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Inbreeding in Rainbow Trout (Salmo gairdneri): Analysis of Lethal Temperature Tolerence

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INBREEDING IN RAINBOW TROUT

(Salmo gairdneri):

ANALYSIS OF LETHAL TEMPERATURE

TOLERANCE

BY

JOSEPH L. MANGIARDI II

A thesis submitted
in partial fulfillment of the requirements for the
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1979

INBREEDING IN RAINBOW TROUT

(Salmo gairdneri):

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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INBREEDING IN RAINBOW TROUT (Salmo gairdneri):
ANALYSIS OF LETHAL TEMPERATURE TOLERANCE

Abstract

JOSEPH L. MANGIARDI II

Inbreeding in rainbow trout (Salmo gairdneri) was evaluated in relation to an altered environment (upper lethal temperature 29 C), at four levels of inbreeding. At each level of inbreeding mean time to death for inbred, female-outbred half-sib, male-outbred half-sib and outbred families were evaluated.

In all cases inbreds were less resistant to lethal temperature than were respective outbred families. Analysis of variance (Snedecor's F) and multiple range analysis (Tukey's "w" procedure) provided inference limitations. Inbred fish were 82% less resistant to lethal temperature as compared to the respective outbred family at 50% inbreeding and 19% less resistant at 25% inbreeding.

How inbreeding practices influence stocks of rainbow trout are summarized below:

A. Domestic stocks of rainbow trout are inbred to an unknown and varying degree.

B. Inbreeding has been common in domestic fish stocks for over 100 years in the United States.

C. Stocking domestic fish in natural waters has destroyed most genetically unique populations probably by

genetic "dilution".

D.   Millions of hatchery fish have been broadcasted
internationally with unknown consequences in regard to
the genetic structure of the original wild populations.

E.   Inbred groups can show increased phenotypic
variation when compared to their outbred countergroups
for some traits.

F.   Highly inbred fish may suffer reduced genetic
variability lowering tolerance to environmental stress.

Introduction

Fish culturists have generally failed to fully understand either the beneficial or detrimental consequences of genetics in relation to fish production efficiency. Two salient features commonly overlooked have been the cumulative effects of inbreeding and the accompanying loss of genetic variability.

Habitat alteration is the most serious threat facing all fish and wildlife resources. Man's activity has affected most rivers and streams of the world (Hynes 1970). It would appear that compromise of habitat suitability is the most common consequence of expanding human activity. The critical importance of water temperature, a component of habitat, in fish well-being is apparent since over 7,000 publications on this topic exist (Beltz et al. 1974). Oxygen requirements of many fish species are high and oxygen saturation points decline with increased temperature.

It seems a human trait to utilize generalizations. This is apparent from the breeding practices throughout fish hatcheries. Hatchery stocks of rainbow trout, because of domestication for some 100 years, are inbred to a degree which varies in different populations. Lack of recorded detail on breeding methods prohibits concise definition of the extent to which this problem exists, but several inferences will follow.

The purpose of this paper is to report on inbreeding in relation to an altered environment by investigating the question: Does inbreeding significantly increase vulnerability of rainbow trout (Salmo gairdneri) populations exposed to elevated temperature? This question has not previously been formally addressed.

Methods and Materials

In 1970 the U.S. Fish and Wildlife Service, Fish
Genetics Laboratory in Beulah, Wyoming embarked on the
program of evaluation inbreeding depression for several
fitness (efficiency in producing healthy adults) traits
related to hatchery production (von Limbach 1970, Bridges
1971, 1972, Kincaid 1976a, 1976b, Kincaid et al. 1976a,
1976b). Depression estimates were obtained from mean
differences between inbred and outbred half-sib families
reared contemporaneously in a standardized environment
(as in the present investigation, see Kincaid 1976a).

Since fertilization in salmonids is external,
separate egg groups can be fertilized by different males.
Likewise, milt from a single male can fertilize eggs
from different females. If one male is related to the
female, the offspring from their mating are inbred to a
degree determined by the closeness of their relation
(Appendices 1 and 5). By dividing sperm of a male to be
used with eggs of a closely, and also distantly related
female, both inbred and outbred offspring can be produced
which contain one haploid chromosome set in common.
Table 1 indicates the mating scheme employed for this
experiment.

The haploid chromosome set in common (Table 1) provides
an inherent control of environmental variance and maternal
effects from different female parents. The mechanical

TABLE 1. Mating Scheme For The Production Of Inbred, Female And Male Outbred Half-Sib Families

A	B	C	D
I_f X I_m	I_f X O_m	O_f X I_m	O_f X O_m
Inbred Family	Female outbred half-sib Family	Male outbred half-sib Family	Outbred Family

Where I_f = Inbred female, I_m = Inbred male

O_f = Outbred female, O_m = Outbred male

course followed in this study was to create several sets of inbred-outbred half-sib families (Table 1) each set being different in the level of inbreeding for the inbred family.

Member samples from each family were subjected to an upper lethal temperature assay to measure "resistance times" as defined by Fry (1947). Then for each level of inbreeding the hypothesis that mean time to death for the families were equal was tested statistically by Snedecor's F and Tukey's "w" procedure tests (Steele and Torrie 1960). One-way analysis of variance was chosen to indicate which means were significantly different within each of the four levels of inbreeding.

