in the System of Records Notice, Permits System, FWS-21.

Disclosure: The information requested in this form is voluntary. However, submission of requested information is required to process applications for permits authorized under the listed authorities. Failure to provide the requested information may be sufficient cause for the U.S. Fish & Wildlife Service to deny the request.

PAPERWORK REDUCTION ACT STATEMENT

We are collecting this information subject to the Paperwork Reduction Act (44 U.S.C. 3501) in order provide the U.S. Fish and Wildlife Service the information necessary, under the applicable laws governing the requested activity, for which a permit is requested. Information requested in this form is purely voluntary. However, submission of requested information is required in order to process applications for permits authorized under the applicable laws. Failure to provide all requested information may be sufficient cause for the U.S. Fish and Wildlife Service to deny the request. According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a currently valid OMB control number. OMB has approved this collection of information and assigned Control No. 1018-0093.

ESTIMATED BURDEN STATEMENT

We estimate public reporting for this collection of information to average 2 hours and 20 minutes, including time for reviewing instructions, gathering and maintaining data and completing and reviewing the form. Direct comments regarding the burden estimate or any other aspect of the form to the Service Information Clearance Officer, Fish and Wildlife Service,
U.S. Department of the Interior, 5275 Leesburg Pike, MS: BPHC, Falls Church, VA 22041-3803, or via email at Info_Coll@fws.gov.

Please do not send your completed form to this address.

University of Memphis – Supplemental Information for US FWS 3-200-43

Emily E. Puckett, PhD
Associate Professor, University of Memphis

Part I- Q5- *Provide a copy of any other applicable Federal, local, or state permissions (e.g., National Wildlife Refuge Special Use Permit, NOAA National Marine Sanctuary permit, etc.) required to conduct your proposed work, OR indicate whether you have applied for, secured, or will apply for such permissions (please provide contact information).*

We are jointly applying for a CITES authorization from Environment Canada. The contact information is:

CITES Permitting Office
Canadian Wildlife Service
Environment Canada
351, St. Joseph Blvd
Gatineau, Quebec K1A 0H3
Email: cites@ec.gc.ca
Fax: 1-855-869-8671

Part I- Q6- A- *Attach a justification for taking an ESA-listed species, and explain why your proposed activities are not appropriate for a similar non-ESA-listed species*

The take we request includes the collection of plucked hair, shed hair, and/or skin biopsy plugs from polar bears (*Ursus maritimus*). We are studying adaptation to the Arctic environment with polar bears as our focal species. Specifically, we are interested in three skin or hair traits that allowed for this species to colonize the Arctic: white hair, black skin, and air-filled hollow hair. We are specifically interested in the underlying genomic changes that produced these traits. We hypothesize that regulatory changes are largely responsible for these traits when compared to the brown bear (sister taxa to polar bears) and/or American black bear (closest living outgroup). Thus, we will compare gene expression within the skin between these three species to test our hypotheses.

Samples can only be collected from live animals as we want to compare RNA profiles (i.e., gene expression) among polar, brown, and American black bears. RNA degrades upon cell death; thus, must be collected from live animals. We have two distinct sets of partners for the collection of samples: Canadian scientists, and North American zoos (in both the USA and Canada). Our Canadian scientific partners do annual monitoring of the west Hudson Bay polar bear subpopulation, which includes taking biopsies. They use the fat of the biopsy to measure mercury load. We will use the skin for our experiments. Given their interest in accessing the fat, they choose to take biopsies from the dorsal rump where there is an excess of fat. They are experienced polar bear handlers and sample collectors, and place high importance on the safety of the bears and humans when collecting the samples. Biopsies collected from the dorsal rump allow for the three biopsy punches per animal needed to obtain sufficient tissue for long-read DNA sequencing, RNA sequencing, histology, and immunohistochemistry.

A secondary source of skin tissue could be obtained from an ear punch. When individual identification tags are affixed to the animal's ears, a small through biopsy is made, and often discarded, in the process. Our collaborators may collect this tissue instead of discarding it as an extra source for DNA and/or RNA sequencing.

We also have partnerships with several accredited North American zoos to either collect plucked or shed hair, or to collect plucked hair and skin biopsies. Shed hair can be collected in a fully non-invasive manner by sweeping up the hair from within the animal's indoor enclosure, as long as multiple polar bears are not housed in the same habitat and we can ensure that each sample represents an independent animal. In contrast, the plucked hair and skin biopsies will be collected only during regularly scheduled health checks that already require anesthesia under a licensed veterinarian and post-anesthesia monitoring by caretakes for the animal. No animals will be put under anesthesia for the sole collection of these samples, instead this is opportunistic sampling during a regular health check.

Our research questions have been explicitly designed around polar bears because of their multiple and synergistic Arctic adaptations. We are interested in understanding the mutations that gave rise to each trait specifically, and any interactive effects among these traits. Part of the power of our study comes from identifying a population of brown bears that contain the hollow hair trait. Thus, we are able to use this variation and the history of polar bear introgression into brown bears, to dissect the genetic architecture of the trait. This would not be possible in other Arctic adapted species, even if they had similar traits (e.g., white or hollow hair), specifically because the causative locus fixes in the species due to strong positive selection in the past. It is the ready comparison among the three species of bears which provides high power to our study.

Bulk RNA sequencing is a method of measuring average gene expression levels for all genes expressed in a tissue. It remains the method of choice to identify statically differentially expressed (SDE) genes between different sample groups, such as skin biopsies from Am. black, brown, and polar bears. Sample size requirements for bulk RNA sequencing comparisons must factor for inherent technical and biological variability.

Despite our efforts to minimize technical variation by providing a detailed collection protocol, skin biopsies will be obtained by different veterinarians, at different times of the hair cycle, and different body locations (wild bears: rump; captive bears: shoulder). Additionally, skin biopsies are a heterogenous mix of cell types, of which the relevant ones, melanocytes and hair matrix keratinocytes, comprise a small (2-5%) fraction. Minority cell populations are prone to compositional variation between samples. Also, hair growth is an active process in adult skin, during which relevant cell populations change in size and transcriptional activity. All of these factors can add noise to detection of gene expression changes between groups and are difficult to account for using statistical packages designed to estimate sample size requirements.

A recent study (Schurch *et al*, 2016) that empirically tested sample size requirements indicated a minimum of 3 biological samples per group are required to detect greater than two-fold differences in gene expression under ideal conditions. The same study recommended a minimum of 6 biological samples per group to identify the majority of SDE genes. We have successfully used bulk RNA sequencing with similar sample sizes to identify key genes underlying coat color variation in skin biopsies collected from non-experimental animals, including the cheetah (Kaelin *et al*, 2012) and the African striped mice (Mallarino *et al*, 2016). Thus, we plan to RNA sequence samples for each bear species.

We will utilize hair proteomics in an exploratory manner to identify proteins with differential abundance between species. Proteomic studies require higher sample sizes due to greater variation among samples for low abundance proteins. Similarly to the other two Arctic adaptations of interest, the hollow hair trait shows a gross phenotypic difference from the brown bear; thus, we expect clear signals of protein abundance within the data.

Part I- Q6- B- *Describe both the short-and long-term anticipated effects of each of your activities alone or cumulatively on the behavior and physiology of the target animals and critical habitat or proposed critical habitat for the species.*

The short-term implications of the biopsy will be a small wound in each animal's shoulder or haunch area where the sampling occurred. In captive bears, the veterinarian will use their discretion and standard procedures from their institution to determine if small dissolvable stiches are warranted. Given the size of polar bears versus the size of the biopsy (5mm), limited complications are expected. Further, at the zoos caretakers will watch for healing or discomfort for the animals. The site should heal within a week; thus, no long-term implications of the biopsy are expected on behavior or physiology.

We are not altering critical habitat in any way.

Part I- Q6- D- *Identify how you would mitigate any potential negative effects.*

The zoo caretakers will monitor the bears and bring any distress to the attention of veterinarians, upon which a treatment plan will be prepared and implemented.

Wild bears are monitored while coming out from anesthesia to prevent attacks from other animals while defenses are down. The presence of humans at a safe distance from the bear deters other animals from disturbing the bear.

Part I- Q8- *Do you plan to conduct your public display, research, or MMPA enhancement activities with* ***MARINEMAMMALS that are CURRENTLY HELD IN A CAPTIVE ENVIRONMENT (including, but not limited to import into the U.S. of captive-held live animals/specimens).***

Table R1 shows the samples for which samples have already been collected or for which collections are pending and that are held in a captive environment.

In addition, hair and skin biopsy samples would be provided from seven wild caught Canadian adult polar bears.

Table R1- Details of known captive animals to be sampled.

Species	Sex	Birth Date	Description	Country of Origin	Source	Current Location of Animal
Ursus maritimus	F	28-Dec-98	ZIMS 3010 (Berit)	USA	Captive bred (and born)	Henry Vilas Zoo
Ursus maritimus	F	3-Dec-15	GAN#PRN15-16557, BZC#6837, SB#1208 (Hope)	USA	Captive bred (and born)	Chicago Brookfield
Ursus maritimus	M	14-Dec-06	GAN#MIG12-29919335, BZC#2356, SB#1165 (Hudson)	USA	Captive bred (and born)	Chicago Brookfield
Ursus maritimus	F	~ Jan 2021	ZIMS 27723109 (Nikita)	Canada	Wild	Toronto Zoo
Ursus maritimus	F	~ Jan 2021	ZIMS MIG12-29695880 (Aurora)	Canada	Wild	Toronto Zoo
Ursus maritimus	M	11-Oct-11	ZIMS MIG12-29714894 (Hudson)	Canada	Captive bred (and born)	Toronto Zoo
Ursus maritimus	F	11-Nov-15	ZIMS YVZ15-07790 (Juno)	Canada	Captive bred (and born)	Toronto Zoo
Ursus maritimus	M	25-Nov-04	GAN: 16451775 (Koda)	USA	Captive bred (and born)	Memphis Zoo
Ursus maritimus	F	15-Nov-02	GAN: 27923658 (Haley)	USA	Captive bred (and born)	Detroit Zoo

Part I- Q12- *Are you requesting to Import Parts/Specimens of/from marine mammals?*

This section is in regards to biopsies from Canadian researchers sampling wild bears

a. The proposed date of import;

i. July 2025 - June 2027

b. The name and address of the foreign exporter, including the country of export;

Evan Richardson,
Environment and Climate Change Canada
234 Donald St, Unit 510, Winnipeg, MB, Canada R3C1M8

c. The current location of the specimens;

a. Environment and Climate Change Canada office in Winnipeg, CA

d. The country of origin of the animals from which the specimens were/will be collected;

a. Canada

e. List the number of animals by species, age class/life stage, and sex from which parts/samples are sought. If you are requesting opportunistic sample import, you may request an unlimited number of samples from a specified number of animals, by taxa (e.g., unlimited samples from up to 100 polar bears annually).

a. *Ursus maritimus* (polar bear), seven animals, adults, 7:0 female: male
b. Samples were collected in March 2024

f. The types of specimens to be imported (e.g., blood, skin biopsy, carcasses, etc.) and number of each type from each animal;

a. From each adult animal,

i. We request a clump of plucked hair (100-500 individual hairs). These will be individually stored in coin envelopes. Thus, we expect 7 envelopes of hair.

ii. We request three 5mm skin biopsies. Each biopsy will be submerged in an individual tube of AllProtect within a 2mL cryotube. As we need three biopsies per animal, we expect 21 tubes with biopsies.

g. The source of the specimens to be imported (wild, captive-bred, or captive born);

a. Wild

h. Were the animals/will the animals be alive or dead at the time of sample collection?

a. Live

i. Provide a detailed description of the source of the specimens to be imported and the manner in which the sample was/will be taken or collected. For example, this might include the following sources:

a. Animals in captivity (samples taken during routine husbandry procedures or under separate authorization; distinguish between permanently captive in public display or research facility and temporarily captive in rehabilitation facility);

i. n/a

i. Animals in foreign countries stranded alive or dead or that died during rehabilitation;

a. n/a

ii. Animals killed during legal subsistence harvests;

a. n/a

iii. Animals killed incidental to legal commercial fishing operations;

a. n/a

iv. Samples from other authorized researchers or collections;

a. Dr. Evan Richardson leads polar bear sampling for Environment and Climate Change Canada, part of Environment Canada. Animals from the western side of Hudson Bay are annually monitored. This includes anesthesia of a subset of bears for the collection of skin and subcutaneous fat from which mercury is measured.

v. Soft or hard parts that are sloughed, excreted, or discharged naturally.

a. n/a

j. Provide a copy of the foreign collecting/capture authorization(s) (if not required, indicate "not required");

a. Please see attached documents provided by Dr. Evan Richardson:

i. Manitoba Species at Risk/Wildlife Scientific Permit SAR23008.

ii. Parks Canada Agency Research and Collection Permit WAPNP-2022-45543.

k. If importing samples from subsistence-hunted marine mammals in foreign countries, describe the subsistence method. Include documentation, if available, that verifies that the taking was/will be conducted in a humane manner (i.e., using the method that involves the least possible degree of pain and suffering);

a. n/a

l. If importing samples from live animals, describe how the samples were/will be collected, including animal handling and sample collection protocols. This should include a description of how the take was humane; and

a. Polar bears will be identified on the landscape by researchers in helicopters flown within Wapusk National Park, Manitoba, Canada. Once a polar bear is spotted, the animal will be anesthetized with Telazol (based on estimated body weight) via dart gun. Bears will not be darted if they show any sign of distress, overheating, or injury, and an animal may not be pursed longer than 3 minutes. Once down, researchers will commence with data and sample collection from the bear. Specific to this permit, three 5mm biopsy samples will be taken from the rump area going all the way through the epidermal and dermal layers of the skin. Biopsies for this study will not include substantial amounts of fat. The

skin punches will be placed into a vial containing AllProtect, then submerged. AllProtect is able to intercalate the sample and preserve DNA, RNA, and proteins. After 24hours at room temperature, the sample can be moved to a refrigerator or freezer for long-term preservation. Following sample collection, researchers will stay with the bear for several hours to prevent any attacks of the animal by others while it is vulnerable. Once the bear begins moving its head and forelimbs, researchers will leave the area.

m. Describe how the specimens will be preserved, shipped, and stored/curated.

a. The biopsy samples will be placed into AllProtect in the field. This reagent preserves DNA, RNA, and proteins. Once preserved, the samples can be refrigerated or frozen, but are also okay at room temperature. Meta-data on bear ID, sex, location, sampling date, and collaborator will be placed into a spreadsheet for future use. International samples will be shipped via FedEx on blue ice to the FWS inspection facility for inspection then pick-up by Emily Puckett.
b. Hair samples will be put into coin envelopes and stored in a dark and dry environment at room temperature. Once collected, samples will be shipped to the University of Memphis if within the USA, or via FedEx to the FWS inspection facility for inspection then pick-up by Emily Puckett.

Part I- Q12- *Are you requesting to Import Parts/Specimens of/from marine mammals?*

This section is in regards to biopsies from Canadian zoo collaborators sampling captive bears (also see table for Question 8)

a. The proposed date of import;

c. July 2025 - June 2027

b. The name and address of the foreign exporter, including the country of export;

Toronto Zoo
361A Old Finch Avenue
Toronto, ON
M1B 5K7
Canada

c. The current location of the specimens;

Toronto, CA

d. The country of origin of the animals from which the specimens were/will be collected;

Canada

e. List the number of animals by species, age class/life stage, and sex from which parts/samples are sought. If you are requesting opportunistic sample import, you may request an unlimited number of samples from a specified number of animals, by taxa (e.g., unlimited samples from up to 100 polar bears annually).

