HUMBOLDT STATE UNIVERSITY

INSTITUTIONAL ANIMAL CARE AND USE PROTOCOL FOR THE HUMANE CARE AND USE OF LIVE VERTEBRATE ANIMALS

This box is for the review of the use by the Institutional Animal Care and Use Committee. Authors should not write or type inside the borders of the box.

Date 1st Received: 2/19/18 Revision 1 Date:_____ Revision 2 Date:_____ No. 17/18.ESM-65-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 (X) Yes ( ) No

Signature, IACUC Member	2/27/18 Date	(✓) Approved	( ) Denied
Signature, IACUC Member	3/5/18 Date	(✓) Approved	( ) Denied
Signature, Campus Veterinarian (if necessary)	Date	( ) Approved	( ) Denied
Signature, IACUC Chair	3.8.18 Date	(✓) Approved	( ) Denied

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

Protocol Edition 2/15/2017

1. **Faculty Project Leader:** Alison O'Dowd

   Department Affiliation: ESM

2. **Project Title:** Trinity River Macroinvertebrate Drift

3. **Email address(es) of the Faculty Project Leader and other corresponding applicants:**

   ap73@humboldt.edu

4. **Names of others handling live animals in the absence of, or not directly supervised by, the faculty project leader, and their qualifications to perform the procedures indicated. (Do not include class rosters here - see 8 below):**

   Staff from the Yurok Tribe, Hoopa Valley Tribe and CA Dept of Fish and Wildlife will be working in conjunction with this overall project. They will be utilizing their existing fish handling permits.

5. **Will the described project be funded? ☒ Yes ☐ No**

   If funded, will the funds be administered by the HSU Sponsored Programs Foundation (SPF)? ☒ Yes ☐ No

   If funded, but not administered by the HSU SPF, then list the unit that will administer the funds:

   Click or tap here to enter text.

6. **Proposed starting date (the starting date cannot precede date of approval, and 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.

   The field work for this project began on February 5, 2018 under the existing IACUC protocol (1718.ESM.32-A). If approved, this IACUC protocol would replace the previously approved one.

   Project start date: Feb 5, 2018 (fieldwork will end around April 30, 2018)

7. **Provide a brief, non-technical, description of the project. Your response should include the proposed goals, general methods, and educational or scientific objectives that the proposed use is designed to meet.**

   The goal of this project is to describe juvenile Chinook diet on the upper Trinity River near macroinvertebrate drift monitoring sites downstream of Lewiston Dam, and assess how fish diet changes with flow management, drift concentrations and between two sites. We will describe and assess the differences in terrestrial invertebrate and benthic macroinvertebrate (BMI) composition, biomass and abundance found in drift and benthos as well as in diets of juvenile Chinook salmon from two sites on the upper Trinity River, below Lewiston Dam. Further comparisons will be made between daily and seasonal (February-April) variation found in drift and benthos and diets. The data collected will help to inform future research on the potential of experimental flow pulses released from Lewiston

Dam to increase BMI drift forage abundance and other food resources available to juvenile Chinook salmon. See response to question 16 for detailed methods.

8. **Is the primary purpose of the project for ☐ instruction, ☒ research, or ☐ both?**

   Based on your answer, please address the relevant questions below.

   <u>If the primary purpose is for instruction</u>, list the course number and write the CRN for this semester (note that this CRN will need to be updated with any future offering of the course covered by this protocol).

   Course # (e.g. ZOOL 356): Click or tap here to enter text.

   CRN: Click or tap here to enter text.

   Will all of the enrolled students in the course denoted by the CRN above participate in the use of animals covered by this protocol? ☐ Yes ☐ No

   If no, then provide a list of the students exposed to, or otherwise using, live vertebrate animals.

   Click or tap here to enter text.

   Describe the learning objectives that justify 1) the use of, and 2) duplication of procedures involving, live animals for instruction.

   Click or tap here to enter text.

