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### Effects of Various Temperature Regimes

on the Incubation of Susitna River Chum and Sockeye Salmon

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#### Abstract

A study was undertaken to assess the potential effects of temperature alteration resulting from hydroelectric development on salmon incubation rates in the Susitna River, Alaska. Chum (<u>Oncorhynchus keta</u>) and sockeye (<u>O. nerka</u>) salmon eggs from Slough 11, Upper Susitna River, were collected and fertilized on three occasions during September, 1982. The eggs were incubated in our laboratory at four different temperature regimes until alevins achieved complete yolk absorption. The four regimes simulated 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.

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We used the concept of <u>average</u> temperature of each regime for subsequent comparisons. Average temperature for the four regimes described above were 2.1, 2.9, 3.9, and 4.0°C, respectively. Complete yolk absorption was delayed by up to two months at our coldest regime, as compared to the regimes having average temperatures of 3.9 and 4.0°C. No meaningful differences were observed between mean length of alevins reared in the four regimes. A regression equation is presented which evaluates development rates at known temperatures. It should be possible to predict the number of days required for Susitna chum and sockeye to hatch and to attain complete yolk absorption if the average incubation temperature of the spawning area can be specified. Effects of Various Temperature Regimes on the Incubation of Susitna River Chum and Sockeye Salmon DRAFT

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#### Introduction

Construction of dams and their resulting reservoirs are known to alter downstream temperature regimes in certain rivers and streams. Hydroelectric development is in the planning or construction stages in several Alaskan rivers. 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 is known that temperature changes within the range 0° to 5°C are more pronounced upon development rates than those between 5° and 10°C (Bams 1967), little additional information is available on egg incubation at temperatures less than 4°C (Dong 1981; Raymond 1981; Alderdice and Velson 1978). There is a regional need for this information as Alaskan salmon often spawn in water temperatures approaching or already less than 4°C. There is a further need for this information for Susitna River stocks due to the hydroelectric dams proposed for Susitna River miles 153 and 184.

The Susitna River in southcentral Alaska (Fig. 1) drains about 19,000 mi<sup>2</sup> into Cook Inlet. It is the sixth largest drainage in Alaska and supports a fishery resource that includes five species of salmon, a few non-anadromous salmonids, and other resident species such as grayling (<u>Thymallus</u> <u>arcticus</u>) and burbot (<u>Lota lota</u>). A host of studies have been undertaken to evaluate potential impacts of hydroelectric development on fish and wildlife. Our study was designed to investigate the incubation of two local stocks of salmon under varying temperature regimes.

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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 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 preproject temperatures in the fall and early winter. To accurately predict the effects of cold-water temperature changes on Susitna River salmon eggs, the following study objectives were developed:

- Construct a laboratory environment to document incubation of salmon eggs and alevins under 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 temperature regime at 4°C.
- 2. Collect and spawn five to seven pairs of chum (<u>Oncorhynchus keta</u>) and sockeye (<u>O. nerka</u>) salmon from a slough in the Upper Susitna on three different dates which include their normal, peak spawning period.
- 3. Record the time of egg hatching and complete yolk absorption in temperature units (TUs) and days for each species incubated under the four temperature regimes in the laboratory. Measure lengths and record weights of alevins at time of hatch and yolk absorption. (Document survival and note any abnormalities during development.)
- Provide a final report to help planners predict how certain temperature regimes will affect incubation and development of Susitna chum and sockeye salmon.

This study was conducted by the National Fishery Research Center (NFRC), U.S. Fish and Wildlife Service, in cooperation with its Division of Ecological Services and the Alaska Department of Fish and Game (ADF&G), Su-Hydro Division. Major funding was provided by the Alaska Power Authority.

### 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 insulated Heath incubators. Control and monitoring of the incubator water temperatures was possible from  $0.5^{\circ}$  to  $12^{\circ}$ C with  $0.1^{\circ}$ C resolution and  $\pm 0.3^{\circ}$ 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  $5^{\circ}$ 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 insured 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.

