Monday, 31 July 2006 11:45 AM DRAFT

Puntledge River High Temperature Study: Influence of High Water Temperature on Adult Summer Chinook Salmon (*Oncorhynchus tshawytscha*) in 2004 and 2005

J.O.T. Jensen, W.E. McLean, T. Sweeten, W. Damon, and C. Berg

Fisheries and Oceans Canada
Science Branch, Pacific Region
Pacific Biological Station
Nanaimo, British Columbia
V9T 6N7

2006

Canadian Technical Report of Fisheries and Aquatic Sciences

Canadian Technical Report of

Fisheries and Aquatic Sciences ####

2006

PUNTLEDGE RIVER HIGH TEMPERATURE STUDY: INFLUENCE OF HIGH WATER TEMPERATURE ON ADULT SUMMER CHINOOK SALMON (*Oncorhynchus tshawytscha*) IN 2004 AND 2005

by

J.O.T. Jensen[a]; W.E. McLean[b]; T. Sweeten[a], W. Damon[a], and C. Berg[c]

[a]Fisheries and Oceans Canada
Science Branch, Pacific Region
Pacific Biological Station
Nanaimo, British Columbia
V9T 6N7

[b]205 McGimpsey Road
Campbell River, British Columbia
V9H 1K8

[c]Fisheries and Oceans Canada
Habitat and Enhancement Branch, Pacific Region
Puntledge Hatchery
PO Box 3111, #38 Powerhouse Road
Courtenay, BC
V9N 5N3

©Her Majesty the Queen in Right of Canada, 2006.
Cat. No. Fs ##-#/####E ISSN ####-####

Correct citation for this publication:

Jensen, J.O.T., W.E. McLean, T. Sweeten, W. Damon, and C. Berg. 2006. Puntledge River High Temperature Study: Influence of High Water Temperature on Adult Summer Chinook Salmon (*Oncorhynchus tshawytscha*) in 2004 and 2005. Can. Tech. Rep. Fish. Aquat. Sci. ####: vi + ##p.

Table of Contents

List of Tables

List of Figures

ABSTRACT

Jensen, J.O.T., W.E. McLean, T. Sweeten, W. Damon, and C. Berg. 2006. Puntledge River High Temperature Study: Influence of High Water Temperature on Adult Summer Chinook Salmon (*Oncorhynchus tshawytscha*) in 2004 and 2005. Can. Tech. Rep. Fish. Aquat. Sci. ####: vi + ## p.

In 2004, pre-spawning mortality of adult Puntledge River summer chinook (Oncorhynchus tshawytscha) held at the Puntledge Upper site in warm water (daily average temperatures exceeding 22 °C for a week in August) exceeded 90 %. This high mortality combined with hydraulic damage to the hatchery barrier fence forced postponement of the controlled temperature experiment in 2004. In 2005 experimental work resumed and returning summer adults were exposed to 3 different temperature regimes from July to October. The overall pre-spawning mortality rate for females at the much warmer Puntledge Hatchery (i.e. daily average temperatures in 2005 reaching 22 °C in August at the Lower and Upper sites) was 57 % while pre-spawning mortality at the cooler Rosewall Creek hatchery (pond temperatures ranged from 8 to 9 °C) was 8 %. The possible effect of two additional factors (i.e. gas supersaturation and the diatom Gomphonema geminata) on adult mortality at Puntledge is considered. No conclusive differences in egg size, egg size variation or in female maturation rates were observed among the 3 temperature regimes. Egg mortality rate ($P<0.001$) and spermatocrits or sperm density ($P<0.02$) were significantly influenced by temperature regime, with lowest egg mortality and the highest sperm density occurring for fish held in the lowest temperature regime at the Rosewall Creek hatchery.

RÉSUMÉ

Jensen, J.O.T., W.E. McLean, T. Sweeten, W. Damon, and C. Berg. 2006. Puntledge River High Temperature Study: Influence of High Water Temperature on Adult Summer Chinook Salmon (*Oncorhynchus tshawytscha*) in 2004 and 2005. Can. Tech. Rep. Fish. Aquat. Sci. ####: vi + ## p.

1.0 INTRODUCTION

This is the third study conducted at Puntledge Hatchery which deals with the influence of high water temperatures on maturing adult salmon. In 2002 pink salmon were exposed to 3 declining temperature regimes (chilled, ambient and heated) in 10-ft circular ponds (2 replicates) to simulate different river conditions. This formal experiment clearly demonstrated the negative effects of warm water on maturation rates, adult holding mortality and egg quality (Jensen et al. 2004). A similar study was attempted in 2003 with chinook. The experiment was successfully initiated in mid August and 3 groups of chinook were exposed to different temperature regimes in the 10-ft circular ponds. However this experiment did not yield clear results. Spurious adult mortality occurred when the water supply was abruptly changed during a maintenance procedure. In fact water supplied from the upper river via the BC Hydro penstock (Figure 1) was turned off and replaced by water pumped from the lower river. It was surmised that some unknown water quality factor related to this change confounded the temperature experiment (Jensen et al. 2005).

Attempts to repeat this experiment in 2004 failed. Flooding in the river damaged the barrier fence making it impossible to capture fish at the lower (main) hatchery site where the experimental setup is located (Figure 1). In addition, although large numbers of fish were captured at the Puntledge Upper Site, there was an extremely high pre-spawning mortality (> 90%) over the summer. This was probably related to the combination of high water temperature and elevated gas supersaturation levels. Because of these problems, no fish were committed to the temperature experiment in 2004.

With these high losses at the upper hatchery site in mind, we dramatically modified the experimental design in 2005. Firstly the bulk of returning summer chinook were intercepted in the lower river and held at the main hatchery site (Figure 1). This site has an aeration tower and so is protected against gas supersaturation. Also, to further reduce the risk of pre-spawning mortality, a portion of these fish were transported by truck 35 km south to Rosewall Creek Hatchery where the high quality groundwater has a constant temperature of 8 °C throughout the summer. These fish were moved in early July before the summer temperature peaks in the Puntledge River to minimize handling stress. At the same time these fish were transported to Rosewall, a sub-sample was removed to 10-ft circular ponds in an attempt to repeat the formal temperature experiment. These are the same ponds used for pinks in 2002 and chinook in 2003 and are supplied with chilled, ambient and heated water. The remainder of the chinook were left at the Main Puntledge Hatchery site in production raceways – a small number were held at the upper site.

In summary, the experiment in 2005 involved groups of summer chinook held in production raceways at Puntledge Upper and Lower sites, Rosewall Creek Hatchery and in 10-ft ponds at Lower site (formal temperature experiment). These sites represented a range of holding conditions – as in previous years, their effects on pre-spawning mortality and gamete quality were assessed.

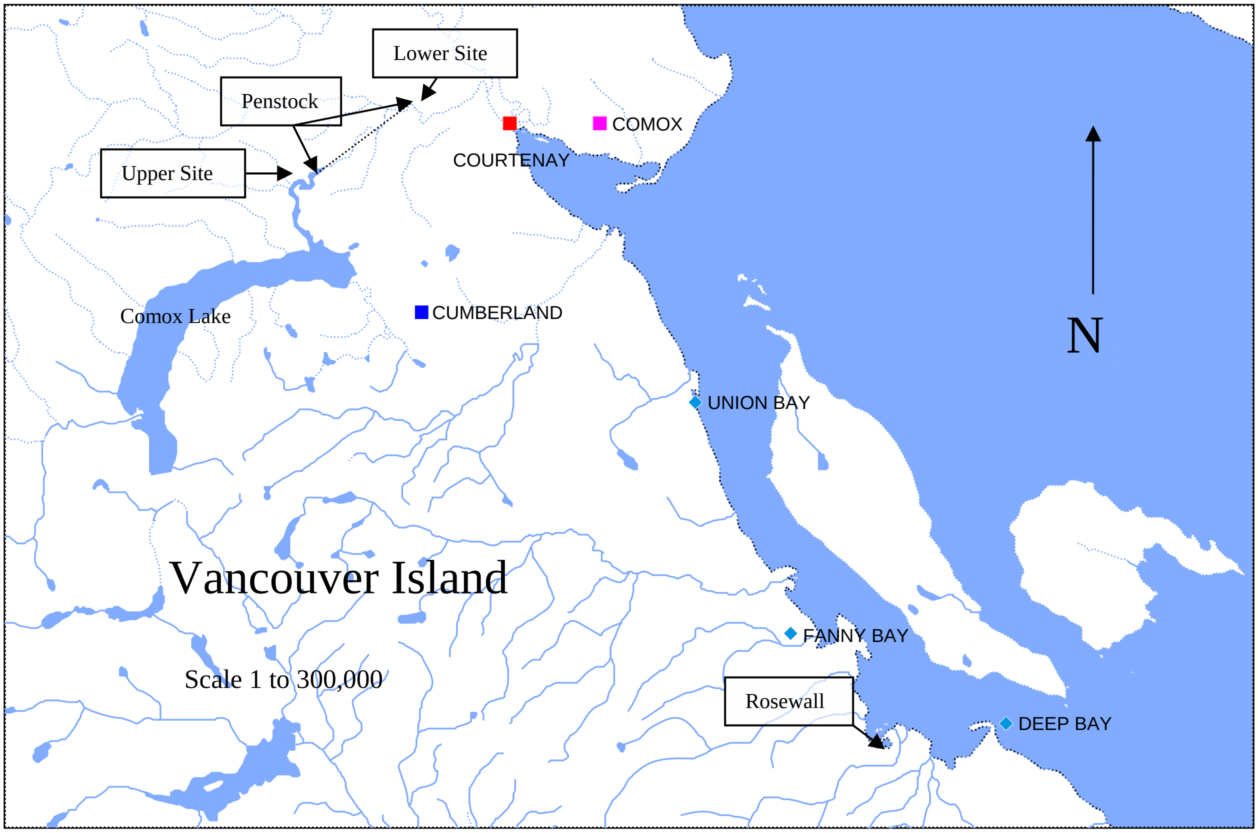

Figure 1. Map showing study area including the Puntledge River draining Comox Lake, Upper and Lower (main) hatchery sites, and a rough representation of the location of the Penstock. The Rosewall Creek hatchery is about 35 km south.

2.0 MATERIALS AND METHODS

The experimental setup in the 10-ft circular ponds at the main Puntledge Hatchery site (lower) in 2005 was the same as for the pink salmon in 2002 and chinook in 2003 (Jensen et al. 2004, 2005). Two replicate ponds were assigned to each of three temperature treatments -- chilled (Ponds 1 and 3), ambient (Ponds 2 and 4) and heated (Ponds 5 and 6). The water to Pond 2 and 4 comes from the “rearing head tank” in the aeration tower building while water to Pond 1, 3, 5 and 6 comes from the “incubation head tank”. Because heating increases total gas pressure, the water to Ponds 5 and 6 passes through additional aeration just before entering the ponds. The load target for each pond was 8 females and 8 males with a water flow of 100 LPM. Jacks (precocious males) were used instead of full sized males to reduce the pond density.

An important difference in 2005 was that ponds were loaded much earlier in the summer before the seasonal temperature peaks. In 2005 fish were loaded between June 28 and July 19 – the bulk of the females were loaded before July 12. To minimize capture and handling stress, fish were trucked to Rosewall Creek Hatchery and placed in the test ponds on these same days. Efforts were made to distribute available fish equally to each pond until the final load had been reached. Note that temperature was not controlled yet -- all the ponds were on ambient water supplied by gravity from the BC Hydro penstock (Figure 1). We planned to turn the chiller and heater on in the first week of August. In contrast the 2003 test fish were loaded in the 3rd week of August and the 3 temperature regimes (chilled, ambient, heated) were already established before the fish were added (Jensen et al. 2005).

Because handling occurred so early in the summer and holding time was prolonged, formalin treatments were required to prevent fungus. Formalin is more toxic at temperatures above 18 °C (Hoskins et al. 1983) so treatment levels for fish in the 10-ft ponds at the Puntledge hatchery were reduced. The standard dose is 100 ppm of Parasite S (supplied by Syndel Laboratories Ltd., 9211 Shaughnessy St., Vancouver, BC) for 1 hour. At Puntledge, a stock solution of Parasite S pumped to the inflow stream for 1 hour resulted in a gradual increase in the pond concentration to a maximum of 40 mg/L. Treatments (twice per week) started on July 19. Fish transported to Rosewall Creek received a higher dose - a “flow-through” treatment once per week resulted in a maximum formalin concentration of 100 ppm at 1 hour.

Throughout the summer of 2005, the ponds were supplied with either penstock water (gravity) or with water pumped from the lower river (Figure 1). The first switch from penstock to river water occurred on July 25 when the penstock was shut down for maintenance by BC Hydro. The resultant increase in flow to the river disturbs materials that have settled and attached to the river bottom substrate. Hence, a significant increase in suspended material, including the benthic algae gomphonema (Jensen et al. 2005), occurs soon after flow increases in the river. Flows to the penstock and the river were recorded.

On July 27 the chiller was turned on cooling the water flowing to Ponds 1 and 3. Because of greater than anticipated mortality at ambient temperature, the heater was not turned on in 2005 and Ponds 5 and 6 remained on ambient water throughout the experiment.

Between June 28 and July 19, 160 chinook (95 females, 52 males and 13 jacks) were transported to two 20 ft diameter circular ponds at Rosewall Creek Hatchery. These ponds had a water depth of 4.7 ft and a flow rate of 400 LPM. (200 LPM new and 200 LPM reused). The ponds had fibreglass covers so the fish were undisturbed over the summer. Groups of chinook were also held over the summer at the upper and lower hatchery sites. Precise numbers were unavailable at these sites because it was impossible to get initial counts. Also unaccountable losses from predation were high and high water temperatures made handling to check for sex and species impossible.

At maturity in the fall, sub-samples of gametes from each group were transported to the Puntledge hatchery lower site in coolers. Eggs were fertilized and incubated from the outset in water chilled below 12 ºC. This guaranteed excellent incubation conditions so that differences in egg mortality would truly reflect differences in the quality of the adults. Approximately 200 eggs from each female were divided into four replicates (50 eggs) and fertilized with 0.25 mL of milt (pooled). Replicates were incubated in individual cells of divided Heath trays (20 cells) and assessed until just after hatch (survival to the alevin stage). Sub-samples of eggs were taken from 30 females at Rosewall, 17 at the Upper Site and 29 at the Lower Site. Because of a shortage of milt at Rosewall, 15 of the females were fertilized with milt from the Lower Site.

Spermatocrit (i.e. % packed cell volume; an indicator of sperm density) was measured as a way of assessing male maturation. Samples of milt were collected in micro-hematocrit tubes (non-heparinized) and spun in a centrifuge for 5 minutes at 13,500 G (7200 rpm) (Bouck and Jacobson, 1976). The packed cell volume was expressed as a % of the total fluid volume. Three replicates were tested per male.

Egg size (mean and standard deviation S) was measured for a number of females. Measurements were made at the "eyed stage" when eggs could be easily handled. The procedure was as follows. 25 batches of 10 eggs each (n = 10) were randomly selected from an incubator containing the eggs of 1 female. These samples were drained and weighed (W mg) and the individual egg weights (mg/egg) were calculated from W/10. The mean egg weight for a female was estimated by taking the average of the 25 samples. The standard deviation of the 25 samples "sd" was used to estimate the standard deviation of the individual egg weights within a female "S": $S = sd * \sqrt{n} = sd * \sqrt{10}$.

As in previous years, dissolved oxygen was measured several times per week at the outflow of the 10-ft circular ponds and at the outflow of the 20 ft ponds at Rosewall Creek. Temperatures in each pond and at Rosewall Creek and at the Upper and Lower Puntledge Hatchery sites were monitored using Onset TidbiT temperature loggers. The logging frequency was every 5 minutes (i.e. 288 measurements per day).

