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Effect of Temperature on Early-Life Survival of Sacramento River Fall- and Winter-Run Chinook Salmon

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EFFECT OF TEMPERATURE ON EARLY-LIFE SURVIVAL OF SACRAMENTO RIVER FALL- AND WINTER-RUN CHINOOK SALMON

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Abstract—Temperature is one of the most important factors controlling early-life survival of Pacific salmon. This report documents two preliminary studies designed to examine Sacramento River chinook salmon early-life temperature tolerances. The first experiment consisted of fall-run chinook salmon incubated through the cleavage egg, embryo, eleutheroembryo, and pre-emergent alevin stages at constant 50, 52, 54, 56, 58, and 60°F. Temperatures were elevated from 56°F to 60-62°F during the eleutheroembryo or pre-emergent alevin stages for additional treatments. A similar experiment using fewer replicates was conducted with winter-run chinook salmon. Fish were incubated at constant 56, 58, 60, 62, and 64°F. Temperatures were also elevated from 56°F to 60-62°F during the embryo, eleutheroembryo, or pre-emergent alevin stages. An alevin rearing phase was conducted under less precise conditions for each experiment. Incubation mortality increased non-linearly with increasing temperature, resulting in 90% fall-run, and 87% winter-run, mortality at 62°F. Incubation at 64°F resulted in 100% winter-run salmon mortality. The fall-run experiment indicated early-life temperature exposure manifested in later alevin mortality. Our data suggest incubation temperatures above 56°F result in significantly higher alevin mortality but temperature tolerance may vary between runs. More precise rearing experimentation is needed to examine the relationship between incubation temperature and later alevin mortality.

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Introduction

Water temperature is one of the most important factors controlling early-life survival and growth of Pacific salmon (*Onchorynchus* spp); incubation temperature as well as magnitude, rate, and timing of temperature change can directly and indirectly influence salmon survival (Murray and Beacham 1986). Numerous laboratory studies have documented the effects of high and low temperatures on chinook salmon embryo and alevin mortality (e.g., Combs and Burrows 1957; Combs 1965; Murray and McPhail 1988). Elevated water temperatures can induce temperature shock (Neitzel and Becker 1985), yolk coagulation (Johnson and Brice 1953), and developmental abnormalities (Seymour 1956). Hatching, emergence (Embody 1934; Alderdice and Velsen 1978), and growth (Fowler 1972; Heming 1982) are also influenced by incubation temperature, sometimes resulting in reduced swimming performance (Bams 1967) and increased vulnerability to predators (Mead and Woodall 1968). Exposure to extreme temperatures may also increase susceptibility to disease (Leitritz 1959).

Previous research on chinook salmon temperature requirements, summarized by Boles (1988) and Velsen (1987), indicated egg and alevin mortality was lowest when water temperatures were maintained between 40°F and 57°F. Seymour (1956) found complete egg mortality of Sacramento River fall-run chinook salmon when water temperature was held at 65°F and complete sac-fry mortality when water temperature was held at 60°F. Sac-fry mortality exceeded 50% at 57.5°F and was lowest between 40°F and 55°F. Healy (1979) found Sacramento River fall-run chinook salmon mortality was lowest at water temperatures of 43.5-57.5°F, and exceeded 80% when temperatures exceed 61°F. No previous temperature tolerance research has been conducted on winter-run chinook salmon in controlled environments. Summer water temperatures on the Sacramento River below Keswick Dam have frequently exceeded 60°F probably contributing to the decline of winter-run chinook salmon (Slater 1963, Hallock and Fisher 1985).

Four distinct runs of chinook salmon are present in the Sacramento River: fall, late-fall, winter, and spring. Runs are named for the season adults begin entering the estuary on their spawning migration; however, incubation and hatching occur 2-6 months later, depending on run (Boles 1988). Of the four runs in the Sacramento River, winter-run chinook salmon are exposed to the highest water temperatures because peak egg hatching occurs during late summer and early autumn.

Historically, winter-run chinook salmon spawned in cooler, higher-elevation areas of the Sacramento and McCloud rivers north of Redding, California (Slater 1963). Most of their historical spawning and rearing habitat became inaccessible with the completion of Shasta Dam in 1943 and Keswick Dam in 1950. Sacramento River winter-run chinook numbers declined after 1944 but recovered during the 1960s. From 1969 until the late 1980s, winter-run numbers passing through Red Bluff Diversion Dam (RBDD) declined

steadily (Hallock and Fisher 1985), leading to their federal listing under the Endangered Species Act as "threatened" in 1989 and reclassification as "endangered" in 1994 (National Marine Fisheries Service 1994). Most of the current winter-run spawning and rearing habitat exists between Keswick Dam and RBDD. Maintaining suitable water temperature in this area has become a management and legislative priority (Sacramento River Winter-Run Chinook Salmon Recovery Team 1996).

Hypolimnetic water released from Shasta Reservoir normally maintains viable temperatures for winter-run chinook salmon rearing between Keswick Dam and Red Bluff. However, when the reservoir becomes depleted, warmer epilimnetic water is released through the power generating gates of Shasta Dam. Low reservoir levels resulted in elevated tailwater temperatures during several years between 1943 and 1987 (U.S. Fish & Wildlife Service 1990). Beginning in 1988, the U.S. Bureau of Reclamation (Reclamation) implemented a "cold-water bypass" action, releasing hypolimnetic water from lower non-power-generating gates of Shasta Dam to control tailwater temperature. USBR is now required, insofar as possible, to maintain main-stem Sacramento River temperatures at ≤56°F between Keswick Dam and Bend Bridge (State Water Resources Control Board 1990). The Reclamation completed construction of an $80 million temperature control device in 1996. The device reduces revenue lost to cold-water release by drawing water from various depths for power generation. Because hypolimnetic water is limited during dry years, cold-water releases must be carefully balanced between power generation and water conservation.

The goals of this study were to evaluate Sacramento River chinook salmon early-life survival under various temperature regimes and to refine procedures for possible future studies. This report documents two separate but similar experiments, one conducted with fall-run and the other with winter-run chinook salmon. The fall-run experiment was conducted to collect preliminary data and establish methodologies in order to minimize unnecessary mortality of endangered winter-run chinook salmon. The winter-run experiment was conducted using fewer available fish and slightly different treatments. The fall-run experiment consisted of treatment temperatures ranging from 50-62°F; however, laboratory conditions precluded precisely maintaining water temperatures below 56°F during summer. Since we were primarily interested in winter-run chinook salmon upper temperature tolerances, the winter-run experiment included temperatures of 56-64°F. We evaluated four stages of embryonic development during the incubation phase of each experiment: cleavage embryo, embryo, eleutheroembryo, and pre-emergent alevin (Balon 1975). Each experiment also included a rearing phase to evaluate alevin survival.

Although the fall- and winter-run experiments are not strictly comparable, they were intended to examine the general consistency of Sacramento River chinook salmon temperature tolerance across runs. Developing specific temperature tolerance models was inappropriate due to the limitations of these experiments. Instead, we examined the

consistency of these data with chinook salmon temperature tolerance information published elsewhere. In general, both experiments coincided with the expected pattern; however, our data suggest differences between fall and winter runs may exist. If specific temperature-mortality relationships are required, more rigorous and controlled experiments should be conducted, for which we have included recommendations.

Methods

Fall-run Experiment

Incubation Phase — Fall-run chinook salmon eggs were obtained from the Coleman National Fish Hatchery, Anderson, California. Eggs from five spawning pairs were fertilized at the hatchery, disinfected in 50 ppm Argentyne® iodophore, and water hardened for one hour. Approximately 250 mL of fertilized eggs from each spawning pair were placed into 2-L Nalgene® bottles with an equal amount of water. Bottles were then placed in an insulated ice chest, maintained at 54-56°F, and transported to the Northern Central Valley Fish & Wildlife Office (NCVFWO) wet laboratory.