From the Toronto Zoo- *Ursus maritimus* (polar bear), four animals, adults, 3:1 female: male

f. The types of specimens to be imported (e.g., blood, skin biopsy, carcasses, etc.) and number of each type from each animal;

a. From the Toronto Zoo-

i. From each animal, we request a clump of plucked hair (100-500 individual hairs). These will be individually stored in coin envelopes. Thus, we expect 4 envelopes of hair.
ii. From each animal, we request three 5mm skin biopsies. Each biopsy will be submerged in an individual tube of AllProtect within a 2mL cryotube. As we need three biopsies per animal, we expect 12 tubes with biopsies.

g. The source of the specimens to be imported (wild, captive-bred, or captive born);

a. Two of the animals (Nikita and Aurora) were wild. They were orphaned as cubs after a hunter killed their mother near Peawanuk, Ontario, Canada in 2001.
b. Two of the animals (Hudson and Juno) were captive-bred (and born) from the same parents (Aurora and Inukshuk) who were orphaned as cubs and brought to the zoo. Hudson was born in 2011 and Juno in 2015.

h. *Were the animals/will the animals be alive or dead at the time of sample collection?*
   a. Live

i. *Provide a detailed description of the source of the specimens to be imported and the manner in which the sample was/will be taken or collected. For example, this might include the following sources:*
   a. *Animals in captivity (samples taken during routine husbandry procedures or under separate authorization; distinguish between permanently captive in public display or research facility and temporarily captive in rehabilitation facility);*
      i. Bears at the Toronto Zoo were sampled during routine health checks occurring between 2022-2025. Anesthesia was applied by the supervising veterinarian, then samples were collected at the end of the exam. The wound site was monitored by the animals' keepers.
   vi. *Animals in foreign countries stranded alive or dead or that died during rehabilitation;*
      a. n/a
   vii. *Animals killed during legal subsistence harvests;*
      a. n/a
   viii. *Animals killed incidental to legal commercial fishing operations;*
      a. n/a
   ix. *Samples from other authorized researchers or collections;*
      a. n/a
   x. *Soft or hard parts that are sloughed, excreted, or discharged naturally.*
      a. n/a

j. *Provide a copy of the foreign collecting/capture authorization(s) (if not required, indicate "not required");*
   a. n/a

k. *If importing samples from subsistence-hunted marine mammals in foreign countries, describe the subsistence method. Include documentation, if available, that verifies that the taking was/will be conducted in a humane manner (i.e., using the method that involves the least possible degree of pain and suffering);*
   a. n/a

l. *If importing samples from live animals, describe how the samples were/will be collected, including animal handling and sample collection protocols. This should include a description of how the take was humane; and*
   a. Bears at the Toronto Zoo were sampled during routine health checks occurring between 2022-2025. Anesthesia was applied by the supervising veterinarian, then samples were collected at the end of the exam. The wound site was monitored by the animals' keepers.

m. *Describe how the specimens will be preserved, shipped, and stored/curated.*
   a. The biopsy samples will be placed into AllProtect at the time of the health check. This reagent preserves DNA, RNA, and proteins. Once preserved, the samples can be refrigerated or frozen, but are also okay at room temperature. Meta-data on bear ID, sex, location, sampling date, and collaborator will be placed into a spreadsheet for future use. Once collected, samples will be shipped to the University of Memphis if within the USA, or via FedEx to the FWS inspection facility for inspection then pick-up by Emily Puckett.

b. Hair samples will be put into coin envelopes and stored in a dark and dry environment at room temperature. Once collected, samples will be shipped to the University of Memphis if within the USA, or via FedEx to the FWS inspection facility for inspection then pick-up by Emily Puckett.

Part I- Q13- *Are you requesting to EXPORT or RE-EXPORT PARTS/SPECIMENS of/from marine mammals?*

a. The types of specimens and quantity of each to be exported/re-exported;

a. Hair samples from eight of the sampled polar bears (*Ursus maritimus*) will be exported to Japan for chemical analysis of melanin. For each animal we estimate 100-200mg of hair will be sent.

b. The complete name and address of person/facility receiving the specimen(s);

Prof. Kazumasa Wakamatsu
Fujita Health University School of Medical Science
Room 302, Building #11
Toyoake, Aichi, Japan, 470-1192

c. A description of the origin of the specimens to be exported/re-exported;

a. The specimens to be exported could be from any of the polar bears sampled during the proposed project (as described in this application).

d. The name(s) of the facility/institution that currently holds the specimens; and

a. Currently the specimens are held by collaborators at the Environment and Climate Change Canada and multiple zoo partners (Memphis Zoo, Brookfield Zoo, and Henry Vilas Zoo). This permit requests these samples be moved to the University of Memphis, which will serve as the institution that ultimately conducts the export to Japan.

e. Whether a portion of the specimen will need to be re-imported following export/re-export.

a. No. The hairs will be consumed by the procedure.

Part III- Q17- *Explain how the proposed research meets the MMPA definition of "bona fide research," i.e., scientific research on marine mammals, the results of which: (A) are likely to be accepted for publication in a referenced scientific journal; (B) are likely to contribute to the basic knowledge of marine mammal biology or ecology; or (C) are likely to identify, evaluate, or resolve conservation problems.*

Our research will identify the genetic basis for three polar bear adaptations to its harsh Arctic environment. These traits include their white hair coloration, black skin coloration, and structural changes to their hair which make them air filled. Our general workflow is to identify potential causative variants using genomic or transcriptomic data. Understand differential protein abundance or RNA expression in the hair or skin, respectively, then demonstrate an evolutionary change. Then conduct experiments to functionally verify the variant, or elucidate the function of the adaptation. Our team includes evolutionary biologists, skin biologists, physicists, and computer engineers. Each team member (see attached resumes) has a record of publishing their results and supporting their labs with grants (for which the granting agencies also expect a publication record). Given our preliminary data, success using these techniques in past research with similar questions, and high public and scientific interest in our questions, we think this will be an incredibly successful project leading to at least six publications on the mechanisms of thermoregulatory adaptations within polar bears.

Our project will contribute to basic knowledge in two ways. First, we have preliminary data that the causative locus for two of our traits of interest are formed from complex structural variants. Our work will create new knowledge on how, mechanistically, structural variants alter gene expression and thus

phenotype. Second, expansion of the hair center, medulla, to the point of hollowing out this structure via cell collapse is a convergent trait in Arctic mammals. The trait is found in multiple different orders including the rabbit, deer, and bear families. Our work identifying how this occurs in bears will offer approaches and causative loci that either we or others can investigate in the future to understand convergent evolution. Beyond contributing to basic knowledge, we will create new long-read genomes with structural variants identified as part of our project. These resources will be publicly available for broader use in the research community.

We believe our results will inspire the public to care about polar bears and their conservation due to their singular evolutionary history. As part of our research, we will do online and in-person science communication around the project. A core focus of this communication will include information on polar bear habitat and conservation.

Part III- Q18- *Provide a detailed description of the proposed project. You may attach a formal research proposal, provided it includes all the requested information, including:*

a. Objectives and hypotheses and associated methodology;

Our objective is to link the genetic architecture of each polar bear trait (white hair, black skin, and air-filled hair) to its cellular and molecular pathology and then to the species evolutionary history.

Hypotheses related to pigmentation

Our hypotheses are not mutually exclusive, as we will investigate the hair and skin color traits together.

H_1- Melanocytes are preferentially located in the skin instead of hair. Thus, the absence of melanocytes would result in white fur due to the lack of pigment producing cells within hair follicles.

H_2- Melanogenesis is downregulated or impaired. Melanocytes present within the hair bulb would produce a reduced or absent amount of melanin.

H_3- Pigment type switching from eumelanin to pheomelanin results in phenotypic change. Melanin is deposited, but it is the red/yellow form instead of the black/brown form observed in closely related bear species.

Pigmentation Methods

We will section biopsies to understand where pigment is deposited, then compare among American black (*U. americanus*), brown (*U. arctos*), and polar bears (*U. maritimus*). We will use immunofluorescence to quantify two markers that denote the distribution of melanocytes between bear species: MITF and KITLG. To understand the molecular mechanism underlying differential melanocyte migration in polar bears, we will utilize RNA-sequencing. We will compare transcript abundance while remaining phylogeny aware of the three taxa. We are specifically interested in highly or lowly expressed transcripts in polar bears compared to Am. black and brown bears. Any hits will be further investigated for coding sequence or surrounding structural variants that are derived in polar bears. For the best resolution, we will create new long-read genomes with structural variants identified, then map reads to this multi-species reference genome.

We expect the above experiments to show differential proteins or expression of a candidate locus we identified for the hair color phenotype. We will specifically work to understand how the variant changes RNA expression by sequencing isoforms of the gene from each of the three bear species. We expect that polar bears will have unique isoforms that are caused due to splicing differences between the untranslated regions and exons of the candidate gene.

We will export hair samples of each species to Japan for quantitative analysis of eu- and pheo-melanin. This analysis will support/refute our hypothesis and preliminary data that polar bears deposit melanin in their hair.

Hair Structural Alterations Methods

H_4- Alterations in structural proteins disrupt cell cohesion. Specifically, cells within the medulla are formed then collapse due to misfolding or weak cross linking. We will compare protein abundance among bear species and phenotypes to identify differential abundance, then identify genomic lesions around candidate genes.

Hair Proteomics- The gross structural differences between hairs with and without structural defects suggests that protein composition will vary between the phenotypes. We will quantify this variation using proteomics. For each species, we will analyze the hair proteome of 10-20 individuals. We will separate out awn type hairs as they are numerous in our samples, and dissect out a section approximately 4cm long that neither includes the root nor tip, thereby focusing on the structural region of the hair. Three awn hair sections will be pooled for each bear. Hairs will be digested with a specialized protocol for mammalian hair (Goecker *et al*, 2021). Extracts are trypsin digested prior to ionization on a liquid chromatography-mass spectrophotometer (LC-MS/MS).

The peptide sequence data will be analyzed by comparing the sequences to a protein sequence database within the software MaxQuant (Tyanova *et al*, 2016). UniProtKB has entries for both polar and Am. black bears; therefore, we will add a reference database for brown bears. We will control for false discovery rate through target-decoy search using ProteinProphet (Nesvizhskii *et al*, 2003). We will compare the relative abundance of proteins across species.

Histology and Immunofluorescence- We will examine the structure of anagen hair follicles using histochemical approaches, techniques we have used to evaluate follicle development in other mammals (Fitch *et al*, 2003; Imsland *et al*, 2016; Kaelin *et al*, 2012). Morphometry will be used to measure the thickness and cell density of the hair follicle layers (medulla, cortex, cuticle, inner root sheath, outer root sheath) on hemotoxylin and eosin-stained sections from bear skin biopsies. The structural integrity of the hair bulb layers (medulla, cortex, and cuticle) that give rise to the hair shaft will be examined. Evidence of keratinocyte cytolysis within a subset of follicular progenitors will highlight the affected cell type and point to a list of candidate genes that underlie the air-filled hair phenotype. There is precedent for our approach in human patients with basal keratinocyte lysis and the blistering disease, epidermolysis bullosa simplex (Bolling *et al*, 2011), while mutations in the suprabasal keratins, induce lysis in a more superficial epidermal cell and lead to epidermolytic hyperkeratosis (Chamcheu *et al*, 2011). In parallel, we will use immunohistochemistry to interrogate the development and differentiation of the hair bulb layers. Antisera specific to medullary (AE15/trichohyalin or KRT6/16 antibodies) or cortex and cuticle (AE13/pan-keratin) progenitors will be applied to thin sections of bear skin containing anagen hair follicles.

b. Background information discussing relevant published literature on the subject of your proposal, with citations;

Polar bears are an iconic Arctic mammal which garner significant scientific research into their ecology and evolution. Their ecology, and particularly their use of summer sea-ice for hunting blubber-rich seals, has made them an indicator species for the impacts of climate change, especially within the vulnerable Arctic (Stirling and Derocher, 2012). Their evolution is of

interest due to the multiple and multi-faceted adaptations they evolved to live in harsh Arctic conditions. These adaptations arose rapidly in evolutionary time, as divergence from brown bears occurred 340-480 kya (Liu *et al*, 2014) and stable isotype analyses from a 110 kya jawbone (Lindqvist *et al*, 2010) estimates they were fully marine mammals by that time. Notably since divergence between polar and brown bears, there were two admixture pulses moving polar bear alleles into brown bears. The first occurred ~100kya; and the second, 20-40kya, affected North American populations more than European brown bears (Wang *et al*, 2022).

While the physiological, morphological, and behavioral adaptations of polar bears are well described, few genomic signatures associated with these changes have been identified (Liu *et al*, 2014; Rinker *et al*, 2019; Samaniego Castruita *et al*, 2020), and none functionally verified. Genes under positive selection in polar bears included functions in lipid metabolism, cardiovascular function, and coat color (Liu *et al*, 2014; Miller *et al*, 2012). Further analysis showed that selection on standing variation was a strong driver of evolution in polar bears, although *de novo* mutations were also positively selected (Samaniego Castruita *et al*, 2020). Of interest in this proposal are three skin and hair traits that contribute to thermoregulation: white hair, black skin, and air-filled hollow hair. Trait mapping to the bear phylogeny shows that each of these is a derived trait in polar bears, as most species have dark hair, pink skin, and intact hairs. Notably, the white and hollow hair traits have evolved in other taxa adapted to Arctic environments; thus, our work to understand the molecular and cellular mechanisms of these traits will provide a starting point to investigate convergence.

c. An explanation of how this study is different from, builds upon, or duplicates past research;

This study is novel with regard to understanding the molecular basis of specific Arctic adaptations in polar bears. However, our work takes advantage of the well-constructed molecular pathways described in humans to hypothesize where we may identify candidate targets of molecular evolution. The hypotheses related to hair and skin coloration build upon an extensive understanding of the pigmentation pathway that has been identified in humans and manipulated within mice. The hypothesis related to structural hair variation again uses our understanding of keratin deposition in humans, as well as skin and hair diseases arising from alterations in keratins, as a framework for how polar bears evolved altered hair structure. Previous work to understand adaptations in polar bears has focused on using genetic sequence analysis and/or dN/dS to identify proteins with an abundance of amino acid changes, then associate those proteins to functional traits. We take an opposite approach, starting with the traits of interest, then using an evolutionary comparative approach and direct measurements of gene expression or protein abundance in tissues to identify the relevant genes causing these adaptive traits. While identifying the specific alleles or mutations is a goal, our work is not predicated on this level of DNA interrogation, and allows for more than amino acid altering alleles to be candidates. Our work will contribute to greater understanding of regulatory variation in polar bears, enabling novel inference beyond the three specific traits.

d. An explanation of how you determined your sample size/take numbers (e.g., based on previous encounter rates or abundance estimates for the study area). If appropriate for your study, include a power analysis or other sample size estimation to show whether the sample size is sufficient to provide statistically significant or otherwise robust results appropriate for your study;

Since we are receiving biopsies opportunistically, we are unable to control the timing of hair growth. The ideal experiment would have us pluck a patch of hair, then sample as the hair began to grow back so that the RNA expression profile of the hair follicle would represent the active growth, aka- anagen, phase. However, that is not conducive to opportunistic sampling and would

result in more stress on each animal as two anesthesias would be required. Instead, we are asking for a slightly larger number of samples, so that we may first assess the proportion of hairs in the anagen phase and remove samples that are not actively growing hair at the time of sampling. Ultimately, we plan to conduct the RNA-sequencing study on six samples. However, the higher number will ensure we have six quality samples in case some are neither in the correct phase and/or were not properly preserved in the AllProtect.

