Independent Scientific Review Panel
for the Northwest Power & Conservation Council
851 SW 6th Avenue, Suite 1100
Portland, Oregon 97204
www.nwcouncil.org/fw/isrp

Memorandum (ISRP 2012-9) **June 15, 2012**

To: Joan Dukes, Chair, Northwest Power and Conservation Council

From: Rich Alldredge, ISRP Chair

Subject: Follow-up Review CRITFC Lamprey Program Objectives 3.3 and 5.1 (2008-524-00)

Background

This review concerns the Columbia River Inter-Tribal Fish Commission's Accord Project, Tribal Pacific Lamprey Restoration Plan Implementation (2008-524-00). This multi-faceted project has undergone several ISRP reviews, including a review of the comprehensive plan/program for which the ISRP requested more detailed descriptions, essentially sub-proposals, for the project's various objectives (ISRP 2010-16). Sub-proposals for these objectives have been submitted for ISRP review over the last two years.

On April 6, 2012 the Council requested that the ISRP evaluate a response to the June 2011 review (ISRP 2011-15) concerning three project objectives (or sub-proposals):

1. Objective 3, Action 3.3, Tasks 3.3A, subtask(i); Assessment of gene flow in Pacific lamprey using microsatellite markers

2. Objective 3, Action 3.3, Tasks 3.3A, subtask(ii); Microsatellite analysis on Pacific lamprey from the Willamette Basin

3. Objective 5, Action 5.1, Task 5.1B; Emerging and legacy contaminants in juvenile Pacific Lamprey in the Columbia River Basin.

Our review follows below with recommendations and comments for each objective.

Recommendations and Comments

I. Objective 3. Monitor and evaluate, collect and disseminate information on lamprey population status, life histories and mainstem habitat.

a. Task 3.3A. Fund analysis of existing juvenile and adult genetic samples to optimize suite of DNA and AFLP markers

Subtask (i) Analyze existing samples to assist in establishing gene flow trends and temporal vs. geographical/spatial differences.

Assessment of Gene Flow in Pacific lamprey using Microsatellite Markers

2012 Recommendation: Does not meet scientific review criteria

Comments:
The ISRP requested clarification of whether the effort to develop Pacific lamprey microsatellites was most efficient and cost effective if undertaken by the identified principal investigator (PI), or if it would be more cost effective to contract this effort to an existing laboratory already set up for such work, thereby streamlining the microsatellite development effort. This question was not answered.

The sponsors have argued that using the FIASCO protocol would be more cost effective than switching to next generation sequencing techniques. The ISRP believes that assertion is reasonable. However, no evidence is provided by the principal investigator that use of the FIASCO protocol is more efficient and cost effective – measured by either cost in dollars or in time spent developing the markers – than using a specialized contractor.

The goal is identified as "Use microsatellite markers to clarify or define populations or aggregations in the Columbia River and along the West coast of North America (range-wide scale) and examine temporal variation." The sponsors need to set the project in the context of management objectives. How will this work lead to lamprey population restoration? The proposal addressed the ISRP's concerns about the spatial aspects of the problem quite well, but no mention is made of sampling to estimate temporal variation and how its significance would be interpreted in management actions.

The ISRP also concluded that the small sample sizes proposed in the study will likely lead to "no effect" due to lack of statistical power. Presumably, these data could be considered as a long-term study where additional samples are collected over time and added to the database with periodic re-analyses.

Below are some additional ISRP comments on specific elements of the sub-proposal.

- Isolate polymorphic microsatellite markers using Fast Isolation by AFLP Sequences Containing repeats (FIASCO)

Justification for the proposed method. DNA microsatellite simple repeats are a suitable genetic marker for estimating genetic variation, genetic population structure, and various biological phenomena (parentage analysis, hybridization, gene exchange, to name a few). The ISRP requested clarification of whether the effort to develop Pacific lamprey microsatellites was most efficient and cost effective if undertaken by the identified principal investigator (PI), or if it would be more cost effective to contract this effort to an existing laboratory set up for such work. This question was not answered. The assertion that using the FIASCO protocol would be more cost effective than switching to next generation sequencing techniques is reasonable but no evidence is provided that development by the PI would be better than using a specialized contractor.

The proposal indicates that FIASCO was used to discover 2 polymorphic microsatellite loci among eleven candidate loci, and informs the ISRP that 130 more candidate DNA sequences are available for development and screening. More information is needed on the level of experience the lab has in developing microsatellites, the number of markers likely from the 130 candidates, or how many markers they believe will be required to provide the precision in analysis of lamprey genetic variation. In addition more discussion on the pros and cons of in-house versus contractor development of these technologies is needed. Based on the information provided, the ISRP is unable to conclude that the anticipated development scheme is efficient and cost effective.

Time frame. A time frame for development and analysis of the 130 candidate sequences was provided. The sponsors anticipate completing the project in 2 years: 6 to 9 months for marker development, 6 to 9 months for genotyping lamprey samples, and 4 months for data analysis and report preparation. These time frames are reasonable.

