Effects of Various Water Temperature Regimes on the Egg and Alevin Incubation of Susitna River Chum and Sockeye Salmon

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Abstract

This study was conducted to assess the potential effects of water temperature alterations (resulting from proposed hydroelectric development) on incubating salmon eggs and alevins in the Susitna River, Alaska. Chum (*Oncorhynchus keta*) and sockeye (*O. nerka*) salmon eggs from Slough 11, Upper Susitna River, were collected and fertilized on site during three occasions in September, 1982. The eggs were incubated in a laboratory in four separate temperature regimes until alevins achieved complete yolk absorption. The four regimes were designed to simulate 1982-83 temperatures in: (1) the main-stem Susitna River; (2) Slough 8A (a known spawning area); (3) a regime 1°C colder than in Slough 8A; and (4) a constant 4°C. Average temperatures for the four regimes described above were 2.1, 3.9, 2.9, and 4.0°C, respectively.

Complete yolk absorption was delayed by up to two months in the 2.1 and 2.9°C temperature regimes, as compared to the regimes having average temperatures of 3.9 and 4.0°C. However, no biologically meaningful differences were observed in the mean size (mm) of alevins reared in any of the four regimes at complete yolk absorption. Regression equations are presented to predict development rates as a function of water temperature. For convenience, these rates have been converted to the number of days required for Susitna chum and sockeye to hatch and to attain complete yolk absorption for a given average incubation temperature.

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Introduction

Hydroelectric development projects are in the planning or construction stage in several Alaskan rivers. Construction of these dams and their resulting reservoirs are known to alter the normal downstream water temperature regimes (Baxter and Glaude 1980). Thermal effects from dam and reservoir operation in Alaska should be most pronounced during the fall and spring when the river's natural rapid cooling and warming may be modified by reservoir discharges. Thus, salmon eggs and alevins incubating downstream of a hydroelectric project may experience alterations from historical water temperatures which, in Alaska, generally range from 0 to 8°C.

Much research has been conducted on the incubation of salmon eggs. While it has been reported that temperature changes within the range 0 to 5°C have a more pronounced effect on development rates than those between 5 and 10°C (Bams 1967), little information is available on egg incubation in water temperatures less than 4°C (Dong 1981; Raymond 1981; Alderdice and Velsen 1978). There is a regional need for this information as Alaskan salmon often spawn in water temperatures approaching or less than 4°C. There is a specific need for this information for Susitna River stocks due to the hydroelectric dams proposed for the Susitna River at river miles 153 and 184.

The Susitna River in southcentral Alaska (Fig. 1) drains about 19,000 mi^2 into Cook Inlet. It is the sixth largest drainage in Alaska and supports a fishery resource that includes five species of salmon and other resident species such as grayling (*Thymallus arcticus*) and burbot (*Lota lota*). Many studies have been undertaken to evaluate the potential impacts of Susitna River hydroelectric development on fish and wildlife. This study was designed to investigate the incubation of eggs and alevins from two species of Susitna salmon under varying temperature regimes.

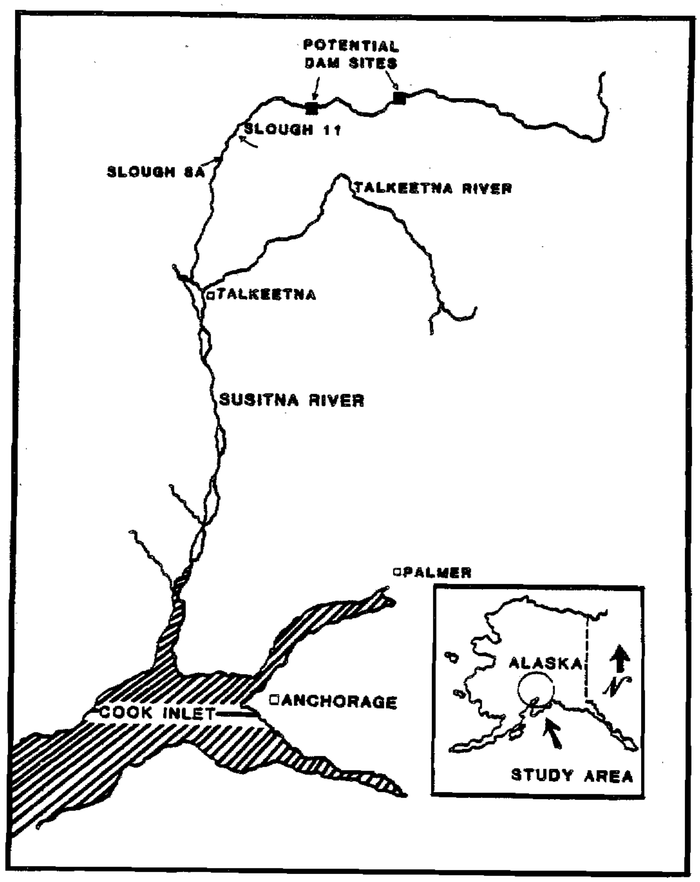


Figure 1. The Susitna River in southcentral Alaska and the location of two sloughs (used by spawning salmon) in relation to proposed hydroelectric dam sites.

While expected post-project river temperatures have not been fully identified at this time, it is understood that water temperatures downstream of the dams will be less than pre-project temperatures in the summer, and greater than pre-project temperatures in the fall and early winter. To help predict the effects of various fall/winter water temperature regimes on Susitna River salmon eggs, the U.S. Fish and Wildlife Service, National Fishery Research Center (NFRC) developed the following study objectives in cooperation with the Alaska Department of Fish and Game (ADF&G) and the Alaska Power Authority (APA):

1. Incubate salmon eggs and alevins under controlled conditions using four temperature regimes which simulate: (1) the main-stem Susitna; (2) a side-channel slough system; (3) a second slough system differing from the first by -1°C; and (4) a constant water temperature of 4°C.

2. Collect and spawn five to seven pairs of chum (*Oncorhynchus keta*) and sockeye (*O. nerka*) salmon from a slough in the Upper Susitna on three different dates which include their normal peak spawning period.

3. Provide data on time to egg hatching and complete yolk absorption in temperature units (TUs) and days for each species. Also, measure lengths and record weights of alevins at time of hatch and yolk absorption and record data on survival and abnormalities of alevins during development.

4. Develop these data into a final report to help planners predict how certain temperature regimes will affect egg incubation and alevin

development of Susitna chum and sockeye salmon.

This study was conducted by the NFRC, U.S. Fish and Wildlife Service, in cooperation with its Division of Ecological Services and ADF&G, Su-Hydro Division. Major funding was provided by APA.

Materials and Methods

Well water was plumbed into eight insulated waterbaths in the NFRC laboratory. Water temperature control was achieved in the baths (heating or chilling) before the water flowed into eight separate Heath incubators. Control and monitoring of the incubator water temperatures was possible from 0.5 to 12°C with 0.1°C resolution and ±0.3°C accuracy. Four temperature regimes, each with a replicate, were monitored with a 10-channel temperature data logger. The data logger provided hourly printouts of water temperature for all eight incubators. The average temperature for 24 hours was used to compute the accumulated temperature units (TUs) for each temperature regime. Thus, if eggs were incubating at an average 5°C for 10 days, they would accumulate 50 TUs.

Incubators were modified prior to egg collections to prevent water from mixing between the two species under study since chum and sockeye salmon were to incubate simultaneously in all eight incubators. These modifications ensured that two water lines from a common source each fed only four of eight egg trays in each incubator, thus providing the ability to incubate each species independently. Because each water line fed only four trays, temperatures did not vary more than 0.1°C as water passed through the trays.

Unusually low water levels resulted in an insufficient adult salmon escapement (for experimental collections) into Slough 8A (RM 125). This site was previously chosen to represent the temperature regime of a typical side-channel slough and provide a location for collecting the experimental salmon stocks. Slough 11 (RM 135.3) had adequate chum and sockeye salmon escapements and provided an alternate site for the egg collections while temperature recording equipment remained in Slough 8A. Thus, three egg collections were made from chum and sockeye salmon at Slough 11 (Fig. 1). For each species at least seven pairs (males and females) were spawned on September 3, 9 and 15. Egg fertilization and handling procedures followed the recommendations of Leitritz and Lewis (1980). After eggs were fertilized and rinsed they were allowed to water harden for one hour in an 18 L bucket filled with water from the slough. Surface water temperatures in Slough 11 ranged from 5.8 to 5.0°C at point measurements taken during the three egg collections. The fertilized eggs were then transported to the NFRC laboratory in Anchorage.

The eggs were measured out volumetrically in the laboratory and distributed into eight equal lots. Each lot of eggs was allowed to acclimate to the incubating water temperature for one hour, before the eggs were placed into incubator trays. Eggs were placed into plastic rings (10 cm diameter x 5 cm depth) which were surrounded by styrofoam within the incubator trays. The styrofoam directed water flow through the rings where eggs (average 4.6 ml eggs/cm^3 ring volume) were held until hatching. Water flow was maintained at about 2.2 L/min throughout the study. Eggs were generally placed into the incubator trays within 10 hrs of fertilization. Dissolved oxygen was measured weekly until hatching was completed and then monthly thereafter.

Because of their larger diameter the average number of chum eggs per tray (1,070) was less than the average number of sockeye eggs per tray (1,400). By the conclusion of the third egg collection, four incubators had three trays each of chum and three trays each of sockeye eggs representing the three egg takes (Table 1) and the four temperature regimes. The four additional incubators were used with an identical design to provide a complete replicate of the study.

Table 1. Egg placement within a given incubator. (Each incubator and its replicate maintained a specific temperature regime.)

Tray		
1	Chum eggs	Collected 09/03
2	Chum eggs	Collected 09/09
3	Chum eggs	Collected 09/15
4	Empty	Water to drain
5	Sockeye eggs	Collected 09/03
6	Sockeye eggs	Collected 09/09
7	Sockeye eggs	Collected 09/15
8	Empty	Water to drain

Two of the four water temperature regimes used in this study simulated natural temperatures in two reaches of the Upper Susitna River. The first regime simulated the record of a thermograph placed in the main-stem Susitna near Gold Creek at river mile 136. We designated this regime as "MS". The second regime simulated temperatures recorded by a thermograph within Slough 8A, a known spawning area for chum and sockeye. We designated this regime as "S2".

Incubator water temperatures were adjusted up to twice weekly during the fall and spring when the greatest river temperature fluctuations occurred. Personnel from ADF&G, Su-Hydro Division, measured and informed NFRC personnel of all field water temperatures.

A third temperature regime (designated "S1") was established as an intermediary regime between MS and S2. S1 differed from S2 by 1°C. The fourth regime (designated 4°) was maintained at a constant 4°C for the duration of the study.

