THE USE OF ALBINISM TO VERIFY GYNOGENESIS AND TO INVESTIGATE THE VIABILITY AND GROWTH RATES OF INBRED CHINOOK SALMON *ONCORHYNCHUS TSCHAWYTSCHA*

by

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in the Department of Biology

We accept this thesis as conforming to the required standard

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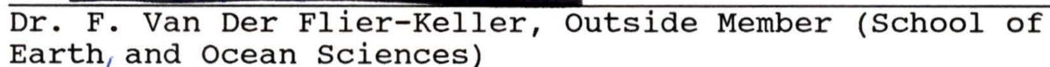

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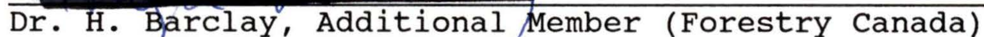

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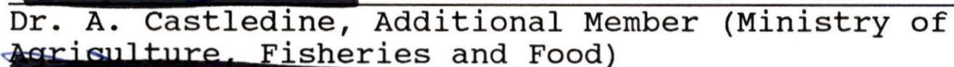

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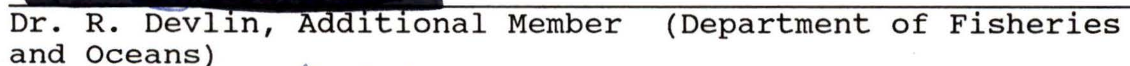

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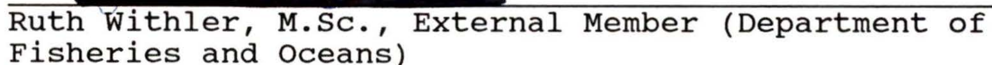

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ABSTRACT

Distinction of possible pleiotropic and inbreeding effects on albino chinook salmon produced from full-sib mating were determined by comparing the viability and growth of albinos with outbred wild-type half-siblings. Phenotypes of crosses between wild-type and albino chinook salmon demonstrate that the allele responsible for albinism in Big Qualicum chinook salmon is recessive. Analysis of growth showed differences between pigment types in freshwater and saltwater phases of culture. Albinos were significantly heavier than their wild-type half-sibs by Day 448 of culture. Albinism had no negative pleiotropic effect on growth for the duration of the study, however increased mortality was evident in both saltwater and freshwater culture. Albino fry exhibited a higher frequency of deformities than wild-type fry. Experiments to produce gynogenetic chinook using albinism as confirmation of purely maternal genetic contribution in offspring were successful. Albino offspring were produced by fertilising albino eggs with ultraviolet (U.V.) treated wild-type sperm and applying pressure shocks of 3 to 6 minutes duration at both 30-40 minutes and 300-330 minutes after fertilisation. The gynogenetic origin of these offspring was confirmed with Southern blot analysis of genomic DNA.

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This research is dedicated to <u>my</u> family, Linda and Tyson for their inspiration and love.

INTRODUCTION

Albinism occurs in many fish species including a shark belonging to the genus *Stegostoma* (Nakaya 1973), the common carp (*Cyprinus carpio*, Johnson 1968), the lake chub (*Kyphosus;* Sgano and Abe 1973), channel catfish, (*Ictalurus punctatus*; Bondari 1984), tilapia (*Oreochromis niloticus*; Scott et al. 1987), rainbow trout (*Oncorhynchus mykiss;* Bridges and Limbach 1972) and paradise fish *Macropodus opercularis* (Kosswig 1935).

In rainbow trout, tilapia, grass carp (*Ctenopharyngodon idella*) and channel catfish, albinism is inherited as a monogenic autosomal trait. In most of these species the trait is recessive to the wild-type, but in Japanese rainbow trout it is dominant (Chourrout 1982;Onozato 1984).

Although the genes controlling albinism in rainbow trout and channel catfish appear to control colour in a simple relationship, the substitution of one allele for another can affect more than one biochemical pathway, and if more than one pathway is affected, more than one trait (e.g. growth rate or viability) can be affected. These secondary effects are termed pleiotropic effects. If the pleiotropic effects include traits such as growth rate, disease resistance, viability, or fecundity, they become economically important, possibly more important than the primary trait. If the trait of albinism is used solely for research purposes it is important to quantify pleiotropic

effects before it is used as a genetic marker, because these effects may confound the quantitative genetic data of the group under investigation.

Albinism in vertebrates is the result of a lack of the tyrosinase enzyme in the melanocytes of the epidermis. Tyrosinase catalyses the oxidation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) which is an intermediary in melanin synthesis. Whiteness of the skin by itself is not diagnostic of albinism in fish, the eyes of the fish must also lack melanin at the back of the retina and appear pink (Purdom 1993).

Albinism in humans is associated with several vision abnormalities, including decreased visual acuity, increased sensitivity to light and astigmatism. In addition, albinism is associated with an increased incidence of skin cancer due to the lack of pigmentation. The human eye requires melanin pigment to develop normal vision although the reason for this requirement is unknown. In albino humans the iris does not have enough pigment to screen excess light and it passes with light from the pupil to the retina. As melanin serves to protect tissues from ultraviolet (U.V.) radiation there is an associated increase in U.V. damage to the retina in albinos (Wynbrandt and Ludman 1991).

The culture and marketing of rare phenotypes of food fish for their unusual appearance is common, as evidenced by the culture and sale of red tilapia (<u>Oreochromis</u>

niloticus) and albino trout (Oncorhynchus mykiss). Interest in pilot-scale culture of albino chinook was expressed by a few local salmon farmers when the current research was initiated. Given the extreme competitiveness of the salmon farming industry and the small margin between profit and loss, it is also important to quantify any effects on growth for any colour variant that may have commercial value, as this will affect the cost of production of this phenotype, and ultimately the profitability of its culture.

Many genes responsible for colour in fish have pleiotropic effects. Wohlfarth and Moav (1970) found that both blue (bb) and gold (gg) common carp have lowered growth rates as a pleiotropic effect. Matricia et al. (1989) concluded that grey tilapia grew faster than red morphs, but comparative strain evaluation was complicated by genotype/environment interaction and by undetected strain variation within colour morphs. Rothbard and Wohlfarth (1993) found that albino grass carp were on average significantly shorter than full-sib wild-types.

Converse to the observations of Wohlfarth and Moav (1970), Wlodek (1968) found that Polish blue common carp grew better than normally pigmented carp. Bondari (1984) provides evidence of a negative pleiotropic effect of the homozygous recessive (aa) genotype channel catfish, in that albinos spawn later, produce smaller egg masses and have a reduced growth rate compared to normally pigmented

catfish. In addition, albino catfish are subject to more predation than the wild-type (Prather 1961). In contrast to these examples, Bridges and Limbach (1972) suggest that no such pleiotropic effects are seen in albino rainbow trout. However, positive pleiotropic effects in terms of 18-27% higher growth rates have been noted for another colour morph, an iridescent metallic blue rainbow trout (Fish Genetics Lab, U.S. Fish and Wildlife Service, Beulah, WY. in Tave 1989).

The impetus for the current research project was provided by anecdotal reports of above-average growth and reduced mortality, of albino chinook compared to normally pigmented (wild-type) chinook (Dave Munday pers comm. President, Regent Seafoods Ltd., Duncan, B.C.). A controlled study to test these observations was the major objective of this project.

The albino phenotype does not appear to be common among chinook salmon in the wild. A single published report of one albino chinook salmon fry in the Columbia River (Dauble et al. 1978) is the only reference to albinism in wild chinook salmon in the literature. The low incidence of albinos in wild populations may reflect increased vulnerability due to a lack of protective colouration and possibly weakened eyesight.

Although the Big Qualicum albinos used in this study lack melanin, production of white pigment by iridiphores or yellow and orange pigments by xanthophores and

erythrophores does not appear to be affected. The fish retain the ability to absorb astaxanthin and canthaxanthin. The result is a pink-fleshed fish with yellow skin colouration on the dorsal surface and white colouration on the ventral surface. Albino chinook adults used as broodstock in this study had pink pupils and yellow irises, and by definition are true albinos.

The deleterious effects of inbreeding on the productivity of cultured fish have been documented in several studies. For example, Aulstad and Kittelsen (1971) reported lethal deformities in fry of rainbow trout (Salmo gairdneri, recently reclassified Oncorhynchus mykiss) associated with 25% inbreeding. Moav and Wohlfarth (1963) observed a 15% reduction in relative growth rate in inbred carp (Cyprinus carpio) and an increased proportion of fish with dorsal-fin anomalies produced from full-sib parents. Kincaid (1976a,1976b,1983) has shown that as the degree of inbreeding increases above 18% in O. mykiss so do the negative effects on phenotypic traits of weight, survival and the proportion of cripples.

The primary effects of inbreeding in the Seaspring Hatchery strain of Big Qualicum chinook salmon were expected to be reduced growth rate and viability. Because they were known to be full-sibs, the albino chinook broodstock used in this project provided a convenient model to examine inbreeding effects, a secondary objective of this study. Offspring from these full-sib albino parents

had an inbreeding coefficient F of 0.25. F is the probability that the two alleles at a given locus are identical copies of a single ancestral allele.

The performance of inbred albino offspring was compared to that of outbred wild-type half-sibs. The wild-type offspring were produced by fertilising half of the eggs from each albino dam with a putatively unrelated wild-type male from the same hatchery strain. It is recognised that this design will confound possible negative pleiotropic effects with possible inbreeding depression effects. The design therefore provides a measure of the combined effects when compared to an outbred control.

Quantitative phenotypes, such as weight and length, are important production traits which can be measured. These traits are controlled by many genes, unlike qualitative traits such as colour which may be controlled by single genes. The heritability of quantitative phenotypes is dependent on the proportion of additive genetic variance (V_a) to the total phenotypic variance (V_p). One objective of the present study was to use analysis of sib variance to compare the estimates of heritability for quantitative traits in these inbred and outbred families.

The distinctive nature of the albino phenotype lends itself to use in chromosome manipulation research such as gynogenesis. Gynogenesis is the parthenogenetic development of an egg, in which cell division is activated by sperm penetration but no genetic contribution is made by

the sperm. Diploidy is restored to the egg by preventing the completion of the second meiotic division or the first mitotic division of the haploid egg. Induced gynogenesis is the critical first step in a process used to produce all female stocks of fish. The ability to produce genetically female stocks has been important to the Salmon farming industry in reducing losses associated with premature male sexual maturation in chinook salmon. If albinism in chinook salmon were verified as a recessive trait with no negative effect on embryonic viability, it could be used in refining techniques for producing gynogens.

The production of gynogenetic offspring verified by the albino phenotype very early in development replaces more difficult and intensive verification methods such as karyology, protein or DNA analysis. Confirmation of gynogenesis on live individuals requires rearing salmon until they can be verified as all females which may be as long as eight months (Donaldson and Hunter 1982), or obtaining tissue samples and using karyological examinations at younger stages. The use of albino eggs allows non-intrusive confirmation of gynogenesis by the eyed-egg stage of development as soon as 16 days after fertilisation at 10°C.

Gynogens are currently produced by fertilising eggs with sperm which has been exposed to gamma or ultraviolet radiation. Diploid salmon gynogens are then produced by a heat, cold or pressure shock applied after fertilisation

with the treated sperm (Fig. 1). A shock administered shortly after fertilisation interrupts the second meiotic division of the oocyte producing embryos with an extra set of maternal chromosomes from the second polar body. Due to meiotic crossing-over in the region between genes and centromere, meiotic gynogens tend to retain a relatively high genomic heterozygosity (Purdom 1993). Gynogens can also be produced by preventing the first mitotic division several hours after fertilisation, which in contrast to meiotic gynogens results in completely genomic homozygous individuals. Clonal lines of fish can then theoretically be created by applying meiotic gynogen techniques to the eggs of mitotic gynogens.

Recessive phenotypes have been used as a check on the success of gynogenesis in other fish. Komen et al.(1988) used female common carp (Cyprinus carpio L.) homozygous for a gene determining scalation (mirror, ss), and fertilised ova from these fish with irradiated milt from heterozygous males (scaled, Ss). The absence of scales in the progeny was considered to be proof of their gynogenetic origin.

Gynogenetic development has also occasionally been observed in crosses between coho salmon (Oncorhynchus kitsutch) and brook trout (Salvelinus fontinalis), and between masu (Oncorhynchus masou) and chum (Oncorhynchus keta) salmon (Yamazaki 1983). The possibility that interspecific crosses could be used to produce gynogens was also examined in the present study.

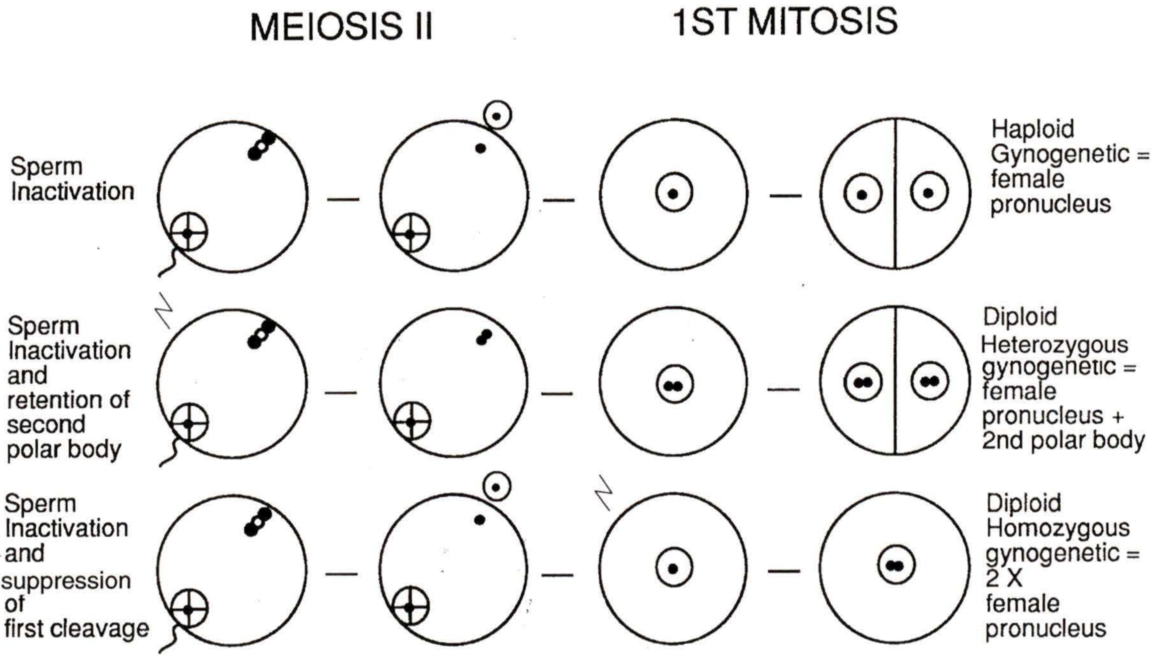


FIGURE 1. Diagram of possible gynogenetic salmon produced with inactivated sperm.

Experiments were performed in this study to assess the utility of albino chinook ova for use in determining the efficiency of hydrostatic gynogenesis techniques. The results of these trials are presented below.

In summary, this research project had several objectives, each of which was linked as a required proof to the next objective: i) confirmation of albinism as a recessive, non-lethal trait in chinook salmon; ii) determination of pleiotropic effects of albinism in chinook by comparing growth (as measured by weight and length) and viability of same-age half-sibs of the wild and albino phenotypes; iii) distinction of negative pleiotropic effects from inbreeding depression in albino offspring from albino parents; iv) comparison of heritability estimates to demonstrate empirically the effect of inbreeding on this calculation; and v) demonstration of the utility of the trait in refining the parameters for hydrostatic gynogen production.