Eggs from each bottle (mating pair) were divided into 11 incubation cups, each cup containing approximately 80-90 eggs. The exact number of eggs in each cup was calculated at the conclusion of incubation by summing mortalities, unfertilized eggs, and surviving fry transferred to rearing units. Eleven incubation units (treatments) received one cup from each mating pair; cups served as replicates. Units were incubated at different temperature regimes as described in Table 1. Incubation units 50-62 were held at constant temperature throughout incubation, while temperatures in incubation units 60E1, 60E2, 62E1, and 62E2 were elevated beginning in the embryo or eleutheroembryo stage. Incubation Unit 56 (56°F) served as control since previous research indicated chinook salmon mortality did not increase significantly in the range of 55-57.5°F (Combs and Burrows 1957; Healy 1979), but began to increase at higher temperatures (Seymour 1956). Incubation units were gradually brought to their prescribed treatment temperatures over the first 72-hours of the study. Approximate developmental phase of the eggs was estimated by monitoring cumulative temperature units (TU; degree-days above 32°F; Leitritz and Lewis 1976; Alderdice and Velsen 1977).

Incubation cups consisted of 2.5-L capacity, 19.1-cm height, and 14.6-cm diameter polyethylene upwelling-flow containers. Cups contained 3-mm plastic mesh screen located 7.6 cm above the bottom of the cup. An additional 1.9-cm plastic mesh screen was used as "substrate" to simulate interstitial spaces in gravel. Upwelling flows of 0.4-0.5 L/min were maintained in each cup.

Incubation units consisted of 126.8-L capacity insulated ice chests. Sacramento River water from the Tehama-Colusa Canal was used throughout the experiment. Water was pumped through sand filters and into head tanks via the single- and dual-purpose canals.

Table 1.—Protocol for temperature (°F) exposure of incubation units for fall-run chinook salmon embryos.

		Incubation unit										
Developmental phase	TU	50	52	54	56	58	60	62	60E1	60E2	62E1	62E2
Cleavage embryos	0-450	50	52	54	56	58	60	62	56	56	56	56
Embryos	451-900	50	52	54	56	58	60	62	56	60	56	62
Eleutheroembryos	901-1350	50	52	54	56	58	60	62	60	60	62	62
Pre-emergent alevins	1351-1800	50	52	54	56	58	60	62	60	60	62	62

Water temperatures were controlled using a Frigid Unit® D1-100 water chiller-circulator and Protec® S5235/PII immersion heaters. Furthermore, water temperatures in each incubation unit were controlled by Corning Vycor® model 33900-095 immersion heaters that were operated by a YSI® model 72 proportional controller. Outflow water temperatures from incubation units were monitored by thermistors connected to a Ryan Instruments Data Mentor®. Thermistors were periodically re-calibrated with a certified mercury thermometer. Water temperature in each incubation unit remained within ±6.5 °F of the target, and mean daily water temperature was within ±1 °F, of the target temperature after the initial 72 hours of the study (Figure 1).

Water was passed through three Aquanetics model 30IL ultra-violet sterilizers prior to entering incubation units to control water quality. Dissolved oxygen was measured weekly using a Hach Portable Dissolved Oxygen Meter® (model 16046). Ammonia (NH_3), alkalinity, water hardness, and pH,were also measured weekly using portable Hach kits.

All egg and fry mortalities were removed from cups daily. Eggs that became white and died but exhibited no sign of development were assumed to be unfertilized and were not included in the analysis. Dead eggs were removed using a bulb pipette and fixed in a clearing solution of glacial acetic acid, salt, and water for 3-5 minutes, then placed into a solution of 2% acetic acid and 7% formalin to determine embryonic development. Fry were transferred to rearing tanks when visual inspection revealed that all fry were swimming and yolk sacs were about 50% absorbed, approximately 1350-1590 TU. Incubation unit 50 was an exception, where fish were moved to rearing unit 1 after only 1330 TU in order to conduct the rearing phase of the experiment concurrently with incubation unit 52 (Appendix 1).

Rearing Phase — Only three temperature regimes were employed in the rearing phase of the experiment since water temperature could not be controlled as precisely in rearing tanks as in the incubation cups (Table 2). Fish from different incubation units were kept in separate compartments within each rearing unit, but all cups from a particular treatment were combined into one compartment. The rearing phase was ended when either 100% mortality occurred or fish reached a mean fork length of 60 mm.

The rearing system consisted of four 352-L capacity fiberglass tanks measuring 1.82 x 0.76 m. Each tank was partitioned into compartments with 3-mm mesh aluminum screening. Water depth within the tanks was maintained at 25.4 cm. Water temperature was controlled using a Frigid Unit® D1-33 1/3 horsepower water chiller-circulator and Corning Vycor® model 33900-095 immersion heaters in head tanks similar to those used in the incubation phase of the experiment. Temperatures were monitored using a Data Mentor® with probes in each of the head tanks and rearing units. Water quality was monitored in the same manner as it was during the incubation phase of the experiment.

Table 2.—Distribution of fall-run chinook salmon alevins in rearing units following incubation and hatching.

Rearing tank	Temperature range (°F)	Incubation units included
1	50-54	50, 52
2	56-58	54, 56, 58
3	60-64	60, 62, 60E1, 60E2
4	60-64	62E1, 62E2

