Evaluation Proposal - Non Capital

Project Name: **ORIGIN OF JUVENILE CHINOOK SALMON IN WRIA 6 MIXED STOCK REARING ENVIRONMENTS**

1. BACKGROUND

Estuary and nearshore habitats are occupied by juvenile salmon during their transition from freshwater spawning and rearing habitats to ocean feeding grounds. One major data gap apparent in efforts to develop a recovery plan for Puget Sound Chinook salmon is information on juvenile Chinook salmon use of estuarine/nearshore habitats in mixed stock rearing environments. This proposal will fill data gaps in WRIA 6 by increasing our understanding of juvenile salmon nearshore habitat use as a function of river of origin.

Salmon productivity depends not on a single habitat or life history stage but rather on the full range of habitats used by fish throughout their entire life cycle. Disruptions of access to or quality of any of the habitats used by salmon throughout their life cycle will significantly reduce the productivity (abundance) and resilience (persistence) of salmon populations (Greene and Beechie 2004; Bottom et al. 2005a).

Emerging research demonstrates that estuarine/nearshore habitat characteristics and the period of estuarine/nearshore residence are critical to the viability of some salmon populations and will directly affect population productivity and abundance of returning adults (Mortensen et al. 2000; Magnusson and Hilborn 2003; Beamer et al. 2005; Bottom et al. 2005a & 2005b; Greene et al. 2005). Duration of estuarine/nearshore residence and attributes of estuarine/nearshore habitats can be important limiting factors in recovery of salmon populations (Beamish et al. 2000, Mortensen et al. 2000; Magnusson and Hilborn 2003; Greene and Beechie 2004; Beamish et al. 2004; Bottom et al. 2005a & 2005b).

Chinook salmon are the most estuarine/nearshore dependent of the pacific salmon species (Healey 1982 & 1991; Simenstad et al. 1982) and therefore the most vulnerable to human alterations of estuarine/nearshore ecosystems.

2. PROBLEM STATEMENT

Decisions about what estuarine/nearshore habitats to restore and models predicting the benefits of these restoration actions are hampered by a lack of knowledge about how different Chinook salmon populations use available estuarine/nearshore habitats. Use of estuarine/nearshore ecosystems by juvenile Chinook salmon will likely vary among populations or regional groups of populations (e.g., south Puget Sound versus north Puget Sound) due to such attributes as size at entry into Puget Sound, origin (what river they come from), and timing (when they enter estuarine/nearshore ecosystems). Population specific information on habitat use, particularly in mixed stock areas like WRIA 6, will make it possible to design restoration actions targeting specific populations. To date, our ability to document differences between Chinook salmon populations in their use of estuarine/nearshore habitats has been limited to coded wire-tagged, hatchery-origin fish in the main basin of Puget Sound (Duffy 2003; Brennan et al. 2005; Fresh et al. 2006). Hatchery origin salmon do not necessarily represent wild salmon life history types and results from the main basin of Puget Sound do not represent other areas throughout Puget Sound.

The proposed assessment will provide the data and analysis to make habitat-type-specific, place-specific, and population-specific priorities for Puget Sound listed Chinook salmon within WRIA 6. In consultation with the WRIA 6 Lead Entity TAG, the results of this assessment will be incorporated into an update of the WRIA 6 salmon recovery plan (SRP) and translated into the lead entity's ranking criteria for restoration and protection projects. The basis for SRP and project ranking updates will be the new information about the biological importance/dependence of particular habitat types and locations within the WRIA 6 nearshore by specific salmon stocks or assemblages of stocks. The priorities will weigh not only biological importance, but also the mandates of local and regional recovery plans. For example, not all populations of Chinook salmon present in the Puget Sound ESU have the same priority for recovery or the same risk of extirpation. This study is part of the Island County 3 year work plan for salmon recovery and is ranked as a high priority to implement. This study will also provide other lead entities within Puget Sound answers about the relative importance of WRIA 6 habitat to Chinook salmon populations originating in their rivers, thus linking river based recovery plans with nearshore recovery plans and addressing a weakness in the Puget Sound Salmon Recovery Plan identified by the TRT. Restoration and protection actions based upon this assessment will be immediately added to the local 3 year plan and implemented in sequence as funds become available.