For hair samples - The sensitive and somewhat inconsistent nature of MS-LC-LC on hair fibers necessitates a higher sample size. We have been advised by Dr. Glendon Parker, a mammalian hair proteome expert and project collaborator, that we will need 20 samples from each of the experimental groups we want to investigate. Thus, this sample size was informed by expert opinion. Additional hair volumes that will not be consumed in the analysis will either be used for HPLC analysis or measured for physical properties such as thermal conductance and tensile strength.

e. If proposing novel procedures, include a discussion on results from pilot studies or studies on other species, if available; and

We are using a number of standard procedures including DNA sequencing, RNA sequencing, Mass Spectrometry with Liquid Chromatography (for proteomics), and immunofluorescence.

f. Disposition of animals or remaining specimen material once your project is complete.

Any remaining biopsy or hair samples will be stored in the Puckett Lab for the duration of the PI's career. Opportunities to use left-over skin tissue, hair, or DNA will be utilized with appropriate approvals to expand the scientific inference we can make with these samples and prevent additional take requests.

Part III- Q19- *Provide the expected research schedule (clearly specify the proposed start date and end date of your research or field season(s) and overall duration of the project). Include the months of the year and frequency of field work/sampling (e.g., number of times per year). If your research extends beyond five years, or is a continuation of previously authorized research, give information about when the research began and when you expect it to end.*

Due to the cost of our research, our start date is contingent upon grant funding. At present (November 2024), we have a proposal in review at the US National Science Foundation. Assuming a positive decision on the current proposal, the earliest we could have funding would be March 2025.

We estimate that the total project work will take four years to complete. We have already begun collecting American black and brown bear samples for this project, and lined up collaborators to obtain the polar bear samples. Our Canadian collaborator, Evan Richardson has a permit to collect polar bear skin and fat biopsies for heavy metals analysis. He has used his permit to collect additional biopsies for this project (see Part I, Q12 response). Thus, once approved by US and Canadian governments, we would be able to ship and obtain the needed polar bear samples to begin project work. We estimate that shipping could occur in July to Oct 2025.

We expect the hair and skin pigmentation questions to require ~3 years of data collection, analysis, and writing. We expect the investigation into the molecular basis and physical properties of the hollow hair trait to take ~4 years. Thus, a reasonable end date to the project would be December 2028, assuming the earliest start date possible.

Part III- Q20- *Indicate which research procedures/activities you will be conducting that will or might result in* ***TAKE or HARASSMENT of TARGET species****, and describe each activity in detail, including the information indicated in a-i, below.*

As detailed in the research plan, I am not personally collecting samples but relying on 1) Canadian researchers handling and sampling wild bears and 2) zoo-housed bears sampled by veterinarians. No additional wild bears will be handled / sampled specifically for this study and the additional samples from captive-held bears will be collected during other, routine, veterinary work.

For captive held bears:

f. Intrusive sampling (e.g., blood, blubber, muscle, skin); include i-xiii below, in your activity description:

i. Will sampling be remote or under restraint?
Biopsy sampling will occur when the bear is already anesthetized for other purposes.

ii. Will local anesthetics be administered?
Individuals will be under a general anesthetic under the direction of a veterinarian during the sampling procedure.

iii. Type of tissues sampled;
Skin.

iv. Size or volume of sample (diameter and depth or total volume);
Three x 5mm skin biopsies from each animal.

v. Target sampling location on body;
Rump area.

vi. Maximum number of samples per animal per day and per year;
Each bear would only be sampled once.

vii. Sampling intervals (e.g., for serial blood or biopsy samples);
N/A

viii. Collection method and equipment/materials used (e.g., dart fired from rifle, dart depth, sterilization/disinfection);
Skin biopsy punch.

ix. If remote, what is the minimum approach distance?
N/A

x. If restrained, describe treatment of site of sample collection (e.g., cleansing, wound left open or closed);
A dissolvable stich maybe used at the attending veterinarian's discretion.

xi. Number of attempts per animal per day (include total number of attempts needed for all work if requesting multiple procedures (e.g., remote tagging and biopsy) on same animal on the same day);
N/A

xii. The names of the personnel who will conduct the sampling; and
The attending veterinarian at each of the zoo's where the sampled polar bears are housed.

xiii. Sample preservation and analysis.
Each biopsy will be submerged in an individual tube of AllProtect within a 2mL cryotube. This reagent preserves DNA, RNA, and proteins. Once preserved, the samples can be refrigerated or frozen but are also okay at room temperature. Analysis will be conducted as described in these supplemental materials.

21. For each procedure/activity, provide the information in a-j, below, including the maximum number of animals of each species expected to be taken by the procedure annually, broken down by sex and age class; the number of takes per animal per year; and an estimate of the number of animals of the study species that might be incidentally harassed (i.e.,# of non-target animals of your study species that might be harassed by your activities). Also, include the time periods and specific locations of the takes.

Procedure	Level A or B	Age Class	Sex	Max. # Animals Per Year	Max. # Takes Per Animal Per Year	Max. non-target conspecifics incidentally harassed
Skin biopsy and hair^ collection (zoo held)	N/A	Adult	Both	9	1	0
Skin biopsy and hair collection (wild)	Level A (however, sampled during other procedures)	Adult	Both	7	1	Unknown*

*These samples have already been collected from wild polar bears in Canada during other research activities.
^These samples will be collected as shed hair swept from individual animals' enclosures.

23. Define each age class listed in your response to question 21(d), above, for each species (i.e., list the range of months or years (or mass for otters) constituting each age class); provide the minimum age (or mass) that animals will be targeted for take activities; and indicate whether females with calves/pups/cubs less than that minimum age will be targeted for take activities?

The ages of captive held animals are provided in Table R1. Wild adult bears are >4 years old.

24. Describe the precautions that will be taken to minimize the likelihood that harassment of non-target individuals of the study species will occur and the actions that will be taken should harassment occur.

The majority of sampled will be obtained from captive bears so no harassment of non-target bears will occur. Other samples will be obtained from bears which are already being captured and the proposed project will not result in additional harassment of non-target individuals.

25. Explain how you determined that your methods involve the least possible degree of pain and suffering and why there are no feasible alternative methods to obtain the desired data or results.

The proposed research requires these types of samples, in the numbers requested, to provide scientifically robust data. Samples are being collected during other procedures, while the bears are under anesthetic which avoids additional pain or suffering.

26. Provide: a) an estimate of the possible number of unintentional deaths or serious injuries that might result from your research activities; b) the number of unintentional and intentional (via euthanasia for humane purposes if an animal is seriously injured) deaths or serious injuries you seek approval for annually; c) the steps you will take to reduce the likelihood of deaths or injuries; and d) if euthanasia might occur, provide the method of euthanasia (e.g., gunshot, drug, etc.) and who would conduct the euthanasia procedure.

No unintentional deaths or serious injuries are expected to result from the proposed research activities.

29. If biological samples are to be collected or received domestically, provide responses to a through j, below, for each individual animal per species. This information, or part of the information, may be provided in table format such as the table below.

This information is provided in Table R2; in response to Q.21; and in response to other questions above.

30. Provide a list of all personnel that will be involved in the project, identifying each as either a principal investigator or co-investigator, their project duties/responsibilities, and a brief description or CV that demonstrates their experience and expertise to perform their designated duties, including knowledge of the marine mammal species that is/are the subject of this application.

See CVs provided.

31. Describe how you will collaborate or coordinate with other researchers in your study area. Who are they? Explain how this will occur and how it will minimize negative impacts on the species. For example, will it involve sharing resources, samples or data; timing surveys to minimize disturbance, etc.?

As described in this application, we are collaborating with zoos who hold captive bears and with scientists conducting on-going research in Canada to obtain samples. This ensures that negative impacts to individuals and the species are minimized.

32. If you intend to conduct research on animals in a captive-holding facility such as a zoo or aquarium, provide documentation showing that the facility(ies) has authorized you to conduct your proposed activities.

All the zoos from which we are obtaining samples from captive bears are licensed under the AWA. No research will be conducted at the facilities.

33. Animal Welfare Act (AWA) Compliance (for research on live animals only): AWA requirements apply to all research facilities, which include institutions, organizations, or people that use or intend to use LIVE animals in research, tests, or experiments; AND, that receive funds under a grant, award, loan, or contract from a department, agency, or instrumentality of the U.S. for the purpose of carrying out research, tests, or experiments, or acquires or transports the animals in commerce. Provide the following documentation:

a. Registration under the AWA as a research facility:
i. Attach a copy of your APHIS certificate of registration as a research facility, or for Federal facilities, a letter from your Institutional Officer that you are compliant with applicable requirements for scientific research under the AWA; OR
ii. If your facility does/will not conduct activities requiring registration under the AWA, attach a letter from APHIS confirming that registration is not required.

b. Institutional Animal Care and Use Committee (IACUC) documentation: If your facility is registered as a research facility under the AWA or is a Federal research facility (see a.i), attach the applicable IACUC documentation from the list in i-iii, below. Please note that all activities that involve an invasive procedure, harm, or materially alter the behavior of an animal under study, even if the activities are carried out in the field, are subject to IACUC review and approval. See (AWA regulations and standards for definition/explanation of covered research activities.):
i. Attach a copy of your final protocols with the IACUC signed approval; OR
ii. Attach a copy of your proposed protocols to be reviewed by your IACUC along with an explanation as to how and when the protocols will be reviewed (Note: A copy of your final signed protocols and certification will be required prior to permit issuance.); OR
iii. Attach the IACUC determination that your research activities are not subject to IACUC review and approval.

c. If your facility is not registered as a research facility under the AWA, please provide an explanation of how your take activities are reviewed and monitored to assure that the proposed takes are humane (i.e., using the method that involves the least possible degree of pain and suffering).

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Manitoba

Wildlife Branch
Box 24 – 14 Fultz Boulevard
Winnipeg MB R3Y 0L6

August 4, 2023

Dr. Evan Richardson
Environment and Climate Change Canada
Unit 510 – 234 Donald Street
Winnipeg, MB R3C 1M8

Dear Dr. Evan Richardson:

As requested in your July 7, 2023 email, enclosed you will find Species at Risk/Wildlife Scientific Permit **SAR23008** authorizing research on Polar Bears (*Ursus maritimus*). Please sign the permit to indicate that you understand the conditions therein.

This permit will expire on March 31, 2026. You are required to submit an annual report that details the activities carried out under authority of this permit, including the number, sex and use of specimens marked or collected and the origin by marking or collecting site, and the results or accomplishments achieved. This report must be received no later than March 31st by the Director, Wildlife Branch. You will also note that the permit conditions require you to notify the local Conservation Officer in Churchill and Gillam prior to commencing field operations.

No wild animal or part thereof may be exported from Manitoba without first obtaining an export permit. Upon completion of your field activities, an export permit must be obtained from one of our regional offices prior to your departure from Manitoba.

Please call Tim Poole, phone 204-803-1523, or email timothy.poole@gov.mb.ca, if you have any questions or if we can be of further assistance.

Yours truly,

Maria Arlt
Director

Manitoba

SAR23008 SPECIES AT RISK/WILDLIFE SCIENTIFIC PERMIT

Subject to the provisions of The Endangered Species and Ecosystems Act, The Wildlife Act, the regulations made thereunder and the conditions set out in this permit:

Permit Holder: Dr. Evan Richardson
Environment and Climate Change Canada
Unit 510 – 234 Donald Street
Winnipeg, MB R3C 1M8

Is hereby authorised to conduct scientific research on the following wildlife or wildlife habitat:

Species: Polar Bear (*Ursus maritimus*)

At, within or on the following location:

Location: Thompson Compliance area sub-districts of Churchill and Gillam including Churchill and Kaskatamagan Wildlife Management Areas

Conditions:

1	Research is limited to the activities described in the original application, proposal, or an amendment thereto, that has been approved by the Director, Wildlife Branch, which form part of this permit. Where there is a difference between the application, proposal or amendment and this permit, this permit prevails.
2	The authority granted by this permit is limited to (i) an employee or subcontractor of the permit holder while engaged in the duties approved or required by the permit holder, or (ii) an associate, or a person supervising or working under the supervision of the permit holder. The permit holder shall provide a person under subclause (ii), when working alone, with a counter-signed and dated photocopy of this permit.
3	Prior to undertaking any field activity, the permit holder shall notify the Conservation Officer in charge of Churchill Sub-District (204-675-8897).
4	The permit holder shall follow recognized protocols and Canadian Council of Animal Care (CCAC) guidelines during the collection, capture, possession, euthanizing or release of any species named herein.
5	The permit holder is authorized annually to: 1) capture up to a maximum of 100 individual polar bears of either sex during the fall season (August 20 – September 30); 2) hold, examine, perform morphological and physiological measurements, and collect body fluid and tissue samples including premolar teeth, 3) mark with ear-tags, lip tattoos and non-permanent dyes/paints; and 4) place GPS, satellite-linked collars on up to 10 adult female bears (selected from the 100 adult animals captured as part of this condition). All individuals are to be released upon completion of measurements and marking at the site of capture. In addition, up to a maximum of 15 family groups may be captured, held, measured, marked, have samples collected, and be released, in the spring season (February 15 – March 31). No bear shall be captured more than once per fall or spring field season, unless special circumstances warrant such action. Captures must not take place between the Nelson River east to the Manitoba/Ontario border.
6	The permit holder shall make a reasonable attempt to visually confirm that family groups are still together 24 hrs after being captured and handled, if continuing field research in the area where the capture occurred.
7	The permit holder shall discontinue chasing a polar bear that shows signs of distress, overheating or injury, unless capture is imminent, and shall not pursue a polar bear for longer than 3 minutes. When a pursuit is discontinued, the polar bear shall not be pursued again for 24 hours.
8	The permit holder shall provide to the Regional Wildlife Manager, the transmitter serial number; transmitter frequency and the animal identification X-number for each bear handled and outfitted with a satellite collar.
9	The permit holder is also authorized annually to collect biopsy samples from an additional 195 individual polar bears of either sex during the fall season through the use of biopsy darts fired from a helicopter.
10	The permit holder will submit annually (no later than March 31) to the Director, Wildlife Branch, a written report describing the activities carried out under authority of this permit, including the number, sex and use of specimens marked or collected and the origin by marking or collecting site, and the results or accomplishments achieved.
11	The permit holder shall endeavour to inform the local communities as to the purpose and scope of research activities, and the results achieved, through newsletters, community newspaper articles, presentations to interested groups, annual public meetings or other recognized methods of engaging the public.

12	If a bear that is involved in the research that is authorized herein dies, the permit holder shall a) report the death immediately to the Conservation Officer in the Churchill Sub-District (204-675-8897); b) within 48 hours, at the expense of the permit holder, have the bear transported to Building D-20, Churchill, or the Gillam Conservation Office, for salvage, biological collection or such other purpose as may be deemed appropriate by the Director, Wildlife Branch unless otherwise directed or exempted in writing by the Director; and c) within 48 hours, submit a written report to the Director, Wildlife Branch detailing the permit holder's interaction with the bear, suspected or known cause of death and recommendations or steps that are being implemented to prevent such further occurrences unless otherwise directed or exempted in writing by the Director.
13	This permit is not valid a) in an ecological reserve, provincial park, special conservation area or a wildlife refuge unless otherwise approved in writing by the Director, Parks Branch, or the Director, Wildlife Branch, respectively; b) on private land without permission of the owner or lawful occupant; or c) in a National Park without written authorization from Parks Canada.
14	The Government of Manitoba shall not be held responsible or liable for any damage, injury or loss sustained to the person or property of the permit holder or for any damage, injury or loss sustained by any other person or the property of any other person as a result of the exercise of a right or privilege granted herein.
15	This permit may be cancelled or the conditions amended at any time.
16	The exercise, by the permit holder, of a right or privilege granted herein shall be construed as acceptance of and agreement to comply with the conditions set out herein.

