

marks  
sharlyn

### Routing Slip for IACUC Protocol Reviews

Please keep this routing slip with the IACUC protocol you are reviewing. Please note, per our PHS Assurance, that reviews take place simultaneously on the same version of the protocol. Reviewers should communicate via phone or email to discuss any changes or concerns with the protocol.

Protocol No. 14/15 . B . 93 - A

Reviewer	Approve	Disapprove (Attach comments)	Date
<u>Reiss</u> (pt)	<input checked="" type="checkbox"/>		
<u>Brown</u>	<input checked="" type="checkbox"/>		

1st Review 5/14/13  
2nd Review 5/28/15  
3rd Review \_\_\_\_\_  
4th Review \_\_\_\_\_

HUMBOLDT STATE UNIVERSITY  
INSTITUTIONAL ANIMAL CARE AND USE  
PROTOCOL ROUTING SLIP

MAY 28 2015

College of Natural Resources & Sciences  
Humboldt State University

The attached protocol for the humane care and use of live vertebrate animals was submitted on

05/13/15  
(date)

by Sharyn Marks  
(faculty project leader)

for BIOL 699  
(course # if appropriate)

Check whether the work described in this protocol will be supported by funding administered by the ( ) HSU Foundation, ( ) another administrative unit -list \_\_\_\_\_, or (X) will be unfunded.

Animals used for this project will be housed in the following facilities (please check all that apply):

( ) Animal Rooms; ( ) Fish Hatchery; ( ) Game Pens; ( ) Telonicher Marine Lab;

( ) Natural History Museum; ( ) Other, specify site and room \_\_\_\_\_

Person / phone number (or e-mail) to contact: Madeline Cooper, mcc31@humboldt.edu

Project Title: Use of permanent and seasonal pools by invasive juvenile bullfrogs (*Lithobates catesbeianus*) on the lower Mad River in Humboldt Co., CA

♦ **ROUTE FIRST TO THE CHAIR OF THE IACUC** BRING THIS FORM TO THE COLLEGE OF NATURAL RESOURCES AND SCIENCES (RM. 101 IN THE FORESTRY BUILDING). Please allow ten working days for review of proposals to conduct minimally invasive procedures and an excess of one month for review of proposals to conduct invasive procedures; note that these time periods are minimal and assume that no revisions will be necessary prior to approval. ALWAYS VERIFY APPROVAL (OFFICE OF THE CHAIR OF THE IACUC; 826-3256) BEFORE STARTING YOUR PROJECT.

**THE REMAINDER OF THIS PAGE IS FOR THE USE OF THE INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE**

Date 1<sup>st</sup> Received 5/13/15

2<sup>ND</sup> REVIEW 5/28/15

No. 14 / 15 B. 93 - A

- ( ) E- Procedures are exempt from full IACUC review because they are purely observational, non-invasive, and produce no perceptible discomfort or they concern only the use of tissues from dead animals. To be considered exempt, tissues from dead animals must be obtained from animals euthanatized or otherwise killed by means, and for purposes, unrelated to the proposed project. The procedure may be approved by the Chair one additional member of the IACUC.
- (X) A- Procedures will be minimally invasive or produce relatively little discomfort. Protocols may involve, bleeding, injections, minimal sampling, anesthesia or humane euthanasia without prior invasive manipulation. The procedure may be approved by the Chair and two additional members of the IACUC. Project topics will be reviewed by the IACUC at the next scheduled meeting.
- ( ) B- Procedures will involve prolonged manipulation or be invasive. Protocols may involve surgical or other stimuli inducing pain or distress, but all pain or distress will be mitigated with appropriate anesthetics or analgesics. The procedure may be initially approved by the Chair, the Campus Veterinarian and one additional member of the IACUC. Protocols will be reviewed by the IACUC at the next scheduled meeting.
- ( ) C- Procedures will be invasive and may cause prolonged physiological or psychological stress. Pain, considerable distress, or discomfort may be induced and not mitigated by anesthesia or adequate analgesia (e.g. LD50 experiments, long-term food or water deprivation, etc.). These protocols will be reviewed thoroughly by the IACUC prior to commencement of the project.

