GROWTH RATE AND SPAWNING TIME IN DIALLEL CROSSES OF THREE STRAINS OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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ABSTRACT

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Factors controlling individual pre-maturation instantaneous growth rate and female spawning date were examined in two complete sets of diallel crosses of three strains of rainbow trout (*Oncorhynchus mykiss*). Hybrids generally performed intermediately to pure strains. For growth rate, the trend among dam strains was opposite to the trend among sire strains. Hybrids grew more like their sire's strain and spawned more like their dam's strain. Males grew faster than females for all strains. Early-maturing individuals weighed more prior to maturation, but grew at the same rate as later-maturing individuals. Spawn date was highly repeatable over seasons. Genetic parameters and breeding values were estimated for 2-year old weight, and 3- and 4-year old spawn dates. All traits had high heritabilities. Negative phenotypic correlations and zero genetic correlations occurred between weight and spawn dates. Recommendations were made for a breeding program that simultaneously increases growth and delays spawning time.

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CHAPTER 1. INTRODUCTION

1.1. GROWTH AND SPAWNING TIME IN RAINBOW TROUT AQUACULTURE

In farming of rainbow trout, two genetically-based traits that influence production are growth and spawning time. Growth is important because as the time to raise fish to market size increases, the production costs rise. Fast growth would reduce the amount of time until fish reach a market weight, thus decreasing costs for the farmer. Spawning time also has a major effect on the farming operation. Salmonids have a seasonal reproductive cycle, and so there are corresponding peaks and lows in farming activity. Reducing these fluctuations should improve the farm operating efficiency.

A number of commercial strains of rainbow trout have been developed in an attempt to maximise profits for the farmer. Interactions among these two production traits have limited the success of these commercial strains. For example, strains with increased growth may spawn only in the fall and winter seasons. Other strains with spring spawning times can suffer from low growth rates.

Knowledge of how growth and spawning time interact for a particular population could help a farmer in developing a more efficient operation. If these traits are heritable, it may be possible to make some predictions of offspring development based on parental performance. This could aid in developing a long-term improvement strategy.

1.2. THESIS OBJECTIVES

Previous studies have compared three pure strains of rainbow trout for various traits, including growth, survival, and maturation. This study expands those findings.

Using data obtained from pure and crossed populations of rainbow trout held at the Alma Aquaculture Research Station, the objectives of this study were:

1. Determine the effects of time of fertilisation on growth and spawning time.
2. Compare strains for growth and spawning time performance.
3. Determine the types of strain interactions that affected growth and spawning time.
4. Determine the effects of sex on growth.
5. Determine the effects of age of maturation on growth and spawning time.
6. Calculate the repeatability of spawning time.
7. Estimate genetic parameters related to growth and spawning time.
8. Determine the most efficient breeding scheme to develop a synthetic strain of rainbow trout with fast growth and a delayed spawning season.

CHAPTER 2. LITERATURE REVIEW

2.1. INTRODUCTION

This literature review focuses on factors that influence growth and spawning time. These are both quantitative traits, and so phenotypes are affected by the simultaneous segregation of many genes combined with variation due to many non-genetic factors (Falconer, 1981).

2.2. GROWTH

2.2.1 Non-genetic (environmental) factors affecting growth

Nutrition

Feed quality is important to growth and generally, increased protein results in faster growth. Rainbow trout require an optimum of 40 to 45% protein and 12 to 14% lipids in their diet (Iwama, 1996). Higher feeding frequency is needed with increased physical activity (Christansen and Jobling, 1990) and high density (Holm et al., 1990). Variation in feed quantity will also affect growth, as shown by Quinton and Blake (1990) who observed that rainbow trout lost weight during a starvation period, but had very high compensatory growth rates when feeding was resumed.

Temperature

Iwama (1996) stated that water temperature is the single most dominant environmental effect on growth in fish, and is a controlling factor for growth. The environmental temperature affects the rate at which chemical reactions take place in poikilotherms (Iwama, 1996). Growth generally increases with higher temperatures,

within species' and strains' ranges of tolerance. Above certain temperatures, feed conversion can become less efficient which may limit growth (R. Danzmann, personal communication). Fluctuations in water temperature can also affect growth rates. Rainbow trout and Atlantic salmon reared in diel high-low water temperature regimes had faster growth than groups held in constant mid-range temperatures (Hokanson et al., 1977; Berg et al., 1990).

Photoperiod and other environmental cycles

Clarke and Shelbourn (1986) increased growth rate in coho salmon by delaying the natural season by 30 days. Saunders and Harmon (1990) found a ratio of 18 hours light to 6 hours dark gave the best growth in Atlantic salmon, with longer lasting effects for 3 months exposure over 2 months exposure. Photoperiod may stimulate the release of growth hormone (Komourdjian et al., 1976, 1989; Bjoernsson et al., 1989). Farbridge and Leatherland (1987) found a 2-week cycle of rapid and slow growth in coho salmon, and suggested that lunar cycles act as a *Zeitgeber* for synchronisation of growth rate rhythms. Wagner and McKeown (1985) showed cycling growth rates in rainbow trout. There is also some evidence of tide cycles affecting growth in salmonids (Iwama, 1996).

Rearing Density

Wedemeyer (1976) recommended an optimum density for rainbow trout of 13 to 61 kg/m^3. Experiments with rainbow trout have shown that in general, growth declines as rearing density increases (Papoutsoglou et al., 1987; Holm et al., 1990). Holm et al. (1990) found that density-linked growth depression still occurred even with continuous feed availability, and suggested that it was a response to stress. Sources of stress may be behaviour (such as a dominance hierarchy established leading to competition for food),

and water quality. These may induce a stress response at some energetic cost, causing an attenuation of growth (Iwama, 1996).

Hormone treatment

Growth can also be affected by administering growth hormone (both somatotropin or its analogues) by ingestion, injection, immersion, or implants. Recombinant bovine somatotropin (RBST) stimulated growth by 20 to 40% over controls in rainbow trout (Schulte et al., 1989). Recombinant fish growth hormone increased growth 100% in rainbow trout (Agellon et al., 1988).

Maternal effects

Maternal effects are not usually considered important in salmonids since these fish do not nurture their offspring (Gjerde, 1988). Maternal effects in salmonids could take the form of egg size and quality, or maternal spawning season. Any maternal effects on growth probably decrease with the offspring's age, as suggested by Klupp (1979). Langholz and Hörstgen-Schwark (1987) found maternal effects were not important components of 9-month and 16-month body weights.

2.2.2. Genetic factors affecting growth

Strain, stock, and family differences

There have been many studies showing differences in growth between salmonid stocks in both natural and common environments (Table 2A.1, see Appendix 2.1). Some differences between strains may be caused by variation in feed utilisation. Kolok (1989) found that a slow-growing strain of rainbow trout needed a higher maintenance ration than did a fast-growing strain. This suggested that fish in the slow-growing strain had higher basal metabolic rates, and therefore could not allocate as much energy to growth

as could those in the fast-growing strain (Kolok, 1989). Danzmann et al. (1987) observed that families with higher levels of heterozygosity used less oxygen and so had greater metabolic efficiencies than families with lower levels of heterozygosity.

Response to selection

Selection experiments in salmonids have generally been successful in improving growth traits. Kincaid et al. (1977) reported a net genetic gain in rainbow trout weight of 30.1% from phenotypic family selection over 3 generations. Family selection over two generations improved rainbow trout for pan-size (Hörstgen-Schwark, 1993). Index selection over two generations also caused significant gains in Atlantic salmon weights (O'Flynn et al., 1999).

Heritability estimates

Published heritability estimates for rainbow trout weights and growth rates are generally over 0.2 (Table 2A.2), indicating that these traits are influenced by additive genetic effects. Across studies, estimated heritability of body weight seems to decrease with age (Table 2A.2), but within studies, no clear relationship has been found between age and the heritability of weight. Gjerde (1986), Gall and Gross (1978A), Elvingson and Johansson (1993), and Su et al. (1996) found increasing heritability estimates for body weight with age, while Crandall and Gall (1993A, 1993B) found heritability estimates decreased with age. Estimates also vary across studies due to different methods used to estimate heritability.

Genetic Correlations

Genetic correlations among growth traits are generally positive and high: over 0.5. This applies to different measurements at the same age (Gjerde and Gjedrem, 1984) as well as measurements at different ages (McKay et al., 1986B; Pohar, 1992).

There does appear to be some genetic correlation between body weight and maturation (Gjerde and Gjedrem, 1984), which would predict indirect maturation responses to selection for weight. There does not appear to be strong unfavourable genetic correlations between weight and egg traits such as egg volume, size and number (Huang and Gall, 1990), nor between weight and important carcass traits such as belly thickness, fat percentage, and meat colour (Gjerde and Schaeffer, 1989).

Non-additive genetic effects

A number of studies have found that there are at least some non-additive genetic effects on growth traits. Gall and Huang (1988A) found the dam variance component was larger than the sire variance component for body weight traits, implying that some degree of non-additive genetic or common environmental effect exists for body weight. Su et al., (1996B) found considerable full-sib family effects that tended to increase with age and concluded that these were partially due to non-additive genetic effects. Pante (1998) confirmed the presence of non-additive variance for body weight in rainbow trout, most of which was dominance variance. In a study of 9- and 16-month body weights, Langholz and Hörstgen-Schwark (1987) found no important reciprocal effects, and calculated only slight heterosis (5 to 10%) for 9-month weight and no heterosis for 16-month weight.

Genotype-Environment Interaction

Several studies have found that some genetic groups of rainbow trout have better growth performance under particular environmental conditions. Significant strain and family interactions have been found with rearing environment (Klupp et al., 1978; Bagley et al., 1994), rearing density (Iwamoto et al., 1986; Bagley et al., 1994), ration levels (Iwamoto et al., 1986), and production system (Sylven et al., 1991).

Inbreeding

Inbreeding in populations may also affect growth. In rainbow trout, Su et al. (1996A) reported a 2.3 to 5.8% decrease in body weight, and Pante et al. (2001) reported 1.6 to 4.5% decrease in body weight per 10% unit increase in inbreeding coefficient. Rye and Mao (1998) reported 0.6 to 2.6% decrease in body weight per 10% increase in inbreeding in Atlantic salmon.

Maturation

The relationship between growth and maturation is usually discussed in the literature with reference to precocious sexual maturation (see Thorpe et al., 1983; Thorpe, 1986). While the growth rate of maturing adult salmonids is retarded by sexual maturity, growth of precociously maturing juveniles is enhanced (Iwama, 1996). Several studies have shown a decline in somatic growth rate with sexual maturation (Taylor, 1989; Lamont, 1990; Jobling and Baardvik, 1991). The decline in growth may be caused by reduced food intake, combined with increased energy demands from developing gonadal material which results in energy being shunted from somatic growth to gonadal development (Rowe and Thorpe, 1990). Tveranger (1986) found that in females the fat

and protein incorporated into developing eggs came from body tissues as well as ingested food.

Ploidy

There does not appear to be any pattern to the effect of ploidy on growth in salmonids. Some studies have shown diploids to grow faster than triploids (Lincoln and Hardiman, 1982, cited by Iwama, 1996; Thorgaard et al., 1982; Solar et al., 1984; Bonnet et al. 1999; Friars et al., 2001), while others have found no difference between diploids and triploids (Oliva-Teles and Kauchik, 1987, 1990; Benfey, 1988; Sutterlin and Collier, 1991). There are also differences within studies: Myers and Hershberger (1991) found one group of triploid rainbow trout initially had slower growth than diploids, then recovered and were more rapid; another group of triploids were initially larger than diploids, but smaller by the end of the study. One possible reason for this lack of consensus is strain or family interactions with ploidy which affect growth (Bonnet et al., 1999; Friars et al., 2001). Detection of such interactions may also depend on the stage of growth under study (Bonnet et al., 1999).

2.3. Spawning Time

2.3.1. Natural Life History

Rainbow trout, like other salmonids, have an annual reproductive cycle. This means animals only release gametes during certain months of the year. These reproductive months are commonly referred to as the spawning season. Rainbow trout in nature occur in both fall-spawning (decreasing daylength and water temperature) and

spring-spawning (increasing daylength and water temperature) strains (Pepper and Crim, 1996).

Rainbow trout will not spontaneously release gametes in culture situations. Gametes must be stripped from animals and eggs are fertilised artificially. Milt may be stripped from males for most of the spawning season. Females only ovulate once during the spawning season, and there is only a short window during which the eggs must be stripped and fertilised. Since the female spawning time is more limiting and can be recorded with greater accuracy, this is the trait that most studies have examined.

2.3.2. Non-genetic (environmental) factors affecting spawning time

Most non-genetic factors are related to altering the external cues regulating the reproductive cycle. These included photoperiod, temperature, hormone manipulation and domestication.

Photoperiod

In the wild, salmonids use changes in photoperiod to set internal circannual clocks so that spawning occurs at the optimal season for reproductive success (Duston and Bromage, 1988). Salmonid spawning time can be adjusted by manipulating photoperiod. Beacham et al. (1994) photoperiod-induced pink salmon to spawn 6 months early. Duston and Bromage (1986) and Bromage et al. (1992) advanced and delayed female rainbow trout spawning up to 5 months by manipulation of photoperiod. Photoperiod manipulation is a commonly used method of changing spawning time in aquaculture (e.g. Buss, 1980), however Purdom (1993) has said that this method can be expensive and unreliable.

Temperature

Ambient water temperature, like photoperiod, is an indicator of time of year. Bromage et al. (1992) found under the same photoperiod, rainbow trout held at seasonal 4°C (Feb) to 16.5°C (July) water had advanced spawning time over those held at constant 8.5°C water.

Domestication

Petersson et al. (1996) found domestication (without selection) has had no significant effect on the date of ovulation of Atlantic salmon and sea trout.

Hormone manipulation

Administration of hormones artificially induces spawning by simulating the natural hormonal changes that take place in response to external cues (Hunter and Donaldson, 1983). Bromage et al. (1992) used treatments of LHRHa, HCG, and pituitary extract to advance female rainbow trout spawning by 2 to 3 weeks.

Purdom (1993) has commented that hormone manipulation may not be suitable for use on a production scale as it only causes small changes in spawning time and can be unreliable. There is also more labour required to administer hormones. Patino (1997), however, contends that hormone manipulation combined with environmental conditions will remain an important method of controlling gametogenesis and spawning.

2.3.3. Genetic factors affecting spawning time

Strain, stock and family differences

Gall and Huang (1988A) found that progeny of photoperiod-manipulated parents spawned during the population's normal season, which demonstrated that spawning season must be controlled by a strong genetic component.

Purdom (1993) stated that different strains of rainbow trout had varying spawning times, and hybrids spawned intermediate of parents. He proposed that the best solution for established fish cultivation industry is use of individual strains at different times of the year.

Response to selection

Producers have attempted to change spawning time through selection. There is anecdotal evidence that a wide range of spawning times have been developed by selection, and a shift of approximately one month can be achieved in 4 to 5 generations of selection (Purdom, 1993).

In published literature, there are few studies on spawn date responses to selection. After six generations of phenotypic selection for early spawn date in female rainbow trout, Siitonen and Gall (1989) found a response of nearly 7 days per generation. After one generation of phenotypic selection for early spawning in fall-spawning rainbow trout, Sadler et al. (1992) found that spawn date moved forward 15 days, however the lack of an unselected control group means this result was confounded with changes due to environment.

Heritability and repeatability estimates

There have been a few studies that have attempted to quantify the genetic components of spawning time in salmonids. Published heritability estimates related to spawning time are generally quite high, but there is a wide range, probably due to the actual traits measured and methods of estimation (Table 2A.3).

Since rainbow trout are repeat spawners, repeatability of spawning time across seasons may also be estimated. Repeatability sets the upper limit of heritability (Falconer,

1981). Sadler et al. (1992) calculated spawn date repeatability of individual female rainbow trout at an Ontario farm as 0.5.

Genetic correlations

The few published genetic correlations of spawning time with body weight have been high and positive: 0.45 with 25-month weight (Huang and Gall, 1990); 0.48, 0.41 (Huang and Gall, 1990) and 0.726 (Su et al., 1997) with post-spawn weight. Genetic correlations with egg traits are high and positive as well: 0.24 (Huang and Gall, 1990) and 0.515 (Su et al., 1997) with egg size, 0.52 (Huang and Gall, 1990) and 0.537 (Su et al., 1997) with egg volume; 0.38 (Huang and Gall, 1990) and 0.249 (Su et al., 1997) with egg number.

