

Circ

MAY 27 2014

College of Natural Resources & Sciences
Humboldt State University

HUMBOLDT STATE UNIVERSITY
INSTITUTIONAL ANIMAL CARE AND USE
PROTOCOL ROUTING SLIP

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

20 May 2014 (date) Tim Mulligan (faculty project leader) by _____ (course # if appropriate)

Check whether the work described in this protocol will be supported by funding administered by the
(X) HSU Foundation, () another administrative unit -list _____, or () 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: Tim Mulligan 826-3684 tjm2@humboldt.edu

Project Title: Baseline Monitoring of Estuarine MPAs on the North Coast

- ♦ **ROUTE FIRST TO THE CHAIR OF THE IACUC**, ASSOCIATE DEAN OF THE COLLEGE OF NATURAL RESOURCES AND SCIENCES (RM. 106C 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 1st Received 5/15/14 REVIEW No. 13/14.F.113-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 and the Campus Veterinarian.
- ☒ 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, the Campus Veterinarian, and one additional member 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.

Peggy J. [Signature] 5/28/14
Signature, IACUC Member Date

☒ Approved () Denied

[Signature] 6/2/14
Signature, IACUC Member Date

☒ Approved () Denied

[Signature] 6-2-14
Signature, IACUC Chair Date

☐ Approved () Denied

Routing slip revision 05/08

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

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, Associate Dean of the College of Natural Resources and Sciences, Forestry Bldg, Room 106C. 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) with questions concerning protocol preparation and submission.

1. Course Number (if applicable).

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

Baseline Monitoring of Estuarine MPAs on the North Coast

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

Name Tim Mulligan

Department Fisheries Biology

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

Dr. Frank Shaughnessy, advanced HSU upper classmen from the Depts. of Fisheries Biology and Biological Sciences, and graduate students, Ms. Katie Osborn and Mr. Zachariah Badaoui. Dr. Shaughnessy and these students have gained much field experience, especially in handling

fishes, during previous research projects, and/or through courses taken in the Departments of Fisheries Biology, Biological Sciences, Wildlife, etc.

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.

10 June 2014

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>Leptocottus armatus</i> , staghorn sculpin		male/female	0-3 yrs
<i>Atherinops affinis</i> , topsmelt		male/female	0-3 yrs
<i>Hypomesus pretiosus</i> , surf smelt		male/female	0-3 yrs
<i>Gasterosteus aculeatus</i> , stickleback		male/female	0-3 yrs
<i>Sygnathus californiensis</i> , bay pipefish		male/female	0-3 yrs
<i>Aulorhynchus flavidus</i> , tubesnout		male/female	0-3 yrs
<i>Platichthys stellatus</i> , starry flounder		male/female	0-6 yrs
<i>Citharichthys stigmaeus</i> , speckled sanddab		male/female	0-3 yrs
<i>Parophrys vetulus</i> , English sole		male/female	0-4 yrs
<i>Citharichthys sordidus</i> , Pacific sanddab		male/female	0-4 yrs
<i>Psettichthys melanostictus</i> , sand sole		male/female	0-3 yrs
<i>Ophiodon elongatus</i> , ling cod		male/female	0-8 yrs
<i>Cymatogaster aggregata</i> shiner surfperch		male/female	0-3 yrs
<i>Amphistichus koelzi</i> , calico surfperch		male/female	0-3 yrs
<i>Dmalichthys vacca</i> , pile surfperch		male/female	0-3 yrs
<i>Embiotoca lateralis</i> , striped surfperch		male/female	0-3 yrs
<i>Scorpaenichthys marmoratus</i> , cabezon		male/female	0-6 yrs
<i>Hemilepidotus spinosus</i> , brown Irish lord		male/female	0-3 yrs
<i>Hemilepidotus hemilepidodus</i> , red Irish lord		male/female	0-3 yrs
<i>Raja binoculata</i> , big skate		male/female	0-6 yrs
<i>Myliobatus californica</i> , bat ray		male/female	0-8 yrs
<i>Triakis semifasciata</i> , leopard shark		male/female	0-6 yrs
<i>Sebastes melanops</i> , black rockfish		male/female	0-12 yrs
<i>Ocella verrucosa</i> , warty poacher		male/female	0-3 yrs
<i>Stellerina xyosterna</i> , pricklebreast poacher		male/female	0-3 yrs
<i>Apodichthys flavidus</i> , penpoint gunnel		male/female	0-3 yrs
<i>Pholis ornata</i> , saddleback gunnel		male/female	0-3 yrs
<i>Lepidogobius lepidus</i> , bay goby		male/female	0-3 yrs

These species are considered target species and may or may not be collected during seasonal sampling. None of these species are listed as protected, threatened or endangered by the USFWS. **Non targeted** anadromous salmonids (potentially juvenile *Oncorhynchus mykiss*,

steelhead & *O. kisutch*, coho) may be collected. If encountered they will be immediately released.