Water from the main-stem Susitna River overtopped Slough 8A during the winter of this study. The overtopping of Slough 8A resulted in cold intragravel temperatures (near 0°C) as determined by point measurements collected after the overtopping occurred. Because this condition did not represent typical slough water temperature and due to the loss of the ADF&G continuous temperature recorder, it was decided to use temperature data obtained from Slough 8A in 1981-1982 (Trihey 1982) as the new model for S2.

Eggs were observed weekly during their early incubation and mortalities were removed from the egg rings. When eggs began to hatch, the alevins were removed from the egg rings daily and placed into additional plastic "alevin rings" within the incubator tray. When 50 percent of the eggs had hatched within any given tray, 30 alevins were removed, anesthetized and then weighed to the nearest 0.01 g and measured (to 0.1 mm) for total length. After 95 percent of the eggs had hatched in any given tray, the styrofoam water block and rings were removed. Subsequently, a sample of 10 alevins was removed and anesthetized to obtain length measurements each week. Alevins removed from incubator trays were not returned to the trays.

Weekly samples continued until the alevins had completed yolk sac absorption. This stage was determined by observing the opening along the alevin's ventral surface which was separated by yolk sac. When the right and left ventral sides had sutured over the remnant yolk sac, the alevin was defined to have completed yolk absorption (CYA). When 50 percent CYA was achieved a sample of 30 alevins was removed and measured for total length.

Data were compiled to provide comparisons for time to hatch and CYA in TUs and days. Mortalities and abnormalities for each temperature regime and egg collection were also noted. The eggs within a few incubator trays experienced lethal stresses due to experimental errors and local power outages. Data from these trays were not used in analysis of length, weight and development rates but have been appended to this report (Appendix 1). For descriptive purposes, data from replicates and the three egg-collection dates were often pooled. When this was done, the raw data were entered into Appendix 1.

A one way analysis of variance (Sokal and Rohlf 1969) for lengths and weights for each species at 50 percent hatch and complete yolk absorption was performed to compare all temperature regimes and egg-collection dates. If a significant difference was found ($P<0.05$) a Duncan multiple comparison test (Nie *et al.* 1975) was performed to combine statistically similar groups ($P=0.01$).

Growth curves were constructed from weekly length measurements to evaluate length as a function of temperature unit accumulation. Comparisons were also made between development rates[1] (at various temperatures) for Susitna River chum and sockeye and those of other chum and sockeye stocks reported in the literature.

Results

Water Temperature

It was not possible to duplicate diurnal or within-week temperature variations taking place in the Susitna River or Slough 8A. However, a rough equivalence of temperature variation was produced during the salmon incubation period. The rapid temperature decline of the main-stem Susitna in the fall, its long winter period of 0°C, and warming in the spring was simulated by the thermal regime designated MS in Fig. 2. The coldest temperature we could maintain was 0.4 to 0.5°C because the water bath began to freeze if lower temperatures were attempted. Because complete yolk absorption was achieved faster in S2 than in S1, the 1°C temperature difference was maintained through late April only. We used intermittent reports from ADF&G to estimate subsequent temperatures for S1.

The accumulated temperature units for all four temperature regimes and for the first and third egg collections (September 3 and 15) are presented in Figs. 3 and 4. The major difference between the two egg-collection dates occurred within the main-stem temperature regime. Due to the declining water temperature, eggs from the third collection that were incubated in the main-stem

[1] Development rate is defined as the reciprocal of days from fertilization to a specific stage, such as complete yolk absorption, multiplied by 1000.

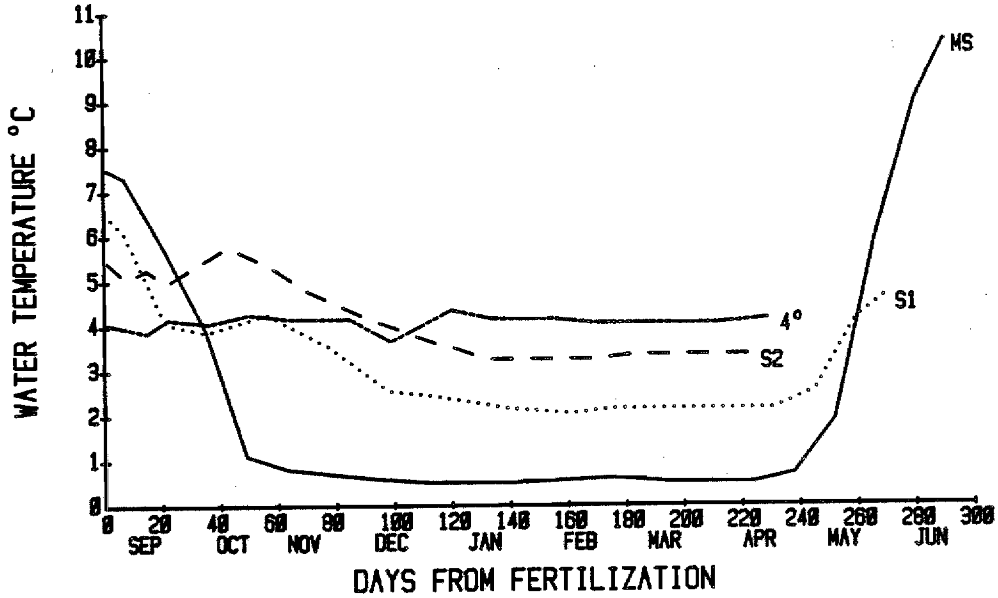


Figure 2. Temperature regimes for the Susitna River egg-incubation study which simulated the main-stem river (MS), Slough 8A (S2), an intermediate regime (S1), and 4°C Constant (4°). Regimes were plotted from 3 September 1982, the first egg collection period.

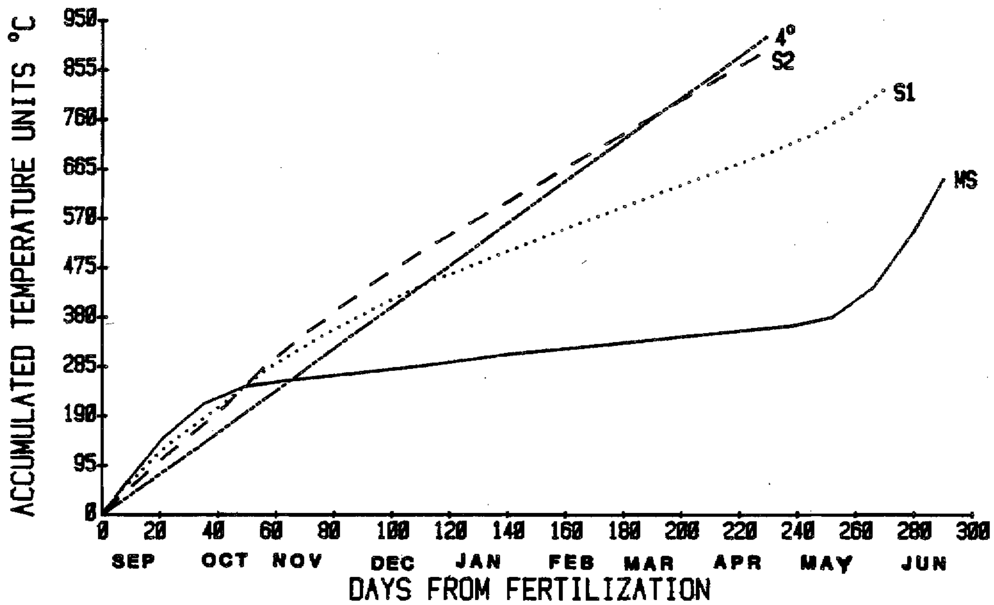


Figure 3. Accumulated temperature units at four different temperature regimes for the Susitna River egg-incubation study. Data were plotted from 3 September 1982, the first of three egg collection dates. The four regimes simulated the Susitna main stem (MS), Slough 8A (S2), an intermediate regime (S1), and 4°C Constant (4°).

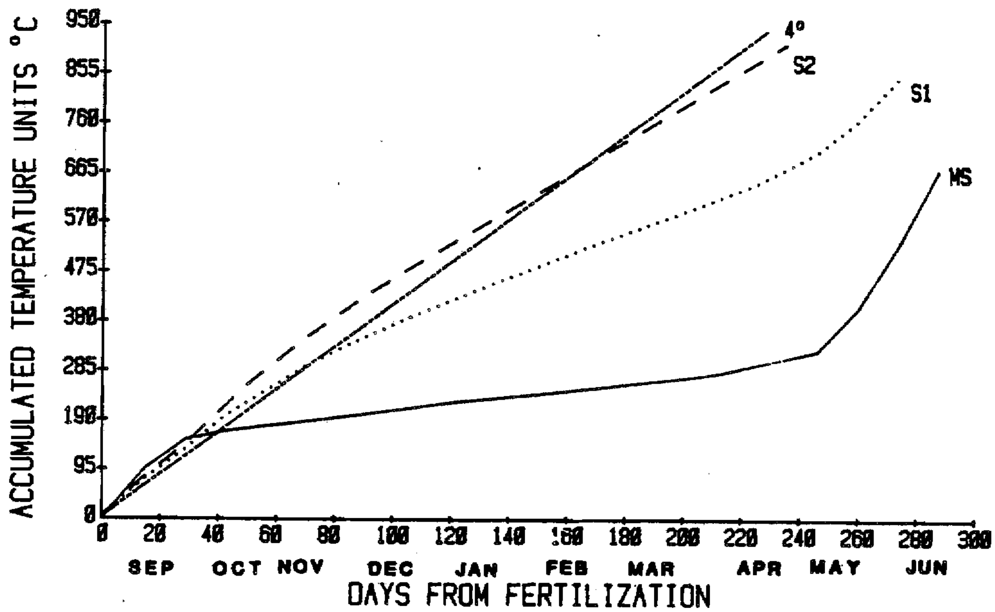


Figure 4. Accumulated temperature units at four different temperature regimes for the Susitna River egg incubation study. Data were plotted from 15 September 1983, the third of three egg collection dates when chum and sockeye ova were fertilized. The four regimes simulated the Susitna main stem (MS), Slough 8A (S2), an intermediate regime (S1), and 4°C Constant (4°).

thermal regime required 88 days to reach 200 TUs, while eggs from the first collection period required only 31 days to reach 200 TUs.

Chum Salmon

Five of the 24 incubator trays containing chum salmon were eliminated from analysis of growth and survival due to uncontrolled (lethal) stresses. These represented S2 (first and second egg collections) and the 4°C Constant replicate for all three egg collections. When 50 percent or more of the eggs remained viable, however, the subsequent data from those trays were included in the evaluations of time to hatch and complete yolk absorption.

Incubation timing and survival:

Computations of the average temperature (for each regime) to various developmental stages allowed for standardized comparisons between temperature regimes. The average water temperature from egg fertilization to complete yolk absorption described a colder to warmer trend between the main stem, S1, S2 and 4°C Constant temperature regimes (Table 2).