A testing apparatus was devised to perform the assay. The materials used were seven glass testing cylinders (20ℓ jars each containing 18ℓ of test water), submerged in a fiberglass body insulated tank ("Min-O-Cool": 20.8 cm X 56 cm X 54 cm = 630ℓ capacity). These test cylinders were surrounded by heated water (29 C) and did not contact each other or the walls of the tank, but rested on the bottom. Heating was accomplished by using four aquarium heaters (Geisler 100 watt). Approximately 400ℓ of the heated water were circulated in external contact with test cylinders by two electric water pumps.

One pump was submerged and forced water lengthwise from one corner along the botton of the tank. The other

pump took bottom water from the diagonally opposite corner and sent it along the surface. This method assured complete and rapid circulation plus efficient heat transfer to water in the test cylinders. Throughout experimentation, water temperature was monitored and maintained at 29 ± 0.5 C.

The test temperature of 29 C was selected for several reasons:

a. The uniform ambient temperature in the laboratory rearing facility was 14.0 ± 0.5 C. Since no acclimatization to other test temperatures was made for any test fish, the upper lethal limit remains uniform.

b. Salmonids usually die at water temperatures of approximately 30 C (Brett 1956). The temperature used (29 C) proved consistently lethal to fish in this experiment. This temperature was selected by testing random samples of fish prior to the actual experiment.

c. Although lethal (all fish usually died within 5 hours), 29 C was determined appropriate since the time-duration of each sample was ideally suited for the experimental design, as discussed below.

Mating occurred in January 1976 and experimentation followed during August 1976. All tests were performed within a short time period (daily over 2 weeks) thus minimizing possible confounding effects due to the changing natural light cycle (Ihssen 1973) at the location

(44 N. latitude). Water quality parameters were obtained
throughout the rearing of the test fish (Appendix 2).

An air pump (1/6 hp, 1725 rpm continuous) provided
oxygen and circulation within each test cylinder. The
air was bubbled from the bottom of each cylinder through
air stones. Air flow was maintained at a sufficient
volume to assure O_2 saturation. Dissolved O_2 was measured
for the first 48 samples (Appendix 3), but was discontinued
thereafter.

The scale of measurement used to record resistance
time was logarithmic since Fry et al. (1946), Fry and
Gibson (1953), and others indicated that the log of the
time to death when plotted against test temperature was
linear. Therefore, after Scott (1964) and Tyler (1966),
times to death were measured directly on a log scale
preset by the base unit of one day:

$$\text{where } 1.0 \le T_i \le 3.0 \ (i = 1,\ldots,30).$$

$$T_0 = 1.0 = 14.4 \text{ minutes,}$$

$$T_1 = 1.1 = 18.1 \text{ minutes,}$$

$$T_2 = 1.2 = 22.8 \text{ minutes, etc.}$$

The assay procedure simultaneously subjected seven
randomized samples of 25 fish per sample to an upper lethal
temperature (29 C) bath to measure thermal resistance times.
Tests were replicated twice for each family using random
sub-samples.

After complete randomization of samples, a schedule

was devised to remove fish from feed 24 hours prior to the execution of the experiment. Sample sets were drawn from inbred, both half-sib and outbred families (Table 1). Four levels of inbreeding were evaluated, F_w = 0.25, 0.406, 0.496, 0.50, (F_w is Wright's coefficient of inbreeding). The calculation of F_w values are figured with diverse form and function (see Wright 1922, Falconer 1960, Kempthorne 1969). Wright's coefficient of inbreeding (called here F_w) should not be confused with Snedecor's F.

Tested fish were removed from their respective rearing tanks and placed into one of the seven test cylinders. As soon as all cylinders contained samples, timing began.

Cessation of opercular movement was selected as the criterion of death (Brett 1952, Ihssen 1973). Although Tyler (1966) found this index inappropriate when testing _Phoxinus_ spp. since those fishes could recover after opercular movement ceased, such was not the case with rainbow trout.

As fish died they were removed from the test cylinder with a dip-net and placed into plastic bags which had been marked to identify the sub-sample and time interval in which they had died. Body weight and length measurements of the fish were obtained after the completion of the trials for all seven samples. A total of 80 samples of 25 fish per sample comprised the experiment. In all,

1,930 fish were used (eight samples were incomplete).
Fish were individually weighed on an electronic balance
to the nearest 0.01 g. These morphometric data were
compared by a stepwise multiple regression technique
(Kim and Kohout 1975) to characterize any weight or
length relationships with time to death.

Immediately after each testing session when
morphometrics had been obtained, the testing apparatus
was thoroughly cleaned, rinsed then filled and heated
to testing temperature before introduction of the next
set of samples.

For statistical analysis the mid-point of each
interval was used as the log time to death for fish which
died in that interval. These values were used in analysis
of variance of the means at each level of inbreeding.
Tukey's multiple range test (Steele and Torrie 1960)
was utilized to measure differences between group means
for each level of inbreeding. Each level of inbreeding
was evaluated separately since parents mated were comprised
of different strain combinations (Appendix 4). All data
were analyzed by an IBM 370/145 computer at South Dakota
State University, Brookings, South Dakota.