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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.

Zero to 100 individuals (usually 0 to 20) of the various species listed above will be collected between 10 June – 10 December 2014 and 1 January - 10 December 2015. Numbers will be determined by sampling site and conditions (temp., light, time of day, tides, wind, etc.). All fish collected will be identified, enumerated, and released alive at the site of capture

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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.

Fishes will be sampled in the Mad River, Big River, Ten Mile and South Humboldt Bay estuaries. The latter three were designated as Marine Protected Areas in December 2012.

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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.

No specimens will be kept in captivity

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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.**

The goal of this project is to compare the abundance and diversity of fishes sampled in estuaries designated as Marine Protected Areas (MPAs) and in one reference site, 10 June – 10 December 2014 and 1 January - 10 December 2015. Fishes will be sampled in all estuaries using a 100'x 6' beach seine with 10 mm mesh and by deploying fyke nets with 1.0 m x 1.7 m mouth openings, 15' bodies, 15' leads, all with 10 mm mesh. Two replicate beach seine hauls will be taken monthly, at ea. of three sites in each estuary (MPA sites and reference site), 10 June – 10 December 2014 and 1 January - 10 December 2015. Two fyke nets will be set overnight, approximately 1800 hrs to 0600 hrs, monthly 10 June – 10 December 2014 and 1 January - 10 December 2015 in one or two sub-estuaries flowing into the Mad, Big River and Ten Mile MPA Estuaries. Fishes will be immediately removed from the beach seine hauls by hand, identified, enumerated, measured and released alive at the site of capture. Fishes will be removed from fyke nets by hand at approx. 0600 hrs after fishing overnight. Fyke net caught fishes will also be identified, enumerated, measured and released alive at the site of capture. Fish collected by either method that show signs of stress (lack of equilibrium, or injury from the nets) will be held for 5 to 10 minutes in aerated 1m x 0.5m x 0.5 m live wells. When fully

recovered they will be released. Any fish that appear moribund or that show obvious signs of severe stress will be euthanized by cranial concussion administered by a small leaded club immediately followed by exsanguination in accordance with the American Veterinary Medical Association's June 2007 Guidelines on Euthanasia. These fish will be fixed in 10% formalin in the field. In approximately 2 weeks they will be preserved in 40% isopropyl alcohol and placed in the HSU Fish Museum for student study or archival purposes.

This study will allow us to compare the abundance and diversity of fishes found in MPA designated estuaries to reference site estuaries and to allow us to examine the overall effectiveness of MPAs.

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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.

Fishes will be sampled in all estuaries using a 100' x 6' beach seine with 10 mm mesh and by deploying fyke nets with 1.0 m x 1.7 m mouth openings, 15' bodies, 15' leads, all with 10 mm mesh. Two replicate beach seine hauls will be taken monthly, at ea. of three sites in each estuary (MPA sites and reference site), 10 June – 10 December 2014 and 1 January – 10 December 2015. Two fyke nets will be set overnight, approximately 1800 hrs to 0600 hrs, monthly 10 June – 10 December 2014 and 1 January – 10 December 2015, in one or two sub-estuaries flowing into the Mad River, Big River and Ten Mile MPA Estuaries. Based on previous site visits to all sampling areas, water depths for fyke net sets will range from 4' (high tide) to a minimum of 1.5' (low tide, 0.0') causing minimum stress to fishes. Fishes will be immediately removed from the beach seine hauls by hand, identified, enumerated, measured to the nearest mm total length on glazed (smooth) fish boards, and released alive at the site of capture. Fishes will be removed from fyke nets by hand at approx. 0600 hrs after fishing overnight. Fyke net caught fishes will also be identified, enumerated, measured to the nearest mm total length on glazed (smooth) fish boards and released alive at the site of capture. If large numbers (>24) of fish are collected in any fyke net sets, or beach seine hauls, they will be placed in 2' x 2' x 2' live wells, secured in flowing water, to minimize stress prior to processing and release. If <24 fish are collected in fyke net sets or beach seine hauls they will be quickly removed from the nets and processed directly. Fish collected by either method that show signs of stress (lack of equilibrium, or injury from the nets) will be held for 5 to 10 minutes in aerated 1 m x 0.5 m x 0.5 m live wells. When fully recovered they will be released. Any fish that appear moribund or that show obvious signs of severe stress will be euthanized by cranial concussion administered by a small leaded club immediately followed by exsanguination in accordance with the American Veterinary Medical Association's June 2007 Guidelines on Euthanasia. These fish will be fixed in 10% formalin in the field. In approximately 2 weeks they will be preserved in 40% isopropyl alcohol and placed in the HSU Fish Museum for student study or archival purposes.