Other genetic factors

Other genetic factors that spawning time are inbreeding, gynogenesis, and molecular genetic structure. Su et al. (1996A) reported that spawning age of females was delayed by 0.53% per 10% increase in inbreeding coefficient, but the spawning age of males was not affected. Quillet (1994) reported that gynogenetic female rainbow trout spawned on average one week later and had a longer reproductive season than diploid controls. Ferguson et al. (1993) found differences in the distribution of mitochondrial DNA haplotypes among fish from the same farm but spawning at different times of the year. Sakamoto et al. (1999) reported thirteen microsatellite markers associated with spawning time, and at least five linkage groups with spawning time quantitative trait loci.

APPENDIX 2.1. LITERATURE REVIEW TABLES

Table 2A.1. Salmonid stock growth comparison studies.

Species/ Trait	Comparisons	Reference
Rainbow trout		
2 year weight	full- and half-sib families	Gjerde and Gjedrem, 1984
96 day weight	homozygous and heterozygous groups	Danzmann et al., 1987
9 month weight 16 month weight	131 full-sib families from 17 stocks	Langholz and Hörstgen-Schwark , 1987
growth rate	10 strains	Smith et al., 1988
body weight caloric growth rate	1 fast- and 1 slow-growing strain	Kolok, 1989
168 to 364 day body weight	3 lines	Su et al., 1996B
2.5 year weight	full- and half-sib families at 8 farms	Sylven et al., 1991
12 month body weight 17 month body weight specific growth rate	16 maternal half-sib diploid and triploid families	Bonnet et al. 1999
weight specific growth rate	diallel crosses of 2 strains	Wangila and Dick, 1996
Atlantic salmon		
2 year weight	strains and full- and half-sib families	Gjerde and Gjedrem, 1984
2 year body weight and length	37 stocks grown at 5 farms (each stock had several full- and half-sib families)	Gunnes and Gjedrem, 1978
5 month weight 7 month weight	full-sib families	Herbinger, C. M., et al. 1999
Chinook salmon		
alevin and smolt body weight growth rate	stocks and families within stocks	Withler et al., 1987
	6 stocks at 5 farms	Kreiberg, 1987; cited by Iwama, 1996
Pink salmon		
2 year weight	full- and half-sib families growing in natural environment	Smoker et al., 1994
Chum salmon		
alevin weight	stocks	Beacham and Murray, 1987
Arctic charr		
weights at various ages	4 morphs in one lake	Jonsson et al, 1988
	2 strains	Delabbio et al., 1990
Brown trout		
17 month body weight 23 month body weight 29 month body weight specific growth rate	16 maternal half-sib diploid and triploid families	Bonnet et al. 1999

Table 2A.2. Heritability estimates (h^2) for body weight and growth rate at different ages in rainbow trout

Trait / Age	$h^2 \pm SE$	Estimation Method	Reference
Body Weight at			
4 months	0.52 ± 0.15	half-sibs (sire variance)	Gall and Huang, 1988A
147 days	0.26	realised h^2	Kincaid et al., 1977
150 days	0.50 ± 0.07		von Limbach, 1970
159 days	0.53 ● 0.14	DFREML animal model	Crandell and Gall, 1993A
168 days	0.052	DFREML animal model	Su et al., 1996B
180 days	0.50± 0.14	DFREML animal model	Crandell and Gall, 1993A
196 days	0.075	DFREML animal model	Su et al., 1996B
224 days	0.083	DFREML animal model	Su et al., 1996B
252 days	0.097	DFREML animal model	Su et al., 1996B
9 months	0.2	full-sibs and diallel crosses	Langholz and Hörstgen-Schwark, 1987
278 days	0.36 ± 0.13	DFREML animal model	Crandell and Gall, 1993A
280 days	0.29 ± 0.20		Aulstad et al., 1972
280 days	0.087	DFREML animal model	Su et al., 1996B
308 days	0.085	DFREML animal model	Su et al., 1996B
334 days	0.82 ± 0.38		Klupp, 1979
335 days	0.41± 0.13	DFREML animal model	Crandell and Gall, 1993A
336 days	0.104	DFREML animal model	Su et al., 1996B
362 days	0.38 ± 0.13	DFREML animal model	Crandell and Gall, 1993A
364 days	0.103	DFREML animal model	Su et al., 1996B
1 year	0.06 to 0.19		Refstie, 1980
1 year	0.38 ± 0.25	half-sibs (sire variance)	Linder et al., 1983
1 year	0.10 ● 0.08	full-sibs (dam variance)	Linder et al., 1983
1 year	0.20 ± 0.11	half-sibs (sire variance)	Gall and Huang, 1988A
1 year	0.20 ± 0.12	half-sibs (sire variance)	McKay et al., 1986B
398 days	0.26± 0.11	DFREML animal model	Crandell and Gall, 1993A
453 days	0.40 ± 0.13	DFREML animal model	Crandell and Gall, 1993A
16 months	0.2	full-sibs and diallel crosses	Langholz and Hörstgen-Schwark, 1987
17 months	0.15	realised h^2	Hörstgen-Schwark, 1993
544 days	0.33± 0.12	DFREML animal model	Crandell and Gall, 1993A
628 days	0.24 ± 0.11	DFREML animal model	Crandell and Gall, 1993A
2 years	0.32 ± 0.11	half-sibs (sire variance)	Gjerde and Gjedrem, 1984
2 years	0.38 ± 0.09	full-sibs (dam variance)	Gjerde and Gjedrem, 1984
2 years	-0.01 to 0.34		Gunnes and Gjedrem, 1981
2 years	0.17 ± 0.12	half-sibs (sire variance)	McKay et al., 1986B
740 days	0.21± 0.13	DFREML animal model	Crandell and Gall, 1993A
25 months	0.18 ± 0.12	half-sibs (sire variance)	Gall and Huang, 1988A
2.5 years	0.21	MIVQUE sire variance	Gjerde and Schaeffer, 1989
2.5 years	0.41	MIVQUE dam variance	Gjerde and Schaeffer, 1989
2.5 years	0.38 ± 0.22	half-sibs (sire variance)	McKay et al., 1986A
2.5 years	0.12 to 0.27	REML animal model	Sylven et al., 1991
4 years	0.27 ± 0.20	half-sibs (sire variance)	McKay et al., 1986A
maturation	0.20 ● 0.10	half-sibs (sire variance)	Gall and Huang, 1988A
slaughter (~2.8kg)	0.43	half-sibs (sire variance)	Pohar, 1992

Trait / Age	$h^2 \pm SE$	Estimation Method	Reference
slaughter (~2.8kg)	0.63	full-sibs (dam variance)	Pohar, 1992
female post-spawning	0.50	full-sibs	Gall and Gross, 1978B
female post-spawning	0.15 ± 0.14	half-sibs (sire variance)	Gall and Huang, 1988B
male post-spawning	0.31	full-sibs	Gall and Gross, 1978B
Growth Rate			
specific	0.27 ± 0.04	half-sibs (sire variance)	Wangila and Dick, 1996
specific	0.88 ± 0.35	half-sibs (dam variance)	Wangila and Dick, 1996
instantaneous 2.5-year	0.29 ± 0.14	half-sibs (sire variance)	McKay et al., 1986A
instantaneous 2.5-4-year	0.10 ± 0.13	half-sibs (sire variance)	McKay et al., 1986A

Table 2A.3. Heritability estimates (h^2) related to female spawning time in rainbow trout.

Trait	$h^2 \pm SE$	Estimation Method	Reference
within-season spawn date	0.53 ± 0.05 to 0.55 ± 0.07	realised h^2	Siitonen and Gall, 1989
within-season spawn date	0.9	realised h^2	Sadler et al., 1992
within-season spawn date	0.494	animal model, control line	Su et al., 1997
within-season spawn date	0.842	animal model, egg size-selected line	Su et al., 1997
within-season spawn date	0.868	animal model, growth-selected line	Su et al., 1997
within-season spawn date	0.652	animal model, pooled lines	Su et al., 1997
age of spawning	0.38 ± 0.17	half-sibs (sire variance)	Gall et al., 1988
age of spawning	0.36 ± 0.26	full-sibs (dam variance)	Gall et al., 1988
age of spawning	0.38 ± 0.001	regression of daughter on dam	Gall et al., 1988
age at spawning	0.034 to 0.153	animal model	Su et al., 1999

CHAPTER 3. DATA ANALYSIS INTRODUCTION

3.1 PURPOSE OF STUDY

The purpose of this study was to compare three pure strains and the nine strain combinations resulting from the diallel crosses for instantaneous growth rate and female within-season spawning time. Strain comparisons included: contrasts of pure strains, contrasts of pure and hybrid strains (heterosis) and contrasts of reciprocal hybrids. Within the strains, the effects of age at sexual maturation will also be tested.

Previous studies have compared these pure strains for various traits, including early growth, survival, and maturation (McMillan and McKay, 1992; McKay and McMillan, 1997). This study expands on the previous findings.

3.2 MATERIALS AND METHODS

3.2.1. Strain descriptions

Three different strains of rainbow trout used in Ontario aquaculture were studied. Strain O was an Ontario commercial strain that has been selected for many generations, with fast growth and a fall spawning season. Strain B was a West Coast commercial strain with intermediate growth and a winter spawning season. Strain G was a newer Ontario commercial strain, only selected for two generations from the wild, with slow growth and a spring spawning season.

Under the study conditions, most females of these strains matured at 3 or 4 years old and most males matured at 2 (precocious) or 3 years old. Precocious males are generally considered undesirable in farm populations.

3.2.2. General husbandry

Rearing Conditions

The study was conducted at the Alma Aquaculture Research Station (AARS), University of Guelph, Guelph, ON. Fertilised eggs from each of the three strains were brought to the research station, where they were raised in same conditions. Rearing conditions at the AARS are discussed in detail by Moccia and Burke (1998). Water temperature remained constant at 8.5 ± 0.3°C and dissolved oxygen was 96% of saturation. Natural photoperiod was maintained. Fertilised eggs were incubated and hatched in vertical-flow stack incubator trays. Fish were housed in 0.7 m, 1.0 m, and 2.0 m semi-square fibreglass tanks and fed a commercial dry salmonid feed manufactured by Martin Feed Mills Ltd. (Elmira, ON) with rations calculated according to that company's charts. Feed was dispensed by automatic vibrating and belt feeders until the beginning of the first female spawning season (3 years of age); after this time all feeding was done with demand feeders.

Tagging

Fish were anaesthetised with tricaine methanesulfonate (MS-222) and AVID© passive integrated transponder (PIT) tags were injected into the abdominal cavity using a hand-held syringe. After tagging, fish were allowed to recover in fresh water.

3.2.3. Experimental design

Samples from each of the three strains were raised to maturity under the same conditions. At maturity, two sets of complete diallel crosses were made: group 1 (94 year class) when the parents were 3 years old, and group 2 (96 year class) when the parents were 5 years old. All reciprocal crosses were made. There was no time when dams from

all three strains spawned at the same time. Therefore, to complete the diallel crosses, matings had to be done over a series of weeks (Table 3.1). Each strain combination in the diallel crosses was made from pooled matings of 2 to 6 randomly-chosen individuals from each dam and sire strain. Twenty-eight females and 29 males parented group 1, and 30 females and 40 males parented group 2. Individual females were used once per group. For group 1, individual males were used up to 5 times over 12 weeks. For group 2, individual males were used up to 6 times over 17 weeks.

Table 3.1. Diallel cross mating schedule, showing fertilisation dates and week numbers for two offspring year classes. The first letter in the strain combination represents the strain of the dam and the second letter represents the strain of the sire. The number of individuals used in each diallel cross is indicated by "*a*×*b*" where *a* is the number of dams and *b* is the number of sires.

Date	Week	Strain Combination								
		OO	**OB**	**OG**	**BO**	**BB**	**BG**	**GO**	**GB**	**GG**
Offspring Group 1 (94 year class)										
94/02/10	1	4×4	4×4	4×4	4×4	4×4	4×4			
94/02/24	3				4×4	4×4	4×4			
94/03/24	7				5×4	5×4	5×4	4×4	4×4	4×4
94/03/30	8					2×4	2×4		3×4	3×4
94/04/27	12							2×4	2×4	2×4
Offspring Group 2 (96 year class)										
95/11/30	1	3×3	3×2	3×4						
95/12/07	2	2×2	2×4	2×2						
95/12/21	4		2×3	4×4						
96/01/09	6	1×3		1×3	3×3		3×3			
96/02/13	11					3×4	1×4			
96/02/27	13				3×3	1×3		3×3	3×3	3×3
96/03/12	15				2×3	2×3	2×3	4×3	4×3	4×3
96/03/27	17							4×5	4×5	4×5

Progeny from each pair of parents were kept in separate incubator compartments. After hatching, equal numbers of fry from the same fertilisation week and strain combination were pooled into 0.7 m tanks. Due to this pooling, the exact parentage of the first offspring cross was unknown. In the second group, microsatellite DNA analysis was done to determine the exact dams and sires of each individual.

Before pooling into 1.0 m tanks, fingerlings were fin-clipped to distinguish the different fertilisation week-strain combination groups. Before pooling into 2.0 m tanks, individuals were PIT-tagged. The parent year class was tagged at 766 to 793 days old. The 94 year class was tagged at 433 to 519 days old. The 96 year class was tagged at 292 to 335 days old.

3.2.4. Data collection

Weight and maturation observations were recorded for individuals from the parental and offspring year classes. Tagging allowed repeated measurements on the same individuals. The record for each individual included: ID number, year class, strain of dam, strain of sire, fertilisation date, individual weights and corresponding weigh dates, sex, age at sexual maturation (a categorical trait: 2, 3, or 4 years old), and spawn dates of females at 3 and 4 years old. The second diallel cross also included pedigree information (ID of dam and sire).

3.2.5. General statistical methods

Instantaneous growth rates and female spawning dates were analysed to find significant strain effects. This was with analysis of variance (ANOVA) using SAS© PROC GLM. Tukey's tests and contrasts from this analysis were performed to find strain differences and any evidence of heterosis.

CHAPTER 4. GROWTH RATE ANALYSIS

4.1. INTRODUCTION

4.1.1. Definition of Terms

Individual instantaneous growth rate has not been well examined in aquaculture genetics studies. This is probably due to the difficulty of tagging individuals early in life and the additional labour and cost involved in following individuals for long periods of time. Growth rate in this project refers to the instantaneous growth rate and is defined as the differential of the growth function at any time point. Growth is exponential early in life, but tapers off in the older animal. Individual weights were recorded at intervals up to the first spawning season. The instantaneous growth rate for each individual was the slope of the regression of the transformed weight on age in weeks. Since early growth in fish generally follows an exponential relationship, the natural log of weight over time follows a linear relationship. In this study, growth rate was measured by calculating the slope of the regression of the natural log of weight on age, the number of days from fertilisation to weighing.

4.1.2. Purposes of study

There were four main purposes for this study. First, to determine the effects of fertilisation week on growth rate. Second, to rank dam and sire strains according to their offspring's growth rate. Third, to determine types of strain interactions that affected growth, such as heterosis or differences between reciprocal crosses, and fourth, to find effects of sex and age of sexual maturation on pre-maturation growth.

4.2. Materials and Methods

4.2.1. Data Collection

"Age" throughout this study refers to number of days or weeks post-fertilisation. All crosses from the 96 offspring year class were weighed every 12 weeks from September 19, 1996 to October 8, 1998 (290 to 1040 days of age). The population was held in 1m and 2m semi-square tanks during this time.

For each individual weighing, fish were anaesthetised in a water bath containing MS-222. For each fish, the PIT-tag was scanned and the id number, weight, forklength, plus any comments on colour and maturation were recorded. The fish were returned to a tank with fresh water for recovery.

4.2.2. Statistical Methods

Data

Each individual had approximately 4 weighings recorded from 280 to 650 days of age. This age range was chosen for several reasons. First, this is a period of exponential growth, so there is a linear relationship between the natural log of weight with time and slope can be calculated easily. Second, the population was not mature at this stage, so any slowing in growth due to maturation had probably not occurred. This period also covers the earliest possible four data points and allows for some missing data points.

The instantaneous growth rate (g) for each individual was defined as the slope of the regression of the natural log of weight on age in weeks. Age in weeks was used because the slope was too small when calculated with age in days. Values of g and intercepts were calculated with PROC REG in SAS. In addition, the fitted equations

were used to predict natural logs of weights at 50, 60, 70, and 80 weeks post-fertilisation for each individual.

Only females that matured at 3 or 4 years old and males that matured at 2 or 3 years old were included in the data set. These were typical maturation ages for the populations reared under the study conditions. Others were considered outliers and removed from the data set. Missing cells caused by these outliers interfered with the analyses, especially with the calculation of interactions. The number of observations in the analyses is summarised in Table 4.1.

Table 4.1. Number of observations for all strain and maturation combinations.

Strain Combination	Maturation Category				Total
	2-year males	3-year males	3-year females	4-year females	
BB	17	16	26	2	61
BG	8	18	25	1	52
BO	22	10	31	0	63
GB	16	18	26	0	60
GG	4	21	20	7	52
GO	12	21	29	2	64
OB	24	9	23	1	57
OG	11	20	40	3	74
OO	20	6	33	2	61
Total	134	139	253	18	544

Homogeneity of Variance and Analyses of Variance

Levene's test was used to test the homogeneity of growth rate variances across all strain combinations and all maturation categories. All of the analyses of variance (ANOVA) were done with the SAS General Linear Models procedure.

Fertilisation Week Effects

The different strains' spawning seasons did not allow all possible strain combinations to be done in one week, so effects of dam strain and fertilisation week

effects were confounded. There was no overlap of O and G dams (i.e. they did not appear in the same fertilisation week). It was therefore difficult to determine if any differences were due to dam strain or to fertilisation week. In an attempt to separate confounded effects, two ANOVA were done to test the effects of fertilisation week within each dam strain. These were as shown in Model 4.1:

Model 4.1. $y_{ijkl} = \mu + S_i + M_j + F_k + e_{ijkl}$

where

y_{ijkl} is the observation of instantaneous growth rate or the natural log of weight at 50, 60, 70, or 80 weeks of age for an individual with sire strain i, maturation category j and fertilisation week k,

μ is the population mean,

S_i is the fixed effect of the sire strain i (i=1, 2, 3),

M_j is the fixed effect of the maturation category j (j=1,..., 4),

F_k is the effect of fertilisation week k (k=1, ..., 4 in O and B dams; k=1, 2, 3 in G dams),

e_{ijkl} is the residual error associated with observation $ijkl$.

The fertilisation effect was tested as a covariate in the first ANOVA, but considered as a fixed effect in the second ANOVA in order to estimate least squares means.

Strain and Maturation Effects

Models 4.2, 4.3, and 4.4 were used to test the effects of strain and maturation on growth rate and estimated weights.

Model 4.2. $y_{ijkl} = \mu + D_i + S_j + M_k + (DS)_{ij} + (DM)_{ik} + (SM)_{jk} + (DSM)_{ijk} + e_{ijkl}$

where

y_{ijkl} is the instantaneous growth rate or the natural log of weight at 50, 60, 70, or 80 weeks of age for an individual with dam strain i and sire strain j in maturation category k,

μ is the population mean,

Di is the fixed effect of the dam strain i (i=1, 2, 3),

S_j is the fixed effect of the sire strain j (j=1, 2, 3),

M_k is the fixed effect of the maturation category k (k=1,..., 4),

$(DS)_{ij}$ is the interaction of the dam and sire strains,

$(DM)_{ik}$ is the interaction of the dam strain and maturation category,

$(SM)_{jk}$ *is* the interaction of the sire strains and maturation category,

$(DSM)_{ijk}$ is the 3-way interaction of dam strain, sire strain and maturation,

e_{ijkl} is the residual error associated with observation *ijkl*.

Type IV Sums of Squares were used in Model 4.2 due to missing cells in the 3-way interaction. Least square means could not be estimated with Model 4.2 due to the unbalanced nature of the data set and some missing interaction cells. Therefore, the 3-way interaction was removed (Model 4.3) to estimate least squares means.

Model 4.3. $y_{ijkl} = \mu + D_i + S_j + M_k + (DS)_{ij} + (DM)_{ik} + (SM)_{jk} + e_{ijkl}$

All variables in Model 4.3 are as defined for Model 4.2. There were no missing cells for Model 4.3 so Type III Sums of Squares were used and least squares means could be estimated.

Heterosis between two strains was tested by using contrasts of the mean performance of pure strains versus the mean performance of hybrids with Model 4.3.

Contrasts between reciprocal hybrid crosses were non-estimable with Model 4.3, so reciprocal contrasts were tested with Model 4.4.

Model 4.4. $y_{ikl} = \mu + C_i + M_k + e_{ikl}$

where

y_{ikl} is the instantaneous growth rate or the natural log of weight at 50, 60, 70, or 80 weeks of age for an individual in strain combination i and maturation category k,

C_i is the fixed effect of the strain combination i (i=1, ..., 9),

e_{ikl} is the residual error associated with observation ikl.

The remaining effects are as defined for Model 4.2. Two types of contrasts were done with Model 4.4: contrasting hybrids for whether a strain is used as a dam or a sire, or contrast reciprocal crosses.

4.3. RESULTS

Complete ANOVA tables are presented in Appendix 4.1 and figures are presented in Appendix 4.2.

4.3.1. Homogeneity of variances

Strain variances were homogeneous, and in addition, sample sizes were comparable, therefore ANOVA was considered appropriate without any further data transformations. Maturation variances were heterogeneous (p=0.0416) and sample sizes were very different across categories (there were especially low numbers in the 4-year maturing female category), so ANOVA may not have been reliable in this case.

4.3.2. Fertilisation week effects

Relationships between growth rate and fertilisation week in offspring of all dam strains are shown in Figure 4.1. Growth rate increased with fertilisation date in offspring of O and G dams, but not in offspring of B dams (Table 4.2). Relationships between weight and fertilisation week for offspring of each dam strain are plotted in Figures 4.2 to 4.4. Natural logs of weight increased with fertilisation date only in offspring of O dams (Table 4.2).

Table 4.2. Model 4.1 ANOVA results. Significance of effects on growth rate (*g*) and natural logs of weight at 50, 60, 70 and 80 weeks within dam strain with fertilisation week as a covariate.

Source	df	*g*	Pr > F 50 weeks	60 weeks	70 weeks	80 weeks
O dams						
Model	6	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Sire strain	2	0.6208	<0.0001	<0.0001	<0.0001	<0.0001
Maturation category	3	0.0152	<0.0001	<0.0001	<0.0001	<0.0001
Fertilisation week	1	0.0003	0.0022	<0.0001	<0.0001	<0.0001
Error	184					
B dams						
Model	6	0.0006	<0.0001	<0.0001	<0.0001	<0.0001
Sire strain	2	0.0007	<0.0001	<0.0001	<0.0001	<0.0001
Maturation category	3	0.0011	<0.0001	<0.0001	<0.0001	<0.0001
Fertilisation week	1	0.4978	0.2779	0.2985	0.3385	0.4028
Error	169					
G dams						
Model	6	0.0003	<0.0001	<0.0001	<0.0001	<0.0001
Sire strain	2	0.0101	<0.0001	<0.0001	<0.0001	<0.0001
Maturation category	3	0.2873	<0.0001	<0.0001	<0.0001	<0.0001
Fertilisation week	1	0.0005	0.6269	0.8236	0.2885	0.0535
Error	170					

4.3.3. Strain Effects

Model 4.2. 2-way and 3-way interactions among main effects

For growth rate, all interactions were non-significant. Dam strain, sire strain and maturation effects were highly significant (Table 4.3). For natural logs of weight, 3-way interactions were never significant, but significant 2-way interactions between dam strain and sire strain and between sire strain and maturation category occurred at higher ages.

Table 4.3. Model 4.2 ANOVA results. Significance of effects on growth rate (*g*) and natural logs of weights at 50, 60, 70, and 80 weeks old.

Source	df	Pr > F				
		g	50 weeks	60 weeks	70 weeks	80 weeks
Model	34	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Dam strain	2	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Sire strain	2	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Maturation	3	0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Dam strain×Sire strain	4	0.1425	0.0792	0.0318	0.0111	0.0044
Dam strain × Maturation	6	0.5922	0.8281	0.7493	0.6306	0.5028
Sire strain × Maturation	6	0.1423	0.1931	0.0494	0.0081	0.0014
Dam strain × Sire strain × Maturation	11	0.3532	0.3323	0.3651	0.4084	0.4532
Error	509					
Corrected Total	543					

Model 4.3. Least squares means and heterosis

Significant dam strain by sire strain interactions occurred for growth rate and natural logs of weights at 70 and 80 weeks old, meaning that the differences between sire strains depended on the dam strain. Therefore, main dam strain and sire strain effects could not be examined. There was also a significant sire strain by maturation interaction for the natural log of weight at 80 weeks old.

Table 4.4. Model 4.4 ANOVA results. Significance of effects on growth rate (g) and natural logs of weights at 50, 60, 70, and 80 weeks old.

Source	df		Pr > F			
		g	50 weeks	60 weeks	70 weeks	80 weeks
Model	23	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Dam strain	2	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Sire strain	2	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Maturation	3	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Dam strain×Sire strain	4	0.0123	0.3776	0.1276	0.0227	0.0029
Dam strain × Maturation	6	0.3245	0.8910	0.7873	0.5939	0.3801
Sire strain × Maturation	6	0.0753	0.4901	0.2517	0.0837	0.0219
Error	520					
Corrected Total	543					

Least squares means for growth rates and natural logs of weights at 50, 60, 70 and 80 weeks of each strain combination are summarised in Table 4.5. The trends of growth rates for dam and sire strains were opposite to each other: those with G dams grew fastest, those with B dams were intermediate, and those with O dams were slowest; this ranking was reversed among the sire strains.

Least squares means for natural logs of weights at 50, 60, 70 and 80 weeks are also plotted in Figures 4.5 to 4.8. In terms of actual weights, the overall between-strain trends were the same: those with O parents (dams or sires) were the largest, those with B parents were intermediate, and those with G parents were the smallest. Hybrids had intermediate weights to the pure strains, except for B-G hybrids after 60 weeks of age.

Table 4.5. Least squares means ± SE for growth rate (*g*) and natural logs of weight at 50, 60, 70 and 80 weeks of age.

			Sire Strain		
			O	B	G
Dam Strain	O	*g*	*0.06089 ±0.0013*	*0.05815 ±0.0015*	*0.058160 ±0.0011*
		50	4.324 ± 0.068	3.751 ± 0.081	3.814 ± 0.060
		60	4.933 ± 0.063	4.332 ± 0.075	4.423 ± 0.056
		70	5.541 ± 0.060	4.914 ± 0.072	5.004 ± 0.054
		80	6.150 ± 0.060	5.495 ± 0.072	5.586 ± 0.053
	B	*g*	*0.06741 ±0.0014*	*0.06227 ±0.1112*	*0.06053 ±0.0015*
		50	3.950 ± 0.077	3.402 ± 0.063	3.306 ± 0.079
		60	4.624 ± 0.072	4.025 ± 0.058	3.911 ± 0.074
		70	5.298 ± 0.069	4.648 ± 0.056	4.517 ± 0.070
		80	5.972 ± 0.069	5.271 ± 0.056	5.122 ± 0.070
	G	*g*	*0.07126 ±0.0012*	*0.06910 ±0.0017*	*0.06370 ±0.0011*
		50	3.877 ± 0.066	3.316 ± 0.089	3.298 ± 0.058
		60	4.589 ± 0.061	4.007 ± 0.083	3.935 ± 0.054
		70	5.302 ± 0.059	4.698 ± 0.079	4.572 ± 0.052
		80	6.014 ± 0.058	5.389 ± 0.079	5.209 ± 0.052

All contrasts were considered significant for $p<0.05$. Least squares means and contrast results for growth rates within and between parent strains are shown in Tables 4.5 and 4.6, respectively. There were no differences in growth rates within O dams. Within B dams, offspring of O sires had a faster growth rate than offspring of B and G sires. For G dams, offspring of O and B sires had a faster growth rate than offspring of G sires. Within offspring of O and B sires, growth rates of offspring of G sires was fastest, offspring of B sires were intermediate, and offspring of O sires were slowest. Within offspring of G sires, offspring of G dams had a faster growth rate than offspring of O dams, yet produced smaller progeny.

Table 4.6. Significance of offspring growth rate contrasts within and between parent strains.

Within Dam Strain Contrast	Pr > F	Within Sire Strain Contrast	Pr > F
within O dam strain		*within O sire strain*	
O sires vs. B sires	0.1306	O dams vs. B dams	0.0001
O sires vs. G sires	0.0774	O dams vs. G dams	<0.0001
B sires vs. G sires	0.9944	B dams vs. G dams	0.0261
within B dam strain		*within B sire strain*	
O sires vs. B sires	0.0035	O dams vs. B dams	0.0157
O sires vs. G sires	<0.0001	O dams vs. G dams	<0.0001
B sires vs. G sires	0.3289	B dams vs. G dams	0.0001
within G dam strain		*within G sire strain*	
O sires vs. B sires	0.2385	O dams vs. B dams	0.1609
O sires vs. G sires	<0.0001	O dams vs. G dams	0.0003
B sires vs. G sires	0.0049	B dams vs. G dams	0.0763

Positive heterosis for growth rate only occurred in crosses of O and G strains (Table 4.7). The O/G hybrids on average grew faster than the average of the pure O and G strains. In these strains, the average weight of the hybrids was not different from the average weight of the pure strains (no heterosis) at 50 and 60 weeks of age, but at 70 and 80 weeks, the average weight of the hybrids was higher than the average weight of the pure strains.

Table 4.7. Significance of heterosis contrasts for growth rate (g) and natural logs of weight at 50, 60, 70 and 80 weeks of age.

Heterosis Contrast		Pr > F			
	g	50 wk	60 wk	70 wk	80 wk
B / G (BB + GG vs. BG + GB)	0.0587	0.4502	0.6646	0.9563	0.7313
B / O (BB + OO vs. BO + OB)	0.1879	0.7921	0.9848	0.7971	0.5930
G / O (GG + OO vs. GO + OG)	0.0102	0.3407	0.1223	0.0314	0.0071

Model 4.5. Reciprocal contrasts

Least squares means for growth rates in the Model 4.5 differed from those in the in the original model. In hybrid crosses, offspring had faster growth when B and G were

used as dam strains than when they were used as sire strains (Table 4.8). Offspring had faster growth when O was used as a sire strain than as a dam strain. Reciprocal crosses had significantly different growth rates. Since reciprocal crosses had different growth rates, even though there was no difference between their weights at 50 weeks, differences between reciprocals' weights increased with time. Reciprocal crosses that had the faster-growing strain as a sire grew faster than those who had that strain as a dam.

Table 4.8. Significance of within-strain dam and sire contrasts and reciprocal contrasts for growth rate (g) and natural logs of weight at 50, 60, 70 and 80 weeks of age.

Contrast		Pr > F			
	g	50 weeks	60 weeks	70 weeks	80 weeks
B (BG+BO vs. GB+OB)	0.0160	0.3157	0.1174	0.0330	0.0087
G (B+GO vs. BG+OG)	<0.0001	0.6874	0.0388	0.0001	<0.0001
O (B+OG vs. BO+GO)	<0.0001	0.1428	0.0002	<0.0001	<0.0001
BG vs. GB	0.0004	0.6626	0.2361	0.0485	0.0069
BO vs. OB	<0.0001	0.0578	0.0005	<0.0001	<0.0001
GO vs. OG	<0.0001	0.9058	0.0760	0.0004	<0.0001

4.3.4. Maturation Effects

Least squares means for maturation levels are summarised in Table 4.9 and Figure 4.9. Contrasts are summarised in Table 4.10. Males grew faster than females. Males that matured at 3 years old grew faster than females that matured at 3 years old. There was no difference in growth rate between ages at maturation within either sex.

Generally, weights were different between categories: males were heavier than females, 2-year maturing males were heavier than 3-year maturing males, and 3-year maturing females were heavier than 4-year maturing females. The differences in weight between females and males that both matured at 3 years old decreased with time. Since

males that matured at 3 years old had a faster growth rate, by 80 weeks of age the weights of these groups were not significantly different.

Table 4.9. Least squares means ± SE for for growth rate (g) and natural logs of weight at 50, 60, 70 and 80 weeks of age in four maturation categories.

Maturation Category	g	LSMean ± SE 50 weeks	60 weeks	70 weeks	80 weeks
2-year males	0.06472 ± 0.00068	3.993 ± 0.037	4.640 ± 0.034	5.288 ± 0.033	5.935 ± 0.033
3-year males	0.06428 ± 0.00063	3.698 ± 0.034	4.341 ± 0.031	4.983 ± 0.030	5.626 ± 0.030
3-year females	0.06159 ± 0.00044	3.823 ± 0.024	4.439 ± 0.022	5.055 ± 0.021	5.671 ± 0.021
4-year females	0.06340 ± 0.00172	3.181 ± 0.092	3.815 ± 0.086	4.449 ± 0.082	5.083 ± 0.082

Table 4.10. Significance of maturation category contrasts for growth rate (g) and natural logs of weight at 50, 60, 70 and 80 weeks of age.

Contrast	g	Pr > F 50 weeks	60 weeks	70 weeks	80 weeks
females vs. males	0.0474	<0.0001	<0.0001	<0.0001	<0.0001
males: 2-year vs. 3-year	0.6361	<0.0001	<0.0001	<0.0001	<0.0001
females: 3-year vs. 4-year	0.3092	<0.0001	<0.0001	<0.0001	<0.0001
3-year: females vs. males	0.0005	0.0024	0.0094	0.0482	0.2143

4.4. Discussion and Conclusions

4.4.1. Fertilisation week

Based on the within-dam strain analyses, no general fertilisation week effect within dam strain could be shown. Any overall time effect for all strains was confounded with dam strain, and including this effect in the model could have artificially reduced the dam strain effect. There was not much overlap in time between dam strains, and the

regression slopes may not have been parallel. There was probably not a large enough time range in each strain to conclude if there was any real within-dam strain pattern.

4.4.2. Strain rankings and interactions

Model 4.3, which included the 3-way interaction between dam strain, sire strain, and maturation category, showed no significant interactions between these effects, and all factors had highly significant effects on instantaneous growth rate. When the 3-way interaction was removed in Model 4.4, significant dam strain by sire strain interactions occurred for growth rate meaning that the differences between sire strains depended on the dam strain and vice versa. Therefore, main dam strain and sire strain effects could not be examined. Least squares means of growth rates of each strain combination showed trends of growth rates for dam and sire strains were opposite to each other. Those with G dams grew fastest, those with B dams were intermediate, and those with O dams were slowest. This ranking was reversed among the sire strains, which was unexpected. Strain rankings for absolute size, however, were consistent with both dam and sires. Offspring with O parents were largest, those with B parents were intermediate, and offpring of G parents were smallest. The larger strains may have had slower growth rates because they were further along a sigmoidal growth curve than the smaller strain, and growth had slowed. It is possible that offspring of G dams could have a faster growth rate if they were still in the steep section of a sigmoidal growth curve and the other strains were past this, but dam and sire strains would still be expected to act in the same way. Differences in weights at the same ages may have been due to groups of the same age being measured at different times of the year. Within-parent strain contrasts for growth rate also showed rankings of strains depended upon whether they were used as a dam or as a sire.

Generally, there were more differences in the within-sire strain contrasts than in the within-dam contrasts (Table 4.6). This suggests that strain of the dam may have had a greater effect on the offspring's growth rate during this period than the strain of the sire, but this contradicts the paternal effects found in the reciprocal cross contrasts. It is more probable that the opposing rankings of parent strains and increased impact of dam strain may have been due to a confounded effect of fertilisation week on growth rate such that growth in groups with earlier fertilisation dates was slowing down and coming out of the exponential phase.

Heterosis

Heterosis for growth rate only occurred in crosses of O and G strains. The O/G hybrids grew faster than the average of the two pure strains, so at 70 and 80 weeks the average weight of the hybrids was more than the average weight of the two pure strains. Any inbreeding depression that may have affected growth in the O strain could have been relieved by crossing with the more wild-type G strain, but this is unlikely since no such interaction was found when O and B strains were crossed.

Reciprocal contrasts

Least squares means for growth rates in Model 4.5 differed from those in the in the original model. All reciprocal crosses had significantly different growth rates. B and G strains produced faster offspring growth rates when used as dams than when used as sires. O produced a faster growth rate as a sire strain than as a dam strain. These results agreed with the opposite trends of growth rates between dam strain and sire strain discussed above. Therefore, it mattered if a strain was used as a sire or as a dam in a cross.

Generally, weight differences between reciprocal crosses increased with time. This agrees with the different slopes found between reciprocals. Reciprocal crosses that had the faster-growing strain as a sire grew faster than those who had that strain as a dam, therefore hybrids appeared to grow more like the strain of their sires. This observation suggests that there may some paternal effect on growth between 280 and 650 days post-fertilisation. Paternal effects have not been observed in other salmonid studies, but one explanation might be the very low recombination rates in male rainbow trout compared with females (Sakamoto et al., 2000). If sire recombination rates were lower than those of dams, then genes transmitted from sires might not be as randomly assorted as genes transmitted from dams. Progeny would then tend to perform more like their sires (R. Danzmann, personal communication).

4.4.3. Maturation Effects

Growth rates differed between sexes such that on average, males grew faster than females. Males that matured at 3 years old had faster growth rates than females that matured at the same age, so even though these males weighed less than these females at 50 weeks of age, at 80 weeks the weights of the two sexes were not significantly different. Time of maturation also caused differences in size: early-maturing fish were heavier than later-maturing ones. Within sexes, there were no differences in growth rate between early- and late-maturing groups. Differential growth related to maturation occurs very early in life, within one year following fertilisation. This was unexpected as it was thought that approaching maturation might cause some differences in growth rate during this time period. During the time period studied, weight was a better indicator of the timing of maturation than instantaneous growth rate.

APPENDIX 4.1. GROWTH ANALYSIS OF VARIANCE TABLES

Table 4A.1. Model 4.2 growth rate ANOVA.

Source	df	MS	F-value	Pr>F
Model	34	0.000315	6.51	<0.0001
Dam strain	2	0.001497	30.87	<0.0001
Sire strain	2	0.000492	10.15	<0.0001
Maturation	3	0.000341	7.04	0.0001
Dam strain × Sire strain	4	0.000084	1.73	0.1425
Dam strain × Maturation	6	0.000037	0.77	0.5922
Sire strain × Maturation	6	0.000078	1.61	0.1423
Dam strain × Sire strain × Maturation	11	0.000054	1.11	0.3532
Error	509	0.000048		
Corrected Total	543			

Table 4A.2. Model 4.2 natural log of weight at 50 weeks of age ANOVA.

Source	df	MS	F-value	Pr>F
Model	34	2.292982	16.48	<0.0001
Dam strain	2	5.549886	39.89	<0.0001
Sire strain	2	7.541935	54.20	<0.0001
Maturation	3	3.188899	22.92	<0.0001
Dam strain × Sire strain	4	0.292683	2.10	0.0792
Dam strain × Maturation	6	0.065886	0.47	0.8281
Sire strain × Maturation	6	0.201963	1.45	0.1931
Dam strain × Sire strain × Maturation	11	0.157755	1.13	0.3323
Error	509	1.139145		
Corrected Total	543			

Table 4A.3. Model 4.2 natural log of weight at 60 weeks of age ANOVA.

Source	df	MS	F-value	Pr>F
Model	34	2.203630	18.41	<0.0001
Dam strain	2	3.953504	33.03	<0.0001
Sire strain	2	8.744788	73.07	<0.0001
Maturation	3	3.415063	28.54	<0.0001
Dam strain × Sire strain	4	0.319033	2.67	0.0318
Dam strain × Maturation	6	0.068976	0.58	0.7493
Sire strain × Maturation	6	0.254011	2.12	0.0494
Dam strain × Sire strain × Maturation	11	0.130724	1.09	0.3651
Error	509	0.119680		
Corrected Total	543			

Table 4A.4. Model 4.2 natural log of weight at 70 weeks of age ANOVA.

Source	df	MS	F-value	Pr>F
Model	34	2.177362	19.81	<0.0001
Dam strain	2	2.656424	24.17	<0.0001
Sire strain	2	10.106031	91.40	<0.0001
Maturation	3	3.709480	33.75	<0.0001
Dam strain × Sire strain	4	0.362132	3.29	0.0111
Dam strain × Maturation	6	0.079551	0.72	0.6306
Sire strain × Maturation	6	0.321664	2.93	0.0081
Dam strain × Sire strain × Maturation	11	0.114426	1.04	0.4084
Error	509	0.109909		
Corrected Total	543			

Table 4A.5. Model 4.2 natural log of weight at 80 weeks of age ANOVA.

Source	df	MS	F-value	Pr>F
Model	34	2.214179	20.16	<0.0001
Dam strain	2	1.658644	15.10	<0.0001
Sire strain	2	11.445666	104.21	<0.0001
Maturation	3	4.072148	37.08	<0.0001
Dam strain × Sire strain	4	0.421980	3.84	0.0044
Dam strain × Maturation	6	0.097610	0.89	0.5028
Sire strain × Maturation	6	0.414921	3.69	0.0014
Dam strain × Sire strain × Maturation	11	0.108860	0.99	0.4532
Error	509	0.109833		
Corrected Total	543			

Table 4A.6. Model 4.3 growth rate ANOVA.

Source	df	MS	F-value	Pr>F
Model	23	0.000440	9.07	<0.0001
Dam strain	2	0.001500	30.87	<0.0001
Sire strain	2	0.000596	12.26	<0.0001
Maturation	3	0.000336	6.91	<0.0001
Dam strain × Sire strain	4	0.000157	3.23	0.0123
Dam strain × Maturation	6	0.000057	1.16	0.3245
Sire strain × Maturation	6	0.000093	1.92	0.0753
Error	520	0.000049		
Corrected Total	543			

Table 4A.7. Model 4.3 natural log of weight at 50 weeks of age ANOVA.

Source	df	MS	F-value	Pr>F
Model	23	3.314178	23.75	<0.0001
Dam strain	2	4.928229	35.32	<0.0001
Sire strain	2	6.855873	49.13	<0.0001
Maturation	3	3.818816	27.37	<0.0001
Dam strain × Sire strain	4	0.147380	1.06	0.3776
Dam strain × Maturation	6	0.053211	0.38	0.8910
Sire strain × Maturation	6	0.126436	0.91	0.4901
Error	520	0.139539		
Corrected Total	543			

Table 4A.8. Model 4.3 natural log of weight at 60 weeks of age ANOVA.

Source	df	MS	F-value	Pr>F
Model	23	3.195020	26.64	<0.0001
Dam strain	2	3.462360	28.87	<0.0001
Sire strain	2	8.125844	67.76	<0.0001
Maturation	3	3.892033	32.46	<0.0001
Dam strain × Sire strain	4	1.215785	1.80	0.1276
Dam strain × Maturation	6	0.063294	0.53	0.7873
Sire strain × Maturation	6	0.156843	1.31	0.2517
Error	520	0.119913		
Corrected Total	543			

Table 4A.9. Model 4.3 natural log of weight at 70 weeks of age ANOVA.

Source	df	MS	F-value	Pr>F
Model	23	3.163984	28.76	<0.0001
Dam strain	2	2.296430	20.88	<0.0001
Sire strain	2	9.514956	86.50	<0.0001
Maturation	3	4.032391	36.66	<0.0001
Dam strain × Sire strain	4	0.315593	2.87	0.0227
Dam strain × Maturation	6	0.084681	0.77	0.5939
Sire strain × Maturation	6	0.205933	1.87	0.0837
Error	520	1.110005		
Corrected Total	543			

Table4A.10. Model 4.3 natural log of weight at 80 weeks of age ANOVA.

Source	df	MS	F-value	Pr>F
Model	23	3.221071	29.33	<0.0001
Dam strain	2	1.430440	13.03	<0.0001
Sire strain	2	11.023208	100.38	<0.0001
Maturation	3	4.239890	38.61	<0.0001
Dam strain × Sire strain	4	0.446802	4.07	0.0029
Dam strain × Maturation	6	0.117373	1.07	0.3801
Sire strain × Maturation	6	0.273704	2.49	0.0219
Error	520	0.109813		
Corrected Total	543			

APPENDIX 4.2. GROWTH FIGURES

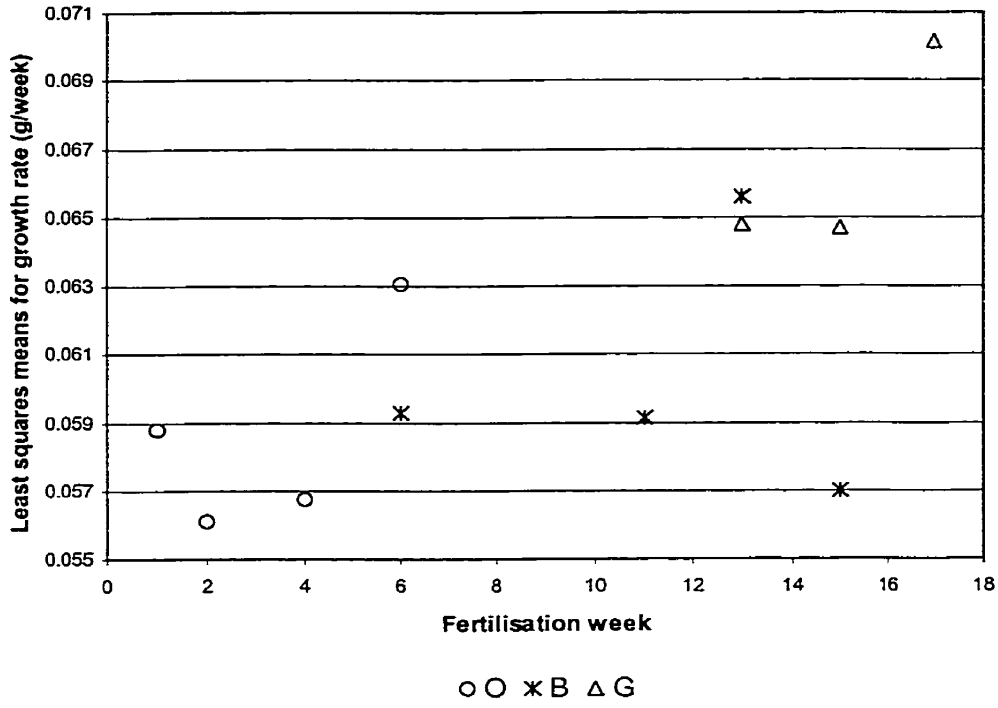

Figure 4. 1. Growth rate in offspring of O, B, and G dams fertilised over time.

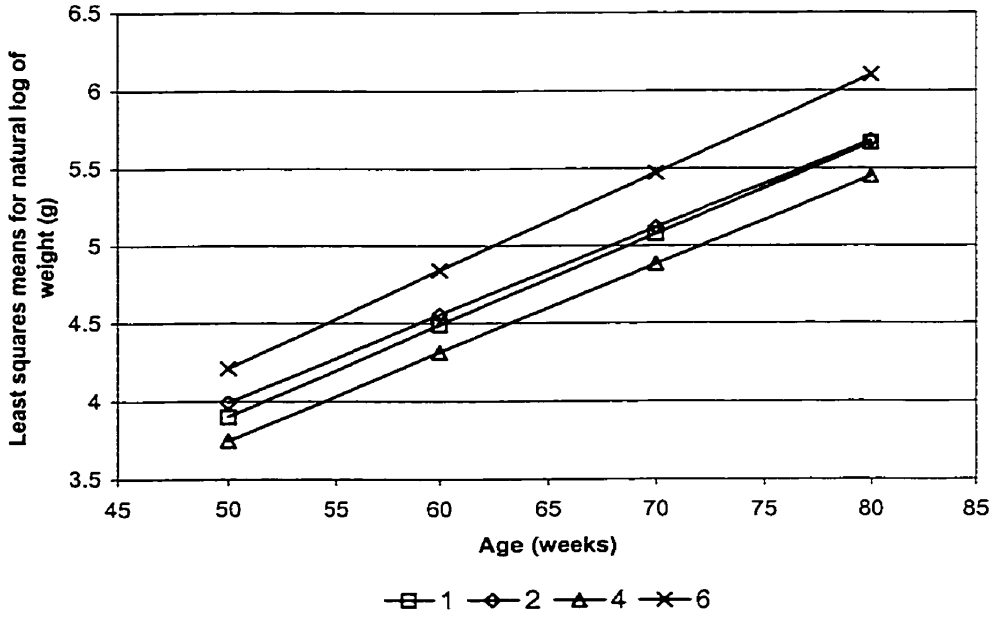

Figure 4.2. Growth in offspring of O dams fertilised in weeks 1, 2, 4, and 6.

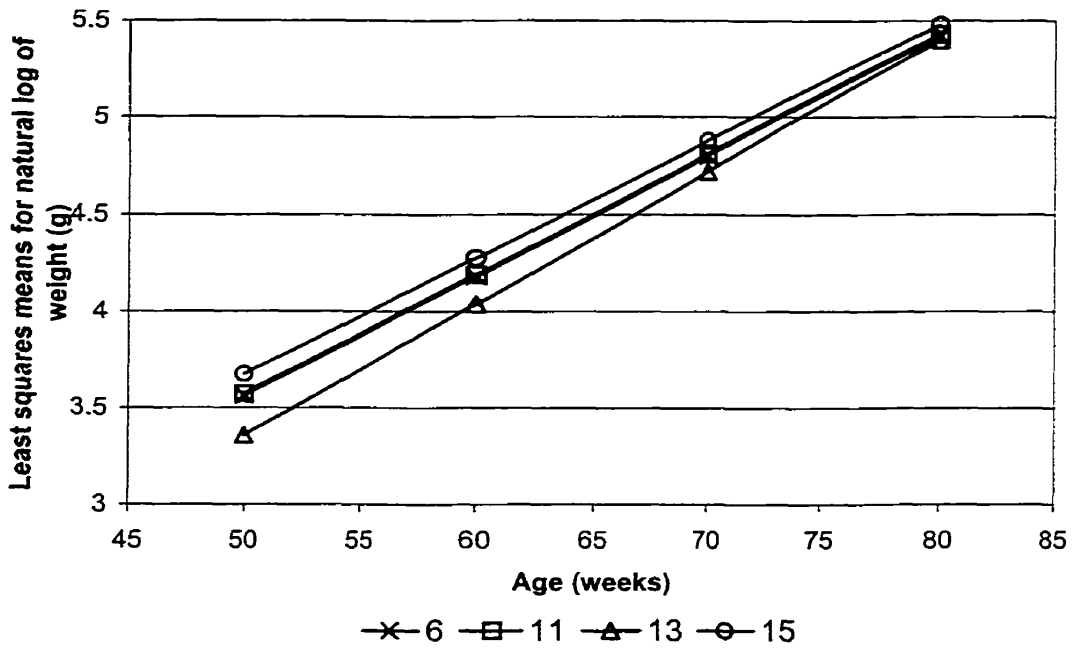

Figure 4.3. Growth in offspring of B dams fertilised in weeks 6, 11, 13, and 15.

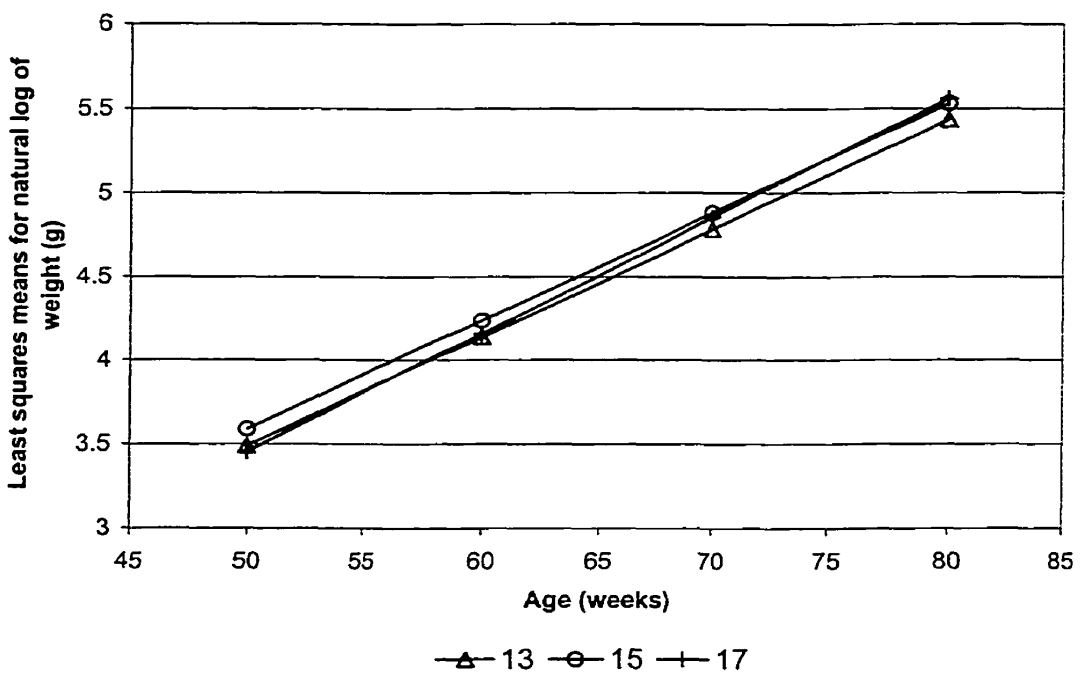

Figure 4. 4. Growth in offspring of G dams fertilised in weeks 13, 15, and 17.

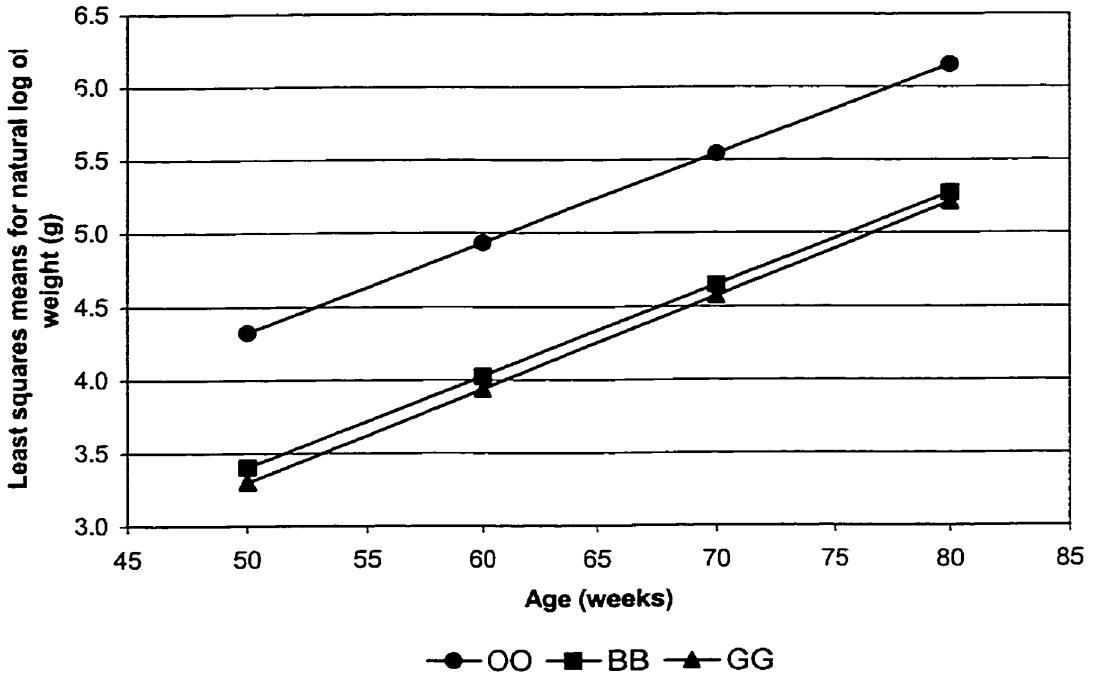

Figure 4. 5. Growth in pure strains.

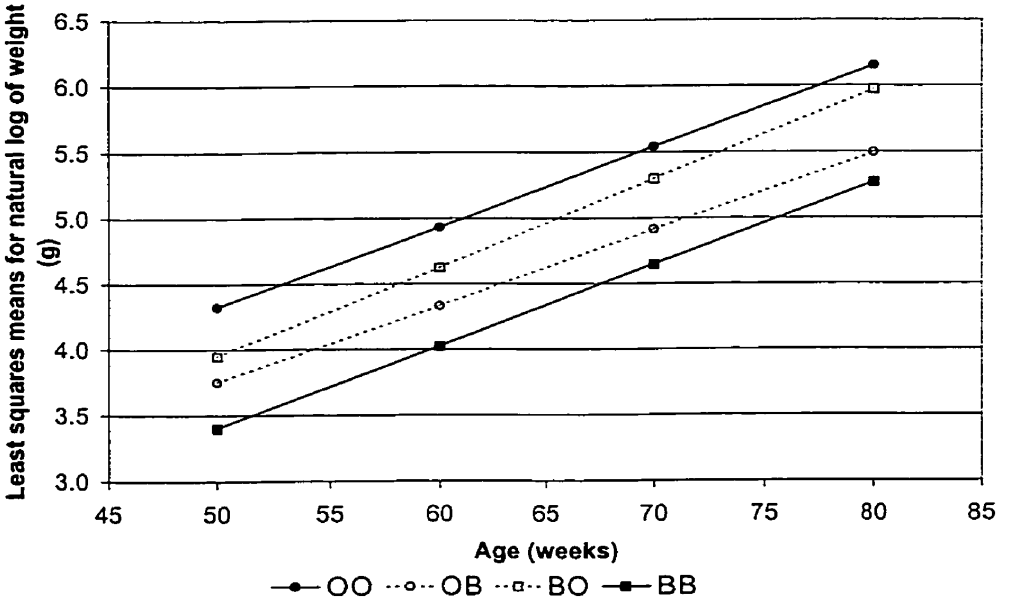

Figure 4. 6. Growth in pure O and B strains and their hybrids.

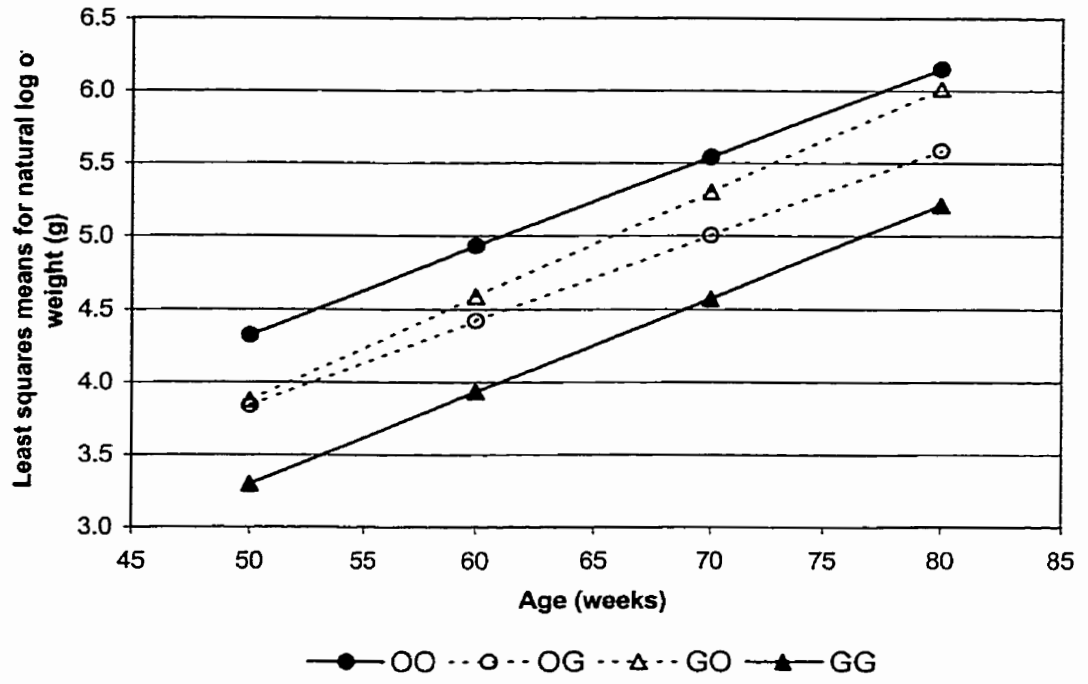

Figure 4. 7. Growth in pure O and G strains and their hybrids.

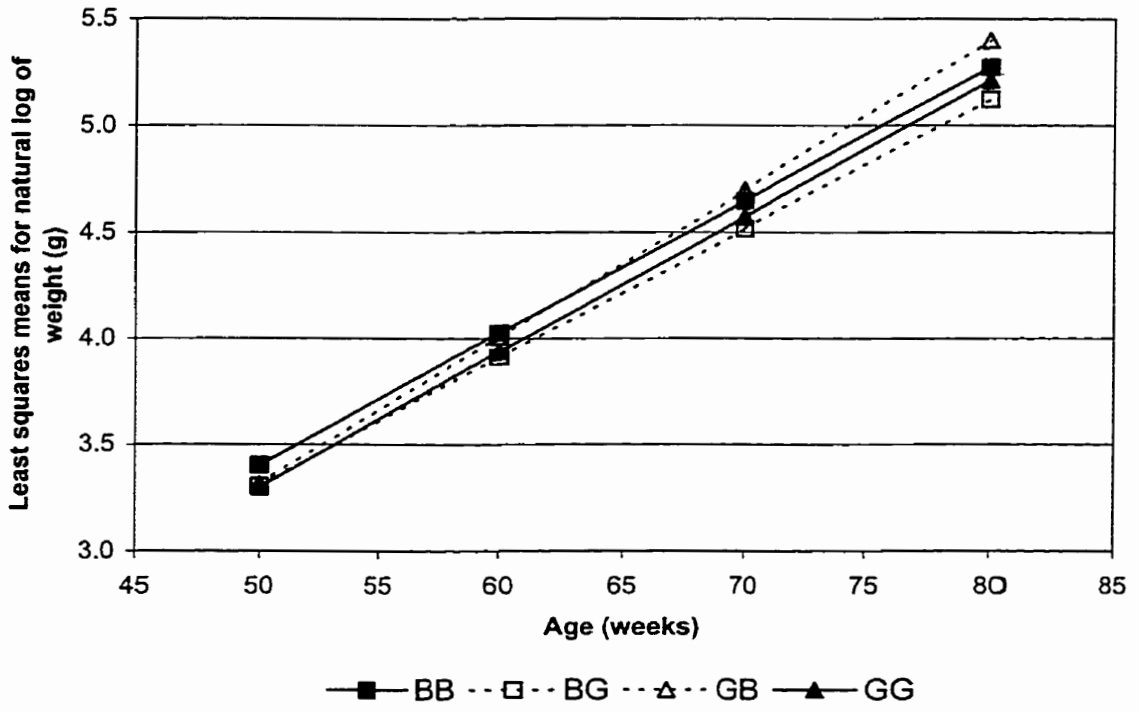

Figure 4. 8. Growth in pure B and G strains and their hybrids.

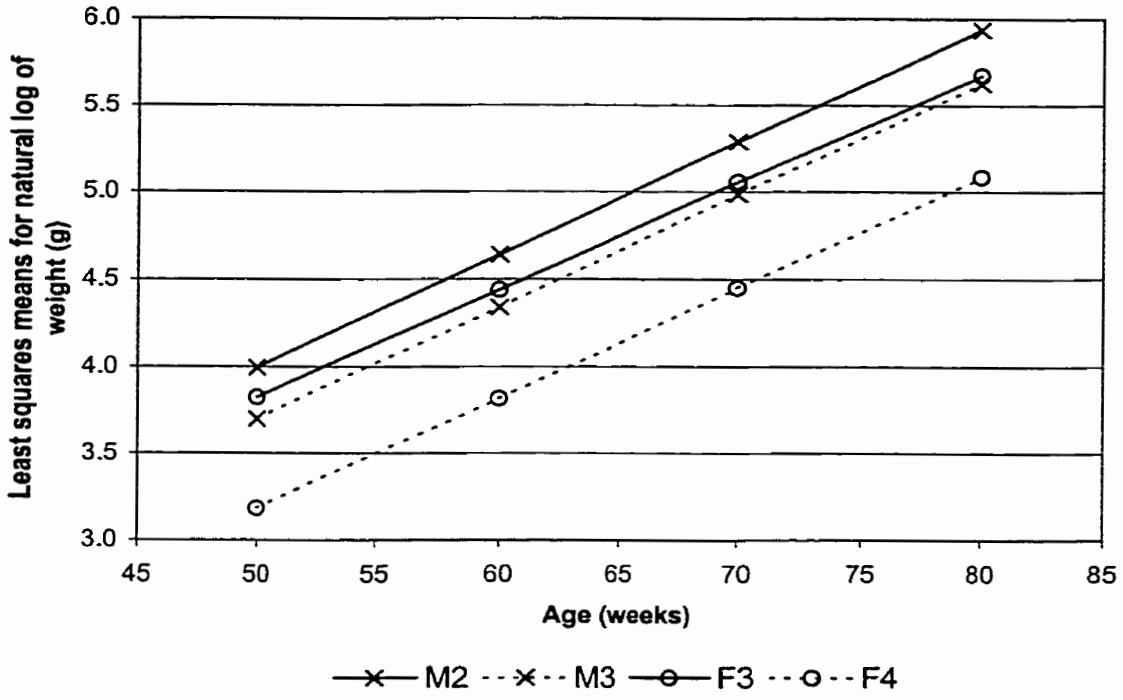

Figure 4. 9. Growth in 2-year maturing males (M2), 3-year maturing males (M3), 3-year maturing females (F3), and 4-year maturing females (F4).

CHAPTER 5. SPAWNING TIME ANALYSIS

5.1. INTRODUCTION

5.1.1. Definition

Female spawning time refers to the time of year when eggs are mature and released from the ovaries. In this population of rainbow trout, females spawned from October to May. Spawning dates were recorded at 3 and 4 years of age for females in the 91, 94 and 96 year classes.

5.1.2. Purposes of study

There were five main objectives for this study. First, to determine the effects of fertilisation week on spawn date. Second, to rank strains according to spawn date. Third, to determine types of strain interactions that affected spawn date, such as heterosis or differences between reciprocal crosses. Fourth, to find effects of age of sexual maturation on spawn date, and fifth, to calculate the repeatability of spawn date from 3 years old to 4 years old.

