2007 Annual Report

Steelhead Kelt Reconditioning and Reproductive Success

Prepared by:

Ryan Branstetter
Jeff Stephenson
Douglas R. Hatch (Principal Investigator)
John Whiteaker
Saang –Yoon Hyun

Columbia River Inter-Tribal Fish Commission
729 NE Oregon Street, Suite 200
Portland, OR 97232

Bill Bosch
Dr. David Fast
Joe Blodgett
Todd Newsome

Yakama Nation
Department of Natural Resources
Fish and Wildlife
401 Fort Road
Toppenish, WA 98948

Lisa M. Hewlett
Jens Lovetang
Mike Gauvin

Confederated Tribes Of the Warm Springs Reservation
Department of Natural Resources
Fish and Wildlife
P O Box C
Warm Springs, OR 97761

Rhonda Dasher
Chris Fisher

Colville Confederated Tribes
Department of Natural Resources
Fish and Wildlife
Fish & Wildlife Department
PO Box 150
Nespelem, WA 99155

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U.S. Department of Energy
Bonneville Power Administration
Division of Fish and Wildlife
P.O. Box 3621
Portland, OR 97283-3621

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ABSTRACT

Iteroparity, the ability to repeat spawn, is a natural life history strategy that is expressed by some species from the family Salmonidae. Current rates of observed steelhead iteroparity rates in the Columbia River Basin are severely depressed due to anthropogenic development, operation of the hydropower system and other factors. Artificial reconditioning, which is the process of culturing post-spawned fish (kelts) in a captive environment until they are able to reinitiate feeding, growth, and redevelop mature gonads, is evaluated in this study as method to restore depressed steelhead populations. To test the efficacy of steelhead kelt reconditioning as a management and recovery tool different scenarios were investigated ranging from very low intensity (collect and transport fish) to high intensity (collect and feed fish in captivity until rematuration). Examinations of gamete and progeny viability were performed for first-time spawners and reconditioned kelt steelhead. Further, a long-term field study is being implemented to evaluate the relative reproductive success of natural-origin, hatchery-origin, and reconditioned kelt steelhead to two streams. Parentage analysis is being performed on juvenile *O. mykiss* from the study streams using 16 microsatellite loci.

Analysis of management scenarios indicated that no-term and short-term reconditioned kelts continue to perform well outmigrating to the ocean but returns from these groups have ranged from 0-12% during 2002-2007. Long-term reconditioning has rebounded to nearly 54% surviving to remature and release. The first successful year of kelt gamete and progeny analysis demonstrated that success fell within the normal range for steelhead gamete and juvenile develop ment. Fertilization rates were lower when compared against the first time spawning but egg and juvenile survival was higher. This year marked the first documented evidence of a steelhead kelt successfully spawning in the wild, we discovered 3 juveniles that were of direct genetic lineage to a steelhead kelt that was released in 2006 at Omak Creek. The management exploration has discovered that river or ocean conditions may be inhibiting the ability of kelts to successfully return from the ocean after release. Secondly the success of long-term reconditioning may be tied to the quality of kelts that are returning from the ocean. The progeny and gamete and reproductive success data are both preliminary but both demonstrate that steelhead kelts have the ability to reproduce successfully and contribute viable offspring.

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Introduction

Oncorhynchus mykiss are considered to have one of the most diverse life histories in Salmoninae (Behnke 1992) with variants that include resident, estuarine, and anadromous ecotypes, widely ranging ages, timing of juvenile and adult migrations, and various reproductive strategies including precocity, semelparity, and iteroparity. This complex array of life history variation is possibly a compensating or bet hedging device for life in stochastic environments (Taborsky 2001). Overlapping generations provide resources in the event of failure of any brood year due to brief catastrophic events. While fluctuating populations and overlapping generations may reduce the effective size (N_e; Waples 2002), persistence of the species may be favored due to these compensating life histories (Narum et al. 2008).

Populations of wild steelhead *O. mykiss* have declined dramatically from historical levels in the Columbia and Snake rivers (Nehlsen et al. 1991; NRC 1996; *US v. Oregon* 1997; ISRP 1999). In 1997 steelhead in the upper Columbia River were listed as endangered under the Endangered Species Act (ESA) (NMFS 1997). Those in the Snake River have been listed as threatened since 1997 (NMFS 1997). Stocks originating in the mid-Columbia were listed as threatened in 1999 (NMFS 1999). The causes of the species decline are numerous and well known. The two that have had the biggest impact are hydropower operations and habitat loss (TRP 1995; NPPC 1986; NRC 1996; ISRP 1999). Regional conservation plans recognize the need to protect and enhance weak upriver steelhead populations while maintaining the genetic integrity of those stocks (NPPC 1995).

Iteroparity, the ability to repeat spawn, is a natural life history strategy that is expressed by *0. mykiss,* with rates estimated to be as high as 79% for populations in the Utkholok River of Kamchatka, Russia 1994-96 (Savvaitova et al. 1996) and as high as 30% for British Columbia (Withler 1966). Historical rates for the Columbia River have not been readily documented but adult emigrating steelhead averaged 58% of the total upstream runs in the Clackamas River from 1956 to 1964 (Gunsolus and Eicher 1970). Current iteroparity rates for Columbia River Basin steelhead are considerably lower, due largely to high mortality of downstream migrating kelts (post-spawn steelhead) at hydropower dams (Evans and Beaty 2001), and potentially inherent differences in iteroparity rate based on latitudinal and inland distance effects (Withler 1966; Bell 1980; Fleming 1998). The highest recent estimates of repeat spawners from the Columbia River Basin were in the Kalama River (tributary of the unimpounded lower Columbia River) having exceeded 17% (NMFS 1996). Farther upstream, 4.6% of the returning summer-run steelhead in the Hood River (above only one mainstem dam) are repeat spawners (J. Newton, ODFW, pers. comm.). Iteroparity rates for Klickitat River steelhead were reported at 3.3% from 1979 to 1981 (Howell et al. 1984). Summer steelhead in the South Fork Walla Walla River have expressed 2% to

9% iteroparity rates (J. Gourmand, ODFW, pers. comm.). Repeat spawners composed only 1.6% of the Yakima River wild run (from data in Hockersmith et al. 1995).

Rationale

Post-spawn steelhead represent a portion of the population that have successfully survived through an entire life cycle and spawning event. Artificially reconditioning these kelts may counter the negative selective forces against iteroparity associated with the hydrosystem, thereby helping to preserve the evolutionary legacy of the species. Kelt reconditioning starts with the introduction of feed which encourages the reinitiation of feeding, thereby enabling kelts to survive and rebuild energy reserves required for gonadal development and repeat spawning. Techniques used in kelt reconditioning were initially developed for Atlantic salmon *Salmo salar* and sea-trout *S. trutta.* A review of these studies and those applicable to steelhead kelts are summarized in Evans *et al*. (2001). Additional reviews of this subject (Hatch et al. 2002 and 2003b) provide strong support of the benefits of kelt reconditioning to address population demographic and genetic issues in steelhead recovery. The ultimate success of kelt reconditioning should be assessed based on the number of individuals that successfully spawn in the wild following reconditioning and release. Although it is difficult to witness individual fish spawning in the wild, and even more difficult to assess the viability and quality of gametes, we are conducting experiments (reproductive success and gamete/progeny viability) to determine the extent to which if reconditioned kelts are contributing to subsequent generations.

Objectives

Management Scenario Evaluation

An evaluation of the reconditioning will be based on two fundamental hypotheses aimed at comparing the relative survival and rematuration rates of program fish

H_o: Repeat spawner rates are similar among all treatments including: in-river release; transport and release; short-term recondition and transport; and long-term recondition and release.

H_o: Rematuration rates are similar among all treatments including: in-river release; transport and release; short-term recondition and transport; and long-term recondition and release.

Yakima In-River Release

For the third year, we systematically collected a portion of the kelts that would have been suitable for reconditioning, and release them immediately back to the Yakima River to act as a control group. These baseline data will also provide an opportunity to compare Hockersmith et. al. (1995) reported respawner rates inferred from steelhead scale pattern analysis from the Yakima River.

No-term Treatment

We continue to directly transport steelhead kelts around the hydro-system to evaluate this management effect on iteroparity rates as well as to observe Lower Columbia River seaward and return spawning migration. Given the high mortality rates of seaward migrating kelts observed during radio telemetry experiments in the Snake and Columbia Rivers (Evans et al. 2001; Evans 2002; Hatch et al 2003a) iteroparity may be augmented by simply transporting kelts around the hydro system, thereby improving access to the marine environment.

The purpose of this objective is to evaluate the lowest cost alternative aimed at increasing steelhead iteroparity. Prior to an implementation of a large-scale kelt steelhead transportation program it is important to consider potential effects on non-target fish. If kelts maintain residence in the estuary rather than migrating to the ocean, they may have a predatory effect on migrating salmonid smolts. It is also important to assess whether transportation impacts the homing ability of these fish. To address these concerns, all steelhead kelts were PIT-tagged with a portion also receiving hydro-acoustic tags. This technology will provide us with the necessary information regarding fish survival, movement, distribution, travel time, velocity, residence time in the estuary, and return rates.

Short-Term Reconditioning Treatment

Successful expression of iteroparity in steelhead may be limited by post-spawning starvation and downstream passage through the mainstem corridor. Thus, short-term reconditioning may augment iteroparity rates by initiating the feeding response while still allowing kelts to naturally undergo gonadal recrudescence in the estuary and marine environments. Short-term reconditioning is defined as the period of time needed (approx. 3-12 weeks) for kelts to initiate post-spawn feeding, followed by the transportation of kelts around mainstem hydroelectric facilities for release, and rematuration in the Pacific Ocean. Since short-term reconditioned fish were also transported and released below Bonneville Dam, PIT-tag and hydro-acoustic tags were used to assess fish survival, movement, distribution, travel time, velocity, as well as residence time in the estuary.

Long-term Reconditioning Treatment

Currently we define long-term reconditioning as holding and feeding post-spawn steelhead until stream/river temperatures begin to fall, typically in mid to late October. The fish are released to over-winter and return to the spawning sites on their own volition. During 2007, we continued with the most efficient and successful of the long-term steelhead reconditioning regimes by repeating the most successful diet and treatment identified during the 2001 and 2002 studies (krill and Moore-Clark pellets) (Hatch et al. 2001 and Hatch et al. 2002). Steelhead kelt reconditioning at the other sites utilized a modified version of the Prosser reconditioning with minor modification to feed types.

Gamete and Progeny Viability

H_o**:** Measures of gamete and progeny viability and quality are similar between first spawning and following artificial reconditioning.

Long-term reconditioning and subsequent captive spawning provides us with means to obtain valuable quantitative data on endocrine function, gonad processes, maturation rates and juvenile survival. Data resulting from this research will greatly contribute to the evaluation of reconditioning as a conservation tool. This experiment utilizes a replicated, repeated measures experimental design, to assess and compare egg and progeny viability of reconditioned vs. first time spawners.

Reproductive Success

H_o: Reproductive success among natural-origin, hatchery-origin, and reconditioned kelt steelhead is equal within and among streams.

The reproductive success of long-term reconditioned kelts needs to be explored to assess the net benefit of this program. Specific questions regarding the success of artificially reconditioning kelt steelhead include: do reconditioned kelts produce viable offspring that contribute to recruitment, how does kelt reproductive success compare with natural first time spawners, and how does kelt reproductive success compare with hatchery origin spawners? We will utilize microsatellite DNA markers and pedigree analysis to help us answer these questions. The answers to these questions will be important in determining if kelt reconditioning is a viable restoration tool that will aid in the recovery of ESA listed steelhead populations in the Columbia River Basin.

Study Area

Kelt Reconditioning

Kelt reconditioning research was conducted at the Prosser Fish Hatchery in Prosser, Washington. Prosser Hatchery is located on the Yakima River at river

kilometer (Rkm) 75.6, downstream from Prosser Dam, and adjacent to the Chandler Juvenile Evaluation Facility (CJEF) where the steelhead kelts are collected for the in-river, no-term, short-term, and long-term treatments (Figure 1). The Yakima River is approximately 344 km in length and enters the Columbia River at Rkm 539. Summer steelhead populations primarily spawn upstream from Prosser Dam in Satus Creek, Toppenish Creek, Naches River, and other tributaries of the Yakima River (TRP 1995). The Prosser Hatchery is operated by the Yakama Nation (YN), with a primary function of rearing, acclimation, and release of fall chinook salmon *O. tshawytscha*, and is also used for coho salmon *O. kisutch* rearing prior to acclimation and release in the upper Yakima River Basin.

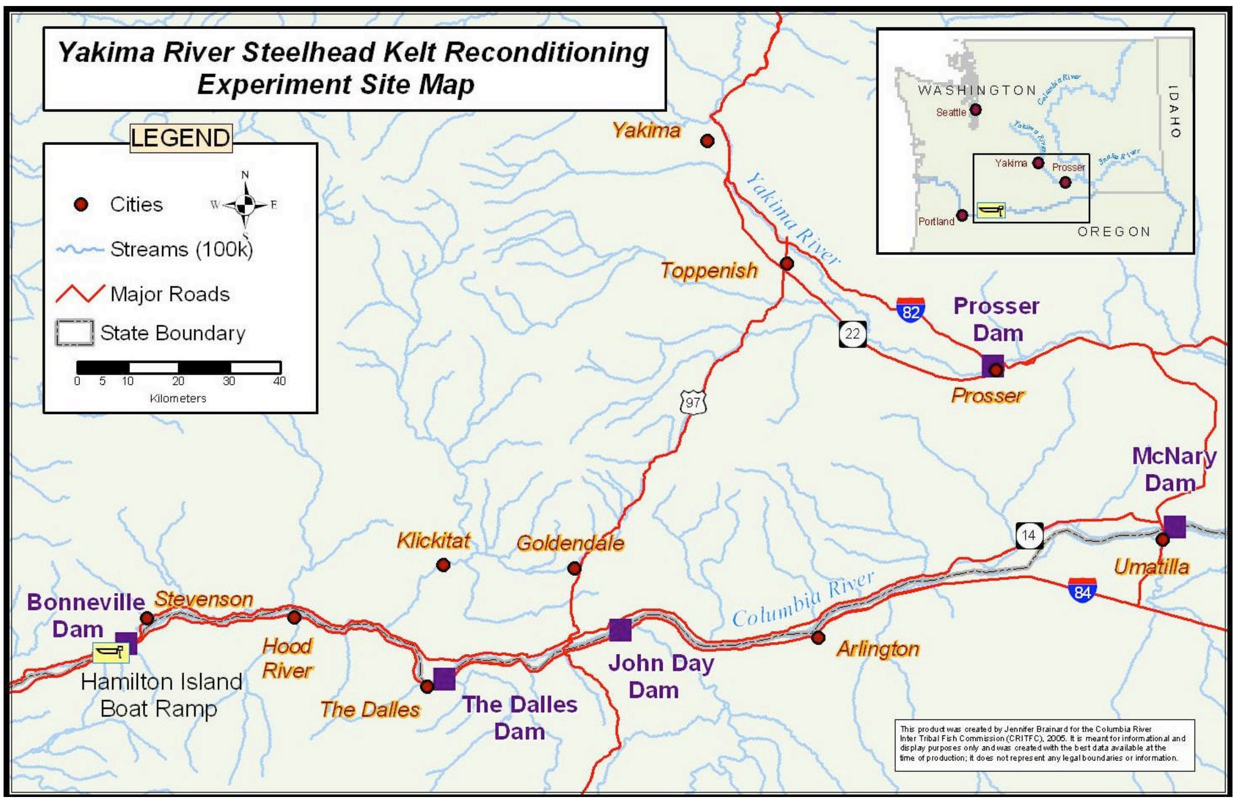

Figure 1: Reconditioning site (Prosser, WA) and release site (Hamilton Is. Boat Ramp) for No-term and Short-term reconditioning experiments.

Steelhead for the Short–term and No-term treatments are released at the Hamilton Island Boat Ramp (rkm 231) which is located downriver from Bonneville Dam on the Washington shore of the Columbia River. The lower Columbia River habitat from approximately rkm 75-0 is typified as an estuarine environment, and is influenced by tidal oscillations from the Pacific Ocean. The migration of experimental groups are monitored in the lower Columbia River using acoustic telemetry technology (Figure 2) (rkm 138 to 0.) (Appendix A). Migration behavior, survival, and timing are evaluated for these treatments.

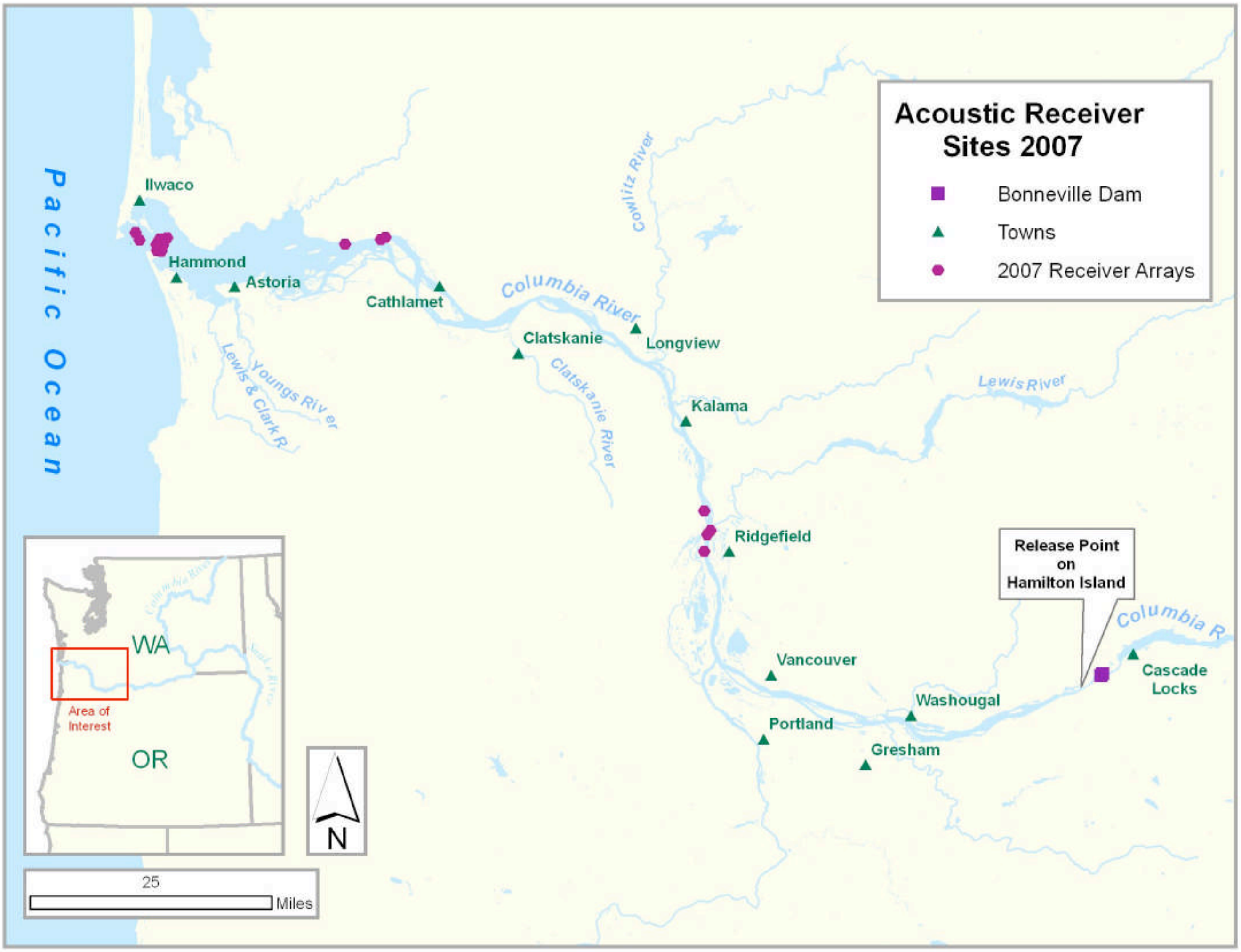

Figure 2: Lower Columbia River acoustic receiver deployment 2007.

Gamete and Progeny Viability

The Hood River is a tributary of the Columbia River in northwestern Oregon. Approximately 40 km long from its mouth to its farthest headwaters on the East Fork, the river descends from wilderness areas in the Cascade Range on Mount Hood and flows through the agricultural Hood River Valley to join the Columbia River in the Columbia River Gorge. Adult steelhead collection for the Parkdale Fish Facility is conducted at Powerdale Dam located on at rkm 6.4 just north of the city of Hood River, Oregon and operated by Oregon Department of Fish and Wildlife (ODFW). Steelhead are spawned then reconditioned at the Parkdale Fish Facility which is co-managed by the Confederated Tribes of the Warm Springs Reservation (CTWS) and Oregon Department of Fish and Wildlife (ODFW) and is located on the Middle Fork of the Hood River (5.6 rkm) near Parkdale, OR (Figure 3). The Middle Fork Hood River is approximately 16 km long, rises in several short branches on the north slopes of Mount Hood, from Coe and Eliot glaciers. It flows north through the upper Hood River Valley.

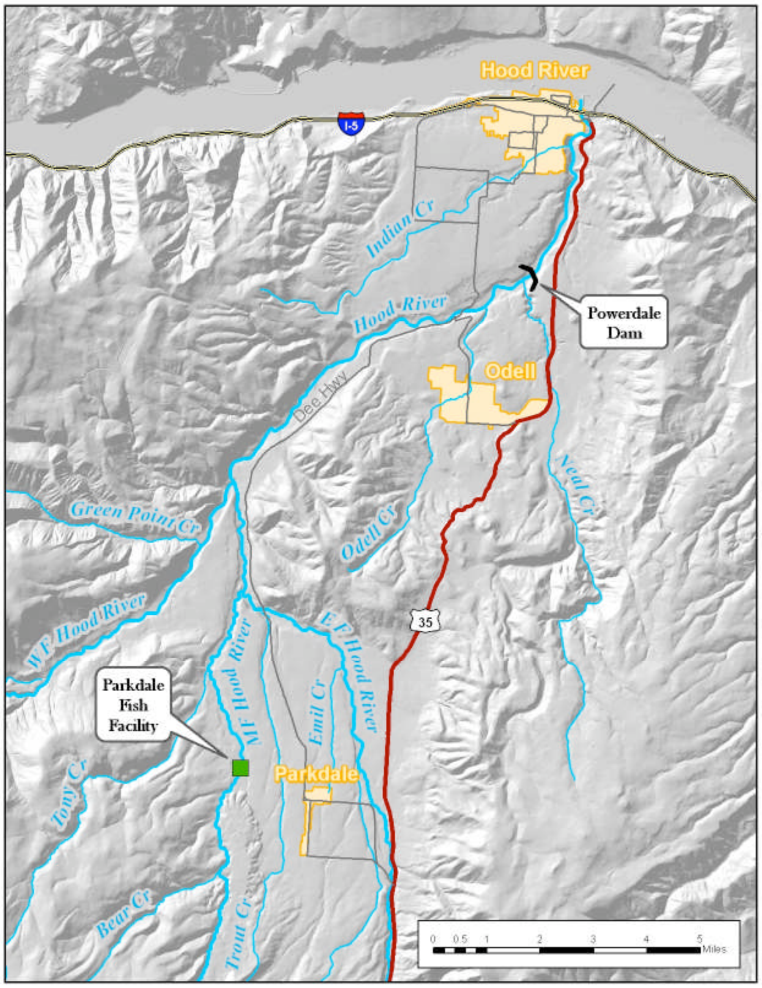

Figure 3: Location of Parkdale Fish Facility and Powerdale Dam/ Fish Trap.

