Columbia River Water Management Program on Lower Crab Creek's Aquatic Ecosystem: Emphasis on Native Salmonids

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Executive Summary

The impetus for this project was the detection of steelhead trout in Red Rock Coulee in 2004 by the U.S. Fish and Wildlife Service and how proposed changes in water supply development activities could affect the Lower Crab Creek (LCC) aquatic ecosystem. We began sampling in LCC in July 2007 and collected data from the biotic and abiotic communities. Initial upstream trapping efforts were developed to capture steelhead migrating into LCC where we had thought they would congregate and spawn. However, the first year of upstream trapping proved to be unsuccessful at capturing steelhead. During 2008 and 2009, our upstream trapping efforts again failed to capture any migrating steelhead. Because adult steelhead are notorious at evading traps, we supplemented our steelhead sampling efforts with a downstream sampling 5-foot rotary screw trap located approximately 3 km upstream from the LCC confluence with the Columbia River. Three years of downstream trapping yielded only two native redband trout.

Interestingly, we captured many Chinook salmon in both our upstream and downstream traps along with spawning and carcass surveys that confirmed Chinook salmon production within the LCC system. Available information indicated Chinook salmon within the LCC system were simply strays from throughout the Columbia Basin and there was little initial interest in their presence. However, preliminary genetic analysis indicated a distinct population of Chinook salmon within LCC. Additional testing of samples collected during the second and third year verified that Chinook salmon within LCC were in fact a distinct genetic population.

Despite the degraded habitat within portions of LCC and water quality parameters that generally did not fit the model with respect to salmonid utilization, sensitive species such as Chinook salmon and native redband trout managed to persist. However, during times when water temperatures were high or considered lethal, these species were not present. One potential reason for these fish to utilize LCC, which was once thought to be unsuitable for a salmonid population, may be attributed to a genetic component not currently identified. WDFW geneticists have indicated they would like to look further at the genetics of LCC salmon to determine if there is an allele absent in other strains of Chinook salmon that make LCC salmon more suited for warm water inhabitation.

Recent findings warrant additional investigations of LCC, specifically regarding the salmon and trout. Furthermore, increased fishing pressure at the confluence of LCC where it flows into the Columbia River has grown exponentially over the past five years and the impacts to LCC bound salmon harvested from this locally concentrated fishery are undocumented. Along with recreational activities, future water management activities could also impact the salmonids of LCC. We would advise a monitoring and evaluation project continue in the area to reduce the likelihood of extirpation of these native fishes from LCC.

I. Introduction

Water quality within Lower Crab Creek has been identified as a concern by the Washington State Department of Ecology (DOE 2004). Additionally, a DOE water quality monitoring station has been set up on Lower Crab Creek near Beverly, WA (Station 41A070). Directly related to water quality are the fishes of LCC, and until this project, no comprehensive studies have been conducted on LCC. The impact to fish, particularly salmon species, associated with water use must be addressed as outlined in RCW 90.03.360 (Controlling works and measuring devices – Metering of diversions – Impact on fish stock), and RCW 90.22.060 (Instream flow evaluations – Statewide list of priorities – Salmon impact). Additionally, the first two objectives of the Northwest Power and Conservation Council (NPCC) Crab Creek Subbasin Plan fish management portion states the need to determine the origin of the Chinook salmon (*Oncorhynchus tshawytscha*) and steelhead (*O. mykiss*) found in the creek (KWA 2004), as prior to this project both species have been observed spawning in Lower Crab Creek and Red Rock Coulee (Bowen et al. 2003).

Because Upper Columbia Steelhead are listed as endangered (Meyers et al. 1998), it was important to collecting pertinent information regarding this species as they had been detected within the LCC system (Appendix 1). The Upper Columbia steelhead ESU was listed as endangered on August 18, 1997. The ESU possesses four known populations of steelhead, the Wenatchee, Entiat, Methow, and Okanogan (UCSRB 2007). Although steelhead have been observed in Crab Creek, this population was not included in the Upper Columbia ESU because of their uncertain origin. Steelhead in Crab Creek may originate from three possible sources. First, they may simply be resident rainbow trout of hatchery (*O. mykiss*) or redband (*O. mykiss gairdnerii*) origin that have begun to exhibit an anadromous life history strategy (*see* e.g. Pascual et al. 2001). Second, steelhead from one of the four recognized populations may stray into Crab Creek to reproduce by themselves or introgress with resident *O. mykiss*. Conversely, they may be native to Crab Creek and therefore should be included in the Upper Columbia ESU. Chinook salmon have also been observed spawning within Lower Crab Creek and Red Rock Coulee (Bowen et al. 2003). Like steelhead, the origin of these fish is not known (KWA 2004). However, the Chinook salmon observed in LCC have always been thought to be strays.

Lower Crab Creek may also be utilized by other sensitive species such as ESA listed bull trout (*Salvelinus confluentus*), white sturgeon (*Acipenser transmontanus*), and lamprey (*Lampetra* spp.), although water quality parameters could greatly limit the times that habitat is adequate. As well as native fishes, it was thought that Lower Crab Creek might support non-native predatory fishes such as smallmouth bass (*Micropterus dolomieu*) and walleye (*Sander vitreus*). Anecdotally, we frequently hear of anglers catching large fish within the Columbia River and literature suggests the presence of predators throughout the system (Beamesderfer and Rieman 1988; Zimmerman 1999). However, it is theorized that the production of non-native deleterious species does not occur within the Mainstem Columbia River. Instead, it is

hypothesized that they originate from up-river reservoirs and lakes through rivers and creeks such as Crab Creek. The entrainment of fishes including predators has been documented from Moses and Banks Lakes, and Crab Creek may be an avenue of non-native fish introductions into the Columbia River via the irrigation and natural waterways.

During the infancy of the LCC project, it became very apparent the habitat, environment, and dangerous nature of the area surrounding LCC would influence our sampling efforts and an adaptive management approach would have to be taken. We could not set our traps in the most optimal locations as the possibility of vandalism and unfavorable interactions with individuals could put property and staff at risk. Despite our best efforts to minimize gear lost, we still experienced a considerable amount of theft and vandalism of gear. Approximately six months prior to the start of the LCC project, there was a large illegal drug growing operation that was ceased by authorities; during the project we found a stolen SUV and two shootings occurred with 2 miles of our trap site. Consequently, personnel safety was paramount, which negated night work.

Despite the problems with sampling LCC, we designed and completed the proposed tasks and in doing so, identified a native population of Chinook salmon not previously described. As previously mentioned, we had anticipated contacting a population of steelhead but did not during our three years of sampling LCC. However, we did collect tissue samples from juvenile rainbow trout that were identified as native redband trout that varied from Upper Crab Creek populations. Due to the presence of sensitive native populations, future work or changes in water operations should be accompanied with an appropriate monitoring and evaluation program to ensure the fauna of LCC is not extirpated.

II. Methods

The four primary components used during the Lower Crab Creek Project are designated as fish sampling; tissue collections and genetic analysis; macroinvertebrate sampling; and water quality and habitat analysis.

Fish Sampling

Upstream Trapping

We used a resistance board weir trap similar to those used by the Alaskan Department of Fish and Game (Tobin 1994; Stewart 2002) to sample upstream migrating fish. Unlike the rocky substrate in Alaskan systems where these traps are primarily deployed, the substrate in LCC consisted of sand and areas of clay. Consequently, considerable modifications were made to our trap throughout the three years of deployment (2007, 2008, and 2009). The basic trap design deployed in LCC used a 7.62 cm on-center layout with 6.1 m long, 4.126 cm outside-diameter schedule 40

pickets and made up an individual panel 91.44 cm wide (Figure 1). In order to block the majority of LCC, we constructed and deployed 13 individual panels that totaled 11.9 m in width. Each panel was fastened to the adjacent panel using an additional 6.1 m schedule 40 tube and attached to the rail system that was in turn secured to the substrate. The trap was located approximately 5 km upstream from the confluence of LCC with the Columbia River.

With any trapping system, there are substantial variations with respect to the box that captures fish migrating into a system. We built a steel cage that was 2.44 m L x 1.22 m W x 1.52 m H to best accommodate the potentially large fish moving through the system. To reduce the likelihood of injury to fish while in the box we also coated it with a rubberized truck bed liner material. The entrance to the box was 100 cm tall x 47 cm wide, which then funneled inside the trap to 25 cm wide. In order to reduce the likelihood of fish entering and leaving the, trap we purchased plastic trigger entrances from Neptune Marine Products Inc. (neptunemarineproducts.com/) that would flex to allow fish to enter but not exit the trap. Beaver and muskrat activity in the area accounted for a significant amount of damage to the entrances, which required they be replaced on a regular basis.

Once the resistance board weir trap was put into place, staff checked, cleaned, and fixed the trap on a daily basis. Because water visibility was generally poor in LCC, visually obtaining fish trapped in the box was not possible and required staff to enter the box and pull a large net through the water to capture fish in the box (Figure 2). Once captured, salmon were identified, measured, scanned with a PIT tag scanner, tagged with a FLOY anchor tag, and a small fin clip was taken for genetic analysis. The original proposal stated we would sedate fish once captured but as there was a potential for fish to fall back into the Columbia River and potentially be harvested by anglers, we could not use any of the anesthetics currently available. Consequently, we minimized the fish handling time and kept fish in the water for as long as possible during processing. Once data collections were complete, the fish were released upstream of the resistance board weir trap.

After captured fish were worked up, staff cleaned the trap and repaired any holes that were located. Cleaning the trap consisted of removing all vegetation and debris stuck above and below the water line (Figure 3). Failure to clean the trap adequately resulted in a change in the hydraulic component associated with the trap and in turn lead to areas of extreme undermining or sinking portions of the floating weir. After cleaning, the entire barrier was slowly walked over to locate areas of undermining; any holes were immediately filled with sandbags. The process of checking, cleaning, and performing maintenance was repeated every day while the resistance board weir trap was deployed.

Figure 1. General resistance board weir trap design that was used during 2007, 2008, and 2009 on Lower Crab Creek. Floating panels, fencing, and fish box are visible in the photograph.

Figure 2. Staff inside box at Lower Crab Creek resistance board weir trap netting captured fish.

Figure 3. The daily task of cleaning debris from the resistance board weir trap in Lower Crab Creek often required staff to wear waders or a dry suit.

Downstream Trapping

Downstream screw trapping was conducted during spring in 2008, 2009, and 2010 in LCC 4 km from the confluence with the Columbia River. A highly modified E.G. solutions 1.52 m (5- foot) rotary screw trap was used to capture out-migrating fishes from LCC (Figure 4). A standard screw trap only has the ability to raise and lower the cone. We designed a frame system suspended with overhead supports that allowed us to raise both the cone and the livewell using aircraft cable and two winches (Figure 5). To maintain a lightweight yet strong system, we constructed the framing from 0.5 cm aluminum square tubing with flat aluminum cross-arms for reinforcement. In addition, we removed the cleaning drum and replaced it with compressed aluminum screening that permitted constant flow to pass through the entire back portion of the livewell. The entire trap was suspended from a highline 8' from the surface of the water and was tethered to the trap with a bridle. A 4-ton pulley was attached to the bridle from the trap and was able to roll freely along the highline in order to reduce wear associated with cable-to-cable contact. However, to prevent the pulley from rolling from shoreline to shoreline, we attached two cable clamps on each side of the pulley that effectively stopped the pulley from rolling great distances. The cable used for this project was 1.3 cm stainless steel aircraft cable with a breaking rating of 12,700 kg.

The first year of sampling was conducted as a pilot study in order to determine the feasibility of the sampling location and trap performance. During the 2009 and 2010 sampling seasons after the appropriate modifications were made to the trap, we sampled continuously from April to mid-July in 2009 and from March to mid-June in 2010. A typical day of sampling consisted of arriving at the trap site early in the

morning then wading onto the trap from the shore. Prior to netting fish from the livewell, we cleared debris from in front of the trap and from the livewell. The trap was then raised half the total distance from the water, which reduced the volume of water within the livewell and made it easier to net fish within the livewell. When fish were netted from the livewell, they were held in a bucket of water until they were worked up.

Once all the fish were removed from the livewell, the trap was raised completely out of the water to verify complete fish removal. Remaining fish were placed in holding buckets and the trap was thoroughly cleaned of debris, silt, and mud. Individual fish were removed from the holding buckets, identified, measured, fin-clipped (trout and salmon only), and released. Tissue samples collected via fin clips were used for genetic analysis.

We used two methods to expand data to estimate the total number of fish that passed the LCC screw trap point on a daily basis. During the 2009 sampling season, we conducted a simple volumetric expansion. In order to complete the volumetric expansion, we needed to determine the volume of water that was passing the trap and the volume of water captured through the trap.

Volume of water through trap= trap area (m^2) * (flow * time sampling)

Total volume (discharge) of water sampled through the screw trap

$$Q_t = A_t * (V * T)$$

Trap area = 0.910449 m^2

The total volume of water passing by trap was estimated using a channel cross-section methodology at a consistent transect at the trap site. Each transect was separated into equidistance sections where the depth, width, and water velocity at 40% depth were collected. The sum of the product of each of the sections yielded the total stream discharge.

$$Q_T = (W_1 * D_1 * V_1) + (W_2 * D_2 * V_3)\ldots$$

Where Q is the total discharge in m^3/s of water passing by the trap, W is the width, D is the depth, and V is the velocity (m/s) of each individual section. The proportion of the water that was sampled was calculated by dividing the volume of water that passed through the trap (Q_t) by the total volume of water of LCC (Q_T).

$$\text{Proportion of water sampled} = Q_t / Q_T$$

Assuming a linear relationship between the number of fish emigrating and the volume of water passing the trapping location, the expanded estimate of fish was calculated

by dividing the total number of fish captured by the mean proportion of water sampled.

During the 2010 sampling season, we calculated emigration using a trap efficiency approach following the protocol in Seiler et al. (2004) to estimate production. In order to complete this task, we marked a large number of captured fish with left ventral fin clips and re-released them upstream. To increase the number of fish marked and released in one event, we secured an inflatable boat behind the trap with a 300-gallon holding tank equipped with a battery/solar powered freshwater pump. Water temperatures dictated holding time, but fish were not held longer than 3 days prior to being released 1 km upstream from the trap. We calculated trap efficiency by:

$$E_i = R_i / M_i$$

Where E is the trap efficiency for a given time, R is the number of fish recaptured, and M_i is the number of fish marked. The expanded number of fish that passed the trap was calculated with the following formula:

$$N_i = C_i / e_i$$

Where N is the estimated number of fish passing the trap during a period of time, C is the number of unmarked fish captured at the trap during the given time period and e is the estimated trap efficiency.

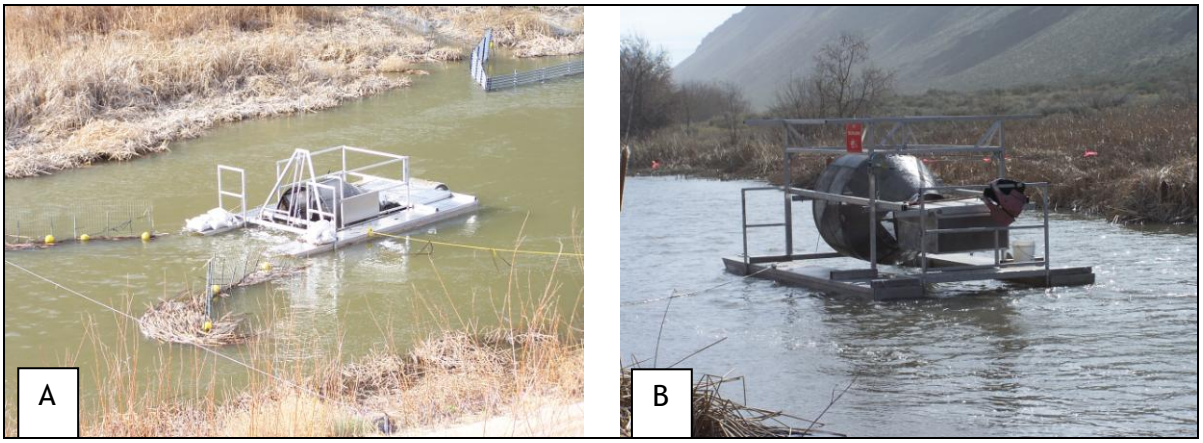

Figure 4. Downstream screw traps on Lower Crab Creek. (A) Stock rotary screw trap. (B) Modified rotary screw trap used during spring 2009.

Figure 5. Modified rotary screw trap suspended above Lower Crab Creek.

Spawning Surveys

Spawning surveys in LCC proved to be quite problematic because of high turbidity and subsequent poor visibility (Figure 6). High turbidity was an issue throughout the entirety of LCC except for the upper 4 km where LCC flowed through U.S. Fish and Wildlife Refuge land. The creek channel from the Red Rock Coulee Rd crossing (T16N R26E SEC 30) to the McManamon Rd crossing (T16N R28E SEC 9) is highly disturbed because of various agricultural practices and grazing. Furthermore, much of this land is privately owned, some by large hunting clubs, and we were advised by WDFW wildlife personnel that this area would be best to avoid. Consequently, we concentrated our efforts on the first 29 rkm of LCC along with the Red Rock Coulee irrigation ditch that entered at the top end of the our survey reach.

Spawning surveys were conducted during the fall and spring using canoes, motorized kayaks, walking the bank, and by retrieving carcasses that had washed onto our resistance board weir trap. Standard equipment used by survey crews included polarized glasses, tape measures, machetes, a DNA sampling kit (alcohol wipes, scissors, and pre-labeled vials), hemostats, scale envelopes, and knives. When conducting a survey, staff visually scanned the water for movement or disturbances that would suggest fish presence. If fish were spotted, a location was recorded using GPS as well as the number of fish present, and whether or not a redd was visible. When salmon carcasses were found, we recorded the total length, species identification, and GPS location. When possible we collected tissue samples and scales that were used for genetic analysis and aging, respectively. Carcasses that we encountered were often degraded, making them unfit for tissue samples and made

accurate scale collections difficult (Figure 7). Once data were collected from a carcass, the tail was cut off using a machete to ensure fish were not counted again during surveys that followed.

Figure 6. The water within Lower Crab Creek was often very turbid which was especially apparent at the confluence with the Columbia River.

Figure 7. Many of the carcasses we located in Lower Crab Creek or Red Rock Coulee had begun to decompose.

To supplement our sampling efforts we also utilized boat electrofishing and several slat traps in LCC. An 18' Smith-Root flat bottom electrofishing boat was used to sample a portion of LCC. To reduce the potential of injury to sensitive species, we only used direct current (DC) at low power (50-500 volts, 30 Hz) and no more than 50 percent of range. Settings were adjusted based on the behavior of fish. If fish displayed severe tetanus, we adjusted our settings to induce taxis and minimize tetanus. We used slat traps baited with cheese at several locations as a means to capture catfish. Catfish are generally very difficult to sample and require a tremendous amount of effort. Consequently, we thought our best chance to contact catfish within LCC was to use slat traps.

Tissue Collections and Genetic Analysis

To prevent cross contamination, WDFW staff followed a strict standard operating procedure (SOP). Tissue samples were collected using scissors or a scalpel. Tools were kept clean by first wiping the tools of any loose material or debris, sprayed with alcohol, and wiped again with an individual sterile wipe. Samples were placed into individually labeled Evergreen 5 ml vials containing a DNA preservation solution that contained anhydrous ethanol. Each vial's number corresponded with the biological data that was used for future reference. Samples and relevant biological data were sent to the WDFW's genetics lab at the end of each sampling season for analysis. Tissue samples of either fin-clips or scales were used for genetics testing. Methods for genetic analysis are summarized below from Small et al. (2011). The complete methods including references are available in Appendix 10.

Tissues were genotyped at the 13 standardized loci for Chinook salmon (GAPS loci). Genomic DNA was extracted from tissue using silica membrane kits (Macherey-Nagel). Microsatellite alleles were amplified by polymerase chain reaction (PCR) using fluorescently labeled primers. The PCRs were conducted in 5-µL volumes employing 1 µL of template with final concentrations of 1.5 mM $MgCl_2$, 200 µM of each deoxynucleotide triphosphate, and 1 x Promega PCR buffer and followed a "touch-down" protocol. After an initial 2 min of denaturing at 94°C, there were three cycles of 94°C for 30 s, annealing at 60°C (temperature stepped down 1°C each cycle) for 30 x, and extension at 72°C for 60 s. These were followed by 36 or 39 cycles of 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 60 s, then a final 10-min extension at 72°C. Microsatellites were detected using an ABI 3730 automated DNA analyzer, and alleles were sized (to base pairs) and binned using an internal lane size standard (GS500Liz from Applied Biosystems) and GeneMapper software (Applied Biosystems). We employed genotypic data from the GAPS database for 11 proximal Chinook salmon collections in our genetic comparisons. All collections were from populations that have summer or fall adult return timing and were from the hatcheries and tributaries that are geographically closest to LCC. We used these collections to test the origin of the LCC Chinook salmon population.

We tested loci for selective neutrality with an F_{ST} outlier test implemented in ARLEQUIN 3.5.1.2. We calculated basic genetic statistics to assess whether the LCC Chinook salmon samples were collected from a single, randomly mating population and to compare our collections with other Chinook salmon collections from the interior Columbia River basin. We used FSTAT 2.9.3 to conduct statistical tests on data per locus and across all loci for conformation to Hardy-Weinberg expectations of genotypic equilibrium (HWE), and expressed by F^{IS} values. Tests for HWE evaluate significant deviations from expected heterozygosity within populations, which may indicate that individuals are from different populations or family groups, that there are large-sized or null alleles that failed to amplify in the PCR, or that there is nonrandom mating in a population (such as breeding among related individuals). We assessed linkage disequilibrium (nonrandom genotypic associations between all possible pairs of loci) using GENETIX. Linkage disequilibrium may arise if the collection included family groups or migrants from another population or there is nonrandom mating or drift due to small population size. We calculated diversity measures, including allelic richness (the average number of alleles corrected for sample size) and gene diversity (expected heterozygosity corrected for sample size) using FSTAT. Genetic diversity provides an indication of the genetic biodiversity in a population and, in general, small and isolated populations are less diverse.

We used self-assignment tests in GeneClass2 to calculate the likelihood that the Chinook salmon collected in LCC originated in a spawning population located in the creek or were strays that originated in other populations. In this analysis, the baseline included Chinook salmon collections from proximal tributaries and hatcheries and the LCC collection. We also conducted a first-generation migrant analysis to determine whether the LCC Chinook salmon collection included recent descendants of migrants from other populations.

Macroinvertebrate Sampling

Macro invertebrates were sampled at seven locations throughout LCC with 14-plate Hester Dendy colonizing samplers. Each 3-inch plate was spaced equally with four 0.065 cm nylon washers. Each sampler had an area of 1,277.42 square centimeters. Hester Dendy samplers were hidden to prevent tampering by members of the public.

1. At trap site below trap. 11T 282412 m E, 5190369.61 m N
2. At trap site above trap. 11T 282412 m E, 5190369.61 m N
3. Washout Bridge. 11T 287097 m E, 5190316 m N
4. Water Quality Site 3. Located at the bridge where Road E SW crosses LCC. 11T 301622 m E 5191050 m N.
5. Water Quality Site 4. Actually in Red Rock Coulee creek where Road E SW crosses creek. 11T 301960 m E 5192268 m N.
6. Water Quality Site 7. Located on Road B SE 400 m north of Water Quality Site 8. 11T 312791 m E 5188008 m N.

7. Water Quality Site 8. Located on Road B SE south of Water Quality Site 7. 11T 312807 m E 5188367 m N

Hester Dendy samplers were collected monthly and the number and species of macroinvertebrates colonizing each was analyzed based on a unit of time (days). During each collection period, staff traveled to the prescribed sites and exchanged a Hester Dendy sampler with a new one. Collected Hester Dendy samplers were immediately placed into containers and transported back to the lab to be cleaned. When time was not available to process Hester Dendy samplers immediately, the containers were stored in a refrigerator and cleaned the following day. Samplers were cleaned under running water into a 163µ sieve and contents preserved in 200 proof reagent alcohol to be analyzed during the winter months. We identified invertebrates to the lowest practical taxon using Pennak (1989) and Merritt and Cummins (1996), and obtained wet weights from taxonomic groups. We calculated species composition by number and weight as well as spatial and temporal analysis relative to species composition.

Water Quality and Habitat Analysis

Water quality and water chemistry data were collected at static locations. In addition, HOBO© Water Temperature Pro loggers were placed at several locations to collect water temperatures continually every hour. Loggers were placed at the following locations:

- Resistance Board Weir and Screw Trap Site. 11T 282412 m E, 5190369.61 m N
- Washout Bridge. 11T 287097.75 m E, 5190316.31 m N
- Water Quality Site 3. 11T 301613.52 m E, 5191056.93 m N
- Water Quality Site 4. 11T 301957.46 m E, 5192306.02 m N
- B Road, North Branch of LCC. 11T 312808.13 m E, 518368.05 m N
- B Road, South Branch of LCC. 11T 312792.51 m E, 5188008.01 m N

Loggers were placed in a manner that reduced the likelihood of them becoming lost during a potential flood event or tampered with by people. Loggers were downloaded in the field using a HOBO© waterproof shuttle, which permitted the logger to remain in place and continue collecting temperature data. Once data from the loggers were transferred to the shuttle, the shuttle was downloaded, and data were stored and converted to MS Excel files.

Seven sites were selected within LCC and its tributaries and were sampled once every two weeks (Table 1) using a YSI 6600 V2 Multiparameter Water Quality Sonde (YSI). The YSI consisted of a YSI 6600 V2 Multiparameter Water Quality Sonde that contained all the necessary probes for data collections, handheld YSI computer, and cord.

Table 1. Static water quality sites in Lower Crab Creek.

Water Quality (WQ) Site	Location Description.	Coordinate
1	Located at the confluence of LCC with the Columbia River Hwy 243 Bridge	11T, 277222.08 m E, 5188788.39 m N
2	First LCC Road Bridge approximately 4 miles east of Beverly	11T, 285232.35 m E 5190280.60 m N
3	Located at the bridge where Road E SW crosses LCC	11T, 301622.26 m E 5191050.97 m N
4	Actually in Red Rock Coulee creek where Road E SW crosses creek	11T, 301960.41 m E 5192268.94 m N
5	Hwy 26 bridge crossing LCC	11T, 319494.78 m E 5187743.77 m N
6	W. McManamon Rd Bridge crossing over LCC	11T, 324501.67 m E 5196148.58 m N
7*	Located on Road B SE 400 m north of WQ Site 8	11T, 312807.71 m E 5188367.52 m N
8*	Located on Road B SE south of WQ Site 7	11T, 312791.32 m E 5188008.16 m N

*Sites 7 and 8 were located between sites 5 and 6 and were added after the initial site selections.