3. PROJECT OBJECTIVES

The principal goal of this project is to inform the next update to the WRIA 6 Salmon Recovery Plan by providing a WRIA-wide analysis and synthesis of salmon habitat use (distribution by habitat type) and salmon origins. Filling these data gaps will empower the Lead Entity to evaluate and prioritize restoration and protection actions based upon known habitat use for a targeted population. Thus, managers will know what they are accomplishing towards salmon recovery with their work plans. The specific objectives of this study are:

Objective 1. Collect wild juvenile Chinook salmon in spring and summer from a range of locations, times, and estuarine habitat types in WRIA 6 using appropriate sampling methods;

Objective 2. Identify the origins of juvenile Chinook salmon obtained from each space/time stratum using microsatellite DNA analyses;

Objective 3. Compile and synthesize all existing use juvenile Chinook salmon data for Island County (Stillaguamish Tribe, Wild Fish Conservancy, NOAA Fisheries) and include these data in final analysis;

Objective 4. Work with WRIA 6 Lead Entity Technical Advisory Group and integrate the findings of this study into their salmon recovery plan and project ranking criteria;

Objective 5. Provide local, regional, and international managers with information on stock-specific use of estuarine habitats by juvenile Chinook salmon within WRIA 6.

4. PROJECT APPROACH

This project assesses juvenile Chinook salmon use of nearshore habitats (intertidal shoreline, estuary, and shallow subtidal habitats) within WRIA 6. This is a landscape scale assessment to determine linkages between nearshore habitats in WRIA 6 and juvenile Chinook salmon populations. This proposal, if funded, will integrate the following information for the WRIA 6 landscape:

- A spatially explicit model of juvenile salmon use of habitats, including estuarine/nearshore/offshore areas by sub-region within WRIA 6 (Figures 1, 2 and 3); and
- A spatially explicit model of the origin of juvenile Chinook salmon using estuarine/nearshore/offshore areas by sub-region within WRIA 6.

We considered other approaches and opportunities to achieve the objectives of this assessment and thus fill this data gap. No existing or past fish sampling efforts can be cobbled together into a credible landscape scale assessment because these efforts collected small amounts of data (limited in space and habitat type) over several years. It is not suitable for assembling into a big picture understanding of juvenile salmon use of habitats throughout WRIA 6 because salmon population dynamics (size and origins) are different each year. Existing coded wire tag data can tell us what adults or subadults are using WRIA 6 but these data are not suitable to draw conclusions about the importance of nearshore habitat to juvenile salmon. Our approach of collecting and compiling data from throughout WRIA 6, in the diversity of habitats present, and over the full time period of salmon use is the only way to gain a specific understanding of the importance of nearshore habitat to the ESA listed Puget Sound origin salmon.

If this project is not implemented at this time, salmon recovery efforts in WRIA 6 will proceed without managers knowing whether their protection or restoration actions are benefiting ESA listed Puget Sound origin salmon. Projects will continue to be driven by opportunity and an eclectic collection of data rather than shaped by a strategy to recover ESA listed Puget Sound origin salmon.

Costs were determined by each project partner. Each project partner has proven experience and capability to deliver the proposed work. This project makes use of a skilled team of scientists, ongoing regional sampling efforts, and federally funded labs. In addition to making good use of previous and ongoing sampling efforts, proposed not-for-profit staff and facilities costs are, on average, 35% less expensive than private consultants.

Other sources of funding were sought for this work, with no success. We have applied for funding to the past two funding cycles of the Pacific Salmon Commission's Southern Fund but have not been awarded funding. The lack of funding by the Southern Fund relates to this project not meshing well with their priorities for research, which typically is directed mostly at Fraser River sockeye populations. See attached consultant's report regarding research into funding options for a similar project in San Juan County (Appendix A).

5. PROJECT METHODOLOGY

Collection of Juvenile Chinook Salmon:

Juvenile Chinook salmon samples will be obtained from different regions, habitats, and time periods. Selection of sampling sites and dates are based upon a stratification scheme using time, space (region), and habitat type. The conceptual foundation for this stratification is based upon results of research from throughout the Pacific Northwest demonstrating that juvenile Chinook salmon use of estuarine and inland coastal landscapes will vary with region, habitat type, and time period. For example, Zhang and Beamish (2000) found a bimodal seasonal abundance curve for wild sub-yearling Chinook salmon in Georgia Strait; each mode was potentially a different group of fish (e.g., different life history strategy). Similarly, Beamer et al. (2005) recently found that differences in time (season or month) and habitat type directly affect the relative abundance of juvenile Chinook salmon life history types within Skagit Bay (Figure 1).

