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COMPARATIVE SURVIVAL OF NATURALIZED STEELHEAD, FERAL KAMLOOPS, AND THEIR HYBRIDS DURING EGG AND FRY STAGES[1]

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Abstract.-- The two strains of anadromous rainbow trout *Oncorhynchus mykiss* currently being stocked in the Minnesota waters of Lake Superior may have the potential to hybridize, thus compromising the genetic integrity of the naturalized population. Although no evidence of natural reproduction has yet been found in the hatchery strain referred to as kamloops, unusual climatic conditions or inadvertent human intervention may enable their propagation and hybridization with the naturalized steelhead strain, since their spawning runs overlap in time and space. Egg viability and fry behavior experiments were undertaken to evaluate the threat of hybridization and to suggest future management of the two strains. Eggs from kamloops were slightly smaller, and displayed higher mortality from spawning to hatch than eggs from steelhead. Differences in feeding behavior and differing tolerances to nitrogen supersaturation caused kamloops to remain healthier in the hatchery environment than steelhead in 1994. Growth and survival of kamloops fry exceeded that of steelhead; hybrids exhibited intermediate traits, but more closely resembled the maternal strain. These differences in size and health appeared to benefit kamloops fry in feeding and predator avoidance trials, but results may be unrelated to growth and survival in natural environments. In feeding trials, kamloops and hybrids consumed more daphnids than pure steelhead, and consumption was directly related to fry length. Predator avoidance abilities by fry of the four mating types were not significantly different. In contrast, steelhead displayed significantly greater wariness than kamloops when startled by movement over their tanks in 1996 trials. Hybrid wariness was intermediate to that of the pure strains, but more closely resembled the maternal strain. The different degrees of wariness were heritable and were indicative of natural selection in steelhead and hatchery selection in kamloops. The reduced survival and self-sustainability potential of kamloops in natural environments, regardless of mechanism, represents a threat to the reproductive potential, genetic integrity, and adaptedness of naturalized steelhead.

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Introduction

Two strains of anadromous rainbow trout *Oncorhynchus mykiss* are currently being stocked into Minnesota waters of Lake Superior to satisfy public demands for a recreational fishery. A potential exists for hybridization between the naturalized steelhead and hatchery kamloops strains, which may compromise the genetic integrity of the naturalized steelhead population. The steelhead strain was introduced from the Pacific coast into Lake Superior over 100 years ago, and has since become naturalized with spawning populations using tributary streams along the north and south shores (Krueger et al. 1994). These "wild" fish spawn in Minnesota tributaries, but barriers near the mouths of most streams limit access to additional areas that are potentially suitable for spawning. Therefore, steelhead adults are captured annually in the French River to obtain additional spawn, eggs are hatched, and unfed swim-up fry are later stocked above tributary barriers. The minimal hatchery influence on these stocked fry reduces (but does not eliminate) artificial selection pressures, while increasing the number of smolts. A limited program to rear and stock yearling steelhead is also in progress for an indeterminate duration.

A second anadromous strain of rainbow trout known as kamloops was introduced into Minnesota waters of Lake Superior in the late 1960s to augment naturalized stocks. Genetic analyses have shown that these fish do not represent the pure form of Kamloops trout originating in the Kamloops area of British Columbia (Krueger et al. 1994), so the strain is referred to in this report in lower case. Due to the perceived threat of hybridization with naturalized steelhead, kamloops stocking has recently been limited to reduce the overlap in spawning runs. Feral adult kamloops in spawning condition, identified by fin clips, are captured annually in the French River, and offspring are raised to yearling size for stocking in the mouths of the French, Lester, and Chester rivers. Because this strain is apparently maintained entirely by stocking, it is referred to as a hatchery strain.

Preserving the genetic integrity of naturalized steelhead stocks will promote self-sustaining populations adapted to local conditions. Genetic variability is necessary if species are to successfully adapt to future natural and human-caused environmental changes (Thorpe et al. 1981; Steward and Bjornn 1990). Artificial selection is inevitable in hatcheries, which increases the risk that hatchery fish will perform poorly under natural conditions. Releases of hatchery fish into areas inhabited by wild stocks can cause a loss of genetic integrity and fitness of the wild stock when wild and hatchery fish interbreed (Waples 1991; Hilborn 1992; Krueger et al. 1994). Hybridization with kamloops could have direct genetic effects reducing the long term fitness and productivity of the steelhead population in Minnesota. Indirect genetic effects of stocking may also occur, such as reductions in size of the wild population due to competition, predation, disease, or other factors (Krueger and Menzel 1979; Waples 1991).

The steelhead fishery in Minnesota reached peak catch per effort in the 1960s, before high levels of stocking took place. However, steelhead catch rates have generally declined over the last 20 years, despite the annual stocking of millions of fry. The North Shore Steelhead Plan (Minnesota Department of Natural Resources 1991) was initiated to investigate the decline of the steelhead fishery, and to gather necessary information to rehabilitate naturalized steelhead stocks. Krueger et al. (1994) investigated the genetic relationships among naturalized and hatchery populations in Minnesota's Lake Superior tributaries. Among naturally spawned yearling fish that were examined, distinct steelhead stocks were identified in many of the Minnesota tributary streams, but no significant contribution from the kamloops strain was found. Thus, there was no evidence that kamloops were successfully reproducing in the wild, or hybridizing with steelhead. In contrast, genetic contributions from a previously stocked Michigan strain were apparent in some locations.

The mechanisms that isolate kamloops and steelhead strains have not been identified, but behavioral or viability differences have been suggested, which could affect spawning adults, incubating eggs, or juvenile fish. Anecdotal evidence suggests that steelhead spawn later, ascend waterfalls better, and spawn further upstream than kamloops. The ability of kamloops to select a spawning site and build a redd, the viability of kamloops and hybrid eggs at ambient river temperature, and the manifestation of appropriate behaviors for survival by fry are also questioned. Behavioral differences manifested by hatchery fish may be caused by psychosensory deprivation in the hatchery environment or by genetic selection for behavior patterns suitable for survival in hatcheries (Olla et al. 1994). Whether isolating mechanisms for steelhead and kamloops are entirely genetic, or a combination of genetic and learned behaviors is uncertain.

Unusual climatic conditions, or inadvertent human intervention could render reproductive isolating mechanisms ineffective. Resulting hybrids could provide a reproductive bridge between the two strains, leading to further interbreeding and reduced genetic adaptedness of steelhead stocks in Lake Superior (Krueger et al. 1994). Preliminary studies in 1977 indicated that hybrid eggs produced from steelhead and kamloops crosses were viable when raised in the French River Coldwater Hatchery at temperatures of 8°C to 10°C (Darryl Bathel, Minnesota Department of Natural Resources, personal communication). Ambient river temperatures at the time of kamloops and steelhead spawning and incubation, however, are quite variable, which could affect survival rates.

The continued lack of successful natural reproduction by kamloops is critical, because it means the population can still be controlled by fisheries managers. Identifying environmental circumstances or behavioral traits that cause differential mortality, or reproductively isolate kamloops and steelhead may help avoid future hybridization. Hybrids could easily and inadvertently be created during hatchery propagation because of a fin clipping error before stocking, or by misidentification of a fin clip, since this is the major feature used for brood stock identification. Other distinguishing traits sometimes used for identification when a clip is ambiguous, such as stockier body shape and smaller eggs in kamloops, are variable and suspect. If hybrid survival can be demonstrated, the practice of continued kamloops stocking should be reevaluated. Central to this issue is the concern that differences manifested by kamloops and steelhead are genetic rather than learned, because learned behaviors are simply corrected by appropriate environmental conditioning, whereas genetic problems cannot be easily or practically corrected. Identical incubation and rearing conditions in this study essentially eliminated the possibility of differential learning.

This study focuses on genetic differences between steelhead, kamloops, and steelhead x kamloops hybrids at the egg and fry stages. The objectives of this study were to test the following hypotheses: 1) eggs resulting from pure steelhead crosses (steelhead x steelhead), pure kamloops crosses (kamloops x kamloops), and hybrid crosses (steelhead x kamloops) are equally viable in French River water at ambient temperatures; 2) steelhead x steelhead, kamloops x kamloops, and steelhead x kamloops fry are equally capable of consuming live food; and 3) steelhead x steelhead, kamloops x kamloops, and steelhead x kamloops fry are equally capable of avoiding predation. Information of this type is timely for managers, who are attempting to develop and justify management strategies that promote healthy, sustainable, economical, and ecologically sound fish populations in Lake Superior (Minnesota Department of Natural Resources 1991).

Methods

Egg Viability Comparisons

The format for these experiments followed a study of lake trout strains by Nelson (1989). Gametes were collected from kamloops and steelhead on five dates from 22 April to 19 May 1994, on days when both strains were being stripped for hatchery propagation. Care was taken to eliminate water (which interferes with postponed fertilization) from gamete samples. Gametes were held on ice until matings were made (within one hour).

The gametes from each parent were subdivided into two parts for diallel crosses. One-half the eggs from each female were fertilized by a male of each strain, and one-half the sperm from each male fertilized eggs of each strain, resulting in a 2 x 2 matrix of four full-sib families as illustrated below:

		Male Parent (m)	
		Steelhead (S)	Kamloops (K)
Female Parent (f)	Steelhead (S)	Sf Sm	Sf Km
	Kamloops (K)	Kf Sm	Kf Km

This procedure was repeated 16 times, making sixteen 2 x 2 diallel sets. Four mating types (SfSm, SfKm, KfSm, KfKm) were thus created with each diallel cross. Two hundred fertilized eggs from each mating type were placed in individual screen-bottomed cartons, and were randomly assigned to locations within an incubator tray. Each tray held eight cartons as shown below:

A	B	C
D		E
F	G	H

front of tray

Eggs were incubated in French River water at ambient temperatures. After eye-up, dead eggs were removed and counted daily from all egg lots until the completion of hatching. Mean egg size was measured volumetrically for each full-sib family. Temperatures in the incubator stack were monitored with a datalogger. Fry with obvious physical deformities at hatching were counted and removed from trays.