Date Issued: August 4, 2023	**Expiry Date:** March 31, 2026
Signature of Permit Holder: Eileen Richardson	**Issued By:** For Minister of Natural Resources and Northern Development

FORM REVISED: MAY 2002

Parks Canada Agency
Research and Collection Permit
(Not Transferable)

Permit Number:	WAPNP-2022-45543
Fieldwork Start Date:	2023-08-15
Fieldwork End Date/Expiry Date:	2026-03-31

Project title: The ecology, population dynamics, and status of polar bears (Ursus maritimus) in relation to environmental change.

Principal Investigator Name: Richardson, Evan (Dr.)

Address: Wildlife Research Division
Science & Technology Branch
Environment and Climate Change Canada
150 – 123 Main Street
Winnipeg, Manitoba R3C 4W2

Telephone: 780-863-5250

E-mail: evan.richardson@ec.gc.ca

Affiliation: Environment and Climate Change Canada

Is hereby authorized to conduct the research project entitled "**The ecology, population dynamics, and status of polar bears (Ursus maritimus) in relation to environmental change.**", Research and Collection Permit Application Number "**56011**", in **Wapusk National Park of Canada**, subject to the terms and conditions set out below and/or attached to and forming part of this Research and Collection Permit.

Members of the Research Team:

Derocher, A. (Dr.)
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Acts, Regulations and Terms and Conditions

Permit issued pursuant to:

National Parks General Regulations: Subsection(s) 7(5), 11(1), 14(2)
National Historic Parks General Regulations: Subsection(s) 3(2), 4(2), 12(3)
National Parks Wildlife Regulations: Paragraph(s) 15(1)(a)
National Historic Parks Wildlife and Domestic Animals Regulations: Subsection(s) 5(1)
Federal Real Property Regulations: Subsection 4(2)

General Terms and Conditions:

Failure to comply with the applicable Heritage Area regulations or the terms and conditions of the permit may constitute grounds to cancel or suspend the permit, refuse to issue future permits, and may be considered as grounds for prosecution under the applicable Act(s) or Regulation(s).

Permit:

All permit holders must be in possession of a valid permit before the fieldwork commences and at other periods as stated on the permit.

Permits are not transferable and each member of the fieldwork team must have a copy of the valid permit in their possession.

The permit is valid only for the geographic location, the period, the activities, and under the terms and conditions described on the permit, unless amended and revalidated by the Superintendent.

Restrictions:

The Superintendent may suspend, cancel, or restrict the scope of the permit.

The permit shall cease to be valid if the fieldwork is not started within six months of the date of issue.

Other Acts and Regulations:

The Principal Investigator must abide by applicable regulations and all other federal, provincial, territorial or municipal regulations applying to the Heritage Area or the Research and Collection Permit.

The Principal Investigator and any team member will identify themselves and show a valid Research and Collection Permit when requested by the Superintendent, an authorized Heritage Area staff member, fisheries officer, Indigenous guardian or a police constable.

Principal Investigator's Responsibilities:

Any damage resulting from a Principal Investigator's activities, or those of their team, shall be reported promptly to the Superintendent. Principal Investigators will be financially responsible for any damage resulting from their activities or those of their team.

A site, or site component(s) that has been excavated or disturbed shall be restored or conserved by the Principal Investigator to the satisfaction of the Superintendent.

The Principal Investigator must advise the Research Coordinator of any adjustments in work location, research plan and methodology, implementation schedule, or main personnel, among others, during the course of the research project.

Principal Investigators working in a Heritage Area are required, as a condition of their permit, to submit, unless otherwise negotiated:

a) A report of progress sixty (60) days following the completion of the fieldwork, unless otherwise agreed with the Research Coordinator;

b) An Investigator's Annual Report (IAR) within one year of signing the permit. In the case of a multi-year permit, the principal investigator will submit an IAR for each year of the research project; and,

c) A final report, in electronic or hard copy form no later than eight (8) months following the completion of the fieldwork, unless otherwise agreed with the Research Coordinator.

The reporting requirements above do not replace any reporting requirements set out in any contract between Parks Canada and the Principal Investigator.

The Principal Investigator will be responsible for all members of their team. All team members must observe all terms and general and specific conditions of the Research and Collection Permit.

The Principal Investigator, and their team, shall at all times indemnify and save harmless the Crown (Canada) from and against all claims, demands, loss, costs, damages, actions, suits, or other proceedings, by whosoever made, sustained, brought or prosecuted, in any manner based upon, occasioned by, or attributable to, anything done or omitted by the Principal Investigator or the project personnel in the fulfillment or purported fulfillment of any of the terms and conditions of the permit. This does not apply to Principal Investigators or permit holders who are employees of Parks Canada.

General Conditions Governing Natural Science Research:

Any natural objects collected under authority of this permit remain the property of the Crown (Canada) and are considered on loan to the permit holder. Final disposition of natural objects must be described in the Project Proposal Form unless amended by the Superintendent. Export of natural objects or specimens requires approval by the Superintendent and is subject to the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), the *Cultural Property Export and Import* Act and the *Export and Import Permits Act*. Intention to export specimens must be indicated in the Project Proposal Form.

Only the natural objects or categories of natural objects indicated on the permit may be collected.

A detailed inventory of material collected will be provided to the Research Coordinator prior to its removal from the Heritage Area by the Principal Investigator.

Fossils or evidence of previous human occupation must be immediately reported to the Superintendent and must be left undisturbed upon discovery until they may be inspected by a Parks Canada palaeontologist or archaeologist.

Specific Conditions:

The following conditions apply to Research & Collection Permits issued for Wapusk National Park and are in addition to those outlined above.

OTHER REGULATIONS

1. Any aboriginal staff employed by the research project that is entitled to exercise Aboriginal or treaty rights in the Province of Manitoba shall not exercise these rights while engaged in research activities in the park.
2. Designated persons to use firearms must have a valid firearm permit.
3. If transportation in the park involves the use of a snowmobile, a valid over-snow permit is required.
4. The Principal Investigator will ensure that all party members understand and comply with the National Park Act and Regulations.
5. Location coordinates of any unexploded ordinance shall be reported within 48 hours to Wapusk National Park Resource Conservation Manager.

The Principal Investigator shall:

6. Provide a copy of the current permit from the Canadian Wildlife Service for authorizing the disturbance of migratory birds if applicable.
7. Provide a copy of the Complete and Approved Protocol Letter from the Animal Care Committee to the research coordinator, if applicable.
8. Provide the names of the members of the research team to the research coordinator prior to commencing field work.

FLYING

9. When engaged in work related to scientific research, flight operations must be in accordance with established protocols for those applicable species. Researchers must provide Parks Canada with anticipated flight requirements (i.e. range of dates, locations, and elevations) associated with their project(s). When research is not being conducted, researchers are expected

to comply with the Standards for Aircraft Flights over Wapusk National Park.

FUEL

10. Approval from Wapusk National Park must be obtained prior to the commencement of field activities for fuel caching. The location, amount, and the type of fuel at each cache are to be outlined in the Fuel Cache Request Form, which will be provided by request from the research coordinator.
11. All fuel caches and empty drums are to be clearly and indelibly marked with the project leads name, fuel type, and year the fuel was drummed/cached.
12. All empty drums and unusable fuel are to be removed from the park by the end of the field season year unless an alternate date is approved in writing. Failure to remove empty drums and unusable fuel may result in the cancellation of this research permit, refusal to approve future research permits and/or the researcher being held responsible for the costs of removing the empty drums and unusable fuel.

WASTE

13. All garbage is to be removed from the park in accordance with the National Park Regulations.
14. Human waste: in field camp situations, human waste is to be collected in sealable metal/plastic containers or sealable bags. The project leader is responsible for ensuring that all containers of human waste are removed from the park for disposal at an appropriate facility. The cost of this disposal is the project leader's responsibility.

POLAR BEAR OCCURRENCES OR ENCOUNTERS

15. A polar bear safety plan must be in place prior to commencing field activities. All members of the research team must be familiar with the polar bear safety plan.
16. All polar bear deterrent actions must be in place prior to commencing field activities. All members of the research team must be familiar with the use of these deterrents.
17. Any polar bear occurrence, especially one that results in the injury/death of the polar bear or human, must be reported immediately to Wapusk National Park Resource Conservation Manager.
18. Location coordinates of any polar bear found dead shall be reported within 12 hours to Wapusk National Park Resource Conservation Manager.
19. Under the authority of the Manitoba Wildlife Act, no wild animal or part thereof may be exported from Manitoba without first obtaining an export permit.
20. A summary report related to polar bears along with completed data sheets are to be submitted to the research coordinator by December 31st of each year.
The information that must be included is:
a. Names of people working on the project and dates as well as locations spent in Wapusk NP.
b. Daily summary of number of bears encounters and occurrences (important to note days where zero bears were encountered).
c. Number of polar bear encounters (observations) and occurrences while conducting work on the ground and completion of the Parks Canada Polar Bear Encounter / Occurrence Form for each occurrence (see definition below).

DEFINITION OF A POLAR BEAR OCCURRENCE

Any situation where deterrent action is taken, including:
• a barrier is challenged (i.e. charging an electric fence, barrier fence, vehicle or pushing on a door / window to gain entry); or
• a barrier is breached (i.e. a bear has gained entry to an area, fence, building or vehicle); or
• a bear actively investigates humans or camps in a determined manner; or
• a bear approaches an individual or group, either out of curiosity or aggressively, and a deterrent is required to stop the bear's advance; or
• a bear physically attacks an individual causing injury or death; or
• significant property damage has occurred

DEFINITION OF A POLAR BEAR ENCOUNTER (OBSERVATION)

Any situation where bear(s) and human(s) come into proximity and there is no interaction between
them. No deterrent action occurs in an encounter. Examples include:
• a bear is seen in the vicinity and is observed to change its direction of movement to avoid the human(s), building, vehicle; or
• a bear or human come upon the other suddenly and the bear immediately leaves the area to avoid contact with the human; or
• a bear is in the vicinity of human(s), but it remains stationary and poses no threat to the Human (e.g. A bear resting near a fenced camp)

CULTURAL RESOURCES

21. If a cultural site is encountered, the Principal Investigator or member of the group shall record the site through digital photographs; GPS location coordinates; and notes (when possible) and report the find to the Park Superintendent and Cultural Resource Management Advisor (sandra.hollender@canada.ca) upon return from the Park.
22. The Principal Investigator and/or any member of their team shall not remove, disturb or displace any cultural site. All cultural sites and objects there within remain the custodial responsibility of the Crown.
23. Should a member of the Principal Investigator's team disturb a cultural site, the Principal Investigator shall immediately cease all activity in progress at the location and contact the
Park Superintendent or Cultural Resources Management Advisor. The Principal Investigator shall only resume activity when permitted to proceed with the authorization of Parks Canada.
24. Where human remains and/or funerary objects are accidentally encountered, the activities in progress at the location must be suspended immediately and the Superintendent notified. The Principal Investigator will await further direction from the Superintendent.
25. The Principal Investigator and/or any member of their team shall not divulge the finding of cultural sites or share photographs unless it is with the express permission of the Park Superintendent.

DEFINITION OF A CULTURAL SITE

Any object, grouping of objects, place, or evidence of past human occupation that may be associated with an aspect of human history and culture within Wapusk National Park.

COMMUNICATION

The permit holder shall endeavour to undertake in person engagement in the communities represented on the Wapusk Management Board and seek opportunities to engage youth in research and understanding of polar bear science during the duration of this permit.

REPORTING

By December 31st of each year, the Principle Investigator is responsible for providing the research coordinator with:

26. A two-page summary of the project (progress report).
27. Five digital photographs that can be used by Parks Canada for presentations, publications and website/social media content. Please include photo citation information such as Location, Date and Photographer. Photos must be in JPEG format with a resolution range of 150 – 300 dpi. The photographer will be given credit for all photos taken.
28. Spatial data collected as part of this project.

Parks Canada staff will follow-up directly with each researcher to determine the data format and discuss data any potential sharing issues. Annual Progress Report templates are available upon request from the research coordinator.

29. Where appropriate, Parks Canada, Wapusk National Park, will be referenced/acknowledged.
30. Data files will be provided to Parks Canada upon request.

TIMELINE FOR DELIVERABLES

December 31 of each year
- Two-Page Summary Report (progress report)
- Five Digital Photos
- Spatial Data
- Polar Bear Summary Report and Data Sheets (see section 20)

March 31 of year after permit expires
- Final Report

Principal Investigator Signature

I, **Richardson, Evan (Dr.)**, the Principal Investigator, accept all the stated Research and Collection Permit terms and conditions.

Evan Richardson	Evan Richardson	2023-08-11
Name	Signature	yyyy-mm-dd

Permit issued and approved by:

Superintendent Name	Superintendent Signature	yyyy-mm-dd

Parks Canada Contact

Wapusk National Park of Canada
Churchill, Manitoba

LeeAnn Fishback
Resource Conservation Manager
LeeAnn.Fishback@pc.gc.ca
204.675.8863

Matthew Webb
Research Coordinator
Matthew.Webb@pc.gc.ca
613.862.4826

1 Mantayo Seepee Meskanow
Churchill, Manitoba
R0B 0E0

Emily E. Puckett

Department of Biological Sciences
333 Ellington Hall
University of Memphis
Memphis, TN 38152

Emily.Puckett@memphis.edu
[redacted]
https://PuckettResearch.org/

RESEARCH EXPERIENCE

2024-*present*	Associate Professor: University of Memphis, Memphis, TN
2018-2024	Assistant Professor: University of Memphis, Memphis, TN
2017-2018	Postdoctoral Associate: Michigan State University, East Lansing, MI (PI: G Bradburd)
2015-2017	Postdoctoral Associate: Fordham University, Bronx, NY (PI: J Munshi-South)
2010-2015	Graduate Assistant: University of Missouri, Columbia, MO (Advisor: LS Eggert)
2008-2010	Research Associate III: Edenspace Systems Corporation, Chantilly, VA
2006-2008	Research Project Assistant: SUNY- College of Environmental Science and Forestry, Syracuse, NY (Advisor: LB Smart)
2005-2006	Research Assistant: University of North Carolina, Chapel Hill, NC (PI: CE Mitchell)

EDUCATION

2015	PhD	University of Missouri, Columbia, MO; Biological Sciences Certificate: Science and Public Policy, 2013
2008	MS	State University of New York- College of Environmental Science and Forestry, Syracuse, NY; Environmental and Forest Biology
2004	BS	North Carolina State University, Raleigh, NC; Botany (*summa cum laude*)

PUBLICATIONS

Puckett Mentees: [1] Undergraduate; [2] Graduate

Pollard, MD[2], W Meyer, and **EE Puckett**. (*Accepted*) Convergent relaxation of molecular constraint in herbivores reveals the changing role of liver and kidney functions across mammalian diets. *Genome Research*.

- *bioRxiv*: https://doi.org/10.1101/2023.11.17.567625

Puckett, EE, IS Davis[1], DC Harper, K Wakamatsu, G Battu, JL Belant, DE Beyer, C Carpenter, AC Crupi, M Davidson, CS DePerno, N Forman, NL Fowler, DL Garshelis, N Gould, K Gunther, M Haroldson, S Ito, D Kocka, C Lackey, R Leahy, C Lee-Roney, T Lewis, A Lutto, K McGowan, C Olfenbuttel, M Orlando, A Platt, MD Pollard[2], ME Ramaker, H Reich, JL Sajecki, SK Sell, J Strules, S Thompson, F van Manen, C Whitman, R Williamson, F Winslow, C Kaelin, MS Marks, and G Barsh. 2023. Genetic architecture and evolution of color variation in American black bears. *Current Biology* 33: 86-97.