5.2. MATERIALS AND METHODS

5.2.1. Data Collection

Imminent female spawning was assessed by colour, look and feel of the abdomen, and vent extension. Females judged close to spawning (within the following 3 weeks) were placed in separate "soon" tanks to be checked every week. Males were also placed in tanks for pheromonal stimulation.

Each ripe female was weighed before and after stripping, and her forklength was measured. Eggs were stripped by applying manual pressure to the abdomen. The eggs were stored in labelled plastic tubs and refrigerated, to be measured later. If not all eggs could be stripped easily, the female was returned to the soon tank and remaining eggs were stripped the following week. The same measurements may or may not have been repeated in the second week, depending on the amount of eggs left over. Generally, if more than 50 ml of eggs were stripped in the second week, all measurements (including body weights and length) were repeated. If 30 to 50 ml were stripped in the second week, only total egg volume was measured. Volumes less than 30 ml were approximated by eye. When all eggs were stripped, the female was placed in a 2m "spent" tank for recovery. Females in the spent tank were left alone for the remainder of the spawning season.

5.2.2. Statistical Methods

Data

Data consisted of female spawning dates at 3 and 4 years of age in parental and in both diallel cross year classes. The numbers of observations used in analyses are summarised in Table 5.1. To simplify analyses, spawning date was defined as the period after October 1 (in days) that a female was ripe.

Table 5.1. Numbers of observations for spawning date analyses. ASM = age at sexual maturation.

Group	Year class	Strain Combination	3-year spawning N ASM = 3	4-year spawning N ASM=3	4-year spawning N ASM=4
Parent	91	B	76	67	17
		G	27	22	13
		O	42	35	8
Offspring	94	BB	21	20	1
		BG	11	11	3
		BO	10	9	3
		GB	6	5	5
		GG	5	4	3
		GO	5	5	5
		OB	3	2	1
		OG	2	2	1
		OO	5	2	2
	96	BB	23	20	3
		BG	25	15	2
		BO	29	25	1
		GB	27	20	0
		GG	19	15	7
		GO	29	25	2
		OB	23	22	2
		OG	39	32	3
		OO	26	18	1

Homogeneity of Variance and Analyses of Variance

Levene's test was used to test homogeneity of spawn date variances across all strain combinations within each year class. All of the analyses of variance (ANOVA) were done with the SAS General Linear Models procedure.

Fertilisation Week Effects

The effects of fertilisation week (see section 4.2.2) on 3-year old female spawn date were examined within dam strains of each progeny year class. In the 94 year class, the O dam strain was only used in fertilisation week 1, so no tests were done for this

group. Model 5.1 was used to test within B and G dam strains in the 94 year class, and within all dam strains in the 96 year class, as follows:

Model 5.1. $y_{ijk} = \mu + S_i + F_j + e_{ijk}$

where

y_{ijk} is the observation of 3-year old spawn date for an individual with sire strain i and fertilisation week j,

μ is the population mean,

S_i is the fixed effect of the sire strain i (i=1, 2, 3),

F_k is the effect of fertilisation week k (k=1, ..., 4 in 94 year class B dams, and 96 year class O and B dams; k=1, 2, 3 in 94 and 96 year class G dams),

e_{ijk} is the residual error associated with observation ijk.

The fertilisation effect was tested as a covariate in the first ANOVA, but considered as a fixed effect in the second ANOVA in order to estimate least squares means. An effect year of maturation was included in the 4-year old spawn date analysis. Three-year old spawning data came only from 3-year maturing females, whereas 4-year old spawning data came from 3-year and 4-year maturing females. The effects of fertilisation week on 4-year old female spawn date were examined using Model 5.2 as follows:

Model 5.2. $y_{ijkl} = \mu + S_i + M_j + F_k + e_{ijkl}$

where

y_{ijkl} is the 4-year old spawn date for an individual with sire strain i, maturation year j and fertilisation week k,

M_j is the fixed effect of maturation year j (j=1, 2),

e_{ijkl} is the residual error associated with observation *ijkl*.

The remaining variables are as defined for Model 5.1.

Strain and Maturation Effects

Model 5.3 was used to test the effects of strain on 3-year old spawn date in all year classes, as follows:

Model 5.3. $y_{ijkl} = \mu + Y_i + D_j + S_k + (YD)_{ij} + (YS)_{ik} + (DS)_{jk} + (YDS)_{ijk} + e_{ijkl}$

where

y_{ijkl} is the 3-year old spawn date for an individual in year class i, with dam strain j and sire strain k,

μ is the population mean,

Y_i is the fixed effect of year class i (i=1, 2, 3),

D_j is the fixed effect of the dam strain j (j=1, 2, 3),

S_k is the fixed effect of the sire strain k (k=1, 2, 3),

$(YD)_{ij}$ is the interaction of the year class and dam strain,

$(YS)_{ik}$ is the interaction of the year class and sire strain,

$(DS)_{jk}$ *is* the interaction of the dam and sire strains,

$(YDS)_{ijk}$ is the 3-way interaction of year class, dam strain and sire strain,

e_{ijkl} is the residual error associated with observation *ijkl*.

Least squares means for Model 5.3 were non-estimable, so spawn dates in the parental and offspring generations were tested separately. Model 5.4 was used to test the effects of strain on 3-year old spawn date in the parental year class, as follows:

Model 5.4. $y_{ij} = \mu + P_i + e_{ij}$

where

y_{ij} is the 3-year old spawn date for an individual in pure strain i,

μ is the population mean,

P_i is the fixed effect of the pure strain i (i=1, 2, 3),

e_{ij} is the residual error associated with observation ij.

Model 5.5 was used to test the effects of strain on 3-year old spawn date in the offspring year classes, as follows:

Model 5.5. $y_{ijkl} = \mu + Y_i + D_j + S_k + (DS)_{jk} + e_{ijkl}$

where

Y_i is the fixed effect of year class i (i=1, 2).

The remaining variables are as defined for Model 5.3. It would have been preferable for offspring models to include all possible interactions between the main effects, however the experiment was not designed to test all interactions. Highly unbalanced data such as this can make analysis of interactions unreliable. Since the two offspring year classes were replicates and were raised in the same manner, no interactions with year class were tested. Model 5.6 was used to test the effects of strain and year of maturation on 4-year old spawn date in the parent year class, as follows:

Model 5.6. $y_{ijk} = \mu + P_i + M_j + (PM)_{ij} + e_{ijk}$

where

y_{ijk} is the 4-year old spawn date for an individual in pure strain i with maturation year j,

M_j is the fixed effect of maturation year j (j=1, 2),

e_{ijk} is the residual error associated with individual ijk

The remaining variables are as defined for Model 5.4. Model 5.7 was used to test the effects of strain and year of maturation on 4-year old spawn date in the offspring year classes, as follows:

Model 5.7. $y_{ijklm} = \mu + Y_i + D_j + S_k + M_l + (DS)_{jk} + (DM)_{jl} + (SM)_{kl} + (DSM)_{jkl} + e_{ijklm}$

where

y_{ijklm} is the 4-year old spawn date for an individual from year class i with dam strain j, sire strain k and maturation year l,

M_l is the fixed effect of maturation year l (l=1, 2),

$(DM)_{jl}$ is the interaction of dam strain and maturation year,

$(SM)_{kl}$ is the interaction of sire strain and maturation year,

$(DSM)_{jkl}$ is the 3-way interaction of dam strain, sire strain and maturation year,

e_{ijklm} is the residual error associated with observation $ijklm$.

The remaining variables are as defined for Model 5.3.

Least squares means were estimated for main effects in Models 5.4 to 5.7. Orthogonal and non-orthogonal contrasts were used to compare main effects in Models 5.4 to 5.7. Heterosis was defined as a significant difference between the average of two pure strains and the average of their hybrids, and was tested by doing contrasts with Models 5.5 and 5.7. Contrasts between reciprocal hybrid crosses were non-estimable with Models 5.5 and 5.7. Reciprocal contrasts for 3- year old spawn date were tested with Model 5.8 as follows:

Model 5.8. $y_{ijk} = \mu + Y_i + C_j + e_{ijk}$

where

y_{ijk} is the 3-year old spawn date for an individual in year class i with strain combination j,

C_j is the fixed effect of strain combination j (j=1, ..., 9),

e_{ijk} is the residual error associated with observation ijk.

The remaining variables are as defined for Model 5.5. Reciprocal contrasts for 4-year old spawn date were tested with Model 5.9 as follows:

Model 5.9. $y_{ijkl} = \mu + Y_i + C_j + M_k + (CM)_{jk} + e_{ijkl}$

where

y_{ijkl} is the 4-year old spawn date for an individual from year class i with strain combination j and maturation year k,

M_k is the fixed effect of maturation year k (k=1, 2),

$(CM)_{jk}$ is the interaction of strain combination and maturation year,

e_{ijkl} is the residual error associated with observation $ijkl$.

The remaining variables are as defined for Model 5.8. Two types of contrasts were done: contrasting hybrids for whether a strain is used as a dam or a sire, and contrasting reciprocal crosses.

Repeatability

Repeatability is a measure of the strength of the relationship between repeated records for a trait (Bourdon, 1997). In this study, female spawn date repeatability was defined as the correlation between spawn date at 3 years old and spawn date at 4 years old. Correlations, however, assume that experimental units are random samples from a bivariate population. Since these strains and their spawning times were chosen deliberately, they were not random samples. A better method for repeatability analysis

was multivariate analysis of variance (MANOVA). Repeatability estimates were partial correlation coefficients from SAS PROC GLM (MANOVA statement). This method removed non-random effects, such as strain, from the analysis. There were 124 and 262 observations in the parent and offspring generations, respectively. Model 5_10 was used for the parent generation, as follows:

Model 5.10. $y_{ij} = \mu + P_i + e_{ij}$

where

y_{ij} is the 3- or 4-year old spawn date for an individual in pure strain i.

Remaining variables are as defined for Model 5.4. Model 5.11 was used for the offspring generation, as follows:

Model 5.11. $y_{ijkl} = \mu + Y_i + D_j + S_k + (DS)_{jk} + e_{ijkl}$

where

y_{ijkl} is the 3- or 4-year old spawn date for an individual in year class i with dam strain j and sire strain k.

Remaining variables are as defined for Model 5.5.

To examine the difference between spawn dates at 3 years and spawn dates at 4 years old, and to determine if there is a shift in spawn date from one year to the next, two methods were attempted. First, differences between 3 and 4 year spawn dates in individuals that spawned in both years (3-year old maturing females) were tested using ANOVA Models 5.4, 5.5 and 5.8, where the dependent variable was defined as the difference between spawn dates at 3 and 4 years (in days) for the individual. This method only included individuals that spawned in both years, a subset of the data. In order to use the complete data set, including females that only spawned at 4 years of age, mixed-model

analyses and contrasts were done using SAS PROC MIXED. There were 307 and 630 observations in the parent and offspring generations, respectively. Model 5.12 was used for the parent generation, as follows:

Model 5.12. $y_{ijkl} = \mu + P_i + E_j + (PE)_{ij} + I_k + e_{ijkl}$

where

y_{ijkl} is the 3- or 4-year old spawn date for an individual k in pure strain i and spawning season j,

E_j is the fixed effect of the spawning season j (j=1, 2),

$(PE)_{ij}$ is the interaction of pure strain and spawning season,

I_k is the random effect of individual k,

e_{ijkl} is the residual error associated with observation $ijkl$.

The remaining variables are as defined for Model 5.4. Model 5.13 was used for the offspring generation, as follows:

Model 5.13. $y_{ijklm} = \mu + Y_i + C_j + E_k + (CE)_{jk} + I_l + e_{ijklm}$

where

y_{ijklm} is the 3- or 4-year old spawn date for an individual l in year class i and strain combination j in spawning season k,

E_k is the fixed effect of the spawning season k (k=1, 2),

$(CE)_{jk}$ is the interaction of pure strain and spawning season,

I_l is the random effect of individual l,

e_{ijklm} is the residual error associated with observation $ijklm$.

5.3. RESULTS

Complete ANOVA and MANOVA tables are presented in Appendix 5.1, and figures are presented in Appendix 5.2.

5.3.1. Homogeneity of variances

Variances of spawn date at 3 years and at 4 years were not significantly different across strain combinations, except for the 94 year class 4 year spawn date variances ($p = 0.0193$). Data appeared to be normally distributed in charts, but no normality tests were done.

5.3.2. Fertilisation week effects

Relationships between spawn date and fertilisation week in daughters from the three dam strains are shown in Figures 5.1 to 5.4. There was no effect of fertilisation week on spawn date in the 94 year class (Table 5.2). In the 96 year class, spawn date increased with fertilisation week in daughters of B dams during in both spawning seasons, and in daughters of O dams during the 3-year old season only (Table 5.2).

Table 5.2. Effects on spawn date within offspring year class and dam strain with fertilisation week as a covariate.

Year Class	Dam Strain	Fertilisation week Pr>F	
		3-year spawn date	4-year spawn date
94	B	0.6522	0.6762
	G	0.5914	0.2624
96	O	0.0021	0.0969
	B	0.0024	0.0081
	G	0.6870	0.4749

5.3.3. Strain main effects

Model 5.3 showed highly significant effects of year class, dam strain, and sire strain on spawn date at 3 years old (Table 5A.1, in Appendix 5.1). Least squares means

could not be estimated with Model 5.3, even with the non-significant 3-way interaction removed. This probably occurred because the parental year class did not have all of the strain combinations and the dam strain was the same as the sire strain. Therefore, subsequent analyses were done on parent and progeny groups separately.

Strain and year class least squares means for Models 5.4 to 5.8 are presented in Table 5.3, and strain comparison results are summarised in Table 5.4. In the parent year class, strain had a highly significant effect on spawn date in both years (Tables 5A.2 and 5A.3), and all strains had significantly different spawn dates such that O females spawned first, B females spawned second, and G females spawned last. In the offspring, year class did not have a significant effect, but dam and sire strains had highly significant effects on spawn date in both years (Tables 5A.4 and 5A.5). In order, daughters of O parents had the earliest spawn dates, daughters of B parents were second, and daughters of G parents had the latest spawn dates. Spawn dates of the daughters from all dam strains and sire strains were significantly different.

Table 5.3. Least squares means and standard errors for strain and year class effects on spawn dates at 3 and 4 years in parent (Models 5.4 and 5.6, respectively) and offspring (Models 5.5 and 5.8, respectively) year classes. All least squares means were significantly different from zero ($p<0.0001$).

Group	Effect	Level	3-year spawn date (d from Oct. 1)		4-year spawn date (d from Oct.1)	
			LS mean	SE	LS mean	SE
Parent	Pure Strain	O	92.143	3.704	62.773	5.978
		B	132.250	2.754	115.839	4.143
		G	172.667	4.620	162.280	5.336
Offspring	Year class	94	117.928	2.547	112.683	3.182
		96	118.369	1.306	114.815	2.624
	Dam strain	O	97.093	2.338	87.687	4.459
		B	117.550	1.885	116.610	3.814
		G	139.802	2.308	136.951	3.239
	Sire strain	O	104.401	2.158	100.515	3.927
		B	120.318	2.126	111.869	4.058
		G	129.727	2.224	128.863	3.578

Table 5.4. Strain comparison results for spawn dates at 3 and 4 years in parent (Models 5.4 and 5.6, respectively) and offspring (Models 5.5 and 5.8, respectively) year classes.

Year class	Comparison	3-year spawn date		4-year spawn date	
		Contrast Pr>F	Tukey's Pr>|t|	Contrast Pr>F	Tukey's Pr>|t|
Parent	O vs. B	<0.0001	<0.0001	<0.0001	<0.0001
	O vs. G	<0.0001	<0.0001	<0.0001	<0.0001
	B vs. G	<0.0001	<0.0001	<0.0001	<0.0001
Offspring	O vs. B Dams	<0.0001	<0.0001	<0.0001	<0.0001
	O vs. G Dams	<0.0001	<0.0001	<0.0001	<0.0001
	B vs. G Dams	<0.0001	<0.0001	<0.0001	0.0002
	O vs. B Sires	<0.0001	<0.0001	0.0448	0.1102
	O vs. G Sires	<0.0001	<0.0001	<0.0001	<0.0001
	B vs. G Sires	0.0012	<0.0001	0.0017	0.0048

5.3.4. Strain interactions

Least squares means estimated for spawn dates of all dam strain by sire strain combinations in the offspring generation are shown in Table 5.5. Hybrids spawned intermediate to pure strains. Contrasts showed significant heterosis only between B and O strains at 3 years old (Table 5.6). On average, O-B hybrids spawned earlier (~97 days) than the average of pure O and B strains (~105 days).