Relative Reproductive Success

Shitike Creek, located on the Warm Springs Reservation, flows into the Deschutes River at rkm 152, and is the river's second largest tributary. The Deschutes River is tributary of the Columbia River and is located in north-central Oregon and is 406 rkm long. Shitike Creek is a fourth order stream with a drainage area of 145.9 square kilometers (Figure 4). Shitike Creek kelt steelhead were reconditioned at Warm Springs National Hatchery, located on the Warm Springs River, approximately 16 kilometers north (downstream on the Deschutes River) of Shitike Creek. The hatchery is operated by the U.S. Fish and Wildlife Service on lands leased from the Confederated Tribes of Warm Springs (CTWS) to propagate and raise wild and hatchery spring Chinook salmon.

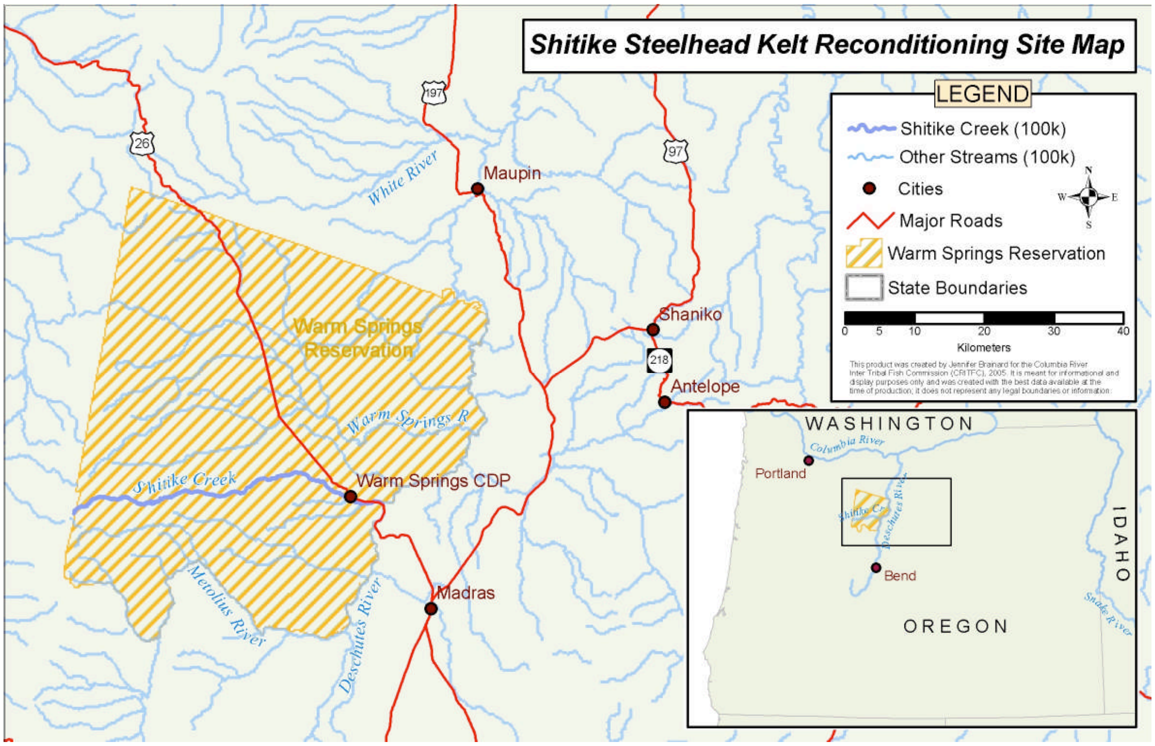

Figure 4: Map showing the location of Shitike Creek and the Confederated Tribes of the Warm Springs Reservation

Omak Creek, a tributary to the Okanogan River, is located in Okanogan County in North Central Washington. It is approximately 35.4 km ending at its confluence with the Okanogan River (Figure 5). It runs entirely within the reservation of the Colville Confederated Tribes (CCT). Omak Creek kelt steelhead were reconditioned at the Cassimer Bar Hatchery located at the confluence of the Okanogan River and the Columbia River downriver of Chief Joseph Dam. Currently the Colville Confederated Tribes operate the Cassimer Bar Hatchery. The facility was originally constructed in 1994, as a sockeye salmon *O. nerka* production facility in an attempt to supplement Lake Osoyoos and is currently utilized for the development of locally-adapted stock to supplement natural production of steelhead in Omak Creek.

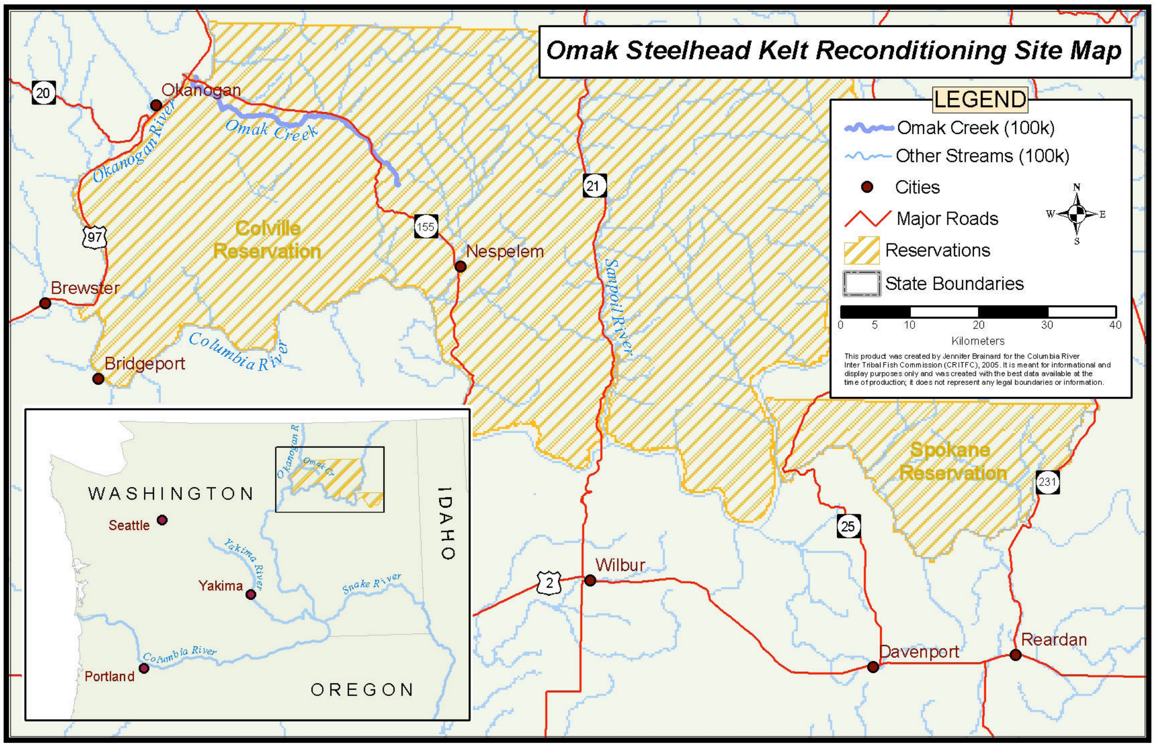

Figure 5: Map showing the locations of Omak Creek as well as the Confederated Tribes of the Colville Reservation.

Methods

Kelt Collection and In-Processing

Yakima River

After spawning naturally in tributaries of the Yakima River, a portion of the steelhead kelts that encounter Prosser Dam during emigration are diverted into an irrigation channel that directly connects to the Chandler Juvenile Evaluation Facility (CJEF). The CJEF diverts migratory fishes away from the irrigation canal to reduce mortality associated with agriculture. Once diverted into the CJEF, emigrating kelts can be manually collected from a fish separation device (a device which allows smaller juvenile salmonids to “fall through” for processing in the juvenile facility while larger fish can be dipnetted off the separator for processing or release back to the river). Yakama Nation (YN) staff monitored the Chandler bypass separator 24 hours a day from March 26 to July 3, 2007.

Figure 6: Chandler Juvenile Evaluation Facility adult separator.

All adult steelhead arriving at the CJEF separator were dipnetted off the separator (Figure 6) and placed into a water-lubricated PVC pipe slide that was directly connected to a temporary holding tank 20’ (l) x 6’ (w) x 4’(h) containing oxygenated well water (57^{0}F or 13.8^{0}C). Post-spawned steelhead kelts were identified (Evans and Beaty 2001) then transferred with a dipnet from the temporary holding tank to a nearby 190-L sampling tank containing fresh river water, and anesthetized in a buffered solution of tricaine methanesulfonate (MS-222) at 60 ppm. All specimens visually determined to be prespawn individuals were immediately returned to the Yakima River.

Following kelt identification, we collected data on weight (collected in pounds but converted to kg for this report), condition (good- lack of any wounds or descaling, fair- lack of any major wounds and/or descaling, poor- major wounds and/or descaling), coloration (bright, medium, dark), and presence or absence of physical anomalies (e.g., head burn, eye damage). Passive Integrated Transponder (PIT) tags (if not already present) were then implanted in the fish’s abdominal cavity for later individual identification The steelhead kelts deemed to be in “good” to “fair” condition were retained for reconditioning while steelhead kelts found to be in “poor” condition and dark in color were released back to the river. A portion of collected steelhead kelts that were found to be in good condition were released back to the river as a in-river treatment to establish baseline data on the natural repeat spawner rate in the Yakima River.

All kelts held for an extended period of time in reconditioning tanks, are susceptible to severe infestation of parasites which can be lethal to cultured

fishes. Formalin is generally (varies to some degree at sites based on fungal growth) administered five times a week at 1:6,000 for 1 hour in all reconditioning tanks to prevent fungal outbreaks. Another concern with holding wild steelhead was susceptibility to Salmincola in such environments. Salmincola is a genus of parasitic copepod that can inhibit oxygen uptake and gas exchange at the gill lamellae/water surface interface by attachment to the lamellae. Recent research by Johnson and Heindel (2000), suggested that Ivermectin™ – a treatment often used to control parasites in swine and cattle – increases the survivorship of cultured fish by killing the adult morph of the parasite. Due to its successful use in treating Salminicola in this project's kelt reconditioning experiments during 2000 (Evans and Beaty 2000), Ivermectin™ was diluted with saline (1:30) and injected into the posterior end of the fish's esophagus using a small (1cc) plastic syringe. All kelts except for in-river release were administered a one-time subcutaneous injection of Oxytetracycline.

<u>In-River Release</u>

A portion of the kelts that would have been suitable for reconditioning, were PIT-tagged, and then released immediately back to the Yakima River (Prosser, WA rkm 75.6) to monitor the rate of natural iteroparity. This data will be compared to iteroparity rates inferred from scale pattern analysis from the Yakima River (Hockersmith et. al. 1995). In-river release specimens were selected proportionally from fish that were collected at the CJEF from 3/26- 7/3/2007.

Omak and Bonaparte Creeks

To increase the total number of kelts available for reconditioning, Kelts were also collected from the Bonaparte Creek weir which is a tributary of the Okanogan River. This stock is being used by the Cassimer Bar Hatchery to develop a naturalized steelhead broodstock for the Okanogan and Omak Rivers (including Omak Creek). Trapping at the Omak semi-permanent weir (Figure 7) located at approximately RKM 1.2 on Omak Creek, started on March 12, 2007 and continued through May 22, 2007. Trapping began in Bonaparte Creek (rkm 0) on March 23, 2007. The first kelt was collected on April 19, 2007 with the peak of kelt collection occurring between May 9 and May 24, 2007. Kelts are collected for reconditioning in either of two ways: 1) males and females collected for broodstock that survive spawning are put into the kelt tank for reconditioning. 2) kelts exiting Omak Creek or Bonaparte Creek are collected at the trap site of the respective creek and transported to the Cassimer Bar hatchery. All anadromous *O. mykiss,* irregardless of up or downstream movement and not selected for broodstock or reconditioning, were sampled for length, condition factor, inspected for tags (PIT or other), and received a PIT tag, sampled for DNA and marked with a fin clip.

Figure 7: Photograph of the resistance board weir located on Omak Creek.

Shitike Creek

The Shitike Creek picket weir began operation on March 1, 2007; it was placed so that it was perpendicular to the flow of the stream (Figure 8). The trap was monitored until June 21, 2007 and at that point operation switched to video monitoring only. The design of the weir incorporated separate trap boxes for upstream and downstream migrants (Figure 9). The location of the downstream trap was situated at the edge of the thalweg to minimize the amount of flow inside the trap box and to reduce any potential for crowding (10'l x 4'w x 3'h). The upstream box was situated in the deepest section of the river where fish were likely to migrate. To reduce potential impacts on the fish due to high flows, a catch pen was constructed next to the downstream box whereby fish would enter the trap and find an opening on the side to access the catch pen.

Fish 508 mm or greater in length (20 inch ODFW standard) were treated as anadromous adult steelhead. Information recorded for each fish captured at the weir included origin, sex, physical condition, coloration, floy tag, PIT tag number, fork length, and weight. Physical condition was recorded as either good (<25% fungus), fair (25-50% fungus), or poor (50-100% fungus). All adipose clipped fish were released downstream.

Figure 8: Shitike Creek weir construction February 2007.

Figure 9: Shitike Creek weir during summer of 2007.

Short-Term Reconditioning

Holding and Feeding

Steelhead kelts retained for the short-term reconditioning program at Prosser Hatchery were held in one of the four 20’ (d) x 4’ (h) circular tanks (Figure 10). Numbers held were well below the 200 fish carrying capacities for these tanks. Short-term reconditioned kelts were fed a diet of krill for the duration (3-5 weeks) of their captivity.

Figure 10: Steelhead kelt reconditioning tanks Prosser, WA.

Long-Term Reconditioning

Steelhead kelts retained for the reconditioning program at Prosser Hatchery were held in either one of four 20’ (d) x 4’ (h) circular tanks (Figure 11). Individual tank carrying capacity was set at a maximum of 200 fish based on the aquaculture experience of YN hatchery staff, and the project goal of maximizing kelt survival in captivity. Tanks were fed oxygenated 13.8C (57.0F) well water at 200 gallons/minute.

One 22’ circular tank was used to recondition Omak Creek steelhead kelts (Figure 11). Water was circulated at 120 gallons/minute at an average temperature of 13.3C (56.0F).

Figure 11: Steelhead kelt reconditioning facilities at Cassimer Bar Hatchery.

Steelhead kelts captured from Shitike Creek are held in a circular kelt reconditioning tank, 16’ (d) x 4’ (h), at Warm Springs National Fish Hatchery (WSNFH) Isolation Rearing Facility (IRF). The tank was installed (Figure 12) exclusively for kelt reconditioning. The tank was fed with Warm Springs River water. Before induction into the reconditioning tank water was filtered and treated with ultraviolet light to reduce pathogens. Water was circulated at 100 gallons/minute and temperatures were maintained by mixing in chilled water to maintain a steady 15.0C (59.0F) through the critical high temperature months (June-September). Artificial lighting replicated the photoperiod.

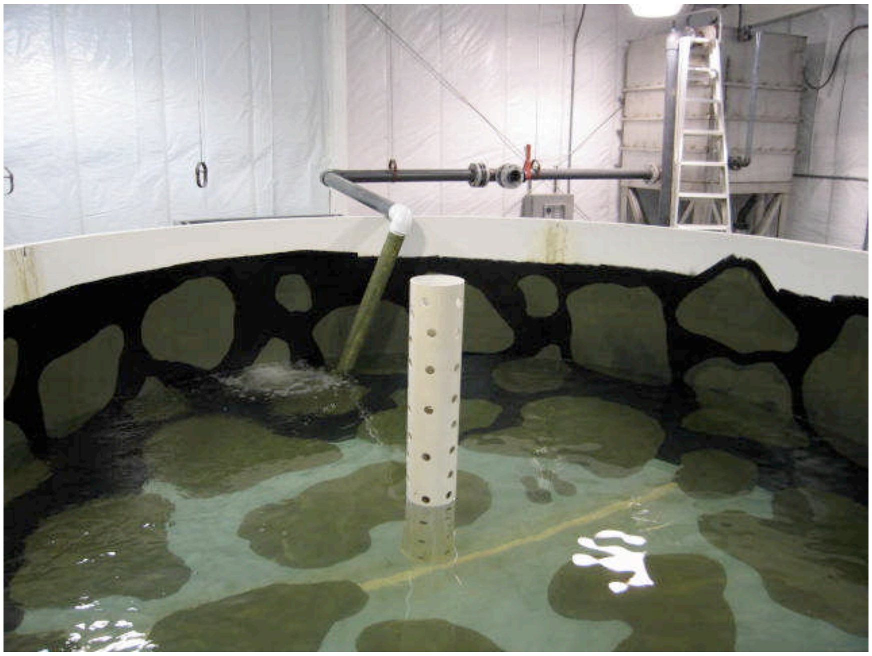

Figure 12: Indoor steelhead kelt reconditioning tank at WSNFH utilizing a camouflage pattern.

Feeding

The long-term reconditioned fish at Prosser Hatchery were initially fed frozen krill for 2.5 months then slowly switched over to Moore-Clarke Trout Broodstock pellets until release. Krill is utilized as a starter feed due to the readiness of kelts to consume this specific feed. Steelhead kelts are then slowly moved over to the Moore-Clark pellets to improve nutrition in the diet.

Modified versions of the feeding and holding protocols developed at Prosser Hatchery are utilized for long term reconditioning at Cassimer Bar Hatchery, Warm Springs National Fish Hatchery, and Parkdale Fish Facility (Hatch et al 2004). Generally fish are fed to satiation 2-3 times a day, and are monitored to prevent overfeeding which causes pollution in the holding tanks and is wasteful of expensive feed.

Hatchery managers are allowed to modify protocols as needed to improve survival. The staff at Cassimer Bar had the most modifications to the diet. Given that ocean going steelhead diets are diverse with such prey as squid, euphausiids, amphipods and various fishes (LeBrasseur 1966) and (Manzer 1968). The CCT fisheries staff provided such diverse food sources as cod liver oil, anchovies, squid, and herring to mimic natural food sources that may be found at sea. When pelletized food is introduced, natural food sources are still kept in rotation to provide trace minerals that have been found to be important in successful gamete production. Steelhead at Parkdale Fish Facility are captured as first time spawners so they do not endure the rigors of spawning, but they are recycled through Oregon Department of Fish and Wildlife's Sport's Fishery

numerous times. These fish are started on high krill to pellet ratio at capture to encourage feeding then are slowly moved off feed about 1-2 months prior to spawning. After spawning they are slowly moved back to a diet with a low krill to pellet ratio. Shitike Creek fish did not accept pellet feed so were maintained on a solitary diet of krill.

Kelt Mortalities

The following data were collected on all kelts that died during the reconditioning process at all facilities. On discovery of a mortality, fish were first examined externally by hatchery personnel to record the suspected time of death, general condition (good, fair, poor), fish color (bright, intermediate, dark), color of the gill arches (red, pink, white), size of the abdomen (fat, thin), presence of any scars or obvious lesions, and any other anomalies. Once the external exam was completed, an internal examination was conducted to record color of muscle tissue (red, pink, white), type of gonads (ovaries, testes), size of gametes (small, large), and presence of any internal anomalies. PIT tags, acoustic tags and radio tags were also removed from mortalities and identification numbers were entered into a computer database along with the growth measurement data. We attempted to reuse viable acoustic or radio tags whenever possible.

Steelhead Kelt Status and Release

Prior to release, all steelhead kelts were scanned for PIT tags, weighed, measured for fork-length. A majority of the no-term and short-term reconditioning releases were fitted with an acoustic receiver to compare releases strategies. All surviving specimens retained for reconditioning at the time of release were classified as either feeding or non-feeding based on weight change during captivity.

Fish in the long-term experiment were released in early October 2007 when river water temperatures match well water temperatures at the hatchery facilities. These long term reconditioned kelts over-winter within the systems they are released, and are able to volitionally return to the spawning grounds in late winter and spring. Release in the fall instead of the spring was prompted by results associated with the potential for early maturation at higher well water temperatures during winter (Branstetter et al 2006, Stephenson et al. 2007).

Prior to release, all surviving steelhead in the long-term experiments were examined with ultrasound equipment (Evans *et al*. 2001) to assess maturation status. Growth measurement data and rematuration status were recorded on all released individuals. Overall success of the reconditioning process was based on the proportion of fish that survived the reconditioning process and specifically for the long-term experiment, the number of fish that successfully rematured (based on ultrasound examinations).

No-Term Release

No-term release steelhead kelts were held for a maximum of one week. These kelts were then transported and released at the Hamilton Island Boat Ramp (rkm 231), below Bonneville Dam. We expect fish to return from this study in 2008 and also 2009.

Short-Term Release

All short-term reconditioned kelts were transported and released at the Hamilton Island Boat Ramp (rkm 231), below Bonneville Dam. We expect fish to return from this study in 2008 and possibly 2009.

Long-term Release

Yakima River
All long-term reconditioned kelts were transported and released at the Mabton Boat Ramp (Yakima River, rkm 96.3). We expect fish to migrate to the spawning grounds during the 2007-2008 spawning migration.

Shitike Creek
Reconditioned steelhead kelts were trucked from the Warm Springs National Fish Hatchery and released into the Deschutes River at the confluence of Dry Creek (rkm 148), approximately 4.5 rkm downstream from Shitike Creek to avoid potentially lethal temperatures that can be present in Shitike Creek at this time of the season.

Omak and Bonaparte creeks
Reconditioned steelhead kelts held at the Cassimer Bar Fish Hatchery were released into the Okanogan River (rkm 1) instead of Omak Creek to avoid low flows and poor water quality that are present in Omak Creek at that particular time of the season.

Comparison of Treatments Using Biotelemetry

The literature does not contain information regarding the impact that artificial reconditioning may have on the homing abilities of steelhead. Data collected by Foster and Schom (1989) provided evidence that the ability to home in Atlantic salmon kelts is imprinted during the fish's juvenile life stage and that reconditioning does not alter natal homing instincts. Based on these data artificial reconditioning should have no deleterious effects on outward migration as well. Steelhead kelts collected at Prosser Dam are wild fish that could have spawned in any of several upstream areas. We cannot know if individuals homed to natal locations prior to spawning. However, use of acoustic telemetry

and PIT tag technologies can provide data on repeat homing, at least to the subbasin level.

Acoustic Telemetry

Most steelhead kelts from the No-term and Short-term reconditioning experiments at Prosser Hatchery had a coded Vemco© V16-4H acoustic transmitter surgically implanted into the body cavity (length 65 mm, weight in water 10g which constitutes on average 0.25% of the fishes total body weight). A biologist trained by a licensed veterinarian performed surgeries to minimize adverse effects associated with handling and surgery and to ensure a high tag retention rate. Each acoustic tag has a unique bandwidth pulse that provides individual identification codes. After release, migration to the Pacific Ocean was tracked using acoustic telemetry arrays that spanned sections of the Columbia River and estuary below Bonneville Dam (Appendix A). The complete array was deployed in mid- April and was retrieved mid October 2007. This year's array placement remained nearly the same as last year's (Branstetter et al 2006) (Appendix A) with an additional receiver placed at the St. Helens (rkm 138) array and the removal of the Miller Sands side channel receiver (38.6 rkm). This arrangement provided us data on survival and timing in-river, to the estuary, and to the ocean. Using acoustic telemetry data we compared No-term and Short-term reconditioning experiments to assess fish survival, movement, distribution, travel time, velocity, as well as residence time in the estuary (Appendix B).

