IACUC Protocols (/iacuc_protocols/)

Community structure of larval fishes off northern California

This Application Has Been Locked ×

IACUC Office

Reviewer Section

Reviewers Signature:

Signature:
Micaela Szykman Gunther
Signature Date:
Friday, September 11, 2020

Project Leader Review Section

Project Leader Comments:

Dear IACUC, I have marked one certification "incorrectly" and only so that submission could be completed, as work on this project has already begun. Until very recently, I simply (as in absent-mindedly) did not consider that this augmented analysis of our archived samples might represent a sufficiently substantial extension of work on collection and identification of larval fishes currently approved under previous and current IACUC protocols (05/06.ML.41-A, 09/10.F.03-A,13/14.M.117.A, and 15/16.M.102-A for the samples considered here, 2020M61 for ongoing collections not included in this project). This project includes analysis of archived samples to identify larval fishes. Visual identification is part of our ongoing work -- what is substantively new here is enhancing that analysis with genetic techniques to parse out identifications for visually cryptic larval rockfishes. In any case, my sincere apologies. This was a simple error and certainly represents no disrespect for the IACUC and the standards it upholds. Please contact me directly by email (epb4@humboldt.edu) or phone (831-247-8277) if I may provide any further clarification. Any reprimands, etc., should be directed at me, and I ask that this not reflect detrimentally on the graduate student (Blair Winnacott) whose work on this project is nearing completion or on my colleague (Andrew Kinziger) in whose lab the genetic analysis work is being done. Respectfully, Eric Bjorkstedt
Copied over from emailed comments: ""Hello Eric, I understand that you will be using archived fish samples and not live specimens, but you still need to provide a detailed list of procedures, otherwise, I cannot really evaluate your project as an E-level protocol. N/A as a response is really only applicable for certain sections. Also, on the species list, I do not see estimates of the # of individuals you expect to find. I know you cannot know exact #s, but are we talking single digits, double or triple digits? Finally, I'm not sure what your note about "incorrectly" checking a box is referring to - can you please be more specific? Thank you, Micaela"
Responses: Thanks for the quick review. To address comments we have added additional information on the (prior) collection of specimens included in our archive, and on the procedures used for genetic identification of larval rockfishes. We have added information on numbers below (in Numbers Justification) and as a preface to the Species List provided as a supplementary document. Regarding "incorrectly" -- this referred to my checking 'yes' to the query that work had not yet been initiated pending IACUC review. Please refer to my original note above regarding my tardy recognition that the proposed work, even though on archived samples, represented a substantial extension of research requiring additional IACUC review. Just being honest that we have already started this work, and are working in good faith to reconcile this. Please let me know if I can provide further clarification & thanks again!

1. Faculty Project Leader and Personnel

Project Leader:

Eric P Bjorkstedt

Project Leader Role: Faculty
Department: Marine Facilities
Other Protocol Participants:

Name:
Blair Winnacott
Email:
Blair.Winnacott@humboldt.edu (mailto:Blair.Winnacott@humboldt.edu)

Name:
Andrew Kinziger
Email:
Andrew.Kinziger@humboldt.edu (mailto:Andrew.Kinziger@humboldt.edu)

2. Project Information

Project Title:
Community structure of larval fishes off northern California
Protocol start date on date of approval:
Yes

4. Non-HSU Collaboration

Is this project part of a collaboration?:
No

5. Funding

Will the described project be funded?:
Yes
If Funded will the funds be administered by the HSU sponsored Programs Foundation (SPF):
No

6. Lay Abstract of Proposed Project

Lay Abstract:
The purpose of this project is to analyze a 12-year (2008-2019) time series of archived ichthyoplankton (larval fish) extracted from samples collected along a cross-shelf transect off northern California to characterize variability in assemblage structure and cross-shelf distributions in the context of seasonal and basin-scale changes in oceanographic conditions. Larval fish will be identified and measured under magnification. A key element of this research will be the application of genetic methods to resolve ambiguity in identification of larval rockfishes (Sebastes spp.) – a first application of this approach off northern California. Our goal is to (1) describe assemblage variability with extra focus on rockfishes; (2) relate variability to oceanographic conditions and (3) identify how ichthyoplankton surveys might yield insight into potential population shifts linked to climate variability (e.g., El Nino and Marine Heatwaves). Results from this work will contribute to filling a substantial spatial gap in our understanding of plankton distributions along the U. S. West Coast, and how the California Current Ecosystem responds to seasonal cycles and interannual climate dynamics.

