



Date: Friday, April 10, 2020 11:48:13 AM

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View: SF: Basic Information

Basic Information

1. * Select research team:

Rico-Guevara

2. * Title of protocol:

Relating Floral Characteristics to Hummingbird Behavioral Ecology

3. * Short title:

Relating Floral Characteristics to Hummingbird Behavioral Ecology

4. * Summary of research:

Hummingbirds demonstrate high metabolic demands, and much of their behavior is associated with meeting these needs. Our research has two goals: (1) to examine how variation in floral traits mediates preferences in different hummingbird species and across different age groups and sexes, and (2) to explore the role of floral traits in determining the social behaviors hummingbirds exhibit towards each other. To do this, we will implant small passive integrated transponder tags (PIT tags) subcutaneously to track individual hummingbird visitations to artificial feeders. These feeders will vary in sugar concentration, volume, flower size, and flower orientation across the field site. We will also video record hovering flight at the feeders using high-speed videography and interactions between different individuals with standard videos. Lastly, we will connect the foraging preferences of hummingbirds to their morphology and physiology by measuring metabolic rates during hovering and the aerodynamic capabilities of these animals.

5. * Principal investigator:

Alejandro Rico-Guevara

6. * What is the intention of the animal protocol?

Field Research

Protocol Team Members

1. Identify each additional person involved in the design, conduct, or reporting of the research:

Name	Role	Involved in Animal Handling	Authorized To Order Animals	E-mail	Phone
David Cuban	Research Assistant/Technician	yes	no	dcuban@uw.edu	
Derrick Jordan Edgar Groom	Postdoctoral Fellow	yes	no	dgroom@uw.edu	
Kristiina Hurme	Research Scientist	yes	no	hurme@uw.edu	
Alyssa J Sargent	Research Assistant/Technician	yes	no	sargena@uw.edu	

2. If veterinary care will be provided by individuals outside of DCM or WaNPRC, provide the name, credentials and contact information below:

Funding Sources

1. Identify each organization supplying funding for the protocol:

Funding Organization	eGC1 Number(s)
View University of Washington	

Scientific Aims

1. * Scientific aims of the research:

Hummingbirds and other hovering animals have high metabolic rates, and are well adapted to efficiently extract nectar from flowers to meet their energetic needs. However, much of our understanding of nectar foraging energetics comes from captive studies focusing solely on the traits of the hummingbird, and do not incorporate the traits of the flowers they feed from. Floral traits may have an impact upon the energetic benefits for their pollinator due to variations in nectar concentration, nectar volume, flower size, and flower orientation. Concentration and volume determine how much sugar the hummingbirds receive from a single meal, while the size and orientation of the flower can interfere with the motions of the wings during hovering, leading to less efficient hovering. Together, variation in floral traits may result in varying amounts of energetic profit recovered from each flower that they forage upon. However, we do not have an understanding of what floral characteristics are considered optimal by hummingbirds, and this likely varies with the species, age, and sex of the hummingbirds. One goal of this study is to determine what combination of floral characteristics result in optimal energetic profit across the different hummingbirds in a free-living system. We hypothesize that variation in morphology and physiology that we see across hummingbird ages, sexes, and species results in different preferred floral characteristics in free-living animals. This will result in differences in flower preferences.

Being on the brink of energetic deficit due to their high metabolic demands, hummingbirds spend a considerable amount of time everyday foraging and defending territories that supply them with their much-needed energy. As such, hummingbirds likely choose and defend flowers and patches that maximize their energetic profit. However, we currently do not know how different floral characteristics may affect the social dynamics between conspecifics, between sexes, and between different species. The second goal of our study is to examine the impact of floral characteristics and how it affects social interactions between hummingbirds. We hypothesize that the most profitable flower traits will result in a greater number of altercations. However, we also hypothesize that flowers that result in higher competition may attenuate the profitability of preferred flowers.

Overall, this research aims to link morphology and physiology to the social behaviors we see in hummingbirds. The highly aggressive nature of some species may stem from their high metabolic demands, and we predict that many of their behaviors are mediated through foraging to meet their daily energetic needs. This work will provide insight into the potential drivers of pollinator behavior.

2. * Using language understandable to non-scientists, describe the goals and significance of the protocol to humans, animals and science:

Hummingbirds have immense sugar needs because their metabolic rates are so high, and they meet these needs by feeding on floral nectars. However, most of our understanding regarding the energy demands for these animals has focused on their individual traits (such as the size of their wings and their body mass) and less so on the traits of the flowers they feed from. Flowers can play an important role in setting the amount of energy that pollinators gain from a single meal, because the volume and concentration of the nectar determines how much sugar they gain, while the size and orientation of the flower determines how much energy they expend to receive it.

Additionally, the preferences for specific flower traits may mediate many of the behaviors

we see in hummingbirds, including aggressive confrontation. This research will give insight into the close association between flowers and the pollinators that feed from them, and the fundamental underpinnings of this unique ecological interaction.

3. * Provide a statement to address the potential harm to the animals on this study (e.g., pain, distress, morbidity, mortality) relative to the benefits to be gained by performing the proposed work:

Our study is primarily interested in the social dynamics and behaviors that free-living hummingbirds exhibit in natural conditions. In order to achieve this goal, we primarily rely on techniques that minimize direct human interventions, reducing distress. Additionally, the PIT tagging used to identify different individuals results in low morbidity and mortality and can provide more reliable data than other tracking techniques. These PIT tags can also provide long-term data for the lifetime of the animal, and complementary studies can be performed on the populations that we tag.

Experiments

Note: If you will be administering cells, cell lines, sera or other biologicals to rodents, contact the Rodent Health Monitoring Program (RHMP, rhmp@uw.edu). Testing may be required prior to administration to rodents.