Table 5.5. Dam strain by sire strain least squares means ± SE (in d from Oct. 1) for 3-year (Model 5.5) and 4-year (Model 5.7) spawn dates in offspring.

			Sire Strain		
			O	**B**	**G**
Dam Strain	**O**	**3-year**	86.915 ± 3.709	92.831 ± 4.061	111.533 ± 3.372
		4-year	77.085 ± 7.963	84.212 ± 7.938	101.763 ± 6.908
	B	**3-year**	101.559 ± 3.267	122.899 ± 3.005	128.192 ± 3.368
		4-year	101.288 ± 6.789	115.784 ± 6.751	132.759 ± 6.272
	G	**3-year**	124.727 ± 3.565	145.224 ± 3.587	149.455 ± 4.153
		4-year	123.173 ± 5.395	135.613 ± 6.303	152.068 ± 5.099

Table 5.6. Significance of heterosis contrasts for 3-year (Model 5.5) and 4-year (Model 5.7) spawn date.

Heterosis Contrast	Pr > F	
	3-year spawn date	4-year spawn date
B / G (BB + GG vs. BG + GB)	0.8795	0.9664
B / O (BB + OO vs. BO + OB)	0.0265	0.6169
G / O (GG + OO vs. GO + OG)	0.9877	0.7419

ANOVA results for Models 5.8 and 5.9 are shown in Tables 5A.6 and 5A.7, respectively. Contrasts (Table 5.7) showed that hybrids that had O dams spawned significantly earlier on average than hybrids that had O sires. Hybrids that had G dams spawned significantly later on average than hybrids that had G sires. OG spawned earlier than GO in both years. BG spawned earlier than GB at 3 years, but not at 4 years.

Table 5.7. Significance of reciprocal cross contrasts for 3-year (Model 5.8) and 4-year (Model 5.9) spawn dates in the offspring generation.

	Contrast	Pr > F	
		3-year spawn date	4-year spawn date
Dam vs. Sire	O (OB+OG vs. BO+GO)	0.0016	0.0053
	B (BG+BO vs. GB+OB)	0.2370	0.2998
	G (GB+GO vs. BG+OG)	<0.0001	0.0531
Reciprocal	OB vs. BO	0.0856	0.1033
	OG vs. GO	0.0047	0.0148
	BG vs. GB	0.0005	0.7484

5.3.5. Age at maturation effects on 4-year spawn date

Models 5.6 and 5.7 showed age at maturation had a significant effect on 4-year spawn date in both parents and offspring (Tables 5A.3 and 5A.5, respectively). Females that matured at 3 years old spawned significantly earlier than females that matured at 4 years old in both parent and offspring groups (Table 5.8).

Table 5.8. Least squares means for 4-year spawn date (d from Oct. 1) of 3- and 4-year maturing females in parent and offspring groups. All least squares means are significantly different from zero ($p<0.0001$).

	Age at maturation	
Generation (Model)	3 years	4 years
Parent (Model 5.6)	103.9459 ± 3.0330	123.3150 ± 5.1929
Offspring (Model 5.7)	105.0920 ± 1.9546	122.4067 ± 4.0985

5.3.6. Repeatability

MANOVA results for parent and offspring generations are summarised in Tables 5A.8 and 5A.9, respectively. Spawn date repeatability estimates (partial correlation coefficients) were 0.7240 ($p<0.0001$) for the parents and 0.8294 ($p<0.0001$) for offspring.

Strain had a significant effect on the difference between spawn dates in the parent generation (Table 5A.11) and year class, dam strain, and strain had significant effects on the difference between spawn dates in the offspring generation (Table 5A.12). Main effects least squares means for the difference between spawn dates in Models 5.4 and 5.5 are presented in Table 5.9. Dam strain by sire strain least squares means from Model 5.5 are presented in Table 5.10.

Table 5.9. Least squares means and standard errors for difference between spawn dates in main effects of Models 5.4 and 5.5. All least squares means were significantly different from 0 ($p<0.0001$).

Model / Generation	Effect	Level	Difference in spawn date (d)	
			LS mean	SE
5.4 Parent	Pure Strain	O	35.0857	3.5718
		B	26.8060	2.5816
		G	19.3636	4.5052
5.5 Offspring	Year class	94	15.6518	1.8892
		96	9.3745	0.9881
	Dam strain	O	17.0974	1.7703
		B	8.7184	1.3935
		G	11.7238	1.7385
	Sire strain	O	15.0589	1.6624
		B	9.5625	1.5124
		G	12.9182	1.6778

Table 5.10. Dam strain by sire strain least squares means ± SE (Pr > |t|) for the difference between 3 year spawn date and 4 year spawn date (d) in offspring (Model 5.5).

		Sire Strain		
		O	**B**	**G**
Dam Strain	**O**	22.9218 ± 3.1038 (p<0.0001)	11.5333 ± 2.6995 (p<0.0001)	16.8371 ± 2.4967 (p=<0.0001)
	B	7.2959 ± 2.3660 (p=0.0023)	6.3381 ± 2.1321 (p=0.0032)	12.5213 ± 2.6823 (p<0.0001)
	G	14.9591 ± 2.5936 (p<0.0001)	10.8161 ± 2.7580 (p=0.0001)	9.3961 ± 3.1932 (p=0.0036)

Contrasts (Table 5.11) showed that there was a greater difference in the O strain than in the G strain in the parent year class. In the offspring group, there was a greater difference in daughters of O dams compared with the other dam strains, and a greater difference in daughters of O sires compared with daughters of B dams. Also, 94 year class had a greater difference between 3- and 4- year spawn dates than did the 96 year class.

Table 5.11. Significance of strain contrasts and Tukey's tests for difference between spawn dates

Year class	Comparison	Contrast Pr>F	Tukey's Pr>|t|
Parent	O vs. B	0.0627	0.1493
	O vs. G	0.0072	0.0195
	B vs. G	0.1544	0.3271
Offspring	O vs. B Dams	0.0001	0.0004
	O vs. G Dams	0.0162	0.0427
	B vs. G Dams	0.1655	0.3472
	O vs. B Sires	0.0087	0.0235
	O vs. G Sires	0.3275	0.5895
	B vs. G Sires	0.1148	0.2551

In the offspring group, hybrids generally had intermediate differences compared with pure strains, except the average difference between 3 year and 4 year spawn dates in

O and B hybrids (~9.4 days) was significantly lower than the average of these pure strains (~14.6 days) (Table 5.12).

Table 5.12. Significance of Model 5.8 heterosis contrasts for difference between 3- and 4-year spawn date in offspring year classes.

Heterosis Contrast	Pr > F
B / G (BB + GG vs. BG + GB)	0.1574
B / O (BB + OO vs. BO + OB)	0.0400
G / O (GG + OO vs. GO + OG)	0.9245

Mixed model analysis of difference between seasons

Results of Models 5.12 and 5.13 are shown in Tables 5A.13 and 5A.14, respectively. Contrasts showed spawn dates at 4 years were earlier than spawn dates at 3 years in all parent strain and offspring strain combination (Tables 5.13 and 5.14). The possible exception was pure G females, where the contrast was of borderline significance.

Table 5.13. Mixed model 5.12 least squares means ± SE and contrast results for parental year class.

Strain	O	B	G
3 year spawn date ± SE	93.72 ± 4.23	135.37 ± 3.12	176.64 ± 5.04
4 year spawn date ± SE	58.92 ± 4.20	109.36 ± 3.04	159.49 ± 4.68
Contrast Pr>F	<0.0001	<0.0001	0.0001

Table 5.14. Mixed model 5.13 least squares means ± SE and contrast Pr>F for offspring year classes.

			Sire Strain		
			O	**B**	**G**
Dam Strain	**O**	**3 years**	90.10 ± 4.12	93.62 ± 4.25	114.49± 3.72
		4 years	69.55 ± 4.36	84.89 ± 4.21	100.95 ± 3.76
		Pr>F	<0.0001	0.0008	<0.0001
	B	**3 years**	102.24 ± 3.65	122.99 ± 3.45	132.41 ± 3.83
		4 years	96.53 ± 3.68	116.35 ± 3.45	120.94 ± 3.92
		Pr>F	0.0146	0.0023	<0.0001
	G	**3 years**	129.08 ± 3.91	145.57 ± 3.96	155.36 ± 4.42
		4 years	116.66 ± 3.85	136.66 ± 4.01	149.27 ± 4.26
		Pr>F	<0.0001	0.0010	0.0502

5.4. Discussion and Conclusions

5.4.1. Fertilisation week

Similar to the growth analyses in Chapter 4, no general fertilisation week effect within dam strain was found for spawn date. Any overall time effect for all strains was confounded with dam strain, and including this effect in the model could artificially reduce the dam strain effect. There was not much overlap in time, and regression slopes may not have been parallel between dam strains. There was not enough data in this study to conclude if there was any real within-dam strain pattern.

5.4.2. Pure strain rankings

At both 3 and 4 years of age, females from the O strain had the earliest spawn date, females from the B strain were second, and females from the G strain had the latest spawn date. Given the known history of these strains, these results were expected.

5.4.3. Strain interactions

Hybrids generally spawned intermediate to pure strains, and the only indication of heterosis for spawn date occurred in hybrids of B and O strains at 3 years old. On average, these hybrids spawned earlier (~97 days) than the average of pure OO and BB groups (~105 days). This difference may have been due to early-spawning OO females not being large enough at a genetically-set spawning time at 3 years old, so spawning was delayed in this first season. When size or growth rate was no longer a factor at 4 years of age, the average of the hybrids was the same as the average of the pure strains. There was no interaction between G and O strains observed in 4 year olds compared to the interaction observed in 3 year olds. This would be expected if the heterosis were solely due to delayed OO spawning.

There were a number of differences in offspring spawn dates dependant upon dam and sire strain origins. Hybrids that had O dams spawned significantly earlier on average than hybrids that had O sires. Hybrids that had G dams spawned significantly later on average than hybrids that had G sires. At 3 and 4 years of age, BG spawned earlier than GB and OG spawned earlier than GO. OB spawned earlier than BO at 4 years, but not at 3 years. The trend among these differences is that the hybrid spawned more similarly to its dam's strain. Hybrids that had an early-spawning dam strain spawned earlier than hybrids that had the same strain as their sire instead.

5.4.4. Maturation effects on 4-year spawn date

At 4 years old, females that matured at 3 years old, and were in their second spawning season, spawned earlier than females that matured at 4 years old, and were in their first spawning season. This supports the idea that there was some delay in spawning at 3 years old due to growth.

5.4.5. Repeatability

Repeatability estimates of spawn date were high, therefore females that spawned early in the season at 3 years old also tended to spawn early in the season at 4 years old. Similarly, animals that spawned late in the season at 3 years old also tended to spawn late in the season at 4 years old.

The reproductive season occurred earlier in the calendar year when the populations were 4 years old than when they were 3 years old. This may have been caused by some change in environmental cues, such as photoperiod, from one year to the next. It is unlikely, however, that the same environmental changes occurred at the same ages in all three year classes to cause similar advances in the spawning season, as

happened in this study. There may be some innate effect, such as a larger body size, which allowed 4-year old females to spawn earlier than 3-year old females. There may also be some strain variation for this effect, since ANOVA found that the fall-spawning O strain had a more delayed spawn date at 3 years old than the other strains. Mixed model analysis also showed spawn dates at 4 years old were earlier than spawn dates at 3 years old, but within-strains differences were not the same as those found with ANOVA. This was probably due to the larger amount of data used in the mixed-model analysis. There were few pure G females that spawned in both years and could be included in the ANOVA, but the mixed-model analysis used records from all individuals, including those with only one spawn record.

APPENDIX 5.1. SPAWNING TIME ANALYSIS OF VARIANCE TABLES

Table 5A.1. Model 5.3 ANOVA for 3-year spawn date in all year classes.

Source	df	MS	F	Pr>F
Model	20	12272.86	27.14	<0.0001
Year class	2	4475.17	9.90	<0.0001
Dam strain	2	14099.28	31.18	<0.0001
Sire strain	2	11142.92	24.65	<0.0001
Year class × Dam strain	2	685.38	1.52	0.2207
Year class × Sire strain	2	350.32	0.77	0.4614
Dam strain × Sire strain	4	539.09	1.19	0.3134
Year class × Dam strain × Sire strain	4	332.27	0.73	0.5685
Error	445	452.13		
Total	465			

Table 5A.2. Model 5.4. ANOVA for 3-year spawn date in parent year class.

Source	df	MS	F	Pr>F
Strain	2	54618.98	94.78	<0.0001
Error	142	576.26		
Total	144			

Table 5A.3. Model 5.6 ANOVA for 4-year spawn date in parent year class.

Source	df	MS	F	Pr>F
Model	5	42920.82	46.11	<0.0001
Strain	2	71838.95	77.18	<0.0001
Maturation	1	9655.42	10.37	0.0016
Strain × Maturation	2	309.73	0.33	0.7174
Error	156	930.76		
Total	161			

Table 5A.4. Model 5.5 ANOVA for 3-year spawn date in offspring year classes.

Source	df	MS	F	Pr>F
Model	9	12562.56	31.64	<0.0001
Year class	1	9.30	0.02	0.8785
Dam strain	2	41597.47	104.78	<0.0001
Sire strain	2	16384.23	41.27	<0.0001
Dam strain × Sire strain	4	717.79	1.81	0.1272
Error	298	397.00		
Total	307			

Table 5A.5. Model 5.7 ANOVA for 4-year spawn date in offspring year classes.

Source	df	MS	F	Pr>F
Model	18	9365.40	14.20	<0.0001
Year class	1	218.67	0.33	0.5653
Dam strain	2	26942.03	40.84	<0.0001
Sire strain	2	9765.01	14.80	<0.0001
Maturation	1	9452.18	14.33	0.0002
Dam strain × Sire strain	4	53.88	0.08	0.9880
Dam strain × Maturation	2	58.34	0.09	0.9154
Sire strain × Maturation	2	1533.94	2.33	0.0997
Dam strain×Sire strain ×Maturation	4	834.34	1.26	0.2841
Error	278	659.75		
Total	296			

Table 5A.6. Model 5.8 ANOVA for 3-year spawn date in offspring year classes.

Source	df	MS	F	Pr>F
Model	9	12562.59	31.64	<0.0001
Year class	1	9.30	0.02	0.8785
Strain combination	8	14079.22	35.46	<0.0001
Error	298	397.00		
Total	307			

Table 5A.7. Model 5.9 ANOVA for 4-year spawn date in offspring year classes.

Source	df	MS	F	Pr>F
Model	18	9365.40	14.20	<0.0001
Year class	1	218.67	0.33	0.5653
Strain combination	8	9596.09	14.55	<0.0001
Maturation	1	9452.18	14.33	0.0002
Strain × Maturation	8	896.67	1.36	0.2145
Error	278	659.75		
Total	296			

Table 5A.8. Model 5.10 MANOVA for 3- and 4-year spawn dates in parent year class.

Source	df	3-year spawn date			4-year spawn date		
		MS	F	Pr>F	MS	F	Pr>F
Strain	2	47124.61	90.92	<0.0001	66981.50	71.44	<0.0001
Error	121	518.495			937.554		
Total	123						

Table 5A.9. Model 5.11 MANOVA for 3- and 4-year spawn dates in offspring year classes.

Source	df	3-year spawn date			4-year spawn date		
		MS	F	Pr>F	MS	F	Pr>F
Model	9	11270.12	29.20	<0.0001	13381.19	22.45	<0.0001
Year class	1	92.02	0.24	0.6258	912.39	1.53	0.2171
Dam strain	2	36937.50	95.70	<0.0001	46542.33	78.10	<0.0001
Sire strain	2	15090.56	39.10	<0.0001	19330.40	32.44	<0.0001
Dam strain × Sire strain	4	897.17	2.32	0.0571	942.51	1.58	0.1797
Error	252	385.97			595.93		
Total	261						

Table 5A.10. Model 5.4. ANOVA for difference between 3- and 4-year spawn dates in the parent year class.

Source	df	MS	F	Pr>F
Strain	2	1744.89	3.91	0.0227
Error	121	446.53		
Total	123			

Table 5A.11. Model 5.5. ANOVA for difference between 3- and 4-year spawn dates in the offspring year classes.

Source	df	MS	F	Pr>F
Model	9	675.52	3.62	0.0003
Year class	1	1583.91	8.50	0.0039
Dam strain	2	1423.85	7.64	0.0006
Sire strain	2	667.94	3.58	0.0292
Dam strain × Sire strain	4	375.18	2.01	0.0931
Error	252	186.35		
Total	261			

Table 5A.12. Mixed model 5.12 results for parental year class.