Full-sib means and variances were calculated for egg size, mortality from fertilization to hatch, and deformity rates. I analyzed results with the MGLH module of SYSTAT version 5.03 (Wilkinson 1988). Mortality rates and deformity rates were arcsine(square root) transformed prior to testing to normalize the data (Sokal and Rohlf 1981; Nelson 1989). Analyses of variance (ANOVAs) were performed on each measured trait. If a particular effect in the ANOVA was found to be significant, multiple comparisons were made between the different levels of that effect in order to detect significant differences between strains. I evaluated the relationship between mortality and egg size with linear regression, and evaluated strain effects in the linear relationships between mortality and spawning date or egg size with analyses of covariance (ANCOVAs). Relationships and trends were further examined graphically. The term significant refers to statistical significance at the $\propto = 0.05$ level.

Egg size and corresponding maternal body size data were obtained from French River Hatchery files for pure strain steelhead and kamloops reared in 1993, and steelhead reared in 1992. Mortality to hatch data were also available for individual egg lots of 1993 steelhead. These file data were analyzed separately from the experimental data discussed above. ANCOVAs were used to determine if the correlation between egg size and maternal length (or weight) differed with strain of the mother. The relationship between egg size and mortality-to-hatch of the 1993 steelhead year class was evaluated by linear regression.

In spring 1996, additional eggs were incubated and hatched in four 39 cm by 39 cm open trays using heated Lake Superior water, and mortality to hatch was calculated for comparison with 1994 eggs. The number of eggs within each tray varied, but density was very low and the eggs covered only about one-half the bottom surface of each tray. The eggs provided fry for comparisons in wariness behavior, and each tray held one of the four mating types.

Behavior Comparisons

Behavioral tests of fry from each of the four mating types in 1994 were designed to mimic natural settings as closely as practical, and to demonstrate behavioral differences between strains. To ensure that fry had food available for their earliest feeding, the fish were transferred from the incubator trays to open troughs prior to swim-up, combining egg lots of like mating types. Fry from each mating type were tested simultaneously to ensure similar developmental stages, and similar temperatures. Mortality after hatching was noted, and total length of at least 10 fish from each mating type was measured on three dates after swim-up during the behavior testing.

Feeding behavior.--I followed the experimental design of Savino et al. (1993). Trials were run in one-liter beakers, each with a black shield to minimize external disturbances. Forty-seven fry from each of the four mating types were tested individually after swim-up, from 20 June to 11 July. Live *Daphnia magna* served as prey, since rainbow trout fry preferentially select live daphnids as food over benthic organisms, other zooplankters, or dead items (Irvine and Northcote 1982, 1983). Daphnid broodstock were obtained from the Environmental Protection Agency Laboratory in Duluth, and 4-6 day old offspring were used in the feeding trials. Individual fry of each mating type were placed in four test chambers and allowed to acclimate for 30 minutes before daphnids (10/test) were added. The fry were allowed to forage undisturbed for 10 minutes, after which their total lengths were measured, and daphnids in their stomachs were counted. Trials were conducted in river water that was filtered to remove other prey, and water temperatures were noted.

Statistical tests were performed using the MGLH and NPAR modules of SYSTAT (Wilkinson 1988). Mean lengths were calculated for each mating type on each of three dates. Differences in total length between mating types on each date were compared using ANOVAs and the Least-Significant Difference test (Wilkinson 1988; Table 1). Differences in capture rate among strains were compared with Kruskal-Wallis nonparametric one-way ANOVAs, and post-hoc pairwise comparisons were made using Wilcoxon's Signed Ranks Test (Sokal and Rohlf 1981). Mortality after hatching was graphed for each mating type.

The number of daphnids eaten was considered as a logistic function of fish length, fitted by an iterative least squares method. The logistic function was used because the proportion eaten was bounded by 0 and 1 and the function was sigmoid. Various logistic functions were compared using a likelihood ratio test (Weisberg 1985). A three parameter model, including an asymptote variable, was compared with a two parameter model; and separate functions for each mating type were compared with the same function for all types together. Temperature and testing date were added to the analyses, both individually and together, and functions were again compared to determine the best fit.

Predator avoidance.--Experimental design was modified from similar studies by Savino and Henry (1991) and Savino et al. (1993). Four troughs (1.5 m length x 41 cm width x 14 cm depth) with rubble substrate (1.3 cm to 11.4 cm diameter), and flowing river water (<15 cm/s) were used to simultaneously test the four mating types. These substrate and flow conditions follow juvenile steelhead trout preferences outlined by Pauley et al. (1986). Plastic screening directly over the troughs and a dark curtain surrounding the troughs minimized disturbances and prevented escape during the trials. Fry were tested after swim-up from 15 June to 24 June, and each fry was used only once. Two year old juvenile lake trout *Salvelinus namaycush* approximately 20 cm in total length served as predators. The predators were introduced to rainbow trout fry several weeks before trials, but food was withheld for 24 hr prior to testing. Four trials were conducted simultaneously, and individual predators remained in the experimental troughs between trials, so trials were run on alternate days. Before each trial, the predator was confined in one end of the trough while 50 rainbow trout fry were placed in the other end of the trough to acclimate. After 30 min, the divider was removed, and the predator was free to forage. After 6 hr, the number of fry remaining in each trough was counted. Each mating type was tested 6 times, rotating among the four troughs. Water temperature during the trials was noted. From preliminary trials in the winter of 1993-1994, I determined the acclimation and testing time periods. Differences in

Table 1. Statistical tests for rainbow trout strain comparisons. (Significance: ns = not significant for $\alpha \leq 0.05$, * = significant for $\alpha \leq 0.05$, ** = significant for $\alpha \leq 0.01$, >> signifies much greater than). Effects noted in parentheses are based on graphic inspection, not strict statistical analyses. Abbreviations for parental strains and mating types are defined as follows: S=steelhead, K=kamloops, f=female, m=male.

Dependent variable	Independent variable	Statistical test	Significance (of each Indep. Variable)	Significant contrasts (between parental strains or mating types)	Hypothesis tested
mortality to hatch	female, male, f*m[a]	ANOVA	*,ns,ns	Kf>Sf	1
"	female, spawning date, f*d	ANOVA	ns,*,*		1
"	female, tray location, f*t	ANOVA	*,ns,ns		1
"	egg size (all 1994 eggs, then by strain)	Regression	ns		1
"	egg size (1993 steelhead eggs)	Regression	*		1
"	female, spawning date	ANCOVA	slopes *		1
"	female, egg size	ANCOVA	ns		1
"	mating type	shown graphically,Figure 3	(*)	(KfKm>KfSm>SfKm=SfSm)	1
egg size	female, male, f*m[a]	ANOVA	*,ns,ns	Sf>Kf	1
"	female, spawning date, f*d	ANOVA	*,ns,ns		1
"	female, spawning date	ANCOVA	slopes =,		1
			intercepts *		1
"	female, length	ANCOVA	"		1
"	female, weight	ANCOVA	"		1
deformity rate	female, male, f*m[a]	ANOVA	ns,ns,ns		1
length of fry on	female, male, f*m[a]	ANOVA			
June 15			ns,ns,*		1
June 20			ns,*,ns	Km>Sm	1
July 15			**,**,ns	K>>S	1
length of fry on	mating type[b]	ANOVA/Least-Significant-Difference pairwise comparisons (see also Figure 6)			
June 15			ns,**,ns,ns,ns,**[b]	SfSm>KfSm; KfSm<KfKm	1
June 20			ns,ns,*,ns,ns,ns[b]	SfS,<KfKm	1
July 15			*,**,**,*,**,*[b]	SfSm<SfKm<KfSm<KfKm -nonadjacent mating types were most different	1
mortality after hatch	strain & mating type	shown graphically, Figure 7	(*)	(SfSm>SfKm>KfSm>KfKm)	extra[c]
capture rate (daphnids/10 min)	length of fry (together, then by mating type)	Kruskal-Wallis,	*		2
		Wilcoxon Signed Ranks, & log functions (Figure 8)	*	SfSm<SfKm=KfSm=KfKm	2
"	temperature	added to above functions	ns		2
"	date of test	added to above functions	ns		2
# fry left in predation trials	mating type[b]	Kruskal-Wallis	ns		3
"	test trough	Kruskal-Wallis	ns		3
wariness[d]	mating type[b]	Kruskal-Wallis,	**		3
		Wilcoxon Signed Ranks	*,**,**,**,**,ns[b]	SfSm>SfKm>>KfSm=KfKm[d]	3

[a] Use of "female" or "male" as independent variables refers to maternal or paternal strain.
[b] All statistical tests using mating type were done in the following order: SfSm*SfKm, SfSm*KfSm, SfSm*KfKm, SfKm*KfSm, SfKm*KfKm, KfSm*KfKm.
[c] Information used in Discussion.
[d] Wariness is inversely related to the number of fish left in the centers of each tray during wariness trials.

capture rate among strains, trials, and test troughs were compared with Kruskal-Wallis nonparametric one-way ANOVAs (Sokal and Rohlf 1981).

