Puntledge River High Temperature Study: Influence of High Water Temperatures on Adult Pink Salmon Mortality, Maturation, and Gamete Viability

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PUNTLEDGE RIVER HIGH TEMPERATURE STUDY: INFLUENCE OF HIGH WATER TEMPERATURES ON ADULT PINK SALMON MORTALITY, MATURATION, AND GAMETE VIABILITY

by

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ABSTRACT

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Adult pink salmon (*Oncorhynchus gorbuscha*) were exposed to three declining water temperature regimes prior to spawning. The mean (range) test temperatures for the chilled, ambient, and heated regimes were 15.1°C (11.6°C-19.4°C), 18.4°C (15.0°C-21.8°C), and 21.3°C (16.6°C-24.0°C), respectively, from August 28 to Sept. 17, 2002. During that period, the adult mortality was 2, 10, and 82 %, respectively. Maturation rates also were affected with 53, 7, and 0 % of females ripe by October 1, 2002, respectively. Thirdly, mean egg mortality was 14, 41, and 60 %, respectively. Hence, the adverse influence of high water temperature during the latter phase of maturation was demonstrated to significantly ($P< 0.05$) increase adult mortality, delay maturation rate, and reduce gamete viability.

RÉSUMÉ

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.

Des saumons roses (*Oncorhynchus gorbuscha*) adultes ont été exposés avant la fraye à trois régimes de baisse de la température. La moyenne (fourchette) de température des essais des trois régimes : refroidissement, température ambiante et réchauffement, étaient respectivement de 15,1°C (11,6°C-19,4°C), 18,4°C (15,0°C-21,8°C) et 21,3°C (16,6°C-24,0°C) entre le 28 août et le 17 septembre 2002. Pendant cette période, la mortalité des adultes a été respectivement de 2, 10 et 82 %. Les taux de maturation ont aussi été affectés, le pourcentage des femelles à maturité au 1er octobre 2002 étant respectivement de 53, 7, et 0 %. De plus, la mortalité moyenne des œufs était respectivement de 14, 41 et 60 %. L'effet néfaste de la hausse de la température pendant la dernière phase de la maturation est donc démontré puisque, de façon significative ($P< 0,05$), cette hausse fait augmenter la mortalité des adultes, elle ralentit la maturation et elle réduit la viabilité des gamètes.

1.0 INTRODUCTION

Returning adult salmon are exposed to high water temperatures in many BC rivers including the Puntledge River. The water temperatures in the Puntledge River at the lower hatchery have been recorded and the daily means and extremes (i.e. averaged minima and maxima) are illustrated in Figure 1.

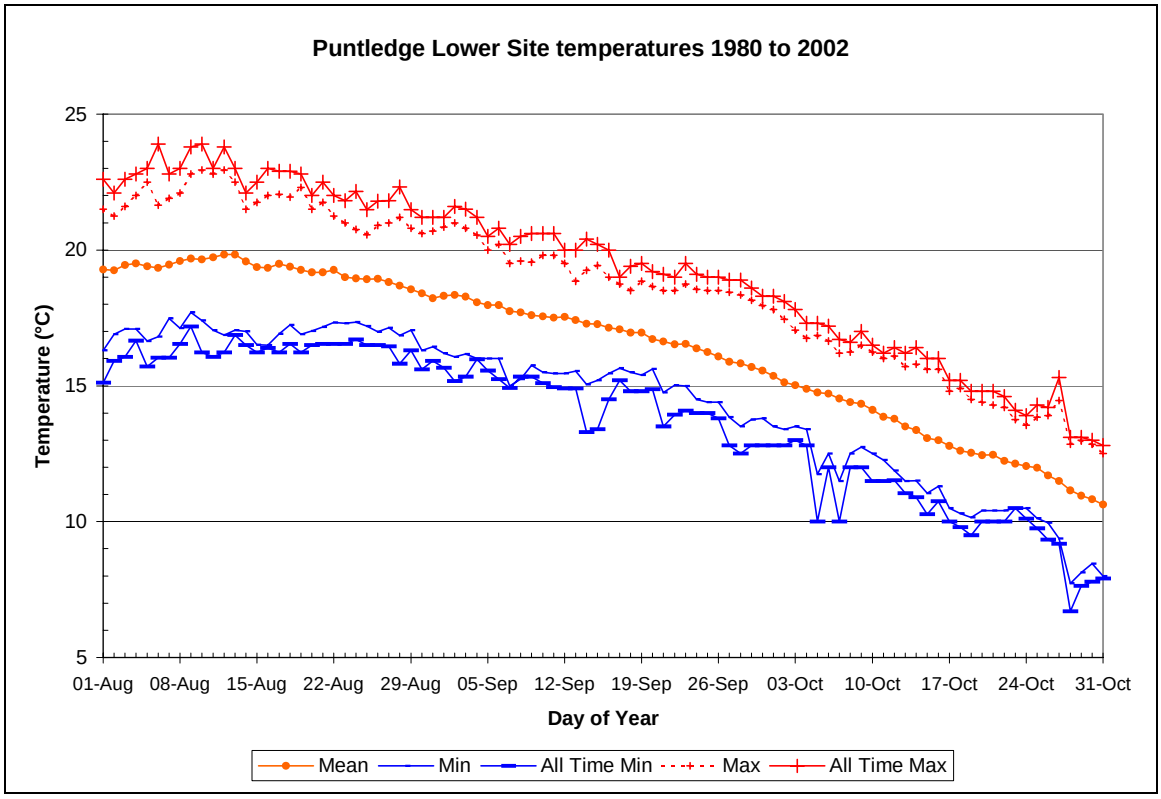

Figure 1. Puntledge River lower site temperature averaged from 1980 to 2002, except for 1997 and 1998, for the period Aug. 1 to Oct. 31. The daily Mean, Min, and Max values are averaged over the 22 years, while the All Time Min and the All Time Max values are the daily extremes for the 22-year period.

Although studies on the acute lethal effects of high temperatures on adult salmon have been reported (Berman, 1990; Servizi and Jensen, 1977), the effect of temperature on latter stages of egg maturation, fertilization success and egg development is complex and poorly understood. The high water temperatures recorded for the Puntledge River in the summer and early fall likely affect the productivity of summer chinook (*Oncorhynchus tshawytscha*) and pink (*O. gorbuscha*) salmon returning to the Puntledge River. The purpose of this study was to capture returning adult pink salmon and to expose them to different temperature regimes for the latter part of maturation and spawning in order to determine the potential impact on maturation rate, adult mortality, and subsequent gamete viability.

2.0 MATERIALS AND METHODS

The Puntledge River is located on east side of Vancouver Island, British Columbia, Canada (Fig. 2).

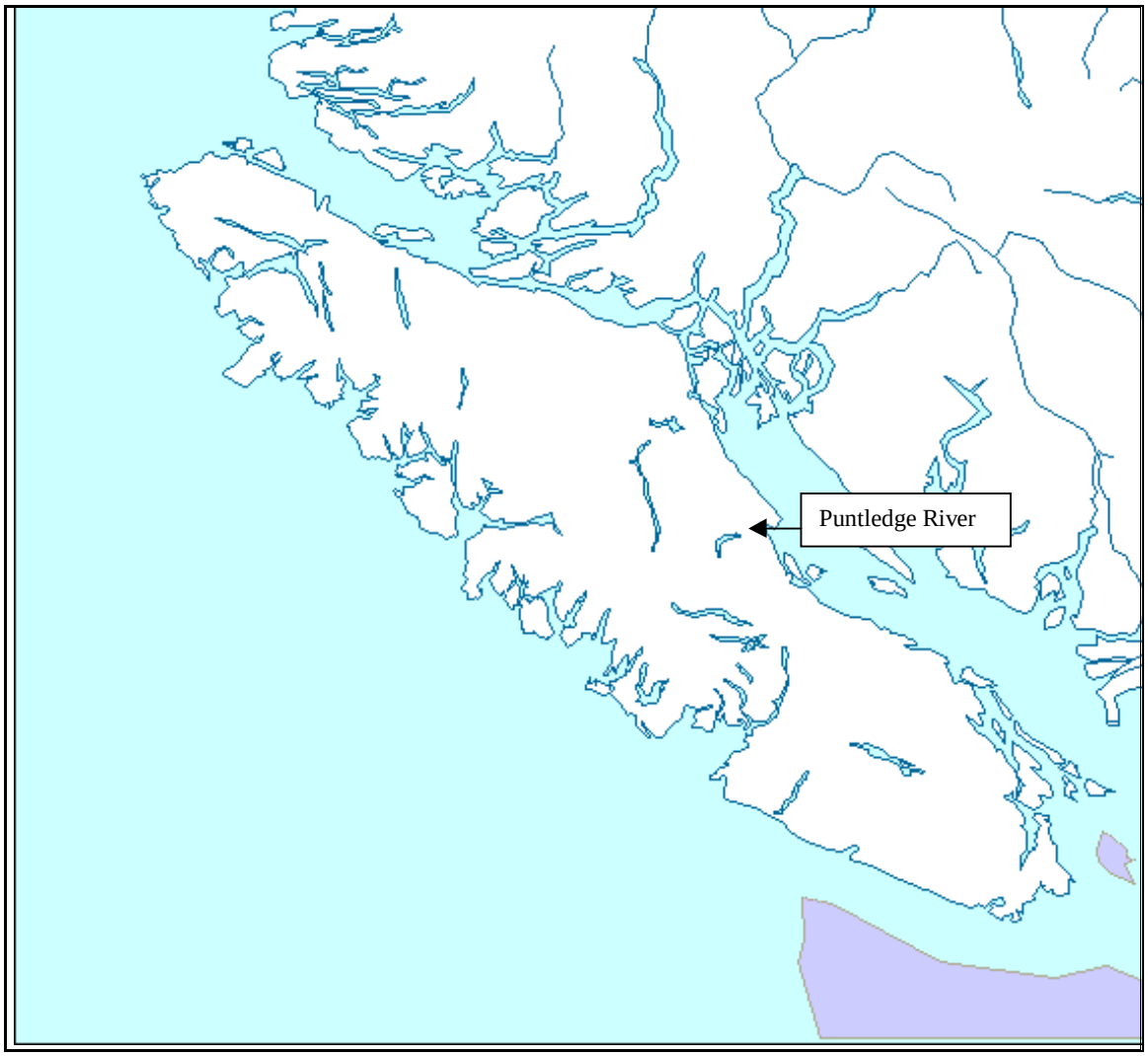

Figure 2. Map of Vancouver Island indicating the location of the Puntledge River.

On August 28, 2002, adult pink salmon were captured using a beach seine downstream of the Puntledge hatchery diversion fence (Fig. 3).

Figure 3. Transportation trucks waiting for adult pink salmon captured downstream of the diversion fence, indicated by arrow.

Additional fish were also obtained from a holding area, by using a dipnet, at the outflow from the hatchery adult raceway (Fig. 4).

Figure 4. Capturing fish by crowding and then using a dipnet.

The fish, totalling 148 (68 females and 80 males), were then randomly distributed among six 3-m diameter circular ponds (Fig. 5) at the Puntledge hatchery.

Figure 5. Circular ponds used for holding adult pink salmon at different temperatures at the Puntledge hatchery.

The volume of each pond was 6.7 m^3 with flows nominally set at 40 litres per minute (LPM). Flow was checked regularly by timed volume measurements and re-set if found to be 5 LPM too low or too high. Supplemental oxygen was also plumbed to each pond in case the fish lowered the dissolved oxygen (DO) below 6 mg/L. DO measurements showed that this system was not required and it was turned off on August 30.

The temperature treatments for adult fish exposure consisted of 3 temperature regimes. The chilled treatment was achieved by running ambient temperature river-water through a refrigeration system, powered by a 25-horsepower motor operated at full capacity, with planned nominal cooling of 2 ºC below ambient. The chiller performed poorly until Sept.4, when repairs were made, increasing chilling capacity dramatically. The second temperature regime was achieved by using ambient temperature river-water. The third temperature regime was achieved by running ambient temperature river-water through a 6-element 180 KW heater with a planned nominal heating of 2 ºC above ambient. Gas supersaturation caused by heating was removed by the use of aeration columns. Each temperature regime exhibited a natural daily fluctuation due to solar heating.

Temperatures in each holding pond were recorded using Onset TidbiT temperature loggers, set to record at 5-minute intervals. Flow rates and dissolved oxygen levels were monitored regularly. The fish were visually inspected daily for signs of maturation and mortalities were recorded, removed, and measured for length and weight. Weights and post-orbital hypural lengths (POHL) were taken before removal of gametes.

Typical hatchery spawning procedures were employed. The water level in each pond was lowered and fish were carefully captured, using a dipnet, and placed in an anaesthetic bath (50 ppm tricaine methanesulfonate). Mature females were dispatched by clubbing. Milt was expressed from mature males,

which were then returned to the holding ponds. Three replicate subsamples of about 100 eggs per female were fertilised with 0.3 ml of pooled (i.e. pooled from all ripe males in a temperature treatment) milt from mature fish for each temperature treatment; the only exception occurred on the last day of spawning where milt from males exposed to ambient temperature was used to fertilise eggs from females exposed to heated temperature, since no males exposed to heated temperature had survived. Chilled water was used for incubation to ensure the best potential for egg survival, since anticipated ambient temperatures were expected to cause increased egg mortality (Velsen, 1987). Egg mortality was monitored to just prior to hatch to assess gamete viability.

Adult mortality, maturation rate, and gamete viability data were analysed by ANOVA and Tukey's or Dunn's multiple comparison testing, using Sigmastat software, to assess the impact of adult exposure to the three temperature regimes.

3.0 Results

3.1 Water temperature measurements

The data used for Fig. 1 are tabulated in Table 1.To allow for comparison of the recorded experimental temperature regimes with historical records the following figure (Fig. 6) was produced from compiled temperature records (Table 1) for the Puntledge River lower hatchery from 1980 to 2002. The records are continuous, except for 1997 and 1998.

Table 1. Puntledge River lower site temperature averaged from 1980 to 2002, except for 1997 and 1998, for the period Aug. 1 to Oct. 31. The Mean, Min, and Max values are averaged over the 22 years, while the All Time Min and the All Time Max values are the all time daily extreme temperatures for the 22-year period. Temperatures >= 23.0 °C are shown in bold.

Day of Year	Mean	Min	Max	stdev	N	All Time Min	All Time Max
01-Aug	19.28	16.30	21.50	1.4440	21	15.11	22.60
02-Aug	19.25	16.90	21.25	1.3274	21	15.92	22.10
03-Aug	19.45	17.09	21.60	1.3515	21	16.07	22.60
04-Aug	19.50	17.09	22.00	1.4354	21	16.66	22.80
05-Aug	19.41	16.65	22.50	1.6571	21	15.70	**23.00**
06-Aug	19.34	16.81	21.65	1.3733	21	16.03	**23.90**
07-Aug	19.46	17.47	21.90	1.2965	21	16.03	22.80
08-Aug	19.60	17.12	22.10	1.2705	21	16.54	**23.00**
09-Aug	19.68	17.71	22.80	1.4294	21	17.18	**23.80**
10-Aug	19.66	17.41	22.95	1.5968	21	16.23	**23.90**
11-Aug	19.73	17.04	22.80	1.5694	21	16.07	**23.00**
12-Aug	19.83	16.86	22.95	1.6545	21	16.23	**23.80**
13-Aug	19.83	17.04	22.50	1.5221	21	16.86	**23.00**
14-Aug	19.58	17.00	21.50	1.3944	21	16.50	22.10
15-Aug	19.38	16.51	21.75	1.4121	21	16.23	22.50
16-Aug	19.34	16.50	22.00	1.3726	20	16.39	**23.00**
17-Aug	19.48	16.91	22.05	1.3615	20	16.23	22.90
18-Aug	19.39	17.24	21.95	1.4276	20	16.54	22.90
19-Aug	19.26	16.90	22.30	1.4360	20	16.23	22.80
20-Aug	19.17	17.02	21.50	1.3895	21	16.50	22.00
21-Aug	19.17	17.16	21.75	1.3786	21	16.54	22.50
22-Aug	19.26	17.33	21.25	1.2784	21	16.54	22.00
23-Aug	19.00	17.30	21.00	1.1470	21	16.54	21.82
24-Aug	18.95	17.35	20.76	1.0899	21	16.70	22.16

Day of Year	Mean	Min	Max	stdev	N	All Time Min	All Time Max
25-Aug	18.92	17.20	20.56	1.0851	21	16.50	21.49
26-Aug	18.93	16.98	20.90	1.0700	21	16.50	21.80
27-Aug	18.82	17.13	21.00	1.1506	21	16.45	21.82
28-Aug	18.68	16.85	21.20	1.2327	21	15.81	22.31
29-Aug	18.55	17.05	20.80	1.0871	21	16.30	21.49
30-Aug	18.40	16.30	20.60	0.9836	21	15.60	21.20
31-Aug	18.22	16.44	20.70	0.9547	21	15.92	21.20
01-Sep	18.31	16.21	20.85	1.2391	21	15.66	21.20
02-Sep	18.34	16.07	21.00	1.3131	21	15.18	21.60
03-Sep	18.28	16.17	20.80	1.2057	21	15.34	21.50
04-Sep	18.07	16.00	20.55	1.1736	21	15.98	21.20
05-Sep	17.97	16.00	20.00	1.1586	21	15.55	20.50
06-Sep	17.97	16.00	20.20	1.2825	21	15.24	20.80
07-Sep	17.75	15.00	19.50	1.2488	21	14.92	20.20
08-Sep	17.70	15.25	19.60	1.2799	21	15.34	20.50
09-Sep	17.59	15.75	19.55	1.1436	21	15.34	20.60
10-Sep	17.55	15.50	19.80	1.1946	21	15.10	20.60
11-Sep	17.51	15.45	19.80	1.1522	21	14.95	20.60
12-Sep	17.54	15.45	19.50	1.0861	21	14.90	20.00
13-Sep	17.43	15.55	18.85	1.0408	21	14.90	20.00
14-Sep	17.28	15.05	19.25	1.1732	21	13.30	20.40
15-Sep	17.27	15.20	19.43	1.1649	21	13.40	20.20
16-Sep	17.14	15.45	19.00	1.0495	21	14.50	20.00
17-Sep	17.07	15.65	18.75	0.9953	21	15.20	19.00
18-Sep	16.96	15.50	18.50	0.9742	21	14.80	19.40
19-Sep	16.97	15.40	18.85	0.9953	21	14.80	19.50
20-Sep	16.72	15.63	18.65	0.9580	21	14.87	19.20
21-Sep	16.63	14.75	18.50	1.0564	21	13.50	19.10
22-Sep	16.53	15.02	18.50	1.0496	21	13.93	19.00
23-Sep	16.54	15.00	18.75	1.1842	21	14.08	19.50
24-Sep	16.37	14.50	18.55	1.2536	21	14.00	19.10
25-Sep	16.24	14.40	18.50	1.2600	21	14.00	19.00
26-Sep	16.09	14.40	18.50	1.1407	21	13.80	19.00
27-Sep	15.89	13.85	18.45	1.2316	21	12.80	18.90
28-Sep	15.83	13.50	18.35	1.2761	21	12.50	18.90
29-Sep	15.70	13.75	18.15	1.2432	21	12.80	18.60
30-Sep	15.56	13.80	17.95	1.1029	21	12.80	18.30
01-Oct	15.37	13.50	17.80	1.1608	21	12.80	18.30
02-Oct	15.13	13.40	17.45	1.0913	21	12.80	18.10
03-Oct	15.02	13.50	17.05	1.0311	21	13.00	17.80
04-Oct	14.89	13.40	16.75	0.9516	21	12.80	17.30
05-Oct	14.76	11.75	16.85	1.1430	21	10.00	17.30
06-Oct	14.71	12.50	16.65	0.9902	21	12.00	17.20
07-Oct	14.52	11.50	16.20	1.1283	21	10.00	16.70
08-Oct	14.40	12.50	16.25	1.0530	21	12.00	16.60
09-Oct	14.34	12.75	16.50	1.0787	21	12.00	17.00
10-Oct	14.12	12.50	16.25	1.1067	21	11.50	16.50
11-Oct	13.87	12.27	16.00	1.1486	21	11.50	16.20
12-Oct	13.78	11.88	16.10	1.1754	21	11.52	16.40
13-Oct	13.50	11.50	15.70	1.1844	21	11.05	16.20
14-Oct	13.37	11.51	15.80	1.1427	21	10.90	16.40