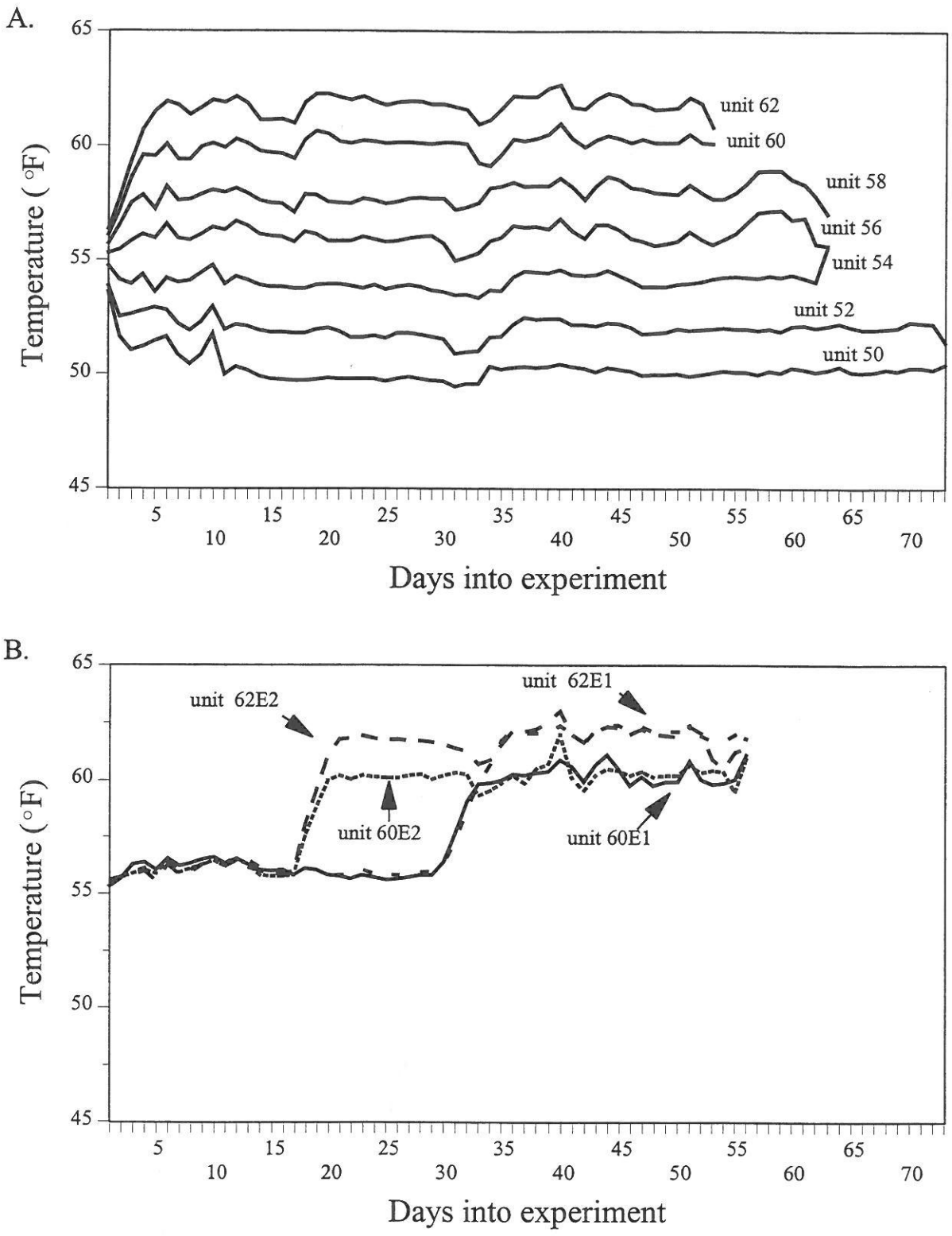


Figure 1.--Fall-run experiment mean daily temperatures (°F) in (A) incubation units (unit) 50-60 and (B) incubation units 60E1, 60E2, 62E1, and 62E2.

Demand-type feeders were attached to each compartment in the rearing tanks. Feeders consisted of a 0.24-L funnel with a 1.6 mm diameter stainless steel rod connecting the funnel to the surface of the water. When contact was made with the rod a small amount of BioDiet® fish food was released from the funnel. Fry transitioned to feeders quickly and survival did not appear affected.

Fry fork lengths were sampled during the rearing phase to assess growth under different temperature regimes. Ten fish from each cup were measured to the nearest mm while being moved to rearing units and 10 fish from each compartment were measured every other week thereafter for the remainder of the rearing phase. All fish were returned to their rearing compartment following measurement.

Winter-run Experiment

Incubation Phase — Winter-run chinook salmon eggs were obtained from the Coleman National Fish Hatchery. Due to low escapement, this experiment was limited to three spawning pairs - the maximum number that spawned on any single day. Eggs from three spawning pairs were fertilized, transported, and incubated using the same methods and equipment described above for the fall-run experiment.

The incubation phase of the winter-run experiment consisted of eleven incubation units (Table 3), but temperature regimes differed from the fall-run experiment. Incubation units 56-64 were held at constant temperatures throughout development with incubation unit 56 serving as control. No incubation units were kept below control temperature, while incubation unit 64 was maintained at warmer temperature than any fall-run incubation unit. Temperatures were elevated beginning in the pre-emergent alevin stage for two treatments in the winter-run experiment (incubation units 60E3 and 62E3), in addition to treatments where temperature was elevated beginning in the embryo or eleutheroembryo stages (incubation units 60E1, 60E2, 62E1, and 62E2). Because only three spawning pairs were available for the winter-run experiment, each incubation unit contained only three replicates (cups).

Water temperatures in incubation units varied by up to 7.5 °F from target temperatures and 1 °F from mean daily target temperatures after initial equilibration (Figure 2). Since development rates were less staggered among incubation units than the fall-run experiment, all fry were moved to rearing tanks on the same day. Fry were moved when visual inspection revealed that most fish in each incubation unit were accepting food, approximately 1,550-1,850 TU (Appendix 2).

Rearing Phase — Fry were moved into rearing units with three different temperature regimes as summarized in Table 4. The same techniques, equipment, and procedures described for the fall-run experiment were used, except that fish were measured less frequently (approximately monthly following the first two weeks) and the experiment was continued until fish reached mean lengths of 85-90 mm.

Table 3.—Protocol for temperature (°F) exposure of incubation units for winter-run chinook salmon embryos.

		Incubation unit										
Developmental phase	TU	56	58	60	62	64	60E1	60E2	60E3	62E1	62E2	62E3
Cleavage embryos	0-450	56	58	60	62	64	56	56	56	56	56	56
Embryos	451-900	56	58	60	62	64	56	56	60	56	56	62
Eleutheroembryos	901-1350	56	58	60	62	64	56	60	60	56	62	62
Pre-emergent alevins	1351-1800	56	58	60	62	64	60	60	60	62	62	62

Table 4.—Distribution of winter-run chinook salmon alevins in rearing units following incubation and hatching.

Rearing tank	Temperature range (°F)	Incubation units included[a]
1	56-58	56, 58
2	58-60	60, 60E1, 60E2, 60E3
3	60-64	62, 62E1, 62E2, 62E3

[a]100% mortality occurred during incubation in unit 64.