Standard water quality parameters collected included temperature, dissolved oxygen (D.O.; mg/L), specific conductance (s/cm), turbidity (NTU), pH, and chlorophyll (µg/L) (Appendix 2). As part of the quality control conditions, the YSI was calibrated following a SOP prior to collecting field data and bench marked immediately upon returning to the office (Appendix 3). As all of our sites were located on road bridges and exposure to traffic was substantial. To reduce the likelihood of vehicle pedestrian incidents, staff wore highly visible orange vests at all times. Prior to recording any data, a 40-second acclimation period with the sonde submerged in the water was necessary. Readings were collected between 0.1 – 0.3 m and data recorded on a data sheet as well as logged into the computer. This process was completed at each site.

Water samples were also collected once a month and sent to Yakima Valley Labs to be tested for total phosphorous (TP) and turbidity. To negate any influences from the Columbia River, we selected water quality Site 2 for our low-end location. The uppermost site selected was water quality Site 6.

Water samples were collected from two locations in LCC using a Van Dorn bottle. Before and after each use the Van Dorn bottle was rinsed with distilled water. In addition, dark bottles were prepared at the lab with the appropriate labels and cleaned with sulfuric acid to prevent contamination from previous samples. From each of the two sites we filled 2-150 ml bottles for turbidity and total phosphorous

analysis and placed samples immediately in a cooler of ice. Once at the lab the samples were placed in a fridge and the following day transported to Yakima Valley Labs for analysis. Yakima Valley Labs are an accredited lab that follows strict SOP's for water and chemical analyses (Appendices 4-6).

In 2006, BioAnalysts Inc. conducted a habitat survey for the U.S. Fish and Wildlife service and the Columbia National Wildlife Refuge on LCC that flowed through refuge land (USFWS 2006). The area they surveyed began at the Hwy 26 crossing upstream to the base of Potholes Reservoir. The area we surveyed during spring 2008 began at the confluence of LCC and extended 29 rkm upstream to E SW road crossing. The portion of LCC that was not surveyed by us or BioAnalysts Inc. is a 17 km reach between the E SW road river crossing upstream and the Hwy 26 road crossing. This portion of LCC is agricultural and the majority of the creek has been converted to highly constrained canals (Figure 8). Sinuosity was measured by dividing 1000 m linear from point to point by the river distance. A ratio close to 1.0 indicated little sinuosity, whereas, ratios less than 1.0 indicated increased deviation from a linear channel.

For comparison purposes, we used methodologies similar to those used by Bioanalysts Inc. (Hawkins et al. 1993; Appendix 7). The portion of LCC we surveyed was divided into 29 1-km reaches and within each reach; a 100-m site was randomly selected for habitat evaluations. Consequently, we surveyed 10% of the habitat in first 29 km of LCC. Access and navigability of LCC was difficult and a canoe was used to access our sites. Originally, we had thought that we could access the creek by paralleling it but vegetation along the riparian zone was frequently too thick and deep to move through. Furthermore, the safety of our field crews would have been reduced, as encounters with ticks and rattlesnakes would have been increased had we hiked in to our selected sites.

Figure 8. Aerial photo of a portion of Lower Crab Creek between E SW road and Hwy 26 where agricultural practices have degraded the natural state of the creek to one of irrigation canals and ditches.

III. Results

Fish Sampling

During the 2007 sampling season, we deployed our trap in a manner identical to other systems where resistance board weir traps have been used (Figure 9). The trap was installed over a three-day period and was fishing by October 1, 2007. Within 24 hours of placing the fish box in LCC, a hole ~4 m across and 2 m deep formed because of bed load movement. Adjustments were made to the trap site with respect to armoring the area where the box was placed, but no modifications proved successful. The entire trap was removed and reconfigured with the box placed on the river right on an armored sandbag apron to prevent continued undermining. Despite the reconfiguration, we still experienced substantial bed load movement along the rail system that created the weir and directed fish towards the box. Along with substrate issues, the trap was also repeatedly damaged by beavers in the area. To rectify this, we hired a professional trapper to remove the animals. Although this issue was tremendously diminished, we still experienced trap damage attributed to several species of Rodentia.

As a result of the catastrophic failure experienced during the fall 2007 trapping efforts, we elected to fish the trap through the winter in order to capture steelhead entering LCC as well as work on perfecting our methodologies. Maintenance on this

trap was continuous, and during this season, we were never completely satisfied with our construction. Furthermore, the winter of 2007-2008 was severely cold and the trap was frozen in place (Figure 10). Despite up to 5 in of ice, the trap experienced minimal damages and after the necessary repairs were completed, we continued to fish until May 6, 2008. During the spring sampling, we captured 110 common carp (*Cyprinus carpio*), a mountain whitefish (*Prosopium williamsoni*), and a yellow perch (*Perca flavescens*).

Figure 9. Resistance board weir trap placement in Lower Crab Creek during the fall 2007 sample period.

Figure 10. During the winter of 2007-2008, the resistance board weir trap was frozen in place in Lower Crab Creek.

Prior to the fall 2008 sampling season, additional modifications were made to the resistance board weir trap, which included armoring the substrate with sandbags and building PVC fences that bordered the weir. Even with these modifications, we still experienced an extensive amount of undermining that required constant repairs. The trap was deployed on August 29, 2008 and fished until November 26, 2008; the first Chinook salmon was captured on September 14 (Figure 11). During the fall trapping efforts, 43 fish were captured migrating upstream (Table 2), the majority of which were Chinook salmon. Two Chinook salmon (7%) originally captured fell back over the trap and were captured a second time.

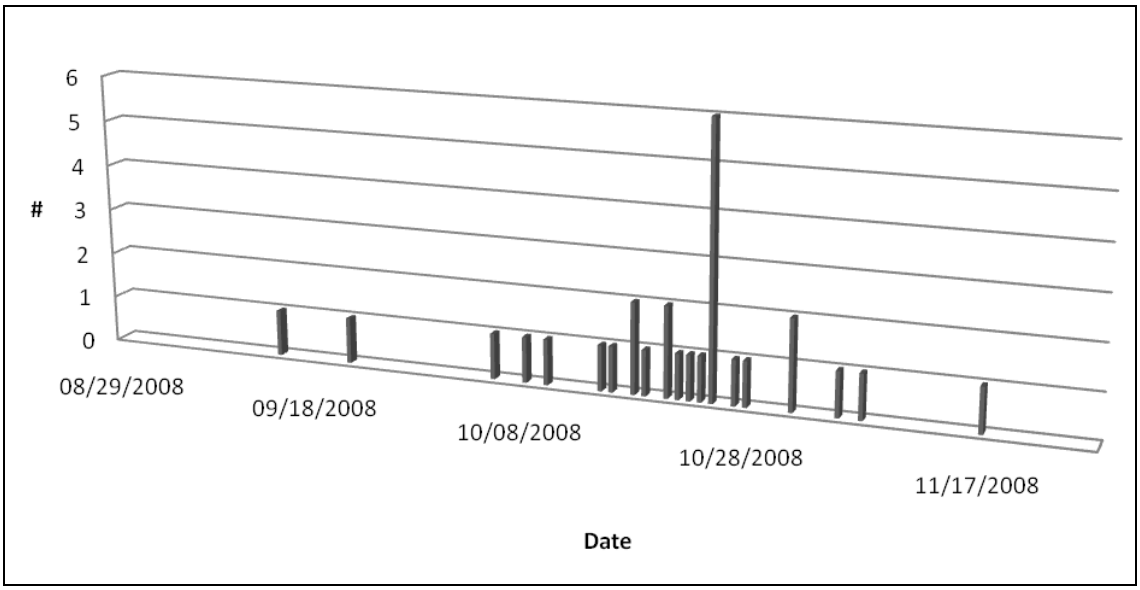

Figure 11. Run timing of adult Chinook salmon captured in the resistance board weir trap in Lower Crab Creek during the fall 2008 sampling season.

Table 2. Number and species composition of fish captured in the resistance board weir trap from August 29, 2008 through November 26, 2008 in Lower Crab Creek.

Species[1]	Number	%
Common Carp	10	23.3
Chinook Salmon	31	72.1
Coho Salmon	1	2.3
Sucker species	1	2.3
Total	**43**	

[1]Scientific names for fish are listed in Appendix 8.

During the fall 2009 sampling season, the resistance board weir trap was deployed from August 26 until November 18. The level of success was greatly increased from 2008 and we captured 74 fish, of which 69 were salmon (Table 3). Run timing was very similar to 2008 with the majority of Chinook salmon entering LCC during November (Figure 12).

Table 3. Number and species composition of fish captured in the resistance board weir trap from August 26, 2009 through November 18, 2009 in Lower Crab Creek.

Species[1]	Number	%
Chinook Salmon	53	71.6
Coho Salmon	16	21.6
Mountain Whitefish	1	1.4
Whitefish Species	4	5.4
Total	**74**	

[1]Scientific names for fish are listed in Appendix 8.

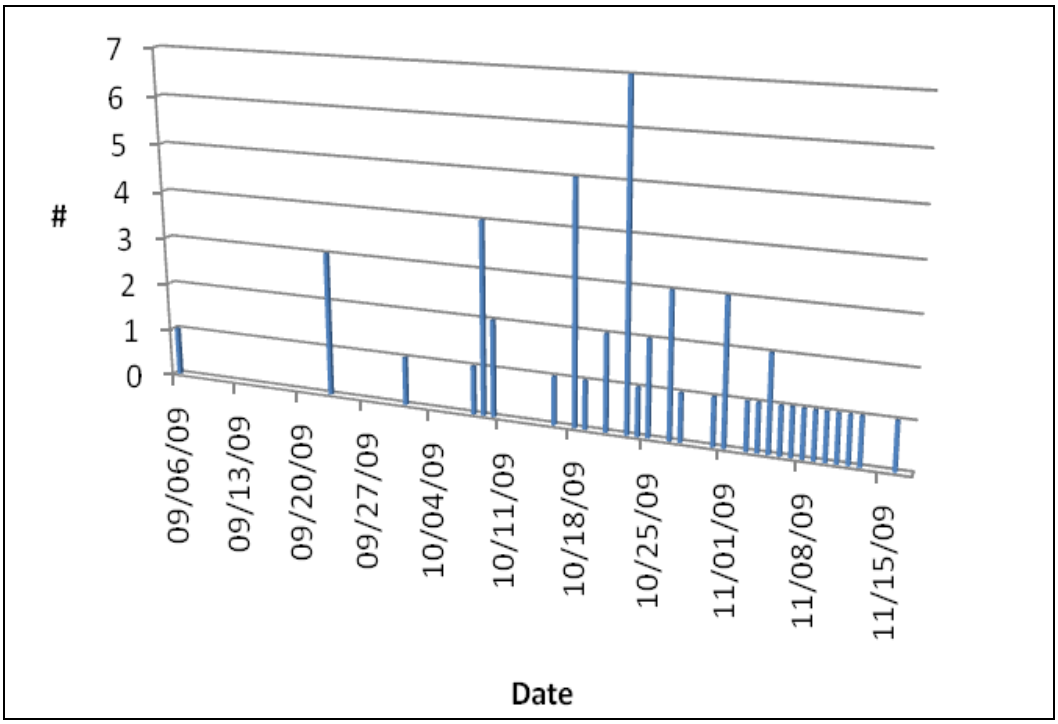

Figure 12. Run timing of adult Chinook salmon captured in the resistance board weir trap in Lower Crab Creek during the fall 2009 sampling season.

In 2008, we deployed the downstream screw trap for 53 days between March 28 and June 10 during which time 19 species were collected (Table 4). During the 2009 and 2010 season, we fished the screw trap 58 and 74 days, respectively. Due to environmental conditions, continuous sampling was not always permitted. Chinook salmon were the most abundant species captured in our trap during the 2008 and 2010 sampling seasons (Table 4). During the 2009 sampling period, largemouth bass (*M. salmoides*) represented the largest percentage of fish captured in our trap (Table 5). In addition, several species of sucker were caught during the 2009 season whereas they were absent from our trap during the 2008 and 2010 seasons. Chinook salmon emigration timing was relatively similar for all year's sampled (Figure 13). During the

2010 season, timing was bimodal with a noticeable spike in mid-April but with the majority of movement occurring during May similar to the 2008 and 09 seasons (Figure 13). During the three years of downstream trapping, we captured 6,120 Chinook salmon. Pooling the data for 2008, 2009, and 2010 indicated a substantial range in the sizes of salmon captured within a given month (Table 6).

Table 4. Number of fishes captured in the Lower Crab Creek downstream screw trap in 2008, 2009, and 2010.

	Year			
Species[1]	**2008**	**2009**	**2010**	**Sample years combined**
Black Crappie	3	5	35	43
Bluegill	8	6	19	33
Bullhead spp.	20	70	16	106
Bridgelip Sucker	1			1
Burbot			1	1
Channel Catfish	7	1	6	14
Chinook Salmon	329	592	3071	3992
Chiselmouth		4	1	5
Coho Salmon	2	4	16	22
Common Carp		8	2	10
Largemouth Bass	1	681	4	686
Largescale Sucker		1		1
Minnow spp.		2		2
Mosquitofish	6	4	8	18
Northern Pikeminnow	71	89	31	191
Rainbow Trout	1	1		2
Peamouth	15	11	23	49
Pumpkinseed		3	3	6
Redside Shiner	1	12	5	18
Salmonid spp.		8	5	13
Sculpin spp.		12	9	21
Sucker spp.		171		171
Smallmouth Bass	8	62	50	120
Sunfish spp.	3	1		4
Threespine Stickleback	30	46	123	199
Unknown spp.	13	13	9	35
White Crappie			1	1
Whitefish spp.	19	36	106	161
Yellow Perch	2	131	25	158
Days Sampled	**53**	**58**	**74**	
Fish per day	**10.2**	**34.0**	**48.2**	

[1]Scientific names for fish are listed in Appendix 8.

Table 5. Species composition (% by number) of fishes captured in the Lower Crab Creek downstream screw trap in 2008, 2009, and 2010. Highest values are highlighted.

	Year		
Species[1]	**2008**	**2009**	**2010**
Black Crappie	0.56	0.25	0.98
Bluegill	1.48	0.30	0.53
Bullhead spp.	3.70	3.55	0.45
Bridgelip Sucker	0.19	0.00	0.00
Burbot	0.00	0.00	0.03
Channel Catfish	1.30	0.05	0.17
Chinook Salmon	60.93	29.99	86.05
Chiselmouth	0.00	0.20	0.03
Coho Salmon	0.37	0.20	0.45
Common Carp	0.00	0.41	0.06
Largemouth Bass	0.19	34.50	0.11
Largescale Sucker	0.00	0.05	0.00
Minnow spp.	0.00	0.10	0.00
Mosquitofish	1.11	0.20	0.22
Northern Pikeminnow	13.15	4.51	0.87
Rainbow Trout	0.19	0.05	0.00
Peamouth	2.78	0.56	0.64
Pumpkinseed	0.00	0.15	0.08
Redside Shiner	0.19	0.61	0.14
Salmonid spp.	0.00	0.41	0.14
Sculpin spp.	0.00	0.61	0.25
Sucker spp.	0.00	8.66	0.00
Smallmouth Bass	1.48	3.14	1.40
Sunfish spp.	0.56	0.05	0.00
Threespine Stickleback	5.56	2.33	3.45
Unknown spp.	2.41	0.66	0.25
White Crappie	0.00	0.00	0.03
Whitefish spp.	3.52	1.82	2.97
Yellow Perch	0.37	6.64	0.70

[1]Scientific names for fish are listed in Appendix 8.

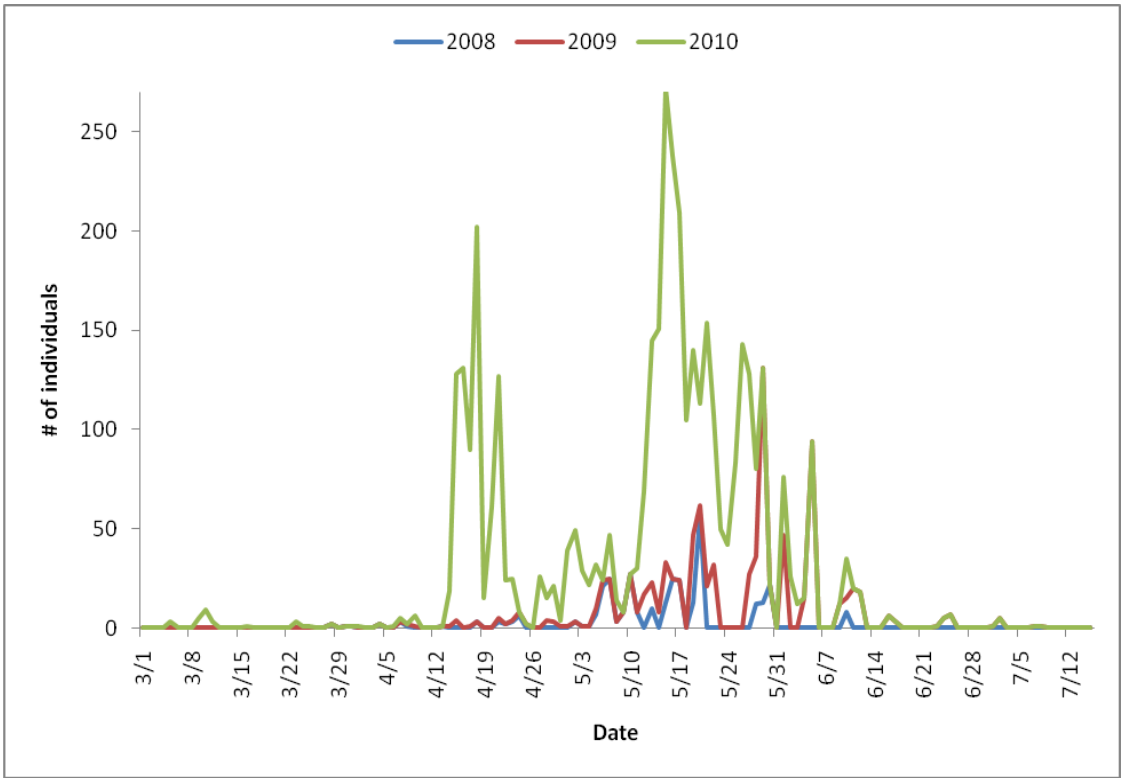

Figure 13. Chinook salmon out-migration timing from Lower Crab Creek during 2008, 2009, and 2010.

Table 6. Size ranges of Chinook salmon captured in the Lower Crab Creek downstream screw trap pooled over three years (2008-2010).

	Total Length (mm)		
Month	**Min L**	**Mean L**	**Max L**
March	35	40	71
April	27	54	139
May	29	79	196
June	49	79	112
July	65	83	103

Using a volumetric method we estimated the screw trap was capturing 10.28% of the water moving through LCC from April 10 – July 15, 2009 in conjunction with LCC discharge estimated using cross section depth and velocity measurements (Figure 14). During this period we captured 1,973 fish and estimated that 19,187 fish passed by the trap. In 2010, we still captured all fish emigrating from LCC but shifted a portion of our focus on Chinook salmon emigrating from LCC. During three separate mark and recapture events, 2,627 Chinook salmon juveniles were handled and of those, 292 fish were marked. Of the marked fish, 26 were recaptured in the screw trap, which

sampled at an efficiency of 8.9%. From April 16 through May 27 2010, we estimated 26,236 Chinook salmon passed by the screw trap site.

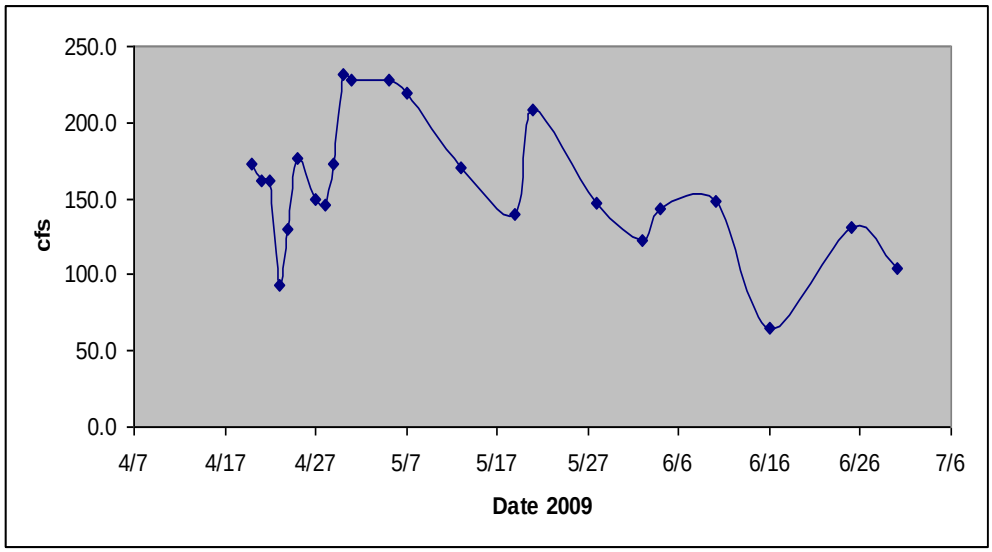

Figure 14. Total discharge (cfs) of water passing by the downstream screw trap in Lower Crab Creek.

Chinook salmon spawning and carcass surveys in 2007 proved to be difficult within LCC due to poor visibility associated with highly turbid water. Consequently, we did not observe any spawning activity within LCC. In addition walking the bank of LCC to visually acquire fish activity was not practical as the riparian zone was impassable due to dense vegetation including invasive purple loosestrife (*Lythrum salicaria*) and Russian olive (*Elaeagnus angustifola*) (Figure 15). Consequently, we focused our efforts on Red Rock Coulee (R26E T16N SEC 25), a tributary to LCC, where we counted 76 Chinook salmon actively spawning or in the vicinity of a redd during November 2007 (Figure 16). Of the 76 Chinook salmon we observed within Red Rock Coulee, we collected data from 37 individuals and of those, we sampled 19 for DNA analysis. We also collected tissue samples from two Chinook salmon carcasses that floated onto our resistance board weir trap. Ideally, we would have liked to collect data from every fish we observed but we did not want to disturb fish that were on the spawning grounds. Consequently, we waited until fish died prior to handling them, which was problematic in its own right because dead fish were not always readily visible, and when they were found their decomposition status often omitted them from DNA tissue sample considerations. The 39 salmon we handled ranged in size from 489 mm to 1,095 mm with a mean of 768.9 mm and 95% confidence intervals (CI) of 49.9.

Figure 15. The riparian zone of Lower Crab Creek was very dense and often impassable because of vegetation growth.

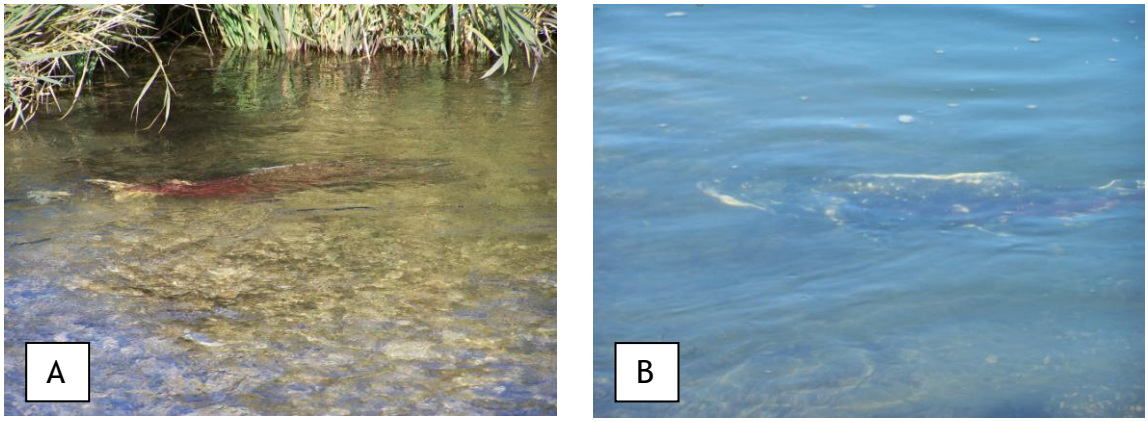

Figure 16. Fall Chinook salmon in Red Rock Coulee, a tributary to Lower Crab Creek. (A) Female Chinook salmon on redd. (B) Male Chinook salmon holding in deeper pool.

In 2008, Chinook salmon spawning survey efforts were repeated in both LCC and Red Rock Coulee. Spawning surveys were conducted from rkm 29 to the confluence on multiple occasions. We used a 15-foot flat-bottomed canoe to conduct surveys in LCC but again, because of the high turbidity of LCC, locating fish and redds proved quite difficult. However, one Chinook salmon was spotted at rkm 16 (11T 0290701, 5190305) and two active spawning areas were located below our trap site in the only available spawning habitat (11T 0280704, 5190043). Fish and activity at these locations were observed but not handled to minimize any possible anthropogenic impacts. The greatest concentration of redds and spawning activity we observed was

again in Red Rock Coulee, the majority of which occurred in the upper 1 km section from the downstream culvert at Water Quality Site 4 to the upstream culvert under Red Rock Coulee Rd. In this reach, we identified 15 spawning sites with multiple redds (Appendix 9) and observed 70 adult salmon and 2 jacks.

Carcasses were collected from Red Rock Coulee during spawning surveys and from the resistance board weir trap in LCC as the trap occasionally collected the carcasses of salmon that had previously passed the trap. Twenty carcasses were sampled and the pertinent data and samples collected. The mean length of Chinook salmon sampled was 720 mm, whereas, the maximum and minimum lengths were 1,070 mm and 465 mm, respectively.

As in previous years, water clarity in 2009 LCC hampered spawning and carcass survey efforts. Consequently, surveys during the 2009 season focused on Red Rock Coulee to maximize data returns. The mean size of Chinook salmon was slightly longer than in the two previous years at 798 mm (Table 7). However, the largest Chinook salmon measured was smaller at 980 mm and the minimum length Chinook salmon was 540 mm. Of the 44 carcasses, we located only four had tags indicating we had sampled them previously in our resistance board weir trap. We continued to record locations of spawning and redd sightings using GPS (Appendix 9) and located areas of both single redds and cluster areas where multiple redds were built. Seven individual redds and seven clusters totaling 24 redds were observed and locations recorded.

Table 7. Descriptive statistics for the three years of Chinook salmon carcass sampling on Lower Crab Creek.