Wariness.--In spring 1996, 200 fry from each mating type were reared in 39 cm by 39 cm open trays (one mating type per tray) in heated Lake Superior water. Whenever fish died in one tray, fish were also removed from each other tray, so all trays always had equal numbers of fish. A 23 cm by 23 cm square outline on the bottom of each tray distinguished the center from the edge, and a camera with a cable release was mounted in a sliding rack above the four trays. The trays, located against a wall at a height of 1.2 m, could be approached in a crouching position to avoid alarming the fish. Differences in wariness exhibited by each of the four mating types were documented in 15 startling trials over a 7 wk period beginning on the date of swim-up and first feeding. Each startling trial proceeded as follows: the camera was slid directly over the appropriate tray, followed by 60 seconds of waiting to insure undisturbed behavior by the fish; one elbow was placed against the tray edge and an open hand was quickly pivoted toward the water surface; the hand was held just above the water surface for 3 seconds; the hand was quickly withdrawn and the cable release was pressed immediately to record positions of the fish (using ambient light). Fish located in the center and edge of the trays were counted from the photographs. Differences in the number of fish located in the center of each tray immediately after startling were compared with a Kruskal-Wallis nonparametric one-way ANOVA, and post-hoc pairwise comparisons were made using Wilcoxon's Signed Ranks test (Sokal and Rohlf 1981).

Results

Egg Viability Comparisons

The spawning runs of steelhead and kamloops were essentially simultaneous (Figure 1), and some eggs from each mating type survived to hatch. Egg size was positively correlated with maternal length and weight, and the function slopes were equal between strains. Steelhead eggs were significantly larger than those of kamloops for females of equal body size, based on 1992-1993 file data (Table 1, Figure 2). Paternal strain had no effect on fertilized egg size, and no significant effect on egg mortality rate. Eggs with maternal steelhead genes showed significantly lower mortality than those without (Table 1; Figure 3 top). KfKm eggs had the highest mortality at nearly 50% and SfSm eggs had the lowest at about 25%. Hybrid egg mortalities tended to fall between the pure strain fish, but more closely resembled the maternal strain. Egg mortality was independent of egg size based on the measurements made in this study (Figure 3 middle). In contrast, mortality and egg size were significantly correlated for 1993 steelhead eggs. Location of the eggs within the incubation tray did not affect mortality rates (Figure 3 bottom). Mortality was significantly higher among eggs spawned at later dates, especially among eggs from kamloops females (Figure 4). Deformity rates were generally low, ranging from 0 to 33% with a mean of 2.6%. There was no significant relationship between deformity rate and parental stock.

River temperatures typically fluctuated 4-5°C daily, and ranged from 1.4-21.1°C during May 1994 (Figure 5). Eggs from later spawning dates developed more quickly in the warmer water than those from earlier spawning dates with incubation periods ranging from 19 to 33 days. Eggs from each spawning date hatched within a 2-3 day period regardless of mating type. Eggs raised in 1996 were held at more constant temperatures (9° - 11.5°C) in heated Lake Superior water, and mortality was lower than that of the 1994 eggs (Table 2).

Table 2. Mortality of eggs reared in 1996 in 39cm x 39cm open trays using heated Lake Superior water. Abbreviations for mating types are defined as follows: S=steelhead, K=kamloops, f=female, m=male.

Mating type	Total number of eggs	Number of dead eggs
SfSm	1076	199 (18.5%)
SfKm	952	184 (19.3%)
KfSm	1574	588 (37.4%)
KfKm	1109	426 (38.4%)

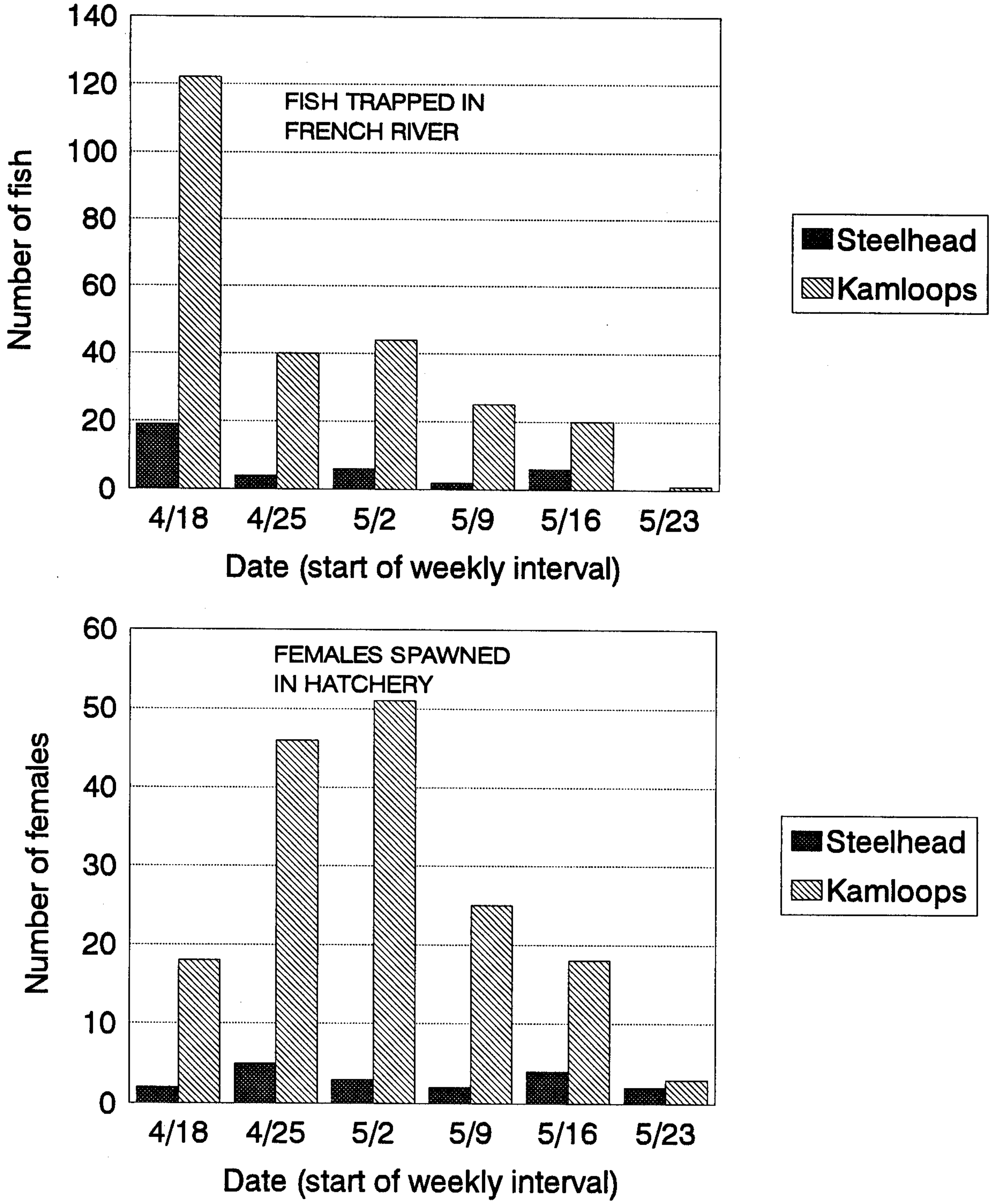

Figure 1. Spawning runs of steelhead and kamloops strain rainbow trout in the French River, spring 1994. The upper graph depicts the weekly totals of steelhead and kamloops captured in the French River trap and brought into the hatchery. The lower graph depicts the weekly totals of females that became ripe for hatchery spawning. Because males are often spawned on several dates, they were not included in the lower graph.