Day of Year	Mean	Min	Max	stdev	N	All Time Min	All Time Max
15-Oct	13.08	11.04	15.60	1.1768	21	10.28	16.00
16-Oct	13.00	11.30	15.60	1.1613	21	10.75	16.00
17-Oct	12.78	10.50	14.80	1.1395	21	10.00	15.20
18-Oct	12.62	10.30	14.90	1.0920	21	9.80	15.20
19-Oct	12.54	10.15	14.50	1.0008	21	9.50	14.80
20-Oct	12.44	10.40	14.40	0.9990	21	10.00	14.80
21-Oct	12.46	10.40	14.30	0.9415	21	10.00	14.80
22-Oct	12.23	10.40	14.20	0.9688	21	10.00	14.60
23-Oct	12.13	10.50	13.75	0.8163	21	10.50	14.10
24-Oct	12.05	10.50	13.55	0.8254	21	10.11	13.90
25-Oct	11.98	10.13	13.85	0.9523	21	9.75	14.30
26-Oct	11.71	9.96	13.90	1.0107	21	9.34	14.20
27-Oct	11.49	9.38	14.45	1.1003	21	9.19	15.30
28-Oct	11.15	7.73	12.85	1.1021	21	6.69	13.10
29-Oct	10.96	8.12	13.00	1.0091	21	7.63	13.10
30-Oct	10.83	8.46	12.85	1.0675	21	7.79	13.00
31-Oct	10.63	8.00	12.50	1.0984	21	7.90	12.80

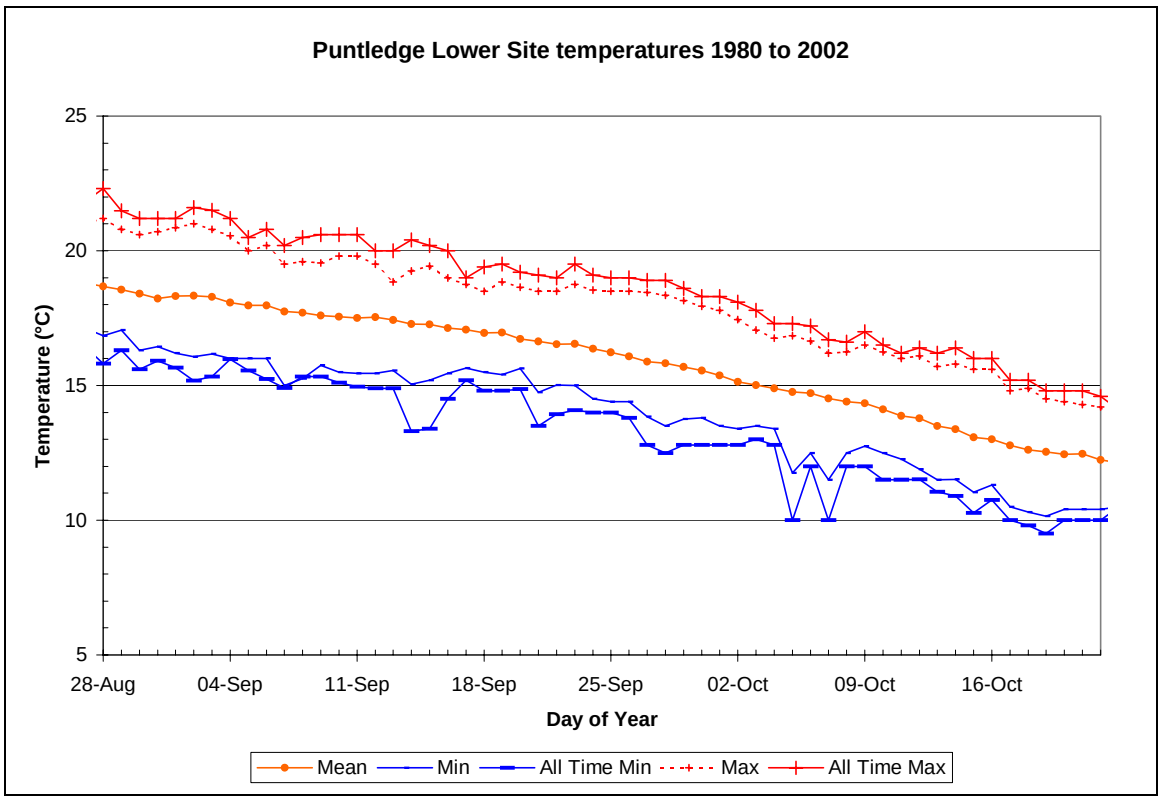

Figure 6. Puntledge River lower site temperature averaged from 1980 to 2002, except for 1997 and 1998, for the period Aug. 28 to Oct. 22. The Mean, Min, and Max values are averaged over the 22 years, while the All Time Min and the All Time Max values are the all time daily extreme temperatures for the 22-year period.

Temperature records were averaged for the 2 ponds for each temperature regime and are presented in Figure 7. The heated treatment was maintained for 21 days; after which heating was terminated mid-day on September 17th due to other water demands in the hatchery.

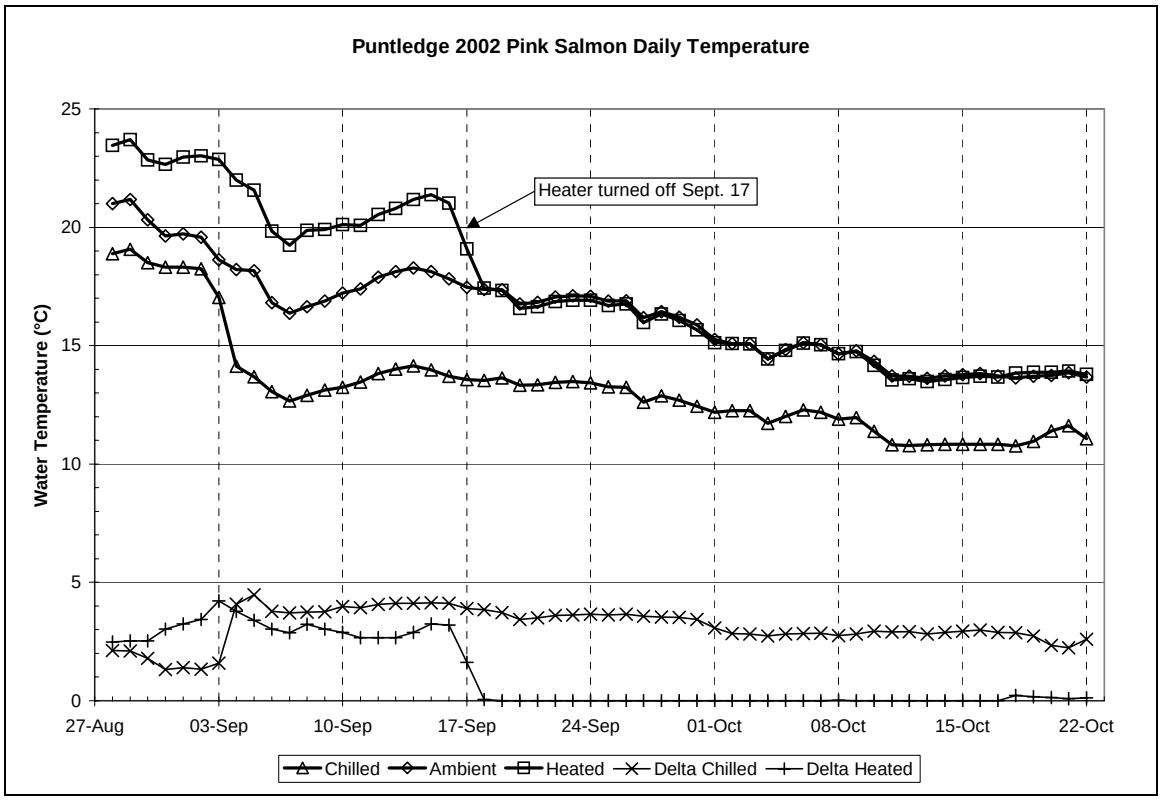

Figure 7. Adult holding temperatures from Aug. 28 to Oct. 22, 2002 for Chilled (Ponds 1&3), Ambient (Ponds 2&4), and Heated (Ponds 5&6) temperature regimes. The difference between Chilled and Ambient (Delta Chilled) and between Heated and Ambient (Delta Heated) is also shown.

Since all temperature regimes were based on the ambient temperature, a normal temperature decline, with variations due to daily weather effects, was observed. Although it was not intended, the 3 temperature treatments of chilled, ambient, and heated were very similar to the all time minimum, mean, and all time maximum, respectively, shown in Fig. 6. A comparison of the 3 temperature treatments with historical daily means and temperature extremes (i.e. all time min and max) for the 21-day period from Aug. 28 to Sept. 17 are shown in Figs. 8-10.

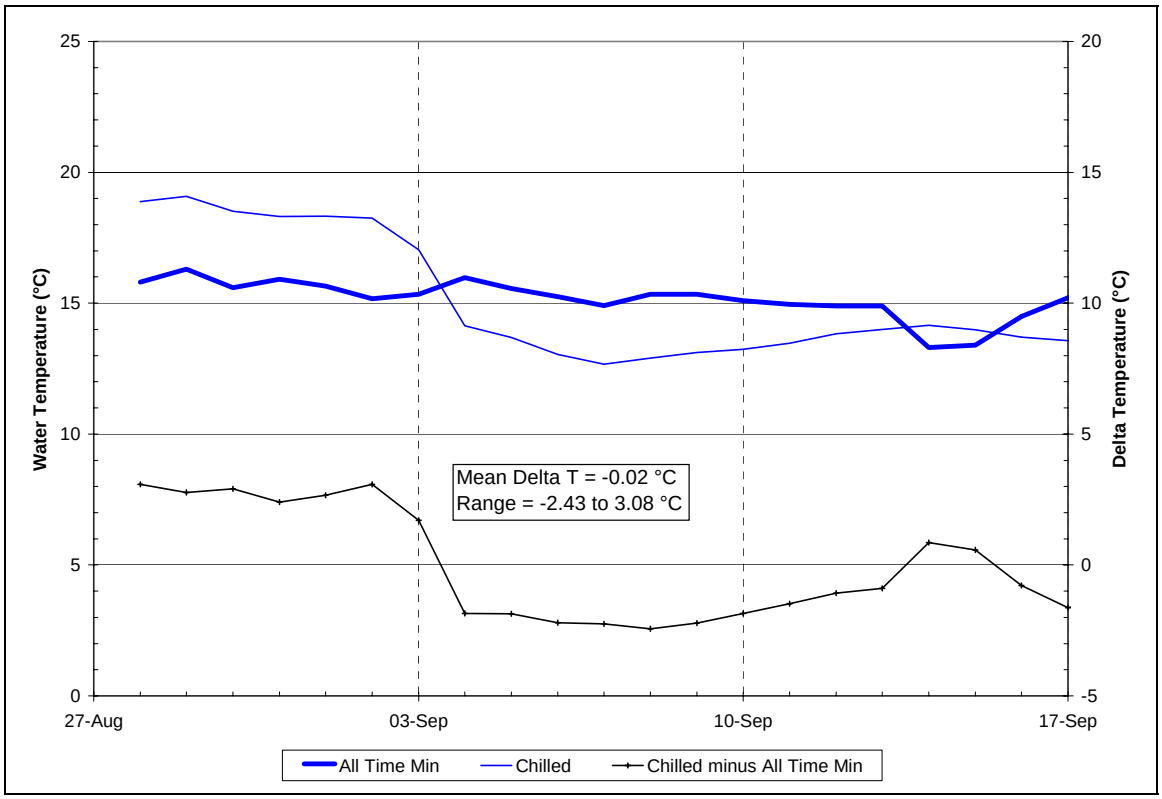

Figure 8. Chilled treatment and All Time Minimum temperature (1980 – 2002) from Aug. 28 to Sept. 17, along with Delta T (Chilled minus All Time Min).

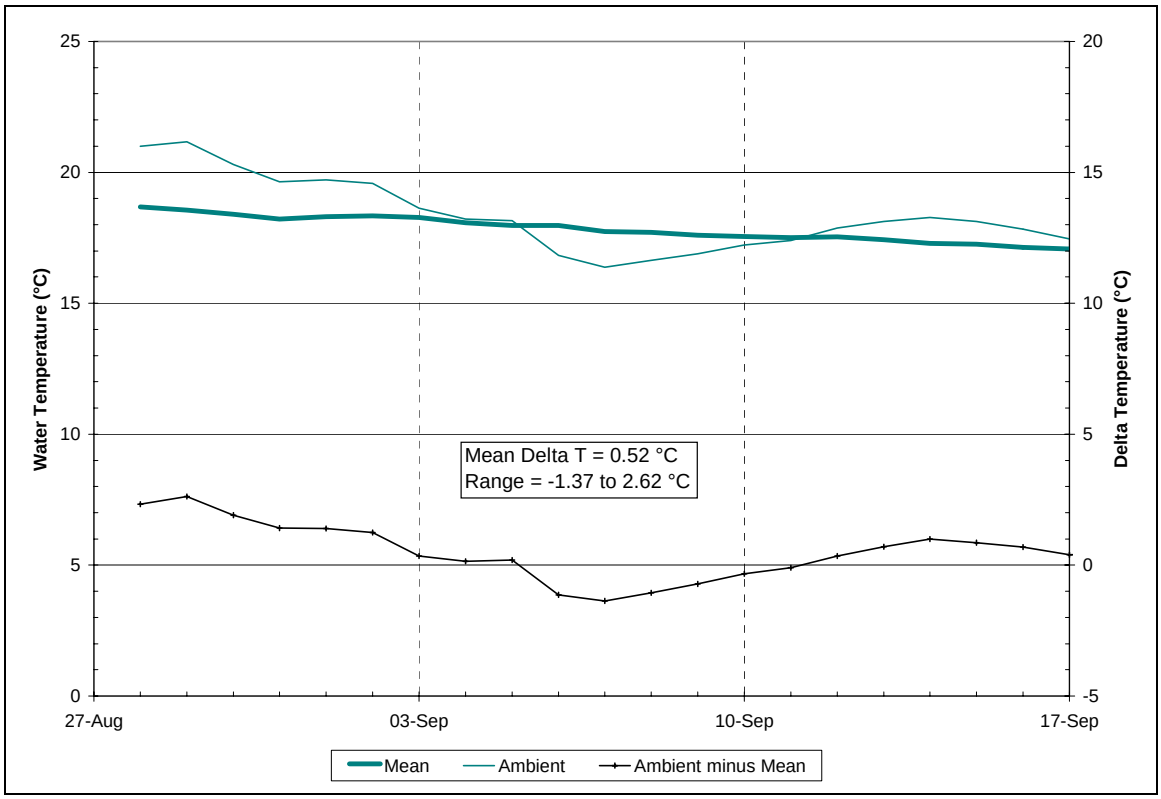

Figure 9. Ambient treatment and Mean temperature (1980 – 2002) from Aug. 28 to Sept. 17 along with Delta T (Ambient minus Mean).

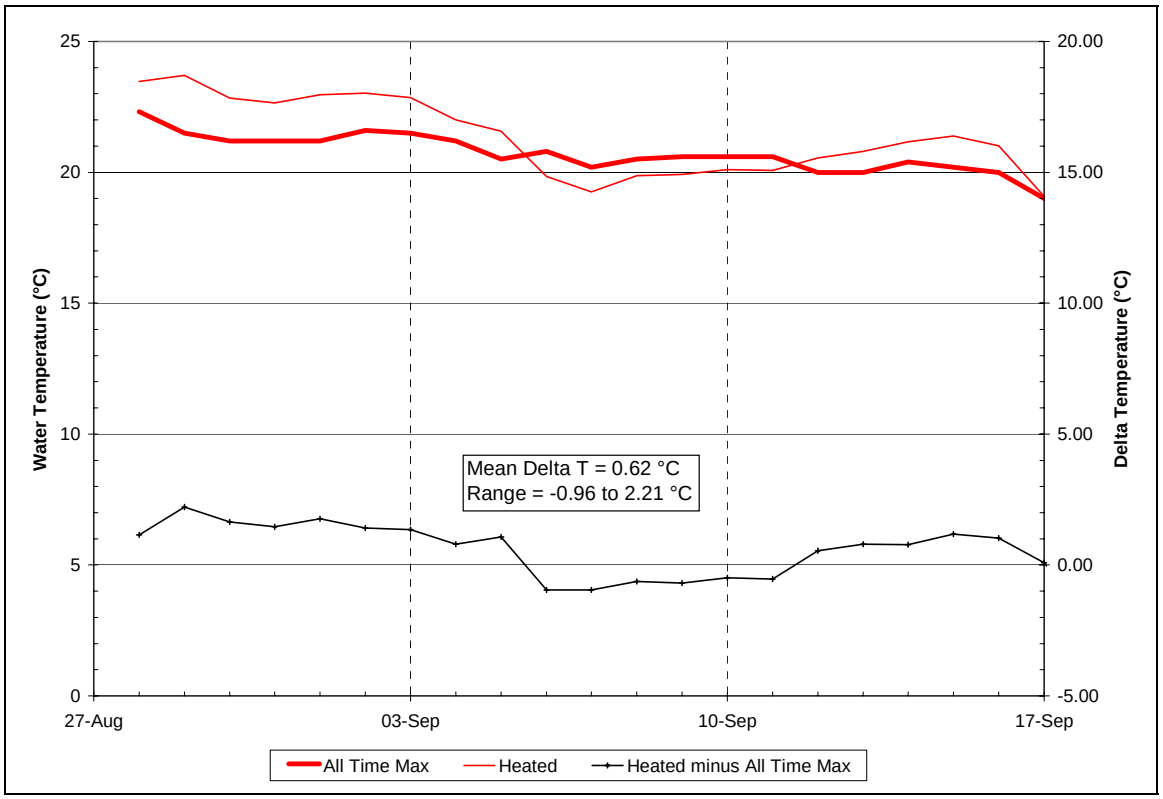

Figure 10. Heated treatment and All Time Maximum temperature (1980 – 2002) from Aug. 28 to Sept. 17, along with Delta T (Heated minus All Time Max).