Results and Discussion

In this study inbred rainbow trout were less tolerant to a severe environmental change than were the respective half-sib families and the outbred family (Table 2). The ability to withstand a lethal temperature (29 C) decreases with increasing inbreeding (Table 2).

At the 25% inbreeding level (Table 2), mean time to death was not found to differ significantly from the outbred half-sib family or the outbred family upon analysis of variance. However, Tukey's "w" procedure indicated otherwise. This test declared the inbred family mean to differ significantly from the outbred family.

At the $F_w = 0.406$ and $F_w = 0.496$ levels of inbreeding analysis of variance rejected the null hypothesis of no difference among the means. Note that Tukey's "w" procedure (Table 2) failed to declare a significant difference between the inbred family and the female-outbred half-sib family for all levels of inbreeding. This is presumed to be the consequence of maternal effects due to the inbred female parent contribution in common with their respective outbred half-sib offspring.

Unfortunately, there were no outbred fish available for the $F_w = 0.500$ level of inbreeding. The partial results obtained were comparable to the $F_w = 0.496$ level of inbreeding.

Tukey's "w" procedure (Table 2) indicated that the

TABLE 2. Summary Of: Means (Expressed As Minutes) For Time To Death; GLM Procedure Computation Of One-Way Analysis Of Variance (Barr et al. 1976) and Tukey's "w" Procedure Multiple Range Tests (Steele and Torrie 1960).

Statistic	Inbreeding Coefficient	A $I_f \text{ X } I_m$	B $I_f \text{ X } O_m$	C $O_f \text{ X } I_m$	$O_f \text{ X } O_m$
Mean Time to Death (min)	0.250	109.80	114.20	128.70	134.50
	0.406	42.50	102.40	61.70	112.50
	0.496	33.58	60.25	46.87	185.89
	0.500	36.10	40.35	79.56	------

Statistic	Inbreeding Coefficient	degrees of freedom	F Statistic
Computed F	0.250	13,3	0.89ns
	0.406	11,3	6.64**
	0.496	3,2	17.86*
	0.500	2,2	5.64ns

Tukey's "w" Procedure

	0.250	A <u>B C D</u>
	0.406	<u>A B</u> <u>C D</u>
	0.496	<u>A B</u> C D
	0.500	<u>A B</u> C

Footnotes: The computed F statistic is tested for significance at the 5% level (one asterisk) and the 1% level (two asterisks). Tukey's "w" procedure was calculated at the 5% level; means not underlined are significantly different.

male outbred half-sib group differed significantly from the inbred group and the female-outbred half-sib group, at the $F_w = 0.500$ level.

From these results the theory that inbreeding may weaken temperature tolerance becomes more apparent with increasing levels of inbreeding. The outbred group's capacities at the $F_w = 0.496$ and $F_w = 0.500$ inbreeding levels are 62% and 82% longer respectively in regard to resistance times.

In the regression analysis, weight accounted for 10% of the variability in time to death. The addition of length to the stepwise equation did not account for a significant increase in time to death variability. The prediction equation to explain time to death was derived as:

$$Y = -0.358 \ (X) + 20.89$$

where Y = time to death and X = weight.

Several groups of fish were found to contain food elements in their stomachs despite the 24 hour off-feed schedule employed. Whether the food elements found were sufficient to account for the variability explained for weight is speculative only.

Lack of knowledge concerning gene action, in terms of breeding and especially variation (genetic, phenotypic and environmental), at the population level may negatively impact the proper management of fish populations. Several

concepts require familiarity to enable fuller appreciation of inbreeding in fish hatcheries and the consequences of reduced genetic variability.

Mutation is the raw material of evolution and genetic variability is the working material of evolution. Several factors can contribute to changes in the genetic variability within a population, these are: migration, mutation, natural selection, artificial selection and inbreeding. Of primary concern to the hatchery manager are migration, artificial selection and inbreeding.

Migration is the movement of genes from one population to another. With the advent of shipping eggs internationally, many original genetic constitutions of fish population have been altered or lost. When immigrants enter a population different in gene and genotype frequencies from the recipient population, the recipient population may become new genetically. Emmigrants who are not a random sample of various genotypic classes cause the following generation to change in a population left (Emmel 1976).

Artificial selection of brood stock causes inbreeding in inverse proportion to the number of individuals chosen as parents (Appendix 1). Inbreeding is common and often results in the loss of genetic variability. Inbreeding occurs naturally in all closed populations. In the hatchery, a closed population, inbreeding can be minimized as explained below. Unfortunately the easiest course

for a hatchery manager to follow when providing eggs for shipment is one where inbreeding accumulates at a swift rate. For example, a female rainbow trout produces approximately 2,000 eggs. Egg shipments are at times small (approximately 20,000 eggs). For purposes of establishing new brood stocks, a hatchery manager might ship one-third more fertilized eggs to offset mortality losses. The simplest way to produce 30,000 eggs is the mating of 1 male with 15 females (30,000 eggs) yielding 13.3% inbreeding (Appendix 1). Inbreeding is cumulative with each generation inbred. Furthermore, eggs shipped at the same stage of development from different parents are apt to be more closely related than other brood stocks of differing spawning times. This contention is supported by ease of change by selection for spawning time indicating a high heritability for time at maturity. Heritability is high when a trait is associated with a high additive genetic variance, or the trait is more genetically than environmentally controlled.