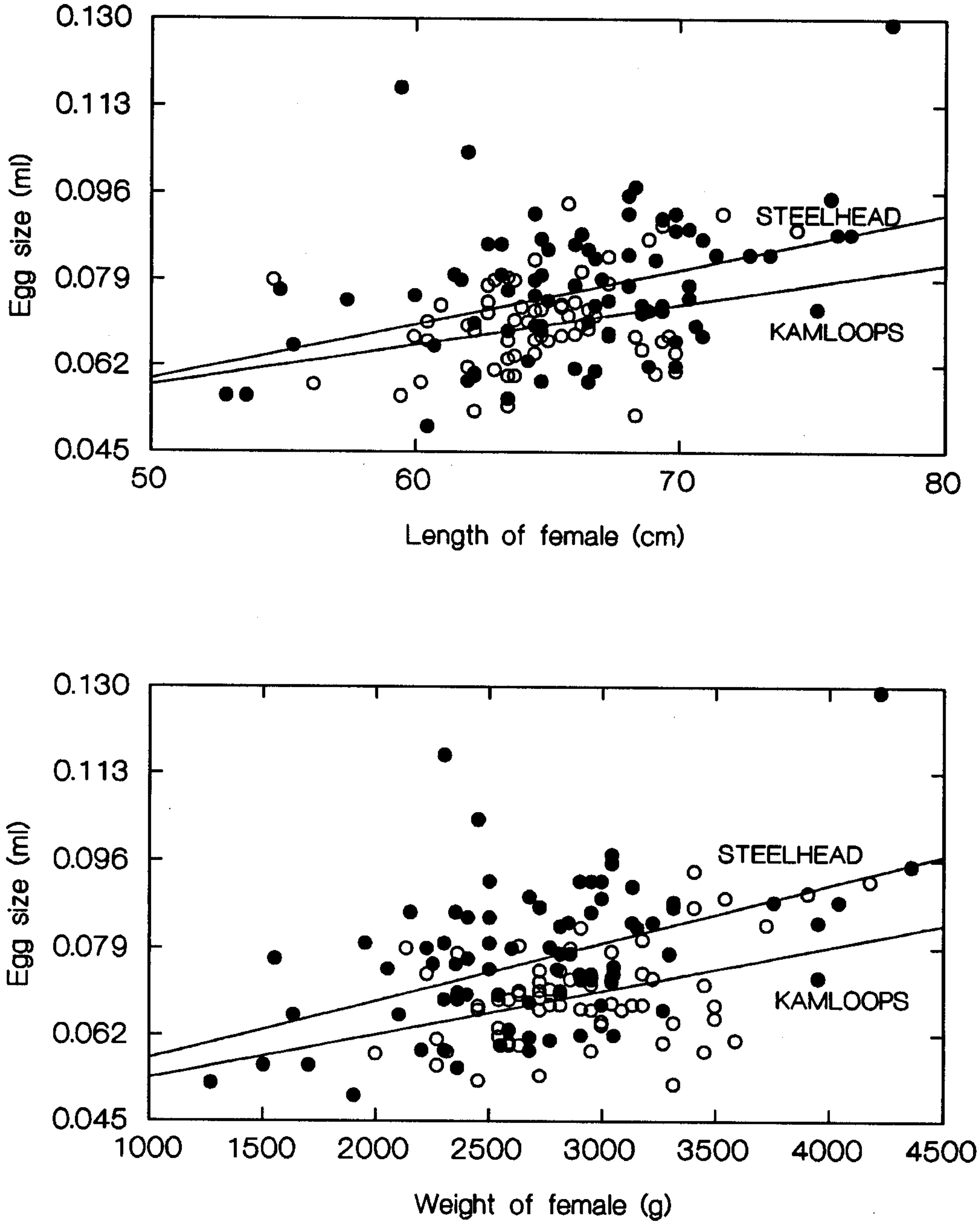

Figure 2. The relationships of egg size to length and weight of female steelhead and kamloops from the French River in 1992-1993 (French River Coldwater Hatchery, file data). Closed circles represent steelhead data; open circles represent kamloops data.

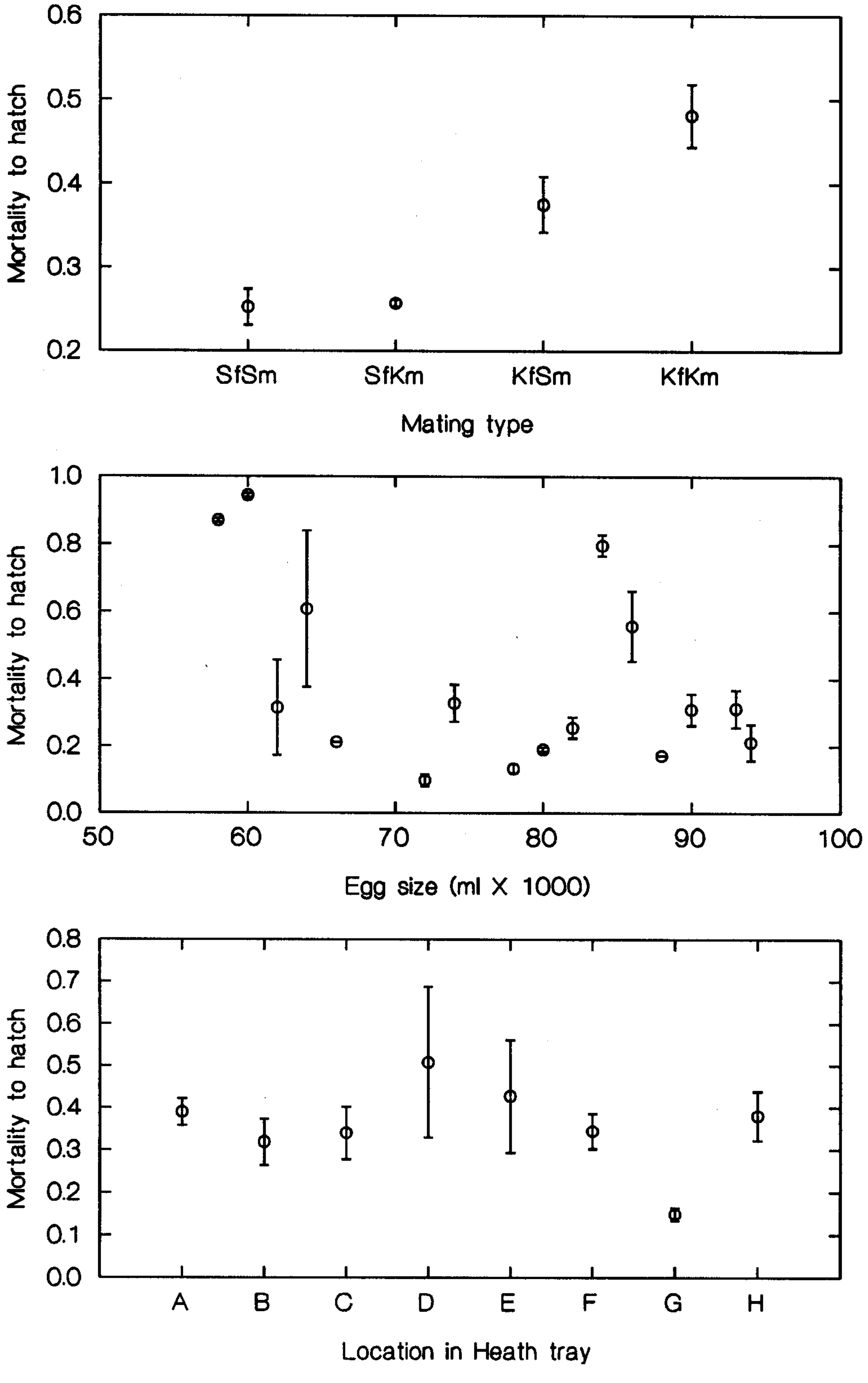

Figure 3. Mean mortality rates versus mating type, egg size, and location of eggs in incubator trays in 1994. Error bars = 95% confidence intervals. Mean mortalities and errors were backtransformed from arcsine(square root) values. Abbreviations for mating types are defined as follows: S=steelhead, K=kamloops, f=female, m=male.

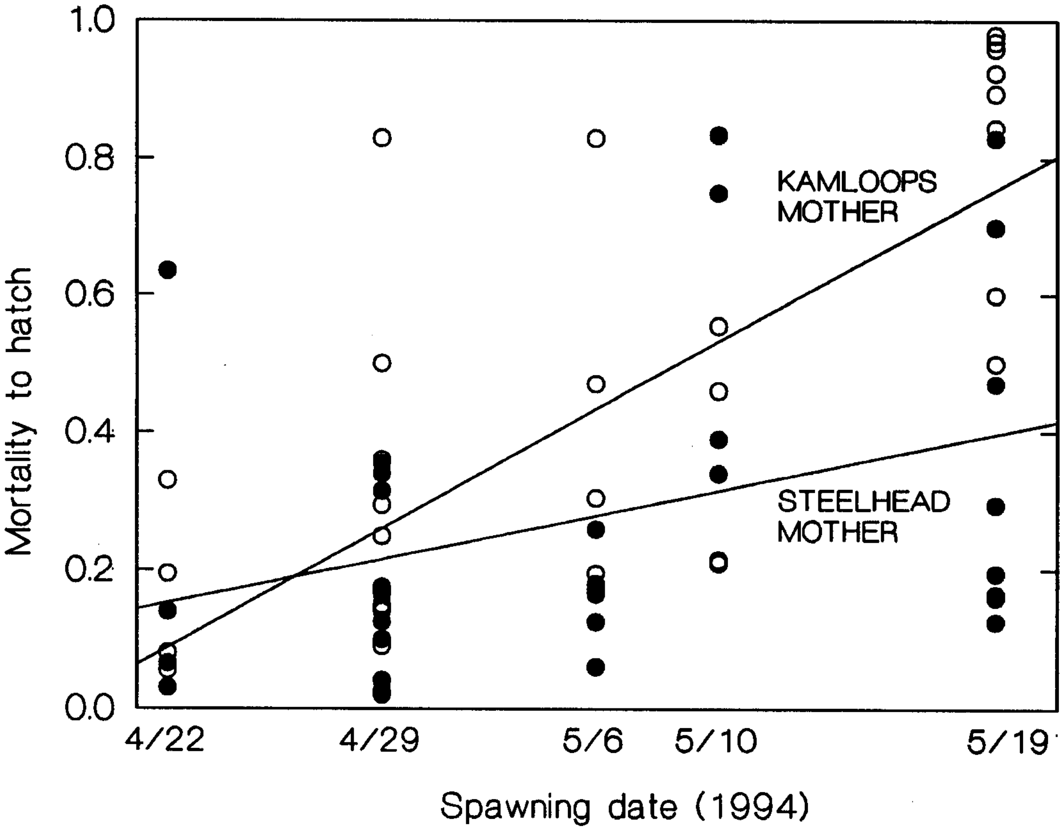

Figure 4. The relationships of egg mortality rates to spawning dates. Closed circles represent steelhead; open circles represent kamloops.