   <u>If the primary purpose is for research</u>, explain how you determined that this protocol does not unnecessarily duplicate previously published observations or experiments; please include:

   1. **the type of literature searches conducted:**
      A thorough literature review was conducted via google scholar, JSTOR, and BioOne to determine if any similar previous efforts were made and it is clear that there have been very few macroinvertebrate and fish diet studies done in the Trinity River. There has not been a macroinvertebrate study in the Trinity River since the 1980s. This research has the potential to guide future flow releases out of Lewiston Dam to increase fish food forage.

   2. **keywords used:**
      benthic macroinvertebrate drift, passive drift, catastrophic drift, behavioral drift, salmonid invertebrates as food

   3. **range of dates searched:**
      1970-present

   4. **other resources used:**
      Worked with collaborators from the Yurok Tribe, United States Fish and Wildlife Service, and Hoopa Valley Tribe to develop methods and write research proposal.

9. **Will any of the animals described in this protocol be housed in an animal facility?**

☐ **Yes** ☒ **No**

If yes, check the appropriate facility below:

☐ **Biological Sciences Animal Rooms**
☐ **Fish Hatchery**
☐ **Samoa Aquaponics**
☐ **Telonicher Marine Lab**
☐ **Wildlife Pens**
☐ **Zebra Fish Development Lab**
☐ **Other**. Please list: Click or tap here to enter text.

9a. Facility managers must be consulted prior to submitting protocol form. Please enter the date the manager was consulted: Click or tap here to enter text.

10. Scientific name, common name, and characteristics of all species to be used. List species separately to explain variation in use. Please also list the total numbers of animals to be used or substantially affected by this project.

For field studies, please list all target species and note their status (not protected = NP; protected, including species of special concern or candidate species = P; considered by the state or federal government to be threatened = T, considered by the state or federal government to be endangered = E); also list non-target species that are likely to be impacted. List the range of numbers of individuals to be used for each species.

TARGET SPECIES - *please attach additional pages if needed*

Latin Binomial(s)	**Common name(s)**	**Sex**	**Age or Wt Range**	**Status**	**Numbers**
Oncorhynchus tshawytscha	Chinook salmon		Juvenile >50 mm in length		Max of 480 individuals will be handled over course of entire project. Max of 100 individuals will be sacrificed to confirm effectiveness of lavage
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NON-TARGET SPECIES – *please attach additional pages if needed*

Latin Binomial(s)	**Common name(s)**	**Status**	**Numbers**
Oncorhynchus kisutch	Coho Salmon		Individuals of O. kisutch may be captured via seine net accidentally. All fish that are not Chinook will be released immediately.
Oncorhynchus mykiss	Steelhead trout		Individuals of O. mykiss may be captured via seine net accidentally. All fish that are not Chinook will be released immediately.
Salmo trutta	Brown trout		Individuals of S. trutta may be captured via seine net accidentally. All fish that are not Chinook will be released immediately.
Click or tap here to enter text.	Click or tap here to enter text.		Click or tap here to enter text.

11. Explain why a smaller number would not allow you to meet your objectives (please provide justification based on statistical or other logical 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. N/A is an inappropriate response unless the protocol covers only the transportation, use, and/or storage of carcasses or tissues.

We will sample 10-20 individuals during each sampling date. There are 28 sampling dates. A sample size smaller than this would not give us enough statistical power to characterize the daily and seasonal variation in the drift of benthic macroinvertebrates or on the diets of Chinook salmon.

12. Source of the animals (or tissues) to be used for captive studies or the location of 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.

The fish lavage samples will be collected at two sites on the upper Trinity River. The first site is downstream of the outflow from Lewiston Dam and the second site will be several miles downstream from Lewiston Dam, downstream of the confluence with Grass Valley Creek.

13. Will live vertebrate animals be maintained in captivity for greater than 12 hours?
☐ Yes ☒ No

If yes, describe where and how the animals will be housed (include all relevant husbandry details):

Click or tap here to enter text.

Who will be responsible for their daily care?

Click or tap here to enter text.

