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Effects of inbreeding coho salmon (Oncorhynchus kisutch) on survival during early life history, growth, feed efficiency, and resistance to gas bubble disease were studied. No effect of inbreeding could be associated with either F=0.25 or F=0.125. Tetraploidy may help explain the absence of inbreeding depression at these levels.

Effects of Inbreeding Coho Salmon (*Oncorhynchus kisutch*)

by

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INTRODUCTION

The coefficient of inbreeding (F) has been defined as the probability that two alleles at a locus are identical by descent (Wright 1921). It also represents the average reduction in heterozygous loci per individual or per population, measured from some base generation before the inbreeding began (Fisher 1949). Depressive effects of inbreeding, particularly on fitness characters, have been described in various animal species by Falconer (1960). Examples of inbreeding depression per 10% increase in F are: pigs litter size at birth 4.6%, sheep fleece weight 5.5%, poultry hatchability 6.4%, and Drosophila subobscura fertility (per pair/day) 12.5% (Falconer 1960). These results suggest that mild inbreeding (F=0.10) can result in significant depressions. Therefore, inbreeding coho salmon at F=0.25 and F=0.125 also might be expected to cause significant depressions in survival, feed efficiency, growth, or resistance to disease.

Moav and Wolfarth (1963) demonstrated a 15% reduction in relative growth rate and an increase in the frequency of dorsal fin anomolies in the offspring of full-sib matings of carp (Cyprinus carpio). Aulstad and Kittleson (1971) reported that inbreeding rainbow trout (Salmo gairdneri) increased the frequency of deformities.

Bridges (1973) demonstrated a 0.4% depression in formalin tolerance in inbred (F=0.10) rainbow trout. Kincaid (1976) found that depression in the number and weight of rainbow trout remaining in a production lot at one year of age was 17.4% and 36.6% after one generation (F=0.25) and 47.9% and 65.4% after two generations (F=0.375) of inbreeding.

The objectives of the present study were to determine effects of inbreeding (F=0.25 and F=0.125) on survival and growth of juvenile coho salmon (Oncorhynchus kisutch) in a hatchery environment. Specifically, the effects of inbreeding were measured for: 1) percent survival at specified life history stages; 2) feed efficiency; 3) growth; and 4) percent survival after exposure to air supersaturated water.

METHODS AND MATERIALS

This study on the effects of inbreeding in coho salmon was begun when the 1969 brood adults returned to Big Creek Hatchery on the lower Columbia River in 1972. At that time, eggs from each of five females were divided into 12 equal lots; and each lot was fertilized by one of 12 males, resulting in a five by 12 factorial mating scheme. The offspring of these 60 matings were marked with unique coded-wire tags and given an adipose fin clip. All families were reared in a single raceway until they were released from the hatchery.

On November 11, 1975, when these fish returned to Big Creek Hatchery, each fish with an adipose-clip was sacrificed and spawned into separate 2 liter plastic containers. The snouts were removed from the carcasses and numbered to correspond to the gamete container. Eggs and sperm were held at $4^{o}C$ until matings were made approximately 12 hours later. Meanwhile, the tags were removed from the snouts and decoded, and the gametes identified to family.

The eggs from four females that had no relatives returning were pooled, and the sperm from two groups of two males each with no relatives returning also were pooled. It was assumed that these fish were related to each other no more than was the average relationship for the population. Matings among these individuals were assumed to produce progeny with no inbreeding or F=0.0. These

males and females were used to produce non-inbred and common male and female controls (Fig. 1). Mating single females simultaneously with both a full-brother and the pooled sperm from two non-related males produced three contemporary inbred (F=0.25) and three common female non-inbred families. The sperm used to produce the inbred families also was used to produce three contemporary non-inbred common male families. A like procedure was used to generate four half-sib families (Fig. 1).

The full-sib matings resulted in three families in which the inbreeding coefficient was 25%, and the half-sib matings resulted in four families in which the inbreeding coefficient was 12.5%. "Common male" families are families sired by an individual male and from pooled eggs of four unrelated females. "Common female" families are those families resulting from the eggs of a single female mated to pooled semen from two unrelated males. For each inbred family, there was a common female family and a common male family.