☒ Requires Health Assurance

Signature, IACUC Member

Date

(X) Approved

( ) Denied

Signature, IACUC Member

Date

(X) Approved

( ) Denied

Signature, IACUC Chair

Date

(X) Approved

( ) Denied

**Final Committee Decision. All protocols must be approved prior to the start of research.**

cc: ( ) Project Leader, ( ) Animal Facility Supervisor, ( ) Department Chair

Routing slip revision 03/27/2015

2<sup>ND</sup>  
5/28

## **PROTOCOL FOR THE HUMANE CARE AND USE OF LIVE VERTEBRATE ANIMALS**

Federal animal welfare regulations require that an Institutional Animal Care and Use Committee (IACUC) review and approve all activities involving the use of vertebrate animals prior to their initiation. This includes any animals used for the development of experimental methodologies, instructional purposes, research, etc. Approved protocols for ongoing and recurrent activities must be reviewed by the IACUC on an annual basis. However, extensions and amendments requiring an abbreviated application process may be granted for a total of three consecutive years. Compliance with animal welfare regulations is mandatory and is the responsibility of all individuals (including faculty and students) who choose to work with live vertebrate animals.

To avoid the proliferation of submissions, please provide generic descriptions (including multiple routes of compound administrations, minor procedural variations, similar laboratory exercises from a single course, routine exercises used in several courses, etc). When multiple vertebrate species are to be used, please clearly describe all procedures, and all variations thereof, to be used with each individual species.

Once completed, signed, and dated, please submit your protocols to the Chair of the IACUC, Dean of the College of Natural Resources and Sciences, Forestry Bldg, Room 101. All protocols should be submitted on the most recent version of the forms. For your convenience, protocol forms are available in several software formats from the Chair of the IACUC, from several department offices and stockrooms, and they can be downloaded from the IACUC web page (<http://www.humboldt.edu/~iacuc>). You can expedite the review process by following these formatting rules: avoid changing the format of the routing slip unless minor reformatting is necessary to keep it to a single page; leave an extra blank line between your answers and the questions; leave questions in bold-face type; type your answers in regular (non-bold) type; and format the final signature page so that it begins with the final question. Please contact the Campus Veterinarian, Dr. Richard Brown, (by phone-826-3320, or e-mail- [RNB2@humboldt.edu](mailto:RNB2@humboldt.edu)) with questions concerning protocol preparation and submission.

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### **1. Course Number (if applicable). BIOL 699**

**Project Title** (note that this title must match the title shown on the routing slip).

Use of permanent and seasonal pools by invasive juvenile bullfrogs (*Lithobates catesbeianus*) on the lower Mad River in Humboldt Co., CA

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### **2. Responsible Faculty Member: Instructor, Principal Investigator or Project Director.**

**Name:** Sharyn Marks

**Department:** Biological Sciences

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### **3. Names of others involved in animal use activity and their qualifications to perform the procedures indicated.**

Madeline Cooper (graduate student) has prior experience (from fieldwork and employment prior to HSU) in safely handling and measuring amphibians and other aquatic species. She has taken Herpetology and Herp Management classes at HSU where she has also learned more about field

methods. She has never used radio-telemetry or Visible Implant Elastomer (VIE) before but she will be trained by Karen Pope (USFS) to use VIE and by Lee Hecker (HSU graduate student) to attach transmitter belts to frogs. They are each very experienced in these areas and have agreed to train her.

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4. **Proposed starting date (the starting date cannot precede date of approval, and note that *all* protocols must be renewed or extended annually).** The Annual Protocol Review Form must be approved on or before the anniversary of the approval date to indicate termination of the project or to request extension of the dates of approval; annual review is automatic and you no longer need to submit an end date.

Date of approval (as soon as possible).

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5. **Scientific name, common name, and characteristics of all species to be used. List multiple species separately to explain variation in use. For field studies, please list all target species, species listed as protected, threatened, or endangered by the USFWS or the state in which the work will be conducted, and any non-target species that are likely to be impacted.**

Latin binomial	Common name	Sex	Age or Weight Range
<i>Lithobates catesbeianus</i>	Bullfrog	both	Juveniles, at least 26g

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6. **Number of animals to be used. Explain why a smaller number would not allow you to meet your objectives (please provide clarification if based on statistical reasoning).** If this is a field project, and you cannot predict the exact number of animals to be sampled, please give your best estimate and an explanation of the variables that will determine your sample size. Write N/A if this protocol covers only the transportation, use, and/or storage of carcasses or tissues.