We hypothesize that juvenile salmon use of the estuarine landscape will vary spatially and temporally because of differences in the migratory pathways and habitats potentially available to source salmon populations. Migratory pathways could be influenced by the shape and diversity of the landscape, distance from natal river mouths, water quality, and water currents. Differences between source population sizes and characteristics (e.g., composition of life history types) could influence the composition of juvenile salmon populations within habitats.

We propose to obtain wild juvenile Chinook tissue samples from 11 different areas within WRIA 6 (Figure 3, Table 1). Each area represents a subset of WRIA 6 estuarine habitat where Chinook salmon stock composition might be unique based on differences in salmon migration pathways and proximity to source population areas like the Skagit, Stillaguamish, or Snohomish Rivers (Figures 2 and 3, Table 1). Within each of the 11 areas, we will sample a diversity of habitat types within the shallow intertidal, subtidal fringe, shallow subtidal, and offshore (Figure 4). Sampling methods will be consistent with those commonly used to sample in Puget Sound (see Beamer et al. 2005 and Fresh et al. 2006).

To account for potential timing differences in fish use in each region and habitats within each region, we will sample during two time periods (February – April; June – August). These two time periods encompass the time of year during which different life history types of juvenile Chinook salmon are expected to use these habitats. For example, juvenile Chinook salmon often use shallow intertidal areas and pocket estuaries early in the year when they are fry-sized. They move progressively off shore and into deeper habitat as they get larger later in the year. This seasonal progression is illustrated in Figure 1. During June – July, fry move out of shallow estuarine habitats into deeper areas while freshwater rearing parr migrants or delta estuary rearing Chinook salmon move from their natal estuary into nearshore rearing areas.

Our goal will be to collect a minimum of 50 unmarked juvenile Chinook salmon samples for each of the 22 space/time strata possible in Table 1. For sample collections, we plan to spend one day per stratum. Although our goal is to collect 50 fish per stratum, additional samples collected beyond our goal of 50 will be archived. Such archived samples may prove useful in exploring sample size issues and finer scale sources of variability in populations using different strata.

All fish catch data will be processed in a standardized fashion. Counts of all species will be obtained, lengths will be obtained on up to 20 individuals of each species, and fin clips (for genetic analysis) obtained on a random sample of at least 50 juvenile Chinook salmon collected from each strata. Genetic mixture samples will be obtained from as many different sites within a stratum as possible. In addition, any juvenile Chinook salmon with a coded-wire tag will be retained.

We will summarize what we observed about the timing, abundance, and size of juvenile Chinook salmon using the 11 different spatial strata within WRIA 6 and compile with other previous data as comparable. We will look for differences in the Chinook salmon variables (timing, abundance, size of fish) between habitat types and spatial strata. The results will be translated into specific hypotheses of juvenile Chinook salmon use for each area and habitat type based on the results of the habitat assessment part of this proposal. Results will be mapped in GIS and applied to all areas within WRIA 6 where spatial strata and habitat type are similar to those sampled.

This assessment will generate data necessary to conduct similar analyses (described above) for other fish species. However, the estimated cost of this study accounts only for analysis of juvenile Chinook salmon usage into specific hypotheses by area and habitat type. It would be relatively easy (low additional cost) to complete the analysis for all salmon species because we have life cycle models (specific knowledge of likely source populations, life stages, and life history types) that we can use to analyze results. For other species, such as forage fish, we would need to develop the conceptual life cycle models to analyze results.

Eric Beamer from Skagit River System Cooperative will lead (1) collecting juvenile samples in mixed stock nearshore areas for genetic analysis, (2) analyzing catch and size data, and (3) reporting these results. Eric Beamer has worked as a scientist for the Skagit River System Cooperative since 1984. He is the principle investigator on several Skagit watershed projects, including monitoring Chinook salmon in the tidal delta and nearshore, studies of the use of non-natal estuaries by juvenile Chinook salmon, and recent research that directly links estuarine and nearshore habitat to recovery of wild Skagit River Chinook salmon populations.