Length (mm)	2007	2008	2009
Mean	768.9	719.9	798.0
Standard Error	24.6	27.2	14.5
Median	768.5	707.0	800.0
Standard Deviation	147.5	121.7	96.4
Sample Variance	21,750.5	14,801.8	9,296.9
Range	606.0	605.0	440.0
Minimum	489.0	465.0	540.0
Maximum	1,095.0	1,070.0	980.0
Count	36.0	20.0	44.0
95% Confidence Interval	49.9	56.9	29.3

Even though we did not capture any steelhead in our upstream resistance board weir trap during the three years of sampling LCC, we still conducted steelhead spring spawning ground surveys. As well as dedicated spawning surveys, we also performed frequent spot checks in areas where a steelhead had been reported in the past. These surveys were generally conducted concurrently with other tasks such as water quality monitoring and temperature logger deployment. During our spring spawning

survey efforts, we located what appeared to be a spawning sized salmonid in Red Rock Coulee. However, we were not able to capture this fish, nor did we locate any areas that appeared to be disturbed because of spawning activity. On March 12, 2008 we observed two fish on what appeared to be a redd. However, this particular redd was a Chinook salmon redd from fall 2007 as indicated by one of our ribbons tied in the riparian zone and the fish on this redd were identified as common carp (Figure 17). We continued to perform steelhead redd surveys as late as July 2, 2008 and we were only able to find colored up common carp on old salmon redds and/or hydraulic anomalies within the Red Rock Coulee (Figure 17). Our downstream screw trapping, which only captured one *O. mykiss* during our spring 2008 efforts, further corroborated the lack of *O. mykiss* production this year within the LCC drainage. Therefore, analysis to determine anadromy, was not practical with such a young fish (<60 mm).

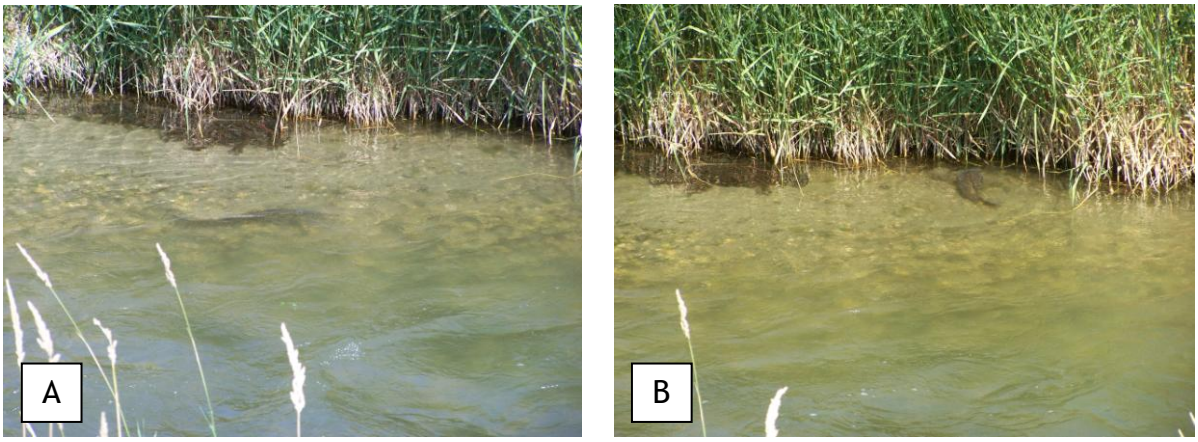

Figure 17. Steelhead spawning surveys. (A) During our spring steelhead surveys we frequently observed colored-up fish on old salmon redds or hydraulic anomalies. (B) After continued observations, we ascertained that the fish in question was not a steelhead but a common carp.

Supplemental fish collection efforts, which included boat electrofishing and slat trapping was very difficult within LCC for a number of reasons. Due to the morphology of LCC, there was only one location identified where we could operate our electrofishing boat safely and efficiently. In addition, we would have rather conducted our surveys during the night hours, but due to gang violence and drug activity in the area, this was not feasible. During our project tenure in LCC, there were two shootings, one of which involved two WDFW agents and a felon. Despite the obvious logistical and safety issues, we were able to boat electrofish and found suckers and carp to be the most abundant species (Table 8).

Table 8. Number and species composition (%) of fish captured via electrofishing in Lower Crab Creek.

Species[1]	Number	%
Bluegill	3	2.5
Bullhead	2	1.7
Chinook Salmon	1	0.8
Carp	36	30.5
Grass Carp	1	0.8
Longnose Sucker	3	2.5
Largescale Sucker	53	44.9
Northern Pikeminnow	1	0.8
Smallmouth Bass	11	9.3
Whitefish	5	4.2
Yellow Perch	2	1.7

[1]Scientific names for fish are listed in Appendix 8.

Slat trapping within LCC proved to be the most effective tool to capture channel catfish albeit with limited success. We captured five channel catfish, the largest being 464 mm. However, within a matter of one month of sampling, all of our traps had been stolen and it was apparent the cost to benefit ratio was not worth the effort and money.

Tissue Collections and Genetic Analysis

The impetus for collecting genetics samples initially was to determine the origin of steelhead within LCC. However, we did not encounter any adult steelhead. Instead we used genetic analyses to examine the hypotheses that Chinook salmon are (1) random strays, (2) a population founded since the 1950s (by wild or hatchery strays), or (3) an established native population. Tissue samples that were collected were sent to the WDFW genetics lab in Olympia Washington for analysis. The text below was summarized from the paper accepted for publication in the peer-reviewed journal Transactions of the American Fisheries Society (Small et al. 2011) The complete results including references are available in Appendix 10.

Allelic richness and gene diversity differed among the Columbia River basin Chinook salmon collections. Allelic richness in LCC was below average, but the difference was not significant (paired *t*-test; $P = 0.317$). Lower allelic richness can be a signal of a past genetic bottleneck (since populations tend to lose rare or low-frequency alleles during bottlenecks) or a signal of sampling error (if the sample was nonrandom). Gene diversity varied less than allelic richness among Columbia River basin collections and was average in LCC.

Pairwise tests confirmed that the 2008 and 2009 Chinook salmon collections from LCC were from the same population. The genetic variance between temporal samples was not significantly different from zero, and the *P*-values for the genotypic tests indicated no significant differences. The LCC Chinook salmon collection was significantly different from all other Columbia River basin Chinook salmon collections but shared significantly greater genetic variance with the collections from the Hanford Reach, Umatilla Hatchery, and lower Yakima River (average pairwise F_{ST} = 0.0087 versus 0.015 for the upper Columbia River; Student's *t*-test, *P* = 0.001 for comparison) versus the collections from the Snake River, Lyons Ferry, and Marion Drain (average pairwise F_{ST} = 0.0125; Student's *t*-test, *P* = 0.024 for comparison). The temporal stability and differentiation from other Columbia River Chinook salmon collections supported the identity of the LCC collection as a discrete spawning aggregate rather than a collection of strays from other populations. Average pairwise F_{ST} values support LCC Chinook salmon membership in the Hanford-Yakima major population group.

The LCC population of Chinook salmon was found to be significantly different from interior Columbia River basin hatchery and wild populations and to have alleles that are absent from proximal populations. Lower Crab Creek Chinook salmon showed no evidence of recent founding and had genetic diversity, allelic richness, and effective population size similar to those of other Columbia River basin Chinook salmon. The data suggest that a genetically distinct, native population of Chinook salmon inhabits LCC that has adapted to the rigorous environment.

Macroinvertebrate Sampling

Countless samplers were either vandalized or stolen during the tenure of the project. The three most abundant macroinvertebrates that colonized the Hester Dendy samplers within LCC were Diptera, Trichoptera, and Ephemeroptera (Table 9). Site 3, located approximately 12 rkm upstream, from the confluence consistently recolonized more macroinvertebrates on average than the six other sites. Trichoptera, Diptera, and Gastropoda represented the largest biomass from all sites and times combined within LCC (Table 10). Site 3 exhibited the most variation for both numbers and biomass of macroinvertebrates between sample times (Figure 18, 19).

Table 9. Taxa composition (% by number) of macroinvertebrates sampled in Lower Crab Creek.

Taxa	% by Number
Diptera	38.13
Trichoptera	27.47
Plecoptera	0.45
Ephemeroptera	21.11
Odonata	0.36
Coleoptera	2.49
Hemiptera	0.01
Arachnids	0.45
Bivalvia	1.43
Gastropoda	2.77
Amphipoda	0.10
Annelida	3.75
Turbellaria	0.69
Hydra spp.	0.15
Daphnia spp.	0.01
Copepoda	0.08
Nematoda	0.52
Lepidoptera	0.01
Collembola	0.01
Nematomorpha	0.01
Ostracoda	0.01

Table 10. Taxa composition (% by weight) of macroinvertebrates sampled in Lower Crab Creek.

Taxa	% by Weight
Diptera	8.84
Trichoptera	63.43
Plecoptera	1.61
Ephemeroptera	5.47
Odonata	2.39
Coleoptera	0.49
Hemiptera	0.00
Arachnids	0.01
Bivalvia	5.25
Gastropoda	8.74
Amphipoda	0.05
Annelida	3.09
Turbellaria	0.41
Hydra spp.	0.00
Daphnia spp.	0.00
Copepoda	0.00
Nematoda	0.19
Lepidoptera	0.02
Collembola	0.00
Nematomorpha	0.01
Ostracoda	0.00

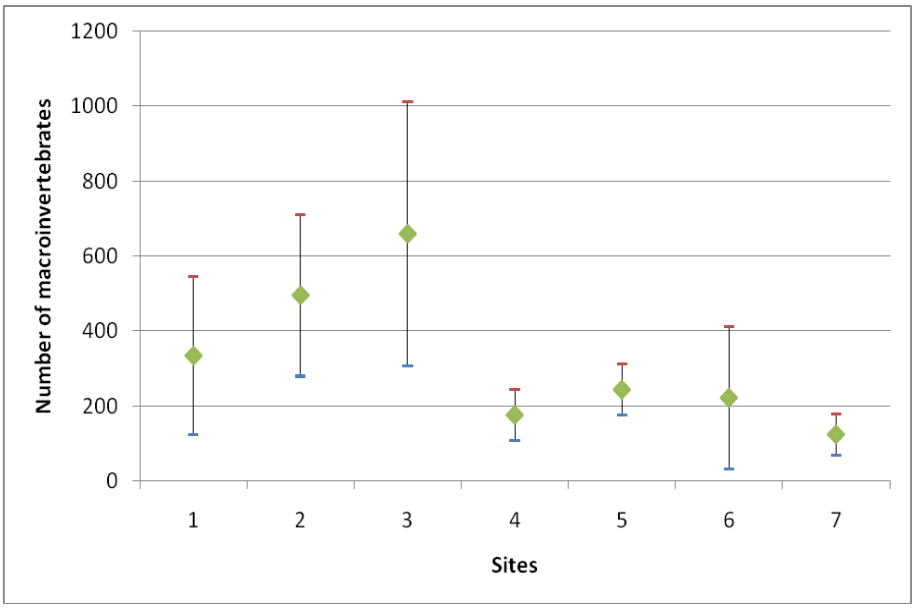

Figure 18. Mean number and 95% confidence intervals of macroinverebrates that colonized Hester Dendy samplers in Lower Crab Creek between visits.

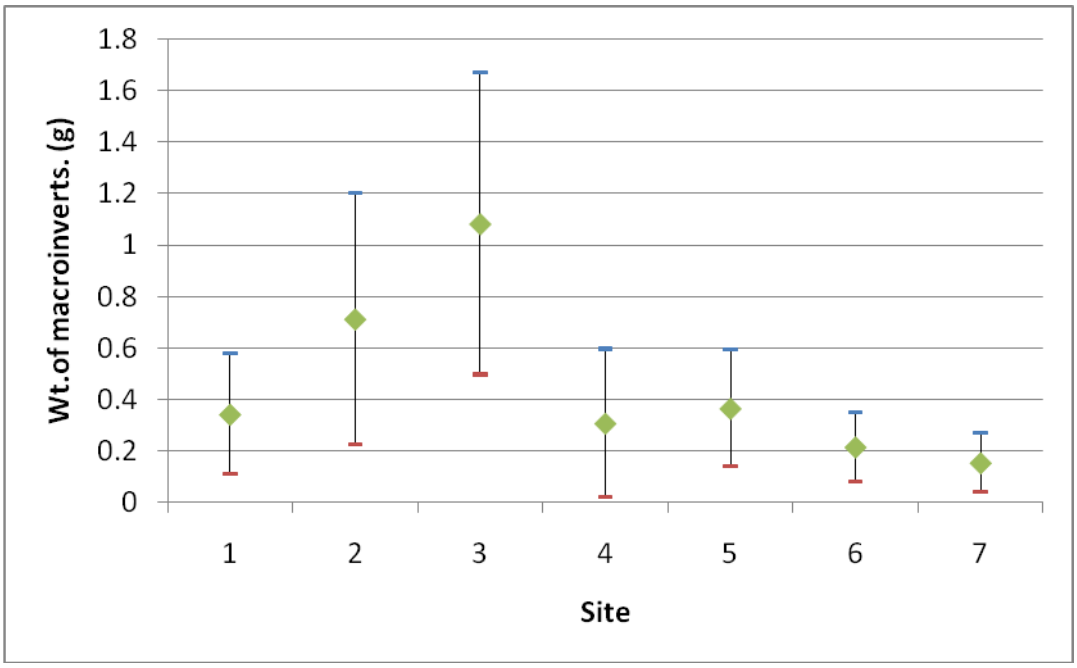

Figure 19. Mean and 95% confidence intervals of macroinvertebrate weights from Hester Dendy samplers in Lower Crab Creek throughout the project.

Water Quality and Habitat Analysis

Temperature

Temperature loggers collected data continuously from the November 2007 through April 2010 (Table 11). Pooled monthly data indicated variations between locations throughout LCC (Figure 20). Despite our best efforts to hide loggers from presumed vandalism, two temperatures loggers were stolen from Washout Bridge and B road North Branch. Consequently, gaps in our data developed, which precludes analysis that incorporates the entire sampling period due to bias in the data between locations (Figures 21-26). However, we performed an ANOVA on data collected from the consistent period of sampling (November 2007 - November 2008) and found there was a significant difference between sites ($F = 89.89$, $P < 0.0001$). A multiple comparison post-hoc Tukey test of unequal samples indicated a significant difference between all locations except sites 1 and 2 (Table 12).

Table 11. Monthly means and standard deviations of temperature data collected from HOBO© Water Temperature Pro loggers placed in six locations throughout the Lower Crab Creek study area from November 2007 through April 2010.

	At trap site		Washout bridge		WQ Site 3		WQ Site 4		LCC RD B north		LCC RD B south	
	4 rkm		12 rkm		29 rkm		30 rkm*		41 rkm north		41 rkm South	
Month	**Mean**	**St. Dev**	**Mean**	**St. Dev**	**Mean**	**St. Dev**	**Mean**	**St. Dev**	**Mean**	**St. Dev**	**Mean**	**St. Dev**
Jan	2.24	1.70	2.47	1.59	2.54	1.33	3.85	1.21	3.03	1.49	1.95	1.41
Feb	5.30	2.00	5.41	2.02	5.51	1.96	5.81	1.65	4.57	1.41	4.66	2.09
Mar	8.40	1.73	8.33	2.33	8.51	1.56	8.61	1.90	7.71	1.68	7.73	2.01
Apr	11.73	2.53	11.80	2.52	11.85	2.29	11.59	2.16	11.85	2.04	11.37	2.83
May	16.99	3.31	16.57	3.33	16.20	3.13	16.13	2.91	16.02	2.66	16.07	3.23
Jun	20.13	2.99	20.29	3.01	19.38	2.48	19.29	2.38	19.00	2.89	19.88	2.71
Jul	23.53	2.32	22.93	3.12	22.03	2.10	22.18	2.04	22.91	1.83	22.74	2.38
Aug	21.77	2.39	21.32	2.50	20.98	1.92	21.41	2.02	21.41	1.83	20.92	2.30
Sep	17.83	2.12	17.29	1.84	17.60	1.82	18.42	1.77	18.17	1.51	17.17	2.15
Oct	11.27	2.26	11.16	2.27	11.92	1.96	12.69	1.89	13.68	2.63	11.07	2.37
Nov	6.64	2.14	6.76	2.16	6.90	2.32	7.88	1.71	8.77	3.01	6.41	2.21
Dec	1.99	2.02	2.29	1.94	2.73	1.53	3.73	1.35	4.66	2.89	1.94	1.96

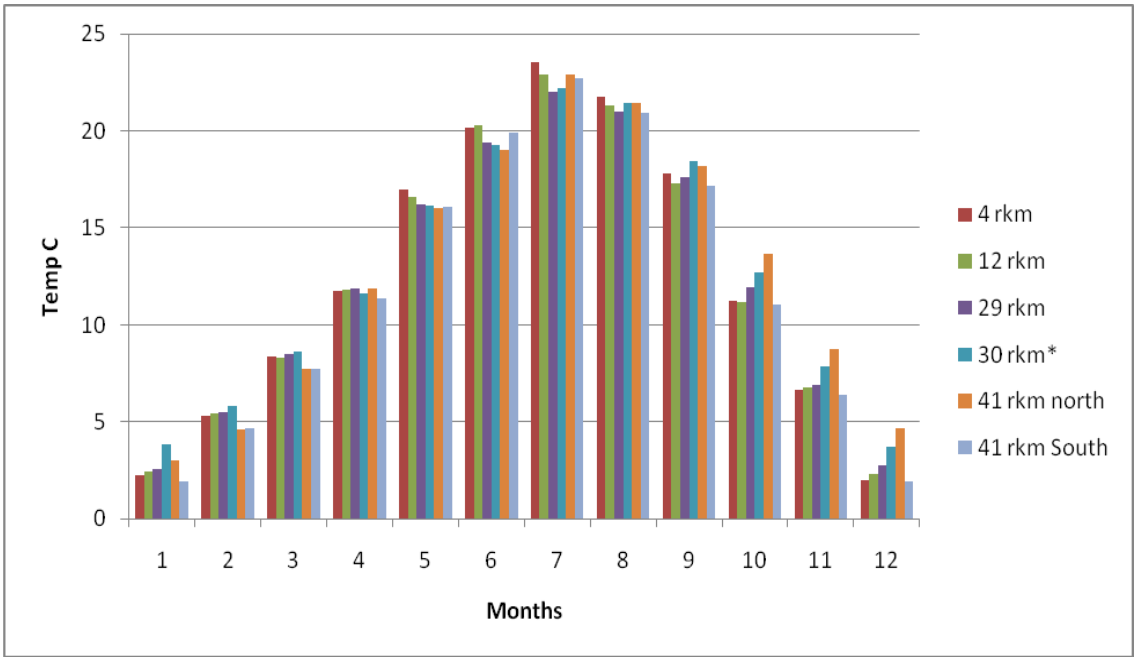

Figure 20. Pooled month and year temperature data collected from November 2007 through April 2010 from each of the six locations in Lower Crab Creek.

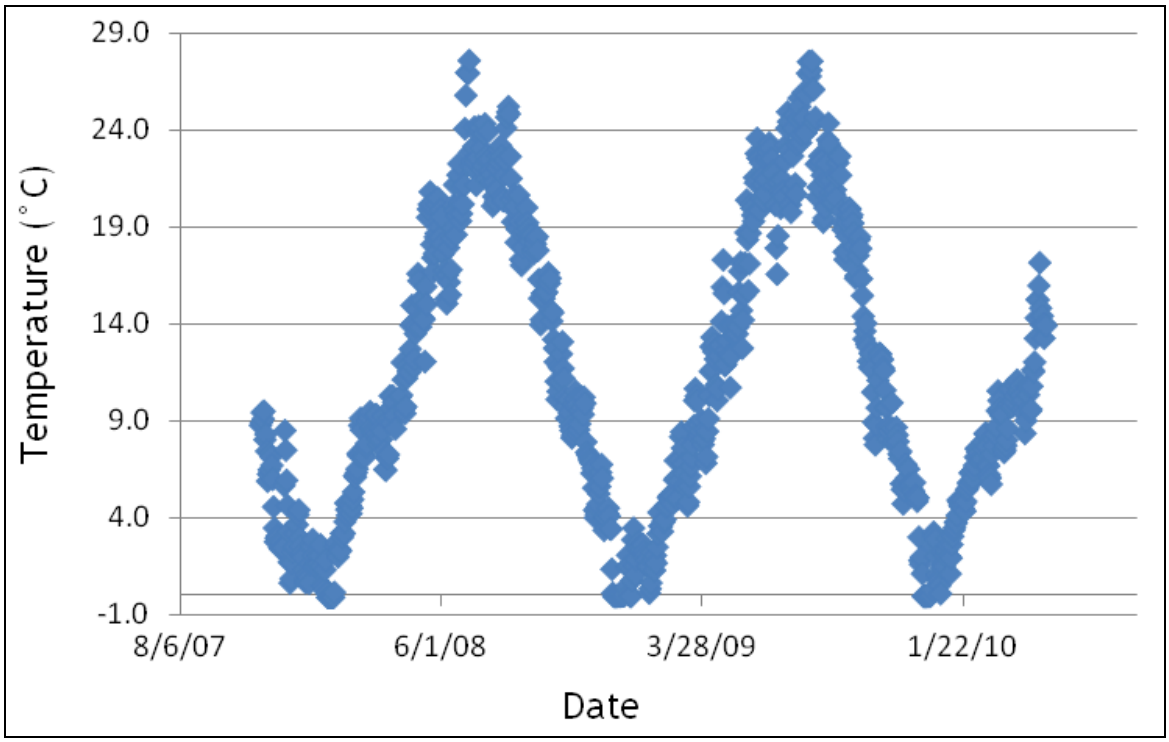

Figure 21. Results of fixed temperature monitoring from the temperature logger located at our trap site on Lower Crab Creek.

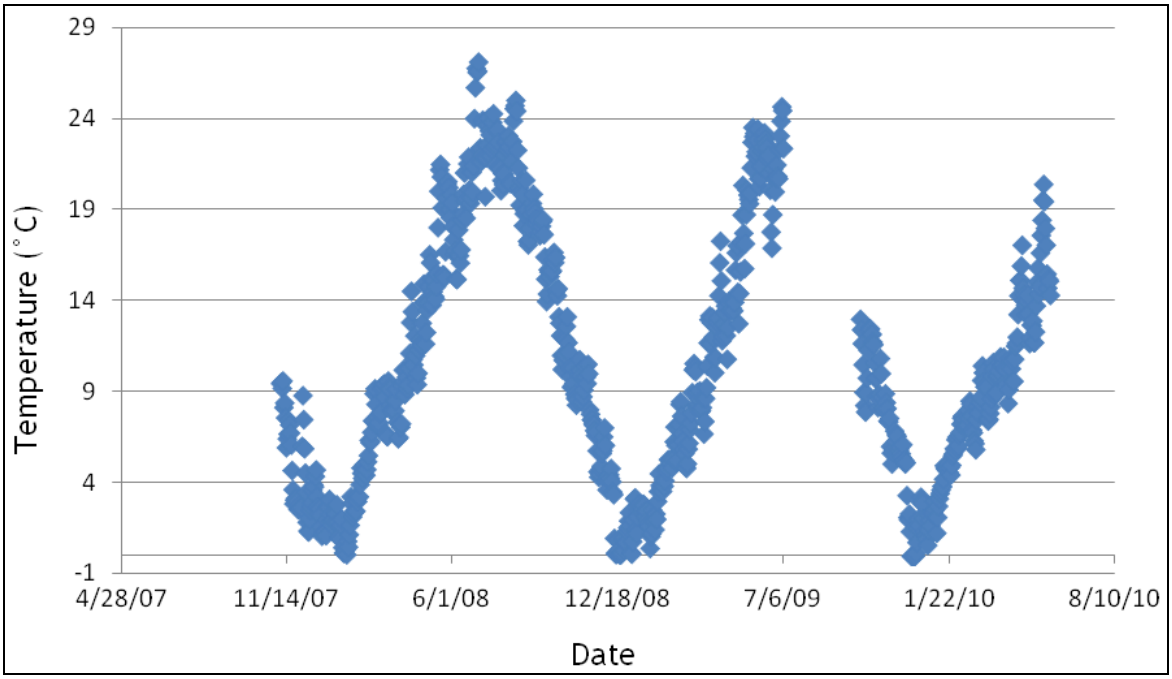

Figure 22. Results of fixed temperature monitoring from the temperature logger located at Wash Out Bridge at rkm 9 above Water Quality Site 2 on Lower Crab Creek.

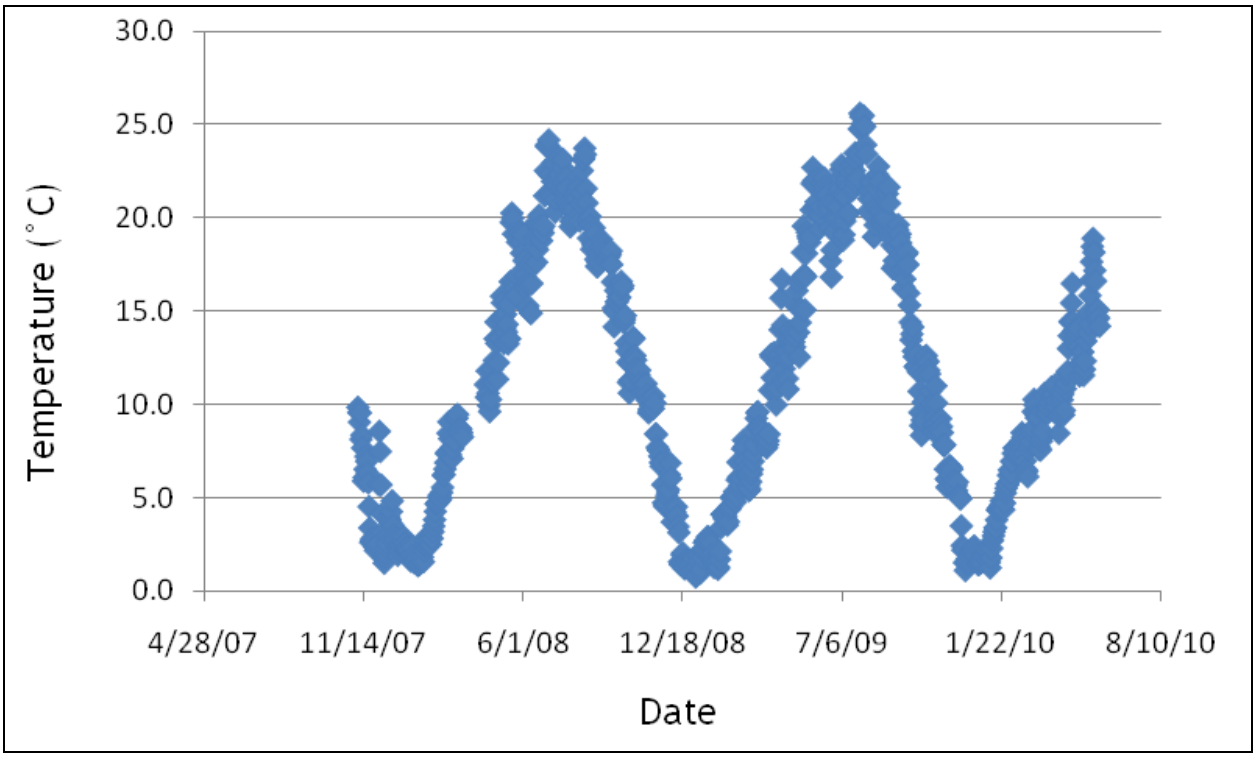

Figure 23. Results of fixed temperature monitoring from the temperature logger located at Water Quality Site 4 approximately 29 rkm from the confluence of Lower Crab Creek with the Columbia River.

