

**PART I: INTRODUCTION**

## **4. Salmon Genetics**

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## 4. SALMON GENETICS

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### EXECUTIVE SUMMARY

The genetic diversity of a salmon species is important to that species abilities to undergo and withstand changes in climate, natural predation, diseases, competition, ocean rearing conditions and natural catastrophes. It is through this resiliency that the species survive.

The National Marine Fisheries Service has provided guidance in the form of three components of a successful restoration strategy (NMFS 1996). Such a strategy should consist of the following elements: (A) *protection and conservation*; (B) *certainty* that the strategy will be implemented and (C) a *comprehensive monitoring program*. NMFS expanded that guidance into three additional fundamental elements ultimately need to be addressed. Those include: (1) "...increased abundance of naturally spawned fish..."; (2) a "...broad geographic distribution of naturally spawned fish..."; and (3) a "...genetic diversity in a pattern and at levels consistent with natural evolutionary processes...". In this document we intend to supply the reader with the information necessary to assist in answering the third element.

There have been several attempts to organize salmon populations along genetic lines. Currently, the National Marine Fisheries Service (NMFS) has organized salmon populations in Evolutionary Significant Units (ESUs). An ESU is best defined as a population (or group of populations) that is (1) reproductively isolated from other conspecific population units (separate from), and (2) represents an important component in the evolutionary legacy of the species (unique). The isolation of a population does not have to be absolute, but strong enough to allow for evolutionary important differences to accrue between populations. The second criteria is best met if the population contributes to the ecological and/or genetic diversity of the species as a whole. (Waples 1991). The boundaries of Puget Sound salmonid ESUs often overlap but differ between species.

Much of the genetic data used to define ESUs has come from information obtained by the Washington Department of Fish and Wildlife (WDFW) and the Western Washington Treaty Indian Tribes (WWTIT). WDFW has organized stocks into genetic diversity units (GDUs) and major ancestral lineages (MALs). In most cases, GDUs are grouped into larger assemblages called Major Ancestral Lineages (Marshall et al. 1995). The GDU/MAL initiative was intended as part of an effort to provide NMFS with pertinent information to assist in ESU designations. Given that much of the genetic data used in the federal ESU determinations were collected and analyzed on behalf of the State of Washington (Marshall et al. 1995), it is not surprising that the Puget Sound chinook MAL contains a group of populations similar to the ESU grouping. This is not always the case with other salmon species as the ESUs and MALs often overlap but have different boundaries.

A summary of stock status, stock history and ESA status is contained in table Gen-1.

Green River origin fall chinook have the largest amount of genetic information available, followed by chum and coho salmon. Steelhead have undergone a similar level of scrutiny but utilizing different investigative techniques. Sockeye salmon populations in the Green River have

not been genetically evaluated. Pink salmon are known to occur in the Green River but are not considered viable by the natural resource management agencies and are not discussed in this document.

<b>Table Gen-1. Salmon Species and Stocks Found in the Green/Duwamish River (WDFW and WWTT 1994). The NMFS Evolutionary Significant Units (ESU) and listed or proposed Endangered Species Act (ESA) listing status are also shown as of October 4, 1999.</b>				
<b>Stock <sup>1</sup></b>	<b>Stock Origin <sup>2</sup></b>	<b>Production Type <sup>3</sup></b>	<b>ESU</b>	<b>ESA Status</b>
Duwamish/Green River Fall Chinook	Mixed <sup>4</sup>	Composite <sup>7</sup>	Puget Sound <sup>10</sup>	Threatened
Newaukum Creek Fall Chinook	Mixed	Wild <sup>8</sup>	Puget Sound <sup>10</sup>	Threatened
Duwamish/Green River Fall Chum	Mixed	Composite	Puget Sound /Strait of Georgia <sup>11</sup>	Not Warranted
Crisp (Keta) Creek Fall Chum	Non-native <sup>5</sup>	Cultured <sup>9</sup>	Puget Sound /Strait of Georgia <sup>11</sup>	Not Warranted
Green River/Soos Creek Coho	Mixed	Composite	Puget Sound/Strait of Georgia <sup>12</sup>	Candidate
Newaukum Creek Coho	Mixed	Composite	Puget Sound/Strait of Georgia <sup>12</sup>	Candidate
Duwamish/Green River Summer Steelhead	Non-native	Composite	Puget Sound <sup>13</sup>	Not Warranted
Duwamish/Green River Winter Steelhead	Native <sup>6</sup>	Wild	Puget Sound <sup>13</sup>	Not Warranted
Duwamish/Green River Early Winter Steelhead (Chambers Ck.)	Non-native	Cultured	Puget Sound <sup>13</sup>	Not Warranted
Green River Sockeye <sup>14</sup>	Unknown	Wild	Not Determined	Uncertain
Green River Bull Trout <sup>15</sup>	Native	Wild	Puget Sound	Proposed Threatened
Green River Coastal Cutthroat Trout <sup>16</sup>	Native	Wild	Puget Sound	Not Warranted
<p>Notes</p> <p>1. As defined in WDFW and WWTT (1994), the fish spawning in a particular lake or stream(s) at a particular season, which fish to a substantial degree do not interbreed with any group spawning in a different place, or in the same place at a different season.</p> <p>2. The genetic history of the stock</p> <p>3. The method of spawning and rearing that produced the fish that constitutes the stock.</p> <p>4. A stock whose individuals originated from commingled native and non-native parents, and/or by mating between native and non-native fish (hybridization) or a previously native stock that has undergone substantial genetic alteration.</p> <p>5. A stock that has become established outside of its original range.</p> <p>6. An indigenous stock of fish that have not been substantially impacted by genetic interactions with non-native stocks, or by other factors, and is still present in all or part of its original range.</p> <p>7. A stock sustained by both wild and artificial production</p> <p>8. A stock that is sustained by natural spawning and rearing in the natural habitat, regardless of parentage (includes native)</p> <p>9. A stock that depends on spawning, incubation, hatching, or rearing in a hatchery or other artificial production facility.</p> <p>10. Meyers <i>et al.</i> (1998).</p> <p>11. Johnson <i>et al.</i> (1997).</p> <p>12. Weitkamp <i>et al.</i> (1995).</p> <p>13. Busby <i>et al.</i> (1996).</p> <p>14. Not listed in WDFW and WWTT (1994)</p> <p>15. Listed in WDFW SaSI (1998).</p> <p>16. Johnson et al (1999).</p>				