The rationale above is common: Billard (1977) introduced a new technique for the artificial insemination of salmonids. The procedure involves mixing 1 ml of sperm (1 male) with 2 ℓ of eggs (many females). He stated: "sire populations in hatcheries can be reduced because 1 male can fertilize 100 females". Significantly, this results in 12.6% inbreeding.

Some effects of inbreeding were realized from breeding animals about 200 years ago (Lush 1948). Fuller appreciation of inbreeding implications were elucidated by East and Jones (1919). Wright's (1921a, b, c, d, e) series of papers on systems of matings further expounded on inbreeding. Lush (1948) defined inbreeding as: The mating of individuals which are related to each other more closely than the average relationship within that population. The intensity of relationship may reach the condition where both alleles at all loci are identical (F = 1.0) in the population, in which case genetic variability cannot exist.

Wright (1921a, b) established the quantitative measures of inbreeding but Malecot (1948) pointed out that genetic identity can arise by two mutually exclusive events:

a. One gene may pair with an ancestral duplicate; these are identical by descent. Two genes identical by descent are identical homozygotes, and arise through inbreeding.

b. Genes identical, but not by descent, are independent. Homozygotes of independent genes are called independent homozygotes or identical in state.

Small closed populations will eventually be comprised of individuals closely related by descent. The study of inbreeding is essentially the study of the consequences of small population size.

In 1908 it was theoretically established that an

indefinitely large, randomly intrabreeding sexual
population, gene and genotype (zygotic) frequencies
will remain in "Hardy-Weinberg equilibrium" generation
after generation (Hardy 1908, Weinberg 1908). The
stipulations are no migration, mutation or selection.
The proportion of each possible genotype for a character
(e.g. AA = 25%, Aa = 50%, aa = 25%) are the genotype
frequencies for that population. From these, particular
gene frequencies can be obtained (Falconer 1960). These
measures genetically describe a population and may
supply indices of genetic change.

For example, if we ignore mutation, migration and
selection but sub-divide a "base" population which is
in Hardy-Weinberg equilibrium into separate small breeding
groups, the consequences of inbreeding should emerge with
time. In relation to the theoretical base population,
these expected changes are summarized, then individually
discussed below:

    a. Differentiation between the groups,

    b. Reduction of genetic variation (a measure of
        genetic uniformity) within the groups,

    c. An overall increase in the occurrence of
        homozygotes at the expense of heterozygotes,

    d. Reduced buffering capacity toward environmental
        change, and

    e. Reduction in mean phenotypic value for fitness

traits (known as Inbreeding Depression). Fitness
is classically defined as the proportionate
contribution an individual makes to the next
generation (Falconer 1960).

a.   Due to the sampling of gametes in small breeding groups,
gene frequency fluctuates in each generation with respect
to one locus among the groups. The direction of this
change is unpredictable but the magnitude is a function of
the original gene frequency and population size. The
net effect is a dispersion of gene frequencies among
the groups called random drift (Wright 1931). As this
process continues, the variance of a gene's frequency
among the groups increase (Falconer 1960). Since the
mean gene frequency in the population as a whole (all
groups) remains unchanged, the groups become spread
apart: Differentiation between the groups.

b.   Continued inbreeding within these groups leads to
eventual limits in inter-group differentiation. Gene
frequency for a particular locus cannot extend past
the limits of zero or one. When a gene frequency
reaches either limit, no return to an intermediate
frequency is possible. Obviously, when a gene reaches
a frequency of 1, it is considered "fixed", and when it
reaches a frequency of zero, it is considered "lost".
This means that all the alleles at a locus are the same
genes (identical by descent) when at a frequency of one

(1.0) with respect to that locus (Emmel 1976). This is precisely the rationale behind highly inbred strains of test animals since one source of experimental variation is presumably removed: Namely that the genetic variation within groups.

This classic expectation of genetics experiences doubt. The apparent contradiction is presumed to be due to the lower buffering capacity among inbreds. Because inbreeding lowers genetic variability, presumably the environment poses more of an influence on individuals of low genetic variability. Such individuals are less able to remain in a stable course of development even when the environment is "controlled". Outbreeding increases genetic variability. Outbreds are better equipped genetically to adjust to environmental change and remain in a stable course of development. At the high levels of inbreeding ($F = 0.406, 0.500$), I found that for time to death the mean coefficient of variation (standard deviation ÷ mean) was higher ($CV = 69.25$) than for the outbred groups ($CV = 32.00$). Further examples of this apparent paradox in salmonids may be found soon because it is far from unknown in other animals (Livesay 1930, Green 1931, Yoon 1932, Wexelsen 1937, Grunberg 1950, Robertson and Reeve 1952, Mather 1953, McLaren and Michie 1956, Lindsey and Harrington 1972).