The mean Delta Ts (i.e. the difference between each treatment temperature and the 21-yr average daily means and extremes) for chilled, ambient, and heated treatments were -.-0.02, 0.52, and 0.62 °C, respectively.

To further describe the declining temperatures, linear regression was used to model the daily mean temperatures for the 21-day exposure period from Aug. 28 to Sept. 17 (Fig. 11 and Table 1).

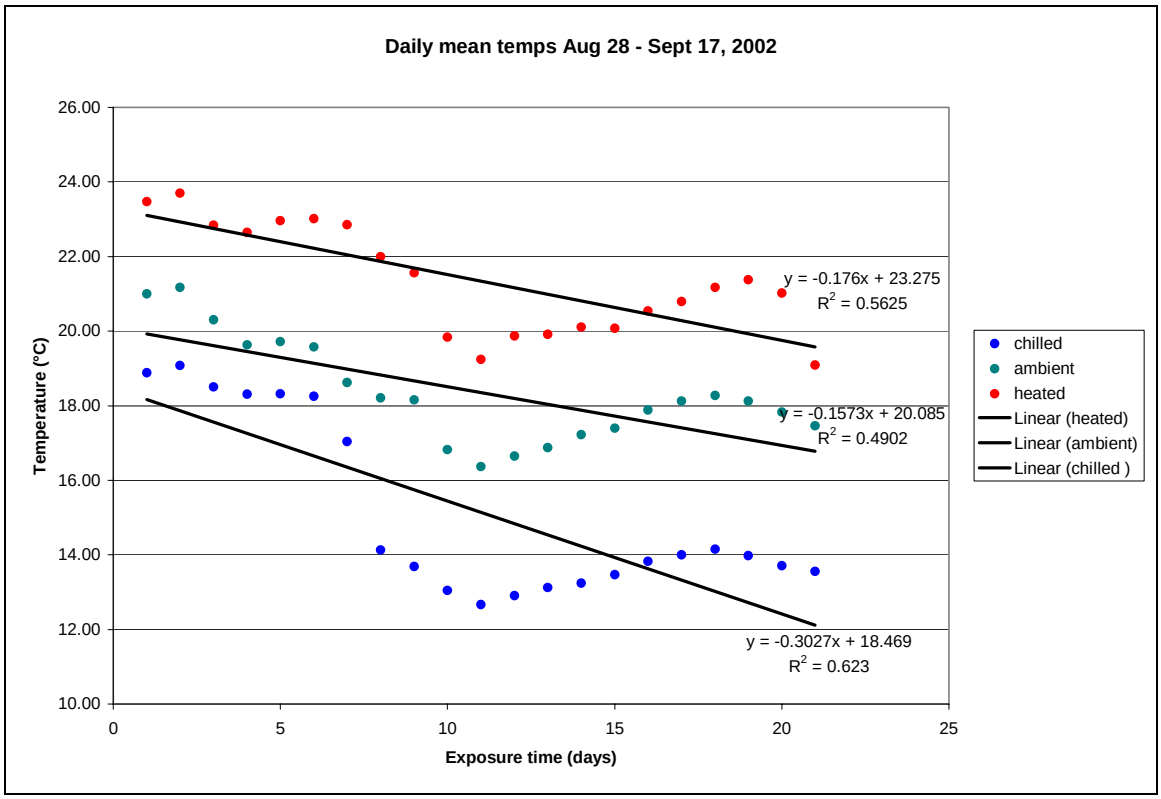

Figure 11. Mean daily temperatures for Chilled, Ambient, and Heated treatments from Aug. 28 to Sept. 17, 2002, with least squares regression equations**.**

Table 2. Linear regression equations, total temperature decline (based on linear equation), and mean (range) temperatures for the 21-day period from Aug.28 to Sept. 17, 2002.

Treatment	Linear Regression (R^2) Y=temperature (°C) X=time (days from Aug. 28)	Total temperature (ºC) decline over 21 days	Mean temperature (ºC) (range)
Chilled	Y = -0.3027X +18.469 (0.6230)	6.4	15.1 (11.6 – 19.4)
Ambient	Y = -0.1573X +20.085 (0.4902)	3.3	18.4 (15.0 – 21.8)
Heated	Y = -0.1760X +23.275 (0.5825)	3.7	21.3 (17.6 – 24.0)

3.2 Water flow and dissolve oxygen measurements.

The water flow rate measurements for the 6 test ponds are tabulated in Table 3.

3.2.1 Pond flow measurements

Table 3. Pond Flow Aug 22/02 to Oct 10/02.

	Litres per minute						Pond 1&3	Pond 2&4	Pond 5&6
	Pond 1	Pond 2	Pond 3	Pond 4	Pond 5	Pond 6	**Avg. chilled**	**Avg. ambient**	**Avg. heated**
22-Aug				43.9				**43.9**	
27-Aug	42.8		46.9		44.6	43.9	**44.9**		**44.3**
3-Sept	42.7	41.8	45.5	48.0	43.1	40.9	**44.1**	**44.9**	**42.0**
6-Sept	43.1		46.4	41.3	45.2	36.9	**44.8**	**41.3**	**41.0**
6-Sept						38.0			**38.0**
9-Sept	41.2	27.6	39.9	28.0			**40.5**	**27.8**	
9-Sept		44.7		44.4				**44.5**	
10-Sept	41.1	42.7	39.8	40.3	41.7	37.8	**40.5**	**41.5**	**39.8**
12-Sept	44.7	41.1	41.2	43.2	44.5	41.0	**43.0**	**42.1**	**42.7**
13-Sept	43.1	37.9	39.3	39.5	45.1	38.2	**41.2**	**38.7**	**41.6**
13-Sept		40.9		42.0				**41.5**	
16-Sept	40.2	45.9	43.6	46.6	45.6	37.6	**41.9**	**46.3**	**41.6**
16-Sept						39.8			**39.8**
17-Sept	42.9	45.9	44.6	47.2	46.0	44.0	**43.7**	**46.6**	**45.0**
19-Sept	43.3	42.0	44.4	41.4	46.3	43.2	**43.8**	**41.7**	**44.8**
21-Sept	40.9	38.8	42.7	40.1	45.9	44.3	**41.8**	**39.4**	**45.1**
23-Sept	41.2	35.4	40.8	38.2	45.7	42.5	**41.0**	**36.8**	**44.1**
23-Sept		41.8		40.8				**41.3**	
26-Sept	38.0	41.6	39.8	35.9	44.4	41.4	**38.9**	**38.8**	**42.9**
26-Sept	42.7		43.6	41.3			**43.1**	**41.3**	
30-Sept	42.7	55.2	45.6	47.1	44.7	40.8	**44.2**	**51.1**	**42.7**
30-Sept		44.2						**44.2**	
2-Oct	43.1	43.5	43.3	44.5	47.1	45.1	**43.2**	**44.0**	**46.1**
10-Oct	44.8	41.5	44.1	43.6	44.1	47.2	**44.4**	**42.6**	**45.6**
N=	17	18	17	20	15	17	**17**	**21**	**17**
Avg=	42.3	41.8	43.0	41.9	44.9	41.3	**42.6**	**41.9**	**42.8**

Figures 12, 13, and 14 (below) show measured flows for chilled (Pond 1 and 3), ambient (Pond 2 and 4) and heated treatments (Pond 5 and 6). Variability in Pond 2 and 4 flows was due to renovations being made to the aeration tower.

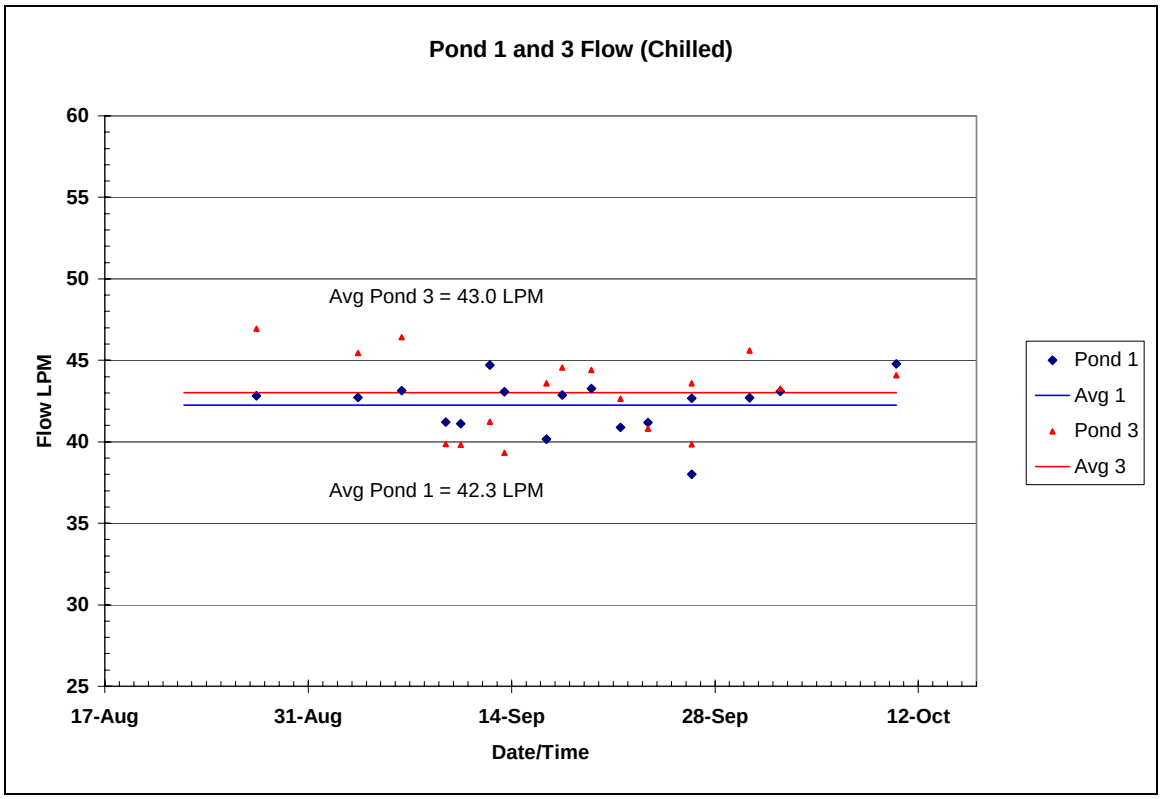

Figure 12. Flow measurements in chilled (ponds 1 & 3) treatment. The average flow for each pond is shown as well.

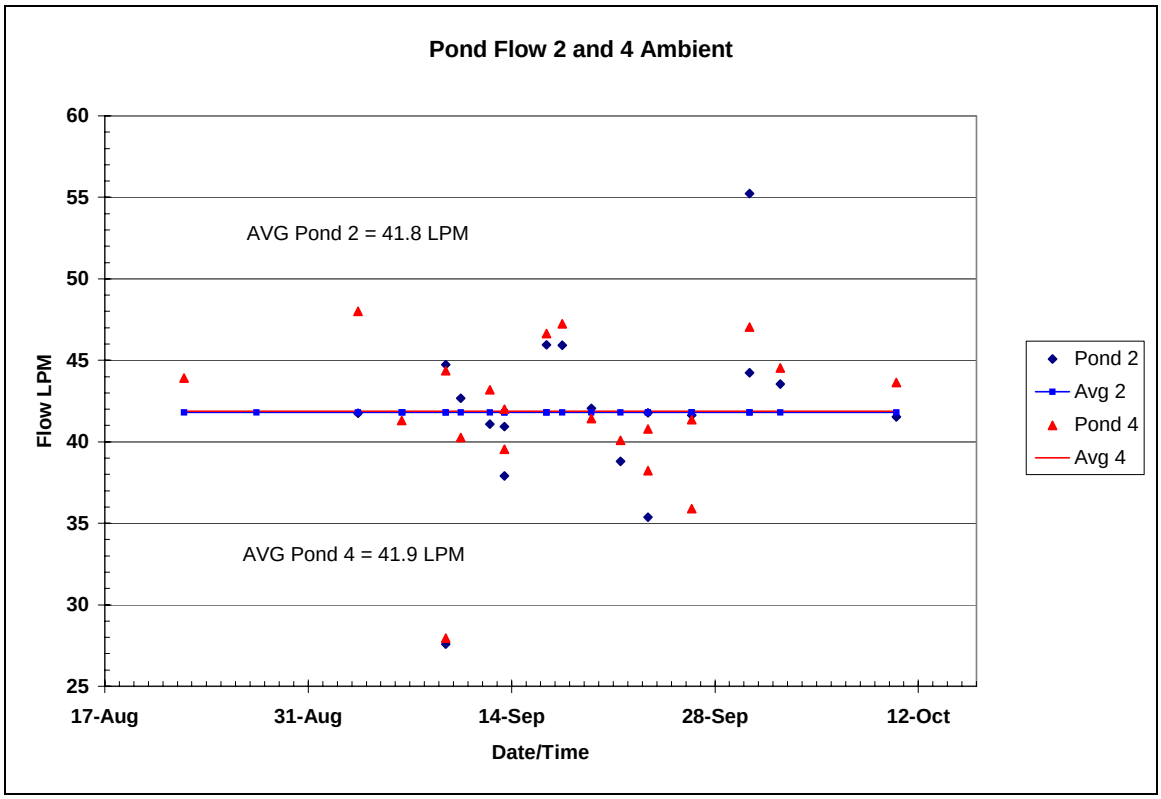

Figure 13. Flow measurements in ambient (ponds 2 & 4) treatment.

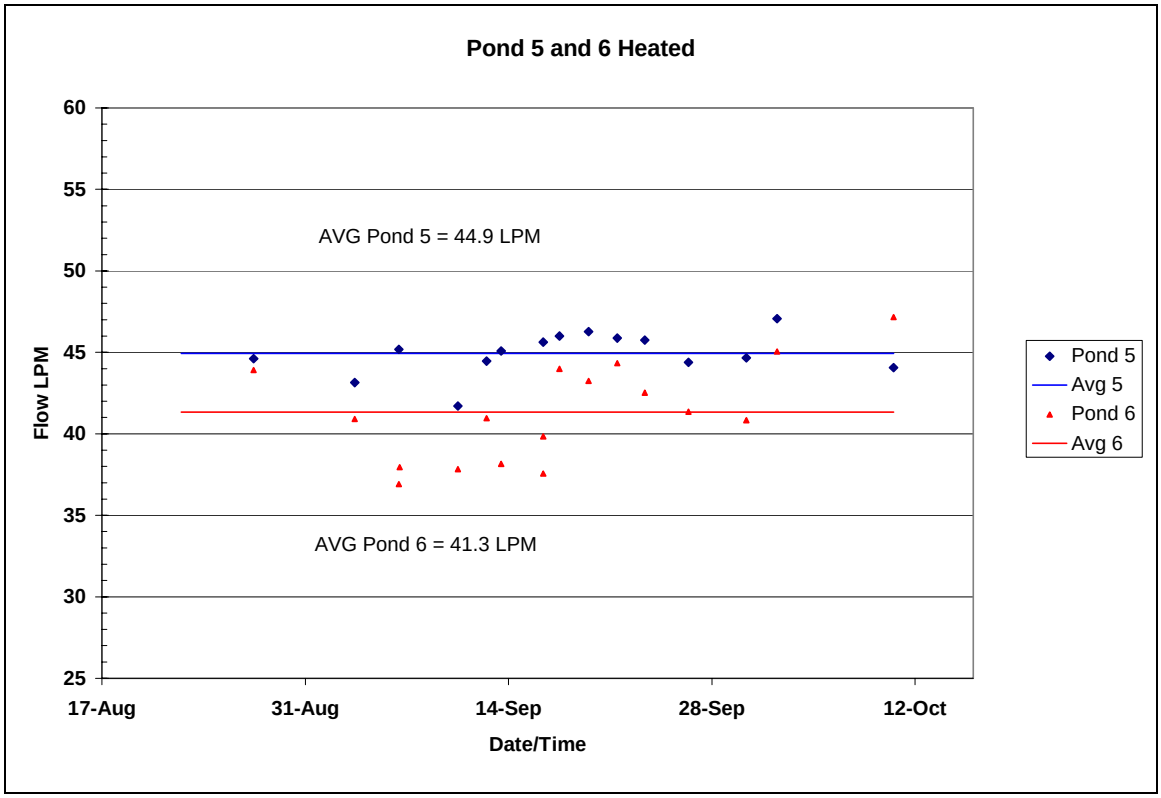

Figure 14. Flow measurements in heated (ponds 5 & 6) treatment.

3.2.2 Dissolved Oxygen measurements

DO measurements recorded from Aug. 30 to Oct. 10 are tabulated in Table 4.

Table 4. Dissolved oxygen (%) at pond outflow.