1. * Define the experiments to be used in this protocol:

Name	Species	USDA Count	Pain Category	Count by Procedures	Husbandry Exception Types
Hummingbird flight energetics, performance, and behaviors	Birds - no Other	200	B: 0 C: 200 D: 0 E: 0	<ul style="list-style-type: none">■ Capture/Trapping: Rico-Guevara - Mist netting (Team)■ Capture/Trapping: Rico-Guevara - Hummingbird Drop Trap v2 (Team)■ Implants: Rico-Guevara - PIT Tag Implantation (Team)■ Other: Rico-Guevara - Maximum flight performance test (Team)■ Other: Rico-Guevara - Feeder Mask Respirometry (Team)	Birds - No husbandry or enrichment exceptions.
Mask-respirometry of Hummingbirds	Birds - no Other	20	B: 0 C: 20 D: 0 E: 0	<ul style="list-style-type: none">■ Capture/Trapping: Rico-Guevara - Mist netting (Team)■ Capture/Trapping: Rico-Guevara - Hummingbird Drop Trap v2 (Team)■ Capture/Trapping: Rico-Guevara - Hummingbird Drop-Door Trap v1 (Team)■ Euthanasia: Rico-Guevara - Drop euthanasia (Team)■ Other: Rico-Guevara - Feeder Mask Respirometry (Team)	Birds - No husbandry or enrichment exceptions.

2. Will any single animal undergo more than one survival surgery? (include any animal that underwent surgery prior to use on this protocol) ☐ Yes ☒ No

Procedure Personnel Assignment

1. * Select the team members who will be performing each procedure:

Procedure	Species	Is USDA Species	Team Members
Capture/Trapping: Rico-Guevara - Hummingbird Drop Trap v2, ver. 1 (Team)	Birds - Other	no	Kristiina Hurme Alejandro Rico- Guevara David Cuban Derrick Jordan Edgar Groom Alyssa J Sargent
Capture/Trapping: Rico-Guevara - Hummingbird Drop-Door Trap v1, ver. 1 (Team)	Birds - Other	no	Kristiina Hurme Alejandro Rico- Guevara David Cuban Derrick Jordan Edgar Groom Alyssa J Sargent
Capture/Trapping: Rico-Guevara - Mist netting, ver. 1 (Team)	Birds - Other	no	Kristiina Hurme Alejandro Rico- Guevara David Cuban Derrick Jordan Edgar Groom Alyssa J Sargent

Procedure	Species	Is USDA Species	Team Members
Euthanasia: Rico-Guevara - Drop euthanasia, ver. 1 (Team)	Birds - Other	no	Kristiina Hurme Alejandro Rico- Guevara David Cuban Derrick Jordan Edgar Groom Alyssa J Sargent
Implants: Rico-Guevara - PIT Tag Implantation, ver. 1 (Team)	Birds - Other	no	Kristiina Hurme Alejandro Rico- Guevara David Cuban Derrick Jordan Edgar Groom Alyssa J Sargent
Other: Rico-Guevara - Feeder Mask Respirometry, ver. 1 (Team)	Birds - Other	no	Kristiina Hurme Alejandro Rico- Guevara David Cuban Derrick Jordan Edgar Groom Alyssa J Sargent
Other: Rico-Guevara - Maximum flight performance test, ver. 1 (Team)	Birds - Other	no	Kristiina Hurme Alejandro Rico- Guevara David Cuban Derrick Jordan Edgar Groom Alyssa J Sargent

2. Team member training:

First Name Last Name Training

David Cuban	Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date	No experience data to display
	Animal Use Laws & Regulations	General	Online	Basic Course	Stage 1	2/20/2020	2/20/2025	
	Animal Use Medical Screening	General	Online	Basic Course	Stage 1	2/26/2020	2/28/2023	
Derrick Jordan Edgar	Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date	No experience data to display
	Animal Use Laws & Regulations	General	Online	Basic Course	Stage 1	2/19/2020	2/19/2025	
	Animal Use Medical Screening	General	Online	Basic Course	Stage 1	2/25/2020	2/28/2023	
Kristiina Hurme	Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date	No experience data to display
	Animal Use Laws & Regulations	General	Online	Basic Course	Stage 1	3/31/2020	3/31/2025	
	Animal Use Medical Screening	General	Online	Basic Course	Stage 1	4/1/2020	4/30/2023	
Alejandro Rico-Guevara	Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date	No experience data to display
	Animal Use Medical Screening	General	Online	Basic Course	Stage 1	10/14/2019	10/31/2022	
	Animal Use Laws & Regulations	General	Online	Basic Course	Stage 1	10/14/2019	10/14/2024	
Alyssa J Sargent	Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date	No experience data to display
	Animal Use Laws & Regulations	General	Online	Basic Course	Stage 1	3/2/2020	3/2/2025	

Animal Details

1. * How are animals acquired?

Captured

2. Describe the acquisition for:

3. Identification of individual animals (other than cage cards):

a. Method(s) (e.g., ear punch/tag, tattoo, tagging/banding, radio collar, etc.)

(Note: If method is implantation (e.g. PIT tag), create or select an Implant procedure to describe the details. If method is surgical (e.g., satellite tag), create or select Survival Surgery procedure to describe the details):

Free-living birds will be identified by their PIT tag.

b. Will external identification be replaced if it falls off/out? If yes, describe the plan for replacement:

No.

c. Will external identification be removed as part of the protocol (e.g., radio collars on field animals)? If yes, describe the plan for removal:

No, as the PIT tags do not negatively affect the hummingbirds.

4. Identify strain/stock for rodents and genetically modified animals:

Species	Is USDA Species	Strain	Genetically Modified Strain	Phenotype Description
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There are no items to display

Animal Number Adjustments

“Animals Identified in Experiments” is the total number of animals per pain category listed in all experiments on this protocol. If more or fewer animals will be used on the protocol (see Help Text for examples), click Update to enter this new number in the corresponding “Adjusted Animal Count” column. ****Only input numeric values in this field; 0 is acceptable.****

If no adjustment is required, the values in the “Animals Identified in Experiments” and “Adjusted Animal Count” columns must match. Click Update in each Pain Category row to input the matching value.

For questions about adjusting animal numbers, contact OAW.