Table 2. Average water temperature during incubation of chum eggs and alevins to 50 percent hatch and complete yolk absorption in four temperature regimes. (Data were pooled from replicates and egg collections.)

	Average water temperature (°C)	
Temperature regime	50 Percent hatch	Complete yolk absorption
Main stem	1.7	2.2
S1	3.6	2.9
S2	4.6	3.9
Constant 4°C	4.0	4.0

The number of days required from egg fertilization to 50 percent hatch and complete yolk absorption (Appendix 1) is inversely proportional to increases in temperature between the four temperature regimes (Fig. 5). Eggs required about 61 more days to reach 50 percent hatch and alevins required 70 more days to complete yolk absorption in the main-stem temperatures, as compared to the Constant 4°C or S2 temperatures.

In contrast, the accumulation of TUs is directly proportional to the increases in temperature between the four study regimes for egg hatching and complete yolk absorption (Fig. 6). Eggs required about 187 fewer TUs to 50 percent hatch and alevins required 239 fewer TUs to complete yolk absorption in the main-stem temperatures as compared to the Constant 4°C or S2 temperatures (Appendix 1).

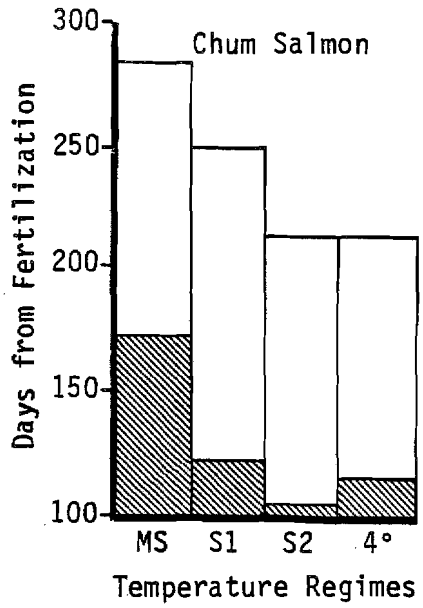


Figure 5. Days from fertilization to 50% hatch (cross-hatched bars) and complete yolk absorption (open bars) for chum salmon at four different temperature regimes which simulated the Susitna main stem (MS), Slough 8A (S2), an intermediary (S1), and 4°C Constant (4°). (Data were pooled from three fertilization dates in September and from study replicates.)

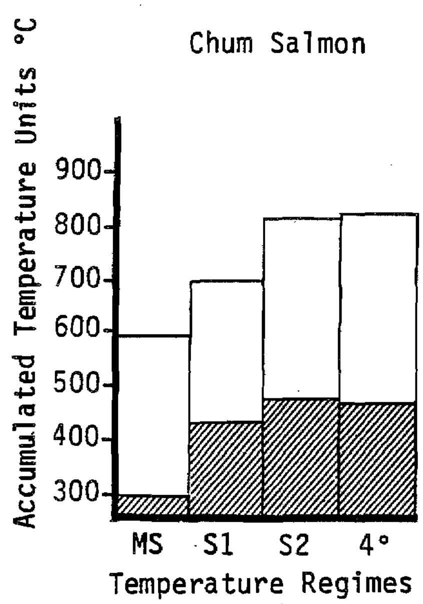


Figure 6. Accumulated temperature units (°C) to reach 50% hatch (cross-hatched bars) and complete yolk absorption (open bars) for chum salmon at four different temperature regimes which simulated the Susitna main stem (MS), Slough 8A (S2), an intermediary (S1), and 4°C Constant (4°). (Data were pooled from three fertilization dates in September and from study replicates.)

Thus, if chum salmon were spawning from early to mid-September in temperature regimes similar to those in this study, hatching would take place from mid-December to mid-March, and complete yolk absorption from early April to late June (Table 3).

Table 3. Dates for hatching and complete yolk absorption for chum eggs and alevins incubated in four temperature regimes based on spawning dates of September 3 and 15.

Temperature regime	Spawning date	25 Percent hatch	50 Percent hatch	75 Percent hatch	Complete yolk absorption
Main stem	03	Feb 06	Feb 11	Feb 16	Jun 16
Main stem	15	Mar 08	Mar 16	Mar 20	Jun 24
S1	03	Dec 28	Dec 29	Jan 01	May 08
S1	15	Jan 20	Jan 22	Jan 24	May 22
S2	03	Dec 15	Dec 17	Dec 18	Apr 06
S2	15	Dec 27	Dec 29	Dec 31	Apr 17
Constant 4°C	03	Dec 29	Dec 31	Jan 02	Apr 07
Constant 4°C	15	Jan 07	Jan 09	Jan 13	Apr 14

Chum egg and alevin survival was greater than 90 percent (Appendix 1) for all four temperature regimes (Fig. 7). Abnormalities noted in the main-stem temperature regime (0-2.2 percent) included curved spines, deformed body parts, and "head-first" hatching. The number of abnormalities noted in the other three temperature regimes was generally less than 0.1 percent in each incubator tray (Appendix 1).

Lengths and weights:

Weight measurements were taken through 50 percent hatch, and for selected alevins at complete yolk sac absorption. However, the weighing process was

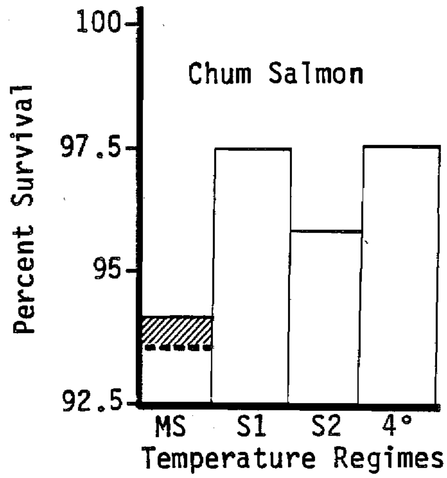


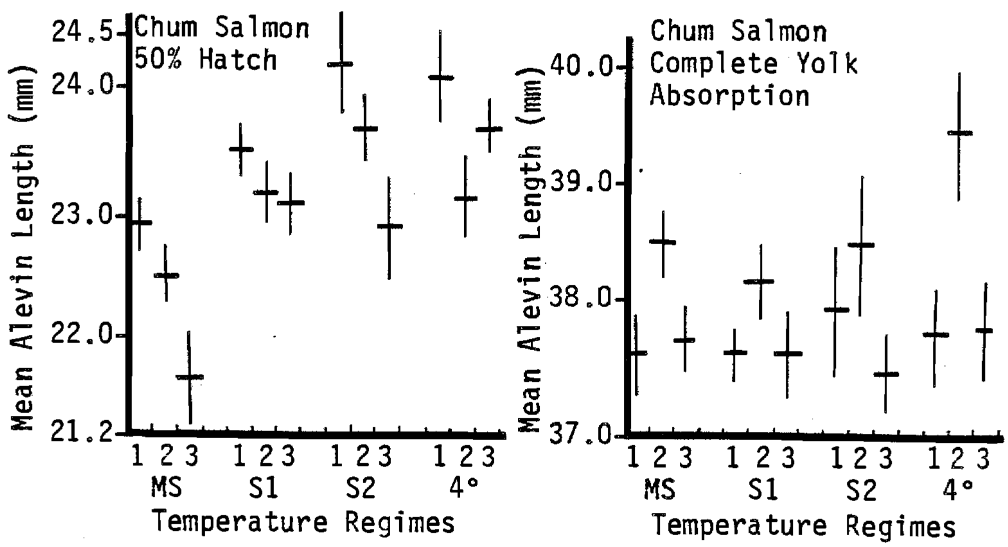
Figure 7. Percent survival for chum salmon eggs and alevins reared to complete yolk absorption at four different temperature regimes which simulated the Susitna main stem (MS), Slough 8A (S2), an intermediary (S1), and 4°C Constant (4°). Cross-hatched area represents percent of abnormalities among survivors. (Data were pooled from three fertilization dates in September and from study replicates.)

time consuming and the results (from blot drying) had little interpretive value between temperature regimes, so the measurements were discontinued. The mean weight for all chum alevins at 50 percent hatch was 0.20 g ± 0.01 g (95 percent CI). No weight analysis was performed between egg collections.

A one-way analysis of variance (ANOVA) for alevin lengths at 50 percent hatch for all temperature regimes and egg collections (minus the deleted trays mentioned earlier) revealed a statistically significant difference existed between the groups ($P<0.01$)($df=11$). With some exceptions, temperature regimes with colder average temperatures resulted in smaller alevin lengths at 50 percent hatch (Fig. 8). A Duncan multiple comparison test ($P=0.01$) combined statistically similar groups which resulted in a separation of the warmest temperature regime from the coldest (Table 4). The difference in length from the smaller to larger alevins was 2.5 mm (11 percent).

Table 4. Mean lengths of chum alevins at 50 percent hatch which are bracketed into statistically similar groups (P=0.01) and their corresponding temperature regime and egg collection. (Data were pooled within replicates.)

Temperature regime	Egg collection date (September)	Average temperature (°C)	Mean length (mm)
Main stem	15	1.5	21.7
Main stem	9	1.7	22.5
Main stem	3	2.0	22.9
S2	15	4.6	22.9
S1	15	3.4	23.1
Constant 4°C	9	4.0	23.1
S1	9	3.6	23.2
S1	3	3.9	23.5
S2	9	4.7	23.7
Constant 4°C	15	4.0	23.7
Constant 4°C	3	4.0	24.1
S2	3	4.7	24.2



Figures 8 and 9. Mean lengths (horizontal lines) of chum salmon alevins at 50% hatch and at total yolk absorption from three fertilization dates (1=September 3; 2=September 9; 3=September 15) within each of four different temperature regimes. The temperature regimes simulated the Susitna main stem (MS), Slough 8A (S2), an intermediary (S1), and 4°C Constant (4°). (Data were pooled from study replicates. Vertical lines represent 95% confidence intervals.)

Length analysis (one-way ANOVA) of chum alevins at complete yolk absorption also revealed a significant difference between the groups of temperature regimes and egg collections ($P<0.01$)($df=11$). Mean lengths and 95 percent confidence intervals of these groups are plotted in Fig. 9. The difference in length between the smallest and largest group was 1.9 mm (5 percent).