Date	Pond 1	Pond 3	Avg. Chilled	Pond 2	Pond 4	Avg. Ambient	Pond 5	Pond 6	Avg. Heated
30-Aug	90	91	85.6	92	94	86.9	94	93	96.7
30-Aug	102	87	85.6	89	79	86.9	84	100	96.7
30-Aug	91		85.6			86.9			96.7
3-Sept	98	79	85.6	86	81	86.9	94	90	96.7
3-Sept	85	88	85.6	88	85	86.9	91	96	96.7
5-Sept	81	82	85.6	82	84	86.9	96	97	96.7
6-Sept	85	83	85.6	88	87	86.9	97	96	96.7
9-Sept	82	80	85.6	80	78	86.9	94	93	96.7
10-Sept	86	85	85.6	90	89	86.9	99	98	96.7
12-Sept	86	83	85.6	88	85	86.9	97	97	96.7
13-Sept	81	78	85.6	84	83	86.9	97	95	96.7
16-Sept	83	81	85.6	88	87	86.9	99	98	96.7
17-Sept	85	83	85.6	90	89	86.9	96	95	96.7
19-Sept	88	85	85.6	89	88	86.9	100	98	96.7
21-Sept	90	85	85.6	90	88	86.9	100	100	96.7
23-Sept	85	83	85.6	85	85	86.9	99	98	96.7
26-Sept	85	81	85.6	87	85	86.9	100	99	96.7
30-Sept	84	82	85.6	87	89	86.9	99	97	96.7
2-Oct	85	82	85.6	87	84	86.9	103	101	96.7
10-Oct	96	91	85.6	95	97	86.9	97	96	96.7
Avg=	87.4	83.6	85.6	87.6	86.2	86.9	96.6	96.7	96.7
n=	20	19	20	19	19	20	19	19	20

DO at the outflows of chilled (pond 1 and 3), ambient (pond 2 and 4) and heated treatments (pond 5 and 6) is shown in the figures (Figs. 15 to 17) below. Individual measurements around the average values are also shown. Heated water was passed through aeration towers before entering the ponds. On average, the TGP dropped from 108 % to 101 %.

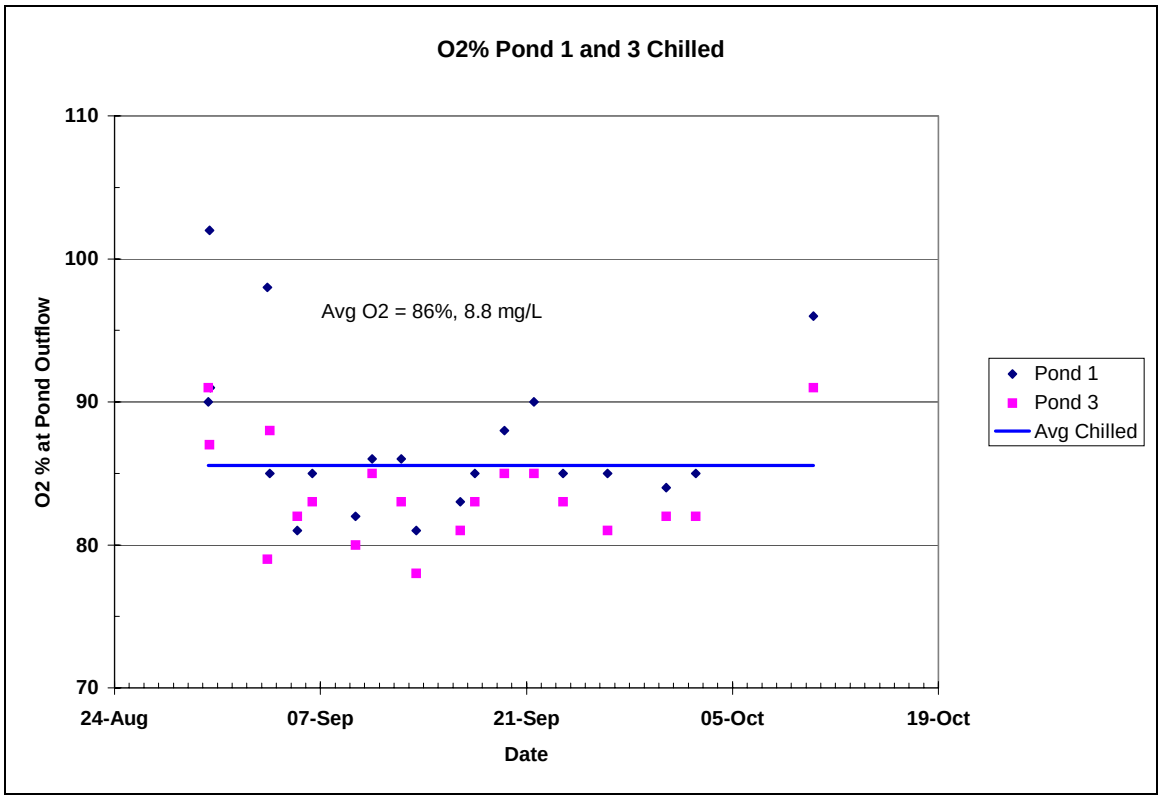

Figure 15. DO (%) measurements in chilled (ponds 1 & 3) treatment.

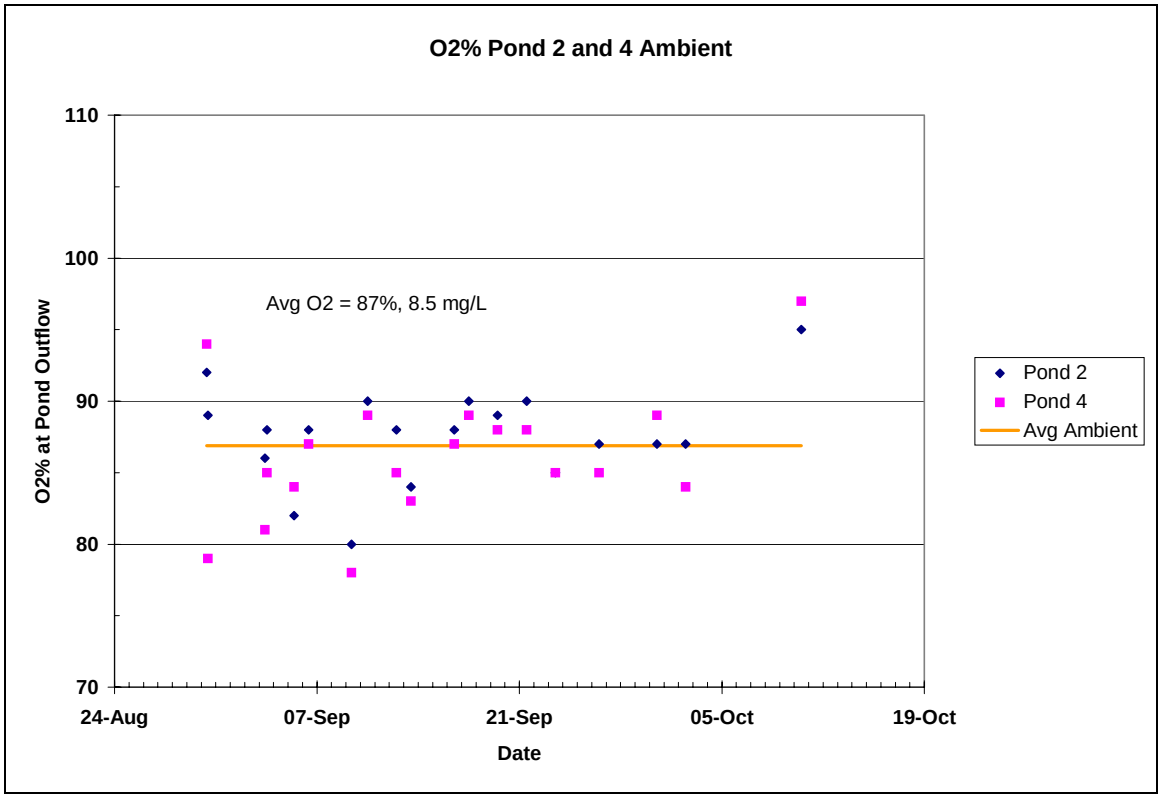

Figure 16. DO (%) measurements in ambient (ponds 2 & 4) treatment.

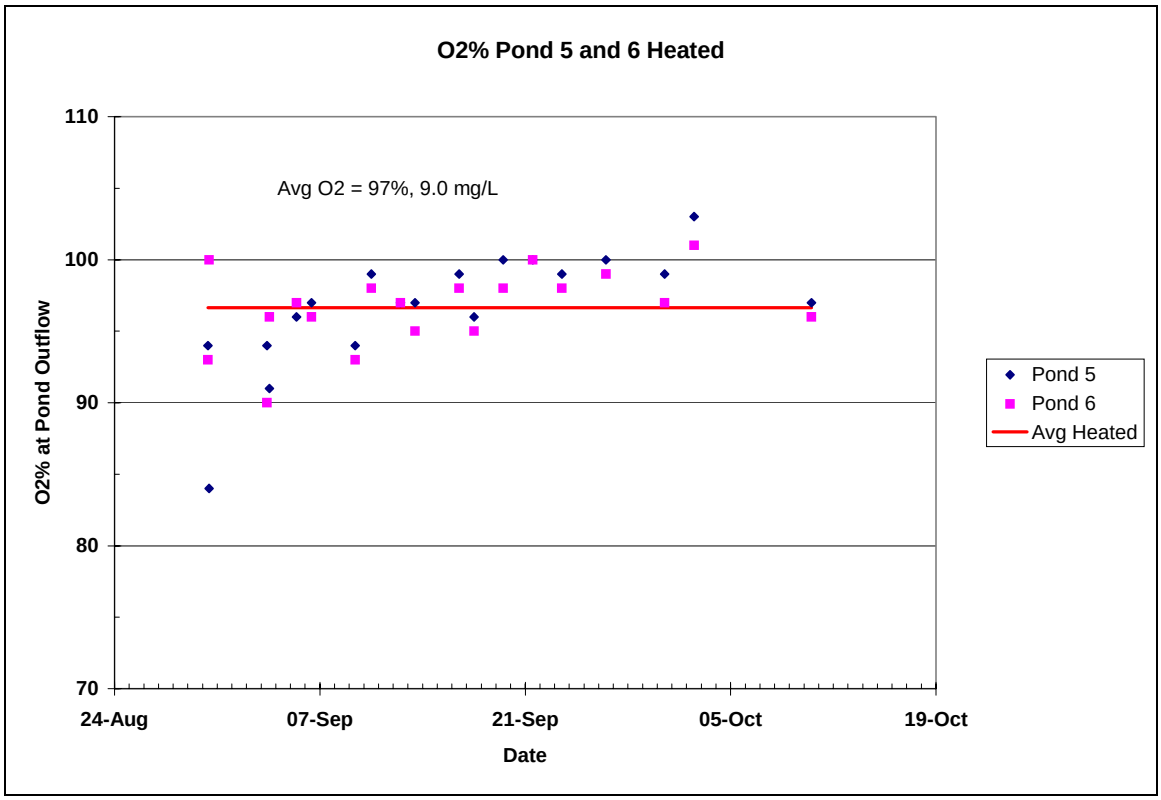

Figure 17. DO (%) measurements in heated (ponds 5 & 6) treatment.

3.3 Post orbital hypural length (POHL, cm) and weight (gm) of female and male pink salmon

Lengths and weights of a representative sample of adult pink salmon are listed in Table 5 and summarised in Table 6.

Table 5 . Post orbital hypural length (POHL, cm) and weight (gm) of adult pink salmon.

Date	Pond	Sex	Condition	Length (cm)	Weight (gm)
01/10/02	1	Female	Spawn	35.1	800
01/10/02	1	Female	Spawn	36.7	1950
01/10/02	1	Female	Spawn	36.4	1000
01/10/02	1	Female	Spawn	36.5	900
01/10/02	1	Female	Spawn	36.7	900
01/10/02	1	Female	Spawn	35	900
01/10/02	3	Female	Spawn	37	1000
01/10/02	3	Female	Spawn	34.5	850
01/10/02	3	Female	Spawn	36.1	900
01/10/02	3	Female	Spawn	35.1	800
01/10/02	3	Female	Spawn	36.3	1000

Date	Pond	Sex	Condition	Length (cm)	Weight (gm)
01/10/02	3	Female	Spawn	38.9	1150
01/10/02	4	Female	Spawn	36	1050
01/10/02	4	Female	Spawn	40.1	1200
08/10/02	1	Female	Spawn	38.5	1150
08/10/02	1	Female	Spawn	36.2	1000
08/10/02	1	Female	Spawn	35.5	950
08/10/02	2	Female	Spawn	36.2	500
08/10/02	3	Female	Spawn	36.9	1200
08/10/02	3	Female	Spawn	36.5	1050
08/10/02	3	Female	Spawn	35.7	800
08/10/02	3	Female	Spawn	35.7	1000
08/10/02	4	Female	Mort	35.3	800
08/10/02	4	Female	Spawn	34.8	850
08/10/02	4	Female	Spawn	34.5	500
08/10/02	4	Female	Spawn	37.2	600
08/10/02	4	Male	Mort	36.2	1050
08/10/02	4	Male	Mort	37.9	1150
15/10/02	1	Female	Mort	36.9	850
15/10/02	1	Male	Mort	38.6	950
15/10/02	2	Female	Spawn	35.9	900
15/10/02	2	Female	Spawn	36.5	900
15/10/02	2	Female	Spawn	37.4	1000
15/10/02	2	Female	Spawn	35.9	950
15/10/02	2	Female	Spawn	37.5	950
15/10/02	2	Female	Spawn	38.9	1200
15/10/02	2	Female	Spawn	35.4	900
15/10/02	2	Male	Mort	41.6	1600
15/10/02	2	Male	Mort	33.2	750
15/10/02	3	Female	Spawn	38.2	1150
15/10/02	3	Male	Mort	37.2	950
15/10/02	3	Male	Mort	37	1000
15/10/02	3	Male	Mort	36.1	950
15/10/02	3	Male	Mort	32.2	650
15/10/02	3	Male	Mort	32.9	600
15/10/02	4	Female	Mort	34.9	800
15/10/02	4	Female	Mort	34.6	850
15/10/02	4	Male	Mort	35.4	950
15/10/02	6	Female	Mort	35	800
15/10/02	6	Female	Spawn	37.2	1150
22/10/02	1	Male	Mort	33.6	1050
22/10/02	1	Male	Mort	35.5	1000
22/10/02	2	Female	Mort	36.4	1100
22/10/02	2	Male	Mort	33.9	1000
22/10/02	2	Male	Mort	35.6	1050
22/10/02	4	Female	Spawn	36.2	900
22/10/02	4	Female	Spawn	35.8	850
22/10/02	4	Female	Spawn	34.2	750
22/10/02	4	Female	Spawn	37.2	1200

Date	Pond	Sex	Condition	Length (cm)	Weight (gm)
22/10/02	4	Male	Mort	34.9	800
22/10/02	4	Male	Spawn	31.8	600
22/10/02	5	Female	Spawn	36.4	950
22/10/02	5	Female	Spawn	34.4	900
22/10/02	5	Male	Mort	35.6	900
22/10/02	6	Female	Spawn	34.7	850
22/10/02	6	Female	Spawn	37.1	1000

Table 6. Mean length (POHL, cm) and weight (gm) of adult pink salmon.

Sex	Mean Length (cm)	Mean Weight (gm)	N
Female	36.3	952.1	48
Male	35.5	944.4	18

The initial loading density in the 6 ponds is tabulated in Table 7, along with summarised values of flows and Dos.

Table 7. Water flow rates (LPM), dissolved oxygen (%), initial number of fish per pond, mean biomass per pond (kg), and load rates (Kg/LPM and Kg/m^3) for the 6 ponds. Note, numbers in brackets indicate number of measurements.

	Chilled Treatment			Ambient Treatment			Heated Treatment		
	Pond 1	Pond 3	Mean	Pond 2	Pond 4	Mean	Pond 5	Pond 6	Mean
Flow (LPM)	42.3 (17)	43.0 (17)	42.6	41.8 (18)	41.9 (20)	41.9	44.9 (15)	41.3 (17)	42.8
DO (%)	87.4 (20)	83.6 (19)	85.6	87.6 (19)	86.2 (20)	86.9	96.6 (19)	96.7 (19)	96.7
No. of fish	23	28	26	24	28	26	24	21	23
Biomass (kg)	21.76	26.49	24.13	22.71	26.49	24.60	22.71	19.87	21.29
kg/LPM	0.51	0.62	0.57	0.54	0.63	0.59	0.51	0.48	0.49
kg/m 3	3.26	3.97	3.62	3.40	3.97	3.69	3.40	2.98	3.19

3.4 Adult mortality

3.4.1 Chilled Treatment

Adult mortality in the chilled treatment (ponds 1 and 3) is listed in Table 8 and illustrated in Figure 18.

Table 8. Adult pink salmon mortality in chilled (pond 1 & 3) treatment.