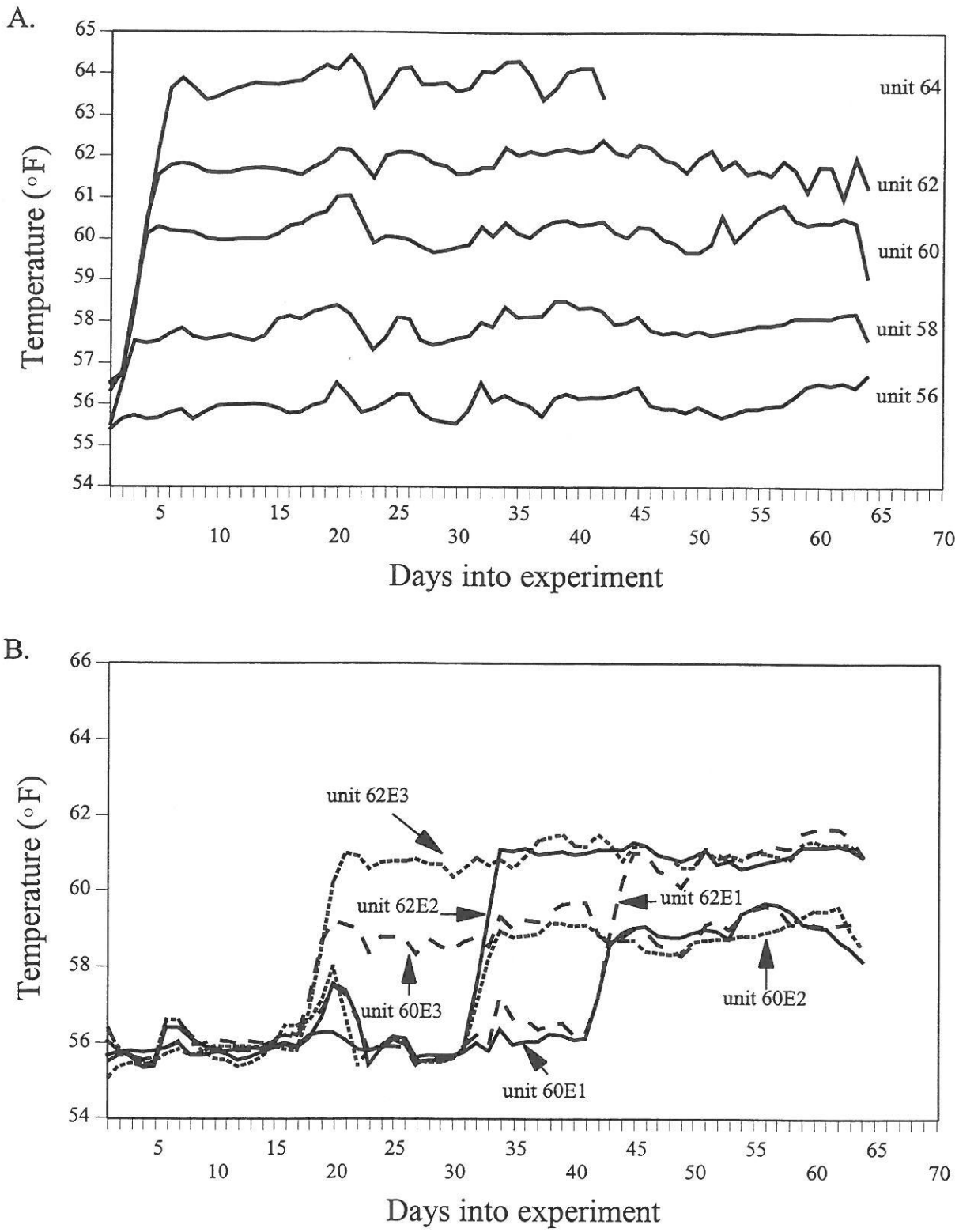


Figure 2.--Winter-run experiment mean daily temperatures (°F) in (A) incubation units (unit) 56-64 and (B) units 60E1, 60E2, 60E3, 62E1, 62E2, and 62E3 .

Data Analysis

For each experiment, incubation unit mean cumulative mortality by life stage was compared by two-way ANOVA arranged in a randomized complete block design (Kuehl 1994). Incubation units served as treatments and the experiments were blocked by cup (mating pair). In order to meet the assumption of equal variance among treatments, mortality fractions were normalized prior to ANOVA using the following arc-sine transformation (Zar 1984):

$$\frac{1}{2}\times\left[\arcsin\sqrt{\frac{X}{N+1}}+\arcsin\sqrt{\frac{X+1}{N+1}}\right]$$

where X = # of mortalities
N = # fertilized eggs in the cup.

Tukey's HSD multiple comparison test was used to identify significant differences among treatment groups. ANOVA was performed on cumulative mortality at each developmental phase. Additionally, incubation units where temperatures were elevated in the embryo, eleutheroembryo, or pre-emergent alevin stages were compared to control groups at the end of incubation with ANOVA and Tukey's HSD. Because replication was not practical in the rearing phase of the experiment, mortality rates within rearing units were tested for heterogeneity with Pearson's χ^2 statistic. We considered $P \leq 0.05$ to be statistically significant for all tests.

Growth assessment was conducted as a descriptive study to complement mortality data rather than as a rigorous experiment. No statistical tests were conducted because fish were moved from incubation cups to rearing units at slightly different developmental stages depending on treatment applied (Appendices 1 and 2). We used mean lengths and SE's as general indicators of temperature related variation in early-life growth rates.

Results

Fall-run Experiment

Incubation — Cumulative mortality increased with incubation temperature and differed significantly among incubation units at the embryo, eleutheroembryo, and pre-emergent alevin stages but did not differ significantly in the cleavage egg stage (Table 5). Incubation unit 62 exhibited significantly higher mortality than incubation units 56-60 following cleavage. Mortality increased non-linearly with increasing temperature during the embryo, eleutheroembryo, and pre-emergent alevin stages; however, high variation obscured differences between most treatments.

Table 5.—Fall-run chinook salmon mean cumulative mortality (M; %) in incubation units 50-62 during four developmental phases. *P* values are for ANOVA on arc-sine transformed mortalities. Bars indicate means that were not significantly different using Tukey's HSD multiple comparison test.

Incubation Unit	Cleavage eggs (0-450 TU)		Embryos (451-900 TU)		Eleutheroembryos (901-1350 TU)		Pre-emergent alevins (1351-1800 TU)	
	M	*P*=0.309	M	*P*=0.000	M	*P*=0.000	M	*P*=0.000
50	3		6		9		-[a]	-
52	4		8		13		13	
54	6		11		15		16	
56	3		10		14		15	
58	6		16		22		32	
60	4		15		20		32	
62	14		37		72		90	

[a] Moved to rearing unit 1 at 1330 TU.

Incubation unit 56 (the control) did not differ significantly from any other incubation unit except 62 at any developmental stage (Table 5).

Incubation unit 60E2 exhibited significantly greater mortality through the pre-emergent alevin stage than incubation unit 60E1, and incubation unit 62E2 significantly greater than incubation unit 62E1 (Table 6), indicating elevated temperatures earlier in development resulted in greater cumulative mortality. Neither incubation units 60E1 or 62E1 differed significantly from unit 56; however, mortality in units 62E1 and 62E2 was nearly twice that in units 60E1 and 60 E2, repectively, suggesting the magnitude of temperature increases was also directly related to mortality.

Rearing — Rearing temperature was directly related to mortality rate (χ^2; P=0.000) among rearing units (Table 7). Within rearing units, not only did cumulative mortality increase with previous incubation temperature, but also mortality during rearing (Table 7). This pattern suggests previous incubation temperature regime influenced later alevin survival. The differences were significant in rearing units 1, 3, and 4, and nearly significant in rearing unit 2 (Table 7).

Temperatures of 52-54°F in rearing unit 1 resulted in slower (differences in mean length >2 SE) growth than temperatures of 56-64°F in rearing units 2-4, which had similar growth rates (Table 8). Mean lengths of fish from different incubation units also differed by >2 SE within rearing unit 1 but did not within rearing units 2-4 (Appendix 5). These data suggest growth rates began to plateau in the range of 54-56°F.

Winter-run Experiment

Incubation — Cumulative mortality increased significantly with increased constant temperature at all developmental stages (Table 9). Mortality exhibited a similar trend to the fall-run experiment, generally increasing non-linearly with increasing incubation temperature. Incubation unit 60 had the lowest temperature that differed significantly from the control at any developmental stage. Incubation unit 64 differed significantly from the control during the first three developmental stages and reached 100% mortality during the eleutheroembryo stage indicating the upper limit for viable incubation was in the range of 62-64°F.

Elevating temperatures within treatments during incubation resulted in greater mortality but did not have a statistically significant effect on winter-run chinook mortality. Incubation units 62E1, 62E2, and 62E3 differed significantly from the control but did not differ significantly from each other (Table 10). There were no significant differences between incubation units 56, 60E1, 60E2, and 60E3 (Table 10). The magnitude of change in cumulative mortality between incubation units 60E1 and 60E2, and between incubation units 62E1 and 62E2 was comparable to that of their counterparts in the fall-run experiment (fall-run incubation units 60E1 vs. 60E2, and 62E1 vs. 62E2)

Table 6.—Fall-run chinook salmon mean cumulative mortality (M; %) at the end of incubation in units where temperatures were elevated (60E1, 60E2, 62E1, and 62 E2) compared to unit 56 (the control). *P* values are for ANOVA on arc-sine transformed mortalities. Bars indicate means that were not significantly different using Tukey's HSD multiple comparison test.

Incubation unit	M	*P*=0.002
56	15	▌
60E1	21	▌
60E2	45	▌

Incubation unit	M	*P*=0.001
56	15	▌
62E1	40	▌
62E2	73	▌

Table 7.—Fall-run chinook salmon cumulative mortality through rearing, and mortality within rearing units 1-4. Pearson χ^2 probabilities are for tests of homogeneous mortality within rearing units.

Rearing unit	Previous incubation unit	Cumulative mortality (%)	Mortality (%) within rearing unit	$P(\chi^2>\chi^2_{\alpha,\nu})$ within rearing unit	Mean cumalitive mortality (%)
1	50	16	7	0.000	23
	52	31	20		
2	54	50	40	0.075	56
	56	57	49		
	58	68	44		
3	60	89	83	0.000	87
	62	100	100		
	60E1	78	72		
	60E2	81	66		
4	62E1	89	82	0.002	94
	62E2	99	95		

Table 8.—Mean length (L, mm) of subsampled fall-run chinook salmon during rearing.