Growth and temperature unit accumulation:

Alevin growth (total length) was plotted versus accumulated temperature units (°C) for all four temperature regimes of the first egg collection (Figs. 10 and 11) and for three temperature regimes of the third egg collection (Fig. 12). Comparative differences (TUs and days to 50 percent hatch and CYA) within a single egg collection were mentioned previously. Variations in growth curves (length vs TUs) are noted here. A greater deflection in the slope of the main-stem growth curves as compared to S1 and S2 was observed (Figs. 10 and 12). The greatest change in slope for the main-stem growth curves appeared at about 380 TUs for the first egg collection and about 315 TUs for the third egg collection. For both egg collections in the main-stem temperature regime the decrease in slope in Figures 10 and 12 represented the effect of increased water temperature (greater than 1°C) which occurred during the first week in May. Thus, while development and growth were faster over time (days) in the 4°C and S2 temperature regimes than the S1 or MS regimes (Figs. 5 and 10), the alevin growth rate increased as a function of accumulated temperature units when water temperature was less than 1°C (Figs. 10 and 12).

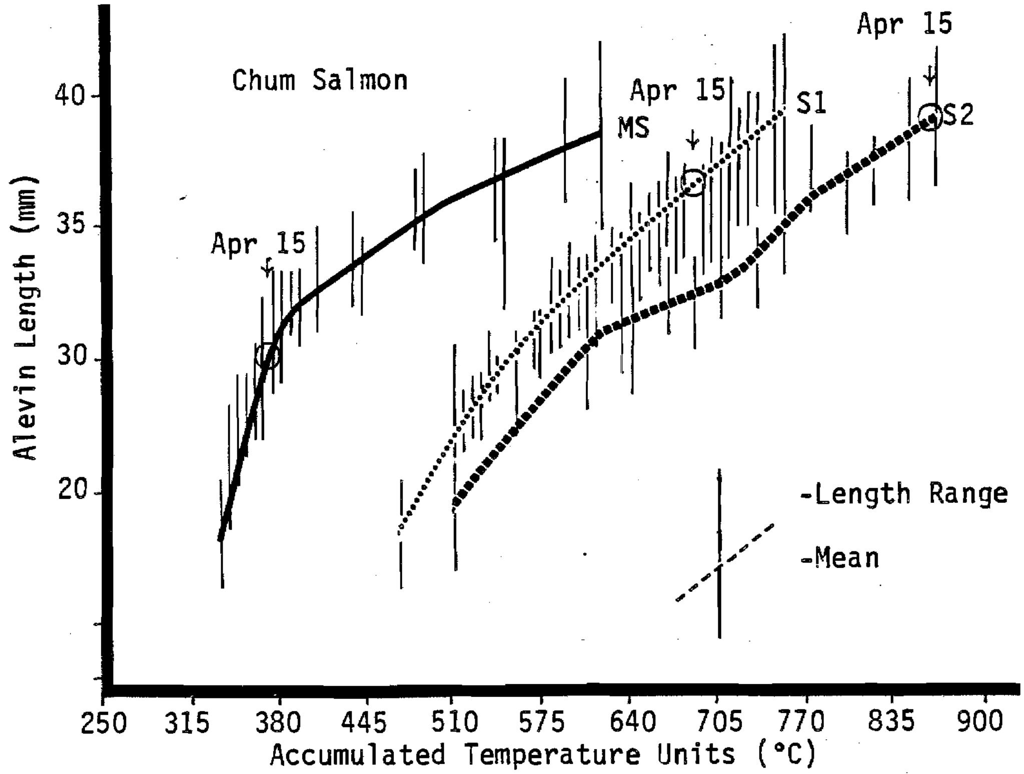


Figure 10. Alevin growth (total length) from 50% hatch to complete yolk absorption for chum salmon incubated at three different temperature regimes. The regimes simulated the Susitna main stem (MS), Slough 8A (S2), and an intermediary (S1). (Data are based on a fertilization date of September 3. Data from replicates were pooled.)

The constant 4°C water temperature regime is presented in a separate figure because of an almost complete overlap of data points with S2. Similarly, the growth curves for S1, S2 and Constant 4°C are almost identical when the first and third egg collections are compared. Differences between the main-stem growth curves for the first and third egg collections are due to the colder average water temperatures experienced by eggs from the third egg collection.

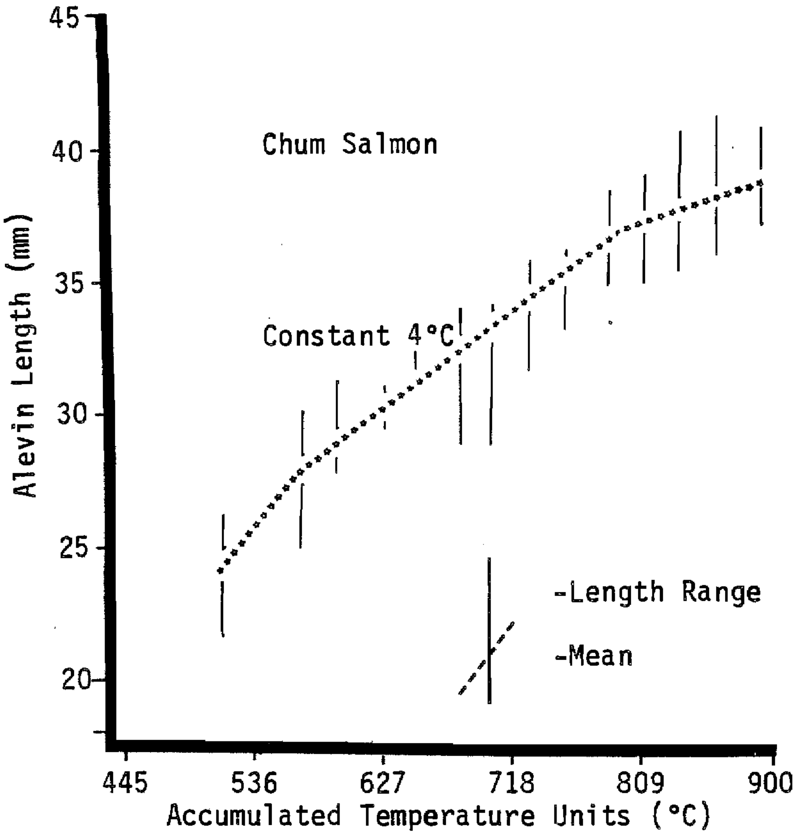


Figure 11. Alevin growth (total length) from 50% hatch to complete yolk absorption for chum salmon incubated at Constant 4°C. (Data are based on a fertilization date of September 3.)

Development rates:

Development rates were computed (to 50 percent hatch and complete yolk absorption) and plotted from results of this study and available literature on chum incubation (Figs. 13 and 14). A regression analysis was performed on the data for each incubation stage. In each regression r is equal to 0.99 (Table 5). Thus chum development rates are predictable for known temperature regimes.

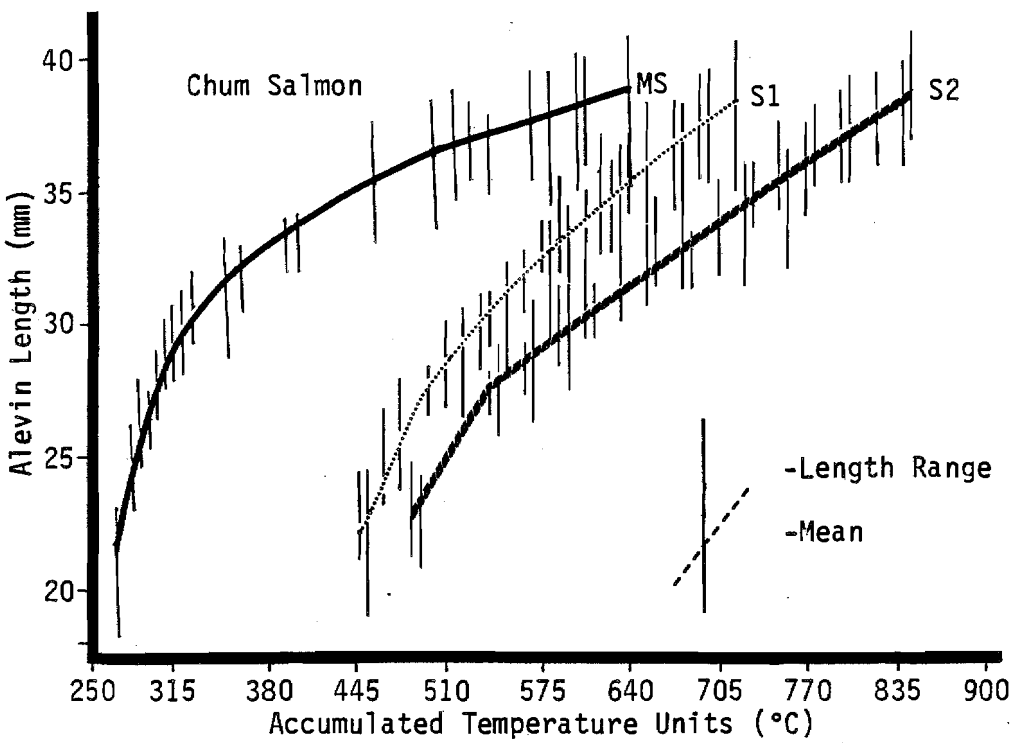


Figure 12. Alevin growth (total length) from 50% hatch to complete yolk absorption for chum salmon incubated at three different temperature regimes. The regimes simulated the Susitna main stem (MS), Slough 8A (S2), and an intermediary (S1). (Data are based on a fertilization date of September 15. Data from replicates were pooled.)

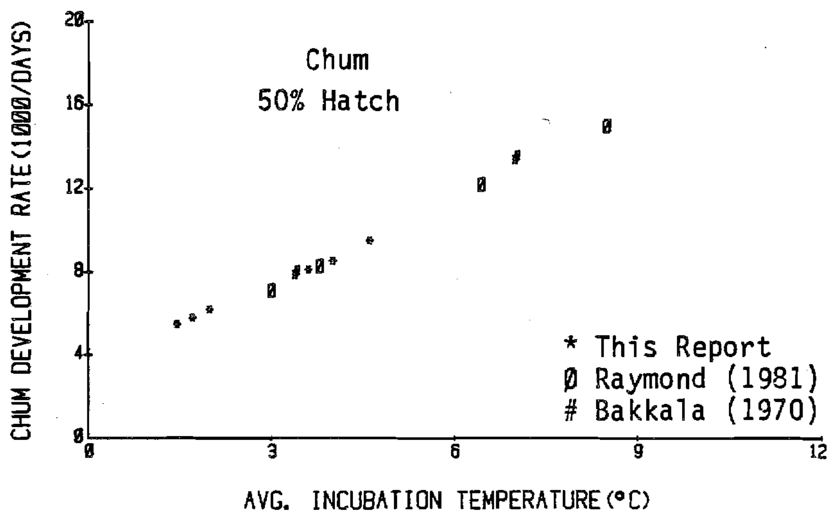


Figure 13. Development rates to 50% hatch for chum salmon incubated at various temperatures (°C). The reciprocal of the days to 50% hatch was multiplied by 1000.