Total gas pressure was continuously monitored at the Upper Site using a gas pressure logger (Common Sensing Model TBO-DL). The instrument was placed at the inflow to the holding pond and recorded excess gas pressure mmHg (ΔP) and the barometric pressure mmHg (BP) every 15 minutes. Spot checks were made using a total gas pressure meter (Point 4 Systems model PT4) in order to check the accuracy of the TBO. The PT4 was also used to measure total gas pressure at the Lower Site. Total gas pressure as read by these instruments (ΔP mmHg) is commonly expressed as a percent: $TGP\% = (BP + \Delta P)/BP * 100\%$.

3.0 RESULTS

3.1 Puntledge Lower site 10-ft Circular Ponds

3.1.1 Initial numbers of fish, Water Flow and Dissolved Oxygen (DO) Measurements.

The 10-ft diameter circular ponds at the Puntledge Lower site were loaded with summer chinook adults between June 28 and July 19. A total of 38 females and 48 males were distributed amongst the 6 ponds. Numbers of fish, biomass, pond flow, and load rate (kg of fish per LPM) at the start of the experiment are shown in Table 1. DO in mg/L was measured routinely at the outflow of each pond – readings were taken in the morning (AM) and afternoon (PM) (Figures 2, 3 and 4). DO decreased as the pond was loaded and increased as biomass decreased due to mortality. Even at maximum load DO was above 7 mg/L.

Table 1. Initial numbers of fish and biomass (kg) loaded to the 10-ft circular ponds at Puntledge Lower Site. Flow (Litres per minute LPM) and load rate (kg of fish per LPM) are also shown. Note that "Heated" treatments were not heated in 2005 (see section 3.1.2).

Location	Treatment	Flow LPM	Female	Male	Jacks	Total	Biomass kg	Load kg/LPM
1	Chilled	94	7	2	5	14	71	0.76
3	Chilled	99	5	3	3	11	63	0.64
Total	Chilled	193	12	5	8	25	134	0.70(avg)
2	Ambient	100	5	4	5	14	62	0.62
4	Ambient	98	9	4	5	18	91	0.93
Total	Ambient	198	14	8	10	32	153	0.77(avg)
5	Heated	103	7	3	7	17	76	0.73
6	Heated	96	5	2	5	12	50	0.52
Total	Heated	199	12	5	12	29	126	0.63(avg)

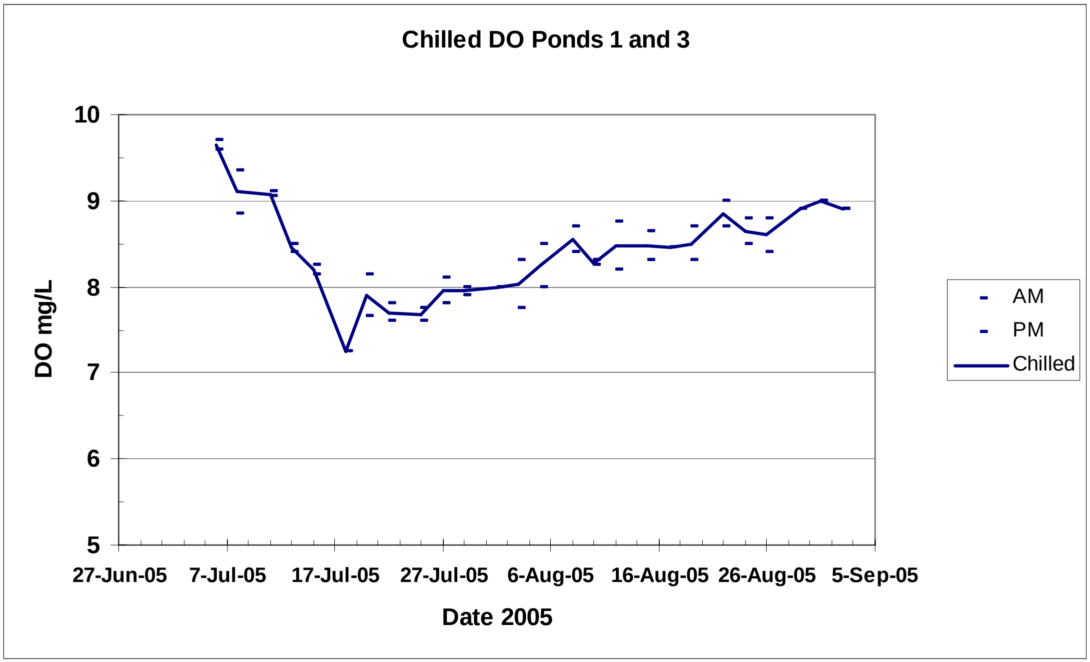

Figure 2. Dissolved oxygen (DO mg/L) in the morning (AM) and afternoon (PM) at the outflow of Ponds 1 and 3 (chilled).

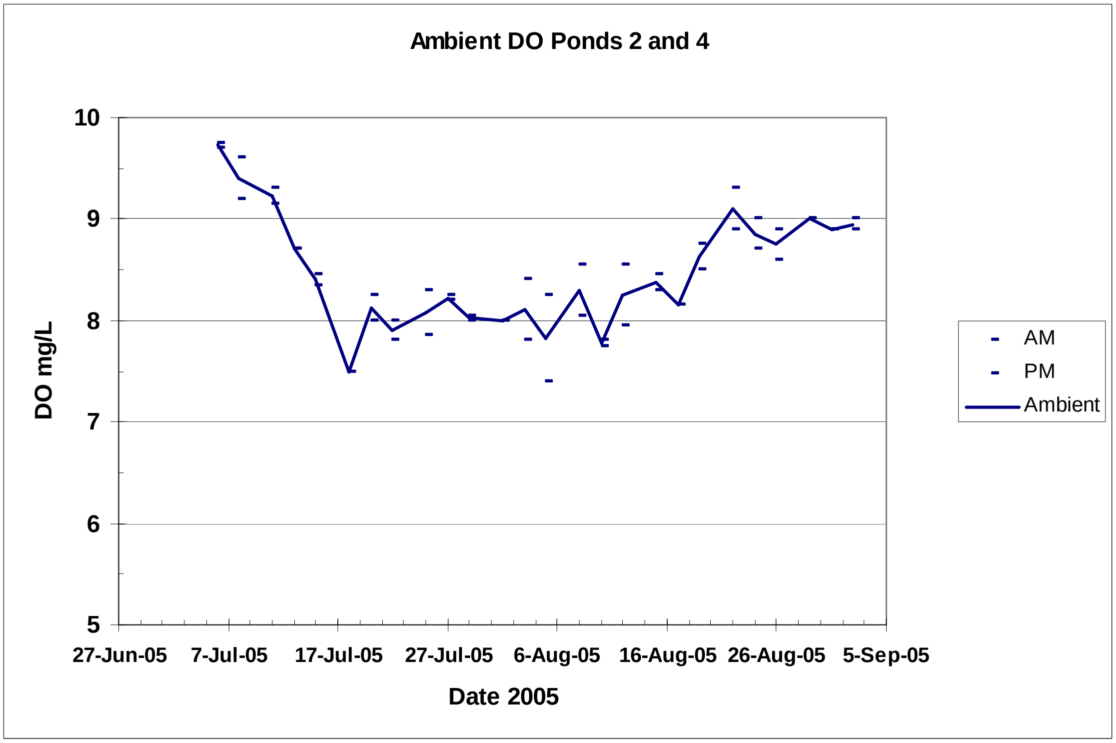

Figure 3. Dissolved oxygen (DO mg/L) in the morning (AM) and afternoon (PM) at the outflow of Ponds 2 and 4 (ambient).

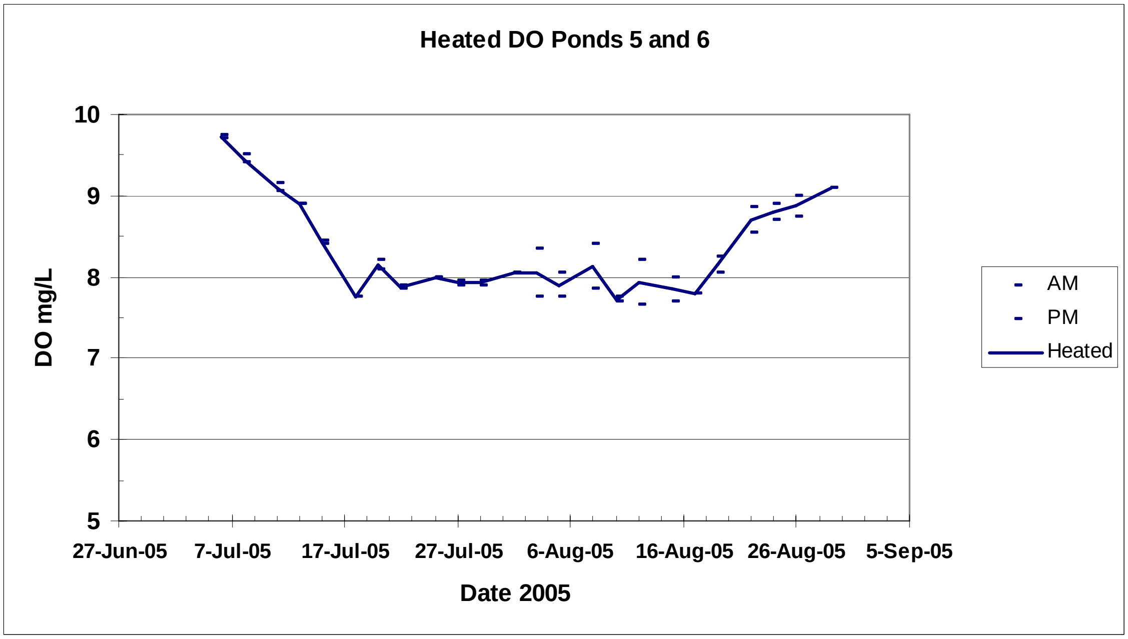

Figure 4. Dissolved oxygen (DO mg/L) in the morning (AM) and afternoon (PM) at the outflow of Ponds 5 and 6 (heated; actually "ambient" temperature, see section 3.1.2).

3.1.2 Water Temperatures

Ponds 1 and 3 were on ambient water until the chiller was started on July 27. The heater was not started during the experiment so Ponds 5 and 6 remained at ambient temperatures. Figure 5 shows the daily temperatures for the chilled (1 and 3) and ambient ponds (2, 4, 5 and 6).

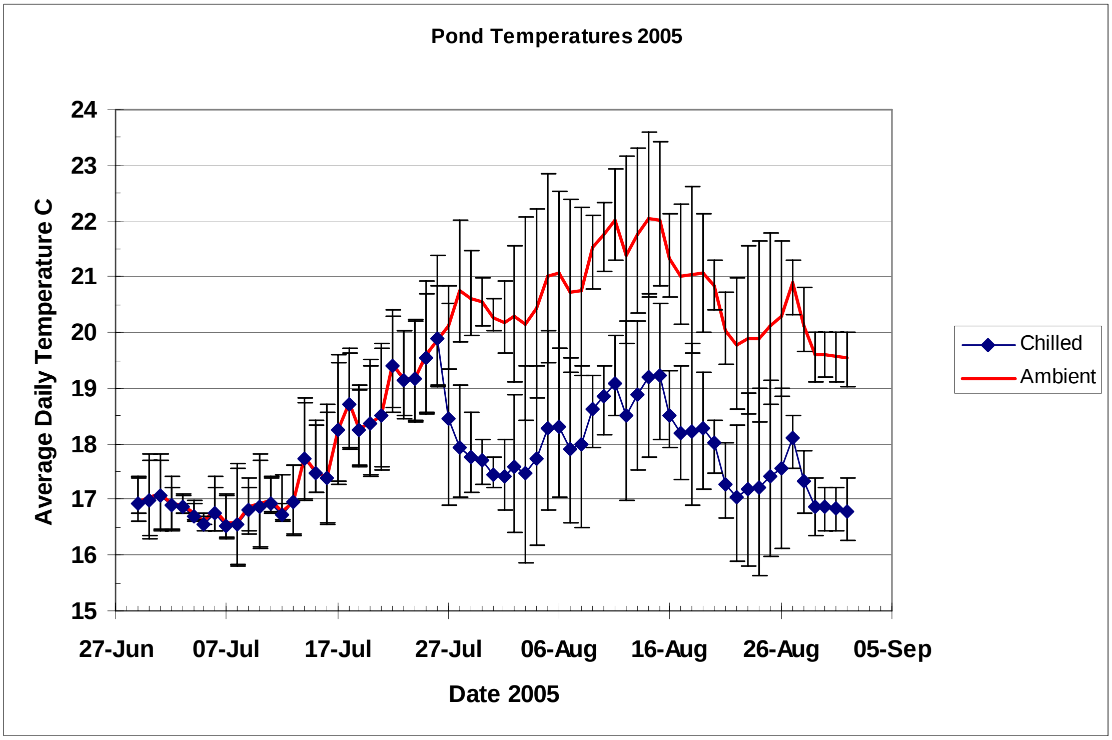

Figure 5. Daily average, maximum and minimum temperatures for chilled (ponds 1 and 3) and ambient ponds (ponds 2, 4, 5 and 6). The heater was not turned on in 2005 and so ponds 5 and 6 remained at ambient temperatures.

3.1.3 Adult Mortality

Cumulative female and male mortality for the 10-ft ponds are shown in Figures 6 and 7 for the chilled, ambient and heated treatments. Although Ponds 5 and 6 have ambient temperature regimes (heater off), they are presented separately and are referred to as the “heated treatment”. The only difference between these ponds and Ponds 2 and 4 is that their inflow stream has additional aeration for removing gas supersaturation.

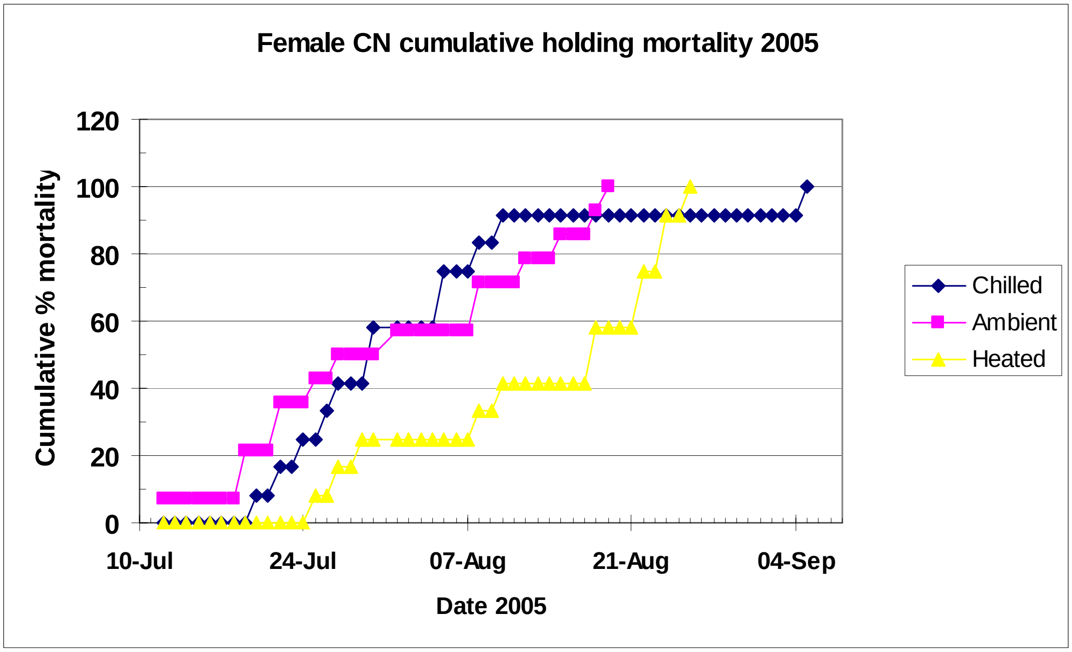

Figure 6. Cumulative mortality (%) for females in 10-ft ponds for chilled (ponds 1 and 3) ambient (ponds 2 and 4) and "heated" (ponds 5 and 6). Note ponds 5 and 6 remained at ambient temperatures in 2005.