Rearing unit	Date measured									
	1/9/92		1/23/92		2/6/92		2/20/92		3/5/92	
	L	SE	L	SE	L	SE	L	SE	L	SE
1	33.8	0.1	37.1	0.3	41.5	0.6	46.6	1.1	54.4	1.3
2	37.8	0.3	46.3	1.5	51.2	0.8	58.3	1.9	65.1	1.2
3	38.8	0.4	44.9	0.7	50.8	1.0	58.8	1.3	70.9	1.4
4	38.1	0.6	43.9	0.8	50.8	1.0	60.6	1.6	74.7	1.0

Table 9.—Winter-run chinook salmon mean cumulative mortality (M; %) in incubation units 56-64 during four developmental phases. *P* values are for ANOVA on arc-sine transformed mortalities. Bars indicate means that were not significantly different using Tukey's HSD multiple comparison test.

Incubation Unit	Cleavage eggs (0-450 TU)		Embryos (451-900 TU)		Eleutheroembryos (901-1350 TU)		Pre-emergent alevins (1351-1800 TU)	
	M	*P*=0.021	M	*P*=0.000	M	*P*=0.000	M	*P*=0.002
56	5		14		15		17	
58	5		14		16		26	
60	5		14		35		78	
62	5		22		62		93	
64	9		74		100		-	-

Table 10.—Winter-run chinook salmon mean cumulative mortality (M; %) at the end of incubation in units where temperatures were elevated (60E1, 60E2, 60E3, 62E1, 62E2, and 62E3) compared to unit 56 (the control). *P* values are for ANOVA on arc-sine transformed mortalities. Bars indicate means that were not significantly different using Tukey's HSD multiple comparison test.

Incubation unit	M	*P*=0.490
56	17	
60E1	23	
60E2	21	
60E3	33	

Incubation unit	M	*P*=0.004
56	17	
62E1	27	
62E2	37	
62E3	66	

suggesting high variation and low replication obscured differences between winter-run incubation units (Tables 6 and 10).

Rearing — Cumulative mortality increased significantly (χ^2; P=0.000) with increasing temperature among rearing tanks (Table 11). Mortality during rearing followed the same general trend as the fall-run experiment - increasing with previous incubation temperature. The significant effect of previous incubation temperature was apparent in rearing unit 1 but unclear in units 2 and 3 because each contained fish from only one incubation unit that was held at constant temperature.

There were few consistent trends in winter-run chinook salmon growth during the rearing phase (Table 12). Within rearing units, fish from different incubation units had similar mean lengths (Appendix 6). Alevins reared in unit 3 were generally >2 SE smaller than those reared in unit 1, while alevins reared in unit 2 had intermediate lengths.

Discussion

Nascent chinook salmon exhibit low and nearly constant mortality within a narrow temperature range; however, mortality increases sharply as water temperature deviates from that range (Seymour 1956; Combs and Burrows 1957; Healy 1979). While sub-optimal temperatures seldom occur in the Sacramento River (U.S. Fish & Wildlife Service 1990), elevated temperatures may result in direct mortality during embryonic development or latent mortality of alevins. Although not strictly comparable, our data followed a similar trend to other studies (Figure 3A). Survival rates, through the pre-emergent alevin stage, of fish incubated at 62-64°F in the winter-run experiment and 62°F in the fall-run experiment were comparable to the studies of Healy (1979) and Seymour (1956). Incubation temperatures of 62-64°F appear to be physiological limit for embryo development resulting in 80-100% mortality prior to emergence.

The latent effect of early-life temperature exposure observed in the fall-run experiment, and less clearly in the winter-run experiment, is also consistent with other studies of fall-run chinook salmon. Several mechanisms for latent mortality have been proposed. Seymour (1956), Combs and Burrows (1957), Olson and Foster (1957), and Jewett (1970) reported increased mortality during later development when embryos were incubated at 58-62°F. They hypothesized embryo development and differentiation were altered by elevated temperatures. Johnson and Brice (1953), and Hinze et al. (1956) also observed latent mortality at elevated temperatures, which they believed was due to yolk coagulation resulting in poor absorption. Heming (1982) reported faster yolk absorption and lower conversion efficiency as temperature increased. Johnson and Brice (1953) and Olson and Foster (1957) reported increased alevin mortality when embryos were incubated at elevated temperatures, even though rearing temperatures were cooled to 54°F or below.

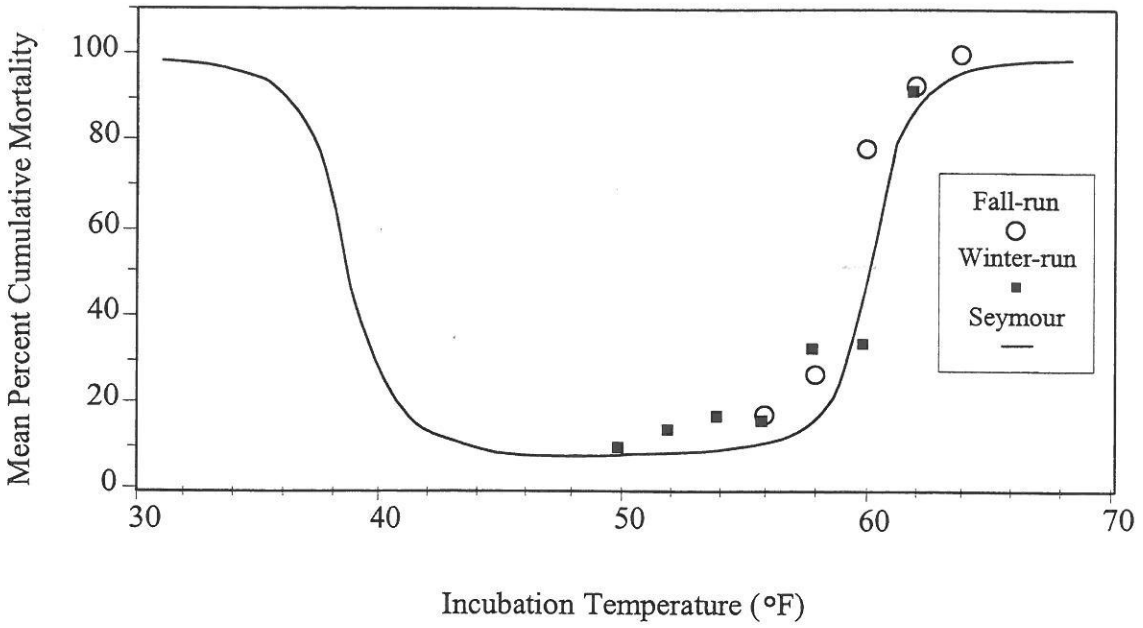
Table 11.—Winter-run chinook salmon cumulative mortality through rearing, and mortality within rearing units 1-3. Pearson χ^2 probabilities are for tests of homogeneous mortality within rearing units.

Rearing unit	Previous incubation unit	Cumulative mortality (%)	Mortality (%) within rearing unit	$P(\chi^2>\chi^2_{\alpha,v})$ within rearing unit	Mean cumalitive mortality (%)
1	56	18	1	0.000	25
	58	33	9		
2	60	79	5	0.349	45
	60E1	31	11		
	60E2	31	13		
	60E3	42	14		
3	62	100	100	0.000	81
	62E1	61	47		
	62E2	84	75		
	62E3	78	38		

Table 12.—Mean length (L, mm) of subsampled winter-run chinook salmon during rearing.