14. List the specific procedures likely to affect the behavior, physiology or wellbeing of live animals.

Seine netting will cause behavioral response to fish in the river reach. Fish temporarily held in buckets may experience some stress. Lavage will also cause stress and potentially harm to fish.

15. Mark the level of expected pain or distress caused by your methods below.

☐ **The methods described are purely observational and non-invasive OR will involve only the tissues or carcasses of dead animals; behavior of live animals will not be influenced intentionally.**

☐ **The methods will affect behavior, but no animals will be captured or handled (e.g. baiting animals, cameras in close proximity to animals, production of noises within normal limits of volume and frequency)**

☐ **The methods involve capture or handling without anesthesia, but only for a brief period for measurement or observation. No samples will be collected.**

☐ **The methods involve capture or handling without anesthesia, and routine samples (hair, blood, etc.) will be collected or euthanasia will be performed; this may involve use of routine pharmaceuticals to promote health (e.g. antibiotics, vitamins, fluids). This work may also involve temporary marking, placement of permanent tags, or fitting with telemetry transmitters or GPS receivers.**

☒ **The methods require use of anesthesia to mitigate distress or facilitate handling, and routine samples (hair, blood, etc.) will be collected or euthanasia will be performed. As above, this work may involve temporary marking, placement of permanent tags, or fitting with**

telemetry transmitters or GPS receivers.

☐ **The methods require use of anesthesia to mitigate pain or distress, and procedures will be invasive enough to require pain killing drugs (analgesics) upon revival. Sampling and marking may be performed as above.**

☐ **The methods will cause pain or considerable distress, but analgesics will not be used to mitigate the pain (e.g. surgeries from which animals are revived without provision of analgesics).**

☐ **The methods will be invasive and cause prolonged physiological or psychological stress without adequate mitigation of pain or distress. This may involve allowing animals to progress to death without provision of euthanasia or analgesia (e.g. LD50 experiments or long-term food or water deprivation).**

16. **Provide a complete and detailed description of all procedures to be performed involving live vertebrate animals. This response should justify comments made in # 13-15 and provide a detailed explanation of all procedures that affect animal behavior, physiology or wellbeing. Your response must address the handling and restraint of animals; deprivation of food or water; use of chemicals or biological agents; sampling methods for removal of biological samples; surgical and post-surgical procedures. N/A is an inappropriate response unless the protocol covers only the transportation, use, and/or storage of carcasses or tissues.**

Drift and diet sample collection
A crew of 6 (3 at each site) will be used to collect fish diet samples. During each of the three flow pulses released from Lewiston Dam, fish diet samples will be collected at 24 hr intervals for 2 hrs at dusk.

On each sampling date, just prior and during macroinvertebrate drift sampling, juvenile Chinook salmon will be captured by snorkel seine netting or other means for gastric lavage to be performed. Stomach contents will be collected to establish the size and species composition of diet. Depending on abundance and size (fish under 50mm will not be included) of juvenile chinook encountered, lavage will be performed on ~10-20 individuals at each sampling date.

Seine nets will be of various sizes and made of nylon or polyester 1/8" mesh. They will be operated by two tribal fisheries biologists or fisheries technicians and there will also be a snorkeler in the water guiding their efforts. Fish will be placed in either an 1/8" mesh enclosure in river, or a plastic tote with river water and aerator on bank. All fish will be handled using dip nets or nitrile gloves. Fish will be anesthetized each day in plastic trays of river water using MS-222 (150 mg/L buffered) in small batches of 3-5 fish. All other species will be released upon identification. Once anesthetized, fork length will be measured, they will be weighed, and gastric lavage will be performed over a 250 micro mesh or solid receptacle by flushing river water into the stomach cavity using pipets or rinse bottle nozzles (<1 cm) inserted in the mouth. After this process, fish will be held in either an 1/8" mesh enclosure in river, or a plastic tote with river water and bubbler on bank until they have recovered. The majority of fish will then be released into the area from which they were captured. A small number of fish (4 fish per individual performing gastric lavage per week, or if no fish are encountered of appropriate size to lavage) will be sacrificed to confirm that stomach contents are being evacuated effectively by the techniques being performed, or to provide stomach contents in the case that no fish large enough for lavage are encountered. The total number of juvenile Chinook Salmon sacrificed during the project will not exceed 100 individuals.