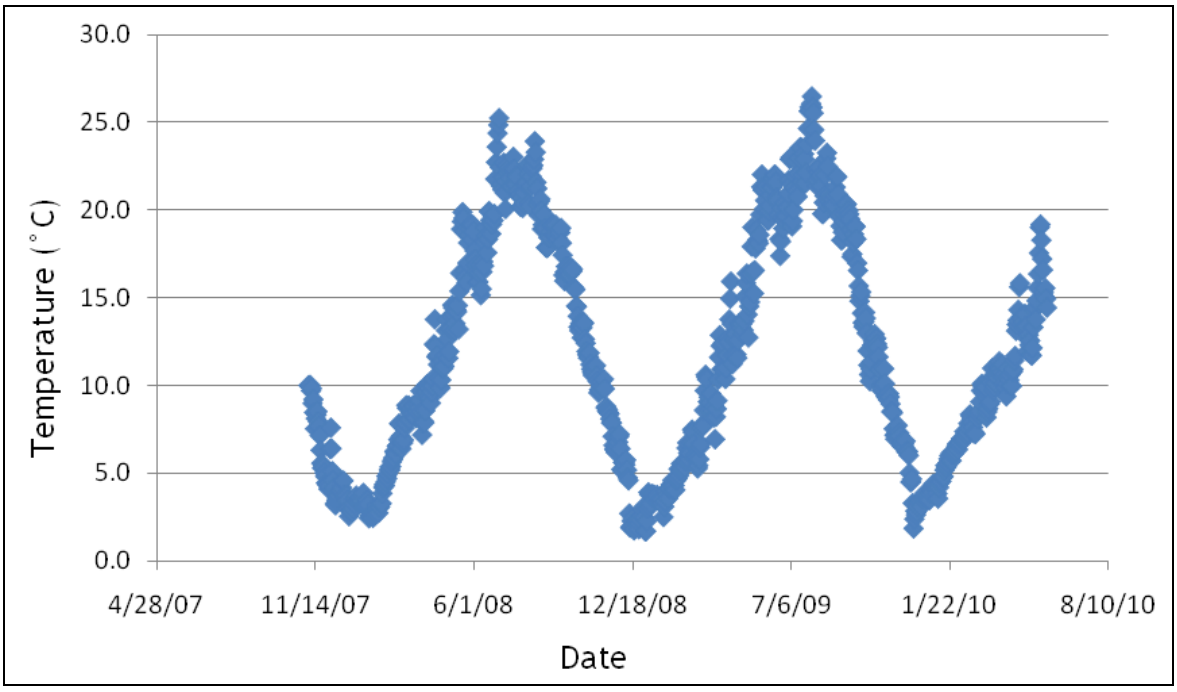

Figure 24. Results of fixed temperature monitoring from the temperature logger located in Red Rock Coulee, a tributary to Lower Crab Creek.

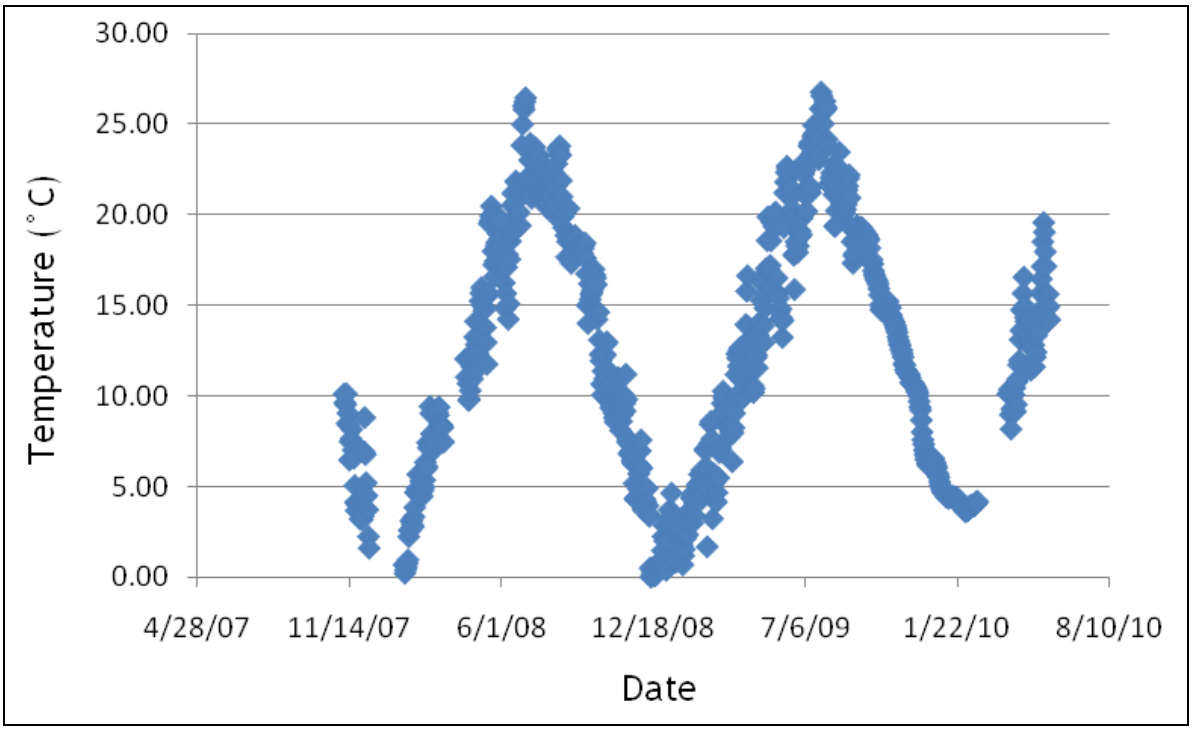

Figure 25. Results of fixed temperature monitoring in Lower Crab Creek from the temperature logger located at B road north crossing.

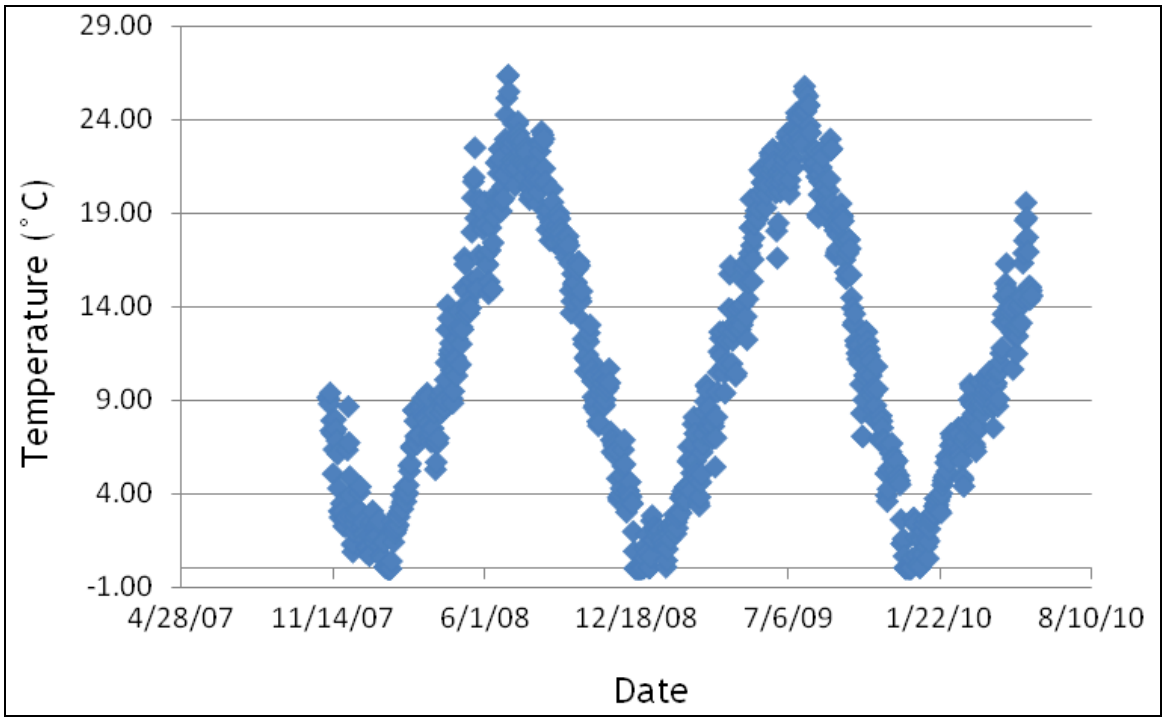

Figure 26. Results of fixed temperature monitoring in Lower Crab Creek from the temperature logger located at B road South crossing.

Table 12. Results of post-hoc Tukey test conducted on ANOVA results comparing data between temperature logger sites in Lower Crab Creek.

Site versus Site	SE	q Calc.	Significance
5v6	0.0816	27.53	yes
5v1	0.0819	23.49	yes
5v2	0.0816	22.86	yes
5v3	0.0823	18.67	yes
5v4	0.0808	17.48	yes
4v6	0.0759	10.97	yes
4v1	0.0763	6.70	yes
4v2	0.0760	5.97	yes
4v3	0.0768	5.91	yes
3v1	0.0779	4.96	yes
3v2	0.0776	4.24	yes
3v6	0.0776	9.13	yes
2v1	0.0771	0.74	no
2v6	0.0768	4.94	yes
1v6	0.0771	4.18	yes

$q_{0.05, \infty, 5}$; critical value 3.858

Although temperature data were collected with a mobile unit during our site visits, these data are relatively insignificant compared to the data collected from our temperature data loggers (Figures 21-26). A temperature logger was not placed at Site 6 and temperature data were only collected during bi-weekly site visits. Comparisons of temperatures between the two sites separated by the longest distance (Site 1 at the confluence and Site 6 just below the start of LCC) did not indicate a significant difference (t = 1.018, P = 0.31, df = 133). However, the temperatures from Site 6 were generally warmer than temperatures from sites located downstream including Site 1 located at the confluence with the Columbia River (Figure 27).

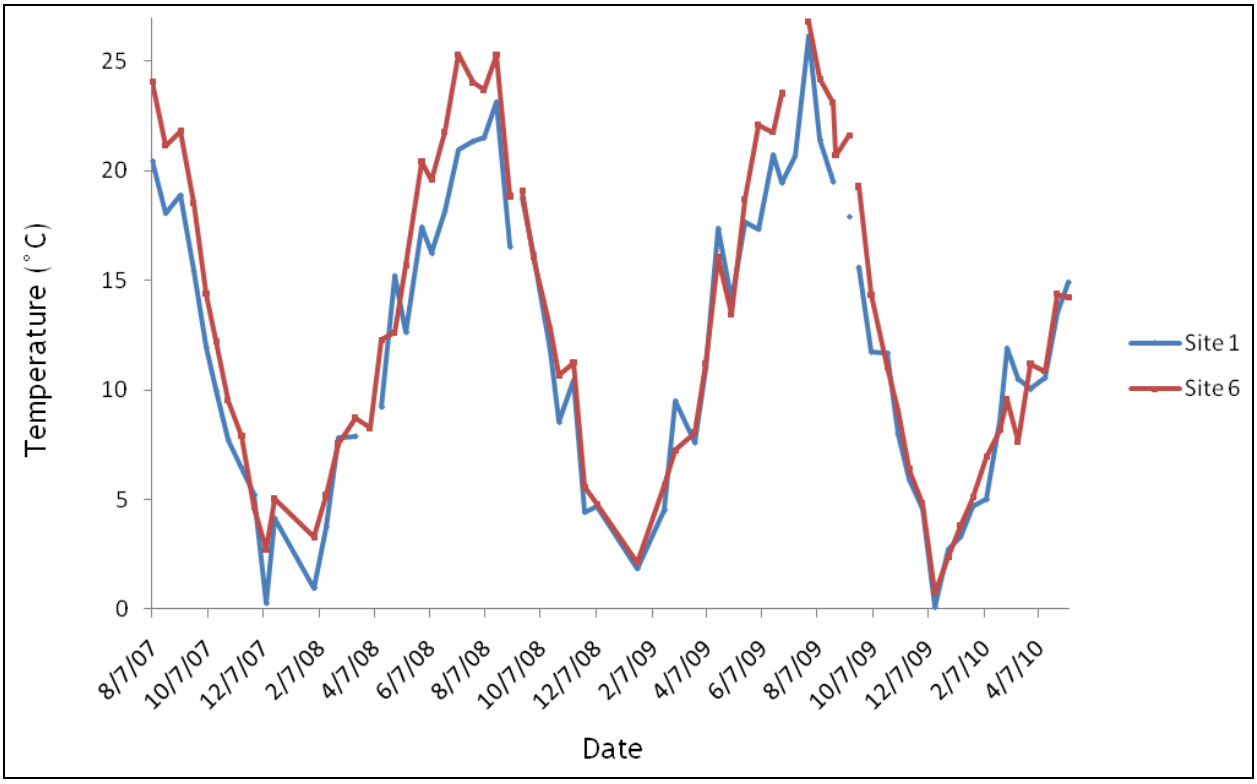

Figure 27. Water temperatures taken from Lower Crab Creek at sites 1 and 6 indicated a cooling trend as the creek flows downstream.

Dissolved Oxygen, Specific Conductivity, pH, and Total Phosphorus

Although an ANOVA of the data indicated a significant difference (F = 3.66, P < 0.001, df = 7) in the D.O. levels between sites throughout the duration of the collections, the lowest D.O. level never below 4.0 mg/L which would have precluded many sensitive fish families such as Salmonidae from LCC (Figure 28). The difference in specific conductance between the uppermost and lower sites appeared substantial (Figure 29). An ANOVA confirmed a highly significant difference in specific conductance

between all sites throughout LCC (F = 46.30, P < 0.0001, df = 7). We used the pH scale to measure the acidity or alkalinity of the water at all eight sites. The lowest mean pH value was 8.2 at Site 1 (Figure 30) and none of the sites reached neutrality (7) during our sampling efforts. Mean total phosphorus (TP) values were considerably higher at Site 2 compared to Site 6 (Table 13). However, out of the 14 times we collected samples for water chemistry, TP was only detected six and four times at sites 2 and 6, respectively. In addition Site 2 was highly variable for both TP and turbidity values.

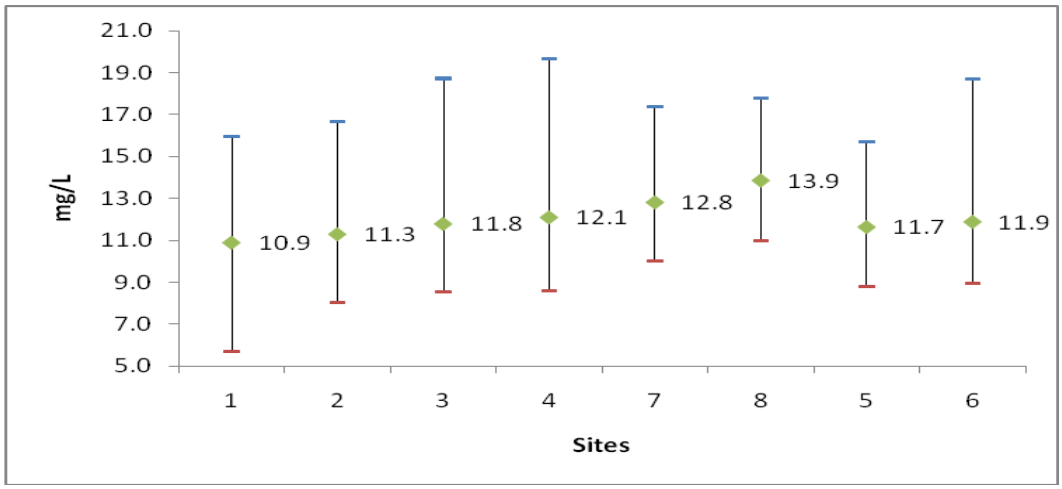

Figure 28. Mean, maximum, and minimum concentration (mg/L) of dissolved oxygen collected from the seven water quality sites in Lower Crab Creek and one in Red Rock Coulee (Site 4).

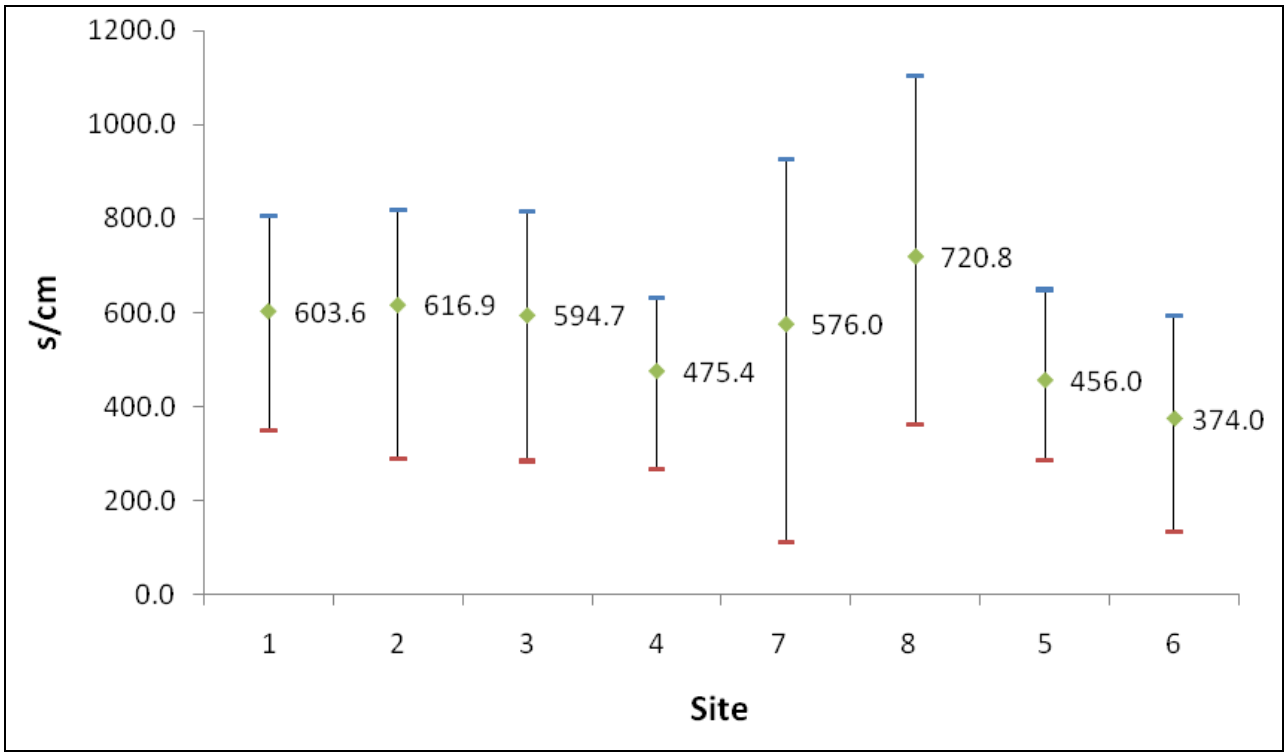

Figure 29. Specific conductance from the seven water quality sites in Lower Crab Creek and one in Red Rock Coulee (Site 4).

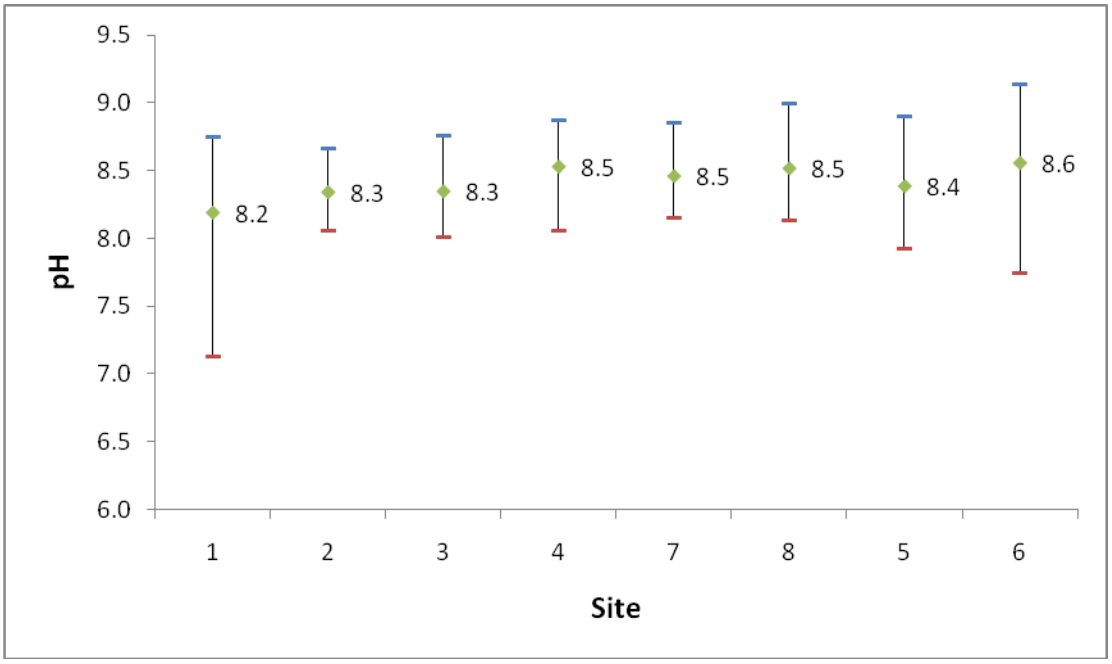

Figure 30. Mean, maximum, and minimum range of pH values from the seven water quality sites in Lower Crab Creek and one in Red Rock Coulee (Site 4).

Table 13. Descriptive statistics of total phosphorus collected at sites 2 and 6 in Lower Crab Creek.

	Site 2	Site 6
Mean	110.00	28.57
Standard Error	69.02	13.42
Standard Deviation	258.25	50.21
Sample Variance	66,692.3	2,520.9
Skewness	3.27	1.46
Range	970.0	140.0
Minimum	0.0	0.0
Maximum	970.0	140.0
Count	14	14
95% Confidence Interval	149.11	28.99

Turbidity

Turbidity varied considerably at almost every location excluding Site 6 located at the headwaters of LCC at the base of O'Sullivan dam (Figure 31). In addition, the mean turbidity value increased from Site 6 downstream to the confluence (Table 14) and a significant difference was detected with an ANOVA between sites ($F = 20.81$, $P < 0.001$, df = 7). At a glance, there appeared to be a difference in the turbidity values collected during water chemistry sampling (samples analyzed by Yakima Valley Labs) between Site 2 (lower) and Site 6 (upper) within LCC (Figure 32). In addition, Site 2 was subject to far more range in turbidity compared to Site 6 (Table 15). Statistical tests confirmed a significant difference in turbidity values between sites 2 and 6 ($t = 4.04$, $P < 0.001$, df = 56).

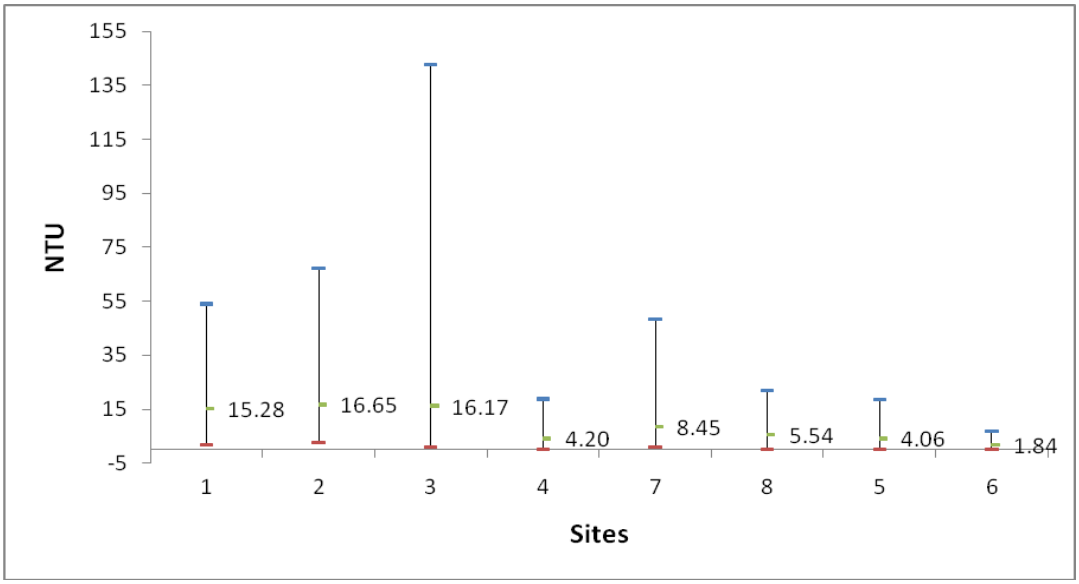

Figure 31. Mean, maximum, and minimum turbidity readings from the seven water quality sites in Lower Crab Creek and one in Red Rock Coulee (Site 4).

Table 14. Descriptive statistics of turbidity values from the seven sites in Lower Crab Creek and one in Red Rock Coulee (shaded).