The SRSC field crew will be instrumental in capturing juvenile Chinook salmon within some mixed stock areas (Table 1). For other sampling areas, we will use contractors with proven experience in nearshore sampling and SRFB grants. These groups include the Wild Fish Conservancy (formerly Washington Trout) to lead sampling on the west side of Whidbey Island, the Stillaguamish Tribe to lead sampling within Port Susan, and Island County WSU Beach Watchers to assist with sampling within Saratoga Passage, Possession Sound, and west Whidbey Island sites.

Jason Griffith has been a Fisheries Biologist at the Stillaguamish Tribe for the past seven years, working on a wide range of research and restoration projects. Whether writing grants, running the Tribe’s smolt trap, or beach seining in the estuary, his work is focused on promoting recovery of Stillaguamish Chinook populations. He has a Bachelors in Fisheries from the UW.

Micah Wait of the Wild Fish Conservancy has six years experience as a conservation biologist working on the recovery of wild fish in Washington. He has managed numerous research and restoration projects in the Puget Sound nearshore, including two years of intensive fish use sampling on the western shoreline of Whidbey Island.

Kurt Fresh has worked as a Fisheries scientist in the Pacific Northwest for over 30 years, the last four years as a Research Biologist at the Northwest Fisheries Science Center (NWFSC). For most of his career, he has investigated the life history and ecology of juvenile salmon in freshwater, estuarine and nearshore habitats in this region. Most recently, his work has focused on obtaining the science needed to support the protection and restoration of estuarine and nearshore habitats needed for salmon recovery.

Genetic Methods:

This study employs Genetic Stock Identification (GSI) techniques using standardized microsatellite DNA loci. GSI methods use a “baseline” genetic database to estimate the stock composition of “mixture” samples. The baseline is the whole set of reference samples representing spawning aggregates in known geographic locations (Figure 5, Table 2). We will use a Washington and British Columbia baseline dataset extracted from the standardized coast-wide database developed by the multi-agency workgroup Genetic Analysis of Pacific Salmonids collaborators (GAPS) (Moran et al. 2005). The “mixtures” are groups of fish derived from different populations in different proportions, which in this study are groups of juvenile Chinook salmon collected within different space/time/habitat strata. By comparing the multilocus genotypes in the mixture samples to the gene frequencies of the reference populations, standard mixed fishery methods are used to estimate the likely proportional contribution from each of the baseline populations. The baseline populations are pooled in various ways to assess stock composition at various levels of geographic hierarchy, e.g., Canadian versus U.S. stocks, and among sub-basins. It is through multivariate analysis of stock compositions mapped onto a broad range of biotic and abiotic variables over space and time and examined at various scales that we expect this study to make significant contributions. All the baseline data needed for this study is already in hand (Figure 5, Table 2) and all the genetic analyses proposed here would be directed toward characterizing mixtures of juvenile Chinook salmon. No new baseline development is proposed in this study.

Before we begin analyzing juvenile mixtures we will expand the power analyses that were conducted to explore the feasibility of this study design. Table 3 shows the results of simulations of juvenile mixtures that might be expected in one of our sampling strata. The simulations show that the variance on the estimated proportional contribution likely to be observed in real mixtures is very small relative to those estimates. It is worth noting that even with quite small mixture samples (a potential concern in this study) the estimates are still quite good (Table 3). Additional power analyses will explore finer-scale reporting units, and will hone our study design. We expect to have excellent power for fine-scale allocation of juvenile Chinook salmon mixtures throughout the WRIA 2 study area. Based on our simulations relative to combined empirical and theoretical results in other systems, we believe we can make definitive inferences about habitat use and migration pathways based on differences in stock composition. However, while we believe we can make definitive inferences about habitat use and migration pathways with the existing GAPS baseline dataset, we expect that during the period of this study new baseline data collected by NWFSC, WDFW, and CDFO will become available. For example, in 2006 the number of populations included in the GAPS baseline for Washington and British Columbia increased from 35 to 63 populations. The inclusion of additional baseline samples during the course of our study will only improve our ability to allocate mixture samples. Again, we are not requesting funding for baseline expansion as part of this proposal.