Fertilized eggs from each family were placed into separate Heath® incubator trays suspended in a trough of flowing water. Trays were labeled and placed into the rack at random. On December 22, 1975, after the eggs had reached the eyed stage of development, each tray was removed, the eggs shocked, and the dead eggs were counted. The number of surviving eggs in each family was estimated volumetrically, and percent survival from fertilization to the eyed stage

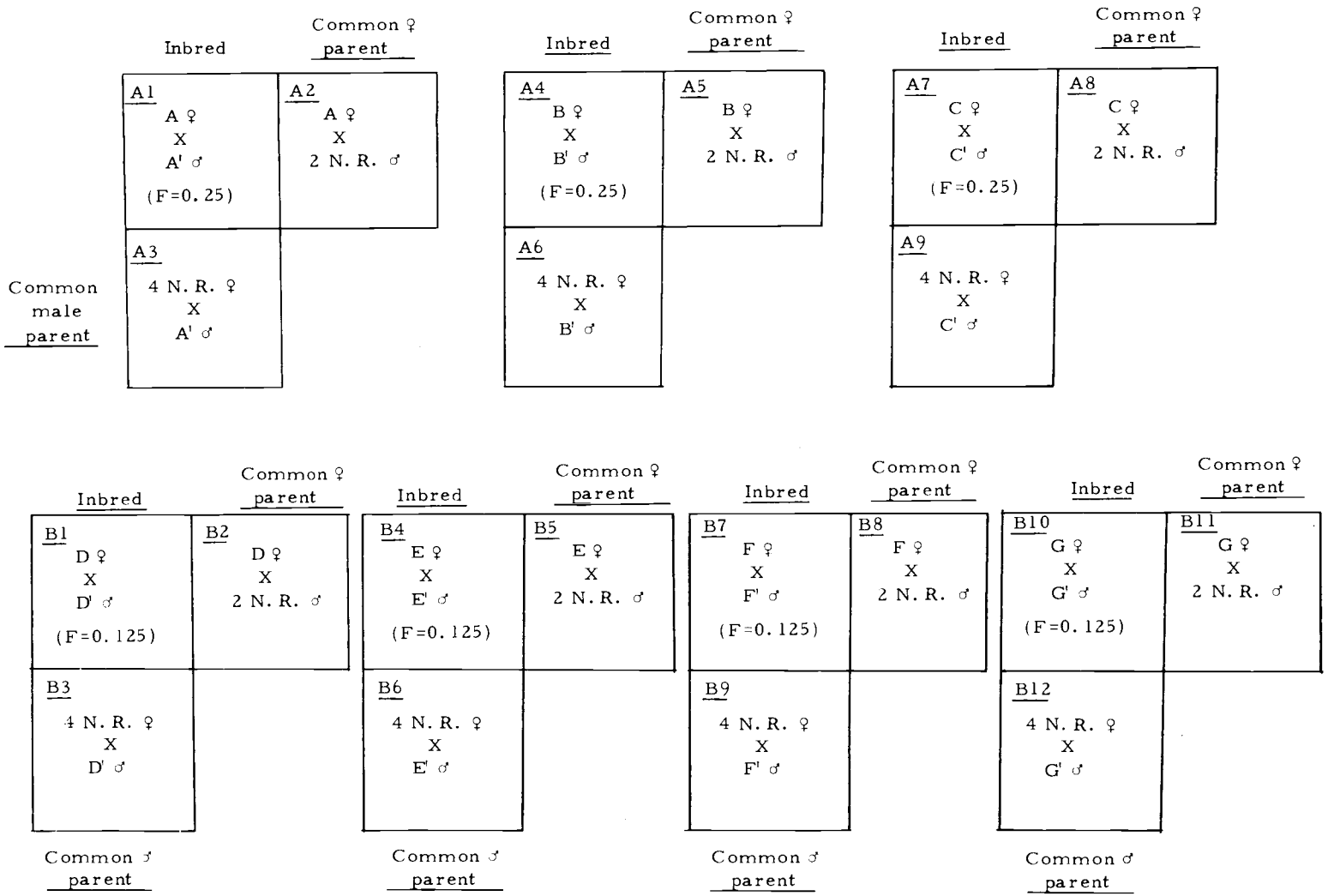

Figure 1. Experimental design used to produce 3 groups of inbred (F=0.25) and 4 groups of inbred (F=0.125) coho salmon and the common male and female controls (N. R. refers to Non-Related).