Twenty-nine frogs will be tracked using radio-telemetry. Telemetry studies on amphibians typically use anywhere from 20 to 150 individuals to increase the power of statistical analysis. It is possible to lose transmitters in the field so tracking more than the minimum number of animals is desired.

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7. **Source of the animals (or tissues) to be used or the study area(s) for field studies.** For transportation, storage, and use of tissues from carcasses, explain the circumstances of death. If this information is unknown, provide the name and contact information for the person or company from which the samples are to be obtained.

All Bullfrogs will be caught from the wild from ponds near the lower Mad River.

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8. **If live animals are to be maintained in captivity for greater than 12 hours, explain where and how the animals will be housed and who will be responsible for their daily care.** If no animals will be maintained in captivity, please clearly state that to be the case. Write N/A if this protocol covers only the transportation, use, and/or storage of carcasses or tissues.

N/A



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**9. Provide a non-technical description of the proposed goals, general methods, and the educational or scientific objectives that the proposed use is designed to meet.**

Bullfrogs (*Lithobates catesbeianus*) are an invasive species in the western United States and 40 other countries around the world. They have been named one of the IUCN's 100 worst invaders because of their ability to quickly outcompete native anurans, prey on fish, and carry the deadly amphibian disease, chytridiomycosis (Global Invasive Species Database, 2005). Bullfrogs were first introduced to California in 1914 and have since spread throughout the state (Moyle, 1973). Although Bullfrogs are frequently present in habitats that have been affected by human disturbance, they have also been found in otherwise pristine habitats around California (Kupferberg, 1997). Introduced Bullfrogs have been shown to have many negative effects on native fauna. Madeline proposes to use radio telemetry to track invasive Bullfrogs on the Mad River to examine use of seasonal pools by juvenile frogs. Specifically, she will (1) determine whether juvenile Bullfrogs use temporary wetland areas that are distinct from adult breeding sites, (2) examine the characteristics of pools used by juvenile Bullfrogs and (3) investigate movement patterns in relation to habitat variables. Tracking juveniles and examining habitat variables will fill gaps in data on juvenile habitat use that could inform habitat modifications to target juvenile Bullfrogs. Radio tracking will also allow me to fill in gaps on the dispersal capabilities of juvenile Bullfrogs. Complete Bullfrog eradication is very difficult, but understanding dispersal capabilities and pairing that information with habitat use data can enable land managers to use habitat modifications to slow population growth and limit colonization of coastal Humboldt County, California.

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**10. Provide a complete and detailed description of all procedures to be performed involving live vertebrate animals.** Your response should address the handling and restraint of non-anesthetized animals; deprivation of food or water for a period that is atypical for this species; use of chemical or biological agents; the drawing of blood; the use of anesthetics, analgesics, sedatives or tranquilizers; surgical procedures; exposure to radioactive materials, known carcinogens, or highly toxic substances; and any post-operative procedures. Write N/A if this protocol covers only the transportation, use, and/or storage of carcasses or tissues.

Using radio-telemetry, Madeline will track 29 frogs captured at pools from May to September of 2015. Bullfrogs are expected to be most active between June and August so this time window will capture both ends of their active season. Capture locations will be spread throughout the lower Mad River based on information from California Department of Fish and Wildlife (CDFW), with several frogs being captured at each pool. She will attach radio transmitters (Wildlife Materials, Murphysboro, IL) to juvenile Bullfrogs. These transmitters weigh 2.6-2.8 grams each and have an estimated battery life of 130 days. Transmitters will not be placed on frogs when the weight of the transmitter would exceed 10% of the individual's body weight (Schmidt et al., 2008; Bloomquist & Hunter Jr., 2007; Muths, 2003; Richards, 1994). To be consistent with previous research, juveniles will be defined as those with a snout-vent length (SVL) of less than 70mm (Gahl et al., 2008; Roninger, 2008; Willis et al., 1956). In Oregon, Bullfrogs less than 70mm weighed between 21.5 and 33.6g (Roninger, 2008). Frogs will be captured by hand or net in mid-May. Transmitters will be epoxied to a small, lightweight aluminum beaded belt that goes around the pelvis of the frog (Burow et al., 2006). All tracked frogs, as well as any additional frogs caught will be marked with less than 1/10 ml of visual implant elastomer (VIE; Northwest Marine Technology Inc., Shaw Island, WA) which will be injected under the skin of the hands and feet. Anesthesia is not needed for this process (Sapsford et al., 2014). During all frog handling exam gloves will be worn to protect frogs. All frogs will be located three to seven times a week. Except for periodic weighing, tracked frogs will not be handled when they are located. Frogs marked with just VIE will be recaptured opportunistically throughout the summer. At the end of the tracking period