MATERIALS AND METHODS

All the albino dams and sires in the family study were the progeny of a single pair of normally pigmented parents. Of the 60 albino individuals within this parental stock, 7 females and 20 males matured sexually in October 1990, and these individuals were crossed to produce the albino offspring used in the family study. The albino offspring of these full-sib crosses were therefore inbred with an inbreeding coefficient (F) of 0.25 (25%). Their growth, mortality and proportion of deformities were compared with outbred wild-type crosses from within the same hatchery population.

i) Family Study

Table 1 lists the steps in the family study, with the date and chronological day. All albino sires and dams were reared as a single population for the first 27 months of their life at Dalmar Seafarms in Nanoose Bay, British Columbia. They were transferred to the Pacific Biological Station for the final eight months of maturation.

The albino fish were then transferred in October 1990 to Seaspring Hatcheries in Duncan and moved from seawater to freshwater. On October 23, 1990, each of six albino dams were randomly mated with one of six albino sires and with one of six wild-type sires (Table 2). The wild-type sires were the same age and hatchery strain as the albinos but

TABLE 1. Chronology of events in family study

Day	Date	Event
0	October 23, 1990	-Spawned 6 albino females with 6 albino males and 6 wild-type males -Egg mass split by weight, 1/2 fertilised with albino, 1/2 with wild-type. 12 families produced.
30	November 23, 1990	-Eggs shocked by siphon drop
31	November 24, 1990	-Dead Eggs removed | designated
32	November 25, 1990	-Dead Eggs removed | - "first egg
42	December 7, 1990	-Dead Eggs removed | pick"
64	December 29, 1990	-Eggs/alevins picked
67	January 1, 1990	-Eggs/alevins picked
77	January 11, 1991	-Alevins picked
87	January 21, 1991	-Alevins and deformities picked
90	January 23, 1991	-450 fry from each of 10 families ponded at Rosewall -3 replicate tanks of 150 fry each for each family -50 alevins from each of 10 families sampled for weight and length from tray population.
132	March 6, 1991	-30 fry from each replicate tank sampled for weight and length. Density reduced by random sample to 100 fry per tank.
173	April 23, 24 1991	-40 fry from each replicate tank sampled for weight and length. -Density reduced by random sample to 75 fry per tank. -20 fry from each tank PIT tagged and returned
197	May 17, 1991	-Inoculated all 30 tanks with Aqua Health Ltd., Furogen B and Vibrogen
227	June 11,12,13	-20 PIT tagged fish from each tank sampled for weight and length and pooled -55 untagged fish sampled
228	June 13, 1991	-Fish introduced to seawater at Pacific Biological Station
341	October 4, 1991	-First saltwater measurement
448	January 19, 1992	-Second saltwater measurement
538	April 18, 1992	-Third saltwater measurement Family experiment terminated

they had different parentage and were reared at Eagles Landing in Sansum Narrows. Morphometric data collected on parental fish included weight and length of both sexes, and weight, volume and number of eggs produced for dams. Eggs taken from each dam were divided into two lots of approximately equal numbers. Each lot was fertilised with either a full-sib albino sire or with a less related wild-type pigmented sire. This procedure produced pairs of half-sib families related by a common dam.

TABLE 2. Morphometric data of parents for the family study

Dam	Dam Weight (g)	Total Egg Weight (g)	Number of Eggs	Mean Egg weight (g) n=50	Sire Phenotype	Sire Weight (g)
1	4820	1103.3	4057	0.259[b*]	Albino	3270
					Wild	4000
2	4300	776.1	3338	0.241[d]	Albino	4620
					Wild	4800
3	5410	1138.9	3673	0.274[a]	Albino	3730
					Wild	6550
4	5230	1065.6	3721	-	Albino	4840
					Wild	3500
5	4560	1100.4	3013	0.263[b]	Albino	4000
					Wild	4200
6	4300	833.5	2901	0.250[c]	Albino	3620
					Wild	4500

* Different superscripts indicate mean egg weights are significantly different (P>.05) using Tukey's Studentized Range Test

All eggs were incubated (10°±0.5°C measured by Peabody Ryan Thermograph) in vertical-stack incubation trays (Heath Technicorp., Seattle, Washington) with water flows of 10-15 L/min. Each half-sib family was randomly assigned to one side of a tray divided in half by a Plexiglas sheet. Standard hygienic treatment of the fertilised eggs included water-hardening the fertilised eggs with an iodophore and biweekly treatment with malachite green (50 mg/L flush treatment) until the eyed-egg stage. The eggs were shocked by a siphon drop 30 days after fertilisation and the dead eggs were removed. True mortalities were distinguished from unfertilised eggs by clearing eggs in Stockard's solution and establishing the presence or absence of embryonic tissue. All losses were recorded throughout the incubation period.

At swim-up stage, all fry with gross abnormalities were counted and culled from all families. Due to limited tank availability, the offspring of Dam 4 were randomly chosen to be eliminated from the experiment at this stage. From the remaining fry, 450 were randomly selected from each family and divided into three replicate groups of 150 fish. Each of these replicates was randomly placed in one of an array of 30 identical 25 L cylindrical tanks at the Department of Fisheries and Oceans research facility at Rosewall Creek. Each tank received equal water flows (4 litres per minute, average temperature 7.8°C; range 8.7°C to 7.2°C) from the same source. All tanks were located

indoors and the photoperiod corresponded to external daylight. All groups were fed to satiation by hand four times daily during daylight hours. Initially the fry were fed Biomoist starter diet (Bioproducts Ltd., Warrenton, Oregon), and were then switched to a Whitecrest grower diet (Campbell River, B.C.). Mortalities were removed and recorded daily from every tank.

Fish in each tank were sampled for weight and length on Days 132 and 173. The fish were sampled randomly and without replacement during these days and the number of fish was reduced to 100 and then to 75 per tank by Day 173.

These withdrawals maintained culture densities in the freshwater phase to between 6.6 and 24 g/L to minimize density effects on growth. During sampling each family tank was removed from the array and all the individuals anaesthetised in 20 mg/L tricaine methane sulphonate (MS-222). Selection of untagged individuals for weight and length within tanks was random.

To facilitate repetitive and non-destructive identification of individuals after they were pooled in a common environment, fish from each tank were tagged with a passive integrated transponder (PIT) tag. On April 23 and 24, at a mean weight of approximately 4 grams, 20 fry from each tank (60 fry per family, 600 in total) were randomly selected, anaesthetised and injected intraperitoneally with a PIT tag. At that time, the 10-digit alphanumeric PIT tag code, wet weight (±0.1 g), fork length (± 1 mm) and family

group identification of each fry was recorded. All fish implanted with PIT tags were externally marked by the removal of the adipose fin. Tagged fish were returned to the tank from which they were sampled.

On June 11 to June 13, all remaining fish were resampled for weight and length. The PIT-tagged fish were pooled and transported to a common environment in a single marine netcage (volume 125 m^3) at the Pacific Biological Station research farm in Nanaimo. The fish were reared on WhiteCrest grower diet during this period. Mortalities were recorded and removed daily.

During this saltwater rearing phase the fish were weighed and measured for length at age 341, 448, and 538 days. In all measurements all fish were dip-netted from the saltwater pen, anaesthetised, identified with a PIT-tag reader, weighed, measured for length, and then returned to the pen. The net pen was temporarily divided into two halves during sampling to separate sampled from unsampled fish, to ensure no fish was sampled twice.

ii) Chromosome Manipulation

One objective of this work was to confirm the utility of the albino phenotype as a convenient marker in verifying the gynogenetic origin of manipulated eggs. A secondary goal was to better define the physical parameters required to produce gynogens using pressure shocking.

The following procedure effectively deactivates nuclear DNA within sperm cells without affecting their ability to

penetrate the egg. Sperm was stripped from each male, checked for motility, divided into two unequal volumes and placed on ice in plastic bags with oxygen. Due to the volume of sperm required for creating clear seminal plasma used for sperm dilution, sperm was pooled from three mon-sex males (genotypic females) for Trial Number 1. Subsequent trials varied in the number of donor males from 1 to 4 males depending on the quantity of eggs used. The greater portion of the sperm was centrifuged at 600 g in transparent 15 ml tubes with an International Equipment Centra-4 centrifuge for 10 minutes. The clear seminal plasma was decanted from the test tube and was used to dilute the stored untreated sperm to a 1:9 sperm/plasma ratio. A portion of this diluted sperm was exposed to a total of 33,000 ergs/mm^2 of short wave ultraviolet light. The U.V. light was provided by three, 40W shortwave U.V. bulbs, and dosage was monitored with a Blak-Ray Model J225 U.V. meter which measures uW/cm^2 at a wavelength of 254 nm. The apparatus was set up in a cold room at 4°C with 5 ml of dilute sperm in an open Petri dish on a rotating plate set at 100 r.p.m. The calculated depth of the diluted sperm in the Petri dish was 0.84 mm. With the exception of the first trial, sperm was shaded from other light sources after irradiation to prevent DNA repair by the activation of the PR (Photo Reactivation) enzyme (Circuns 1990).

Sperm was checked before treatment and after treatment to ensure non-motility prior to and sufficient motility

after activation with water. The eggs from an albino female were randomly divided into three lots, two lots were fertilised with U.V.-treated sperm and one lot with untreated sperm. Approximately 0.5 ml of treated sperm was used for every 100 eggs. After the sperm was added to the eggs, 10°C water was added to the mixture and this was designated as the moment of fertilisation (time zero of incubation). The eggs were then incubated at 10°C for specified times and then placed into a stainless steel pressure cell.

Each shock treatment consisted of placing approximately 100 eggs in the pressure cell, (5.07 cm^2 [0.786 $inch^2$] piston-face surface area), filling the cell with 10°C water and sealing it with a piston. The pressure level was raised to the target pressures within seconds using a laboratory scale hydraulic press (Carver Lab Press Model C).

Pressure within the cell was not measured directly but read from a gauge on the press. Due to the mechanical advantage of this apparatus, internal cell pressure was higher than gauge pressure and target pressures were exceeded. Gauge pressures of 5.5, 6.2, 6.9, 7.6 and 8.3 kPa (per Teskeredzic et al., 1993) equivalent to 563, 633, 703, 773 and 844 kg/cm^2 were converted to cell pressure by multiplying gauge pressure of the hydraulic press by a factor of 1.273. The corresponding cell pressures were to 717, 805.5, 895, 984, and 1074 kg/cm^2.

The three treatments for each experiment were: 1) fertilisation with U.V.-treated sperm and no pressure shock; 2) fertilisation with treated sperm and pressure shocked; 3) and fertilisation with untreated sperm and no pressure shock. These treatments were anticipated to produce gynogenetic haploids, gynogenetic diploids and normal diploids respectively. The percentage of albino and normally pigmented alevins in a treated group was the measure of the efficiency of both the ultraviolet and pressure shock treatments used in the present study.

For the experiment to produce gynogens using interspecific crosses, donated gametes collected from salmonid species at several federal hatcheries were used. The gametes used were approximately 24-hours old and were held at 4°C in a sealed bag with pure oxygen until fertilised. No treatment was applied to the sperm. Half of the fertilised eggs were pressure-treated at 895 kg/cm^2 for 6 minutes, 30 minutes after fertilisation using the techniques described above.

iii) <u>Analysis of Genomic DNA by Southern Hybridisation</u>

Verification of the gynogenetic origin of albino offspring was done using the analysis of genomic DNA. The materials and methods for DNA Extraction from chinook tissue are modifications of Sambrook et al. (1989) per Devlin et al. (1991). A detailed description of the methods is included in Appendix 1.

RESULTS

i) Inheritance of Albinism

Wild phenotype offspring resulted from every wild by albino cross (Table 3). Albino offspring resulted from every albino by albino cross (Fig. 2). The parental albino fish used in this study were all the product of a cross between normally pigmented parents. The ratio of offspring phenotypes from that initial cross was approximately 3:1 (wild-type:albino) which is the expected theoretical ratio for a heterozygous cross of dominant and recessive alleles.

TABLE 3. Progeny phenotypes at the eyed-egg stage from albino and wild-type crosses

Parents				Progeny phenotype (eyed-egg stage)			
Number of Matings	Phenotype male	female	Total eyed eggs	Wild no.	%	Albino no.	%
8	albino X	albino	9121	0	0	9121	100
16	wild X	albino	9149	9149	100	0	0
12	albino X	wild	2143	2143	100	0	0
1*	wild X	wild	3678	2678	72.8	1000	27.2

* These data were obtained from hatchery records of the parental mating from which the adults used in this study were produced. N.B. The number of albinos in this cross represents an approximate value.

Prior to this study, successful crosses of chinook, which are homozygous for this trait, had not been accomplished. The present results indicate that sexually

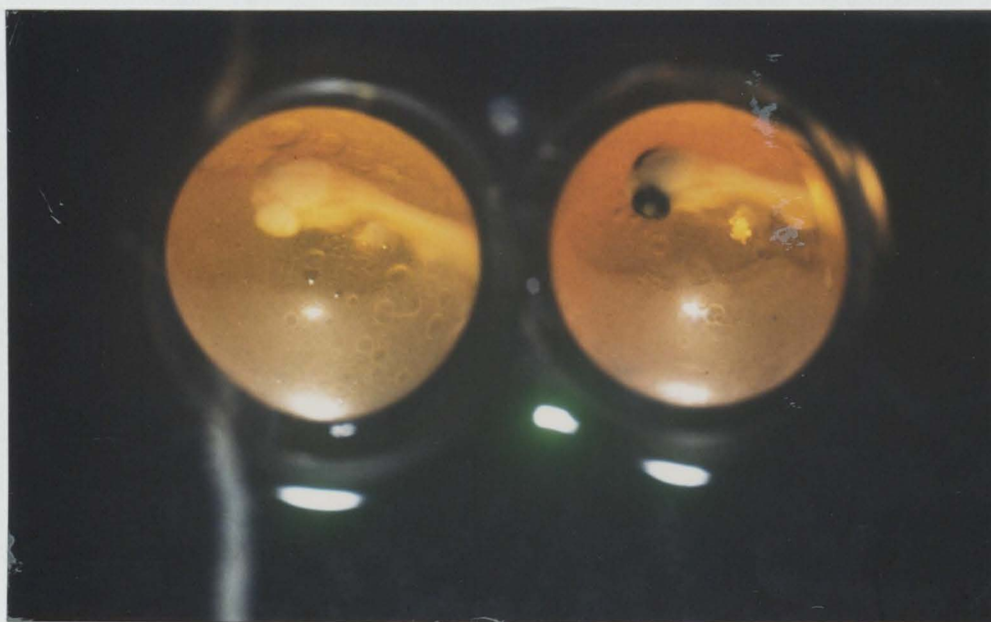


FIGURE 2. Albino and wild-type eyed-eggs of chinook salmon.