was calculated. All surviving eggs from each family were placed into plastic containers packed in ice and transported to Corvallis, Oregon. In Corvallis, the eggs for each family were placed into separate Heath® incubator trays supplied with well water of the following characteristics: temperature 11°C, dissolved oxygen 8.8 ppm, hardness ($CaCO_3$) 99 mg/l, pH 7.3, and conductivity 241 μmo. Every two days all dead eggs were removed and counted. By January 3, 1976, hatching was approximately 98% complete. Percent survival from the eyed stage to hatch was calculated for each family at that time. The next day all families were reduced to 320 alevins per tray, except that excessive early mortality during development necessitated maintaining families A7, A8, and B8 (Fig. 1) at 172, 164, and 42 alevins, respectively. Alevins removed from all families (including inbreds) were pooled into two extra incubator trays and reared for use in later experiments.

Throughout the remainder of the experiment, daily mortality records were maintained. Equal numbers of fish were maintained in each experimental block (except for families A7, A8, and B8) by randomly removing excess fry to compensate for mortality. This procedure assured equal densities within blocks. Live fish removed during this process were added to the extra groups for use in later experiments.

By February 5, 1976, yolk sac absorption was complete, and the percent survival from hatch to swim-up was calculated. All families were reduced to 300 swim-ups except A7, A8 and B8 which contained 148, 150, and 41, respectively. Swim-ups were weighed by dipping all 300 fish from the incubator tray with a fine mesh aquarium net, allowing excess water to drain, placing the fish into a tared container of water and recording the weight to the nearest 0.001 g. Each family was placed into a 0.61 meter diameter, circular fiberglass tank where they were reared throughout the food conversion experiment.

Twenty mg of Oregon moist pellet per gram of fish per day was fed. Fish received half of this ration in the morning and half in the afternoon for the first four weeks. Subsequent feeding was once a day. Feeding was done slowly to assure that all offered food was consumed. Every two weeks for fourteen weeks, each family group was weighed, and the ration was adjusted to accommodate for the weight gain during the previous two weeks. In addition, each family was counted, and adjustments were made to maintain equal numbers of fish per experimental block.

Once every two weeks, 25 live fish were removed from the extra group and a 25 g sample of the ration was collected. Fish were sacrificed and individually weighed to the nearest 0.001 g and placed into a drying oven at 80^{o}C along with the food sample. After drying,

samples were cooled to room temperature in a vacuum desiccator. Fish and food were then weighed to the nearest 0.001 g. Percent water for the 25 fish and the 25 gram sample of food was used to adjust the wet weights of both fish and food to a dry weight basis. Feed efficiency (FE) was calculated by the following formula:

$$FE = \frac{\text{Dry weight gain}}{\text{Dry weight fed.}}$$

This procedure was followed at each two week weighing. The ration was maintained at 20 mg/g/day until March 22 when it was increased to 30 mg/g/day and held at this level throughout the remainder of the food conversion study.

Comparisons among inbred and non-inbred groups were calculated for each characteristic as the difference between the average for the inbred and the average for the non-inbred families for each common male and female parent. This difference was divided by the non-bred family average and multiplied by 100.

A one-way analysis of variance was used to analyze survival data (Tables 3 and 4). Feed efficiency and mean weight data were analyzed using the analysis of variance for a randomized block, split-plot design (Snedecor and Cochran 1967).