Sites	Mean	SE	Median	Mode	Standard Deviation	Variance	95% CI
1	15.28	1.37	12.40	18.90	11.03	121.69	2.73
2	16.65	1.43	14.70	4.90	11.85	140.31	2.85
3	16.17	2.64	12.20	15.10	21.60	466.49	5.27
4	4.20	0.49	3.15	1.00	3.85	14.81	0.98
7	8.45	0.94	6.35	6.10	7.12	50.71	1.87
8	5.54	0.80	3.70	0.90	5.80	33.60	1.60
5	4.06	0.42	3.20	3.00	3.37	11.39	0.84
6	1.84	0.21	1.30	0.70	1.55	2.40	0.41

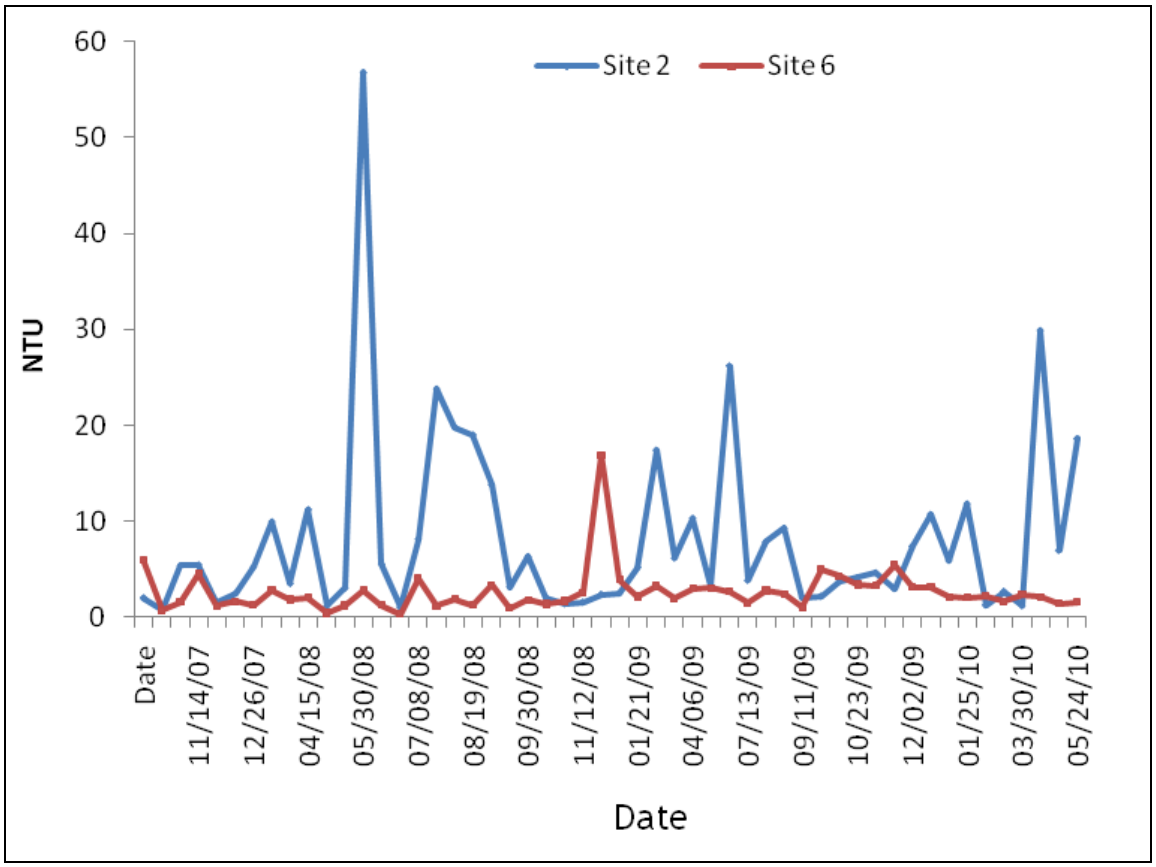

Figure 32. Turbidity values from sites 2 and 6 on Lower Crab Creek. Water chemistry samples were analyzed by Yakima Valley Labs.

Table 15. Descriptive statistics of turbidity values from sites 2 and 6 in Lower Crab Creek.

	Site 2	Site 6
Mean	8.261	2.554
Standard Error	1.372	0.328
Standard Deviation	9.800	2.344
Sample Variance	96.043	5.494
Kurtosis	11.521	28.630
Skewness	2.952	4.766
Range	56.010	16.570
Minimum	0.790	0.330
Maximum	56.800	16.900
95% Confidence Interval	2.756	0.659

Habitat Analysis

At each site, we collected habitat data from three transects in 50 m intervals (Appendix 7). Generally, the habitat types within the portion of LCC we surveyed were homogenous. Of the 87 transects surveyed, 83 were identified as run / glide habitat and 54, 14, and 4, of the substrate types were identified as clay, sand, and clay/sand, respectively. Except for the bottom three reaches, most of the channel widths and wetted widths were also similar (Figure 33). Using ArcMap and aerial photos we measured the sinuosity at six locations within LCC: four within the relatively undisturbed portion of the creek and two sites adjacent to agricultural use (Figures 34-39). Estimates of sites within the agricultural portions of the creek were closer to 1.0 than other sites indicating channelization of LCC with areas of agricultural use (Table 16). Water operations greatly influenced discharge within LCC as indicated by data from a remote station located just upstream from Beverly, Washington (Figure 40).

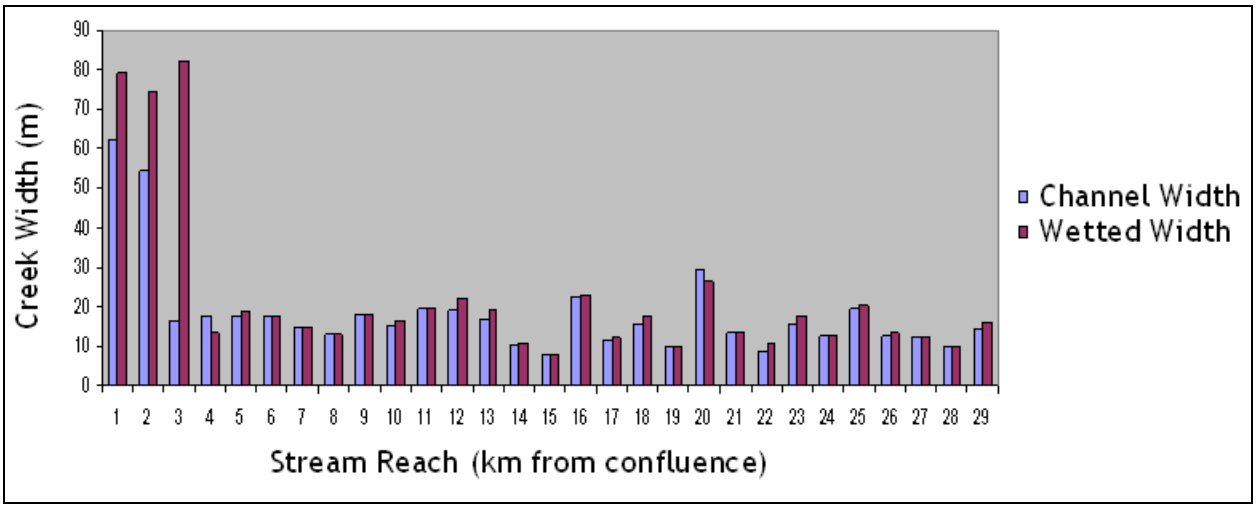

Figure 33. Mean channel and wetted widths of the 29 reaches surveyed on Lower Crab Creek.

Figure 34. Aerial photo of Lower Crab Creek at river kilometer 0 used to determine sinuosity.

Figure 35. Aerial photo of Lower Crab Creek at river kilometer 10 used to determine sinuosity.

Figure 36. Aerial photo of Lower Crab Creek at river kilometer 19 used to determine sinuosity.

Figure 37. Aerial photo of Lower Crab Creek at river kilometer 29 used to determine sinuosity.

Figure 38. Aerial photo of Lower Crab Creek at river kilometer 37 used to determine sinuosity.

Figure 39. Aerial photo of Lower Crab Creek at river kilometer 43 used to determine sinuosity.

Table 16. Sinuosity estimation for six sites on Lower Crab Creek. Those proportions closer to 1.0 indicate areas of greater stream channelization and less sinuosity.

River Km from Confluence	Actual River Distance	Proportion (1,000/River Distance)
0	1,607	0.62
10	1,145	0.87
19	1,119	0.89
29	1,136	0.88
37	1,003	0.99
43	1,095	0.91

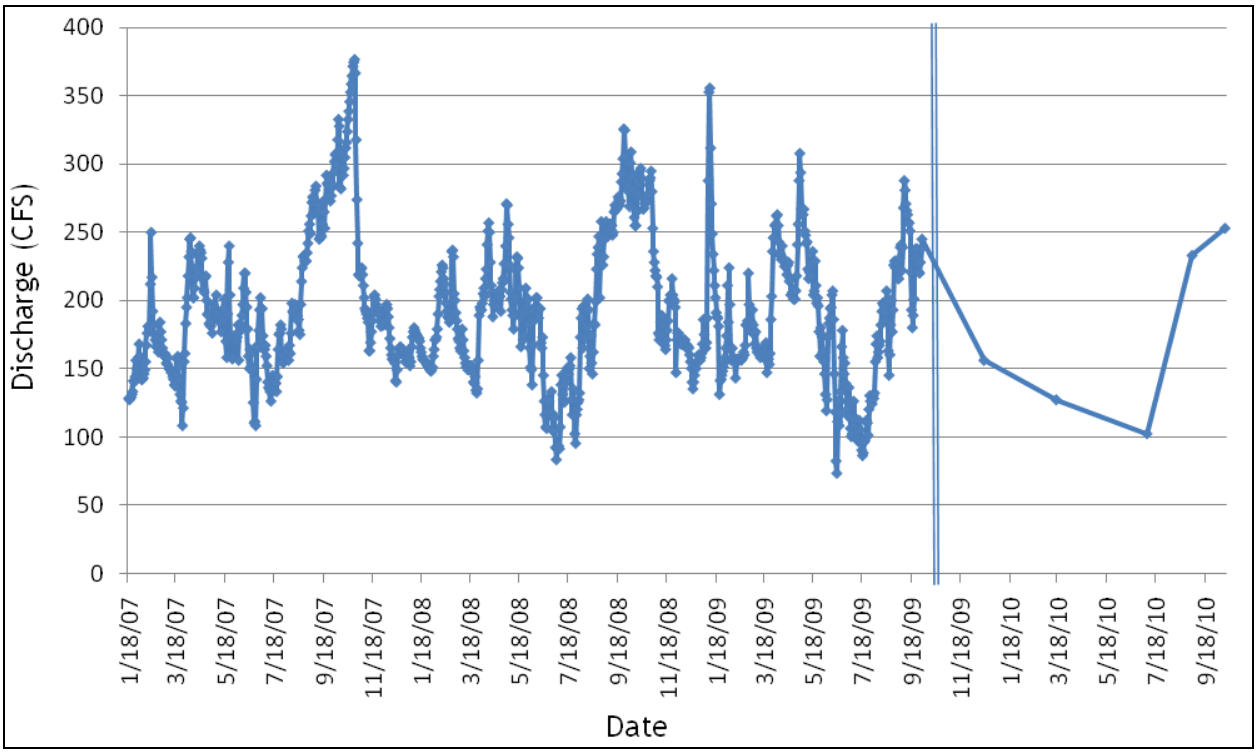

Figure 40. Calculated discharge from the DOE water quality monitoring station on Lower Crab Creek near Beverly, WA (Station 41A070).

IV. Discussion and Recommendations

The Crab Creek drainage is over 13,000 square kilometers and extends from the Columbia River near Beverly to Davenport, WA. Although some feel LCC is simply a product of the Columbia Basin Irrigation Project (CBIP), historical information indicates LCC is a natural system that predates the CBIP, which began in 1955. Prior to the construction of the CBIP, portions of LCC were naturally impounded and flowed subsurface. However, it was believed that many of the natural blockages were breached during high flow events. Today the lower portion of Crab Creek begins as seeps from below O'Sullivan Dam, which impounds Potholes Reservoir. Additional water enters LCC via both surface and sub-surface irrigation returns. The influence of the CBIP is not refuted, as water levels within LCC fluctuate relative to water operations within the Basin.

Lower Crab Creek is a unique system and because of this, comparisons within the region are difficult. We conducted extensive literature searches and have not located any information regarding other desert systems of like size and that experience the same amount of manipulation that support a potentially distinct population of fall Chinook salmon within the mid-upper Columbia River Basin. An initial assessment of LCC would suggest salmonid utilization would not be probable as our data indicated temperatures were often over 20°C and even above 25°C, which is the upper lethal limit for Chinook salmon (Quinn et al. 1991). However, the warm temperatures within LCC are ideal for aquatic macroinvertebrate production, which was also corroborated within our analysis. The abundance and presence of certain orders of invertebrates such as mayflies (Ephemeroptera) and caddisflies (Trichoptera) was surprising as our habitat surveys did not detect large areas of cobble or rocky substrate which is preferred by many aquatic macroinvertebrates.

One likely impact of the CBIP is the manipulation of the water table associated with water operations. Since the inception of the CBIP, not only has the amount of area inundated by water increased, but we can assume so has the amount of subsurface flow within the Columbia River basin. Although not significantly different, our data indicated during the summer months water temperatures in the lower portions of the creek were often cooler than the upper portions of LCC. The longitudinal cooling of a lotic system is uncommon unless influenced by cooler surface or subsurface water. Although there are numerous surface returns and withdrawals throughout LCC, none would be expected to lower the water temperatures. Consequently, the cooler temperatures within the lower sites of LCC can only be attributed to cooler subsurface water additions. Site 2 for both the thermograph and YSI temperature sampling frequently recorded temperatures during the summer months that were lower than temperatures at sites upstream. The area to the north of LCC is higher in elevation and the irrigated land in this area would increase both surface and subsurface water that flows into the LCC basin (Figure 41). Consequently, areas where cooler ground water enters LCC would create temperature micro-refuges where sensitive fishes could escape sub-optimal temperatures elsewhere in the creek.

The influx of water has greatly altered the historical habitat and inundated areas that typically never held water, which has been both beneficial and detrimental to both wildlife and fishes in the drainage. The increase in water that passes through the Columbia Basin has increased the amount of available fish habitat. However, inundating land has decreased terrestrial habitat and reduced potential migratory routes for terrestrial animals.

Figure 41. The Lower Crab Creek channel is contained within black boundary bars. Black arrows indicate the decrease in elevation toward the creek channel; the area outside of channel is generally higher in elevation. The area in the upper portion of the photo is agricultural land with substantial irrigation. Run-off and areas where springs may exist are also visible.

The following discussion on varying sizes of juveniles was summarized from the paper accepted for publication in the peer-reviewed journal Transactions of the American Fisheries Society (Small et al. 2011) The complete discussion including references are available in Appendix 10. During the screw trap sampling, we captured varying sizes of Chinook salmon. Such variation could be attributed to varied growth rates of the same cohort, Chinook salmon from other systems coming into LCC to over-winter and out-migrate the following year, or yearling LCC Chinook salmon that spent a year in the system. Variation in growth within a system can be expected but not to the degree we observed. Therefore, the variation in size may have been attributed to strays from other systems coming into LCC to over-winter and migrate out the

following year. If the Chinook salmon in LCC were random strays or a collection of strays from a wild or hatchery population, we would expect them to be assigned to their collection(s) of origin in the assignment test and share all of their alleles with other collections. However, LCC Chinook salmon were assigned back to the LCC collection with high fidelity, and that collection possesses alleles that are absent from other Columbia River basin Chinook salmon populations. In fact, one of these alleles has not been detected in any other GAPS population. Few strays were detected in the assignment test, and nearly all confidently assigned juveniles originated in the LCC spawning aggregate. Consequently, the variation in size-classes could not be attributed to strays over-wintering in LCC.

Due to the sometimes-lethal temperatures within LCC, Chinook salmon residency for a year in LCC was not thought to be likely. Fish might avoid some of the water temperature problems since adults move into the creek in the fall when temperatures are declining and smolts exit the creek in the spring before temperatures rise to lethal levels of summer. However, the abundance of larger-size-class juveniles in LCC (at least 20% each year) and the difference between the daily minimum and maximum sizes suggests that it is common for LCC Chinook salmon juveniles to overwinter and migrate as yearlings. A variation in size-classes might arise if some juveniles emerged earlier and grew fast in the warm water with abundant food while other juveniles continued to emerge through spring, maintaining the smaller size-class. Juvenile emergence might have a bimodal distribution if the spawning season is bimodal owing to the water temperature or flow in LCC or if spawning occurs over an extended period. Connor et al. (2002) noted that in warmer waters in the Snake River fall Chinook salmon fry emerged earlier and growth was faster and that cold water fostered slower growth and stream-type juveniles. Juveniles might survive the summer in cool groundwater upwellings obscured by turbidity; the size of LCC juveniles was within the published range of stream-type juveniles (Waknitz et al. 1995) and overlapped the size range for rare stream-type juveniles identified in fall Chinook salmon from the Wenatchee River (Steven Schonning, WDFW, unpublished data).

Another possibility that is not mutually exclusive with the others is that LCC Chinook salmon have adapted to warmwater environments; smaller redband trout in desert streams have been found to be more tolerant of thermal stress (Rodnick et al. 2004), and juvenile Chinook salmon may be similarly tolerant. Collecting scale data for juveniles to examine whether larger-sized individuals are yearlings and a thermal profile of the tributary would help us distinguish between the various possibilities. Genetic differentiation from nearby populations combined with poor-quality water and high temperatures in parts of its habitat suggest that the LCC population has developed adaptations to environmental conditions that are unusual for Chinook salmon. The run timing in LCC fish is most similar to that of the nearby Hanford-Yakima major population group (within the upper Columbia ESU substructure; Appleby et al. 2010) and they share the most genetic variance with this group, but their apparent bimodal juvenile life history would be unique within the upper Columbia River ESU.

Lower Crab Creek is not only important to resident native salmonids, but may also be used by non-native fishes from the Columbia River. In addition, the warmer temperatures would have allowed for increased food production for both native and non-native fishes within LCC. The conditions in the spring are not only optimal for native fishes, but also for non-native fishes such as bass, which was further corroborated during the 2009 sampling season when largemouth bass were the most abundant fish captured in our downstream trap. The mechanism for which largemouth bass moved down stream, whether volitional or involuntary associated with water operations was not determined. However, due to the small average size of largemouth bass captured in our trap (~35 mm) and the susceptibility of small fish to become entrained (Devine Tarbell & Associates, Inc. 2005) the latter of the two possibilities is most likely the case. The concern with non-native species, such as large- and smallmouth bass moving down LCC is the possibility of them reaching the Columbia River where they may negatively impact native and listed species. Although one study has shown bass impacts on salmon are minor (Tabor et al. 2007), other studies have indicated predation by non-native fishes such as bass can have negative impacts on salmon (Rieman et al. 1991; Fritts and Pearsons 2004).

Even minute impacts by non-native fishes within the Columbia River may be considered unacceptable losses by the National Oceanic and Atmospheric Administration (NOAA). "No net impacts" agreements to out-migrating salmon have minimal tolerances regarding percentage of mortality within the Mid Columbia River (Grant PUD 2005). Losses associated with predation in other systems deemed minimal may not be the case within the Columbia River. Case in point, the WDFW's LLRT is currently finishing a Grant Co. PUD funded three-year predation / survival study with the USGS and Blue Leaf Environmental to quantify the source of salmon and steelhead mortality adjacent to PUD projects. Although too early to present concrete results, predation has not been ruled out as a contributor to mortality rates between projects that are unacceptable by federal standards. Therefore, any impacts by native and non-native predators may be deemed deleterious.

As part of the original scope of work, we have developed management recommendations for the WDFW regarding LCC.

Implement a comprehensive LCC Chinook salmon M&E program.

a. Continue to trap and collect tissue and scale samples of migrating salmonids through the LCC system. Develop multiple trap locations.
b. Closely monitor the recreational harvest of salmon on the mainstem Columbia River between Priest Rapids and Wanapum dams using a creel survey to determine what percentage of LCC salmon are harvested each year.

Although only anecdotal information is available, the recreational fishery between Priest Rapids and Wanapum dams has grown considerably over the past five years and

now includes professional fishing guides (Figure 42); therefore, justifying investigations on LCC that encompass the Mainstem Columbia River.

Through personal communications and publications, several agencies such as the Yakama Nation (personal communication), NOAA (Dale Bambrick, personal communication), USFWS, USBOR, and the Grant Co. PUD (KWA 2004) have expressed an interest in information from LCC. Even though LCC Chinook salmon are part of the Upper Columbia River Summer-/Fall-Run Chinook ESU, which are not federally listed, the population within LCC is unique as a spawning population within a desert stream. Therefore, we propose that investigations within the LCC system continue.

Figure 42. Recreational sport fishery near Wanapum Dam approximately 4 km upstream from the confluence of Lower Crab Creek.

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VI. Appendices

Appendix 1. Memo from USFWS indicating steelhead presence in Red Rock Coulee, Washington.

United States Department of the Interior
Fish and Wildlife Service
Mid-Columbia River Fishery Resource Office
7501 Icicle Road
Leavenworth, WA 98826
Phone: (509) 548-7573
Fax: (509) 548-5743

Memo to file

March 9, 2004 - Columbia Refuge Red Rock Coulee Steelhead

1. Met with Randy Hill and Justin Bader to discuss methods to collect redd data.
2. Assisted Refuge personnel on setting up steelhead redd surveys.
3. Visited Red Rock Coulee to locate potential site of surveys.
4. Small site maybe 200 m long. The Refuge is unsure of boundary locations.
5. Area is just downstream of Red Rock Lake, system is small but has substrate located downstream of where it passes under the roadway. Substrate is hard and encrusted, but looks like once the surface is broken may provide good substrate.
6. Identified 2 steelhead redds. Downstream stream redd is just above large boulders no fish located. Second redd is upstream river left, with 3 fish on redd. Looked like 2 adult steelhead (colored up) and one smaller silver fish. An additional fish was observed just downstream moving upstream. Water temp 9.9 deg C at 12:35 pm.
7. Deployed 2 thermographs in that area.

Appendix 2. Standard Operating Procedures for Water Quality Data Collection.

Purpose
To provide guidelines for conducting water quality surveys.

Area of Applicability
For WDFW LLRT personnel conducting water quality surveys.

Materials needed

- YSI 6600 V2 Multiparameter Water Quality Sonde, handheld YSI computer and cord
- Secchi disk and line
- Anchor and line
- Bucket
- Static sites
- Data sheets and pencils

Procedures

1. Arrive at specific site using GPS coordinates. Throw anchor and make sure the boat* is not moving. Fill bucket 2/3 full of water from the body of water to be sampled. Use the YSI sonde to measure the water quality parameters.
2. Before sampling begins, hold the YSI sonde just below the surface of the water for 40 seconds prior to recording any data to acclimate the YSI sonde and allow it to clean the optic ports. The first reading can then be taken at the surface and then at each meter until the bottom is reached (try not to touch the bottom).
3. Parameters are logged on the handheld YSI computer and recorded on the water quality data sheets (Figure 1). The parameters include depth (m), temperature (°C), specific conductivity (s/cm), dissolved oxygen (mg/L), pH, turbidity (NTU) and chlorophyll (µg/L). Also, record the barometric pressure at the first site.
4. After the last reading is recorded at the bottom of the water column, slowly pull the YSI sonde up and out of the water. Place the YSI sonde in the bucket of water that was previously filled. The bucket should be dumped and refilled periodically throughout the day.
5. Secchi depths are taken at each site. Sunglasses and hats should be removed when taking readings. The Secchi disk is lowered into the water on the shaded side of the boat. Once the disk disappears, pull it back up until it reappears again. Raise the disk up and down until the exact vanishing point is found and record the depth (m) on the data sheet.
6. After all data are recorded and equipment is secured, pull anchor and proceed to the next site*.

**If sites are not reached by boat, simply follow the same methods while on dry land.*

W.Q. Data Sheet
Page: _____ Project: _____

Date: _____ B.P. _____ Initials: _____

Field data check _____
Office data check _____
Bio data check

Location	Depth (m)	Temp.	SpC	D.O. mg/L	pH	Turbidity NTU	Chl (µg/L)	Secchi (m)	Zoop. Pull Depth (m)

Calibration date:

Comments

Appendix 3. Calibration Instructions for the YSI 6600 V2 Multiparameter Water Quality Sonde.

Purpose
To provide guidelines for calibration methods to ensure the YSI 6600 V2 Multiparameter Water Quality Sonde is accurate for specific bodies of water.

Area of Applicability
For WDFW LLRT personnel calibrating the YSI 6600 V2 Multiparameter Water Quality Sonde.

Materials needed
- YSI 6600 V2 Multiparameter Water Quality Sonde, YSI computer and cord
- Distilled water
- Known turbidity standard (<0.1, 10, 20, and 40 NTU)
- 4, 7, and 10 pH standards
- Known conductivity solution
- KimWipes®
- Paper towels
- Calibration data sheet

Procedures
1. Fill out a calibration data sheet for the specific body of water to be sampled and gather the proper standards for the calibration.
2. Connect the YSI sonde to the handheld YSI computer with its field cord. Turn on the handheld YSI computer and bring up the calibration menu. Remove the black cap from the calibration cup on the YSI sonde and fill 1/3 of the calibration cup with distilled water. Replace the cap and swish gently to rinse. Empty the distilled water and repeat the rinse step. For the calibration of conductivity and pH, the calibration cup will be attached to the YSI sonde and the probes are pointing up when standards are poured. For the calibrations turbidity, chlorophyll, and dissolved oxygen, the calibration cup is removed from the YSI sonde and inserted so that the probes are now pointing down.
3. On the calibration menu, scroll to the conductivity option and press enter. Choose the SpCond option for specific conductivity and press enter. Enter the value of standard used in this calibration (for most of our water quality surveys we calibrate at 0.5 µm/s). Fill the calibration cup to cover the sensor. Pay close attention that air bubbles are removed from the sensor for an accurate reading. On the handheld YSI computer, press enter to start the calibration and allow some time for the sensor to give an accurate reading. Record this reading in the in the "actual" box on the calibration data sheet and then press the enter key again to calibrate the sensor. A new number should read on the screen. Record this number in the "after calibration" box. The standard should then be transferred into a clean, labeled bottle for benchmarking after

the survey is completed. Rinse the calibration cup and sensors with distilled water for the next calibration.

