EFFECTS OF INBREEDING AND FAMILY ORIGIN ON SIZE OF CHINOOK SALMON (*ONCORHYNCHUS TSHAWYTSCHA*) FRY

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# Effects of Inbreeding and Family Origin on Size of Chinook Salmon (*Oncorhynchus tshawytscha*) Fry[1]

ABSTRACT: We cultured separate lines of chinook salmon fry of Chickamin River, Southeast Alaska, ancestry in seven common garden enclosures. A parentage analysis based on variation of microsatellite alleles showed that within these lines seven brother-sister matings created 35 inbred fish from 7 families (F = 0.25) and 37 outbred fish resulted from 10 matings between segregated lines. Outbred and inbred fish did not differ in length (P = 0.42), weight (P = 0.86), or condition factor (P = 0.16). There was significant variation among families for length (P = 0.01) and weight (P < 0.01), but not for condition factor (P = 0.48). Because variation among families can be large, it can potentially confound the effects for which a study was 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.

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# TABLE OF CONTENTS

## LIST OF FIGURES



## LIST OF TABLES

## LIST OF APPENDICES

## ACKNOWLEDGMENTS

Thank you to the Rasmuson Foundation and to the University of Alaska Fairbanks for fellowship awards that supported this research. Thank you to the National Marine Fisheries Service's (NMFS) Auk Bay Laboratory for financial support. Thanks to Douglas Island Pink and Chum for donating laboratory space. Thank you to everyone who volunteered their time to assist in the field and laboratory work. The coauthors of this work are John Joyce, fisheries biologist with the NMFS Auke Bay Laboratory, who cultured fish from spawning until the inception of the experiment at Little Port Walter Research Station, Baranof Island, Southeast Alaska, with other NMFS staff. Cara Rodgveller reared fish while in common garden tanks, and performed DNA isolations. Andrew Grey, fisheries geneticist with the NMFS Auke Bay Laboratory, completed the amplifications, electrophoresis and detection of alleles. Andrew and Cara cooperatively analyzed the genetic data. Cara Rodgveller completed statistical analysis. Transport and rearing permits P-02-008 & 02J-1008 were granted by Alaska Department of Fish and Game. That you to my advisory committee, Dr. William Smoker, Dr. A.J. Gharrett, and Dr. Milo Adkison, for their time and guidance.

# INTRODUCTION

## Inbreeding Depression

Information on inbreeding depression in salmon is important for hatchery managers raising fish for ocean ranching and supplementation of endangered stocks. Inbreeding is also a concern for conservation biologists managing small populations. If inbreeding causes depression of fitness-related traits in salmon to the degree that some studies have reported in rainbow trout *(O. 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. Conservation biologists would also benefit from more knowledge of the effects of inbreeding depression on fitness. Wild populations with small effective population sizes have a great potential for inbreeding, and in turn inbreeding depression of fitness related traits.

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. Inbreeding depression is the reduction in the performance of a trait relative to non-inbred individuals in a population. Inbreeding depression can result from a loss of genetic variation or deleterious alleles, reducing their fitness (Falconer and Mackay 1996). Although inbreeding has been shown to cause depression of fitness-related traits in many species, there is little knowledge of

how inbreeding manifests itself 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 show an increase in the number of crippled fry (Aulstad and Kittelsen 1971), and egg hatchability, feed conversion efficiency, and fry survival decrease (Kincaid 1976a). Juveniles and adults had a slower growth rate (Kincaid 1976b) and fish stocked in a pond had lower survival rates (Kincaid 1983). When inbred chinook salmon (F = 0.25) are exposed to *Myxobolus cerebralis*, the parasite that causes whirling disease, the probability and severity of infection increase (Arkush et al. 2002).

When F is increased to 0.375 (two generations of full-sib mating) and 0.5 (three generations of full-sib mating), many developmental traits show an intensified depression (Kincaid 1976a). Gjerde et al. (1983) also reported inbreeding depression in rainbow trout of the survival of eyed eggs, alevin and fry, and adult growth at inbreeding levels of 0.25-0.5.

At lower levels of inbreeding (average F = 0.064, range=0-0.195) female rainbow trout exhibited a decreased spawning age, egg number, fertility, spawning body weight, and egg hatchability (Su et al. 1996). In addition, depression of female body weight was stronger from 165 days until spawning. Su et al. (1996) did not report which stock their experimental fish originated from, so the stock of trout could have experienced inbreeding before the inception of experiment (adding up to an inbreeding coefficient

higher than 0-0.195). In another study of rainbow trout, the effects of low levels of inbreeding (F<0.125) were moderate for growth until harvest (Pante et al. 2001). That experiment 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 levels of depression were not high enough to cause any serious impact to selective breeding programs.