On July 6, 1976, all families resulting from full-sib matings were pooled into one tank as were all fish resulting from half-sib and all non-inbred matings. A random sample of 100 fish from each

of the three groups was transported to the Western Fish Toxicology Station in Corvallis. Each group was placed into a separate compartment of a cage suspended in a 3.66 meter diameter fiberglass tank supplied with air supersaturated water (127-139%) at 11°C. Fish were observed at regular intervals, and dead fish were removed. The time to death of fish at each inbreeding level was recorded. Death was determined by the cessation of opercular movement. Exposure was continued until 76% of the F=0.25 inbred fish were dead. Mortality (percentage) was transformed to arcsin $\sqrt{\text{percentage}}$ and regressed against time to death in minutes. This transformation was used to linearize death with time. Slopes of the regression lines (Fig. 3) for full-sib and non-inbred offspring were compared using the methods described by Snedecor and Cochran (1967).

RESULTS

Percent inbreeding depression for survival of the full-sib groups ranged from 0.9% to 13.4% and averaged 6.0% (Table 1). Percent inbreeding depression for survival of the half-sib groups ranged from 0.2% to 0.6% and averaged 0.15% (Table 2). Inbreeding at F=0.25 resulted in average depressions of 2.1% and 9.9% for common female and common male groups, respectively. However, there were no statistically significant differences in survival between inbred and non-inbred families (Tables 3 and 4).

The large standard deviations associated with percent of eggs reaching the eyed stage for common female and both inbred groups (F=0.25 and F=0.125) are due to the decreased survival of eggs from one female. The matings (A7, A8, B7, B8) in which this female was involved all showed a reduction in survival when compared to all other matings (Fig. 2).

Percent inbreeding depressions for feed efficiency for the full-sib groups averaged 9.3%. The common female percent inbreeding depression was 0.5% and the common male 18.0%. Inbreeding at F=0.125 showed that common male and female families had no inbreeding depression for feed efficiency (Table 2). Feed efficiency increased significantly over time for both full and half-sib groups (Tables 5 and 6). The feed efficiency values for inbred, common

male, and common female groups differed significantly for inbreeding at F=0.25 (Table 5). An LSD test (Snedecor and Cochran 1967) revealed that the significant F value was due to the difference between common male and inbred families (Table 1).

The inbreeding depression values for full-sib groups at two week intervals to 195 days averaged 5.0% for common male families and zero for common female families. An LSD test revealed that the significant F value for inbred, common male and common female families (F=0.25) (Table 7) was due to the difference between common male and inbred families. Mean weights for inbred (F=0.125), common male, and common female groups (Table 8) showed no statistical significance.

Fifty percent mortality in air supersaturated water occurred after 36.9, 42.5, and 41.3 hours for full-sib, half-sib, and non-inbred offspring, respectively. Thus, there was a 10.5% depression in time to 50% mortality for full-sib offspring compared to non-inbred offspring. The difference was not significant ($\alpha = 0.05$), but if it is in fact a real difference, then since the Columbia River is at times supersaturated at the mouth, this could present a problem for inbred coho salmon during the downstream migration.