- Media Coverage: New York Times, Cottage Life Magazine, Bear Hunting Magazine

Puckett, EE and IS Davis[1]. 2021. Spatial patterns of genetic diversity in eight bear (Ursidae) species. *Ursus* 32e20.

Puckett, EE, S Murphy, and G Bradburd. 2021. Phylogeographic analysis delimits three evolutionary significant units of least chipmunks and identifies unique genetic diversity within *Neotamias minimus atristriatus*, an imperiled population. *Ecology and Evolution* 11: 12114-12128.

Pedersen, MW, B De Sanctis, NF Saremi, M Sikora, **EE Puckett,** Z Gu, KL Moon, JD Kapp, L Vinner, Z Vardanyan, CF Ardelean, J Arroyo-Cabrales, JA Cahill, PD Heintzman, G Zazula, RDE MacPhee, B Shapiro, R Durbin, and E Willerslev. 2021. Environmental genomics of American black bear and the extinct short-faced bear. *Current Biology* 31: 2728-2736.

- Media Coverage: Popular Mechanics, Smithsonian Magazine, Science Daily

Sjodin, B, **EE Puckett**, R Irvine, G Howald, J Munshi-South, M Russello. 2021. Global origins of invasive brown rats (*Rattus norvegicus*) in the Haida Gwaii archipelago. *Biological Invasions* 23: 611-623.

Puckett, EE, E Sherratt, M Combs, E Carlen, W Harcourt-Smith, and J Munshi-South. 2020. Variation in brown rat cranial shape shows directional selection in nasal and tooth row lengths over 120 years in New York City. *Ecology and Evolution* 10(11): 4739-4748.

Puckett, EE, D Orton, and J Munshi-South. 2020. Integrating phylogeography and zooarchaeology of commensal rats to understand connections between human societies. *BioEssays* 42: 1900160.

- Cover Article

Puckett, EE, E Magnussen, LA Khylap, TM Strand, A Ludkvist, and J Munshi-South. 2020. Genomic analyses reveal three independent introductions of the invasive brown rat (*Rattus norvegicus*) to the Faroe Islands. *Heredity* 124 (1): 15-27.

- Cover Article
- Media Coverage: Heredity Podcast

Burkhart, JJ, **EE Puckett**, CJ Kroese, CN Sholy, RD Semlitsch, and LS Eggert. 2019. Post-Pleistocene differentiation in a Central Interior Highlands endemic salamander. *Ecology and Evolution* 9(19): 11171-11184.

Puckett, EE and J Munshi-South. 2019. Brown rat demography reveals pre-commensal structure in eastern Asia prior to expansion into Southeast Asia. *Genome Research* **29**: 762-770.

Puckett, EE, O Micci-Smith[1], and J Munshi-South. 2018. Genomic analyses identify multiple Asian origins and deeply diverged mitochondrial clades in inbred brown rats (*Rattus norvegicus*). *Evolutionary Applications* 11 (5): 718-726.

Kristensen, TV, **EE Puckett**, EL Landguth, JL Belant, JT Hast, C Carpenter, JL Sajecki, J Beringer, M Means, JT Cox, LS Eggert, D White Jr, KG Smith. 2018. Genetic structuring in American black bears (*Ursus americanus*) can be explained by more than female philopatry: interactions of density, genetic diversity, and sex-biased dispersal. *Heredity* 4: 329-341.

- Cover Article

Combs, M, **EE Puckett**, JR Richardson, D Mims[1], and J Munshi-South. 2018. Spatial population genomics of the brown rat (*Rattus norvegicus*) in New York City. *Molecular Ecology* 27: 83-98.

- Media Coverage: NPR, The Atlantic, Popular Science

Puckett, EE. 2017. Variability in total project and per sample genotyping costs under varying study designs including with microsatellites or SNPs to answer conservation genetic questions. *Conservation Genetics Resources* 9 (2): 289-304.

Puckett, EE, J Park, M Combs, MJ Blum, JE Bryant, A Caccone, F Costa, EE Deinum, A Esther, CG Himsworth, PD Keightley, A Ko, A Lundkvist, LM McElhinney, S Morand, J Robins, J Russell, TM Strand, O Suarez, L Yon, and J Munshi-South. 2016. Global population divergence and admixture of the brown rat (*Rattus norvegicus*). *Proceedings of the Royal Society B* 283: 20161762.

- Media Coverage: New York Times, Popular Science, and Business Insider

Puckett, EE, DC Kesler, and DN Greenwald. 2016. Taxonomic class, petitioning agency, and lawsuits affect time spent awaiting listing under the Endangered Species Act. *Biological Conservation* 201: 220-229.

- Media Coverage: Huffington Post

Wilton, CM, J Beringer, **EE Puckett**, LS Eggert, and JL Belant. 2016. Spatio-temporal capture-recapture biases in black bear detection probability. *Journal of Mammalogy* 97: 266-273.

Puckett, EE and LS Eggert. 2016. Comparison of SNP and microsatellite genotyping panels for spatial assignment of individuals to natal range: A case study using the American black bear (*Ursus americanus*). *Biological Conservation* 193: 86-93.

Puckett, EE, PD Etter, EA Johnson, and LS Eggert. 2015. Phylogeographic analyses of American black bears (*Ursus americanus*) suggest four glacial refugia and complex patterns of post-glacial admixture. *Molecular Biology and Evolution* 32: 2338-2350.

Wilton, CM, **EE Puckett**, J Beringer, B Gardner, LS Eggert, and JL Belant. 2014. Trap array configuration influences estimates and precision of black bear density and abundance. *PLoS One* 9(10): e111257.

Puckett, EE, TV Kristensen, CM Wilton, SB Lyda, KV Noyce, PM Holahan, DM Leslie, Jr, J Beringer, JL Belant, D White, Jr, and LS Eggert. 2014. Influence of drift and admixture on population structure of American black bears (*Ursus americanus*) in the Central Interior Highlands, USA 50 years after translocation. *Molecular Ecology* 23: 2414-2427.

Puckett, EE, MJ Serapiglia, AM DeLeon[1], S Long, R Minocha, and LB Smart. 2012. Differential expression of genes encoding phosphate transporters contributes to arsenic tolerance and accumulation in shrub willow (*Salix* spp.). *Environmental and Experimental Botany* 75: 248-257.

Hudson, CM, **EE Puckett**, M Bekaert, JC Pires, and GC Conant. 2011. Selection for higher gene copy number after different types of plant gene duplication. *Genome Biology and Evolution* 3: 1369-1380.

Mitchell, CE, D Blumenthal, V Jarosik, **EE Puckett**, P Pysek. 2010. Controls on pathogen species richness in plants' introduced and native ranges: roles of residence time, range size and host traits. *Ecology Letters* 13 (12): 1525-1535.

PREPRINTS

Pollard, MD[2] and **EE Puckett**. 2022. Evolution of degrees of carnivory and dietary specialization across Mammalia and their effects on diversification rate at different taxonomic levels. *bioRxiv*: https://doi.org/10.1101/2021.09.15.460515

BOOK CHAPTERS

Puckett, EE and LS Eggert. 2020. "Using genetics in the conservation management of the American black bear (*Ursus americanus*) in Missouri." IN: J Ortega and JE Maldonado (eds.), *Conservation Genomics in Mammals*: *integrative research using novel approaches*. Springer, New York.

IN REVIEW

de Jong, MJ**,** M Awan, N Lecomte, **EE Puckett**, AP Crupi, and A Janke. (*In Review*) Population-genomics reveals the dual ancestry of grizzly bears.

MANUSCRIPTS

Puckett, EE. (*In Revision*) Spatial and temporal analyses identify two introgression events between brown and American black bears.

Clendenin, HR[2], MD Pollard[2], and **EE Puckett**. (*In Prep*) Linking measures of inbreeding and genetic load to demographic histories across three species of bears.

Douchinsky, PR[2], AP Crupi, Y Kwon, JN Waite, **EE Puckett**. (*In Prep*) Harvest data analysis to determine population metrics in sympatric bear populations in Southeast Alaska.

Tocco, NS, D Martin, C Garrett, **EE Puckett**, E Reinhardt, M Ruszczyk, and N Mishra. (*In Prep*) GM1-gangliosidosis in three juvenile American black bears (*Ursus americanus*) in Connecticut between 2020 and 2023.

DISSERTATION & THESIS

Phylogeography and Population Genomics of the American Black Bear (*Ursus americanus*). PhD Dissertation, University of Missouri, 2015, p 190.

Molecular Basis for Differential Uptake and Sensitivity to Arsenic among Clones of Shrub Willow (*Salix* spp.). MS Thesis, SUNY-ESF, 2008, p 168.

GRANTS

2024 NIH- National Institute on Aging
Hair-greying and Melanocytic Regulation in a Non-model Organism- [redacted]

2022 National Fish and Wildlife Foundation
Building a Future for the Louisiana Black Bear through Habitat Restoration, Public Relations, and Genomic Rescue Planning- [redacted]

2021 Alaska Department of Fish and Game
Landscape Genomics within a Sympatric Population of Brown (*Ursus arctos*) and American Black (*U. americanus*) Bears in Southeast Alaska- [redacted]

2020 College of Arts and Sciences, University of Memphis
Using Whole Genome Resequencing to Identify Low Diversity Regions of the Genome in Louisiana Black Bear (*Ursus americanus luteolus*) Subpopulations to Target during Genetic Rescue-[redacted]

2018 New Mexico Department of Game and Fish and US Fish and Wildlife Service
Range-wide assessment of genomic diversity, demography, and subspecies status for the least (*Tamias minimus*) and Colorado (*T. quadrivittatus*) chipmunks-[redacted]

RESEARCH TALKS

Invited

Duquesne University: Pittsburgh, PA	2024
Arkansas State University- UandI-DEECoDE: Jonesboro, AR	2024
University of Florida: Gainesville, FL	2023
HudsonAlpha: Huntsville, AL	2023
National Genomics Center for Wildlife and Fish Conservation: *Virtual*	2022
Mississippi State University: Starkville, MS	2022
University Tennessee Health Science Center: Memphis, TN	2021
Canadian Society for Ecology and Evolution: Edmonton, AB	*Canceled Covid-19*
University of Mississippi, Biology Department: Oxford, MS	2019
Association of Southeastern Biologists: Memphis, TN	2019
University of Memphis: Memphis, TN	2018
Evolution Meeting, Evolution in Urban Environments Symposium: Portland, OR	2017
Yale University, DNA Analysis Facility: New Haven, CT	2017
24th International Bear Association: Anchorage, AK	2016
Fordham University, Biology Department: Bronx, NY	2016
Missouri Chapter of The Wildlife Society: Bois D'Arc Conservation Area, MO	2013
Missouri Department of Conservation: Columbia, MO	2013

Contributed

28th International Bear Association: Edmonton, AB, Canada	2024
Evolution Meeting: Cleveland, OH	2022
27th International Bear Association: *Virtual*	2021
Evolution Meeting: *Virtual*	2021
26th International Bear Association: Kalispell, MT	*Canceled Covid-19*
Evolution Meeting: Austin, TX	2016
SMBE-GAP Genetics of Admixed Populations: San Antonio, TX	2016
NY Area Population Genomics Workshop: Princeton, NJ	2016
University of Missouri, Life Sciences Fellows: Columbia, MO	2014
Evolution Meeting: Snowbird, UT	2013
University of Missouri, EcoLunch: Columbia, MO	2013

Public

NerdNight Memphis, Memphis, TN 2024
Biology on Tap, Lansing, MI 2018

POSTER PRESENTATIONS

SMBE: Puerto Vallarta, Mexico 2024
14th Western Black Bear Workshop: Jackson, WY 2024
SMBE- Molecular Evolution in Small Populations: Princeton, NJ 2023
25th Eastern Black Bear Workshop: Trego, WI 2023
SMBE: *Virtual* SMBEverwhere (S1) 2022
American Genetics Association: Snowbird, UT 2021
Evolution Meeting: Providence, RI 2019
24th Eastern Black Bear Workshop: Potosi, MO 2019
13th Western Black Bear Workshop: Grand Junction, CO 2018
23rd Eastern Black Bear Workshop: Ligonier, PA 2017
Bronx Science Consortium: Bronx, NY 2016
Ecological and Evolutionary Genomics, Gordon Research Conference: Biddeford, ME 2015
12th Western Black Bear Workshop: Canamore, AB 2015
22nd Eastern Black Bear Workshop: Louisville, MS 2015
12th Ecological Genomics Symposium: Kansas City, MO 2014
Life Sciences Week, University of Missouri: Columbia, MO (3rd place- Best EEB Poster) 2014
11th Ecological Genomics Symposium: Kansas City, MO 2013
Life Sciences Week, University of Missouri: Columbia, MO 2013
10th Ecological Genomics Symposium: Kansas City, MO 2012
Evolution Meeting: Ottawa, ON 2012
11th Western Black Bear Workshop: Coeur d'Alene, ID 2012
Life Sciences Week, University of Missouri: Columbia, MO 2012
Ecological and Evolutionary Genomics, Gordon Research Conferences: Biddeford, ME 2011
20th Eastern Black Bear Workshop: Hendersonville, NC 2011
Northeast Section, American Society of Plant Biologists: Storrs, CT 2008
Symposium in Plant Biology, University of Massachusetts: Amherst, MA 2007
Ecological Society of America: San Jose, CA 2007

TEACHING EXPERIENCE

Instructor on Record- University of Memphis

Spring	BIOL 3072: General Genetics
Fall- Even Yrs	BIOL 4090/ANTH 4992/POLI 3700: Conservation Biology, People, and Policy Co-taught with faculty from Anthropology or Political Science
Fall- Odd Yrs	BIOL 7104/8104: Speciation
Fall 2024	BIOL 7000/8000: Orientation to Graduate School
Spring 2020	UNHP 1100: Honors Forum: Women in Science

Guest Lectures

2022 Phylogeography, BIO 4983: Molecular Ecology, University of Missouri
2019 Phylogeography, BIOL 4096: Molecular Ecology, University of Memphis
2017 RADseq, American Museum of Natural History
2017 Molecular Phylogenetics, BISC 3244: Evolution, Fordham University
2017 Natural Selection, BISC 3244: Evolution, Fordham University
2016 Genomic Evolution, BISC 7501: Population Biology, Fordham University
2015 Evolution of Sex, BISC 3244: Evolution, Fordham University
2013 Phylogeography, BIO 4983: Molecular Ecology, University of Missouri

Teaching Assistant

2014-2015 Teaching Assistant, BIO 2200: General Genetics, University of Missouri
2008 (Spring) Teaching Assistant, EFB 531: Plant Physiology Laboratory, SUNY-ESF
2003 (Fall) Teaching Assistant, BCH 451: Principles of Biochemistry, NC State University

GRADUATE MENTORING

2021-2024 Philip R. Douchinsky
MS Thesis: *Comparative landscape genetics and dynamics in demography within sympatric ursids in Southeast Alaska*
2020-*present* Heather R. Clendenin
2019-2024 Matthew D. Pollard
PhD Dissertation: *Mammalian trait evolution: Macroevolutionary and comparative genomic analyses of diet and Ursid-specific adaptations*

GRADUATE COMMITTEE SERVICE- University of Memphis

2023-2024 Sam Drewry (PI: Jennifer Mandel)
2023-2024 Serena Blais (PI: Jennifer Mandel)
2020-2021 Paige Murin (PI: Jennifer Mandel)
2019-2020 Steven M. Ballou (PI: Jennifer Mandel)
2018-2019 Caroline Melton (PI: Bernie Daigle)
2018-2023 Avery Tucker (PI: Shawn Brown)