1. * Click Update to adjust the number of animals to be used or produced for this protocol:

	USDA Species Covered Species	Pain Category	Animals Identified in Experiments	Adjusted Animal Count
View	Birds - no Other	Pain Category B	0	0
View	Birds - no Other	Pain Category C	220	200
View	Birds - no Other	Pain Category D	0	0
View	Birds - no Other	Pain Category E	0	0

2. If you adjusted the number of animals for this protocol, explain why:

Animals undergoing the captive mask-respirometry experiment will also undergo PIT tagging.

3. If you will be using animals to train personnel or to practice procedures included in this protocol, describe below:

NA

4. Supporting documents:

Document Name	Date Modified
There are no items to display	

Alternatives and Duplication Searches

Display Procedures that cause pain or distress:

- [Implants: Rico-Guevara - PIT Tag Implantation, ver. 1 \(Team\)](#)

1. Record all searches for any previous research that this protocol might duplicate:

Search Date	Searched Databases	Other
View 3/26/2020	Scopus Web of Science (searches multiple databases)	Google Scholar

2. Briefly describe the results of your searches and why you can or cannot incorporate the findings. Or, if a literature search was not performed, describe the methods used to determine that alternatives are not available or feasible:

During the search process, I have found two other methods of tracking hummingbirds (not recapturing): using radiotelemetry and visual markers.

1) Radiotelemetry tags currently on the market are nearly 10-times heavier than PIT tags and do not last from season to season because they rely on internal batteries, and they do not provide life-time identification. Radiotelemetry also does not have the spatial resolution of PIT tags, which would be problematic for closely-spaced feeders proposed in this study.

2) Visual markers, such as back or leg "flags" (color bands would not be visible in hummingbirds) require constant observation from researchers, leading to missing data or significantly scaling back on the number of feeders to match the number of observers. Video identification of individuals based on color combinations of the flags is limited by the color combinations that would fit in tiny flags and the camera resolution, changes in daylight, etc. which makes it an unreliable identification technique.

The use of PIT tags as we have proposed will increase the amount of data that is being collected while minimizing the amount of additional weight that the hummingbirds will carry. It will also permit the deployment of multiple feeders for the detection of PIT tags across a larger landscape.

Of the papers that have used PIT tagging methods in hummingbirds, none have reported combining PIT tagging technology with respirometry and high-speed videography as we propose, and none has been performed in tropical species. Thus, our work will not result in unnecessary duplication of previous work.

3. Confirm that you have made every effort to ensure that this protocol is not unnecessary duplication of previous research: ☒

Field Research Details

1. * Where will these studies take place?

King, Snohomish, Skagit, Whatcom, Island, San Juan, Jefferson, and Kitsap Counties, Washington state.

2. * Will these animals be housed for 12 or more hours?

☐ Yes ☒ No

3. If yes, describe duration, type of enclosure, and monitoring plan:

Hummingbirds will be temporarily housed in mesh, collapsible cages (approximately 0.6 x 0.6 x 0.9 m) with access to a hummingbird feeder. All hummingbirds will be housed for less than 12 hours in duration.

4. Upload pictures and/or diagrams of housing location and enclosures, as appropriate:

Name	Category	Date Modified
Collapsible cage		3/31/2020 4:14 PM

5. Describe your monitoring plan(s) during and after procedures, including frequency and specific behavioral and clinical signs to be monitored:

After PIT tag implantation, hummingbirds will be put into a recovery cage and offered sugar water. Once flight has returned to normal, the hummingbird will be released.

6. Describe how animals will be transported to and from this location, including container and route (Note: Use of private vehicles requires IACUC approval):

All procedures will be done at the field site.

7. * Describe your plan if animals become distressed or injured:

If an animal becomes distressed, as indicated by lethargy, fluffed feathers, labored breathing, and closed eyes, all procedures on that animal will be stopped. The individual will immediately be isolated and offered sugar water (20-25% sugar). Upon recovery, the animal will be released.

If severely injured, the animal will be euthanized.

8. * Identify required permits to conduct the field research:

Washington State Department of Fish and Wildlife - Scientific Collection Permit
US Fish and Wildlife Service - Scientific Collection Permit

Disposition

1. Disposition plans for the animals when this research is complete:

(check all that apply)

Other (e.g., adoption, release; provide explanation below)

2. If other, provide an animal disposition description:

Hummingbirds will be released at the site of capture following PIT tag implant.

3. If protocol involves fixing tissues, list agents (e.g., paraformaldehyde, formalin):

NA

Refinement, Replacement and Reduction

- 1. Describe below how the three R's (refinement, replacement and reduction) have been employed on this project. Include alternatives that were considered for the procedures above that cause pain or distress:**

*** Refinement (use of methods to decrease animals' sensitivity to pain)**

We are primarily using capture methods that reduce the chance of capturing non-target species. We are also primarily using techniques that utilize free-living hummingbirds, reducing the amount of handling each individual bird undergoes.

PIT tagging with a local anesthesia leads to quick recovery and faster release of the animal.

*** Replacement (include in vitro tests, use of less sentient animals)**

As our study specifically examines social dynamics and morphological/physiological capabilities of hummingbirds, replacements are unavailable.

*** Reduction (use of fewer animals to attain statistical significance)**

The use of free-living hummingbirds will significantly reduce the stress that an animal experiences compared to using a captive environment. However, the social dynamics at the field site are largely uncontrolled, and one target species (Rufous hummingbirds) are less dominant. We will receive far fewer measurements from these individuals. In order to capture as many inter- and intraspecific interactions as possible, including from the more submissive individuals, we will need to tag as many hummingbirds as possible.

- 2. Describe the rationale for using animals and the appropriateness of the species proposed:**

Hummingbirds display wide variation in flight performance, morphology, social behaviors, and combative capabilities.