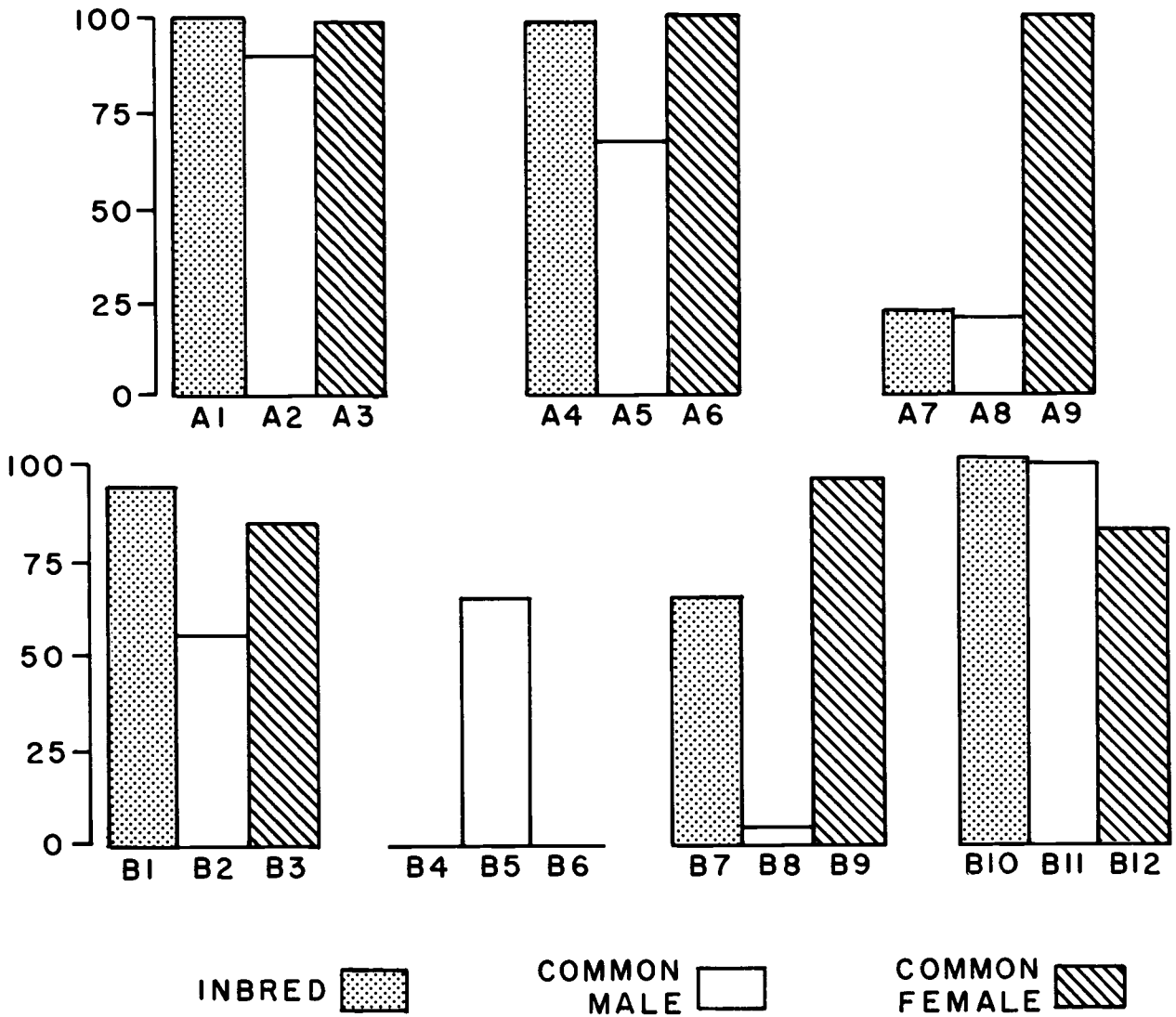

Figure 2. Percent survival from fertilization to eye for inbred (F=0.25 and F=0.125) and non-inbred coho salmon.

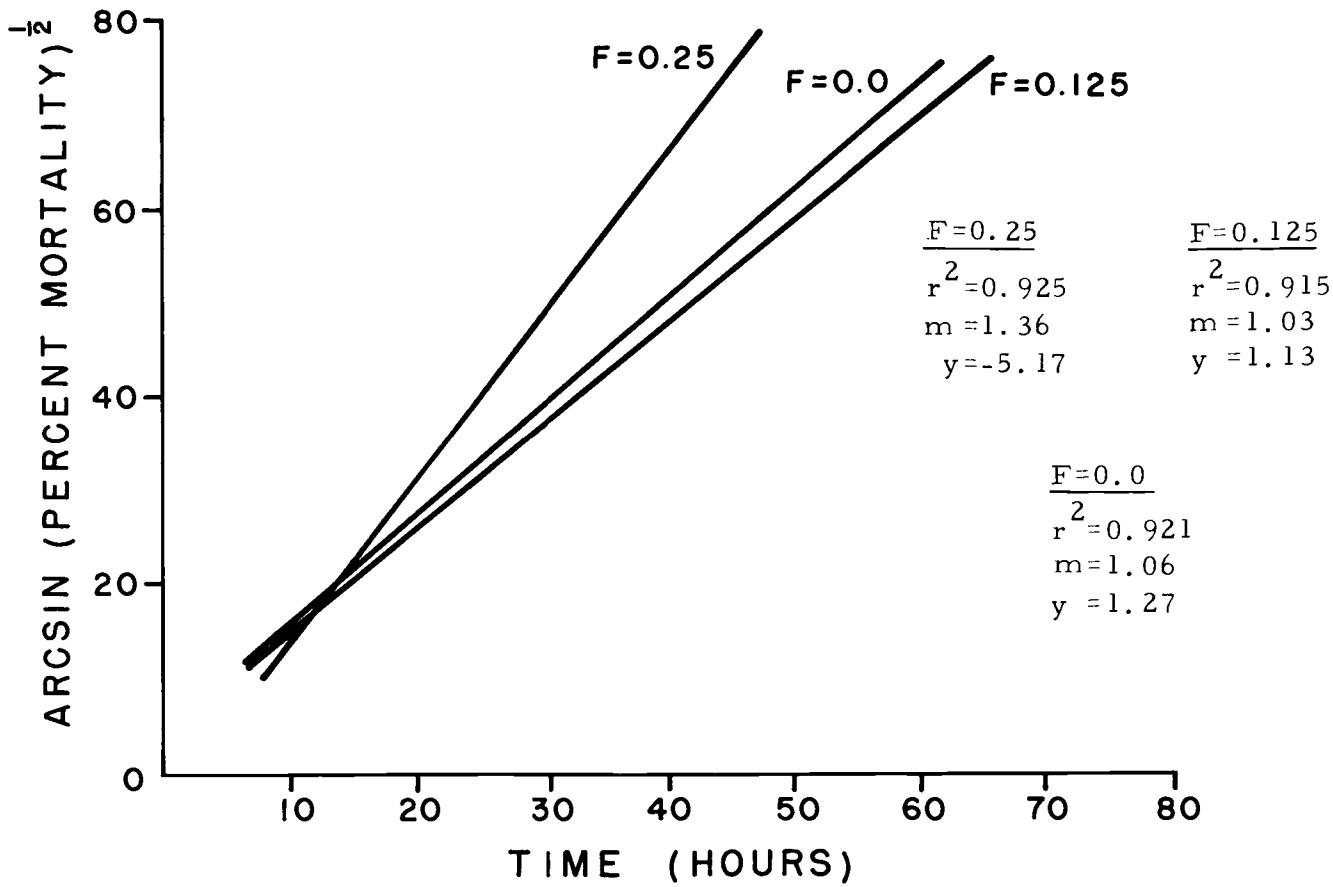

Figure 3. Regressions of mortality on time for inbred (F=0.25 and F=0.125) and non-inbred coho salmon exposed to air supersaturated water.

Table 1. Inbreeding depression [inbred mean - non-inbred mean] / non-inbred mean X 100 for several characteristics of inbred (F=0.25), common male, and common female groups of coho salmon. Absence of a value indicates that no depression was observed. These values were assumed to be equal to zero for calculations of average percent depression. (± indicate one standard deviation).

Characteristic	Common female parent		Common male parent		Average percent depression	Inbred mean
	Non-inbred mean	Percent depression	Non-inbred mean	Percent depression		
Eggs eyed	58.7 ± 29.3		97.6 ± 0.1	26.7	13.4	71.5 ± 36.1
Eggs hatched	99.7 ± 0.3	7.1	99.8 ± 0.1	7.2	7.2	92.6 ± 9.7
Swim-ups	99.8 ± 0.1	1.2	99.2 ± 0.6	0.6	0.9	98.6 ± 1.4
Survival 195 days	91.4 ± 6.9		98.0 ± 1.1	5.0	2.5	93.1 ± 7.0
Mean feed efficiency	0.197 ± 0.08	0.5	0.239 ± 0.09	18.0	9.3	0.196 ± 0.09
Mean Wt. in grams:						
97 days	0.437 ± 0.022		0.428 ± 0.018			0.464 ± 0.040
139 days	0.925 ± 0.157		1.146 ± 0.120	3.3	1.7	1.108 ± 0.075
195 days	3.70 ± 0.462		4.61 ± 0.302	11.7	5.9	4.070 ± 0.110

Table 2. Inbreeding depression [inbred mean - non-inbred mean] / non-inbred mean X 100 for several characteristics of inbred (F=0.125), common male, and common female groups of coho salmon. Absence of a value indicates that no depression was observed. These values were assumed to be equal to zero for calculations of average percent depression. (± indicate one standard deviation).

Characteristic	Common female parent		Common male parent		Average percent depression	Inbred mean
	Non-inbred mean	Percent depression	Non-inbred mean	Percent depression		
Eggs eyed	53.2 ± 37.3		85.7 ± 3.2			86.1 ± 13.9
Eggs hatched	98.1 ± 1.9		99.8 ± 0.1	0.4	0.2	99.4 ± 0.9
Swim-ups	99.1 ± 1.1		99.0 ± 1.3			99.6 ± 0.1
Survival 195 days	94.5 ± 2.6	0.2	94.9 ± 1.6	0.6	0.4	94.3 ± 4.7
Mean feed efficiency	0.205 ± 0.07		0.205 ± 0.08			0.205 ± 0.06
Mean Wt. in grams:						
97 days	0.457 ± 0.042		0.498 ± 0.005	7.2	3.6	0.462 ± 0.017
139 days	1.127 ± 0.099	2.1	1.169 ± 0.138	5.6	3.9	1.103 ± 0.020
195 days	4.41 ± 0.73	8.4	4.42 ± 0.20	8.6	8.5	4.04 ± 0.21

Table 3. Analysis of variance for survival (percentage) of full-sib groups through four stages of development.

Source of variation	DF	Mean Square	F
Fertilization to eye	2	118.0	1.1
Error	6	108.0	
Eye to hatch	2	51.8	1.1
Error	6	47.5	
Hatch to swim-up	2	1.1	1.0
Error	6	1.2	
Swim-up to 195 days	2	34.7	0.7
Error	6	46.8	

Table 4. Analysis of variance for survival (percentage) of half-sib groups through four stages of development.

Source of variation	DF	Mean Square	F
Fertilization to eye	2	1073.0	1.3
Error	6	798.0	
Eye to hatch	2	2.3	1.1
Error	6	2.1	
Hatch to swim-up	2	0.4	0.3
Error	6	1.4	
Swim-up to 195 days	2	0.3	0.02
Error	6	15.7	

Table 5. Analysis of variance for gain per unit of feed for full-sib groups. "C♂ " and "C♀ " refers to common male and common female groups, respectively.

Source of variation	DF	Mean Square	F
Time	6	425.0	31.5**
Inbred, C♂ , C♀	2	126.3	9.4**
Time X Inbred, C♂ , C♀	12	43.4	3.2**
Blocks	2	292.3	21.7**
Time X Blocks	12	70.3	5.2**
Blocks X Inbred, C♂ , C♀	4	18.6	1.4
Error	24	13.5	

* Significant $\alpha = 0.05$
** Significant $\alpha = 0.01$

Table 6. Analysis of variance for gain per unit of feed for half-sib groups. "C♂ " and C♀ " refers to common male and common female groups, respectively.

Source of variation	DF	Mean Square	F
Time	6	345.2	14.21**
Inbred, C♂ , C♀	2	1.5	0.06
Time X Inbred, C♂ , C♀	12	12.0	0.49
Blocks	2	7.0	0.29
Time X Blocks	12	15.8	0.65
Blocks X Inbred, C♂ , C♀	4	13.4	0.55
Error	24	24.3	

* Significant $\alpha = 0.05$
** Significant $\alpha = 0.01$

Table 7. Analysis of variance for mean wet weight of full-sib groups. "C♂" and "C♀" refers to common male and common female groups, respectively.