Date	Pond 1	Pond 1	**Pond 1**	Pond 3	Pond 3	**Pond 3**			
Initial numbers	Female	Male	**Total**	Female	Male	**Total**	total females	total males	
28-Aug-02	10	13	**23**	13	15	**28**	23	28	
DATE							CHILLED Female mortality (%)	CHILLED Male mortality (%)	CHILLED Total male and female mortality (%)
29-Aug-02	0	0	**0**	0	0	**0**	0.0%	0.0%	0.0%
30-Aug-02	0	0	**0**	0	0	**0**	0.0%	0.00%	0.0%
31-Aug-02	0	0	**0**	0	0	**0**	0.0%	0.00%	0.0%
01-Sept-02	0	0	**0**	0	0	**0**	0.0%	0.00%	0.0%
02-Sept-02	0	0	**0**	0	0	**0**	0.0%	0.00%	0.0%
03-Sept-02	0	0	**0**	0	0	**0**	0.0%	0.00%	0.0%
04-Sept-02	0	0	**0**	0	0	**0**	0.0%	0.00%	0.0%
05-Sept-02	0	0	**0**	0	0	**0**	0.0%	0.00%	0.0%
06-Sept-02	0	0	**0**	0	0	**0**	0.0%	0.00%	0.0%
07-Sept-02	0	0	**0**	0	0	**0**	0.0%	0.00%	0.0%
08-Sept-02	0	0	**0**	0	0	**0**	0.0%	0.00%	0.0%
09-Sept-02	0	0	**0**	0	0	**0**	0.0%	0.00%	0.0%
10-Sept-02	0	0	**0**	0	0	**0**	0.0%	0.00%	0.0%
11-Sept-02	0	0	**0**	0	0	**0**	0.0%	0.00%	0.0%
12-Sept-02	0	0	**0**	0	1	**1**	0.0%	3.57%	2.0%
13-Sept-02	0	0	**0**	0	0	**0**	0.0%	3.57%	2.0%
14-Sept-02	0	0	**0**	0	0	**0**	0.0%	3.57%	2.0%
15-Sept-02	0	0	**0**	0	0	**0**	0.0%	3.57%	2.0%
16-Sept-02	0	0	**0**	0	0	**0**	0.0%	3.57%	2.0%
17-Sept-02	0	0	**0**	0	0	**0**	0.0%	3.57%	2.0%
18-Sept-02	0	0	**0**	0	1	**1**	0.0%	7.14%	3.9%
19-Sept-02	0	0	**0**	0	0	**0**	0.0%	7.14%	3.9%
20-Sept-02	0	0	**0**	0	0	**0**	0.0%	7.14%	3.9%
21-Sept-02	0	0	**0**	0	0	**0**	0.0%	7.14%	3.9%
22-Sept-02	0	0	**0**	0	0	**0**	0.0%	7.14%	3.9%
23-Sept-02	0	0	**0**	0	0	**0**	0.0%	7.14%	3.9%
24-Sept-02	0	0	**0**	0	0	**0**	0.0%	7.14%	3.9%
25-Sept-02	0	0	**0**	0	0	**0**	0.0%	7.14%	3.9%
26-Sept-02	0	0	**0**	0	0	**0**	0.0%	7.14%	3.9%
27-Sept-02	0	0	**0**	0	0	**0**	0.0%	7.14%	3.9%
28-Sept-02	0	0	**0**	0	0	**0**	0.0%	7.14%	3.9%
29-Sept-02	0	0	**0**	0	0	**0**	0.0%	7.14%	3.9%
30-Sept-02	0	0	**0**	1	0	**1**	4.3%	7.14%	5.9%
01-Oct-02	0	0	**0**	0	0	**0**	4.3%	7.14%	5.9%

Date	Pond 1	Pond 1	**Pond 1**	Pond 3	Pond 3	**Pond 3**			
Initial numbers	Female	Male	**Total**	Female	Male	**Total**	total females	total males	
28-Aug-02	10	13	**23**	13	15	**28**	23	28	
DATE							CHILLED Female mortality (%)	CHILLED Male mortality (%)	CHILLED Total male and female mortality (%)
02-Oct-02			**0**			**0**	4.3%	7.14%	5.9%
03-Oct-02			**0**			**0**	4.3%	7.14%	5.9%
04-Oct-02			**0**			**0**	4.3%	7.14%	5.9%
05-Oct-02			**0**			**0**	4.3%	7.14%	5.9%
06-Oct-02			**0**			**0**	4.3%	7.14%	5.9%
07-Oct-02			**0**			**0**	4.3%	7.14%	5.9%
08-Oct-02			**0**			**0**	4.3%	7.14%	5.9%
09-Oct-02			**0**			**0**	4.3%	7.14%	5.9%
10-Oct-02			**0**			**0**	4.3%	7.14%	5.9%
11-Oct-02			**0**			**0**	4.3%	7.14%	5.9%
12-Oct-02			**0**			**0**	4.3%	7.14%	5.9%
13-Oct-02			**0**			**0**	4.3%	7.14%	5.9%
14-Oct-02			**0**			**0**	4.3%	7.14%	5.9%
15-Oct-02	1	1	**2**	0	5	**5**	8.7%	28.57%	19.6%
16-Oct-02			**0**			**0**	8.7%	28.57%	19.6%
17-Oct-02			**0**			**0**	8.7%	28.57%	19.6%
18-Oct-02			**0**			**0**	8.7%	28.57%	19.6%
19-Oct-02			**0**			**0**	8.7%	28.57%	19.6%
20-Oct-02			**0**			**0**	8.7%	28.57%	19.6%
21-Oct-02			**0**			**0**	8.7%	28.57%	19.6%
22-Oct-02		2	**2**			**0**	8.7%	35.71%	23.5%
final % mortality	**10.0**	**23.1**	**17.4**	**7.7**	**46.7**	**28.6**	**8.7%**	**35.71%**	**23.5%**

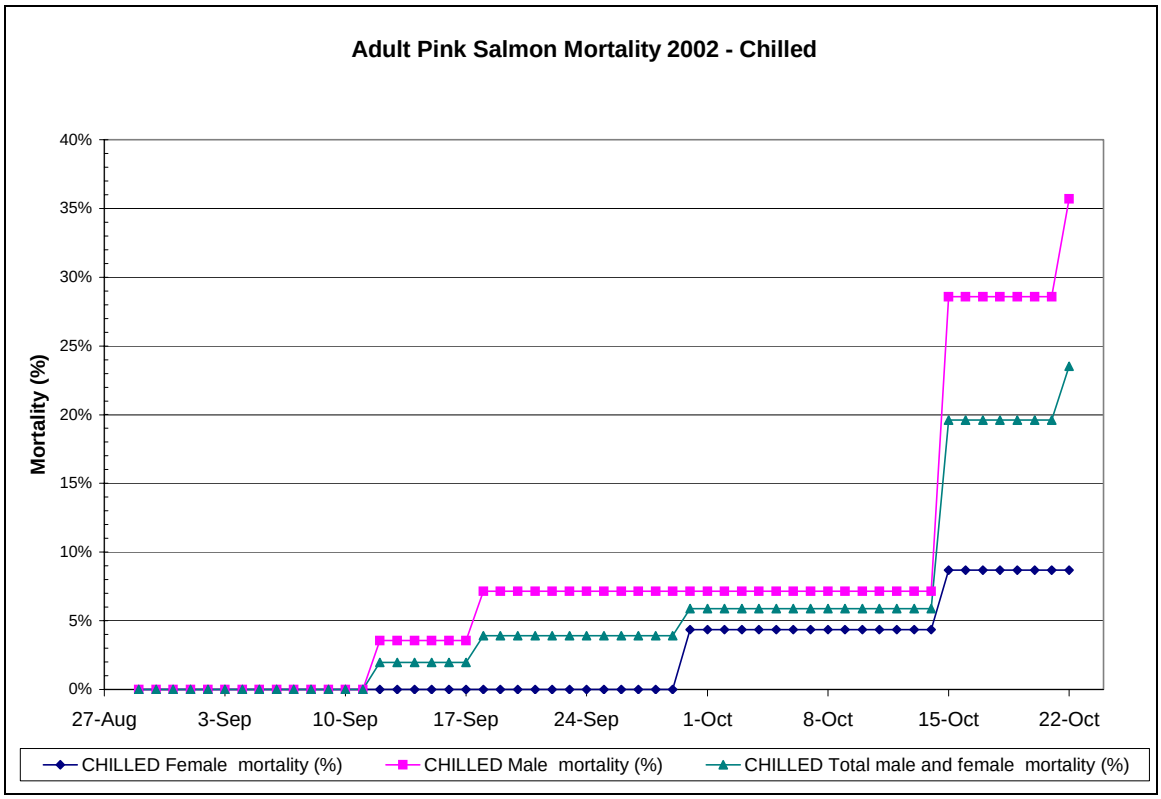

Figure 18. Adult mortality in the chilled treatment.

3.4.2 Ambient Treatment

Adult mortality in the ambient treatment (ponds 2 and 4) is listed in Table 9 and illustrated in Figure 19.

Table 9. Adult pink salmon mortality in ambient (ponds 2 & 4) treatment.

Date	Pond 2	Pond 2	**Pond 2**	Pond 4	Pond 4	**Pond 4**			
initial numbers	Female	Male	**Total**	Female	Male	**Total**	total females	total males	
28-Aug-02	11	13	**24**	14	14	**28**	25	27	
DATE							AMBIENT Female mortality (%)	AMBIENT Male mortality (%)	AMBIENT Total male and female mortality (%)
29-Aug-02	2	1	**3**	1	1	**2**	12.0%	7%	9.6%
30-Aug-02	0	0	**0**	0	0	**0**	12.0%	7.41%	9.6%
31-Aug-02	0	0	**0**	0	0	**0**	12.0%	7.41%	9.6%
01-Sept-02	0	0	**0**	0	0	**0**	12.0%	7.41%	9.6%
02-Sept-02	0	0	**0**	0	0	**0**	12.0%	7.41%	9.6%
03-Sept-02	0	0	**0**	0	0	**0**	12.0%	7.41%	9.6%
04-Sept-02	0	0	**0**	0	0	**0**	12.0%	7.41%	9.6%
05-Sept-02	0	0	**0**	0	0	**0**	12.0%	7.41%	9.6%
06-Sept-02	0	0	**0**	0	0	**0**	12.0%	7.41%	9.6%
07-Sept-02	0	0	**0**	0	0	**0**	12.0%	7.41%	9.6%

Date	Pond 2	Pond 2	**Pond 2**	Pond 4	Pond 4	**Pond 4**			
initial numbers	Female	Male	**Total**	Female	Male	**Total**	total females	total males	
28-Aug-02	11	13	**24**	14	14	**28**	25	27	
DATE							AMBIENT Female mortality (%)	AMBIENT Male mortality (%)	AMBIENT Total male and female mortality (%)
08-Sept-02	0	0	**0**	0	0	**0**	12.0%	7.41%	9.6%
09-Sept-02	0	0	**0**	0	0	**0**	12.0%	7.41%	9.6%
10-Sept-02	0	0	**0**	0	0	**0**	12.0%	7.41%	9.6%
11-Sept-02	0	0	**0**	0	0	**0**	12.0%	7.41%	9.6%
12-Sept-02	0	0	**0**	0	0	**0**	12.0%	7.41%	9.6%
13-Sept-02	0	0	**0**	0	0	**0**	12.0%	7.41%	9.6%
14-Sept-02	0	0	**0**	0	0	**0**	12.0%	7.41%	9.6%
15-Sept-02	0	0	**0**	0	0	**0**	12.0%	7.41%	9.6%
16-Sept-02	0	0	**0**	0	0	**0**	12.0%	7.41%	9.6%
17-Sept-02	0	0	**0**	0	0	**0**	12.0%	7.41%	9.6%
18-Sept-02	0	0	**0**	0	0	**0**	12.0%	7.41%	9.6%
19-Sept-02	0	0	**0**	0	0	**0**	12.0%	7.41%	9.6%
20-Sept-02	0	0	**0**	0	0	**0**	12.0%	7.41%	9.6%
21-Sept-02	0	0	**0**	0	0	**0**	12.0%	7.41%	9.6%
22-Sept-02	0	0	**0**	0	0	**0**	12.0%	7.41%	9.6%
23-Sept-02	0	0	**0**	0	0	**0**	12.0%	7.41%	9.6%
24-Sept-02	0	0	**0**	0	0	**0**	12.0%	7.41%	9.6%
25-Sept-02	0	0	**0**	0	0	**0**	12.0%	7.41%	9.6%
26-Sept-02	0	0	**0**	1	0	**1**	16.0%	7.41%	11.5%
27-Sept-02	0	0	**0**	0	0	**0**	16.0%	7.41%	11.5%
28-Sept-02	0	0	**0**	0	0	**0**	16.0%	7.41%	11.5%
29-Sept-02	0	0	**0**	0	0	**0**	16.0%	7.41%	11.5%
30-Sept-02	0	1	**1**	0	0	**0**	16.0%	11.11%	13.5%
01-Oct-02	0	0	**0**	0	0	**0**	16.0%	11.11%	13.5%
02-Oct-02			**0**			**0**	16.0%	11.11%	13.5%
03-Oct-02			**0**			**0**	16.0%	11.11%	13.5%
04-Oct-02			**0**			**0**	16.0%	11.11%	13.5%
05-Oct-02			**0**			**0**	16.0%	11.11%	13.5%
06-Oct-02			**0**			**0**	16.0%	11.11%	13.5%
07-Oct-02			**0**			**0**	16.0%	11.11%	13.5%
08-Oct-02		1	**1**	1	3	**4**	20.0%	25.93%	23.1%
09-Oct-02			**0**			**0**	20.0%	25.93%	23.1%
10-Oct-02			**0**			**0**	20.0%	25.93%	23.1%
11-Oct-02			**0**			**0**	20.0%	25.93%	23.1%
12-Oct-02			**0**			**0**	20.0%	25.93%	23.1%
13-Oct-02			**0**			**0**	20.0%	25.93%	23.1%
14-Oct-02			**0**			**0**	20.0%	25.93%	23.1%
15-Oct-02		2	**2**	2	1	**3**	28.0%	37.04%	32.7%
16-Oct-02			**0**			**0**	28.0%	37.04%	32.7%
17-Oct-02			**0**			**0**	28.0%	37.04%	32.7%
18-Oct-02			**0**			**0**	28.0%	37.04%	32.7%
19-Oct-02			**0**			**0**	28.0%	37.04%	32.7%

Date	Pond 2	Pond 2	**Pond 2**	Pond 4	Pond 4	**Pond 4**			
initial numbers	Female	Male	**Total**	Female	Male	**Total**	total females	total males	
28-Aug-02	11	13	**24**	14	14	**28**	25	27	
DATE							AMBIENT Female mortality (%)	AMBIENT Male mortality (%)	AMBIENT Total male and female mortality (%)
20-Oct-02			**0**			**0**	28.0%	37.04%	32.7%
21-Oct-02			**0**			**0**	28.0%	37.04%	32.7%
22-Oct-02	1	2	**3**		1	**1**	32.0%	48.15%	40.4%
final % mortality	**27.2**	**53.8**	**41.7**	**35.7**	**42.9**	**39.3**	**32.0%**	**48.15%**	**40.4%**

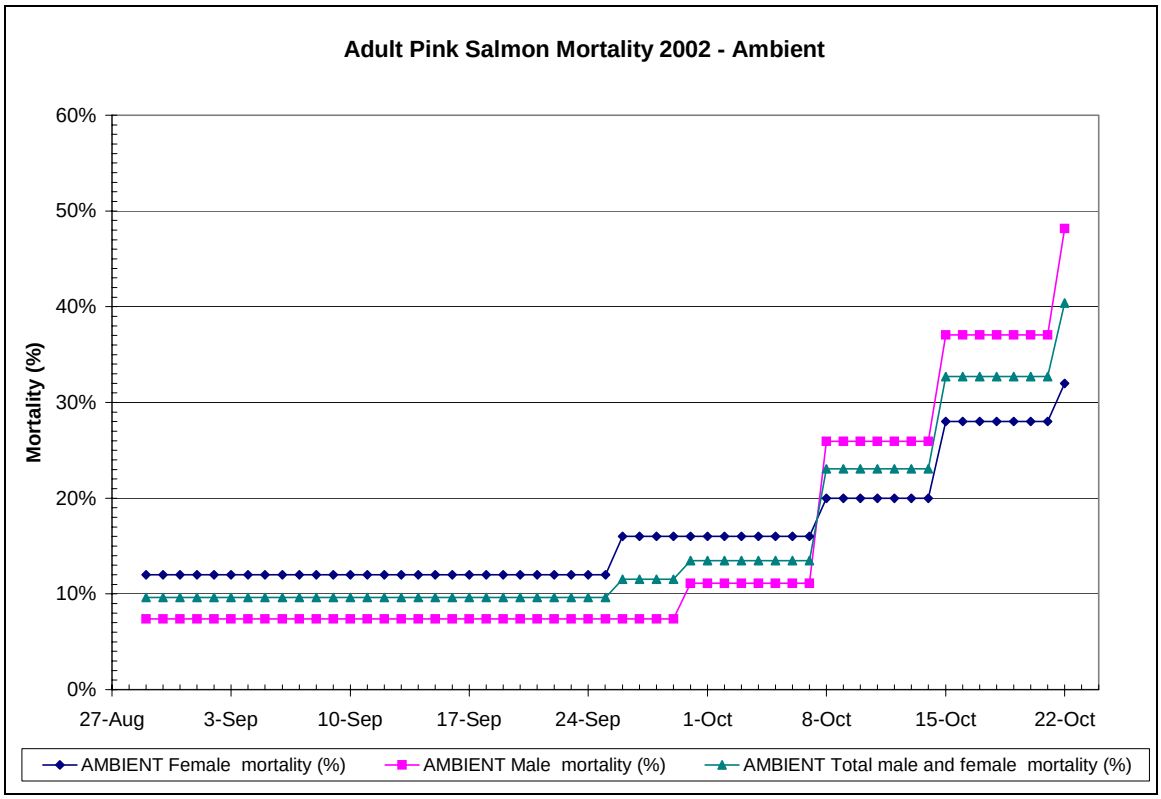

Figure 19. Adult mortality in the ambient treatment.

3.4.3 Heated Treatment

Adult mortality in the heated treatment (ponds 5 and 6) is listed in Table 10 and illustrated in Figure 20.

Table 10. Adult pink salmon mortality in heated (ponds 5 & 6) treatment.