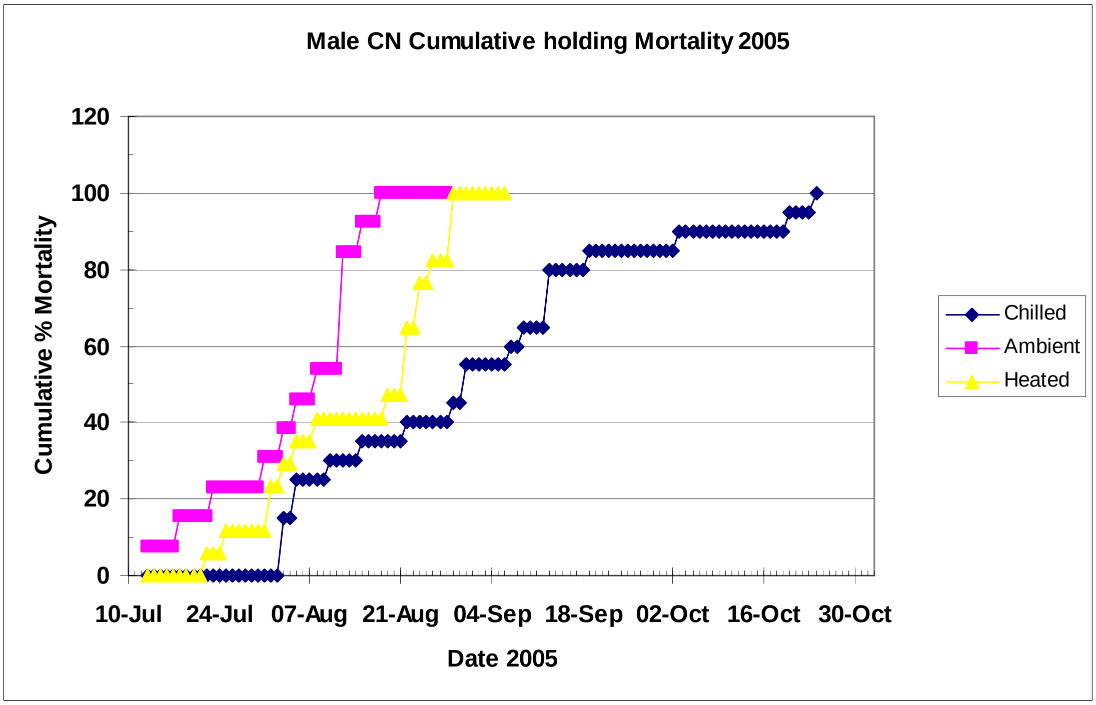

Figure 7. Cumulative mortality (%) for males in 10-ft ponds for chilled (ponds 1 and 3) ambient (ponds 2 and 4) and "heated" (ponds 5 and 6). Note ponds 5 and 6 remained at ambient temperatures in 2005.

By September 1 all the females were dead. The dates to 50 % mortality for females and males are shown for each treatment in Table 2.

Table 2. Dates for 50% mortality.

Treatment	Females	Males
Chilled	July 30	August 31
Ambient	July 28	August 12
Heated	August 18	August 22

3.1.4 Chiller Performance

When the water temperature was high (23 °C), the chiller reduced an inflow stream of 280 LPM by 3.1 °C. This is a cooling capacity of Q*ΔT = 280*3.1 = 869 LPM °C or approximately 61 kw. As the inflow temperature dropped, ΔT decreased. Data from 2002, 2003 and 2005 were pooled (Figure 8) to obtain a relationship between capacity "Cap" (LPM °C) and the inflow temperature T (°C): $Cap = a * T^2 + b*T + c$ where $a = -0.7385$, $b = 67.836$, $c = -300.92$, $R^2 = 0.8026$ and $N = 156$.

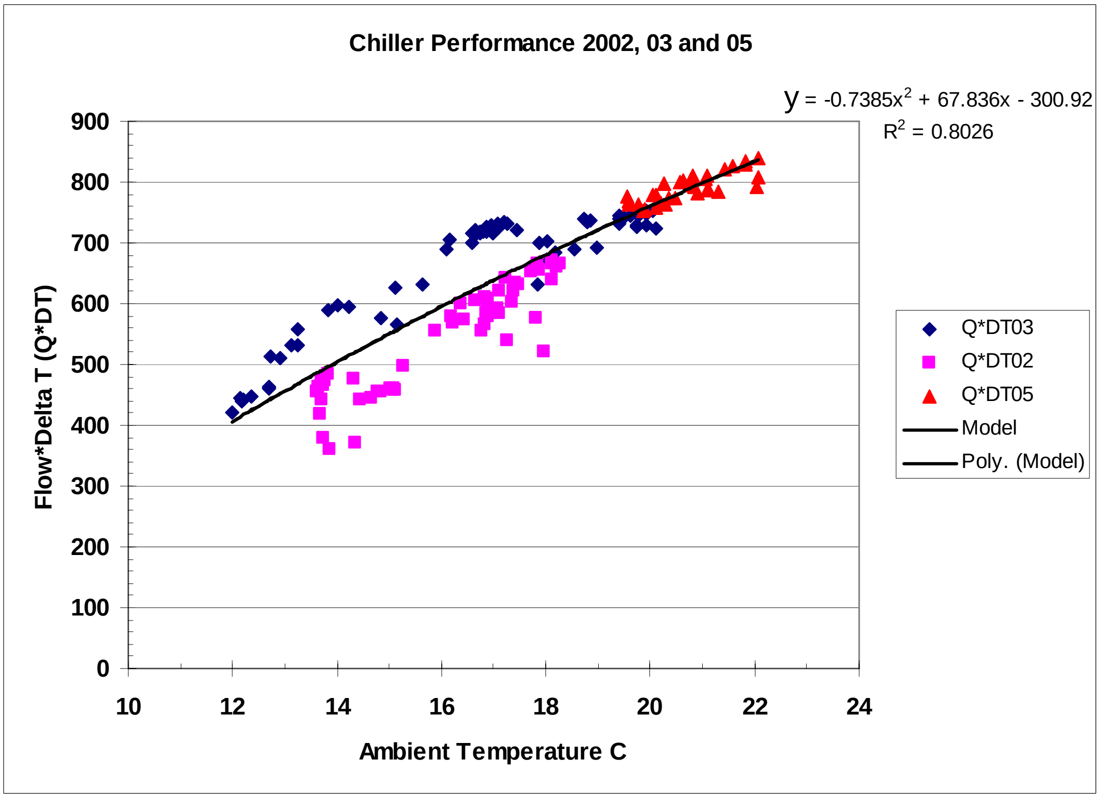

Figure 8. Chiller capacity (i.e. Cap) at Puntledge Hatchery for 2002, 2003 and 2005. The product of water flow Q (LPM) and temperature drop (ΔT °C) or Q*ΔT is plotted against the ambient (or inflow) temperature °C.

3.1.5 Length Weight Relationship

Weight (W kg) and post-hypural length (POHL cm) measurements were taken as fish died and were removed from the ponds (Figure 9). The "W vs POHL" relationship was derived using Table Curve 2D (SPSS Inc., Chicago IL., USA) and is: $W = a* (POHL)^b$ where: $a = 3.26901*10^{-5}$, $b = 2.9051$, $R^2 = 0.9706$, $N = 85$.

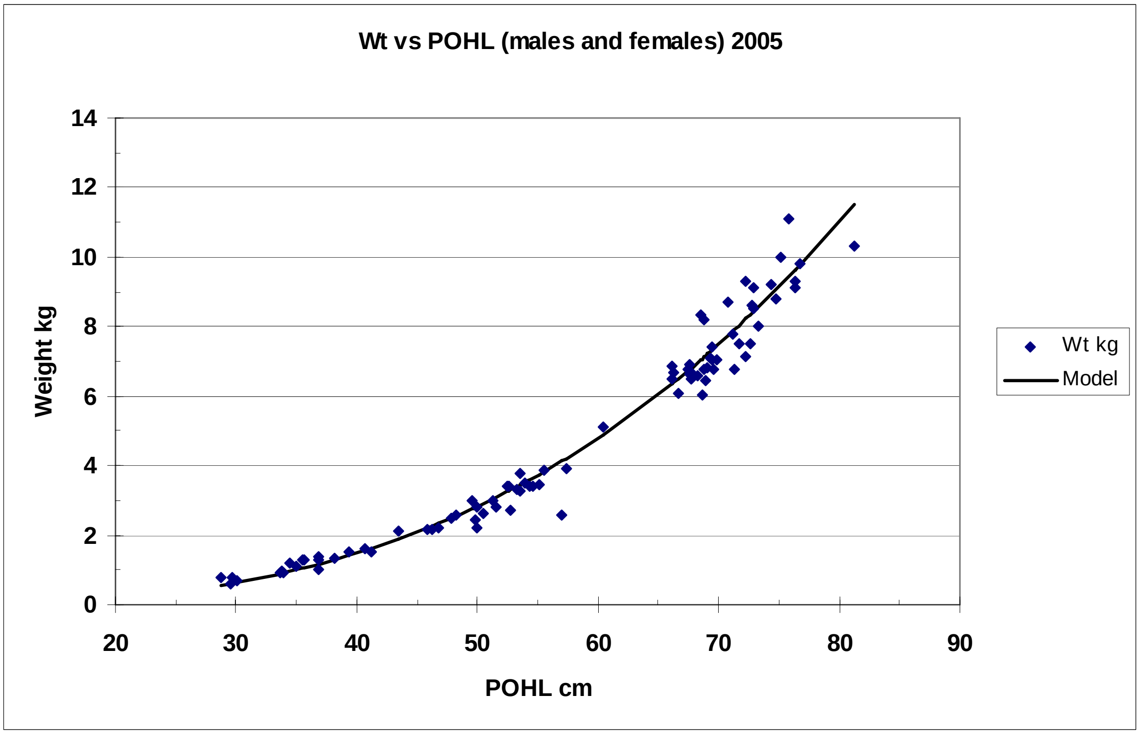

Figure 9. Weight (kg) vs post-orbital hypural length (POHL cm) for all fish in the 10-ft ponds.

3.1.6 Formalin Treatments.

A formalin stock solution (405 mL to 12 L water or 33,750 ppm) was pumped (200 mL/min) to the pond inflow stream (~100 L/min) for 1 hour. Figure 10 shows the calculated concentration profile (assuming the pond behaves as a mixed flow reactor). A peak value of 40 ppm was achieved at the end of 1 hour while the average concentration over the first 2 hours was 25 ppm. This treatment was much less than the recommended treatment of 100 ppm for 1 hour because of concerns over the high water temperatures.

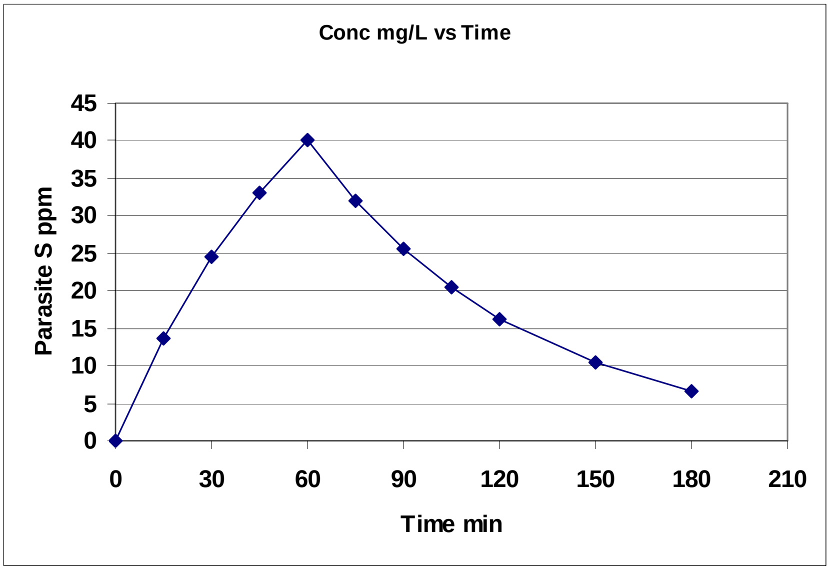

Figure 10. Calculated formalin (Parasite S) concentration (ppm) vs time (minutes) for treatments carried out in 10-ft ponds.

Each pond was treated 9 times between July 25 and August 25. The average water temperature during treatment of the chilled ponds was 17.0 °C (range 15.3 °C to 18.8 °C) and 19.4 °C (15.4 °C to 21.8 °C) for the ambient ponds. In all cases dissolved oxygen was above 80% of saturation.

3.1.7 Abrupt Changes in the Water Supply.

Over the summer, the water supply to the Lower Site (and the 10-ft ponds) alternated between "penstock" and "pumped". The penstock supply is gravity fed from the upper river while the pumped supply originates from the lower river (Figure 1). In both cases the water passes through an aeration tower before reaching the fish.

Switching water supplies resulted in abrupt changes in the water source (penstock vs pumped). Figure 11 shows the pumped and total flows – the difference (total – pumped) is the penstock flow. For a few days in mid-August both supplies were operating and the flow to the aeration tower reached approximately 17000 USGPM.

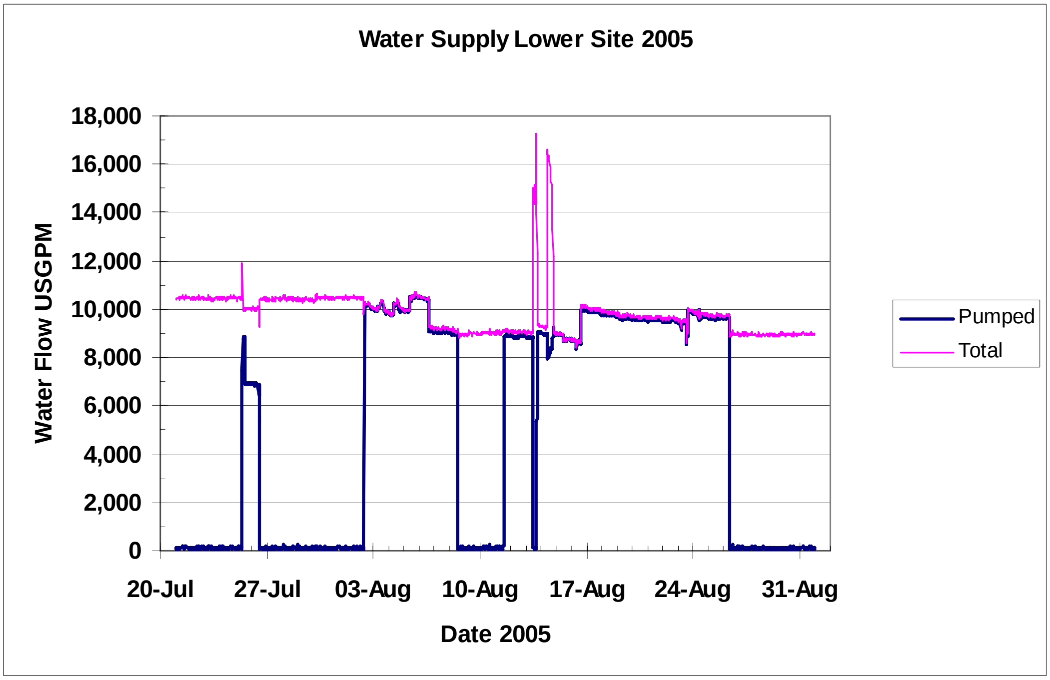

Figure 11. Water supply for Puntledge Lower Hatchery site over the summer of 2005. "Pumped" and Total flow (USGPM) are plotted – the difference (Total – Pumped) is the "penstock" flow.

3.2 Rosewall Creek Hatchery

3.2.1 Initial Numbers of Fish and Environmental Conditions

Summer chinook adults (160 fish) were transported from Puntledge Hatchery and held in two 20 ft ponds at Rosewall Creek. Average daily water temperatures at the inflow and outflow of the ponds are shown in Figure 12.