Supporting Documents

1. Attach supporting files:

Document Name

Date Modified

There are no items to display

Procedures Appendix:



View: Custom SF: Procedure Identification

Procedure Identification: Rico-Guevara - Feeder Mask Respirometry

1. * Name of the procedure or surgery:

Rico-Guevara - Feeder Mask Respirometry

2. * Select procedure type:

Other

3. * Species:

Birds - Other

4. * Will administering this procedure cause any more than momentary pain or distress? Yes No

If yes,

i. Identify expected symptoms from administering this procedure:

ii. Identify criteria under which animals will be removed from research:

Other

1. Description of Procedure:

Mask respirometry is a technique for measuring an animal's metabolic rate, by determining the rates of oxygen consumption and carbon dioxide production of an animal. The mask is in a fixed location, and the hummingbird flies to, and hovers at, the mask to receive a meal of sugar water. While it is hovering at the mask, the respired gases are collected and the concentration of the gases is determined by gas analyzers.

The mask is constructed of a custom-made long tube approximately the same length as the head of the hummingbird. The width of the mask is approximately 10-20% wider than the width of the head of the hummingbird, ensuring that the head of the animal does not get stuck in the mask. The mask is fitted with an outlet situated close to the nares of the hummingbird that withdraws air using an air pump, and a small tube at the end of the mask that provides a 20% sucrose solution (see attached figures).

Hummingbirds are first trained to use the respirometry mask before data collection occurs. At the beginning of training, hummingbirds will be guided to the mask by hand to receive a nectar meal. Once it consistently feeds while being handled, a perch will be placed in front of the respirometry mask to encourage the animal to receive a nectar meal of its own will. After they are able to freely feed inside the mask while perched, the perch will be gradually moved away from the mask until the hummingbird maintains hovering flight while they receive the sucrose meal.

During training, hummingbirds will be continuously monitored. Hummingbirds will be fed every 45 minutes, either from the mask or from a nectar syringe by hand if they are not able to feed from the mask on their own.

Hummingbirds will be trained four times per week until they are able to feed inside the mask while hovering. Each training session will be a maximum of 4 hours in duration. Based on previous experience, hummingbirds are able to freely hover at the mask within 1-2 training sessions.

On testing days, hummingbirds will be given access to feed from the mask respirometer at least once an hour.

Procedure Documents

1. Supporting documents:

Document Name	Date Modified
Hummingbird Hovering - Mask Resp.png	2/25/2020 2:07 PM



View: Custom SF: Procedure Identification

Procedure Identification: Rico-Guevara - Mist netting

1. * Name of the procedure or surgery:

Rico-Guevara - Mist netting

2. * Select procedure type:

Capture/Trapping

3. * Species:

Birds - Other

4. * Will administering this procedure cause any more than momentary pain or distress? Yes No

If yes,

i. Identify expected symptoms from administering this procedure:

None

ii. Identify criteria under which animals will be removed from research:

None

Capture/Trapping

Obtaining required permits (State, Federal, and International) is the responsibility of the PI and is required prior to the start of the project.

1. Describe Capture/Trapping Protocol or Procedure:

Mist net: Netting captures birds passively as they move. Non-lethal.

As per "The North American Banders' Manual for Hummingbirds," 2019, Stephen Russell and Ruth Russell:

Place one or more nets (24-mm mesh) perpendicular to flight paths where hummingbirds are seen moving predictably. Placing feeders strategically may attract birds to sites where they can more conveniently be captured. Nets set in lines are most effective when the air is still and temperatures moderate. Light wind makes the nets more visible, and stronger wind causes the netting to become taut, increasing the chances of injury. Nets placed in shadows and against dark backgrounds are less visible and may be more productive.

If conditions are extreme, i.e., windy, in sun, or temperatures <20 C (68 F) or >32 C (90 F), net checks will occur more frequently or will be closed if conditions could harm a bird.

2. How frequently will traps or capture equipment be monitored?

At least every 15 min in most conditions. Every 5 minutes in more extreme conditions.

3. Duration animals will be in traps, restrained or immobilized:

At most, 15 minutes.

4. Indicate non-target species that may be inadvertently captured:

Any species not listed under relevant permits.

5. Disposition of target and non-target animals (e.g., euthanized, released, etc.):

Target: released, with subcutaneous passive integrative transponder (PIT) tag

Non-target: Immediately released.

6. Could the same animal be captured more than once?

Yes, however they will be released immediately upon capture.

7. * Provide scientific justification for choice of capture or trapping methods:

From "The North American Banders' Manual for Hummingbirds," 2019, Stephen Russell and Ruth Russell:

Nets may be useful where feeders are not used, in situations with high numbers of birds at concentrations of food, such as at flowering trees or in meadows with many hummingbird-pollinated flowering plants.

8. In the event of accidental injury to an animal during capture, list euthanasia criteria and method:

Any animal injured will be humanely euthanized.

Hummingbirds can become more tangled with longer times in the net, potentially causing injury, our rate of checking will result in a low probability of injury.

Injured animals will be euthanized using an overdose of isoflurane followed by decapitation.

Procedure Documents

1. Supporting documents:

Document Name

Date Modified

There are no items to display



HoverBoard

View: Custom SF: Procedure Identification

Procedure Identification: Rico-Guevara - Maximum flight performance test

1. * Name of the procedure or surgery:

Rico-Guevara - Maximum flight performance test

2. * Select procedure type:

Other

3. * Species:

Birds - Other

4. * Will administering this procedure cause any more than momentary pain or distress? Yes No

If yes,

i. Identify expected symptoms from administering this procedure:

NA

ii. Identify criteria under which animals will be removed from research:

NA

Other

1. Description of Procedure:

This procedure tests for the maximum take-off performance of a hummingbird, as described by Chai et al. (1997; J. Exp. Biol 200: 921-929). Here, we encourage the hummingbird to fly with an increasing amount of weight until it flies with its maximum total mass.

An elastic harness fitted attached to a long chain of threaded beads is placed over the head of the animal. The total mass of the threaded beads + harness should be greater than 20x the mass of the animal, well in excess of the flight capabilities of the bird, to ensure that the bird does not fly away and escape with the harness.

The animal is released from the bottom of the cage, eliciting the escape response and a vertical take off. Because the mass of the beads is in excess of the maximum flight capabilities of the hummingbird, the bird will return and land on the cage floor. The entire flight is recorded using high-speed videography.