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Three egg collections were made for chum and sockeye salmon at Slough 11 (RM 135.3) on the Susitna River (Fig. 1). For each species at least seven pairs (males and females) were spawned on September 3, 9 and 15. The fertilized ova were brought to our lab. Egg fertilization and handling procedures followed the recommendations of Leitritz and Lewis (1980).

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The eggs were measured out volumetrically in the lab and distributed into eight equal lots. 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<sup>3</sup> ring volume) were held until hatching. Water flow was maintained at about 2.2 L/min throughout the study. 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). At the conclusion of the third egg collection, four incubators each had three trays of chum and three trays of sockeye eggs representing the three egg takes (Table 1) and the four temperature regimes. The four additional incubators were added with an identical design to provide a complete replicate of the study.

Two of the four water temperature regimes used in our study simulated natural temperatures in two reaches of the Upper Susitna River. The first simulated 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 (RM 125), a known spawning

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area for chum and sockeye. We designated this regime as "S2". Incubator water temperatures were adjusted as much as twice weekly during fall and spring when the greatest river temperature fluctuations occurred. Personnel from ADF&G, Su-Hydro Division, determined all field water temperatures.

Table 1. Egg placement within a given incubator during our study. (Each incubator maintained a specific temperature regime or its replicate.)

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

A third temperature regime (designated "SI") 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.

Because high water inundated Slough 8A during the winter of our study, the ADF&G thermograph was lost. Thus, it was necessary to use temperature data obtained from 1981-1982 (Trihey 1982) as the new model for Slough 8A (S2).

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 mea-

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sured (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 and 10 alevins were sampled for length measurements each week.

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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 closed over the remnant yolk sac, the alevin was determined to have completed yolk absorption (CYA). A sample of 30 alevins was removed and measured for total length when 50 percent CYA was achieved.

Data were compiled to provide comparisons for time to hatch and complete yolk absorption 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. The partial results from these trays were removed from analysis of length, weight and development rate but have been appended to this study (Appendix 1). For descriptive purposes data from replicates and all three egg collection dates were often pooled. When this was done, the raw data was 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 with the accumulation of temperature units. Comparisons were also made between development rates<sup>1</sup> (at various temperatures) for Susitna River chum and sockeye and those of other chum and sockeye stocks as cited in the literature.

### Results

### <u>Water Temperature</u>

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Lab water temperature control was reliable throughout the course of the study. It was not possible to duplicate diurnal or within-week temperature variations taking place in the field (Susitna River), but a rough equivalence was produced during the salmon incubation period. The rapid temperature decline of the main-stem Susitna in the fall, its long winter period of  $0^{\circ}$ 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. The water bath would begin to freeze if we attempted to maintain temperatures below 0.4 to 0.5°C. 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 the field 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

<sup>1</sup> Development rate is defined as the reciprocal of number of days from fertilization to specific stage, such as complete yolk absorption, multiplied by 1000.

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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 1983, the first egg collection period when ova of chum and sockeye salmon were fertilized.



Figure 3. Accumulated temperature units at four different temperature regimes for the Susitna River egg incubation study. Data were plotted from 3 September 1983, the first 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°).

occurred within the main-stem temperature regime. Due to the declining water temperature, eggs from the third collection that were incubated in the mainstem thermal regime required 88 days to reach 200 TUs, while eggs from the first collection period required only 31 days to reach 200 TUs.



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°).

### <u>Chum Salmon</u>

Five of the 24 incubator trays containing chum salmon were eliminated from analysis of growth and survival due to uncontrolled lethal stresses. These represented Slough 2 (first and second egg collections) and the 4°C Constant

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replicate for all three egg collections. When 50 percent or more of the eggs remained viable however, the subsequent data from those trays was included in the evaluations of time to hatch and complete yolk absorption.

#### Incubation timing and survival:

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Computations of the average temperature (for each regime) to various developmental stages allowed for standardized comparisons between study temperatures. The average temperature to complete yolk absorption showed the 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 wat	ter 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 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

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Constant 4°C or S2 temperatures.