Several studies have reported that inbreeding at various levels is correlated with depression of fitness-related traits in trout. Despite the long history of artificial culture of Pacific salmon and reliance on cultured salmon, little is known about inbreeding depression in Pacific salmon. In addition, little is known about the manner in which inbreeding depression affects different life history stages in any salmonid. In particular, very few experiments have studied inbreeding in chinook salmon, and few studies have examined the first few months of growth. Consequently our objective was to examine moderate levels of inbreeding depression in chinook salmon fry by crossing two full sibs (F = 0.25) and examine growth of their offspring in common garden enclosures.

**Family Variation**

Heritability of size in salmon fry can be very high. For example, heritability for size of coho salmon pre-smolts (*O. kisutch*) was estimated to be 0.5 to 0.7 (Silverstein and Hershberger 1994). Heritability of length of chinook fry can be up to 0.74 (Winkelman et al. 1992). Coho salmon weight at saltwater entry has an estimated

heritability of 0.98 (Swift et al. 1991), and the weight of coho 57 days post swim up have an estimated heritability of $0.63 \pm 0.31$ (mean $\pm$ standard error) (Iwamoto et al. 1982).

Because estimates of heritability of size at the pre-smolt stages are high, sizes of salmon fry should be expected to vary among families. If few families are included in studies of salmon size, even if the sample size is large, family variation could confound the effects for which a study was designed to estimate. Because of the potential for drawing improper conclusions, it is important to be certain that many families are sampled in an experimental design, and/or to include family as an explanatory variable in an experimental analysis so that the variation can be partitioned appropriately. If this is done then results will not be confounded by family variation. We included family origin in the analysis of fry size so that family variation could be accounted for.

# MATERIALS AND METHODS

## Common Garden Growth Experiment

From July-September 2002 we conducted a common garden growth experiment in seven 114-liter circular tanks at the University of Alaska Fairbanks wet laboratory space in Douglas Island Pink and Chum's (DIPAC) Macaulay Hatchery, Juneau, Alaska. On July 1-3, 2002 we stocked 7 tanks at standard densities with 150 Chickamin River chinook fry that had been cultured for 77 days after first feeding in a growth trial intended for other research (Appendix A). 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 two subsets of the fish to detect differences related to inbreeding.

We identified one set of fish as outbred because they were derived from outcrosses between two experimental lines of Chickamin River chinook salmon. Both lines had previously been reared, uniquely identified with coded wire tags, and released from the National Marine Fisheries Service's (NMFS) Little Port Walter Research Station (LPW) on Baranoff Island, Southeast Alaska, since they were collected from the Chickamin River. One line was established five generations previously and the other one generation prior to these matings. These fish are an appropriate outbred comparison for the inbred fish because even they were themselves inbred, one generation of outbreeding eliminates inbreeding in the resulting generation. If the fry were created from mating 4$^{th}$

cousins (the fish in the two lines having originated from the same parents 5 generations previously), their inbreeding coefficients would be less than 0.1%.

The outbred group would not be expected to show outbreeding depression, a decline in the fitness of offspring from genetically different parents, because the two lines are derived from the same gene pool and the two stocks have been in culture for only five or fewer generations. We would not expect to see ecological outbreeding depression due to additive genetic effects, because at its inception the more recently founded line grew and survived as well in the hatchery as the line that was founded earlier (unpublished data, J. E. Joyce). That is, the outbred group would not experience ecological outbreeding depression of growth and survival unless the line that was in the hatchery for five generations differed phenotypically from the other line.

Outbreeding depression due to the disruption of coadapted genomes, or the disruption of genes working together to produce a favorable phenotype, also would not be expected because the two experimental lines do not have different genetic compositions, having been recently derived from the same natural population. This type of outbreeding depression would require that the two lines have different evolutionary histories and therefore different genetic material, or the introduction of new genes into at least one of the lines (Waples 1992, Lynch 1991, Emlen 1991). No new genetic material was introduced into the two lines after their inception, and in only five generations there has not been enough time for mutation to change the genotypes of these fish. Additionally, the lines have the same evolutionary histories, so we conclude that depression due to outbreeding would not have occured in these outcrossings.

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 the inbred fish to have an inbreeding coefficient higher than 0.25 if the fishes' 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 cannot assume an inbreeding coefficient greater than 0.25.

We randomly assigned the tanks over four benches in the wet laboratory and randomly assigned placement on each of the benches to randomize effects of position in the room. Each bench had its own water line from a common source supplied from Macaulay Hatchery culture water, from Salmon Creek. At this point in the experiment, family origin was unknown, so each tank did not necessarily include every family. We used automatic feeders to feed fish to satiation 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 preserved a caudal fin clip of at least 0.1 grams in 100% ethanol for a genetic parentage analysis.

**Microsatellite Parentage Analysis**

We analyzed 434 of the fin clips preserved in ethanol for parentage using microsatellites (Appendix A). Many of these fish were not used in the final analysis

because the parentage study showed that the fish were from a group not being studied, or because we were not able to deduce the parentage.