The entire procedure, from attaching the harness to recording the flight, should take no longer than 5 minutes.

Procedure Documents

1. Supporting documents:

Document Name

Date Modified

There are no items to display



HoverBoard

View: Custom SF: Procedure Identification

Procedure Identification: Rico-Guevara - Hummingbird Drop Trap v2

1. * Name of the procedure or surgery:

Rico-Guevara - Hummingbird Drop Trap v2

2. * Select procedure type:

Capture/Trapping

3. * Species:

Birds - Other

4. * Will administering this procedure cause any more than momentary pain or distress? Yes No

If yes,

i. Identify expected symptoms from administering this procedure:

ii. Identify criteria under which animals will be removed from research:

Capture/Trapping

Obtaining required permits (State, Federal, and International) is the responsibility of the PI and is required prior to the start of the project.

1. Describe Capture/Trapping Protocol or Procedure:

Hummingbird feeders containing a 20-25% sucrose solution (using white, refined sugar) will be made available to attract hummingbirds to the trapping location. A ratio of 4 parts water to 1 part sugar is recommended, though in winter conditions a 3:1 ratio may be appropriate. This sucrose solution will be changed 3 times per week, or any point at which the solution becomes cloudy. Feeders will be removed to be cleaned at least once a week, with extra feeders on hand to replace them, in order to avoid disrupting the hummingbirds. Feeders will be occasionally soaked in a solution of one part bleach to ten parts water, full-strength white vinegar or full strength (3%) hydrogen peroxide; a bottle brush or pipe cleaner will be used to remove any mold or grime. Disinfected feeders will be thoroughly rinsed and allowed to dry before next use.

A hummingbird feeder will be housed inside an open-sided, collapsible netting trap. The outside of this cylindrical hanging trap is made of netting that falls to the base when the trap is triggered; while in use, the trap will be opened 1/3 to 1/2 way. Birds may approach the central hanging feeder from 360 degrees and may come and go unrestricted until the trap is triggered, either manually or remotely. The netting is attached to a monofilament line (i.e. fishing line) that keeps the mesh bundled as long as the line is taut. Release of the line causes the mesh to drop.

During trapping operations, the trapper will hold the line to keep the mesh up. Upon entry of a hummingbird, the trapper will release the line, causing the trap to fall around the hummingbird. The release of the line will only occur once the hummingbird is feeding from the hummingbird feeder. The trapper will then retrieve the hummingbird from the trap and bring the animal into captivity.

When the trap is not in use, only the frame (no curtain) may be hung for pre-baiting purposes, to allow birds to become accustomed to approaching a feeder with an object above their heads.

2. How frequently will traps or capture equipment be monitored?

Traps will be continuously monitored during trapping efforts. Trap frames may remain deployed when trapping is completed for the day.

3. Duration animals will be in traps, restrained or immobilized:

As the traps are continuously monitored, hummingbirds will be immediately retrieved from the trap.

4. Indicate non-target species that may be inadvertently captured:

As only hummingbird species arrive at a hummingbird feeder, we do not expect non-target species to be inadvertently captured.

5. Disposition of target and non-target animals (e.g., euthanized, released, etc.):

Non-target animals will be immediately released.

Target animals will be immediately assessed and brought into captivity.

6. Could the same animal be captured more than once?

Yes. However, non-target individuals will be immediately released.

7. * Provide scientific justification for choice of capture or trapping methods:

This method of trapping provides minimal harm to hummingbirds compared to other trapping methods. The soft mesh is gentle and incredibly fine, minimizing any risk of hummingbird injury and negating the possibility of any birds becoming tangled. By ensuring that birds are feeding at the center of the trap prior to triggering the mesh drop, the possibility of injury is lowered even further. When hummingbirds are frightened, they tend to fly upwards, which complements the use of a drop-trap.

This use of a baited trap is different from traditional bird capture techniques, which usually relies on mist nets. The drop trap allows for more efficient retrieval of birds compared to mist netting, minimizing stress and handling time.

8. In the event of accidental injury to an animal during capture, list euthanasia criteria and method:

In the unlikely event of injury caused by the trap, the hummingbird will be brought into captivity and monitored. A veterinarian will be immediately consulted for assessment.

Euthanasia will be delivered through an overdose of inhaled isoflurane followed by cervical dislocation.

Procedure Documents

1. Supporting documents:

Document Name	Date Modified
Hummingbird drop trap.docx	3/4/2020 1:34 PM



View: Custom SF: Procedure Identification

Procedure Identification: Rico-Guevara - Hummingbird Drop-Door Trap v1

1. * Name of the procedure or surgery:

Rico-Guevara - Hummingbird Drop-Door Trap v1

2. * Select procedure type:

Capture/Trapping

3. * Species:

Birds - Other

4. * Will administering this procedure cause any more than momentary pain or distress? Yes No

If yes,

i. Identify expected symptoms from administering this procedure:

ii. Identify criteria under which animals will be removed from research:

Capture/Trapping

Obtaining required permits (State, Federal, and International) is the responsibility of the PI and is required prior to the start of the project.

1. Describe Capture/Trapping Protocol or Procedure:

Hummingbird feeders containing a 20-25% sucrose solution will be made available to attract hummingbirds to the trapping location. This sucrose solution will be changed 3 times per week.

A hummingbird feeder will be housed inside a metal cage, with a single door that closes due to gravity. The door is attached to a monofilament line (ie. fishing line) that keeps the door open as long as the line is taut. Release of the line causes the door to shut.

During trapping operations, the trapper will hold the line to keep the trap door open. Upon entry of a hummingbird, the trapper will release the line, causing the trap door to close upon the hummingbird. The release of the line will only occur once the hummingbird is feeding from the hummingbird feeder. The trapper will then retrieve the hummingbird from the trap and bring the animal into captivity.

When the trap is not in use, the door will be locked open.

2. How frequently will traps or capture equipment be monitored?

Traps will be continuously monitored during trapping efforts. Traps will remain deployed with the drop door locked open when trapping is completed for the day.