Source of variation	DF	Mean Square	F
Time	7	15.659	921.12**
Inbred, C♂, C♀	2	0.766	45.06**
Time X Inbred, C♂, C♀	14	0.084	4.94**
Blocks	2	0.409	24.06**
Time X Blocks	14	0.033	1.94
Inbred, C♂, C♀ X Blocks	4	0.157	9.24**
Error	28	0.017	

* Significant $\alpha = 0.05$
** Significant $\alpha = 0.01$

Table 8. Analysis of variance for mean wet weight of half-sib groups. "C♂" and "C♀" refers to common male and common female groups, respectively.

Source of variation	DF	Mean Square	F
Time	7	17.029	567.63**
Inbred, C♂ , C♀	2	0.096	3.20
Time X Inbred, C♂ , C♀	14	0.018	0.60
Blocks	2	0.494	16.47**
Time X Blocks	14	0.064	2.13
Inbred, C♂ , C♀ X Blocks	4	0.029	0.97
Error	28	0.030	

* Significant $\alpha = 0.05$
** Significant $\alpha = 0.01$

DISCUSSION

Differences in survival, feed efficiency, and growth between inbred and non-inbred groups could not be attributed to inbreeding, and average inbreeding depression estimates were smaller than inbreeding depression estimates for the rainbow trout (Kincaid 1976). Kincaid demonstrated that inbreeding at F=0.25 in eight pairs of fall spawning rainbow trout produced a significant depressive effect on survival and growth. Utter et al. (1973) has shown that the genus Oncorhynchus has significantly less biochemical genetic variation than is present in the rainbow trout, genus Salmo. Estimates based on protein variation for percent of polymorphic loci and average heterozygosity have yielded values of 0.13 and 0.18, respectively, for coho salmon and 0.26 and 0.37, respectively, for rainbow trout.

Inbreeding will result in a reduction in heterozygous loci in proportion to F. If coho salmon have half as many heterozygous loci as rainbow trout, then inbreeding at a given level of F will change twice as many loci to the homozygous form in rainbow trout as coho salmon. Inbreeding measures the decrease in homozygosity and this decrease in homozygosity is assumed to be responsible for the deleterious effects associated with inbreeding.

Ohno et al. (1969) suggested that the family Salmonidae may have evolved by the process of genome duplication, the process by

which all genetic information is duplicated, and presented evidence to this effect from studies of chromosomes. His hypothesis is that both Oncorhynchus and Salmo are tetraploid. According to his report, first meiotic metaphase preparations from coho salmon contained mostly bivalents and only a few quadrivalents. It appeared that they have almost completed the process of diploidization. Other species, such as the rainbow trout, showed extensive Robertsonian polymorphism within the species. Ropers et al. (1973) have mentioned that "salmonid fish appear tetraploid with respect to DNA content per cell and the number of chromosomes as compared to other members of the order Isospondylii."

A diploid organism has two alleles at each locus while a tetraploid organism would have four. This difference in the number of alleles at a locus forms the basis of the different responses of these two types of organisms to the effects of inbreeding. Since inbreeding affects the viability of an organism by decreasing the number of heterozygous loci, greater levels of inbreeding would be necessary to change the four alleles of a tetraploid to the homozygous state than to change the two alleles of a diploid to the homozygous form.

Busbice and Wilsie (1966) presented evidence from alfalfa (Medicago sativa) that inbreeding in a tetraploid may show only 17% as much depression as the same level of inbreeding in a diploid. This effect is thought to be the result of the interaction of alleles at

a single locus. In the diploid (AA) there is only one interaction possible between the two alleles. In the tetraploid (AAAA) there are six possible interactions between the four alleles at one locus.

If coho salmon are tetraploid and have not completed the process of diploidization, then inbreeding at F=0.25 may not change heterozygous loci to the alternate homozygous form at a rate sufficient to produce a significant depression in survival and growth. Rainbow trout are presumed to have already completed the process of diploidization and have again developed allelic heterozygotes via mutation (Ohno, et al., 1969). Ryman (1970) observed a decrease in the frequency of recapture of Atlantic salmon (Salmo salar) that were inbred at F=0.25. This suggests that the effects of inbreeding might not be expressed until after release from the hatchery.

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