Project ID: 35039
Title: The influence of hatcheries and their products on the health and physiology of naturally rearing fish

Sponsor: USGS-CRRL
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Responses to ISRP comments:

1. **Methodology for detection of *Renibacterium salmoninarum* (Rs) in large water samples is uncertain (but expected to be functional within a year).**

   Although selection of the final methodology is planned to occur during the first year of this project after methods comparisons are complete (task 1a), preliminary research indicates that the most likely procedure will be a modified version of the membrane filtration-fluorescent antibody technique (MF-FAT) that is currently used for quantification of Rs in water samples (McKibben and Pascho 1999, Dis. Aquat. Org. 38:75-79). The current MF-FAT cannot be used to determine Rs viability. The modified method would use filters and a filtering manifold system suitable for filtering larger sample volumes, and bacteria on filters would be stained with both a fluorescein-labeled antibody for specific identification of Rs, and a second fluorochrome to assess the viability of detected Rs cells. Such rapid procedures are now commonly used for detection of specific, active bacteria in environmental samples (see review by McFeters et al. 1999, J. Appl. Microbiol. Symp. Suppl. 85:193S-200S). We are evaluating several commercially available bacterial viability fluorochromes for selection of a stain most suitable for viability assessment of Rs. The final procedure should greatly reduce or eliminate the need for Rs viability assessment by bacteriological culture, which is time-consuming due to the long incubation period required (6+ weeks), and is often unreliable for environmental samples because Rs is frequently overgrown by contaminants even when a selective medium is used.

2. **Performance of tasks 3b-3h (determination of Rs levels in water and resident fish in areas naturally or artificially stocked with adult salmon carcasses) is contingent on results of task 3a (determination of effects of freezing on Rs in adult salmon carcasses).**

   If freezing of adult salmon carcasses in a hatchery freezer (as is practiced at some locations) is determined to be an effective means for destruction of Rs, stocking of previously frozen carcasses into streams for nutrient replenishment may present little risk of Rs transmission to wild fish. In this case, the investigations described under tasks 3b-3h would indeed be unnecessary as stated in the proposal, and an appropriate budget adjustment would be made. However, it might still be necessary to conduct additional carcass freezing experiments to further define the conditions necessary for

destruction of Rs. Such experiments are not described in the present proposal, and their implementation would require further modifications to the methods and budget.

3. **It is not certain that the three proposed hatchery sites meet the stated (5) criteria. Added justification of the sites should be provided and the proponents should seek Regional input regarding these sites before implementing the study.**

The final selection of the three hatchery sites for water sampling is planned for the second year of this project (FY 04), and sampling is planned to occur during FY 04 and FY 05. We will seek Regional input and guidance before the final selections are made, and are currently gathering information from the appropriate agencies about the suitability of the tentative selections (primary and alternate hatcheries) with respect to the stated selection criteria: (1) at least all adult female salmon are screened for Rs levels, (2) wild or naturally rearing salmonids are found in the adjacent stream, (3) historical data records on fish characteristics, common practices, disease history, etc., exist for the hatchery, (4) there is reasonable access to sampling sites and sampling logistics are fairly straightforward, and (5) hatcheries are located in three different areas of the Columbia River basin. Regarding the tentative choices for our three proposed hatchery sites (i.e., Cle Elum, Carson, and the Methow hatcheries), all of the sites meet the 5 criteria. This is based on our own personal knowledge from previous work at these facilities and has been confirmed after discussions with facility managers or fish health personnel. Specifically, details on how the sites meet the criteria are as follows:

1. Cle Elum: all females screened for Rs and other pathogens; chinook salmon spawning and rearing in the Yakima river and its tributaries; good records kept for this relatively new, conservation hatchery; good access to water sampling sites; located in Central Washington. Two of the co-PI's on our proposal, Dave Fast and Todd Pearsons, are very familiar with the Cle Elum facility and have confirmed these criteria.

2. Carson: all females screened for Rs; steelhead spawning and rearing in the Wind River; historical records maintained on this relatively older, production hatchery; good access to water sampling sites; located in south-central Washington on a tributary of the lower Columbia River. We have discussed these criteria with personnel from the Lower Columbia Fish Health Center and are awaiting discussions with the hatchery manager.

3. Methow: all females screened for Rs; chinook and steelhead spawning and rearing in the Methow River and tributaries; hatchery records maintained; good access to sampling sites; located in north-central Washington. These criteria have been confirmed by Bob Jateff, hatchery manager, and he believes this facility would be a good place to conduct this work.