3. Duration animals will be in traps, restrained or immobilized:

As the traps are continuously monitored, hummingbirds will be immediately retrieved from the trap.

4. Indicate non-target species that may be inadvertently captured:

As hummingbirds are the sole species that arrive at a hummingbird feeder, we do not expect non-target species to be inadvertently captured.

5. Disposition of target and non-target animals (e.g., euthanized, released, etc.):

Non-target animals will be immediately released.

Target animals will be immediately assessed and brought into captivity.

6. Could the same animal be captured more than once?

Yes. However, non-target individuals will be immediately released.

7. * Provide scientific justification for choice of capture or trapping methods:

This method of trapping provides minimal harm to hummingbirds compared to other trapping methods.

This method of trapping is also different from traditional bird capture techniques, which usually relies on mist nets. The drop-door trap allows for faster retrieval times from the trap compared to mist netting. Further, the possibility of injury is very low, as the trap door

is only closed when the hummingbird is far from the drop-door when they are feeding from the feeder.

8. In the event of accidental injury to an animal during capture, list euthanasia criteria and method:

In the unlikely event of injury caused by the trap, the hummingbird will be brought into captivity and monitored. A veterinarian will be immediately consulted for assessment.

Euthanasia will be delivered through an overdose of inhaled isoflurane followed by cervical dislocation.

Procedure Documents

1. Supporting documents:

Document Name

Date Modified

There are no items to display



HoverBoard

View: Custom SF: Procedure Identification

Procedure Identification: Rico-Guevara - Drop euthanasia

1. * Name of the procedure or surgery:

Rico-Guevara - Drop euthanasia

2. * Select procedure type:

Euthanasia

3. * Species:

Birds - Other

4. * Will administering this procedure cause any more than momentary pain or distress? Yes No

If yes,

i. Identify expected symptoms from administering this procedure:

N/A

ii. Identify criteria under which animals will be removed from research:

N/A

Euthanasia

1. * Method of euthanasia:

Cervical Dislocation

2. Describe procedure:

Drop a cotton ball soaked with isoflurane to the bottom of a deep 50mL container with a twist cap lid. In a well ventilated area and while holding the animal, place the head of the animal within the container making sure that the cotton ball remains at the bottom of the container.

Upon recumbency, the bird is removed from the chamber and a surgical plane of anesthesia is confirmed by lack of response to toe pinch, change in respiratory character and rate.

Then, cervical dislocation will be performed. Only certified protocol personnel will perform this procedure.

This work is conducted within a certified fume hood. Isoflurane is pharmaceutical grade.

Isoflurane is an irritant and may cause reproductive problems in women. Refer to Occupational Health Recommendations.

3. * Will anesthesia be used? Yes No

4. Describe how death will be confirmed:

Death will be confirmed by lack of respirations and heartbeat.

5. Is this method approved by the AVMA Guidelines on Euthanasia (2013)?

Yes No

Procedure Documents

1. Supporting documents:

Document Name

Date Modified

There are no items to display



View: Custom SF: Procedure Identification

Procedure Identification: Rico-Guevara - PIT Tag Implantation

1. * Name of the procedure or surgery:

Rico-Guevara - PIT Tag Implantation

2. * Select procedure type:

Implants

3. * Species:

Birds - Other

4. * Will administering this procedure cause any more than momentary pain or distress? Yes No

If yes,

i. Identify expected symptoms from administering this procedure:

This procedure is expected to cause minor discomfort that should be relieved by local anesthesia. Please see procedure description and/or experimental description for monitoring plan.

ii. Identify criteria under which animals will be removed from research:

All birds will be monitored for signs of pain, distress and other abnormal behaviors. Signs of compromise may include: eyes closed, feathers fluffed as if cold, body limp and tongue protruding, excessive vocalization, or labored breathing, especially a 'jerky' spasmodic motion with each breath (which can also be an indication of aspiration). If a bird shows any above signs, the bird will immediately be removed from the research process (see section on monitoring).

Implants

1. Type, including approximate size and weight:

Biomark Mini HPT10 PIT Tag:

Size: 10.3mm axial length by 1.41mm diameter, capsule shaped

Weight: 35mg (< 1% of body mass of hummingbirds)

2. Site:

Subcutaneous on back, just right of the centerline

3. Maintenance and care of chronic implants:

These tags do not require maintenance. Previous work has shown that in avian studies, long-term PIT tag retention rate is high. In a study on pied flycatchers, there were no measurable negative short-term or long-term effects on individuals that retained subcutaneously implanted PIT tags (Ratnayake et al., 2014; Ornis Fennica 91: 244-255).

4. Method used to sterilize implants:

Sterilized and individually packed by the supplier.

5. Describe implant procedure (if the implant is surgical, create a new Survival Surgery procedure and refer to it here):

This is a two person procedure. One person will secure the bird while the second person performs the implantation.

The bird will be held in the fingertip hold (thumb and second finger on either side of the bird on top of the wings to prevent flapping, and the first finger on the back - this hold allows the bird to remain in a natural, upright position).

For the implantation:

Identify the tag insertion area, just to the side of the centerline of bird and between the scapulae, making sure to avoid any superficial blood vessels.

Clean site with 70% alcohol to clean the area. Apply Bactine or similar product (Lidocaine 2.5% + 0.13% benzalkonium chloride) as a local anesthetic to the area with a cotton swab. Wait two minutes for the local anesthetic to take effect.

Attach the preloaded PIT tag needle to the implanter syringe and push the plunger until it makes contact with the PIT tag in the needle, advancing the tag until it is just inside of the needle orifice.

Using forceps, gently lift the skin. Insert the needle of the PIT tag implanter under the skin at a shallow angle. The needle will be inserted with the bevel of the needle facing "up", as opposed to facing towards the body when inserted. The needle should be inserted deeply enough so that the orifice on the bevel is just inside of the skin.

Inject the pit tag into the bird and remove the needle.

Apply pressure to the injection site to control any bleeding. Apply silver nitrate or styptic powder if necessary.

