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 Based on a preliminary genetic analysis, we have identified Big Beaver animals as *R. luteiventris* (unpublished data).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