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 used six microsatellites: *Ots100, Ots104, Ots107* (Nelson and Beacham 1999), *Ogo4* (Olsen et al. 1998), *Omy325* (O'Connell et al. 1997), and *Ssa289* (McConnell 1995) for parentage analysis. In general, PCR conditions were as follows: 1 cycle at 95°C for 3 minutes; 28 cycles at 95°C for 30 sec., *x*°C for 15 sec., and 72°C for 15sec.; and finally 1 cycle at 72°C for 1 minutes; where *x* is the annealing temperature (58°C, *Ots*100, *Ots*104, Ots107, *Ogo*4, *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 deduce the parentage of the experimental fish. We used a mixed model procedure (PROC MIXED) in SAS to analyze variation of lengths, weights and condition factors at the end of the experiment. The mixed model is appropriate because it accommodates for unequal family sample sizes as well as correlations that can occur between family members. In the mixed model procedure, the random and fixed effects are distinguished from each other (Little et al. 1996). The effects of inbreeding, tank, and family origin were analyzed with this model:

$$Y_{ijkl} = \mu + T_i + G_j + T_i * G_j + F_{ijk} + e_{ijkl}$$

Where $Y_{ijkl}$ is the length, weight or condition factor at the end of the experiment, $\mu$ is theoretical population mean, $T_i$ is the tank, $G_j$ is the group (inbred or outbred), and $T_i * G_j$ is the effect of the interaction between tank $i$ and group $j$. $F_{ijk}$ is the effect of family within group $j$ and tank $i$, and $e_{ijkl}$ is the random error. Tank and group and the

interaction term of tank and group are treated as fixed effects and family as a random effect. The variance of the random parameter, family, became the covariance parameter for the model (Little et al. 1996).

We calculated the minimum effect size that our data could detect for length and for weight at 95% power. To calculate the effect size 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, p 189).

## RESULTS

With the parentage analysis we observed 35 inbred individuals from 7 families and 37 outbred individuals from 10 families (Figures 1-3). There was no significant difference of average length (inbred = 77.74mm, outbred = 77.38mm), weight (inbred = 5.27g, outbred = 5.36g), or condition factor (inbred = 1.12, outbred = 1.14) between inbred and outbred fish (Figures 1-3, Table 1). There were no detectable tank effects on length, weight, or condition factor (Table 1). The tank by group interaction was not significant for length, weight, or condition factor (Table 1). In contrast, family origin was a highly significant explanatory variable for variation of length or weight, but not for condition factor (Table 2).

The results indicate that there was no measurable effect of inbreeding on growth of Chickamin River chinook fry from swim up to 114 days post swim up. The 95% power analysis showed that our data has the power to detect a difference as small as 3.56 mm (4.6% of average = 77.56mm) and 0.88 grams (16.6% of average = 5.12g; Table 3).

# DISCUSSION

## Inbreeding

In this experiment we did not detect depression of size during the first summer of growth. We conducted a 95% power analysis that demonstrated we could detect a difference of 3.56 mm or 0.88 grams or greater (Table 3), however we did not include the variance due to family origin in the power analysis, therefore the true detectable differences were probably slightly greater than our estimates. Since studies have shown that size differences as large as 10 mm may not be biologically meaningful (Beckman et al. 2003; Quinn and Patterson 1996), the difference between our estimated and actual power is probably insignificant.

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 salmonids fry. In a comparison between small chinook fry $\leq$75 mm, and large fry$\geq$85mm, groups smolted at the same time, had similar movement patterns and behavior, and $Na^+$ -$K^+$ -ATPase activities, when the diet was not restricted (Beckman et al. 2003). In a similar study comparing the over-winter survival of small (70-79mm) and large (>89mm) 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 gene frequencies due to inbreeding may take longer than in completely diploid organisms. Because of this, salmon may be buffered to the effects of inbreeding (Allendorf and Thorgard 1984). However, inbreeding depression has been observed in rainbow trout (eg. Kincaid 1976a), so the benefits of tetraploidy may not be substantial.

**Initial Weights**

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 include them in the analysis (Appendix A). That is, we chose not to include egg weight as a proxy for weight at the beginning of the experiment because, although egg size can affect size at a young age, several studies have reported that in as little as 4-18 weeks after emergence there are no significant effects of egg weight on length and weight of salmonids (Heath et al. 1999; Kelley 1994; Springate and Bromage 1985; Fowler 1972). Beacham et al. (1985) reported that the size of alevins is no longer related to egg size as soon as 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**

Family variation was significant in the model (Figures 1-3, Table 2, Appendices D, E); in both length and weight, its magnitude was approximately as large as the variation among individuals. 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 comparative studies consider the number of families sampled, or the amount of variation that can be attributed to family origin. Inadequate sampling across many families, or failure to consider the variation caused by family of origin can confound the results of an experiment. 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, 21 fish were collected from a hatchery and 21 wild fish were collected by angling for the observation, but 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 which may have been important. In a study comparing sizes of

several brain structures of wild and hatchery-bred rainbow trout, 35 and 16 fish from two hatchery strains and 37 and 11 fish from two 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 or 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, twenty pairs of coho were placed into three 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 their results. If individuals within a family are correlated, and if there is variation between 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 three 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 make certain that many families will be sampled, but it makes the confounding of family effects less probable.