Close the incision using VetBond (veterinary grade cyanoacrylate glue).

The bird will be offered sugar water and released into a small cage for observation. Once normal flight behaviors have resumed, the bird will be released.

6. Monitoring protocol:

All birds will be monitored for signs of compromise during handling. Signs of compromise may include: eyes closed, feathers fluffed as if cold, body limp and tongue protruding, loss of contour body or tail feathers, excessive vocalizations, or labored breathing, especially a 'jerky' spasmodic motion with each breath (which can also be an indication of aspiration). If a bird shows any signs of distress, the implantation process will be suspended immediately and sugar water offered.

If the bird does not drink and improve, then the bird will be placed in a container where it can recuperate. As per the North American Banding Manual for Hummingbirds, a "mini hospital" chamber will be made from a large round plastic container, with a large circle cut out of the lid and replaced with a fine mesh to allow for adequate ventilation within the chamber. A hand warmer pack may be placed in the container, so long as the warming pack is covered with a thin sock or some material to ensure that the bird cannot come in direct contact with it. A compromised bird will be placed in the container and supported so that the head is upright. The container and bird will be placed in a warm dry place away from noise, wind or other disturbance. The bird's condition will be checked every 5-15 minutes and sugar water may be offered once the bird is able to hold its head upright and is alert enough to feed. Once the bird is able to feed, and is no longer showing signs of distress, it will be immediately released.

If recovery is not likely, the animal will be humanely euthanized in the field.

7. Will it be necessary to surgically remove implants, or to re-implant or repair implants if they fail? If so, describe the circumstances and the maximum number of replacements if applicable:

No, as failure of the implants will not have a negative effect on the health of the animal. A 2014 study (Ratnayake et al.) revealed no measurable negative short-term or long-term effects on Pied Flycatcher individuals that retained subcutaneously implanted PIT tags.

Procedure Documents

1. Supporting documents:

Document Name	Date Modified
MiniHPT8 16 gauge needle image	3/16/2020 1:23 PM
MiniHPT8 datasheet	3/16/2020 1:21 PM
MiniHPT8 image	3/16/2020 1:20 PM
PIT tag implanter	3/16/2020 1:24 PM

1. * Select the funding organization:

University of Washington

If Other was selected in question 1, provide Funding Organization:

2. * All animal use projects must be reviewed for scientific merit prior to initiating animal use. Choose the required reviews for this project:

Has been conducted by my department or school and has been found to be scientifically meritorious

3. Provide name of the committee or the department reviewer (Required if

“Has been conducted by my department or school and has been found to be scientifically meritorious” was selected):

4. eGC1 Number(s):(assigned internally)

Experiments Appendix:

Hummingbird flight energetics, performance, and behaviors

1. * Experiment name:

Hummingbird flight energetics, performance, and behaviors

2. * Species:

Birds - Other

3. If other was selected, provide a species:

Anna's hummingbird (*Calypte anna*) and Rufous hummingbird (*Selasphorus rufus*)

4. What is the scientific goal of this experiment:

The goal of this experiment is to monitor and track feeder usage, social interactions, biomechanics, and energetics at feeders across different species and sexes.

5. * Describe the animal experience in the experiment, from enrollment in the study to the final endpoint, including all procedures in chronological order and the minimum time between procedures. We encourage using bullet points, timeline, table, or a flow chart as appropriate:

1.

Hummingbirds will be baited to the field site using hummingbird feeders filled with 20-25% sucrose solution. The solution to attract the hummingbirds will be replaced every other day, or as needed.

2.

Hummingbirds will be captured at the field site using drop-net traps, drop-door traps, or mist nets (See procedures).

3.

Upon capture, morphological measurements are taken of the wings, bill, and tail using a ruler and calipers followed by photographs of the wings, bill, and tail using against graph paper.

4.

Perform the max test trial to determine the peak performance of each hummingbird.

5.

The PIT tag is implanted subcutaneously on the back of the animal, and the animal is released.

6.

A variety of hummingbird feeders will be made available at the field site. These feeders will vary in sugar concentration from 10-60% and will be changed every other day. These feeders will also be mounted with RFID antennas that will detect PIT tagged hummingbirds while they visit different feeders spread throughout the field site. High-speed and conventional cameras will be mounted at various feeders to record their flight and social interactions while in the area of these feeders. Adjacent to the feeders will be monitors that will play videos of hummingbirds, to induce social behaviors from hummingbirds attempting to feed from the feeders.

7.

One hummingbird feeder will also include a port that will sample the respired gases of a hummingbird to measure oxygen consumption and carbon dioxide production rates while hovering to feed. This respirometer/hummingbird feeder will be open to all hummingbirds, and will include cameras and an RFID antenna similar to (5). The rate of sugar solution delivery to the hummingbird will be varied to encourage longer feeding durations while at the feeder.

8.

As the cameras will be set up at the feeders which are open to non-target species, incidental video recordings of other animal species will be made. Other species that may be recorded include birds, bees, bats, and butterflies. None of these species will be captured and handled at any time while they visit the feeders.

Animal Sex:

Female

Male

Animal Ages:

Juveniles and Adults

Animal Size:

4.3 g (Anna's Hummingbird); 3.3 g (Rufous Hummingbird)

6. Select experimental procedures:

Name	Type	Version	Scope
Rico-Guevara - Hummingbird Drop Trap v2	Capture/Trapping	1	Team
Rico-Guevara - Mist netting	Capture/Trapping	1	Team

Name	Type	Version	Scope
Rico-Guevara - PIT Tag Implantation	Implants	1	Team
Rico-Guevara - Feeder Mask Respirometry	Other	1	Team
Rico-Guevara - Maximum flight performance test	Other	1	Team

7. Monitoring protocol, including frequency and specific behavioral and clinical signs to be monitored. Include humane endpoints (criteria for euthanasia):

Animals will be continuously monitored when captured and while undergoing PIT tag implantation.