## KEY FINDINGS AND DATA GAPS

### FALL CHINOOK HIGHLIGHTS

- The Green River has had a fall chinook hatchery program for the last 95 years.
- Green River fall chinook have played an important part in a number of hatchery programs throughout Puget Sound.
- Green River hatchery and Newaukum wild fall chinook populations are genetically indistinguishable.
- Draft data indicates that the contribution of natural spawned adults to escapement at the Soos Creek Hatchery is approximately 39 percent (range: 1 to 76 percent).
- Draft data indicates that the contribution of natural spawned adults to escapement in the Newaukum River is approximately 45 percent (range: 15 to 79 percent).
- Draft data indicates that the contribution of natural spawned adults to escapement in the Green River is approximately 56 percent (range: 25 to 83 percent).

### FALL CHINOOK DATA GAPS

- The exact contribution of hatchery fall chinook to mainstem Green River natural escapement is not yet fully known.
- In the Green River Basin, the ramifications genetic flow between the hatchery and wild populations is unknown.

### CHUM HIGHLIGHTS

- The Green River has had a chum hatchery program since 1976.
- Green River chum salmon are geographically isolated from other chum salmon populations in Puget Sound.
- Two chum salmon stocks exist within the Green River Basin.

### CHUM DATA GAPS

- The extent of chum salmon straying in the Green River Basin is unknown.
- In the Green River Basin, the ramifications genetic flow between the hatchery and wild populations is unknown.

## COHO HIGHLIGHTS

- Green River hatchery and Newaukum coho are genetically similar.
- Green River Basin coho are listed as a Candidate for listing under the ESA.

## COHO DATA GAPS

- The contribution of hatchery coho to natural escapement is unknown. The reverse is also true.
- In the Green River Basin, the ramifications genetic flow between the hatchery and wild populations is unknown.
- The actual extent of any temporal separation in timing between Green River and Newaukum Creek coho is unclear in terms of defining separate stocks.

## WINTER STEELHEAD HIGHLIGHTS

- Green River origin winter steelhead are a part of the larger wild Puget Sound winter-run steelhead stocks.

## WINTER STEELHEAD DATA GAPS

- In the Green River Basin, the ramifications genetic flow between the winter steelhead hatchery and wild populations is unknown.
- Because of timing differences the genetic flow between these stocks is believed to be low.

## INTRODUCTION

As human populations have increased in the Green River Basin, tremendous pressure has been put on salmon and steelhead stocks. Demand for these fish has increased as their habitat base has been reduced. Several years of unfavorable marine conditions have reduced their survival. Management planning has considered issues such as harvest, reduced marine survival and freshwater productivity, but until recently has not considered the magnitude and importance of the genetic diversity of these fish.

The knowledge of the genetic stock structure of salmon and steelhead is now recognized as a fundamental issue for their conservation and management. These fish exhibit a tremendous amount of genetic diversity that is revealed through ecological, life history and molecular genetic variability. The genetic diversity that these fish contain is a major contributor to current productivity and, potentially more important, a resource for adaptive change. It is the genetic diversity available for adaptive change that will promote future productivity and survival.

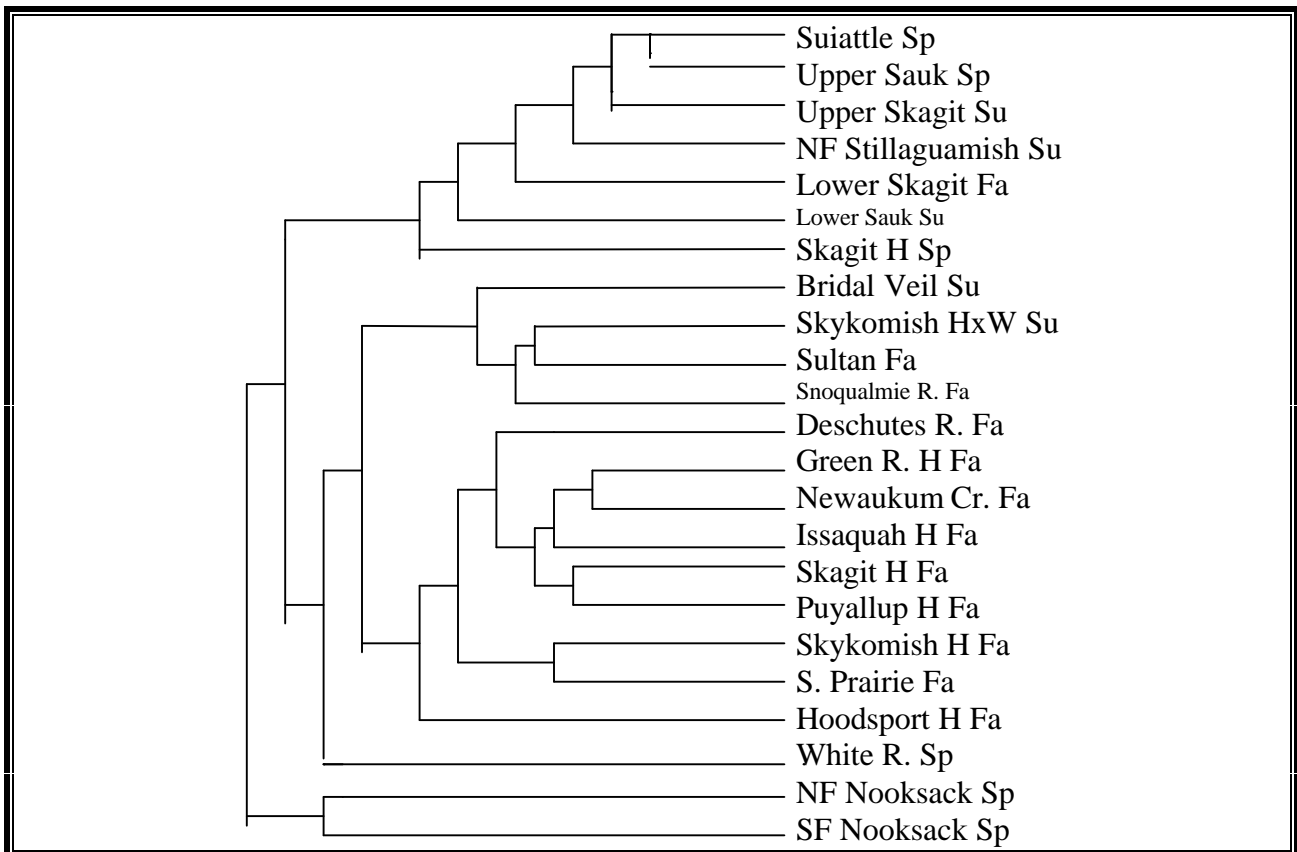
To understand and conserve genetic diversity it is important to understand the existing amount and pattern of genetic diversity. This paper is an attempt to provide the reader with an overview of current genetic issues of Green River Basin salmon and steelhead. Scientists are still investigating the genetics of Green River salmon and steelhead stocks, as well as others within the region. It is possible; that as new information becomes available the views presented below may change.

## BACKGROUND

In this paper we present the reader with biochemical and molecular genetic evidence that has been used to define reproductively isolated populations of Green River origin chinook, chum and coho salmon, along with winter steelhead. The methods by which much of this data is processed is very technical, the details of which are not necessary for the purposes of the information presented below. However, it is useful to know that the bulk of the data consists of frequencies of protein variants (allozymes) or mitochondrial DNA identified through electrophoresis. After the allozymes are identified, several standard statistical methods are used to analyze the molecular genetic data in order to test various hypothesis of reproductive isolation. These methods are applied within and between populations. A finding of significant frequency differences between populations may be evidence of reproductive isolation.

There are additional methods of measuring the genetic isolation between populations. These methods calculate genetic differences from allele-frequency estimates and may use one or more of several genetic distance measures. It is unclear if one method is always superior to the others as each method has its own inherent strengths and weaknesses. There are several technical papers available that discuss the different approaches (Nei 1978; Hillis et al 1996; and Rogers 1991). A method commonly employed is to place genetic data from stocks on a chart, called a dendrogram, which resembles a branching family tree. This method of viewing genetic data suggests similarities and differences between groups or samples. A typical dendrogram can be found in Figure Gen-1.

**Figure Gen-1. Dendrogram Resulting from Cluster Analysis among Puget Sound Chinook Populations (Source: Marshall 1995).**



H = Hatchery W = Wild Sp = Spring-run Su = Summer-run Fa = Fall-run

**NATIONAL MARINE FISHERIES SERVICE**

The Endangered Species Act allows the listing of “distinct population segments”. The National Marine Fisheries Service (NMFS) has developed a policy on this issue for anadromous Pacific salmonids that considers a population distinct if it represents an Evolutionary Significant Unit (ESU). An ESU is defined as a population (or group of populations) that is reproductively isolated from other conspecific population units, and (2) represents an important component in the evolutionary legacy of the species. Definitions for the terms “Threatened,” “Endangered,” “Candidate,” and “Not Warranted for Listing” are contained in the Glossary.

NMFS has used data collected by others as well as NMFS geneticists in an effort to analyze the biochemical and molecular genetic evidence that might be used to define reproductively isolated populations of salmonids. Through this analysis coupled with data on life-history differences they have identified distinct population segments.

**ESU STATUS**

Puget Sound chinook were listed as a threatened species on March 24, 1999. The ESU included Green River origin naturally spawned populations. Chinook salmon from the Green River Hatchery were not included in the listing. The Puget Sound steelhead, chum and odd year pink



salmon ESUs were determined to “Not Warrant” listing as threatened or endangered. Puget Sound coho have been listed as a candidate species that warrants further consideration. The Green River is not part of a sockeye salmon ESU. Actual ESU boundaries vary by species and the details of those ESU boundaries can be found below.

## **WASHINGTON DEPARTMENT OF FISH AND WILDLIFE**

In 1985, the Washington Department of Fish and Wildlife (WDFW) began actively characterizing the genetic diversity among salmonid stocks in Washington state (Busack 1995). Through the use of collected genetic data, coupled with life history traits, ecological and physiological data, these stocks were initially described as Genetic Conservation Management Units GCMUs by Leider et al. (1994). The development of GCMUs was meant to parallel the NMFS Evolutionary Significant Units (ESUs) and the Washington Department of Wildlife (WDW) Draft Steelhead Management Plan.