4. Select pH from the calibration menu on the handheld YSI computer. Choose the 3-point calibration option from the menu. Start with the pH 7 standard (press 7 on the numeral keypad). Pour a small amount of the pH 7 standard over the sensor and then swish to rinse out any existing distilled water and empty. Fill the calibration cup with pH 7 standard to cover the sensor. On the handheld YSI computer, press the enter key and allow some time for the sensor to give an accurate reading. Record the actual reading along with pH MV Buffer reading on the data sheet in the appropriate boxes. Press enter to accept calibration, and record the next readings in the "after calibration" boxes. Once calibration is successful, pour the pH 7 standard into a clean, labeled bottle for benchmarking after the survey is completed. Rinse out the calibration cup with distilled water for the next standard. Repeat step 4 for using pH standards 4 and 10.
5. Next, choose turbidity 3-point calibration on the handheld YSI computer. The calibration will start with a 0 value. To begin, rinse the sensors and calibration cup with distilled water and dry with KimWipes®. Pay close attention to dry between the sensors. The calibration cup should be removed and completely disassembled to dry all parts. Reassemble the calibration cup and pour in a small amount of the <0.1 NTU standard to rinse the calibration cup and the YSI sonde sensors. The calibration cup and instruments must be dried again. Reassemble the calibration cup with the black lid attached to the bottom. Pour <0.1 NTU standard into the calibration cup until it is about 1/3 full. Immerse the sensors carefully into the standard (make sure no bubbles are on the sensor); if the standard does not completely cover the bottom of the sensor add a small amount of <0.1 NTU standard to the calibration cup. Press the enter key to give the actual reading. There will be an option to clean optics; select this for the best calibration results. Once the cleaning is through, record the reading in the in the "actual" box on the calibration data sheet, accept the calibration, and record the reading again in the "after calibration" box on the calibration data sheet. Repeat this process for the next two turbidity calibrations.
6. Rinse the calibration cup and the YSI sonde sensors with distilled water. Choose the chlorophyll option from the calibration menu and then the 1-point calibration from the next menu. Fill the calibration cup 1/3 full with distilled water and immerse the YSI sonde into the calibration cup. Run the optic cleaner to remove any bubbles or debris. Record the actual reading and accept the calibration. Record the next reading also. Empty the distilled water from the calibration cup.
7. Return to the calibration menu and select dissolved oxygen and then %Saturated. Fill the calibration cup 1/3 full with distilled water and set the sensors into the calibration cup. Make sure no water droplets are on the dissolved oxygen membrane and the water level is not touching the membrane. Enter the barometric pressure on the handheld YSI computer located at the

bottom of the screen and select enter. Let the meter sit up to ten minutes and then record the actual and calibrated values.

8. Empty the water, replace the wet sponge in the calibration cup, and screw it back onto the YSI sonde. Pack all the components back into the travel bag.
9. Upon return from the water quality sample period, benchmark each standard. Test each standard with the YSI sonde to document the values after the survey. Use distilled water to rinse between each standard. The turbidity samples must be benchmarked with the same procedures as in step 5. The calibration cup and the YSI sonde must be cleaned and dried before each turbidity standard. Each benchmark value should be recoded in the proper space on the calibration data sheet.

Appendix 4. Yakima Valley Labs Standard Operating Procedures for Turbidity Analysis.

SOP 0180

Turbidity

Revision Number: 1.0

Effective Date of SOP: 10/01/2003

Approved: Bennett K. Osborne, Laboratory Director

1) SOP – GENERAL

1.0. Turbidity

1.1. METHOD(S):

SM 2130B; EPA 180.1

1.2. SUMMARY:

The method is based upon comparison of intensity of light scattered by the sample under defined conditions with the intensity of light scattered by the standard sample under the same conditions. The higher the intensity of scattered light, the higher the turbidity. Readings, in nephelometric turbidity units (NTU), are made on a nephelometer. The styrene-divinylbenzene copolymer is manufactured by Hach and approved by the EPA as the primary turbidity standard sample used to calibrate the nephelometer.

1.3. SCOPE AND APPLICATION:

This method is applicable to drinking, surface, and saline waters in the range of turbidity from 0.1 to 40 NTU. Higher values may be obtained with dilution of the sample.

Clarity of water is important in producing products destined for human consumption and in many manufacturing operations. Beverage producers, food processors, and potable water treatment plants drawing from surface water source commonly rely on fluid-particle separation processes such as sedimentation and filtration to increase clarity and insure an acceptable product. The clarity of a natural body of water is an important determinate of its conduction and productivity.

1.4. DEFINITIONS:

NTU – nephelometric turbidity units.

Turbidity – an expression of optical property that causes light to be scattered and absorbed by suspended and colloidal matter, such as clay, silt, finely divided organic and inorganic matter, and plankton or other microscopic organisms, rather than transmitted with no change in direction or flux level through the sample.

True Color – water color due to dissolved substances that absorb light.

Other definitions of terms are given in SOP 0099.

2) SOP – SPECIFIC

2.0. INTERFERENCES:

The presence of debris and rapidly settling coarse sediment will give low turbidity readings. Dirty glassware and the presence of air bubbles will give false results. The presence of true color can cause measured turbidities to be low, although this effect is generally not significant in treated waters.

2.1. SAFETY:

The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials or procedures. VEL has a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) is made available to all personnel involved in the chemical analysis. All applicable components of the VEL Safety Manual shall be strictly adhered to.

EQUIPMENT AND SUPPLIES:

Hach 5000 Turbidimeter
Turbidity tubes. (NOTE: These must be clean inside and out. New tubes should be indexed and calibrated according to manufacturer instructions. Discard when they become scratched or etched. DO NOT handle them on the sides. Store in their protective case. Clean smudges on the outside of the tubes with water or a 2% solution of concentrated Ammonium Hydroxide. Wipe dry with a Kimwipe)

REAGENTS AND STANDARDS:

Primary standards:

1.0 NTU & 10.0 NTU Hach Formazin Solution. Store at room temperature.

Turbidity-free water: Culligan distilled water. (Filtering through 0.45μ filter does not lower turbidity, therefore filtering is not necessary.)

2.4. CALIBRATION AND STANDARDIZATION:

Daily Calibration:

Select a secondary sealed standard in the range of the samples to be tested. Most drinking water samples are in the 0-1.0 NTU range. Align the indexing arrow on the tube with the indexing arrow on the meter and insert the tube in the meter chamber. Close the lid and push the READ button. If the displayed value is not within ±2% of the true value labeled on the sealed standard tube then recalibrate according to manufacturers instructions (page 17 of LaMotte 2020 instruction manual). Record the reading in the "Turbidity and Color' data book. (When using the Monitek, follow instructions on page 3-1 of the operating manual.)

Quarterly Calibration:

Use the 10.0 NTU primary standard to calibrate the instrument Fill the turbidity tube in the same manner as samples. Record about five readings in the data book. Record about five readings of the 10.0 NTU secondary standard and calculate the average. Label the secondary standard with the average reading. This is its true value. Recalibrate the instrument using the 1.0 NTU primary standard and calibrate the 1.0 NTU secondary standard in the same way as the 10.0 NTU secondary standard. The instrument is usually calibrated with the 1.0 NTU standard since this is the range most samples are in.

2.5. POLLUTION PREVENTION:

Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The USEPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option. The quantity of chemicals purchased should be based on expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability. For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Management for Waster Reduction," available from the American Chemical Society's Department of Government Regulations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036, (202) 872-4477. VEL pollution prevention is described in SOP 0003.

2.6. WASTE MANAGEMENT:

Due to the nature of this method there is little need for waste management. No large volumes of solvents or hazardous chemicals are used. However, the U.S. Environmental Protection Agency requires that laboratory waste management practices conducted be consistent with all applicable rules and regulations. Excess reagents, samples, and method process wastes should be characterized and disposed of in an acceptable manner. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with all solid and hazardous waste regulations, particularly hazardous waste identification rules and land disposal restrictions. For further information on waste management consult the "Waste Management Manual for Laboratory Personnel," available from the American Chemical Society. VEL waste management policy described in SOP 0003.

3) SAMPLE HANDLING

3.0. COLLECTION / SHIPMENT:

Minimum sample needed:	100 mL
Normal size/type bottle used:	Plastic or Glass
Preservation used before sampling:	N/A
Preservation needed after sampling:	N/A
Must be shipped at 4°C?	Yes
Other Special Requirements:	N/A

3.1. HOLDING TIMES:

First extraction holding time: NA
Second extraction holding time: NA
Holding time for analysis: 48 h

3.2. STORAGE: Must be stored at 4°C.

4) QUALITY CONTROL - SEE SOP 0004

5) PROCEDURE

Allow the nephelometric instrument to warm up for 30 min.

For the daily calibration, select a secondary sealed standard in the range of the samples to be tested. Most drinking water samples are in the 0-1.0 NTU range. Align the indexing arrow on the tube with the indexing arrow on the meter and insert the tube in the meter chamber. Close the lid and push the READ button. If the displayed value is not within ±2% of the true value labeled on the sealed standard tube then

recalibrate according to manufacturers instructions. Record the reading on the Turbidity bench sheet.

It may be necessary to perform a quarterly calibration. The procedure for the quarterly calibration is to use the 10.0 NTU primary standard to calibrate the instrument. Fill the turbidity tube in the same manner as samples. Record about five readings in the data book. Record about five readings of the 10.0 NTU secondary standard and calculate the average. Label the secondary standard with the average reading. This is its true value. Recalibrate the instrument using the 1.0 NTU primary standard and calibrate the 1.0 NTU secondary standard in the same way as the 10.0 NTU secondary standard. The instrument is usually calibrated with the 1.0 NTU standard since this is the range most samples are in.

5.4. Allow samples to reach room temperature and mix gently.

5.5. Rinse turbidity tube twice with about 5 ml of distilled water (blank) or sample and shake out excess.

5.6. Fill the tube to the neck with sample by carefully pouring down the side of the tube to avoid creating bubbles. Cap the tube and wipe it dry with a Kim-Wipe.

5.7. Align the indexing arrow on the tube with the indexing arrow on the nephelometric meter and insert the tube in the meter chamber. Close the lid and push the READ button. Record the readings in NTU. If samples exceed 40 NTU then dilute them with distilled water.

5.8. Subtract appropriate turbidity tube correction factors (written on turbidity tube) and multiply sample readings by appropriate dilution. Report results as follows:

NTU Range of Sample:	Report to Nearest:
0.0-1.0	0.05
1.0-10.0	0.1
10-40	1
40-100	5
100-400	10
400-1000	50
>1000	100

6) CALCULATIONS

6.0. Calculate turbidity as follows:

Subtract appropriate turbidity tube correction factors (written on turbidity tube) and multiply sample readings by appropriate dilution.

7) METHOD PERFORMANCE – N/A

8) CORRECTIVE ACTIONS - SEE SOP 0007

9) REFERENCES

9.0. EPA Methods for Chemical Analysis of Water and Wastes, Method 180.1 (1983).

9.1. Standard Methods for the Examination of Water and Wastewater, 20th Edition, p 2-9, Method 2130B (1998).

Appendix 5. Yakima Valley Labs Standard Operating Procedures for Phosphorus, Total and Reactive, Analysis.

SOP 0365

PHOSPHORUS, TOTAL AND REACTIVE

Revision Number: 1.0

Effective Date of SOP: 03/01/2004

Approved: Bennett K. Osborne, Laboratory Director

SOP – GENERAL

TOTAL PHOSPHORUS

1.1. Method(s): EPA 365.2; SM 4500P – B&E; HACH 8190

1.2. Summary:

Phosphorus occurs in natural waters and wastewaters almost solely as phosphates, and arises from a variety of sources. Some are added to water supplies during treatment, while others are applied to agricultural or residential cultivated land as fertilizers and are carried into surface waters by storm runoff.

1.3. Scope & Application:

Phosphorus is essential to the growth of organisms and can be the nutrient that limits the primary productivity of a body of water. In instances where phosphate is a growth-limiting nutrient, the discharge of raw or treated wastewater can stimulate the growth of aquatic micro- and macro-organisms in nuisance quantities.

Total Phosphorus samples are preserved with 1 mL conc HCl/L to pH<2, stored at 4^{o}C, and must be analyzed within 28 days.

1.4. Definitions:

TP – Total Phosphorus
P – Phosphorus
Other definitions in SOP 0099

2) SOP – SPECIFIC

2.0. INTERFERENCES:

High Iron may cause precipitation and loss of Phosphorus. If Arsenate is higher than the level of P it may also interfere.

2.1. SAFETY:

The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials or procedures. VEL has a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) is made available to all personnel involved in the chemical analysis. All applicable components of the VEL Safety Manual shall be strictly adhered to.

EQUIPMENT AND SUPPLIES:

HACH Test n Tube w/sulfuric acid
HACH 2010 Spectrophotometer
COD Reactor at 150^{o}C

2.3. REAGENTS AND STANDARDS:

Test n Tube w/H_2SO_4
Potassium Persulfate (HACH Potassium Persulfate PP)
1.54 N NaOH
Ascorbic acid, Ammonium molybdate, Antimony potassium tartrate (HACH PhosVer 3 PP)
Phosphate std stock solution – 1.0 mg/L as P

2.4. CALIBRATION AND STANDARDIZATION:

Internal calibration table in HACH 2010 must be confirmed with at least three standards usually 1.0, 0.50, and 0.10 mg/L and make curve using %T or Abs. Verify calibration for each batch with 0.10 or 0.33 mg/L CCV.

2.5. POLLUTION PREVENTION:

Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The USEPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option. The quantity of chemicals purchased should be based on expected usage during its shelf life and disposal cost of unused

material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability. For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Management for Waster Reduction," available from the American Chemical Society's Department of Government Regulations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036, (202) 872-4477. VEL pollution prevention is described in SOP 0003.

2.6. WASTE MANAGEMENT:

Due to the nature of this method there is little need for waste management. No large volumes of solvents or hazardous chemicals are used. However, the U.S. Environmental Protection Agency requires that laboratory waste management practices conducted be consistent with all applicable rules and regulations. Excess reagents, samples, and method process wastes should be characterized and disposed of in an acceptable manner. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with all solid and hazardous waste regulations, particularly hazardous waste identification rules and land disposal restrictions. For further information on waste management consult the "Waste Management Manual for Laboratory Personnel," available from the American Chemical Society. VEL waste management policy described in SOP 0003.

3) SAMPLE HANDLING

3.0. COLLECTION / SHIPMENT:

Minimum sample needed:	20 mL
Normal size/type bottle used:	Pyrex Glass or Plastic (acid cleaned)
Preservation used before sampling:	2 mL conc. Sulfuric acid/L
Preservation needed after sampling:	4^{o}C
Must be shipped at 4 C?	YES
Other Special Requirements:	NA

3.1. HOLDING TIMES:

Holding time for analysis:	28 days

3.2. STORAGE: Store at 4^{o}C

4) QUALITY CONTROL – Standard QC as described in SOP 0004:
3 standards or CCV at MRL (0.1 mg/L); LCS spike (1/batch); LMB (1/batch); dup (10%)
Curve > 0.95 r^2; CCV +/- 20%; LCS +/- 20%; LMB < MRL; dup within 10%

5) PROCEDURE

Standards are prepared by diluting 2 mL of the 50 mg/L stock solution to 100 mL for 1.0 mg/L, then volumetrically diluting 5 & 25 mL to 50 mL to make 0.1 and 0.5 mg/L standards. Prepare a blank from distilled water. Digest by persulfate method along with samples.

Rinse any glassware used with dilute NaOH before having them washed. Pipette 5 mL of sample into HACH Test 'N Tube. Digest standards, blank, and samples @ 150^{o}C for 30 minutes in COD reactor that was preheated. Remove from reactor and allow cooling to room temperature. Add 2.0 mL of 1.54N NaOH to each tube. For each sample, place tube holder in HACH 2010, place tube in holder, place cap on tube holder and hit zero. Add PhosVer 3 PP, shake and let color develop for 2-8 minutes, place tube back in tube holder, cap and read and record mg/L, %T and Abs. @ 890nm no later than 8 minutes after adding the color reagent.

6) CALCULATIONS

HACH internal calibration or linear curve. Adjust for dilutions. Samples must be diluted before digestion if necessary.

7) METHOD PERFORMANCE

MDL - 0.03 mg/L (BKO)

8) CORRECTIVE ACTIONS - SEE SOP 0007

9) REFERENCES

HACH 8190

EPA 365.2

Appendix 6. Yakima Valley Labs Standard Operating Procedures for Orthophosphate Analysis.

SOP 4500P

ORTHOPHOSPHATE

Revision Number: 1.0

Effective Date of SOP: 03/10/2004

Approved: Bennett K. Osborne, Laboratory Director

1) SOP – GENERAL

Orthophosphate (Reactive Phosphorus)

1.1. METHOD(S):

SM 4500P E; Hach 8048

1.2. SUMMARY:

Orthophosphate is determined through a reaction with ammonium molybdate and potassium antimonyl tartrate to form phosphomolybdic acid, which is reduced to molybdenum blue by ascorbic acid and read at 890 nm.

1.3. SCOPE AND APPLICATION:

This method is suitable for orthophosphate concentrations as low as 10 μg/L under ideal conditions in drinking and surface waters or domestic and industrial wastes.

1.4. DEFINTIONS:

Orthophosphate (o-PO_4) as P is the term for the units reported in mg/L.
Other definitions of terms are found in SOP 0099.

2) SOP – SPECIFIC

2.0. INTERFERENCES:

Arsenates react with the reagent occasionally to produce a blue color similar to orthophosphate. Nitrite and Cr (VI) can produce interferences if greater than 1 mg/L.

2.1. SAFETY:

Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials or procedures. VEL has a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) is made available to all personnel involved in the chemical analysis. All applicable components of the VEL Safety Manual shall be strictly adhered to.

EQUIPMENT AND SUPPLIES:

Hach 2010 Spectrophotometer with 10 mL sample cells

2.3. REAGENTS AND STANDARDS:

Distilled Water – Culligan Distilled Water has been certified.
Color Reagent – HACH PhosVer 3 Phosphate Powder Pillow (PP)
Orthophosphate Standard Solution – 50 mg/L as PO_4^{3-} HACH

2.4. CALIBRATION AND STANDARDIZATION:

Run 3 standards at levels of 0.50, 0.10 and 0.05 mg/L and record %T and Abs. Create curve on Excel spreadsheet or use internal calibration in Hach 2010. Verify calibration by analyzing a second source QC sample with each batch. (+/- 20%)

2.5. POLLUTION PREVENTION:

There are no known hazardous chemicals used in this procedure that cannot be poured down the drain. VEL pollution prevention is described in SOP 0003.

2.6. WASTE MANAGEMENT:

Very little sample and chemicals are used in this procedure. No specific waste management procedures required. VEL waste management policy described in SOP 0003.

3) SAMPLE HANDLING

3.0. COLLECTION / SHIPMENT:

Minimum sample needed:	20 mL
Normal size/type bottle used:	Plastic or Glass
Preservation used before sampling:	None
Preservation needed after sampling:	4°C
Must be shipped at 4°C?	Yes
Other Special Requirements:	N/A

3.1. HOLDING TIMES

Holding time for analysis: 48 hours

3.2. STORAGE: Store at 4°C.

4) QUALITY CONTROL – See SOP 0004

5) PROCEDURE

Turn on HACH 2010 and enter 490 for the program number.

Rotate the wavelength dial until the display shows 890nm. Approach wavelength from higher number.

For each sample, blank, and standard:

Fill one 10 mL sample cell with 10 mL of sample
Add the contents of one PhosVer 3 Phosphate PP
Shake the cell with stopper in place and start timer for 2 minutes
Fill the second matched cell with 10 mL of fresh sample and place into cell holder of 2010
Press Zero
Remove stopper from sample and place the prepared sample into cell holder of 2010.
Press Read.
Read and record spec conc, %T and Abs. at 890 nm within 20 minutes of addition of PP.

6) CALCULATIONS

6.0. Calculate concentration of Orthophosphate in the with a linear calibration curve by plotting the absorbance value of standards versus the corresponding Orthophosphate concentrations. Obtain concentration value from standard linear curve or read directly from HACH 2010 if all QC is OK.

7) METHOD PERFORMANCE

MCL = N/A
Reporting Limit = 0.1 mg/l (SRL is 0.1 mg/L)
MDL = 0.01 mg/l
Accuracy = 99.9%
Precision (standard deviation) = 0.0001 mg/L

8) CORRECTIVE ACTIONS

8.0. REPLICATES:

If the acceptance criteria are not met, repeat the replicates. If the problem continues, identify the source of the problem and correct.

CALIBRATION DRIFT CHECK STANDARD:

If the control criteria are not met, the standard analysis must be repeated. If the standard still does not meet the criteria the entire standardization procedure is to be repeated.

8.1. Procedures for out-of-control QC or QA – SEE SOP 0004

8.2. Corrective actions for out-of-control data – SEE SOP 0004

8.3. Contingencies for handling unacceptable data – SEE SOP 0004

9) REFERENCES

Standard Methods for the Examination of Water and Wastewater, 20th Edition, 4-146-147, Method 4500P E (1997).

Appendix 7. Criteria used to qualify and quantify habitat in Lower Crab Creek, Washington.

Habitat	From Hawkins et al. 1993		
Level 1	Level 2	Level 3	
Fast water	Turbulent	Cascade	A riffle with a stream gradient > 10%
		Rapid	A riffle with a stream gradient < 10-3%
		Riffle	Riffle with stream gradient less than 3%
	Non-turbulent	Run/Glide	Laminar flow, homogenous
		Sheet	Very shallow with bedroock or hardpan substrate
Slow water / Pool	Dam	Beaver	Beaver dam
		Debris	Debris dam creates pool
		Landslide	Landslide creates pool
	Scour	Convergence	Converging tributary
		Lateral Scour	Water flows into bank causing scour
		Mid-Channel	Partial stream blockage creates pool
		Plunge	Waterfall
		Trench	Where a trench is located in the middle of another habitat unit

The Process

1. Randomly select 50 m site within each reach
2. Catalog habitat type within each site and record length of each habitat type.
3. Collect depths at each 50m interval starting at 0 m.
4. Depth transects consist of 3 measurements. Middle then half the distance to each shore.

Substrate	
Type	Size
sand	<2 mm
gravel	2-64 mm
cobble	64-256 mm
boulder	256-4,096 mm
bedrock	> 4,096 mm

Width

Channel width: width of creek where water travels unblocked
Wetted Width: Entire width of stream what flows through. Includes flow through brush etc.
Bankfull: Width of stream that occurs during high flow.
Flood plain: Width measured at two times the Bankfull depth

Reach: Static. 29 reaches within Lower Crab
Sites: Randomly selected within each reach. A site is 100 meters long

Flows at each transect taken at 60% depth

Appendix 8. Fish Species Common and Scientific Names.

Black Crappie	*Pomoxis nigromaculatus*
Bluegill	*Lepomis macrochirus*
Bullhead spp.	*Ameiurus* spp.
Bridgelip Sucker	*Catostomus columbianus*
Burbot	*Lota lota*
Channel Catfish	*Ictalurus punctatus*
Chiselmouth	*Acrocheilus alutaceus*
Coho Salmon	*Oncorhynchus kisutch*
Grass Carp	*Ctenopharyngodon idella*
Largescale Sucker	*Catostomus macrocheilus*
Longnose Sucker	*Catostomus catostomus*
Mosquitofish	*Gambusia affinis*
Northern Pikeminnow	*Ptychocheilus oregonensis*
Peamouth	*Mylocheilus caurinus*
Pumpkinseed	*Lepomis gibbosus*
Redside Shiner	*Richardsonius balteatus*
Sculpin spp.	*Cottus* spp.
Sucker spp.	*Catostomus* spp.
Threespine Stickleback	*Gasterosteus aculeatus*
White Crappie	*Pomoxis annularis*

Appendix 9. Major Chinook salmon spawning locations within the Lower Crab Creek drainage observed during fall 2008. GPS coordinates in UTM, map datum WGS 84.

Stream	GPS location of Redd Sites
Lower Crab Creek	0290701 / 5190305
Lower Crab Creek	0279002 / 5190006
Red Rock Coulee	0302016 / 5192184
Red Rock Coulee	0302044 / 5192226
Red Rock Coulee	0302051 / 5192284
Red Rock Coulee	0302049 / 5192311
Red Rock Coulee	0301983 / 5192456
Red Rock Coulee	0301972 / 5192489
Red Rock Coulee	0301911 / 5192662
Red Rock Coulee	0301905 / 5192778
Red Rock Coulee	0301688 / 5192594
Red Rock Coulee	0301748 / 5193671
Red Rock Coulee	0301761 / 5193720
Red Rock Coulee	0301856 / 5193928
Red Rock Coulee	0301889 / 5193952
Red Rock Coulee	0301939 / 5194022
Red Rock Coulee	0301969 / 5194119

Appendix 10. LCC genetics paper in print.

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SPECIAL SECTION: GENETIC ADAPTATION

Does Lower Crab Creek in the Eastern Washington Desert Have a Native Population of Chinook Salmon?

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Abstract

Lower Crab Creek (LCC) in eastern Washington is a groundwater-fed tributary to the Columbia River at river kilometer 661. The creek traverses agriculturally modified desert habitat, and in several reaches the water quality is poor, summer water temperatures are lethal to fish, and stream habitat is degraded. The creek was thought to be unsuitable for salmonids, yet fall-run Chinook salmon *Oncorhynchus tshawytscha* spawn and rear in it. The origin of these fish is uncertain since it is unclear whether LCC was a perennial creek prior to the hydrologic changes in the Columbia River basin stemming from the Columbia Basin Irrigation Project in the 1950s. We used genetic analyses to examine the hypotheses that these fish are (1) random strays, (2) a population founded since the 1950s (by wild or hatchery strays), or (3) an established native population. Chinook salmon juveniles and adults were collected in LCC and genetically characterized with the Chinook salmon microsatellite DNA locus suite in the Genetic Analysis of Pacific Salmonids database. The LCC population of Chinook salmon was found to be significantly different from interior Columbia River basin hatchery and wild populations and to have alleles that are absent from proximal populations. Lower Crab Creek Chinook salmon showed no evidence of recent founding and had genetic diversity, allelic richness, and effective population size similar to those of other Columbia River basin Chinook salmon. The data suggest that a genetically distinct, native population of Chinook salmon inhabits LCC that has adapted to the rigorous environment.