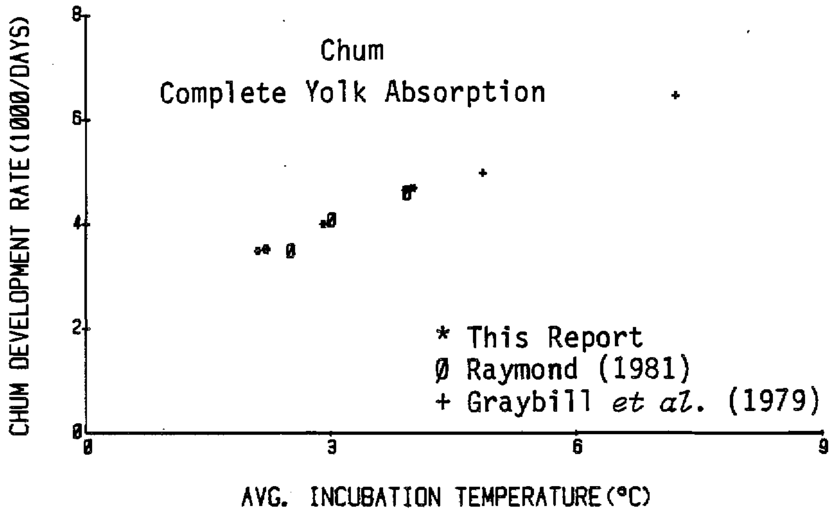


Figure 14. Development rates to complete yolk absorption for chum salmon incubated at various temperatures (°C). The reciprocal of the days to complete yolk absorption was multiplied by 1000.

Table 5. Regression analysis of development rates at various average incubation temperatures for chum alevins to 50 percent hatch and complete yolk absorption from data in Figs. 13 and 14.

Incubation stage	Analysis	n	Slope	Y intercept	r	t-statistic
50 percent hatch	y = mx + b	12	1.40	3.23	0.99	P<0.001
Complete yolk absorption	y = mx + b	11	0.59	2.25	0.99	P<0.001

Sockeye Salmon

Six of the 24 incubator trays containing sockeye salmon were eliminated from analysis of growth and survival due to uncontrollable (lethal) stresses. These represented the thermal regimes from S1 (second egg collection), S2 (second egg collection), S2 replicate (all three egg collections) and the Constant 4°C replicate (second egg collection). As with our analysis for chum salmon, when 50 percent or more of the eggs remained viable, the subsequent data from those trays were included in the evaluation of time to hatch and complete yolk absorption.

Incubation timing and survival:

Average temperature (for each regime) was calculated (Appendix 1) to make comparisons between temperature regimes and timing to 50 percent hatch and complete yolk absorption (Table 6).

Table 6. Average water temperature during incubation of sockeye eggs and alevins to 50 percent hatch and complete yolk absorption in four temperature regimes. (Data were pooled from replicates and egg collections.)

	Average water temperature (°C)	
Temperature regime	50 Percent hatch	Complete yolk absorption
Main stem	1.5	2.1
S1	3.3	3.0
S2	4.2	3.9
Constant 4°C	4.0	4.0

Sockeye eggs in the main-stem temperature regime required about 71 more days to reach 50 percent hatch and alevins required 56 more days to complete yolk absorption as compared to the Constant 4°C or S2 temperatures (Fig. 15).

In comparison, eggs required about 291 fewer TUs (Appendix 1) to 50 percent hatch and alevins required 301 fewer TUs to complete yolk absorption in the main-stem temperatures than in the Constant 4°C or S2 temperatures (Fig. 16).

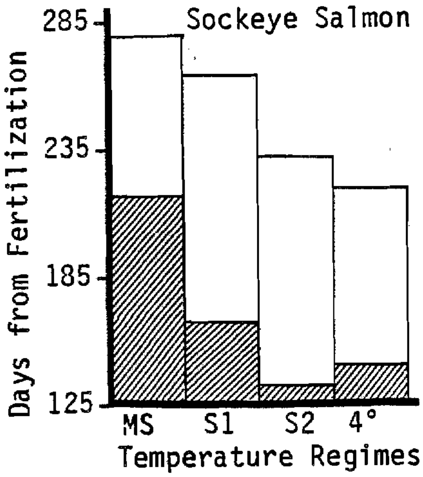


Figure 15. Days from fertilization to 50% hatch (cross-hatched bars) and complete yolk absorption (open bars) for sockeye salmon at four different temperature regimes which simulated the Susitna main stem (MS), Slough 8A (S2), an intermediary (S1), and 4°C Constant (4°). (Data were pooled from three fertilization dates in September and from study replicates.)

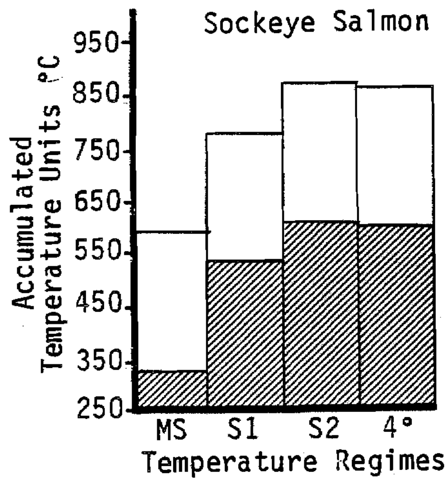


Figure 16. Accumulated temperature units (°C) to reach 50% hatch (cross-hatched bars) and complete yolk absorption (open bars) for sockeye salmon at four different temperature regimes which simulated the Susitna main stem (MS), Slough 8A (S2), an intermediary (S1), and 4°C Constant (4°). (Data were pooled from three fertilization dates in September and from study replicates.)

Therefore, if sockeye salmon were to spawn from early to mid-September in temperature regimes similar to those in this study, hatching would take place from late January to early May and complete yolk absorption would take place from mid April to late June (Table 7).

Sockeye egg and alevin survival was greater than 90 percent (Appendix 1) for all temperature regimes (Fig. 17). Developmental abnormalities noted in incubator trays with main-stem and Constant 4°C temperatures ($\leq$0.3 percent)

Table 7. Dates for hatching and complete yolk absorption for sockeye eggs and alevins incubated in four temperature regimes based on spawning dates of September 3 and 15.

Temperature regime	Spawning date	25 Percent hatch	50 Percent hatch	75 Percent hatch	Complete yolk absorption
Main stem	03	Mar 24	Mar 29	Apr 05	Jun 14
Main stem	15	May 01	May 05	May 10	Jun 25
S1	03	Feb 05	Feb 11	Feb 15	May 26
S1	15	Mar 01	Mar 05	Mar 09	Jun 09
S2	03	Jan 16	Jan 20	Jan 23	Apr 14
S2	15	Feb 04	Feb 10	Feb 12	May 05
Constant 4°C	03	Jan 28	Feb 01	Feb 04	Apr 12
Constant 4°C	15	Feb 11	Feb 14	Feb 17	Apr 26

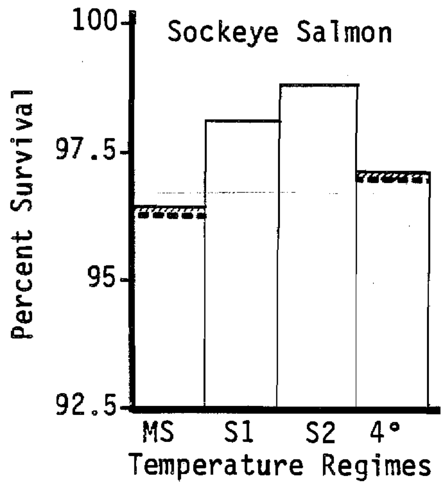


Figure 17. Percent survival for sockeye salmon eggs and alevins reared to complete yolk absorption at four different temperature regimes which simulated the Susitna main stem (MS), Slough 8A (S2), an intermediary (S1), and 4°C Constant (4°). Cross-hatched area represents percent of abnormalities among survivors. (Data were pooled from three fertilization dates in September and from study replicates.)

included curved spines, twinning, double heads and tails, and head-first hatching (Appendix 1). Abnormalities in S1 and S2 temperature regimes remained less than 0.1 percent in all incubator trays.

Lengths and weights:

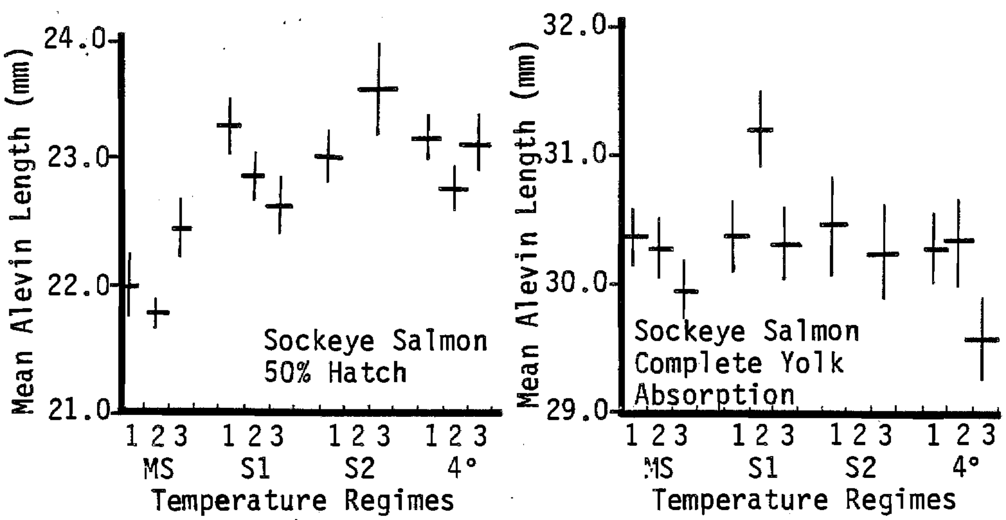
Analysis of sockeye weights at 50 percent hatch provided little interpretive information. The mean weight of sockeye at 50 percent hatch was 0.11 g. No variation was observed between temperature regimes or egg collections when evaluated by a one-way ANOVA and a Duncan multiple comparison test (P=0.01) (df=10).

A one-way ANOVA for alevin lengths at 50 percent hatch for all temperature regimes and egg collections (minus the deleted trays mentioned earlier) revealed a statistically significant difference between the groups (P<0.01) (df=10). Similar to the chum lengths at 50 percent hatch, a trend was observed wherein colder average temperatures resulted in smaller alevin lengths (Fig. 18). A Duncan multiple comparison test (P=0.01) combined statistically similar groups which resulted in the separation of the coldest and warmest temperature regimes (Table 8). The difference in length from the smaller to larger alevins was 1.8 mm (8 percent).

Length analysis (one-way ANOVA) of sockeye alevins at complete yolk absorption also revealed a significant difference between the groups of temperature regimes and egg collections (P<0.01)(df10). No trend was observed between mean lengths of alevins within the four regimes (Fig. 19). The difference in length between the smallest and largest groups was 1.7 mm (5.7 percent). (If those two groups were eliminated the range difference would be 0.6 mm.)