- Figure 5. Days from fertilization to 50% hatch (crosshatched 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°C). 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.)

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 less TUs to 50 percent hatch and alevins required 239 less TUs to complete yolk absorption in the main-stem temperatures as compared to the Constant 4°C or S2 temperatures (Appendix 1).

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 at four temperature regimes based on spawning dates of September 3 and 15.

Temperature regime	Spawning date	50 Percent hatch	Complete yolk absorption
Main stem	03	February 11	June 16
Main stem	15	March 16	June 24//
S1	03	December 29	May 08 🦟
S1	15	January 22	May 22
S2	03	December 17	April 06
S2	15	December 29	April 17
Constant 4°C	03	December 31	April 07
Constant 4°C	15	January 09	April 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 included curved spines, deformed body parts, and "headfirst" hatching.

Lengths and weights:

Weight measurements were taken through 50 percent hatch and for some alevins with complete yolk sac absorption. But the weighing process was 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  $\pm$  0.01 g (95 percent CI). No weight analysis was performed between egg collections.



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 intermeddiary (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.)

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 differences between the groups (P<0.01). 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) resulted in groupings which separated the alevin lengths of the warmest temperature regimes from the coldest (Table 4). The difference in length from the smaller to larger alevins was 2.5 mm (11 percent).

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). Mean lengths and 95 percent confidence



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	22.5-7
<b>Angen</b>	Main stem	3	2.0	22.9
	S2	15	4.6	22.9
	S1	15	3.4	23.1
مدرية.	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

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vth\_and temperature unit accumulation:

vi growth (total length) was plotted versus accumulated temperature units
c) for all four temperature regimes of the first egg collection (Figs. 10
d 1) and for three temperature regimes of the third egg collection (Fig.
2) - Comparative differences (TUs and days to 50 percent hatch and CYA)
ithin a single egg collection were mentioned previously. Variations in
iro th curves are noted here. A greater deflection in the slope of the mainstem growth curves as compared to S1 and S2 is observed in Figs. 10 and 12.
Th. greatest change in slope for the main-stem growth curves appears at about 30° TUs for the first egg collection and about 300 TUs for the third egg collection. For both main-stem temperature regimes this represents the
i creased water temperatures greater than 1°C which occurred during the first week in May.

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 related to the colder average water temperatures experienced by eggs from the third egg collection.

Development rates:

Development rates were computed (to 50 percent hatch and complete yolk ab-

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sorption) 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 equation  $R^2$  was greater than 0.92 (Table 5). Thus chum development rates are predictable for known temperature regimes.



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.)

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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.)

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

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Incubation stage 50 percent hatch Complete yolk absorption	R <sup>2</sup>	Slope	Y intercept				
50 percent hatch	0.959	0.63	-1.4				
Complete yolk absorption	0.927	1.51	-2.9				

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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.)

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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.

Sockeye Salmon

Six of the 24 incubator trays containing sockeye salmon were eliminated from analysis of growth and survival due to uncontrolled lethal stresses. These represented the thermal regimes 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).

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

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

Thus, 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).





Figure 15.



- Accumulated temperature Days from fertilization Figure 16. units (°C) to reach 50% hatch (cross-hatched to 50% hatch (crosshatched bars) and combars) and complete yolk plete yolk absorption absorption (open bars) (open bars) for sockeye salmon at four different for sockeye salmon at four different temperatemperature regimes which ture regimes which simusimulated the Susitna lated the Susitna main main stem (MS), Slough 8A stem (MS), Slough 8A (S2), an intermediary (S1), and 4°C Constant (S2), an intermediary (S1), and 4°C Constant (4°). (Data were pooled from three fertilization (4°). (Data were pooled from three fertilization dates in September and from study replicates.) dates in September and from study replicates.)
- Table 7. Dates for hatching and complete yolk absorption for sockeye eggs and alevins incubated at four temperature regimes based on spawning dates of September 3 and 15.