PIT Tags

All fish in this study received a PIT tag in the abdominal cavity at the time of capture. Each tag is unique and identifies an individual fish to assess performance throughout the reconditioning process and to determine the fate of kelts after release by measuring movement, timing, and survival. Sites that are relied upon for detection are located throughout the Columbia Basin on the Yakima, Omak, Shitike, Warm Springs and Columbia (hydropower dams) watersheds. Automatic adult PIT-tag detectors are present in all ladders at Bonneville Dam, McNary Dam, 2 of 3 ladders at Prosser Dam, and weirs on smaller systems. The Colville Tribes employ a Crump weir antenna located approximately ½ mile downstream of the semi-permanent weir on Omak Creek. The Crump weir antenna is a system that does not impede migration and detects the PIT tags when fish passed over the structure. There are some efficiency problems with the crump weir system when stream flows are unseasonably high.

Gamete and Progeny Viability

Parkdale Fish Facility

Pre-spawn steelhead captured for this experiment, were trapped from August 25, 2006 to October 22, 2006 at the Powerdale Dam trap located on Hood River. Fish were then transported to the Parkdale Fish Facility and held until ripened for

air spawning. Male gametes were collected manually and cryogenically stored prior to use. Female gametes were collected using the air-spawning technique (Leitritz and Lewis 1980) and mixed with thawed milt. Eggs were held until certified as disease free (Infectious Hematopoietic Necrosis virus (IHNV) and Bacterial Kidney Disease (BKD)) to allow shipment across state lines. Eyed eggs were then transported to the University of Idaho Aquaculture Research Institute for gamete and progeny analysis. Surviving females were reconditioned at the Parkdale Fish Facility and spawned a second time with cryopreserved milt from the same males to assess the effect of reconditioning.

The following parameters and variables were measured to assess and compare gamete and progeny viability from first and second spawnings: The proportion of eggs that reach keel stage (around 10 eggs were collected on day 15 after fertilization and preserved in Stockard's solution to assess percent fertilized). Also, the proportion of eggs that successfully hatched by cold shocking the eggs and then picking unfertilized and/or dead eggs from the live ones. After eggs are hatched juvenile survival and weight is monitored over time.

Prosser Hatchery

2006

After the lack of spawning success of reconditioned kelts at Section Corner Creek in 2005 (Branstetter et al. 2005) we retained 10 long-term reconditioned kelts in 2006 to test for any potential negative effects that winter feeding may have on egg quality during final maturation. These fish were split evenly with one group taken off of feed and another group which continued to receive standard feed rations. It was intended that kelts were to be sacrificed in mid-February and eggs evaluated but they perished prior to that time. This was the last year that this experiment was conducted at Section Corner Creek due to the high predation rates and the lack of detectable steelhead kelt contribution to juvenile populations.

2007

In earlier experiments, where long-term reconditioned females from Prosser Hatchery along with first-time spawners were outplanted to Section Corner Creek, the lack of reproductive contribution by the reconditioned kelts may be attributed to their exposure to relatively higher winter water temperatures at the hatchery (Branstetter et al. 2005 and Stephenson et al. 2007). In 2007, we retained 6 males and 100 females to study the effect of water temperature on the final maturation of long term reconditioned kelts. The fish were split into two groups, a chilled group (well water chilled 47^{o}F or river water < 50^{o}F) and a non chilled group (well water 57^{o}F) with all males going to the chilled group. Males were placed in the chilled tank as our assumption was that chilled water treatment would likely produce quality male gametes versus the warmer well water. We assume that male hormones have a negligible effect on the development of female gametes whereas diel patterns, nutrition, and water

temperatures are likely decisive factors in the formulation of female gametes. Water in both systems continued to be treated with formalin for the prevention and reduction of fungal infections.

An unfortunate accident resulted in the loss of all the fish in the chilled group (6 males and 50 females) within days of being transferred to the chilled tank. Twenty five of the remaining 50 kelts were transferred to the chilled tank to continue the experiment. Kelts from both tanks will be periodically checked for ripeness. Ripe eggs will be collected via air spawning technique and fertilized with cyropreserved milt from Skamania Stock males. These eggs will be held in the Prosser Hatchery incubation trays until they pass disease screens then transferred to the University of Idaho for analysis. We will assess gamete and progeny viability at the Prosser Hatchery as is currently conducted at the Parkdale Fish Facility experiment. In addition, blood samples will be collected from these individuals to determine hormonal levels of reconditioned steelhead kelts during spawning season.

Reproductive Success

Reproductive success will be assessed by genetic contribution of adult steelhead to the juvenile progeny. This is accomplished using microsatellite DNA markers and pedigree analysis. We conducted this work in Omak and Shitike creeks.

Sampling Scheme

A general sampling scheme was designed to concentrate our sampling effort on collecting 3, 4, and 5 year-old adult fish with 2 to 4 year freshwater residency. To include one pretreatment / control and three temporal replicates plans were made to continue this project for a 17-year study period. This allows for temporal and spatial replication of the study to the F3 generation. To expedite results, juveniles have been included as they offer an earlier measurement of reproductive success. In particular, age classes of juveniles representing brood years that potentially included reconditioned kelts were targeted.

Adult *O. mykiss* Collection

Tissue samples are were collected from all adults interrogated at the collection weirs on Shitike and Omak creeks. Sampling was conducted regardless of status (prespawner or kelt) or condition. A small caudle fin punch was collected and recorded for genetic analysis.

Juvenile *O. mykiss* Collection

<u>Omak Creek</u>
Emigrating smolts in Omak Creek were collected utilizing a screwtrap. Additional juveniles of unknown anadromous status were collected by electrofishing. Due to poor trapping conditions experienced in 2006 the rotary screw trap was moved

approximately a ¼ mile upstream in 2007. The trap was deployed on April 4, 2007 and continued until May 20, 2007 when flows decreased. Additional samples above the screwtrap were collected via electrofishing and included both resident rainbow trout and anadromous juveniles.

Shitike Creek
Shitike Creek juveniles were collected using a screw trap located downstream of the adult trap at RKM 0.9. The screw trap was operated in spring March 6, 2007 to June 29, 2007 and fall October 9, 2007 to November 30. 2007. There was approximately 3 days of total deactivation due to high water or debris jams. While collection of anadromous steelhead progeny was the goal, it was expected that resident rainbow trout progeny would also be captured.

Resident *O. mykiss* Population Collection

Omak Creek
Resident trout in Omak Creek were collected as unknowns via electrofishing. It is thought that fish greater than or equal to 180mm in length and captured after the spring migration belong to the resident population. However, population assignment of juvenile fish to either the resident or anadromous population was based solely on genetic parentage or population tests.

Shitike Creek
Adult residents (Rainbow Trout) in Shitike Creek were collected during the upstream migration that overlapped with the upstream migration of adult anadromous steelhead. Fish under 508 mm in length (20 inch ODFW standard) were considered resident rainbow trout.

Genetic Analysis

Samples were collected and stored in ethanol for preservation of DNA. Genetic analysis was conducted at the Hagerman Fish Culture Experiment Station in Hagerman, ID. DNA was extracted from tissue samples using standard manufacture's protocols from Qiagen® DNeasy™ in conjunction with a Qiagen® 3000 robot. The polymerase chain reaction (PCR) was used to amplify 16 microsatellite loci; Ogo 4 (Olsen et al. 1998), Oki 23 (GenBank Accession #AF272822), Omm 1036 (GenBank Accession #AF346686), Omm 1046 (GenBank Accession #AF346693), Oke 4 (Buchholz et al. 1999), Omy 1001, Omy 1011 (P. Bentzen pers. comm.), Omy 7 (K.Gharbi, pers. comm.), One 102 (Olsen et al. 2000), One u14 (Scribner et al. 1996), Ots 100 (Nelson and Beacham 1999), Ots 3M (Greig and Banks 1999), Ots 4 (Banks et al. 1999), Ssa 289 (McConnell et al. 1995), Ssa 407 and Ssa 408 (Cairney et al. 2000). PCR products were genotyped using manufacture's protocols with an Applied Biosystems® model 3730 genetic analyzer and scored using Genemapper v3.7 Software.

Prior to statistical analysis, confirmed duplicate samples, samples with incomplete genotypes and non-target species samples were omitted and are not included in the results. Expected and observed heterozygosity were calculated using Excel Microsatellite Toolkit (Park 2001). The number of alleles and allelic richness were calculated using HP-Rare (Kalinowski 2005). Deviation from Hardy-Weinberg equilibrium and genotypic linkage disequilibrium between all pairs of loci was evaluated using exact tests (Haldane 1954, Weir 1990, Guo and Thompson 1992) implemented in GENEPOP v3.4 (Raymond and Rousset 1995). Corrections to the significant value were made using the Bonferroni method (Rice, 1989). Parentage analysis was performed using CERVUS v 3.0 (Marshall et al. 1998, Kalinowski et al. 2006). Information on fish gender was not included in the analysis.

To help infer population structure in Omak Creek, the program STRUCTURE v.2.0 (Pritchard et al. 2000, Falush et al. 2003) was used. Aside from the known adult anadromous steelhead, samples in Omak Creek were expected to be mixed collections of the anadromous juveniles and resident populations. In an attempt to separate the two expected population groups, K=2 was used with the population containing the majority of anadromous adult steelhead referred to as the anadromous group. The alternative population was labeled the resident group. Samples with assignment probabilities lower than 0.70 were treated as unassigned.

Juvenile sampling in 2007 in Omak Creek was preferentially targeted at age 1 fish. This was done to increase the chance of detecting the progeny of reconditioned kelts released in 2005 and potentially returning to spawn in 2006. One male and two females were released in October 2005, but only the male was confirmed to have passed upstream of picket weir when it was handled on March 30, 2006. It was not detected when it passed the crump weir, nor was it detected again during a downstream migration. One female was detected entering Omak Creek at the crump weir on March 23, 2006 and again on April 17, 2006, but was not confirmed to have passed above the picket weir. The second female was not detected after the October release. While two of the three kelts were not detected above the weir, their return and contribution to spawning in Omak Creek cannot be ruled out as weir operations in 2006 were subject to disturbances from high water flows. At Shitike Creek there have been no subsequent PIT-tag detections of previously released kelts.

Seven juveniles collected in spring of 2006 via the screwtrap were assigned to two of the reconditioned kelts, but at lengths consistent with the first time spawning event occurring in the hatchery. To separate first time and reconditioned kelt spawning events, age histograms were created for age one and two juveniles. Lengths of juveniles with known parents were first used to create the histograms. Age of juveniles assigned to parents that potentially spawned as both a first time spawner and a reconditioned kelt were then determined by comparison to the histograms.

Results

General Population Characteristics

Yakima River

A total of 567 live kelts were captured between March 26 and July 3, 2007. Of these captures, 11 were culled due to poor condition, 53 were used in the Yakima River in-river release and 38 were used for the no-term release, leaving 465 for reconditioning efforts. Collection followed migrational waves with the peak collection date occurring on April 20 (Figure 13). The total number of kelts captured represented 36.9% (567 of 1,537) of the previous Yakima River spawning migration based on fish ladder counts obtained from Prosser Dam for the period July 1, 2006 to July 3, 2007. It's likely that this number does not represent the total kelt emigration from the Yakima River due to some kelts passing over the spillway at Prosser dam and facility shutdowns due to debris jams

The overwhelming majority of kelts captured were female which is consistent with previous findings. Based on visual observations, 87.0% (511) of the kelts were classified as female, whereas 76 (13.0%) were identified as male in 2007. This may be indicative of an evolutionary advantage to female iteroparity.

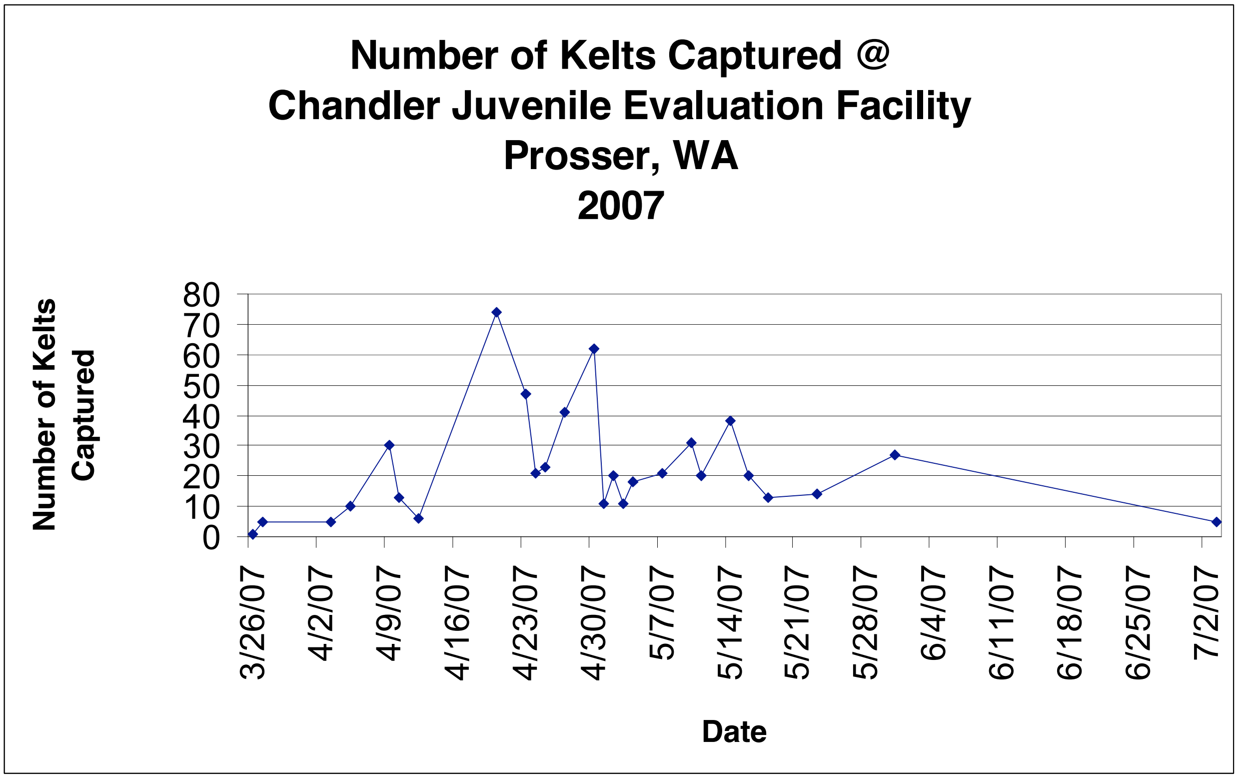

Figure 13: Steelhead kelt collection dates and numbers of fish removed from the CJEF in 2007.

Most kelts collected during 2007 were classified as being in good (38.1%) or fair (57.0%) condition, with the remaining fish classified as poor (4.7%). Coloration was predominately bright (51.2%), or intermediate (45.4%) with few dark (3.2%) fish.

Shitike Creek

A total of 90 upstream migrating summer steelhead (61 wild and 29 adipose clipped) were trapped at the weir in 2007 (Table 1). All wild fish were passed above the weir. One reconditioned kelt from 2006 was released in October but has not been detected at the trap. Of the adipose clipped steelhead, 6 were passed upstream of the weir and 23 were released downstream. Forty-one summer steelhead were processed in the downstream trap (33 wild and 8 adipose clipped), 19 kelts in fair condition were released downstream, 14 kelts were transported to WSNFH for reconditioning, and there were 8 mortalities (Table 2).

Table 1: Summary of upstream migrating hatchery and wild summer steelhead captured at picket weir, Shitike Creek March- June 2007.

Clip*	Sex	
	Male	Female
NM	29	32
AD	8	14
ADLV	1	2
ADLM	2	1
ADRM	0	1
Total	40	50

*note: clip marks are adipose (Ad), adipose left ventral (ADLV), adipose left maxillary (ADLM), adipose right maxillary (ADRM), and wild (NM)

Table 2: Summary of hatchery and wild steelhead collected in the downstream trap at the Shitike Creek weir, March - June 2007.

	Total Captured	Transported to WSNFH for reconditioning	Released Downstream	Total Mortalities
Wild	33	14	12	7
Hatchery	8	0	7	1
Total	41	14	19	8

*note: clip marks are adipose (Ad), adipose left ventral (ADLV), adipose left maxillary (ADLM), and wild (NM)

In 2007, approximately 32% of upstream migrants were of hatchery origin and 68% were of natural origin. The condition of upstream wild steelhead migrants was 56% good, 30% fair, and 15% in poor condition. The condition of upstream hatchery steelhead was 34% good, 38% fair, and 28% poor (Table 3).

Table 3: Condition of hatchery (AD) and wild (NM) steelhead caught in the upstream trap at the Shitike Creek weir (%).

Condition	AD	NM
Good	34%	56%
Fair	38%	30%
Poor	28%	15%

Omak Creek

The semi-permanent weir was installed in Omak Creek on March 12, 2007. The first fish was enumerated on March 23, 2007 and the last fish released upstream was on May 22, 2007. The first kelt was collected on April 26, 2007 with peak collection occurring between April 29, 2007 and May 14, 2007. A total of 97 fish were processed at the adult trap in Omak Creek during 2007 compared to 67 fish in 2006. Samples of DNA were taken on all but 4 fish. Of the 97 fish sampled 43

had existing PIT tags, and 54 did not. Ten of these fish were transported to Cassimer Bar hatchery for broodstock while the others were released above the weir to spawn naturally. The ratio of males to females in Omak Creek was 1.2 males per female. There were 4 unclipped, untagged females and 12 unclipped untagged males, comprising 16.4% of the return to the creek. A total of 38 post-spawned steelhead were captured in the downstream trap. We retained 10 kelts for reconditioning, bypassed 14 poor condition kelts and recovered 14 mortalities that floated downriver. The two reconditioned kelts that were released in 2006 were not caught at the weir. It is possible that these fish may have spawned below the weir or bypassed the weir undetected.

There were 168 steelhead enumerated at the Bonaparte weir in 2007. Of these fish, 66 had PIT tags and 102 did not. The ratio of males to females in Bonaparte Creek was approximately 1.75 males for every female. There were 9 unclipped, untagged males and 10 unclipped, untagged females that comprised 11.3% of the return to the creek. Trapping began in Bonaparte Creek on March 23, 2007 and ended, May 30, 2007. The first kelt was collected on April 19, 2007 with the peak of kelt collection occurring May 9, 2007 through May 24, 2007. There were a total of 43 kelts captured and retained for reconditioning with 18 poor quality fish passed down river and an additional 11 that were counted as mortalities moving downriver.

In-River, No-term, and Short term capture, treatment, and release

A total of 53 kelts from the Yakima River were collected, processed, PIT tagged and released as part of the in-river treatment between April 12, 2007 and May 31, 2007 (Table 4).

There were two No-term release groups. Both groups were collected, processed, PIT tagged and acoustic tagged in the same manor. The first group was collected and released on April 20, 2007. The second group was collected on May 23, 2007 and released two days later (Table 4).

Short-term reconditioned kelts were captured on April 25, 2007, held for reconditioning, and then processed, PIT tagged, acoustic tagged, and released on May 30, 2007 (Table 4). Chandler Dam Sluiceway shutdowns resulted in reduced kelt collections therefore, we allocated only one group to the short-term reconditioning this year.

Table 4: Release group results by treatment

Treatment	In River	No-term 1	No-Term 2	Short-term
Release Date	4/12/07-5/31/2007	4/20/07	5/25/07	5/25/07
No. Collected	53	33	10	41
No. Released	53	28	10	38
No. with acoustic tags	0	25	8	27
Mean Capture Weight (kg)	4.35	4.19	4.15	4.32
Mean Release Weight (kg)	4.35	4.19	4.15	3.97

Mortality

We assumed that we had high immediate survival for the In-River Release group as handling was minimal and fish were quickly released into the river.

All known mortalities for the No-term experiment occurred shortly after capture, fish processing, and subsequent tagging surgery. We had 5 mortalities at the time of the first release and no mortalities at the second release. We were unable to determine the source of mortality as all sampling and tagging procedures occurred within 48 hours after capture.

The Short-term release group had three observed mortalities directly after acoustic tag surgeries. The post surgery mortalities are likely caused by compounded stress from handling and surgery but an exact cause is unknown. Fish in the short-term release group lost an average of 9.7% body weight in captivity.

Long-Term Reconditioning and Survival to Release

Yakima River 2006

Fish captured for the long-term reconditioning were captured from March 29, 2006 to May 22, 2006. After induction into the reconditioning program 46.4% of the mortalities at the Prosser Hatchery occurred within 4 weeks. The rest of the mortalities occurred at a steady rate until release. A total of 85 fish were successfully reconditioned by October 17, 2006. Based on ultrasound images 92.9% of these individuals were likely mature. Of the surviving 85 Long-term reconditioned fish 44 were allocated to the feed/no-feed trial and gamete analysis, and 41 were released into the Yakima River at the Mabton boat launch (rkm 96.3) (Table 5).

Table 5: Long-term reconditioning results by tank for 2006.

Tank:	C1	C2	Long-term Total
Held for Reconditioning	110	168	279*
Released	8	32	41*
Retained for No Feed Trial/Egg Viability	11	31	44*
Survival Rate	17.2%	37.5%	30.5%
Rematuration Rate			92.9%
Pct with wt gain	78.0%	79.0%	76.4%
Avg. wt gain (lbs)	1.09	1.10	1.10
* Includes Fish that lost PIT-tag so no rearing tank or capture weight data is available. .			

Yakima River 2007

Kelt steelhead were collected from March 26 to July 3, 2007. After induction into the Prosser Hatchery reconditioning facility, 29% of the mortalities occurred within 4-weeks. The rest of the mortalities occurred steadily over time until fish were released. A total of 52.4% of the fish collected for long-term reconditioning survived to October 11, 2007 at which time 115 individuals were released into the Yakima River. The remaining 106 fish were retained for female gamete development analysis (Table 6). Based on ultrasound scans 87% of this year's release were likely mature. Fish were released at the Mabton boat launch (rkm 96.3).

Table 6: Long-term reconditioning results by tank 2007.

Tank:	C1	C3	S4	Long-term Total
Held for Reconditioning	190	190	42	422
Released or	6	94	15	115
Retained for Chill/non-chill study	80	21	5	106
Survival Rate	45.3%	60.5%	47.6%	52.4%
Rematuration Rate				87%
Pct with wt gain	78.5%	71.5%	80.0%	75.1%
Avg. wt gain (lbs)	1.17	0.43	0.39	0.72

Shitike Creek

Of the 14 (12 female and 2 male) kelts transported to the WSNFH for Long Term Reconditioning in 2007, only one female survived to be released back into the river the following fall. All of the 13 carcasses had a necropsy performed in an attempt to determine suspected causes of mortality. Fish mortality was attributed to lack of eating, copepods, fungus, and unexpected jump outs. Nearly half of the mortalities occurred within the first three weeks of reconditioning

On October 12, 2007, CRITFC and CTWS personnel released one steelhead kelt into the Deschutes River at the confluence of Dry Creek (rkm 148), approximately 4.5 rkm downstream from Shitike Creek. Due to the apparent infestation of copepods an additional dose of Ivermectin ™ (5cc 1:30 diluted) and Oxytetracycline (5cc) were administered. The ultrasound showed that the reconditioned female had a small undeveloped egg mass. The fish gained 0.8 kilograms (kg) in the reconditioning facility.

Omak and Bonaparte creeks

A total of 10 kelts were transported from Omak Creek and 43 kelts transported from Bonaparte Creek to the Cassimer Bar Hatchery for long term reconditioning. Eight kelts survived reconditioning and were on station at the hatchery (7 females, one male) until release on October 5, 2007. At the time of release, all fish exhibited a strong feeding response, were bright in color, average weight increased by 28%, and there was little to no copepod presence. Necropsy results showed the primary cause of death for Bonaparte fish to be irritation of the gill tissue. The primary cause of death for Omak Creek fish was due to lack of feeding. The secondary cause of death for kelts overall, was due to lack of feeding and secondary infections.