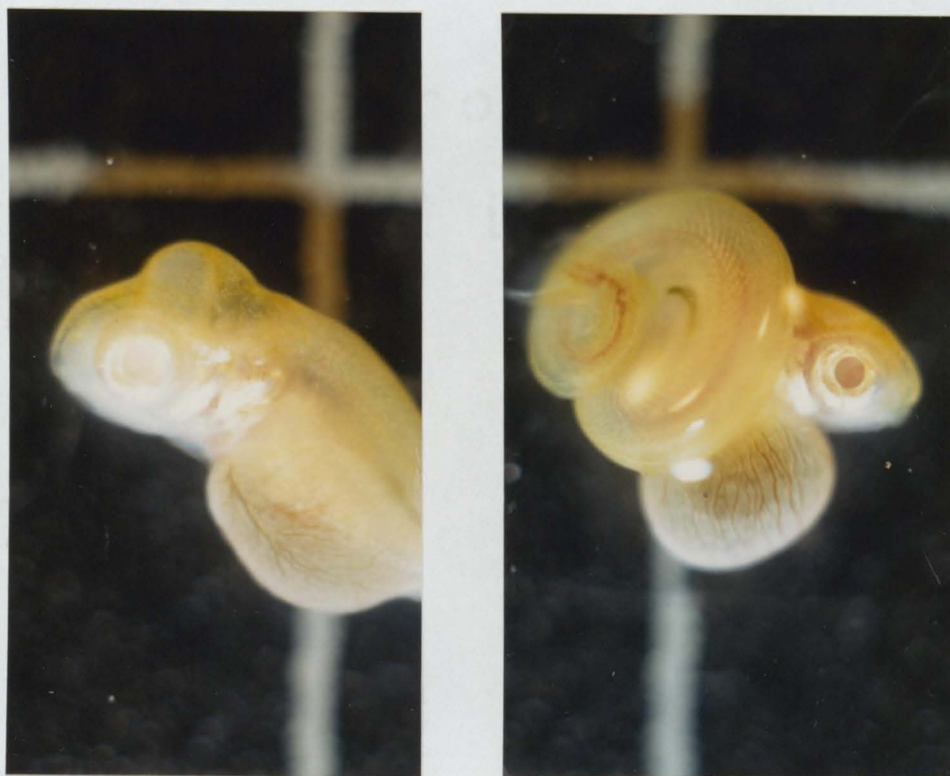


FIGURE 3. Typical lethal cranial and spinal deformities of fry culled from family study.

mature and fertile albino individuals of both sexes can be produced. To prove definitively that albinism is determined by a single allele, attempts were made to rear and accelerate the sexual maturity of wild-type heterozygotes produced to perform the backcross of albino X (wild-type X albino). These attempts were unsuccessful due to plankton blooms and equipment failure that killed the heterozygous fish.

Albinism in Big Qualicum chinook is recessive to the wild-type trait and it is likely inherited as a monogenic autosomal trait.

ii) <u>Pleiotropic Effects of Albinism</u>

The nearly identical survival rates to hatching (Table 4) of half-sib families 1,2,4,and 6 indicate that albinism does not affect hatching success. The mortalities at first egg-pick represent unfertilised or infertile eggs. The low survival at the first and subsequent egg-picks of both phenotypes in Family 2 are indicative of poor egg quality in Dam 2. The differences in survival evident between the half-sibs in Families 3 and 5 are likely due to differences in the fertilisation success of the males.

A Tukey-type test was performed on arcsine transformed proportions of post-first-pick mortalities (eyed-eggs and alevins which died during development). Proportions indicated by different superscripts in Table 4 are significantly different. In contrast to unfertilised eggs the post-first-pick mortalities represent true mortalities.

TABLE 4. Cumulative mortality and survival to ponding at Day 90

Dam	Family	Total Eggs	Mortalities: First Egg Pick	%	Post First Pick	%	Total	Survival %
1	Albino	2027	55	2.71	43	2.18[a*]	98	95.17
1	Wild	2030	52	2.56	39	1.97[a]	91	95.52
2	Albino	1631	410	25.46	307	25.14[d]	717	56.04
2	Wild	1707	443	25.95	297	23.50[d]	740	56.65
3	Albino	1767	74	4.19	62	3.67[b]	136	92.30
3	Wild	1906	622	32.63	40	3.11[b]	662	65.27
4	Albino	1836	46	2.51	39	2.18[a]	85	95.37
4	Wild	1885	50	2.65	50	2.72[a]	100	94.69
5	Albino	1482	290	19.57	41	3.44[b]	331	77.67
5	Wild	1531	54	3.53	37	2.51[a]	91	94.06
6	Albino	1434	72	5.02	91	6.68[c]	163	88.63
6	Wild	1467	110	7.50	105	7.74[c]	215	85.34
Total								
	Albino	10177	947		583		1530	75.66
	Wild	10526	1331		500		1899	69.31

* Different superscipts indicate percent mortality are significantly different (P>0.05) using Tukey's test

Significant maternal effects are evident in the differences in proportion of survival between dams (Table 4) but no effects of pigment within dams are evident. The lack of pigment effects confirms a lack of negative pleiotropic or inbreeding effects in the post-first-pick mortalities.

In all half-sib family pairs, except Dam 3, the albinos had a greater number of deformed alevins and fry than wild-types up to day 90 (Table 5).

TABLE 5. Numbers of deformed alevins and fry to ponding at Day 90

Dam	Family	Ponded Fry	Post first Egg-Pick Mortalities Total	Number Deformed	% Deformed of Mortalities (Ponded Fry)
1	Albino	1919	43	8	18.6 (0.42)
1	Wild	1939	39	1	2.6 (0.05)
2	Albino	914	307	25	8.1 (2.7)
2	Wild	967	297	9	3.0 (0.9)
3	Albino	1631	62	0	0 (0)
3	Wild	1244	40	4	10 (0.3)
4	Albino	1751	39	3	7.7 (0.17)
4	Wild	1785	50	0	0 (0)
5	Albino	1151	41	7	17.1 (0.61)
5	Wild	1440	37	0	0 (0)
6	Albino	1271	91	9	9.9 (0.71)
6	Wild	1252	105	2	1.9 (0.16)
	Albino	8619	583	52	8.9 (0.6)
	Wild	8627	568	16	2.8 (0.18)

The majority of deformities consisted of spinal curvatures and cranial abnormalities (Fig. 3). A total of 8.9% of the albino mortalities were deformed compared to 2.8% of wild type mortalities. As a proportion of all albino fry ponded at day 90 the deformities equal 0.6%. This is an increase of 0.42% when compared to wild-type alevins and fry (Table 5). This is a significantly greater number of deformed albino fry than wild-type fry as tested by Chi-square ($Z=4.74$ $P<0.01$).

In all half-sib pairs, except dam 6, the albino families experienced an increase in mortality relative to their wild-type half-sibs in the first freshwater culture

interval from Day 90 to Day 132 (Table 6). The difference in proportions of total mortalities to survivors between albinos and wild-types was not significant (Chi-square Z=1.936 0.10<P<0.05). No difference in mortality between pigment types for the second and third intervals of freshwater culture is evident (Table 6).

TABLE 6. Cumulative fry mortality from ponding to Day 228

Dam	Family	Day 90 to Day132 n_1=450	% Dead of n_1	Day 133 to Day 174 n_2=300	Day 175 to Day228 n_3=225
1	Albino	7	(1.5%)^{a*}	0	0
1	Wild	0	(0%)^{c}	0	0
2	Albino	11	(2.4%)^{a}	1	0
2	Wild	6	(1.3%)^{a}	0	0
3	Albino	4	(0.9%)^{b}	2	0
3	Wild	2	(0.5%)^{b}	0	0
5	Albino	1	(0.2%)^{b}	1	0
5	Wild	0	(0%)^{c}	0	0
6	Albino	3	(0.7%)^{b}	1	1
6	Wild	6	(1.3%)^{a}	2	2
Total					
	Albino	26	(1.2%)	5(0.3%)	1(0.08%)
	Wild	14	(0.6%)	2(0.1%)	2(0.17%)

* Different superscipts indicate percent mortality are significantly different (P>0.05) using Tukey's test

All families showed a low mortality rate for all intervals in the freshwater phase of culture (Table 6).

Mortalities during saltwater culture (Day 228 to Day 538) were the same between and within pigment types in the first and third culture interval (Table 7).

TABLE 7. Mortality during saltwater culture

Dam	Family	Day 228 to Day 341 (113 days)	Day 342 to Day 448 (107 days)	Day 449 to Day 538 (90 days)	Total
1	Albino	3 (5%)^{b}	22 (38.6%)^{a}	1 (2.9%)^{a}	26
1	Wild*1	11 (18.3%)^{a}	7 (14.3%)^{b}	2 (4.8%)^{a}	20
2	Albino*2	16 (27.6%)^{a}	19 (45.2%)^{a}	1 (4.3%)^{a}	36
2	Wild	17 (28.3%)^{a}	18 (41.9%)^{a}	1 (4.0%)^{a}	36
3	Albino*2	16 (27.6%)^{a}	6 (14.3%)^{b}	0 (0)^{a}	22
3	Wild	13 (22.0%)^{a}	4 (8.5%)^{c}	0 (0)^{a}	17
5	Albino	7 (12.0%)^{b}	11 (21.0%)^{b}	0 (0)^{a}	18
5	Wild*1	3 (5.0%)^{b}	6 (11.0%)^{c}	1 (2.0%)^{a}	10
6	Albino	16 (26.6%)^{a}	12 (27.3%)^{b}	0 (0)^{a}	28
6	Wild	12 (20.0%)^{a}	11 (23.0%)^{b}	1 (2.7%)^{a}	24
Albino		58/296 (19.6%)	70/238 (29.4%)	2/168 (1.2%)	
Wild		56/299 (18.7%)	46/243 (19.0%)	5/197 (2.5%)	

Initial numbers equal 60 except; *1 n=59, *2 n=58
Proportions with the same superscript are not significantly different, (P>0.05).

However, during the second interval of saltwater growth the albino families incurred a significantly higher proportion of mortalities (Chi-square Z=2.32 0.05<P<0.02) compared to wild-types (Table 7). The sample at Day 341 was taken during a period of elevated surface water temperatures (18°C) and many mortalities were noted immediately post-sample. It is possible that the albinos, particularly those families from Dams 1 and 5, may have a lower tolerance to high water temperatures. This suggestion is consistent with inbreeding theory, in that inbred lines are known to respond differently to identical environments and may be more sensitive than outbred

populations to specific stressors (Falconer 1989). Of note is the generally high mortality in Dam 2 half-sib families, noted previously, (Tables 5 and 6), which suggests a chronic maternal effect in these families.

The albino half-sibs of every pair incurred greater mortality than the wild-type by Day 538 (Table 7). Total mortality was 43.9% for albinos and 35.7% for wild-types during saltwater culture. This difference in total mortality between albino and wild-types is mainly due to the difference in mortality during the second growth interval.

iii) Distinction of Pleiotropic and Inbreeding Effects

Figures 4 through 7 illustrate the difference in growth rates between families for the albino and wild-types in both fresh and saltwater culture. A comparison of the means and confidence limits (Tables 8,9) and coefficient of variation (C.V.) (Tables 10,11) indicates that the albinos have a greater mean weight and a larger coefficient of variation for the majority of half-sib families and sampling periods. This trend is evident during freshwater culture by Day 228 for the majority of Dams (Fig. 4 and Table 8). The exceptions to this trend are the sample at Day 341 for all families and the half-sibs of Dam 6 up until Day 341 (Fig. 5 and Table 9). For Dam 6 at Day 228 the weight of the wild-types was greater than the albinos, however, the difference in mean weight of untagged fish (Table 8) was not

significant and the least of all half-sib pairs. Different sire breeding values may have influenced the relative rankings of phenotypes within this half-sib family. The results also indicate that the inbred albino families have a greater phenotypic variance than the outbred wild-types. The greater variance is also reflected in larger confidence limits and is graphically illustrated by comparing confidence limits in Figures 5 and 7 and the data for Day 448 and Day 538 in Table 9.

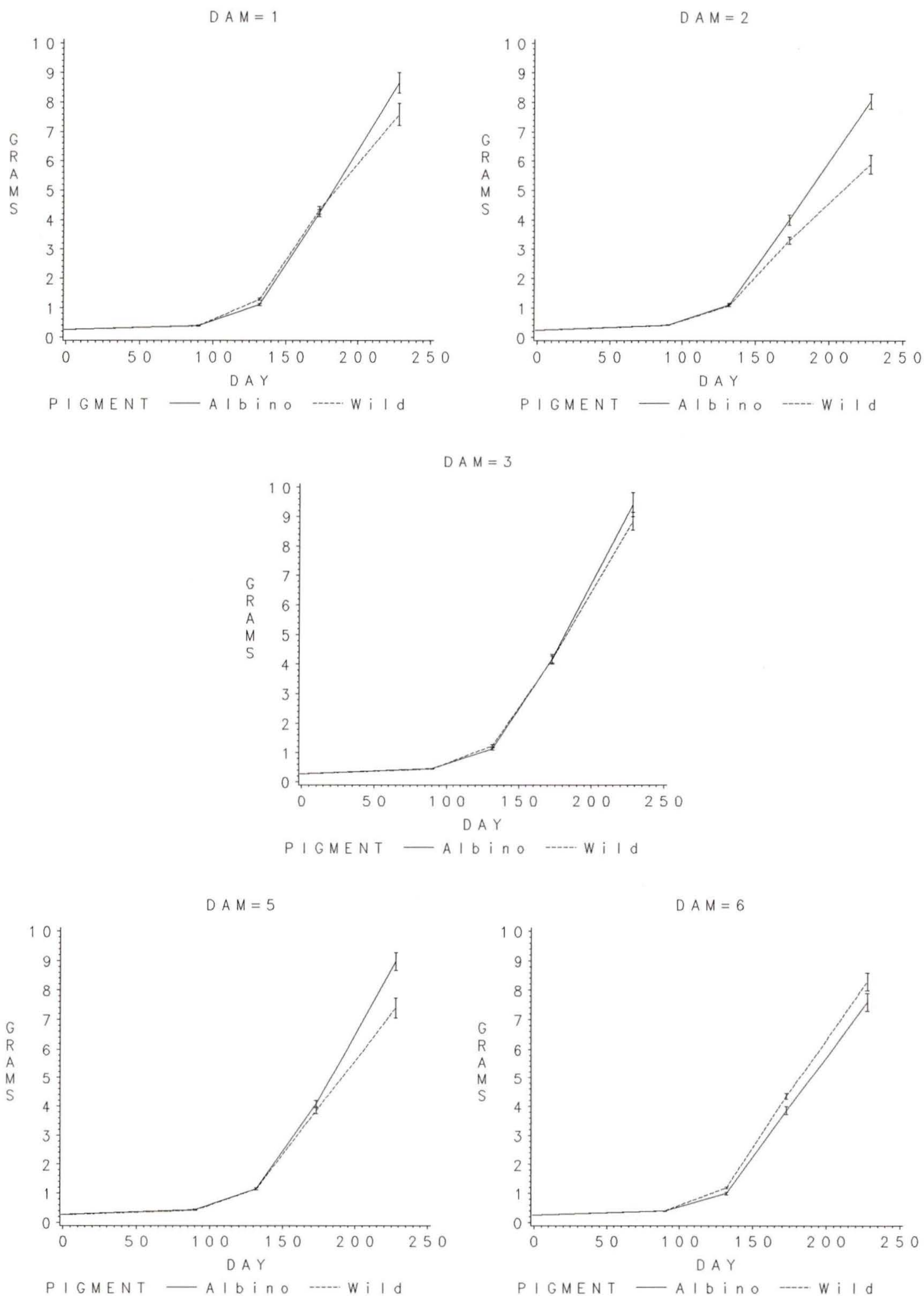


FIGURE 4. Weight changes in families during freshwater culture by dam($\bar{x}$±95% C.L.)