Temperature regime	Spawning date	50 Percent hatch	Complete yolk absorption
Main stem	03	March 29	June 14
Main stem	15	May 05	June 25
S1	03	February 11	May 26
S1	15	March 05	June 09
S2	03	January 23	April 14
S2	15	February 10	April 12
Constant 4°C	03	February 01	April 12
Constant 4°C	15	February 10	April 26

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Sockeye egg and alevin survival was greater than 90 percent (Appendix 1) for all temperature regimes (Fig. 17). Abnormalities noted in main-stem and Constant 4°C temperatures included curved spines, twining, double heads and tails, and head-first hatching.



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.)

Lengths and weights:

Analysis of sockeye weights at 50 percent hatch offered little 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).

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 differences between the groups (P<0.01). 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) resulted in the grouping of alevin lengths in an order which separated the coldest and warmest temperature regimes (Table 8). The difference in length from the smaller to larger alevins was 1.8 mm (8 percent).

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Table 8. Mean lengths of sockeye alevins at 50 percent hatch which are bracketed into 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

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). No trend was observed between mean lengths of alevins within the four regimes (Fig. 19). The difference in

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length between the smallest and largest group was 1.7 mm (5.7 percent). (If those two groups are eliminated the range difference is 0.6 mm.)

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Figures 18 and 19. Mean lengths of sockeye salmon alevins at 50% hatch and at total yolk absorption (horizontal lines) 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.

درین یرین یرین 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 had not reached 50 percent hatch until early May, when water temperatures were already higher than 1°C.



Figure 20. Alevin growth (total length) from 50% hatch to complete yolk absorption for sockeye 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.)

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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 Constant 4°C water temperature regime is presented separately (Fig. 21) 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.



Figure 22. Alevin growth (total length) from 50% hatch to complete yolk absorption for sockeye 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.)

Development rates:

Development rates were computed (to 50 percent hatch and complete yolk absorption) and plotted from the results of 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  $R^2$  value for each regression line was greater than 0.97 (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 for sockeye eggs to 50 percent hatch and alevins to complete yolk absorption from data in Figs. 23 and 24.

Incubation Stage	R <sup>2</sup>	Slope	Y intercept
50 Percent hatch	0.977	0.78	-1.6
Complete yolk absorption	0.973	1.07	-1.3

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 (S2)(Table 2) 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 under altered temperature regimes so that yolk absorption is achieved at the most optimal time during spring (Dong 1981). The similarities in incubation time (TUs and days) between S2 and  $4^{\circ}$ C (constant) for chum and sockeye to reach complete yolk absorption can be explained by the similar average incubating temperature to yolk absorption within each regime (Figs. 3 and 4 and Tables 2 and 6). The slower development rates which occurred within our coldest regimes (MS and S1) are also proportional to the number of TUs accumulated. Thus, we find no evidence that chum and sockeye from Slough 11 have the ability to regulate their development rates to accomodate temperature alterations from 2.1° to 4°C.

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It is possible that the <u>average</u> 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 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. Such an increase (for example, 4.5°C <u>average</u>) could result in an earlier emergence as compared to our warmest temperature regime (4°C constant).

Secondly, fluctuations in winter discharge in the main stem could result in increased channel ice formation. Increased ice staging of the river may divert main stem flows and inundate sloughs with 0°C water. If completion of the incubation process were delayed by one to two months (as occurred in our main stem and S1 temperature simulations), it may have a significant effect on the smoltification of the chum. The smolting process, which is critical to the successful ocean adaptation of salmon, can dysfunction is it occurs "out of phase" to the historical smolt timing as when temperature has decreased or increased alevin development (Folmar et al. 1982). The effect on sockeye salmon is less clear as they will be rearing for another one to two years outside of the slough habitat before their outmigration as smolts.

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Our results with Susitna River salmon indicate increases in mortalities and abnormalities begin when average incubation temperatures to hatch are less then 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.

Baily and Evans (1971) reported an increase in mortalities for pink salmon (<u>0. gorbuscha</u>) when initial incubation was below  $4.5^{\circ}$ C, and complete mortality occurred when initial incubation was below  $2.0^{\circ}$ C. Studies with coho salmon (<u>0. kisutch</u>) have also noted 100 percent mortality when eggs were initially incubated in temperatures below  $1.0^{\circ}$ C (Dong 1981).