## SUMMARY

After examining the growth of inbred and outbred chinook salmon fry that originated from the Chickamin River, we did not find any differences in length, weight or condition factor. We did detect a significant effect of family origin on the length and weight of the fry at the end of the experiment. The results indicate that family origin can be a large source of variation, and should be accounted for in studies like this.

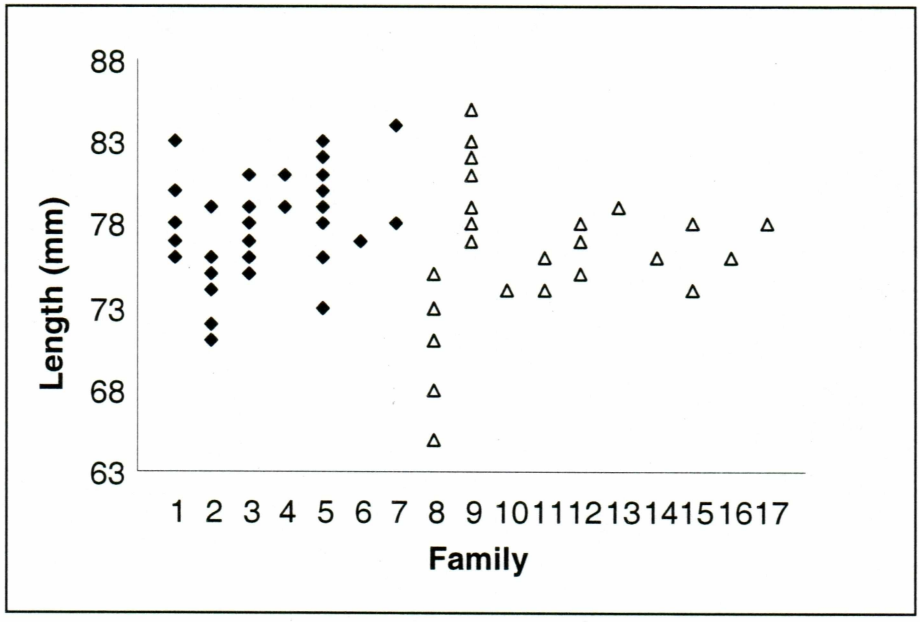


Figure 1. Lengths of inbred and outbred chinook fry native to the Chickamin River, Alaska raised in the laboratory in a 'common-garden' culture. Inbred individuals are offspring of brother-sister matings, designated by a filled diamond; outbred individuals are offspring of parents taken from two lines that have been separated for 5 generations, designated by open triangles.

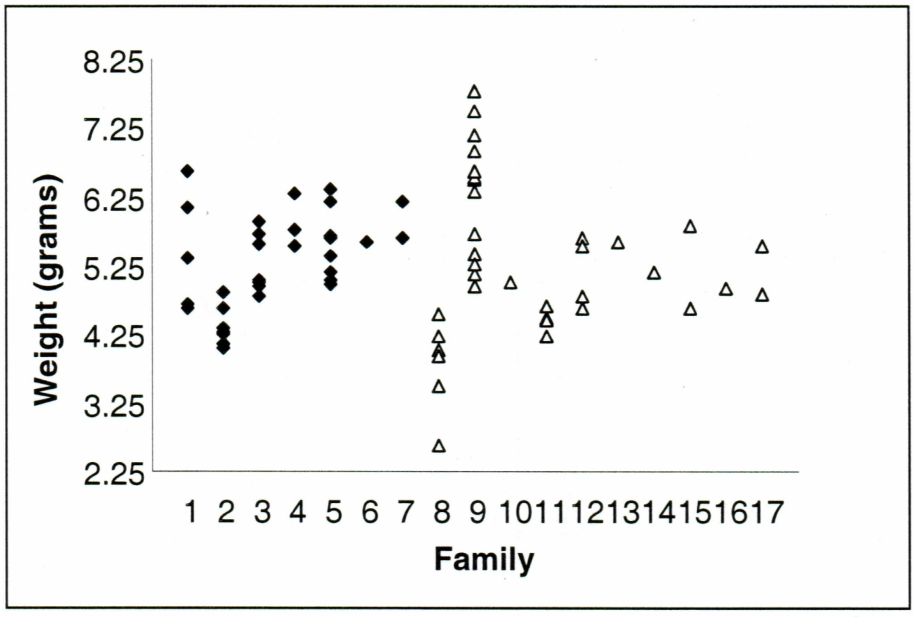


Figure 2. Weights of inbred and outbred chinook fry native to the Chickamin River, Alaska raised in the laboratory in a 'common-garden' culture. Inbred individuals are offspring of brother-sister matings, designated by a filled diamond; outbred individuals are offspring of parents taken from two lines that have been separated for 5 generations, designated by open triangles.

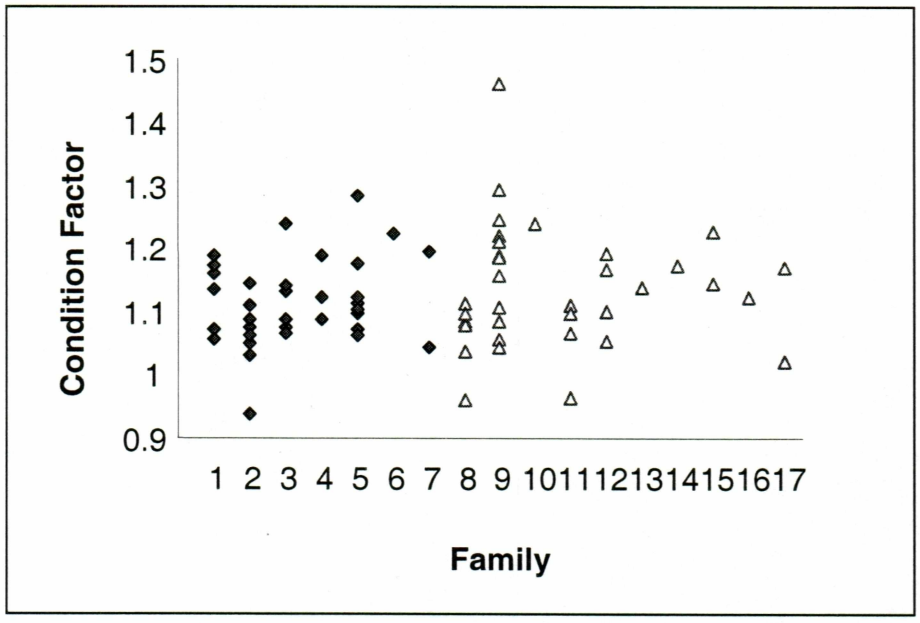


Figure 3. Condition factors (100,000*weight/length$^3$) of inbred and outbred chinook fry native to the Chickamin River, Alaska raised in the laboratory in a 'common-garden' culture. Inbred individuals are offspring of brother-sister matings, designated by a filled diamond; outbred individuals are offspring of parents taken from two lines that have been separated for 5 generations, designated by open triangles.

Table 1. Analysis of lengths, weights, and condition factors of inbred and outbred chinook fry using the mixed model procedure (PROC MIXED) in SAS. The fry were native to the Chickamin River, Alaska and raised in the laboratory in a 'common-garden' culture. Inbred individuals are offspring of brother-sister matings; outbred individuals are offspring of parents taken from two lines that have been separated for 5 generations. The degrees of freedom (df), the *F* statistic and the probability associated with it (P) are listed for group (inbred or outbred), tank, and tank by group interaction (Tank * Group) when length in millimeters, weight in grams, or condition factor ($100{,}000*\text{weight}/\text{length}^3$) is the response variable.

| | *F* | df | P |
|---|---|---|---|
| Length | | | |
| Group | 0.67 | 1,36 | 0.42 |
| Tank | 0.63 | 6,36 | 0.71 |
| Tank * Group | 0.40 | 4,36 | 0.81 |
| Weight | | | |
| Group | 0.03 | 1,36 | 0.86 |
| Tank | 0.71 | 6,36 | 0.64 |
| Tank * Group | 0.62 | 4,36 | 0.66 |
| Condition Factor | | | |
| Group | 2.06 | 1,36 | 0.16 |
| Tank | 0.98 | 6,36 | 0.45 |
| Tank * Group | 0.63 | 4,36 | 0.64 |

Table 2. SAS mixed model procedure (PROC MIXED) estimates of the random effect parameter family. The estimate of the variance, standard error (se), Z statistic, and probability (P) associated with the Z statistic when length (millimeters), weight (grams), or condition factor (100,000*weight/length$^3$) is the response.

| | variance | se | Z | P |
|---|---|---|---|---|
| Family Length | 7.14 | 2.79 | 2.56 | 0.011 |
| Family Weight | 0.47 | 0.16 | 2.86 | 0.004 |
| Family Condition factor | 0.0008 | 0.00011 | 0.71 | 0.475 |

Table 3. Power analysis of weight (grams), length (millimeters) and condition factor (100,000*weight/length$^3$) of inbred and outbred chinook fry native to the Chickamin River, Alaska raised in the laboratory in a 'common-garden' culture. Inbred individuals are offspring of brother-sister matings; outbred individuals are offspring of parents taken from two lines, originally from the Chickamin River, that have been separated for 5 generations. The mean ($\bar{x}$), standard deviation (sd), and minimum effect size (effect) detectable by our statistical analysis are listed when the power in 95%.