8. If there is expected mortality (spontaneous death) in this experiment:

a. Procedure/condition associated with mortality:

Stress related to capture and PIT tagging may result in mortality. Careful monitoring of all animals and providing access to sugar solutions will avoid nearly all cases.

b. Estimated mortality rate, i.e. percentage of animals expected to die spontaneously (not via euthanasia) or need to be euthanized as a result of the procedure. (Be sure to account for this in your animal number calculations):

< 0.1%

c. Explain why euthanasia is not possible or appropriate:

9. Will some animals live out their natural lifespan as part of this experiment? If so, indicate their use and describe the monitoring plan for aged animals (e.g., rodents >18 months of age), including frequency, behavioral and clinical signs to be monitored and criteria for euthanasia.

No.

10. * Total number of animals used in this experiment:(including all the animals to be produced)

200

a. Justify total number of animals used in this experiment:

We will maximally tag 100 individuals per species. Across two different species, we will tag a maximum of 200 animals.

The redetection rate for PIT tagged hummingbirds is around 70% following implantation, as many hummingbirds can be transient in the region due to migration or competitive exclusion. Because the social dynamics at the site are unpredictable, reducing the frequency of feeder visitation in less dominant individuals, we need to maximize the number of hummingbirds that are tagged to ensure proper representation across all species.

11. Number of animals by pain and distress category:(include each animal only once in the highest pain category)

B: 0

C: 200

D: 0
E: 0

a. Justify the need for any animals in pain category E:

12. * Identify husbandry exceptions:

Exception Type	Description and Justification
View Birds - No husbandry or enrichment exceptions.	

13. Supporting documents:

Document Name	Date Modified
Hummingbird feeder example	3/31/2020 4:31 PM

1. * Exception type:

Birds - No husbandry or enrichment exceptions.

2. Description and justification:

Mask-respirometry of Hummingbirds

1. * Experiment name:

Mask-respirometry of Hummingbirds

2. * Species:

Birds - Other

3. If other was selected, provide a species:

Anna's hummingbird (*Calypte anna*) and Rufous hummingbird (*Selasphorus rufus*)

4. What is the scientific goal of this experiment:

In order to measure the metabolic rates of all hummingbirds, and not just those most dominant at the feeders, a subset of captured hummingbirds will be held in captivity for < 12 hours to record hovering metabolic rates. This allows us to measure metabolic rates across the various species.

5. * Describe the animal experience in the experiment, from enrollment in the study to the final endpoint, including all procedures in chronological order and the minimum time between procedures. We encourage using bullet points, timeline, table, or a flow chart as appropriate:

1.

Hummingbirds will be baited to the field site using hummingbird feeders filled with 20-25% sucrose solution. The solution will be replaced every other day, or as needed.

2.

Hummingbirds will be captured at the field site using drop-net traps, drop-door traps, or mist nets (see procedures).

3.

Upon capture, hummingbirds will be brought into a 0.9 x 0.9 x 0.9m mesh cage and provided a 20-25% sugar solution. The hummingbird will be isolated until the animal settles down in the cage. This is normally achieved within 30 minutes to an hour. The bird will be checked every 10-15 minutes.

4.

Once the hummingbird has calmed within the cage, the bird will be immediately trained on the respirometer and respirometric data will be collected (See procedure). Training and data collection will be capped at 6 hours. If data collection is not complete by the prescribed time, the animal will be immediately released.

5.

The bird will be undergo morphological measurements and PIT tagging as described in “Free-living hummingbird feeder dynamics” and then released.

Animal Sex:

Female

Male

Animal Ages:

Juveniles and Adults

Animal Size:

4.3 g (Anna's Hummingbird); 3.3 g (Rufous Hummingbird)

6. Select experimental procedures:

Name	Type	Version	Scope
Rico-Guevara - Hummingbird Drop Trap v2	Capture/Trapping	1	Team
Rico-Guevara - Hummingbird Drop-Door Trap v1	Capture/Trapping	1	Team
Rico-Guevara - Mist netting	Capture/Trapping	1	Team
Rico-Guevara - Drop euthanasia	Euthanasia	1	Team
Rico-Guevara - Feeder Mask Respirometry	Other	1	Team

7. Monitoring protocol, including frequency and specific behavioral and clinical signs to be monitored. Include humane endpoints (criteria for euthanasia):

Monitored every 10-15 minutes following initial capture.

Continuous monitoring when undergoing respirometry trials.

8. If there is expected mortality (spontaneous death) in this experiment:

a. Procedure/condition associated with mortality:

Capture is associated with some distress in hummingbirds. Frequent monitoring for signs of significant distress, such as fluffed feathers, labored breathing, and closed eyes, will result in immediate removal from the study, recovery with sugar water, and release.

Close monitoring of all individuals will avoid nearly all cases of mortality.

b. Estimated mortality rate, i.e. percentage of animals expected to die spontaneously (not via euthanasia) or need to be euthanized as a result of the procedure. (Be sure to account for this in your animal number calculations):

< 0.1%

C. Explain why euthanasia is not possible or appropriate:

9. Will some animals live out their natural lifespan as part of this experiment? If so, indicate their use and describe the monitoring plan for aged animals (e.g., rodents >18 months of age), including frequency, behavioral and clinical signs to be monitored and criteria for euthanasia.

No.

10. * Total number of animals used in this experiment:(including all the animals to be produced)

20

a. Justify total number of animals used in this experiment:

We will measure metabolic rates using mask respirometry across 10 individuals per species. With 2 species at the field site, we will need a total of 20 individuals. As we are only seeking a mean measurement for each species, 10 individuals per species will allow us to estimate a species-level mean.

11. Number of animals by pain and distress category:(include each animal only once in the highest pain category)

B: 0

C: 20

D: 0

E: 0

a. Justify the need for any animals in pain category E:

12. * Identify husbandry exceptions:

Exception Type	Description and Justification
View Birds - No husbandry or enrichment exceptions.	

13. Supporting documents:

Document Name	Date Modified
There are no items to display	

1. * Exception type:

Birds - No husbandry or enrichment exceptions.

2. Description and justification: