Population Analysis of Coastal Cutthroat Trout in the Bypass Reach of the Sultan River, Summer 2007

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EXECUTIVE SUMMARY

The abundance and size structure of coastal cutthroat trout *Oncorhynchus clarkii clarkii* in the bypass reach (area between Culmback Dam and the Diversion Dam) of the Sultan River was assessed during August 2007 using direct observation snorkel surveys. Single pass dive counts were conducted in 10 shallow pools, 10 flatwaters (glides), and 10 riffles, and calibrated with 15 replicate count surveys using the Method of Bounded Counts (MBC) protocols. In addition, five deep pools were surveyed using single-pass dive counts. Qualitative electrofishing was conducted at two locations in order to identify trout species, assess length-frequency distributions, and collect genetic samples.

All trout clearly observed while diving or captured by electrofishing appeared to possess morphometric characteristics consistent with rainbow trout *O. mykiss*, and no positive identifications of cutthroat trout were made. Results from the subsequent genetic evaluation of tissue samples collected from 64 individuals confirmed these visual observations, and indicated that all genetic samples were from rainbow trout. While no coastal cutthroat trout were identified, data collected during this survey was further processed to estimate the abundance and size distribution of rainbow trout in the bypass reach. However, an applied population viability assessment for rainbow trout was not conducted.

A total of 128 rainbow trout were counted during diving efforts. These fish ranged in length from approximately 30 mm to 220 mm. Electrofishing yielded a catch of 69 trout (57 from the lower electrofishing site and 12 from the upper site) ranging in length from 30 mm to 150 mm. Other fishes observed included sculpin *Cottus spp*. and mountain whitefish *Prosopium williamsoni*.

Estimates of abundance and density were calculated for three size classes of rainbow trout based on the combined length-frequency distributions derived from fish observed: fry (< 70 mm), juveniles (70-130 mm), and adults (> 130 mm). Abundance estimates calculated represent approximately 5.2 miles of the habitat that was available for sampling in the bypass reach, or approximately 76% of the total reach length (estimates exclude cascades, extreme gradient areas, and other areas where sampling was not possible). Estimated abundance was greatest for trout classified as adult with 850 (± 484, 95% C.I.), followed by fry with 578 (± 333) and juveniles with 424 (± 207). Overall rainbow trout densities were low, with maximum densities observed in riffles (0.23 fish/100ft^2, all size classes combined) and minimum densities observed in deep pools (0.01 fish/100ft^2). These population estimates are likely conservative due to relatively poor sampling conditions (visibility) and fish activity levels associated with cool temperatures.

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1.0 STUDY OBJECTIVE AND DESCRIPTIONS

The Public Utility District No.1 of Snohomish County (District) owns and operates the Jackson Hydroelectric Project (Project) in the Sultan River watershed. The Project includes Culmback Dam and downstream powerhouse, and the Diversion Dam that distributes water to and from Lake Chaplain.

As part of the District's ongoing efforts to relicense the Project with the Federal Energy Regulatory Commission (FERC), the District has entered into consultation with many different stakeholders. As a part of the relicensing process, the USDA Forest Service (USFS) filed a request for a study to assess the viability of the coastal cutthroat trout *Oncorhynchus clarkii clarkii* population isolated in the Sultan River between Culmback Dam and the Diversion Dam. This 6.8-mile river reach is referred to as the "bypass reach" and is located on the Mt. Baker-Snoqualmie National Forest which is administered by the USFS Region 6.

Since 1983, a minimum flow of 20 cfs has been released from Culmback Dam into the bypass reach to provide habitat for resident salmonids upstream of the Diversion Dam. However, these releases consist of cold water from the reservoir hypolimnion which may affect fish growth and condition in the bypass reach. An assessment of the cutthroat trout population in the bypass reach was recommended to determine whether the minimum instream flow release is providing suitable habitat conditions for the long-term persistence of this species.

Since there is limited information regarding the status of the coastal cutthroat trout population in the bypass reach, a thorough evaluation of the population abundance and size structure was proposed. This information would help to determine whether current Project operations are providing conditions suitable for the long-term viability of the cutthroat trout population, and may help to determine whether any future proposed operations would have negative effect on the viability of the population in the bypass reach. The specific objectives of this study were to:

- Assess the population abundance and size distribution of coastal cutthroat trout in the bypass reach of the Sultan River;
- Estimate the proportion of cutthroat trout, rainbow trout *O. mykiss*, and hybrids in the population; and
- Assess the overall viability of the cutthroat trout population in the bypass reach.

2.0 BACKGROUND INFORMATION

Coastal cutthroat trout are found from Northern California to Alaska, and exist in both anadromous and non-migratory resident populations (Johnson et al. 1999; Trotter 1989). They can generally be identified by red streaks on either side of the lower jaw, dense spotting along the body and tail, and the presence of basibranchial teeth (Pollard et al. 1997). Anadromous populations may reside in freshwater for up to six years before leaving for the ocean (Wydoski and Whitney 2003). In their freshwater stage or resident forms, cutthroat trout generally reside in streams with temperatures ranging from 10 – 20^o C and dissolved oxygen concentrations greater than 5 mg/L (Wydosky and Whitney 2003). Juvenile fish are opportunistic feeders that rely mostly on benthic and drift insects. Larger adult individuals prey on small fish, insects and crustaceans. Coastal cutthroat trout are iteroparous, and time of sexual maturity varies among populations but occurs typically in 2 to 4 years (Hart 1973). Spawning can occur from December through May, and is dependent upon water conditions. Fry emerge in the spring after 6-7 weeks of incubation (depending on water temperature).

Coastal cutthroat trout are a species of concern in many streams in western Washington (Johnson et al. 1999). They have been referred to as a “canary in a mine”, due to their sensitivity to changes in their physical habitat or to changes in species composition (Behnke 2002). Native stocks of cutthroat trout are subject to loss of genetic identity through interbreeding with hatchery stocks or through hybridization with rainbow trout, and may be further depressed through competition with rainbow trout or brook trout *Salvelinus fontinalis*, which are more tolerant of degraded or altered stream conditions (Behnke 2002; Wydosky and Whitney 2003).

The Sultan River is located in northwest Washington approximately 20 miles east of the city of Everett, and is a tributary to the Skykomish River (Figure 2.1). The Sultan River has a drainage area of approximately 77.1 mi^2 and is impounded by Culmback Dam (RM 16.5) and the Diversion Dam (RM 9.7) for Lake Chaplain. The 6.8-mile section of river between the two impoundments is referred to as the bypass reach and was the focused study area for our population assessment. This section of river is highly confined with a drainage area of approximately 7.9 mi^2. The lowermost portion of the reach (2.5 miles) consists predominately of a low gradient (1.0%), wide stream channel, and can be accessed from either the Diversion Dam or from two access trails located at approximately RM 10.1 and 11.7.

Further upstream, the channel gradient increases to an average of 1.4% in the upper 3.6 miles. The stream channel is narrower than the lower portion of the bypass reach and consists largely of high gradient riffles and long deep pools bordered by steep bedrock cliffs. Access into this upper portion of the river reach is limited to two access points, the EK-92 or “Stringer Bridge” trail at RM 14.4 and the 6122 River Access trail at RM 15.4. The 0.7-mile section of river immediately below Culmback Dam consists predominately of large boulder substrate and high gradient cascades. This uppermost portion of river was deemed too hazardous for sampling and, therefore, was not sampled due to safety concerns.

A year-round release of 20 cfs from Culmback Dam provides a base flow within the reach, however accretion through lateral inflows can be significant and typically results in summertime flows of 30-40 cfs at the Diversion Dam. Prior to the construction of

Culmback Dam, historical summer (June, July, August) daily flows averaged 541 cfs, with a range of 51 to 5,050 cfs, (USGS Gaging Station, Sultan River near Startup, pre-1965). However, because Culmback Dam effectively captures most spring runoff, high flow events are now relatively rare in the bypass reach and only occur during heavy rainfall or uncontrolled spill events. The most recent spring spill event occurred on 25 March, 2007 with a daily flow of 3,300 cfs (USGS Gaging Station, Sultan River below Diversion Dam). Prior to this fish population study, flow releases from Culmback Dam were increased from 20 cfs to 165 cfs on 28 June 2007 and 300 cfs on 29 June for an instream flow study.

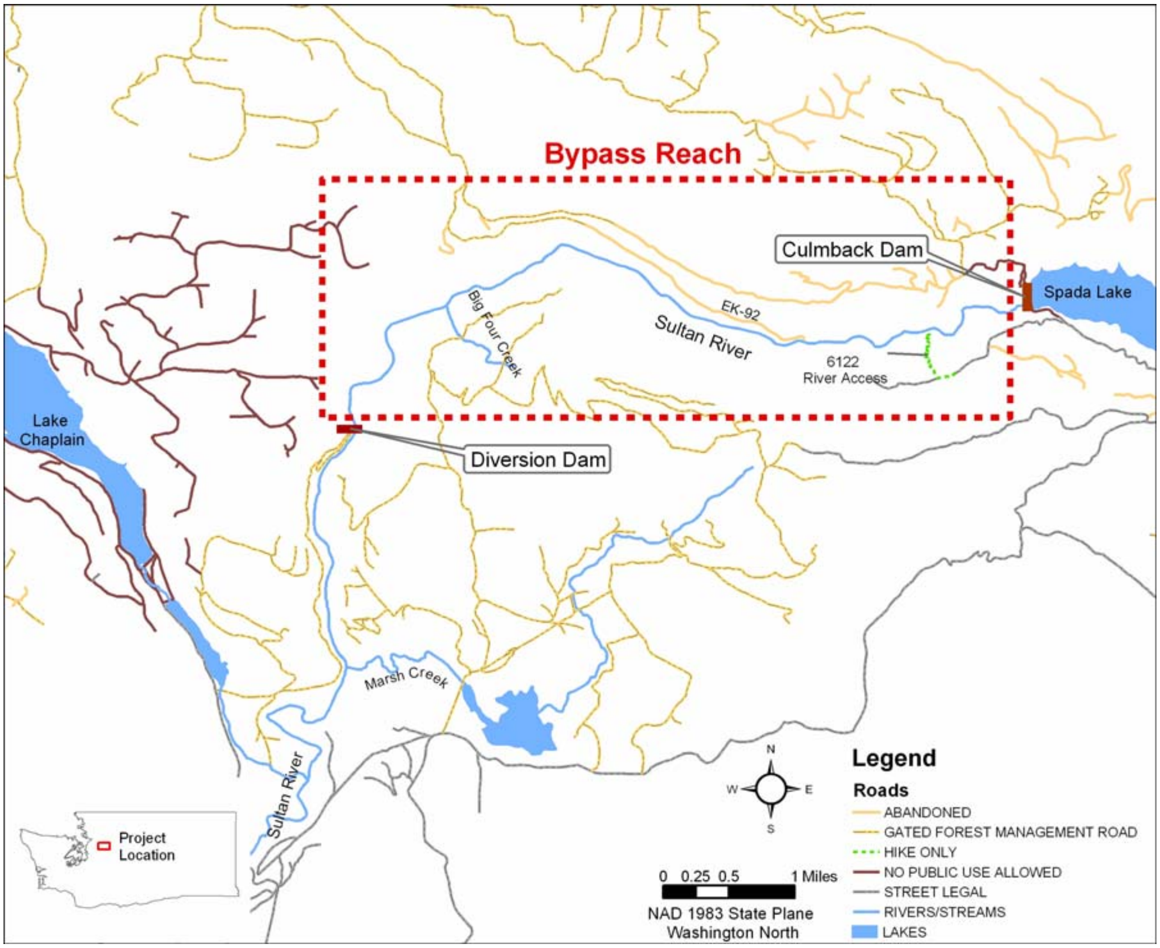

Figure 2.1 The bypass reach of the Sultan River in relation to Culmback Dam located in Western-Washington.

3.0 METHODS

The original study plan approved by FERC in October 2006 suggested a fish sampling design which employed two methodologies. The first suggested methodology was to use removal-depletion electrofishing in the downstream, low gradient portion of the bypass reach, while the second suggestion was to use angling/snorkeling mark-resight methodology in the upstream, high gradient river section. During initial site visits and discussions with District Biological Staff and other personnel working in the bypass reach, it was clear that electrofishing would have limited applicability and be generally ineffective, if not impossible, due to the abundance of deep water habitat and limited access. The angling/snorkeling mark-resight methodology was also deemed infeasible due to the low density of trout and relatively poor water visibility, which would make the capture of a sufficient number of trout and the re-identification of the marked trout highly improbable.

Consequently we proposed an alternative methodology that utilized the Method of Bounded Counts (MBC), a method designed for low density populations that relies on snorkel counts and is less dependent upon electrofishing (Mohr and Hankin, *in press*). The MBC is a recent modification to the Hankin and Reeves (1988) protocol that utilizes an improved unit selection procedure, incorporates habitat-fish correlations to improve estimator precision, and increases the number of samples through increased emphasis on rapid dive counts and reduced emphasis on slower electrofishing procedures. Like the Hankin and Reeves (1988) protocol, the MBC was developed primarily for use in small streams or sampling units which contain relatively low numbers of fish, and is particularly suited for locations where electrofishing is undesirable (*e.g.,* when endangered species are present) or infeasible (*e.g.,* remote canyon areas).

Because conventional dive counts represent an index estimate of abundance and not an estimate of total abundance, the MBC procedure requires a random subsample of the units sampled by diving to be re-sampled. This is done to calibrate the dive count index estimates and allows calculation of total abundance estimates. To accomplish this, the MBC utilizes a simple estimator previously applied in wildlife studies that uses four independent counts to calibrate a subsample of single pass dive counts (referred to as second-stage calibration units; Hankin and Reeves 1988). An important aspect of the MBC application is a bias adjustment factor. In its development, the adjusted estimator was robust and accurate for juvenile coho salmon *O. kisutch* when counts were less than 20 fish per sampling unit (Moyer 2001). When the second stage calibration units contain more than 20 fish (per species and life-stage), electrofishing is recommended to calibrate the single pass count. In areas not feasible for electrofishing (*e.g.,* most of the bypass reach), the four-pass dive count is used for calibration regardless of the number of fish in the sample unit. In terms of fish densities and sampling feasibility, the bypass reach was a suitable candidate for applying the MBC.

Although poor water visibility and low stream temperatures do not provide an ideal environment for the application of snorkeling-based protocols, agreement was reached between PUD and USFS biologists to utilize the MBC methodology to estimate the population abundance and size structure of coastal cutthroat trout in the bypass reach.

3.1 Habitat Stratification and Unit Selection

The use of a consistent methodology allowed the bypass reach to be treated as a single reach and not split into low and high gradient portions, as originally proposed. This change in methodology was made because snorkel counts are typically less limited by depth and velocity than electrofishing.

Prior to the initiation of this study, stream habitat within the bypass reach was mapped and divided into discrete habitat units (see Revised Study Plan 18: Riverine, Riparian and Wetland Habitat Assessment of the Sultan River below Culmback Dam). The uppermost 0.7 mi (approximately 10% by length) of the bypass reach was deemed unsafe and unsuitable for sampling due to extreme gradient. Therefore the study area encompassed the lower 90% of the bypass reach. Revised Study Plan 18 delineated the bypass reach into five main habitat types: high gradient riffles, low gradient riffles, cascades, glides (or flatwaters), and pools. To better account for expected habitat-related differences in fish densities and fish observability (*i.e.*, the proportion of fish counted by divers to the total number of fish present), we redefined the original study area habitat map into four habitat types which were deemed suitable for fish sampling and constituted 84% of the study area (Table 3.1). The remaining 16% of the habitat in the bypass study area was deemed unsuitable for sampling and included cascades (6%), split channels (1%), artificial habitats (4%, *e.g.,* the pool immediately upstream of the Diversion Dam), and habitats which were deemed too hazardous to sample (5%). Pools were divided into shallow and deep units based on a 6ft maximum depth criterion, which was determined from a site visit conducted just prior to the fish sampling. This was intended to separate pools that could be effectively surveyed by snorkeling (*i.e.,* most of the substrate was clearly visible) versus pools where a significant portion of the substrate was not visible.

Habitat units were further modified by partitioning long, high velocity habitats into shorter, more manageable sampling units. This was done to minimize the selection of extremely long habitat units that would require an inordinate amount of time to sample. Only riffles and flatwaters longer than 200 ft were partitioned into subunits of up to 150 ft in length; riffles and flatwaters less than 200 ft in length and pools were not partitioned.

Table 3.1 Relative proportions (by length) of habitat types sampled in the bypass reach of the Sultan River, summer 2007.

Habitat types[a]	Length (ft)	Proportion
Deep pools (> 6ft in depth)	5,309	0.16
Shallow pools (< 6ft in depth)	5,684	0.18
Flatwaters (or glides)	7,107	0.22
Riffles[b]	9,226	0.28
Habitat not sampled	5,155	0.16

[a] Habitat types exclude the upper 0.7 mi of high gradient channel below Culmback Dam (mapping data modified from RSP 18).
[b] Includes both low and high gradient riffles.

Partitions within a selected habitat unit were made at naturally occurring gradient breaks. Such gradient breaks combined with the relatively fast currents characteristic of some habitat types, serve to reduce the ability of fish to move upstream and out of the sampling unit during the dive count. In pools however, the lack of gradient breaks allow considerable fish movement, and could lead to less reliable counts. Only after the unit was partitioned did the crew randomly select which subunit to sample. This ensured that the crew could not partition a unit in such a way as to bias which area was surveyed. Each partitioned subunit was treated as an independent habitat unit for the purposes of unit selection. Although it was possible that more than one subunit of a specific habitat unit could be randomly selected for sampling, it did not occur during this study.

Ten habitat units, either full original units or partitioned subunits, were randomly selected for each habitat type for a total of 40 habitat units; 10 riffle, 10 flatwater, 10 shallow pool, and 10 deep pool. Habitat units were selected for dive counts were located using a detailed habitat map while walking upstream. A laser rangefinder was used to verify unit locations and lengths. If a riffle or flatwater longer than 200 ft was selected, partitioning and selection of the actual subunit to be sampled was not performed until the field crew was on site and potential gradient breaks could be identified. Of the 10 habitat units selected, five units were randomly selected for diver calibration using the multiple-pass dive count methodology described in the MBC protocols (Mohr and Hankin, *in press*). To reduce potential bias, habitat units selected for calibration were not disclosed to divers until after the initial dive count was completed.

3.2 Dive Counts

Depending on the unit width, three to four divers surveyed each selected habitat unit using a single pass dive count. Divers cautiously entered pre-specified dive lanes in the lower end of each habitat unit, taking care to avoid displacing fish downstream and out of the unit. When in place, each diver referred to a wrist-mounted ruler to calibrate size estimates. The accuracy of the divers at estimating the length of a fish was tested periodically using plastic fish models of differing lengths. These tests allowed individual divers to identify personal biases in size estimation and were intended to improve the accuracy of subsequent length classifications. The dive team proceeded upstream in unison to the top of the habitat unit while counting and identifying the species of all fish that passed downstream within each diver's lane. The accuracy of dive count data was maximized by verbal communications between divers either during or immediately after a pass through a habitat unit. If fish passed downstream over a lane boundary, the two adjacent divers compared counts to ensure that all fish were observed and that data regarding each fish was recorded by only one diver. As the divers approached the top of the unit, they looked ahead to count any fish that moved upstream and out of the sample unit. Each diver tallied the species and estimated length of each fish on an underwater slate, and then total fish counts were transferred to a data sheet after each dive. The summed counts from a single pass were used to estimate an index of trout abundance for that habitat unit.

After conducting the single-pass dive count, the divers determined if the sampling unit was selected for a second-stage calibration survey by removing a previously recorded and concealed label containing a "yes" or "no" for each randomly selected unit. If the unit was not selected for calibration, the divers continued upstream to the next selected unit. If the habitat unit was selected for calibration, the divers conducted three more independent dive counts according to the MBC protocols. Each repetitive count was conducted under similar visibility conditions as the initial sample. Additional information collected at each habitat unit included starting and ending dive times, water temperature, and underwater visibility. A digital photograph was taken of each sampled habitat unit.

Underwater visibility was monitored during dive counts by estimating the distance a diver could clearly see an artificial trout approximately the size of a large fry. Water visibility was measured perpendicular to flow and in the same lighting (*i.e.,* sun or shade) that was predominant within the surveyed habitat unit. In general, larger, adult sized trout can be identified at a distance approximately twice that of smaller, fry sized trout (TRPA 2005).

3.3 Electrofishing

To collect fish for genetic samples and to physically inspect trout for possible species differentiation, electrofishing was employed at two locations within the bypass reach. Qualitative, single-pass electrofishing was employed in the lower portion of the bypass reach approximately 0.5 mile upstream of the Diversion Dam, and in the upper portion approximately 1 mile downstream of Culmback Dam. At both locations, approximately 700 ft of river was sampled.

A Smith-Root Model 11A backpack shocker was deployed with two netters to collect all stunned fish. A setting of 500 volts at a frequency of 60 Hz was required to effectively shock fish for capture. Captured fish were held in buckets of aerated water, and then anesthetized using clove oil. The fork length (FL) of all fish were measured to the nearest mm, and all fish were visually assessed for cutthroat trout characteristics such as maxillary size, head length, spotting patterns, basibranchial teeth, and throat slash (Weigel *et al*. 2002). [It should be noted that some of these physical characteristics, such as the presence of basibranchial teeth, are only reliable indicators for fish larger than 100 mm (Leary *et al.* 1996)]. For all trout over 50 mm FL, a small sample of the upper lobe of the caudal fin was clipped for genetic analysis. Fin clips were placed in individually labeled vials and preserved for later analysis.

3.4 Data Analysis

3.4.1 Estimation of Fish Abundance

We combined length frequency distributions from electrofishing data and the estimated length obtained from dive counts to develop size classes that could be used to evaluate population abundance. The length-frequency distributions were visually assessed to stratify the dive counts into three size classes, hereafter termed "fry" (likely 0+ fish), "juveniles" (likely 1+ and 2+), and "adults" (likely 3+ and older). Since the length frequency distributions contained considerable overlap, the fry, juvenile, and adult classifications should not be considered as rigorous definitions for young-of-year, juvenile, and mature trout.

The abundance and density (number/100ft^2 of stream channel) of trout by size class was estimated within each individual habitat unit and for each habitat type as a whole within the entire bypass reach (excluding the habitat that could not be sampled). Pooled reach estimates were calculated by summing the independent estimates from the four habitat types and the three size classes. For individual habitat units, single pass dive counts were used to estimate an index of abundance of fish per unit. For estimation of fish abundance and densities at the reach scale, dive counts calibrated by MBC in shallow pools, flatwaters, and riffles were used according to the equations presented in Appendix A. Habitat unit length was used as an auxiliary variable in the ratio estimators for all reach estimates because we expected a positive correlation between numbers of fish and habitat unit size. A high, positive correlation will increase the precision of ratio estimators and thus improve the ability to detect differences among spatial and temporal scales. For deep pools, where single pass dive counts were not calibrated, and index of abundance was estimated using Simple Random Sampling (SRS) formulas (Cochran 1977).

3.4.2 Population Viability

After determining the population size and age structure, the viability of the coastal cutthroat trout population in the bypass reach was assessed. Combined length frequency distributions (from dive counts and electrofishing) were compared to determine if there were apparent gaps in the age class distribution.

4.0 RESULTS

4.1 Overview

Upon initial sampling of the bypass reach, it became evident that diving was not effective in the deep pool habitat (> 6 ft maximum depth) due to the poor water visibility. Consequently, only five deep pools were retained for sampling and only single-pass index counts were conducted. As a result, 35 individual habitat units (or subunits) were sampled by direct observation snorkel counts over a 10 day period in August 2007 (Table 4.1). Flow released from Culmback Dam during the survey was approximately 20 cfs. A detailed map delineating the habitat units sampled within the bypass reach along with individual photos of each habitat unit is located in Appendix B.

A total of 6,153 linear feet of channel was surveyed, representing approximately 19% of the total length of the bypass study area (Table 4.1.). Water temperatures recorded at each sampled habitat unit ranged from 7^{o} C to 12^{o} C, with a mean of 9.5^{o} C. Estimated visibility ranged from 4 to 6 ft, with the lowest values occurring at units located in the upper portion of the reach.

In total, 128 trout ranging in length from approximately 30 mm to 260 mm were observed during diving efforts (Figure 4.1). The number of trout counted within a single habitat unit ranged from zero to 15 fish (Table 4.1). Other fishes observed during diving efforts included sculpin *Cottus* spp. and mountain whitefish *Prosopium williamsoni*. Electrofishing in two locations yielded a catch of 69 trout (57 from the lower electrofishing site and 12 from the upper site) ranging in length from 30 mm to 150 mm.

No positive identifications of cutthroat trout were made during the dive counts or during the electrofishing efforts. Several of the large trout captured by electrofishing (>100 mm) possessed denser spotting than other fish, and one individual possessed a maxillary that extended to the posterior edge of the orbital socket, but none clearly exceeded the eye. Based on tactile inspection, none of the larger fish appeared to possess basibranchial teeth. Instead, all fish appeared to possess the morphometric characteristics that were more consistent with rainbow trout than with coastal cutthroat.

Results of the subsequent genetic analysis confirmed our field observations, and indicated that all tissue samples collected in the lower and upper bypass reach were exclusively rainbow trout (Appendix C).

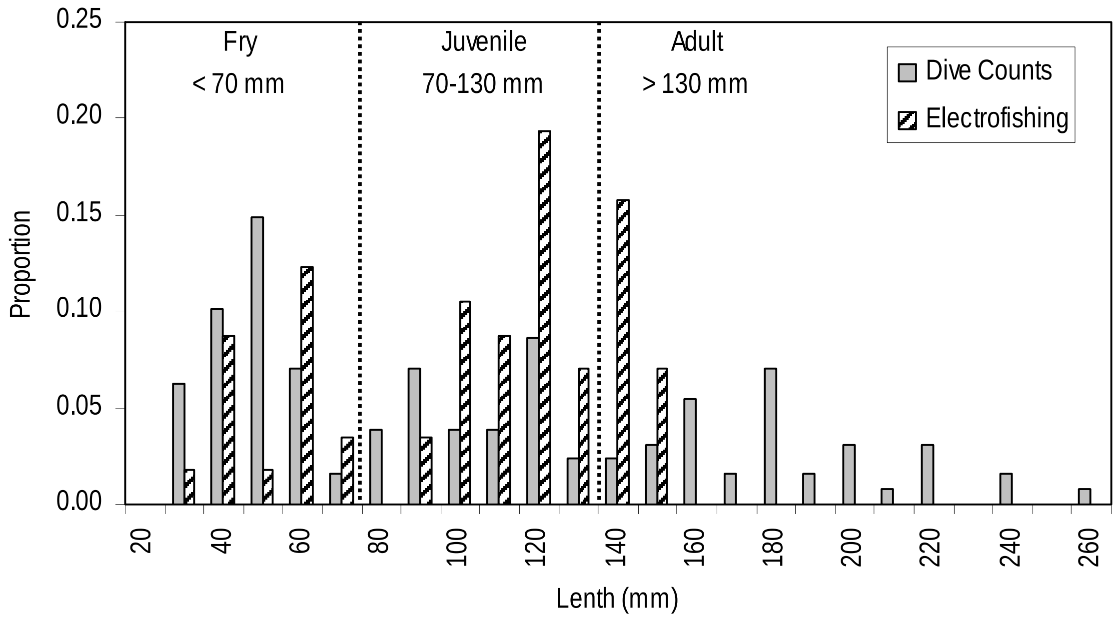

Figure 4.1 Length frequency distribution of trout observed during dive counts and collected during electrofishing in the Sultan River. Dotted lines indicate estimated age-class delineations.

Table 4.1 Summary of habitat and dive count statistics for units sampled in the bypass reach of the Sultan River.

Habitat Type	Unit NSO No.[a,b]	Unit Length	Mean Width	Dive Time	Water Temp °C	Water Visib. ft	No. Trout Counted [c]			Other Fish
							Fry < 70 mm	Juvenile 70-130 mm	Adult >130 mm	
Deep pools	31	519	63	1320	-	-	0	1	2	
	44	267	45	1130	10	-	1	1	1	
	46	312	43	1412	10	5	0	2	1	3 whitefish
	75	282	52	1409	9.5	5.5	0	0	0	
	122	168	56	1230	-	-	0	0	0	
		1548					1	4	4	
Shallow pools	8	126	68	904	11	5	4	0	2	
	57*	50	39	955	9	4.5	0	0	0	3 whitefish
	82*	139	25	1351	8.5	5	0	0	1	
	98*	180	48	1403	10	4	0	0	0	
	130	125	41	1216	8.5	5.5	0	0	0	
	131*	129	40	1220	-	-	1	0	0	
	134*	111	59	1248	7.5	-	0	0	0	
	139	340	56	1132	-	-	0	0	1	
	141	264	44	1152	-	-	0	0	0	
	145	697	107	1037	7	5	0	0	3 [d]	
		2161					5	0	7	
Flat-waters	11b	80	72	943	-	-	1	0	0	
	30c	78	76	1301	11	5.5	2	0	0	
	37c*	160	58	1100	10	5	0	0	0	1 sculpin
	42	140	48	1110	9	5	4	6	2	1 whitefish
	50*	122	63	1505	11	6	3	2	0	2 whitefish
	59b*	81	49	1054	9	-	0	1	1	
	68a	99	43	1215	9.5	5	0	0	0	
	78*	155	35	1212	8	-	0	0	0	
	99*	190	54	1409	10	4	0	0	0	
	119b	90	31	1220	-	-	0	0	0	
		1195					10	9	3	
Riffles	18b	106	78	1106	11.5	5	6	1	1	
	20d*	108	59	1204	-	-	2	2	1	
	24	192	54	1345	11	-	5	2	6	
	27*	131	70	1450	11	5	0	2	3	
	29d*	132	74	1430	12	-	10	4	1	2 whitefish
	71*	102	70	1301	-	-	0	2	0	
	77	108	33	1145	8.5	5	0	0	1	
	112	142	36	1113	9	4	0	1	1	
	132c*	75	45	1341	8.5	-	0	1	0	
		1249					23	15	14	

[a] Letters indicate subunits (e.g., a=1st subunit, b=2nd subunit, etc.)
[b] Asterisk indicates unit selected for MBC calibration
[c] Only first-pass count data shown, an additional 33 trout were counted during calibration dives.
[d] Unit subsampled due to wide channel, count expanded to estimate whole unit

Table 4.2 Estimated diver observation probabilities for trout by size-class and habitat type, based on MBC replicate dive counts.

Trout size classes	Habitat types	n[a]	Estimated Observation Probability		
			min	mean	max
Fry < 70 mm	Shallow pools	1	0.33	0.33	0.33
	Flatwaters	2	-0.27	-0.13	0
	Riffles	3	0.31	0.58	0.76
Juvenile 70 – 130 mm	Shallow pools	0	-	-	-
	Flatwaters	2	0.80	0.83	0.87
	Riffles	5	-0.33	0.51	1.00
Adult >130 mm	Shallow pools	4	-1.0	-0.17	0.33
	Flatwaters	1	0.86	0.86	0.86
	Riffles	4	-0.13	0.22	0.67

[a] Represents the number of calibration units where counts exceed zero (max = 5 units).

4.2 Population Abundance

Since all of the trout observed during the dive counts (or from DNA collected from trout captured by electrofishing) were identified as exclusively rainbow trout, we were unable to estimate population abundance for cutthroat trout or hybrids. To provide supplementary information, a population abundance estimate was instead made for rainbow trout in the bypass reach.

Diver observation probabilities were calculated for each size class using the calibration units designated within each habitat type. Therefore, diver observation probabilities were calculated using repeated counts from 5 shallow pools, 5 flatwaters, and 5 riffles. However the low fish densities resulted in relatively few units where observation probabilities could be reliably estimated (Table 4.2). Overall, observation probabilities varied among trout size class distinctions and habitat types, but in general were typically highest for larger fish in flatwaters and for fry in riffles.

The overall estimated abundance of rainbow trout in the bypass reach totaled 1,852 (±622, 95% C.I.) fish. This estimate excludes the non-sampled habitat in the study area (*e.g.,* cascades) and the upper 0.7 mi of high gradient channel below Culmback Dam (Table 4.3). Of this total abundance estimate, an estimated 578 (±333) trout were classified as fry (< 70 mm), 424 (±207) were classified as juveniles (70-130 mm), and 850 (±484) were classified as adults (>130 mm; Figure 4.2). Overall density was 0.108 (±0.05) trout/100ft^2, with a density of 0.034 (±0.019) fry/100ft^2, 0.025 (±0.012) juveniles/100ft^2, and 0.05 (±0.028) adults/100ft^2 (Table 4.3; Figure 4.3).

The estimated abundance and the estimated density of all size classes of rainbow trout were highest in riffles, often by a factor of two or more (Table 4.3). Densities of fry and juveniles were lowest in pools followed by flatwaters, whereas densities of adult trout were highest in shallow pools followed by flatwaters. Because of the expected differences in diver observation probabilities between trout size classes and habitat types, we did not attempt to assign statistical significance to the observed differences in abundance or density. The wide confidence intervals associated with most estimates would further limit the distinction of significant differences among fish size or habitat type strata.

4.3 Population Viability

Since none of the trout observed or collected during electrofishing were positively identified as coastal cutthroat trout, a comparison of size classes within the observed length frequency distribution was not completed and no determination of population viability was made.

4.4 Other Observations

The effectiveness of dive counts in the bypass reach was indirectly assessed by comparing a single-pass electrofishing survey in one shallow pool habitat with a single-pass dive count conducted in the same unit three days later. The electrofishing pass was conducted as part of the qualitative assessment to collect trout for genetic samples and was not intended to estimate abundance. The electrofishing pass was conducted in late afternoon at a water temperature of 12^{o} C, and resulted in the capture of seven trout between 59 mm and 152 mm in length. The single pass dive count was conducted in the morning (at 0904) three days later at a temperature of 11^{o} C, when six trout ranging from 50 mm to 220 mm were observed. The similarity in results from this single example suggests that the dive counts in the bypass reach may produce estimates similar to those derived from electrofishing.

Rainbow trout appeared to be distributed disproportionately throughout the bypass reach, with the highest densities occurring in the lower 2.5 mile portion of the reach. As previously indicated, the bypass reach was treated as a single reach, and not split into upper and lower portions for a statistical comparison. However, based on dive counts of individual habitat units, over 87% of the fish observed occurred in habitat units located within the lower 2.5 mile portion of the reach (16/35 units). Also, a comparison of length-frequency distributions from the lower 2.5 mile portion of the bypass reach with distributions from the upper portion showed a higher proportion of fry-sized (< 70 mm), presumably young-of-year, rainbow trout in the lower section. The reasons for these differences are unknown, but may be attributed to more suitable habitat in the lower portion of the reach. The lower gradient and less confined channel in the lower portion may offer more spawning gravels than in the upper, steeper portion. Also water temperatures were slightly warmer in the lower reach during the surveyed period, which may have resulted in greater fish activity and a higher likelihood of observing fish in the lower portion of the bypass reach.

Table 4.3 Population abundance and density statistics for rainbow trout by size class and habitat type in the bypass reach of the Sultan River

Size Class	Statistic	Deep pools	Shallow pools	Flatwaters	Riffles	All habitats*
Fry < 70 mm	Abundance (Y)	4	62	137	375	578
	Variance (Y)	11	173	2,739	23,688	26,610
	95% C.I.	9	30	118	348	333
	Density (no./mi)	2.9	46.9	102	215	101
	Var (no./mi)	6.1	98.3	1,500	7,802	809
	95% C.I.	6.9	22.4	88	200	58
	Density (no./100ft2)	0.001	0.015	0.036	0.072	0.034
	Var (no./100ft2)	0.0	0.00001	0.00019	0.00086	0.00009
	95% C.I.	0.002	0.007	0.031	0.066	0.019
Juvenile 70-130 mm	Abundance (Y)	15	0	106	303	424
	Variance (Y)	37	0	1,210	9,056	10,303
	95% C.I.	17	0	79	215	207
	Density (no./mi)	11.5	0.0	78.5	173.8	73.9
	Var (no./mi)	21.5	0.0	662.6	2,982.8	313.1
	95% C.I.	12.9	0.0	58.2	123.5	36.1
	Density(no./100ft2)	0.004	0.000	0.028	0.058	0.025
	Var (no./100ft2)	0.0	0.00000	0.00008	0.00033	0.00004
	95% C.I.	0.004	0.000	0.021	0.041	0.012
Adult > 130 mm	Abundance (Y)	15	296	35	503	850
	Variance (Y)	37	12,956	232	43,018	56,244
	95% C.I.	17	257	34	469	484
	Density (no./mi)	11.5	223.4	26.2	288.6	148.1
	Var (no./mi)	21.5	7,371.4	127.3	14,169.2	1,709.2
	95% C.I.	12.9	194.2	25.5	269.3	84.3
	Density (no./100ft2)	0.004	0.072	0.009	0.096	0.050
	Var (no./100ft2)	0.0	0.00076	0.00002	0.00156	0.00019
	95% C.I.	0.004	0.062	0.009	0.089	0.028
All Trout	Abundance (Y)	34	358	279	1,181	1,852
	Variance (Y)	85	13,129	4,181	75,762	93,157
	95% C.I.	26	259	146	623	622
	Density (no./mi)	26	270	206	678	323
	Var (no./mi)	49	7470	2290	24954	2831
	95% C.I.	19	240	133	439	148
	Density (no./100ft2)	0.009	0.087	0.074	0.225	0.108
	Var (no./100ft2)	0.00001	0.0008	0.0003	0.0028	0.0003
	95% C.I.	0.006	0.077	0.047	0.146	0.050

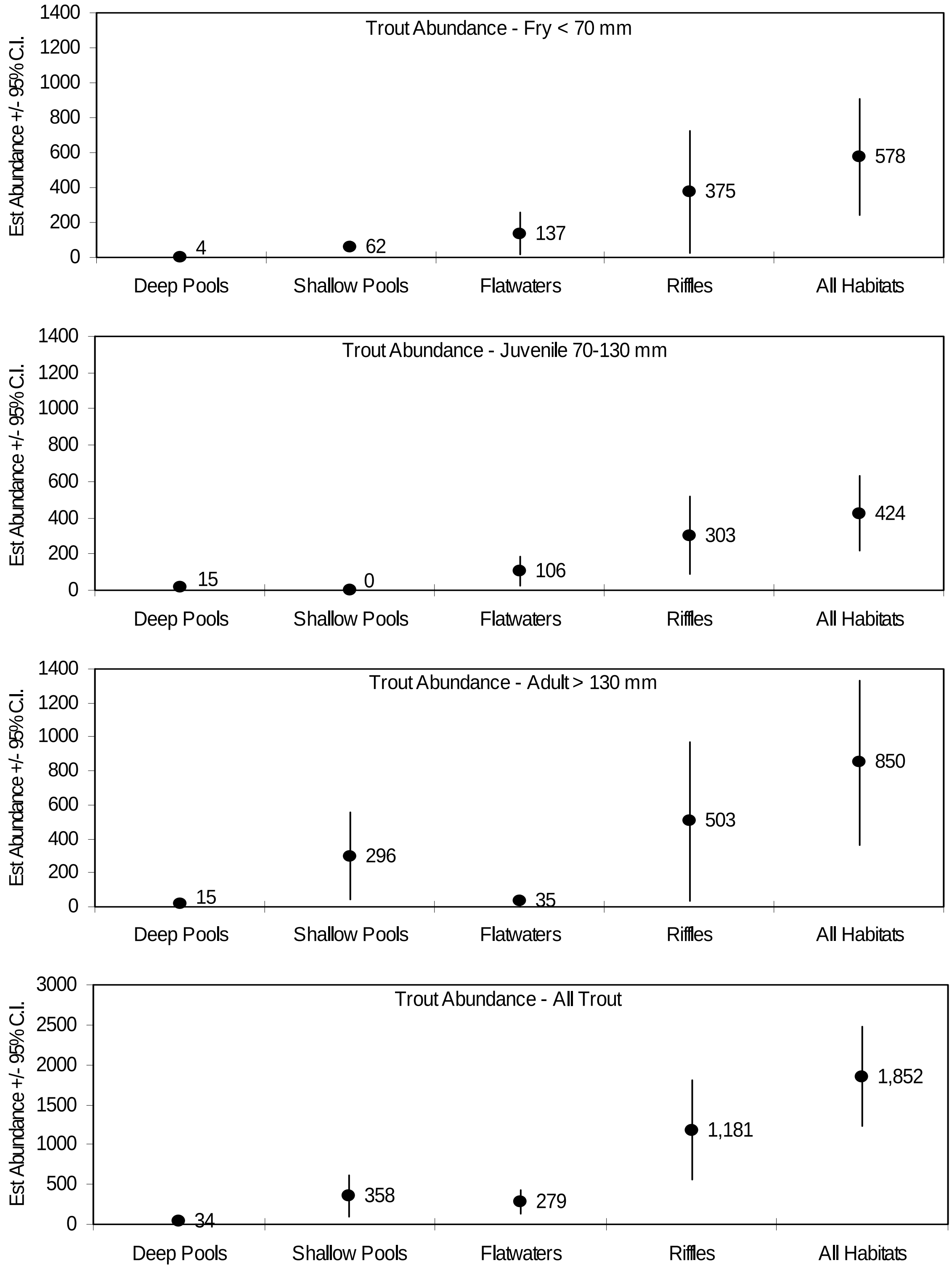

Figure 4.2 Estimated abundance of trout in the bypass reach of the Sultan River according to size class and habitat type.

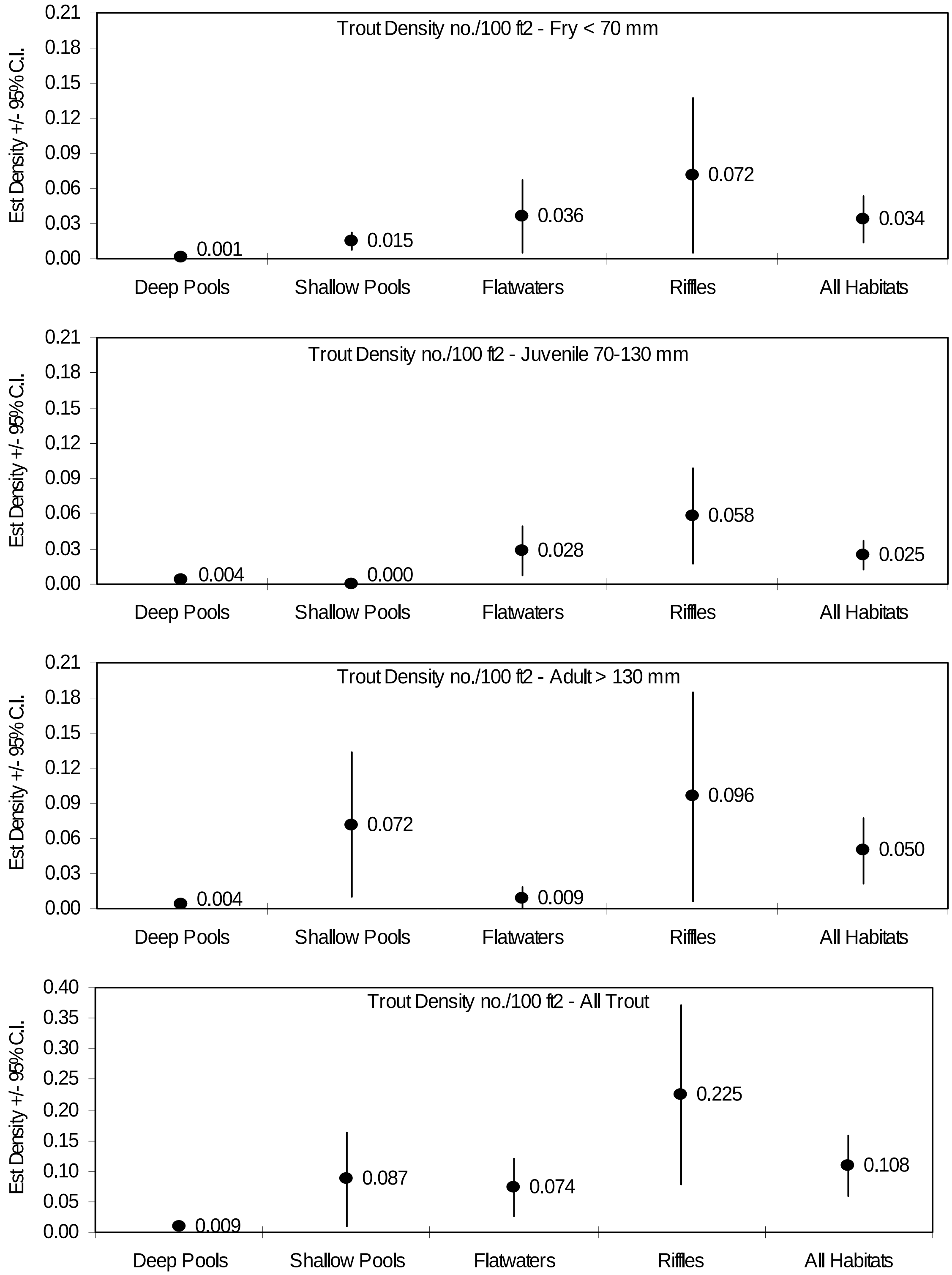

Figure 4.3 Estimated density (no./100 ft^2) of rainbow trout in the bypass reach of the Sultan River according to size class and habitat type.

To evaluate the potential effects of water temperature on fish observability, an impromptu evaluation was conducted. Two habitat units were selected and surveyed by diving twice in one day, first in the morning at cooler temperatures, then again in the late afternoon at warmer temperatures. Only one trout was observed at each unit (FW 37 and FW 42) during the morning dive count, whereas during the subsequent afternoon dive count, a greater number of trout were observed. In the case of unit 42, the number of trout observed increased from one to 12 individuals while in unit 37 the number of trout observed increased from one to three fish.

5.0 DISCUSSION

While coastal cutthroat trout are known to occur in the Sultan River below the Diversion Dam, and historically were found in waters upstream of Culmback Dam (Pfeifer et al. 1998), the results of this study suggest that coastal cutthroat trout are not currently present in the bypass reach of the Sultan River. Although the poor water clarity prevented divers from clearly identifying the species of some trout, most trout observed during the extensive dive counts appeared to have physical characteristics resembling rainbow rather than cutthroat trout. Furthermore, the capture and visual inspection of 69 trout collected by electrofishing did not reveal any fish that exhibited physical attributes that were characteristic of cutthroat trout. These observations were validated with the results of the genetic analysis (Appendix C), which found that all tissue samples collected from trout in the bypass reach were exclusively rainbow trout.

An applied population viability analysis of cutthroat trout in the bypass reach could not be accomplished in this study because of the lack of positive identifications of cutthroat trout. While it is theoretically still possible that a small remnant population of pure or hybridized cutthroat trout exists in the bypass reach and was not observed or collected, the likelihood of this is low. Furthermore, given the relatively low abundance of trout observed in the bypass reach, it is unlikely that an undetected population exists at a level that maintains long-term genetic viability (Rieman and Allendorf 2001). Despite the lack of an applied population viability analysis, the length-frequency distribution of rainbow trout does indicate the presence of several age classes, signifying successful recruitment in 2007 and during previous years. The expected decline in abundance with age is also suggested, with an estimated 578 fry, 424 juveniles, and 850 adults (assumed to represent two or more age classes).

Supplementary results of this study indicate that the rainbow trout densities in the bypass reach are relatively low at approximately 0.11 (±0.05) fish/100ft^2. Comparative fish density data based on dive counts in western Cascade streams similar in size to the bypass reach is scarce. However, densities of trout (from electrofishing) over an 11-year period in a small (< 20 ft wide) stream in western Oregon ranged between 2.9 and 6.7 fish/100ft^2 (House 1995). Mean electrofishing based densities of trout from 33 streams along the coasts of Oregon and Washington ranged from 0.08 to 10.2 trout/100ft^2, but only six of these estimates showed minima less than 0.02 trout/100ft^2 (Platts and McHenry 1988). These limited comparisons suggest that trout densities in the bypass reach in 2007 were lower than typical densities in most other small to medium-sized streams in the western Cascades.

The low estimated densities of rainbow trout in the bypass reach may be due in part to the difficulty of detecting trout by diving in cold water with poor visibility. Direct observation snorkel surveys were judged to be the only feasible alternative; however the cold water temperatures and poor water clarity may have reduced confidence in the dive counts. Water temperatures in the bypass reach remained cool throughout this study, and likely resulted in some degree of hiding behavior by smaller trout. Water temperatures below 10^{o} C have been shown to elicit substrate hiding behavior among juvenile salmonids (Chapman and Bjornn 1969; Meyer and Griffith 1997). This behavior makes observations of concealed fish less likely. In the bypass reach, large substrates (*e.g.,* cobbles and boulders) were relatively common and afforded many interstitial hiding places, which made observation difficult or impossible. Poor visibility in the water is also directly related to the likelihood of a fish being observed. This combined with the relatively wide stream channel of some units (in comparison to the small crew size), undoubtedly resulted in some fish avoiding detection by the dive team, and consequently higher variability among abundance estimates.

Timing of dive counts may have also biased the abundance estimates low and resulted in wide confidence intervals. The potential effects of water temperature on the likelihood of observing fish was indirectly assessed in two habitat units that were surveyed by diving twice in one day, first in the morning at cooler temperatures, then again in the late afternoon at slightly warmer temperatures. In both units more fish were observed in the afternoon dive counts than in the morning dive counts. While it is difficult to draw any strong conclusions from these observations, they do indicate temporal differences in fish behavior existed. This would further suggest that our estimates of abundance are likely conservative and the true abundance is probably higher.

The factors associated with cold water temperatures, poor visibility, and possible temporal differences in fish behavior likely contributed to the high variability observed in the calibration dives, and may have violated one of the assumptions of the MBC protocol, namely that every fish has the potential of being seen by a diver. The estimation of diver observation probabilities in the MBC calibration units gives an indication of how consistently divers could count fish during the calibration dives. The low number of calibration units (5 shallow pools, 5 flatwaters, and 5 riffles) and the low fish densities resulted in relatively few units where observation probabilities could be reliably estimated. Negative observation probabilities (from repeat counts where the variance exceeded the mean) occurred in several individual calibration units, and resulted in a negative mean probability for fry in flatwaters and for adults in shallow pools (note: the bias adjustment procedures that rely on estimated observation probabilities were not used to calculate these abundance estimates). High mean probabilities (> 0.50) occurred for fry and juveniles in riffles, and for juveniles and adults in flatwaters. Mohr and Hankin (*in press*) concluded that the bias adjustment procedure resulted in reliable abundance estimates when the mean observation probabilities exceed 0.4, a condition that was met for only four of the eight datasets in this study.

The high variability observed within the calibration dives suggests that the estimates contained in this report should be interpreted as indices of total abundance, rather than as true estimates of total abundance. Indices of abundance, although less desirable than estimates of true abundance, can be compared to other data in a rigorous and statistically valid manner if the survey methodologies (*e.g.,* sampling design, diving protocols, etc.) and stream conditions (*e.g.,* temperatures and visibility, etc.) are consistent between surveys.

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APPENDIX A

Derivation of Method of Bounded Counts Expected Value and Variance

<u>M</u>ethod of <u>B</u>ounded <u>C</u>ounts Estimators (from Mohr & Hankin *in press*)

For the habitat units that were calibrated with repeat dive counts (shallow pools, flatwaters, and riffles), the estimation of "true" abundance in those habitat types was calculated by the MBC as:

$\tilde{y}_B = D_{[m]} + (D_{[m]} - D_{[m-1]})$ where $\tilde{y}_B$ is the bounded count estimate of true abundance,

$D_{[m]}$ is the largest of the four dive counts, and $D_{[m-1]}$ is the second largest of the four dive counts.

Estimated Abundance of Trout

To estimate the abundance of trout for each size class and within each habitat type (excluding non-accessible or non-sampleable areas) in the project reach, we used the following definitions:

Definitions (per size class & habitat type)	**Variable**
total number of available dive units	N
number of dive units sampled	n_1
number of calibrated dive units	n_2
mean diver counts in sampled dive units	$x_{1\ bar}$
mean diver counts in calibrated dive units	$x_{2\ bar}$
mean "true" abundance in calibrated dive units	$y_{2\ bar}$
total length of all available dive units	$z_{u\ bar}$
total length of all sampled dive units	$z_{1\ bar}$
total length of all calibrated dive units	$z_{2\ bar}$
ratio of true abundance to 1st pass counts in calibrated dive units	B_x

Habitat unit length was used as an auxiliary variable for all shallow pool, flatwater, and riffle estimators due to their expected correlation with dive counts.

The estimated abundance of trout ($\hat{t}_{y,DA}$) was calculated as:

$$\hat{t}_{y,DA} = N\bar{y}_2[\frac{\bar{x}_1}{\bar{x}_2} + \frac{\bar{z}_U - \bar{z}_1}{\bar{z}_2}]$$

with a variance of:

$$\hat{V}(\hat{t}_{y,DA}) = N^2(1-\frac{n_1}{N})(\frac{\bar{z}_U}{\bar{z}_1})^2 \frac{s_e^2(\bar{y}_{2,z})}{n_1} + N^2(1-\frac{n_2}{n_1})(\frac{\bar{x}_1}{\bar{x}_2})^2 \frac{s_e^2(\bar{y}_{2,x})}{n_2}$$

where

$$s_e^2(\bar{y}_{2,z}) = \sum_{k=1}^{n_2} (y_k - \hat{B}_z z_k)^2 / (n_2 - 1) \quad \text{with} \quad \hat{B}_z = \bar{y}_2 / \bar{z}_2$$

and

$$s_e^2(\bar{y}_{2,x}) = \sum_{k=1}^{n_2} (y_k - \hat{B}_x x_k)^2 / (n_2 - 1) \quad \text{with} \quad \hat{B}_x = \bar{y}_2 / \bar{x}_2$$

An important component of the MBC methodology is the estimation and correction of the negative bias that is associated with dive counts. To estimate bias, the diver observation probability is first required:

Definitions	**Variable**
diver observation probability in unit k	p_k
the i^{th} diver count in unit k	D_{ik}
number of repeat counts (=4)	m_D
overall diver observation probability	p
number of calibration pools where D_k bar is >0	n_2*

$$\hat{p} = \frac{1}{n_2^*} \sum_{k=1}^{n_2^*} \hat{p}_k \quad \text{where} \quad \hat{p}_k = 1 - \frac{s_k^2(D)}{\bar{D}_k}$$

and

$$\bar{D}_k = \sum_{i=1}^{m_D} D_{ik} / m_D \quad \text{and} \quad s_k^2(D) = \sum_{i=1}^{m_D} (D_{ik} - \bar{D}_k)^2 / (m_D - 1)$$

Bias is then calculated using:

Definitions	**Variable**
original bounded count estimate	$\tilde{y}_B$
bias-corrected bounded count estimate	$\tilde{y}_B$ *
number of repeat counts	m

$$\tilde{y}_B * = \tilde{y}_B - \sum_{u=0}^{\tilde{y}_B - 1} \hat{F}(u)^{m-1} (m - (m+1)\hat{F}(u)) \quad \text{where} \quad \hat{F}(u) = \sum_{j=0}^{u} \binom{\tilde{y}_B}{j} \hat{p}^j (1 - \hat{p})^{\tilde{y}_B - j}$$

The bias-corrected dive count estimate, or $\tilde{y}_B$ *, is then inserted back into the equations for $\hat{t}_{y,DA}$ and its variance to produce a bias-corrected estimate of abundance and variance for each stream.

95% confidence intervals for the stream abundance estimates were calculated as:

$$\hat{t}_{y,DA} \pm t_{n1-1}\sqrt{\hat{V}(\hat{t}_{y,DA})}$$

Estimates pooled among habitat types and/or size classes were simply sums of the independent estimates of abundance and variance. Confidence intervals for the pooled estimates were calculated as above but with the degrees of freedom as:

$$(n_{DP}\text{-}1) + (n_{SP}\text{-}1) + (n_{FL}\text{-}1) + (n_{RF}\text{-}1)$$

Dive counts were not calibrated with MBC for deep pools, since much of the bottom was not visible to divers and an estimate of *true* abundance was not possible. Instead, an *index* of abundance of trout in deep pools was calculated using <u>S</u>imple <u>R</u>andom <u>S</u>ampling formulas in Cochran (1977), as:

$$\hat{Y} = N \sum^{n} x_i/n$$

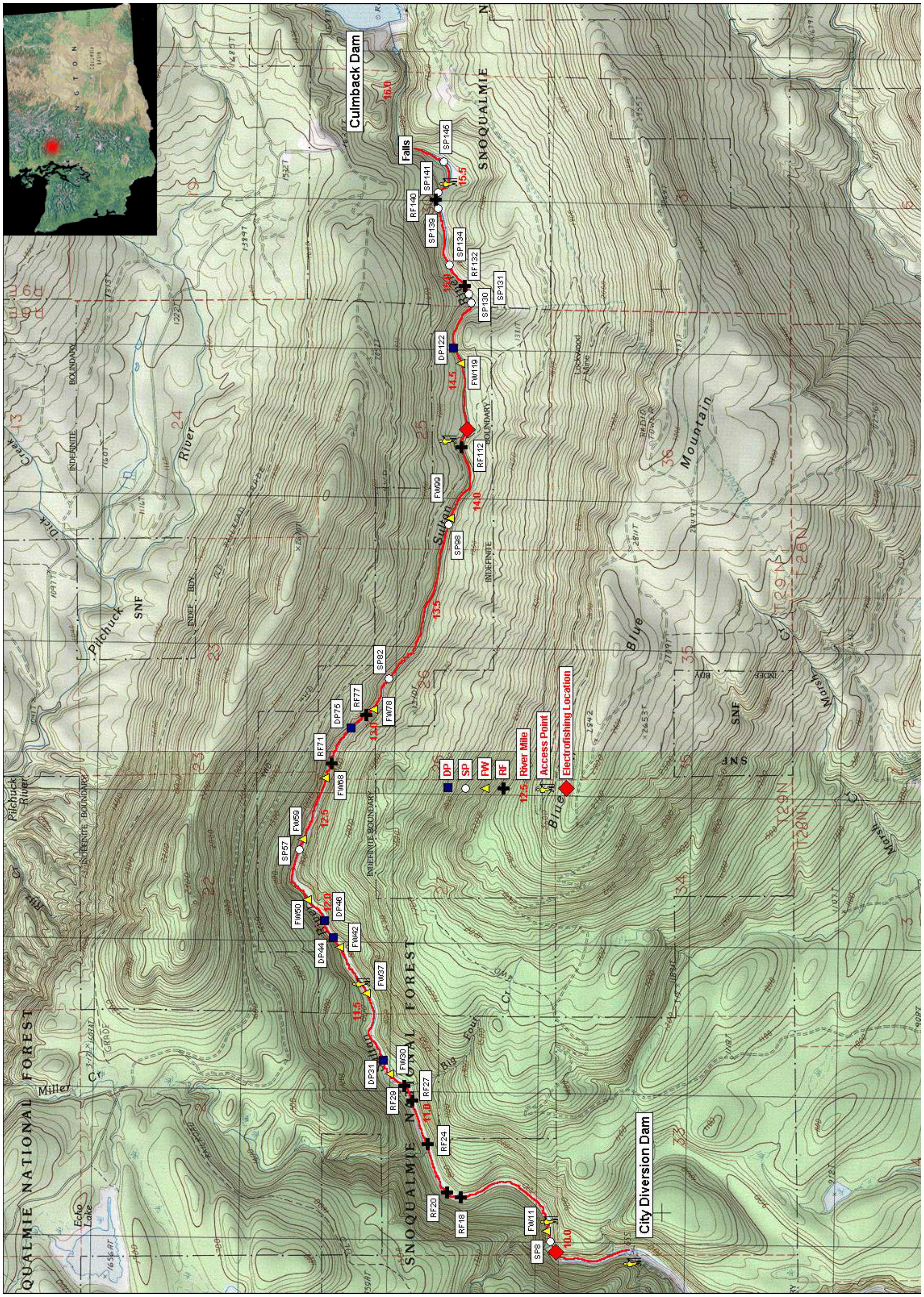
With a variance of:

$$v(\hat{Y}) = \frac{N^2 s^2}{n} \left(\frac{N-n}{N}\right)$$

The 95% confidence interval for deep pool index estimates were calculated as for the other habitat types, described above.

APPENDIX B

Detailed Map of Habitat Units Sampled in the Sultan River Along With Photographs, 2007



Detailed map of Sultan River bypass reach showing sampled habitat units and other features (Habitat Type codes are DP=deep pool, SP=shallow pool, FW=flatwater, RF=riffle).


Unit SP8


Unit FW11


Unit RF18


Unit RF24


Unit RF27


Unit RF29


Unit FW30+DP31


Unit FW37


Unit FW42


Unit DP44


Unit DP46


Unit FW50


Unit SP57


Unit FW59


Unit FW68


Unit RF71


Unit DP75


Unit SP98+FW99


Unit RF112


Unit FW119


Unit DP122


Unit SP130


Unit SP131


Unit RF132

APPENDIX C

Sultan River Resident Fish Genetic Analysis

United States Department of the Interior

WESTERN FISHERIES RESEARCH CENTER
BIOLOGICAL RESOURCES DIVISION
U.S. GEOLOGICAL SURVEY
6505 NE 65th St
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Sultan River Resident Fish Genetic Analysis: Report on the Genetic Results
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January 29, 2008

Summary

Four species-specific nuclear DNA markers (OM-55, OCC-16, OCC-34, and OCC-42) and mitochondrial DNA (mtDNA) marker (ND2) were screened through 63 individuals. Nuclear DNA markers are useful for identifying hybrid individuals and estimating the percentage of admixture (foreign genes) within a population, and mtDNA markers are useful for identifying the maternal lineage of individuals, since mtDNA is maternal inherited in trout. Genetically, all trout samples analyzed resembled rainbow trout. The conclusion is that the resident trout community within the Sultan River between Culmback Dam and the City of Everett's Diversion Dam (the Bypass Reach) is composed of rainbow.

Results

Sixty-four individual sample tubes were received, and one sample tube (# 30) did not contain a fin tissue sample. DNA was extracted from the 63 individual fin tissue samples; 51 samples from the lower reach and 12 from the upper reach. The DNA quality from one individual from the lower reach (# 28) was poor yielding spurious results, and this individual was not included in the analysis. Thus, 62 total fin tissues were included in the analysis; 50 samples from the lower reach and 12 from the upper reach.

Nuclear DNA

The analysis of species-specific, nuclear DNA markers revealed that all individuals at both sample locations were homozygous for the rainbow trout allele for all four markers. In other words, every individual contained a pair of rainbow trout genes at each locus. The attached file contains the genotype for each individual at each locus (RR refers to an individual homozygous for the rainbow trout allele, CC refers to an individual homozygous for the cutthroat trout allele, and RC refers to an individual heterozygous for the cutthroat and rainbow trout allele).

Hybrid Index:

The hybrid index score for each individual was 8, indicating all individuals were rainbow trout.

Percentage of introgressed cutthroat tour nuclear DNA alleles (P_{CT}) = (N_{CT}/2LN) x 100:

N_{CT} = 0 (the number of cutthroat trout alleles observed within a location)
L = 4 (the number of diagnostic loci)
N = 12 upper reach, 50 lower reach (the total number of individuals within a location)
P_{CT} upper reach = 0
P_{CT} lower reach = 0

Power analysis:

A power analysis was conducted to determine how effective the genetic analysis was for detecting a 1% genetic contribution of cutthroat trout within the rainbow trout sample set using four species-specific markers. For this analysis, the upper and lower reaches were considered as a single population.

$q = 0.01$
$N = 62$
$x = 4$
$\alpha = (1 - q)^{2Nx}$
$\alpha = (1 - 0.01)^{(2)(62)(4)} = 0.007$

The power for detecting a 1% contribution of cutthroat trout DNA within the rainbow trout population in this analysis was 99.3%.

Mitochondrial DNA

Mitochondrial DNA from 61 individuals was identified as rainbow trout. The mtDNA from one individual, # 44, could not be determined due to a low yield of purified DNA. The attached file contains the mtDNA lineage for each individual (R refers to rainbow trout mtDNA and C cutthroat trout mtDNA).

Conclusion

Genetic analysis of the trout community in the Sultan River between Culmback Dam and the City of Everett's Diversion Dam (the Bypass Reach) indicates that the population is composed exclusively of rainbow.

Materials and Methods

DNA extractions were performed using Qiagen spin columns. The genetic composition (nuclear DNA) for each individual was determined using four PCR primers (OM-55, OCC-16, OCC-34, and OCC-42) that amplify species-specific products differentiating rainbow and coastal cutthroat trout. Procedures for PCR amplification, electrophoresis, and product visualization followed the methods of Ostberg and Rodriguez (2002, 2004).

At each locus, each individual was scored as homozygous (RR for rainbow trout alleles or CC for cutthroat trout alleles) or heterozygous (RC). A hybrid index score describing the composite genotype for each individual was generated by summing the number of RBT nuclear alleles over all loci. For example, at each locus CC received a score of 0, RC received a score of 1, and RR received a score of 2. The hybrid index for each individual was then summed across all loci, four in this case. So, a cutthroat trout received a hybrid index score of 0, a rainbow trout received a hybrid index score of 8, and a first generation hybrid (F1) received a hybrid index score of 4.

The percentage of introgressed cutthroat trout nuclear alleles (P_{CT}) at each location was calculated using the equation $P_{CT} = (N_{CT}/2LN) \times 100$, where N_{CT} = the number of cutthroat trout alleles observed within a location, L = the number of diagnostic loci, and N = the total number of individuals within a location (USFWS 2003). The P_{CT} can be used as a baseline measurement within each location for future comparisons to determine if introgression has increased or decreased with time or management action.

A power analysis was conducted to determine the effectiveness of the genetic analysis. The equation $\alpha = (1 - q)^{2Nx}$ (Kanda et al. 2002) was used to determine the power for detecting 1% cutthroat trout DNA within the sample set; where, α is equal to 1 minus the probability of detection, q is the desired frequency of cutthroat trout DNA to detect (in this case 0.01), N is the number of fish sampled, and x is the number of species-specific markers applied in the analysis (in this case 4).

The maternal lineage of each individual was differentiated by amplifying the ND2 region of the mtDNA genome and digesting the PCR product with the restriction enzyme Csp6I (Ostberg et al. 2004, Ostberg and Rodriguez in 2006).

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Genetic results on the resident trout community within Sultan River between Culmback Dam and the City of Everett's Diversion Dam. For each nuclear DNA locus (OCC-16, OCC-36, OCC42, and OM-55), RR refers to the homozygous genotype for the rainbow trout allele. For the mtDNA marker (ND2), R refers to the rainbow trout mtDNA. For the species designation, RBT refers to rainbow trout

Sample #	OCC-16	OCC-36	OCC-42	OM-55	ND2	Hybrid Index	Species Designation
Lower Reach							
1	RR	RR	RR	RR	R	8	RBT
2	RR	RR	RR	RR	R	8	RBT
3	RR	RR	RR	RR	R	8	RBT
4	RR	RR	RR	RR	R	8	RBT
5	RR	RR	RR	RR	R	8	RBT
6	RR	RR	RR	RR	R	8	RBT
7	RR	RR	RR	RR	R	8	RBT
8	RR	RR	RR	RR	R	8	RBT
9	RR	RR	RR	RR	R	8	RBT
10	RR	RR	RR	RR	R	8	RBT
11	RR	RR	RR	RR	R	8	RBT
12	RR	RR	RR	RR	R	8	RBT
13	RR	RR	RR	RR	R	8	RBT
14	RR	RR	RR	RR	R	8	RBT
15	RR	RR	RR	RR	R	8	RBT
16	RR	RR	RR	RR	R	8	RBT
17	RR	RR	RR	RR	R	8	RBT
18	RR	RR	RR	RR	R	8	RBT
19	RR	RR	RR	RR	R	8	RBT
20	RR	RR	RR	RR	R	8	RBT
21	RR	RR	RR	RR	R	8	RBT
22	RR	RR	RR	RR	R	8	RBT
23	RR	RR	RR	RR	R	8	RBT
24	RR	RR	RR	RR	R	8	RBT
25	RR	RR	RR	RR	R	8	RBT
26	RR	RR	RR	RR	R	8	RBT
27	RR	RR	RR	RR	R	8	RBT
29	RR	RR	RR	RR	R	8	RBT
31	RR	RR	RR	RR	R	8	RBT
32	RR	RR	RR	RR	R	8	RBT
33	RR	RR	RR	RR	R	8	RBT
34	RR	RR	RR	RR	R	8	RBT
35	RR	RR	RR	RR	R	8	RBT
36	RR	RR	RR	RR	R	8	RBT
37	RR	RR	RR	RR	R	8	RBT
38	RR	RR	RR	RR	R	8	RBT
39	RR	RR	RR	RR	R	8	RBT
40	RR	RR	RR	RR	R	8	RBT
41	RR	RR	RR	RR	R	8	RBT
42	RR	RR	RR	RR	R	8	RBT
43	RR	RR	RR	RR	R	8	RBT
44	RR	RR	RR	RR	no result	8	RBT
45	RR	RR	RR	RR	R	8	RBT

Sample #	OCC-16	OCC-36	OCC-42	OM-55	ND2	Hybrid Index	Species Designation
46	RR	RR	RR	RR	R	8	RBT
47	RR	RR	RR	RR	R	8	RBT
48	RR	RR	RR	RR	R	8	RBT
49	RR	RR	RR	RR	R	8	RBT
50	RR	RR	RR	RR	R	8	RBT
51	RR	RR	RR	RR	R	8	RBT
52	RR	RR	RR	RR	R	8	RBT
Upper Reach							
53	RR	RR	RR	RR	R	8	RBT
54	RR	RR	RR	RR	R	8	RBT
55	RR	RR	RR	RR	R	8	RBT
56	RR	RR	RR	RR	R	8	RBT
57	RR	RR	RR	RR	R	8	RBT
58	RR	RR	RR	RR	R	8	RBT
59	RR	RR	RR	RR	R	8	RBT
60	RR	RR	RR	RR	R	8	RBT
61	RR	RR	RR	RR	R	8	RBT
62	RR	RR	RR	RR	R	8	RBT
63	RR	RR	RR	RR	R	8	RBT
64	RR	RR	RR	RR	R	8	RBT

APPENDIX D

Responses to Stakeholder Comments on Draft Report

	Tulalip Tribes – Review of Study Plan 2 – Letter Transmitted to District March 10, 2008	
1.	**Point 1. Figure 2.1** Culmback Dam is spelled incorrectly in the balloon identifying the location of the dam.	This has been corrected.
2.	**Point 2.** The Revised Study Plan for the cutthroat population analysis indicates that genetic “samples of pure rainbow trout, pure cutthroat trout, and potential hybrid cutthroat x rainbow trout will be collected throughout Bypass Reaches 1a and 1b”. However, the draft report indicates that genetic samples were collected from only two locations, one at the upstream and one at the downstream end of the bypass reach. The draft report fails to justify this departure from the originally-stated methods. Why were these genetic samples determined to be representative of the entire reach? The reason for the study plan to require genetic samples to be collected throughout the bypass reach is that cutthroat trout are often distributed in patches within a given watershed due to their relatively specific habitat requirements. As Latterell et al. 2003 point out, “Within individual river basins, trout distribution may further reflect variation in temperature (Roper et al. 1994), channel size (Hartman and Gill 1968; Platts 1979) and gradient (Bozek and Hubert 1992; Kruse et al. 1997), species interactions (Fausch et al. 1994), habitat patch size (Reiman and McIntyre 1995), and migratory behavior (Trotter 1989). Within streams and among reaches, dispersal barriers (Nelson et al. 1992; Kruse et al. 1997), catastrophic disturbances (e.g., debris torrents), and spatial variation in factors limiting persistence (e.g., interspecific competition, refugia, nutrient availability, prey abundance, or spawning and rearing habitat) may further regulate trout distribution.” In addition, it can be very difficult to identify the difference between small rainbow trout and cutthroat trout during a snorkel survey, which means that	The individual tissue samples were collected from 64 fish captured at two locations within the bypass reach: RM 10.7 and RM 14.3. These locations were easily accessible and conducive to electrofishing. Much of the bypass reach is difficult to access, not conducive to electrofishing, or both. Having a two man crew travel down the river to randomly sample representative habitats is not feasible. The District and consultants believe that these individual tissue samples accurately represent the fish population in the bypass reach and that further sampling and testing are not warranted. These samples were collected following a logical and rational approach. The consultants had no indication that 64 (100%) of the samples would prove to be rainbow trout with no indication of hybridization.

	visual identification of rainbow trout throughout the reach does not necessarily mean a remnant cutthroat population does not exist. Fortunately, a relatively simple remedy of this genetic sampling shortfall exists. Conducting a stratified random survey of representive habitat types throughout the bypass reach could be conducted by a two-man crew with a backpack electrofishing unit in a matter of a few days in Summer 2008. Samples could be worked up by the same genetic lab and would further justify or refute the conclusion that cutthroat trout are absent from the entire bypass reach.	
3.	**Point 3.** The Tulalip Tribes are very concerned about the results of this study that suggest that “coastal cutthroat trout are not currently present in the bypass reach of the Sultan River.” If this is true, we expected to see implications and recommendations sections of the report that would identify the management implications of a non-viable cutthroat trout population and recommend actions necessary to re-establish a cutthroat population in the bypass reach of the Sultan River. At a minimum, we would like to see a description of conditions necessary for re-establishment of a viable cutthroat population in this reach (e.g., flows, temperatures, habitat, etc.) and whether a pure genetic source of Sultan River cutthroat trout is available from another mainstem reach or a tributary reach to aid potential re-establishment.	The results of RSP 2 indicate that coastal cutthroat trout are not present in the bypass reach of the Sultan River. These results are consistent with the results of the bull trout presence/absence survey conducted in the bypass reach in 2004. The reasons for their absence are unclear, although in numerous studies where cutthroat and rainbow trout or steelhead occupied the same watersheds; cutthroat trout have been found primarily in the headwater tributaries, while steelhead and rainbow trout occupied the larger river reaches (Johnson et al. 1999). It is also known that the volume of water in sea-run cutthroat trout spawning streams seldom exceeds 10 cfs during the summer low flow period, with most averaging less than 5 cfs (Johnston 1982, Trotter 1991). Competitive interactions with rainbow trout, high bypass reach spawning flows (in excess of 10 cfs), the lack of suitable spawning tributaries, habitat fragmentation, and other factors may contribute to the lack of cutthroat in the bypass reach. The presence and persistence of a self sustaining rainbow trout population indicates that suitable and hospitable conditions do exist for this species. This trout population was first sampled in 1979 by the Washington Department of Game and deemed to be self-sustaining at that time. Twenty-eight years later (2007) this population continues to persist within the bypass reach.

	USDA Forest Service – Comments on SP2, SP15 and SP18 – Letter Dated March 14, 2008	
4.	In general, the USDA Forest Service concurs with the methodologies of the various studies except where noted below,	The District appreciates your support of the studies and your participation in Jackson Project relicensing.
5.	**Study Report for Study Plan #2: Bypass Reach Cutthroat Trout Population Analysis** The 2007 field surveys did not find coastal cutthroat trout, and the genetic analysis identified all tissue samples as rainbow trout. While no cutthroat were identified, the data was processed to estimate the abundance and size distribution of rainbow trout in the bypass reach, but did not conduct an applied population viability assessment. Rainbow trout are a Mt. Baker-Snoqualmie National Forest Management Indicator Species, and while this species does not have the same status as coastal cutthroat trout, the Forest Service is still interested in how Project operations and facilities might affect this population. As a result the Forest Service requests that a viability assessment be completed for the rainbow population, and if appropriate, recommend measures to protect the viability of the population.	The District believes that the best testament to the viability of the rainbow trout population in the bypass reach is its persistence over time. In 1982, the Washington Department of Game and the District compiled the results of several years of studies conducted prior to Stage II of the Project. This study is available on the District's relicensing website. Within that report, the results of sampling (electrofishing and snorkeling) in the bypass reach (1979 & 1980) indicated the presence of multiple size classes of trout. The conclusion, at that time, was that "it appears that the trout population in this section of the river is maintaining itself under present conditions". Subsequent sampling in 2004 and 2007 (24 to 28 years later) corroborates these findings. The District believes that these data indicate that measures are already in place to protect the viability of this population, and additional studies or viability assessments are not warranted.