Date	Pond 5	Pond 5	**Pond 5**	Pond 6	Pond 6	**Pond 6**			
initial numbers	Female	Male	**Total**	Female	Male	**Total**	total females	total males	
28-Aug-02	9	15	**24**	11	10	**21**	20	25	
DATE							HEATED Female mortality (%)	HEATED Male mortality (%)	HEATED Total male and female mortality (%)
29-Aug-02	0	2	**2**	0	0	**0**	0.0%	8%	4.4%
30-Aug-02	0	1	**1**	0	0	**0**	0.0%	12.00%	6.7%
31-Aug-02	0	4	**4**	3	3	**6**	15.0%	40.00%	28.9%
01-Sept-02	4	4	**8**	4	3	**7**	55.0%	68.00%	62.2%
02-Sept-02	2	0	**2**	0	2	**2**	65.0%	76.00%	71.1%
03-Sept-02	0	3	**3**	0	1	**1**	65.0%	92.00%	80.0%
04-Sept-02	1	0	**1**	0	0	**0**	70.0%	92.00%	82.2%
05-Sept-02	0	0	**0**	0	0	**0**	70.0%	92.00%	82.2%
06-Sept-02	0	0	**0**	0	0	**0**	70.0%	92.00%	82.2%
07-Sept-02	0	0	**0**	0	0	**0**	70.0%	92.00%	82.2%
08-Sept-02	0	0	**0**	0	0	**0**	70.0%	92.00%	82.2%
09-Sept-02	0	0	**0**	0	0	**0**	70.0%	92.00%	82.2%
10-Sept-02	0	0	**0**	0	0	**0**	70.0%	92.00%	82.2%
11-Sept-02	0	0	**0**	0	0	**0**	70.0%	92.00%	82.2%
12-Sept-02	0	0	**0**	0	0	**0**	70.0%	92.00%	82.2%
13-Sept-02	0	0	**0**	0	0	**0**	70.0%	92.00%	82.2%
14-Sept-02	0	0	**0**	0	0	**0**	70.0%	92.00%	82.2%
15-Sept-02	0	0	**0**	0	0	**0**	70.0%	92.00%	82.2%
16-Sept-02	0	0	**0**	0	0	**0**	70.0%	92.00%	82.2%
17-Sept-02	0	0	**0**	0	0	**0**	70.0%	92.00%	82.2%
18-Sept-02	0	0	**0**	0	0	**0**	70.0%	92.00%	82.2%
19-Sept-02	0	0	**0**	0	0	**0**	70.0%	92.00%	82.2%
20-Sept-02	0	0	**0**	0	0	**0**	70.0%	92.00%	82.2%
21-Sept-02	0	0	**0**	0	0	**0**	70.0%	92.00%	82.2%
22-Sept-02	0	0	**0**	0	0	**0**	70.0%	92.00%	82.2%
23-Sept-02	0	0	**0**	0	0	**0**	70.0%	92.00%	82.2%
24-Sept-02	0	0	**0**	0	0	**0**	70.0%	92.00%	82.2%
25-Sept-02	0	0	**0**	0	0	**0**	70.0%	92.00%	82.2%
26-Sept-02	0	0	**0**	0	0	**0**	70.0%	92.00%	82.2%
27-Sept-02	0	0	**0**	0	0	**0**	70.0%	92.00%	82.2%
28-Sept-02	0	0	**0**	0	0	**0**	70.0%	92.00%	82.2%
29-Sept-02	0	0	**0**	0	0	**0**	70.0%	92.00%	82.2%
30-Sept-02	0	0	**0**	0	0	**0**	70.0%	92.00%	82.2%
01-Oct-02	0	0	**0**	0	0	**0**	70.0%	92.00%	82.2%
02-Oct-02			**0**			**0**	70.0%	92.00%	82.2%
03-Oct-02			**0**			**0**	70.0%	92.00%	82.2%

Date	Pond 5	Pond 5	**Pond 5**	Pond 6	Pond 6	**Pond 6**			
initial numbers	Female	Male	**Total**	Female	Male	**Total**	total females	total males	
28-Aug-02	9	15	**24**	11	10	**21**	20	25	
DATE							HEATED Female mortality (%)	HEATED Male mortality (%)	HEATED Total male and female mortality (%)
04-Oct-02			**0**			**0**	70.0%	92.00%	82.2%
05-Oct-02			**0**			**0**	70.0%	92.00%	82.2%
06-Oct-02			**0**			**0**	70.0%	92.00%	82.2%
07-Oct-02			**0**			**0**	70.0%	92.00%	82.2%
08-Oct-02			**0**			**0**	70.0%	92.00%	82.2%
09-Oct-02			**0**			**0**	70.0%	92.00%	82.2%
10-Oct-02			**0**			**0**	70.0%	92.00%	82.2%
11-Oct-02			**0**			**0**	70.0%	92.00%	82.2%
12-Oct-02			**0**			**0**	70.0%	92.00%	82.2%
13-Oct-02			**0**			**0**	70.0%	92.00%	82.2%
14-Oct-02			**0**			**0**	70.0%	92.00%	82.2%
15-Oct-02			**0**	1		**1**	75.0%	92.00%	84.4%
16-Oct-02			**0**			**0**	75.0%	92.00%	84.4%
17-Oct-02			**0**			**0**	75.0%	92.00%	84.4%
18-Oct-02			**0**			**0**	75.0%	92.00%	84.4%
19-Oct-02			**0**			**0**	75.0%	92.00%	84.4%
20-Oct-02			**0**			**0**	75.0%	92.00%	84.4%
21-Oct-02			**0**			**0**	75.0%	92.00%	84.4%
22-Oct-02		1	**1**			**0**	75.0%	96.00%	86.7%
final % mortality	**77.8**	**100.0**	**91.7**	**72.7**	**90.0**	**81.0**	**75.0%**	**96.00%**	**86.7%**

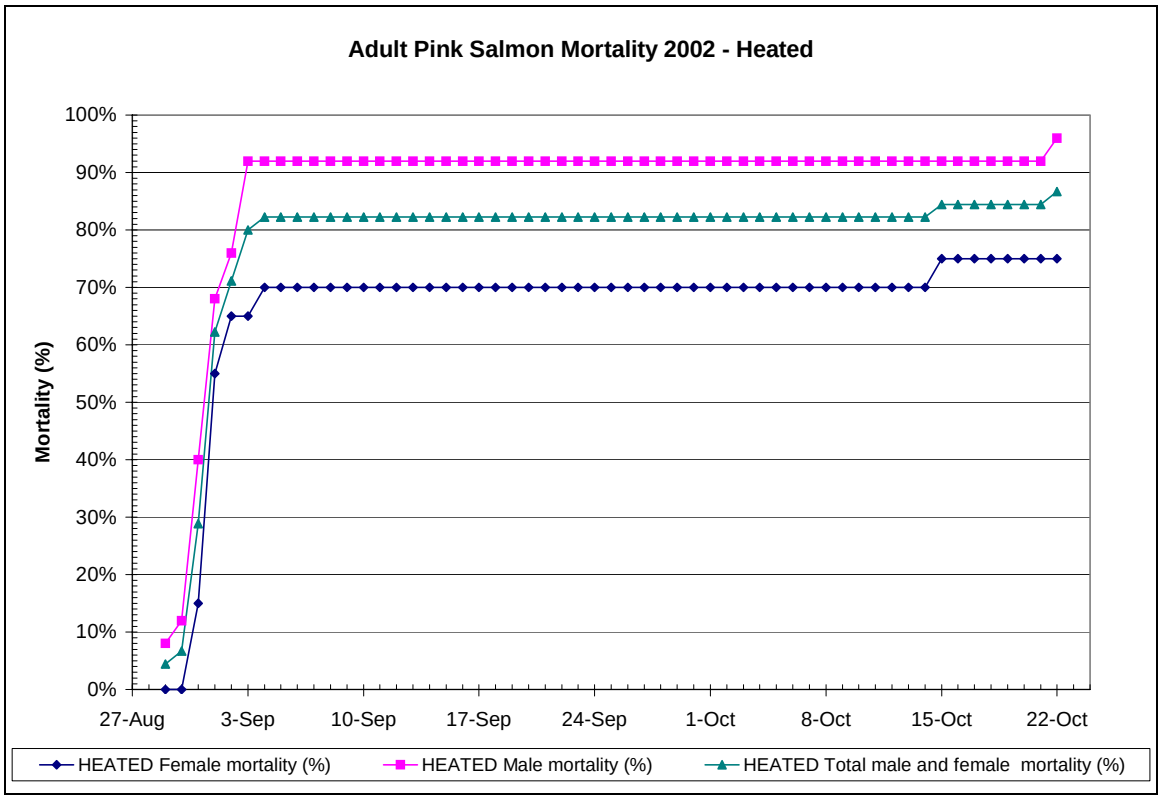

Figure 20. Adult mortality in the heated treatment.

Adult mortality was averaged for the 2 test ponds per treatment and is summarised in Figure 21.

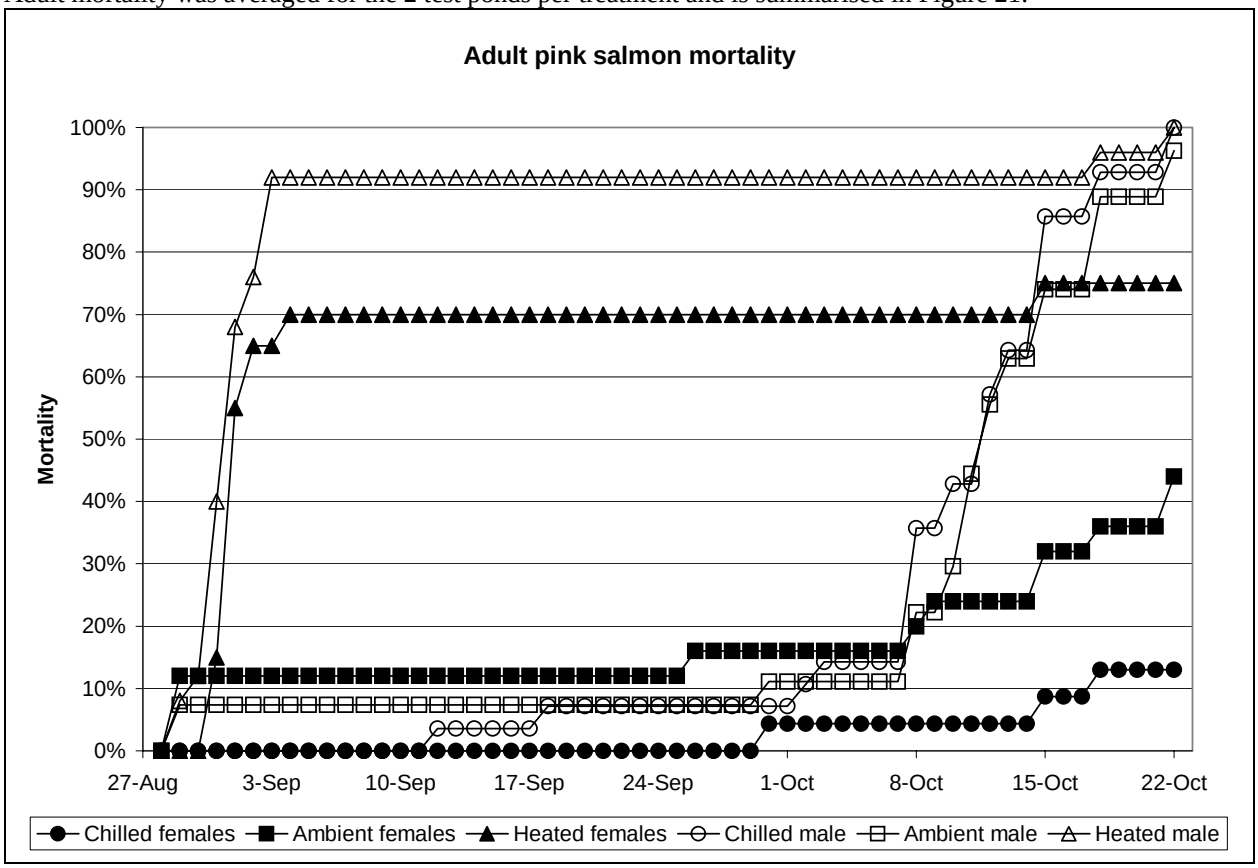

Figure 21. Mean adult mortality of pink salmon exposed to the chilled, ambient, and heated temperature regimes.

Notice that the majority of mortality in the heated treatment occurred within the first week of exposure. Recall also that the heated treatment was terminated mid-day on Sept. 17, after which the temperature treatment was the same as the ambient treatment. The total (i.e. both male and female) adult mortality as of Sept. 17 was 2, 10, and 82 % for the chilled, ambient, and heated treatments, respectively. A two way ANOVA (see Appendix for details), with mortality averaged weekly for each temperature treatment, and Tukey's multiple comparison showed that female mortality increased significantly ($P<0.05$) when exposed to chilled , ambient, and heated temperature regimes, respectively (Table 11). In addition, pairwise comparison of adult mortality averaged over weeks of exposure showed significant differences ($P<0.05$) for week 8 versus week 4 and earlier. Similarly, a two way ANOVA (see Appendix for details) of male mortality also showed a significant ($P<0.05$) increase in mortality when fish were exposed to the heated temperature regime, while differences were not significant between chilled and ambient treatments (Table 11). The pairwise comparison of male mortality averaged over weeks of exposure was similar to the female mortality with significant differences ($P<0.05$) in mortality for week 8 versus week 4 and earlier.

Table 11. Comparison of mean female and male adult mortality proportions indicating significant ($P<0.05$) differences between each treatment.

	Female mortality	Male Mortality
Comparison	P<0.05	P<0.05
Heated vs Chilled	Yes	Yes
Heated vs Ambient	Yes	Yes
Ambient vs Chilled	Yes	No

3.5 Maturation rate

Female maturation (i.e. the number of mature females in each treatment pond and percent based on the initial number of females in a pond) is summarised in Table 12.

Table 12. Number of mature females and percent (based on initial number of females in pond) female maturation from Oct. 1 to Oct. 22, 2002.

Date	Chilled Treatment		Ambient Treatment		Heated Treatment	
	Pond 1 (n=10)	Pond 3 (n=13)	Pond 2 (n=11)	Pond (n=14)	Pond 5 (n=9)	Pond 6 (n=11)
01/10/02	6	6		2		
08/10/02	3	4	1	4		
15/10/02	1		7	2		1
22/10/02			1	4	2	2

Date	Chilled Treatment			Ambient Treatment			Heated Treatment		
	Pond 1 (n=10)	Pond 3 (n=13)	**Avg.**	Pond 2 (n=11)	Pond (n=14)	**Avg.**	Pond 5 (n=9)	Pond 6 (n=11)	**Avg.**
01/10/02	60%	46%	**53%**	0%	14%	**7%**	0%	0%	**0%**
08/10/02	90%	77%	**83%**	9%	43%	**26%**	0%	0%	**0%**
15/10/02	100%	77%	**88%**	73%	57%	**65%**	0%	9%	**5%**
22/10/02	100%	77%	**88%**	82%	86%	**84%**	22%	27%	**25%**

Spawning commenced on October 1, 2002 and continued for 3 weeks, ending on October 22, 2002. It was apparent on the first day of spawning that the 3 temperature treatments had influenced maturation, with 53%, 7%, and 0% of females ripe by October 1, 2002, in chilled, ambient, and heated treatments, respectively. The complete maturation response of female pink salmon (i.e. the percent of the initial number of females) is illustrated in Figure 22.

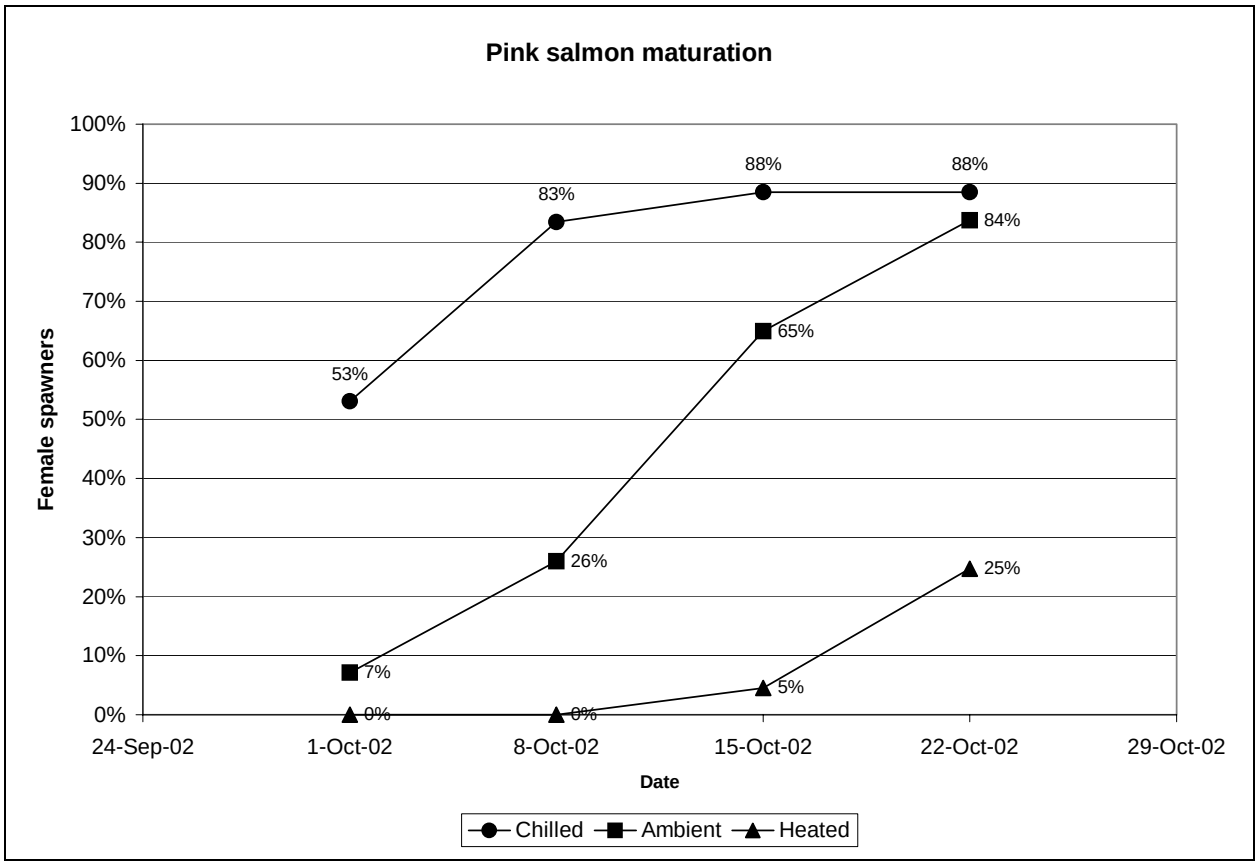

Figure 22. Mean pink salmon female maturation (i.e. female spawners, %) over a 21-day period in chilled, ambient, and heated temperature regimes.

A two-way ANOVA (see Appendix for details) indicated that the percent of female spawners was significantly ($P<0.05$) affected by both spawning time and temperature regimes.

3.6 Egg mortality data

3.6.1 Chilled Treatment

Individual female egg mortality, with 3 replicates per female, from adult fish exposed to the chilled treatment is listed Table 13.

Table 13. Total egg mortality (%) based on egg counts (live, dead) for each subsample (group ID; T=pond # and F=fish #; 3 replicates) of ~300 eggs from female pink salmon exposed to the chilled temperature treatment.

