



Licence Number: XRSF 8 2015
File Number: SARA 358
Valid From: 01-May-2015
Expiry Date: 30-Apr-2016

This licence and/or permit is issued under the authority of SECTION 73 OF THE SPECIES AT RISK ACT.

This licence and/or permit authorizes the person(s) listed below, subject to the following terms and conditions, to collect the species and quantity of fish identified below for: Scientific Research Relating to the Conservation of the Species purposes. Non-compliance with any condition of this licence and/or permit may result in the cancellation of this licence and/or permit.

Licence/Permit Activity Description:

The first component of proposed works (measuring visual acuity and neuromast density) involves a study of neurological and sensory differences between Limnetic and Benthic Sticklebacks in Priest and Paxton lakes, relating to the impact of water quality on mate recognition. The second component (genotyping of fin clips) will continue efforts begun in 2013 and 2014 to estimate the rate at which hybrids between limnetics and benthics are produced, and the rate at which they are removed by natural selection. Fin-clips will also contribute to approximate mark-recapture population estimates. A large number of morphological measurements will also be made on all specimens used to confirm species identity and for general comparative and monitoring purposes.

Licence Holder:

FIN: 121909
UNIVERSITY OF BRITISH COLUMBIA DEPT OF ZOOLOGY 6270 University
Blvd.
Vancouver BC V6T 1Z4

Schluter, Dolph

Contact Number: 604-822-2387
Fax Number: 604-822-2416

Individuals or groups assisting with the authorized activity:

Monica Yau, Shaun McCann, Diana Rennison, Kieran Samuk, Ashwin Bhandiwad, Dr. Catherine Peichel, Dr. Joseph Sisneros, Dr. Tracy Larson, and University of British Columbia undergraduate students.

Species, Quantity of Fish, Area(s) and Gear:

Species: Paxton Lake Benthic and Limnetic Threespine Stickleback species pairs (*Gasterosteus aculeatus*).
Vananda Creek Benthic and Limnetic Threespine Stickleback species pairs (*Gasterosteus aculeatus*).

Areas of Activity: Collection zones in Priest and Paxton Lakes (Texada Island) as delineated in the "Guidelines for the collection and in situ scientific study of stickleback species pairs (*Gasterosteus* spp.)" (Recovery Team for Non-Game Freshwater Fish Species in BC, 2008).

Gear: "Gee" minnow traps, holding containers/buckets, Prime or Amquel drops, 2-litre plastic bags, coolers, 100-litre aquariums, scissors, MS-222 (Tricaine methanesulfonate), Artemia and frozen bloodworms, cloth wrap, custom-built cylindrical acrylic tanks, ethanol, 0.025% 2-(4-(dimethylamino)styryl)-Nethylpyridinium iodide (DASPEI; Invitrogen), zebrafish embryo medium (1 mM MgSO₄, 120 μM KH₂PO₄, 74 μM Na₂HPO₄, 1 mM CaCl₂, 500 μM KCl, 15 μM NaCl, 500 μM NaHCO₃), Sodium hydroxide, DNA processing equipment (e.g. Qubit Fluorometer, Pst1 restriction enzyme, agarose gel, Qiagen gel, alizarin red stain, Potassium hydroxide), microscope, cameras.

Anticipated Number of Fin Clips:

Benthic Paxton Lake Stickleback adult females: 75
Benthic Paxton Lake Stickleback adult males: 75
Benthic Paxton Lake Stickleback juveniles: 150
Limnetic Paxton Lake Stickleback adult females: 75
Limnetic Paxton Lake Stickleback adult males: 75



Limnetic Paxton Lake Stickleback juveniles: 150
Benthic Vananda Creek Stickleback adult females: 75
Benthic Vananda Creek Stickleback adult males: 75
Benthic Vananda Creek Stickleback juvenile: 150
Limnetic Vananda Creek Stickleback adult females: 75
Limnetic Vananda Creek Stickleback adult males: 75
Limnetic Vananda Creek Stickleback juveniles: 150

Anticipated Number of Mortalities:

Benthic Paxton Lake Stickleback adult females: 57
Benthic Paxton Lake Stickleback adult males: 57
Benthic Paxton Lake Stickleback juveniles: 16
Limnetic Paxton Lake Stickleback adult females: 57
Limnetic Paxton Lake Stickleback adult males: 57
Limnetic Paxton Lake Stickleback juveniles: 16
Benthic Vananda Creek Stickleback adult females: 57
Benthic Vananda Creek Stickleback adult males: 57
Benthic Vananda Creek Stickleback juveniles: 16
Limnetic Vananda Creek Stickleback adult females: 57
Limnetic Vananda Creek Stickleback adult males: 57
Limnetic Vananda Creek Stickleback juveniles: 16

Additional Information:

Authorized Activities

Trapping and Fin-clipping: Gee-type minnow trapping will occur in May (adults) and October or November (juveniles), 2015. Fish caught in traps will be decanted into buckets containing fresh lake water. Fish brought back to the lab will be transferred to fresh buckets and eventually to coolers and transported by truck. Fish used in the genetic analysis of fin clips will be removed one by one from the holding bucket and gently held in one hand with the tail splayed open. A sharp pair of scissors will be used to remove the top 1/8th of the tail fin. Each fish will be placed into a fresh bucket where it will be inspected for any signs of stress, then gently released at the location of capture. Per Condition #5 of this permit, additional trapping and release will be carried out in order to generate approximate mark-recapture population estimates using fin-clipped individuals. Conditions #2, 3, 4 and 7 of this permit are intended to reduce impacts to eggs/larvae as a result of capturing potential nest-guarding males. In the rare event of animal injury, the fish will be immediately euthanized with an overdose of MS222 and preserved in pure ethanol; these fish will still be useful for morphological study and will also be included in the genetic analysis.

Transport and housing: Fish will be transported and kept alive in the wet lab at the University of British Columbia (UBC). Fish will be transported from the field in 2-litre plastic bags filled to about 20% with fresh lake water (about 5 individual fish per bag). A few drops of Prime or Amquel will be added to condition the water and remove ammonia. Pure oxygen will be used to inflate the bags, which will then be sealed tightly. The bags will be packed into coolers with an ice pack wrapped in newspaper on the bottom. No more than 40 fish will be packed into each cooler placed into a truck and driven the same day to UBC. Upon arrival, fish will be transferred to stand-alone 100-litre aquariums provided with filtration and aeration (no more than 20 fish per tank). Bags will be removed from the coolers and floated unopened in aquaria for at least 20 minutes. Bags will be opened and slowly filled with aquarium water before being inverted to release the fish into the tank. The lab will be maintained at a temperature of 17-18 degrees Celsius. Fish will be fed twice daily with live Artemia and frozen bloodworms.

Visual Acuity Laboratory Trials: An optokinetic response assay will be used to measure visual acuity of adult fish collected in May over the 2 weeks after collection (neuromast densities will be measured on live fish over the 8 subsequent weeks). Adult fish will be immersed in a 1.6 x 10⁻⁴ mg/mL solution of MS-222 until opercular movements cease for 2 minutes. Anesthetized fish will be measured, then restrained via a cloth wrap. The head, including the eyes



and gills, will not be restricted. Fish will be revived by running fresh dechlorinated water over the gills until the ventilation rate increases to approximately once per five seconds. Awake and restrained fish will be placed into a plastic holder and then a custom-built cylindrical acrylic tank (approximately 2 cm from the edge) and allowed to acclimate for five minutes. After acclimation and return of ocular movement, the experiment is initiated.

Projections will be made on the wall of the tank and the optokinetic response of the fish will be measured using a camera. The procedure will be repeated 20 times on each fish with a 1-2 minute randomized inter-trial interval. During this interval, the water will be aerated and the new stimulus will be prepared for projection. After the last trial, the fish will be removed from the experimental cylindrical tank and replaced into the holding tank.