GRADUATE COMMITTEE SERVICE- Other

2023-*present* Josh Robinson (PI: Jan Janecka; Duquesne University, Pittsburgh, PA)
2023-*present* Faezeh Azimi Chetabi (PI: Aaron Shafer; Trent University, Peterborough, ON)

UNDERGRADUATE MENTORING ([1]- denotes co-author)

2023-*present* Kat Harris
2023 Samantha Lewis
2023 Karrah Van Horn
2023-*present* Delaney Viner
Adaptation among the eastern lineage of American black bears
2023 Kayla Moscon

2023	(Hiyab) Abby Williams
2023	Lauren McNary
Summer 2022	Danielle McConnell (HS student Mississippi School of Science)
2022-*present*	Alexandria Kerr
	Characterizing the hollow medulla trait within brown bear hair
	UofM Student Research Forum 2023- Poster (1st place)
	Honors College Summer Research Fellow- 2023
	UofM Works in Progress Symposium 2023- Oral Talk (2nd place)
	Posters at the Capital- Poster
	Biological Sciences Faculty Award- 2024
2021-*present*	Brian Wentzloff
	Characterizing the hollow medulla trait within brown bear hair
	UofM Student Research Forum 2023- Poster (1st place)
2021-2022	Caleigh Holley
	Genetic basis of black bear chest blazes
	UofM Student Research Forum 2022- Poster (1st place)
	Chi Beta Phi Science Award- 2022
2020-2021	Samhitha Swarna
2020-2021	Heather Thomasovich
2020	Ravi Lipman
2018-2022	Isis Davis[1]
	Global spatial genetic variation across Ursidae
	NSF GRF 2022- Recipient
	Biological Sciences Faculty Award- 2022
	U of Memphis Student Research Forum 2020- Oral Talk (1st place)
	TLSAMP Annual Research Conference 2020- Oral Talk (2nd place)
2017- 2018	Sarah Frocillo
	Variation in isolation-by-distance in marine species along the Scotian shelf
	Michigan State University Undergraduate Research and Arts Forum 2018- Poster
2016- 2017	Olivia Micci-Smith[1]
	Geographic origin of inbred brown rats using genomic data
	Evolution Meeting 2017- Poster
Summer 2016	Destiny Mims[1]
	Sex-biased dispersal of brown rats in New York City
	Evolution Meeting 2017- Poster
2011- 2013	Michelle Anderson
	Geographic variation in *CLOCK* and *FTO* in American black bears
	University of Missouri- Life Sciences Week 2013- Poster
2011- 2012	Jessica (Philbrick) Wayhart
	Mitochondrial phylogeography of American black bears
	University of Missouri- Life Sciences Week 2012- Poster
2007- 2008	Alyssa DeLeon[1]
	Molecular analysis of genes related to arsenic uptake and detoxification in

sensitive and tolerant varieties of shrub willow (*Salix* spp.)
American Society of Plant Biologists 2009- Poster

PROFESSIONAL SERVICE

2024-*present* Secretary, International Association for Bear Research and Management
2024 Panelist, NSF advisory panel
2023-*present* Associate Editor, *Ursus*
2020 Panelist, NSF advisory panel
2019 Panelist, NSF advisory panel
2019-*present* Associate Editor, *Ecology and Evolution*
2016-2020 Member, IUCN Bear Specialist Group
2012-*present* Journal Reviewer (35 peer reviewed journals)

U of MEMPHIS DEPARTMENT of BIOLOGICAL SCIENCES SERVICE

2024-*present* Member, Tenure and Promotion Committee
2023-*present* Member, Graduate Studies Committee
2021-*present* Founder and Senior Drill Sergeant, GRFP Bootcamp
2021-*present* Chair, Seminar Committee
2019-2021 Co-chair, Seminar Committee
2019-*present* Member, Center for Biodiversity Research (CBio)
2019-2020 Founding Member and Advisory Board, Center for Biodiversity Research (CBio)
2018-2023 Member, Communications Committee

U of MEMPHIS COLLEGE of ARTS & SCIENCES SERVICE

2023 Dean's Working Group ASCEND Strategic Plan
Goal 7 (Steward & Generate Financial Resources)

U of MEMPHIS COLLEGE of COMMUNICATION & FINE ARTS

2023 Panelist, STEAM Collaborations

U MISSOURI DEPARTMENTAL SERVICE

2014-2015 Member, Systems Biology Search Committee, Biological Sciences
2011-2014 Member, Biology Seminar Organizing Committee, Biological Sciences
2013 Graduate Student Representative, Divisional Council, Biological Sciences
2010-2015 Secretary (2011-2013) and Member, Biology Graduate Student Association

SUNY-ESF DEPARTMENTAL SERVICE

2008 Member, Graduate Advisory Council

NCSU DEPARTMENTAL SERVICE

2001-2004 President, Botany Club

OUTREACH

2021 Presenter (Virtual), *Kingdom of Rodentia*, 4th and 5th grade students in Shelby County Schools Creative Learning in a Unique Environment Program

2013-2018 Founder and blogger at WildlifeSNPits (http://wildlifesnpits.wordpress.com)
As of October 2018: wrote 53 posts as EEPuckett & 126 posts as WildlifeSNPits

2002-2004 Volunteer, Young Scientists Program: Weekly science tutoring with a fourth grade student in Raleigh, NC

PATENTS

Gampala, S, D Lee, **EE Puckett**, R Nair, and F Chumley. Poplar genomic DNA sequences that regulate gene expression in plants and uses thereof. Patent Filing date: June 4, 2009.

SCHOLARSHIPS and HONORS

2023 Early Career Researcher Award, University of Memphis

2012 TransWorld Airlines Scholarship, U Missouri

2010-2014 Life Science Fellow, U Missouri

2008, 2006 Lowe-Wilcox Graduate Scholarship, Department of Environmental and Forest Biology, SUNY-ESF

2004 Outstanding Botany Senior Award, Department of Botany, NC State University

2004 University Scholars Program, NC State University

2004 Honors Program, College of Agriculture and Life Sciences, NC State University

2003 Larry A. Whitford Scholarship, Department of Botany, NC State University

2001 Promising Young Botanist Award, Department of Botany, NC State University

PROFESSIONAL AFFILIATIONS

2023-*present* Society for Molecular Biology and Evolution (SMBE)

2012-2023 Society for the Study of Evolution (SSE)

2011-*present* International Association for Bear Research and Management (IBA)

2003 Phi Beta Kappa

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Kaelin, Christopher Bryan

eRA COMMONS USER NAME (credential, e.g., agency login): KAELIN.CHRISTOPHER

POSITION TITLE: Senior Scientist, HudsonAlpha Institute for Biotechnology; Scientist, Stanford University

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of California, Davis	B.A.	06/1994	Genetics
Stanford University, Stanford, CA	Ph.D.	06/2005	Genetics
Stanford University, Stanford, CA	Postdoc	08/2008	Genetics

A. Personal Statement

My introduction to genetics and molecular biology was as an undergraduate at UC Davis, and motivated a difficult decision to switch from pre-veterinary tract to a research emphasis. My graduate work focused on the role of melanocortin signaling in neural circuits that control appetite and metabolism, where I used mouse transgenesis and BAC recombineering to visualize and measure levels of gene expression in hypothalamic cell populations. As a postdoctoral fellow, I studied the mechanisms responsible for canine coat color and pattern variation, using molecular genetic and pharmacologic approaches. As a scientist at HudsonAlpha, I am applying genetic and genomic approaches, including linkage mapping, genome-wide association studies, and transcriptomic methods, including single-cell approaches to study the basis of color patterns in mammals, as a means of exploring how periodic structures arise in biology. My training and work experience spans both experimental and computational biologic approaches. I helped to conceive and implement the scientific questions and aims that are the subject of this proposal.

I have also invested time in fostering collaborative relationships within and outside the scientific community to enable work with non-model organisms. This includes coordinating large-scale DNA collection within the cat breeding community that have populated a biobank with more than 2000 samples with corresponding images of color patterns, and fetal tissue collection in conjunction with Trap-Neuter-Return spay/neuter clinics. I have also engaged conservation research scientists at various institutes, including De Wildt Cheetah Center, Wild Cat Education and Conservation Fund, the Audubon Nature Institute, and the African Parks Network.

In addition, I have been an active member of the companion animal genetics and genomics community, attending, presenting at, and helping to organize related conferences and serving for two years on a grant review board for the EveryCat Health Foundation (formerly Winn Health Foundation). Within our research group at Stanford and HudsonAlpha, I have maintained productive working relationships with colleagues for nearly 20 years, and I have mentored several scientists, ranging in experience from high school students to post-doctoral fellows.

Citations:

1. **Kaelin CB**, McGowan KA, Barsh GS. Developmental Genetics of Color Pattern Establishment in Cats. Nat Commun. 2021 Sep 7;12(1):5127. doi: 10.1038/s41467-021-25348-2. PMID: 34493721 PMCID: PMC8423757

2. Sagar V, **Kaelin CB**, Natesh M, Reddy PA, Mohapatra RK, Chhattani H, Thatte P, Vaidyanathan S, Biswas S, Bhatt S, Paul S, Jhala YV, Verma MM, Pandav B, Mondol S, Barsh GS, Swain D, Ramakrishnan U. High frequency of an otherwise rare phenotype in a small and isolated tiger population. Proc Natl Acad Sci U S A. 2021 Sep 28;118(39):e2025273118. doi: 10.1073/pnas.2025273118. PMID: 34518374 PMCID: PMC8488692
3. Bannasch DL, **Kaelin CB**, Letko A, Loechel R, Hug P, Jagannathan V, Henkel J, Roosje P, Hytönen MK, Lohi H, Arumilli M; DoGA consortium, Minor KM, Mickelson JR, Drögemüller C, Barsh GS, Leeb T. Dog colour patterns explained by modular promoters of ancient canid origin. Nat Ecol Evol. 2021 Aug 12. doi: 10.1038/s41559-021-01524-x. Online ahead of print. PMID: 34385618 PMCID: PMC8484016
4. Graff EC, Cochran JN, **Kaelin CB**, Day K, Gray-Edwards HL, Watanabe R, Koehler JW, Falgoust RA, Prokop JW, Myers RM, Cox NR, Barsh GS, Martin DR; 99 Lives Consortium. PEA15 loss of function and defective cerebral development in the domestic cat. PLoS Genet. 2020 Dec 8;16(12):e1008671. doi: 10.1371/journal.pgen.1008671. eCollection 2020 Dec. PMID: 33290415 PMCID: PMC7723247

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2019-present	Grant Review Board Member, EveryCat Health Foundation (formerly WINN feline health)
2008-present	Senior Scientist, HudsonAlpha Institute for Biotechnology, Huntsville, AL
2008-present	Senior Scientist, Stanford University, CA

C. Contributions to Science

1. Biology of melanocortin signaling:

As a graduate student, I contributed to characterizing the hypothalamic circuits involved in appetite and metabolism, by identifying and studying neuronal population producing the orexigenic neuropeptide Agouti-related protein (Agrp), an inverse agonist of melanocortin signaling in brain that transmits peripheral signals into a neuronal response. As a postdoc, I studied the genetic and biochemical function of a beta-defensin, a paracrine antagonist of melanocortin 1 receptor in the skin responsible for black coloration and brindle markings in dogs. More recently, I identified and characterized the gene responsible for sex-linked color pattern, calico, which encodes an intracellular effector of melanocortin signaling in pigment cells.

a) **Kaelin CB**, Xu AW, Lu XY, Barsh GS (2004). Transcriptional regulation of *Agouti-related protein* (*AgRP*) in transgenic mice. *Endocrinology* 145(12):5798-806. doi: 10.1210/en.2004-0956.

b) **Kaelin CB**, Gong L, Xu AW, Yao F, Hockman K, Morton GJ, Schwartz MW, Barsh GS, MacKenzie RG (2006). Signal transducer and activator of transcription (stat) binding sites but not stat3 are required for fasting-induced transcription of agouti-related protein messenger ribonucleic acid. *Molecular Endocrinology* 20(10):2591-602. doi: 10/1210/me.2006-0107.

c) **Kaelin CB**, Cooper GM, Sidow A, and Barsh GS (2007). Mammalian Comparative Sequence Analysis of the *Agrp* Locus. *PLoS ONE* 2(8): e702. PMCID: PMC1931611.

d) **Kaelin CB**, Candille SI, Cattanach BM, Yu B, Thompson DA, Nix MA, Kerns JA, Schmutz SM, Millhauser GL, and Barsh GS (2007). A beta-defensin mutation causes black coat color in domestic dogs. *Science* 318(5855):1418-23. PMCID: PMC290662.

2. Developmental and evolutionary genetics of color patterns:

Formation of repeated structures is a common theme in developmental biology. Color patterns in mammals represent a visually apparent readout of natural (or artificially selected) variation in periodic pattern formation.

Because the laboratory mouse does not have spots or stripes, I have focused on non-model organisms to study how periodic color patterns form in the skin. Early contributions in this area include my postdoctoral research on the mechanism underlying the formation of brindle patterns in the dog, which is caused by an epigenetically unstable mechanism affecting the expression of a beta-defensin gene. Domestic cats and related felids have periodic patterns that vary within and across species. Using forward genetics approaches in pedigreed cats and transcriptomic approaches in fetal cat skin, my research has identified essential components of an evolutionarily conserved processes that shape different pattern types, including those that set up patterns (Transaminopetidase Q and Dkk4) and those that implement patterns as hair grows (Endothelin 3). Additionally, transcriptomic analysis of fetal cat skin has revealed a molecular pre-pattern of genes expressed in spots and stripes that may represent in Turing mechanism in felids and other species.

a) **Kaelin CB**, McGowan KA, Barsh GS. Developmental Genetics of Color Pattern Establishment in Cats. Nat Commun. 2021 Sep 7;12(1):5127. doi: 10.1038/s41467-021-25348-2. PMID: 34493721 PMCID: PMC8423757.

b) Sagar V, **Kaelin CB**, Natesh M, Reddy PA, Mohapatra RK, Chhattani H, Thatte P, Vaidyanathan S, Biswas S, Bhatt S, Paul S, Jhala YV, Verma MM, Pandav B, Mondol S, Barsh GS, Swain D, Ramakrishnan U. High frequency of an otherwise rare phenotype in a small and isolated tiger population. Proc Natl Acad Sci U S A. 2021 Sep 28;118(39):e2025273118. doi: 10.1073/pnas.2025273118. PMID: 34518374 PMCID: PMC8488692.

c) **Kaelin CB** et al. (2012) Specifying and sustaining pigmentation patterns in domestic and wild cats. Science 337: 1536-1541, PMID: 22997338, PMCID: PMC3709578.

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/1-uMZ8hUsPD/bibliography/public/

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Barsh, Gregory Stefan

eRA COMMONS USER NAME (credential, e.g., agency login): barsh.gregory

POSITION TITLE: Professor of Genetics, Stanford University; Faculty Investigator, HudsonAlpha Institute

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of California, Irvine, CA	B.A.	06/1977	Biology
University of Washington, Seattle, WA	M.D. Ph.D.	06/1984	Human Genetics, Pathology
University of California, San Francisco, CA	Postdoc	11/1989	Genetics

A. Personal Statement

My graduate and medical training was in the area of molecular and medical genetics of human Mendelian disease; as a faculty member at Stanford, my research group used forward- and reverse-genetic approaches in laboratory mice to study gene action and interaction. Most of this work used color variation as a model system, and led to the discovery of a new set of paracrine mediators, new insight into the physiology of body weight regulation, novel aspects of membrane remodeling relevant to neurodegenerative disease, and an unexpected connection between the innate immune system and melanocortin receptor signaling.