c. Obviously, as closely related individuals continue

to mate with each other, their offspring will become more closely related. Genetically this is phrased as an increased likelihood that alleles at a locus are identical by descent. The cumulative probability of these events are measured as "F_w" the coefficient of inbreeding. F_w expresses the amount of heterozygosity lost, or the cumulative effect of random drift. For example, an F_w value of 0.500 indicates a 50% probability that alleles at a locus are identical homozygotes. Three generations of full sib matings yield this degree (F_w = 0.500) of inbreeding among the progeny of diploids, although triploidy in rainbow trout and other fishes has been noted (Ceullar and Uyeno 1972, Swarup 1959, Valenti 1975, Allen and Stanley 1978). The coefficient of inbreeding is useful only in relation to a specified base population with an assumed F_w value of zero, since all life has a presumed common ancestor (Darwin 1859).

Summarized, continued inbreeding may yield: An increase in the occurrence of homozygotes at the expense of potential heterozygotes.

d. Successful populations have met several requirements. One requirement is sufficient habitat. Loss of sufficient habitat means loss of a population. Another requirement is sufficiency of genetic variability to permit adjustment to environmental change.

Individual and population environmental alteration

evokes adaptive potential to maintain the welfare of the unit. Thus homeostatic mechanisms continually react providing life with a relationship to the environment (resulting in adaptedness). Lerner (1953) stated:

"Homeostasis refers to the property of an
organism to adjust itself to variable conditions,
or the self-regulatory machanisms of the organism
which permit it to stabilize itself in fluctuating
inner and outer environments."

Genetic variability is greatest when all loci are heterozygous (Falconer 1960). Genetic homeostatis refers to the phenomenon of Mendelian population genetic constitution equilibrium. A Mendelian population is a sexually reproducing community sharing a common gamete resource or gene pool. Lerner (1953) proposed a selective advantage for heterozygotes because of a better "buffering capacity" to environmental changes thus reducing deviation from "phenotypic optimum fitness". Table 2 provides information for better understanding of this contention in relation to fishes.

Finally, another requirement of population is uniformity of adaptive phenotypic expression needed to avoid excessive reproductive waste. Seemingly in contradiction to the genetic variability between individuals Lerner (1953) pointed out that the heterozygote has a better ability to stay within the bounds of

"canalized embryologic development" or developmental
homeostasis. Therefore the loss of heterozygotes leads
to another theoretical implication of lowered genetic
variability by inbreeding: Reduced buffering capacity
to environmental change.

e. "Inbreeding depression" is the most salient observable
result of continued inbreeding. The depression is in
terms of a decline in mean phenotypic value shown in
fitness traits such as reproductive capacity and physio-
logical efficiency. The reason for the depression is
seen in the difference of genotypic value between
homozygotes and heterozygotes when measured phenotypically.
Theoretically if genes which increase the value of a
trait are dominant over alleles which reduce value, then
inbreeding will result in a reduction of the mean. The
change is generally directed toward the more recessive
alleles (Falconer 1960). From more recessive alleles
and the observable reduction in fitness traits, we are
reminded of the generalization that deleterious genes
tend to be recessive and "uncovered" through inbreeding.
From the loss of heterozygotes due to inbreeding we find
an observable phenomenon: Reduction in mean phenotypic
value for fitness characters (inbreeding depression).
See Kincaid (1976a,b) for examples in rainbow trout.

Though inbreeding is usually harmful for a population,
some merit exists. Crossing two highly inbred lines often

produces offspring (F_1) whom exhibit heterosis.   Two
highly inbred lines are each highly homozygous.   At
representative loci between the two lines crossed, it
is improbable that the new alleles are identical homo-
zygotes.   Furthermore, natural selection purges deleterious
alleles expressed as recessive homozygotes, prior to
crossing the two lines.   Upon crossing, the offspring
should be alloted by chance, a scrambled assortment of
alleles with heterozygosity.   Successive generations
produced from the F_1 generation are once again subject to
inbreeding and merit typically declines.   This apparent
reciprocation of inbreeding depression provides an
avenue of plant and animal improvement (Gowen 1952, Srb
et al. 1965).   No experimentation with heterosis was
conducted in this report.

What these results imply when a manager stocks
highly inbred fish is poorly understood.   However, the
importance of genetic variability in maintaining viability
should now be clear.   Recognition of the environmental
framework in which genetic variability operates is
important.   Environments for hatchery fish change
spacially and temporally.   Figure 1 provides visual
evidence of the contention that inbreds suffer reduced
tolerance to environmental change, in this case
elevated temperatures to a lethal limit.