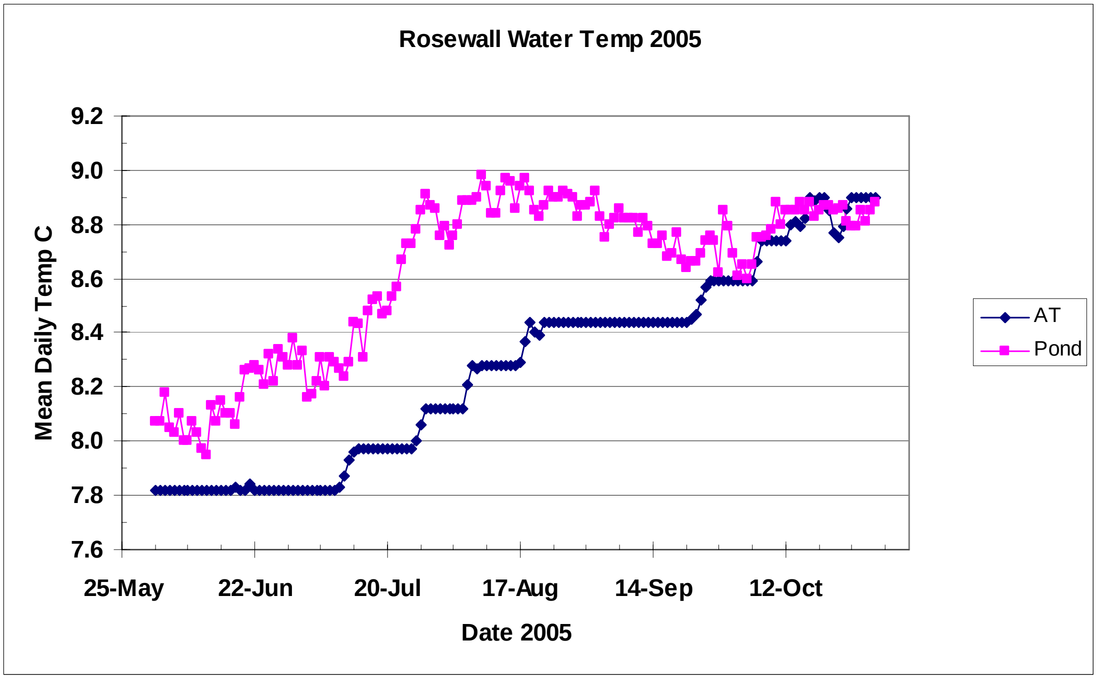

Figure 12. Average daily water temperature at the inflow (Aeration tower AT) and outflow of a 20-ft pond used to hold summer chinook brood stock at Rosewall Creek hatchery over the summer of 2005.

Dissolved oxygen concentrations were measured in the morning and afternoon at the pond outflows (Figure 13). Temperatures in the ponds were between 8 and 9 °C while the pond oxygen was above 9 mg/L over the holding period.

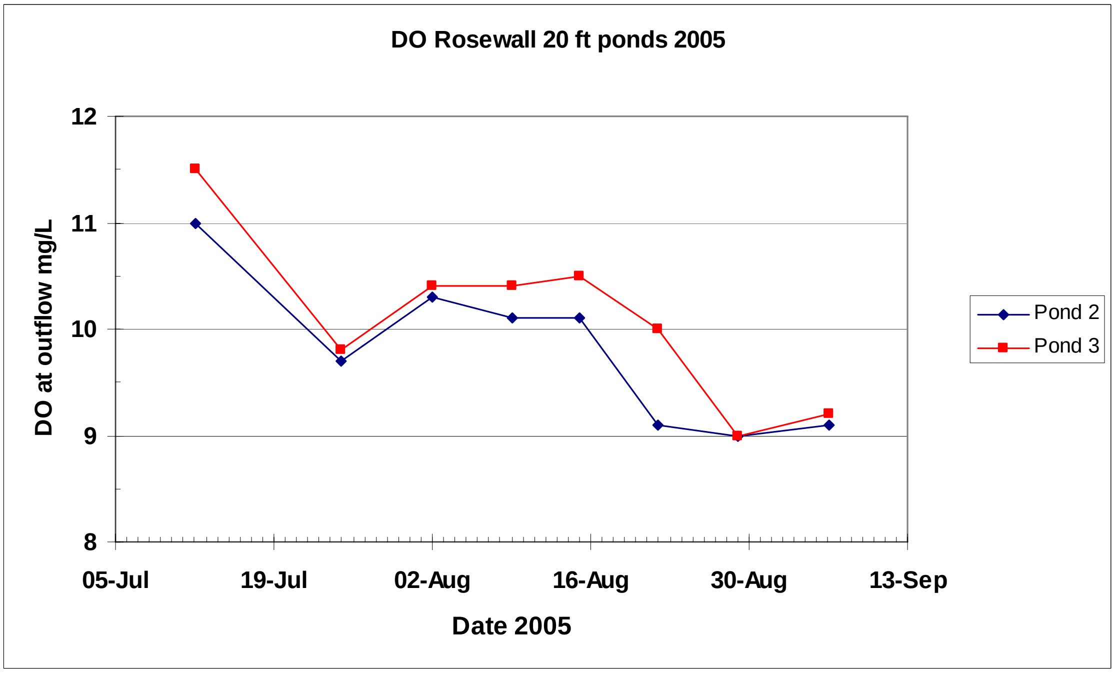

Figure 13. DO (mg/L) at the outflow of the 20 ft ponds holding summer chinook at Rosewall.

3.2.2 Adult Mortality

Out of 95 females moved to Rosewall, 8 died before spawning (8 %). Some the dead were due to accidents (jumpers etc) and therefore not due to holding environment. Mortality of males was 15 out of 65 (23%). This mortality is likely less important than female mortality; also, a portion of the male mortalities may be due to over-maturity.

3.2.3 Gamete Quality

Eggs were taken from 86 females on October 5 (37 females), Oct 11 (35), Oct 18 (11) and Oct 24 (3). From a plot (includes data from both Rosewall Creek and Puntledge) of the cumulative % maturity vs date, approximately 50 % of the population was mature on October 6 (Figure 14).

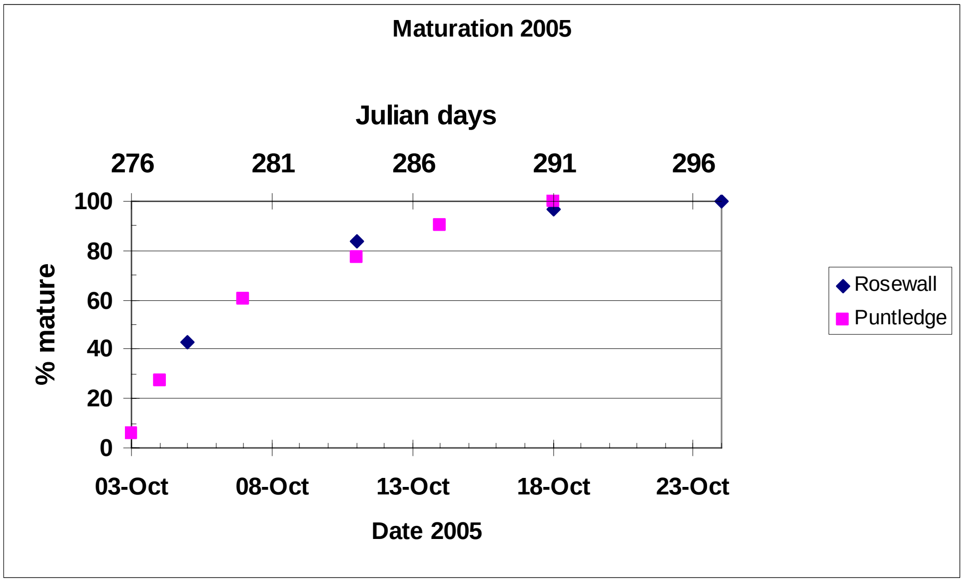

Figure 14. Cumulative maturation % for summer chinook females held at Rosewall and the Puntledge Upper and Lower facilities.

Sub-samples of eggs were taken from 10 females on each of the first 3 egg takes and transported to Puntledge. Four replicates (~ 50 eggs) per female were fertilized and incubated to the alevin stage. The average egg mortality for 30 females was 3.1 % (Table 3).

Table 3. Egg mortality for females held at Rosewall Creek. The mortality for each replicate (~ 50 eggs) and the average mortality per female are shown. Milt was obtained from males held at Rosewall and from Puntledge Lower site.

Egg Take Date 05	Source of Milt	Fish #	Mort %	Avg Mort Per female
05-Oct	Rosewall	1	8.33	
05-Oct	Rosewall	1	15.22	
05-Oct	Rosewall	1	7.41	
05-Oct	Rosewall	1	11.54	10.62
05-Oct	Rosewall	2	2.74	
05-Oct	Rosewall	2	5.41	
05-Oct	Rosewall	2	1.28	

05-Oct	Rosewall	2	2.82	3.06
05-Oct	Rosewall	3	0.00	
05-Oct	Rosewall	3	2.04	
05-Oct	Rosewall	3	0.00	
05-Oct	Rosewall	3	2.04	1.02
05-Oct	Rosewall	4	1.52	
05-Oct	Rosewall	4	1.30	
05-Oct	Rosewall	4	3.45	
05-Oct	Rosewall	4	1.27	1.88
05-Oct	Rosewall	5	4.44	
05-Oct	Rosewall	5	2.04	
05-Oct	Rosewall	5	0.00	
05-Oct	Rosewall	5	1.72	2.05
05-Oct	Rosewall	6	30.19	
05-Oct	Rosewall	6	30.00	
05-Oct	Rosewall	6	31.82	
05-Oct	Rosewall	6	14.55	26.64
05-Oct	Rosewall	7	1.47	
05-Oct	Rosewall	7	1.27	
05-Oct	Rosewall	7	10.67	
05-Oct	Rosewall	7	1.28	3.67
05-Oct	Rosewall	8	2.27	
05-Oct	Rosewall	8	4.08	
05-Oct	Rosewall	8	2.50	
05-Oct	Rosewall	8	4.76	3.40
05-Oct	Rosewall	9	0.00	
05-Oct	Rosewall	9	1.85	
05-Oct	Rosewall	9	0.00	
05-Oct	Rosewall	9	0.00	0.46
05-Oct	Rosewall	10	3.57	
05-Oct	Rosewall	10	1.92	
05-Oct	Rosewall	10	0.00	
05-Oct	Rosewall	10	0.00	1.37
11-Oct	Puntledge Lower site	11	3.28	
11-Oct	Puntledge Lower site	11	6.90	
11-Oct	Puntledge Lower site	11	3.70	
11-Oct	Puntledge Lower site	11	5.08	4.74
11-Oct	Puntledge Lower site	12	0.00	
11-Oct	Puntledge Lower site	12	1.67	
11-Oct	Puntledge Lower site	12	6.45	
11-Oct	Puntledge Lower site	12	5.33	3.36
11-Oct	Puntledge Lower site	13	0.00	
11-Oct	Puntledge Lower site	13	1.59	
11-Oct	Puntledge Lower site	13	1.64	
11-Oct	Puntledge Lower site	13	3.45	1.67
11-Oct	Puntledge Lower site	14	0.00	
11-Oct	Puntledge Lower site	14	0.00	
11-Oct	Puntledge Lower site	14	0.00	
11-Oct	Puntledge Lower site	14	0.00	0.00
11-Oct	Puntledge Lower site	15	5.08	
11-Oct	Puntledge Lower site	15	1.54	
11-Oct	Puntledge Lower site	15	0.00	

11-Oct	Puntledge Lower site	15	3.51	2.53
11-Oct	Puntledge Lower site	16	0.00	
11-Oct	Puntledge Lower site	16	0.00	
11-Oct	Puntledge Lower site	16	3.77	
11-Oct	Puntledge Lower site	16	4.92	2.17
11-Oct	Puntledge Lower site	17	1.79	
11-Oct	Puntledge Lower site	17	0.00	
11-Oct	Puntledge Lower site	17	0.00	
11-Oct	Puntledge Lower site	17	0.00	0.45
11-Oct	Puntledge Lower site	18	6.25	
11-Oct	Puntledge Lower site	18	11.11	
11-Oct	Puntledge Lower site	18	1.49	
11-Oct	Puntledge Lower site	18	1.37	5.06
11-Oct	Puntledge Lower site	19	0.00	
11-Oct	Puntledge Lower site	19	10.00	
11-Oct	Puntledge Lower site	19	6.58	
11-Oct	Puntledge Lower site	19	2.74	4.83
11-Oct	Puntledge Lower site	20	0.00	
11-Oct	Puntledge Lower site	20	2.04	
11-Oct	Puntledge Lower site	20	0.00	
11-Oct	Puntledge Lower site	20	2.27	1.08
18-Oct	Rosewall	21	0.00	
18-Oct	Rosewall	21	2.74	
18-Oct	Rosewall	21	0.00	
18-Oct	Rosewall	21	0.00	0.68
18-Oct	Rosewall	22	0.00	
18-Oct	Rosewall	22	1.64	
18-Oct	Rosewall	22	0.00	
18-Oct	Rosewall	22	2.90	1.13
18-Oct	Rosewall	23	1.69	
18-Oct	Rosewall	23	0.00	
18-Oct	Rosewall	23	0.00	
18-Oct	Rosewall	23	0.00	0.42
18-Oct	Rosewall	24	0.00	
18-Oct	Rosewall	24	0.00	
18-Oct	Rosewall	24	4.69	
18-Oct	Rosewall	24	5.88	2.64
18-Oct	Rosewall	25	4.05	
18-Oct	Rosewall	25	2.20	
18-Oct	Rosewall	25	5.75	
18-Oct	Rosewall	25	2.53	3.63
18-Oct	Puntledge Lower site	26	2.17	
18-Oct	Puntledge Lower site	26	0.00	
18-Oct	Puntledge Lower site	26	1.67	
18-Oct	Puntledge Lower site	26	0.00	0.96
18-Oct	Puntledge Lower site	27	0.00	
18-Oct	Puntledge Lower site	27	1.92	
18-Oct	Puntledge Lower site	27	0.00	
18-Oct	Puntledge Lower site	27	0.00	0.48
18-Oct	Puntledge Lower site	28	0.00	
18-Oct	Puntledge Lower site	28	5.00	
18-Oct	Puntledge Lower site	28	1.96	

18-Oct	Puntledge Lower site	28	0.00	1.74
18-Oct	Puntledge Lower site	29	0.00	
18-Oct	Puntledge Lower site	29	0.00	
18-Oct	Puntledge Lower site	29	0.00	
18-Oct	Puntledge Lower site	29	0.00	0.00
18-Oct	Puntledge Lower site	30	0.00	
18-Oct	Puntledge Lower site	30	0.00	
18-Oct	Puntledge Lower site	30	2.08	
18-Oct	Puntledge Lower site	30	0.00	0.52
			Rosewall Average	**3.08**
			Count	**30**

Spermatocrit values were measured at Rosewall Creek on 4 males during the October 18 egg take (3 replicates per fish). The average value was 40.0 % (Table 4). Spermatocrits were also measured on sperm samples taken from male chinook at Puntledge Lower and Upper sites during the spawning season. The average value for Puntledge Lower site was 36.0 % and for Puntledge Upper site was 27.9 %. An ANOVA (see Appendix 7.1.2) showed that sperm density was significantly greater for males held at Puntledge Lower Site versus Upper Site (P=0.017) and at Rosewall Creek versus Puntledge Upper Site (P=0.025).

Table 4. Spermatocrits (3 replicates and mean % packed cell volume) for males held at Puntledge Upper and Lower sites and at Rosewall Creek.