Over the last decade, I have become interested in the opportunities provided by genome sequencing and technology to study the biology and evolution of periodic color patterns. In 2009, I accepted a faculty position at HudsonAlpha, which has made it possible to extend pigmentary genetics and genomics into non-model organisms, including the domestic dog, the domestic cat, and several wild felid species.

The arrangement between HudsonAlpha and Stanford allowed me to maintain an active emeritus appointment and mouse genetics colony at Stanford, and provided a unique opportunity to pursue unsolved mysteries in developmental biology and evolution using mammalian color patterns as an entry point. In 2023, I relocated back to Stanford, maintaining a 15% appointment at HudsonAlpha.

The subject of this application builds on work we have carried out for the last decade and that was partially supported by NIH 5R01AR067925 (PI: Barsh, 04/01/2015-02/29/2021, Melanocortin signaling: genetic insight from X-linked color mutations in non-model organisms). This application also builds on a longstanding relationship with the Bengal cat community and collaboration with Anthony Hutcherson of Jungletrax cats. Additional citations relevant to pigmentary genomics and evolution in non-model organisms include:

Anderson TM, vonHoldt BM, Candille SI, Musiani M, Greco C, Stahler DR, Smith DW, Padhukasahasram B, Randi E, Leonard JA, Bustamante CD, Ostrander EA, Tang H, Wayne RK, **Barsh GS**. Molecular and evolutionary history of melanism in North American gray wolves. Science. 2009; 323(5919) 1339-1343. doi:10.1126/science.1165448. PubMed PMID: 19197024. PMCID: PMC2903542.

Mallarino R, Henegar C, Mirasierra M, Manceau M, Schradin C, Vallejo M, Beronja S, **Barsh GS**, Hoekstra HE. Developmental mechanisms of stripe patterns in rodents. Nature. 2016; 539(7630) 518-523. doi:10.1038/nature20109. PubMed PMID: 27806375. PMCID: PMC5292240.

Imsland F, McGowan K, Rubin CJ, Henegar C, Sundström E, Berglund J, Schwochow D, Gustafson U, Imsland P, Lindblad-Toh K, Lindgren G, Mikko S, Millon L, Wade C, Schubert M, Orlando L, Penedo MC, **Barsh GS**, Andersson L. Regulatory mutations in TBX3 disrupt asymmetric hair pigmentation that underlies Dun camouflage color in horses. Nat Genet. 2016; 48(2) 152-158. doi:10.1038/ng.3475. PubMed PMID: 26691985. PMCID: PMC4731265.

Larison B, Kaelin CB, Harrigan R, Henegar C, Rubenstein DI, Kamath P, Aschenborn O, Smith TB, **Barsh GS**. Population structure, inbreeding and stripe pattern abnormalities in plains zebras. Mol Ecol. 2021; 30(2) 379-390. doi:10.1111/mec.15728. PubMed PMID: 33174253.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2020-2023	Smith Family Chair in Genomics, HudsonAlpha Institute for Biotechnology, Huntsville, AL
2016-2023	Faculty Chair, HudsonAlpha Institute for Biotechnology, Huntsville, AL
2013-present	Adjunct Professor, University of Alabama at Birmingham, AL
2011-present	Professor (emeritus, recalled to active duty), Stanford University, Stanford, CA
2009-present	Investigator, HudsonAlpha Institute for Biotechnology, Huntsville, AL
2009-present	Editor In Chief, PLoS Genetics
1989-present	Assistant, Associate, and Full Professor, Stanford University School of Medicine

Honors

2014	Myron Gordon Award, International Federation of Pigment Cell Societies
2010	American Skin Association Achievement Award
2008	Seiji Award, International Society for Pigment Cell Research
2005	Aaron B. Lerner Award, International Society for Pigment Cell Research
2003	E Mead Johnson Award, Society for Pediatric Research
1999	Takeuchi Medal, International Society for Pigment Cell Research

C. Contributions to Science

1. Biology of melanocortin signaling: As a junior faculty member at Stanford, I started my research program using mouse coat color mutations that affect the switch between melanin subtypes as a model system for studying how paracrine signaling triggers a cell biological switch. We identified Agouti signaling protein (Asip) as a novel endogenous antagonist of the melanocortin 1 receptor, and showed that neomorphic alleles of Asip elicit pleiotropic effects through ectopic action on other melanocortin receptors. We have continued to use mutations that affect pigment type-switching to show that Attractin (Atrn), a novel single transmembrane-spanning protein serves as an accessory receptor for Asip, and that beta-defensins function as neutral antagonists for melanocortin receptors. This work has provided fundamental insight into mechanisms whereby a hormone 7-TM receptor interaction triggers a cell biological switch.

a) Duhl DM, Vrieling H, Miller KA, Wolff GL, **Barsh GS**. Neomorphic agouti mutations in obese yellow mice. Nat Genet. 1994; 8(1) 59-65. doi:10.1038/ng0994-59. PubMed PMID: 7987393.

b) Ollmann MM, Lamoreux ML, Wilson BD, **Barsh GS**. Interaction of Agouti protein with the melanocortin 1 receptor in vitro and in vivo. Genes Dev. 1998; 12(3) 316-330. doi:10.1101/gad.12.3.316. PubMed PMID: 9450927. PMCID: PMC316484.

c) He L, Gunn TM, Bouley DM, Lu XY, Watson SJ, Schlossman SF, Duke-Cohan JS, **Barsh GS**. A biochemical function for attractin in agouti-induced pigmentation and obesity. Nat Genet. 2001; 27(1) 40-47. doi:10.1038/83741. PubMed PMID: 11137996.

d) Candille SI, Kaelin CB, Cattanach BM, Yu B, Thompson DA, Nix MA, Kerns JA, Schmutz SM, Millhauser GL, **Barsh GS**. A -defensin mutation causes black coat color in domestic dogs. Science. 2007; 318(5855) 1418-1423. doi:10.1126/science.1147880. PubMed PMID: 17947548. PMCID: PMC2906624.

2. Pigmentary genetics and human disease: We have also used color mutations in laboratory mice as a platform to investigate important aspects of mammalian physiology and pathophysiology. We showed that the ability of ectopic Asip to cause obesity was due to a homologous protein that we named Agouti-related protein (Agrp) and that acts as an endogenous antagonist for the melanocortin 3 and 4 receptors. We also discovered that Asip signaling through the Mc1r and Mc4r requires Atrn as well as an E3 ubiquitin ligase that we named Mahogunin (Mgrn), whose absence leads to spongiform encephalopathy. In a different line of investigation based on mutations that cause dark skin in mice, we discovered that loss of function alleles in Galphaq proteins causes dermal melanocytosis, leading to the discovery of an important human melanoma gene. Finally, we discovered that loss-of-function mutations in ribosomal protein genes cause epidermal melanocytosis, providing some of the first insight into the pathophysiology of so-called ribosomapathies.

a) Ollmann MM, Wilson BD, Yang YK, Kerns JA, Chen Y, Gantz I, **Barsh GS**. Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein. Science. 1997; 278(5335) 135-138. PubMed PMID: 9311920.

b) He L, Eldridge AG, Jackson PK, Gunn TM, **Barsh GS**. Accessory proteins for melanocortin signaling: attractin and mahogunin. Ann N Y Acad Sci. 2003; 994 288-298. PubMed PMID: 12851328.

c) Van Raamsdonk CD, Fitch KR, Fuchs H, de Angelis MH, **Barsh GS**. Effects of G-protein mutations on skin color. Nat Genet. 2004; 36(9) 961-968. doi:10.1038/ng1412. PubMed PMID: 15322542. PMCID: PMC7341985.

d) McGowan KA, Li JZ, Park CY, Beaudry V, Tabor HK, Sabnis AJ, Zhang W, Fuchs H, de Angelis MH, Myers RM, Attardi LD, **Barsh GS**. Ribosomal mutations cause p53-mediated dark skin and pleiotropic effects. Nat Genet. 2008; 40(8) 963-970. doi:10.1038/ng.188. PubMed PMID: 18641651. PMCID: PMC3979291.

3. Developmental and evolutionary genetics of color patterns: The developmental and evolutionary genetic mechanisms that underlie color patterns in warm-blooded animals remain unsolved mysteries in biology. Using variation in domestic animals and natural populations, we have started to chip away at some of these mysteries. We developed a genomic approach to evaluate expression in cheetah spots and zebra stripes in the absence of a reference genome. Using forward genetics in domestic cats, we identified a new component of periodic pattern that is responsible for tabby patterning in the domestic cat, Taqpep, and that is an important component upon which the current application builds. More recently, we worked together with Danika Bannasch and Tosso Leeb to understand the regulatory architecture and evolution of dog color pattern. One of the most important breakthroughs that provides the foundation of the current application is our recent discovery that the secreted Wnt inhibitor encoded by *Dickkopf4* (*Dkk4*) is a central component of a Turing reaction-diffusion mechanism that underlies establishment of felid color pattern.

a) Hong LZ, Li J, Schmidt-Küntzel A, Warren WC, **Barsh GS**. Digital gene expression for non-model organisms. Genome Res. 2011; 21(11) 1905-1915. doi:10.1101/gr.122135.111. PubMed PMID: 21844123. PMCID: PMC3205575.

b) Kaelin CB, Xu X, Hong LZ, David VA, McGowan KA, Schmidt-Küntzel A, Roelke ME, Pino J, Pontius J, Cooper GM, Manuel H, Swanson WF, Marker L, Harper CK, van Dyk A, Yue B, Mullikin JC, Warren WC, Eizirik E, Kos L, O'Brien SJ, **Barsh GS***, Menotti-Raymond M. Specifying and sustaining pigmentation patterns in domestic and wild cats. Science (New York, NY). 2012; 337(6101) 1536-1541. doi:10.1126/science.1220893. PMID: 22997338 PMCID: PMC3709578.

c) Bannasch DL, Kaelin CB, Letko A, Loechel R, Hug P, Jagannathan V, Henkel J, Roosje P, Hytönen MK, Lohi H, Arumilli M, DoGA C, Minor KM, Mickelson JR, Drögemüller C, **Barsh GS***, Leeb T. Dog

colour patterns explained by modular promoters of ancient canid origin. Nat Ecol Evol. 2021; 5(10) 1415-1423. doi:10.1038/s41559-021-01524-x. PubMed PMID: 34385618. PMCID: PMC8484016.

d) Kaelin CB, McGowan KA, **Barsh GS**. Developmental genetics of color pattern establishment in cats. Nat Commun. 2021; 12(1) 5127. doi:10.1038/s41467-021-25348-2. PubMed PMID: 34493721. PMCID: PMC8423757.

*corresponding author

4. National and international service: I served for two terms as a regular member and then chair of the NIH study section on Genetics of Health and Disease, and since 2019 have served on the NHGRI Board of Scientific Counselors. Since 2009, I have been an Editor-in-Chief (together with Greg Copenhaver) of PLOS Genetics.

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/48977803

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: McGowan, Kelly A.

eRA COMMONS USER NAME (credential, e.g., agency login): MCGOWAN.KELLY

POSITION TITLE: Senior Scientist, HudsonAlpha Institute for Biotechnology; Scientist, Stanford University

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Stanford University, Stanford, CA	B.S.	6/1990	Biology
University of Washington, Seattle, WA	M.D.	6/1995	Medicine
University of California, San Francisco, CA		6/1996	Internal Medicine
Stanford University, Stanford, CA		6/1999	Dermatology
Stanford University, Stanford, CA	Ph.D.	6/2007	Genetics
Stanford University, Stanford, CA	Postdoc	1/2010	Genetics, Hematology/Oncology

A. Personal Statement

I was introduced to research in developmental and cell biology during my formal medical training: I spent one year during medical school in Dr. Hynda Kleinman's lab at the NIH as a Howard Hughes Medical Institute Research Scholar and an additional year during my residency in Dr. Peter Marinkovich's lab at Stanford. These experiences motivated me to pursue my doctorate degree after completing my dermatology training. During graduate school I used forward genetics in mice to study a new class of pigmentation mutant characterized by dark skin. I extended this work during my postdoctoral fellowship to study the role of ribosomal proteins and p53 in myelodysplasia and Diamond Blackfan anemia using mouse genetics and cell culture approaches. Portions of my graduate work and postdoctoral fellowship were funded by an NIH Clinical Investigator Award (K08).

As a scientist at HudsonAlpha, I am using genetics and genomics, as well as developmental and cell biologic techniques in mice and non-model organisms (zebra, horse, cheetah) to study the mechanisms that give rise to coat color patterns in mammals. I have been an employee at HudsonAlpha since 2010, but am physically based at Stanford University. The infrastructure at Stanford (Veterinary Service Facility, Mass Spec Facility, Cell Sciences Imaging Facility, Fluorescence Activated Cell Sorting Facility) has enabled me to carry out experimental approaches that complement the genomic work done in the lab. I helped to design and implement the scientific questions and aims that are the subject of this proposal.

For the last five years, I have also fostered collaborative relationships with groups that enable our work with non-model organisms. Multiple Trap-Neuter-Return spay-neuter clinics throughout California facilitate fetal cat tissue collection, and taxidermists and breeders from Zambia, Namibia, Iceland and the United States provide skin, hair and blood samples from zebras, horses and wild cats.

Finally, I am a member of the American Board of Dermatology and have maintained a relationship with the dermatology community at Stanford, where I attend weekly research seminars in the Department of Dermatology. Within our research group and the Department of Genetics at Stanford, I have had productive

working relationships with colleagues for nearly 20 years. In addition, I have mentored several scientists, ranging in experience from high school students to postdoctoral fellows.

Citations:

1. Kaelin CB, **McGowan KA**, Barsh GS. Developmental Genetics of Color Pattern Establishment in Cats. Nat Commun. 2021 Sep 7;12(1):5127. doi: 10.1038/s41467-021-25348-2. PMID: 34493721 PMCID: PMC8423757.

2. Imsland F, **McGowan K**, Rubin CJ, Henegar C, Sundström E, Berglund J, Schwochow D, Gustafson U, Imsland P, Lindblad-Toh K, Lindgren G, Mikko S, Millon L, Wade C, Schubert M, Orlando L, Penedo MC, Barsh GS, Andersson L. Regulatory mutations in TBX3 disrupt asymmetric hair pigmentation that underlies Dun camouflage color in horses. Nat Genet. 2016 Feb;48(2):152-8. doi:10.1038/ng.3475. PMID: 26691985. PMCID: PMC4731265.

3. **McGowan KA**, Li JZ, Park CY, Beaudry V, Tabor HK, Sabnis AJ, Zhang W, Fuchs H, de Angelis MH, Myers RM, Attardi LD, Barsh GS. Ribosomal mutations cause p53-mediated dark skin and pleiotropic effects. Nat Genet. 2008 Aug;40(8):963-70. doi: 10.1038/ng.188. PMID: 18641651. PMCID: PMC3979291.

4. **McGowan KA**, Pang WW, Bhardwaj R, Perez MG, Pluvinage JV, Glader BE, Malek R, Mendrysa SM, Weissman IL, Park CY, Barsh GS. Reduced ribosomal protein gene dosage and p53 activation in low-risk myelodysplastic syndrome. Blood. 2011 Sep 29;118(13):3622-33. doi: 10.1182/blood-2010-11-318584. PMID: 21788341. PMCID: PMC3186336.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2008-present	Senior Scientist, HudsonAlpha Institute for Biotechnology, Huntsville, AL
2008-present	Senior Scientist, Stanford University, Stanford, CA
2000-present	Diplomat, American Board of Dermatology

C. Contributions to Science

1. Pigmentary genetics and human disease:
As a graduate student and postdoctoral fellow, I used mutations that cause dark skin in laboratory mice as an entry point to study aspects of mammalian physiology and pathophysiology. Specifically, I identified mutations in two keratin genes, keratin 1 and keratin 4, that produce phenotypes in mice that recapitulate the dominantly-inherited diseases epidermolytic hyperkeratosis and white sponge nevus, respectively. In addition, I found that loss-of-function mutations in ribosomal proteins cause p53-dependent epidermal melanocytosis and provide insight into the role of p53 in human ribosomopathies such as Diamond Blackfan anemia and 5q-myelodysplasia.