| | inbred $\bar{x}$ | inbred sd | outbred $\bar{x}$ | outbred sd | effect |
|---|---|---|---|---|---|
| Length | 77.74 | 3.19 | 77.37 | 4.42 | 3.56 |
| Weight | 5.26 | 0.69 | 5.36 | 1.16 | 0.88 |
| Condition Factor | 1.12 | 0.07 | 1.14 | 0.10 | 0.08 |

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# APPENDIX A

Background and Complications: previous work with Chickamin River chinook salmon

The NMFS at their research station at LPW, Baranof Island, has developed a geographically isolated chinook broodstock for the Alaska salmon enhancement program. The broodstock was taken from a wild population of spring chinook salmon in the Chickamin River in 1976, and has been in artificial culture for at least 5 generations. Gametes have been taken at LPW, fertilized and incubated there, and the resulting fry, parr and smolts have been cultured there for more than a year of each generation's life cycle before being released into the ocean.

In 1996 NMFS scientists collected gametes from wild chinook in the Chickamin River and produced $F_1$ hybrids between the hatchery-raised and wild fish, and control crosses (wildXwild and hatcheryXhatchery). In 2001, new artificial matings were created but no new gametes were taken from the wild. In 2001 "wild fish" means fish that were spawned in the hatchery in 1996, spent 1 year of their life cycle in the hatchery, and returned to LPW in 2001 when they were spawned to create 2001 crosses. In 2001 the wild and hatchery controls were maintained, reciprocal $F_1$ hybrids were recreated and reciprocal $F_2$ hybrids were created, and are now in the hatchery. $F_1$ hybrids were recreated because the adults used to create the $F_1$ hybrids in 1996 were raised in different environments, the wild fish reared in the Chickamin River and the hatchery fish were cultured at LPW. The $F_1$ recreations of 2001 have wild and hatchery parents that were

raised in the same environment (Appendix C). The purpose of the NMFS research is to detect effects of domestication in the hatchery after 5 generations of culture.

**Laboratory Common Garden Growth Study**

I used the chinook salmon hybrid and control crosses to conduct a laboratory growth and survival at DIPAC's Macaulay Hatchery. On June 3, 2002, we transported fry from the 6 experimental groups (Appendix B) to Macaulay Hatchery from LPW. Over the course of 6 days, I marked the fish to identify them to a cross type with photonic tags (New West Technology), small colored microbeads that are propelled through the skin by pressurized air.

From July-September 2002 I conducted a common garden growth experiment in 12 114-liter circular tanks at Macaulay Hatchery. On July 1-3 I created seven tanks at a low-density of 150 fish per tank and 5 high-density tanks with 300 fish per tank. Each tank included equal numbers of the 6 experimental crosses (Appendix B).

On September 22-24, 2002 I euthenized all fish with MS-222® (Tricaine methansulfonate), weighed them to the nearest hundredth of a gram, measured them from the tip of the nose to the end of the caudal fin in millimeters, and examined them for photonic tags. I preserved a caudal fin clip in 100% ethanol for future genetic analyses.

**Twin Lakes Mesocosm Experiment**

The Twin Lakes are small, shallow reservoirs that are fed by culverts directing runoff from upland suburban neighborhoods. They are closed off to other sources of water. The lakes were developed from tidal mud flats and marsh in the late 1970's after construction of Egan Drive. The Alaska Department of Fish and Game contracts with the DIPAC hatchery to stock Twin Lakes with approximately 10,000 8-10" chinook salmon each spring for recreational fishing. My preliminary trials in 2001 with coho parr demonstrated that growth and survival of salmon can be observed in the naturalistic environment of Twin Lakes by marking and recapturing salmon juveniles during the summer months. For this trial I stocked 3,000 coho fry into the Twin Lakes with out separating the two lakes. During the experiment the northern lake was drained and an indeterminate number of experimental fish were lost. In early October, I recaptured fry in the southern lake over a period of two days, and recovered 109 fish. During this trial I refined tagging, measuring and recapture techniques and demonstrated substantial growth of salmon fry during a brief 2 months' liberty.

Because the twin lakes have different physical attributes (i.e. depth, substrate, size), we separated them by a ¼" seine net and we conducted identical experiments simultaneously in each lake. On June 21-22, 2002 I marked 5000 fish of wild (WW) and 5000 fish of hatchery origin (HH) with a photonic tagging gun. I used the gun to mark the fish for their cross type and release location (north or south Twin Lake). I also clipped a pelvic fin to differentiate our experimental chinook from DIPAC'S chinook broodstock, and to double mark them for their cross type.

On June 28, 2002 I released 2,500 of each cross type (wild and hatchery) into each of the Twin Lakes, 5000 fry in each lake. To keep fish from migrating between lakes we placed a 28' by 8' seine with ¼" mesh between the lakes for the duration of the experiment.