- Estimate levels of genetic diversity and degree of spatial genetic differentiation

The ISRP had concerns about the statistical design of the work. The sponsors state they would like to "Examine variations between years and multiple-years at some geographic locations." The sponsors should clarify if they mean "within years" rather than "between years."

The sponsors should also clarify what they mean by the objective "to determine at what scale differences exist." Detection of differences in allele frequencies are largely driven by sample sizes with small sample sizes having poor power to resolve differences in allelic frequencies and large samples able to detect trivial differences. Similarly "scale" is ill defined – is this river kilometer, as the crow flies distance, or stream order differences? Given the relatively small sample sizes proposed, only vaguely identified in the proposal, the ISRP concludes that the sponsors will not be able to achieve this objective regardless of scale or whether the intent is to evaluate variation within years or between years.

The proposed QA/QC using replicate analysis of some individuals and double scoring all individual samples is a good idea. However, the sponsors do not state how many individuals will have replicate analyses.

The sponsors state, "We will make estimates of statistically significant differences … among populations …" Clarification on what these populations are is needed. Estimates of the differences should also be computed for all pairs of populations, regardless if they are "statistically significant."

b. Task 3.3A. Fund analysis of existing juvenile and adult genetic samples to optimize suite of DNA and AFLP markers
Subtask (ii) Analyze potential for subpopulation gene flow in the Willamette subbasin.

Microsatellite analysis on Pacific lamprey from the Willamette Basin

2012 Recommendation: Does not meet scientific review criteria

Comments:
This sub-proposal *Microsatellite analysis on Pacific lamprey from the Willamette Basin* and the sub-proposal *Assessment of Gene Flow in Pacific lamprey using Microsatellite Markers* still do not seem to be integrated.

The sponsors need to more effectively frame the project in the context of management objectives. How will results of the work lead to lamprey population restoration? The ISRP noted that the objective is to relate genetic variation to migration behaviors (see page 4 of the proposal). However, the proposed methods under genetic sampling (page 5) discuss comparing allelic frequencies among various populations but do not address how the proposal will actually investigate the stated goal.

Below are some additional ISRP comments on specific elements of the sub-proposal.

- <u>Details on the genetic analysis of adult Willamette lamprey tagged in 2009 and 2010</u>

The proposal briefly, but adequately, identifies the number of lamprey tagged in 2009 and 2010, and also identifies the number that were located during post-tagging migration. The proposal also identifies that the Willamette samples will be compared with other regions from British Columbia to California that were considered in analyses by Docker et al. 2011. The question of comparing the genetic structure among Willamette River tributaries is not sufficiently addressed. The proposal states that comparisons will be made between samples in 2009 and 2010 and if they are not significantly different, they will be pooled for comparison with other samples. A cogent discussion of the options for analysis of the fish tagged at Willamette Falls, and either detected or undetected elsewhere, was not provided. The proposal identifies the number of tagged lamprey subsequently detected, but does not identify the

numbers in specific tributaries or the numbers in different holding/migrating behaviors or habitats. As a logical starting point the sponsors should discuss the options for analysis of samples taken at Willamette Falls to detect structure within the Willamette River population.

The response provided in the joint comments indicates that the "microsatellite data may show stock structure among the holding habitat types, migration behaviors…" but does not provide sufficient details.

- Integration with the proposal "Monitoring the relative abundance of ammocoetes in the Willamette River Basin" submitted by Dr. Carl Schreck

The proposal sufficiently identifies that tissues from larval sampling by Dr. Schreck will be available for analysis by this effort.

- Relationship between the sub-proposals: *Assessment of Gene Flow in Pacific lamprey using Microsatellite Markers* and *Microsatellite analysis on Pacific lamprey from the Willamette Basin*

The ISRP is concerned that the two projects still do not seem to be appropriately integrated and collaborative. The Willamette proposal does not indicate consideration of using new microsatellite markers developed by the coastwide proposal. Neither proposal specifically identifies collaboration or sharing tissues. The Willamette proposal identifies that data will be combined with earlier work by the Docker team, but there is no identification of sharing data and analysis with the coastwide project.

The two groups should be exchanging samples from the same fish, sending each other the specifics of their PCR methods, and the methods used to identify the genotypes of each lamprey they examine. They should be comparing the allele frequencies in the same microsatellite loci that their labs are able to detect. Laboratory procedures will certainly be different but they should have about 90% agreement. The UBC group, headed by David Close, could benefit the University of Manitoba group, headed by Susan Docker, if additional variable microsatellite loci can be found as that would help her group determine if separate populations of lamprey exist in the Willamette basin. Conversely, the Manitoba group also has expertise in microsatellite discovery. Perhaps they could be assisted in the search for additional microsatellites, if samples outside of the Willamette basin were shared by the Close Lab. Closer collaboration would aid lamprey genetics research needed for management decisions.

- Details of study design and benefits to management

The Willamette proposal presents a very general scheme for analyzing the data; one that is not aimed at identifying subdivision with the Willamette River subbasin. A thorough presentation is needed of the potential analyses, the hypotheses the analyses address, and the management questions that can be addressed with these data.

The ISRP noted that the goal is to relate genetic variation to migration behaviors (see page 4 of the proposal). However, the proposed methods under genetic sampling (page 5) discuss comparing allelic frequencies among various populations and do not address how the proposal will actually investigate the stated goal.