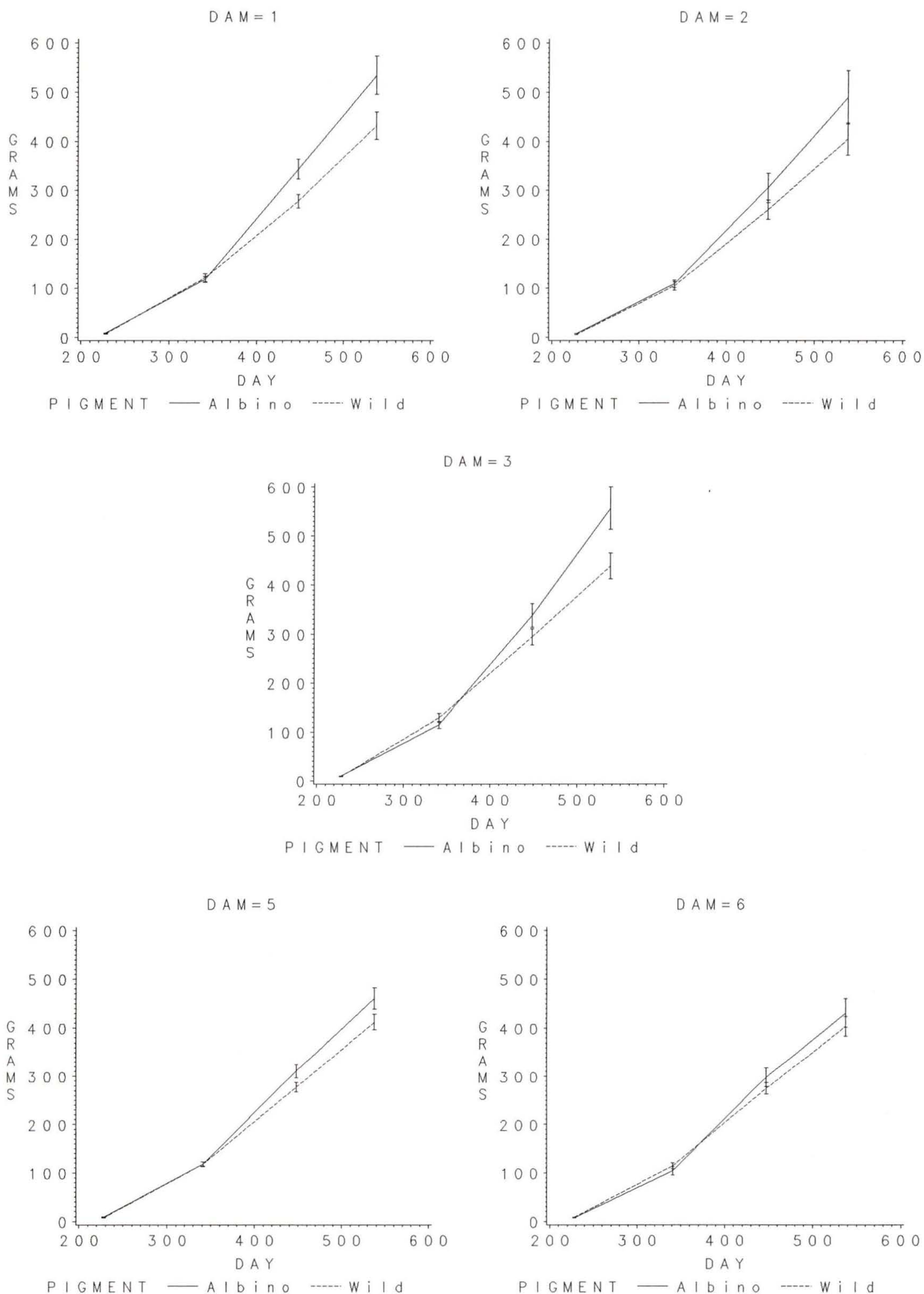
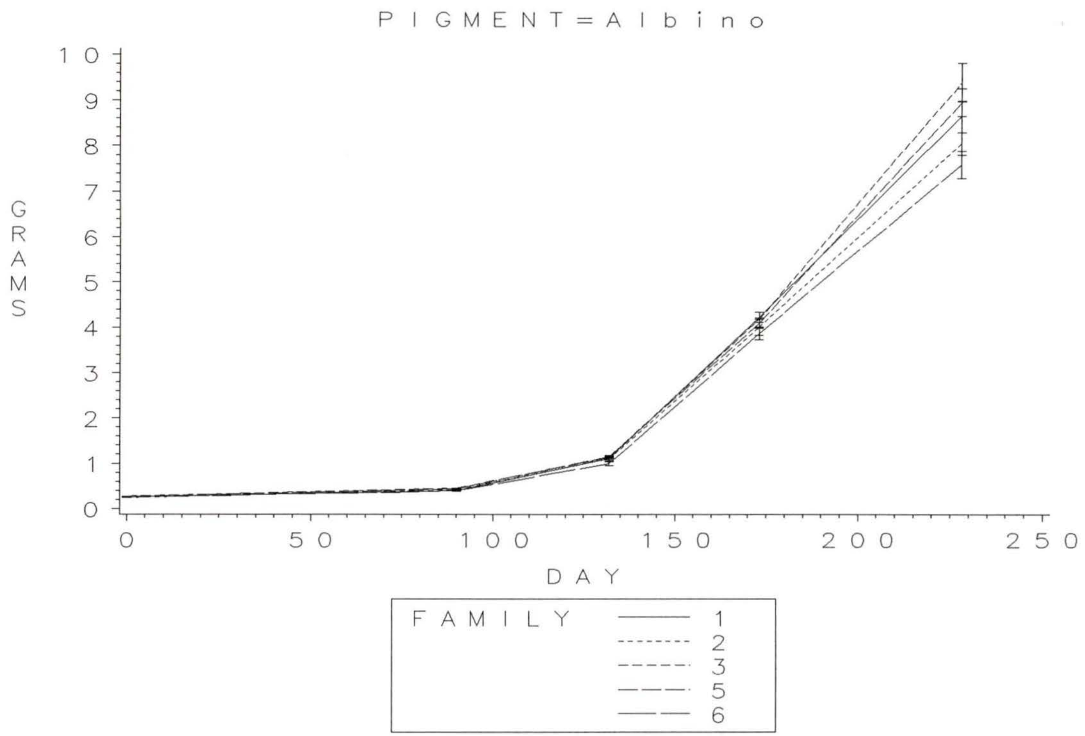


FIGURE 5. Weight changes in families during saltwater culture by dam($\bar{x}$±95% C.L.)



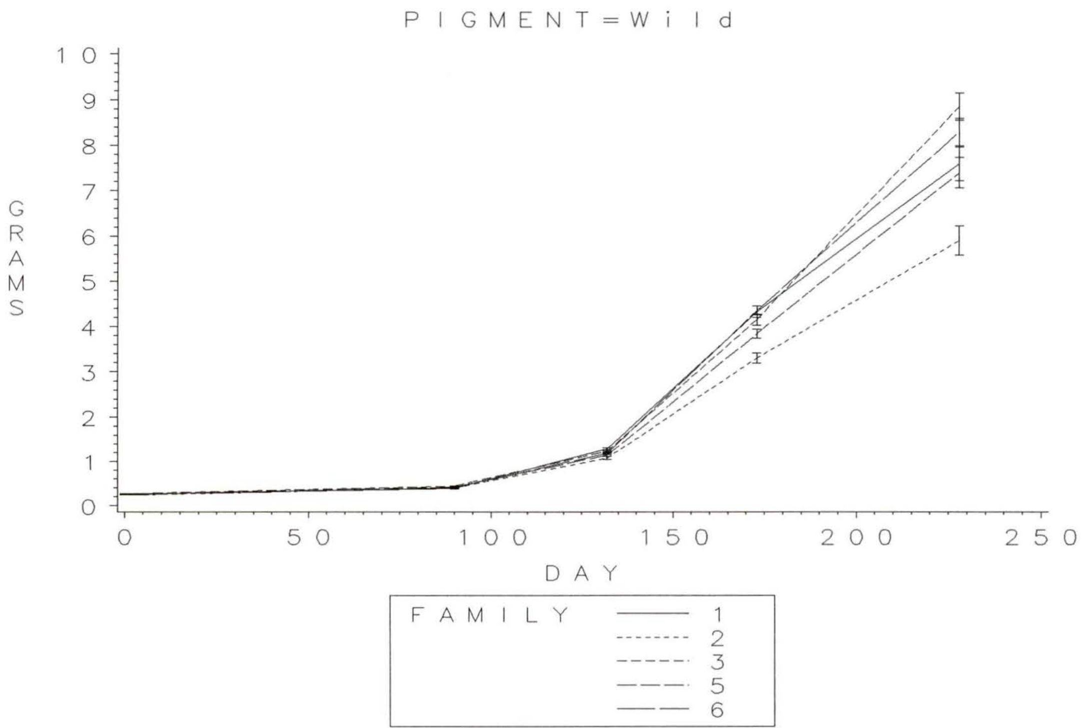
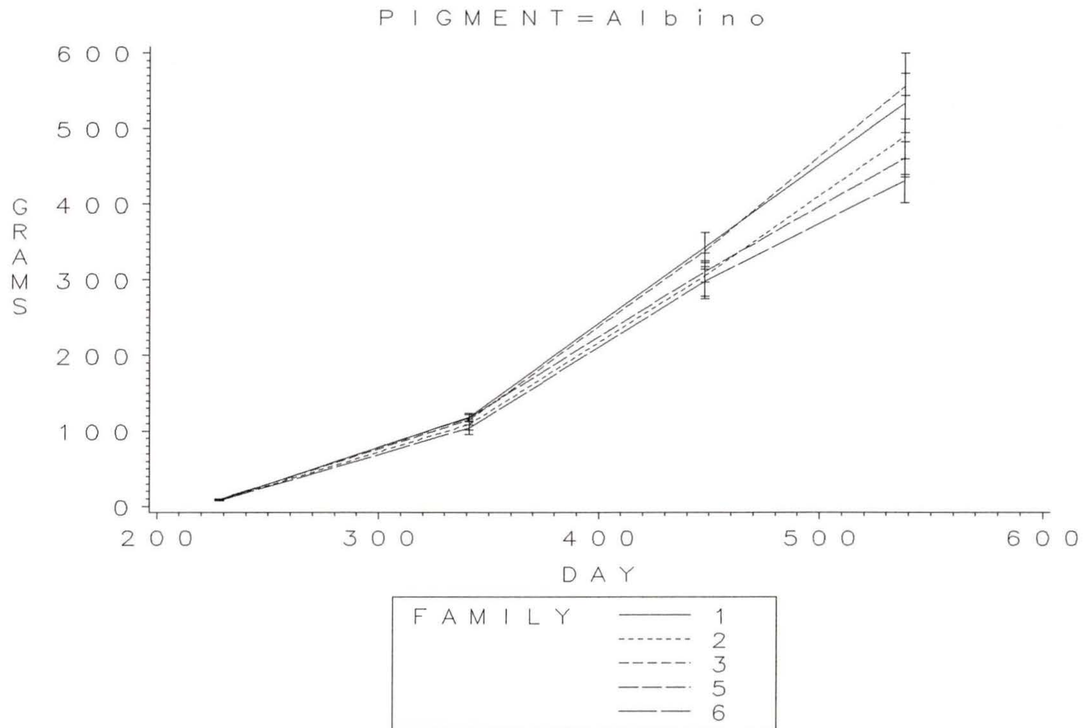


FIGURE 6. Weight changes in families during freshwater culture by pigment($\bar{x}$±95% C.L.)



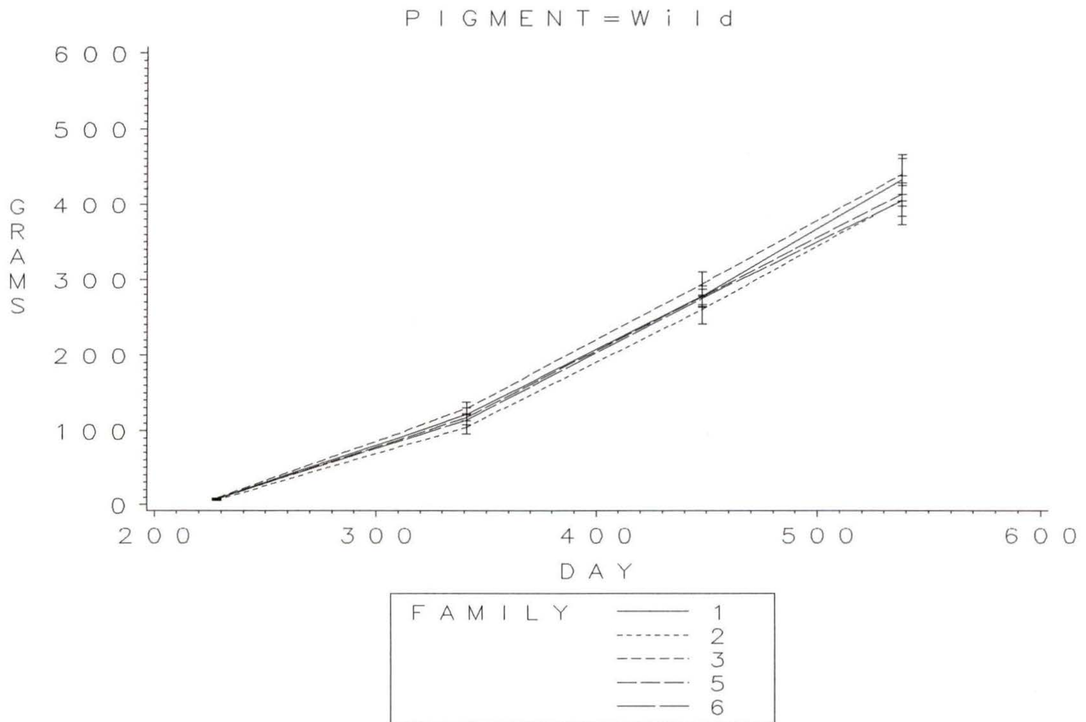


FIGURE 7. Weight changes in families during saltwater culture by pigment($\bar{x}$±95% C.L.)