Also in 1994, WDFW undertook an effort to systematically summarize and analyze the data produced by previous efforts to document the genetic diversity of salmonid species. Through the development of the WDFW Wild Salmonid Policy (WSP) and the 1994 effort to further analyze previously collected data new terminology evolved. This data led to organizing stocks of salmon into assemblages of biologically and genetically similar groups called Genetic Diversity Units (GDUs). In most cases, GDUs are grouped into larger assemblages called Major Ancestral Lineages (MALs) (Marshall et al. 1995). Given that much of the genetic data used in the federal ESU determination were collected and analyzed on behalf of the State of Washington (Marshall et al. 1995), it is not surprising that the Puget Sound chinook MAL contains a group of populations similar to the ESU grouping. The Puget Sound chinook ESU, as defined by NMFS (Myers et al. 1998), includes all of the Puget Sound GDU's, but also includes populations in Strait of Juan de Fuca streams from the Elwha River east. The Strait of Juan de Fuca is a migratory corridor for Puget Sound chinook, and appears to be a natural transition zone between coastal and interior chinook populations.

A Genetic Diversity Unit (GDU) is defined as: “A group of genetically similar stocks that is genetically distinct from other such groups. The stocks typically exhibit similar life histories and occupy ecologically, geographically and geologically similar habitats. A GDU may consist of a single stock” (Busack 1995).

GDUs are subdivisions of MALs and a MAL may be comprised of several GDUs. Stocks within a GDU may be genetically similar, but are not identical. Within a GDU there may be measurable genetic differences and low levels of natural gene flow among stocks. The reader is cautioned that GDU designations are only an initial attempt to group current patterns of diversity seen in these species and additional analyses may change both the groupings and terminology. Finally, these GDU designations are a picture of what we know today and may not be representative of what these fish looked like 150 years ago or may look like 150 years into the future.

While a GDU is based on similarities and differences currently exhibited by these fish, a Major Ancestral Lineage (MAL) is based on groups that are so different genetically that they are reflective substantial reproductive isolation over extended periods of time. Busack (1995) defined a MAL as: “A group of one or more genetic diversity units (GDUs) whose shared

genetic characteristics suggest a distant common ancestry, and substantial reproductive isolation from other MALs. Some of these groups are likely the result of colonization and diversification preceding the last period of glaciation.”

## GREEN RIVER FALL CHINOOK SALMON GENETICS

### **NATIONAL MARINE FISHERIES SERVICE**

NMFS (Meyers 1998) determined that there are fifteen ESUs of chinook salmon in California, Oregon, Washington and Idaho. Based in part on genetic evidence presented in Marshall et al. (1995), the NMFS has drawn the boundaries of the Puget Sound Chinook ESU.

The Puget Sound ESU extends from the Nooksack River in the north through Puget Sound and west into the Strait of Juan de Fuca to Elwha River. The Elwha River chinook stocks are somewhat intermediate between Puget Sound and Coastal ESU but their marine distribution more closely matches that of Puget Sound stocks. Spring-, summer- and fall-run chinook wild and some hatchery stocks are included in this ESU. The proposed Puget Sound ESU for chinook salmon is similar in geographic coverage to ESUs for steelhead but differs from that proposed for chum, coho and odd-year pink salmon.

The naturally spawning component of the Green River chinook run contains a mixture of wild and hatchery chinook. The major question pertaining to the status of Green River chinook is the contribution of hatchery chinook to the natural escapement. Draft run-reconstruction information for the years 1989 – 1997 inclusive indicates approximately 56 percent (range: 25 to 83 percent) of the natural escapement in the mainstem Green River of being from hatchery reared and released fish (Cross, pers. comm.1999). It is not possible to determine to what extent the remaining approximate 40 percent of the mainstem Green River escapement has its ancestry from hatchery origin fish that have spawned for one or more generations in the wild. For the same time period, in Newaukum Creek, the origin of adult chinook is approximately 45 percent (range: 15 to 79 percent) of hatchery origin (Cross, pers. comm.1999).. Additionally, draft data, for the same time period, indicates approximately 39 percent (range: 1 to 76 percent) of the adult chinook returning to the hatchery rack are progeny of natural spawning adults. Newaukum and Soos Creek data is probably quite reliable since sampling rates are relatively high (30 percent and 98 percent respectively) (Cross, pers. comm.1999). The Green River mainstem sampling rate was roughly 4 percent due to difficulties in locating samples in the large river and is probably less reliable. Sampling efforts in the mainstem Green River were increased beginning in 1998 but the data has not yet been analyzed. Additional details surrounding this issue were addressed previously in Chapter 4.

The Green River chinook salmon belong to a group of Puget Sound spawning populations that are genetically distinguishable from other chinook populations outside the region (Utter et al. 1989). In two subsequent studies (Marshall et al. 1995, Myers et al. 1998), the chinook populations of Puget Sound were also identified as being more similar to one another than to populations outside the region, based on comparisons of genetic characters. Among chinook outside the Puget Sound region, populations in southern British Columbia, Canada are genetically most closely related. The exact geographic boundaries of Puget Sound chinook, as

drawn on the basis of genetic evidence, differ somewhat among sources. Based in part on genetic evidence presented in Utter et al. (1989) and in Marshall et al. (1995), NMFS has drawn the boundaries of the Puget Sound Chinook ESU at the eastern Strait of Juan de Fuca, including the Elwha River, to the Canadian border, including the Nooksack River (Myers et al. 1998). It should be noted that genetic data for naturally spawning Green River chinook was lacking from the federal analyses, which included Green River (Soos Creek) hatchery stock data collected between 1981 and 1990. A genetic baseline on Green River natural spawners from Newaukum Creek is available (Marshall et al. 1995). Genetic data from chinook in Newaukum Creek indicates that there is no significant difference between chinook spawning naturally in Newaukum Creek and chinook from the Soos Creek Hatchery Rack (Marshall 1995).