Site: Puntledge Summer Chinook Upper Site					
Date	Fish No.	Rep1	Rep2	Rep3	Mean
03-Oct	1	21.9	21.0	22.2	21.7
03-Oct	2	34.5	34.4	33.9	34.3
03-Oct	3	27.0	26.2	27.0	26.7
03-Oct	4	21.7	19.7	22.2	21.2
11-Oct	5	28.6	29.0	28.1	28.6
11-Oct	6	29.0	29.5	30.0	29.5
11-Oct	7	37.9	38.7	38.7	38.5
11-Oct	8	24.6	24.2	23.3	24.1
11-Oct	9	27.0	25.8	26.7	26.5
				Mean	27.9
				Standard Deviation	5.66
				N	9

Site: Puntledge Summer Chinook Lower Site					
Date	Fish No.	Rep1	Rep2	Rep3	Mean
03-Oct	1	30.0	29.2	29.0	29.4
03-Oct	2	32.8	32.8	34.4	33.3
11-Oct	3	30.0	32.8	30.4	31.0
11-Oct	4	36.5	38.2	36.8	37.2
11-Oct	5	42.3	41.9	42.6	42.3
11-Oct	6	48.0	48.7	48.2	48.3
11-Oct	7	25.8	26.8	25.0	25.8
11-Oct	8	40.7	39.0	41.4	40.3

11-Oct	9	41.9	44.6	45.6	44.1
11-Oct	10	35.0	36.2	35.1	35.4
11-Oct	11	37.5	37.9	39.3	38.2
11-Oct	12	28.6	29.5	29.5	29.2
11-Oct	13	37.3	38.9	37.7	38.0
11-Oct	14	32.8	31.6	32.3	32.2
11-Oct	15	43.4	44.7	44.9	44.3
11-Oct	16	25.8	25.8	25.4	25.7
11-Oct	17	47.5	48.6	48.4	48.2
11-Oct	18	22.6	22.8	22.2	22.5
11-Oct	19	40.7	39.7	41.5	40.6
11-Oct	20	29.3	29.4	30.9	29.9
11-Oct	21	21.0	21.0	21.3	21.1
11-Oct	22	35.8	35.8	35.2	35.6
11-Oct	23	30.6	29.5	32.3	30.8
11-Oct	24	30.6	30.2	27.7	29.5
11-Oct	25	36.2	37.7	37.1	37.0
11-Oct	26	47.7	45.0	47.2	46.6
14-Oct	27	39.0	38.7	38.7	38.8
14-Oct	28	32.3	32.8	30.6	31.9
14-Oct	29	34.9	35.9	39.0	36.6
14-Oct	30	30.2	30.2	31.3	30.5
14-Oct	31	36.5	35.8	37.5	36.6
14-Oct	32	45.8	45.3	45.0	45.4
14-Oct	33	39.3	40.0	40.3	39.9
14-Oct	34	34.4	33.8	34.9	34.4
14-Oct	35	39.7	38.1	38.7	38.8
14-Oct	36	38.1	38.7	38.1	38.3
14-Oct	37	42.0	34.4	33.9	36.7
14-Oct	38	49.2	49.1	50.0	49.4
18-Oct	39	27.7	27.4	27.4	27.5
18-Oct	40	34.9	34.4	34.4	34.6
18-Oct	41	44.3	43.3	43.5	43.7
18-Oct	42	27.4	25.8	27.0	26.7
18-Oct	43	44.3	43.5	43.3	43.7
				Mean	36.1
				Standard Deviation	7.12
				N	43

Site: Puntledge Summer Chinook Rosewall Creek Hatchery					
Date	Fish No.	Rep1	Rep2	Rep3	Mean
18-Oct	1	55.6	57.4	56.8	56.6
18-Oct	2	35.9	35.5	35.5	35.6
18-Oct	3	33.7	32.3	32.8	32.9
18-Oct	4	42.9	31.3	30.6	34.9
				Mean	40.0
				Standard Deviation	11.12
				N	4

Egg weight was measured on 15 females (Table 5). At the eyed stage 25 batches of 10 eggs each were randomly selected, drained and weighed. The average for all females was 258 mg per egg (N = 15). The egg size variation within a female “S” was calculated from the standard deviation of the 25 samples “sd”: S = √10 * sd. The average value of S for the 15 females tested at Rosewall was 24.5 mg (Table 5).

Table 5. Mean, minimum and maximum egg weights (mg) at the eyed stage for females held at Puntledge Upper and Lower sites and at Rosewall Creek. The standard deviation of individual egg weights within each female is also shown (S).

Puntledge Upper Site

	Date	Date		Egg Weight mg			
Female	Egg Take	Sampled	Mean	Min	Max	sd	S
1	03-Oct	01-Nov	268.0	254.4	288.9	7.96	25.16
2	03-Oct	01-Nov	296.2	260.1	314.7	14.14	44.72
3	03-Oct	01-Nov	286.2	266.5	298.0	6.80	21.49
4	03-Oct	01-Nov	337.6	317.5	349.8	8.98	28.41
12	11-Oct	21-Nov	263.9	245.7	271.0	5.97	18.87
13	11-Oct	21-Nov	306.7	290.2	321.5	8.08	25.56
14	11-Oct	21-Nov	302.0	284.6	319.9	7.54	23.84
15	11-Oct	21-Nov	286.3	274.4	308.6	9.61	30.40
16	11-Oct	21-Nov	319.6	302.8	333.5	9.36	29.60
		Average	296.2				27.6
		SD	23.6				

Puntledge Lower Site

	Date	Date		Egg Weight mg			
Female	Egg Take	Sampled	Mean	Min	Max	sd	S
1	03-Oct	01-Nov	275.1	252.0	289.2	8.84	27.94
2	03-Oct	01-Nov	204.6	188.3	210.4	4.83	15.29
3	03-Oct	01-Nov	248.0	233.9	260.1	7.09	22.40
4	03-Oct	01-Nov	247.3	212.5	257.8	9.44	29.85
5	03-Oct	01-Nov	336.6	323.3	344.2	4.55	14.39
6	03-Oct	01-Nov	280.1	263.1	292.7	6.26	19.78
7	03-Oct	01-Nov	294.3	276.1	306.9	6.58	20.82
8	03-Oct	01-Nov	344.0	333.4	363.4	7.41	23.43
9	03-Oct	01-Nov	291.5	279.8	302.3	6.21	19.62
10	03-Oct	01-Nov	296.4	287.1	305.2	5.00	15.82
11	03-Oct	10-Nov	273.5	256.1	291.4	10.77	34.07
12	07-Oct	10-Nov	207.9	200.6	214.7	3.80	12.02
13	07-Oct	10-Nov	286.0	276.1	296.3	4.61	14.58
14	07-Oct	10-Nov	271.4	259.1	281.1	6.15	19.46
15	07-Oct	10-Nov	295.9	281.3	307.2	6.79	21.47
		Average	276.9				20.7
		SD	38.9				

Rosewall Creek

	Date	Date		Egg Weight mg			
Female	Egg Take	Sampled	Mean	Min	Max	sd	S
1	05-Oct	09-Nov	292.0	278.8	308.3	7.92	25.05
2	05-Oct	09-Nov	206.7	183.6	223.0	11.76	37.18
3	05-Oct	09-Nov	299.1	287.6	310.8	6.87	21.73
4	05-Oct	09-Nov	199.5	186.2	209.4	6.34	20.06

5	05-Oct	09-Nov	305.8	286.0	317.3	7.47	23.61
6	05-Oct	09-Nov	285.1	268.1	299.0	6.69	21.14
7	05-Oct	09-Nov	203.8	185.9	212.7	5.92	18.71
8	05-Oct	09-Nov	357.9	345.7	367.4	5.33	16.86
9	05-Oct	09-Nov	257.4	246.0	268.7	5.70	18.04
10	05-Oct	09-Nov	259.7	236.4	274.1	9.54	30.18
11	11-Oct	21-Nov	210.3	199.4	220.8	5.48	17.34
12	11-Oct	21-Nov	247.6	227.7	260.7	6.85	21.66
13	11-Oct	21-Nov	296.9	283.3	310.0	7.04	22.26
14	11-Oct	21-Nov	259.7	234.6	281.6	15.17	47.98
15	11-Oct	21-Nov	189.6	167.7	204.4	8.24	26.06
		Average	258.1				24.5
		SD	48.9				

3.3 Puntledge Hatchery Production Raceways

3.3.1 Upper Site 2004

The bulk of the summer chinook returning to Puntledge Hatchery in 2004 were held in the channel at the Upper Site. Temperatures at the inlet of the channel are shown in Figure 15.

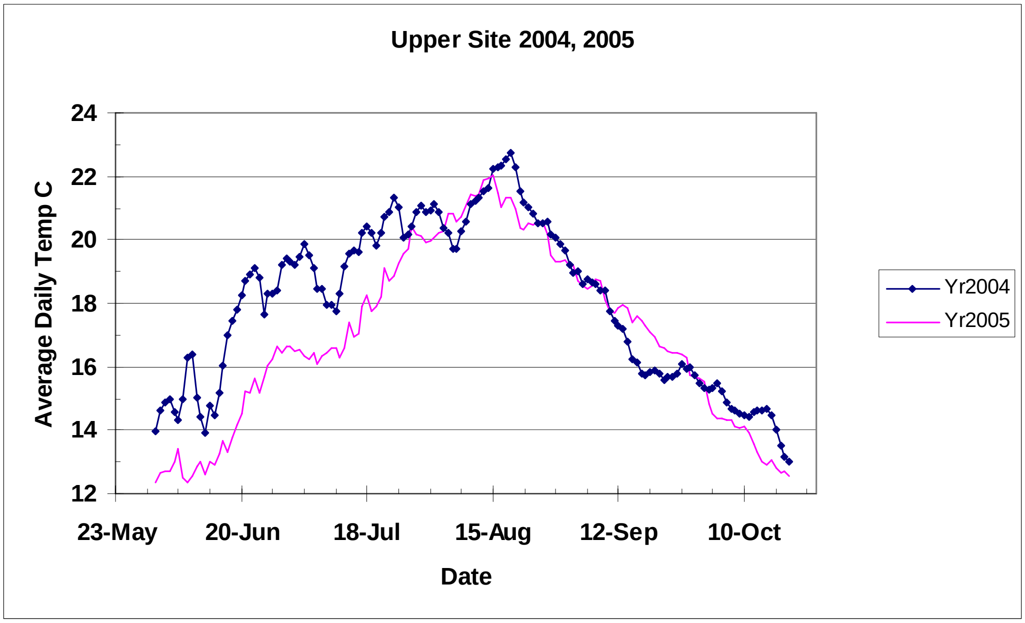

Figure 15. Average daily water temperature at the inlet of the brood stock channel at Puntledge Upper site over the summer of 2004 and 2005.

High temperatures contributed to the excessive mortality rate (> 90%). Only 6 females survived to maturity of the 900 fish (approximate) that entered the channel. A small group holding at the Lower Site had a much higher survival rate over the same time period. These fish also experienced high temperatures. One difference between the groups is that the Lower Site has an aeration tower and therefore lower total gas pressure (TGP). It was speculated that the combination of elevated TGP and high temperature caused the high mortality rates seen at the Upper Site in 2004.

3.3.2 Total Gas Pressure 2005

Average daily ΔP values calculated from continuous gas pressure readings at the Upper Site and spot checks at the Lower Site over the summer of 2005 are shown in Figure 16.

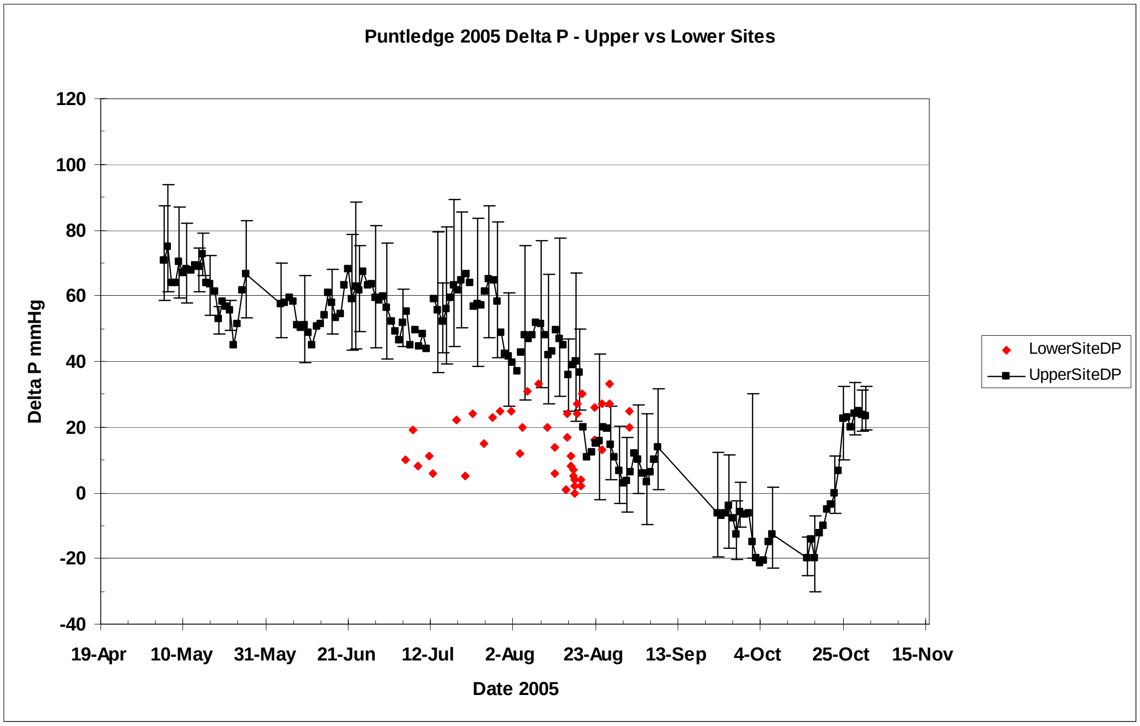

Figure 16. Total gas pressure (ΔP mmHg) at the Upper and Lower sites over the summer of 2005. Daily average, minimum and maximum values (from continuous readings) are shown for the Upper site. Spot checks were made at the Lower site.

The highest value at the Upper Site occurred on May 6 and was ΔP = 75 mmHg or TGP = 110% (at BP = 747 mmHg). The average for July was ΔP = 55 mmHg (TGP = 107 % at BP = 753 mmHg). ΔP dropped slowly over July and August as the summer water temperatures stabilized and then quickly decreased as temperatures started to drop in mid August. With steadily decreasing temperatures in the autumn ΔP becomes negative (Figure 16). ΔP at the Lower Site was much lower because the aeration tower removes excess gas. The highest measured value at the Lower Site was ΔP = 30 mmHg (TGP = 104 % at BP = 760 mmHg) on August 26.

3.3.3 Adult Mortality 2005

At least 73 females (accountable mortality) died before spawning at the Upper and Lower sites. This is an underestimate of the actual mortality and is simply the number of dead females that were retrieved and counted from the holding raceways. Since 85 females survived to maturity (eggs taken), the relative mortality rate was 46% (73/158). The cumulative female mortality is plotted in Figure 17 – half the mortality occurred by August 4. A similar analysis for males (accountable mortality = 109) showed that half the mortality occurred by August 14 (Figure 17).

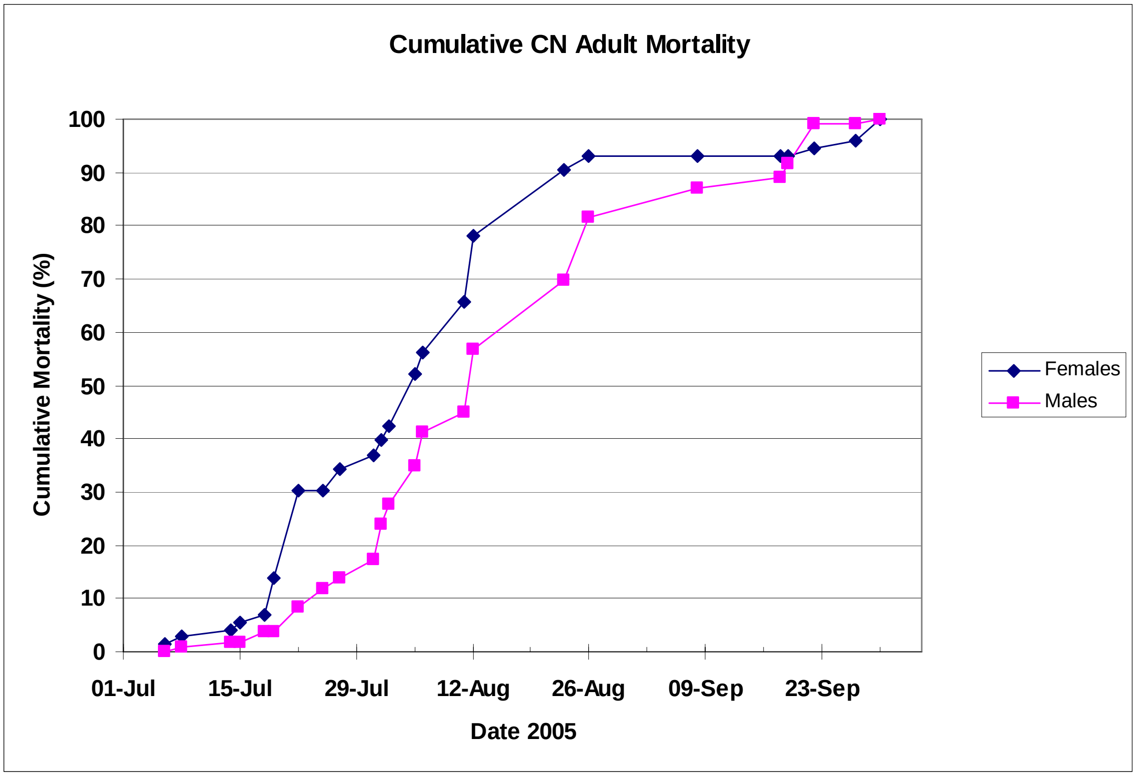

Figure 17. Cumulative female and male mortality % (accountable) for the combined Upper and Lower sites.

3.3.4 Gamete Quality 2005

Eggs were taken from 84 females from the Upper and Lower sites – egg take dates were October 3 (5 females), Oct 4 (18), Oct 7 (28), Oct 11 (14), Oct 14 (11) and Oct 18 (8). One fish on October 18 was rejected (immature). From a plot of the cumulative % maturity (Figure 14), polynomial trend-line models (Figure 18) were used to compare maturation rates at 280 and 287 Julian days (Table 6). These models illustrate that the maturation rates for fish held at Rosewall Creek and the Puntledge hatchery were virtually identical.

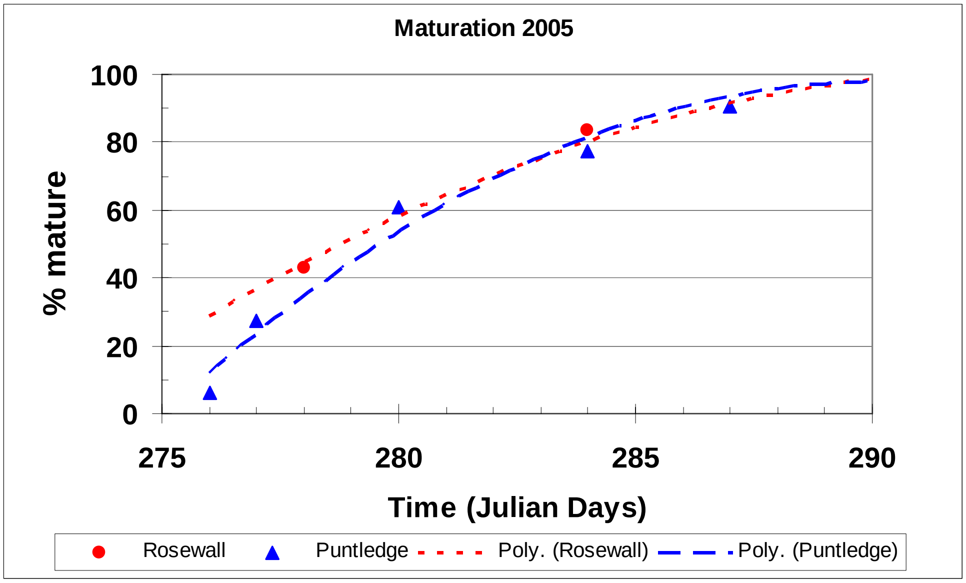

Figure 18. Cumulative maturation polynomial models for females at Rosewall ($y = -0.2385x^2 + 140.01x - 20444$; $R^2 = 0.9856$) and Puntledge (Upper and Lower Sites; $y = -0.4258x^2 + 247.15x - 35765$; $R^2 = 0.9804$).

Table 6. Predicted maturation rates (%) for fish held at Puntledge and Rosewall at 280 and 287 Julian days.

Julian Days	Puntledge Maturation (%)	Rosewall Maturation (%)
280.0	54.3	60.4
287.0	94.3	93.9

Sub-samples of eggs were taken from 17 females at the Upper Site (Table 7) and from 29 females at the Lower Site (Table 8) and incubated in divided Heath trays. Four replicates (~ 50 eggs) per female were fertilized and incubated to the alevin stage. Tables 7 and 8 show egg mortality for each replicate and the average for each female. The average egg mortality for the Upper and Lower sites was 13.4 % (N = 17) and 11.8% (N = 29) respectively.

Table 7. Egg mortality for females held to maturity at Puntledge Upper site. The mortality for each replicate (~ 50 eggs) and the average mortality per female are shown.

Egg Take Date 05	Fish #	Mortality %	Average per female
03-Oct	1	20.63	
03-Oct	1	23.21	
03-Oct	1	22.03	
03-Oct	1	16.07	20.49
03-Oct	2	10.42	
03-Oct	2	15.69	
03-Oct	2	8.00	

03-Oct	2	14.81	12.23
03-Oct	3	4.00	
03-Oct	3	11.54	
03-Oct	3	8.33	
03-Oct	3	15.69	9.89
03-Oct	4	22.22	
03-Oct	4	16.00	
03-Oct	4	18.75	
03-Oct	4	17.07	18.51
07-Oct	5	8.89	
07-Oct	5	13.46	
07-Oct	5	2.44	
07-Oct	5	2.17	6.74
07-Oct	6	8.62	
07-Oct	6	8.06	
07-Oct	6	4.35	
07-Oct	6	7.14	7.04
07-Oct	7	8.70	
07-Oct	7	13.04	
07-Oct	7	24.53	
07-Oct	7	13.04	14.83
07-Oct	8	24.49	
07-Oct	8	7.84	
07-Oct	8	12.24	
07-Oct	8	12.82	14.35
07-Oct	9	18.64	
07-Oct	9	13.21	
07-Oct	9	24.53	
07-Oct	9	20.41	19.20
07-Oct	10	2.44	
07-Oct	10	13.33	
07-Oct	10	11.11	
07-Oct	10	9.76	9.16
07-Oct	11	4.65	
07-Oct	11	12.00	
07-Oct	11	6.38	
07-Oct	11	12.00	8.76
11-Oct	12	13.21	
11-Oct	12	4.69	
11-Oct	12	4.05	
11-Oct	12	3.45	6.35
11-Oct	13	11.54	
11-Oct	13	10.34	
11-Oct	13	6.76	
11-Oct	13	4.69	8.33
11-Oct	14	2.13	
11-Oct	14	3.85	
11-Oct	14	14.29	
11-Oct	14	8.57	7.21
11-Oct	15	46.94	
11-Oct	15	54.55	
11-Oct	15	52.46	

11-Oct	15	55.56	52.37
11-Oct	16	2.00	
11-Oct	16	14.29	
11-Oct	16	3.77	
11-Oct	16	3.45	5.88
14-Oct	17	3.13	
14-Oct	17	13.16	
14-Oct	17	9.21	
14-Oct	17	2.53	7.01
		Upper Site	
		Average	**13.43**
		Count	**17**

Table 8. Egg mortality for females held to maturity at Puntledge Lower site. The mortality for each replicate (~ 50 eggs) and the average mortality per female are shown.

Egg Take Date 05	Fish #	Mortality %	Average per female	Egg Take Date 05	Fish #	Mortality Mort %	Average per female
03-Oct	1	12.96		07-Oct	12	25.33	
03-Oct	1	9.23		07-Oct	12	28.95	34.46
03-Oct	1	3.92		07-Oct	13	5.88	
03-Oct	1	8.47	8.65	07-Oct	13	18.33	
04-Oct	2	9.72		07-Oct	13	0.00	
04-Oct	2	26.32		07-Oct	13	5.77	7.50
04-Oct	2	16.05		07-Oct	14	4.62	
04-Oct	2	18.07	17.54	07-Oct	14	9.84	
04-Oct	3	8.06		07-Oct	14	9.26	
04-Oct	3	24.62		07-Oct	14	8.16	7.97
04-Oct	3	5.66		07-Oct	15	12.28	
04-Oct	3	10.34	12.17	07-Oct	15	2.08	
04-Oct	4	5.45		07-Oct	15	14.00	
04-Oct	4	6.45		07-Oct	15	4.44	8.20
04-Oct	4	4.69		07-Oct	16	4.17	
04-Oct	4	3.28	4.97	07-Oct	16	2.17	
04-Oct	5	4.35		07-Oct	16	7.55	
04-Oct	5	4.76		07-Oct	16	3.64	4.38
04-Oct	5	10.00		07-Oct	17	12.50	
04-Oct	5	9.30	7.10	07-Oct	17	13.75	
04-Oct	6	20.97		07-Oct	17	16.13	
04-Oct	6	9.26		07-Oct	17	8.70	12.77
04-Oct	6	16.98		07-Oct	18	13.21	
04-Oct	6	22.81	17.50	07-Oct	18	6.25	
04-Oct	7	5.00		07-Oct	18	0.00	
04-Oct	7	9.09		07-Oct	18	8.16	6.91
04-Oct	7	8.70		07-Oct	19	5.88	
04-Oct	7	12.82	8.90	07-Oct	19	2.70	
04-Oct	8	4.76		07-Oct	19	12.77	
04-Oct	8	19.05		07-Oct	19	2.13	5.87
04-Oct	8	8.51		07-Oct	20	14.55	
04-Oct	8	8.33	10.16	07-Oct	20	15.87	
04-Oct	9	5.66		07-Oct	20	14.93	

04-Oct	9	5.88		07-Oct	20	5.77	12.78
04-Oct	9	6.00		11-Oct	21	29.38	
04-Oct	9	6.12	5.92	11-Oct	21	18.34	
04-Oct	10	18.75		11-Oct	21	23.16	
04-Oct	10	32.08		11-Oct	21	39.34	27.56
04-Oct	10	23.53		11-Oct	22	4.58	
04-Oct	10	20.00	23.59	11-Oct	22	6.40	
07-Oct	11	10.20		11-Oct	22	10.16	
07-Oct	11	13.21		11-Oct	22	5.66	6.70
07-Oct	11	10.00		11-Oct	23	6.47	
07-Oct	11	12.73	11.53	11-Oct	23	1.63	
07-Oct	12	55.88		11-Oct	23	2.33	
07-Oct	12	27.69		11-Oct	23	6.52	4.24

Egg Take Date 05	Fish #	Mortality %	Average per female
11-Oct	24	23.85	
11-Oct	24	16.04	
11-Oct	24	14.78	
11-Oct	24	20.59	18.82
11-Oct	25	13.59	
11-Oct	25	9.52	
11-Oct	25	21.62	
11-Oct	25	20.95	16.42
11-Oct	26	12.00	
11-Oct	26	14.55	
11-Oct	26	14.91	
11-Oct	26	12.80	13.56
11-Oct	27	10.09	
11-Oct	27	3.92	
11-Oct	27	5.26	
11-Oct	27	7.41	6.67
11-Oct	28	1.04	
11-Oct	28	3.03	
11-Oct	28	0.93	
11-Oct	28	2.52	1.88
11-Oct	29	13.11	
11-Oct	29	18.60	
11-Oct	29	19.40	
11-Oct	29	18.60	17.43
		Lower Site	
		Average	**11.80**
		Count	**29**

Egg mortality was compared for the 3 groups (i.e. Puntledge Upper and Lower Sites and Rosewall Creek) by Kruskal-Wallis One Way Analysis of Variance on Ranks (see Appendix 7.1.1) a significant difference (P = <0.001) was found between eggs from adults held at Rosewall Creek compared to both Lower and Upper Puntledge sites. There was no significant difference in egg mortality between the 2 Puntledge sites. To further illustrate that egg quality was much better from females held at Rosewall Creek prior to spawning, a frequency histogram for the 3 holding sites was constructed (Figure 19). Notice that 90% of the eggs from Rosewall Creek-held fish were in the 5% egg mortality category, while eggs from Puntledge–held fish showed a much poorer egg mortality distribution, with the Upper site exhibiting the highest mortality (i.e. one fish in the 55% egg mortality category). This is further illustrated by the descriptive statistics in Table 9. For example, notice that egg mortality ranged from 0 to 26.6% at Rosewall Creek compared to 5.9 to 52.4 % at the Puntledge Upper Site.

Table 9. Descriptive statistics of chinook mean egg mortalities from fish held at Rosewall Creek, Puntledge Upper Site, and Puntledge Lower Site

Descriptive Statistics	*Rosewall Creek Mean Mortality (%)*	*Puntledge Upper Site Mean Mortality (%)*	*Puntledge Lower Site Mean Mortality (%)*
Mean	3.1	13.4	11.8
Standard Deviation	4.9	11.1	7.5
Minimum	0.0	5.9	1.9
Maximum	26.6	52.4	34.5
Count	30	17	29
Confidence Interval (95.0%)	1.8	5.7	2.8

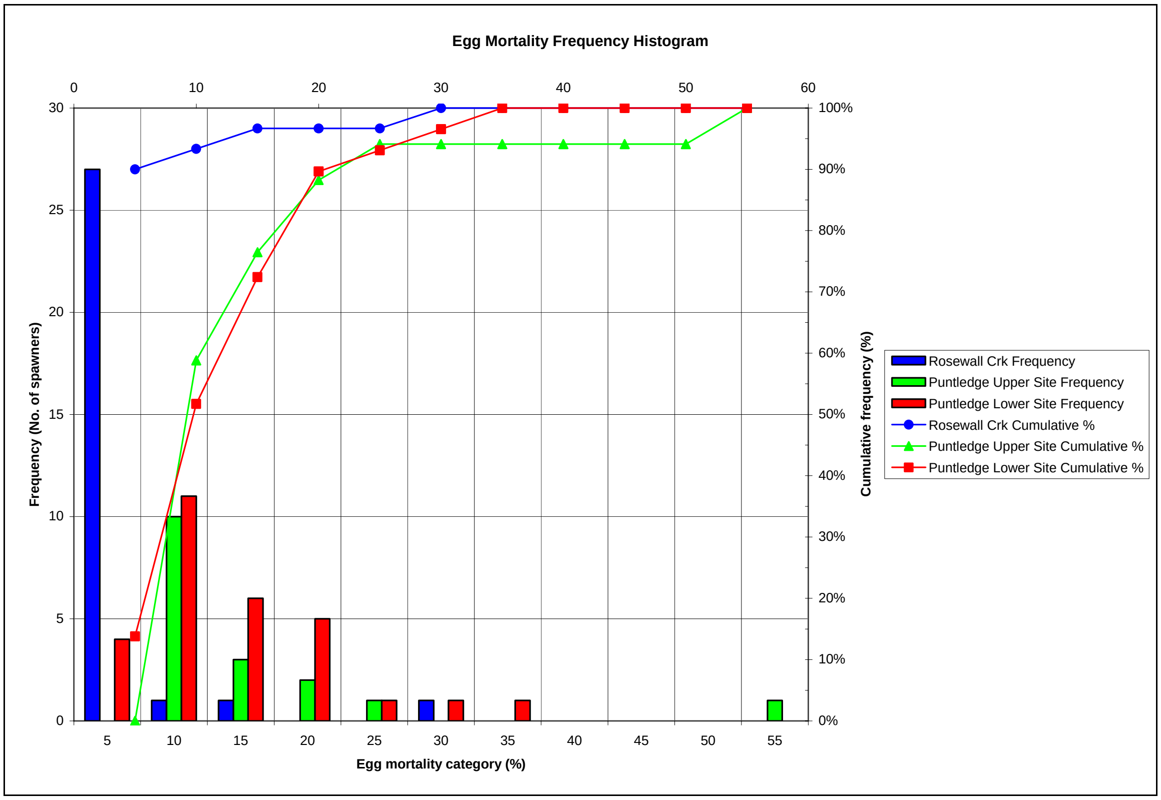

Figure 19. Frequency histogram of chinook egg mortality from fish held at Rosewall Creek, Puntledge Upper Site, and Puntledge Lower Site.