7. Purpose of Project

Purpose of Project:
Student Research
1. the type of literature searches conducted::
Google Scholar
2. keywords used::
larval fish, ichthyoplankon, California Current
3. range of dates searched::
1990-2020

8. Animal Housing

Will Live Vertebrate animals be maintained in captivity for greater than 12 hours?:
No

9. Animals

Target Species:

Latin Binomial(s):
See attached documentation
Sex:
Unspecified

10. Numbers Justification

Explain why a smaller number would not allow you to meet your objectives::
We will conduct genetic analysis on all larval rockfishes recovered from samples (up to a maximum of 50 per sample). Based on visual identifications of corresponding formalin-preserved samples, we expect that the total number of individuals used for this analysis will be on the order of 2000, spread across approximately 500 samples (including samples with no rockfish larvae). It is necessary to do comprehensive analysis of individuals in sample (or a substantial subsample) in order to adequately characterize species diversity in each sample. Processing fewer individuals will introduce substantial uncertainty and compromise estimates of species composition in our samples. Note that the maximum number to be identified for those rare samples that include many rockfish larvae is constrained by funding, as samples of up to 100-300 are often needed to detect rare taxa in plankton community analysis. This analysis requires no new collections or handling of live animals. Specimens/tissues are from archived collections obtained in accordance with IACUC protocols 05/06.ML.41-A, 09/10.F.03-A, 13/14.M.117.A, and 15/16.M.102-A.

11. Source of Animals

Source of the animals (or tissues):
Specimens/tissues are from archived collections obtained along the Trinidad Head Line (41.05 N, 124.3-125 W). in accordance with IACUC protocols 05/06.ML.41-A, 09/10.F.03-A, 13/14.M.117.A, and 15/16.M.102-A.

12. Procedures Likely to Affect Animal Behavior and Welfare

List the specific procedures likely to affect the behavior, physiology or wellbeing of live animals.:
N/A with respect to use of live animals. All specimens/tissues are taken from archived collections.

13. Level of expected pain or distress

The methods described are purely observational and non-invasive.:
No
The methods will involve only the tissues or carcasses of dead animals not originally collected for this study; behavior of live animals will not be influenced intentionally. And I certify that the animals from which tissues were collected were not killed for the purpose of this study.:
Yes
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 or frequency).:
No
The methods involve capture or handling without anesthesia, but only for a brief period for measurement or observation. No samples will be collected.:
No
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.:
No
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 preformed, A above, this work may involve temporary marking, placement of permanent tags, or fitting with telemetry transmitters or GPS receivers.:
No
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 or marking may be preformed as above.:
No
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).:
No
The methods will be invasive and cause prolonged physiological or psychological stress without adequate mitigation of pain or distress.This may allow animals to progress to death without

provision of euthanasia or analgesics (e.g. LD50 experiments or long term food or water deprivation).:
No

14. Detailed Description of the Procedures

Complete and detailed description:
N/A with respect to use of live animals. All specimens/tissues are taken from archived collections. Details of (prior) collection and genetic analysis to be performed on archived samples are provided below. Larval fish to be used in this study are being removed from archived samples collected under IACUC protcols 05/06.ML.41-A, 09/10.F.03-A, 13/14.M.117.A, and 15/16.M.102-A. Larval fishes are collected monthly from five stations along the Trinidad Head Line (TH-line; 41.05° N), a high-frequency coastal transect (HFCT). Hydrographic data and plankton samples have been collected monthly along this transect since 2007 to serve as a source of information on ecosystem state and response to climate forcing in the northern California Current. Sampling is conducted on Humboldt State University's R/V Coral Sea. Larval fishes are collected using a 505 um and 335 um mesh bongo net, towed obliquely from a depth of 5 meters above the sea floor at shallow stations and to 100 meters at deeper stations. After collection, samples from the 335 um net are preserved in 95% ethanol and samples from the 505 um net are preserved in 5% formalin at sea. In lab, all larval fishes are sorted from plankton samples and preserved in ethanol. Laboratory protocol for this project covers only the use of carcasses and tissues for purposes of genetic identification of larval rockfishes. All larval fishes previously collected under IACUC-approved field protocols and have been identified down to the lowest possible taxonomic level based on visual characteristics (Matarese et al. 1989, Moser 1996). Unfortunately, the rockfishes (Sebastes spp.) cannot reliably be identified to species using visual assessment, except for a few rare exceptions. Genetic techniques have allowed the ability to identify previously unknown larval fishes, and have been particularly useful in applications to Sebastes (Taylor et al. 2004, Taylor 2004, Harvey et al. 2017, Thompson et al. 2017, Johansson et al. 2018). Genetic identification of larval rockfishes will therefore be conducted and based on comparison of 625 base pair sequences of the cytochrome b gene to an existing reference database of adult rockfish sequences. Genetic sequencing of larval rockfishes requires the use of tissue to act as template DNA for Polymerase Chain Reaction (PCR). To extract tissue from larval rockfishes, individual rockfish are placed in water under a microscope, photographed, and an eyeball or tissue from the caudal fin is extracted from the fish using forceps. Individual fish are retained in ethanol in individual vials as part the archived collection. Extracted tissues are stored in 70% ethanol in individual eppendorf tubes prior to genetic sequencing. Polymerase chain reaction (PCR) was used to amplify a 625 base pair region of the mtDNA cytochrome b gene using the primers GLURF2 and CB3RF2 (Rocha-Olivares et al. 1999). Each PCR reaction was conducted with 6.25 µl of GoTaq Hotstart Master Mix (Promega), 0.5 µl of 10 µM of each primer, 4.25 µl of