Green River natural chinook are included in the Puget Sound ESU.

## **WASHINGTON DEPARTMENT OF FISH AND WILDLIFE**

In addition to the ESU, the Green River chinook stock also appears in another system of salmon stock classification based largely on genetic data. WDFW has organized chinook into geographic assemblages of genetically similar groups known as Genetic Diversity Units (GDU's). The Puget Sound chinook ESU, as defined by NMFS (Myers et al. 1998), includes all of the Puget Sound GDU's, but it also includes the Strait of Juan de Fuca streams from the Elwha River east. The Strait of Juan de Fuca, which serves as a migratory conduit between these two geographic regions, is a natural zone of transition between coastal ocean and interior Puget Sound chinook populations.

Puget Sound has five GDUs encompassing wild and hatchery populations:

- South Puget Sound, Hood Canal and Snohomish Summer + Fall, (fall chinook in the Skagit, Nooksack and Samish hatcheries are included) chinook;
- South Puget Sound Spring (White River) chinook;
- Stillaguamish and Skagit (Skagit wild spring, summer, fall and hatchery springs; and all Stillaguamish) chinook;
- South Fork Nooksack Spring chinook; and
- North Fork Nooksack Spring chinook.

Green River chinook are included in the first of these GDU's, and transfers of Green River stock to other watersheds have helped to determine the geographic localities of this GDU.

## **NATURAL GREEN RIVER CHINOOK IN RELATION TO OTHER CHINOOK STOCKS**

The Green River fall chinook reside at the geographic center of their ESU/MAL, and they are genetically very similar to a number of hatchery and naturally spawning stocks distributed throughout Puget Sound. For example, natural spawning populations genetically closely related to the Green River Hatchery chinook are found in the Skykomish River (summer), Bridal Veil Creek (summer), Wallace, Sultan and Snoqualmie rivers (fall), and South Prairie Creek in the

Puyallup River (fall) (Myers et al. 1998). However, these samples include hatchery strays and when those fish are removed from the sample, the Snohomish chinook (Sultan River, Snoqualmie River, and Bridal Veil Creek) stocks are significantly different (Smith pers. comm. 1999). Historically, Puget Sound hatchery stocks apparently derived from the Green River Hatchery stock were found at the Skagit Hatchery (summer and fall), Skykomish Hatchery (fall), the Deschutes Hatchery (fall), and the Hoodspout Hatchery (fall) based on the data presented in Myers et al. (1998). Snohomish Hatchery fall chinook stocks were also founded with Green River Hatchery stock, however stocks of Green River Hatchery origin are no longer released from the Skagit and Snohomish hatcheries into their respective basins. In the WDFW studies, genetic baselines for the Green River natural and hatchery populations were closely related to Skykomish hatchery, Issaquah hatchery, Hood Canal hatchery, Puyallup natural and hatchery, and Deschutes hatchery (Marshall et al. 1995).

It is noteworthy that the Green River hatchery stock has played a role in the geographic distribution of the GDU's. The geographic boundaries of Puget Sound GDUs are overlapping. Populations in a GDU may be found in relatively diverse localities due, at least in some cases, to past and current transfers of fish among hatcheries. For example, the northern Puget Sound fall chinook in the Skagit, Nooksack and Samish hatcheries are part of the South Puget Sound, Hood Canal and Snohomish Summer and Fall chinook GDU due to the influence of Green River chinook (Marshall et al. 1995). Twenty hatcheries throughout Puget Sound regularly release Green River origin chinook, according to Marshall et al. (1995). As of 1998 operations, direct transfers of Green River hatchery chinook are more limited, as hatchery programs rely more on local stocks (Kimbel 1999).

It is also noteworthy that the reverse has not been the case. There have not been significant transfers of chinook stocks into the Green River from outside the ESU.

#### POSSIBLE CHANGES IN A GENETICALLY HERITABLE CHARACTER

The geographic boundaries of the ESU/MAL and the content of the GDU's were determined in part by analyzing a combination of heritable characters. Reproductive isolation is studied by using heritable characters not thought to be subject to natural selection, such as frequencies of neutral genes determined from analysis of tissue protein variants (allozymes), mitochondrial DNA and microsatellite loci (Myers et al. 1998). Differences in heritable physical characters subject to natural selection, such as size at age, timing of adult migrations and spawn timing, are less useful for identifying reproductive isolation. Nonetheless, changes in these heritable physical characters through time and within a population are useful indicators of changes in the genetic character of the population.

Data were available to study changes in timing of arrival at the Soos Creek hatchery rack for Green River chinook. Timing of rack returns was studied by testing annual mean date of rack return at Soos Creek hatchery for significant linear trend. A significant negative slope indicates the timing is probably getting earlier, and a significant positive slope indicates timing is probably becoming later. Mean date of annual rack returns from 1960 to 1997 varied from September 23 to October 13, with a grand mean of October 4. There was a small but statistically significant ( $p < 0.05$ ) negative slope on the regression of mean date of annual rack return on year. The timing of rack returns of chinook to the Soos Creek hatchery rack became about one week earlier

over a 38-year period. A limitation of this analysis is that the rack return data were summed on a weekly basis, so there is a measurement error of plus or minus one week. A change of a week or less could be due to measurement error, even though the time trend in date of rack return was statistically significant. These results indicate that an analysis of daily rack return data should be conducted, if these data can be found.