Management Scenario Evaluation

Acoustic Telemetry

Acoustic arrays were placed strategically throughout the lower Columbia River (Appendix A) to monitor and measure outmigration and return survival of the two release strategies (no-term release and short-term reconditioning). We can use these data to determine if short-term reconditioning is beneficial to steelhead kelts or if simply bypassing the hydrosystem is more beneficial to kelt survival. Kelts in these release groups are released at the Hamilton Island Boat Ramp (rkm) (Appendix A).

No-term Treatment Release Detections and Run Timing

There were a total of 25 kelt steelhead in the first release and 8 kelts in the second release. A total of 21 (84%) from the first release and 7 (87.5%) fish from the second release were detected at the St. Helens array. Of these, 13 (52%) from the first release and 4 (50%) fish from the second release were detected (Table 7) entering the upper estuary at either the Welch Island or Pillar Rock telemetry array. Travel time from the release to the ocean averaged 6.67 days for the first release and 4.58 days for the second release group. (Tables 7 & 8). Estuary residence averaged 47:14 hours for the first release and 23:01 hours for the second. We detected 10 kelts (40% of total tagged) from the first release and 4 kelts (50%) from the second release at arrays at the mouth of the Columbia River. We assumed that kelts that were not detected again were mortalities.

Table 7: Number detected, travel time and average travel speed to detection array from time of release of No-term release group 1, N=25. Released 4/23/2007.

St Helens Detection		Time to St. Helens	Time to Estuary	Time to Ocean From Release	Avg. km/hr from Hamilton Is. To St. Helens	Avg. km/hr from Bonneville to Estuary	Avg. km/hr from Bonneville to Ocean
21	River Only Detected Fish Avg. (hr: min: sec)	88:54	*	*	1.06	*	*
Estuary Detection 13	Estuary Only Detected Fish Avg. (hr: min: sec)	95:14	138:29	*	1.00	1.37	*
Ocean Detection 10	Ocean Detected Fish Avg. (hr: min: sec)	94:18	112:56	160:10	1.01	1.68	1.4

Table 8: Number detected, travel time and average travel speed to detection array from time of release of No-term kelt release group 2, N=10. Released May 25, 2007.

St Helens Detection		Time to St. Helens	Time to Estuary	Time to Ocean From Release	Avg. km/hr from Hamilton Is. To St. Helens	Avg. km/hr from Bonneville to Estuary	Avg. km/hr from Bonneville to Ocean
7	River Only Detected Fish Avg. (hr: min: sec)	35:29	*	*	2.67	*	*
Estuary Detection 4	Estuary Only Detected Fish Avg. (hr: min: sec)	44:34	102:36	*	2.13	1.85	*
Ocean Detection 4	Ocean Detected Fish Avg. (hr: min: sec)	52:19	87:51	110:52	1.81	2.15	2.09

<u>No-term Release Survival Probability</u>

Survival and detection probability for kelts in the No-term treatment is calculated by reach (Table 8). The first reach is from release at Hamilton Island (Rkm 233) to the St. Helens array (Rkm 140). The second reach is from the St. Helens array to the arrays in the upper estuary (Rkm 53). The final reach is from the upper estuary to the mouth of the Columbia River. In the final reach, we cannot separate survival from detection probability by derivation, therefore, for this reach we report a quantity termed lambda which is the product of survival and detection probability for this reach. If the detection probability is assumed to equal 1.0 for the detection array located at the mouth of the river, then reach survival would equal lambda. rkm 138. Survival once again this year was good for both no-term release groups but was slightly lower than previous years. Survival was very good to reach one with an average of 85% (Table 8). The first release group though not as successful as the second group survival rate at 84%, still did considerably well with an average of 77% of individuals surviving to the second reach (Table 8). The second release group did phenomenally well when considering the lambda value while the first release group faired well with lambda value that is within the typical average.

Table 8: Survival Probability of No-term release 1 on 4/23/2007 and No-term 2 on May 25, 2007.

No-term Release 1					
Reach	Survival (S)	std. deviation	Detection (P)	std. deviation	Lambda
Reach 1	0.840	0.073	1.000	0.000	0.840
Reach 2	0.774	0.119	0.800	0.127	0.619
Reach 3	-	-	-	-	0.615
No-term Release 2					
Reach 1	0.875	0.117	1	0	0.875
Reach 2	0.857	0.132	0.667	0.193	0.572
Reach 3	-	-	-	-	1

Short-term Release Detection and Run Timing

A total of 27 short-term reconditioned kelts received acoustic tags to assess their emigration behavior, survival, and timing after release from below Bonneville Dam to the Pacific Ocean. Fish were released on May 30, 2007 below Bonneville Dam (Rkm 233) at Hamilton Island. Telemetry arrays were deployed at various locations in the lower Columbia River (described in detail in the No-term section above). Twenty-four of the 27 kelts from the Short-term treatment were heard at the St. Helens array and 19 of 27 were heard entering the ocean (Table 9). The kelt steelhead that reached the ocean traveled 2.38 km/hr on average and took slightly more than 4 days to complete the trip (Table 9). On average the kelts spent just over 22 hours in the estuary.

Table 9: Number detected, travel time and travel speed to detection array from time of release of Short-term kelt release N= 27. Released on 5/30/2007.

St Helens Detection		Time to St. Helens	Time to Estuary	Time to Ocean From Release	Avg. km/hr from Hamilton Is. To St. Helens	Avg. km/hr from Bonneville to Estuary	Avg. km/hr from Bonneville to Ocean
24	River Only Detected Fish Avg (hr: min: sec)	65:24:25	*	*	1.45	*	*
Estuary Detection 17	Estuary Only Detected Fish Avg. (hr: min: sec)	33:21:00	62:43	*	2.87	3.04	*
Ocean Detection 19	Ocean Detected Fish Avg. (hr: min: sec)	42:06:50	75:25	97:26	2.26	2.5	2.38

Short-term Release Survival Probability

Steelhead kelts assigned to the Short-term treatment performed well migrating through the lower Columbia River to the ocean with little mortality incurred during the outmigration (Table 10). Survival through the river and into the ocean was the highest that we have recorded over the last 4 years of study (Table 10). The probability of detection at the various arrays was similar to other release groups and ranged from 73% to 92% (Table 10).

Utilizing the survival probability formula (Appendix B) to compute no-term survival probability, results suggest that the short-term release group of steelhead kelts successfully navigated the lower Columbia River to the ocean with little mortality incurred (Table 10). This release had exceedingly high survival rates to Reach 1 and Reach 2 (Table 10). The lambda rate for Reach 3 is second only to no-term release 2.

Table 10: Survival Probability of Short-term Release, 5/30/2007

Short-term Release					
Reach	Survival (S)	std. deviation	Detection (P)	std. deviation	Lambda
Reach 1	0.970	0.037	0.917	0.056	0.889
Reach 2	0.990	0.078	0.733	0.102	0.726
Reach 3	-	-	-	-	0.722

Gamete and Progeny Viability

Parkdale Fish Facility

The steelhead that was captured and spawned at Parkdale Fish Facility in 2006 was air-spawned again as a reconditioned kelt in May of 2007. The female did not survive the second spawning. In both years, the eggs were fertilized with cryopreserved milt from the same 2 males collected in 2006 to remove any male effect on the experiment. Three experimental groups were created with two single pairings (Male 1 and Male2) and one combined male cross (Combined). Overall fertilization success for the reconditioned kelt was low for a subset of eggs tested for keel development relative to the first time this female was spawned (Table 11). The reconditioned kelt had an average of 13% of a subset of eggs in the keel stage relative to an average of 47% from the her first spawning (Table 11). The remaining live egg survival was greater for the reconditioned kelt than the first time she was spawned with no mortality of eggs after they were transported to University of Idaho (Appendix D).

Table 11: The proportion of eggs that successfully developed to keel stage for a subsample of eggs collected from a female (K06) that was spawned in 2006 and again as a reconditioned kelt in 2007. This female was spawned both times with cryopreserved milt from two males (Male 1, Male 2) and with a "Combined" milt group from Male 1 and Male 2.

Female	Milt Group	Spawn Date	Eggs	# Keel	% Keel	Spawn Date	Eggs	# Keel	% Keel
K06	Male 1	4/10/2006	26	13	50%	5/17/2007	10	2	20%
K06	Male 2	4/10/2006	25	10	40%	5/17/2007	10	0	0%
K06	Combined	4/10/2006	24	12	50%	5/17/2007	10	2	20%
Average					47%				13%

This, steelhead kelt also had higher survival rates for the fry from her second spawning which averaged 96% survival rate relative to her first spawning which averaged an 80% survival rate (Table 12).

Table 12: Fry survival at three months for the first and second spawning of a female (K06) that was paired with three cryopreserved male groups (Male 1, Male 2, and Combined Male1/Male2).

Female	Male	Spawn Date	Starting Number	Ending Number	% Survival	Spawn Date	Starting Number	Ending Number	% Survival
K06	Male 1	4/10/2006	94	78	83%	5/17/2007	58	55	95%
K06	Male 2	4/10/2006	93	65	70%	5/17/2007	57	53	93%
K06	Combined	4/10/2006	93	81	87%	5/17/2007	52	52	100%
Average					80%				96%

2007 First-time Collection and Spawning

A total of 21 female and 24 male steelhead were collected from Powerdale Dam on the Hood River and held at Parkdale Fish Facility until they were ready to spawn in 2007. Milt was collected from all males and cryopreserved for future pairings of first time spawning steelhead and reconditioned kelts. Of the 21 females, 15 were used in experimental crosses (2 single male matings and a combined male mating) to test for gamete and progeny viability. Shortly after spawning all but one of the females being reconditioned at the Parkdale Fish Facility died likely from copepod infestation. This female was reconditioned and will be spawned again in 2008.

For the experimental cross groups used in the 2007 first time spawning gamete and progeny analysis, 8% to100% of the eggs survived to the keel stage (average of 65%) and fry survival ranged from 41% to 100% (average of 78%). Individual egg keel and fry survival data, along with growth data for all crosses can be found in Appendix (D).

2008 First-time Collection and Spawning

Currently there are 14 male and 16 female first time spawners that were captured at the Powerdale Dam trap which are being held for experimental crosses that will be used in the spring of 2008. We have had no mortalities to date. After spawning these fish will be reconditioned and subsequently spawned again in 2009.

Prosser Hatchery

2006

Ten kelt steelhead from the long-term reconditioning treatment were held in tanks to evaluate the effect of feeding on maturation. All of these fish died in late January 2007. Hatchery staff observed that one of the females in the no-feed trail appeared to be dripping good quality eggs. Necropsies by hatchery staff

were inconclusive as to any difference in gamete quality between the group on feed and the group being held without feed.

<u>2007</u>

To investigate the effect of water temperature on maturation, a total of 106 reconditioned kelts were split into two groups. One group of 50 females were held in well water and the second group of 49 females and 7 males were held in chilled well water. Within 72 hours of putting fish into the chilled, no chill groups a system malfunction resulted in the loss of all 56 fish in the chilled group. Upon mortality staff conducted necropsies that showed the fish were exhibiting various levels of gamete development. No gamete development was found in 8 fish One fish had very small discolored eggs. Twenty-three females had small egg skeins (Figure 14) and 15 had relatively large egg skeins (Figure 15). Large testes were found in 7 males (Figure 16).

Figure 14: Example of female with small egg skein discovered by necropsy of kelt mortalities from the chilled water group.

Figure 15: Example of female with relatively large egg skein discovered by necropsy of kelt mortalities from the chilled water group.

Figure 16: Example of male with large testes discovered by necropsy of kelt mortalities from the chilled water group

Hatchery staff continue to monitor reconditioned kelts for maturation status and females will be spawned as they mature. This portion of the experiment is currently ongoing and is anticipated to be finished by March 2008.

Reproductive Success

Omak Creek

Statistical analysis was performed on 1079 samples collected in Omak Creek between 2004 and 2007. Sample numbers and population statistics are reported for each sample collection in Table (13). Departures from Hardy-Weinberg equilibrium (critical level =0.05 /16 loci = 0.00313) and or linkage disequilibrium (critical level =0.05 /120 pairwise comparisons = 0.00042) were seen in all population collections. All collections except the 2004 anadromous adults had a heterozygote deficit. Additional statistical analysis proceeded as normal as population admixture is not unexpected, and Hardy-Weinberg equilibrium is not needed for the additionally analyses to be completed.

Table 13: Population statistics for Omak Creek, Washington. Each population is reported in terms of sample size (n), expected heterozygosity (H_E), observed heterozygosity (H_O), average number of alleles per locus (A), allelic richness (AR), number of loci out of Hardy-Weinberg equilibrium (HW), and number of pairwise loci comparisons in linkage disequilibrium (LD).

Population	N	H_E	H_O	A	AR	HW	LD
Anad. Adult: 2004	92	0.8113	0.8121	12.1	10.2	11	90
Anad. Adult: 2005	105	0.8156	0.7934	13.6	10.8	0	25
Anad. Adult: 2006	89	0.8166	0.8118	13.6	11.1	0	6
Anad. Adult: 2007	86	0.8263	0.8103	14.1	11.4	2	20
Electrofish: 2005	99	0.8307	0.7954	13.1	10.7	9	50
Electrofish: 2006	138	0.8355	0.8034	14.3	11.4	7	36
Electrofish: 2007	93	0.8437	0.8322	14.4	11.8	1	8
Screwtrap: 2006	96	0.8386	0.7968	13.9	11.3	5	30
Screwtrap: 2007	281	0.8265	0.8117	15.8	11.2	10	53

Relative success of both STRUCTURE and CERVUS are reported in Table (14). All anadromous adults assigned to either the first group, subsequently referred to

as anadromous, or intermediate to the anadromous group and the alternative resident group. Assignment to the anadromous population varied in the juvenile collections between 37.7% and 86.1%. A small percentage of samples had parentage assignments to an anadromous adult, but were not assigned to the anadromous population when using Cervus. These samples are reported as combination mismatches in Table (14).

Table 14: Population Assignments. Portion of each collection group assigned to either the anadromous (Anad.), resident (Res.) or unknown (Unk.) group via STRUCTURE and CERVUS (if assigned to an anadromous adult). Combination mismatch refers to juvenile samples that were assigned to an anadromous adult by parentage assignment, but to an unknown population with population assignment.

	Structure			Parentage			Combination
	Anad.	Res.	Unk.	Anad.	Res.	Unk.	Mismatch
Anad. Adult: 2004	0.989	0.000	0.011				
Anad. Adult: 2005	1.000	0.000	0.000				
Anad. Adult: 2006	1.000	0.000	0.000				
Anad. Adult: 2007	0.988	0.000	0.012				
Anad. Adult: Sum	0.995	0.000	0.005				
Electrofish: 2005	0.556	0.323	0.121	0.535	0.000	0.465	0.030
Electrofish: 2006	0.370	0.507	0.123	0.246	0.000	0.754	0.014
Electrofish: 2007	0.613	0.194	0.194	0.591	0.000	0.409	0.097
Electrofish: Sum	0.494	0.364	0.142	0.430	0.000	0.570	0.042
Screwtrap: 2006	0.656	0.208	0.135	0.485	0.000	0.515	0.000
Screwtrap: 2007	0.861	0.089	0.050	0.647	0.000	0.353	0.003
Screwtrap: Sum	0.809	0.119	0.072	0.606	0.000	0.394	0.003

Lengths of known age fish (by parentage analysis) varied from 62mm for young of year fish collected in the fall via electrofishing to 197mm for age two fish collected in the spring via the screwtrap. Figure (17) shows the age histogram for age one and two fish collected in the spring. There was length overlap between age classes albeit at low frequency. Three juveniles collected in 2007 were progeny of the male first returning as a first time spawner in 2005 and again as reconditioned kelt in 2006. These juveniles were consistent with the age one size class, and therefore were considered to be age one and thus progeny of the second (reconditioned kelt) spawning event.

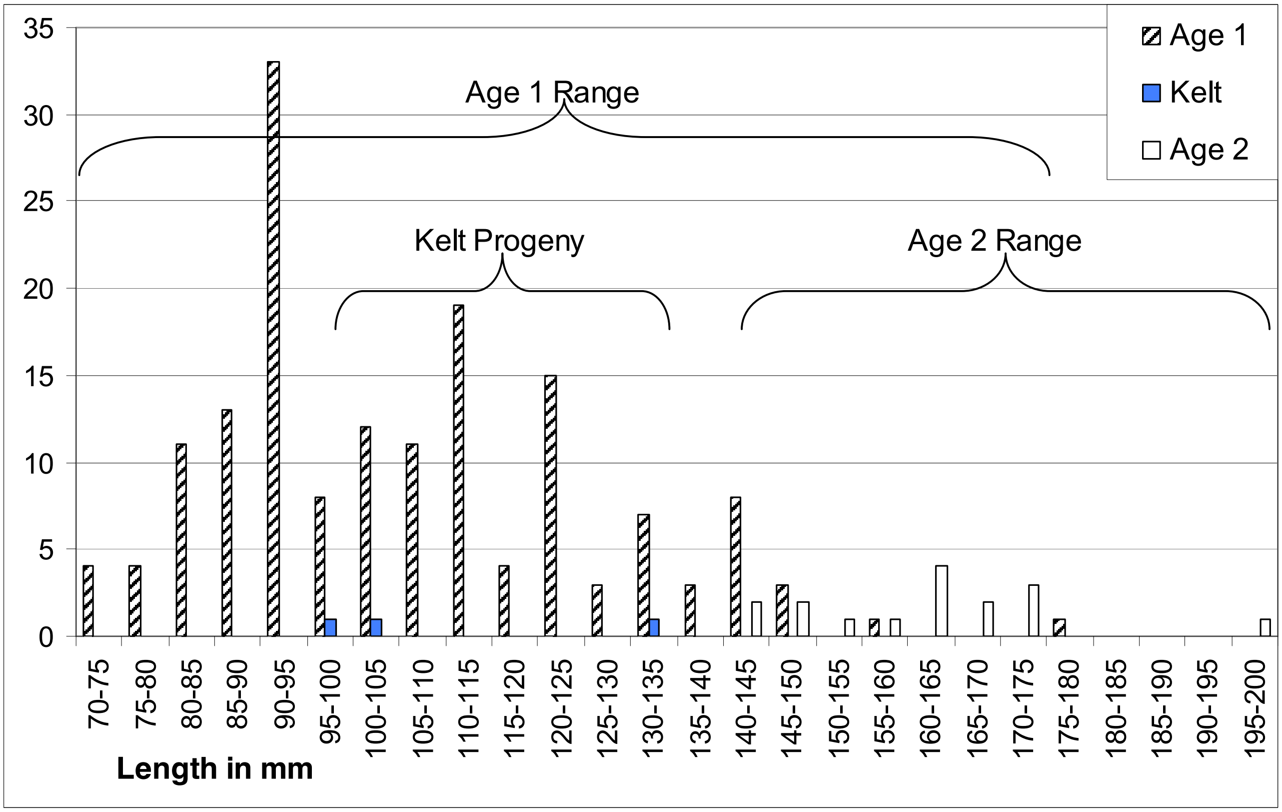

Figure 17: Length Frequency Histogram. Number of fish for length classes is reported for known age (via parentage) juveniles captured in the screwtrap. Kelt progeny assignments are based on lengths corresponding with other age 1 juveniles.

Shitike Creek

Statistical analysis was performed on 791 samples collected from Shitike creek between 2005 and 2007. Sample numbers and population statistics are reported for each sample collection in Table (15).

Table 15. Population Statistics for Shitike Creek steelhead. Each population is reported in terms of sample size (n), expected heterozygosity (H_E), observed heterozygosity (H_O), average number of alleles per locus (A), allelic richness (AR), number of loci out of Hardy-Weinberg equilibrium (HW), and number of pairwise loci comparisons in linkage disequilibrium (LD).

Population	n	H_E	H_O	A	AR	HW	LD
Anad. Adult: 2005	61	0.8207	0.8005	14.5	12.3	0	2
Anad. Adult: 2006	39	0.8176	0.8091	13.2	12.3	1	8
Anad. Adult: 2007	109	0.8147	0.8032	15.6	12.0	1	4
Screwtrap: 2005	60	0.7103	0.7196	11.8	10.0	0	1
Screwtrap: 2006	139	0.7160	0.7117	14.0	10.4	0	5
Screwtrap: 2007	130	0.7898	0.7686	15.5	11.9	2	4
Resident: 2005	150	0.7107	0.7094	13.4	10.0	1	2
Resident: 2006	50	0.7164	0.7195	11.7	10.2	0	1
Resident: 2007	53	0.7219	0.7344	11.3	9.9	0	1

Self assignment rates in Shitike Creek using GENECLASS were high for both anadromous steelhead (246 of 253) and resident rainbow trout (197 of 203). Assignment of juveniles to the anadromous population was nonexistent in 2005 (0 of 60), low in 2006 (11 of 135) and higher in 2007 (71 of 123). Figure (18) shows the relationship of sample date (x-axis) and length (y-axis) between individuals assigned to either the anadromous or resident populations.

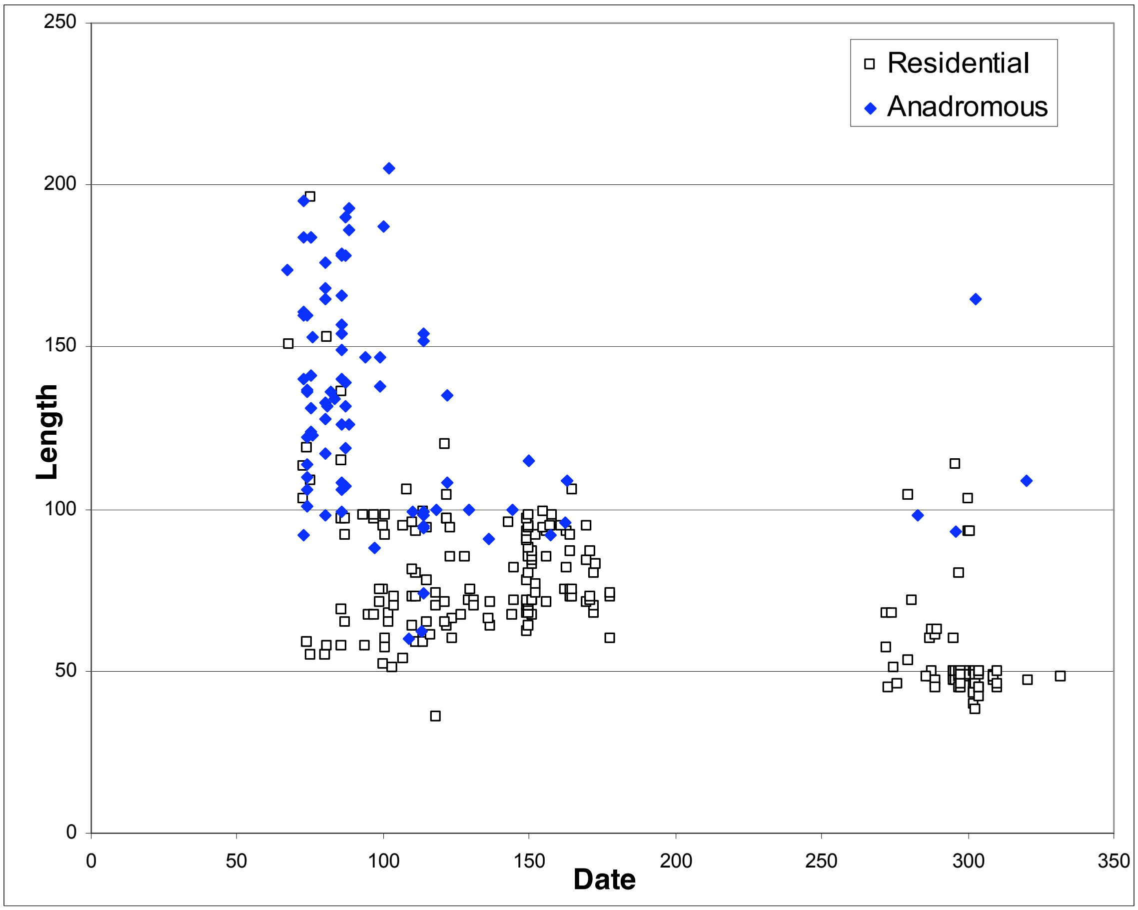

Figure 18: Population Assignments. The relationship of date (X-axis) and length (Y-axis) for anadromous (diamond) and resident (square) juveniles as assigned by GENECLASS.