Neuromast Density Measurements: To visualize neuromasts, live fish will be placed in 0.025% 2-(4-(dimethylamino)styryl)-Nethylpyridinium iodide (DASPEI; Invitrogen) in zebrafish embryo medium for 15 minutes, rinsed with clean embryo medium, then anesthetized with fresh 0.013% MS-222 in embryo medium, adjusted to pH 7.2 with NaOH. Microscopy will then be performed, and images recorded.

Genetic Estimation of Assortative Mating and Natural Selection: Next generation genotyping-by-sequencing will be used to genotype previously sampled fin clips at tens of thousands of markers randomly distributed across the genome. Total genomic DNA will be isolated from fin clips using a standard proteinase K Pheno-chloroform protocol. These data will be used to develop a model of gene flow and selection based on an Ancestry Coefficient estimated from genotypes using the program Structure.

Morphological measurements: A standard series of morphological measurements will be made on planned mortalities. One pectoral fin will be removed from each fish specimen and kept in ethanol to retain a DNA sample for future use. A geometric morphometric approach will be taken to digitize landmarks that highlight important differences in shape between specimens.

Method of Euthanasia: Fish housed in the laboratory are killed with MS222 (1 part MS222 per 20,000 parts water, buffered to a neutral pH using sodium bicarbonate). Fish are then preserved in 95% ethanol for genetic analysis.

Terms and Conditions:

1. All of the proposed activities must be carried out using the techniques outlined in the attached "Guidelines for the Collection and In Situ Scientific Study of Stickleback Species Pairs (*Gasterosteus* spp.)" (Recovery Team for Non-Game Freshwater Fish Species in BC, 2008).
2. Traps must not be set for more than 2 hours at a time.
3. Known nesting sites must not be targeted for trapping.
4. To the extent possible, the least colorful males captured will be retained for laboratory study.
5. Fin clipping procedures must be conducted in such a way that allows for approximate mark-recapture population estimates to be calculated and provided to sara@pac.dfo-mpo.gc.ca in an interim report by December 31, 2015.
6. If the maximum number of permitted mortalities (57 each of Benthic Paxton Lake adult females and adult males; 57 each of Limnetic Paxton Lake adult females and adult males; 57 each of Benthic Priest Lake adult females and adult males; 57 each of Limnetic Priest Lake adult females and adult males; 16 each of Limnetic and Benthic Paxton Lake juveniles; 16 each of Limnetic and Benthic Priest Lake juveniles) is exceeded, work must be discontinued immediately and Martin Nantel must be contacted at sara@pac.dfo-mpo.gc.ca to report the occurrence and to determine any potential for further works that may affect Paxton Lake or Vananda Creek Sticklebacks.
7. Reasonable efforts must be made to coordinate trapping at Priest and Paxton Lakes with other permit holders working in the area to reduce the likelihood of harm to Paxton Lake and Vananda Creek Stickleback Species Pairs.
8. The permit is only valid for the activities described within. It (or a copy) must be carried by a member of the field crew and be made available to a Fishery or Conservation Officer upon request.
9. The permit does not replace any other permits required under provincial or federal legislation.
10. An interim report (by December 31, 2015), and a final comprehensive report (by December 31, 2016) must be filed detailing any activity, authorized by this permit, which results in killing, harming, harassing, capturing or taking of any individuals of the affected species above. If the individuals were captured and are being held then the permit holder



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must also indicate where they are being held and what is planned for these captured individuals (death, release, etc.). The final comprehensive report must include a plan for developing robust Paxton and Priest Lake Stickleback Species Pairs population estimates in the 2016 field season. The completed report(s) must be sent to:

SARA Regional Manager
200 - 401 Burrard Street
Vancouver, BC, V6C 3S4
sara@pac.dfo-mpo.gc.ca

By signing on this document, the person(s) listed below, agree to be bound by the terms and conditions that pertain to each person as an individual and to the group as a whole.

121909

FIN	Licence Holder - Print Name	Signature	Date
			MAR 25 2015

Issued by: Bonnie Antcliffe, Regional Director, Ecosystems Management Branch
Fisheries and Oceans Canada

Licence Issued: 25 March 2015

Licence Printed: 25 March 2015