TABLE 8. Mean weight (g) ±95% C.L. of untagged albino and wild-type half-sib families during freshwater culture by Dam

		Day			
Dam	Family	90	132	173	228
1	Albino	0.40±0.006 n=48	1.11±0.032 n=90	4.2±0.11 n=119	8.9±0.21 n=163
1	Wild	0.39±0.007 n=50	1.28±0.036 n=90	4.3±0.13 n=119	7.7±0.21 n=165
2	Albino	0.41±0.005 n=50	1.10±0.047 n=90	4.0±0.10 n=119	8.7±0.29 n=167
2	Wild	0.40±0.005 n=50	1.08±0.037 n=90	3.3±0.12 n=119	6.5±0.18 n=166
3	Albino	0.45±0.005 n=44	1.13±0.029 n=90	4.2±0.16 n=119	9.5±0.22 n=167
3	Wild	0.44±0.005 n=50	1.24±0.037 n=90	4.2±0.12 n=120	8.6±0.20 n=166
5	Albino	0.44±0.007 n=50	1.15±0.0293 n=90	4.1±0.11 n=119	8.8±0.19 n=165
5	Wild	0.42±0.006 n=49	1.19±0.0218 n=90	3.8±0.10 n=120	7.3±0.18 n=165
6	Albino	0.39±0.007 n=49	0.99±0.043 n=89	3.9±0.13 n=120	7.8±0.18 n=165
6	Wild	0.40±0.006 n=50	1.19±0.029 n=90	4.4±0.10 n=120	8.1±0.22 n=163

TABLE 9. Mean weight (g) ± 95% C.L. of tagged albino and wild-type half-sib families during saltwater culture by Dam

Dam	Family	Day 228	341	448	538
1	Albino	8.7±1.3 n=60	118.3±5.7 n=57	342.8±20.2 n=35	534.0±29.2 n=27
1	Wild	7.6±1.4 n=59	122.4±8.2 n=49	278.8±13.6 n=42	432.0±28.1 n=39
2	Albino	8.3±0.5 n=60	108.2±7.4 n=42	304.5±27.5 n=23	490.0±54.3 n=20
2	Wild	5.9±0.3 n=59	104.6±8.7 n=43	260.2±19.5 n=25	405.1±32.6 n=24
3	Albino	9.4±0.4 n=58	114.7±6.8 n=42	338.2±24.0 n=36	556.6±43.4 n=36
3	Wild	8.8±0.3 n=60	129.2±8.2 n=48	294.2±16.3 n=43	439.2±26.2 n=43
5	Albino	9.0±0.3 n=60	117.5±4.5 n=53	312.8±14.3 n=42	460.9±21.8 n=41
5	Wild	7.4±0.3 n=60	117.7±4.8 n=56	277.3±10.0 n=50	412.7±15.3 n=48
6	Albino	7.6±0.3 n=60	103.8±8.8 n=42	297.1±19.0 n=32	431.3±29.0 n=31
6	Wild	8.3±0.3 n=60	114.2±6.4 n=48	275.1±12.0 n=37	404.1±20.3 n=36

TABLE 10. Mean weight (g), standard error (S.E.) and coefficient of variation (C.V.) of albino and wild-type half-sib families during freshwater culture by pigment

Phenotype	Day 90	132	173
Albino	0.42 n=243 S.E.=0.002 C.V.=7.51	1.10 n=449 S.E.=0.009 C.V.=17.53	4.7 n=596 S.E.=0.032 C.V.=19.05
Wild	0.41 n=247 S.E.=0.0018 C.V.=5.71	1.19 n=450 S.E.=0.008 C.V.=14.24	4.0 n=598 S.E.=0.030 C.V.=18.53

TABLE 11. Mean weight (g), standard error (S.E.) and coefficient of variation (C.V.) of albino and wild-type half-sib families during saltwater culture by pigment

Pigment	Day			
	228	341	448	538
Albino	8.6 n=297 S.E.=0.085 C.V.=17.02	113.3 n=234 S.E.=1.46 C.V.=19.67	342.8 n=168 S.E.=4.83 C.V.=18.26	495.4 n=162 S.E.=7.99 C.V.=20.53
Wild	7.6 n=299 S.E.=0.073 C.V.=16.58	117.7 n=241 S.E.=1.62 C.V.=21.36	278.8 n=197 S.E.=3.08 C.V.=15.49	420.1 n=190 S.E.=5.31 C.V=17.41

The ability to identify individual mortalities allows for comparison of their size to the mean weight of their full-sib family at the beginning of the growth interval in which they died. This comparison gives an indication of what effect any selection pressures, such as stress, have had in the size distribution of a family and whether it has skewed the relative performances of pigment phenotypes.

Comparing the mortality data in Table 12 with that in Table 11 it is evident that, in general, the mean weight of the mortalities was less than the initial mean for each pigment type. Table 12 also reveals that although the average weight by pigment type is similar, albino mortalities were heavier for the interval from Day 228 to 341. The last growth interval is an exception in that the overall average initial weight of the albino mortalities was less than wild-type. The median value of mortalities (Table 12) in all families was nearly equal to the mean, confirming a normal distribution of mortality weight.

On average, smaller individuals of both pigment types were subject to increased mortalities in saltwater culture.

The distribution of weight in all families at Day 344 can be derived by removing the stress episode mortalities from the Day 341 dataset (Table 13). The comparison of the Day 344 data with Day 341 suggests that the mortalities during saltwater culture have not biased the relative ranking of albino and wild-types by selecting against large wild-type or small albinos preferentially.

TABLE 12. Initial mean weight(g)±SD and (Median) of mortalities incurred during saltwater rearing intervals

Dam	Family	Interval 228-341	341-343	343-448	448-538
1	Albino	8.4±2.0 (8.1)	111±16.1 (110.5)	114.1±16.8 (113)	293.3
1	Wild	7.6±2.2 (7.6)	146	129.9±26.3 (121)	274.7±19.6
2	Albino	6.9±2.3 (6.3)	106.3±25.8 (102)	105.9±26.3 (110)	138.6
2	Wild	5.2±1.2 (5.2)	88.6±28.1 (86.5)	93.6±32.1 (98)	212.8
3	Albino	9.7±1.5 (9.5)	120.6±9.5 (123.3)	123.8±9.0 (126.5)	-
3	Wild	9.0±1.2 (8.5)	131 (131)	125.8±26.0 (134.5)	-
5	Albino	9.6±0.9 (9.9)	122.5±11.0 (122.5)	116.5±17.0 (117.5)	-
5	Wild	6.4±2.3 (7.3)	125.8±11.7 (124)	115.3±14.6 (114.3)	222.5
6	Albino	7.5±0.9 (7.5)	81.6±34.9 (81.5)	87.7±34.9 (82)	-
6	Wild	8.0±0.8 (8.0)	101.0±14.7 (99)	95.5±20.0 (99)	237.6
Albino		8.3±2.0	105.4±26.5	108.5±25.6	216.0±77.4
Wild		7.2±2.3	101.6±28.2	105.2±30.2	244.4±28.7

TABLE 13. Mean weight (g) ± 95% C.L. of tagged albino and wild-type half-sib families before and after mortalities due to handling stress

Dam	Family	Day 341	Day 344
1	Albino	118.3±5.7 n=57	120.88±7.0 n=42
1	Wild	122.4±8.2 n=49	121.13±8.3 n=48
2	Albino	108.2±7.4 n=42	108.75±8.2 n=32
2	Wild	104.6±8.7 n=43	110.14±9.5 n=32
3	Albino	114.7±6.8 n=42	114.1±7.4 n=38
3	Wild	129.2±8.2 n=47	128.6±8.5 n=46
5	Albino	117.5±4.5 n=53	117.3±4.6 n=51
5	Wild	117.7±4.8 n=56	117.3±5.0 n=53
6	Albino	103.8±8.8 n=42	109.1±8.1 n=36
6	Wild	114.2±6.4 n=48	115.7±6.9 n=43

In the majority of families the mean weight remained approximately the same in all families at Day 344; the wild-types weighed as much or more than their albino half-sibs (Table 13). In Dam Families 1,3,5 and 6, in which the relative rankings of the phenotypes by size had changed by Day 448 (Fig. 5), the relative rankings had not changed by Day 344. These data indicate that the mortalities due to this handling stress did not affect the relative ranking of the half-sibs within Dams.

The albinos incurred greater mortality after handling

compared to wild-types (Table 14). From Day 343 to Day 448 total albino mortalities were less than those incurred in the first 48 hours after handling. From Day 343 to Day 448 the proportion of albino mortalities was similar to wild-types within most half-sib families. On average the fish that died were significantly smaller, (t-test $p<0.025$, Albino 105.4 g, Wild-type 101.6 g) than the total average of either pigment type on Day 341 (Albino 113.3 g, Wild-type 117.7 g: Table 12).

TABLE 14. Comparison of handling stress and post-stress mortalities

Dam	Family	Days 341-343 48 hours	Days 343-448 post 48 hours
1	Albino	15/57 (26.3%)	7/42 (16.0%)
1	Wild	1/49 (2.0%)	6/48 (12.5%)
2	Albino	10/42 (23.8%)	9/32 (28.1%)
2	Wild	11/43 (25.6%)	7/32 (21.9%)
3	Albino	4/42 (9.5%)	2/38 (5.3%)
3	Wild	1/47 (2.1%)	3/37 (8.2%)
5	Albino	2/53 (3.8%)	9/51 (17.6%)
5	Wild	3/56 (5.4%)	3/53 (5.7%)
6	Albino	8/44 (18.2%)	4/36 (11.1%)
6	Wild	5/48 (10.4%)	6/43 (13.9%)
	Albino	39/238 (16.4%)	31/199 (15.6%)
	Wild	21/243 (8.6%)	25/213 (11.7%)

In summary, the albino half-sibs were larger at the end of the freshwater and by the end of the saltwater phase of culture than their wild-type half-sibs. The albino phenotypes were more variable on average than the wild-type as shown by a greater coefficient of variation than wild-types for all sample periods except Day 341.

In every interval of saltwater culture, the mean of the initial weights of fish that died in each pigment type was less than the mean weight of that pigment type at the start of the culture interval. The average weight of albino mortalities was greater than the average wild-type for intervals from Day 228 to Day 448.

Significantly more albinos died during the handling stress at Day 341 than wild-types. Mortalities during saltwater culture in the intervals before and after Day 341 are similar for both pigment types. The mortalities caused by this stress episode did not affect the relative ranking of pigment types within half-sib families and therefore should not have affected the subsequent relative performance of half-sibs.

iv) <u>Statistical Analyses of Quantitative Phenotypes</u>

There were significant differences in the weights of eggs between the albino females as demonstrated by the analysis of variance (Table 15). Tukey analysis of mean egg weight (Table 2) also confirms this difference and ranks Dam 3 as having produced the heaviest average eggs and Dam 2 as having produced the lightest.

It is interesting to note that the relative ranking of Dam 3 is retained from egg to the end of the freshwater phase and to the end of the saltwater phase in both pigment types (Figs. 6 and 7). Similarly, the relative ranking of pigment type by weight for Dam 2 and Dam 6 are maintained to a lesser degree throughout the experiment. This result

is consistent with that of Fowler (1972) who found a positive correlation of egg size and weight in juvenile chinook salmon.

TABLE 15. ANOVA for egg weight data

Source of Variation	SS	DF	MS
Total	0.05926	249	
Female	0.03269	4	0.008174
Error	0.02656	245	0.000108

Ho: The mean egg weights from all females are the same
Ha: The mean egg weights from all females are not the same

$F=75.37$; $F_{0.05(1),4,245}=3.035$; $p<0.0001$; Reject Ho

Analysis of Weight Data

A mixed model including a fixed effect for pigment and random effects for females by pigment was used to test for differences between pigments for weight and length at each measurement for the measurements within the hatchery:

Model A

Y_{ijkl}= Pigment + Dam + Pigment*Dam + Tank(Dam*Pigment)

Where Y_{ijkl}= weight or length of the ith fish of the jth pigment of the kth dam of the lth tank

For lenght the sample in the seawater phase of culture the model is reduced to:

Model B

Y= pigment + dam + pigment*dam

Where Y_{ijk}= weight or length of the ith fish of the jth pigment of the kth dam.

Least square means for pigment at each measurement were calculated using General Linear Model (GLM) Analysis of the data performed on SAS for PC, adjusting for unequal sample size (SAS,1985) under Model III using the test option which performs F-tests with the corrected error term. Tables 16,17 and 18 summarise the results of these GLM analyses. Complete Analysis of Variance tables are provided in Appendix 2.

TABLE 16. Probabilities from General Linear Model Analysis of weight during freshwater culture (untagged fish)

Source	Day			
	90	132	174	228
Dam	0.008*	0.42	0.43	0.39
Pigment	0.19	0.07	0.73	0.05
Pigment*Dam	0.0001*	0.0006*	0.0001*	0.0003*
Tank(Pigment*Dam)	-	0.02*	0.002*	0.0001*

* Significant difference $p<0.05$

TABLE 17. Probabilities from General Linear Model Analysis of weight during saltwater culture (tagged fish)

Source	Day			
	228	341	448	538
Dam	0.35	0.12	0.13	0.13
Pigment	0.13	0.19	0.0062*	0.01*
Pigment*Dam	0.0002*	0.22	0.31	0.07
Tank(Pigment*Dam)	0.0008*	0.14	0.053	0.16

* Significant difference p<0.05

TABLE 18. Probabilities from General Linear Model Analysis of length during saltwater culture (tagged fish)

Source	Day			
	228	341	448	538
Dam	0.26	0.29	0.044*	0.32
Pigment	0.096	0.62	0.001*	0.018*
Pigment*Dam	0.0001*	0.0013*	0.46	0.16

* Significant difference p<0.05

Significant difference (p<0.05) between Dams exist both for egg weight and ponding weights (Day 90) but not for any sampling period beyond that time. No significant difference in weight difference was found during the freshwater phase of culture between pigment types. As the data in Table 8 show, by Day 228 of freshwater culture the mean weights of albinos in 4 of the 5 half-sib pairs are greater than the wild-type. A comparison of the tagged and untagged fish at Day 228 indicates that the PIT-tagged individuals are a representative sample of the available population of fish (Table 8 and 9). The results of GLM analysis for PIT-tagged and untagged individuals (Appendix 2) are similar for all effects, confirming the adequacy of

the PIT-tagged sample.

Significant effects of pigment on weight are evident at Day 448 and Day 538 and significant Pigment by Dam interaction is evident for untagged fish at Day 228 and for tagged fish at Day 538. It is interesting to note that there is significant tank-effect (replicate) in all the freshwater samples, but that this effect is not evident after the first saltwater measurement at Day 341.

Statistical analysis indicates that the weight of eggs had a significant effect on the subsequent weight of half-sibs in both pigment types. There were significant differences in mean weights between replicate hatchery family-tanks, but no significant weight difference between pigment types until Day 448. The difference was also significant at the end of the experiment at Day 538.

v) Heritability Estimates

It is possible to estimate the intraclass correlations using data from the experiment to estimate the heritabilities of weight at specific ages. A half-sib analysis of heritabilities, commonly used on hierarchical mating designs such as this study, is invalidated due to inbreeding in the albino families. Valid heritability estimates can only be calculated for the wild-type families using full-sib analysis while accounting for the relatedness of the female parents. The offspring are first cousins in the wild-type families and inbred double first cousins in the albino families. Valid interclass

correlations and theoretically invalid heritability estimates are presented for the albino families for comparison purposes. Calculations of interclass correlation (t) and heritability (h^2) and their standard errors are per Becker (1984).
The model used for this calculation is as follows;

Random Model II

$$Y_{ik}=u+a_i+e_{ik}$$

where: u is the common mean

a_i is the effect of the ith mating

e_{ik} is the uncontrolled environmental and genetic deviations attributable to individuals within single pair matings

Weight=family+replicate(family)+ error

The wild phenotype offspring are single first cousins therefore the coefficients (r) of Additive variance (Va) and dominance variance (Vd) are 1/8 and 0 respectively.

$$h^2=\frac{rVa+rVd}{Vp} \; ; \; h^2=\frac{rVa}{Vp} \; ; \; h^2=t/r$$

where Vp= phenotypic variance

TABLE 19. Interclass correlation and heritability of weights at specific times during culture

Day	Weight (g)	Pigment	Interclass Correlation (t)	Heritability	SE
132	1.1	Albino	0.083	(0.664)*1	
	1.2	Wild	0.173	1.388	0.86
174	4.1	Albino	0.01	(0.08)	
	4.0	Wild	0.306	2.44	1.24
228	8.7	Albino	0.126	(1.008)	
	7.6	Wild	0.267	2.13	1.32
341	113.3	Albino	0.053	(0.424)	
	117.7	Wild	0.093	0.74	0.58
448	320.4	Albino	0.082	(0.656)	
	278.7	Wild	0.039	0.314	0.35
538	495.4	Albino	0.193	(1.544)	
	420.1	Wild	0.021	0.16	0.26

*1 the heritability estimates for the albino families are not valid

For the freshwater phase of culture the interclass correlation (t) is calculated as follows;

$$t = \frac{s^2_{family}}{s^2_{family} + s^2_{within} + s^2_{replicate}}$$

where s^2family=variance between families

s^2within=variance within families

s^2replicate=variance between replicates

For saltwater culture where the animals are pooled the equation reduces to;

$$t = \frac{s^2_{family}}{s^2_{family} + s^2_{within}}$$

From this equation it can be seen that interclass correlation and heritability will vary in direct proportion to the ratio of between-family, to within-family variance.