Parentage assignment in Shitike Creek was successful in identifying at least one parent for 24 of the 71 (as determined by GENECLASS assignments) anadromous juveniles collected in 2007. One of the 24 samples assigned to two parents with the exception of a single mismatch at Ots-100. As the genotypes for both the mismatching parent and the juvenile were homozygous, a null allele is a possibility. Seventeen of the juveniles were assigned to eight parents spawning in 2005 (Table 16). Lengths of Brood year 2005 fish varied from 136-187mm. One juvenile assigned to an adult that potentially spawned as both a first time spawner in 2005 and a reconditioned kelt in 2006. The remaining six juveniles were assigned to six parents spawning in 2006 and varied in length from 60-

115mm. Two additional samples were assigned to an anadromous parent with CERVUS, but to the resident population with GENECLASS.

Table 16. Parentage Assignments. Biological data along with brood year for all juveniles with at least one successful parental assignment. Length, weight and date of sample refer to the juvenile sample. Brood year refers to the return year of the adult as determined by parentage assignment.

Juvenile sample	Length (mm)	Weight (grams)	Date of sample	Brood Year
SHSEJU14	60	2.5	4/19/2007	2006
SHSEJU48	96	9.5	6/12/2007	2006
SHSEJU52	98	9.6	4/24/2007	2006
SHSEJU63	100	10.7	5/24/2007	2006
SHSEJU73	107	12.8	3/27/2007	2006
SHSEJU83	115	17.9	5/30/2007	2006
SHSEJU105	136	26.5	3/14/2007	2005
SHSEJU108	137	26.5	3/14/2007	2005
SHSEJU111	139	28.5	3/27/2007	2005
SHSEJU112	140	26.1	3/13/2007	2005
SHSEJU115	141	26.8	3/15/2007	2005
SHSEJU117	147	33.1	4/9/2007	2005
SHSEJU120	152	35.5	4/24/2007	2005*
SHSEJU121	153	37.7	3/16/2007	2005
SHSEJU123	154	35.4	3/26/2007	2005
SHSEJU125	157	39.2	3/26/2007	2005
SHSEJU127	160	36.5	3/13/2007	2005
SHSEJU128	161	41.3	3/13/2007	2005
SHSEJU129	165	42.8	3/20/2007	2005
SHSEJU131	166	45.4	3/26/2007	2005
SHSEJU135	178	50.6	3/27/2007	2005
SHSEJU136	178	57.4	3/26/2007	2005
SHSEJU137	179	53	3/26/2007	2005
SHSEJU142	187	55.1	4/10/2007	2005

***Adult potentially spawned as both a first time spawner in 2005 and a reconditioned kelt in 2006.**

Discussion

Steelhead Kelt Reconditioning

We continued to investigate steelhead reconditioning strategies including; in-river release, no-term, short-term, and long-term treatments in 2007. Additionally we conducted gamete and progeny viability as well as relative reproductive success studies on kelt steelhead. The goal of this program is to evaluate management strategies that could restore steelhead populations and promote iteroparity.

Survival and rematuration of long-term reconditioned kelt steelhead held at Prosser Hatchery nearly doubled in 2007 compared to 2006 at 46% compared to 27%, respectively. Survival and rematuration rates exceeded the average across years by more than 25% (Appendix C). In addition no-term and short-term treatments groups had extremely high survival rates to the ocean (80.7% and 72.2 %). We assume that the in-river release group also survived well but no monitoring system is place to detect these fish prior to their return migration. In past years at Bonneville Dam, we have detected in-river, no-term, and short-term treatment fish returning during the first fall. To date, three fish (5.7% of the release) from the in-river release were detected moving upstream at Bonneville Dam. None of the 2007 released no-term or short-term treatment fish have been detected yet at Bonneville Dam. Overall steelhead kelts entering the Prosser Hatchery were in extremely good condition likely resulting in the high survival of all treatments. Favorable environmental conditions during the previous migration year and spawning may play an important role influencing the survival of kelt steelhead in all treatments.

Lower Columbia River acoustic analysis continues to provide data that demonstrates that kelts move rapidly to the ocean with relatively short estuary residence time. Survival and travel time are plotted and measured from the time of release to the detection arrays for the no-term and short-term treatments releases over the last 4 years (Figure 19). Survival is negatively correlated (R^2=0.52 for no-term and 0.62 for short-term) with travel time for each treatment. We found that with increased travel time there is a decrease in survival regardless of detection point.

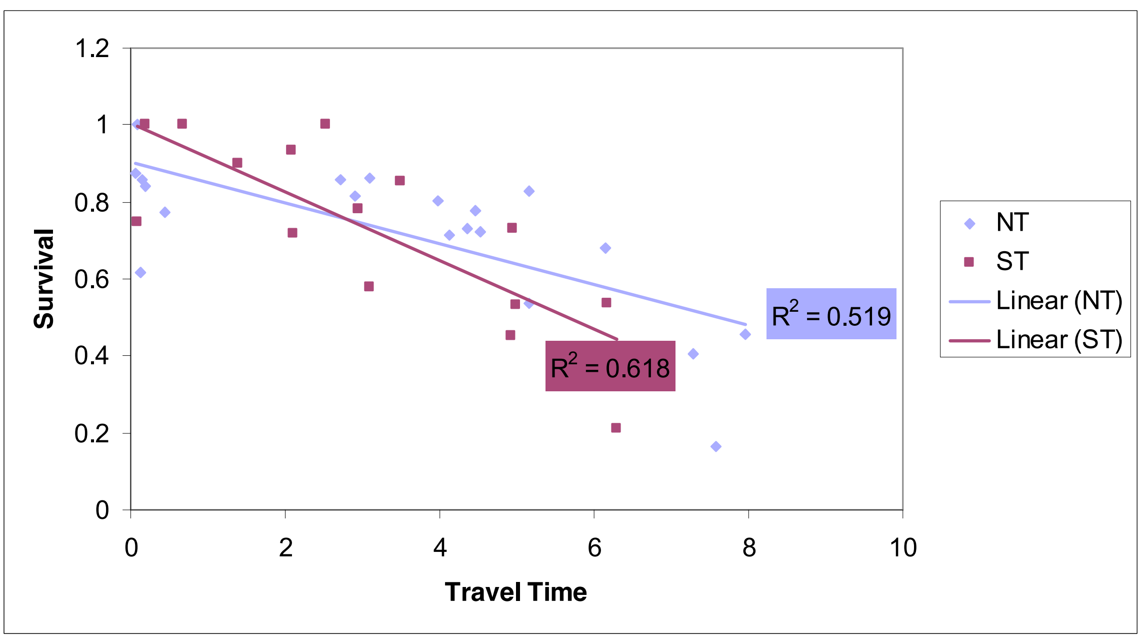

Figure 19: Steelhead kelt outmigration survival as a function of travel time (in days) for no-term (NT) and short-term (ST) treatment releases from 2004 through 2007.

Previously it was thought that the river environment played an important role in the survival of steelhead kelts migrating in the lower Columbia River. Prior to the 2007 release group, kelt steelhead migration survival to ocean appeared to be positively related to river flow with a stronger correlation of (R^2=0.525). However survival in 2007 release groups were quite high (S range 60 to 100%) at flows that previously were associated with lower survival (S range 0 to 54% excluding one release in 2006) (Figure 20). Adding the 2007 data to the survival as a function of flow weakens the correlation to R^2=.345.

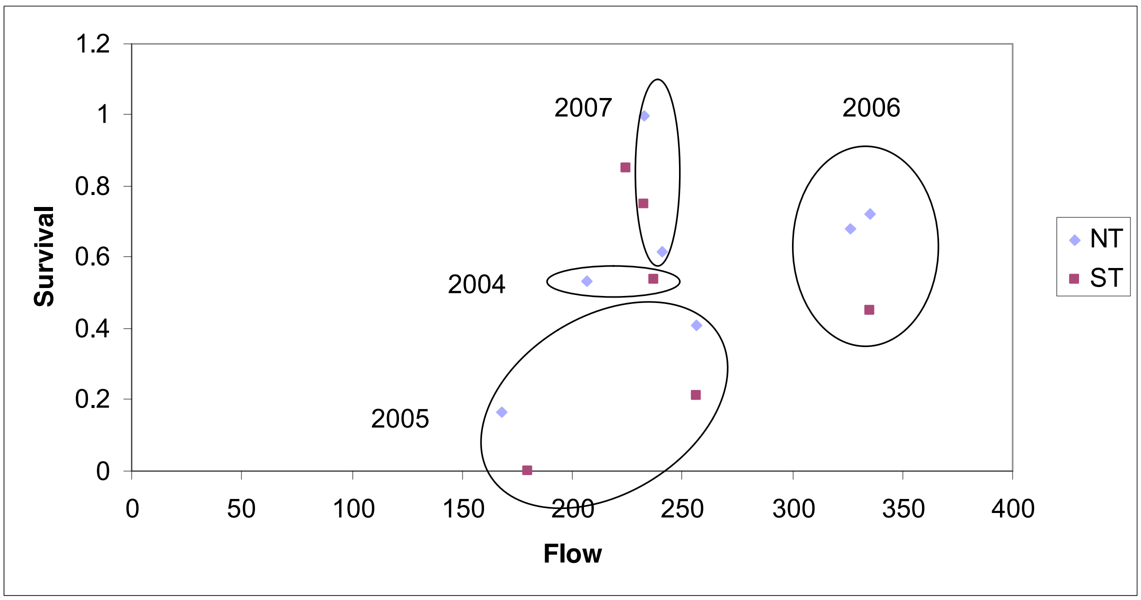

Figure 20: No-term (NT) and short-term (ST) release groups from 2004 through 2007 survival as a function of Columbia River flow (kcfs) measured at Bonneville Dam. The one point not included within an ellipse is from 2006.

It was also assumed that water temperature would affect survival but the effect appears to be minimal until reaching a critical level between 16 and 18C. The lowest survival was a short-term group released in July of 2005, which also was at the highest water temperature of all releases (Figure 21).

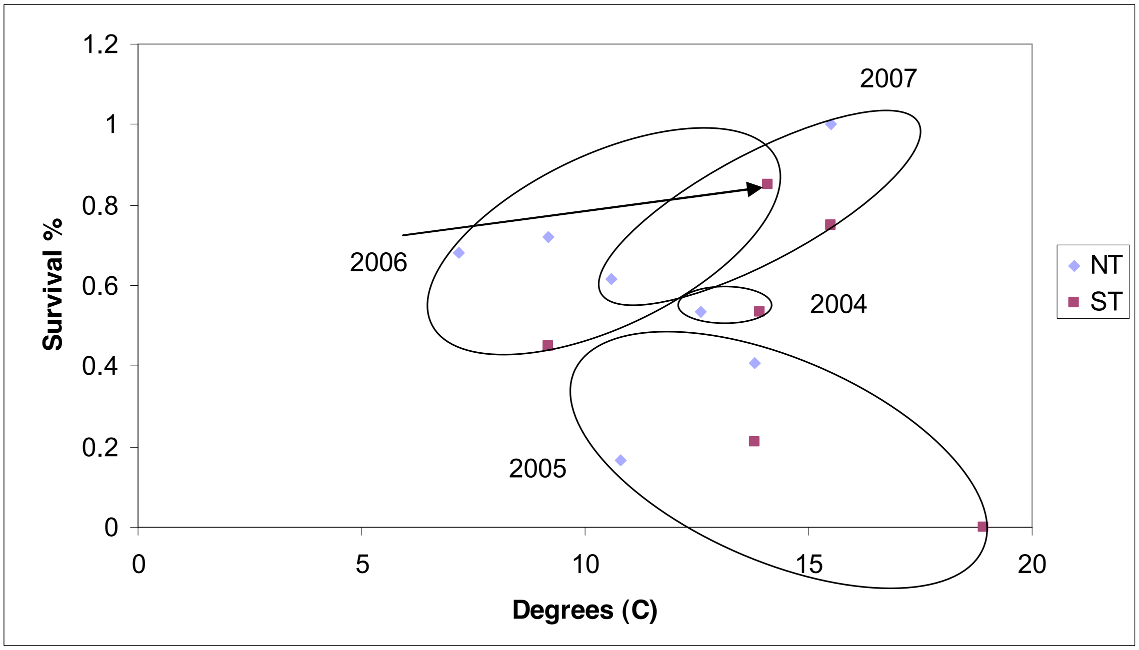

Figure 21: Survival of No-term (NT) and short-term (ST) release groups, in the lower Columbia River, from 2004 through 2007 as a function river temperature. One NT release from 2007 and one ST release from 2006 did not group in their respective ellipses.

Steelhead Kelt Returns

Acoustic Telemetry

In years with such high survival to the ocean, it would be expected that a portion of the steelhead kelts from the no-term and short-term treatments would return with the following (within year) spawning migration. For the 2007 releases, there have been no returns detected in the acoustic arrays, although it's possible that some portion will return during the 2008 spawning migration year (Table 17). There were 3 (6.1%) detections from the 2006 No-term release which has been the highest return for acoustic tagged fish since the project started (Table 17).

Table 17: Acoustic tag return migration detection in the lower Columbia River.

Release Year	Treatment	Total Tagged	Total Observed Returning to Columbia River	Return Year	Number Detected within Return Year	% of total Tagged
2007	No-Term	33	0	2007	0	0.0%
2007	Short-term Release	27	0	2007	0	0.0%
2006	No-Term	49	3	2007	3	6.1%
-	-	-	-	2006	0	-
2006	Short-term Release	50	0	2006	0	0.0%
2005	No-Term	57	1	2007	0	-
-	-	-	-	2006	1	1.5%
-	-	-	-	2005	0	-
2005	Short-term Release	56	1	2007	0	-
-	-	-	-	2006	1	1.5%
-	-	-	-	2005	0	-
2004	No-Term	24	1	2006	0	-
-	-	-	-	2005	1	1.5%
-	-	-	-	2004	0	-
2004	Short-term Release	26	0	2004	0	0.0%

PIT-tag Detections

Overall the highest PIT tag returns at Bonneville Dam for all treatments occurred in 2002 (Short-term 12%) and 2004 (Short-term 6%), although the returns from this year's In-river release were very high at 5.7% (Table 18). Early in the study it appeared that Short-term reconditioning may have had a survival advantage but subsequent releases have shown the other treatments to survive at a higher rate. The surprising trend since 2005 is the high number of steelhead from the In-river releases that are detected at Bonneville Dam. For these years, this treatment had the highest survival of all treatments (Table18). The proportion of steelhead detected returning to the Yakima River at Prosser dam is understandably lower than Bonneville Dam but the trend is consistent with no one treatment showing higher survival in multiple years (Table 19).

Table 18: Total PIT-tag detections for return spawning migration at Bonneville Dam 2002-2007.

Release Year	Treatment	Total PIT-tagged	Total Observed Returning to Bonneville	Return year	Number Detected Returning within Year	% of total Release
2007	In-River Release	53	3	2007	3	5.7%
2007	No-Term	38	0	2007	0	0.0%
2007	Short-term Release	38	0	2007	0	0.0%
2006	In-River Release	52	1	2006	0	1.9%
				2007	1	
2006	No-Term	49	2	2006	0	4.1%
				2007	2	
2006	Short-term Release	52	0	2006	-	0.0%
2005	In-River Release	67	3	2005	3	4.5%
-	-	-	-	2006	0	-
-	-	-	-	2007	0	-
2005	No-Term	96	0	2005	0	0.0%
2005	Short-term Release	99	1	2005	1	1.0%
-	-	-	-	2006	0	-
-	-	-	-	2007	0	-
2004	No-Term	63	3*	2004	3	4.8%
-	-	-	-	2005	0	-
-	-	-	-	2006	0	-
2004	Short-term Release	83	5	2004	3	6.0%
-	-	-	-	2005	2	-
-	-	-	-	2006	0	-
2003	Short-term Release	187	7	2003	4	3.7%
-	-	-	-	2004	3	-
-	-	-	-	2005	0	-
2002	Short-term Release	332	12	2002	29	12.0%
-	-	-	-	2003	11	-
-	-	-	-	2004	1	-
In Branstetter et al. 2006 this was reported as 5 and has been corrected.						

Table 19: Total PIT- tag Detection By Release Year at Prosser 2004-2007.

Release Year	Treatment	Total PIT-tagged	Total Observed Returning to Prosser	Return Year	Number Detected within Return Year	% of total Release
2007	In-River Release	53	0	2007	0	0.0%
2007	No-Term	38	0	2007	0	0.0%
2007	Short-term Release	38	0	2007	0	0.0%
2006	In-River Release	52	1	2006	0	1.9%
		-	-	2007	1	-
2006	No-Term	49	1	2006	0	2.0%
-	-	-	-	2007	0	
2006	Short-term Release	52	0	0	0	0.0%
2005	In-River Release	67	3	2005	3	4.5%
-	-	-	-	2006	0	-
-	-	-	-	2007	0	-
2005	No-Term	96	0	0	0	0.0%
2005	Short-term Release	99	0	0	0	0.0%
2004	No-Term	63	0	0	0	0.0%
2004	Short-term Release	83	4	2004	2	4.8%
-	-	-	-	2005	1	-
-	-	-	-	2006	1	-

Long- Term Detections

We have had a total of three of the long-term release fish that have managed to be detected at Bonneville Dame attempting to return during 2001-2007. Two of the three detections occurred before the PIT-tag readers at Prosser were operational (2001 and 2002), with the last detection occurring at Bonneville with no further upstream detection in 2006.

Gamete and Progeny Viability

Parkdale Fish Facility

The first time spawning of 15 female steelhead in 2007 for the gamete and progeny viability study produced a wide range of baseline fertilization and initial fry survival data. Since each female was mated up to 3 times (2 single males and a combined milt group), a total of 38 matings were obtained. A subsample of eggs from each pairing was used to determine the initial embryo survival rate by enumerating the proportion of eggs that reached the keel stage. These rates

ranged from 8% to 100% with an average of 65% for all matings. The surviving embryos were reared to the fry stage at the University of Idaho which produced fed fry survival rates which ranged from 41% to 100% with an average of 78% survival. The female that was spawned for the first time in 2006 had and average of 47% of her eggs keel and a fed fry survival rate that averaged 80%, which is slightly above the 2007 averages. This baseline first time spawner data will be valuable in evaluating the gamete and progeny viability of artificially reconditioned steelhead kelts.

The use of cryopreserved milt in all experimental matings allows us to spawn the same female with the same males in multiple years to evaluate any reconditioning effect on a female kelt's egg viability and early juvenile survival. Only one female was collected and spawned in 2006 and fortunately survived the reconditioning process to be spawned a second time in 2007 as a reconditioned kelt. The average keel rate for the second spawning was 13% (range 0-20%) compared to 47% (range of 40-50%) from the first spawning. This reduced fertilization rate for the second spawning was followed extremely high survival of surviving eggs and fry. Average fry survival for the second spawning was 96% (range 93-100%) compared with 80% (range 70-87%) for the initial spawning. Based on the data from a single female, it's difficult to make any conclusions about any potential kelt reconditioning effect on gamete and progeny quality or viability.

Results from the second spawning did show a reduced embryo survival rate from the initial spawning, although it does fall within the range of first time spawner data collected in 2007 and this female was able to produce viable offspring. Similar low initial survival of embryos followed by very little mortality has been seen in rainbow trout and could be an indication that once the development reaches a critical point the surviving embryos can have high survival (Stoddard *et al*. 2005) There are several factors that could give these results including, poorly mixing of gametes during the second spawning, reduced number of viable eggs after reconditioning, problems with the cryopreserved milt, or natural variation in fertilization rates. McLean *et al.* (2007) found a wide range of variation in the reproductive success of Forks Creek Washington steelhead in a hatchery setting. On average egg to smolt survival rates ranged from 64% to 95% with 40% of the males and 27 % of the females producing no offspring. The ability of this female to produce viable offspring and the high survival of the viable eggs does show promise for reconditioned steelhead kelts to contribute to the reproduction, although an increased sample size is needed to come to any kind of conclusion.

Yakima River
Reconditioned kelts being held in chilled and unchilled tanks for the gamete and progeny viability analysis are currently being spawned with cryopreserved milt as they mature. The unfortunate early loss of the kelts from this group did allow us

to dissect those individuals to visually assess the maturation status. From these observations it was determined that all the individuals were at varying stages of maturation including 15 females appeared that were in advanced stages of egg development relative to others in the group. However, it is possible that vitellogenesis is advanced in the early fall and slowly progresses over the winter. Such is the case with Rapid River spring Chinook salmon (Penny Swanson, personal communication). The group with less developed eggs may experience accelerated vitellogenesis in the winter or mature in the following year. Currently the source of the asynchronous maturation is unknown and needs research.

Reproductive Success

Shitike Creek

In 2007, an effort was made to identify progeny of the anadromous population in Shitike Creek. The juvenile screwtrap was operated at an earlier time, and genetic analysis was focused on larger sizes of juveniles. As a result, many more juvenile samples (57.7%; 71 of 123) were assigned to the anadromous population in 2007 than the previous two years (0.0% and 8.1%, respectively). The majority of samples assigning to the anadromous populations are seen early in the spring at lengths greater than 100mm (Figure18). Parentage assignment of these samples allows further clarity through the assignment to brood years and age classes. Age two (BY2005) individuals were predominant at lengths of 136-187mm, while age one individuals (BY2006) were seen at lengths of 60 to 115mm. While one female potentially spawned as both a first time spawner and a reconditioned kelt, the length of the juvenile progeny was consistent with the age class associated with the first time spawning event.

While successful, parentage assignment was still limited in 2007. Of the 71 samples assigned to the anadromous populations, only 24 had successful assignment to at least one parent. This is not completely unexpected as weir operation in 2005 did not allow for a high capture rate of migrating adults. Improvements to the weir operation should increase the capture rate although complete sampling of the adult population is unlikely due to the dynamic conditions of water flows during migration. Adult migration in Shitike Creek may begin as early as January and is dependant upon high water flows, which are conditions that make weir operation difficult. Still, improvements in weir operation and the corresponding increase in the percentage of sampled adults should result in an increased success rate for parentage assignment tests.

Concordance between the parentage and population tests is high, only two juveniles with anadromous parents were assigned to the resident population. This is a positive sign that reproductive success analysis is possible in Shitike Creek. Utilization of migration timing and length data will also allow genotyping to concentrate on anadromous juveniles. If the majority of juveniles migrate at age 2, 2008 should provide greater opportunities to determine the reproductive success of the reconditioned kelt that could have spawned in 2006.