FIGURE 1. Histogram Of Times To Death Over All Levels Of
Inbreeding For Outbred (A), Male Outbred Half-
Sibs (B), Female Outbred Half-Sibs (C) and
Inbred (D) Families.
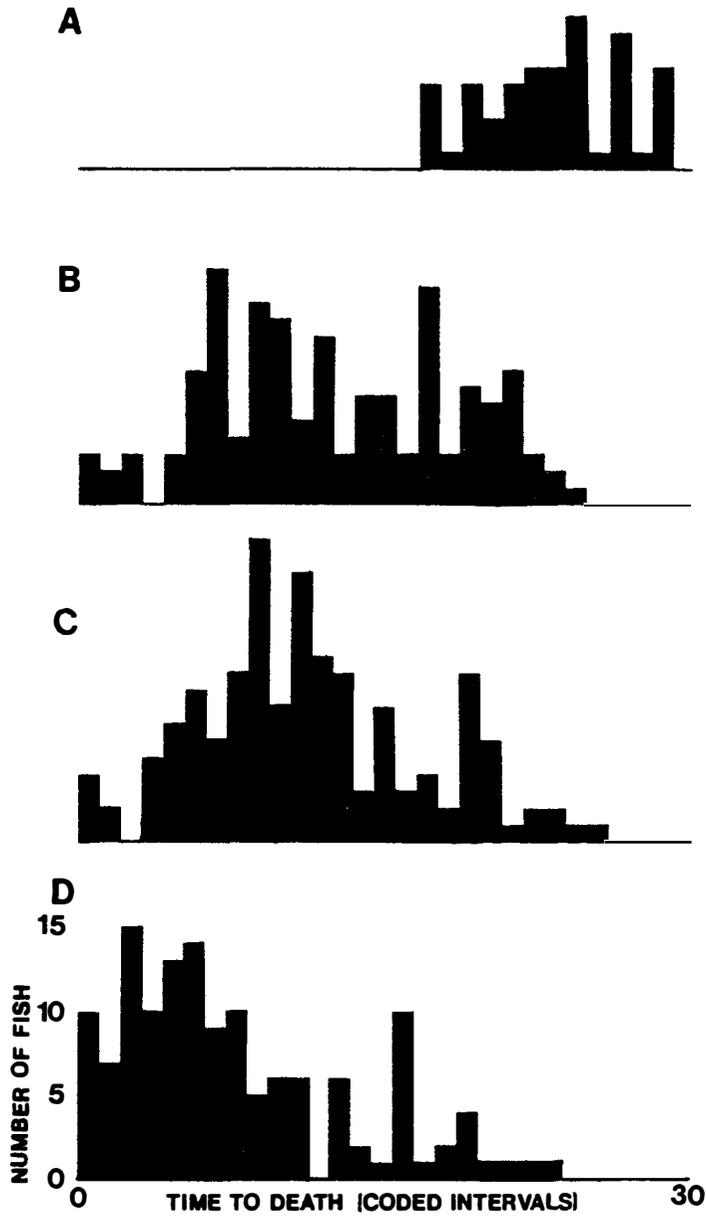
Conclusion

Genetics is the science of explaining variation in biological populations. Inbreeding may seriously affect genetic variability, but many of the consequences concerning fish remain unknown. Fuller knowledge of genetic parameters may greatly aid all aspects of fish culture and management. Inbreeding is but one parameter.

Historically, fish genetics has been largely neglected despite the fact that private groups, state and federal governments have been culturing fish for over 100 years in the United States. The traditional objective of fish culture has primarily been concerned with the production of fish for stocking depleted waters. Recently this perspective has experienced change toward regarding fish as "true" farm animals. A true farm animal is a domestic organism cultured with the intent of optimizing production traits, and the word "trait" implies genetics.

Some salient examples of the use of genetic principles and procedures includes inbreeding as discussed above, the implementation of electrophoresis in management (Utter et al. 1976), heritability estimates and selective breeding especially in relation to fish farming and restoration (Lewis 1944, Donaldson and Olson 1957, Donaldson and Menasueta 1961, Gall 1969, Calaprice 1969, 1970, Simon 1970, Pardom 1972, Millenbach

1973, Moav and Wohfarth 1973, Calaprice and Hagsett
1975, Gjedrem 1975, 1976, Longwell 1976, Pardom 1976).
This interest may have developed because of the increased
need for excellent protein sources, or because fish are
the optimum vertebrate genetic tool or both.

Finally it is important to address the question:
"What can a hatchery manager do about inbreeding?"

There are several ways for a fish culturist to
minimize inbreeding. The simplest is to utilize random
mating from a large population. A number of one-to-one
matings are made to the extent where inbreeding is
minimized to the point desired (Appendix 1). From
examination of Appendix 1, 50 randomly selected males
mated with 50 randomly selected females holds inbreeding
to 0.5% per generation. This is a recommended level.
Of course this suggested level may be inadequate or too
demanding. However, large hatcheries can easily utilize
100 of each sex so that inbreeding is restricted to 0.2%
per generation. Also, it is the sex used in the least
numbers that contribute most to inbreeding. The
advantage of using large numbers in matings (100 ♂ X 100 ♀)
are derived from inbreeding depression estimates for
rainbow trout (Kincaid 1976a, b).

Examination of the available literature on inbreeding
depression estimates make clear the economic need to
devote more cost and time to avoid inbreeding. Kincaid

(1976b) stated: "Application of the total effects of inbreeding on the number of fish remaining and the weight of fish remaining in a production lot at 1 yr of age indicate losses of 17.4 and 36.6% after one generation (F = 0.25) and 47.9 and 65.4% after two generations (F = 0.375) of brother-sister mating."