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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 blastopore.

The mean length of chum and sockeye alevins was significantly smaller at hatching when incubated in colder average temperature regimes (Tables 4 and 8). These results have also been noted for variations of other incubating 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 result earlier depending upon incubating conditions (Garside 1959), and Hayes et al. (1953) suggested cold water temperatures would be expected to promote early hatching (relative to development stage). Additionally, the mean alevin lengths at complete yolk absorption (Figs. 9 and 19) did not reveal the differences between temperature regimes found at hatching. 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

at an earlier stage. 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 similar in our study temperatures, the growth rate did not. A clear increase in growth rates resulted from increasing water temperatures above 1.0°C in the main-stem temperature regimes for both species (Figs. 10, 12, and 20). Temperatures greater than 1.0 to 4.0°C resulted in similar slopes when growth in length was plotted over TUs.

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. The development rates can be calculated for either species given an average incubation temperature and the information in Tables 5 and 9.

If for example, a model of post-project water temperature predicts that a slough with incubating salmon eggs will have an <u>average</u> temperature of 4.5°C (September to May) the number of days required to complete yolk absorption for chum could be calculated as follows:

y = mx + b, where

y = average water temperature (°C)

m = slope of chum yolk absorption regression line (Table 5)

x = chum development rate (to be calculated)

b = y intercept (Table 5).

Solving the equation for x calculates a development rate for chum (at  $4.5^{\circ}$ C) of 4.9. Dividing 1000 by 4.9 results in a prediction of 204 days from fertilization to complete yolk absorption. That would result in a decrease in the chum incubation time of about none days from our warmest temperature regime (4°C constant).

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Altering the thermal regimes and discharges of a major river will have many effects beyond those observed on salmon eggs and alevins. The results from this study indicate incubation timing is an important factor which will be affected by altered water temperatures. But all salmon life stages will be directly 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). Resource planners will be able to use data from this report and plan management, mitigation or enhancement objectives to the extent that post-project temperature regimes can be predicted.

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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.)

	Hate Ster							s1												Constant A*C								
Incubator			ria		2 <b>14</b>							l							JL					1.003				
Tray	1	2	3		5	6	1	1	2	3		5	6	1	1	2	3		5	6	7	1	2	3		5	6	1
ក្រ ក្ TU's <sup>1</sup>	320	293	262		349	321	299	457	446	444		552	558	535	491	508	478		606	607	613	476	473	471		610	618	595
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
TU's <sup>1</sup>	614	635	638	1	594	583	616	733	746	721		793	818	810	860	•	842		8 <b>83</b>	-	895	865	863	871		889	875	909
Days since	287	286	284		285	281	282	246	253	250		264	269	268	218	-	216		225	-	232	216	213	214		222	216	223
Percent	94	91	92		95	98	97	96	98	97		98	-	99	-	-	97		99	-	99	97	98	98		97	98	97
B Percent	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		Mali	n Stei	n Repl	licat	e		S1 Replicate						S2 Replicate							Constant 4°C Replicate							
Tray	1	2	3		5	6	1	1	2	3		5	6	7	1	2	3		5	6	7	1	2	3		5	6	7
, តួ <sup>រប</sup> 's <sup>1</sup>	320	292	266		344	320	294	457	444	435		546	534	521	493	486	478		620	616	622	-	473	472		602	605	607
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
Tu's <sup>1</sup>	594	604	606		584	584	648	731	730	708		791	772	792	849	854	834		876	938	919	-	856	843		889	<b>90</b> 6	910
Days since	285	283	281		284	281	285	249	252	249		266	262	266	213	216	213		222	241	238	-	212	208		223	224	224
Percent survival	96	95	94		96	98	98	98	98	99		98	99	98	97	96	93		-	-	-	-	-	-		98	92	98
e 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

<sup>1</sup> TU's - accumulated temperature units (°C).

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

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