Omak Creek

Departures from Hardy-Weinberg equilibrium and linkage disequilibrium were common in Omak Creek collections. In juvenile collections this is likely due to the presence of both the anadromous and resident component of *O. mykiss*. While no reference collection of adult residents is available, results from population assignment tests support the presence of multiple populations. Furthermore, the resident population has apparent substructure between samples collected above and below Mission falls (Stephenson et al. 2007) due to the falls acting as a migration barrier. Samples collected via electrofishing in 2007 had fewer incidences of departures from Hardy-Weinberg equilibrium and linkage disequilibrium, likely due to the absence of samples collected above the falls during 2007.

In adult collections, departures from Hardy-Weinberg equilibrium were seen in 11 of 16 loci in 2004, and 2 of 16 loci in 2007. Linkage disequilibrium was also high in 2004 when it was seen in 90 of 120 loci pairs. The cause of this is unknown, however, sample records from 2004 are incomplete and the initial broodstock used to re-establish the Omak Creek population may have included multiple populations.

Population self assignment rates were high for anadromous adults with 99.5% of samples assigning to the anadromous group. Although resident samples were only collected as unknowns, the high consistency with which anadromous adults were assigned to as single group supports the divergence of the anadromous and resident populations.

Assignment rates to the anadromous group varied among years and methods for juveniles. Within the electrofishing sample collections, the percent of juveniles assigned to the anadromous group varied from 37.0% in 2006 to 61.3% in 2007 with an intermediate of 55.6% seen in 2005. The discrepancy between years is related to the portion of samples collected above Mission Falls, with few above falls samples collected in 2005 and none collected in 2007. This suggests that the section of Omak Creek above Mission Falls is predominately resident. This is expected as Mission Falls is a potential barrier to upstream migration of anadromous adults. While both parentage and population assignments supported the presence of anadromous juveniles above the falls in 2005, there was no evidence seen in 2006. Either juveniles were sampled from different locations not including anadromous progeny, or high water flows in 2006 limited the upstream migration of adult steelhead. Juveniles from above Mission Falls were not analyzed in 2007.

The proportion of juveniles assigned to the anadromous population is higher in juveniles captured via the screwtrap, 80.9% versus 49.4% via electrofishing. The high rate in 2007 (86.1%) can be partially attributed to at least two factors. First,

the screwtrap was moved to a location further upstream. Secondly, age 1 juveniles were targeted for genetic analysis.

Parentage assignment was most successful in 2007 with 64.7% of juveniles collected via the screwtrap being assigned to at least one known adult. The lowest amount of success was 24.6% seen in samples collected via electrofishing in 2006. The large discrepancy is the result of relative proportions of anadromous and resident juveniles present in each sample collection. As demonstrated by population assignment, resident progeny are more common in samples collected via electrofishing than via the screwtrap. As adult residents were not targeted in the sampling effort, minimal parentage assignment success is expected for progeny of residents.

All but 15 samples assigned to a known parent with CERVUS, were assigned to the anadromous population via STRUCTURE. All 15 were assigned to only a single parent and 14 were collected via electrofishing. One possible explanation is interbreeding between the anadromous and resident populations in Omak Creek.

It is apparent that the single male reconditioned kelt that passed above the Omak Creek picket weir in 2006 successfully spawned. The three 2007 juvenile samples assigned to it through parentage analysis fit within the age 1 size category. Although there is some overlap in length between the age classes (Figure 17), the progeny in question were in a size range only seen in age 1 fish. The final status of the other two kelts is unknown, and it is possible that one or both passed upstream of the picket weir without being detected. Additionally, unsampled adults can spawn below the picket weir, and even below the screwtrap.

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Appendix

Appendix A. Lower Columbia River Array Locations

Figure 1: Lower Columbia Acoustic Receiver Arrays 2007.

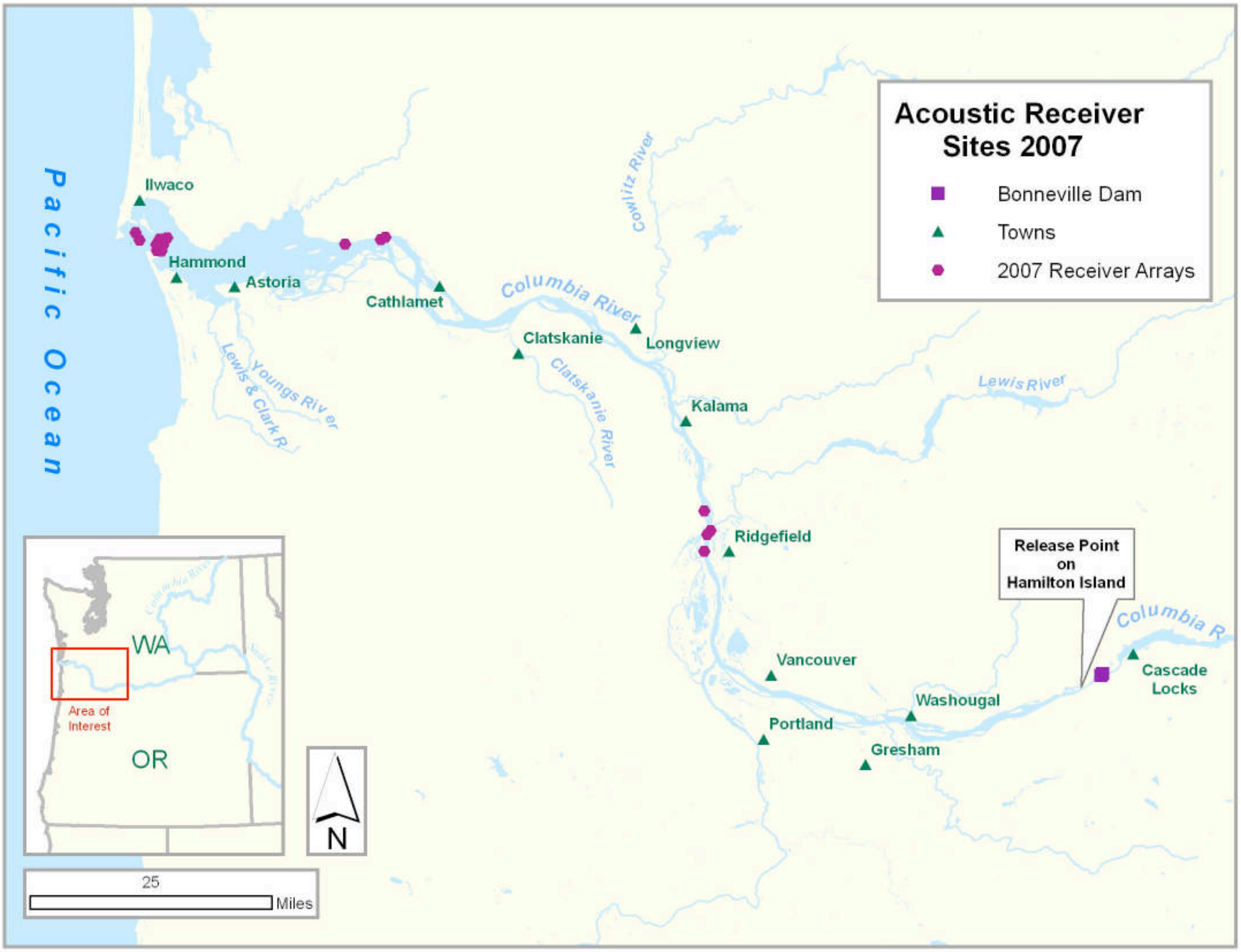


Figure 2. St Helens Array (rkm 138) 2007.

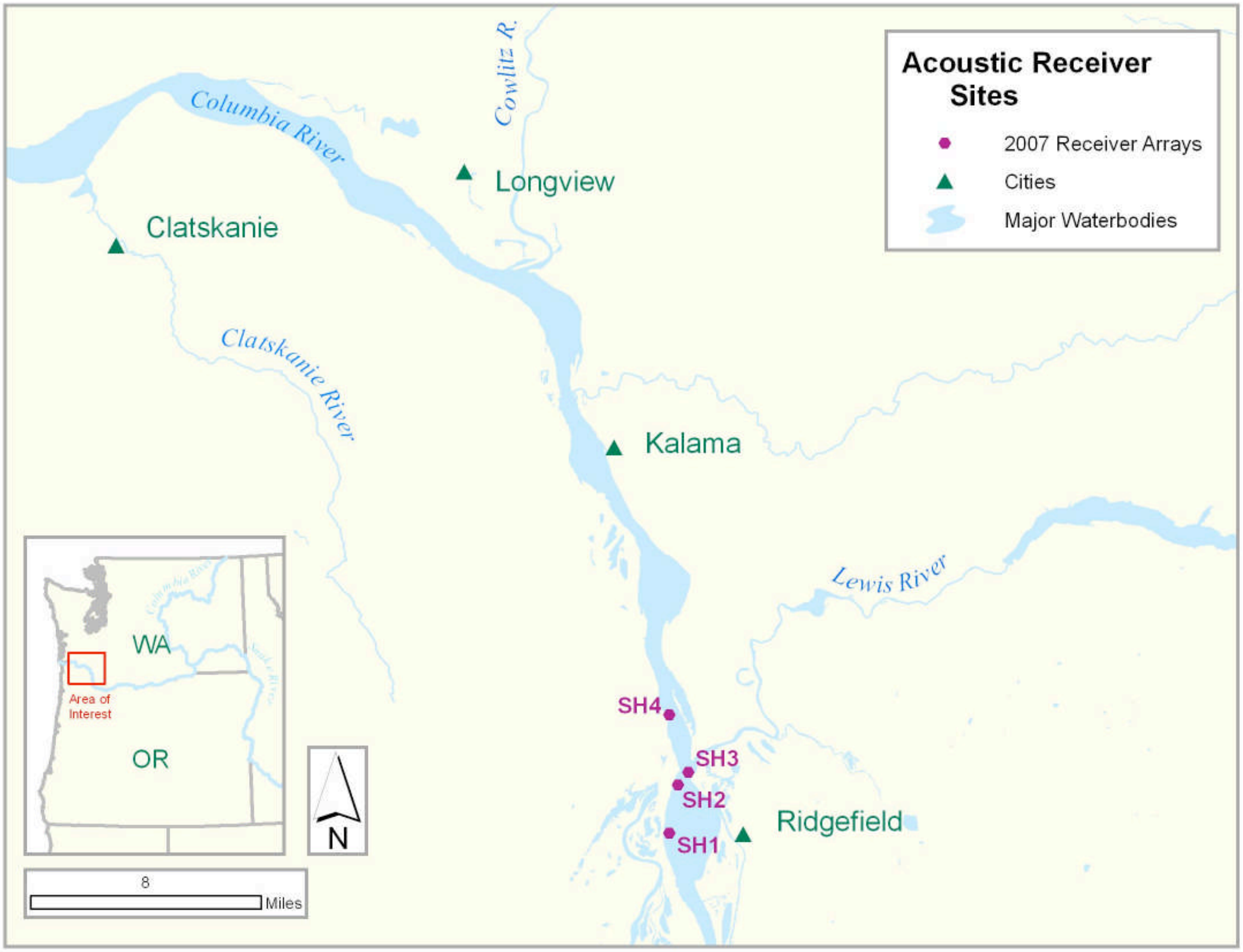
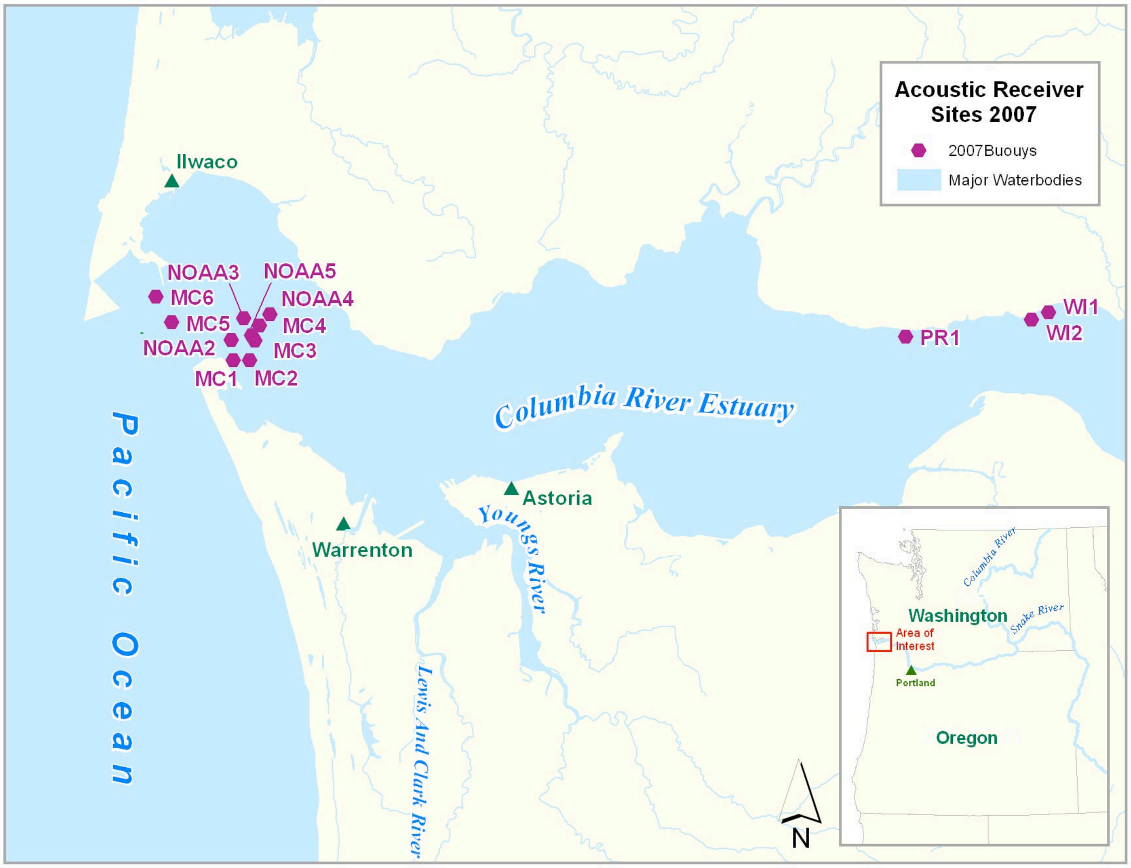


Figure 3: Estuary Acoustic Array Sets (Mouth of the Columbia RM 0 and Estuary rkm 45).



Appendix B: Likelihood functions of parameters

For a tagged fish to be detected at receivers after release, the fish should not only survive to the receiver location but also be successfully detected by receivers. Thus, the number of fish detected in a location involves two parameters: fish survival and receivers' detection rate.

We describe estimation of parameters in a generalized setting where two detection locations are considered (Fig. 1). Even when there are more than two detection locations, the estimation principal remains the same. First, we arrange available data as in Table 1. In case of the experiment design in Fig. 1, numbers of fish released at stage 1 and then detected at stage 2 or 3 become multinomial random variables.

$$(n_{g12}, n_{g13}) \sim Multinomial(R_{g1}, \theta) \quad (1)$$

where parameter vector $\theta = (s_{g1}p_1, s_{g1}(1-p_1)s_{g2}p_2)$. First element of vector θ (i.e., $\theta_{(1)} = s_{g1}p_1$) means the probability that a fish from stage 1 survives to next stage, and also is detected at the next stage. The second element $\theta_{(2)}$ (= $s_{g1}(1-p_1)s_{g2}p_2$) indicates the probability that a fish from stage 1 survives to stage 2, is not detected at stage 2, survives from stage 2 to stage 3 and finally is detected at stage 3.

Also, when considering that the number of fish detected at stage 2 is the new release number, the number of fish detected at stage 2 being detected at stage 3 again becomes a binomial random variable.

$$n_{g23} \sim Binomial(n_{g12}, s_{g2}p_2) \quad (2)$$

where $s_{g2}p_2$ means the probability that a fish from stage 2 survives to stage 3 and then is detected at stage 3. However, so-called success/failure probability in the binomial mass function in eq. 2 consists of two

parameters of s_{g2} and p_2, and such two parameters cause an over-parameterization problem because a success/failure parameter in a binomial mass function is only one. That is, we cannot separately estimate s_{g2} and p_2 and thus express the product as one parameter, say λ_g (Table 1). The expression of λ_g is not problematic in this study, because our ultimate goals are to compare two fish groups (control vs. treatment) not to estimate receivers' detection rates. A difference in λ_g between two fish groups is due to only fish survival s_{g2} not receivers' detection rate p_2 (Table 1). So, comparing two fish groups based on estimates of λ_g is justifiable.

Further these multinomial and binomial events do not affect each other, so they are independent. That is, the probability of those two events is the product of the respective probabilities.

$$p(n_{g12}, n_{g13}, n_{g23} \mid \theta, \lambda_g) = p(n_{g12}, n_{g13} \mid \theta) \cdot p(n_{g23} \mid \lambda_g)$$
$$= Multinomial(R_{g1}, \theta) \cdot Binomial(n_{g12}, \lambda_g) \qquad (3)$$

By definition, the likelihood function of parameters, $L(\theta, \lambda_g \mid n_{g12}, n_{g13}, n_{g23})$ is eq. 3. Ignoring constants with respect to parameters, the likelihood function of parameters is

$$L(\theta, \lambda_g) \propto \theta_{(1)}^{n_{g12}} \cdot \theta_{(2)}^{n_{g13}} \cdot (1 - \theta_{(1)} - \theta_{(2)})^{R_{g1} - n_{g12} - n_{g13}} \cdot \lambda_g^{n_{g23}} \cdot (1 - \lambda_g)^{n_{g12} - n_{g23}} \qquad (4)$$

Note that this likelihood function has three parameters as variables: s_{g1}, p_1, and λ_g. For convenience of the calculation of maximum likelihood estimates (MLEs) of those three parameters and the variances, we take the natural logarithm for the likelihood function of eq. 4. The conversion to the log likelihood form is straightforward so we don't show it here. Finally, implementing the log likelihood function to software, Automatic Differentiation Model Builder (ADMB) (Fournier 2000), we differentiate the log likelihood function with respect to parameters to obtain the MLEs, and further calculate the Hessian matrix for calculation of the variances. We can provide our ADMB codes and executable file for the calculation of the MLEs and variances on request.

Reference

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Table 1. Notations. Release and detection stages are illustrated in Fig. 1.

Index	
g	Fish group (control or treatment).
i, j	Stage index.
Data	
R_{gi}	The number of fish from group g being released at stage i.
n_{gij}	The number of group g-fish being released at stage i and then detected only at stage j. For example, n_{g13} = the number of group g-fish being released at stage 1, and then detected not at stage 2 but at stage 3.
Parameters	
s_{gi}	Probability of a fish from group g surviving from stage i to next stage (i +1).
p_i	Probability of a fish released from stage i to be successfully detected at next stage (i +1). Note that this parameter depends only on receivers' detection ability not on fish. So it does not have subscript, g.
λ_g	Product of s_{g2} and p_2 (i.e., $\lambda_g = s_{g2} \times p_2$).

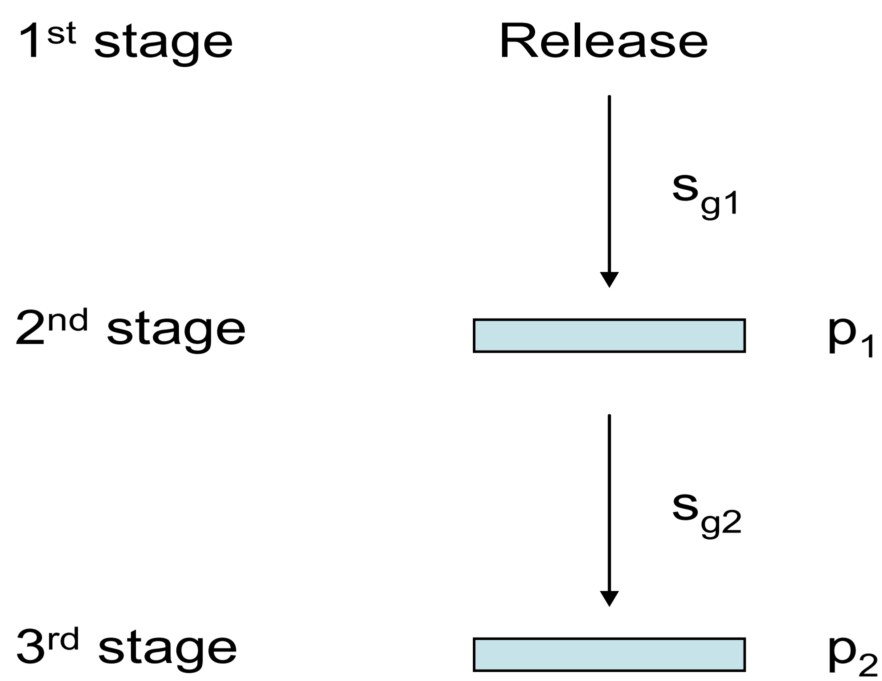


Figure 1. Parameters in the process of fish being released and detected in two different locations. A flat rectangle indicates a detection location. When there are two detection locations, four parameters of s_{g1}, p_1, s_{g2}, and p_2 are involved (Table 1).

Appendix C. Detections History by Treatment and Release Year For Yakima River Steelhead Kelt Releases.

Table 1: Yakima In River Releases and Returns.

Collection Year	In	Survived to Release	Returned to Bonneville	Survived & Returned
2002	--	--	--	--
2003	--	--	--	--
2004	--	--	--	--
2005	67	67	3	4%
2006	52	52	1	2%
2007	53	53	3	6%

Table 2: Collection and Return Migration History of No-term Yakama Releases

Collection Year	In	Survived to Release	Returned to Bonneville	% Detected Returning to Bonneville
2002	--	--	--	--
2003	--	--	--	--
2004	75	63	5	7%
2005	98	96	1	1%
2006	55	49	2	4%
2007	43	38	0	0%
2004-07 weighted average				3%

Table 3: Collection and Return Migration History of Short-term Reconditioned Yakama Releases

Collection Year	In	Survived to Release	Returned to Bonneville	% Detected Returning to Bonneville
2002	479	334	43	9%
2003	208	187	8	4%
2004	105	83	5	5%
2005	106	96	0	0%
2006	56	50	0	0%
2007	40	38	0	0%
2002-04 weighted average				7%
2002-07 weighted average				*6%*

Table 4: Collection and Return Migration History of Long-term Reconditioned Yakama Releases

Collection Year	In	Survived to Release	Mature @ Release	% Mature @ Release
2000	512	91	42	8%
2001	551	197	108	20%
2002	420	140	76	18%
2003	482	298	254	53%
2004	662	253	216	33%
2005	386	86	75	19%
2006	279	85	79	28%
2007	422	221	202	48%
2002-06 weighted average				*31%*
2000-06 weighted average				*26%*

Appendix D. First Time Spawner Analysis for Steelhead Captured at Parkdale 2007.

Table 1. Milt collection 2006.