4. **What artificial streams would be used in objective 2?**

The artificial streams will in fact be large fiberglass raceways. Our goal is to build raceways as large as possible given the constraints of our site location. Tentatively, they'll be at least 50 feet long, 6 feet wide, and 4 feet deep. The location for these streams has adequate water from the Big White Salmon River, is secluded yet close to our laboratory, and can accommodate from 6-8 streams of this size. All streams will be filled with cobble and gravel substrates, have pool-riffle sequences, and will contain an appropriate density of woody debris. To assist us in designing realistic and efficacious artificial streams, we plan on discussing the setup of these streams with colleagues from the NMFS conducting NATURES research.

As an aside, for our planned work with artificial streams in objective 1, they will be constructed in a similar manner but the water source will be from the Cle Elum Supplementation facility.

5. **What will be the statistical methods of analysis?**

Standard published methods will be used to test for differences in prevalence and levels of Rs among groups of fish (see e.g., Elliott et al. 1997, J. Aquat. Anim. Health 9:114-126). To compare relative frequencies of Rs-positive fish (ELISA-positive fish) among groups, chi-square analysis will be used. For comparisons of Rs antigen levels in fish testing positive for Rs by the ELISA, a log transformation will first be applied to ELISA absorbance values to achieve common variances of means among various data groups. Then, analysis of variance (ANOVA) will be used as appropriate for comparisons of ELISA values among groups.

The selection of statistical methods for analysis of Rs counts in water samples will depend on the concentrations of Rs present in the samples. If mean Rs concentrations are low (see e.g., Zar 1974, Biostatistical Analysis, Prentice-Hall, p. 305) such that their occurrence might be described by a Poisson distribution, a chi-square test for random distribution or a mean square successive difference test for serial randomness will be applied as appropriate to data collected from different sample sites at a given time or to data collected from a given sample site at different times. If Rs concentrations are relatively high, ANOVA will be used for comparisons of mean Rs concentrations in the various samples.

<u>Responses to Action Agency/NMFS RME comments:</u>

1. **Studies could also be combined with the heritability studies on disease resistance and immune function.**

One of the principal investigators responsible for proposal 35039 (Diane Elliott) is also a principal investigator for BPA Project No. 2000-072-00 (Heritability of Disease Resistance and Immune Function in Chinook Salmon) and therefore has access to data from that project as it is being generated. It is not likely that experiments from the two projects could be combined because fish experiments for Project 2000-072-00 would be completed before the scheduled start dates of fish

experiments for Proposal 35039 (and use different fish holding systems). Nevertheless, it is expected that data from Project 2000-072-00 would be used to assist in the design of the detailed protocols for fish experiments described in Proposal 35039 under objectives 1 and 2. Furthermore, as described in Proposal 35039, it is expected that the combined data from the two projects would be useful for determining the relative importance of genetic considerations and Rs infection levels in the optimization of hatchery broodstock maintenance programs to enhance the fitness and survival of progeny.

2. Could this proposal examine other pathogens at the same time?

Rs was selected as the principal pathogen of interest because of its high prevalence and known negative impact on hatchery-reared salmonid populations in the Columbia River basin, and because of the availability of methods for quantification of Rs in water samples. However, we expect this research to yield some information about other pathogens as well. Fish populations will be examined for several pathogens during regular agency screening programs at the hatcheries selected for water sampling. Fish in the hatchery /wild Rs exposure studies in objectives 1 and 2 can also be examined for other pathogens during the periodic health and physiological analyses planned for these experiments.

The examination of water samples for pathogens other than Rs would require the availability of reliable methods for specific identification and quantification of viable organisms in the samples. A procedure has been developed for detection of viable *Aeromonas salmonicida* in large water samples (Ford 1994, Aquaculture 122:1-7), but the described procedure is relatively labor-intensive and involves culture of samples on a medium that does not support the growth of Rs, so separate analyses would be required. Polymerase chain reaction (PCR) methods have been developed for specific detection of several fish pathogens in water samples, but the procedures presently available for environmental samples are not quantitative and cannot distinguish live from dead organisms.

We expect this project to serve as a model that could be used for monitoring of water for fish pathogens other than Rs. During the selection of hatcheries for water sampling in objective 1, we will determine whether monitoring for other pathogens in water might be useful based on the hatchery disease histories. Monitoring of water for other pathogens could be added to the project depending on the availability of suitable methods at the time the Rs water sampling is planned (FY 04 and FY 05). For example, if high-quality specific antisera are available for identification of pathogens of interest by immunofluorescence techniques, these procedures may be suitable for combination with viability staining in MF-FAT procedures similar to methods described for Rs. Analysis of samples for additional pathogens by these techniques would require the preparation and analysis of multiple filters from each water sample, and might only be practical if rapid, sensitive automated scanning procedures such as solid phase laser cytometry are available (see e.g. Lemarchand et al. 2001, Aquat. Microb. Ecol. 25:301-309). Implementation of such procedures

would require a budget adjustment to accommodate the increased workload and equipment cost.