Tank variability was low in the wild-type family replicates and therefore the high heritabilities, (h^2>1; greater than unity and therefore invalid), likely reflect non-additive and maternal effects which were important from ponding to Day 228. The values for heritability of weight in wild-type families at Day 174 although high, are comparable to those reported by Withler et al. (1987), who reported a heritability for weight of 1.93 (SE 1.06) for Big Qualicum chinook of the same size. High values of heritability of weight for juvenile salmonids have been reported by many salmonid studies (Gall and Huang 1988; Iwamoto et al. 1982; Withler et al. 1987) and are attributed to maternal and non-additive effects. The calculations reveal an increasing heritability for weight in the albino phenotype from Day 341 to Day 538 which correlates to increasing between-family variance evident in Figure 7.

The calculations for heritability of weight indicate that there are two distinct trends in the pigment types. The wild-type shows a decreasing trend in heritability with age in saltwater culture, whereas the albinos have an increasing heritability, which reflects the increased ratio of between-family variance to within-family variance.

vi) Utility of Albinism in gynogenesis

The results of the first late-pressure-shock trial are presented in Table 20. The results are useful in indicating which parameters might be used to attempt mitotic gynogenesis. Egg survival from all three females was best when shocked with 895 kg/cm^2 hydrostatic pressure at 330 minutes post- fertilisation.

All survivors were wild-type indicating inadequate U.V. treatment of the wild-type sperm.

TABLE 20. Survival of eyed-eggs treated with 4-minute duration late-pressure-shocks after fertilisation and incubation at 10°C.

Time Post-Fertilisation (min.)		Survival to eyed-egg stage Pressure treatments (kg/cm^2) 895			984			1074		
270	f_1	0	0	0	0	0	0	0	0	0
	f_2	0	0	0	2	0	50	0	0	0
	f_3	0	0	0	0	0	0	0	0	1
300	f_1	11	25	39	1	1	0	0	0	0
	f_2	0	0	0	0	0	0	0	0	0
	f_3	0	0	1	0	0	0	0	0	0
330	f_1	43	34	12	1	0	0	0	0	0
	f_2	3	10	19	3	0	0	0	0	0
	f_3	23	15	0	0	0	1	0	0	0

Note: Albino female donors used for pressure treatment denoted f1,f2,f3
Treated eggs (n=55).

The results of the second gynogenesis trial are shown in Table 21. The results from the gynogenesis trials demonstrate that the albino phenotype can be used to

TABLE 21. Gynogenetic albinos produced with ultraviolet light-treated sperm, pressure shocked and incubated at 10°C (Hydrostatic pressure = 895 kg/cm^2)

Time Post-fertilisation (min.)	Shock Duration (min.)	Total Number of alevins hatched	Alevin Phenotype Albino	Wild
Controls* n=100				
1	0	94	0	94
2	0	9	8	1
Early Pressure Shock n=100				
30	4	23	21	2
30	8	0	0	0
40	4	33	32	1
40	8	0	0	0
Late Pressure Shock n=200				
210	3	0	0	0
210	4	0	0	0
210	5	0	0	0
270	3	0	0	0
270	4	0	0	0
270	5	0	0	0
330	3	3	2	1
330	4	4	2	2
330	5	0	0	0

* Control 1 - Untreated sperm and no pressure shock
Control 2 - U.V.-treated sperm and no pressure shock
N.B. Eggs from albino female donor: f4

confirm successful gynogenesis. The most successful trial resulted in 32% survival in meiotic gynogens and 1% survival in mitotic gynogens.

Table 22 shows the results of the third gynogenesis trial. The low values reported in Table 22 reflect poorer egg quality as demonstrated by the poor survival of the

TABLE 22. Gynogenetic albinos produced with ultraviolet light-treated sperm, pressure shocked and incubated at 10°C

Time Interval (min.)	Pressure (kg/cm^2)	Shock Duration (min.)	Number of alevins hatched	Alevin Phenotype Albino	Wild
Controls n=50					
1	0	0	35	0	35
2	0	0	0	0	0
Early Pressure Shock n=50					
30	805	4	0	0	0
30	805	6	1	0	1
30	895	4	0	0	0
30	895	6	0	0	0
30	984	4	0	0	0
30	984	6	0	0	0
30	717	4	4	2	2
40	717	4	3	3	0
40	805	4	5	5	0
40	805	6	1	1	0
40	895	4	4	2	2
40	895	6	0	0	0
40	984	4	0	0	0
40	984	6	0	0	0
Late Pressure Shock n=150					
300	805	3	0	0	0
300	805	4	0	0	0
300	895	3	0	0	0
300	895	4	0	0	0
300	984	3	0	0	0
300	984	4	0	0	0
330	805	3	0	0	0
330	805	4	0	0	0
330	895	3	0	0	0
330	895	4	0	0	0
330	984	3	0	0	0
330	984	4	0	0	0
360	805	3	0	0	0
360	805	4	0	0	0
360	895	3	0	0	0
360	895	4	0	0	0
360	984	3	0	0	0
360	984	4	0	0	0

* Control 1 - Untreated sperm and no pressure shock
Control 2 - U.V.-treated sperm and no pressure shock
N.B. Eggs from albino female donor: f5

wild-type diploid control. The results are within the range of values reported in the literature for survival to first feeding for meiotic gynogenetic salmonids. Reported yields range from 27% survival (Chourrout 1984) to 81% (Lou and Purdom 1984) for meiotic gynogens of rainbow trout produced using hydrostatic pressure and U.V.-treated sperm. With the higher pressures (984 and 1074 kg/cm^2) many of the eggs emerged from the treatment entirely opaque or with opaque foci instead of their normal translucent appearance (Table 20). The physical shock at higher pressures may have ruptured the yolk membrane of these eggs. The initial pressure parameter estimates were based on work with eggs from wild stock coho salmon (O. kisutch) using the same apparatus (Mr. Igor Solar pers. comm., Fish Genetics Laboratory. Fisheries and Oceans, West Vancouver). Subsequent trials using lower pressures showed positive results from 717 and 895 kg/cm^2. This range of pressure shocks is within the range used by other researchers (Onozato 1984) on Oncorhynchus species.

Mitotic gynogens were also produced from this treatment (Table 21). The percentage survival to alevins was low at 1%, however, it is similar to other reported results, such as Onozato (1984) 5% and Chourrout (1984) 8%.

In Trial 2 (Table 21) gynogens were produced both with early pressure shocks (30 to 40 min. after fertilisation) and with late pressure shocks (330 minutes after fertilisation). The gynogenetic origin of the fish may be

confirmed at an early stage by examinating the eye within the developing egg (see Fig 2). The gynogenetic origin of both these albinos is supported by Southern blot analysis of genomic DNA as shown in Figures 8 and 9. Sperm was pooled from four wild-type mono-sex males to fertilise eggs from the albino female parent f4.

Figure 8 is an autoradiograph of a Southern blot of genomic DNA of the gamete donors and the offspring of Trial 2. In Figure 8 it can be seen that all the wild-type male donors had bands at 7.126 kilobase (kb) and at 7.081 kb. The female parent had the 7.126 band but not the 7.081 kb band, this is the same condition for all the nine albino offspring tested. These results support a gynogenetic origin of the offspring. In Figure 9 the albino female parent and the late-pressure-shock albino offspring share a band at 7.0 kb not shown by the males. The female parent and offspring do not share any lighter bands at 2.0 to 3.0 kb range. These results support a gynogenetic and suggest a mitotic origin of the late-pressure-shock albino.

Interspecific crosses and gynogenesis

Heterologous sperm is commonly used in the production of gynogens, and with chinook quite often O. mykiss sperm treated with U.V. light is used (Levanduski et al. 1990). The resulting viable offspring usually must be tested for diagnostic allozymes to confirm their all-maternal inheritance. Albinism offers an alternative method that can quickly confirm the gynogenetic or hybrid origin of

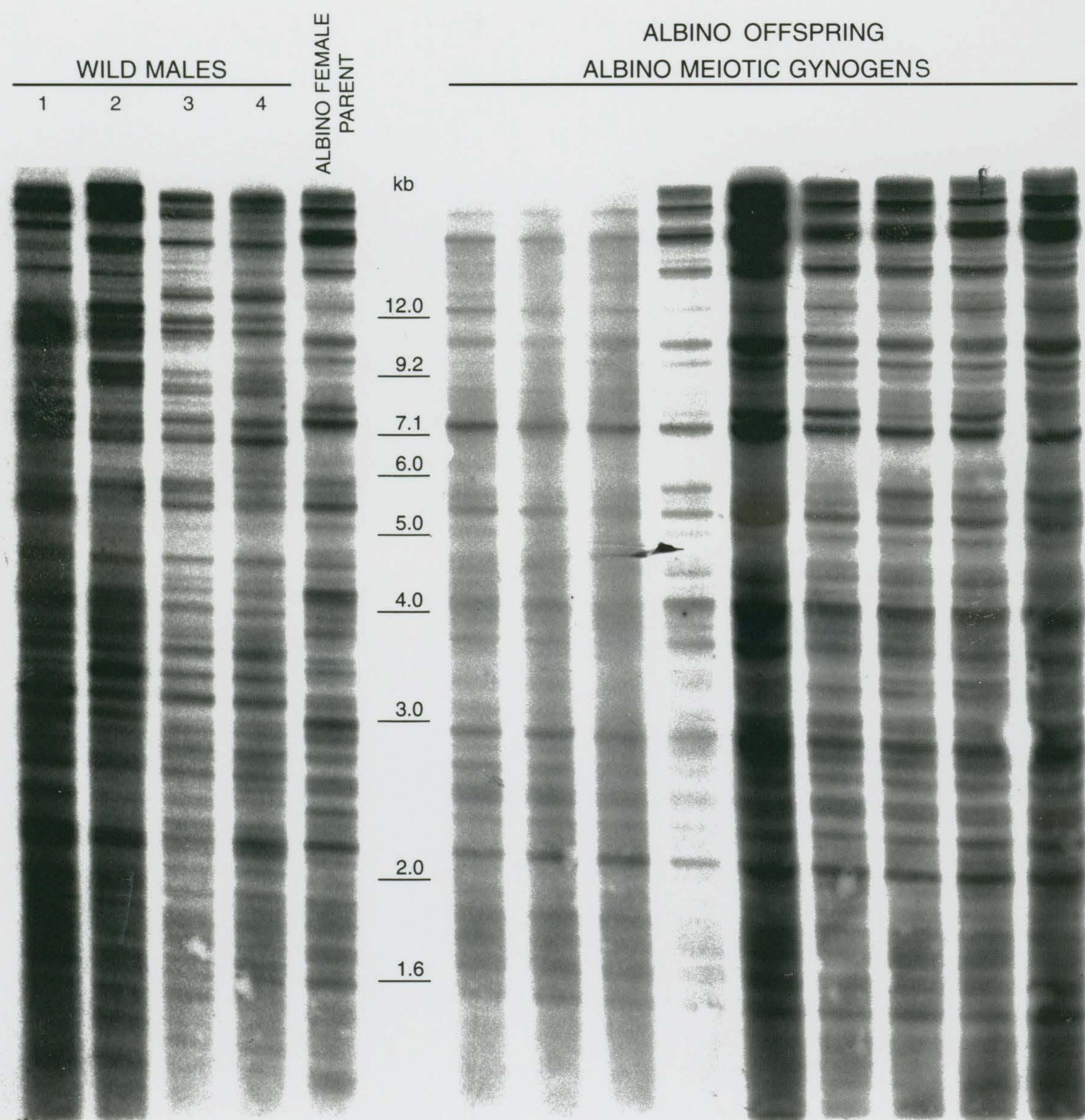


FIGURE 8. Autoradiograph, produced with Hae III restriction endonuclease digests and YN24 oligonucleotide probe, of the wild-type male sperm donors, the albino female parent and the early-pressure-shock albino gynogens

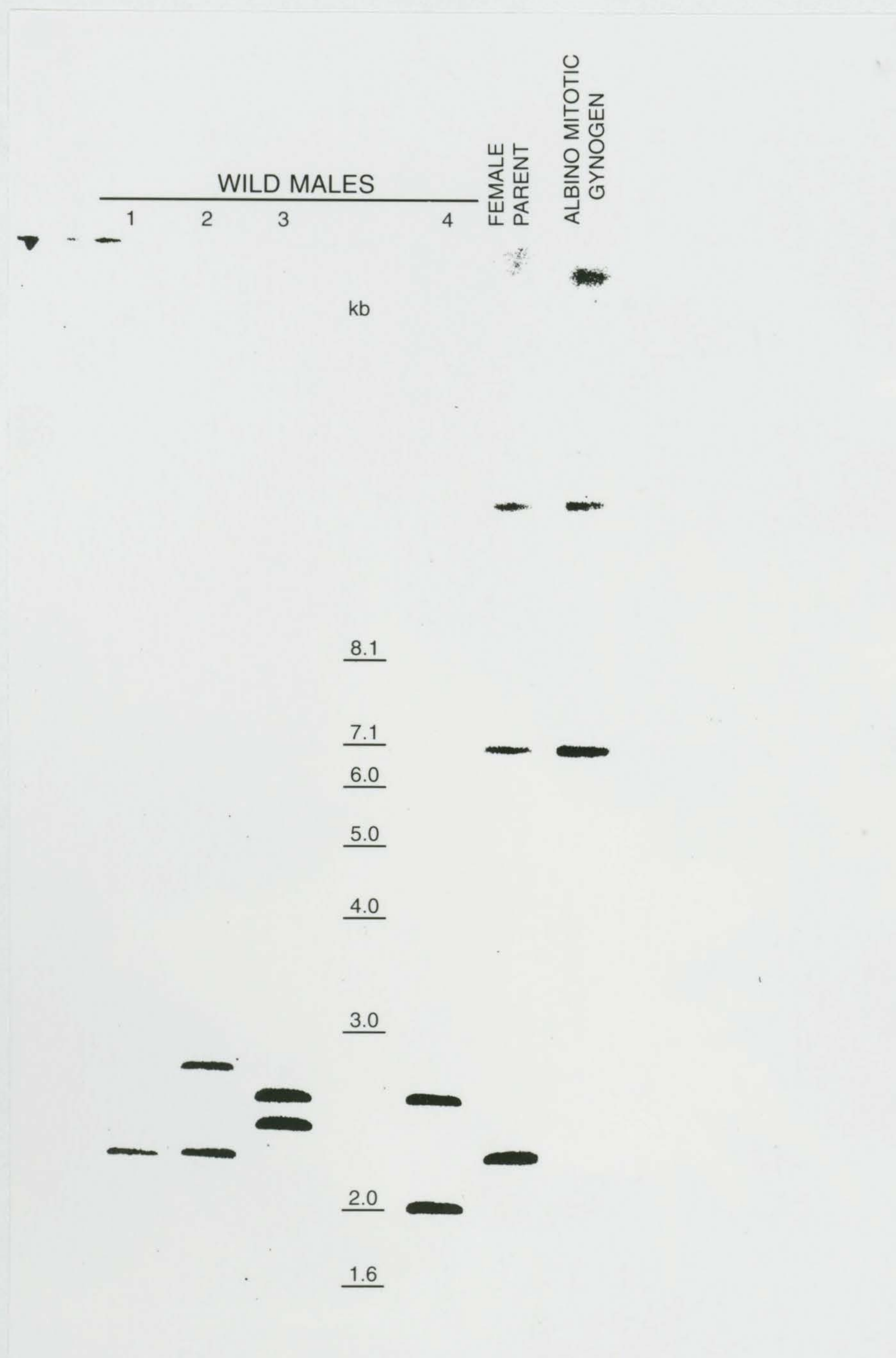


FIGURE 9. Autoradiograph, produced with Hae III restriction endonuclease digests and OtSL1 probe, of the wild-type male sperm donors, the albino female parent and the late-pressure-shock albino gynogen.

offspring from an interspecific mating. Using eggs from an albino female parent and wild-type heterologous male sperm, viable albino offspring would confirm gynogenesis, and wild-type offspring would confirm successful hybridisation. In the hybridisation trial, (Table 23) gametes from individual fish were crossed and the eggs were subsequently examined for fertilisation and hatching success. Given the cursory nature of this experiment only the hatching success and phenotype of viable offspring is presented.

