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ABSTRACT: We cultured separate lines of Chinook salmon fry *Oncorhynchus tshawytscha* of Chickamin River, Southeast Alaska ancestry in 7 common garden enclosures. A parentage analysis based on variation of microsatellite alleles showed that within these lines, 7 brother-sister matings ($F=0.25$) had created 35 inbred fish in 7 families [bred from 6 females (dams) and 6 related males (sires)], and other matings of unrelated fish had created 37 outbred fish in 10 familes (bred from 7 females and 6 males.) There was no measurable effect of inbreeding on growth of Chinook salmon fry through 114 days post swim-up. A general linear model showing the effects of dam, sire, and the interaction of dam and sire explained a significant amount of the variation of length and weight, but not of condition factor. However, analysis of a mixed model showed that only the interaction between dam and sire explained a significant amount of the variation of lengths and weights. Because variation among individuals from different families can be large, effects of individuals can potentially be confounded with the effects for which a study is designed. To avoid drawing improper conclusions, studies should estimate the amount of variation that can be attributed to family origin, or be certain that many families are sampled.

INTRODUCTION

Inbreeding Depression

Inbreeding is a concern for conservation biologists managing small populations and for hatchery managers raising salmonids for ocean ranching and supplementation of endangered stocks. If inbreeding depresses fitness in salmon to the degree that some studies have reported in rainbow trout *Oncorhynchus mykiss* (e.g., Aulstad and Kittelsen 1971; Kincaid 1976a; Kincaid 1976b; Kincaid 1983), then salmon hatchery managers must become more aware of the potential hazards of inbreeding and adopt methods to minimize potential inbreeding in the hatchery.

Inbreeding is the mating of individuals that share a recent common ancestor. The primary effects of inbreeding are a decrease in heterozygosity and an increased probability of the fixation of an allele at a locus, and therefore, a loss of genetic diversity. Inbreeding depression is the reduction in the performance of a trait relative to non-inbred individuals in a population. This reduction in performance can be due to a loss of genetic variation or an increased probability of receiving deleterious alleles from parents (Falconer and Mackay 1996). Although inbreeding has been shown to depress fitness in many species, there is little knowledge of how inbreeding is manifested in fish at different life stages, and very little knowledge about inbreeding in salmon populations.

Several studies have demonstrated that rainbow trout show signs of depression at various levels of inbreeding. After one generation of full-sib mating (inbreeding coefficient, $F=0.25$), rainbow trout families showed an increase in the number of crippled fry (Aulstad and Kittelsen 1971), diminished egg hatchability, feed conversion efficiency, and fry survival (Kincaid 1976a). Juveniles and adults grew more slowly (Kincaid 1976b) and fewer fish stocked in a pond survived (Kincaid 1983). When inbred Chinook salmon ($F=0.25$) were exposed to *Myxobolus cerebralis*, the parasite that causes whirling disease, the probability and severity of infection increased (Arkush et al. 2002).

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When F is increased to 0.375 (2 generations of full-sib mating) and 0.5 (3 generations of full-sib mating), many developmental traits are more intensely depressed (Kincaid 1976a). Gjerde et al. (1983) also reported depression of several traits such as the survival of eyed eggs, alevins, fry, and of adult growth of rainbow trout at inbreeding levels of 0.25–0.5.

At lesser inbreeding (average $F = 0.064$, range = 0–0.195) female rainbow trout exhibited decreased spawning age, egg number, fertility, spawning body weight, and egg hatchability (Su et al. 1996). In addition, female body weight was less for inbred lines from 165 days until spawning. However, Su et al. (1996) did not report which stock their experimental fish originated from, so the trout could have experienced inbreeding before the inception of experiment. In another study of rainbow trout, the effects of low rates of inbreeding ($F < 0.125$) were moderate for growth until harvest (Pante et al. 2001). For that experiment, the authors also assumed that at "generation 0" all fish had an $F = 0$, but the fish used were from a hatchery stock with no pedigree prior to generation 0. Pante et al. (2001) concluded that the difference in growth was not great enough to cause any serious detriment to selective breeding programs.

Because little is known about the effects of inbreeding on salmon (particularly Chinook salmon) during the first few months of growth, our objective was to compare the growth of offspring of full sibs ($F = 0.25$) to outbred offspring from the same native population of Chinook salmon in common garden enclosures. Because variation of growth and size between families of salmon fry can be considerable (Iwamoto et al. 1982; Swift et al. 1991; Winkelman et al. 1992; Silverstein and Hershberger 1994) and might be confounded with apparent differences between inbred fish and outbred controls, we included the effect of family on growth in the analysis.

MATERIALS AND METHODS

Common Garden Growth Experiment

From July through September 2002, we cultured Chinook fry of Chickamin River ancestry in a common garden growth experiment in seven 114-liter circular tanks. On July 1–3, 2002 we stocked each tank with 150 fry that had been cultured for 77 days after first feeding in a growth trial intended for other research (Rodgveller 2004). We cultured the fish for approximately 65 further days, which was approximately 737 degree days. During this time the fry grew from an average length of 44.9 mm to 77.6 mm. We examined 2 subsets of these fish to detect differences related to inbreeding.

We identified one set of fish as outbred because they were derived from outcrosses between 2 experimental lines of Chickamin River Chinook salmon. The lines had been reared, identified with coded-wire tags, and released from the National Marine Fisheries Service's (NMFS) Little Port Walter Research Station (LPW) on Baranof Island, Southeast Alaska, for either one or 5 generations after they were collected from the Chickamin River. These fish are an appropriate outbred control for inbred fish because even if they were themselves inbred, one generation of outbreeding eliminates inbreeding in the resulting generation (Falconer and Mackey 1996). If the outcrossed fry happened to have been created from mating fourth cousins (the fish in the 2 lines having originated from the same parents 5 generations previously), their inbreeding coefficients would be less than 0.1%. These outbred fish would not be expected to show outbreeding depression—a decline in the fitness of offspring of genetically different parents—because the 2 lines were derived from the same local population and the 2 stocks have been in culture for only 5 or fewer generations. Ecological outbreeding depression, due to additive genetic effects, would not be expected in these outbred fish because at its inception the more recently founded line grew and survived as well in the hatchery as the line that was founded earlier (J. E. Joyce, NMFS Auke Bay Laboratory, Juneau, Alaska, personal communication). Outbreeding depression due to the disruption of coadapted genomes also would not be expected because the 2 experimental lines do not have different genetic compositions, having been recently derived from the same natural population (Emlen 1991, Lynch 1991, Waples 1992). No new genetic material was introduced into the 2 lines after their inception, and in only 5 generations there has not been enough time for mutation to change the genotypes of these fish.

We identified a second set of fish as inbred with an inbreeding coefficient of at least 0.25 because they resulted from brother-sister matings. There is potential for these inbred fish to have an inbreeding coefficient higher than 0.25 if their grandparents and great grandparents (and so on) were related. For example if the fish were from 2 generations of full-sib matings, then $F = 0.375$. Because the pedigrees were not available, we assume a minimum inbreeding coefficient of 0.25.

The mating design for the larger NMFS experiment consisted of creating both full-sib families (one female crossed with one male) and half-sibs (one female crossed with 2 males, or the reciprocal). For this

analysis, we used families that were either full-sib, or half-sib families. In a couple of cases a male was crossed with 2 females, one to create an inbred cross and another to create an outbred cross. In the larger NMFS experiment, the inbred families were created from 7 females and 6 males, the outbred line was created from 10 females and 6 males.

We randomly assigned the tanks over 4 benches in the laboratory and randomly assigned placement on each of the benches to randomize effects of position in the room. At the beginning of the experiment, family origin was unknown, so each tank did not necessarily include every family. Each bench's water was supplied from Salmon Creek, Juneau, Alaska, which is used for salmon culture in Macaulay Hatchery. We used automatic feeders to feed fish to satiation each day and we illuminated each tank with individual light bulbs regulated by timers set at ambient day length.

On September 22–24, 2002 we euthanized all the fish with MS-222® (Tricaine methansulfonate), weighed them to the nearest hundredth of a gram, and measured them from the tip of the nose to the end of the caudal fin in millimeters. We used these lengths and weights to calculate a condition factor ($100{,}000 \times \text{weight}/\text{length}^3$). We preserved a caudal fin clip of at least 0.1 grams in 100% ethanol for a genetic parentage analysis.

Microsatellite Parentage Analysis

We isolated total DNA with DNeasy Tissue Kit (QIAGEN, Inc., Valencia, Ca.). Polymerase chain reaction (PCR) amplification was done in 96-well microtitre plates in a DNA Engine (MJ Research, Inc., Reno, NV). We performed reactions in 10 µL volumes [10 mM Tris-HCl at pH 8.3, 50 mM KCl, 25 mM $MgCl_2$, 2.5 mM each deoxyribonucleotide triphosphate (dNTP), 0.5 units Taq polymerase, 0.1–0.5 µM each primer, and 50–100 ng DNA template]. In addition to unlabeled forward and reverse primers for each locus, each mixture included a forward primer labeled with an infrared-sensitive dye, IRDye™ (LI-COR, Inc., Lincoln, NE).

We first used four microsatellites: *Ots100, Ots107* (Nelson and Beacham 1999), *Ogo4* (Olsen et al. 1998), and *Omy325* (O'Connell et al. 1997) for parentage analysis. When the parentage could not be used with 100% probability we also identified *Ssa289* (McConnell 1995) and *Ots104* (Nelson and Beacham 1999, Table 1). In general, PCR conditions were as follows: one cycle at 95°C for 3 min; 28 cycles at 95°C for 30 sec., *x*°C for 15 sec., and 72°C for 15 sec.; and finally, one cycle at 72°C for 1 min; where *x* is the annealing temperature (58°C, *Ots100*, *Ots104*, *Ots107*, *Ogo4*, *Omy325,* and 55°C for *Ssa289)*. After amplification, we denatured DNA products by adding an equal volume of stop buffer (95% formamide, 0.1% Bromophenol Blue) and heating for 3 minutes at 95°C. We loaded 1µl of PCR product onto polyacrylamide denaturing gels composed of 6.5% KB^{Plus} gel matrix (LI-COR, Inc., Lincoln, NE) in a reaction catalyzed by ammonium persulfate and TEMED (N,N,N′,N′ tetramethylethylenediamine).

We separated alleles electrophoretically and detected them on a LI-COR automated sequencer (LongReadIR 4200™, LI-COR, Inc., Lincoln, NE) in 1X TBE running buffer, with running parameters 31.5 W, 1500 V, 35 mA, and 50°C plate temperature. We used Saga™ Generation 2 automated microsatellite software (Version 3.0, LI-COR, Inc., Lincoln, NE) to estimate the sizes of microsatellite alleles. We determined allele sizes by comparing allele band patterns with IRD700™ or IRD800™ standard ladders (LI-COR, Inc., Lincoln, NE) and standardized using reference alleles.

Analysis

We used PROBMAX (Version 1.2, Danzmann 1997) to determine the parentage of the experimental fish. To ensure that fish were not being assigned to the incorrect parents due to the unknown presence of null alleles, we compared 2 analyses in PROBMAX to come up with our final assignments (Dakin and Avise 2004). The first analysis was run assuming that all homozygous loci were actually heterozygotes with one null allele. We ran the second parentage assignment in PROBMAX assuming that all homozygous loci were in fact homozygous for the allele. If there were differences between the 2 analyses in parent assignments for an individual fish, we compared the alleles for all the pos-

Table 1. Allele variation at 6 microsatellite loci in 2 experimental lines of Chinook salmon that were native to the Chickamin River, Alaska and raised in the laboratory in 'common-garden' culture. *Ots100* and *Ssa289* were added to the parentage analysis of a portion of the fish when the parentage assignment could not be concluded with certainty. The other 4 loci were analyzed for all fish. N_a is the number of alleles at each locus, bp is the range of allele sizes at a locus.

Statistic	*Ots100*	*Ssa289*	*Ots107*	*Ots104*	*Omy325*	*Ogo4*
n_a	12	7	10	9	8	6
bp	262–382	156–192	188–356	200–270	83–149	136–166

sible parents to the progeny to determine which parents matched with 100% probability. In the cases where several parental pairs were possible for a progeny the individual was excluded from the analysis.

We used a general linear model analysis of variance (GLM) and a mixed model procedure, both available in SAS, to analyze variation of lengths, weights, and condition factors at the end of the experiment. In the mixed model, the random and fixed effects are distinguished from each other. The mixed model fits the data using a maximum likelihood approach. The mixed model is appropriate for this analysis because it accommodates unbalanced data, or unequal family sizes, as well as data with correlated individuals (Little et al. 1996). We included the general linear model to further explore the data. The effects of inbreeding, tank, dam, and sire were analyzed with this full model:

$$Y_{ijklm}=\mu+T_i+G_j+T_i\times G_j+D_{jk}+S_l+D_{jk}\times S_l+T_i\times D_{jk}+T_i\times S_l+T_i\times D_{jk}\times S_l+S_l\times G_j+S_l\times G_j\times T_i+e_{ijklm,}$$

where Y_{ijkml} is the length, weight, or condition factor at the end of the experiment, μ is the theoretical population mean, T_i is the tank, G_j is the group (inbred or outbred), and $T_i\times G_j$ is the effect of the interaction between tank *i* and group *j*. D_{jk} is the effect of dam within group *j*. Dam was nested within group because the dams used to create the inbred and outbred lines were from different lines. S_l is the effect of sire; sire was not nested within group because the sires used to create the inbred and outbred lines were from one line. $D_{jk}\times S_l$ is the effect of the interaction between dam and sire, $T_i\times D_{jk}$ is the effect of the interaction between dam and tank, $T_i\times S_l$ is the effect of the interaction between tank and sire; $T_i\times D_{jk}\times S_l$ is the effect of the interaction between tank, dam and sire. $S_l\times G_j$ is the interaction between group and sire, $S_l\times G_j\times T_i$ is the interaction between sire, group and tank, and e_{ijklm} is the random error. Because 2 lines of dams were used create the inbred and outbred groups, dam cannot have an interaction with group. Tank and group and the interaction term of tank and group were treated as fixed effects. Dam, sire, and the interaction terms that include these terms were treated as random effects in the mixed model.

We calculated the minimum effect that our data could detect for length, weight and condition factor at 95% power. To calculate the effect we used the number of fish as the sample size and the residual variance estimate from the mixed procedure model as the variance (Zar 1999).

RESULTS

We observed 35 inbred individuals from 8 families created from 6 dams and 6 sires, and 37 outbred individuals from 8 families created from 7 dams and 6 sires (Table 2; Figure 1). Both groups included half-sib families and 3 males were used to create crosses in both the outbred and inbred lines (Table 2).

Because of sparse data, the full model and many of the reduced models could not provide estimates for all of the parameters. Additionally, in the full and reduced models, tank and the interaction terms that included tank were always insignificant; therefore we removed all terms in the model that included tank. The sire-by-group interaction terms also were insignificant and were taken out of the reduced model:

$$Y_{jklm} = \mu + G_j + D_{jk} + S_l + D_{jk}\times S_l + e_{jklm}$$

Because negative variance estimates were not meaningful, we ran the PROC MIXED model with bounds on the estimates (no negatives). Because the estimates of sire effect were sometimes negative in

Table 2. Dams and sires crossed to create experimental inbred and outbred lines of Chinook salmon.

Inbred Crosses			Outbred Crosses		
Females ID	Male ID	Family #	Females ID	Male ID	Family #
2219	1243[b]	1			
2048	1038[a]	9			
2350	1248[b]	2	2133	1038[a]	10
2465	1412	3	2298[a]	1243[b]	11
2487[a]	1291[b]	4	2298[a]	1248[b]	12
2487[a]	1419[a]	5	2306	1250[a]	13
2515[a]	1291[b]	6	2311	1250[a]	14
2515[a]	1419[a]	7	2396	1291[b]	15
2589	1455	8	2098	1114	16

[a] The individual was used more than once to create half-sib families within one line.
[b] The individual was used to create crosses in both lines.

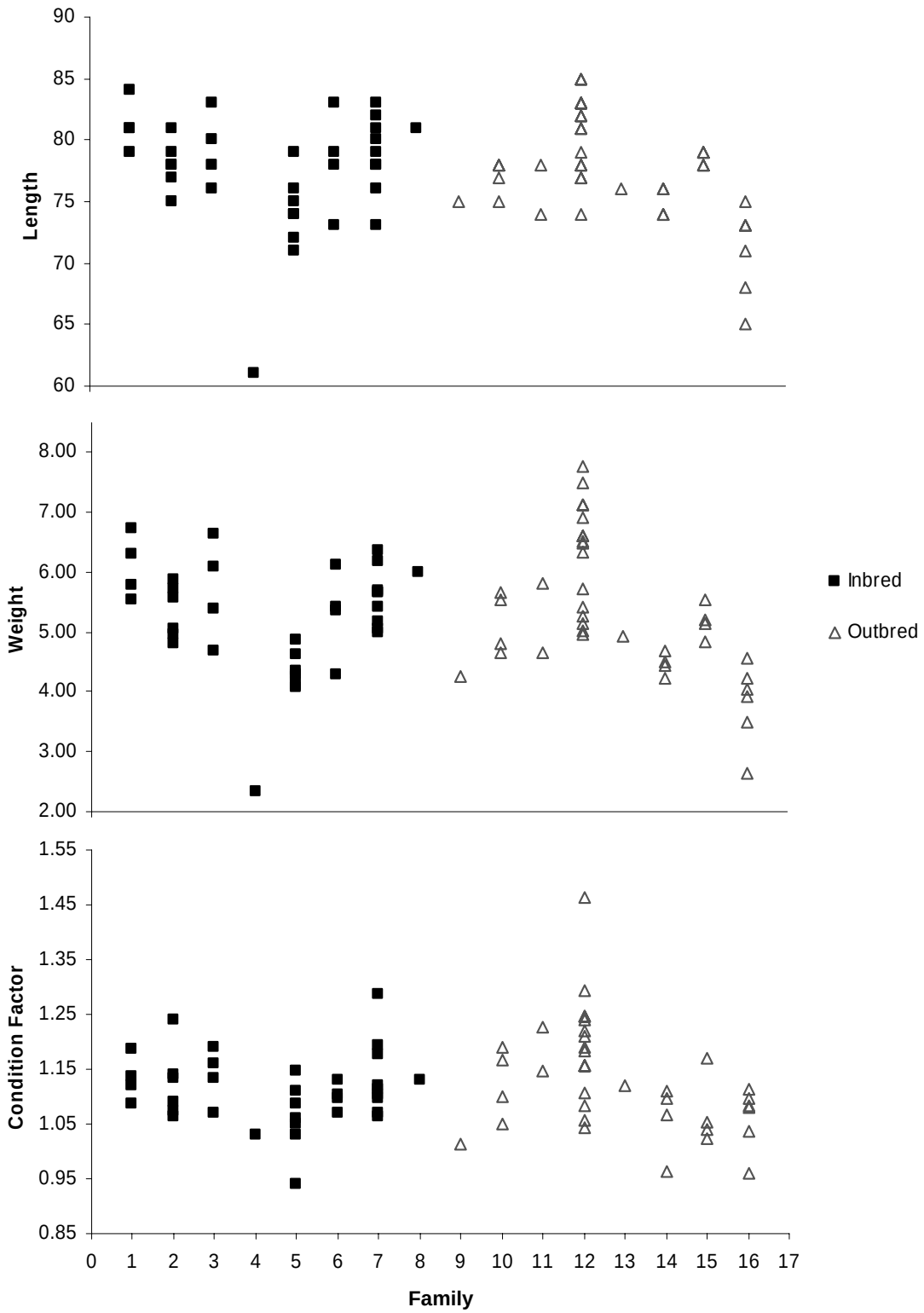

Figure 1. Lengths (millimeters), weights (grams) and condition factors (100,000 × weight/length^3) of inbred and outbred Chinook fry native to the Chickamin River, Alaska, raised in the laboratory in common-garden culture. Inbred individuals are offspring of brother-sister matings, designated by a filled square; outbred individuals are offspring of parents taken from two lines that have been separated for 5 generations, designated by open triangles.

non-bound models, the bounded models reported estimates of variance due to effects of sire of 0. The *Z* statistic used for hypothesis testing, the standard error, and the *P*-value were not estimated in this case (Table 4). Even though there were no estimates for the effect of sire, the model still converged successfully and the estimates for all the parameters in the model are valid.

There was no measurable effect of inbreeding on growth of Chickamin River Chinook fry 114 days after swim up in any of the analyses (Tables 3, 5, 6). In the GLM, the effects of dam, sire, and the interaction of dam and sire explained a significant amount of the variation of length and weight, but not of condition factor (Table 5). However, the mixed model analysis indicated that only the interaction between dam and sire explained a significant amount of the variation of length and weight (Table 4).

The power analysis showed that our experiment had the power to detect a difference between outbred and inbred groups as small as 3.56 mm (4.8% of average = 77.53mm), 0.87 grams (16.5% of average = 5.29g) and 0.07 condition factor units (16.3% of average = 1.12; Table 6).

Table 3. SAS mixed model analysis of lengths, weights, and condition factors of inbred and outbred Chinook fry. The fry were native to the Chickamin River, Alaska and raised in the laboratory in common-garden culture. Inbred individuals were offspring of brother-sister matings; outbred individuals were offspring of parents taken from two lines that have been separated for 5 generations. The degrees of freedom (*df*), the *F*-statistic and the significance of the test (*P*) are listed for group (inbred or outbred when length in millimeters, weight in grams, or condition factor (100,000 × weight/length^3) is the response variable.

	F	*df*	*P*
Length			
Group	0.36	1,1	0.65
Weight			
Group	0.69	1,1	0.56
Condition Factor			
Group	0.00	1,1	0.99

Table 4. SAS mixed model estimates of variance and standard error (*se*) of the random parameter "family," when length (mm), weight (g) or condition factor (100,000×weight/length^3) of Chinook salmon parr was the response variable. Group (inbred or outbred), dam, sire, and dam×sire (interaction of dam and sire) are the explanatory variables, and length (millimeters), weight (grams), and condition factor (100,000 × weight/length^3) are the responses. The *Z*-statistic (*Z*) used for hypothesis testing is calculated by dividing the estimate by the standard error, the significance of the *Z*-test (*P*) is also reported. N/a signifies that the parameter was not estimated.

	variance	*se*	*Z*	*P*
Length				
Dam	5.50	6.74	0.82	0.21
Sire	0	n/a	n/a	n/a
Dam×Sire	11.00	8.26	5.18	0.00
Weight				
Dam	0.37	0.29	1.29	0.10
Sire	0	n/a	n/a	n/a
Dam×Sire	0.27	0.26	1.04	0.00
Condition Factor				
Dam	0.0014	0.0015	0.92	0.18
Sire	0.0002	0.0010	0.21	0.42
Dam×Sire	0	n/a	n/a	n/a

DISCUSSION

Inbreeding

We did not detect effects of inbreeding on the growth of Chinook salmon fry during the first summer. Our power analysis demonstrated that we could detect a difference of 3.72 mm or 0.87 grams or greater (Table 6). However the power analysis did not account for the variance due to family origin, therefore the true detectable differences were probably slightly greater than these estimates. Since studies have shown that size differences as large as 10 mm may not be biologically meaningful (Quinn and Patterson 1996; Beckman et al. 2003), this bias is probably not meaningful.

Several studies have shown that size differences much larger than the differences detectable in this study have little or no influence on the physiology, behavior and survival of salmon fry. In a comparison between small Chinook fry ≤75 mm, and large fry ≥85 mm, both groups smolted at the same time, had similar movement patterns and behavior, and Na^+-K^+-ATPase activities, (Beckman et al. 2003). In a similar study comparing the over-winter survival of small (70–79 mm) and large (>89 mm) wild juvenile coho salmon fry, the small size class had approximately 55% survival, and the large size class had approximately 58% survival (Quinn and Patterson 1996). Even when the size groups differed by at least 10 mm, there was not an important difference in survival.

A possible reason why we did not detect inbreeding depression of size is because salmon are partial tetraploids. In organisms that are tetraploids, changes in heterozygosity due to inbreeding may take longer than in completely diploid organisms. Because of this, salmon may be buffered against the effects of inbreeding (Allendorf and Thorgard 1984). However, inbreeding depression has been observed in rainbow

trout (e.g., Kincaid 1976a), so the effects of tetraploidy may not be substantial.

Although we did not detect any deleterious effects of inbreeding in the first summer of growth, several studies reported that inbred rainbow trout experience decreased survival at later life stages as well as decreased reproductive fitness (e.g., Kincaid 1983; Su et al. 1996). In addition, many studies have detected inbreeding depression in several wild and domesticated species (Frankham et al. 2002). The Chinook salmon used in this experiment may also have exhibited deleterious effects of inbreeding at later stages. It is also possible that we would have observed inbreeding depression if we had observed the fish in a wild environment. The additional stresses of the natural environment can contribute to an increase in inbreeding depression (Crnokrak and Roff 1999).

Table 5. SAS general linear model *F*-test of inbred and outbred Chinook salmon fry raised in common garden enclosures. Group (inbred or outbred), dam, sire, and dam×sire (interaction of dam and sire) are the explanatory variables, and length (millimeters), weight (grams), and condition factor (100,000 × weight/length^3) are the responses. *df* is the degrees of freedom, *F* is the *F*-statistic, and *P* is the significance probability of the test.

	df	*F*	*P*
Length			
Group	1,57	1.30	0.26
Dam	6,57	12.07	0.00
Sire	2,57	9.95	0.00
Dam × Sire	1,57	14.75	0.00
Weight			
Group	1,57	1.32	0.26
Dam	6,57	7.22	0.00
Sire	2,57	7.19	0.00
Dam × Sire	1,57	5.01	0.03
Condition Factor			
Group	1,57	2.17	0.15
Dam	6,57	0.26	0.77
Sire	2,57	1.32	0.26
Dam × Sire	1,57	0.01	0.94

Table 6. Minimum detectable effect by our statistical analysis with 95% power. The mean length (millimeters), weight (grams), and condition factor (100,000 × weight/length^3) of inbred and outbred salmon descended from the Chickamin River, Alaska, population. The average for each group and its standard deviation (*sd*) are also reported.

	Inbred mean (*sd*)	Outbred mean (*sd*)	Detectable Effect
Length	77.67 (4.37)	77.39 (4.38)	3.72
Weight	5.24 (0.88)	5.32 (1.15)	0.87
Condition Factor	1.11 (0.06)	1.13 (0.10)	0.07

Maternal Effects

Because we could not associate individual lengths and weights taken at the beginning of the experiment with measurements at the end of the experiment, we did not analyze individual growth records (Rodgveller 2004). Average egg weight in the mother might have been used as a proxy for individual weight at the beginning of the experiment. However, although egg size can affect body size at a young age, several studies have reported that in as little as 4 weeks after emergence there are no significant effects of egg weight on size of salmonids (Fowler 1972; Springate and Bromage 1985; Kelley 1994; Heath et al. 1999). Beacham et al. (1985) reported that the size of alevins is no longer related to egg size soon after exogenous yolk is absorbed. Maternal effects—which include the effect of egg size and the quality of the yolk—on growth and survival also diminish during the development of the embryo and after hatching (Kanis et al. 1976; Aulstad et al 1972).

Family Variation

Either dam, sire, or dam-by-sire interaction effects were statistically significant in all of the analyses (Tables 4, 5). Because we could not analyze the full models due to sparse data, it is difficult to fully understand the effects of dam and sire, but it is clear that there is a genetic effect, or an effect of family origin, that explains much of the length and weight differences. The large variation between families demonstrates that in some cases much of the variation may be attributed to family origin, and not to the treatment being tested.

Few similar studies have considered the number of families sampled, or the amount of variation that can be attributed to dam, sire, or family origin. Inadequate sampling across many families or failure to consider the variation caused by family of origin can cause the effects of the treatment to be confounded by family effects. For example, several behavioral studies comparing wild and hatchery-bred salmonids compared relatively few samples and did not track the number of families, potentially confounding the studies' results. In a comparison of hatchery-bred and wild cutthroat trout *O. clarki* habitat use, feeding, and aggression, only 42 individuals were observed and the family origin of each fish was unknown (Mesa 1991). In a comparison of wild and hatchery-wild hybrid steelhead trout's *O. mykiss* willingness to forage in areas that exposed them to predators, 11 half-sib families were created. Instead of rearing the families separately, the authors pooled the fish before the experiment. The

family origin of the fish became unknown, and the number of families observed in each trial was unknown (Johnsson and Abrahams 1991).

Many studies in other areas of salmonid research also failed to estimate the family component of variation. In a recent study comparing sizes of several brain structures of wild and hatchery-bred rainbow trout, 35 and 16 fish from 2 hatchery strains and 37 and 11 fish from 2 wild strains were sampled. The number of families that were sampled per strain was, however, unknown (Marchetti and Nevitt 2003). In a comparison of wild, hybrid and hatchery-bred brook trout *Salvelinus fontinalis* survival and growth, an unknown number of families were stocked in several lakes, so the number of families and the family of origin of each fish was unknown (Lachance and Magnan 1990). In a study of breeding success of hatchery and wild coho salmon, 20 pairs of coho were placed into 3 fenced off natural stream beds of varying sizes and viewed while courting and mating. The number of families and the family origin of each fish were unknown (Fleming and Gross 1993).

Because these studies did not determine how many families were sampled, they potentially confounded the treatment effects with differences between families. If individuals from a specific dam, sire, or family are correlated and if there is variation among the families and few families are sampled, improper conclusions can be drawn. An apparent difference between wild and hatchery fish, or any other treatment groups, may actually just be variation between families that reveals itself because too few families have been sampled. And although all of the studies discussed here reported significant differences, the results may have been explained by family differences.

There are 3 solutions to this potential problem. First, researchers could artificially create and segregate the families observed in a study and track family throughout the rearing of fish and the experiment, either by keeping them separated or by marking fish with individual or family-identifying marks. Second, researchers could conduct a genetic parentage analysis, as described here. In these cases, family would become an explanatory variable and the variation attributed to family effects would be partitioned from treatment effects. The third solution would be to use a large sample size in hopes of sampling many of the available families, although in many studies large sample sizes are not practical. Large sample size is not a definite solution because it does not ensure that many families will be sampled, but it makes the confounding of family effects less probable.

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