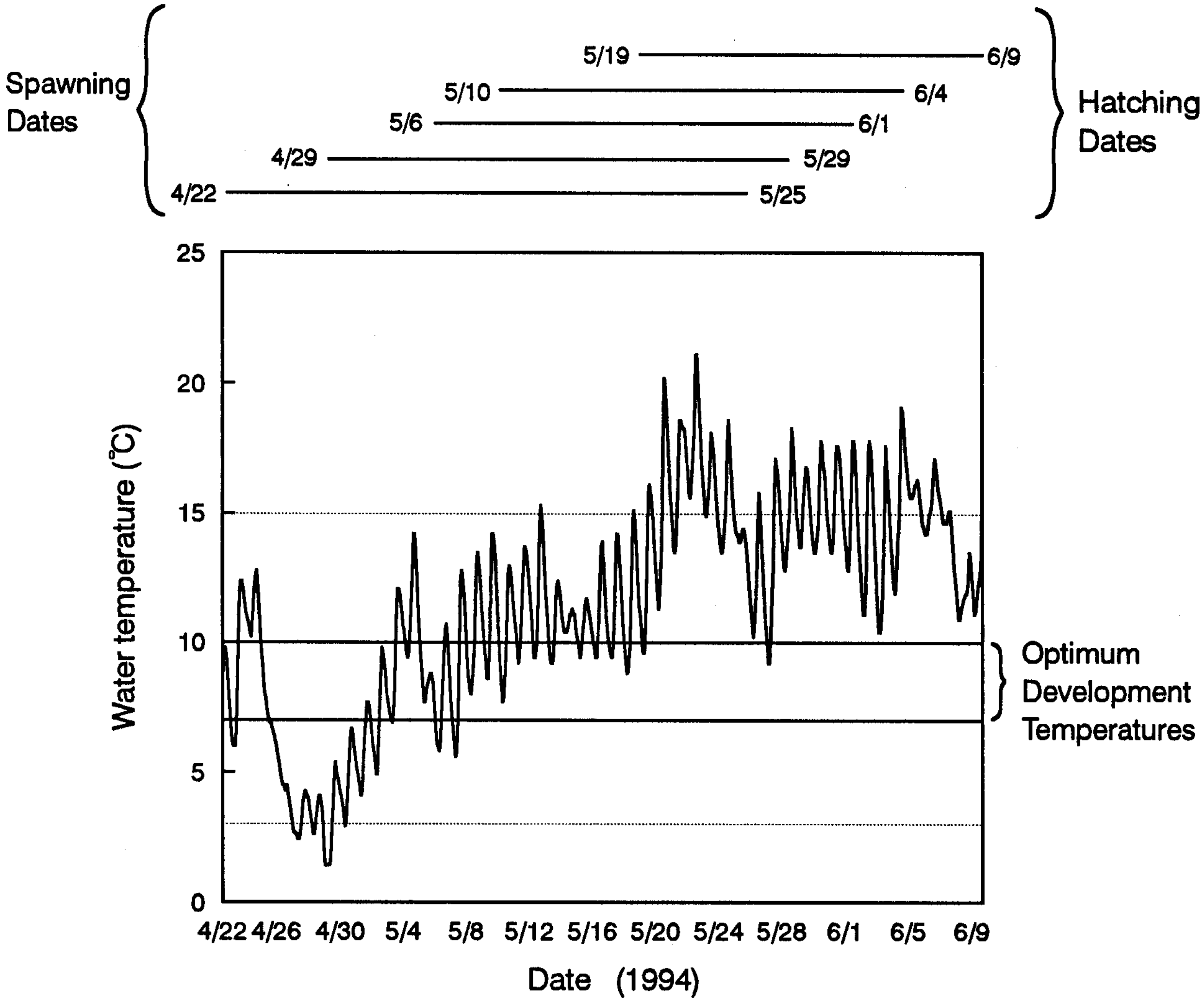

Figure 5. French River water temperatures recorded during egg incubation. The five lines above the graph represent the time periods of egg development for five spawning dates. Optimum development temperatures of 7°C to 10°C (solid lines), and high mortality temperature extremes of 3°C and 15°C (dotted lines) were reported for rainbow trout by Kwain (1975).

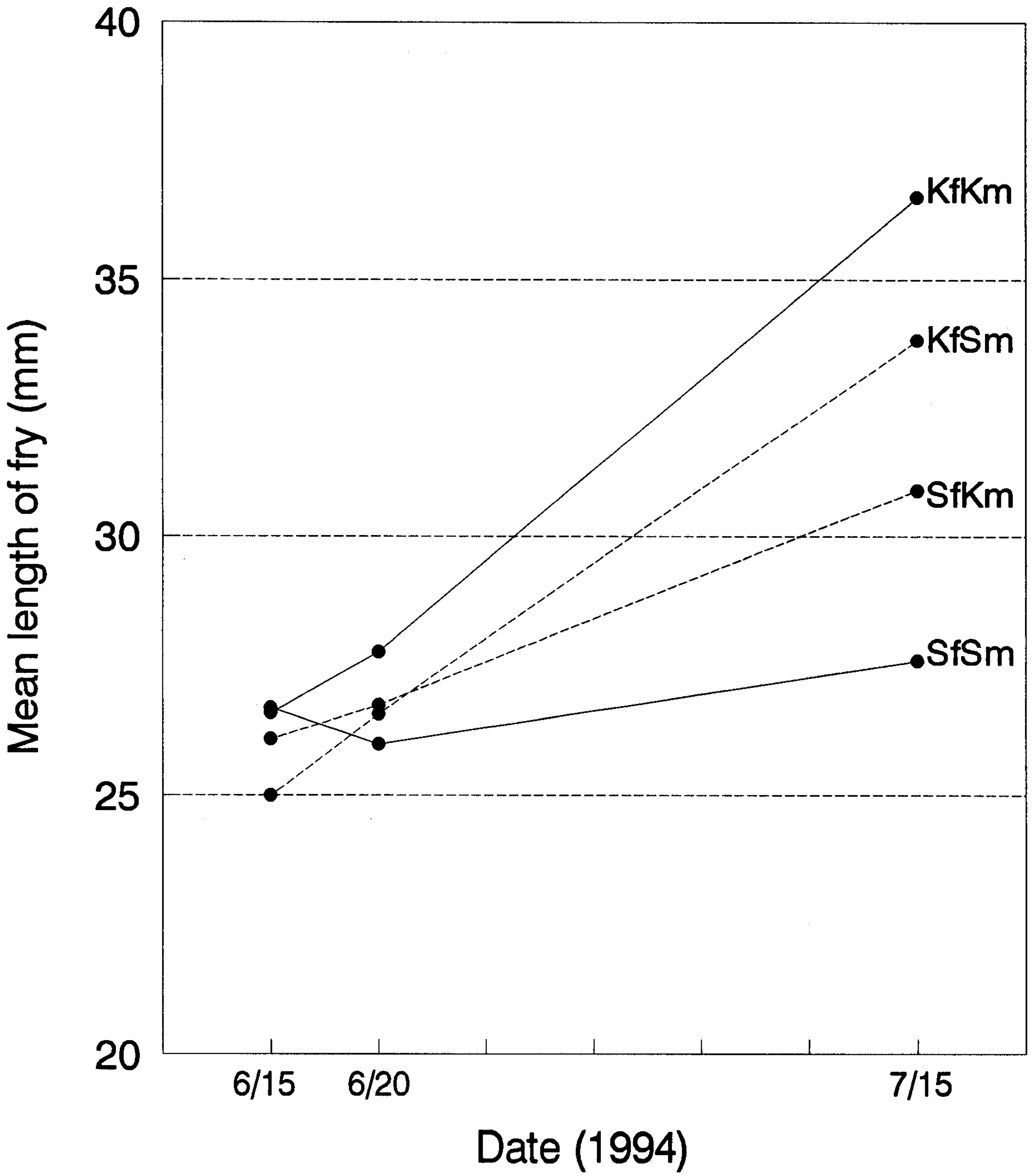

Figure 6. Growth after swim-up of rainbow trout fry from four mating types in the hatchery. Abbreviations for mating types are defined as follows: S=steelhead, K=kamloops, f=female, m=male.