Rearing unit	Date measured													
	8/4/92		8/18/92		9/21/92		10/29/92		11/25/92		12/22/92		1/21/93	
	L	SE	L	SE	L	SE	L	SE	L	SE	L	SE	L	SE
1	33.5	0.1	37.8	0.4	51.4	0.7	68.9	0.9	78.8	1.3	86.7	1.5	91.2	1.7
2	32.7	0.1	35.0	0.3	50.3	0.7	69.2	1.0	74.8	0.8	85.1	1.1	88.8	1.0
3	32.2	0.2	34.6	0.3	44.8	0.6	63.4	1.0	74.8	0.9	84.3	1.2	86.9	1.3

A. Incubation temperature versus cumulative mortality at the end of the fall-run and winter-run incubation phases compared to the relationship reported by Seymour (1956).



B. Incubation temperature versus cumulative mortality at the end of the fall-run and winter-run rearing phases.

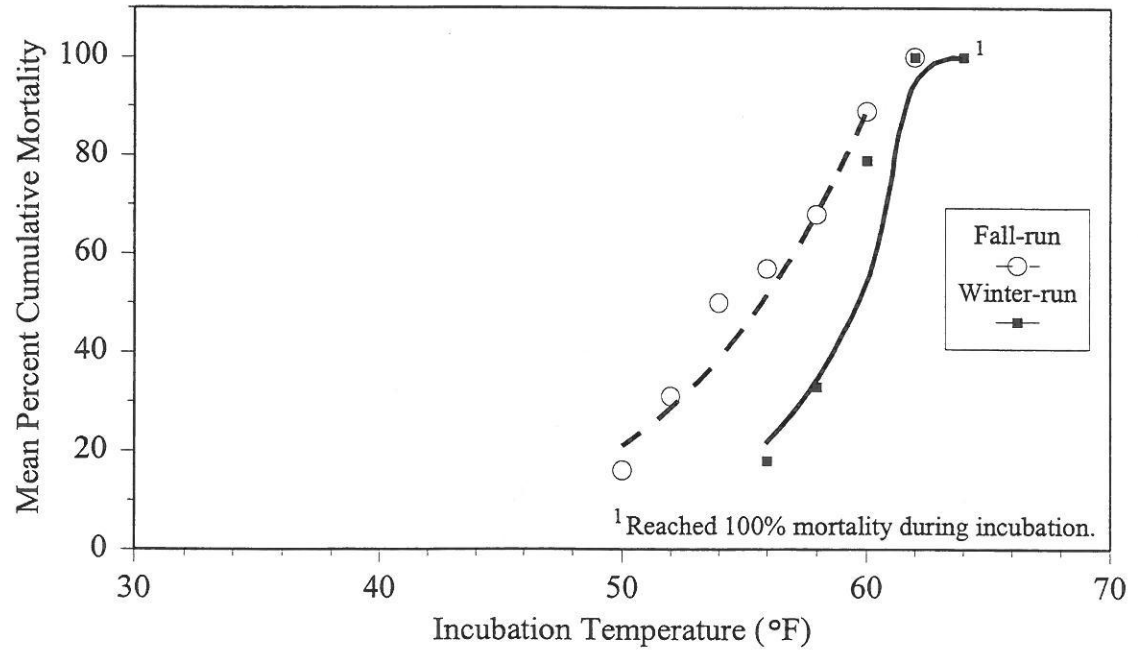


Figure 3.--Mean percent cumulative mortality of fall- and winter-run chinook salmon versus incubation temperature after (A) incubation phases, compared with relationship developed by Seymour (adapted from Seymour 1956), and (B) rearing phases of each experiment. **Note these experiments were not conducted and controlled under the same conditions - this figure illustrates general trends only.**

Although the rearing phases of both experiments in this study were not precisely controlled, precluding strict comparisons, the relation between incubation temperature and mortality during rearing suggests latent mortality occurs well into the alevin stage. Mortality during incubation did not differ significantly between 50°F and 56°F for fall-run chinook salmon but the significant Chi-square test for rearing unit 1 (Table 7) also suggests delayed mortality effects may begin at lower temperatures (somewhere between 50 and 52°F) than previously reported. The significant Chi-square test for winter-run rearing unit 1 indicates higher alevin mortality can be expected for winter-run chinook salmon at temperatures between 56°F and 58°F, although mortality at 56°F (18%) was low and similar to fall-run chinook salmon mortality at 50°F (16%). Because mortality of our lowest treatment temperatures differed significantly during rearing for both experiments, we were unable to experimentally demonstrate the ranges in which no later alevin mortality occurs as a result of temperature (Figure 3B).

The lack of uniformly controlled rearing treatments also precluded testing differences in alevin survival between runs, but may serve as pilot data for future investigations. Our data suggest winter and fall-run chinook salmon may have different tolerances to moderately elevated temperatures. Mortality rates between runs were similar through incubation at all temperatures except 60°F, where winter-run salmon mortality was substantially higher (Figure 3A). However, comparing mortality prior to the alevin stage may be misleading. Winter-run mortality during the rearing phase was low - Despite a rearing period about twice as long as that of the fall-run experiment, cumulative mortality was less than half that of fall-run chinook salmon at 56°F and 58°F (Figure 3B). Seymour (1956) reported eventual fry mortality rates >50% for chinook salmon incubated at 57.5°F, compared to 18% mortality at 56°F for winter-run chinook salmon in this study. Although fall-run chinook salmon temperature tolerances appear to be similar across river systems (Seymour 1956, Hinze 1959), differences between seasonal runs have not been investigated. Slater (1963) suggested winter-run chinook salmon adapted to river temperatures of 50-57°F but did not believe their tolerance to be different than that of other runs. However, Beacham and Murray (1989) reported interior-spawning chinook salmon stocks were more tolerant of low incubation temperatures than coastal-spawning stocks in British Columbia. They hypothesized different tolerances reflected evolutionary adaptation to thermal conditions experienced during development. While Sacramento River winter-run chinook salmon exhibited similar mortality rates to other stocks at relatively high incubation temperatures (60-62°F), winter-run alevins may have adapted to the slightly elevated summer and early-fall water temperatures (56-58°F) they commonly encounter.

The relatively small mean size of fall-run fish reared at 50-52°F and winter-run fish reared at 60-64°F compares closely with Seymour's (1956) research. He reported maximum growth at 54°F with declining growth at higher and lower temperatures. Because larval fish size is related to survival (Blaxter 1969), incubation temperatures that maximize survival of embryos while slowing growth can be a trade-off. While optimum

temperatures for early-life survival and growth of winter-run salmon coincide well, low temperatures that resulted in the greatest survival of fall-run chinook salmon appeared to retard growth. Growth rates may also indirectly influence survival by altering smolting and migration timing. Timing is potentially important to survival because seasonal variation in rearing environments is high. More precise rearing experiments may better quantify temperature-growth relationships.

Research Recommendations

Further temperature tolerance work should be conducted using a modified experimental design. Because the effect of incubation temperature on later alevin survival appears biologically significant, rearing should be conducted in a more controlled environment. Incubation and rearing should be conducted at the same constant temperature, except where experimental protocol calls for elevated temperatures, to allow for comparisons of alevin mortality using ANOVA. Preferably, incubation and rearing should be conducted in the same aquarium.

Since all cups in a particular treatment were placed in the same incubation unit, the fall- and winter-run experiments were pseudoreplicated (Hurlbert 1984). A split-plot design, as outlined in Table 13, would properly replicate the experiment while removing variation associated with mating pairs.

Future experiments should examine long-term tolerance to low and moderately elevated temperatures (48-58°F; Table 13). Water temperatures in the mid 50's resulted in low initial mortality (Tables 5 and 9) but high alevin mortality rates (Tables 7 and 9). The effect of extreme temperatures on embryo development has been well documented; the effect of moderately warm temperatures on later alevin survival has not. Although maintaining river temperatures below 56°F may be practically limited, expected mortality rates at temperatures salmon are likely to encounter could be better quantified. Since significant rearing mortality occurred between 50 and 52°F (Table 5), we recommend a lower control temperature (48°F). Additional laboratory experiments should first focus on creating an accurate and complete model of temperature tolerances without regarding the range that actual river temperatures can be manipulated. When developed, such a model could be used to better predict the consequences of water management decisions.