If intensive selection is intended for certain traits, rotation line crossing can be utilized. Rotation line crossing is more money and time intensive but further minimizes inbreeding. Here, the entire population is subdivided into three independently maintained lines (Figure 2).

Male gamete contributions are represented by dashed lines (diagonal) and females by solid lines (vertical). To produce A' individuals in generation 1, males from line C are mated with females from line A. Each new line is again maintained separately. When these reach spawning age, the procedure is repeated. Rotation line crossing is twice as effective as random mating when the same numbers of fish are utilized (one-third coming from each line).

Rotation line crossing and random mating success in minimizing inbreeding is governed by the population size, the number of generations the population remains closed and how much inbreeding existed when the program is implemented. All closed populations will experience

FIGURE 2. Rotation Line Cross Breeding Scheme For
          Minimization Of Inbreeding In Hatchery Fishes.

GENERATION   LINE

                    I   II   III

0   A   B   C

1   A'   B'   C'

2, etc.   A"   B"   C"

- - - - Male gamete contributions are represented
         by dashed lines (diagonal)

_____   Female gamete contributions are
         represented by solid lines (vertical)
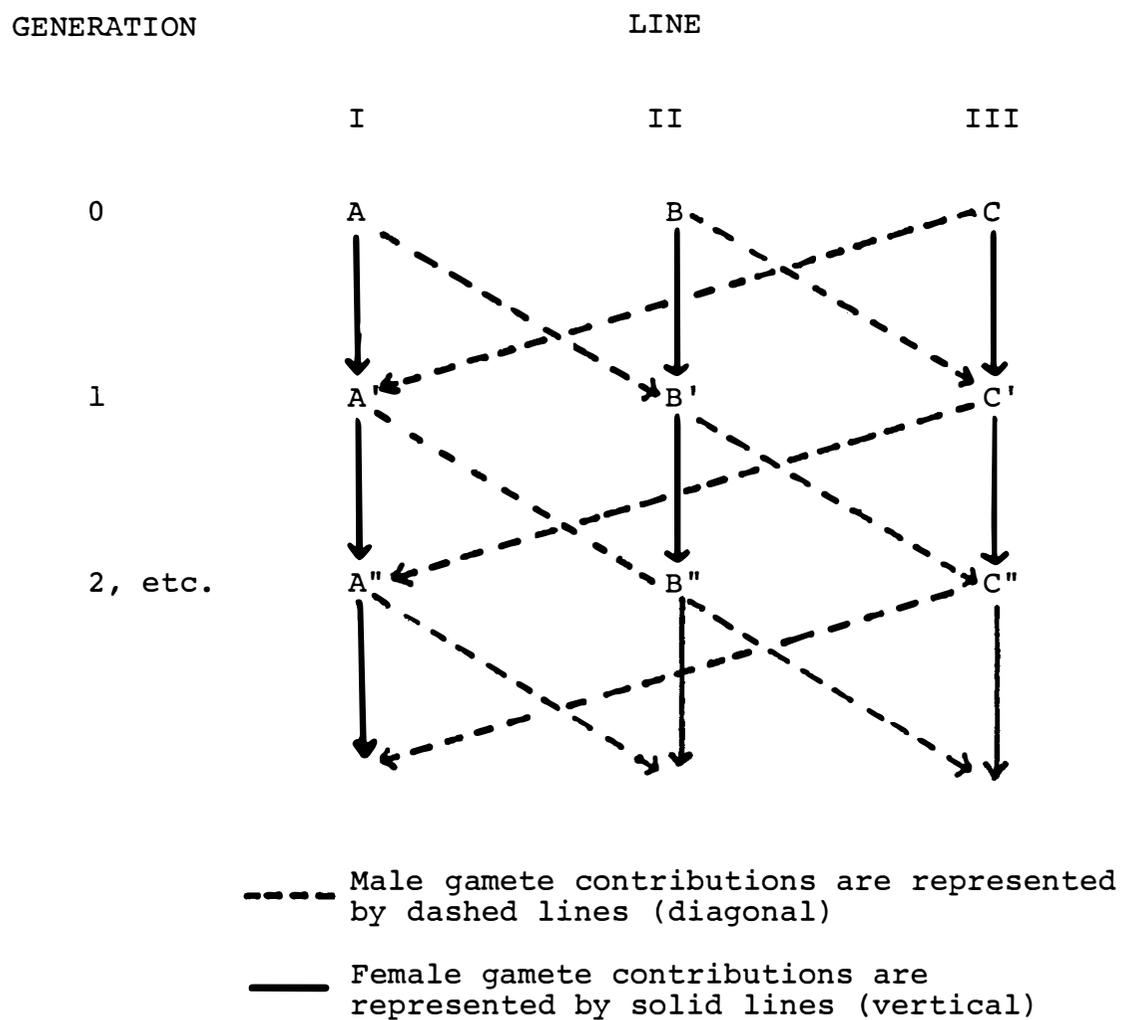
inbreeding. The utilization of hybrid populations and
occasional introduction of sufficiently different stocks
disrupt genetic conditions responsible for inbreeding
depression. However, selection of new stocks for out-
breeding require scrutiny. The new stock should be
free of undesirable traits, compliment the existing
strain, yet be sufficiently different to attain heterosis.
The manager then has many options for dealing with
inbreeding and what course he or she chooses depends
on the hatchery capacity, the amount of inbreeding to
be tolerated and the availability of outside strains.