Mixture samples will be genotyped at 13 microsatellite loci that were selected for standardization by the GAPS collaborators. Mixture analyses as well as simulations related to power analysis will be carried out using the program Genetic Mixture Analysis (GMA) (Kalinowski 2003). This software was developed to meet the specific needs of NWFSC scientists; however, the algorithms (Smouse et al. 1990; Ranalla and Mountain 1997) are based on accepted methods (e.g., the widely-used GeneClass2 and SPAM programs).

Genetic analysis will be conducted by Paul Moran of NOAA Fisheries. Paul Moran has worked as a molecular geneticist at the NWFSC for 13 years. He has developed projects in diverse areas of salmon ecological genetics, including population genetics and zoogeography, life-history evolution, and the application of new molecular and analytical methods to support conservation efforts. Paul has considerable experience leading multi-agency collaborations such as coast-wide standardization of Chinook salmon microsatellite data by the GAPS consortium. Paul's role in this project will be to provide continuity between this and related projects including coast-wide standardization, baseline, and geospatial database development. He will also participate in data compilation, QA/QC of baseline and mixture data, analysis of juvenile mixtures, interpretation of results, and reporting and publication.

<u>Primary Application of Results</u>

6. TASKS AND TIME SCHEDULE

<u>Year 1</u>

February:	Scout potential sampling sites, finalize sampling design
February - April:	Collect initial set of mixture samples
May - July:	Power analysis using currently available data
June - August:	Collect second set of mixture samples
October - December:	Genotyping of mixture samples
December:	Complete entry of all catch, habitat type, environmental, and individual fish (e.g., size, weight, marks) data into database

Year 2

January - April:	Continue genotyping
April - September:	Analysis of unknown stock mixture compositions
September - December:	Preparation of final report and manuscripts for publication Work with Island County TAG to update WRIA 6 salmon recovery plan and prioritization process per results of this project

7. CONSTRAINTS AND UNCERTAINTIES

Unable to meet target sampling goals in some strata

While we expect to be able to meet our space/time strata sampling goals, variability in such factors as environmental conditions and escapement may result in fewer than 50 fish in some strata. If this occurs, we will not increase the amount of sampling unless new or alternative funding becomes available. We will streamline catch processing in the field so we only focus on catching Chinook salmon during the time we are in the field. We have shown with our simulations that estimates can be good even with small mixture samples. The variance on the estimated proportional contribution likely to be observed in real mixtures is very small relative to those estimates.

Sampling assumptions

We have assumed that smaller scale differences in habitat and timing (e.g., tidal stage, time of day) are less important in defining population composition than the larger scale strata we have established. Though our collective experience supports this assumption, we propose to test the validity (and limits) of our spatial, temporal, and habitat type assumptions for determining stock composition of juvenile Chinook salmon at the scale of WRIA 6. We will do this by analyzing stock composition of juvenile Chinook salmon samples collected much more intensively in space, time, and habitat type within the Whidbey Basin by SSRC and NOAA Fisheries (Figure 3). We will compare Chinook salmon stock composition results from two sub-areas (representative of the size range of the 11 areas listed in Table 1) within WRIA 6 to Chinook salmon stock composition results drawn from a sub-sample that represents the simplified temporal and habitat type approach proposed for Whidbey Basin. If we find that smaller scale attributes are important in some cases, we will frame our results within that context and discuss what this means for interpretation of results. Although we would be unable to go back and collect additional samples in this performance period, we would look in our database and archived samples for opportunities to explore finer scale sources of variability.

Equipment breakdown

It is possible that the contractor's large tow net boat or our other small boats may break down. While minor repairs are budgeted for as part of this project, major repairs are not. It will be a condition of the subcontract to deliver a vessel for tow net sampling that is both adequate in vessel capability and time to implement this project. We also have other small boats available on a short term basis to collect shoreline samples and have other methods (e.g., lampera seines using small vessels) that we would employ to try and collect offshore samples in the event of lengthy delays.

Permits cannot be obtained in time

We will be applying for collection permits at the time of final submittal of this proposal. We intend to permit this activity through amendment of the Tribal Research Plan. Given that we do not have to kill fish to obtain DNA samples, we expect no problem in obtaining these permits. It will be necessary to kill CWT hatchery fish that we encounter in order to obtain the CWT.

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