Table 8. Mean lengths of sockeye alevins at 50 percent hatch which are bracketed into statistically similar groups (P=0.01) and their corresponding temperature regime and egg collection. (Data were pooled within replicates.)

Temperature regime	Egg collection date (September)	Average temperature (°C)	Mean length (mm)
Main stem	09	1.5	21.8
Main stem	03	1.7	22.0
Main stem	15	1.3	22.5
S1	15	3.1	22.7
Constant 4°C	09	4.0	22.8
S1	09	3.3	22.9
S2	03	4.3	23.1
Constant 4°C	15	4.1	23.2
Constant 4°C	03	4.0	23.2
S1	03	3.4	23.3
S2	15	4.2	23.6



Figures 18 and 19. Mean lengths (horizontal lines) of sockeye salmon alevins at 50% hatch and at total yolk absorption from three fertilization dates (1 = September 3; 2 = September 9; 3 = September 15) within each of four different temperature regimes. The temperature regimes simulated the Susitna main stem (MS), Slough 8A (S2), an intermediary (S1), and 4°C Constant (4°). (Data were pooled from study replicates. Vertical lines represent 95% confidence intervals.)

Growth and temperature unit accumulation:

Alevin growth (total length) was plotted versus accumulated temperature units (°C) for all four temperature regimes of the first egg collection (Figs. 20 and 21) and for three temperature regimes of the third egg collection (Fig. 22). The greatest change in growth-curve slope for the alevins of the first egg collection occurred in the main-stem temperature regime at about 380 TUs. This coincides with the increase in incubation temperature above 1°C.

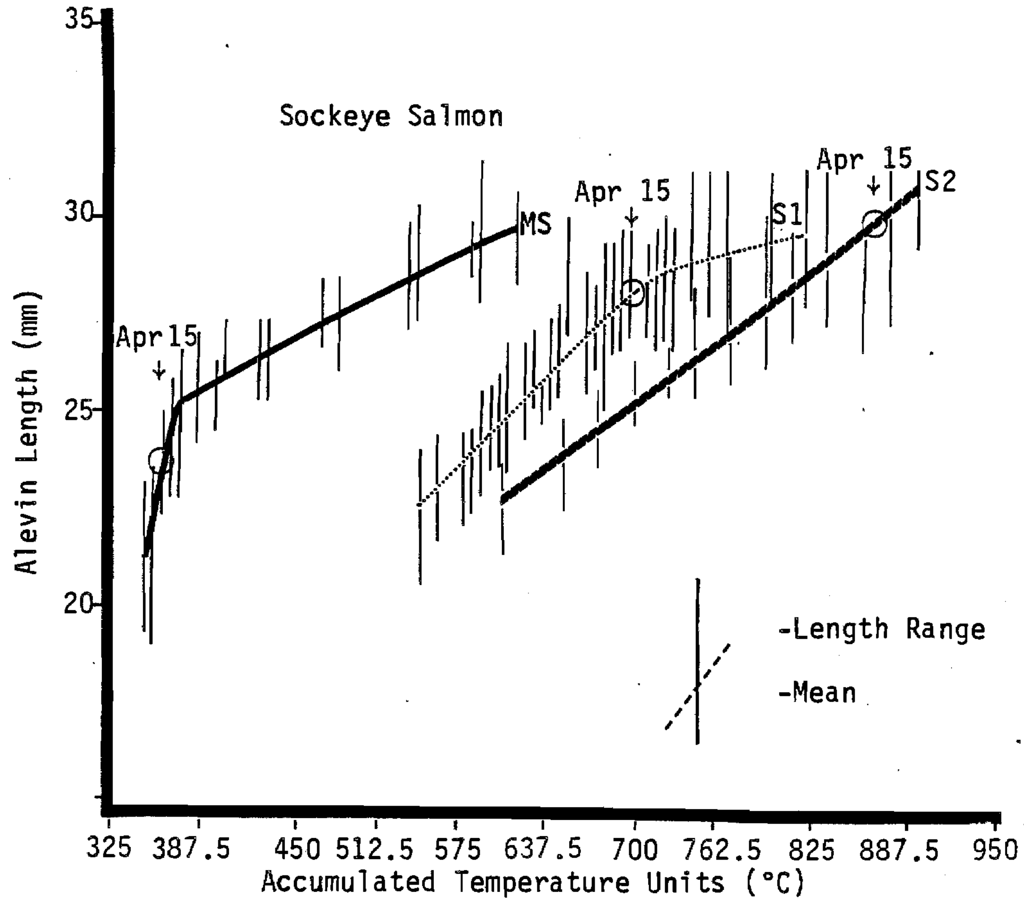


Figure 20. Alevin growth (total length) from 50% hatch to complete yolk absorption for sockeye salmon incubated at three different temperature regimes. Regimes simulated Susitna main stem (MS), Slough 8A (S2) and an intermediary (S1). (Data are based on a fertilization date of September 3. Data from replicates were pooled.)

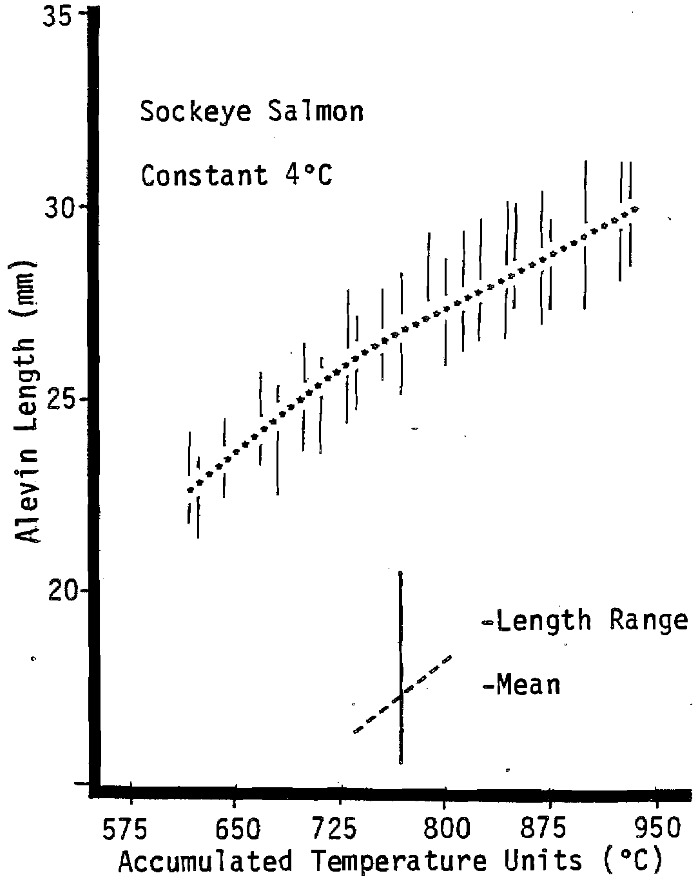


Figure 21. Alevin growth (total length) from 50% hatch to complete yolk absorption for sockeye salmon incubated at Constant 4°C. (Data are based on a fertilization date of September 3.)

The largest change in growth-curve slope for the alevins of the third egg collection occurred in the main-stem temperature regime at about 530 TUs. This coincides with an increase of water temperature above 10°C. The eggs of this regime (MS) had not reached 50 percent hatch until early May, when water temperatures were greater than 1°C (Fig. 22).

The Constant 4°C water temperature regime is presented separately (Fig. 21) because of an almost complete overlap of data points with S2. Similarly, the

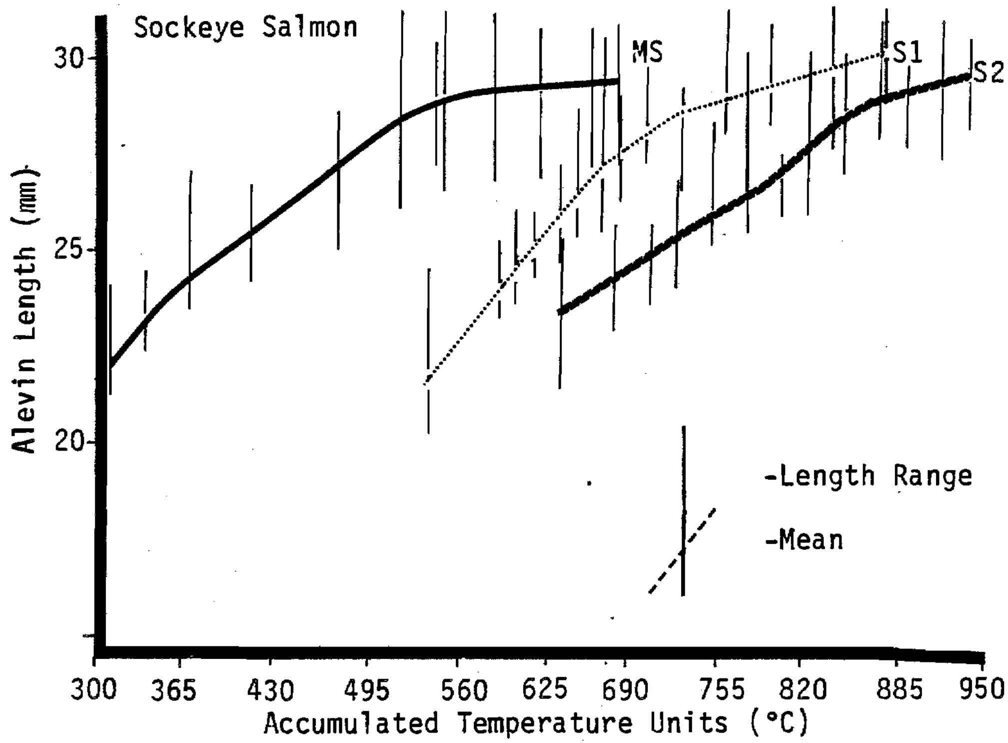


Figure 22. Alevin growth (total length) from 50% hatch to complete yolk absorption for sockeye salmon incubated at three different temperature regimes. Regimes simulated Susitna main stem (MS), Slough 8A (S2), and an intermediary (S1). (Data are based on a fertilization date of September 15. Data from replicates were pooled.)

growth curves for S1, S2 and Constant 4°C are almost identical when the first and third egg collections are compared.

Development rates:

Development rates were computed (to 50 percent hatch and complete yolk absorption) and plotted for data from this study and from data available in the literature (Figs. 23 and 24). A regression analysis was performed on the data for each incubation stage. The r value for each regression line was equal to 0.99 (Table 9).