Fall- and winter-run chinook salmon cannot be tested in the same experiment due to differences in run timing; however, if experiments on the two runs were conducted as consistently as possible a reasonable comparison of run-specific temperature tolerance could be made. In the absence of better data, fall-run should not be used as a surrogate for winter-run chinook salmon in temperature studies - tolerances may differ between runs.

Table 13.—Proposed split-plot design for chinook salmon temperature tolerance experiment. Letters represent cups from a single mating pair.

Aquarium (Replication)	Treatment Temperature (°F)					
	48	50	52	54	56	58
1	A	A	A	A	A	A
	B	B	B	B	B	B
	C	C	C	C	C	C
	D	D	D	D	D	D
	E	E	E	E	E	E
2	A	A	A	A	A	A
	B	B	B	B	B	B
	C	C	C	C	C	C
	D	D	D	D	D	D
	E	E	E	E	E	E
Replications 3-5 repeat as above.						

To better quantify the latent effect of temperature exposure and identify a "critical stage" if one exists, a second experiment should be performed using a series of treatments where temperatures are elevated for a single developmental stage (Table 14) and the same split-plot design outlined above (Table 13). The treatments where temperatures were elevated in the fall- and winter-run experiments did not allow us to differentiate between the effect of temperature during a critical developmental period and the effect of total accumulated temperature units on cumulative mortality.

Management Recommendations

—Retain the ≤56°F temperature requirement between Keswick Dam and Bend Bridge - winter-run chinook salmon cumulative mortality through rearing nearly doubled between 56°F and 58°F.

—Spread cold-water release over the warmest months when water is limited, even if temperatures exceed 56°F. Because mortality increases non-linearly with increasing constant temperature, slightly elevated temperatures over a long period will result in lower salmon mortality than dramatic temperature increases when cold-water is exhausted.

A less desirable alternative would be to maintain water temperatures at 56°F or below only during the peak period of winter-run chinook salmon embryonic development. Because early-life temperature exposure, manifesting in later-life mortality, appears to be an important source of temperature related mortality, this strategy would prevent most of the run from being exposed to elevated temperatures during their most sensitive developmental period. However, there is considerable variation in timing of winter-run chinook salmon incubation and development and fish developing outside of the peak period might suffer high mortality rates. Salmon from other runs would also be negatively impacted if this strategy were employed.

Table 14.—Proposed treatment protocol (°F) for chinook salmon elevated-temperature experiment.

		Treatment						
Developmental phase	TU	1	2	3	4	5	6	7
Cleavage embryos	0-450	60	48	48	48	48	48	48
Embryos	451-900	48	60	48	48	48	48	48
Eleutheroembryos	901-1350	48	48	60	48	48	48	48
Pre-emergent alevins	1351-1800	48	48	48	60	48	48	48
Alevin I	1801-2250	48	48	48	48	60	48	48
Alevin II	2251-2700	48	48	48	48	48	60	48
Alevin III	4951-5400	48	48	48	48	48	48	60

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Appendix 1.— Weekly summary of cumulative temperature units (degree-days above 32°F) applied to fall-run chinook salmon in incubation units 1-11 and subsequently in rearing units. Fish were moved to rearing tanks during shaded weeks.

Date	Incubation Unit										
	1	2	3	4	5	6	7	8	9	10	11
10/29/91	22	22	23	23	24	24	24	23	24	24	23
11/05/91	156	166	177	191	202	214	225	193	192	192	191
11/12/91	285	307	332	361	383	410	432	363	361	362	360
11/19/91	409	446	485	529	562	607	641	531	545	530	550
11/26/91	533	583	638	697	741	803	850	697	742	697	759
12/03/91	658	719	789	862	922	998	1,057	886	938	890	964
12/10/91	786	861	946	1,033	1,105	1,197	1,268	1,085	1,137	1,101	1,176
12/17/91	913	1,001	1,101	1,201	1,288	1,394	1,477	1,282	1,335	1,312	1,387
12/24/91	1,039	1,141	1,256	1,370	1,471	1,599	1,686	1,482	1,535	1,521	1,592
12/31/91	1,166	1,281	1,416	1,543	1,654	1,810	1,897	1,693	1,746	1,725	1,796
01/07/92	1,293	1,421	1,580	1,707	1,818	2,022	2,109	1,905	1,958	1,927	1,999
01/14/92	1,422	1,553	1,743	1,870	1,981	2,227	2,314	2,110	2,163	2,133	2,204
01/21/92	1,556	1,687	1,917	2,044	2,155	2,435	2,522	2,318	2,371	2,342	2,413
01/28/92	1,690	1,821	2,081	2,208	2,320	2,631	2,718	2,514	2,567	2,538	2,610
02/04/92	1,824	1,955	2,256	2,383	2,494	2,839	2,926	2,722	2,775	2,746	2,817
02/11/92	1,962	2,093	2,429	2,556	2,667	3,049	3,136	2,932	2,986	2,957	3,029
02/18/92	2,095	2,226	2,598	2,725	2,836	3,253	3,340	3,136	3,189	3,162	3,234
02/25/92	2,235	2,366	2,771	2,898	3,009	3,462	3,549	3,344	3,398	3,372	3,443
03/03/92	2,367	2,498	2,945	3,072	3,183	3,668	3,755	3,551	3,605	3,577	3,648
03/10/92	2,504	2,635	3,130	3,257	3,368	3,886	3,973	3,769	3,823	3,795	3,866
03/17/92	2,635	2,766	3,301	3,428	3,539	4,084	4,171	3,967	4,020	3,992	4,064
03/24/92	2,780	2,911	3,477	3,604	3,715	4,292	4,379	4,175	4,228	4,200	4,272

Appendix 2.—Weekly summary of cumalitive temperature units (degree-days above 32°F) applied to winter-run chinook salmon in incubation units 1-11 and subsequently in rearing units. All fish were moved to rearing tanks on 8/4/92.

Date	Incubation Unit										
	1	2	3	4	5	6	7	8	9	10	11
06/02/92	16	16	16	16	16	16	16	16	15	16	16
06/09/92	182	194	208	215	221	182	184	182	180	183	184
06/16/92	349	374	405	423	442	350	349	349	347	349	351
06/23/92	517	557	605	632	667	532	522	518	533	522	525
06/30/92	685	736	800	841	890	718	689	684	734	687	691
07/07/92	853	918	997	1,050	1,114	906	868	852	935	875	861
07/14/92	1,022	1,102	1,195	1,261	1,329	1,097	1,057	1,024	1,140	1,078	1,035
07/21/92	1,190	1,282	1,391	1,471	1,464	1,284	1,242	1,213	1,343	1,281	1,235
07/28/92	1,357	1,463	1,590	1,679	1,575	1,475	1,430	1,404	1,545	1,483	1,438
08/04/92	1,528	1,645	1,787	1,886	1,686	1,665	1,621	1,592	1,749	1,687	1,644
08/11/92	1,701	1,820	1,975	2,090	-	1,850	1,807	1,778	1,953	1,890	1,847
08/18/92	1,876	1,994	1,994	2,164	-	2,041	1,996	1,969	2,158	2,095	2,052
08/25/92	2,047	2,164	2,351	2,497	-	2,228	2,183	2,155	2,360	2,297	2,255
09/01/92	2,219	2,336	2,538	2,700	-	2,416	2,371	2,342	2,563	2,500	2,457
09/08/92	2,396	2,513	2,725	2,905	-	2,603	2,560	2,531	2,768	2,705	2,662
09/15/92	2,572	2,689	2,912	3,107	-	2,790	2,745	2,716	2,970	2,907	2,866

Appendix 3.—Fall-run experiment number of mortalities by developmental stage and number of fertilized and unfertilized eggs in each cup. CE=cleavage embryo (0-450 TU), E=embryo (451-900 TU), EL=eleutheroembryo (901-1350 TU), and PE-A=pre-emergent alevin (1351-1800 TU).