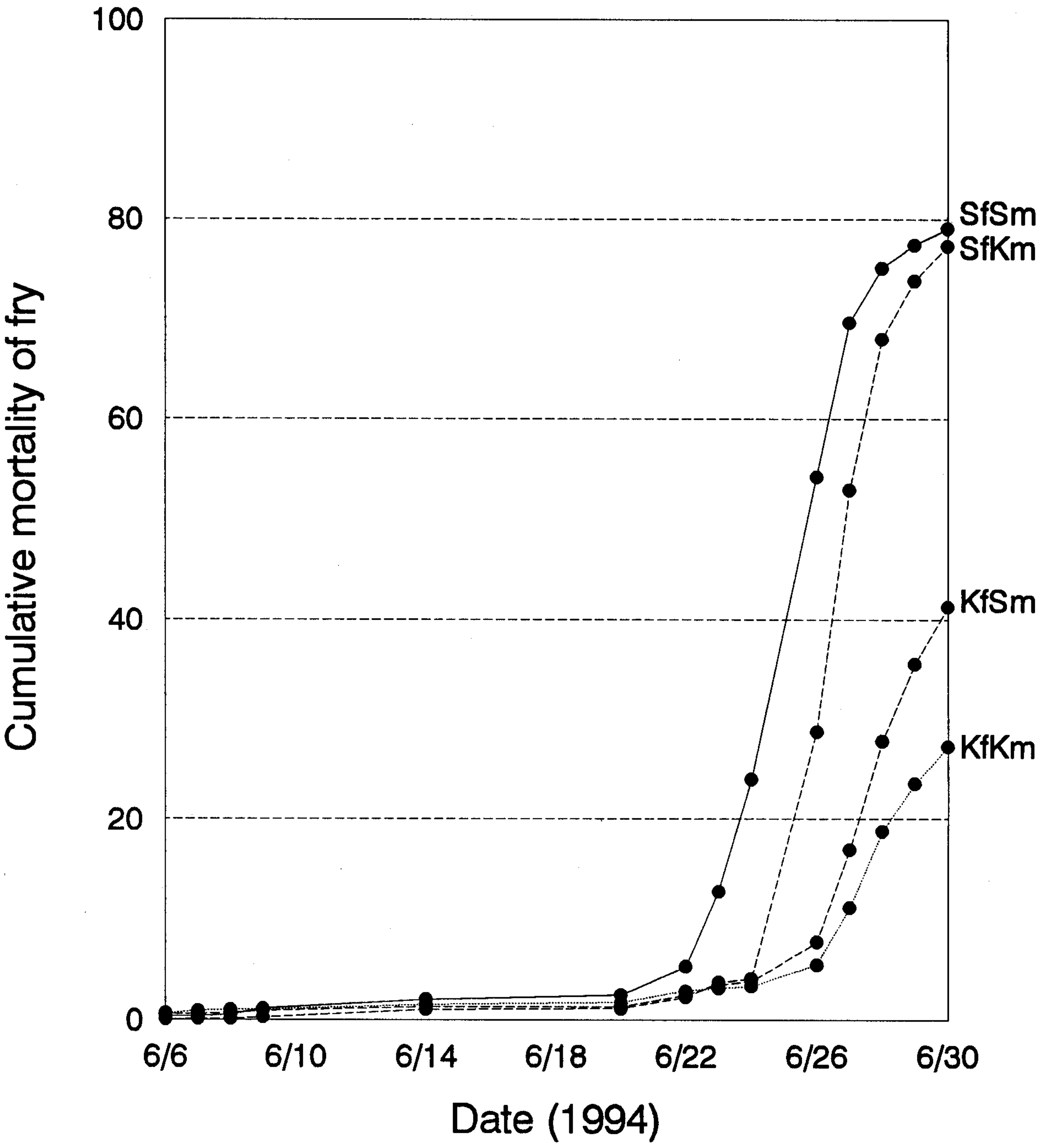

Figure 7. Cumulative mortality of rainbow trout fry from four mating types after hatch in the hatchery. Swim-up had occurred for all fry by 6/14. Abbreviations for mating types are defined as follows: S=steelhead, K=kamloops, f=female, m=male.

The trend of lowest mortality in pure steelhead eggs, highest mortality in pure kamloops eggs, and intermediate mortality resembling that of the maternal strain for hybrids was repeated.

Behavior Comparisons

After swim-up, growth (Figure 6) and survival (Figure 7) of kamloops were superior to that of steelhead in the hatchery conditions. Growth and survival of hybrids were intermediate to the pure strain fish, but more closely resembled their maternal strain. Pure steelhead fry exhibited a distinct startle reaction and hiding response to movement over their trough during food distribution. This reaction appeared to adversely affect steelhead feeding, resulting in slower growth. In contrast, pure kamloops developed a distinct attraction to food distribution, racing to the surface to eat. Hybrid behavior was intermediate to the pure strains, more closely resembling the maternal strain.

A nitrogen supersaturation event (104%), caused a sudden steep rise in mortality beginning on about 21 June 1994. Pure steelhead were least tolerant of the reduced water quality, kamloops were most tolerant, and hybrids were intermediate. Symptoms of nitrogen supersaturation were occasionally noted during feeding trials (primarily in SfSm fry), and these fish invariably consumed little.

Feeding behavior.--Analyses of the feeding experiments showed that KfKm and hybrid fish ate significantly more daphnids than SfSm fry, and significantly more daphnids were consumed by longer fish. The fitted logistic functions also demonstrated that SfSm fry appeared less voracious at most lengths than the other three mating types (Figure 8). The three parameter model, with an asymptote variable, was better than a two parameter model, and separate functions for each mating type provided a better fit than the same function for all types together. There were differences among the other mating types, but not in a consistent direction. The effect of fish length may be somewhat confounded by effects of temperature or date. However, the fit was not significantly improved by the addition of temperature or date to the preferred model.

Predator avoidance.--Predator avoidance experiments were discontinued after six trials because the large numbers of fry used in the trials quickly depleted the dwindling supply of test fish. Results of the predation trials showed no significant differences in the number of fry remaining for any mating type or test trough. The lake trout predators quickly learned to forage very efficiently, even extracting fry from rock crevices, which appeared to overshadow any minor differences in escape capability by the fry. The numbers of fry remaining in the troughs after the first trial ranged from 19 to 37, while the numbers in the last trial ranged from 2 to 15.

Wariness.--Analyses of the wariness experiments showed that significantly fewer SfSm fry remained in the center of the tray after startling (Tables 1&3; Figure 9) than any other mating type. SfKm remained in the center significantly more than SfSm, but less than KfSm or KfKm. KfSm and KfKm fry were most numerous in the tray centers, and were not significantly different from each other. The total number of fish in each tray declined from 200 to 175 during the course of these experiments.

Table 3. Number of fry remaining in the center section of trays after startling in 15 wariness trials. The total numbers of fish in each tray declined from 200 to 175 during the course of these experiments, but numbers of fish in each tray were equal on each date. Abbreviations for mating types are defined as follows: S=steelhead, K=kamloops, f=female, m=male.

Mating type	Mean	95% Confidence interval	Range
SfSm	28.9	±5.23	11 - 54
SfKm	42.1	±7.23	15 - 61
KfSm	59.1	±6.68	35 - 84
KfKm	61.7	±11.57	27 - 103

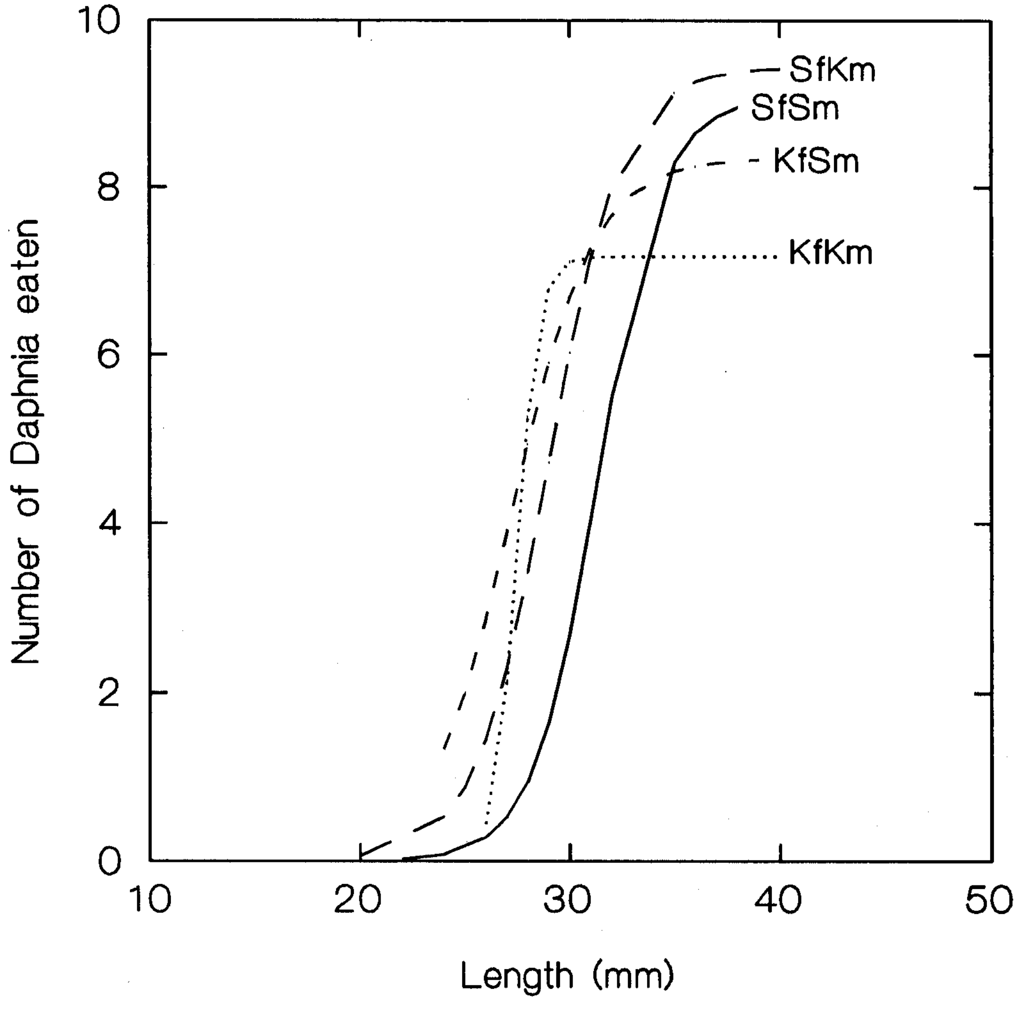

Figure 8. The numbers of daphnids eaten in feeding trials versus lengths of fry of four mating types, expressed as logistic functions fitted by an iterative least squares method. Abbreviations for mating types are defined as follows: S=steelhead, K=kamloops, f=female, m=male.