Egg take Date	Group ID	Live	Dead	Mortality %
01-Oct	T1F1	134	3	2.2
01-Oct	T1F1	115	5	4.2
01-Oct	T1F1	133	2	1.5
01-Oct	T1F3	101	10	9.0
01-Oct	T1F3	107	17	13.7
01-Oct	T1F3	117	12	9.3
01-Oct	T1F6	119	6	4.8
01-Oct	T1F6	159	6	3.6
01-Oct	T1F6	98	15	13.3

Egg take Date	Group ID	Live	Dead	Mortality %
01-Oct	T1F18	0	92	100.0
01-Oct	T1F18	0	116	100.0
01-Oct	T1F18	0	99	100.0
01-Oct	T1F19	110	3	2.7
01-Oct	T1F19	97	3	3.0
01-Oct	T1F19	180	6	3.2
01-Oct	T1F20	110	17	13.4
01-Oct	T1F20	114	24	17.4
01-Oct	T1F20	91	16	15.0
01-Oct	T3F1	116	11	8.7
01-Oct	T3F1	109	7	6.0
01-Oct	T3F1	98	9	8.4
01-Oct	T3F2	135	3	2.2
01-Oct	T3F2	108	2	1.8
01-Oct	T3F2	107	4	3.6
01-Oct	T3F4	127	5	3.8
01-Oct	T3F4	129	10	7.2
01-Oct	T3F4	183	3	1.6
01-Oct	T3F5	121	52	30.1
01-Oct	T3F5	99	45	31.3
01-Oct	T3F5	95	47	33.1
01-Oct	T3F16	122	10	7.6
01-Oct	T3F16	108	9	7.7
01-Oct	T3F16	97	7	6.7
01-Oct	T3F17	169	5	2.9
01-Oct	T3F17	104	7	6.3
01-Oct	T3F17	95	9	8.7
08-Oct	T1F4	138	7	4.8
08-Oct	T1F4	100	6	5.7
08-Oct	T1F4	144	6	4.0
08-Oct	T1F11	126	5	3.8
08-Oct	T1F11	126	11	8.0
08-Oct	T1F11	160	1	0.6
08-Oct	T1F13	90	25	21.7
08-Oct	T1F13	108	12	10.0
08-Oct	T1F13	170	10	5.6
08-Oct	T3F4	144	8	5.3
08-Oct	T3F4	161	5	3.0
08-Oct	T3F4	150	4	2.6
08-Oct	T3F5	129	27	17.3
08-Oct	T3F5	118	12	9.2
08-Oct	T3F5	136	20	12.8
08-Oct	T3F6	181	19	9.5
08-Oct	T3F6	150	12	7.4
08-Oct	T3F6	161	16	9.0
08-Oct	T3F8	201	7	3.4
08-Oct	T3F8	174	5	2.8
08-Oct	T3F8	153	5	3.2
15-Oct	T3F6	138	0	0.0
15-Oct	T3F6	100	17	14.5

Egg take Date	Group ID	Live	Dead	Mortality %
15-Oct	T3F6	91	33	26.6
15-Oct	T3F6	99	6	5.7
15-Oct	T3F6	59	41	41.0
15-Oct	T3F6	82	25	23.4

3.6.2 Ambient Treatment

Individual female egg mortality, with 3 replicates per female, from adult fish exposed to the ambient treatment is listed Table 14.

Table 14. Total egg mortality (%) based on egg counts (live, dead) for each subsample (group ID; T=pond # and F=fish #; 3 replicates) of ~300 eggs from female pink salmon exposed to the ambient temperature treatment.

Egg take Date	Group	Live	Dead	Mortality %
01-Oct	T4F4	87	23	20.9
01-Oct	T4F4	85	23	21.3
01-Oct	T4F4	91	27	22.9
01-Oct	T4F11	115	2	1.7
01-Oct	T4F11	119	8	6.3
01-Oct	T4F11	127	8	5.9
08-Oct	T4F4	88	73	45.3
08-Oct	T4F4	95	27	22.1
08-Oct	T4F4	130	29	18.2
08-Oct	T4F16	115	6	5.0
08-Oct	T4F16	109	8	6.8
08-Oct	T4F16	165	13	7.3
08-Oct	T2F16	174	22	11.2
08-Oct	T2F16	102	24	19.0
08-Oct	T2F16	113	22	16.3
15-Oct	T2F4	105	32	23.4
15-Oct	T2F4	134	1	0.7
15-Oct	T2F4	90	68	43.0
15-Oct	T2F5	63	39	38.2
15-Oct	T2F5	76	45	37.2
15-Oct	T2F5	82	28	25.5
15-Oct	T2F7	133	36	21.3
15-Oct	T2F7	117	32	21.5
15-Oct	T2F7	123	65	34.6
15-Oct	T2F8	123	9	6.8
15-Oct	T2F8	132	10	7.0
15-Oct	T2F8	125	26	17.2
15-Oct	T2F13	9	159	94.6
15-Oct	T2F13	12	147	92.5
15-Oct	T2F13	11	143	92.9
15-Oct	T2F14	109	37	25.3
15-Oct	T2F14	119	40	25.2
15-Oct	T2F14	100	47	32.0
15-Oct	T2F15	99	129	56.6

Egg take Date	Group	Live	Dead	Mortality %
15-Oct	T2F15	50	164	76.6
15-Oct	T2F15	26	130	83.3
22-Oct	T4F3	1	190	99.5
22-Oct	T4F3	30	127	80.9
22-Oct	T4F3	82	197	70.6
22-Oct	T4F4	81	83	50.6
22-Oct	T4F4	69	74	51.7
22-Oct	T4F4	40	109	73.2
22-Oct	T4F2	5	159	97.0
22-Oct	T4F2	6	171	96.6
22-Oct	T4F2	3	163	98.2
22-Oct	T4F5	106	42	28.4
22-Oct	T4F5	55	69	55.6
22-Oct	T4F5	35	92	72.4

3.6.3 Heated Treatment

Individual female egg mortality, with 3 replicates per female, from adult fish exposed to the heated treatment is listed Table 15.

Table 15. Total egg mortality (%) based on egg counts (live, dead) for each subsample (group ID; T=pond # and F=fish #; 3 replicates) of ~300 eggs from female pink salmon exposed to the ambient temperature treatment.

Egg take Date	Group	Live	Dead	Mortality %
15-Oct	T6F3	114	39	25.5
15-Oct	T6F3	155	10	6.1
15-Oct	T6F3	127	24	15.9
15-Oct	T6F1	98	23	19.0
15-Oct	T6F1	136	13	8.7
15-Oct	T6F1	143	4	2.7
22-Oct	T6F2	19	179	90.4
22-Oct	T6F2	14	150	91.5
22-Oct	T6F2	6	192	97.0
22-Oct	T5F2	0	206	100.0
22-Oct	T5F2	0	167	100.0
22-Oct	T5F2	0	162	100.0
22-Oct	T5F3	44	88	66.7
22-Oct	T5F3	24	137	85.1
22-Oct	T5F3	8	155	95.1

3.6.4 Descriptive Summary Statistics of Egg Mortality Data

Descriptive statistics of egg mortality data for the 3 temperature treatments are listed in Table 16.

Table 16. Descriptive statistics of egg mortality (%) for the chilled, ambient, and heated treatments.

Descriptive Statistics			
	Chilled	Ambient	Heated
Mean	13.56651788	40.84439041	60.23920253
Standard Error	2.679452553	4.590985689	10.60589986
Median	7.194244604	26.91646192	85.0931677
Mode	100	#N/A	100
Standard Deviation	21.26749532	31.80728188	41.07647354
Sample Variance	452.3063571	1011.703181	1687.276678
Kurtosis	11.50416106	-0.998256837	-1.925161662
Skewness	3.365819973	0.630999153	-0.393977398
Range	100	98.73569905	97.27891156
Minimum	0	0.740740741	2.721088435
Maximum	100	99.47643979	100
Sum	854.6906264	1960.530739	903.5880379
Count	63	48	15

There was an increase in variance with an increase in temperature (i.e. variance values from chilled, ambient, and heated treatments were 452, 1012,and 1687, respectively). To further illustrate differences in the data distribution among the temperature treatments, egg mortality frequency histograms are shown below (Figs. 23 – 25).

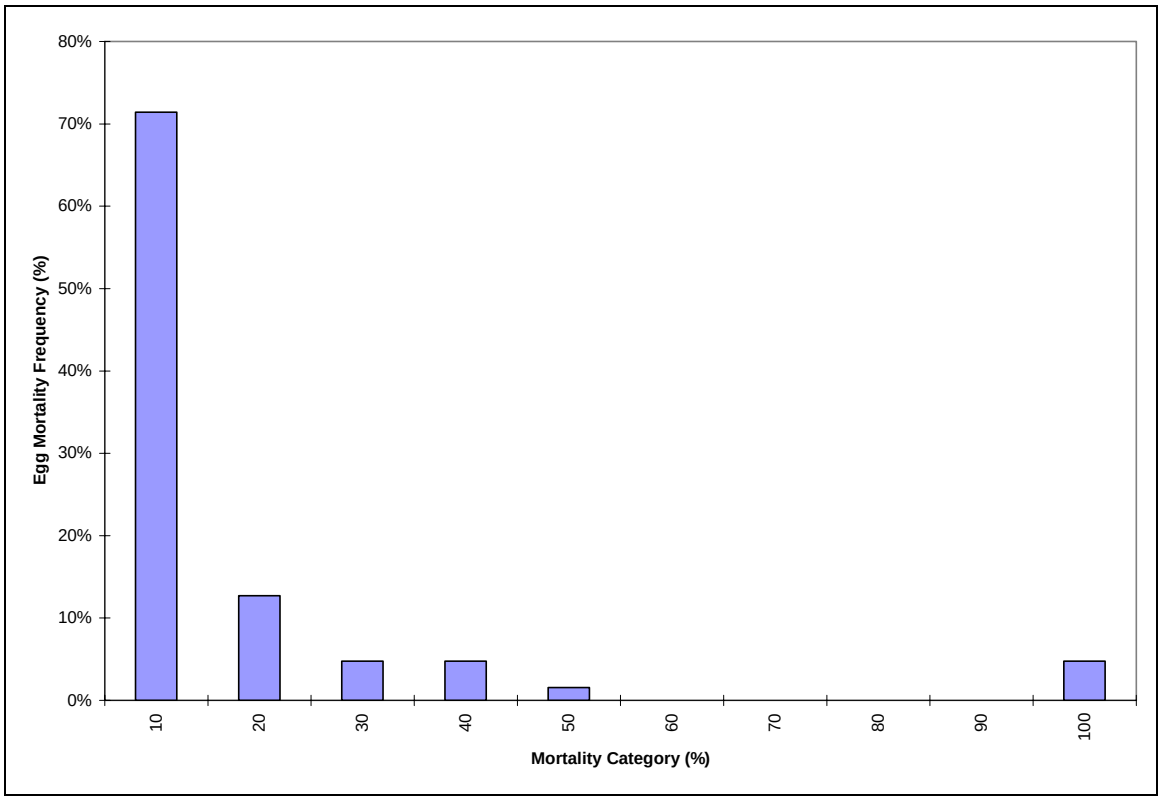

Figure 23. Egg mortality frequency histogram for the chilled treatment.

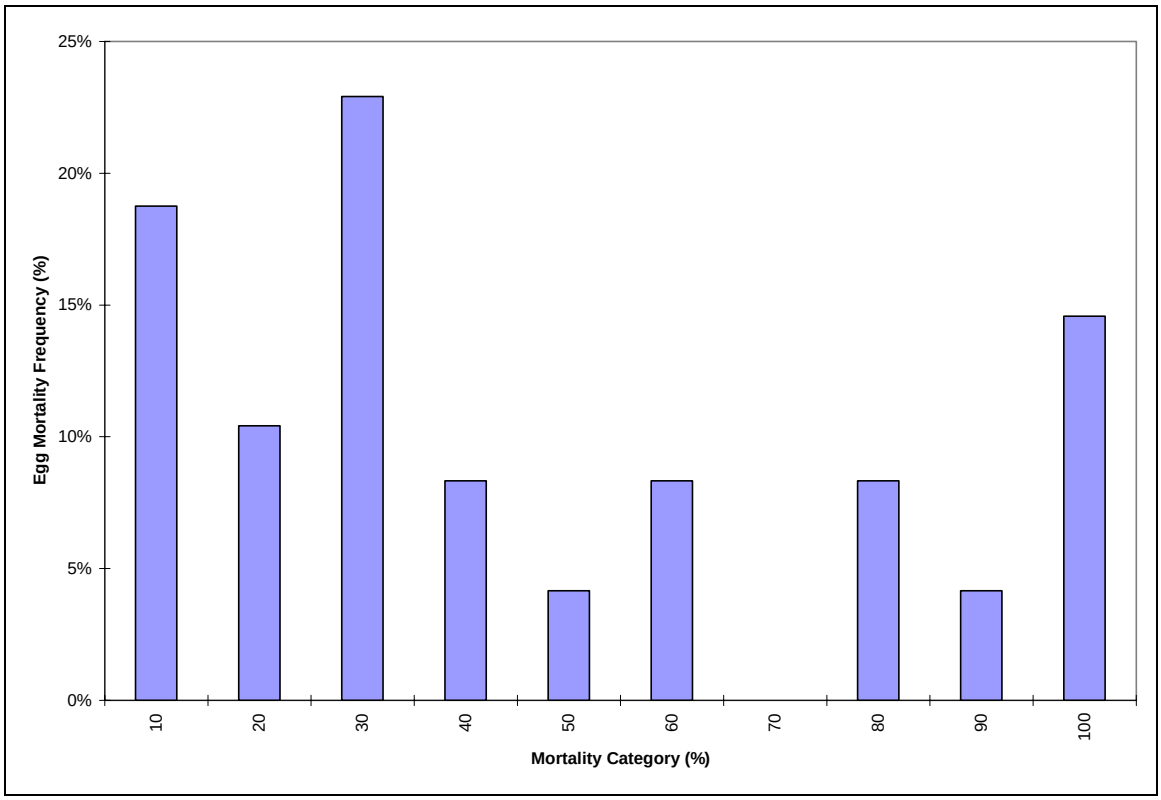

Figure 24. Egg mortality frequency histogram for the ambient treatment.

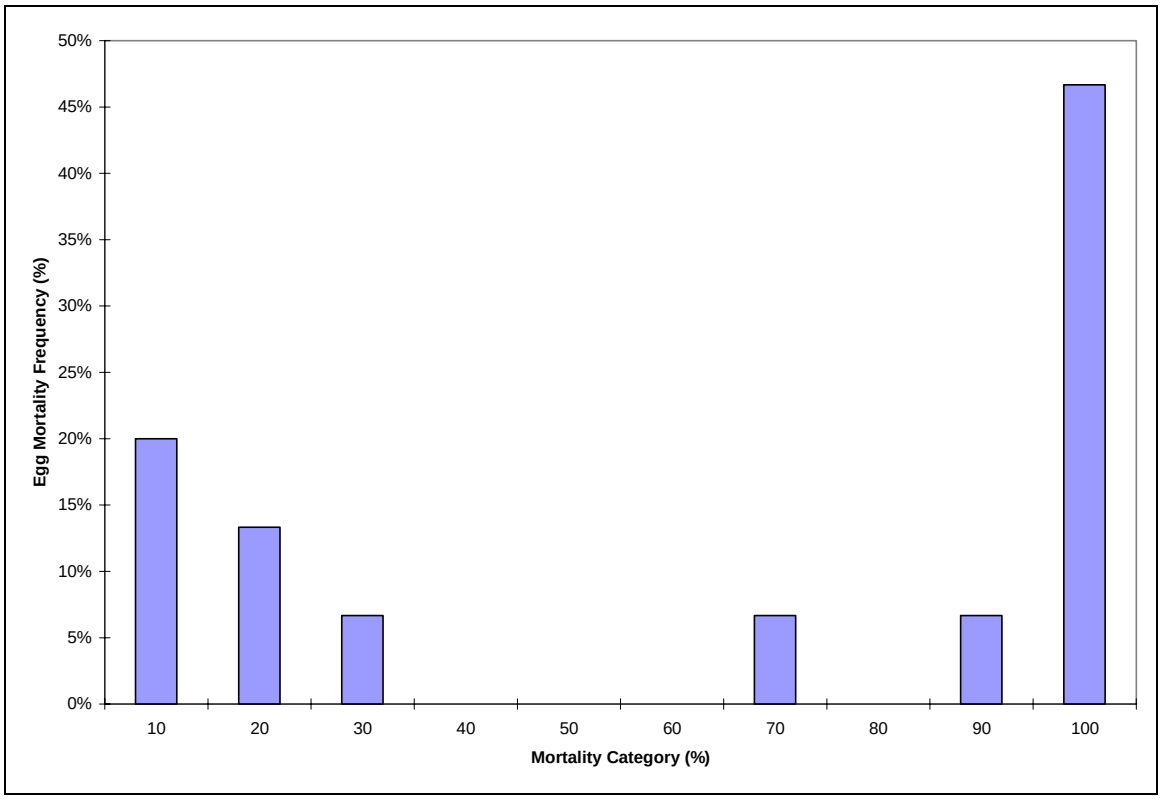

Figure 25. Egg mortality frequency histogram for the heated treatment.

Mean egg mortality (Table 16) from fish held in chilled, ambient, and heated temperature regimes was 13.6, 40.8, and 60.2 %, respectively. ANOVA (see Appendix for details) showed that egg mortality from fish exposed to the chilled temperature regime was significantly ($P<0.05$) lower than egg mortality from fish exposed to both ambient and heated temperature regimes. However, egg mortality was not significantly ($P<0.05$) different between ambient and heated treatments, likely because of the small number of spawners (i.e. 5) with subsequently greater variance, surviving in the heated treatment.

4.0 Discussion

Controlling the chilled and heated temperature treatments proved more difficult than anticipated. Initially, the refrigeration system did not provide enough cooling. This was resolved after 1 week. Similarly, the heated treatment approached acutely lethal levels during the first few days of exposure. In spite of these initial problems, the chilled and heated treatments generally were close to historical Puntledge River temperatures (Figs. 8 to10). Since all the temperature treatments were affected by normal seasonal cooling, each temperature regime gradually declined during the adult pink salmon exposure period (Fig. 11). This decline was modelled for the initial 21 days of exposure using linear regression (Table 2). The mean temperatures (range) for this period for the chilled, ambient, and heated treatments were 15.1°C (11.6°C-19.4°C), 18.4°C (15.0°C-21.8°C), and 21.3°C (16.6°C-24.0°C), respectively.

It must be emphasised from this study that we do not know the minimum exposure time that will influence adult mortality, maturation rate, and gamete viability. The total exposure time for the high temperature treatment was 21 days and for the ambient and chilled treatments was 56 days. Also, due to unforeseen set-up problems and availability of fish, the adult fish were not captured and placed in the experimental treatments until Aug. 28, several weeks later than originally planned. Therefore, the tests reported herein missed the warmest part of the holding period that adult pink salmon are typically exposed to during early to mid-August. Hence, even though the “heated treatment” may look extreme (relative to typical September temperatures) historically fish actually experienced temperatures very close to these in August (Fig. 1 and Table 1).