The shrub-steppe desert of the Columbia River Plateau in eastern Washington is a large basin of plateau basalts overlain by Pleistocene-origin sediments. The basalt aquifer supports Crab Creek, the only natural perennial stream flowing through the desert basin to its confluence with the Columba River at river kilometer (rkm) 661 (Figure 1). The fish assemblage in the creek includes Chinook salmon

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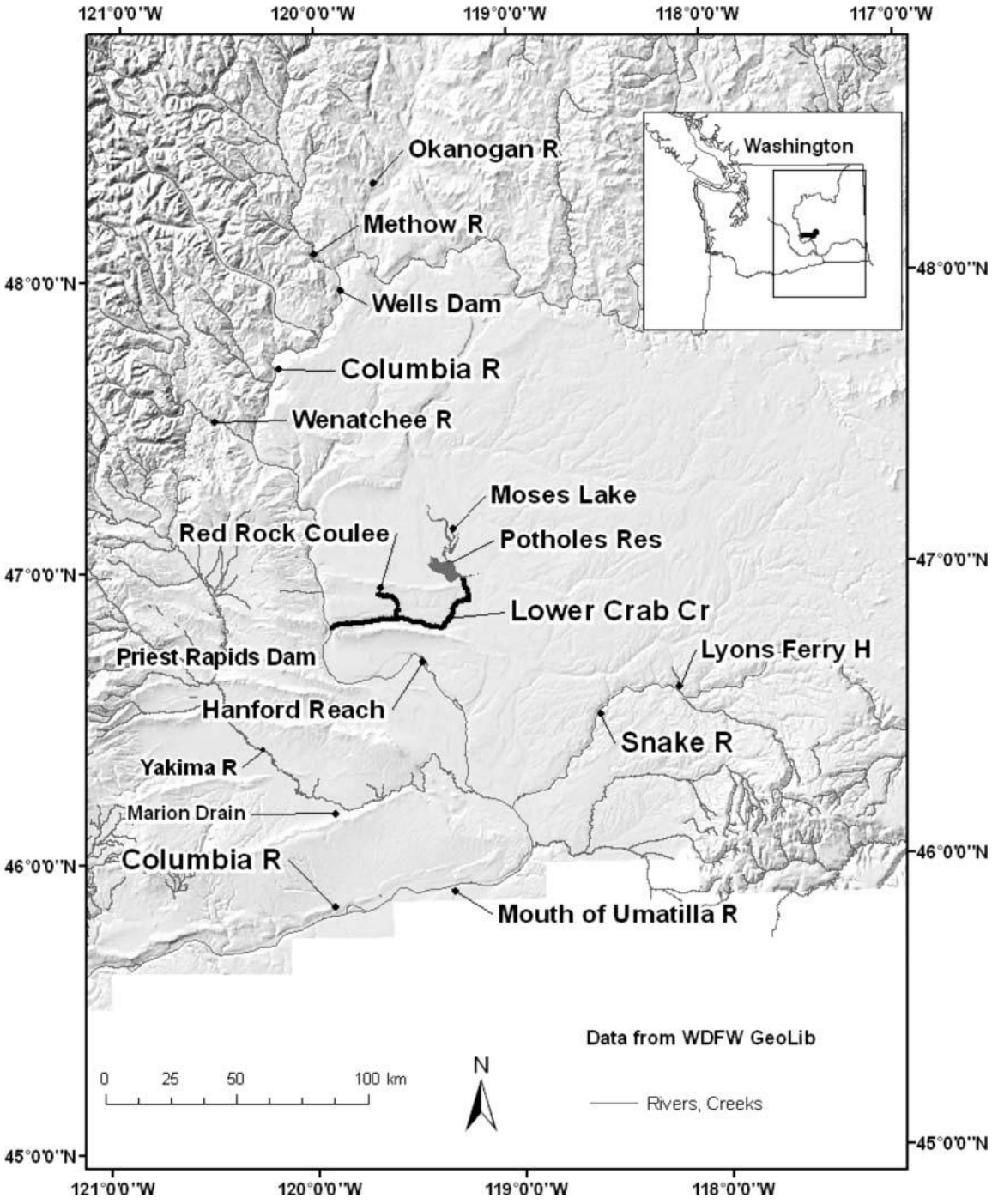


FIGURE 1. Map of the Crab Creek study area. Lower Crab Creek and Red Rock Coulee are highlighted in black below Potholes Reservoir. The tributaries and main stems where the Chinook salmon collections in the GAPS data set were made are labeled.

Oncorhynchus tshawytscha as well as coho salmon *O. kisutch* (Washington Department of Fish and Wildlife [WDFW], unpublished data) and steelhead) and steelhead *O. mykiss* (Bowen et al. 2003; WDFW, unpublished data). Although Chinook salmon are known to spawn and rear in a variety of habitats in various ecoregions (Waknitz et al. 1995; Myers at al. 1998; Waples et al. 2004), the presence of Chinook salmon in Crab Creek is interesting because the habitat was thought to be

unsuitable for salmonids (detailed below). However, anthropogenic changes to the environment of the greater Columbia River basin during the mid-1900s may have fostered Chinook salmon colonization in Lower Crab Creek by providing spawning habitat (KWA 2004) for natural-origin strays or strays from nearby hatchery programs. In this study, we explored whether the Chinook salmon in Lower Crab Creek recently established themselves following anthropogenically induced environmental changes in the Columbia River basin or are a locally adapted native spawning group.

The Crab Creek drainage was created during the Pleistocene Epoch by catastrophic outflows of proglacial lakes that formed when continental glaciers blocked the Columbia River (USGS 2010a). The historical flow of the creek is uncertain since shifting sands periodically blocked at least portions of the creek. Because the creek was a perennial source of water in the desert, Native American activities were centered in the area (KWA 2004). European settlers in the mid-1800s reported native trout and northern pikeminnow *Ptychocheilus oregonensis* in Crab Creek (Strong 1906, cited within KWA 2004), indicating that there was flow out to the Columbia River (which was the source of colonizing native fish). Currently, Lower Crab Creek (LCC) starts as multiple seeps below O'Sullivan Dam, which impounds Potholes Reservoir, which in turn stores water for the Columbia Basin Irrigation Project (CBIP). Lower Crab Creek, a low-gradient drainage, flows roughly 60 km westward through both federal refuge land and substantial agricultural lands to the Columbia River. Over the first 29 km from its confluence with the Columbia River it traverses an undisturbed area supporting an intact riparian zone. However, from rkm 30 to rkm 43 LCC is contained in irrigation ditches and in this segment water quality decreases, sediment load increases, and turbidity decreases visibility to roughly 20 cm. Owing to the relatively degraded nature of LCC and localized high summer water temperatures (up to 26.3°C) that are lethal to salmon (Scott and Crossman 1973), a naturally producing run of Chinook salmon was considered unlikely. The LCC reach is the only portion of Crab Creek accessible to anadromous fish and is influenced by the CBIP and other agricultural practices. When the irrigation season begins, water tables throughout the project area rise via groundwater seepage (KWA 2004), and variations in LCC flow can be attributed to water operations (USGS 2010b). KWA Ecological Sciences hypothesized that Chinook salmon spawning habitat developed in LCC in the 1950s because of increased groundwater from the CBIP (KWA 2004). The Chinook salmon reported historically in LCC were thus thought to be strays that were unsuccessful due to poor habitat. However, Chinook salmon redds were documented in Red Rock Coulee, a tributary of LCC with good spawning habitat (Bowen et al. 2003), and Chinook salmon smolts have been captured in LCC (this study).

Chinook salmon have adapted to a range of environmental conditions and this adaptability may have fostered their establishment and survival in LCC. Chinook salmon express various complex life histories (Myers et al. 1998), including different return times (spring, summer, fall, and winter) and juvenile rearing strategies (ocean-type individuals out-migrate as subyearlings and stream-type individuals as yearlings; Healey 1991). Run timing and juvenile rearing were included as criteria with which to delineate the five Chinook salmon evolutionarily significant units (ESUs) in the interior Columbia River basin east of the Cascade Mountain crest (Myers et al. 1998). The summer and fall runs in the Columbia River basin (the Chinook salmon populations nearest to LCC and of same putative run type) and the fall run in the Snake River basin have ocean-type life histories, and the spring run in the Columbia River basin and the summer run in the Snake River basin have stream-type life histories (Myers et al. 1998). While juvenile rearing is a key feature of Chinook salmon ESUs, the trait has some plasticity. In some basins, juveniles out-migrate both as subyearlings and yearlings; the proportions vary with environmental conditions, warmer, more productive waters promoting subyearling migration (Myers et al. 1998; Connor et al. 2002). However, environmental conditions in the Columbia River basin changed throughout the 1900s; human activities impacted Chinook salmon population structure and ecology when main-stem and tributary dams blocked access to spawning areas and created a series of impoundments. Salmon hatcheries were built to mitigate salmonid losses due to dams, and hatchery salmon were released below dams, often in great quantities. In particular, Priest Rapids Hatchery downstream of LCC (Figure 1), constructed in 1963, releases an average of 6.6 million fall-run Chinook salmon subyearlings (HSRG 2009). Priest Rapids Hatchery fish constitute up to 33% of the fish spawning naturally in the Hanford Reach (Figure 1), a productive main-stem spawning reach for fall-run Chinook salmon near LCC (Evenson et al. 2002). Wells Hatchery upstream from LCC produces over 2 million juvenile summer Chinook salmon (Appleby et al. 2010). Under some conditions, hatchery fish stray into

natural spawning areas (Quinn et al. 1991; Crateau 1997), and hatcheries in the Columbia River basin above and below LCC are potential sources of hatchery strays.

Chinook salmon use LCC despite its poor habitat, but the status of their spawning aggregate is uncertain (Appleby et al. 2010). We examined three hypotheses for this aggregate: (1) the Chinook salmon spawning in LCC are random strays rather than an established population, (2) the aggregation was founded by a nearby natural spawning population or hatchery strays when flow increased after 1950, and (3) a native spawning aggregate was established before anthropogenic changes to the Columbia River basin. To address these hypotheses, juvenile and adult Chinook salmon were collected in LCC over 2 years and compared genetically to samples of proximal natural and hatchery Chinook salmon populations available in the Genetic Analysis of Pacific Salmonids (GAPS; see Seeb et al. 2007) database (data courtesy of Scott Blankenship, WDFW). We considered genetic evidence and adult and juvenile life history information to characterize the LCC Chinook salmon spawning aggregate.

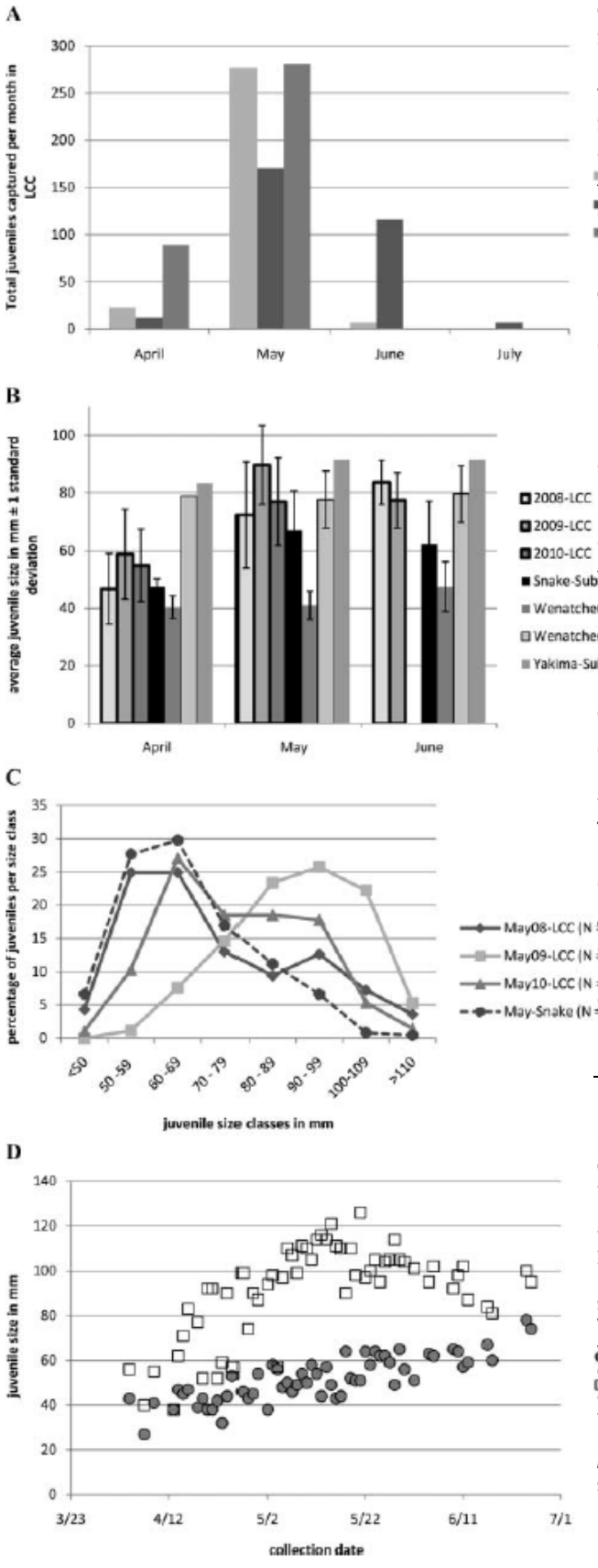
METHODS

The high turbidity in LCC made sampling difficult. Tissue samples (fin clips) were collected from Chinook salmon (Table 1) in 2008 (N = 78) [mixed adults and juveniles]) and 2009 (N = 292) [juveniles only]) in LCC (see Figure 1). Juveniles were collected from April to July (Figure 2) using an E. G. Solutions rotary screw trap modified to allow cleaning of the high-debris load. Adult samples were collected from September to November from carcass surveys throughout LCC and the capture of live adults at a modified resistance board weir trap. We removed carcass tails to identified sampled

TABLE 1. Genetic statistics for fall- and summer-run collections of Chinook salmon from Lower Crab Creek and the interior Columbia River basin. The river kilometers (rkm) column shows the approximate distance from Lower Crab Creek to the river mouth, hatchery, or main-stem reach. Gene diversity (Gene div) is the expected heterozygosity corrected for collection size. Allelic richness (Rich) is the average number of alleles per locus corrected for collection size and scaled to 54 individuals, the minimum number of individuals with complete genotypes in a collection. The Hardy-Weinberg equilibrium value, as expressed by F_{IS}, was calculated over 13 loci; significant differences from zero were calculated with 1,000 permutations (significance before and after Bonferroni correction is indicated by bold italics and underlining, respectively). Garza and Williamson's (2001) M ratio and its variance were calculated over 11 loci, the number of loci with a 2-base-pair or greater repeat unit. Linkage disequilibrium (Link) is the number of locus pairs (out of 78) in 1,000 permutations in which 5% or fewer of the permuted linkage disequilibrium values were greater than the actual value (we also report the number for 1% of the permuted linkage disequilibrium values since there was no Bonferroni correction for multiple tests). The effective number of breeders (N_b) and its 95% confidence interval (CI) were calculated using a linkage disequilibrium method excluding alleles below a frequency of 0.02.

									Link			
Collection	N	rkm	Gene div	Rich	F_{IS}	P-value	M ratio	M variance	5%	1%	N_b (0.02)	95% CI
Lower Crab	370	0	0.8675	19.12	***0.020***	0.0001	0.818	0.028	68	41	170[a]	158–184
Hanford Reach	195	80	0.8744	21.20	***0.010***	0.0459	0.846	0.083	4	3	297	263–339
Priest Rapids Hatchery	81	23	0.8724	20.60	0.015	0.0747	0.744	0.067	3	1	248	195–336
Lower Yakima	103	122	0.8719	20.18	0.000	0.5123	0.819	0.022	8	2	154	132–181
Umatilla Hatchery	96	194	0.8693	21.11	–0.003	0.6208	0.770	0.073	8	3	240	198–300
Wenatchee	135	93	0.8512	19.19	0.005	0.2771	0.834	0.028	5	4	294	237–381
Methow	121	182	0.8581	19.55	–0.017	0.9778	0.818	0.010	5	1	193	169–223
Wells Hatchery	144	200	0.8575	19.54	–0.017	0.9807	0.854	0.012	5	1	272	229–332
Okanogan	91	198	0.8566	19.95	–0.009	0.8199	0.818	0.018	4	2	2,775	798–∞[b]
Marion Drain	190	250	0.8782	19.82	<u>0.022</u>	0.0008	0.874	0.004	13	4	189	171–211
Lyons Ferry Hatchery	186	228	0.8641	19.46	0.013	0.0329	0.840	0.020	5	1	539	427–721
Snake	210	138	0.8708	20.53	–0.001	0.6291	0.814	0.081	11	4	586	478–736
Average			0.8660	20.02								

[a] Only juvenile samples were used for the calculation of N_b in Lower Crab Creek population.
[b] The confidence interval overlapped infinity.



carcasses and prevent resampling. During sampling, we recorded biological data, including length and sex. Implementing the adult trap was complicated by the sandy nature of the substrate (the trap was undermined repeatedly and captured adults only after modifications). Although these were large fish spawning in a small creek, the spawning surveys attempted throughout LCC were mostly foiled by turbidity (the exception was Red Rock Coulee, which has clear water.)

Genotyping.—Tissues were genotyped at the 13 standardized loci for Chinook salmon (GAPS loci; Seeb et al. 2007; Table 2). Genomic DNA was extracted from tissue using silica membrane kits (Macherey-Nagel). Microsatellite alleles were amplified by polymerase chain reaction (PCR) using fluorescently labeled primers (see Table 2 for detailed PCR information). The PCRs were conducted in 5-µL volumes employing 1 µL of template with final concentrations of 1.5 mM $MgCl_2$, 200 µM of each deoxynucleotide triphosphate, and 1 x Promega PCR buffer and followed a "touch-down" protocol. After an initial 2 min of denaturing at 94°C, there were three cycles of 94°C for 30 s, annealing at 60°C (temperature stepped down 1°C each cycle) for 30 x, and extension at 72°C for 60 s. These were followed by 36 or 39 (see Table 2) cycles of 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 60 s, then a final 10-min extension at 72°C. Microsatellites were detected using an ABI 3730 automated DNA analyzer, and alleles were sized (to base pairs) and binned using an internal lane size standard (GS500Liz from Applied Biosystems) and GeneMapper software (Applied Biosystems).

FIGURE 2. Panel (**a**) shows total captures of juvenile fall Chinook salmon in Lower Crab Creek per capture month over the period 2008-2010. Panel (**b**) shows the average ± SD (where SD is available) sizes of juvenile fall Chinook salmon in Lower Crab Creek (2008-2010), the Snake River (2005-2006), the Wenatchee River (2005-2010), and the Yakima River (1983-1985). The designation "sub" indicates subyearlings and the designation "year" yearlings. In the Wenatchee River data, fewer than 1% of the juveniles were yearlings. Year data are presented separately for Lower Crab Creek; years were combined for the Snake, Wenatchee, and Yakima rivers. Panel (**c**) shows the number of juvenile fall Chinook salmon per size category that were collected in Lower Crab Creek in May 2008-2010 and the Snake River in May 2005-2006. Panel (**d**) shows the minimum and maximum sizes of juvenile fall Chinook salmon per collection day in Lower Crab Creek with 3 years of data combined.

We employed genotypic data from the GAPS database for 11 proximal Chinook salmon collections in our genetic comparisons (see Table 1). All collections were from populations that have summer or fall adult return timing and were from the hatcheries and tributaries that are geographically closest to LCC. We used these collections to test the three hypotheses described above regarding the origin of the LCC Chinook salmon population.

Genetic Analysis.—We tested loci for selective neutrality with an F_{ST} outlier test implemented in ARLEQUIN 3.5.1.2 (Excoffier et al. 2009). We calculated basic genetic statistics to assess whether the LCC Chinook salmon samples were collected from a single, randomly mating population and to compare our collections with other Chinook salmon collections from the interior Columbia River basin. We used FSTAT 2.9.3 (Goudet 2001) to conduct statistical tests on data per locus and across all loci for conformation to Hardy-Weinberg expectations of genotypic equilibrium (HWE), and expressed by F^{IS} values. Tests for HWE evaluate significant deviations from expected heterozygosity within populations, which may indicate that individuals are from different populations or family groups, that there are large-sized or null alleles that failed to amplify in the PCR, or that there is nonrandom mating in a population (such as breeding among related individuals). We assessed linkage disequilibrium (nonrandom genotypic associations between all possible pairs of loci) using GENETIX (Belkhir et al. 2004). Linkage disequilibrium may arise if the collection included family groups or migrants from another population or there is nonrandom mating or drift due to small population size. We calculated diversity measures, including allelic richness (the average number of alleles corrected for sample size) and gene diversity (expected heterozygosity corrected for sample size) using FSTAT. Genetic diversity provides an indication of the genetic biodiversity in a population and, in general, small and isolated populations are less diverse. AGARST (Harley 2001) was used to calculate Garza and Williamson's (2001) *M* ratio as an estimator of recent population bottlenecks (within about 50 generations). This ratio is the ratio of the number of alleles to the size range of alleles across multiple loci. If alleles are missing from the full range of alleles, this can be an indication that the population lost low-frequency alleles by chance during a population bottleneck, such as a founding event.

Since most of the LCC collection consisted of juveniles, we estimated family relationships (full and half siblings; Wang 2009; Wang and Santure 2009) using COLONY 2.0.0.0 (Wang 2008 http://www.zsl.org/science/research/software/). We also calculated the effective number of breeders (N_b) that produced the fish in each Chinook salmon collection using a linkage disequilibrium method (Waples 2006) implemented in the program LDNe (Waples and Do 2008).

TABLE 2. Information for multiplexes and loci, including annealing temperature, number of cycles at each temperature, and primer concentration for GAPS microsatellite DNA loci. Multiplex names (e.g., *Ots-M*) are internal laboratory designations for the combining of specific microsatellite loci within a single PCR reaction. References for primer sequences are given in the last column.

Multiplex	Locus	Annealing temperature (cycles)	Concentration (μM)	Reference
Ots-M	*Ots-201b*	60°C (3×); 50°C (36×)	0.35	M. Banks, Oregon State University, unpublished
	Ots-208b		0.20	Grieg et al. (2003)
	Ssa-408		0.20	Cairney et al. (2000)
Ots-N	*Ogo-2*	60°C (3×); 50°C (36×)	0.15	Olsen et al. (1998)
Ots-O	*Ogo-4*	60°C (3×); 50°C (39×)	0.18	Olsen et al. (1998)
	Ots-213		0.18	Grieg et al. (2003)
	Ots-G474		0.16	Williamson et al. (2002)
Ots-R	*Omm-1080*	60°C (3×); 50°C (36×)	0.26	Rexroad et al. (2001)
	Ots-3M		0.12	Banks et al. (1999)
Ots-S	*Ots-212*	60°C (3×); 50°C (36×)	0.30	Grieg et al. (2003)
	Ots-9		0.10	Banks et al. (1999)
Ots-T	*Oki-100*	60°C (3×); 50°C (39×)	0.40	K. Miller, Department of Fish and Oceans, unpublished
	Ots-211		0.38	Grieg et al. (2003)

We used pairwise F_{ST} tests in FSTAT to compare LCC Chinook salmon with proximal Chinook salmon. The tests calculated the amount of genetic variance between two collections in comparison with the total genetic variance in the data set and assessed whether the variance was significantly greater than zero with 1,000 permutations. Pairwise genotypic tests examined whether genotypic distributions were significantly different between collections and assessed the significance with 1,000 permutations. Significant genetic differences arise when populations are spatially or temporally isolated and exchange few to no spawners per generation. We viewed the genetic differences among Columbia River basin Chinook salmon collections in a factorial correspondence analysis (FCA) plot implemented in the program GENETIX (Belkhir et al. 2004). The FCA seeks the combination of allele frequencies that describes the most genetic variation among individuals. The program generates a plot of individuals in three dimensions according to their genotype (the first two dimensions generally encompass most of the genetic variance). To view population centers, the program calculated the genetic center (centroid) of each collection (the *sur populations* option in GENETIX).

We used self-assignment tests in GeneClass2 (Piry et al. 2004) to calculate the likelihood that the Chinook salmon collected in LCC originated in a spawning population located in the creek or were strays that originated in other populations. In this analysis the baseline included Chinook salmon collections from proximal tributaries and hatcheries and the LCC collection. Using the Rannala and Mountain (1997) algorithm, each fish was assigned a likelihood of being observed in each baseline collection based on the genotype of the fish and the allele frequencies of the baseline collections (each fish was removed in turn from its home collection during estimation). The collection with the highest likelihood was presented as the assignment. Relative likelihoods were constructed that were ratios of the highest likelihood over the sum of the likelihoods. If the relative likelihood for the highest assignment was at least 90%, the assignment was rated positive and unambiguous. If the assignment was highest but less than 90%, it was considered positive but ambiguous. We present results with and without the 90% relative likelihood criterion.

We also conducted a first-generation migrant analysis (Paetkau et al. 2004) to determine whether the LCC Chinook salmon collection included recent descendants of migrants from other populations. Using the Rannala and Mountain (1997) algorithm, GeneClass2 calculated the likelihood of drawing an individual's genotype from the collection where it was sampled based on the allele frequencies of the baseline collections (with the individual in question removed) and divided this likelihood by the highest assignment likelihood to any baseline collection (Paetkau et al. 2004). The program then computed the probability that each individual was a resident in its home collection with a Monte Carlo simulation. The simulation created 10,000 individuals for each baseline collection to simulate the genotypes likely to be encountered for that collection. Then it computed assignment likelihoods for the simulated individuals and compared the likelihood of the genotype of the actual fish being tested to the distribution of the likelihoods for their simulated home collection. Fish were hypothesized to be first-generation migrants if their probability of originating in their home collection was represented in less than 1% of the simulated values of their home collection.

RESULTS

Juvenile and adult Chinook salmon were captured over 2 years in LCC during downstream and upstream migrations in the spring and fall, respectively. The capture of juveniles with the screw trap was complicated by turbidity and debris, and when we were testing the efficiency of the screw trap high temperatures killed fish held for upstream release and recapture. In 2008, high mortalities precluded trapping past early June. In 2009, modifications to the screw trap allowed trapping to continue into early July. Despite the challenges, screw trap efficiency was calculated at roughly 8% and productivity estimates are planned for future research. In light of the trapping difficulties, we present and interpret data cautiously; juvenile captures were highest in May (Figure 2a) suggesting that juveniles out-migrated in late spring. In May 2008, juvenile size was 72 ± 18.3 mm (mean ± SD; Figure 2b); the size distribution was somewhat bimodal, with peaks around 60 and 100 mm (Figure 2c). In May 2009, juvenile size was 90 ± 13.7 mm; juveniles larger than 90 mm (largest size, 121 mm) were most abundant, with no bimodal distribution being apparent. We also had size data for juveniles captured in May 2010: juvenile size was 77 ± 15.2 mm, and size distribution was somewhat bimodal, with peaks around 60 and 90 mm. The differences in mean size and variance were significant in comparisons between May 2008 and May 2009 and between May 2008 and May 2010 ($P < 0.01$ for Student's *t*-tests

and *F*-tests). Mean size was significantly larger in May 2009 than in May 2010 (Student's *t*-test; $P < 0.01$), but the variance was not significantly different (*F*-test; $P = 0.16$). The difference could be due to sampling or environmental variation. Combining the juvenile size data and plotting the minimum and maximum sizes per day produces a bimodal size distribution with an average difference of 39 mm (Figure 2d).