Table 4 shows spermatocrit values for males held at the Upper and Lower sites. Averages for each site were 27.9 % (Upper) and 36.1 % (Lower) – this difference is significant (P < 0.05; see ANOVA results in Appendix 7.1.2). Spermatocrits from

Rosewall Creek-held males were also higher (40.0%), and even though only 4 males were sampled, this was significantly different from Upper site-held fish.

Egg weights for females held at the Upper and Lower sites were measured at the eyed stage using the procedure described for Rosewall Creek. Average values for the two sites were 296 mg (N = 9) and 277 mg per egg (N = 15) respectively (Table5). The egg size variation within a female "S" was calculated from the standard deviation of the 25 samples "sd": $S = \sqrt{10} * sd$. The average "S" for the 9 females at the Upper Site was 27.6 mg while the average "S" for the 15 females at the Lower Site was 20.7 mg (Table 5).

There was no significant difference ($P < 0.05$; see ANOVA results in Appendix 7.1.3) in mean egg weight or in egg size variation "S" for females held at the Upper Site, Lower Site or Rosewall Creek.

4.0 DISCUSSION

The dramatic difference in pre-spawning mortality between the Puntledge and Rosewall hatcheries illustrates the overriding importance of water temperature. All 38 females moved in early July to the 10-ft circular ponds at the Puntledge Lower site died before spawning while 86 females out of the 95 transported to Rosewall survived. Water temperature is the most important difference between the 2 sites – Rosewall was 8 to 9 °C while the 10-ft ponds were 17-19 °C during that period (Figure 20).

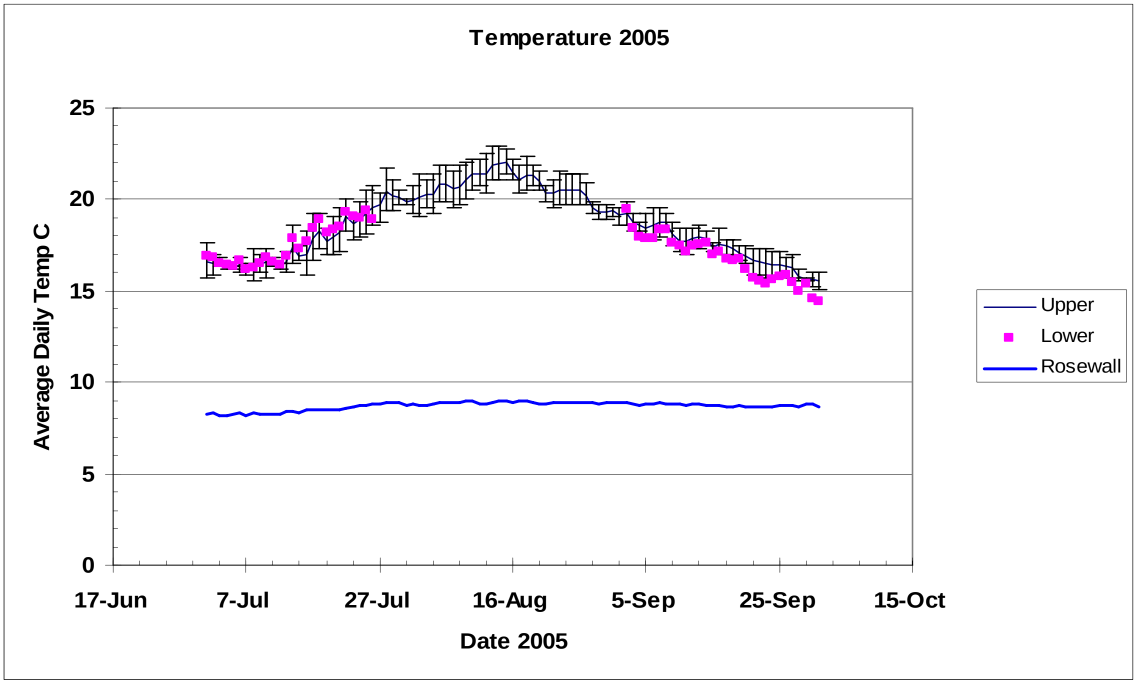

Figure 20. Average daily water temperature at the Upper and Lower sites (inlets) and for the 20 ft pond (outlet) at Rosewall Creek over the summer of 2005. Daily minimum and maximums for the Upper Site are also shown.

Note that fish transported to Rosewall experienced more stress than fish transported to the 10-ft ponds. Although capture and handling techniques were identical, transportation to Rosewall (35 km away) took about 1 hour. The 10-ft ponds on the other hand are 30 meters from the capture area and transport time was only a few minutes. Furthermore, only fish in optimum condition were moved to the 10-ft ponds -- fish with injuries (from seals, otters etc.) were rejected and transported to Rosewall. The recovery of these injured fish at Rosewall was the most outstanding difference between these sites.

Although formalin was used at both sites to control fungus, the high water temperatures at Puntledge made treatment extremely problematic. Too high a dose results in toxicity while too low a dose allows fungus to proliferate. The reduced treatment (40 ppm) was a compromise. However, because of the lower temperature of Rosewall water, formalin treatment was straightforward and effective.

The chiller (to ponds 1 and 3) was not started until July 27 and did not have any sparing effect – 50% mortality occurred by July 30 in Ponds 1 and 3 and by July 28 in Ponds 2 and 4 (Figures 4 and 5). Females in ponds 5 and 6 survived longer – 50 % mortality occurred on August 18. The only difference was that these ponds had additional aeration (3 ft of bio-rings) to strip gas supersaturation caused by the water heater. The heater was not turned on in 2005 and the reason for the longer survival time is not known.

All the females in the 10-ft ponds were dead by the end of August. The overall mortality rate for females at Puntledge (Lower, Upper and 10-ft ponds) was 57 % while pre-spawning mortality at Rosewall was 8 %.

Two other more subtle factors may also have affected pre-spawning mortality at Puntledge. The first possibility is Total Gas Pressure (TGP). TGP is caused by solar heating in the spring and summer and probably contributed to the extreme mortality at the Upper Site in 2004 (Jensen et al., 1986). That year fish at the Upper Site suffered over 90 % mortality over the summer while a small group of chinook held at the Lower Site had little mortality. The Lower Site is protected by an aeration tower while the Upper Site has no protection against TGP. Unfortunately TGP was not measured in 2004 but values can be inferred from measurements made in 2005. TGP is directly rated to the rate of warming (Figure 21) and warming rates were higher in 2004 than in 2005 (Figure 15). Therefore it is probable that TGP in 2004 was higher than the values shown in Figure 16.

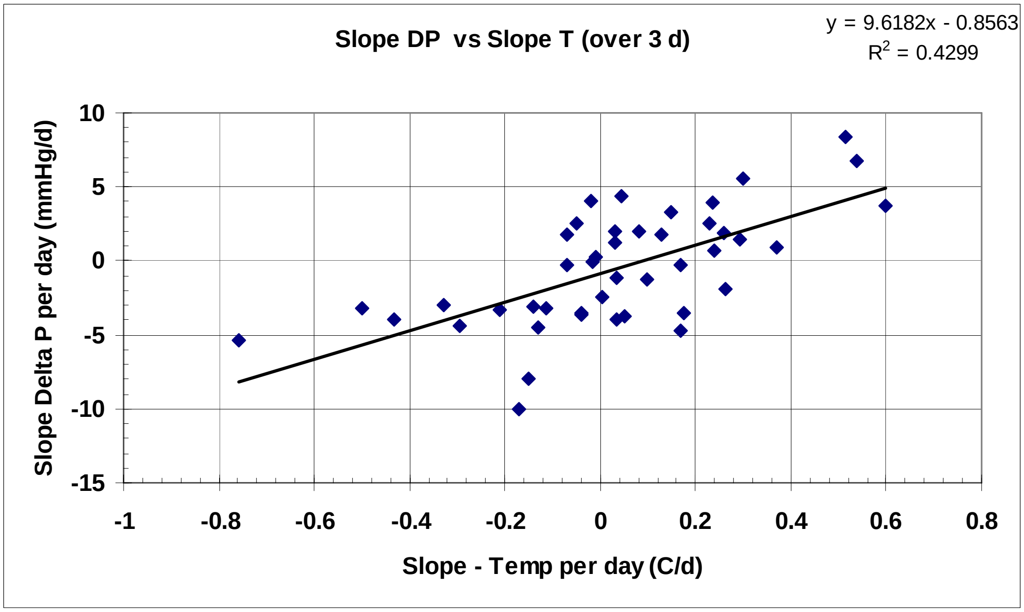

Figure 21. Change in DP mmHg/d (slope over 3 days) vs change in temperature °C/d (slope over 3 days). There is a significant relationship (0.01 level) between DP and the rate of warming (R = 0.656, N = 49).

The average daily ΔP value from July 5 to August 15, 2005 for the Upper Site was 52 mmHg and the peak daily value averaged 75 mmHg (TGP = 107 % and 110 % respectively at BP = 755 mmHg). This compared to an average ΔP at the Lower Site (post aeration) of 16 mmHg (102 %). On two occasions both the penstock and pumped supply were used simultaneously and the flow to the aeration tower reached 17,000 USGPM. Performance of the tower would probably have decreased during this flow overload and TGP at the ponds would have increased. Unfortunately no TGP measurements were made during these periods.

The second factor possibly affecting pre-spawning mortality is the presence of the benthic algae gomphonema (Jensen et al. 2005). It likely affected the Lower Site in 2003 when the water source was switched from "penstock" to "pumped" in the first week of September. Gomphonema blankets the river bottom above the pumping station. Thus the "pumped" supply is vulnerable when algae mats are disturbed and frustules become suspended in the water column (Figure 1). These sharp siliceous fragments are reported to irritate the eyes of swimmers and probably irritate fish gills (Rieberger 1991). The "pumped" supply was used many times during the summer of 2005 (Figure 11) due to penstock maintenance by BC Hydro. Unfortunately suspended sediment or turbidity were not monitored over the summer and so this impact remains speculative.

As well as lower pre-spawning mortality, the cooler water at Rosewall also resulted in better gamete quality. Although there was no conclusive differences in egg size, egg size variation or in female maturation rates between sites there was a highly significant difference in spermatocrits or sperm density ($P<0.02$) and in egg mortality rate ($P< 0.001$). Eggs from females held at Rosewall had a lower mortality rate (3.07 %) than eggs from females held at Puntledge Upper (13.4 %) or Lower (11.8 %) sites. Note that all the eggs were incubated at Puntledge on the same water supply (chilled) so that differences in egg survival truly reflect female holding conditions. Also note that Rosewall eggs survived equally well whether they were fertilized with milt from Rosewall or from Puntledge Lower site (Table 3). This indicates that temperature has a more deleterious effect on egg quality rather than milt quality.

5.0 ACKNOWLEDGEMENTS

The authors thank the staff at Puntledge and Rosewall Creek Hatcheries, especially Chris Beggs, Les Clint, Brian Munro, Dale Fetzner, Bob Addy, Jim Campbell, Allan Robertson, Laura Terry, Cheryl Burroughs, and Bill Jackson for assistance with this work. They were instrumental in capturing, monitoring, and spawning the fish and in the operation of the water chiller/heater. We would also like to thank Mel Sheng and Angus McKay for securing the funding for this research from the Pacific Salmon Commission-Southern Boundary Restoration & Enhancement Fund. Finally, special thanks go to Hanne McLean for her helpful manuscript revisions.

6.0 REFERENCES

Bouck, G.R., and Jacobsen, J. 1976. Estimation of salmonid sperm concentration by microhematocrit technique. Trans. Am. Fish. Soc. 105: 534–535.

Jensen, J.O.T., McLean, W.E., Damon, W., and Sweeten, T. 2005. Puntledge River high temperature study: Influence of high water temperature on adult chinook salmon (Oncorhynchus tshawytscha). Can. Tech. Rep. Fish. Aquat. Sci. 2603: v + 27p.

Jensen, J.O.T., W.E. McLean, W. Damon, and T. Sweeten. 2004. Puntledge River high temperature study: Influence of high water temperatures on adult pink salmon mortality, maturation, and gamete viability. Can. Tech. Rep. Fish. Aquat. Sci. 2523: vi + 50 p

Jensen, J.O.T., J. Schnute and D.F. Alderdice. 1986. Assessing juvenile salmonid response to gas supersaturation using a general multivariate dose-response model. Can. J. Fish. Aquat. Sci. 43: 1694-1709.

Hoskins, G. E., C.J. West and A. E. Kling. 1983. Computer management of fish health problems in hatcheries. Can. Spec. Publ. Fish. Aquat. Sci. 68: 42 p.

Rieberger, K. 1991. The distribution of the diatom Gomphonema geminata in Vancouver Island streams. Work term report in partial fulfilment of the requirements of the biology Co-op Program, summer 1991, University of Victoria. Performed at Ministry of the Environment, Nanaimo B.C. ISBN 0-7726-1493-6. 61p.

7.0 APPENDICES

7.1 Statistical Analyses.

7.1.1 Egg Mortality ANOVA

One Way Analysis of Variance

Data source: 2005 Egg mortality %

Normality Test: Failed (P = <0.001)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks

Data source: 2005 Egg mortality %

Group	N	Missing	Median	25%	75%
Rosewall Crk	30	0	1.811	0.685	3.404
Upper Site	17	0	9.160	7.035	15.749
Lower Site	29	0	8.902	6.692	16.675

H = 40.968 with 2 degrees of freedom. (P = <0.001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :

Comparison	Diff of Ranks	Q	P<0.05
Upper Site vs Rosewall Crk	34.886	5.204	Yes
Upper Site vs Lower Site	2.836	0.420	No
Lower Site vs Rosewall Crk	32.051	5.573	Yes

Note: The multiple comparisons on ranks do not include an adjustment for ties.

7.1.2 Spermatocrit ANOVA

One Way Analysis of Variance

Data source: Spermatocrit data

Normality Test: Passed (P > 0.050)

Equal Variance Test: Passed (P = 0.824)

Group Name	**N**	**Missing**	**Mean**	**Std Dev**	**SEM**
Rosewall spermatocrit	4	0	40.021	11.116	5.558
Lower Site Puntledge spermatocrit	43	0	36.054	7.117	1.085
Upper Site Puntledge spermatocrit	9	0	27.884	5.663	1.888

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Groups	2	604.347	302.174	5.813	0.005
Residual	53	2754.871	51.979		
Total	55	3359.218			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.005).

Power of performed test with alpha = 0.050: 0.782

The power of the performed test (0.782) is below the desired power of 0.800.
You should interpret the negative findings cautiously.

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	**Diff of Means**	**t**	**Unadjusted P**	**Critical Level**	**Significant?**
Lower Site P vs. Upper Site P	8.170	3.092	0.003	0.017	Yes
Rosewall vs. Upper Site P	12.137	2.801	0.007	0.025	Yes
Rosewall vs. Lower Site P	3.967	1.053	0.297	0.050	No

7.1.3 Egg Weight ANOVA

One Way Analysis of Variance

Data source: Egg weight Data

Normality Test: Passed (P > 0.050)

Equal Variance Test: Passed (P = 0.139)

Group Name	**N**	**Missing**	**Mean**	**Std Dev**	**SEM**
Egg wt Rosewall	15	0	258.072	48.856	12.615
Egg wt Lower Site Puntledge	15	0	276.855	38.867	10.036
Egg wt Upper Site Puntledge	9	0	296.245	23.555	7.852

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Groups	2	8380.754	4190.377	2.557	0.092
Residual	36	59005.372	1639.038		
Total	38	67386.126			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.092).

Power of performed test with alpha = 0.050: 0.298

The power of the performed test (0.298) is below the desired power of 0.800.
You should interpret the negative findings cautiously.