II. Objective 5. Evaluate contaminant accumulation and other water quality impacts on lamprey

Task 5.1B. Through funding partnerships with USGS, EPA and others, evaluate juvenile contaminants in 2-3 tributaries in 2010 and expand in future years.

Emerging and Legacy Contaminants in Juvenile Pacific Lamprey in the Columbia River Basin

2012 Recommendation: Meets scientific review criteria (qualified)

A two to three year preliminary study is justified to develop data on the sensitivity of the methods and the likelihood of detecting patterns of differences of contaminant levels in juvenile lamprey (ammocoetes and macropthalmia) and sediments. The study duration may depend on the time needed to complete chemical analyses and subsequent data analyses. The preliminary data should be used to improve the sampling program, and ISRP comments on the study design and sampling strategy should be taken into account. Consideration should also be given to sampling adults.

A report on the preliminary study, once completed, should be submitted to the ISRP for review before further work is conducted.

Comments:

Sample locations and coordinated studies. The response provided an improved description of some elements of the study design. With the analytical costs so high, it is important, even in a reconnaissance, to strategically choose sites for the study to gain maximum useful information. The concept of obtaining contrasting contaminant information from areas with different land uses (agriculture vs. no agriculture) and human population densities with river flow, the latter which was mentioned, can be a very useful initial approach. The 15-mile Creek area with a known historic spill was an excellent site to choose for the initial study. It is also very useful to collect paired samples of sediment and juvenile lamprey at the various locations. Sampling sediment permits the potential calculation of a bioaccumulation factor (BAF), which is very useful information, but the sampling strategy has limitations as described below.

The ISRP believes that considerable efficiency and some cost savings can be gained with effective coordination with the various components of the “Tribal Pacific Lamprey Plan Implementation” (2008-524-00). Other studies under the plan are handling lamprey for genetic studies, and even evaluating juvenile lamprey distribution, abundance, and habitat selection

(e.g., Objective 3, Task 3B). There needs to be strong coordination with these other projects including sharing of information and perhaps sample collection. A potential evaluation of juveniles by size was mentioned and could also provide meaningful information on contaminant bioaccumulation. Opportunistically analyzing samples that become available seems short of fully taking advantage of this opportunity to better understand contaminants, thus, perhaps some additional field time strategically collecting a few additional samples at key areas may pay great dividends. This strategy may become more important as the two-three year study suggested by the ISRP progresses.

The list of contaminants to be analyzed was provided as requested, along with the analytical techniques to be used.

The collection of adults, which is important, was only mentioned as an opportunistic sampling goal. It would seem that to put juvenile findings into perspective, the series of contaminants being evaluated in juveniles in this study should also be evaluated in some adults, especially using the same analytical techniques. It would be important to compare contaminant levels in returning adults with that of macropthalmia departing the Columbia River Basin**.**

The data collected in this study will be useful when deciding which contaminants may be candidates for controlled lab studies. It was rightfully pointed out that lab studies are beyond the scope of this reconnaissance effort.

Statistical design. The ISRP had serious concerns about the statistical design for the project **–** in general, objectives are stated as goals and not well defined or measurable. For example, the sponsors want to develop BAF based on the paired sediment and tissue sampling. However, the proposal earlier indicates that the ammocoetes move several times (presumably only in a downstream direction) so the chemical concentration in the sediment where sampled may not reflect the exposure and accumulation process. For example, ammocoetes from a highly contaminated sample move downstream. Some arrive at a “pristine” site and some at a “contaminated” site. Both tissue samples would have similar concentrations of the chemicals, yet the sediment concentrations would be vastly different. If the presumed life history is correct, it may be worthwhile to collect sediment and tissue samples in a more systematic fashion moving from low-concentration to high-concentration areas, particularly if the tissues samples can be taken from “aged” ammocoetes. If the goal is to determine a BAF, then sampling should be designed to ensure a maximum contrast possible in sediment contamination loads.

It is not clear how “river flow data, wastewater data” will play a part in the sampling design or analysis.

There is a 30 sample limit, but Table 1 does not show how many (composited) samples will be taken at each site. There are 15 locations, so if 1 composited sample is taken at each site, there are 15 samples left. Will some be used to investigate individual variation? If so, how will

information on individual variation be used? The BAF will use the averages based on the composite sampling to create the regression line. Individual data are not used directly.

With only 30 samples and a huge number of chemicals measured, principle components analysis is unlikely to be very useful and observed components will likely be sampling artifacts. Some consultation with a biochemist is in order to establish a priori which components likely load together.

The proposal states, “Separate statistical analyses will likely be performed for each sampling location,” but sample sizes will be very small (only 1 or 2 from each location) so this approach is unlikely to work. The rationale on using “blocking factors” to account for confounding differences is also unlikely to work.

The proposal would benefit greatly by conducting a power analysis to determine the consequences of using only 30 samples.

The rationale for a three-year sampling program was not provided. What are the factors in the data collection, sample analysis, and data analysis that require a three-year study? For example, the ISRP understands that the time to conduct sample analysis can be variable.