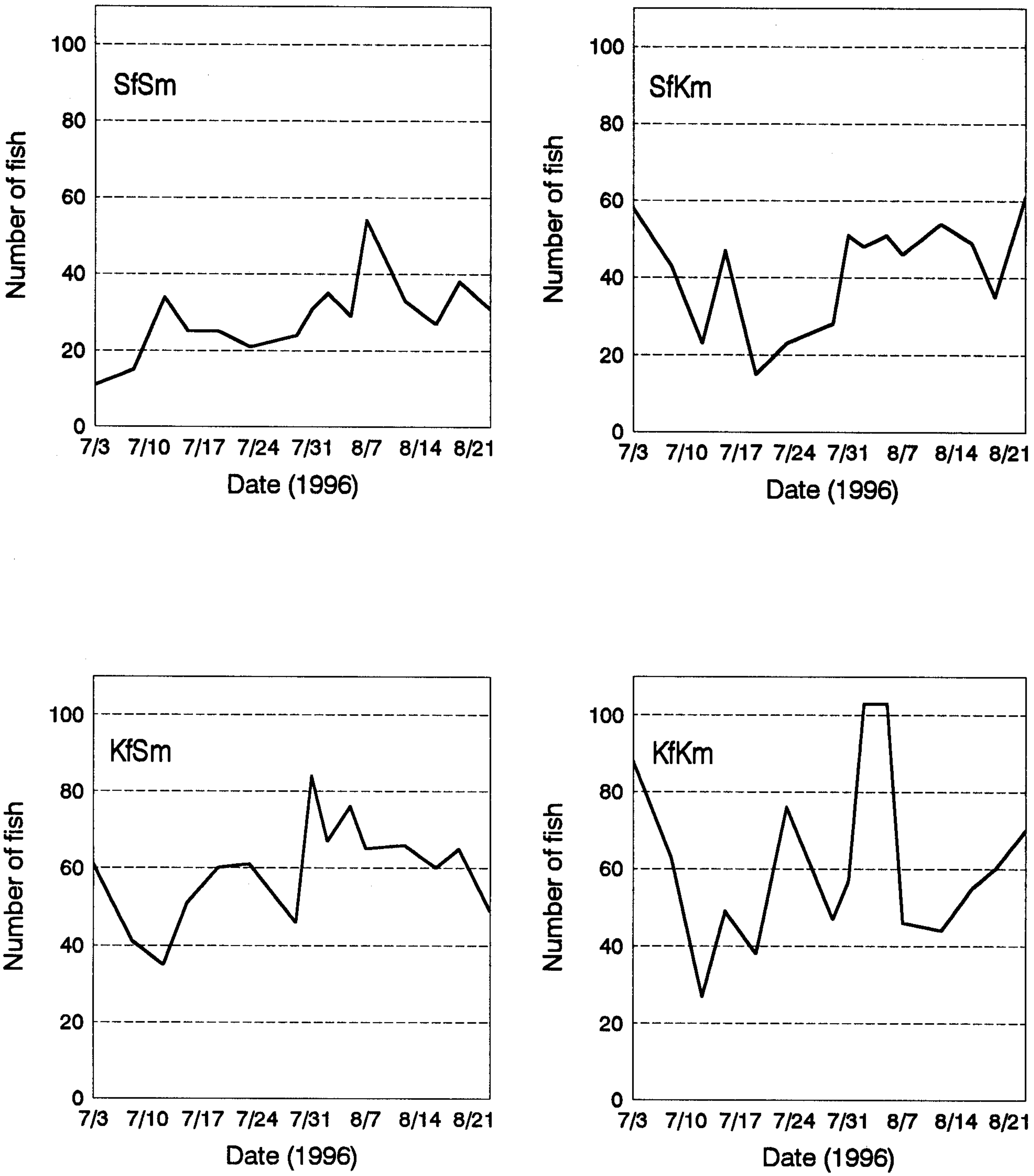

Figure 9. Numbers of fish located in the centers of each tray after being startled in wariness trials. The total numbers of fish in each tray declined from 200 to 175 during the course of these experiments, but numbers of fish in each tray were equal on each date. Abbreviations for mating types are defined as follows: S=steelhead, K=kamloops, f=female, m=male.

Discussion

Egg Viability Comparisons

Egg strain influenced relative mortality rates in both 1994 and 1996. Pure steelhead eggs showed lowest mortality, pure kamloops eggs had highest mortality, and hybrids were intermediate but most similar to their maternal strain. Larger steelhead eggs showed lower mortality than small steelhead eggs in 1993 file data, but this difference was not statistically significant in 1994 experimental data. Development rates and hatching time appeared to be dictated by temperature, not strain, since hatch of all eggs from a particular spawning date was confined to a 3 day period regardless of strain.

Temperature fluctuations appeared to influence egg mortality, but that influence varied by strain. River temperatures in 1994 frequently exceeded optimum rearing temperatures of 7-10°C (Kwain 1975; Figure 5). Temperature extremes of 3° and 15°C, reported to cause significant mortality in rainbow trout eggs (Kwain 1975), were also exceeded. Mortality of kamloops eggs spawned at later dates increased, and mortality of steelhead eggs varied little regardless of spawning time, which suggests that kamloops have a greater sensitivity to warmer temperatures and rapid development. Higher development temperatures (15°C versus 7°C) are known to reduce vertebral and most fin ray counts, increase pectoral fin ray and gill raker counts, and increase the incidence of fused vertebrae in rainbow trout embryos (Kwain 1975). The extent of these or other developmental differences could vary with strain which may influence mortality. Egg mortality was lower for all mating types in 1996 when temperatures were kept more constant.

Along the north shore of Lake Superior, survival of kamloops offspring has not been demonstrated in natural streams. The temperatures that cue adult kamloops to spawn may not promote egg and fry viability. This strain has been sustained for generations through hatchery propagation in water between 8° and 12°C, while water in Minnesota's Lake Superior tributaries can vary 20°C or more during incubation and rearing. The higher mortality of kamloops eggs in widely fluctuating temperatures may be evidence of adaptation to hatchery rearing, while the lower mortality of steelhead eggs at ambient stream temperatures may be an important key to their naturalization. Wide overlaps in ranges of optimum and preferred temperatures have been reported for rainbow trout depending on strain, size, location, and acclimation temperature (Coutant 1977; Jobling 1981; Pauley et al. 1986; Wismer and Christie 1987). Lower avoidance temperatures range from 6°C to 15°C, final preferenda range from 11.4°C to 22.2°C, and upper avoidance temperatures range from 19°C to 27°C. The relationships between temperature, egg mortality, and meristic characteristics of steelhead, kamloops, and hybrids can only be evaluated through additional rearing studies of each strain at precisely controlled temperatures.

Eggs resulting from pure steelhead crosses (steelhead x steelhead), pure kamloops crosses (kamloops x kamloops), and hybrid crosses (steelhead x kamloops) were not equally viable in French River water at ambient temperatures, or in heated Lake Superior water. Thus, hypothesis #1 is rejected based on the results of these experiments. Fertilized eggs with maternal steelhead genes survived at a significantly greater rate than those with maternal kamloops genes, but some eggs from each mating type were viable. Inter-strain spawning, and the reduced survival of eggs with kamloops genes, could provide an explanation for declining numbers of steelhead in Minnesota's Lake Superior tributaries. Further study of spawning interactions is needed to validate the plausibility of this hypothesis.

Behavior Comparisons

The 1994 behavior trials testing consumption of *Daphnia* and predation by lake trout were compromised by poor condition in some fry due to reduced feeding efficiency, and to reduced water quality since the trials coincided with the nitrogen supersaturation event. The startle response which caused steelhead to hide during food distribution, and the attraction response which brought kamloops toward the surface during food distribution appeared to affect feeding efficiency in the hatchery, resulting in a divergence in growth rates between mating types. Pure steelhead fry demonstrated the slowest growth, pure kamloops fry grew fastest, and hybrids were intermediate. Because feeding efficiency was directly correlated with length of the fry, condition prior to the behavior trials is implicated as a major influence on trial outcome.

Post hatch differences in growth and survival observed in these experiments reflect varied adaptation to different environments. The startle response of steelhead was a disadvantage, while the attraction response by kamloops was an advantage in the hatchery where aggressive feeding in the absence of predators promoted growth and survival. Similar responses have been noted by hatchery managers, who report that steelhead fail to thrive as well as kamloops due to behavior and feeding differences (Fred Tureson, Minnesota Department of Natural Resources, personal communication). In contrast, the same responses may reflect superior adaptation by steelhead for survival in the wild, where predator avoidance must temper feeding behavior. The behaviors noted in 1994 led to the design of the wariness experiments in 1996.

Wariness experiments were included in this study to document and quantify behavior differences between pure steelhead and kamloops fry reared under identical conditions, and to demonstrate the effects of hybridization on the expression of that behavior. Results in 1996 concurred with 1994 observations that pure steelhead displayed the greatest wariness, pure kamloops showed the least wariness, and hybrids were intermediate but resembled the maternal strain more closely. The results of these experiments demonstrate the heritability of behavioral traits. Vincent (1960) photographed similar behavior in brook trout, but the differences between wild and hatchery strains were not quantified. Studies by Johnsson (1993) suggested that size-selection in hatcheries favored fish that foraged readily with little regard for predation risk. Reisenbichler and McIntyre (1977) demonstrated significantly greater survival among offspring of wild steelhead compared with hatchery x wild progeny stocked in natural streams. Studies of foraging behavior by steelhead/domesticated trout hybrids (Johnsson and Abrahams 1991) showed that the hybrid juvenile fish exhibited less caution in the presence of a predator than pure steelhead juveniles, indicating that behaviors may be heritable. Dellefors and Johnsson (1995) found that hatchery-reared and wild brown trout foraged with equal frequency in the presence of a predator, but wild fish avoided the predators more when not feeding. In studies by Vincent (1960) and Moyle (1969), wild brook trout fry demonstrated an affinity for aquarium substrates, while domestic fry moved readily throughout the water column. Subsequent survival trials in wild environments demonstrated reduced survival and persistent lack of wariness in domestic brook trout (Vincent 1960). Other salmonines have shown differences in swimming or feeding ability between wild and hatchery strains, but few differences were demonstrated at the fry stage (Bams 1967; Sosiak et al. 1979; Savino et al. 1993).

Steelhead x steelhead, kamloops x kamloops, and steelhead x kamloops fry were not equally capable of consuming live food in the laboratory setting. Thus, hypotheses #2 was rejected. Pure kamloops fry appeared to feed best, pure steelhead fry fed least, and the hybrids were intermediate. These abilities were directly correlated to length of the fish, and may be contrary to feeding efficiencies in wild environments.