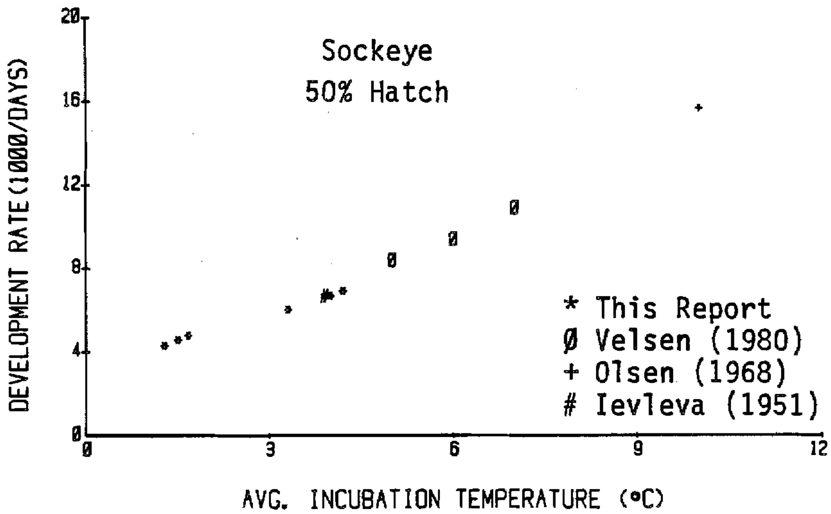


Figure 23. Development rates to 50% hatch for sockeye salmon incubated at various temperatures (°C). The reciprocal of the days to 50% hatch was multiplied by 1000.

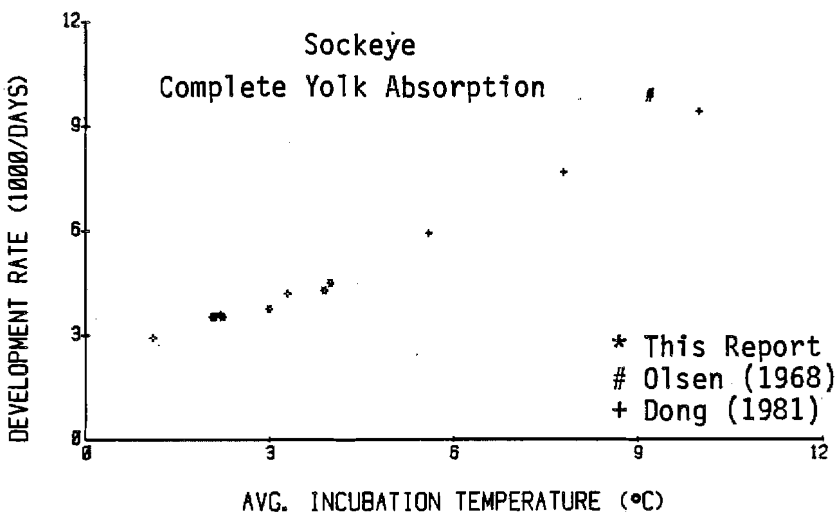


Figure 24. Development rates to complete yolk absorption for sockeye salmon incubated at various temperatures (°C). The reciprocal of the days to complete yolk absorption was multiplied by 1000.

Table 9. Regression analysis of development rates at various average incubation temperatures for sockeye alevins to 50 percent hatch and complete yolk absorption from data in Figs. 23 and 24.

Incubation stage	Analysis	n	Slope	Y intercept	r	t-statistic
50 percent hatch	lny = lnb + mx	11	0.15	3.71	0.99	P<0.001
Complete yolk absorption	lny = lnb + mx	13	0.14	2.61	0.99	P<0.001

Thus, sockeye development rates are highly predictable within known temperature ranges.

Discussion

Field observations indicate that chum and sockeye fry achieve complete yolk absorption in Susitna sloughs by early April, and mid to late April, respectively (ADF&G 1983). These findings agree well with our results (Tables 3 and 7) for the S2 (Slough 8A simulation) and Constant 4°C temperature regimes.

Our study shows that Susitna River chum salmon achieve complete yolk sac absorption within 218 days of fertilization (Fig. 5) when incubated at an average 3.9°C (see S2, Table 2) in a regime which simulated temperatures in Slough 8A. Incubation at a constant temperature of 4°C produced essentially the same result. Incubation at our two coldest regimes (S1 and MS), however, lengthened the amount of time required for complete yolk absorption by about one and two months, respectively. These findings were similar for Susitna sockeye salmon which were incubated simultaneously within the same four temperature regimes (Fig. 15 and Table 6).

It has been suggested that some salmonids can regulate development rates (which determines incubation time) when subjected to altered temperature regimes (Dong 1981). The ability of an egg or alevin to compensate their development rate for temperature changes could assist in achieving yolk absorption at the most optimal time for fry emergence. In this study the similarities in incubation time (TUs and days) between S2 and 4°C (constant) for chum and sockeye to reach complete yolk absorption is explained by the similar average incubating temperature to yolk absorption within each regime (Figs. 3 and 4 and Tables 2 and 6). The longer incubation periods (30-60 days) for alevins to complete yolk absorption within our coldest regimes (MS and S1) were also proportional to the decrease in the number of TUs accumulated. Therefore, it is evident that chum and sockeye from Slough 11 do not have the ability to regulate their development rates to result in a similar number of days to complete yolk absorption when average incubation temperatures vary from 2.1 to 4°C (as simulated by MS and 4°C). However, temperature compensation is noted for growth as a function of _accumulated temperature units_ (particularly below 1°C, see Figs. 10 and 20).

It is possible that the average post-project temperature regime will be outside the range we evaluated in this study (2.1 to 4°C). As our data are directly applicable to a range at or below 4°C, it would be useful if a predictive model could estimate incubation timing for chum and sockeye beyond this range. This information is presented below. First however, we will suggest two possible temperature scenarios (which may result from reservoir construction) so that subsequent data presentations may be placed into perspective.

First, a slight increase in the annual average water temperature of the Susitna main stem may elevate winter ground-water temperatures in the sloughs. A warmer average, post-project temperature (for example, 4.5°C) could result in an earlier fry emergence as compared to our warmest temperature regime (4°C Constant).

Conversely, fluctuations in winter discharge in the main stem could result in increased channel ice formation. Increased ice staging in the river may divert main stem flows and inundate sloughs with 0°C water. This event occurred naturally in Slough 8A during the winter of this study. If completion of the incubation process were delayed by one to two months (as occurred in our main stem and S1 temperature simulations), smoltification of the chum can also be expected to be delayed. Consequently, ocean survival of salmon can be reduced if the parr-smolt transformation occurs "out of phase" to the historical smolt timing because temperature has decreased or increased alevin development (Folmar *et al.* 1982). The effect on sockeye salmon is less clear as this species will rear for another one to two years outside of the slough habitat before their outmigration as smolts.

Our results with Susitna River salmon indicate slight increases in mortalities and abnormalities begin when average incubation temperatures to hatch are less than 3.4°C for chum and sockeye, or when initial incubation temperatures are equal to 4°C for sockeye (Figs. 7 and 17, Tables 4 and 8). Our coldest temperature regime was represented by the egg collection of 15 September incubating in the main-stem simulation. Chum and sockeye eggs were incubating for 31 days with about 156 TUs (5°C average) before water temperatures were decreased to 1.4°C in this regime. Sockeye eggs are noted for being vulnerable to temperature stress before closure of the blastopore, which occurs at about 28 days and 140 TUs (5°C average)(Velsen 1980; Bams 1967; Combs 1965). Thus, our coldest temperature regime did not subject the eggs to thermal stresses before they could successfully adjust.

Bailey and Evans (1971) reported an increase in mortalities for pink salmon (*O. gorbuscha*) when initial incubation was below 4.5°C, and complete mortality occurred when initial incubation was below 2.0°C. Studies with coho salmon (*O. kisutch*) have also noted 100 percent mortality when eggs were initially incubated in temperatures below 1.0°C (Dong 1981).

This type of cold-water stress (early in the incubation process) does not appear to be a likely post-project event in the Susitna. The inundation of sloughs later in the winter however, could quickly reduce incubating temperatures by 2 to 3°C. While this would definitely alter the timing of development processes, it appears that salmonid eggs are not lethally stressed by such water-temperature changes when they are past closure of their blastopores.

The mean length of chum and sockeye alevins was significantly smaller at hatching when incubated in colder average temperature regimes (MS and S1 vs S2 and 4°C)(Tables 4 and 8). These results have also been noted for variations of other environmental conditions such as volume of eggs per incubator, dissolved oxygen levels, substrate type and water flow (Kapuscinski and Lannan 1983; Fuss and Johnson 1982; Peterson *et al.* 1977; Garside 1959; Hayes *et al.* 1953). Hatching can be advanced or delayed depending upon incubating conditions (Garside 1959), and Hayes *et al.* (1953) suggested cold water temperatures would be expected to promote early hatching (relative to alevin length development). In addition, the mean alevin lengths at complete yolk absorption for chum and sockeye (Figs. 9 and 19) did not reveal the corresponding differences between temperature regimes found at 50 percent hatch. Therefore, we did not observe a less efficient development process in cold water at hatching, but rather, the alevins in colder water temperatures had hatched earlier relative to length development. Dong (1981) also concluded that the metabolic efficiency in coho alevins was comparable or higher at 1.3°C as opposed to 4.0 or 6.1°C. Temperatures higher than 6.1°C resulted in smaller alevin lengths at complete yolk absorption (Dong 1981).

While the metabolic efficiency appears to be similar in our study temperatures, the growth rate did not. A clear increase in growth rates (as a function of days) resulted from increasing water temperatures above 1.0°C in the main-stem temperature regimes for both species (Figs. 10, 12, and 20; note April 15 in Figs. 10 and 20). The increased growth rate with increased temperature overshadows the compensation that takes place with growth rates as a function of accumulated temperature units.

A literature review and results from this study have produced a useful predictive tool for estimating the number of days needed from fertilization to hatching or complete yolk absorption for chum and sockeye salmon in the Susitna River System. Development rates have been calculated for both species given several average incubation temperatures and the information in Tables 5 and 9. The development rates were then converted to produce estimates of days to 50 percent hatch and complete yolk absorption (Table 10).

Table 10. Estimates of days to 50 percent hatch and complete yolk absorption (CYA) for Susitna River chum and sockeye salmon for a range of average incubating temperatures.