On September 28-29, 2002 I seined both of the Twin Lakes by repeated baited hauls of a beach seine along the shore and recaptured 106 (54% HHxHH, 45% WWxWW, 1% unknown) experimental fish from only the southern lake with 28' by 8' beach seines with ¼" mesh. I did not recover any fish from the northern Twin Lake. I held all recaptured experimental fish in a 4' cubic net pen. On the 30th of September 2002, I removed the fish from the pens and euthanized them with MS-222. I noted the color of the photonic tag, and measured the weight (to the nearest hundredths of a gram) and length (to the nearest mm) of each fry.

**Complications Arise**

In September 2002, before the completion of the experiment, I realized that the colored photonic marks used to identify the experimental cross types had begun to fade. 85-90% of the tags remained, but may not have faded randomly. Also in September 2002, NOAA geneticists noticed that the experimental groups are not completely comprised of the cross they are supposed to be. For example, what I thought was a control cross of a wild fish could have been any of the other 5 cross types. This accidental mixing of experimental groups occurred at LPW sometime during the spawning or raising of the experimental fish in 2001.

**Parentage Analysis**

Because of these two issues I cooperatively began a genetic parentage analysis to determine which group the experimental fish really belonged to. Because this process is time consuming and expensive, I decided to perform the genetic analysis solely on fish from the low-density tanks. The additional information of parentage also enabled me to include family origin to the analysis, so that the variation attributed to family can be accounted for.

October 2002- March 2003 we performed a parentage analysis of the wild fish and the fish designated as unknown (because their colored mark had faded) and found that the wild fish used in the low-density tanks are actually a mixture of wild fish and a ¾ wild ¼ hatchery hybrid (due to an accidental mating of a WWxWH fish). We also found that many of the wild fish are inbred (brother-sister matings, F = 0.25). The high amount of inbreeding happened by chance because few wild families returned to spawn at LPW in 2001. This was a problem because inbred fish may show inbreeding depression (or depressed levels of fitness related traits such as growth and survival). This would confound the effects that the experiment was designed to look at, the effect of 5 generations of culture on the hatchery line, and hatchery-wild hybrids.

In order to examine inbreeding in the chinook fry from the low-density common garden tanks, I changed my objective to examine the effects of one generation of brother-sister mating on the growth of the 2001 wild Chickamin river chinook salmon fry. I also

examined whether differences in growth can be attributed to inbreeding depression or to family effects.

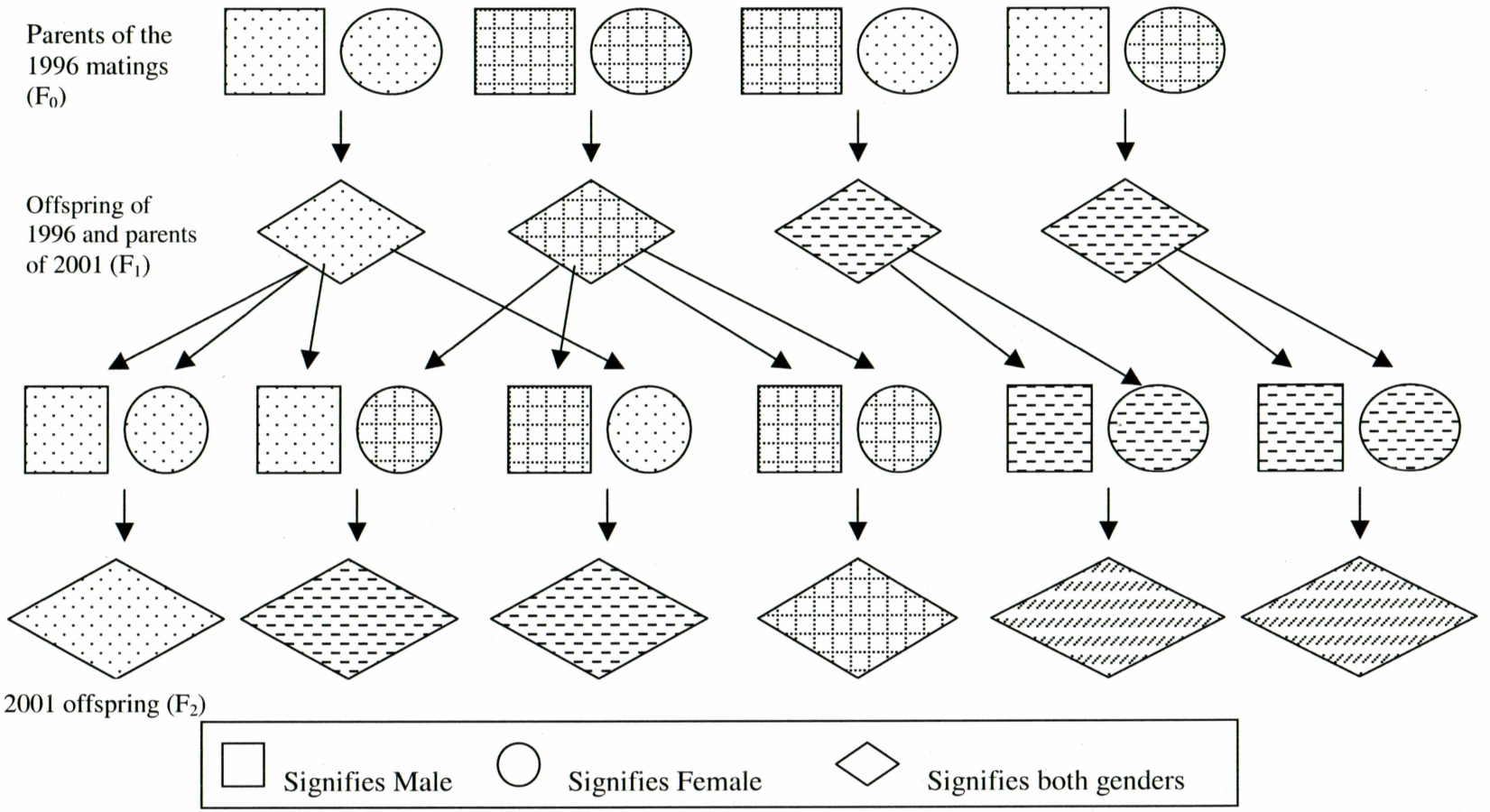
To accomplish the new objective we continued the parentage analysis on the WWxHH cross type from March 2002-July. The WWxHH cross served as the outbred comparison because there is not a possibility of this group being inbred, it was mated from 2 different groups.