Incubation unit	Cup	Fertilized eggs	Unfertilized eggs	Incubation phase mortality				Rearing phase mortality
				CE	E	EL	PE-A	
50	1	82	3	3	2	4	-[a]	62
	2	79	2	6	5	3	-[a]	
	3	72	0	2	0	1	-[a]	
	4	80	1	1	4	4	-[a]	
	5	79	1	1	1	0	-[a]	
52	1	79	1	6	0	4	0	124
	2	89	0	6	14	1	1	
	3	81	0	0	1	8	0	
	4	80	0	5	2	7	0	
	5	77	0	0	0	0	0	
54	1	70	0	2	1	3	0	186
	2	76	0	13	10	0	0	
	3	82	0	5	3	1	0	
	4	70	0	3	5	9	1	
	5	78	0	0	0	2	1	

[a] Moved to rearing unit 1 at 1330 TU.

Appendix 3 (continued)

Incubation unit	Cup	Fertilized eggs	Unfertilized eggs	Incubation phase mortality				Rearing phase mortality
				CE	E	EL	PE-A	
	1	74	0	5	3	2	0	
	2	81	0	4	11	3	2	
56	3	78	0	0	2	1	3	223
	4	82	3	4	12	6	0	
	5	76	1	0	0	2	1	
	1	106	0	6	12	1	6	
	2	91	0	18	9	0	12	
58	3	76	0	2	5	17	14	260
	4	72	0	1	2	5	3	
	5	76	0	0	5	1	5	
	1	72	0	2	6	12	8	
	2	79	0	8	15	0	5	
60	3	83	0	4	4	2	17	341
	4	74	0	1	14	5	5	
	5	77	0	1	3	0	12	
	1	81	0	2	8	59	12	
	2	76	0	15	30	20	8	
62	3	76	0	2	23	22	25	378
	4	70	0	31	12	8	6	
	5	75	0	1	14	26	19	

Appendix 3 (continued)

Incubation unit	Cup	Fertilized eggs	Unfertilized eggs	Incubation phase mortality				Rearing phase mortality
				CE	E	EL	PE-A	
	1	78	0	3	4	3	1	
	2	81	0	12	7	0	1	
60E1	3	84	0	1	5	3	11	313
	4	78	1	6	13	9	0	
	5	81	0	1	2	1	2	
	1	84	0	5	6	5	19	
	2	78	0	7	14	7	11	
60E2	3	65	0	2	2	24	11	314
	4	81	0	3	17	9	10	
	5	80	0	0	3	2	5	
	1	82	1	2	7	4	14	
	2	79	1	9	14	4	11	
62E1	3	79	0	1	4	7	30	355
	4	78	0	1	22	1	13	
	5	80	1	0	3	4	7	
	1	75	0	0	5	21	22	
	2	64	0	13	18	6	11	
62E2	3	71	0	2	3	25	20	351
	4	78	0	0	21	15	12	
	5	68	0	1	20	36	6	

Appendix 4.—Winter-run experiment number of mortalities by developmental stage and number of fertilized and unfertilized eggs in each cup. CE=cleavage embryo (0-450 TU), E=embryo (451-900 TU), EL=eleutheroembryo (901-1350 TU), and PE-A=pre-emergent alevin (1351-1800 TU).

Incubation unit	Cup	Fertilized eggs	Unfertilized eggs	Incubation phase mortality				Rearing phase mortality
				CE	E	EL	PE-A	
	1	92	0	1	1	1	1	
56	2	90	0	0	23	2	3	3
	3	89	1	10	3	0	0	
	1	89	0	0	5	0	0	
58	2	83	1	10	17	2	0	17
	3	89	0	13	2	2	28	
	1	87	0	0	4	10	58	
60	2	89	0	0	14	25	13	3
	3	87	0	12	7	19	43	
	1	88	0	0	9	39	37	
62	2	86	0	0	23	21	32	18
	3	88	0	14	16	46	12	
	1	92	0	6	45	41	-	
64	2	90	0	3	66	21	-	-[a]
	3	86	0	5	63	8	-	

[a] Reached 100% mortaltiy during incubation.

Appendix 4 (continued)

Incubation unit	Cup	Fertilized eggs	Unfertilized eggs	Incubation phase mortality				Rearing phase mortality
				CE	E	EL	PE-A	
	1	90	0	2	0	2	0	
60E1	2	91	0	0	9	3	0	23
	3	87	0	17	3	0	25	
	1	91	0	0	2	0	0	
60E2	2	100	0	0	18	0	1	29
	3	94	0	5	11	4	18	
	1	89	0	4	2	0	5	
60E3	2	87	0	0	13	3	3	24
	3	87	0	14	7	1	34	
	1	90	0	0	1	1	8	
62E1	2	87	0	0	16	0	1	92
	3	88	0	7	6	4	26	
	1	91	0	0	2	0	24	
62E2	2	92	0	0	11	3	3	129
	3	88	0	5	4	1	46	
	1	85	0	0	1	2	42	
62E3	2	79	0	1	22	9	8	36
	3	92	0	11	12	20	43	

Appendix 6.—Mean length (L, mm) of subsampled winter-run chinook salmon at transfer to rearing tanks and during rearing.

Rearing unit	Previous incubation unit	Date													
		8/4/92		8/18/92		9/21/92		10/29/92		11/25/92		12/22/92		1/21/92	
		L	SE	L	SE	L	SE	L	SE	L	SE	L	SE	L	SE
1	1	33.7	0.14	35.9	0.28	52.3	1.06	68.9	1.30	80.9	1.55	87.5	2.08	93.4	2.00
	2	33.3	0.20	35.6	0.70	50.5	0.73	68.9	1.22	76.6	1.92	85.9	2.17	88.9	2.73
	mean	33.5	0.12	37.8	0.37	51.4	0.66	68.9	0.87	78.8	1.30	86.7	1.48	91.2	1.73
2	3	31.5	0.28	34.1	0.31	49.4	1.42	67.8	1.41	72.5	0.85	85.1	2.64	88.5	1.30
	6	33.0	0.27	35.6	0.52	50.2	1.63	69.1	1.80	75.4	1.57	83.9	1.31	90.3	2.27
	7	33.0	0.22	36.0	0.58	54.3	0.83	75.9	1.94	78.7	1.22	85.6	1.38	93.2	1.93
	8	33.1	0.18	34.5	0.40	47.3	1.01	64.0	1.23	72.6	1.50	85.8	2.92	83.2	1.25
	mean	32.7	0.13	35.0	0.26	50.3	0.73	69.2	1.04	74.8	0.76	85.1	1.06	88.8	1.02
3	9	31.9	0.31	35.2	0.57	43.8	0.98	59.7	1.76	75.3	1.98	81.5	1.86	86.5	3.01
	10	32.6	0.24	34.5	0.54	45.8	1.32	64.7	0.90	73.6	1.19	84.2	2.10	85.3	1.52
	11	33.2	0.18	35.0	0.52	43.5	1.52	65.7	1.75	75.6	1.54	87.2	2.27	88.8	2.08
	4	30.0	0.31	33.6	0.54	46.0	0.92	-[a]	-	-	-	-	-	-	-
	mean	32.2	0.17	34.6	0.28	44.8	0.61	63.4	0.98	74.8	0.91	84.3	1.24	86.9	1.30

[a] 100% mortality occured.