PCR clean water, and 1 µl of chelex supernatant containing DNA template. Thermal cycling conditions of PCR included an initial denaturation at 94 C for 2 min, followed by 35 cycles of 94 C for 45 sec, 57 C for 1.5 min, and 72 C for 1.5 min. A final extension was carried out at 72 C for 3 min. A negative control was included in each PCR reaction to test for the possibility of contamination. PCR products were then shipped to McLab Inc. (South San Francisco) for PCR clean-up and sequencing. Cleaned PCR products were sequenced in one direction using the primer CBINR3. Sequences were edited and aligned using the DNA sequence analysis software Sequencher v.4.8 (GeneCodes). Unknown sequences were compared to a reference data set of 374 independent haplotype sequences representing 67 species of identified adult Sebastes (Hyde and Vetter 2007). Resulting species (or species-complex) assignments are used to estimate density of larvae (expressed as number under 10 m2 of ocean surface) at each station along the transect. Data on larval fish community structure and density is then related to oceanographic conditions and climate forcing to understand drivers of variability in coastal ecosystems.

15. Consideration of Alternate Procedures

1. Alt web was searched (http://altweb.jhsph.edu/resources/searchalt):
Yes
2. keywords used::
larval fish, rockfish, Sebastes, genetic identification
3. other resources/methodes used to determine alternative procedures::
consultation with expert colleagues

16. Alternate Procedures Considered and Rejected

Explanation of alternative procedures rejected:
N/A with respect to use of live animals. All specimens/tissues are taken from archived collections. There are no alternative methods for resolving species identifications for larval rockfish in general, although some species can be distinguished visually.

17. Human Health Risks

Human health risks:

Minimal exposure to ethanol vapors during specimen handling and exposure to reagents used in genetic analysis.

Describe steps taken to mitigate risks:

Appropriate steps (e.g., safe lab practices, use of appropriate PPE [gloves, safety glasses]) are taken to reduce exposure to ethanol and genetic sequencing reagents.

18. Fate of Animals Upon Protocol Completion

Describe the fate of animals upon completion of the protocol:

All specimens will be returned to archived collection.

19. Certification

I have read and agree to abide by the "<ins>Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training at HSU,</ins>" 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. https://iacuc.humboldt.edu/iacuc-policy/:

Yes

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.:

Yes

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.:

Yes

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.:

Yes

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.:

Yes

My study does not unnecessarily duplicate previous studies using live vertebrate animals, as determined through literature database searches.:
Yes
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.:
Yes
Animal Welfare Act:
No
State of California Fish and Game Commission (Title 14) - Scientific Collecting Permit(s):
Yes
Endangered Species Act:
No
Fishery Conservation and Management Act:
No
Lacey Act:
No
Marine Mammal Protection Act:
No
Convention on International Trade in Endangered Species of Wild Fauna and Flora:
No
Other:
No

Supplemental Documentation

Supplement:
thl_specieslist.pdf (https://hsu-forms.humboldt.edu/iacuc_protocols/system/files/thl_specieslist.pdf)

(/)

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