## APPENDIX B

Experimental crosses of hatchery-bred and wild chinook salmon from the Chickamin River, Alaska, 2001. H designates hatchery-bred fish; W designates fish of wild ancestry.

| | |
|---|---|
| F1 hybrid recreation | HH X WW |
| F1 hybrid recreation | WW X HH |
| Control | HH X WW |
| Control | WW X WW |
| F2 hybrid | HW X HW |
| F2 hybrid | WH X WH |

## APPENDIX C




Breeding design of wild (W) and hatchery (H) bred chinook salmon from the Chicamin River, Southeast Alaska in 1996 and 2001. The subscript 0 signifies that these fish were parents of the 1996 matings. The offspring of the 1996 matings have a subscript of 1 ($F_1$). In 2001, the $F_1$ were used to create the second generation ($F_2$), which have a subscript of 2. There are two rows of $F_1$ to show that the $F_1$ were created in 1996 when the $F_0$ were crossed, and to show that the $F_1$ were used to create the $F_2$ in 2001.

# APPENDIX D

SAS output for the mixed parameter model (PROC MIXED) when weight is the response variable.

| Cov Parm | Estimate | Standard Error | Z Value | Pr Z |
|---|---|---|---|---|
| family(Tank*Group) | 0.4653 | 0.1627 | 2.86 | 0.0042 |
| Residual | 0.3844 | 0.09599 | 4.01 | <.0001 |

Null Model Likelihood Ratio Test

| DF | Chi-Square | Pr > ChiSq |
|---|---|---|
| 1 | 16.79 | <.0001 |

Type 3 Tests of Fixed Effects

| Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|
| Tank | 6 | 36 | 0.71 | 0.6439 |
| Group | 1 | 36 | 0.03 | 0.8597 |
| Tank*Group | 4 | 36 | 0.66 | 0.623 |

# APPENDIX E

SAS output for the mixed parameter model (PROC MIXED) when length is the response variable.

| Cov Parm | Estimate | Standard Error | Z Value | Pr Z |
|---|---|---|---|---|
| family(Tank*Group) | 7.1410 | 2.7940 | 2.56 | 0.0106 |
| Residual | 7.7048 | 1.9056 | 4.04 | <.0001 |

Null Model Likelihood Ratio Test

| DF | Chi-Square | Pr > ChiSq |
|---|---|---|
| 1 | 12.33 | 0.0004 |

Type 3 Tests of Fixed Effects

| Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|
| Tank | 6 | 36 | 0.63 | 0.7080 |
| Group | 1 | 36 | 0.67 | 0.4192 |
| Tank*Group | 4 | 36 | 0.40 | 0.8101 |

# APPENDIX F

SAS output for the mixed parameter model (PROC MIXED) when condition factor is the response variable.

| Cov Parm | Estimate | Standard Error | Z Value | Pr Z |
|---|---|---|---|---|
| family(Tank*Group) | 0.000815 | 0.001142 | 0.71 | 0.4751 |
| Residual | 0.006093 | 0.001400 | 4.35 | <.0001 |

Null Model Likelihood Ratio Test

| DF | Chi-Square | Pr > ChiSq |
|---|---|---|
| 1 | 0.59 | 0.4424 |

Type 3 Tests of Fixed Effects

| Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|
| Tank | 6 | 36 | 0.98 | 0.4520 |
| Group | 1 | 36 | 2.06 | 0.1598 |
| Tank*Group | 4 | 36 | 0.63 | 0.6426 |