1. **McGowan KA**, Li JZ, Park CY, Beaudry V, Tabor HK, Sabnis AJ, Zhang W, Fuchs H, de Angelis MH, Myers RM, Attardi LD, Barsh GS. Ribosomal mutations cause p53-mediated dark skin and pleiotropic effects. Nat Genet. 2008 Aug;40(8):963-70. doi: 10.1038/ng.188. PMID: 18641651. PMCID: PMC3979291.

2. **McGowan KA**, Pang WW, Bhardwaj R, Perez MG, Pluvinage JV, Glader BE, Malek R, Mendrysa SM, Weissman IL, Park CY, Barsh GS. Reduced ribosomal protein gene dosage and p53 activation in low-risk myelodysplastic syndrome. Blood. 2011 Sep 29;118(13):3622-33. doi: 10.1182/blood-2010-11-318584. PMID: 21788341. PMCID: PMC3186336.

3. **McGowan KA**, Aradhya S, Fuchs H, de Angelis MH, Barsh GS. A mouse keratin 1 mutation causes dark skin and epidermolytic hyperkeratosis. J Invest Dermatol. 2006 May;126(5):1013-6. doi: 10.1038/sj.jid.5700241. PMID: 16528356.

4. Fitch KR, **McGowan KA**, van Raamsdonk CD, Fuchs H, Lee D, Puech A, Hérault Y, Threadgill DW, Hrabé de Angelis M, Barsh GS. Genetics of dark skin in mice. Genes Dev. 2003 Jan 15;17(2):214-28. doi: 10.1101/gad.1023703. PMID: 12533510. PMCID: PMC195979.

2. Developmental and evolutionary genetics of color patterns:
Coat color patterns in mammals are a visual display of periodic pattern formation and can be used to explore how periodic structures arise in other contexts. Pattern formation in the skin is a three-step process: (1) establishment of pattern identity during development, (2) implementation of the pattern by pigment cells within hair follicles; and (3) maintenance of the pattern as the animal grows. Using histochemical, morphometric and transcriptomic approaches in fetal cat skin, I (in collaboration with Christopher Kaelin) have identified a cellular and molecular pre-pattern during development that is important for establishing the pigmentation pattern observed in adult cats. In addition, my histochemical and transcriptomic experiments have helped to confirm that Transaminopetidase Q and Dkk4 shape the molecular pre-pattern in felids, and Tbx3 and Edn3 are essential component of the implementation program in equine and felid species, respectively.

1. Kaelin CB, **McGowan KA**, Barsh GS. Developmental Genetics of Color Pattern Establishment in Cats. Nat Commun. 2021 Sep 7;12(1):5127. doi: 10.1038/s41467-021-25348-2. PMID: 34493721. PMCID: PMC8423757.

2. Imsland F, **McGowan K**, Rubin CJ, Henegar C, Sundström E, Berglund J, Schwochow D, Gustafson U, Imsland P, Lindblad-Toh K, Lindgren G, Mikko S, Millon L, Wade C, Schubert M, Orlando L, Penedo MC, Barsh GS, Andersson L. Regulatory mutations in TBX3 disrupt asymmetric hair pigmentation that underlies Dun camouflage color in horses. Nat Genet. 2016 Feb;48(2):152-8. doi:10.1038/ng.3475. PMID: 26691985. PMCID: PMC4731265.

3. Kaelin CB, Xu X, Hong LZ, David VA, **McGowan KA**, Schmidt-Küntzel A, Roelke ME, Pino J, Pontius J, Cooper GM, Manuel H, Swanson WF, Marker L, Harper CK, van Dyk A, Yue B, Mullikin JC, Warren WC, Eizirik E, Kos L, O'Brien SJ, Barsh GS, Menotti-Raymond M. Specifying and sustaining pigmentation patterns in domestic and wild cats. Science. 2012 Sep 21;337(6101):1536-41. doi: 10.1126/science.1220893. PMID: 22997338. PMCID: PMC3709578.

4. Zhang ET, Hannibal RL, Badillo Rivera KM, Song JHT, **McGowan K**, Zhu X, Meinhardt G, Knöfler M, Pollheimer J, Urban AE, Folkins AK, Lyell DJ, Baker JC. PRG2 and AQPEP are misexpressed in fetal membranes in placenta previa and percreta. Biol Reprod. 2021 Jul 2;105(1):244-257. doi: 10.1093/biolre/ioab068. PMID: 33982062. PMCID: PMC8256106.

3. Cutaneous biology:
My research during medical school and residency employed cell culture approaches. In medical school, I examined the effects of estrogen on endothelial cell behavior and angiogenesis. During residency, my work focused on metalloproteinase-processing of extracellular matrix.

1. Veitch DP, Nokelainen P, **McGowan KA,** Nguyen TT, Nguyen NE, Stephenson R, Pappano WN, Keene DR, Spong SM, Greenspan DS, Findell PR, Marinkovich MP. Mammalian tolloid metalloproteinase, and not matrix metalloprotease 2 or membrane type 1 metalloprotease, processes laminin-5 in keratinocytes and skin. J Biol Chem. 2003 May 2;278(18):15661-8. doi: 10.1074/jbc.M210588200. PMID: 12473650.

2. Morales DE, **McGowan KA**, Grant DS, Maheshwari S, Bhartiya D, Cid MC, Kleinman HK, Schnaper HW. Estrogen promotes angiogenic activity in human umbilical vein endothelial cells in vitro and in a murine model. Circulation. 1995 Feb1;91(3):755-63. doi: 10.1161/01.cir.91.3.755. PMID: 7530174.

3. Kim-Schulze S, **McGowan KA**, Hubchak SC, Cid MC, Martin MB, Kleinman HK, Greene GL, Schnaper HW. Expression of an estrogen receptor by human coronaryartery and umbilical vein endothelial cells. Circulation. 1996 Sep15;94(6):1402-7. doi: 10.1161/01.cir.94.6.1402. PMID: 8822999.

Specimen Report

Species360 13726610 **Local ID**: MADISON / 3010
GAN
Ursus maritimus Polar bear
Studbooks EAZA, WAZA, AZA, JAZA
Order Carnivora **Family** Ursidae
IUCN Vulnerable (VU) **CITES** II
Start Date Jan 01, 1800 **End Date** Nov 12, 2024

Basic Animal Information

No Local Data Differences Found

Sex - Contraception	Female -	**Status**	Alive
Birthdate - Age	Dec 28, 1998 - 25Y,10M,15D	**Preferred ID**	MADISON / 3010
Origin	Denver Zoological Garden	**Rearing**	Parent
Birth Type	Captive Birth/Hatch	**Hybrid Status**	Not Hybrid
Sire	785219 (DENVER / 10441)	**Dam**	MIG12-28893737 (DENVER / 10442)
Current Collection	Main Institution Animal Collection	**Collection Trip**	
Clutch / Litter		**Enclosure**	Bears

Visit History

Date in	Acquisition - Vendor/Local ID	Phy	Own	Reported By	Disposition - Recipient/Local ID	Phy	Own	Date Out
Dec 28, 1998	Birth/Hatch	In	In	DENVER / 980456	Sale CINCINNAT/100065	Out	Out	Jun 28, 2000
Jun 30, 2000	Purchase DENVER/980456	In	In	CINCINNAT / 100065	Loan Out To MADISON/3010	Out	-	Nov 21, 2016
Nov 21, 2016	Loan In From Sender: CINCINNAT/100065 Vendor: CINCINNAT/100065	In	-	MADISON / 3010		-	-	
		-	-	CINCINNAT / 100065	Donation to (ownership only) MADISON/3010	-	Out	Mar 07, 2022
Mar 07, 2022	Donation From Vendor: CINCINNAT/100065	-	In	MADISON / 3010		-	-	

Identifiers

Reported By	Effective Date	Type	Identifier	Location	Status	Comments
MADISON	Nov 21, 2016	Local ID	3010		Active	
CINCINNAT	Feb 04, 2015	Undetermined	100065 / Berit		Active	
CINCINNAT	Nov 19, 2002	Regional Studbook Number	AZA/1122		Active	Legacy SLocation: AZA Legacy Comment:
CINCINNAT	Aug 30, 2000	International Studbook Number	1642		Active	
CINCINNAT	Jul 22, 2000	Transponder	00-01BE-1C63		In-use	
CINCINNAT	Jul 01, 2000	House name	Berit		Active	
CINCINNAT	Jun 30, 2000	Local ID	100065		Active	
DENVER	Feb 18, 1999	House name	BERIT		Active	
DENVER	Dec 28, 1998	International Studbook Number	1642		Active	
WAZA	Dec 28, 1998	International Studbook Number	1642		Active	Studbook: Ursus maritimus
DENVER	Dec 28, 1998	Regional Studbook Number	AZA/1122		Active	Legacy SLocation: AZA Legacy Comment:
AZA	Dec 28, 1998	Regional Studbook Number	AZA/1122		Active	Studbook: Ursus maritimus
DENVER		Local ID	980456		Active	
MADISON		Old accession number	CQS16-00029-IA			

Sex Information

Reported By	Date	Sex	Comments
MADISON	Nov 21, 2016	Female	
CINCINNAT	Jun 30, 2000	Female	
DENVER	Dec 28, 1998	Female	

Parent Info

Reported By	In ZIMS	Parent Info	Type / Probability	Birth Date	Comments
CINCINNAT	Yes	MIG12-28893737 [DENVER / 10442]	Dam/100%	Nov 29, 1985	
CINCINNAT	Yes	785219 [DENVER / 10441]	Sire/100%	Dec 04, 1985	
DENVER	Yes	MIG12-28893737 [DENVER / 10442]	Dam/100%	Nov 29, 1985	
DENVER	Yes	785219 [DENVER / 10441]	Sire/100%	Dec 04, 1985	

Ancestry Information (calculated by Species360 from shared data)

% Pedigree Known	% Pedigree Certain	Taxonomic Inconsistencies	No. Identified Ancestors
100.00%	100.00%	No	10

Rearing Information

Reported By	Start Date	End Date	Rearing	Comments
CINCINNAT	Jun 30, 2000		Parent	

No Life Stage Information Found

Enclosure History

Enclosure Name	Date Moved In	Date Moved Out	Transfer Reason	Comments
MADISON	Nov 21, 2016	Nov 21, 2016	Acquisition Event	
Bears	Nov 21, 2016		Acquisition Event	

No Management Plan Found

Permits

Name	ID	Authority	Assigned	Details
08868C	Polar Bear Transport Authorization	US Fish and Wildlife Service [General]		

Specimen Report

Species360 13726610 **Local ID**: MADISON / 3010
GAN
Ursus maritimus Polar bear
Studbooks EAZA, WAZA, AZA, JAZA
Order Carnivora **Family** Ursidae
IUCN Vulnerable (VU) **CITES** II
Start Date Jan 01, 1800 **End Date** Nov 12, 2024

Basic Animal Information

No Local Data Differences Found

Sex - Contraception	Female -	**Status**	Alive
Birthdate - Age	Dec 28, 1998 - 25Y,10M,15D	**Preferred ID**	MADISON / 3010
Origin	Denver Zoological Garden	**Rearing**	Parent
Birth Type	Captive Birth/Hatch	**Hybrid Status**	Not Hybrid
Sire	785219 (DENVER / 10441)	**Dam**	MIG12-28893737 (DENVER / 10442)
Current Collection	Main Institution Animal Collection	**Collection Trip**	
Clutch / Litter		**Enclosure**	Bears

Visit History

Date in	Acquisition - Vendor/Local ID	Phy	Own	Reported By	Disposition - Recipient/Local ID	Phy	Own	Date Out
Dec 28, 1998	Birth/Hatch	In	In	DENVER / 980456	Sale CINCINNAT/100065	Out	Out	Jun 28, 2000
Jun 30, 2000	Purchase DENVER/980456	In	In	CINCINNAT / 100065	Loan Out To MADISON/3010	Out	-	Nov 21, 2016
Nov 21, 2016	Loan In From Sender: CINCINNAT/100065 Vendor: CINCINNAT/100065	In	-	MADISON / 3010		-	-	
		-	-	CINCINNAT / 100065	Donation to (ownership only) MADISON/3010	-	Out	Mar 07, 2022
Mar 07, 2022	Donation From Vendor: CINCINNAT/100065	-	In	MADISON / 3010		-	-	

Identifiers

Reported By	Effective Date	Type	Identifier	Location	Status	Comments
MADISON	Nov 21, 2016	Local ID	3010		Active	
CINCINNAT	Feb 04, 2015	Undetermined	100065 / Berit		Active	
CINCINNAT	Nov 19, 2002	Regional Studbook Number	AZA/1122		Active	Legacy SLocation: AZA Legacy Comment:
CINCINNAT	Aug 30, 2000	International Studbook Number	1642		Active	
CINCINNAT	Jul 22, 2000	Transponder	00-01BE-1C63		In-use	
CINCINNAT	Jul 01, 2000	House name	Berit		Active	
CINCINNAT	Jun 30, 2000	Local ID	100065		Active	
DENVER	Feb 18, 1999	House name	BERIT		Active	
DENVER	Dec 28, 1998	International Studbook Number	1642		Active	
WAZA	Dec 28, 1998	International Studbook Number	1642		Active	Studbook: Ursus maritimus
DENVER	Dec 28, 1998	Regional Studbook Number	AZA/1122		Active	Legacy SLocation: AZA Legacy Comment:
AZA	Dec 28, 1998	Regional Studbook Number	AZA/1122		Active	Studbook: Ursus maritimus
DENVER		Local ID	980456		Active	
MADISON		Old accession number	CQS16-00029-IA			

Sex Information

Reported By	Date	Sex	Comments
MADISON	Nov 21, 2016	Female	
CINCINNAT	Jun 30, 2000	Female	
DENVER	Dec 28, 1998	Female	

Parent Info

Reported By	In ZIMS	Parent Info	Type / Probability	Birth Date	Comments
CINCINNAT	Yes	MIG12-28893737 [DENVER / 10442]	Dam/100%	Nov 29, 1985	
CINCINNAT	Yes	785219 [DENVER / 10441]	Sire/100%	Dec 04, 1985	
DENVER	Yes	MIG12-28893737 [DENVER / 10442]	Dam/100%	Nov 29, 1985	
DENVER	Yes	785219 [DENVER / 10441]	Sire/100%	Dec 04, 1985	

Ancestry Information (calculated by Species360 from shared data)

% Pedigree Known	% Pedigree Certain	Taxonomic Inconsistencies	No. Identified Ancestors
100.00%	100.00%	No	10

Rearing Information

Reported By	Start Date	End Date	Rearing	Comments
CINCINNAT	Jun 30, 2000		Parent	

No Life Stage Information Found

Enclosure History

Enclosure Name	Date Moved In	Date Moved Out	Transfer Reason	Comments
MADISON	Nov 21, 2016	Nov 21, 2016	Acquisition Event	
Bears	Nov 21, 2016		Acquisition Event	

No Management Plan Found

Permits

Name	ID	Authority	Assigned	Details
08868C	Polar Bear Transport Authorization	US Fish and Wildlife Service [General]		