Average Incubating Temperature (°C)	Chum		Sockeye	
	50 percent hatch	CYA	50 percent hatch	CYA
1.5	188	319	217	313
2.0	166	292	200	292
2.5	149	269	186	272
3.0	135	249	172	254
3.5	123	232	160	238
4.0	113	217	148	222
4.5	105	204	138	207
5.0	98	193	128	193

Most water temperature scenarios that could occur downstream of operational dams on the Susitna River should be addressed by the range of average incubating temperatures in Table 10. If, for example, a model of post-project water temperature predicts that a slough (with incubating salmon eggs) will have an average temperature of 4.5°C from September to May, the number of days required for chum and sockeye to reach complete yolk absorption would be estimated at 204 and 207 days (Table 10), respectively. This is 13 days earlier for chum and 15 days earlier for sockeye as compared to an average 4°C incubating regime (Table 10). Acceleration of the incubation process by 15 days would not be considered deleterious to chum and sockeye populations spawning within Susitna River sloughs. Those populations are spawning naturally for over a 30-day period with a large percent of the population in good spawning condition for over 15 days (ADF&G 1983). Conversely, ice processing may redirect main-stem flows which could inundate sloughs and reduce average incubating temperatures to 2.0°C (as an example). If this were to occur, complete yolk absorption would be delayed about 75 days for chum alevins and 70 days for sockeye alevins as compared to an average 4°C temperature regime (Table 10).

It is reasonable to assume that delaying the incubation process beyond 15 to 20 days will begin to adversely affect the chum population within the Susitna River sloughs. One hypothesis suggests that adult salmon run timing and subsequently their spawning time is genetically controlled (Ricker 1972) and adapted to historical water temperatures in their specific drainage (Miller and Brannon 1981). This hypothesis also identifies the selection pressure for a specific spawning period as the optimum time for fry emergence for each species. If the timing of chum fry emergence is altered beyond the range of historical emergence dates, and the run timing is genetically controlled, it would seem the population may not be able to rapidly compensate for any resulting poor survival of smolt. As mentioned previously, the timing of the parr-smolt transformation is known to play a role in the successful ocean survival of salmon smolt. It may be possible to indirectly quantify chum smolt survival as it is affected by various emergence times via salt water challenge tests. This stress test is referenced as an indicator of smolt viability (Wedemeyer and McLeay 1981).

An adverse effect on sockeye fry survival would also be anticipated if the preceeding hypothesis is correct. However, quantification of survival for sockeye fry from Susitna sloughs would be difficult as they generally move out of the sloughs after emergence and rear for one to two years throughout the Susitna drainage prior to outmigrating as parr/smolt (ADF&G 1983).

Altering the thermal regimes and discharges of a major river may have many effects beyond those observed on salmon eggs and alevins. The results from this study indicate incubation timing is a factor which will be affected depending on the degree of water temperature alteration. All salmon fresh water life stages will be affected by changes in temperature regimes which act in concert with other factors as physiological regulators (metabolism and growth) and behavioral stimuli (migration and overwintering strategies). To the extent that post-project temperature regimes can be predicted, resource planners should be able to use data from this report to determine if incubation timing will be affected and to attain management, mitigation or enhancement objectives.

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Literature Cited

Alaska Department of Fish and Game. 1983. Susitna Hydro Aquatic Studies Phase II Final Data Report Vol. 2 and 3, 1982.

Alderdice, D.F. and F.P.J. Velsen. 1978. Relation between temperature and incubation time for eggs of chinook salmon (*Oncorhynchus tshawytscha*). J. Fish. Res. Board Can. 35:69-75.

Bailey, J.E. and D.R. Evans. 1971. The low-temperature threshold for pink salmon eggs in relation to a proposed hydroelectric installation. Fish. Bull. Vol. 69(3).

Bakkala, R.G. 1970. Synopsis of biological data on the chum salmon, *Oncorhynchus keta* (Walbaum). FAO Fisheries Synopsis No. 41. U.S. Fish Wild. Serv. Circ. 315.

Bams, R.A. 1967. A review of the literature on the effects of changes in temperature regime or developing sockeye salmon eggs and alevins. J. Fish. Res. Board Can., MS, 949:14-22.

Baxter, R.M. and P. Glaude. 1980. Environmental effects of dams and impoundments in Canada: experience and prospects. Can. Bull. Fish. Aquat. Sci. 205:34 p.

Combs, B.D. 1965. Effect of temperaturezon the development of salmon eggs. Prog. Fish. Cult. 27(3).

Dong, J.N. 1981. Thermal tolerance and rate of development of coho salmon embryos. M.S. thesis, Univ. Washington.

Folmar, L.C., W.W. Dickhoff, C.V.W. Mahnken, and F.W. Waknitz. 1982. Stunting and parr-reversion during smoltification of coho salmon (*Oncorhynchus kisutch*). Aquaculture 28:91-104.

Fuss, H.J. and C. Johnson. 1982. Quality of chum salmon fry improved by incubation over artificial substrates. Prog. Fish. Cult. (44)4.

Garside, E.T. 1959. Some effects of oxygen in relation to temperature on the development of lake trout embryos. Can. J. Zool. Vol. 37.

Graybill, J.P., R.L. Burgner, J.C. Gislason, P.E. Huffman, K.H. Wyman, R.G. Gibbons, K.W. Kruko, Q.J. Stober, T.W. Fagnan, A.P. Stayman, and D.M. Eggers. 1979. Assessment of the reservoir-related effects of the Skagit project on downstream fishery resources of the Skagit River, Washington. FRI-VW-7905. Univ. of Washington, Seattle.

Hayes, F.R., D. Pelluet, and E. Gorham. 1953. Some effects of temperature on the embryonic development of the salmon (*Salmo salar*). Can. J. of Zool. Vol. 31.

Ievleva, M.Ya. 1951. Morphology and rate of embryonic development of Pacific salmon. Jzvestiya Tikhookeanskogo nauchno Vol. 34, pp. 123-130.

Kapuscinski, A.R.D., and J.E. Lannan. 1983. On density of chum salmon (*Oncorhynchus keta*) eggs in shallow matrix substrate incubators. Can. J. Fish. Aquat. Sci. 40:185-191.

Leitritz, E. and R.C. Lewis. 1980. Trout and salmon culture (hatchery methods). Calif. Fish Bull. No. 164. University of California, Berkeley.

Miller, R.J. and E.L. Brannon. 1981. The origin and development of life history patterns in Pacific salmonids, p. 296-309. *In:* E.L. Brannon and E.O. Salo, editors, Proceedings of the salmon and trout migratory behavior symposium. School of Fisheries, Univ. Wash., Seattle, Washington.

Nie, N.H., C.H. Hull, J.G. Jenkins, K. Steinbrenner and D.H. Bert. 1975. Statistical package for the social sciences. McGraw-Hill Book Co.

Olsen, J.C. 1968. Physical environment and egg development in a mainland beach area and an island beach area of Iliamna Lake. *In:* Further Studies of Alaska Sockeye Salmon. Univ. Wash. Pub. in Fisheries. New Series Vol. III, Robert L. Burgner (ed.).

Peterson, R.H., H.C.E. Spinney, and A. Sreedharan. 1977. Development of

Atlantic salmon (*Salmo salar*) eggs and alevins under varied temperature regimes. J. Fish. Res. Board Can. 34:31-43.

Raymond, J.A. 1981. Incubation of fall chum salmon (*Oncorhynchus keta*) at Clear Air Force Station, Alaska. Alaska Dept. of Fish and Game Info. Leaflet No. 189.

Ricker, W.E. 1972. Hereditary and environmental factors affecting certain salmonid populations, p. 19-160. *In:* R.C. Simon and P.A. Larkin, editors, The stock concept in Pacific salmon. H.R. Mac Millan Lectures in Fisheries. Univ. of British Columbia, Vancouver.

Sokal, R.R. and F.S. Rohlf. 1969. Biometry. State Univ. of New York at Stony Brook. W.H. Freeman and Co., San Francisco.

Trihey, E.W. 1982. 1982 Winter temperature study. Acres American Inc. Buffalo, New York.

Velsen, F.P.J. 1980. Embryonic development in eggs of sockeye salmon (*Oncorhynchus nerka*). Canadian Special Publication of Fisheries and Aquatic Sciences 49.

Wedemeyer, G.A. and D.J. McLeay. 1981. Methods for determining the tolerance of fishes to environmental stressors. *In:* A.D. Pickering, editor, Stress and Fish. Academic Press.

Appendix 1. Experimental design and associated data for chum (trays 1-3) and sockeye (trays 5-7) salmon to achieve 50% hatch and complete yolk absorption at four different temperature regimes. Regimes (each was replicated) simulated the main-stem Susitna River, Slough 8A (S2), an intermediary regime (S1), and a Constant 4°C. (Eggs for this study were fertilized on three occasions during September, 1982.)

Incubator	Main Stem							S1							S2							Constant 4°C						
Tray	1	2	3		5	6	7	1	2	3		5	6	7	1	2	3		5	6	7	1	2	3		5	6	7
50% hatch: TU's[1]	320	293	262		349	321	299	457	446	444		552	558	535	491	508	478		606	607	613	476	473	471		610	618	595
50% hatch: Days since fertilization	161	172	182		211	217	235	117	122	131		161	173	172	105	112	106		140	142	147	119	117	116		152	152	146
Complete yolk absorption: TU's[1]	614	635	638		594	583	616	733	746	721		793	818	810	860	-	842		883	-	895	865	863	871		889	875	909
Complete yolk absorption: Days since fertilization	287	286	284		285	281	282	246	253	250		264	269	268	218	-	216		225	-	232	216	213	214		222	216	223
Complete yolk absorption: Percent survival	94	91	92		95	98	97	96	98	97		98	-	99	-	-	97		99	-	99	97	98	98		97	98	97
Complete yolk absorption: Percent abnormalities	0	0.9	1.8		<0.1	<0.1	0.1	0	0	0.2		0	-	<0.1	-	-	0.1		0	-	0	0	0.1	0		0	0	0.3

Incubator	Main Stem Replicate							S1 Replicate							S2 Replicate							Constant 4°C Replicate						
Tray	1	2	3		5	6	7	1	2	3		5	6	7	1	2	3		5	6	7	1	2	3		5	6	7
50% hatch: TU's[1]	320	292	266		344	320	294	457	444	435		546	534	521	493	486	478		620	616	622	-	473	472		602	605	607
50% hatch: Days since fertilization	162	173	182		204	215	230	118	122	128		161	164	168	105	104	105		145	147	149	-	117	117		151	150	150
Complete yolk absorption: TU's[1]	594	604	606		584	584	648	731	730	708		791	772	792	849	854	834		876	938	919	-	856	843		889	906	910
Complete yolk absorption: Days since fertilization	285	283	281		284	281	285	249	252	249		266	262	266	213	216	213		222	241	238	-	212	208		223	224	224
Complete yolk absorption: Percent survival	96	95	94		96	98	98	98	98	99		98	99	98	97	96	93		-	-	-	-	-	-		98	92	98
Complete yolk absorption: Percent abnormalities	0.3	1.5	2.2		0.2	0	0	0	0	0		0	<0.1	0	0	0	0		-	-	-	-	-	-		0	0	<0.1

[1] TU's - accumulated temperature units (°C).

- Dashes indicate that data were lost due to uncontrolled stresses.