transmitters will be removed and frogs released at their last capture point. Throughout the tracking season Madeline will record data for the frogs being tracked, and for each capture location. Upon initial capture, she will record weight and snout-vent length (SVL). These data will be used to control for the fact that larger frogs are expected to move farther than smaller frogs. Frogs will be weighed throughout the tracking season to verify that transmitters are not resulting in weight loss. Frogs will not be anesthetized when handled, as is standard for marking and measuring frogs. Equipment (nets, boots, buckets) will be disinfected with 10% bleach and allowed to completely dry between visits to different sites. This will reduce the spread of chytrid, should it be present at these sites.

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- 11. Will any of these procedures cause pain or distress (other than that necessitated by collection, injection, and otherwise mild, momentary discomforts)? If so, please explain. Write N/A if this protocol covers only the transportation, use, and/or storage of carcasses or tissues.**

When transmitters of appropriate size (<10% body weight) are attached properly they should not cause pain or distress (Burow et al., 2006; Schmidt et al., 2008; Bloomquist & Hunter Jr., 2007; Muths, 2003; Richards, 1994). Frogs will be regularly monitored for signs of weight loss or skin abrasions under the belt; if these symptoms are observed, the transmitter will be removed and placed on a new frog. Injection of VIE causes mild momentary discomfort at the injection site.

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- 12. For researchers, explain how you determined that this protocol does not unnecessarily duplicate previously published observations or experiments (cite the type of literature searches as well as any other resources used). For instructors, explain the value of the lesson that merits using live animals. Write N/A if this protocol covers only the transportation, use, and/or storage of carcasses or tissues.**

There are relatively few studies of Bullfrog movements in the literature (Willis et al., 1956; Currie & Bellis, 1969; Roninger, 2008; Stinner et al., 1994), especially considering the invasive status of these frogs and their documented negative impacts on native fauna. Juveniles are probably the main dispersers in this species, yet all but one of these studies (Roninger, 2008) looked at movements of adult Bullfrogs. Two of these studies (Willis et al., 1956; Currie & Bellis, 1969) used mark-recapture techniques, which do not provide the same detailed movement data that radio telemetry does. Literature searches were done on the Humboldt State University library website from 09/2014 to 05/2015. Key words used included: radio telemetry, frog, anuran, tracking, visual implant elastomer, Bullfrog, *Lithobates catesbeianus*, movement patterns, landscape use, mark recapture.

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- 13. Provide alternative procedures that were considered and rejected as well as a brief explanation of why the alternative procedures were rejected. Write N/A if this protocol covers only the transportation, use, and/or storage of carcasses or tissues.**

Mark-recapture by itself was considered as a way to track juvenile Bullfrog movements but because of low recapture probabilities this will not provide the detailed data on movements that radio telemetry will. The additional frogs marked with VIE will supplement movement data obtained from radio tracking, but not replace it.

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- 14. Identify serious human health risks (expected exposures to disease agents, toxic chemicals used, dangerous environmental conditions, etc.) to which any participants might be exposed during the routine performance of the duties proposed herein, and describe steps**

taken to mitigate those risks.

Researchers will be walking in and around ponds and wetland areas so slipping is a possibility. Hiking boots with traction will be worn and care will be taken when walking. Work will be done during the summer, so sunburn and dehydration are also possible. Sunscreen and wide-brimmed hats will be worn. Water will always be taken into the field. There may be poison oak in some areas that every attempt will be made to avoid. If poison oak is unavoidable than a Tecnu shower will be taken at the end of the day.

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15. **Describe the fate of the animals upon completion of the protocol. Include the procedure for euthanasia (if chemical, include drug, route, and dosage) and the method of verification (whether necessary as an experimental termination or in the case of unanticipated, accidental injury).** Note (1) that you must justify the scientific necessity for any variations from the established guidelines for euthanasia (AVMA Guidelines for the Euthanasia of Animals: 2013 Edition as published online by the American Veterinary Medical Association or its replacement in the Code of Federal Regulations), (2) that you must report unexpected deaths to the IACUC as soon as possible to consider options, and (3) that you may write N/A only if this protocol covers only the transportation, use, and/or storage of carcasses or tissues.