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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.**

Fishes may become distressed during the collection process. We try to minimize this by processing fish as quickly and efficiently as possible. Any fish showing obvious signs of stress (lack of equilibrium, or injury from the nets) will be held for 5 to 10 minutes in aerated live wells. When fully recovered they will be released.

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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 is no published (peer reviewed) life history data, or current monitoring, of fishes inhabiting all of the proposed study sites. Data bases searched: Fish, Fisheries & Aquatic Biodiversity Worldwide (recommended by Katia Karajova, HSU Science librarian) and Biosis. These searches were done in August 2013 and again in May 2014. Search terms included: "fishes of the proposed study sites" & "fish surveys/monitoring of the proposed study sites". The scientific objective of the study is to compare the abundance and diversity of fishes found in estuaries designated as Marine Protected Areas (MPAs) to reference site estuaries and to allow us to examine the overall effectiveness of MPAs.

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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.**

Direct observation through SCUBA or snorkeling was considered. However this approach was determined to be inappropriate in cold, occasionally rough waters, with extremely poor visibility. The logistics of using SCUBA or snorkeling to obtain the necessary information is simply not practical in the proposed study areas.

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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.**

Formalin is a carcinogen - protective gloves and eyewear will be worn by all researchers handling this substance.

Students will be instructed in the proper usage of this chemical.

Although the tidal turnover in each of the proposed study sites is not great (approx. 2-4', except in Humboldt Bay) researchers and all students will be instructed of the potential dangers associated with working in tidal currents. Prior to sampling they will "walk" both incoming

and outgoing tides to become more experienced in working flowing waters.

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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 (2000 Report of the AVMA Panel on Euthanasia as published in the Journal of the American Veterinary Medical Association, 2001, 218(5): 669-696 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.**

All fish will be released at the capture site immediately after processing. Euthanasia may be required in cases of accidental injury. For example, it is possible that fish may be damaged by the mesh of the net, or injured by other animals, (i.e. crabs) captured in the net. In these cases, fish will be euthanized by cranial concussion administered by a leaded club immediately followed by exsanguination in accordance with the American Veterinary Medical Association's June 2007 Guidelines on Euthanasia. These fish will be fixed in 10% formalin in the field. In approximately 2 weeks they will be preserved in 40% isopropyl alcohol and placed in the HSU Fish Museum for student study or archival purposes.

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 _____

Tom Mulligan

Signature, Responsible Faculty Member

22 May 2014

Date

Review by the IACUC Attending Veterinarian (if necessary):

Signature, HSU Veterinarian

Date

☐ Approved

☐ Denied

Explanation of denial:

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

Ja 2

Signature, IACUC Chair

Date

6-2-14

☒ Approved

☐ Denied

Explanation of denial:

CITI Collaborative Institutional Training Initiative (CITI)

IACUC Module for faculty and students Curriculum Completion Report
Printed on 7/6/2013

Learner: Timothy Mulligan (username: quilback)
Institution: Humboldt State University-Sponsored Programs Foundation
Contact Information Department: Fisheries Biology
Email: tjm2@humboldt.edu
IACUC Module for faculty and students:

Stage 1. Stage 1 Passed on 07/06/13 (Ref # 10748880)

Required Modules	Date Completed	
Introduction to Working with the IACUC	07/05/13	no quiz
Working with the IACUC	07/05/13	3/3 (100%)
Federal Mandates	07/05/13	2/4 (50%)
The Veterinary Consultation	07/05/13	1/1 (100%)
Getting Started	07/06/13	5/5 (100%)
Alternatives	07/06/13	8/8 (100%)
Avoiding Unnecessary Duplication	07/06/13	1/1 (100%)
USDA Pain/Distress Categories	07/06/13	8/8 (100%)
Personnel Training and Experience	07/06/13	3/3 (100%)
Making Changes after You Receive Approval	07/06/13	1/1 (100%)
Reporting Misuse, Mistreatment, or Non-Compliance	07/06/13	no quiz
Final Comments	07/06/13	no quiz
Euthanasia	07/06/13	1/1 (100%)
Occupational Health and Safety	07/06/13	1/1 (100%)

For this Completion Report to be valid, the learner listed above must be affiliated with a CITI participating institution. Falsified information and unauthorized use of the CITI course site is unethical, and may be considered scientific misconduct by your institution.

Paul Braunschweiger Ph.D.
Professor, University of Miami
Director Office of Research Education
CITI Course Coordinator

Return