State collection permits were received for these activities by the Yurok Tribe and we are covered under the Trinity River Restoration Program section 10 permit for "take" of ESA listed Coho salmon that may be encountered.

Sample sorting and characterization
Once drift and diet samples have been obtained, they will be examined by the HSU River Institute laboratory so that drift concentration and juvenile Chinook diet can be characterized. Drift samples will then be divided into those portions that are representative of Chinook juvenile forage. This will allow the proportion of the drift that constitutes prey for juvenile salmon to be evaluated for how it is affected by flow management actions.

A graduate student from HSU (Thomas Starkey-Owens) will work with field technicians from US Fish and Wildlife Service, Hoopa Valley Tribe and/or Yurok Tribe to collect macroinvertebrate drift samples and fish gut content samples, collected via lavage in conjunction with each drift sampling date. The HSU graduate student will work with additional students and faculty at HSU laboratories to sort and identify the invertebrates collected in the drift samples. Samples will be sorted and identified to Family for insects and Class or Family for non-insect taxa under a dissecting microscope, and separated into that which represents juvenile Chinook salmon forage and that which does not. Biomass estimates will also be made from sorted invertebrates using length-weight regressions.

17. Use of animals for teaching or research requires consideration of alternative procedures to reduce the number of animals used and the pain and suffering caused by animal use. Explain how you determined whether alternative procedures were feasible for your study.

Please refer to the Altweb website (http://altweb.jhsph.edu/resources/searchalt/), which provides links links to search engines and provides general information on alternatives, for help in searching for alternatives to animal use.

1. **the type of literature searches conducted:**
   Online literature search (Altweb) to evaluate alternatives

2. **keywords used:**
   fish lavage to determine feeding rates, determing fish feeding rates without lavage samples

3. **range of dates searched:**
   2000-present

4. **other resources/methods used to determine alternative procedures:**
   Click or tap here to enter text.

18. Describe alternative procedures that were considered and rejected and a brief explanation of why the alternative procedures were rejected. N/A is an inappropriate response unless the protocol covers only the transportation, use, and/or storage of carcasses or tissues.

We could do the project without collecting and assessing fish gut contents, but then we would only know if there was a change in benthic macroinvertebrate drift, but not the effect on Chinook feeding or know which invertebrates the fish were feeding on; which is the real question we are interested in. Primary alternatives to lavage are either not reliable (e.g. bioenergetics models) or lethal.

19. Identify serious human health risks (<u>non-routine</u> exposures to risk, disease agents, toxic

chemicals, dangerous environmental conditions, etc.,) to which any participants might be exposed during the routine performance of the duties proposed herein.

Contact with MS 222 can be harmful. Wading in swift, cold water can be dangerous.

Describe steps taken to mitigate risks.

The material safety data sheet for MS 222 indicates that handlers should wear gloves and eye protection while preparing the stock solution.

Precautions will be taken to limit contact with high water and do do collections in slower water areas.

20. **Describe the fate of the animals upon completion of the protocol. Include (1) the procedure for euthanasia whether necessary as an experimental termination or in the case of unanticipated, accidental, injury whenever animals will be confined or handled and (2) the method of verification of death. Chemical euthanasia methods must include an appropriate, pharmaceutical-grade, drug, the route, and the dose to be used. Applicants should review the current Guidelines for Euthanasia (or its replacement in the Code of Federal Regulations), and justify any variations from the approved methods. Note that the Responsible Faculty Member must report unexpected deaths to the IACUC immediately and that N/A is an inappropriate response unless the protocol covers only the transportation, use, and/or storage of carcasses or tissues.**

(1) Euthanasia procedure for accidentally injured individuals will be overdose with buffered MS 222 (250 mg/L) until death.
(2) Death will be confirmed by lack of opercular ventilation for 10 minutes.