At the end of the tracking period transmitters will be removed and frogs released at their last capture point. No animals will be euthanized for this study. In the event that an animal does need to be euthanized MS-222 will be kept on hand. MS-222 will be buffered to a neutral pH with sodium bicarbonate and frogs will be bathed in a water bath with 5-10g/L of MS-222 until death occurs (approximately one hour). Once breathing has stopped the frog will be double pithed to verify death (AVMA Guidelines for the Euthanasia of Animals, 2013)

### Works Cited

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- Burow, A., Herrick, A., Geffre, A., & Bartlet, P. (2006). A fully adjustable transmitter belt for ranids and bufonids. *Herpetological Review*, 43(1), 66-68.
- Bury, R., & Whelan, J. (1985). *Ecology and Management of the Bullfrog*. U.S. Fish and Wildlife Service, Resource Publication 155, Washington, DC, USA.
- Currie, W., & Bellis, E. (1969). Home range and movements of the Bullfrog, *Rana catesbeiana* Shaw, in an Ontario pond. *Copeia*, (4), 688-92.
- Gahl, M., Calhoun, A., & Graves, R. (2009). Facultative use of seasonal pools by American Bullfrogs (*Rana catesbeiana*). *Wetlands*, 29(2), 697-703.
- Global Invasive Species Database (2005). *Lithobates catesbeianus*. Available from: <http://www.issg.org/database/species/ecology.asp?si=80&fr=1&sts=&lang=EN> [Accessed 2<sup>nd</sup> October, 2014.]
- Kupferberg, S. (1997). Bullfrog (*Rana catesbeiana*) invasion of a California river: the role of larval competition. *Ecology*, 78(6), 1736-1751.
- Muths, E. (2003). A radio transmitter belt for small ranid frogs. *Herpetological Review*. 34(4): 345-348.
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Area.

- Sapsford, S., Roznik, E., Alford, R., & Schwarzkopf, L. (2014). Visible implant elastomer marking does not affect short-term movements or survival rates of the treefrog *Litoria rheocola*. *Herpetologica*, 70(1), 23-33.
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- Stinner, J., Zarlinga, N., & Orcutt, S. (1994). Overwintering behavior of adult Bullfrogs, *Rana catesbeiana*, in northeastern Ohio. *The Ohio Journal of Science*, 94(1), 8-13.
- Willis, Y., Moyle, D., & Baskett, T. (1956). Emergence, breeding, hibernation, movements and transformation of the Bullfrog, *Rana catesbeiana*, in Missouri. *Copeia*, (1), 30-30.

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16. I certify that the above information is accurate and complete, that I have read and agree to abide by the "Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training at HSU," that I will make copies of these principles and other pertinent guidelines available to those persons who work under my supervision, and that deviations from this protocol, including any unanticipated injuries or death of animals, will be reported to the IACUC. Further, my level of supervision will be such that these procedures will be carried out in a humane and a scientifically acceptable manner as described herein. I understand that, as the research supervisor, I take responsibility for the conduct of anyone working under this approved protocol, and I will supervise the research to ensure that no work is conducted that is not covered herein or in a separate approved protocol. I am aware that my research might require permits from federal and/or state agencies that regulate the harassment, capture, transport, captive maintenance, handling and manipulation of live vertebrate animals, and I have marked all boxes pertaining to the relevant laws (and state permits) governing the species used in my research. I certify that my research will be conducted in accordance with all relevant federal and state laws.

I am aware that the following Acts apply to my study (check all that may apply):

- ☒ Animal Welfare Act  
☒ State of California Fish and Game Commission (Title 14) - Scientific Collecting Permit(s)  
☐ Endangered Species Act  
☐ Fishery Conservation and Management Act  
☐ Lacey Act  
☐ Marine Mammal Protection Act  
☐ Convention on International Trade in Endangered Species of Wild Fauna and Flora  
☐ Other: please list \_\_\_\_\_



Signature, Responsible Faculty Member

5/28/15

Date

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Review by the IACUC Attending Veterinarian (if necessary):

\_\_\_\_\_  
Signature, HSU Veterinarian

\_\_\_\_\_  
Date

☐ Approved

☐ Denied



Explanation of denial:

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**All protocols must be approved prior to the start of research.**

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Section 5 Protocol Revision 3/27/2015