Table 23. Results of gynogenesis trials using interspecific crosses with and without hydrostatic pressure treatments (895 kg/cm^2 initiated 30 minutes post-fertilisation, 6 minutes duration)

Crosses Male	Female	Pressure Shocked	Offspring Phenotype	Control (unshocked)	Offspring Phenotype
		Number and (%) of alevins hatched/eggs fertilised			
OtA-albino	Ot1-albino	179/200 (89.5%)	albino	286/333 (85.8%)	albino
OtB-wild	Ot1-albino	184/200 (92.0%)	wild	314/330 (95.2%)	wild
Oki-wild	Ot1-albino	14/189 (7.4%)	wild*1	251/310 (80.9%)	wild
Oke-wild	Ot1-albino	0/210		0/333	
On -wild	Ot1-albino	0/204		0/349	
OtA-albino	On -wild	25/273 (9.2%)	wild	52/473 (10.9%)	wild
OtA-albino	Oke -wild	169/200 (84.5%)	wild	211/332 (63.4%)	wild
OtA-albino	Ot2*2-wild	1/226 (0.4%)	wild	5/454 (1.1%)	wild
OtA-albino	Oki -wild	126/235 (53.6%)	wild	210/394 (53.3%)	wild

Coding:
Ot- O. tshawytscha (male A and B: female 1 and 2)
Oki- O. kisutch
Oke- O. keta
On - O. nerka

OtA and Ot1 -albino chinook male and female other parents were wild-type phenotypes.
N.B. All treatments were made using untreated sperm.
*1 massively deformed alevins
*2 very poor viability of the eggs is likely the cause of the low hatch success

With the exception of the albino male by female cross, no albino offspring were produced, indicating that no gynogens were produced using heterologous sperm.
In addition, the use of albino sperm with eggs from other species produced only wild-type offspring when viable crosses were produced.

Of note is the small increase in mortality caused by the pressure treatment in most crosses where viable offspring were produced. This validates the use of this set of parameters in the previously described gynogenesis experiments. Many of the hybrid alevins produced were extremely deformed and unlikely to survive. The experiment demonstrates that the use of untreated heterologous sperm from these crosses does not produce gynogens. The wild-type offspring of the male O. kisutch by albino female O. tshawytscha indicates that a true hybrid was produced from this cross. The results also indicate that O. kisutch sperm treated with U.V. light may be useful in the production of O. tschawytscha gynogens.

Conversely, should gynogens of O. keta, O. kisutch or O.

nerka be desired, the results indicate the use of U.V.-treated O. tshawytscha sperm may prove effective.

vii) Summary of Results

Crosses between, and within, phenotypes demonstrate that albinism is a recessive trait in Big Qualicum chinook. In the family study a higher frequency of deformities was evident in albino fry compared to their wild-type half-sibs. Significantly higher mortality rates were evident only in the second period of saltwater culture and this was attributed to differences in mortality due to handling stress. No statistically significant difference in weight between pigment types existed at the end of freshwater culture, this may be attributed to the variation of chinook salmon growth between replicate family tanks. Significant differences in weight between pigment types were only evident at Days 448 and 538, although albino half-sibs were generally heavier for most of the experiment.

Gynogenetic offspring were produced using hydrostatic shock treatment as confirmed by albinism in offspring and by Southern blot analysis of genomic DNA. No gynogens were produced using albino eggs, untreated heterologous sperm and hydrostatic treatments, but hybrids were produced as confirmed by the wild phenotypes of the offspring.

DISCUSSION

i) Inheritance of Albinism

Albinism in chinook salmon appears to be inherited as a simple autosomal recessive trait. Crosses of albino parents produced all albino offspring. Any cross of a wild-type parent of either sex with an albino parent resulted in all wild-type offspring. The albino parents used in the experiment were produced from normally pigmented adults presumptive heterozygous fish (AaxAa) and resulted in a 3:1 ratio of offspring. These results are considered to be conclusive evidence of the recessive nature of the trait and evidence of its utility for gynogenetic trials.

ii) Pleiotropic Effects

Fertility

The fecundity and the fertility of the albino females used as parental stock average 3450 eggs and 72% respectively which is well within the averages for this hatchery stock (Dr. Dave Groves, pers. comm. President, Sea Spring Salmon Farms Ltd.). The proportion of fertilisation success of albino eggs by albino males was on average higher that of the wild-type males, therefore no obvious decrease in fertility is associated with albinism.

Viability

In terms of viability, the post-first-pick mortalities suggest that hatching success for both wild and albino

half-sibs are the same, and that albinism is not a sub-viable trait. These results justify the use of viability to hatching as a measure of success of the treatments in the gynogenesis experiments. This contrasts with findings for guppies (Lebistes reticulatus), in which albinism is a sub-viable recessive trait and results in approximately 60% mortality of albinistic offspring prior to birth (Gordon 1957).

iii) Distinction of Pleiotropic and Inbreeding Effects

Effects on Viability

The albinos had slightly higher average mortality than the wild-type for the duration of freshwater culture (0.25% increase from eyed-egg to alevin ponding, and 0.66% increase from ponding of swim-up to day 228, a period of 138 days). This contrasts with Gjerde et al. (1983) who found an 8.9% decrease in eyed-egg survival, a 2.8% decrease in alevin survival and a 9.1% decrease in fry survival with an inbreeding coefficient (F) of 0.25 in rainbow trout. Kincaid (1976b) estimated a 12.6% decrease in survival from swim-up fry to 168 days of age in inbred (F=0.25) rainbow trout. The results of the present study also contrast with the findings of Bondari (1984), who found consistent significantly higher mortality rates, ranging from 8 to 22%, for albinos compared to their wild-type full-sibs during several phases of culture.

Mortality was only significantly different between pigment types during the second saltwater culture period

and was specific to three families. High mortality in specific albino families after an episode of handling stress suggests that inbreeding and not pleiotropic effects were responsible for mortality during the second saltwater culture period. This is confirmed by the similar mortality rates of both pigment types for the duration of the second saltwater growth interval. The depression in viability, (inclusive of the stress episode) for the duration of the saltwater culture phase was 8.1%, which is similar to the 7.7% depression, found for rainbow trout with the same inbreeding coefficient (0.25) for approximately the same culture period (Kincaid 1976a).

Mortality and Mean Weight

Natural selection, favouring survival of the fittest has been suggested as a possible reason for low inbreeding effects on growth in other studies (Gjerde et al. 1983). Mortality acting preferentially on the smallest individuals would tend to underestimate the inbreeding depression for growth, but should result in increased inbreeding depression for survival in the same period. As the results in Table 12 suggest, there was higher mortality of smaller individuals in both pigment types. Average weight of mortalities incurred by the albino families was greater than the average of wild families. However the albino families were all heavier than their wild-type half-sibs by the end of the study. Therefore the mortalities incurred did not bias the relative weight ranking of the albino to

wild-type phenotypes.

The mean weights of the majority of albino half-sib families were consistently larger than those of the wild-type half-sib families. The only exception to this was on the first sample after saltwater introduction (Day 341) in which the mean weight of the wild phenotype averaged across all families was larger. In three of the five half-sib families the weight ranking of pigment types was reversed in the first interval (Days 228 to 341) after saltwater introduction. Mortalities during this period (Table 7) were equivalent between pigment types. This result was unexpected as larger smolts are typically more successful at saltwater adaptation and generally have better growth. As such, the albino fish should have maintained their relative weight ranking. It is possible that the albinos were experiencing some increased physiological stress during saltwater adaptation which may have reduced their weight gain.

In the family study, the first exposure of the fish to natural light was after transfer of the fish to the saltwater cages. It is probable that the albinos were adapting to increased light intensity and to exposure to ultraviolet light during this period. The presumed increase in retinal damage may, as an additional stress, account in part for their reduced weight gain in the period from saltwater introduction to Day 341, relative to their wild-type half-sibs. Any visual impairment that the albino

fish may have incurred during this period has not affected their ability to compete for feed pellets, as evidenced by their superior weight gain after Day 341.

Tank Effects on Growth

During the freshwater phase, efforts were made to standardise rearing conditions in all trays and tanks. The GLM analysis of the data indicates that significant differences exist within replicates as early as Day 132 (Table 16). It is possible that sampling errors occurred in the initial distribution of fry to the replicate family tanks. This possibility is low, however, given the random distribution of fry within replicate tanks and the initially high proportion (23% to 49%) of available fry within families that were used in the experiment.

Analysis of the family results indicates that the albino half-sibs were not significantly different from wild-type half-sibs until Day 448 of sampling. The mean weight of all albinos was, however, consistently greater than that of the wild-type half-sibs during most of the experiment.

The influence of environmental factors (Ve) or tank effect and pigment by dam interaction, account for the significant potion of variability in growth within each family during the freshwater phase of culture. Although the tank effect complicates interpretation of results it does highlight the necessity for having replicate tanks in the design for animals that are to be tagged. It also indicates the desirability of tagging fry at the lowest

practicable weight so that families may be pooled as early as possible to reduce environmental variance (Ve). Before PIT tagging, the only method of family identification was to keep families in separate tanks. If all the members of an untagged family had been placed into only one tank then any significant difference in growth or survival between families, prior to tagging, may have been due to environmental and not genetic differences. Pooling of samples from replicate family tanks will tend to average out any variability due to environment when the fish are placed into a common environment. The effect of tanks was found to be not significant by the first saltwater measurement at Day 341.

Relative Rankings of Phenotypes

Albinos were heavier on average at the end of the freshwater culture period, and significantly heavier at the termination of the experiment. Since both groups of sires came from the same strain and hatchery, and both groups had the same rearing conditions this result is unexpected. Some possible explanations for this result include:

1) The albino allele or closely linked loci may have a positive effect on growth. This pleiotropic effect could be at the physiological or behavioural level and warrants further investigation. Although this is a possibility, as suggested by the results, the best experimental design to test for a positive pleiotropic effect on growth would be to compare the growth of

full-sibs of both phenotypes from a series of heterozygous crosses.

2) There may be a correlation of mortality and growth. Increased mortality within albino families due to the increase in lethal or sub-viable recessive traits may have resulted in a positively skewed distribution of size during time. Conversely, differential mortality within the wild-type families may have resulted in a negatively skewed distribution of size during time. For the sampling intervals in which the initial size of mortalities occurring in that interval can be determined, the average size of mortalities was smaller than the mean in both pigment groups.

3) There may have been initial selection of albino sires with higher breeding values for growth than the selected wild-type sires. The probability of randomly selecting five albino sires each with higher breeding values than five wild-type sires is approximately 0.03.

Increased Abnormalities

The albino families had significantly higher average fry deformities than wild-types ($p<0.001$). However, the proportion of deformities in the albino full-sib cross is much lower (0.6% average, range of 2.7 to 0%) than that noted in other inbreeding studies with the same degree of inbreeding. Aulstad and Kittelsen (1971) reported an average of 11.4% fry deformity in rainbow trout in full-sib crosses ($F=0.25$). Kincaid (1976a) found an average of 7.8%

(F=0.5) crippled fry in rainbow trout with full-sib crosses and 3.6% in the outbred controls.

In a closed hatchery population such as the Sea Spring Big Qualicum hatchery strain, some level of inbreeding (F>0) probably existed in the parental population for this experiment. Selection had occurred for three generations on this hatchery strain and recessive colour variants, i.e. albinos, appeared within the population, which supports such a theory. A low rate of inbreeding would have paired lethal and sub-viable genes within the stock and tended to remove them from the population. Inbreeding depression tends to increase in proportion to the inbreeding coefficient during the initial inbreeding generations as reproductive capacity and viability are lost. As lines are lost, the surviving lines become a selected population and, the prediction of an increasing rate of inbreeding depression from an increasing inbreeding level are no longer directly applicable (Falconer 1989). If some level of inbreeding exists in a closed population, the inbreeding depression resulting from a full-sib mating will probably not be as great as theory predicts. This is likely the case in the albino full-sib crosses in this experiment.

iv) Heritability Estimates and Inbreeding

The trend from Day 132 to Day 341 is toward a higher interclass correlation of weight for the wild phenotype relative to the albino families. This reflects the relatively larger amount of variation between families for

the wild-type families compared to the albinos of the experiment. The increase in between-family variance is due to the poor performance of the wild-type offspring of Dam 2 (see Fig. 6). The interclass correlations of weight on Days 448 and 538 reflect a reversal in rank in relation to growth between pigment types and reflect an increasing between and within-family variation in the albino families. This increase of within-family variation is confirmed by the confidence limits in Figure 7. This is representative of inbred lines, as they typically show greater phenotypic variance even though homozygosity is increased within lines (Tave 1993). The delay for this trend to become evident may be related to the cubic nature of weight gain, and to the difficulty the albino families may have experienced in saltwater adaptation. The relative increase in between-family variation in the albino families compared to wild-types may reflect a magnification of initial weight differences of the inbred albino families lines as the fish grew. Magnification of initial difference of weight among groups has been noted by Wohlfarth and Moav (1972).

This explanation is consistent with the theoretical increased genetic drift due to inbreeding and the random fixation of alleles that has occurred within the albino families relative to wild-type families.

These results represent a graphic demonstration of the possibilities of calculating spurious heritabilities of traits in an inbred population.

v) Utility of Albinism in Gynogenesis

The results of the gynogenetic work are not detailed enough or sufficiently replicated to be useful for refining parameters for hydrostatic production of chinook gynogens. Both meiotic and mitotic gynogens were produced during the trials. The results confirm the utility of the albino phenotype in quickly screening species as candidates for hybrid fertilisation and gynogenesis. The Southern blot analysis of genomic DNA supports the gynogenetic origin of both the meiotic and mitotic gynogens, however, it is not a conclusive proof. In Figure 8 the wild type male sperm donors and differ from the albino female by only the presence of one band (7.081 kb) in the Southern blot. Theoretically all four males could have been heterozygous for this section of DNA and in every case, not passed this DNA to any of the nine albino offspring. This probability is of this occuring is low (P=0.0078).

Similarly the lack of a 2.3 kb band and the presence of the 7.1 and 9 kb bands (Figure 9) in the albino offspring confirm its gynogenetic nature. The 2.3 kb band may have been lost in the second polar body or to a cross-over during meiosis in the egg, in either case the offspring would not contain the band and therefore its mitotic nature cannot be confirmed.

The results highlight several other interesting features including the variation within and between females in eyed-egg survival for the same treatments (Table 20).

The trials indicate that the optimal pressures for inducing mitotic gynogenesis are less than 895 kg/cm^2 and near 330 minutes post-fertilisation at 10°C.