It is possible that timing of rack return for Soos Creek hatchery chinook has become earlier over the past 38 years. Annual mean dates of rack return later than October 4 are much less (three times) common in the past 10 years (1988 – 1997) than in the first 10 years (eight times) (1960 – 1969). This change coincides with changes in hatchery operational procedures that were initiated as a result of concerns expressed over genetic changes. One of the first guidance documents for WDWF hatchery genetics was published during the early 1980s (Hershberger and Iwamoto, undated). Spawning guidelines were provided to all Washington Department of Fisheries hatchery programs in 1983 (Seidel 1983). Both of these resulted in significant operational changes to hatchery spawning techniques that were intended to minimize any alteration to run timing. Additional factors such as water flow in Soos Creek, harvest patterns and changes in ocean rearing conditions could factor into this observation. However, should the differences in timing be real, this would indicate a change in the gene frequencies in the hatchery population over time.

## **GREEN RIVER CHUM SALMON GENETICS**

### **NATIONAL MARINE FISHERIES SERVICE**

Based in part on genetic evidence presented in Phelps et al (1995), the National Marine Fisheries Service (NMFS) has drawn the boundaries of the Puget Sound Chum Evolutionarily Significant Unit (ESU). NMFS (Johnson 1997) determined that there are two major genetic groups of chum salmon in central and southern British Columbia, Washington and Oregon. The smaller of these two groups consists of summer-run chum salmon in Hood Canal and the Strait of Juan de Fuca. The second, and much larger group, consists of fall-, winter- and summer-run chum salmon in other areas of British Columbia, Washington and Oregon. This last group was further divided into: 1) coastal populations along the outer coast of Washington and Oregon, and 2) the remaining populations in British Columbia and Washington. Green River fall-run chum salmon are in this last category.

The proposed Puget Sound/Strait of Georgia ESU for chum salmon is similar in geographic coverage to ESUs for coho and odd-year pink salmon but differs for chinook and steelhead.

### **WASHINGTON DEPARTMENT OF FISH AND WILDLIFE**

The Washington Department of Fish and Wildlife (WDFW) places Green River chum salmon in a group of Puget Sound spawning populations that are genetically similar to other chum populations of central Puget Sound chum salmon (Phelps et al. 1995). The origin of portions of the Green River chum salmon is believed to be the result of transfers of Cowling Creek Tribal Hatchery (eastern Kitsap Peninsula) chum salmon (Phelps et al. 1995) into the Green River in an effort to establish chum runs in this system. This was one factor that influenced Phelps et al

(1995) to place Green River chum into the same Genetic Diversity Unit (GDU) with eastern Kitsap Peninsula chum.

One method by which chum salmon populations may be separated into GDU's is to examine differences in life histories. Life histories include geographic distribution of migration routes, timing of adult entry into freshwater, body size, coloration and time to maturation. Habitat differences include natal river origin (e.g., glacial vs. non-glacial), stream gradient, stream elevation and size of estuaries. Generally, these criteria can contribute to or reflect isolation between stocks or groups of stocks of salmon. Data has been collected on body size but they have not yet been analyzed for regional or stock differences.

The Puget Sound Region is separated into four major areas (Phelps et al. 1995):

- North Puget Sound (the Snohomish, Stillaguamish, Skagit and Nooksack rivers);
- South Puget Sound (the Puyallup and Nisqually rivers and independent tributaries of the southern Puget Sound inlets);
- Hood Canal (the Dosewallips, Duckabush and Hamma Hamma rivers); and
- Strait of Juan de Fuca (the Dungeness and Elwha rivers).

The chum salmon that enter the Green River are part of the South Puget Sound area (Phelps et al. 1995). They are further separated into two stocks (SASSI 1992); Green River fall-run chum and Crisp Creek (also referred to as Keta Creek) fall-run chum salmon. The origin of Green River fall-run chum is an East Kitsap/wild remnant mix, while the Keta Creek fall-run stock is of East Kitsap (Cowling Creek broodstock whose origin is from Chico Creek) origin (Dorn pers. comm. 1999).

Chum salmon spawning ground survey data from the Green River are limited. However, these data indicate that the Green River chum are fall spawners, spawn in mainstem side-channel and tributary habitats (SASSI 1992 and WDFW Spawning Ground Survey Database), share similar adult entry to freshwater timing and time of maturation to stocks on the eastern side of the Kitsap Peninsula. They are separated from chum found on the eastern side of the Kitsap Peninsula by the width of Puget Sound between Bainbridge Island and Elliot Bay. Because of the separation across Puget Sound, it seems unlikely that there is significant opportunity for substantial interchange of spawners. Chum salmon in South Puget Sound inlets exhibit straying between tributaries of an inlet but the exchange of spawners between inlets does not happen to the same degree (Phelps et al. 1995).

Another method by which chum salmon may be placed in GDU's is based largely on genetic data. WDFW (Phelps et al. 1995) has organized chum salmon into geographic assemblages of genetically similar groups known as GDU's.

Puget Sound has five chum salmon GDU's encompassing wild and hatchery populations (Phelps et al. 1995):

- North Puget Sound fall-run GDU;

- Nooksack, Skagit, Stillaguamish and Snohomish rivers and smaller independent tributaries flowing into major bays;
- Central/South Puget Sound summer-run GDU;
- Smaller independent tributaries to Puget Sound;
- Central/South Puget Sound fall-run GDU
- Duwamish/Green River, Puyallup/White (excluding Keta Creek) rivers and small independent tributaries to Puget Sound;
- South Puget Sound winter-run GDU;
- Nisqually River and independent tributaries in WRIA 11;
- Hood Canal fall-run GDU; and
- All Hood Canal streams.