Excluding the effect of temperature, the holding conditions, as indicated by water flow, DO, and initial load rates (Table 7), are considered to have been very good, since the fish that survived to spawn were generally in very good condition. Of particular note was the lack of external fungus lesions that are often observed when maturing adult salmon are handled and kept in captivity in artificial containers.

Adult holding temperature had a significant detrimental ($P<0.05$) effect on adult mortality (Tables 8 to 10 and Figs. 18 to 20), maturation rate (Table 12 and Fig. 22), and egg mortality (Table 16 and Figs. 23 to 25). As expected, the high temperature groups had the highest adult mortality and egg mortality rates. More surprising were the significant differences between the ambient and chilled groups. Total female mortality in the chilled treatment (13%) was significantly ($P<0.05$) lower than in the ambient treatment (44%). Also, maturation rate in the chilled treatment occurred significantly ($P < 0.05$) earlier than in either the ambient or heated treatments. This increase in mortality is likely because the males were returned to the holding ponds after being checked for maturation and expressing milt. Therefore many males were subjected to more stress than the females.

Egg mortality (Table 16) in the chilled treatment (13.6%) was significantly ($P<0.05$) lower than ambient (40.8%) and heated (60.2%) treatments. In addition, of importance, is the increase in egg mortality variance and greater frequencies of higher egg mortality in the ambient and heated treatments illustrated when figures 23 to 25 are compared. These effects are all indicative of the adverse effect of high temperatures on the final stages of maturation. Therefore, even in a year with moderate temperatures similar to the mean Puntledge temperatures from 1980 to 2002 (Fig. 1), adult pink salmon will be significantly affected, resulting in increased egg mortality.

This conclusion was further substantiated by observations made during production egg takes at Puntledge River and Quinsam River hatcheries in 2002.

In 2002, gametes collected at the Quinsam hatchery, were divided into two groups. One group was kept at Quinsam and the other was transported to the Puntledge hatchery for incubation. The eyed egg mortalities of these groups of eggs, as well as mean production eyed egg mortalities, are summarised in Table 17. The Quinsam adult holding temperatures (10.6 – 12.2 °C) are noticeably colder than the Puntledge adult holding temperatures (16.5 – 16.6 °C)

Table 17. Mean adult holding and incubation temperatures (°C) at Quinsam and Puntledge River hatcheries and subsequent eyed egg mortality (%) in 2002.

Source	Incubation Site	21-day adult mean temperature (°C)	Eyed mortality (%)
Quinsam	Quinsam	11.9	3.5
Quinsam	Quinsam	11.6	3.5
Quinsam	Quinsam	11.0	3.5
Quinsam	Puntledge	12.2	8.9
Quinsam	Puntledge	12.0	8.9
Puntledge	Puntledge	16.6	28.0
Puntledge	Puntledge	16.5	27.8
Quinsam	Puntledge	10.6	9.3

Eggs transported from Quinsam did experience additional stress and mortality. Mean mortality for this group was 9.0 % (Table 17). In spite of the transport stress, the eggs from Quinsam still had much lower mortality than eggs taken from fish held at Puntledge, with mean mortality of 27.9%. Water temperature is the only known water-quality difference between the two sites. Holding density and fish culture techniques are similar. Even stock differences are minimal because, over the years, large numbers of eggs have been transplanted from Quinsam. Therefore, the difference in egg mortality between the two sites likely occurred because the holding temperatures at Puntledge were significantly higher.

Finally, to aid water use planning, the model ln(y) = -6.3256 + 3.4153*ln(x); where y = egg mortality % and x = mean adult holding temperature C, was developed (Fig. 26) by relating mean egg mortality to the corresponding adult holding water temperature (Table 2) to which adult pink salmon were exposed (i.e. 21-day exposure).

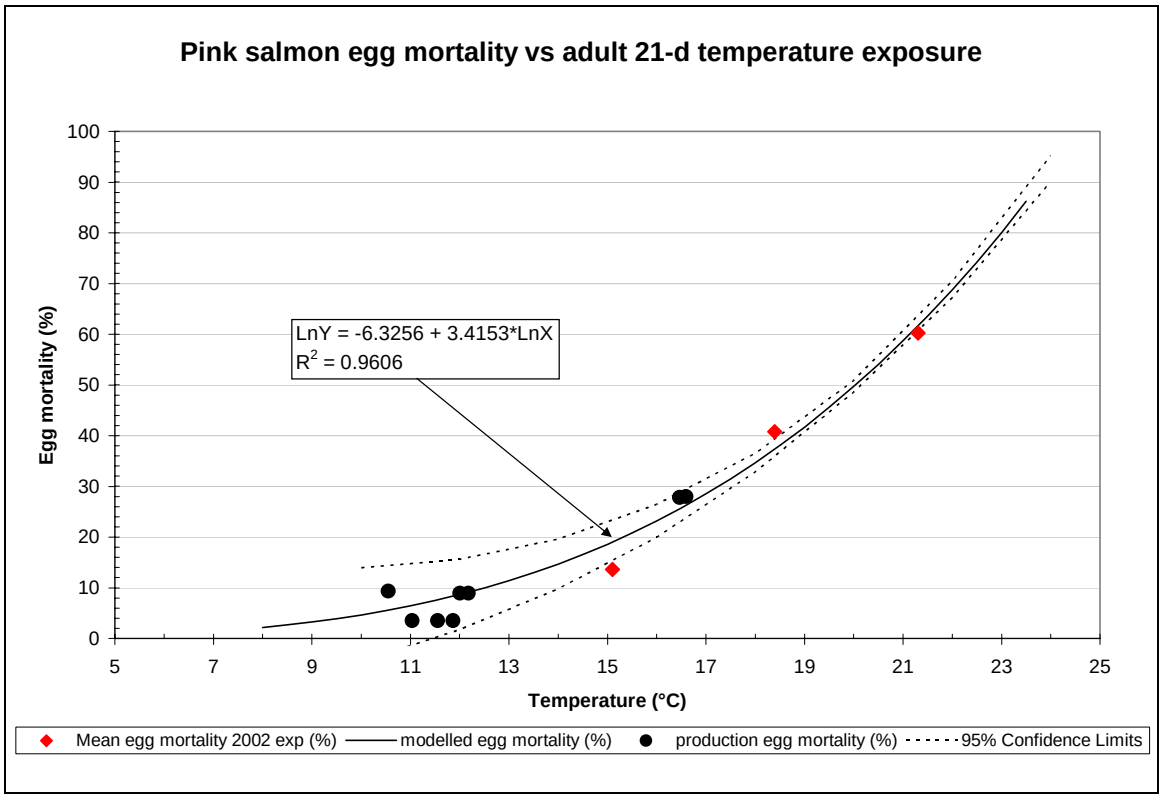

Figure 26. Modelled egg mortality with 95 % confidence limits (solid line and dotted lines, based on mean egg mortality vs mean temperature for 21-d of adult exposure), mean egg mortality from this experiment (solid diamond), and production egg mortality points (solid circle, egg mortality after 21-d adult exposure from Table 17).

The model shown in Fig. 26 clearly illustrates the relationship between exposure of adult pink salmon to various temperatures and gamete viability, as indicated by increased egg mortality at higher temperatures. The addition of the production egg mortality data (listed in Table 17) to the figure adds even further support to this model, since the data appear to fit the modelled curve very closely.

This study has shown that typical water temperatures occurring during late August and early September in the Puntledge River adversely affect the final stages of pink salmon maturation. The consequence is increased adult mortality, delayed maturation rate, and reduced gamete viability.

5.0 Acknowledgements

We gratefully acknowledge the funding by BC Hydro for this study. We also thank the staff at the Puntledge hatchery; especially Lorne Frisson and Bob Addy for capturing the fish, and Christine Berg and Cheryl Burroughs for monitoring and tabulating egg mortality. We are grateful to Joan Bennett and Lorne Frisson for providing the hatchery pink salmon production data used in the discussion. Finally, we thank Mel Sheng for securing funding for this research.

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7.0 Appendix

7.1 ANOVAs

7.1.1 Female mortality

Two Way Analysis of Variance

Dependent Variable: asinsqrt(female mortality proportion)
Data

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Week	7	0.236	0.0337	6.214	0.002
Temperature	2	3.048	1.524	281.220	<0.001
Residual	14	0.0759	0.00542		
Total	23	3.359	0.146		

The difference in the mean values among the different levels of Week is greater than would be expected by chance after allowing for effects of differences in Temperature. There is a statistically significant difference (P = 0.002). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Temperature is greater than would be expected by chance after allowing for effects of differences in Week. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Week**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
8.000 vs. 1.000	0.283	8	6.665	0.006	Yes
8.000 vs. 3.000	0.265	8	6.246	0.010	Yes
8.000 vs. 2.000	0.265	8	6.246	0.010	Yes
8.000 vs. 4.000	0.265	8	6.246	0.010	Yes
8.000 vs. 5.000	0.177	8	4.155	0.137	No
8.000 vs. 6.000	0.159	8	3.746	0.217	Do Not Test
8.000 vs. 7.000	0.0645	8	1.517	0.953	Do Not Test
7.000 vs. 1.000	0.219	8	5.148	0.041	Yes
7.000 vs. 3.000	0.201	8	4.729	0.069	No
7.000 vs. 2.000	0.201	8	4.729	0.069	Do Not Test
7.000 vs. 4.000	0.201	8	4.729	0.069	Do Not Test
7.000 vs. 5.000	0.112	8	2.638	0.592	Do Not Test
7.000 vs. 6.000	0.0947	8	2.229	0.756	Do Not Test
6.000 vs. 1.000	0.124	8	2.919	0.478	No
6.000 vs. 3.000	0.106	8	2.500	0.649	Do Not Test
6.000 vs. 2.000	0.106	8	2.500	0.649	Do Not Test
6.000 vs. 4.000	0.106	8	2.500	0.649	Do Not Test
6.000 vs. 5.000	0.0174	8	0.409	1.000	Do Not Test
5.000 vs. 1.000	0.107	8	2.510	0.645	Do Not Test
5.000 vs. 3.000	0.0889	8	2.091	0.806	Do Not Test
5.000 vs. 2.000	0.0889	8	2.091	0.806	Do Not Test
5.000 vs. 4.000	0.0889	8	2.091	0.806	Do Not Test
4.000 vs. 1.000	0.0178	8	0.419	1.000	Do Not Test
4.000 vs. 3.000	1.110E-016	8	2.612E-015	1.000	Do Not Test
4.000 vs. 2.000	1.110E-016	8	2.612E-015	1.000	Do Not Test
2.000 vs. 1.000	0.0178	8	0.419	1.000	Do Not Test
2.000 vs. 3.000	0.000	8	0.000	1.000	Do Not Test
3.000 vs. 1.000	0.0178	8	0.419	1.000	Do Not Test

Comparisons for factor: **Temperature**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
heated vs. chilled	0.863	3	33.149	<0.001	Yes
heated vs. ambient	0.546	3	20.994	<0.001	Yes
ambient vs. chilled	0.316	3	12.154	<0.001	Yes

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

7.1.2 Male mortality

Two Way Analysis of Variance

Dependent Variable: asinsqrt(male mortality proportion)

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Week	7	0.450	0.0642	4.175	0.011
Temperature	2	4.773	2.387	155.099	<0.001
Residual	14	0.215	0.0154		
Total	23	5.438	0.236		

The difference in the mean values among the different levels of Week is greater than would be expected by chance after allowing for effects of differences in Temperature. There is a statistically significant difference (P = 0.011). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Temperature is greater than would be expected by chance after allowing for effects of differences in Week. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Week**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
8.000 vs. 1.000	0.412	8	5.750	0.019	Yes
8.000 vs. 2.000	0.412	8	5.750	0.019	Yes
8.000 vs. 4.000	0.322	8	4.494	0.092	No
8.000 vs. 3.000	0.322	8	4.494	0.092	Do Not Test
8.000 vs. 5.000	0.300	8	4.196	0.131	Do Not Test
8.000 vs. 6.000	0.236	8	3.292	0.344	Do Not Test
8.000 vs. 7.000	0.0976	8	1.362	0.973	Do Not Test
7.000 vs. 1.000	0.314	8	4.387	0.105	No
7.000 vs. 2.000	0.314	8	4.387	0.105	Do Not Test
7.000 vs. 4.000	0.224	8	3.132	0.399	Do Not Test
7.000 vs. 3.000	0.224	8	3.132	0.399	Do Not Test
7.000 vs. 5.000	0.203	8	2.833	0.512	Do Not Test
7.000 vs. 6.000	0.138	8	1.929	0.859	Do Not Test
6.000 vs. 1.000	0.176	8	2.458	0.666	Do Not Test
6.000 vs. 2.000	0.176	8	2.458	0.666	Do Not Test
6.000 vs. 4.000	0.0861	8	1.203	0.986	Do Not Test
6.000 vs. 3.000	0.0861	8	1.203	0.986	Do Not Test
6.000 vs. 5.000	0.0648	8	0.904	0.997	Do Not Test
5.000 vs. 1.000	0.111	8	1.554	0.947	Do Not Test
5.000 vs. 2.000	0.111	8	1.554	0.947	Do Not Test
5.000 vs. 4.000	0.0214	8	0.299	1.000	Do Not Test
5.000 vs. 3.000	0.0214	8	0.299	1.000	Do Not Test
3.000 vs. 1.000	0.0899	8	1.255	0.983	Do Not Test
3.000 vs. 2.000	0.0899	8	1.255	0.983	Do Not Test
3.000 vs. 4.000	0.000	8	0.000	1.000	Do Not Test
4.000 vs. 1.000	0.0899	8	1.255	0.983	Do Not Test
4.000 vs. 2.000	0.0899	8	1.255	0.983	Do Not Test
2.000 vs. 1.000	0.000	8	0.000	1.000	Do Not Test

Comparisons for factor: **Temperature**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
heated vs. chilled	1.007	3	22.960	<0.001	Yes
heated vs. ambient	0.870	3	19.843	<0.001	Yes
ambient vs. chilled	0.137	3	3.117	0.105	No

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

7.1.3 Female spawners

Two Way Analysis of Variance

Dependent Variable: female spawners (proportion mature)

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Spawn day	3	0.703	0.234	17.887	<0.001
Temp treatment	2	2.022	1.011	77.191	<0.001
Spawn day x Temp treatment	6	0.292	0.0487	3.721	0.025
Residual	12	0.157	0.0131		
Total	23	3.175	0.138		

The difference in the mean values among the different levels of Spawn day is greater than would be expected by chance after allowing for effects of differences in Temp treatment. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Temp treatment is greater than would be expected by chance after allowing for effects of differences in Spawn day. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

The effect of different levels of Spawn day depends on what level of Temp treatment is present. There is a statistically significant interaction between Spawn day and Temp treatment. (P = 0.025)

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Spawn day**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
21.000 vs. 0.000	0.456	4	9.752	<0.001	Yes
21.000 vs. 7.000	0.292	4	6.242	0.004	Yes
21.000 vs. 14.000	0.130	4	2.786	0.252	No
14.000 vs. 0.000	0.325	4	6.967	0.002	Yes
14.000 vs. 7.000	0.161	4	3.457	0.121	No
7.000 vs. 0.000	0.164	4	3.510	0.114	No

Comparisons for factor: **Temp treatment**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
chilled vs. heated	0.710	3	17.556	<0.001	Yes
chilled vs. ambient	0.329	3	8.134	<0.001	Yes
ambient vs. heated	0.381	3	9.422	<0.001	Yes

Comparisons for factor: **Temp treatment within 0**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
chilled vs. heated	0.531	3	6.561	0.002	Yes
chilled vs. ambient	0.460	3	5.678	0.005	Yes
ambient vs. heated	0.0715	3	0.884	0.810	No

Comparisons for factor: **Temp treatment within 7**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
chilled vs. heated	0.835	3	10.312	<0.001	Yes
chilled vs. ambient	0.575	3	7.099	<0.001	Yes
ambient vs. heated	0.260	3	3.213	0.099	No

Comparisons for factor: **Temp treatment within 14**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
chilled vs. heated	0.839	3	10.367	<0.001	Yes
chilled vs. ambient	0.236	3	2.910	0.141	No
ambient vs. heated	0.604	3	7.457	<0.001	Yes

Comparisons for factor: **Temp treatment within 21**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
chilled vs. heated	0.637	3	7.871	<0.001	Yes
chilled vs. ambient	0.0470	3	0.581	0.912	No
ambient vs. heated	0.590	3	7.291	<0.001	Yes

Comparisons for factor: **Spawn day within chilled**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
14.000 vs. 0.000	0.354	4	4.368	0.041	Yes
14.000 vs. 7.000	0.0500	4	0.618	0.971	No
14.000 vs. 21.000	0.000	4	0.000	1.000	Do Not Test
21.000 vs. 0.000	0.354	4	4.368	0.041	Yes
21.000 vs. 7.000	0.0500	4	0.618	0.971	Do Not Test
7.000 vs. 0.000	0.303	4	3.750	0.086	No

Comparisons for factor: **Spawn day within ambient**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
21.000 vs. 0.000	0.766	4	9.465	<0.001	Yes
21.000 vs. 7.000	0.577	4	7.136	0.002	Yes
21.000 vs. 14.000	0.188	4	2.329	0.391	No
14.000 vs. 0.000	0.578	4	7.136	0.002	Yes
14.000 vs. 7.000	0.389	4	4.807	0.024	Yes
7.000 vs. 0.000	0.189	4	2.329	0.391	No

Comparisons for factor: **Spawn day within heated**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
21.000 vs. 7.000	0.248	4	3.058	0.189	No
21.000 vs. 0.000	0.248	4	3.058	0.189	Do Not Test
21.000 vs. 14.000	0.202	4	2.496	0.335	Do Not Test
14.000 vs. 7.000	0.0455	4	0.562	0.978	Do Not Test
14.000 vs. 0.000	0.0455	4	0.562	0.978	Do Not Test
0.000 vs. 7.000	0.000	4	0.000	1.000	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

7.1.4 Egg mortality

One Way Analysis of Variance

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	**N**	**Missing**	**Median**	**25%**	**75%**
arcsin chilled	63	0	0.272	0.186	0.374
arcsin ambient	48	0	0.545	0.435	1.008
arcsin heated	15	0	1.174	0.420	1.384

H = 36.858 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**
arcsin heated vs arcsin chilled	46.340	4.417	Yes
arcsin heated vs arcsin ambient	9.529	0.882	No
arcsin ambient vs arcsin chilled	36.811	5.261	Yes

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