7.2 Pictures from 2005.

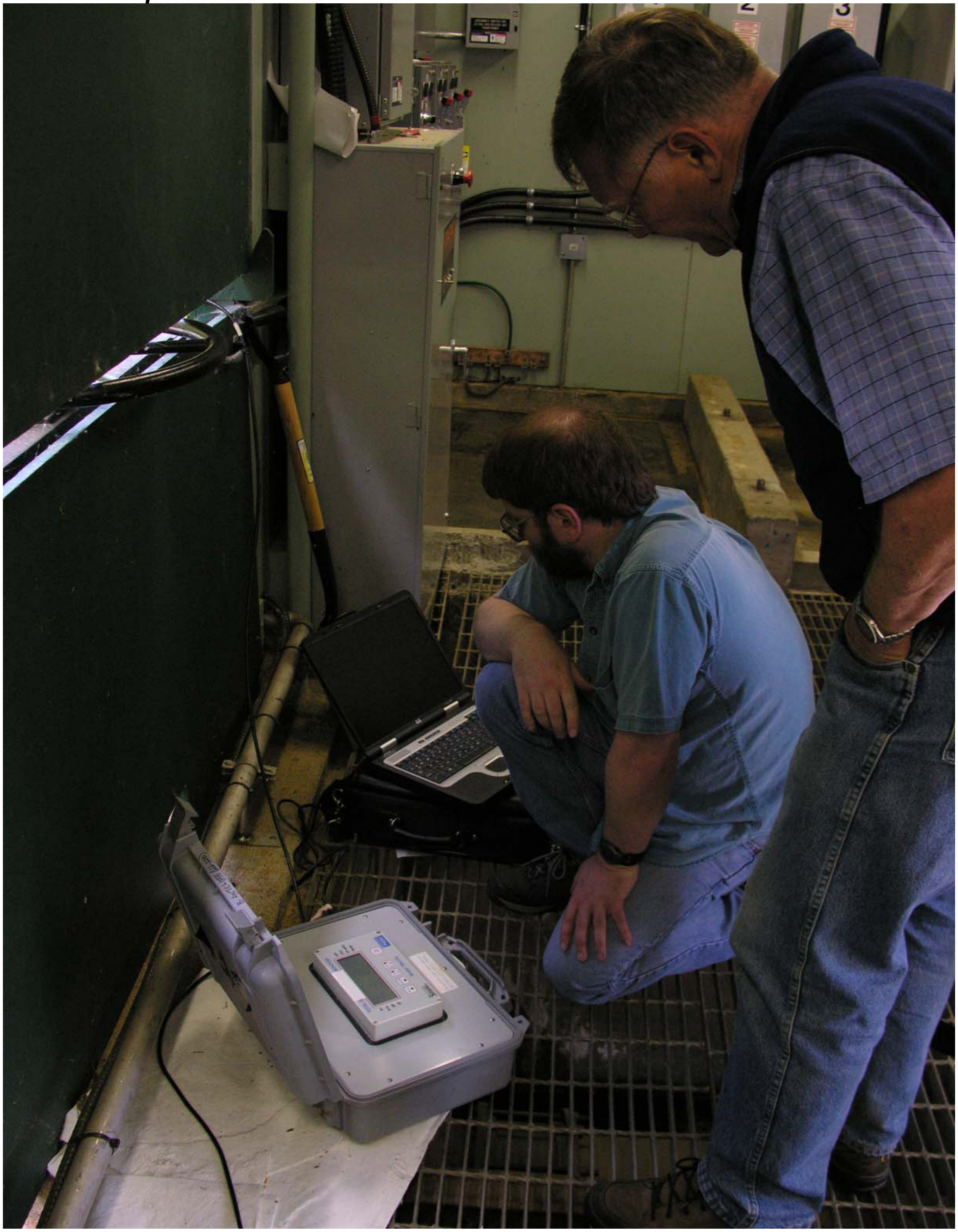

Figure 22. Transferring TGP data files from the Common Sensing meter at the Puntledge Upper Site pump house (i.e. holding channel intake).

Figure 23. Transferring summer chinook from Puntledge Lower Site to a fish transport truck destined for the Rosewall Creek hatchery.

Figure 24. Loading summer chinook into cage to be hoisted to the fish transport truck.

Figure 25. Capturing summer chinook for transport to Rosewall Creek hatchery

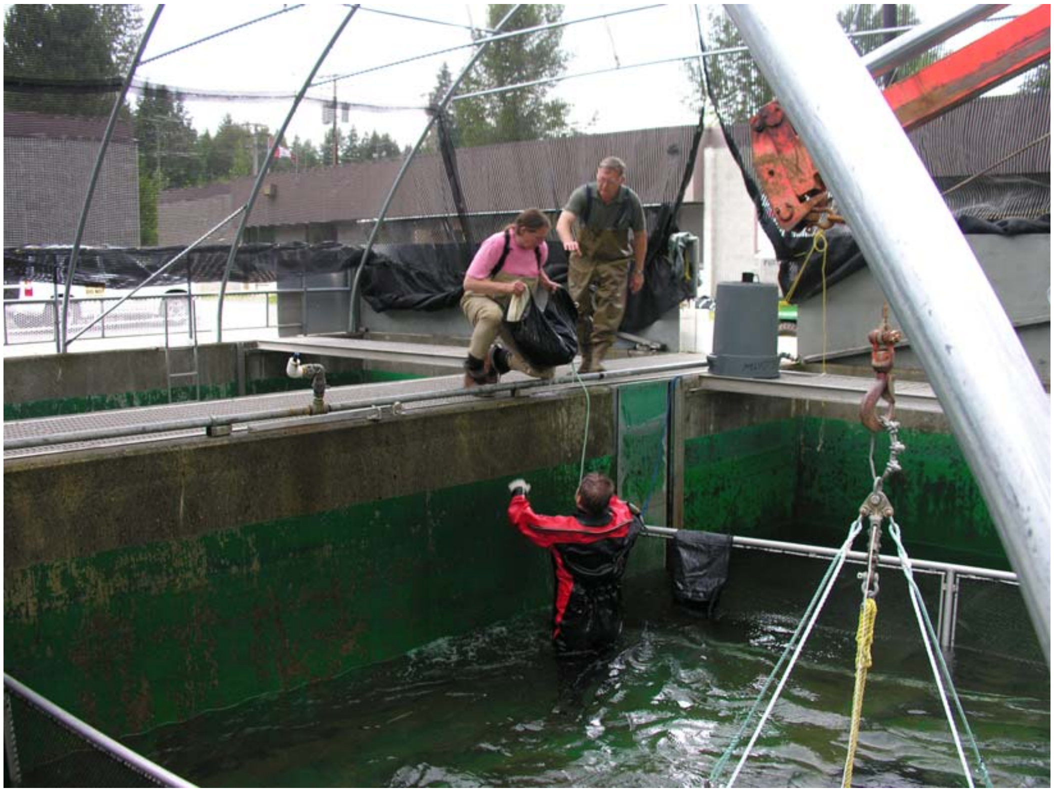

Figure 26. Transferring summer chinook to 10-ft diameter tanks using "soft" transport bag.

Figure 27. Releasing fish into 10-ft diameter tank.

Figure 28. 10-ft diameter fish holding tanks at Puntledge Lower Site.

Figure 29. Tanks 5 and 6 (10-ft diameter at Puntledge Lower Site)) showing extra aeration head tank.

Figure 30. Close-up of black aeration pots for tanks 5 and 6 at Puntledge Lower site.

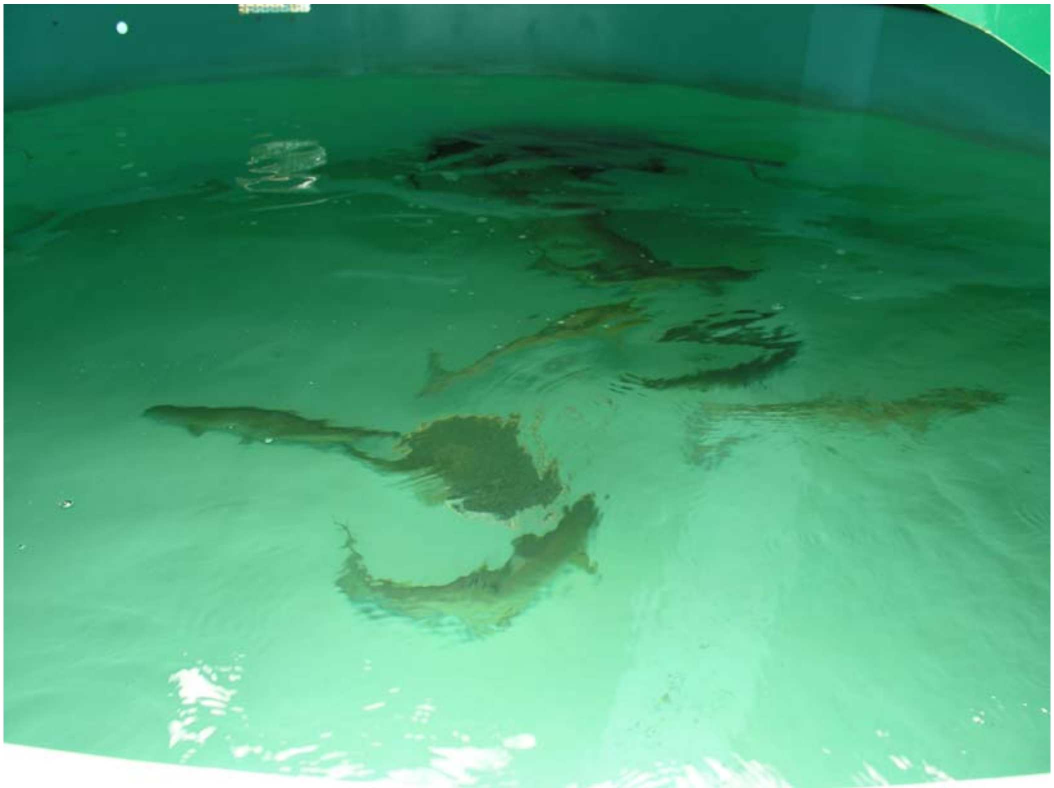

Figure 31. Inside 10-ft diameter tank, illustrating low loading density.

Figure 32. Summer chinook being selected for spawning at the Puntledge Upper Site holding channel.

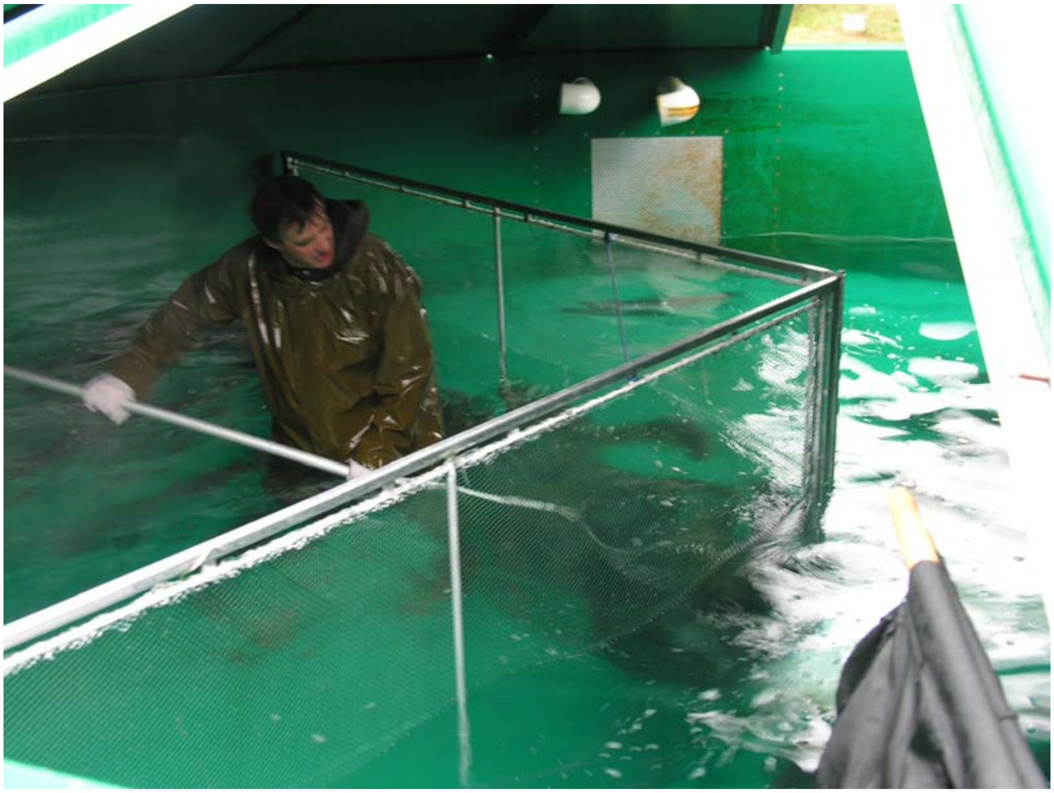

Figure 33. Summer chinook being selected for spawning at the Rosewall Creek hatchery.

Figure 34. Summer chinook being removed from tank for spawning at the Rosewall Creek hatchery.

Figure 35. Spawning of summer chinook at Rosewall Creek hatchery.

Figure 36. Removing all viable eggs from an individual chinook female.

Figure 37. Dividing 4 sub-samples of eggs per female into small Plexiglas compartments to be placed in a standard vertical flow incubation tray.

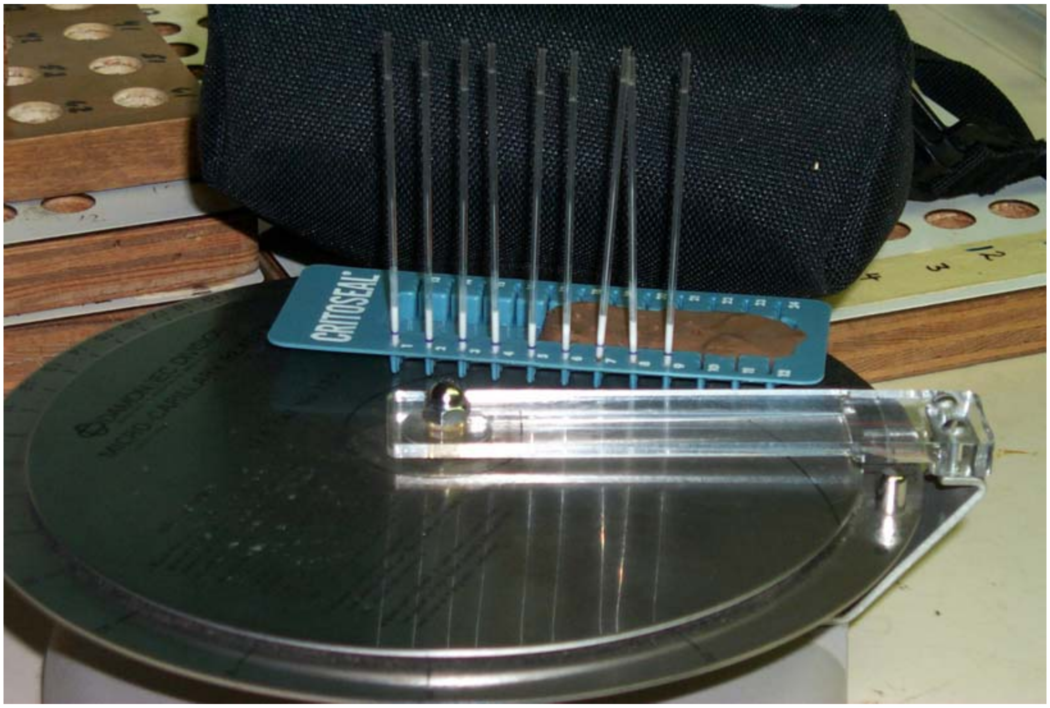

Figure 38. Micro-capillary tubes with packed sperm volume after being spun in a centrifuge, ready for spermatocrit determination.

Figure 39. Measuring sperm density (i.e. spermatocrit; % packed sperm cell volume).

Figure 40. 20-ft diameter holding tanks (5 tanks on the left) at Rosewall Creek hatchery.