Status Report 11/00

Seattle City Light
Wildlife Research Program
Status Report: Species Identification and Genetic Differentiation Among Ranid Frog Populations in the Skagit River Watershed, North Cascades National Park
November 6, 2000

In 1996 and 1997, amphibian surveys of North Cascades National Park were conducted by Ron Holmes and Reed Glesne as part of a four year program to inventory amphibians in the Pacific Northwest. Surveyors were unable to positively identify ranid frogs found in the Big Beaver Valley of the Skagit River watershed. These animals had a combination of field markings from three species present in the Skagit River watershed: *Rana aurora*, *Rana cascadae*, *and Rana luteiventris* (Holmes and Glesne 1998). Based on a preliminary genetic analysis, we have identified Big Beaver animals as *R. luteiventris* (unpublished data). However, this analysis was of mitochondrial DNA (mtDNA), which is maternally inherited. Therefore, it is impossible to determine if these animals are pure *R. luteiventris*, or hybrids between female *R. luteiventris* and males of another ranid species. In 1999, Seattle City Light awarded a grant to our lab to: 1) isolate species specific biparentally inherited nuclear markers that will positively identify the frogs in the Big Beaver Valley and 2) assess the level of genetic variation of ranid populations in the Skagit River watershed relative to other populations within each species range. This report summarizes the work to date for both species identification and genetic variation assessment for the ranids in the Skagit River watershed.

Sample Collections

In the summer of 1997, 11 samples were collected from unidentifiable animals in the Big Beaver Valley. An additional three unidentifiable animals were collected in 1999 from McMillan Creek, 12 km west of the Big Beaver Valley site. In the summer of 1998, 25 *R. cascadae* were collected from Illabot Creek, northwest of the Big Beaver Valley. Between the summers of 1998 and 2000, 80 *R. luteiventris* were collected from Dagger Lake, and four putative *R. cascadae* were collected from the wetland associated with Dagger Lake, within the North Cascades National Park. It is important to note that the four putative *R. cascadae* possessed some *R. luteiventris* field markings, suggesting they may be hybrids. Additionally, we have collected samples from 12-15 populations from both *R. luteiventris* and *R. cascadae* from throughout each species range. Unfortunately, we were unable to collect *R. aurora* from the Skagit River watershed. All samples, with the exception of the three from McMillan Creek, were collected by toe clipping and releasing adult frogs.

Nuclear Marker Isolation and Analysis

In order to isolate species specific nuclear markers, genomic libraries were made from type specimens of *R. aurora*, *R. cascadae*, and *R. pretiosa*, a closely related species of *R. luteiventris*. Initial screening for markers was done in the *R. pretiosa* library, and 10 clones were isolated as potential species specific markers. Primers were synthesized to amplify all 10 of these loci in the polymerase chain reaction (PCR). Amplification was performed on all three ranid species (*R. aurora*, *R. cascadae*, *R. luteiventris*). Three of

these loci, RP49, RP 82, and RP105, amplify in multiple individuals across each species range. In order to determine if the base pair composition of each locus was species specific, all PCR product was used in a Single Strand Conformation Polymorphism Analysis (SSCP, Orita et al. 1989). This technique allows efficient determination of sequence differences between DNA fragments of the same size because unique sequences of DNA will yield different banding patterns on a nondenaturing polyacrylamide gel. All three loci consistently distinguish between *R. aurora*, *R. cascadae*, and *R. luteiventris* across each species range.

The unknown ranids from Big Beaver Valley and McMillan Creek were used in PCR amplifications for RP49, RP82, and RP105, and then run through an SSCP gel next to standards of each species. All 14 animals, previously unidentifiable by field markings, were identified as *R. luteiventris* using this technique. These data agree with the earlier mtDNA analysis identifying these animals as *R. luteiventris*.

Genetic Differentiation Assessment

Currently, we only have genetic differentiation data for one species in the Skagit River watershed, *R. cascadae*. Two mtDNA markers (a portion of the D loop, and a portion of the ND1 gene) were used in an SSCP analysis to determine unique sequences among individuals from Illabot Creek, WA. The SSCP banding patterns were compared to banding patterns from 323 other *R. cascadae* from 10 populations throughout the species range. Our previous analysis of *R. cascadae* populations has divided this species into three distinct genetic areas: the Olympic Peninsula, the Central Cascades of Washington and Oregon, and Northern California (unpublished data). All individuals from Illabot Creek are fixed at both markers for alleles that are unique to the Central Cascades group.

Future Work

We plan to develop at least two more species-specific nuclear markers that will distinguish *R. aurora*, *R. cascadae*, and *R. luteiventris*. Once these markers are developed, they will be used to reconfirm the *R. luteiventris* identity given to the animals of Big Beaver Valley and McMillan Creek. These markers will also be used to positively identify the four putative *R. cascadae* collected from the wetland of Dagger Lake.

We also plan to assess genetic differentiation of *R. luteiventris* in the Skagit River watershed relative to other populations throughout the species range. Because we were not able to collect *R. aurora* in the Skagit River watershed, it will not be possible to conduct a genetic differentiation analysis of this species.

Literature Cited

Holmes, R, RS Glesne. 1998. NOCA NRPP amphibian inventory bridge creek watershed 1997 – Progress Report

Orita, MS, Y Suzuki, T Sekiya, K Hayashi. 1989. Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics 5:874-879