Straw #	Mot %	Originally Collected	straw inventory as of 3/12/2007	collect /freeze date	Spawning Date(s)	Grouping (s)
K-01	90	15	11	4/4/2006	4/10/2006	1
K-02	90	30	26	4/4/2006	4/10/2006	2
K-C	90	20	16	4/4/2006	4/10/2006	C

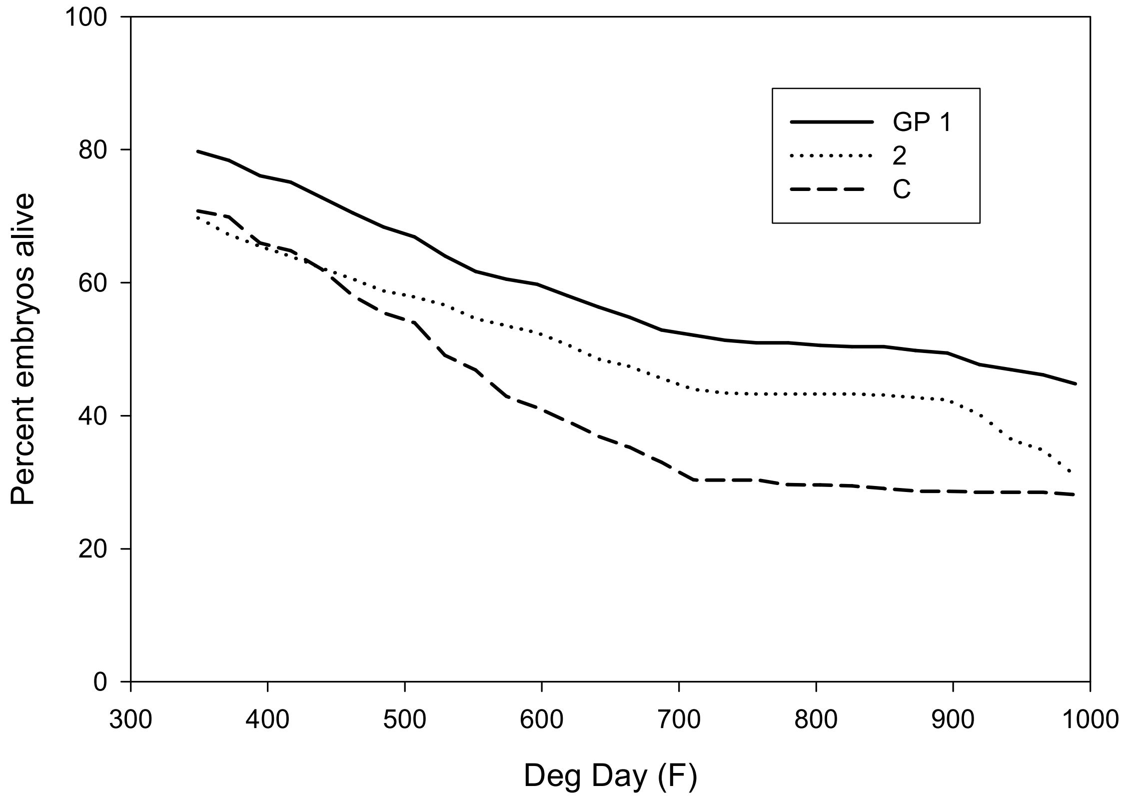
Fertilized eggs were maintained at Parkdale until disease free certification could be made. Embryos were then preserved in a formalin-based Stockard solution on April 28, 2006 for keel stage determination. (Preserved samples were analyzed on June 6, 2006.) Keel stage success:

Table 2. Number of Keel Developed for 2006 Female.

Male	+ Keel	- Keel	% Keel
1	13	13	50
2	10	15	40
C	12	12	50
		Avg.	47%

Eyed eggs were shipped to UI on May 17, 2006. Eggs were placed into trays of a Heath incubation system at Aquaculture Research Institute (ARI) at the University of Idaho.

Figure 1. Daily egg mortality assessed for 2006 Female.



Hatching began on May 26, 2006. Fry were transferred to Heath growth system at the ARI and aquaria at 10C in Gibb Hall on 14 June. On that date, there were 234, 172 and 150 fry from fertilizations with males 1, 2, and C still alive. 100 fry (4 groups of 25) from each fertilization group were transferred to the Heath system at ARI (12 containers). There were 50 fry from each fertilization group were transferred to the aquaria in Gibb Hall. All other fry were euthanized.

Progeny Viability

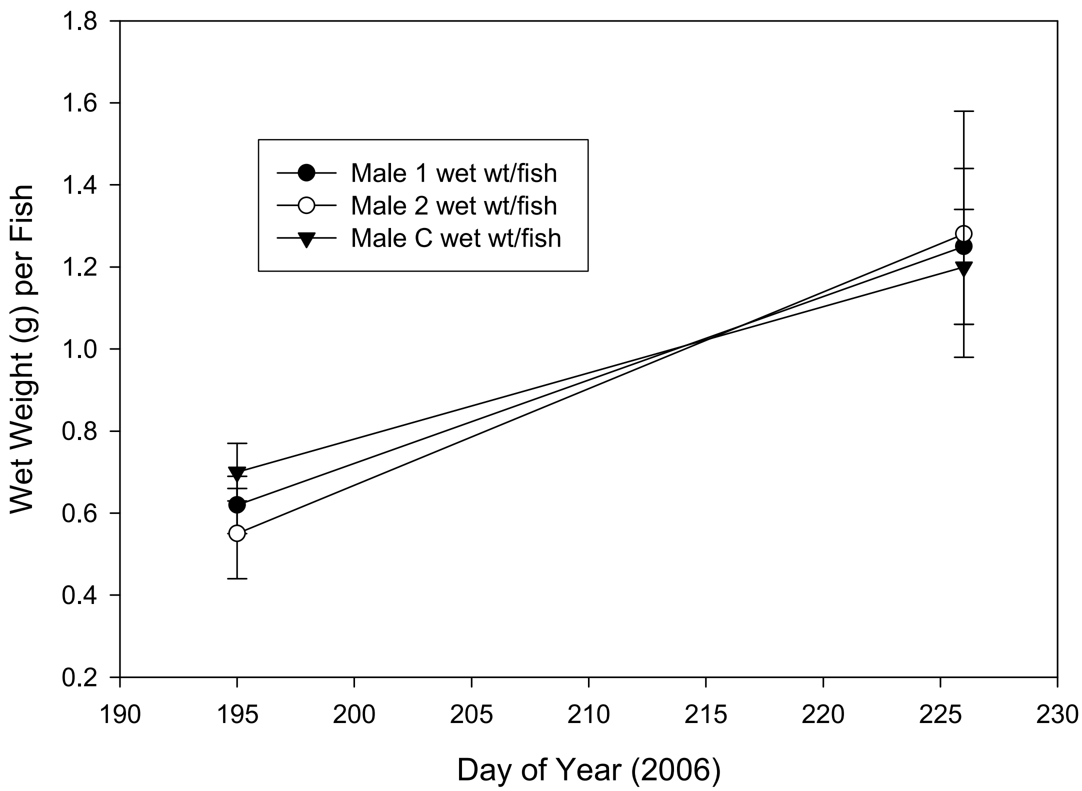
Table 3. 2006 Female, H system mortality:

	Fry to H system, replicate	% Survival at 97 days	Mean
Group 1	25	84.0	82.8
	24	91.7	
	22	72.7	
	23	82.6	
Group 2	24	75.0	69.9
	22	72.7	
	24	70.8	
	23	60.9	
Group C	24	91.7	87.0
	23	87.0	
	23	82.6	
	23	87.0	

Fry were in H system from 15 June – 20 Sept 2006.

Fish were weighed as a group live and then returned to their aquaria. The wet weight of the fish on July 14 (DoY 195) and Aug 14, 2006 (DoY 226): 31 days

Figure 2 . Wet weight of 2006 Female Progeny.



Second Spawning as Reconditioned Kelt

Gametes from this female were successfully collected again in 2007 but she perished shortly thereafter likely from heavy copepod infestation or infection due to heavy exfoliation of dermal layer on snout caused by excessive rubbing on corner of concrete raceway.

Table 4. Keel stage success of 2006 Female Kelt.

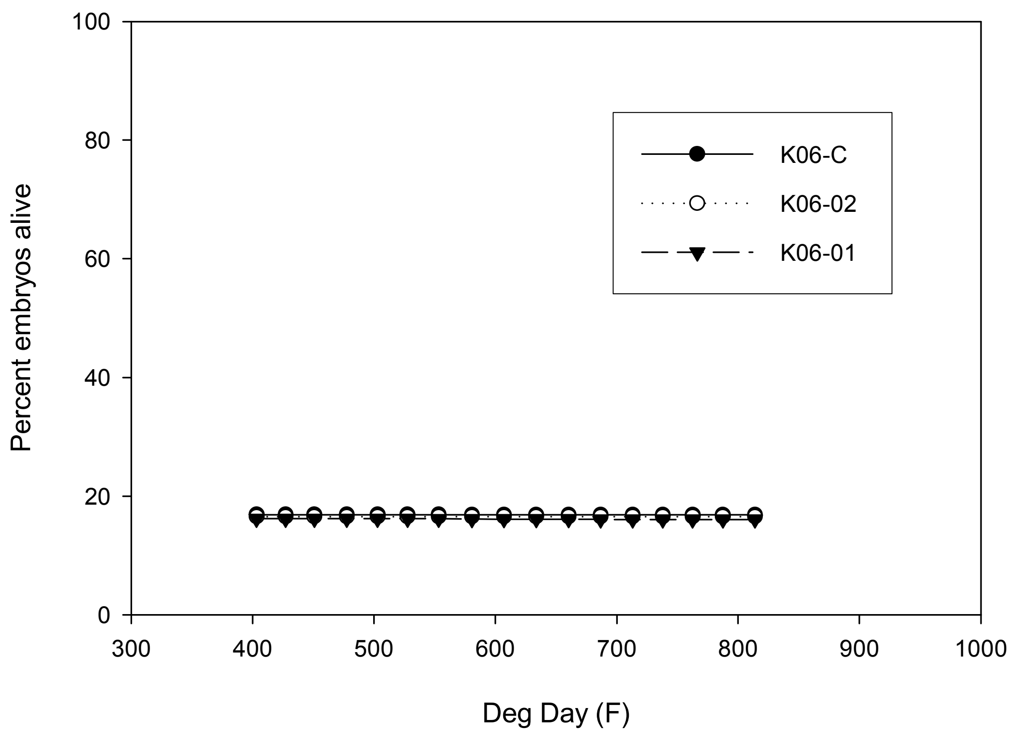
Date Collected	Female	Group	Keel/Eggs	% Keel
5/17/2007	K06	C	2/10	20
	K06	1	2/10	20
	K06	2	0/10	0

Due to possible embryonic mortality factors mentioned in (Stoddard et al. 2005) the percentages of fertilized eggs at keel development were much lower than last year.

Mortality of Embryos in incubator at Aquaculture Research Institute, UI:

Six groups of eyed eggs were shipped to UI (then split into two groups); daily mortality assessed:

Figure 3. Daily egg mortality assessed for 2006 Female Kelt. **Group** received on 11 June 2007 (162):



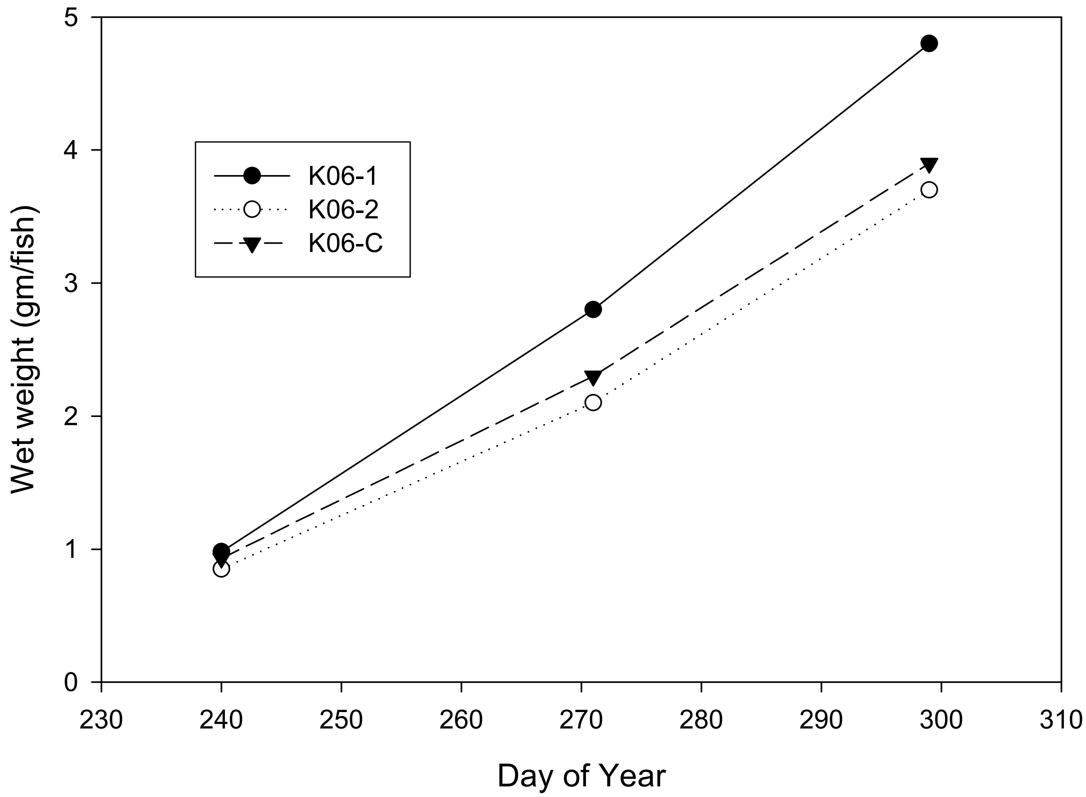
Hatching not recorded. Fry were transferred to H growth system 29 June (180).

Table 5. 2006 Female, H system mortality:

		Fry trans to H sys	Morts	Survival in H sys
Gp 5	K06-1	58	3	95%
Rcv:	K06-2	57	4	93%
6/11	K06-C	52	0	100%

Growth of Fry (wet wt/fish):

Figure 5. Wet weight of 2006 Female Kelt Progeny**.** Received on 11 June 2007 (162): 40 days



2006-2007
Collection
There were a total of 24 males and 21 females collected for progeny and gamete analysis.

Sperm cryopreservation:
Steve Patton made 3 trips to Bonneville to cryropreserve sperm.

Table 6. Milt collection 2006.

Location: Parkdale Fish Facility/ Skamania Steelhead								
CRITFC #	Straw #	Mot %	Originally Collected .5 ml straws	straw inventory as of 3/12/2007	collect /freeze date	stored	Spawning Date(s)	Grouping (s)
146	NPT-271-05	80	10	10	2/15/07	CRITFC ST #3		
067	NPT-272-05	80	19	7	2/15/07	CRITFC ST #3	2/20/2007, 3/8/2007	A,D
090	NPT-273-05	90	20	12	2/15/07	CRITFC ST #3	2/20/2007, 3/8/2007	A
071	NPT-274-05	90	20	11	2/15/07	CRITFC ST #3	2/20/2007, 3/8/2007	B
088	NPT-275-05	80	20	11	2/15/07	CRITFC ST #4	2/20/2007, 3/8/2007	B
072	NPT-276-05	80	20	15	2/15/07	CRITFC ST #4	2/20/2007	C
081	NPT-277-05	90	20	11	2/15/07	CRITFC ST #4	2/20/2007, 3/8/2007	C,D
063	NPT-278-05	80	20	34	3/8/07	CRITFC ST #3	3/15/2007, 3/22, 3/29, 4/5	E
148	NPT-279-05	70	20	34	3/8/07	CRITFC ST #3	3/15/2007, 3/22, 3/29, 4/5	E
49190	NPT-280-05	90	20	34	3/8/07	CRITFC ST #3	3/15/2007, 3/22, 3/29, 4/5	F

092	NPT-281-05	70	20	34	3/8/07	CRITFC ST #3	3/15/2007, 3/22, 3/29, 4/5	F
123	NPT-282-05	70	20	36	3/8/07	CRITFC ST #3	3/15/2007, 3/22, 4/5	G, J
250	NPT-283-05	80	20	33	3/8/07	CRITFC ST #3	3/15/2007, 3/22, 3/29, 4/5	G, H, I
075	NPT-284-05	70	20	39	3/8/07	CRITFC ST #3	3/29/2007	I, J
080	NPT-295-05	90	40	40	3/22/07	CRITFC ST #3	not used in 2007	
091	NPT-296-05	80	35	35	3/22/07	CRITFC ST #3	not used in 2007	

Keel stage success:

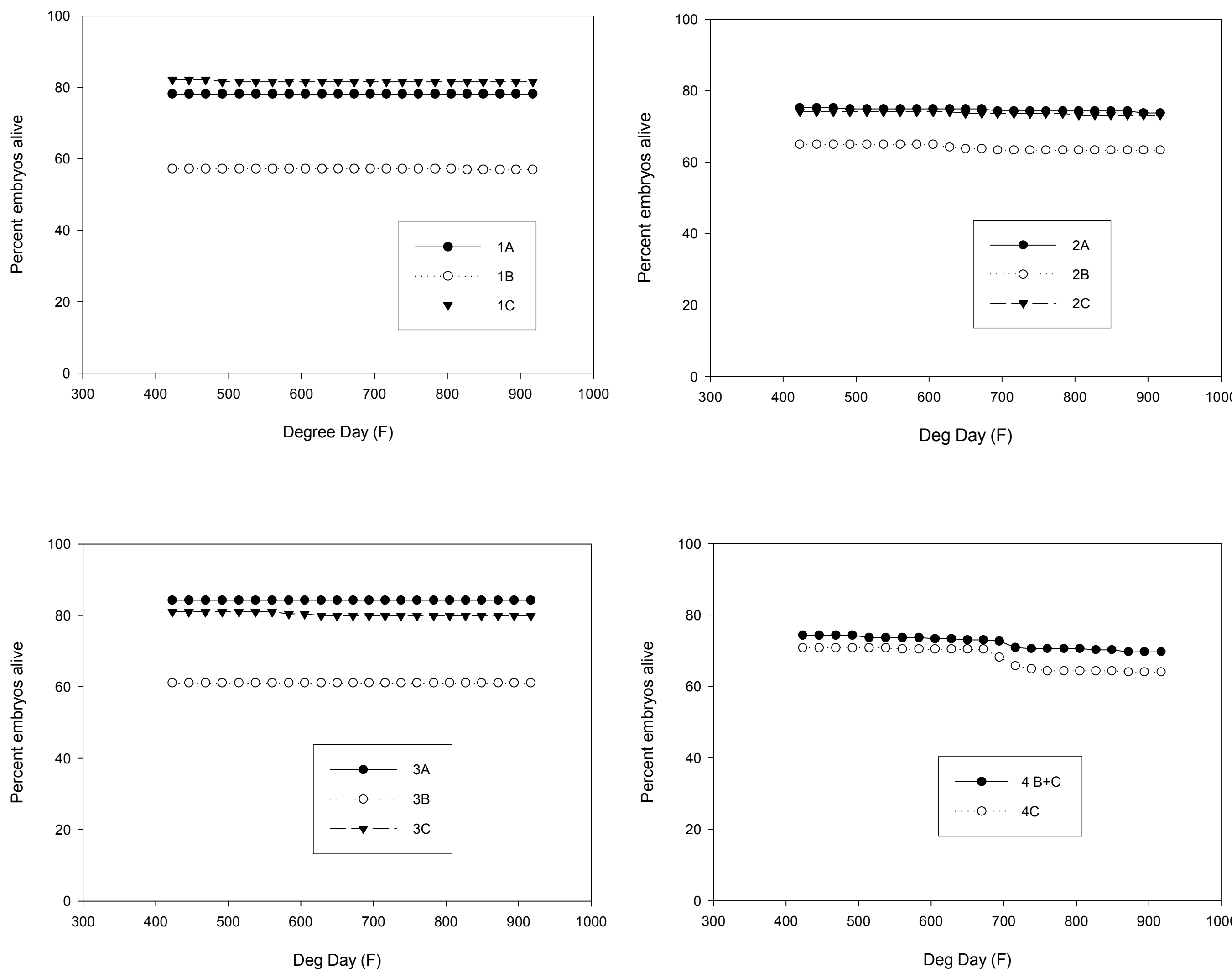
Table 7. Number of Keel Developed for 2007 Females.

Date Coll.	Female	Group	Keel/Eggs	% Keel
3/8/2007	1	A	5/10	50
3/8/2007	1	B	8/10	80
3/8/2007	1	C	9/10	90
3/8/2007	2	A	6/10	60
3/8/2007	2	B	8/10	80
3/8/2007	2	C	5/10	50
3/8/2007	3	A	9/10	90
3/8/2007	3	B	6/10	60
3/8/2007	3	C	8/10	80
3/8/2007	4	B+C	8/10	80
3/8/2007	4	C	7/10	70
3/23/2007	6 (96)	A	8/9	89
3/23/2007	6 (96)	B	10/10	100
3/23/2007	6 (96)	D	6/10	60
3/23/2007	7 (94)	A	8/10	80
3/23/2007	7 (94)	B	8/10	80
3/23/2007	7 (94)	D	9/10	90
3/23/2007	8 (83)	A	8/10	80
3/23/2007	8 (83)	B	8/10	80
3/23/2007	8 (83)	D	9/10	90
4/6/2007	10 (93)	E	9/10	90
4/6/2007	10 (93)	F	8/10	80
4/6/2007	10 (93)	G	7/10	70
4/13/2007	11 (82)	E	6/10	60
4/13/2007	11(82)	F	4/10	40
4/13/2007	11 (82)	H	6/10	60
4/13/2007	12 (68)	E	1/10	10
4/13/2007	12 (68)	F	2/10	20
4/13/2007	12 (68)	I	3/10	30
4/20/2007	(13)87	A	10/10	100
4/20/2007	(13)87	B	7/10	70
4/20/2007	(13)87	C	9/10	90
4/20/2007	14 (84)	A	6/10	60
4/20/2007	14 (84)	B	7/10	70
4/20/2007	14 (84)	C	8/10	80
5/30/07	15	A2	1/10	10
5/30/07	15	B2	1/12	8
5/30/07	15	E2	2/15	13

Mortality of Embryos in incubator at Aquaculture Research Institute, UI:

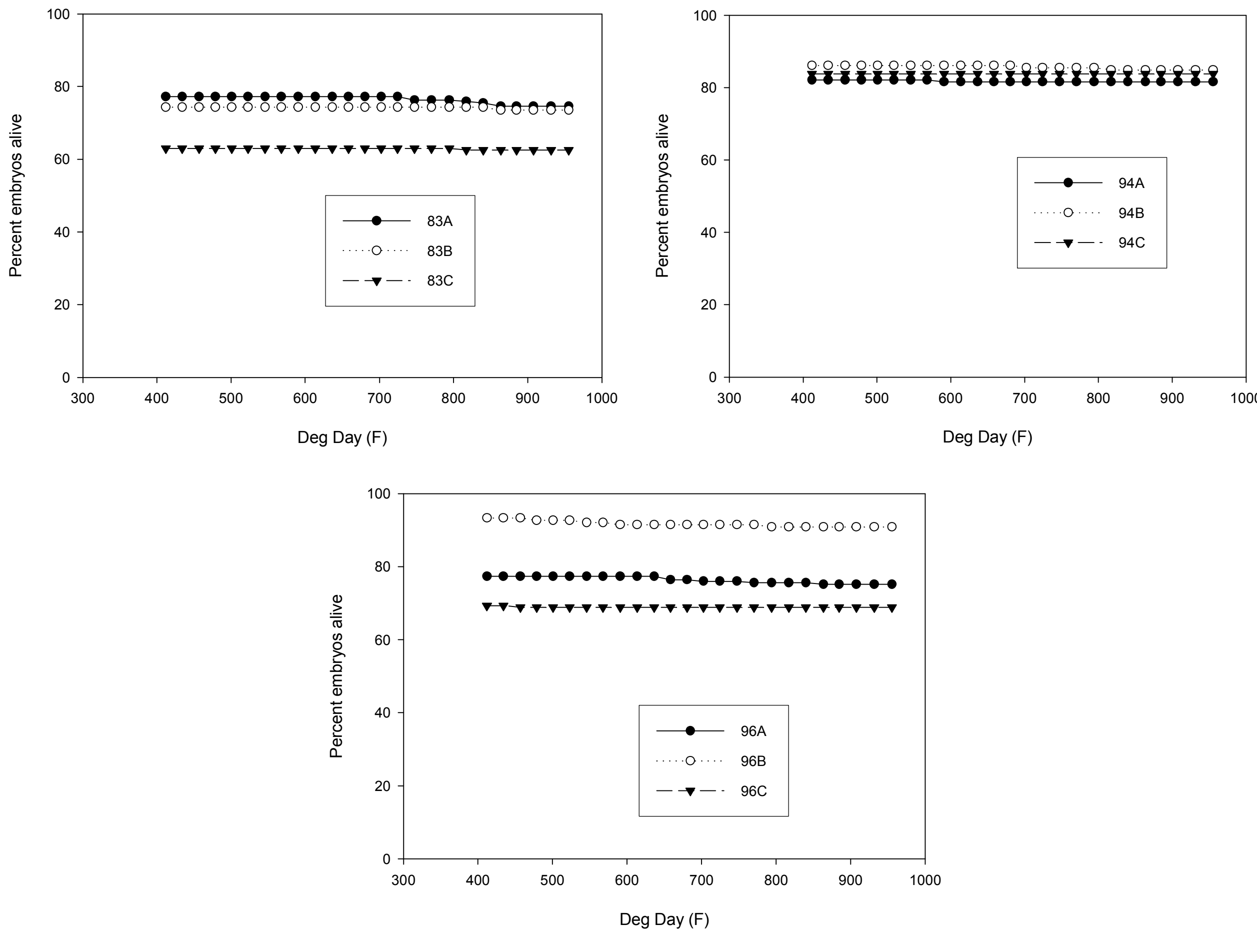
Five (does not include kelt group) groups of eyed eggs were shipped to UI (then split into two groups); daily mortality assessed:

Figure 6. Group 1 received on 6 April 2007 (day of year: 96):



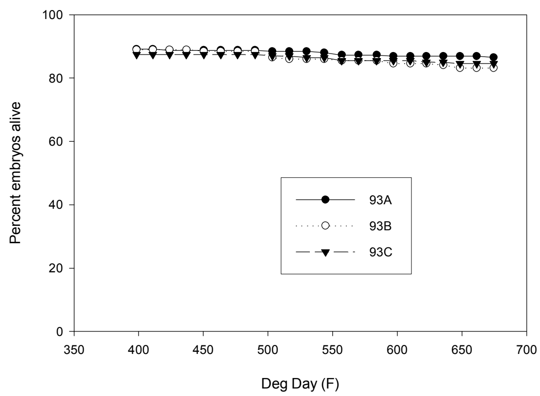
Degree days based on ^{o}F: (F-32.5). Hatching started 15 April (day of year: 105). Fry were transferred to H growth system on 30 April (120).