Final disposition for most individuals will be release.

21. **I certify by checking each of the boxes below, that all of the following are true:**

☒ **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," and 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 *immediately* to the IACUC.**

☒ **My level of supervision will be such that the procedures outlined in this protocol will be carried out in a humane and a scientifically acceptable manner as described herein.**

☒ **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 will ensure that no work described in this protocol will begin until the protocol has been fully approved by the IACUC, and that I will adhere to all deadlines and procedure outlined in the HSU ANIMAL WELFARE ASSURANCE in accordance with the PHS Policy for Humane Care and Use of Laboratory Animals.**

☒ **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.**

☒ **My research will be conducted in accordance with all relevant federal and state laws.**

☒ **My study does not unnecessarily duplicate previous studies using live vertebrate animals, as determined through literature database searches.**

☒ **I have considered the use of less invasive procedures, use of fewer numbers of animals and have determined that the methods proposed in this protocol are justified for the research and/or instructional objectives described herein.**

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** Click or tap here to enter text.

Signature, Responsible Faculty Member **Date**

Cover Sheet for IACUC Protocol Proposal

2-19-18

This protocol was originally approved as **1718.ESM.32-A.** However, we would like to make changes that are not allowed as an amendment because we would like to increase the number of individuals in the study by more than 10%.

In the original proposed protocol, **1718.ESM.32-A,** it stated "a small number of fish will be sacrificed to confirm that stomach contents are being evacuated effectively by the techniques being performed, or to provide stomach contents in the case that no fish large enough for lavage are encountered (only fish >60mm will be lavaged). The total number of juvenile Chinook salmon sacrificed during the project will not exceed 50 individuals."

We would like to submit a new protocol proposal in which the total number of juvenile Chinook salmon sacrificed during the project **will not exceed 100 individuals,** and **only fish >50mm will be lavaged** to collect diet contents (maximum of n=480 handled and n=100 sacrificed). **These changes were suggested and agreed upon in consultation with Dr. Darren Ward, who serves on the IACUC Board.**

Because the proposed increase in animal numbers exceeds 10%, we are submitting a new protocol proposal. See new protocol proposal attached.

The HSU graduate student working on this project has taken the appropriate CITI training and will be trained by Yurok and Hoopa field technicians in the appropriate fish handling protocols.

HSU's work on this project is under our control based on the approved protocol and not that of other individuals or agencies not part of the approved protocol.

HSU graduate student:

Thomas Starkey-Owens

trs338@humboldt.edu

(707) 685-7334

HSU ID: 012002923

FEB 19 2018

College of Natural Resources & Sciences
Humboldt State University

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

HUMBOLDT STATE UNIVERSITY

INSTITUTIONAL ANIMAL CARE AND USE PROTOCOL FOR THE HUMANE CARE AND USE OF LIVE VERTEBRATE ANIMALS

INSTRUCTIONS

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.

Please submit your protocols to the Dean's Office, College of Natural Resources and Sciences, Forestry Bldg, Room 101. All protocols should be submitted on the most recent version of the forms downloaded from the IACUC web page (http://www.humboldt.edu/iacuc). You can expedite the review process by following these formatting rules: leave an extra blank line between the questions and your responses; leave questions in bold-face type; type your answers in regular (non-bold) type; and do not delete anything from the questions. Please contact the Campus Veterinarian, Dr. Rick Brown, (by phone, 826-3320, or e-mail, RBrown@humboldt.edu) or the Chair of the IACUC, Dr. Rick Zechman (by phone, 826-3546, or by email Rick.Zechman@humboldt.edu) with questions concerning protocol preparation and submission.

- 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. Authors of protocols should contact the Campus Veterinarian, the Chair of the IACUC or Violet McCrigler in the CNRS Dean's Office, if they haven't heard any news after 10 days following protocol submission.