While the available biological data indicates that Green River and central Puget Sound streams of the Kitsap Peninsula should be a distinct GDU, the genetic differences between these streams was small. Based on the genetic similarities, the Green River chum are included in the Central/South Puget Sound fall-run GDU.

## GREEN RIVER COHO SALMON GENETICS

### NATIONAL MARINE FISHERIES SERVICE

The National Marine Fisheries Service (NMFS) has drawn the boundaries of the Puget Sound Coho Evolutionarily Significant Unit (ESU) based in part on genetic data and on life history/ecological differences. NMFS (Weitkamp 1995) determined that there are six major genetic groups of coho salmon for the west coast of North America. The Puget Sound/Strait of Georgia ESU includes the drainages of Puget Sound, Hood Canal, the eastern Olympic Peninsula (east of Salt Creek) and portions of British Columbia. Green River coho salmon are in this ESU.

NMFS technical staff have collected allozyme data over a 10 year period from over 100 salmon samples to form the basis of Genetic Stock Identification (GSI) studies. Samples from the Green River Hatchery on Soos Creek were collected in 1982 and again in 1992. The analysis of this data yielded seven major “clusters” that were largely distinct geographically (Weitkamp 1995). One cluster included all coho populations of Puget Sound and British Columbia (except for two Fraser River samples and one sample from the Big Qualicum Hatchery).

The proposed Puget Sound/Strait of Georgia ESU for coho salmon is similar in geographic coverage to ESUs for chum and odd-year pink salmon but differs for chinook and steelhead.

## **WASHINGTON DEPARTMENT OF FISH AND WILDLIFE**

The Washington Department of Fish and Wildlife (WDFW) has not scrutinized coho populations in Puget Sound to the same level as chinook or chum salmon. In the south and mid Puget Sound river systems, coho are generally managed for hatchery rather than natural production. The Green River receives substantial releases of hatchery origin coho annually. The release of yearling coho started in the 1950's and continues today. Regular releases of fingerlings occurred from 1952 to 1962 and from the mid 1970's until 1997. Releases of juvenile coho occur onsite at the Soos Creek Hatchery and offsite in various tributary streams both above and below Howard Hanson Dam. The exchange of genetic material between these hatchery-released coho and wild Green River coho is unknown. At present there is no effective genetic research tool for these fish

One method by which coho salmon populations may be separated is to examine differences in life histories. Life histories include geographic distribution of migration routes, timing of adult entry into freshwater, body size, coloration and time to maturation. Habitat differences include natal river origin (e.g., glacial vs. non-glacial), stream gradient, stream elevation and size of estuaries. Generally, these criteria can contribute to or reflect isolation between stocks or groups of stocks of salmon. Data has been collected on body size but they have not yet been analyzed for regional or stock differences.

The coho salmon that enter the Green River Basin are separated into two stocks (SASSI 1992), Green River coho and Newaukum Creek coho. Of particular interest is that significant differences exist in spawn timing between these stocks that might be indicative of genetic differences. Coho returning to the Green River typically spawn to mid-November. Newaukum Creek coho may spawn into mid-January (SASSI 1992 and WDFW Spawning Ground Survey Database).

As of the date of this writing an analysis for coho salmon populations has not been completed.

## **GREEN RIVER STEELHEAD GENETICS**

### **NATIONAL MARINE FISHERIES SERVICE**

NMFS (Busby 1996) determined that there are fifteen ESUs of west coast steelhead in California, Oregon, Washington and Idaho. Past steelhead genetic studies (Allendorf 1975; Allendorf and Utter 1979; Utter et al. 1980; Parkinson 1984) had identified two major groups along the west coast of North America. They were referred to as the coastal and inland forms. The ESUs identified by NMFS (Busby 1996) includes 12 for coastal steelhead and 3 for inland forms. Summer and winter steelhead were included in the NMFS status review of west coast steelhead (Busby 1996).

The NMFS boundaries for the Puget Sound steelhead ESU is based in part on genetic data and on life history/ecological differences. The Puget Sound ESU extends from the Nooksack River in the north through Puget Sound and west into the Strait of Juan de Fuca to Elwha River and is similar to that of chinook. The ESU includes populations of both winter- and summer-run steelhead.



There is very little information regarding the abundance of naturally produced summer-run steelhead in the Green River basin. While their numbers historically have been small, they represent a substantially different life history strategy from that exhibited by winter-run steelhead.

Green River wild winter-run steelhead are included in the Puget Sound steelhead ESU.

## **WASHINGTON DEPARTMENT OF FISH AND WILDLIFE**

WDFW has not separated steelhead stocks into the same types of units that salmon are grouped into. This is due in part that in Washington State, Pacific salmon have been studied in greater detail than steelhead.

Phelps (1994) first reported the genetic inventory and analysis of Puget Sound steelhead stocks. That study focused on four stocks identified as critical or depressed (SASSI 1992) or for which special concerns had been identified.

There are a number of methods (Cavalli-Sforza and Edwards 1967; Nei 1978; and Saitou and Nei 1987) by which genetic differences among populations may be graphically visualized. These methods utilize different statistical analyses in which estimates of genetic distance can be displayed. Phelps (1994) found that at the first level of analysis there are three main “clusters” of steelhead stocks in Washington. This result was consistent with findings from earlier studies (Allendorf 1975; Schreck et al. 1986).