We compared the LCC juvenile size data with data for juvenile fall Chinook salmon collected from April to June in the Snake River (Anne Marshall [WDFW] and William Connor [U.S. Fish and Wildlife Service], unpublished data), the Wenatchee River (Steve Schonning [WDFW], unpublished data), and the Yakima River (Fast et al. 1986). The average size of Snake River fish in May was significantly less than that of LCC fish for each collection year (Student's *t*-test; all $P < 0.01$). The size distribution of Snake River juveniles peaked around 60 mm, and there were few larger-sized individuals (Figure 2c). Wenatchee River juveniles were collected from 2005 to 2010 and sorted into subyearling ($N = 14,279$) and yearling ($N = 89$) categories using scale data. The subyearlings were roughly 35 mm smaller than the yearlings; in May the subyearlings were 41 ± 4.9 mm long and the yearlings 78 ± 9.9 mm (Figure 2b). Because 84% of the Wenatchee River juveniles were smaller than 50 mm, their size distributions were not compared graphically. In the Yakima River, two size-classes of Chinook salmon juveniles were intercepted at Prosser Dam in 1983-1985; the smaller ones (May average = 91.7 mm; no discrete size data available) were classified as fall subyearlings and the larger ones (May average = 129 mm) as spring yearlings (Fast et al. 1986). Thus, if any Yakima River fall Chinook salmon juveniles out-migrated as yearlings they would have been classified as spring Chinook salmon based on size. The abundance of larger-size-class juveniles in LCC (at least 20% each year; Figure 2c) and the difference between the daily minimum and maximum sizes suggests that it is common for LCC juveniles to overwinter and migrate as yearlings.

Adult capture and detection were compromised by turbidity. Tagging efforts failed to estimate adult capture efficiency at the weir since visibility was often less than 5 cm and tagged fish were rarely reencountered (the exception was two recaptures in Red Rock Coulee). Efficiency was presumed to be low since fluctuating water levels allowed some fish to bypass the weir. After armoring the sandy banks with hundreds of sandbags and increasing the number of weir panels, we captured 28 Chinook salmon in 2008 and 52 in 2009, with the run peaking at the end of October and beginning of November. The weir also trapped three spawner carcasses that washed downstream in 2008. Low visibility in most of LCC compromised spawning surveys and redd counts (in some areas swirling, turbid water suggested fish movements but these were unconfirmed visually). Consequently, we focused the majority of our efforts on carcass collection and spawning surveys in Red Rock Coulee, a tributary to LCC and the only spawning area with clear water. During the 2007-2009 fall spawning surveys, we counted as many as 76 individual Chinook salmon on a given survey day. We also conducted fall redd counts, but individual redds were often difficult to distinguish owing to superimposition in the narrow stream channel. In 2007, we identified nine redd clusters that consisted of 17 individual redds. In 2008, we located two regions of spawning activity in LCC and 14 redd clusters with multiple superimposed redds. In 2009, we located 14 redd clusters that included 7 individual redds.

The F_{ST} outlier test indicated that no loci were under selection (all *P*-values > 0.05). The LCC Chinook salmon collection had seven unique alleles (data not shown) in comparison with the 11 proximal Chinook salmon collections used in this study. Six of these alleles were present in other Chinook salmon GAPS database collections that were not used for comparisons. One allele at *Ots-208b* (allele 336) in the LCC collection was unique to the sanctioned GAPS allele data set; this allele may be found in other Chinook salmon populations but as yet is unsanctioned by the curator for the locus *Ots-208b*, underscoring its rarity. (The GAPS consortium requires that all alleles for GAPS loci to go through a certification process involving verification with holotypes before inclusion in the standard GAPS allele set.)

We combined the 2008 and 2009 LCC Chinook salmon collections since they were undifferentiated in pairwise tests (data not shown). The combined collection (and each collection year) departed from HWE at two loci (*Ogo-4* and *Ots-208*) and over all loci combined (Table 1). The departure from HWE over all loci was due solely to the locus *Ogo-4*, since without *Ogo-4*, the collection was in equilibrium ($F_{IS} = 0.004$, $P = 0.21$). MicroChecker (Van Oosterhout et al. 2004) suggests that there is a null allele at *Ogo-4* because it detected excess homozygotes for most allele size-classes. There was a high percentage (over 50%) of locus pairs in linkage disequilibrium at the 5% and 1% levels

(Table 1). The juvenile portion of the collection generated the linkage signal; with collection years separated, the juvenile collection from 2009 had the same number of locus pairs in linkage disequilibrium and few were linked in the 2008 adult collection. The high linkage was due to family groups: pairwise sibship analysis in COLONY detected 156 full-sibling and 658 half-sibling relationships in the juvenile portion of the collection (less than 2% of the total 42,486 pairwise relationships were half siblings). There were 44 full-sibling families ranging in size from two siblings (24 families) to eight siblings (1 family). We removed all but one individual from each full-sibling family and linkage disequilibrium decreased (at the 1% level) from 43/78 to 11/78 locus pairs, or 14% (two linked pairs involved *Ogo-4* and *Ots-208*). This was still greater than expected by chance. However, the COLONY analysis also detected 276 half-sibling families in the collection, ranging in size from 2 to 44 half siblings per family cluster. These half-sibling relationships probably generated the linkage disequilibrium remaining after the full siblings were removed. Comparisons with other Columbia River basin collections showed some linkage disequilibrium in the Marion Drain and Snake River collections (Table 1).

Allelic richness and gene diversity differed among the Columbia River basin Chinook salmon collections (Table 1). Allelic richness in LCC was below average, but the difference was not significant (paired *t*-test; $P = 0.317$). Lower allelic richness can be a signal of a past genetic bottleneck (since populations tend to lose rare or low-frequency alleles during bottlenecks) or a signal of sampling error (if the sample was nonrandom). Gene diversity varied less than allelic richness among Columbia River basin collections and was average in LCC.

AGARST indicated that *M* ratios were unreliable for two loci, *Oki-100* and *Ots-201b*, where the repeat length was estimated at a single base pair because allele sizes shifted from even to odd numbers. The calculation requires clear-cut repeat units and single-base-pair units could compromise estimation of the ratio. We thus removed the two loci before calculating *M* ratios. In LCC and all other collections, *M* ratios (see Table 1) were above 0.68, the threshold value established by Garza and Williamson (2001) for recently bottlenecked populations (with the exception, perhaps, of the Priest Rapids Hatchery collection [lower variance interval = 0.68]).

We calculated N_b in the LCC collection only for the 2009 juvenile portion (all siblings left in), since this was a single age-class. We used the estimates from LDNe with 0.02 as the lowest allele frequency for calculations to avoid possible bias from low-frequency alleles (Waples 2006). For comparative purposes, we also calculated N_b for LCC using the pairwise sibship method (Wang 2009) and obtained the same value. Other Columbia River basin samples included multiple brood years that probably distorted the N_b calculations (possible downward bias), but the values were calculated for comparative purposes (Table 1). The value of N_b for LCC Chinook salmon was lower than the average for the Columbia River basin; relative to the N_b values for other natural spawner collections, that for LCC was higher than the estimate for the Yakima River collection, comparable to the estimates for the Methow River and Marion Drain collections, and lower than the estimates for the Hanford Reach and Snake River collections.

Pairwise tests confirmed that the 2008 and 2009 Chinook salmon collections from LCC were from the same population (data not shown). The genetic variance between temporal samples was not significantly different from zero, and the *P*-values for the genotypic tests indicated no significant differences. The LCC Chinook salmon collection was significantly different from all other Columbia River basin Chinook salmon collections (Table 3) but shared significantly greater genetic variance with the collections from the Hanford Reach, Umatilla Hatchery, and lower Yakima River (average pairwise $F_{ST} = 0.0087$ versus 0.015 for the upper Columbia River; Student's *t*-test, $P = 0.001$ for comparison) versus the collections from the Snake River, Lyons Ferry, and Marion Drain (average pairwise $F_{ST} = 0.0125$; Student's *t*-test, $P = 0.024$ for comparison). The factorial correspondence analysis supported the distinction of LCC (Figure 3a, b). While the plot of individuals (Figure 3a) showed some overlap among all the Chinook salmon in Columbia River basin collections, the LCC formed a discrete cluster whose population centroid was distinct from those of the others (Figure 3b). The temporal stability and differentiation from other Columbia River Chinook salmon collections supported the identity of the LCC collection as a discrete spawning aggregate rather than a collection of strays from other populations. Average pairwise F_{ST} values support LCC Chinook salmon membership in the Hanford-Yakima major population group (MPG; Appleby et al. 2010).

TABLE 3. Pairwise F_{ST} and genotypic test results for comparisons between fall Chinook salmon collections from the Columbia River basin. The upper triangular matrix contains *P*-values for pairwise genotypic tests (values in bold italics were not significant), and the lower triangular matrix contains pairwise F_{ST} values (values in bold italics were not significantly different from zero; the results for the boxed value differed between the F_{ST} and genotypic tests). The significance of test values was based on a corrected α of 0.00076 (0.05/66).

Collection	Lower Crab	Hanford Reach	Priest Rapids Hatchery	Lower Yakima	Umatilla Hatchery	Methow	Wells Hatchery	Wenatchee	Okanogan	Marion Drain	Lyons Ferry Hatchery	Snake
Lower Crab		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Hanford Reach	0.0087		***0.0525***	***0.3912***	***0.5944***	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Priest Rapids Hatchery	0.0107	***–0.0005***		***0.0101***	***0.4222***	0.0001	0.0004	0.0001	0.0001	0.0001	0.0001	0.0001
Lower Yakima	0.0079	***–0.0006***	***0.0000***		***0.3666***	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Umatilla Hatchery	0.0073	***–0.0005***	***–0.0004***	***–0.0008***		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Methow	0.0141	0.0045	0.0019	0.0044	0.0046		***0.1031***	***0.3320***	***0.5267***	0.0001	0.0001	0.0001
Wells Hatchery	0.0146	0.0040	0.0015	0.0045	0.0048	***0.0008***		***0.0129***	***0.3218***	0.0001	0.0001	0.0001
Wenatchee	0.0154	0.0056	0.0026	0.0063	0.0052	***0.0005***	***0.0012***		0.0001	0.0001	0.0001	0.0001
Okanogan	0.0154	0.0060	0.0037	0.0065	0.0069	***0.0012***	***0.0020***	***0.0021***		0.0001	0.0001	0.0001
Marion Drain	0.0143	0.0064	0.0067	0.0054	0.0061	0.0134	0.0130	0.0161	0.0180		0.0001	0.0001
Lyons Ferry Hatchery	0.0134	0.0077	0.0092	0.0063	0.0062	0.0172	0.0156	0.0187	0.0180	0.0074		0.0001
Snake	0.0097	0.0022	0.0027	0.0018	0.0011	0.0093	0.0084	0.0105	0.0108	0.0060	0.0029	

In the assignment test, we used a relative assignment probability of 90% as a cutoff value for a positive, unambiguous assignment, and assignment values below 90% were considered positive but ambiguous (and put in the "unassigned" category; Table 4). Since we were concerned about family members skewing the assignments, we conducted the analysis with and without full-sibling families; the percentage of correct assignments remained the same (data shown for full data set). With a 90% probability threshold, 194 fish were assigned back to the LCC collection (96.5% of the fish with unambiguous assignments), 1 each to the Hanford Reach, Okanogan, and Priest Rapids Hatchery collections, and 4 to the lower Yakima River collection (Table 4). The LCC temporal collections differed in sample quality and in the proportion that were assigned with 90% or greater probability back to the LCC collection (39% of the 2008 collection versus 52% of the 2009 collection). The 2008 collection included both fresh tissue from juveniles and degraded tissue from spawner carcasses, and some of the carcass samples lacked complete genotypes, compromising assignment. The 2009 samples were high-quality juvenile tissues. If we considered all positive assignments, 264 of the 401 in the LCC collection were assigned back to that collection (Table 4); the next highest numbers of assignments went to the Hanford Reach (22) and lower Yakima River collections (21).

TABLE 4. Assignment summary for Lower Crab Creek Chinook salmon to a baseline of Chinook salmon collections from the greater Columbia River basin. Assignments were made using the Rannala and Mountain (1997) algorithm implemented in GeneClass2 (Piry et al. 2004). The top row shows assignments for which the relative assignment value (highest assignment likelihood/sum of likelihoods) was at least 90%. If the relative assignment likelihood was below 90%, the individual was unassigned (ambiguous). The second row shows assignments with the highest likelihood regardless of the relative assignment score. With a lower stringency threshold, all fish were assigned.

Assignment criterion	Lower Crab	Hanford	Priest Rapids Hatchery	Lower Yakima	Umatilla Hatchery	Wenatchee	Methow	Wells Hatchery	Okanogan	Marion Drain	Lyons Ferry Hatchery	Snake	Unassigned
>90%	194	1	1	4	0	0	0	0	1	0	0	0	200
Highest likelihood	262	22	10	21	12	6	11	6	10	11	11	19	0

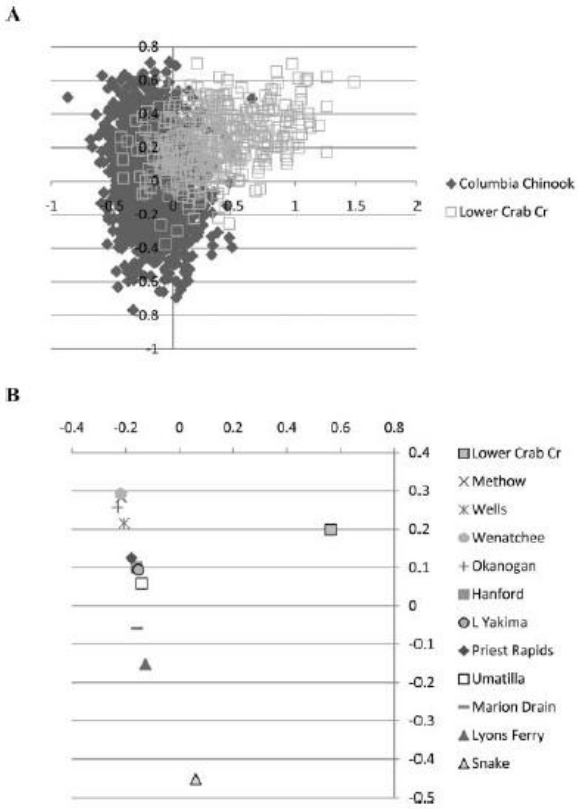


FIGURE 3. Factorial correspondence analysis plots for (**a**) individuals in the Columbia River and Lower Crab Creek and (**b**) various population centers.

Given the low power of the GAPS loci to resolve population differences, this is strong support for the unique identity of the LCC collection. We calculated a chi-square value for the positive assignments that showed that significantly more fish were assigned back to the LCC collection than would be expected by chance (X^2 = 1,399.63, df = 9, P < 0.0001).

First-generation migrant test indicated that all of the juveniles collected in 2009 were offspring from the LCC aggregate. Migrant tests differ from assignments since they test the hypothesis that a fish originated in their home collection even if their genotype is more similar to that of another baseline collection (an unusual but possible genotype, given the potential allele combinations in their home collection). Five fish collected in 2008 appeared to have originated outside the LCC aggregate since their genotypes had less than a 1% chance of arising from the LCC spawning aggregate (P = 0.008 for four of these fish and 0.003 for one). Of these fish, one had a positive assignment to the lower Yakima River collection, a geographically close natural-spawning population. However, the fish was missing three loci from its genotype, which diminished the assignment power.

DISCUSSION

The interior Columbia River basin is bordered by the Cascade Mountains to the west, the Blue Mountains to the south, and the Rocky Mountains to the east. Rivers originate from snowmelt in these mountains and support spawning populations of Chinook salmon with cold, clear water. Crab Creek, in the central plateau of the Columbia River basin, presents a unique habitat for Chinook salmon. The low-elevation creek, flows through a shrub-steppe and agriculturally modified desert (the Snake River and its tributaries traverse the desert but originate in the mountains). Crab Creek is fed by groundwater from the Columbia Plateau regional aquifer supplemented by groundwater infusions from the CBIP. Irrigation fro agriculture introduces sediments, pesticides, herbicides, and fertilizers into the creek. During the summer, water temperatures in the creek increase to levels lethal to salmon.

However, we suspect that springs throughout the creek provide acceptable water quality and thermal refuges for fish. Thus, while Chinook salmon were unexpected in the creek, close inspection found them spawning in good habitat in Red Rock Coulee (Bowen et al. 2003), a tributary of LCC, and hundreds of juveniles were collected over 3 years.

Chinook salmon were noted historically in Crab Creek, but the time frame for the establishment of a Chinook salmon spawning aggregation is uncertain. KWA Ecological Sciences hypothesized that LCC became a perennial creek following implementation of the CBIP in the 1950s because increased groundwater from irrigation created spawning habitat in the formerly ephemeral Red Rock Coulee (KWA 2004). During the mid-1900s, the multiple hatchery programs developed in the greater Columbia River basin may have contributed founders and strays. However, native trout and northern pikeminnow were in Crab Creek prior to the CBIP (supporting a historical connection to the Columbia River), and there may be other spawning areas in LCC that predate the CBIP but that are now obscured from view by agricultural sediments. We considered several hypotheses to explore the status of the Chinook salmon spawning and rearing in LCC, namely, that they are strays, recently introduced, or native fish.

If the Chinook salmon in LCC were random strays or a collection of strays from a wild or hatchery population, we would expect them to be assigned to their collection(s) of origin in the assignment test and share all of their alleles with other collections. However, LCC Chinook salmon were assigned back to the LCC collection with high fidelity, and that collection possesses alleles that are absent from other Columbia River basin Chinook salmon populations. In fact, one of these alleles has not been detected in any other GAPS population. Few strays were detected in the assignment test, and nearly all confidently assigned juveniles originated in the LCC spawning aggregate.

If the LCC spawning aggregate developed from a recent introduction, the closest fall Chinook salmon sources are the Hanford-Yakima MPG (Appleby et al. 2010). This MPG includes main-stem spawning aggregations in the Hanford Reach and lower Yakima River as well as fish of Hanford Reach origin maintained at Priest Rapids Hatchery and released below Priest Rapids Dam. The Hanford-Yakima MPG component of the upper Columbia River summer-fall ESU expresses solely fall run timing and ocean-type juvenile out-migration (Appleby et al. 2010). Other possible sources of strays or founders are fish from the upper Columbia River MPG (Wells Hatchery and the Wenatchee, Okanogan, and Methow rivers; Appleby et al. 2010); fewer than 1% of the Wenatchee River juveniles are stream type (Steven Schonning, WDFW, unpublished data). The run timing in LCC peaks at the end of October, making them fall run, but they differ from the rest of the upper Columbia River ESU populations in that they appear to express both ocean- and stream-type juvenile out-migration, with possibly 20% or more stream-type out-migration. Snake River fall-run Chinook salmon have a minority of stream-type juveniles (Connor et al. 2002), but they would be a source relatively remote from LCC with different run timing, and Snake River fish were genetically distant from LCC. The run timing in LCC fish is most similar to that of the nearby Hanford-Yakima MPG and they share the most genetic variance with this MPG, but their apparent bimodal juvenile life history would be unique within the upper Columbia River ESU.

Given the difficulties studying the tributary and the lack of juvenile-scale data to confirm stream-type life history, we present other possibilities for the bimodal juvenile sizes besides life history variation. A bimodal distribution might arise if some juveniles emerged earlier and grew fast in the warm water with abundant food while other juveniles continued to emerge through spring, maintaining the smaller size-class. Juvenile emergence might have a bimodal distribution if the spawning season is bimodal owing to the water temperature or flow in LCC or if spawning occurs over an extended period. Connor et al. (2002) noted that in warmer waters in the Snake River fall Chinook salmon fry emerged earlier and growth was faster and that cold water fostered slower growth and stream-type juveniles. Smaller LCC juveniles might simply inhabit coldwater upwellings. Environmental conditions impacting screw trap function might also affect juvenile size distributions. Future research will include collecting scale data for juveniles to examine whether larger-sized individuals are yearlings.

We also compared the LCC population of Chinook salmon with the mid and upper Columbia River and Snake River spring-summer populations. We found considerably greater differentiation between the LCC population and these populations (e.g., the pairwise F_{ST} between LCC and Methow River spring Chinook salmon = 0.08) than between the LCC population and the populations indicated in

Table 1, ruling out the possibility that they were odd-timed spring-run Chinook salmon. Since the LCC collection included family groups, we also considered the possibility that we were simply looking at some successful families in LCC and that fish were being assigned back to a family group rather than a population. This might explain the strong distinction of the collection. However, when we removed all but one member from each full-sibling family and reran the assignment test, the result was the same: LCC fish were assigned to LCC. The pairwise tests (and similar genetic diversity to other fall Chinook salmon populations) further supported the status of the LCC population as a temporally stable, discrete spawning aggregate rather than a collection of strays—the temporal collections from LCC were indistinguishable from each other but differentiated from other Columbia River basin collections. Further, genetic distances and run timing support including the LCC population in the Hanford-Yakima MPG, as hypothesized in Appleby et al. (2010).

While the data suggest an independent status for the LCC Chinook salmon, the time frame and manner of their establishment remains uncertain. If the LCC aggregate was established in the 1950s from a small founder group and thereafter remained isolated from their founders, genetic drift could drive the distinction in the assignment tests, pairwise tests, and factorial correspondence analysis (Ramstad et al. 2004). For instance, Chinook salmon populations founded in New Zealand in the early 1900s have a similar amount of genetic variance from their source population (F_{ST} = 0.015; Kinnison et al. 2002) as LCC fish have with other Columbia River basin ocean-type fall Chinook salmon. Yet the New Zealand populations were genetically depauperate in comparison with their source population, whereas LCC Chinook salmon showed diversity similar to that of other Columbia River basin populations. We would expect a founder effect within the pas 20 generations to leave a signature such as decreased genetic diversity, significantly lower allelic richness, high linkage disequilibrium, or an *M* ratio below 0.68 (Garza and Williamson 2001). However, with the exception of high linkage disequilibrium, the LCC population bears none of these revealing attributes (linkage was due to family groups in the collection). It is possible that LCC receives a constant infusion of strays that could boost its genetic diversity, in contrast to the isolated New Zealand populations. But in that case we would expect more LCC fish to be assigned back to their source populations rather than to the LCC population. We would also expect genetic signals indicating mixture in the LCC collection (e.g., the Wahlund effect).

Part of the mystery surrounding LCC Chinook salmon arises from the turbidity that conceals fish as well as the unlikely nature of the tributary as Chinook salmon habitat. Although with close inspection we found 14 redd clusters, we suspect that there are other spawning sites in the creek obscured by turbidity (e.g., areas with swirling waters encountered during spawning surveys) that support the effective number of breeders (170) calculated for the juvenile collection. Turbidity alone is not enough to dismiss Chinook salmon spawning habitat, since salmon spawn in turbid glacial-origin tributaries (Murphy et al. 1989), but the degraded nature of the habitat and high water temperatures were thought to preclude salmonid rearing into the summer. Fish might avoid some of the water temperature problems since adults move into the creek in the fall when temperatures are declining and smolts exit the creek in the spring before temperatures rise to lethal levels of summer. However, the sizes of some LCC Chinook salmon juveniles suggest that they remain through summer and out-migrate the next spring as yearlings. Juveniles might survive the summer in cool groundwater upwellings obscured by turbidity; the size of candidate juveniles was within the published range of stream-type juveniles (Waknitz et al. 1995) and overlapped the size range for rare stream-type juveniles identified in fall Chinook salmon from the Wenatchee River (Steven Schonning, WDFW, unpublished data). Further, coho salmon juveniles were collected in LCC; juveniles of this species typically spend 18 months in freshwater before out-migrating (Weitkamp et al. 1995) and thus might rear in thermal and water quality refuges in LCC. Alternatively, as suggested above, food abundance may be such that juveniles of both species grow quickly and out-migrate as large subyearlings. Another possibility that is not mutually exclusive with the others is that LCC Chinook salmon have adapted to warmwater environments; smaller redband trout in desert streams have been found to be more tolerant of thermal stress (Rodnick et al. 2004), and juvenile Chinook salmon may be similarly tolerant. Scale age data for juveniles and a thermal profile of the tributary would help us distinguish between the various possibilities.

Lower Crab Creek is different from other main-stem and tributary spawning habitats in the Columbia River basin. Although data suggest that there are thermal refugia in the creek, the

contemporary gauntlet of poor habitat conditions and the desert nature of the habitat were thought to exclude salmonids. However, Chinook salmon are adaptable (Waples et al. 2004), as evidenced by their varied life history strategies (Myers et al. 2008), life history shifts in response to anthropogenic environmental changes in the Columbia River (Connor et al. 2005; Waples et al. 2007), and successful introduction into novel environments (e.g., feral Chinook salmon population in the Lake Michigan watershed; Weeder et al. 2005). This adaptability may have fostered their persistence in LCC as the habitat changed with the CBIP and agriculture in the watershed. While we considered only neutral genetic variation in examining LCC Chinook salmon, future work may find phenotypic divergence (Ramstad et al. 2010) or genotypic divergence at loci under selection (Narum and Campbell 2010). Phenotypic plasticity is an unlikely explanation, as temperatures in portions of LCC are well into the lethal range for any known salmonid. However, as discussed above, LCC fish may thrive within thermal refuges. Other investigative methods might support the proposition that adaptation to the unique conditions in LCC is an isolating mechanism fostering genetic divergence. The bimodal juvenile out-migration size suggests possible adaptation to their environment: yearling migrants are rarely detected among fall Chinook salmon juveniles in the Wenatchee River, and the abundance of putative yearling in LCC could indicate that this trait has some selective advantage in the rigors of the LCC environment. While environmental factors influence smolt timing (Taylor 1990; Beckman and Dickhoff 1998; Connor et al. 2002, 2005), Chinook salmon populations with stream-type juveniles are more common in Columbia River headwaters and in tributaries above 55°N (Taylor 1990; Waknitz et al. 1995), as well as in the Snake River, and typically have spring run adult return timing (summer run in the Snake River). Lower Crab Creek Chinook salmon have fall run timing and mid-Columbia River ancestry and have had no intervention beyond habitat modifications associated with agriculture. Perhaps the apparent varied life history expression in LCC juveniles was once more common in Columbia River basin Chinook salmon and the LCC spawning aggregate is a repository of important genetic biodiversity that enabled the population to respond to unusual environmental conditions. Further research documenting the long-term abundance of the spawning aggregate, the stability of its life history expressions, and the role of genetic-based adaptations will determine whether this interesting group merits status as a population under the guidelines for viable salmonid populations (McElhany et al. 2000).

CONCLUSIONS

In summary, the data support the hypothesis that the Chinook salmon spawning aggregate in Lower Crab Creek consists of native fall Chinook salmon. The population is genetically distinct, has a genetic diversity and effective population size similar to those of other fall-run Chinook salmon populations in the interior Columbia River basin, and shows no evidence of any genetic bottleneck. The population's founding may predate changes in hydrology resulting from the Columbia Basin Irrigation Project. Genetic differentiation from nearby populations combined with poor-quality water and high temperatures in parts of its habitat suggest that the LCC population has developed adaptations to environmental conditions that are unusual for Chinook salmon.

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