Hypothesis #3 was rejected because the behavioral differences between strains demonstrated in the wariness experiments suggest differential adaptations to hatchery and natural environments. These adaptations may leave pure kamloops and hybrids more vulnerable to predation in streams. Further experimentation to investigate predator avoidance could include the following: 1) use a shorter testing period, as some fry may be able to avoid predators for a limited time in a confined space; 2) expose fry to predators once before testing, as predator avoidance may be learned to some extent (Olla et al. 1994), and some fry may learn better than others; or 3) use sculpins as predators, since they are prevalent in many tributaries of Lake Superior, they can be significant predators of trout eggs and fry (Savino and Henry 1991), and they may provide a realistic clue to differential survival in Minnesota's streams.

Conclusions

A long-term management goal of the Minnesota Department of Natural Resources (MNDNR 1991) is to preserve and enhance self-sustaining stocks of anadromous rainbow trout in Lake Superior. However, wild stocks generally continue to decline when supplemented by hatchery propagation (Steward and Bjornn 1990), and this trend is evident in Minnesota waters. The reduced viability of pure kamloops and hybrid eggs at ambient river temperatures, and the different wariness responses in fry, represent genetic differences that may provide clues to the apparent lack of naturally reproduced kamloops or hybrids in Lake Superior tributaries.

Natural and hatchery selection processes may fortuitously reduce hybrid or "inappropriate" strain survival, but the uncertainty of this hypothetical selection poses a threat to steelhead genetic integrity. Hybrids or pure steelhead inadvertently created during kamloops yearling propagation may not survive the intense hatchery selection, due to less aggressive feeding, or they may simply grow more slowly reducing their survival potential in Lake Superior. Hybrids or pure kamloops inadvertently created during steelhead fry propagation may be eliminated in the streams, due to a lack of wariness. However, some hybrids may be able to survive in the environment to which their maternal strain is adapted. Also, certain hatchery practices threaten to reduce the behavioral differences that distinguish steelhead and kamloops. For example, rearing steelhead to yearling size in hatcheries produces a domesticated product that is no longer "wild." Hatchery selection necessarily favors those individuals that will thrive under hatchery feeding regimes, so successive generations of domesticated steelhead yearlings will likely resemble kamloops yearlings in behavior and survival.

The adaptability of rainbow trout in general increases the likelihood of eventual interbreeding and survival of some hybrids in the two strains whose breeding overlaps in time and space. A spring season with especially favorable temperatures or flows for egg and fry survival, a stream section with particularly abundant food or protective cover, or a spawning season with high water allowing easier passage upstream for adults could increase the likelihood for hybridization or fry survival. Even if hybrids do not survive to pose a genetic threat, the reproductive products of steelhead could still be wasted in the process of unsuccessful breeding with kamloops, reducing their reproductive potential.

Management Implications

1) The wide overlap in egg sizes between steelhead and kamloops renders this trait highly unreliable as a method of strain identification when fin clips are ambiguous. If ambiguity exists, it would be safer to eliminate the fish rather than to risk the creation of hybrids.

2) Hatchery selection necessarily favors those individuals that will thrive under hatchery feeding regimes, so successive generations of domesticated steelhead yearlings will no longer be "wild," but will likely resemble kamloops yearlings in behavior and survival.

3) If steelhead are stocked, they should only be reared to the fry stage to reduce the extent of hatchery selection, maintain reproductive and survival potential, and preserve wild genotypes as much as possible. Since domesticated steelhead yearlings resemble kamloops in behavior, there is a greater probability of interbreeding between these two strains, which would increase the risk of future hybridization, loss of survival potential, and loss of wild genotypes.

4) The apparent reduced potential for self-sustainability in kamloops, regardless of whether it occurs at the egg stage, during stream life, in the reproductive process of adults, or through competitive interactions, represents a threat to the genetic integrity, adaptedness, and reproductive capacity (from

both wasted gametes, and offspring with poor survival) of wild steelhead stocks.

References

Bams, R. A. 1967. Differences in performance of naturally and artificially propagated sockeye salmon migrant fry, as measured with swimming and predation tests. Journal of the Fisheries Research Board of Canada 24:1117-1144.

Coutant, C. C. 1977. Compilation of temperature preference data. Journal of the Fisheries Research Board of Canada 34:739-745.

Dellefors, C., and J. I. Johnsson. 1995. Foraging under risk of predation in wild and hatchery-reared juvenile sea trout (*Salmo trutta* L.). Nordic Journal of Freshwater Research 70:31-37.

Hilborn, R. 1992. Hatcheries and the future of salmon in the Northwest. Fisheries 17(1):5-8.

Irvine, J. R., and T. G. Northcote. 1982. Significance of sequential feeding patterns of juvenile rainbow trout in a large lake-fed river. Transactions of the American Fisheries Society 111:446-452.

Irvine, J. R., and T. G. Northcote. 1983. Selection by young rainbow trout (*Salmo gairdneri*) in simulated stream environments for live and dead prey of different sizes. Canadian Journal of Fisheries and Aquatic Sciences 40:1745-1749.

Jobling, M. 1981. Temperature tolerance and the final preferendum - rapid methods for the assessment of optimum temperatures. Journal of Fish Biology 19:439-455.

Johnsson, J. I. 1993. Big and brave: size selection affects foraging under risk of predation in juvenile rainbow trout, *Oncorhynchus mykiss*. Animal Behavior 45:1219-1225.

Johnsson, J. I., and M. V. Abrahams. 1991. Interbreeding with domestic strain increases foraging under threat of predation in juvenile steelhead trout (*Oncorhynchus mykiss*): an experimental study. Canadian Journal of Fisheries and Aquatic Sciences 48:243-247.

Krueger, C. C., and M. W. Menzel. 1979. Effects of stocking on genetics of wild brook trout (*Salvelinus fontinalis*) populations. Transactions of the American Fisheries Society 108:277-287.

Krueger, C. C., D. L. Perkins, R. J. Everett, D. R. Schreiner, and B. May. 1994. Genetic variation in naturalized rainbow trout (*Oncorhynchus mykiss*) from Minnesota tributaries to Lake Superior. Journal of Great Lakes Research 20:299-316.

Kwain, W. 1975. Embryonic development, early growth, and meristic variation in rainbow trout (*Salmo gairdneri*) exposed to combinations of light intensity and temperature. Journal of the Fisheries Research Board of Canada 32:397-402.

Minnesota Department of Natural Resources. 1991. North Shore steelhead plan. Minnesota Department of Natural Resources, Section of Fisheries. St. Paul.

Moyle, P. B. 1969. Comparative behavior of young brook trout of domestic and wild origin. The Progressive Fish-Culturist 31:51-56.

Nelson, D. M. 1989. Application of diallel crosses to lake trout (*Salvelinus namaycush*) stock evaluation. Master's thesis, University of Minnesota, St. Paul.

Olla, B. L., M. W. Davis, and C. H. Ryer. 1994. Behavioural deficits in hatchery-reared fish: potential effects on survival following release. Aquaculture and Fisheries Management 25 (Supplement 1):19-34.

Pauley, G. B., B. M. Bortz, and M. F. Shepard. 1986. Special profiles: life histories and environmental requirements of coastal fishes and invertebrates (Pacific Northwest) -- steelhead trout. U.S. Fish and Wildlife Service Biological Report 82(11.62). U.S. Army Corps of Engineers, TR EL-82-4.

Reisenbichler, R. R., and J. D. McIntyre. 1977. Genetic differences in growth and survival of juvenile hatchery and wild steelhead trout, *Salmo gairdneri*. Journal of the Fisheries Research Board of Canada 34:123-128.

Savino, J. F., and M. G. Henry. 1991. Feeding rate of slimy sculpin and burbot on young lake charr in laboratory reefs.

Environmental Biology of Fishes 31:275-282.

Savino, J. F., M. G. Henry, and H. L. Kincaid. 1993. Factors affecting feeding behavior and survival of juvenile lake trout in the Great Lakes. Transactions of the American Fisheries Society 122:366-377.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry. W. H. Freeman and Company, New York.

Sosiak, A. J., R. G. Randall, and J. A. McKenzie. 1979. Feeding by hatchery-reared and wild Atlantic salmon (*Salmo salar*) parr in streams. Journal of the Fisheries Research Board of Canada 36:1408-1412.

Steward, C. R., and T. C. Bjornn. 1990. Supplementation of salmon and steelhead stocks with hatchery fish: a synthesis of published literature. Part 2 *in* W. H. Miller, editor. Analysis of salmon and steelhead supplementation. U.S. Department of Energy, Bonneville Power Administration, Division of Fish and Wildlife, Technical Report 1990. Portland, OR.

Thorpe, J. E., J. R. Koonce, D. Borgeson, B. Henderson, A. Lamsa, P. S. Maitland, M. A. Ross, R. C. Simon, and C. J. Walters. 1981. Assessing and managing man's impact on fish genetic resources. Canadian Journal of Fisheries and Aquatic Sciences 38:1899-1907.

Vincent, R. E. 1960. Some influences of domestication upon three stocks of brook trout (*Salvelinus fontinalis* Mitchill). Transactions of the American Fisheries Society 89:35-52.

Waples, R. S. 1991. Genetic interactions between hatchery and wild salmonids: lessons from the Pacific Northwest. Canadian Journal of Fisheries and Aquatic Sciences 48(Suppl.1):124-133.

Weisberg, S. 1985. Applied Linear Regression. John Wiley and Sons, New York.

Wilkinson, R. S. 1988. SYSTAT: the system for statistics. SYSTAT, Evanston, Illinois.

Wismer, C. A., and A. E. Christie. 1987. Temperature relationships of Great Lakes fishes: a data compilation. Great Lakes Fishery Commission Special Publication 87-3, Ann Arbor, MI.