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APPENDIX 1.   Percent Inbreeding Resulting From Various Numbers Of
              Parents In A Broodstock (Rounded In First Decimal Place)

Number of Male Parents

		1	2	3	5	10	15	25	35	50	100	200
	1	25.0	18.8	16.7	15.0	13.8	13.3	13.0	12.9	12.8	12.6	12.6
	2	18.8	12.5	10.4	8.8	7.5	7.1	6.8	6.6	6.5	6.4	6.3
	3	16.7	10.4	8.3	6.7	5.4	5,0	4.7	4.5	4.4	4.3	4.2
	5	15.0	8.8	6.7	5.0	3.8	3.3	3.0	2.9	2.8	2.6	2.6
Number of Female Parents	10	13.8	7.5	5.4	3.8	2.5	2.1	1.8	1.6	1.5	1.4	1.3
	15	13.3	7.1	5.0	3.3	2.1	1.7	1.3	1.2	1.1	1,0	0.9
	25	13.0	6.8	4.7	3.0	1.8	1.3	1.0	0.9	0.8	0.6	0.6
	35	12.9	6.6	4.5	2.9	1.6	1.2	0.9	0.7	0.6	0.5	0.4
	50	12.8	6.5	4.4	2.8	1.5	1.1	0.8	0.6	0.5	0.4	0.3
	100	12.6	6.4	4.3	2.6	1.4	1.0	0.6	0.5	0.4	0.3	0.2
	200	12.6	6.3	4.2	2.6	1.3	0.9	0.6	0.4	0.3	0.2	0.1

$$\% \ \text{Inbreeding} = \frac{1}{8N_m} + \frac{1}{8N_f} \cdot 100 \ \text{where}$$

N_m = number of male parents;

N_f = number of female parents (Falconer 1960).

Percentages are for one generation of matings and should be
added to those resulting from subsequent and previous generations.

APPENDIX 2. Rearing Water Parameters Obtained* Monthly For Fish Used In This
            Study At The United States Fish Genetics Laboratory, Beulah,
            Wyoming.

<u>1976</u>

	Jan 12	Feb 10	March 11	April 20	May 20	June 15	July 1
Colon bacilli, per 100 cc M.P.N.	< 2.2	5.1	< 2.2	< 2.2	< 2.2	< 2.2	< 2.2
Hardness, parts per million ($CaCO_3$)	480	470	440	440	425	420	410
Total dissolved solids, parts per million	540	568	548	532	600	524	500
Sulfates, parts per million	230	230	230	230	250	230	230
Nitrates, parts per million	1.1	1.4	2.1	0.9	1.6	1.1	1.0
Total carbonates, parts per million							147

* Sample analysis was conducted by: Wyoming Department of Agriculture,
   Chemical and Bacteriology Laboratory, P.O. Box 3228, Laramie, Wyoming 82070.

APPENDIX 3. Dissolved Oxygen (ppm.)* Of Water In Test
            Cylinders Obtained Immediately After
            Completed Trials

Number of Trials Measured 48

Dissolved Oxygen (mean) . . . . 7.35 ppm.

Standard Deviation. . . . . 0.12 ppm.

Coefficient of Variation. 0.0164

*Azide modification of the Winkler method (American
Public Health Association 1973).

APPENDIX 4. Strain Compositions Of Fishes Employed At Each Level
              Of Inbreeding (0.25, 0.406, 0.496 and 0.50).

Coefficient of Inbreeding (F)   Strain (rainbow trout)

        0.25   . . . .   Fishlake

        0.406   . . . .   3/4 Sandcreek, Wy.⎫   grandparental
                                     1/4 New Zealand   ⎬   generation
                                                       ⎭
        0.496   . . . .   Sandcreek, Wy.

        0.50   . . . .   New Zealand

APPENDIX 5. Inbreeding Coefficients Under Various Systems
            Of Mating.   After Falconer 1960.

Generation (t)	A	B	C
0	0	0	0
1	.500	.250	.125
2	.750	.375	.219
3	.875	.500	.305
4	.938	.594	.381
5	.969	.672	.449
6	.984	.734	.509
7	.992	.785	.563
8	.996	.826	.611
9	.998	.859	.654
10	.999	.886	.691
11		.908	.725
12		.926	.755
13		.940	.782
14		.951	.806
15		.961	.827
16		.968	.846
17		.974	.863
18		.979	.878
19		.983	.891
20		.986	.903

Column	System of Mating
A	Self-fertilization, or repeated back-crosses to highly inbred line
B	Full brother X sister, or offspring X younger parent: inbreeding coefficient
C	Half-sib (females half sisters)