Figure 7. Group 2 received on 20 April 2007 (110):



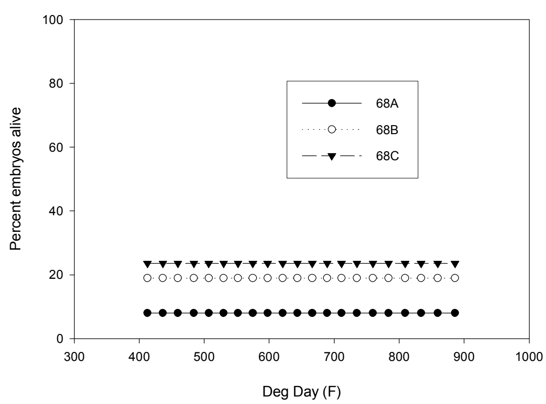
Hatching started 27 April (117). Fry were transferred to H growth system 16 May (136).

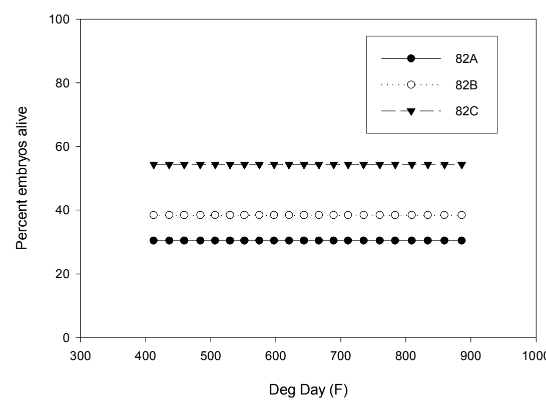
Figure 8. Group 3 received on 3 May 2007 (123). ("Loss has not been removed.")

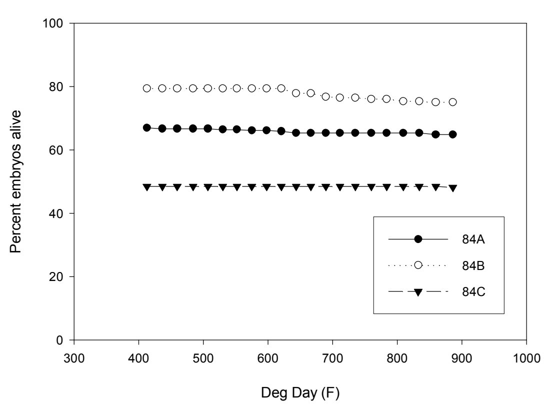


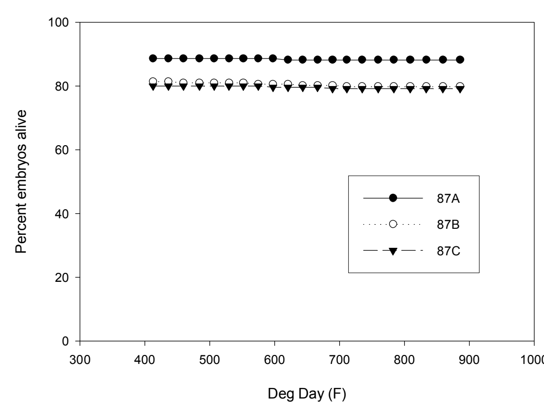
Hatching started 11 May (131). Fry were transferred to H growth system 25 May (145).

Figure 9. Group 4 received on 14 May 2007 (134):



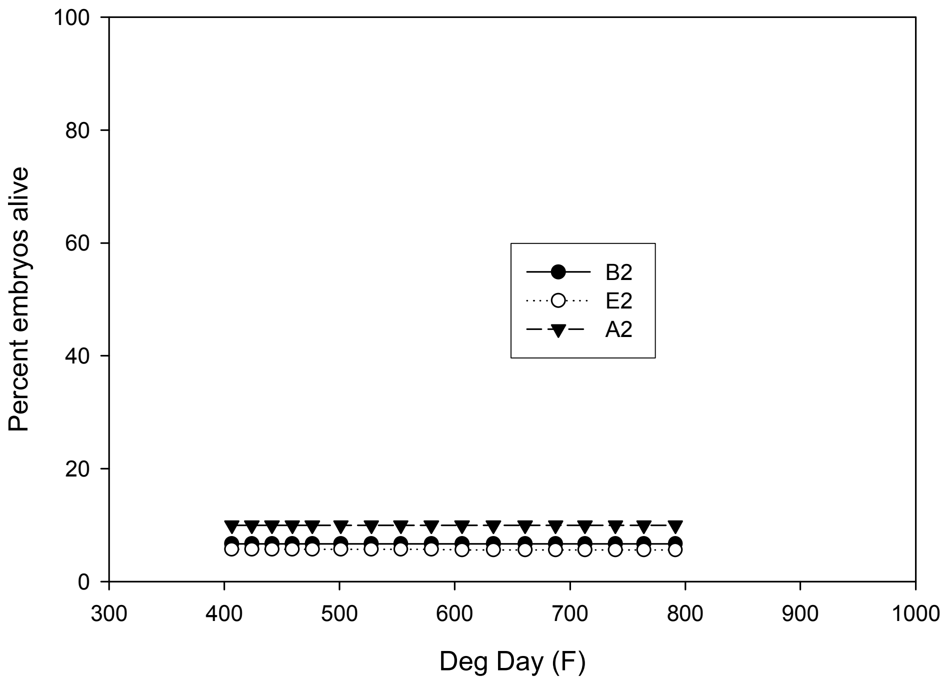






Hatching started 21 May (141). Fry were transferred to H growth system 5 June (156).

Figure 10. Group 5 received on 29 June 2007 (180):



Incubators had been shut down when these arrived; embryos maintained in Gibb incubator at 10C until ARI incubator reactivated on June 6 (157). Hatching started 6 July (187). Fry were transferred to H growth system 18 July (199). Number of dead/unfertilized eggs removed prior to transfer to UI was not reported; percent alive represents mortality of eggs transferred.

(These results appear consistent with those of Stoddard et al. [Repro Fert & Devel 17:785,'05] which indicated that embryonic mortality parallels events at or shortly after fertilization; that is, embryo mortality is highest at fertilization and relatively low and stable thereafter.)

Mortality of Fry upon transfer to growth system (H system); onset of feeding:

Table 8. 2007 Females, H system mortality:

		Fry trans to H sys	Morts	Survival in H sys			Fry trans to H sys	Morts	Survival in H sys
Gp 1 Rcv: 4/6	1A	150	36	76%	Gp 3 Rcv: 5/3	93A	203	30	85%
	1B	131	38	71%		93B	153	39	75%
	1C	115	30	74%		93C	153	20	87%
	2A	185	81	56%	Gp 4 Rcv: 5/14	68A	18	1	94%
	2B	102	28	73%		68B	45	15	67%
	2C	120	71	41%		68C	57	14	75%
	3A	118	29	75%		82A	76	0	100%
	3B	116	39	66%		82B	88	10	89%
	3C	117	64	45%		82C	145	0	100%
	4B+C	168	60	64%		84A	164	0	100%
	4C	147	40	73%		84B	162	7	96%
Gp 2 Rcv: 4/20	83A	132	30	77%		84C	93	0	100%
	83B	136	39	71%		87A	172	36	79%
	83C	97	20	79%		87B	162	35	78%
	94A	142	18	87%		87C	160	46	71%
	94B	118	25	79%	Gp 5 Rcv: 6/29	A2	30	13	57%
	94C	88	11	88%		B2	17	3	82%
	96A	133	3	98%		E2	20	6	70%
	96B	139	15	89%					
	96C	106	22	79%					

Growth of Fry (wet wt/fish):

Figure 11. Group 1 received on 6 April 2007 (96):

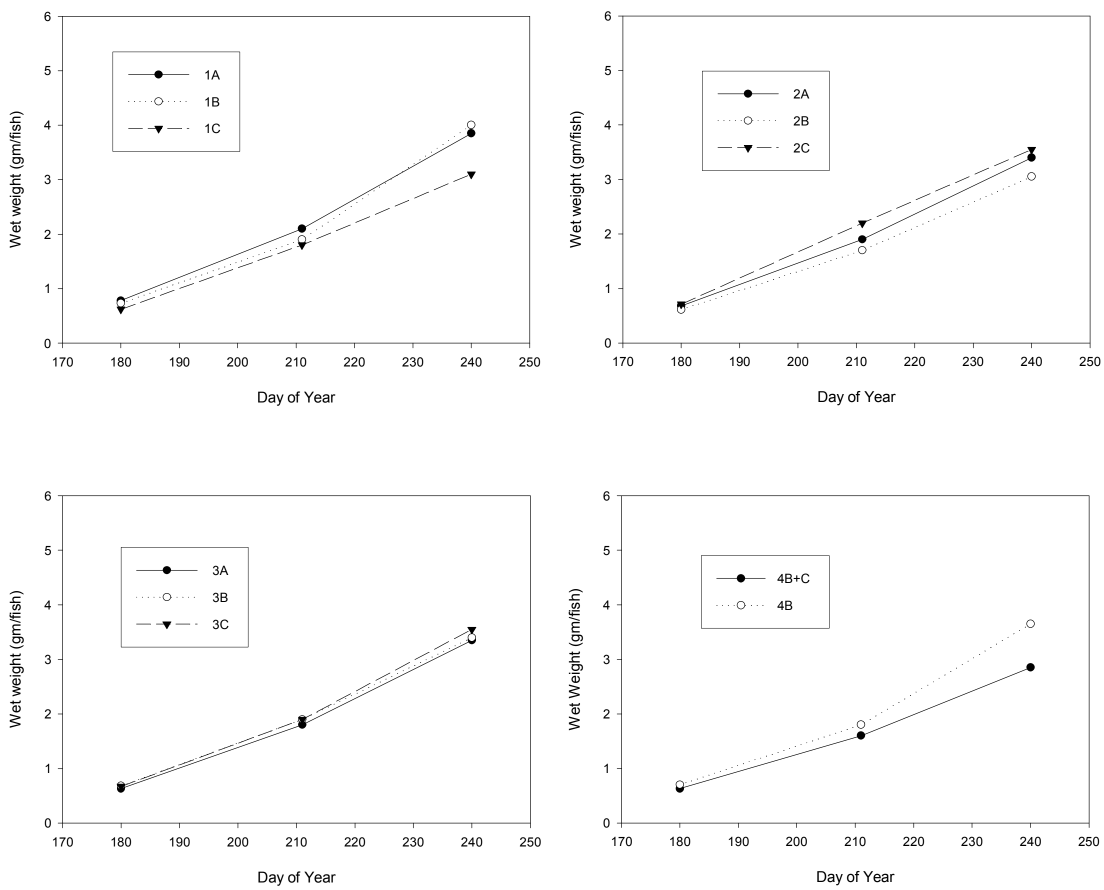
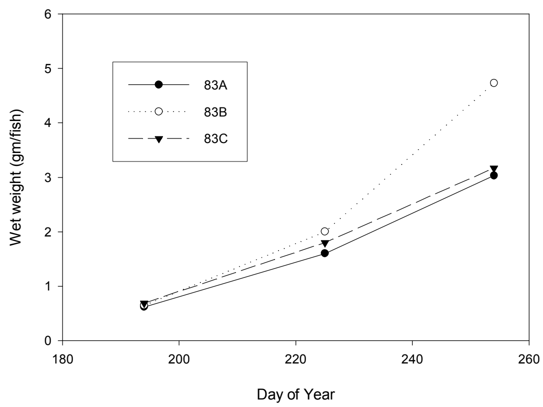
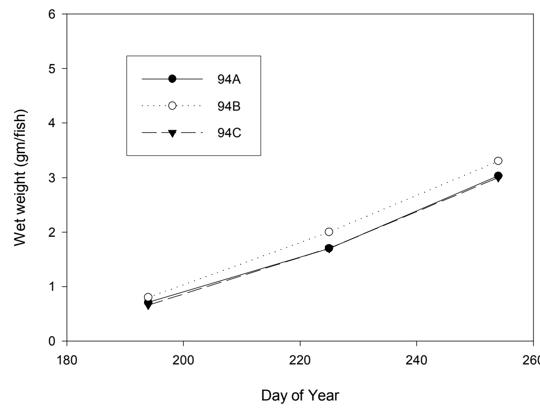


Figure 12. Group 2 received on 20 April 2007 (110):





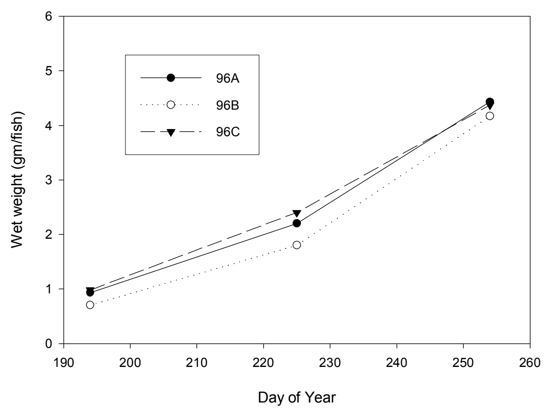


Figure 13. Group 3 received on 3 May 2007 (123):

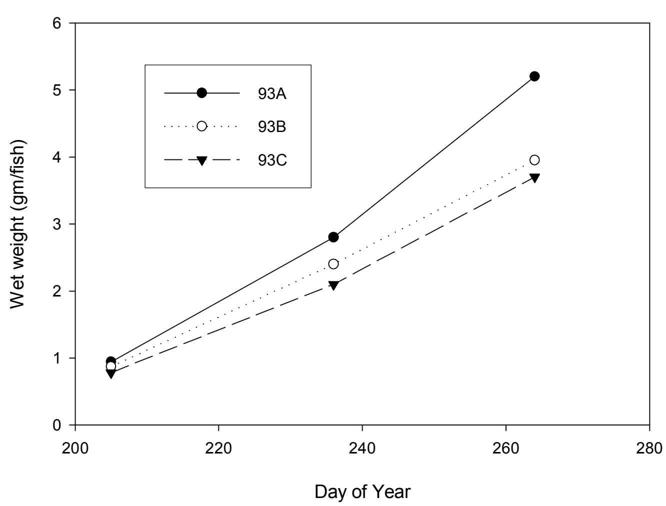


Figure 14. Group 4 received on 14 May 2007 (134):

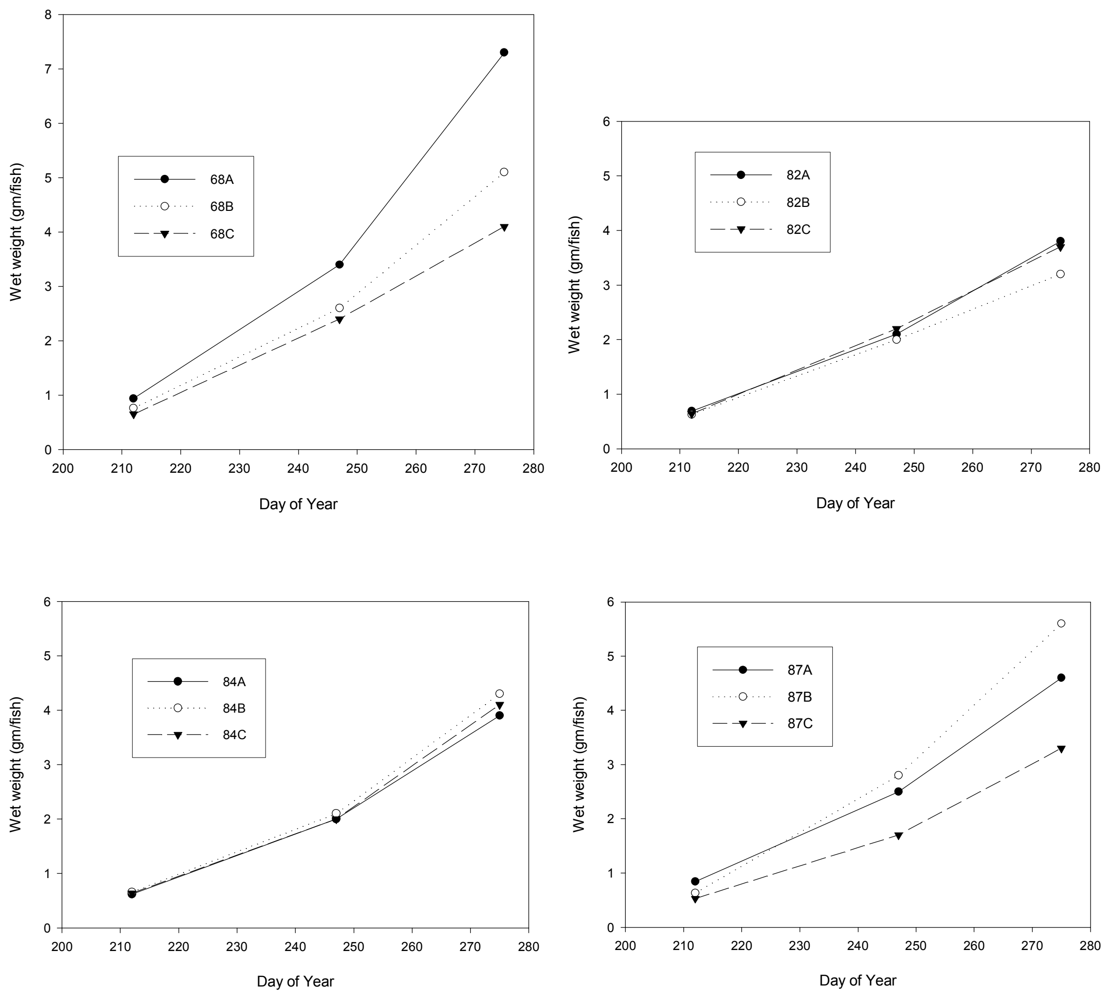
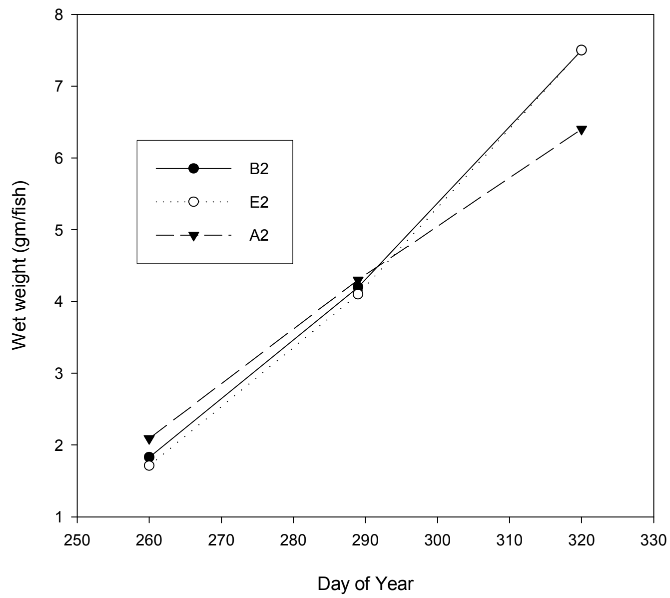


Figure 15. Group 5 received on 29 June 2007 (180):



Post Spawning

Any males surviving were terminated after milt collection as the focus of this study is the fecundity of the females after kelting since they are represented in higher numbers and will see the biggest benefit from reconditioning inland. Disease screening resulted in positive test results for 2 of the females for IHN. These females were promptly terminated and disposed of. There was a loss of 6 female prespawn mortalities. The majority of the mortalities (9) occurred about 3 months after spawning due to heavy copepedic infestation with 3 additional mortalities in August-September likely attributed to the same cause. We aggressively treated with Ivermectin but it's likely that the infestation had taken its toll and the 3 later mortalities could not be averted. We currently have one remaining fish from this spawning year for kelt analysis in 2008. We intend to be more proactive and treat with Ivermectin when fish are brought into the facility and administer another dosage after air spawning along with an oxytetracycline dosage to boost fish immune response after handling and spawning.

2007-2008

Currently 14 males and 16 females are being held at Parkdale for first-time spawning in spring of 2008. We have aggressively treated for copepods using Ivermectin to prevent catastrophic outbreaks of the parasite that occurred directly after spawning in 2007.

Summary

While our first and second year reconditioning numbers have been low (1st year low number of first-time spawners, 2nd year copepod outbreak) we are optimistic that we will have more kelts for comparison in the 2009 spawning year. While it is always difficult to infer much from such a small sample size (N=1) for our kelt comparison, what it does suggest is that kelts are capable of reproduction in the long-term reconditioning. This one individual that was successfully reconditioned appears to have had an initial egg survival it's first spawning albeit egg survival dropped sharply after the first couple of degree days. After the kelt spawning the eggs had a low initial rate of successful fertilization but survival remained steady until hatch. Juvenile survival was decent to good in 2006 with good growth, but in 2007 survival was excellent with exceptional growth. This may be partly explained by low amount of egg fertilization which in turn led to lower stocking densities per tank thus having a positive effect on juvenile survival in 2007. This year it will be imperative for the increased survival of kelts after spawning so that we can increase our sample size to more accurately measure kelt reproductive effort.