Additional analyses, based on differences through genetic analysis and displayed differently by Phelps (1994) found up to six major “clusters” of steelhead stocks in Washington state. These clusters consisted of:

- Hatchery-run strains;
- Wild Puget Sound winter-run;
- Western Washington hatchery and wild summer-run;
- Wind and Washougal rivers stocks;
- Big White Salmon and Klickitat rivers stocks; and
- Satus Creek and Wells Hatchery stocks.

The Green River wild stock was identified as being a portion of the wild Puget Sound winter-run (#2) cluster.

## GREEN RIVER SOCKEYE GENETICS

### **NATIONAL MARINE FISHERIES SERVICE**

NMFS (Gustafson 1997) summarized the presence of riverine origin spawning sockeye at several locations in the Green River and other river systems in western Washington. The Biological Review Team convened by NMFS to assess sockeye ESUs concluded that the evidence was insufficient to determine whether sockeye salmon observed spawning in rivers, including the Green River, without lake-rearing habitats were distinct populations. The status of these populations was determined to be “Uncertain” (Gustafson 1997).

Note: A single juvenile sockeye has been reported captured in the middle Green River in 1998 (Hickey pers. comm.).

### **WASHINGTON DEPARTMENT OF FISH AND WILDLIFE**

The natural resource co-managers do not manage sockeye salmon, in the Green River, as a viable, self-sustaining population. There is the general perception that in years of sockeye abundance in the Lake Washington Basin, there are more sockeye observed in neighboring river systems.

WDFW has not initiated a genetic review of sockeye in the Green River.

## GREEN RIVER BULL TROUT GENETICS

### **U.S. FISH AND WILDLIFE SERVICE**

The U.S. Fish and Wildlife Service includes the Green River basin inside the present geographic range of bull trout in the contiguous United States. There has not been any data collected by which to genetically characterize bull trout in this basin.

### **WASHINGTON DEPARTMENT OF FISH AND WILDLIFE**

Information on the presence, abundance, distribution, utilization and life history of bull trout in the Green River basin is either unavailable or extremely limited. Mongillo (1993) suggested the need for additional data collections. Investigations (Watson and Toth 1994, Tacoma Water HCP 1999 Draft) have not provided any evidence of bull trout spawning in the Green River Basin.

Two bull trout are reported to have been recovered in the lower river. A single bull trout was reported captured at the Soos Creek Hatchery rack in the 1956 (Beak 1996). There is no supporting data regarding this reported individual fish. This information is attributed to a personnel communication from Fred Goetz (1994).

An adult bull trout was captured by the Muckleshoot Indian Tribe at approximately RM 5 during juvenile beach seining sampling efforts in 1994 (R. Malcom. pers comm. 1999). This later fish was analyzed by the University of Washington and confirmed to be a bull trout. It is uncertain if

these fish were of Green River basin origin, were of non-Green River Basin fish temporarily rearing in the Green River Basin, or were strays attempting to recolonize the basin.

No genetic samples have been obtained from this basin and the stock status can only be described as unknown. Field studies by which scientists could characterize or assess bull trout populations in the Green River basin are lacking or unavailable.

Mongillo (1993) suggested the need for additional data collections

## GREEN RIVER COASTAL CUTTHROAT GENETICS

### **NATIONAL MARINE FISHERIES SERVICE**

NMFS includes the Green River basin inside the present geographic range of coastal cutthroat. This geographical range corresponds roughly with the Puget Lowland ecoregion. This region includes all streams in Puget Sound and the Strait of Juan de Fuca west to the Elwha River inclusive. A northern boundary is unclear but unpublished data lend support to the hypothesis that it would extend into southern British Columbia (Johnson 1999). These southern and western boundaries are similar to those for chinook, coho, chum and pink salmon and steelhead. The northern boundary differs from the one for chinook, coho, pink and chum salmon.

The NMFS Biological Review Team (BRT) was unable to reach consensus on the risk for extinction of this ESU. A majority believed that the Puget Sound ESU is not presently in danger of extinction nor is it likely to be in the foreseeable future. A minority believed that the ESU is likely to become endangered in the foreseeable future.

### **WASHINGTON DEPARTMENT OF FISH AND WILDLIFE**

Coastal cutthroat trout are managed under a species complex scenario by WDFW. This is at least in part due to multiple interacting life history trajectories. Cutthroat are found in most fish bearing waters of the Green River basin from high mountain streams downstream to estuarine habitats. Resident and anadromous forms are both found in the Green River basin.

Anadromous cutthroat trout have a freshwater life history similar to steelhead. Typically, anadromous cutthroat trout smolt at two years of age and migrate in the spring into the estuary and marine near-shore habitats. These anadromous cutthroat trout may move up rivers with daily tidal fluctuations to opportunistically feed. Their ability to physiologically handle transitions between salt water and freshwater during this life phase is unique to cutthroat. Remaining in the saltwater environment for two years, these fish again migrate into their natal stream to spawn. Spawning typically occurs from January through June in small headwater streams. First time spawners typically deposit approximately 700 eggs into small gravels for incubation. Adults then return to nearshore habitats to rear again and have been known to spawn up to five times. The repeat spawners are critical to reproductive success of the species as they produce larger and more numerous eggs (Peoples 1988). These repeat spawners also provide for the exchange of genetic material between brood years.

The genetic picture for coastal cutthroat is somewhat unclear at this time. There is evidence that suggests populations are based on geographic proximity and that the Green River population is part of a larger Puget Sound population similar to what NMFS has suggested as their ESU definition.

#### LIST OF FIGURES

Table Gen-1. Summary of stock status, stock history and ESA status.

Figure Gen-1. Dendrogram resulting from cluster analysis among Puget Sound chinook populations.