The interspecific crosses performed did not produce gynogens. The high fertilisation success of the coho male with chinook female suggests that treated coho sperm may be useful in chinook gynogen production.

CONCLUSIONS

This study confirms that there is no negative pleiotropic effect of albinism on growth in Big Qualicum chinook for the period examined under these specific culture conditions. This is significant for aquaculturists and researchers in that the costs and growth performance of this phenotype should be comparable to the wild-type.

While inbreeding depression effects appear to be evident in the increased number of deformities and juvenile mortalities in the albino families, the level of inbreeding tested does not appear to negatively affect growth. Albino viability is slightly reduced but not statistically different from that of wild-type half-sibs for most of the study. The reduction in viability for albinos is similiar to the outbred wild-types for the freshwater phases, and less than values reported in the literature for the same level of inbreeding. This result suggests that some level of inbreeding existed in the parental hatchery population

and that many of the lethal or sub-viable genes have been previously paired and eliminated from this hatchery population.

A more conclusive examination of pleiotropic effects for traits such as viability and growth would be achieved using full-sib families from heterozygous parents. These could be achieved with the cross of heterozygous parents, or preferably with an aa by Aa cross to produce equal numbers of phenotypes within families. Analysis of quantitative phenotypes would allow for the separate examination of pleiotropic effects because inbreeding coefficients would be equal for both pigment phenotypes.

The results confirm albinism is a recessive trait, and the data also suggest that albinism is not a lethal or sub-viable trait in these chinook. It can, therefore, be used in gynogen production with confidence that results are not biased by such factors. Albino offspring were produced using both early and late-pressure-shock treatments, indicating that both meiotic and mitotic gynogens were produced. The results of Southern blot analysis of genomic DNA support these conclusions. Hybridisation trials using untreated heterologous sperm did not produce gynogens, however the production of wild-type offspring from wild-type coho sperm and albino chinook eggs confirms a true hybrid, and the high fertilisation success (80%) indicates that U.V.-treated sperm of coho may be useful in chinook gynogen production.

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APPENDIX 1.

SOUTHERN HYBRIDISATION METHODS

1.1 Tissue and Blood Samples

For each sample, 100 mg of tissue (liver or alevins with yolk sac removed) were chopped into small pieces (1-3 mm) with a clean sterile razor blade. For blood samples, 100 ul was used. To each type of sample, 5 ml of proteinase K buffer (10 mM Tris pH 8.0, 10 mM EDTA (TE), 1% SDS) in a 15 ml disposable tube was added. Proteinase K was added at 200 ug/ml of digest (1 mg for 5 ml of digest). This digest was incubated at 37°C with gentle orbital rocking until all tissue disappeared.

a) For tissue samples, NaCl was added to final concentration of 1.5 M (for 5 ml of extract, add 2.15 ml of 5 M NaCl), mixed thoroughly by inversion, and after 5 minutes at room temperature, centrifuged at 10000 X g for 10 minutes. The supernatant was extracted with PCI (phenol:chloroform:isoamyl alcohol [50:50:1]) and centrifuged at 10000 X g for 10 minutes. This was repeated as necessary until no material was seen at the interface.

b) For blood samples NaCl was added to final concentration of 1.5 M (for 5 ml of extract, add 2.15 ml of 5 M NaCl), and vigorously shaken for 15 seconds, centrifuged at 10000 X g for 10 minutes and the supernatant was simply transferred and precipitated as below.

The aqueous phase was precipitated with 2.5 volumes of 70% ethanol. The DNA precipitate was removed to 5 ml of 70% ethanol and centrifuged at 3750 X g for 5 minutes. The DNA pellet was dried under vacuum then redissolved in 200 ul to 1 ml of TE pH 8.0.

1.2 DNA Quantification

DNA quantification was done using a Hoefer Scientific DNA Fluorometer Model TKO 100. A calf thymus standard (CLONETECH 500 ug/ml) was used as the DNA standard. The fluorometer was calibrated with 2 ul of standard into 2 ml of 1xTNE (10 mM Tris,1 mM EDTA, 0.1 M NaCl) and a DNA fluorescing dye Hoechst 33258(10 ul/100 ml) to a concentration of 500 ug DNA/ml. The machine was zeroed using 2 ml of TNE. The relative concentration of DNA in the samples was measured by adding 2 ul of sample into 2 ml of TNE.

1.3 Restriction Enzyme Digestion

Restriction enzyme digests were prepared with sample DNA solutions, RNase, restriction enzyme, restriction enzyme buffer and distilled deionized water. Volumetric equivalents of 5 ug of sample DNA were used for each digest, fixed volumes of the enzymes and buffer and variable volumes of distilled water were used to bring the final volume of the digest to 30 ul. Digest components were added to 1.5 ml disposable Eppendorf tubes in the

following order and volumes: 10xREACT Hae III buffer (3.00 ul); RNase A (1 ul); dH_2O (variable volume), BRL or Promega Hae III 10 -16 Units/ul(3 ul) and sample DNA (5 ug volumetric equivalents). The digest was incubated in a 37°C water bath for 4 hours. Following incubation, each digest was mixed with 7 ul of a gel-loading buffer (0.25 bromophenol blue, 0.25 xylene cyanol FF, 30% gycerol in water). A portion (35 ul) of this final solution was loaded into the agarose gel well.

1.4 Electrophoresis

All agarose gels were made with 2.5 g of ultrapure Agarose and 500 ml of 1xTBE(Tris-borate/EDTA) with 25 ul of 10 mg/L ethidium bromide added as a intercalating dye. The agarose gel was poured into a 20x25 cm mold with a 15 sample well comb. The 1xTBE was used as the eletrophoresis buffer. Voltage was applied at 45 volts until both the bromophenol blue and the xylene cyanol had migrated out of the gel. Upon completion, the gels were examined under ultraviolet light and a reference photograph taken.

1.5 DNA Transfer

Initially the gels were placed in an acid bath for 15 minutes in 3 litres of 0.25 M HCl, followed by immersion in a solution of 1.5 M NaCl and 0.5 M NaOH for 45 - 60 minutes. This technique was modified to immersion for 25 to 30 minutes in 0.25 M HCl, followed by immersion in

denaturing buffer for 1 hour (the buffer was replaced every 1/2 hour) and then into a neutralising solution (1.5 M NaCl and 0.5 M Tris ,0.001 M EDTA) for 15 minutes.

1.6 Southern blots

The DNA from these gels was transferred to a Hybond nylon blotting membrane via capillary action. The capillary blot is a standard arrangement with the exception that it lacks any transfer buffer. Saran Wrap was taped to the bench top, with a wick of two layers of 3 mm Whatman paper saturated with blotting buffer (20xSSC [175.3 g sodium chloride and 88.2 trisodium citrate /litre]) on top. The gel was placed on top of the wick with the gel tray surface upwards. A sheet of Hybond N the exact size of the gel was placed on top of the gel. Any air bubbles were squeezed out with a glass rod. Two sheets of 3 MM paper cut to size and wetted with 20xSSC were placed on top of the nylon membrane. A 2 cm stack of absorbent paper cut to the size of the gel was placed on top, and additional 7 cm stack of paper towel on top of that. A gel tray and a beaker with 500 ml of water were placed on top of of the paper towels. Transfers proceeded overnight. The membranes were removed and washed in 2xSSC for 2 to 5 minutes, then blotted on 3 mm Whatman and dried at 80°C for 2 hours.

1.7 Hybridisation of DNA attached to Nylon Membrane

Prehybridisation:

The nylon membrane was wetted from beneath with 0.2xSSC. The wet filter was placed into a heat-sealable bag with 30 ml of prehybridisation solution (Church and Gilbert 1984). As much air as possible was removed from the bag. The bags were incubated submerged at 68°C for 1 to 2 hours in a rotating bath.

Hybridisation:

The oligonucleotide was then end labelled with ^{32}P using T_4 kinase which attaches ^{32}P to the end of the oligonucleotide (per Heath et al. in press). The following mixture was incubated for 30 minutes at 37°C and 5 minutes at 65°C:

100 ng Oligonucleotide (YN24)	1 ul
buffer	2 ul
50 mCi of alpha ATP	5 ul
dH2O	10 ul

A corner was cut from the bag and the prehybridisation media was drained out. The hybridisation solution containing 22.5 ml of prehybridisation solution was placed along with 18 ul of oligonucleotides in the bag, (hybrid media plus the oligonucleotide). The bag was then incubated at 41°C overnight. After incubation, the filter was removed and submerged in a tray containing 0.2X SSC and

0.1% SDS. This rinse was performed twice for 30 minutes each: the first was a fast rinse with 500 ml at room temperature, the second rinse with 1000 ml. A final bath in 1 litre of 0.2 X SSC and 1% SDS was done at 40° C for 25 minutes. The filter was then chilled with 1 litre of the same solution at room temperature. The filter was wrapped in Saran Wrap, and exposed to X-ray film to obtain an autoradiograph.

Prehybridisation solution (per 100 ml)

0.5m Na_2HPO_4- 52.6 ml

0.5m EDTA* 200 ul

20% SDS 47 ml

1 g Bovine Serum Albumin

* EDTA -ethylenediaminetetracetate

For labelling methodology of the OtSL1 probe refer to Heath et al. 1994

APPENDIX 2.
GENERAL LINEAR MODEL RESULTS

2.1 Probabilities from General Linear Models Procedure Tests of hypotheses for mixed model Analysis of Variance during freshwater phase of culture

DEPENDENT VARIABLE=WEIGHT

Source	DF	Type III MS	Denominator DF	Denominator MS	F value	Pr>F
Day 90 untagged N=490						
Dam*1	4	0.0527	4	0.002857	18.433	0.0077
Pigment*2	1	0.0071	4	0.002856	2.483	0.1902
PigmentxDam*3	4	0.0029	480	0.004318	6.616	0.0001
Day 132 untagged N=899						
Dam*1	4	0.4694	4	0.379699	1.236	0.4210
Pigment*4	1	2.1589	4	0.379698	5.686	0.0756
PigmentxDam*5	4	0.3797	20	0.049451	7.678	0.0006
Tank(PigmentxDam)*3	20	0.04955	869	0.028949	1.708	0.0271
Day 174 untagged N=1194						
Dam*1	4	13.6435	4	11.57744	1.178	0.4387
Pigment*6	1	1.5994	4	11.57735	0.138	0.7292
PigmentxDam*7	4	11.5774	20	1.051286	11.01	0.0001
Tank(PigmentxDam)*3	20	1.0513	1164	0.484365	2.17	0.0021

DEPENDENT VARIABLE = LENGTH

Source	DF	Type III MS	Denominator DF	Denominator MS	F value	Pr>F
Day 174 untagged N=1194						
Dam*1	4	549.86	4	261.6043	2.102	0.2448
Pigment*6	1	100.52	4	261.6024	0.384	0.5689
PigmentxDam*7	4	261.60	20	30.76606	8.503	0.0004
Tank(PigmentxDam)*3	20	30.77	1164	18.3087	1.680	0.0306

Error Terms

*1 Error:MS(PigmentxDam)
*2 Error:0.9996xMS(PigmentxDam) + 0.0004xMS(Error)
*3 Error:MS(Error)
*4 Error:1xMS(PigmentxDam)+395E-8MS(Error)
*5 Error:1xMS(Replicate(PigmentxDam))+206E-7MS(Error)
*6 Error:1xMS(PigmentxDam)+76E-7xMS(Error)
*7 Error:0.9999xMS(Replicate(PigmentxDam))+527e-7MS(Error)

Appendix 2.2 Results of General Linear Models Procedure Tests of hypotheses for mixed model Analysis of Variance for weight measurement during saltwater phase of culture.

DEPENDENT VARIABLE=WEIGHT

Source	DF	Type III MS	Denominator DF	Denominator MS	F value	Pr>F
Day 228 Untagged N=1628						
Dam*1	4	92.043	4	63.3484	1.327	0.3952
Pigment*2	1	505.50	4	69.347	7.289	0.0541
PigmentxDam*3	4	69.35	20	7.927	8.748	0.0003
Tank(PigmentxDam)*4	20	7.928	1618	1.8111	4.377	0.0001
Day 228 Tagged N=596						
Dam*1	4	60.7	4	40.130	1.513	0.3490
Pigment*5	1	142.7	4	40.129	3.556	0.1324
PigmentxDam*6	4	0.13	20	4.180	9.601	0.0002
Tank(PigmentxDam)*4	20	4.18	566	1.778	1.358	0.0008
Day 341 Tagged N=479						
Dam*1	4	4232.99	4	1176.364	3.598	0.1213
Pigment*7	1	2923.35	4	1172.325	2.494	0.1890
PigmentxDam*8	4	1176.36	20	751.898	1.565	0.2218
Tank(PigmentxDam)*4	20	753.885	449	555.100	1.358	0.1382
Day 448 Tagged N=365						
Dam*1	4	18456.02	4	5238.116	3.523	0.1250
Pigment*9	1	135099.4	4	5134.474	26.31	0.0062
PigmentxDam*10	4	5238.1	21	2579.077	1.267	0.3147
Tank(PigmentxDam)*4	20	3956.49	335	2473.930	1.60	0.0531
Day 538 Tagged N=352						
Dam*1	4	83287.9	4	24641.17	3.38	0.1326
Pigment*11	1	434354.2	4	23781.93	18.26	0.0121
PigmentxDam*12	4	24641.2	21	9871.94	2.49	0.0736
Tank(PigmentxDam)*4	20	9962.282	322	7493.40	1.329	0.1576

Error Terms

*1 Error:MS(PigmentxDam)
*2 Error:1xMS(PigmentxDam) + 232E-7xMS(Error)
*3 Error:0.999xMS(Replicate(PigmentxDam))+0.0001xMS(Error)
*4 Error:MS(Error)
*5 Error:0.9935xMS(PigmentxDam) + 349E-7xMS(Error)
*6 Error:0.999xMS(Replicate(PigmentxDam))+0.0003xMS(Error)
*7 Error: 0.9935xMS(PigmentxDam) + 0.0065xMS(Error)

Error Terms

*8 Error: 0.99xMS(Replicate(PigmentxDam))+0.01xMS(Error)
*9 Error: 0.9625xMS(PigmentxDam) + 0.0375xMS(Error)
*10 Error: 0.970xMS(Replicate(PigmentxDam)+0.0297xMS(error)
*11 Error: 0.9499xMS(PigmentxDam) + 0.0501xMS(error)
*12 Error:0.9634xMS(Replicate(PigmentxDam))+0.036xMS(Error)

2.3 Results of General Linear Model Procedure Tests of hypotheses for mixed model Analysis of Variance during the saltwater phase of culture

DEPENDENT VARIABLE LENGTH

Source	DF	Type III MS	Denominator DF	Denominator MS	F value	Pr>F
Day 538 Tagged N=352						
Dam*1	4	2681.97	4	1610.03	1.67	0.3166
Pigment*2	1	22245.94	4	1581.29	14.07	0.0180
PigmentxDam*3	4	1610.03	342	977.81	1.65	0.1621

Error Terms
*1 Error: MS(PigmentxDam)
*2 Error: 0.9546xMS(PigmentxDam) + 0.0454xMS(error)
*3 Error: MS(Error)

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Carswell B.L., R. Deegan, J. Willow and S. Cross. 1992. Ensiling Salmon Mortalities. Ministry of Agriculture Fisheries and Food.

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Title of Thesis: The use of albinism to verify gynogenesis and to investigate the viability and growth rates of inbred chinook salmon Oncorhynchus tschawytscha

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Jan 25, 1995