Effect	df	F	Pr>F
Season	1	162.55	<0.0001
Strain	2	125.96	<0.0001
Season × Strain combination	2	5.15	0.0070

Table 5A.13. Mixed model 5.13 results for offspring year classes.

Effect	df	F	Pr>F
Year Class	1	0.63	0.4294
Season	1	145.01	<0.0001
Strain combination	8	38.00	<0.0001
Season × Strain combination	8	3.01	0.0030

APPENDIX 5.2. SPAWNING TIME FIGURES

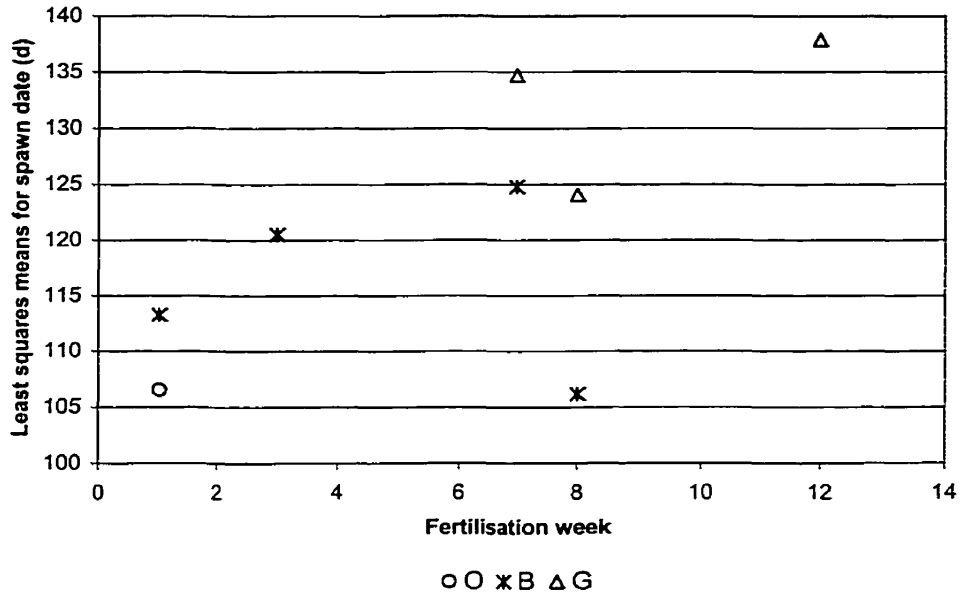

Figure 5. 1. 3-year spawn dates for 94 year class daughters of O, B, and G dams fertilised over time.

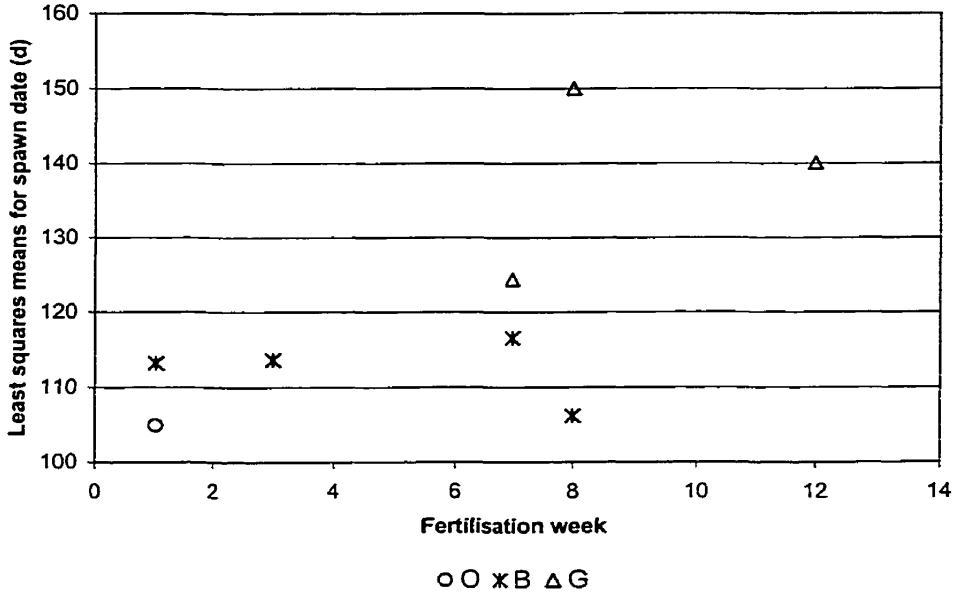

Figure 5. 2. 4-year spawn dates for 94 year class daughters of O, B, and G dams fertilised over time.

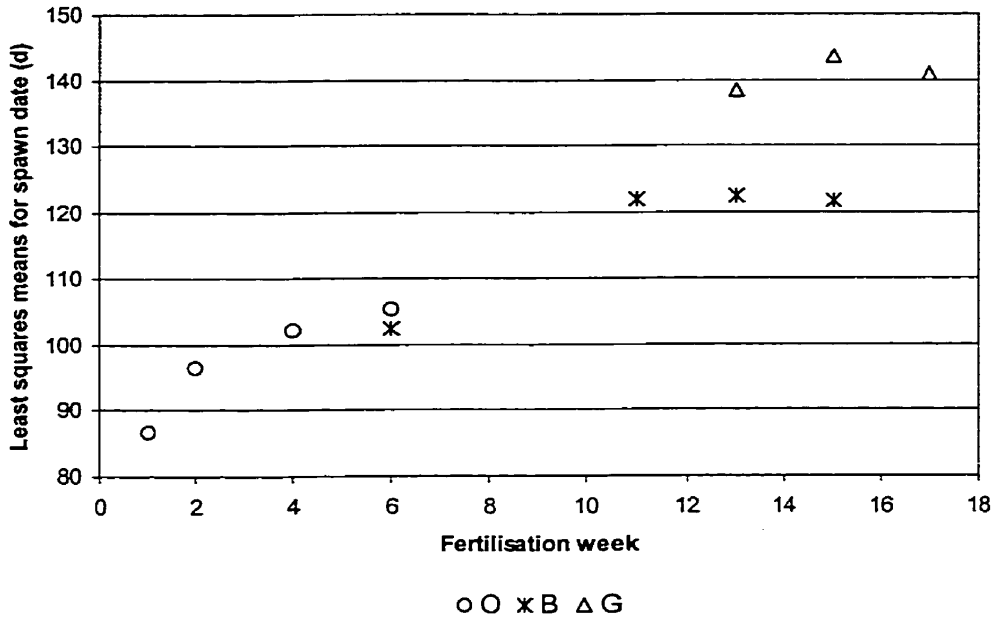

Figure 5. 3. 3-year spawn dates for 96 year class daughters of O, B, and G dams fertilised over time.

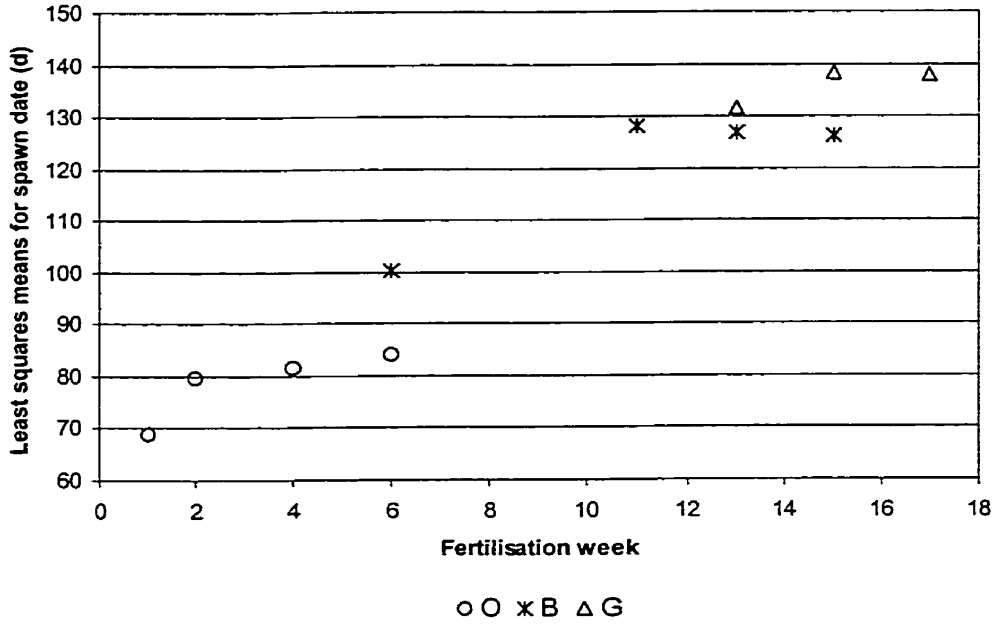

Figure 5. 4. 4-year spawn dates for 96 year class daughters of O, B, and G dams fertilised over time.

CHAPTER 6. ESTIMATION OF GENETIC PARAMETERS

6.1. INTRODUCTION

The goals of this study were to estimate genetic parameters for growth and spawning date and to use these to determine the most efficient breeding scheme to develop a synthetic strain of rainbow trout with fast growth and a delayed spawning season.

Heritability in the narrow sense is an estimate of the proportion of phenotypic variation that is due to additive genetic effects. This allows prediction of selection response. Correlations, both phenotypic and genetic, indicate how traits are related and how a change in one might affect another. Knowledge of genetic parameters can indicate the effectiveness of various breeding schemes. If a trait is highly heritable, then selection should have some response; if not, crossbreeding schemes, which make use of non-additive genetic effects, may be more suitable. If traits are genetically correlated, then selection for one trait will cause a response in the other. Negative genetic correlation suggests that improvement of both traits may not be possible.

6.2. MATERIALS AND METHODS

6.2.1. Phenotypic data

Individual weight and female spawn date data were collected from individuals in the parental and 96 year classes. The progeny year class came from pooled mating in which several females of a strain were mated with several males of another (see methods in Chapters 3, 4, and 5). Therefore, the exact dams and sires were unknown. Exact

parentage of 96 year class individuals was determined using microsatellite DNA methods (McDonald, 2001). There were 29 maternal half-sib families, 38 paternal half-sib families, and 182 full-sib families. The data set contained 408 parental records and 566 offspring records. Descriptive statistics are summarised in Table 6.1.

The specific traits measured were: weight at 2 years of age, spawn date at 3 years old, and spawn date at 4 years old. In the parent year class, 2 year old weight was the individual's body weight measured on May 11 to 15, 1993. In the offspring year class, 2-year old weight had to be estimated from individual weights taken between 570 and 970 days old (approximately 4 weighs per fish). For each individual, a linear regression of weight on age in days was done to calculate a slope and intercept, which were used to estimate weight at 730 days old. Three-year and 4-year spawn dates were defined as in the Chapter 5. Spawning date at 3 years and spawning date at 4 years were treated as separate traits. This is a common method of analysing repeated measures (e.g. dairy lactations, litter size), for several reasons. First, different genes may have been involved in spawning in different years, which is unlikely. Second, if growth influenced spawn date, it would more likely have affected 3-year data than 4-year data. Third, there was a shift to earlier spawning at 4 years old, (see the repeatability analysis in Chapter 5). Strain and sex were recorded for all individuals.

Table 6.1. Descriptive statistics of data used to estimate genetic parameters and breeding values.

Trait	Group	N	Mean	SD	Min	Max
2-year weight (g)	Parent females	209	753.2	533.1	91.3	2077.4
	Parent males	197	751.9	501.2	30.7	2163.4
	Offspring females	292	772.1	290.1	33.3	1553.0
	Offspring males	275	737.6	273.8	86.1	1476.0
3-year female spawn date (d)	Parent	145	128	36	42	222
	Offspring	252	116	29	47	196
4-year female spawn date (d)	Parent	162	106	47	13	207
	Offspring	223	107	36	31	209

6.2.2. Statistical methods

Genetic parameters and individual estimated breeding values (EBVs) were calculated using VCE4 (Neumaier and Groeneveld, 1998) with restricted maximum likelihood (REML) and best linear unbiased prediction (BLUP) animal model, Model 6.1 as follows:

Model 6.1. $y_{ijkl} = \mu + GS_i + X_j + A_k + e_{ijkl}$

where

y_{ijkl} is an observation of 2-year old weight, 3-year old spawn date, or 4-year old spawn date for an individual k in generation-strain i of sex j,

μ is the population mean,

GS_i is the fixed effect of the animal's combined generation and strain i (i=1, ..., 12),

X_j is fixed effect of the animal's sex j (j=1,2),

A_k is the additive genetic value of individual k,

e is the residual error associated with observation $ijkl$.

Individuals' EBVs (or additive genetic values) from Model 6.1 were adjusted by adding their generation-strain effect solutions. This adjustment was required by the large genetic and phenotypic variation in the population. All subsequent EBV analyses were done using these adjusted values.

The correlations between EBVs and performances for the traits were partial correlation coefficients from a multivariate analysis of variance (SAS© GLM procedure, MANOVA statement). Parent and offspring year classes were analysed together. Model 6.2 correlated weight performance and EBV as follows:

Model 6.2. $y_{ijk} = \mu + GS_i + X_j + (GSX)_{ij} + e_{ijk}$

where

y_{ijk} is the performance or EBV of an individual in generation-strain i of sex j,

$(GSX)_{ij}$ is the interaction between generation-strain and sex,

e_{ijk} is the residual error of observation ijk.

The remaining variables are as defined for Model 6.1. Model 6.3 correlated female spawn date performance and EBVs as follows:

Model 6.3 $y_{ij} = \mu + GS_i + e_{ij}$

where

y_{ij} is the performance or EBV of an individual in generation-strain i,

e_{ij} is the residual error of observation ij.

The remaining variables are as defined for Model 6.1.

6.3. RESULTS

6.3.1. Genetic parameters

Estimated genetic parameters are summarised in Table 6.2 and 6.3. Two-year old weight and spawning date at 3 and 4 years old were highly heritable. Weight and spawning date had negative phenotypic correlations, but genetic correlations were close to 0. Plots of the relationships between performance for 2-year old weight and spawn dates at 3 and 4 years of age shown in Figures 6.1 and 6.2.

Table 6.2. Estimated genetic, environmental, and phenotypic variances and heritabilities ± SE for 2-year old weight and female spawning dates at 3 and 4 years old.

Trait	σ_G^2	σ_E^2	σ_P^2	$h^2 \pm$ SE
2-year weight (g)	26102.06	27151.80	53253.86	0.490 ± 0.047
3-year spawn date (d from Oct. 1)	351.99	182.01	534.00	0.659 ± 0.100
4-year spawn date (d from Oct. 1)	403.01	476.66	879.68	0.458 ± 0.120

Table 6.3. Estimated correlations (± SE) and covariances between 2-year old weight and female spawning dates at 3 and 4 years old. Correlations are above the diagonal and covariances are below the diagonal. Genetic, environmental, and phenotypic values appear in the upper, middle, and lower rows respectively.

	2-year weight	3-year spawn date	4-year spawn date
2-year weight		-0.002 ± 0.106	-0.019 ± 0.128
		-0.477 ± 0.095	-0.305 ± 0.087
		-0.20	-0.17
3-year spawn date	-6.460		0.916 ± 0.073
	-1061.168		0.739 ± 0.066
	-1067.628		0.82
4-year spawn date	-62.503	344.972	
	-1096.199	217.703	
	-1158.702	562.675	

6.3.2. Estimated breeding values

Fixed effect solutions and summary statistics for adjusted EBVs are summarised in Tables 6.4 and 6.5, respectively. Relationships between EBVs for 2-year old weight and for spawn dates at 3 and 4 years of age are plotted in Figures 6.3 and 6.4.

Relationships between EBVs and performances are plotted in Figures 6.5 to 6.7. Multivariate analysis of variance results for Models 6.2 and 6.3 are shown in Tables 6A.1 to 6A.3 in Appendix 6.1. Partial correlation coefficients between EBVs and actual performances were 0.915 for 2-year weight, 0.959 for 3-year spawn date, and 0.863 for 4-year spawn date (all coefficients had $p<0.0001$).

Table 6.4. Fixed effect solutions from BLUP analysis.

Effect		2-year Weight (g)	3-year Spawn Date (d)	4-year Spawn Date (d)
Generation-Strain				
Parent	B	-245.14	14.3	3.1
Parent	G	-351.40	56.5	53.1
Parent	O	745.94	-26.2	-46.3
Offspring	BB	-173.83	-1.6	6.1
Offspring	BG	-267.68	16.2	21.6
Offspring	BO	116.69	-19.9	-9.5
Offspring	GB	-183.30	25.0	34.1
Offspring	GG	-393.59	36.5	45.4
Offspring	GO	120.82	6.9	8.1
Offspring	OB	143.80	-27.4	-22.9
Offspring	OG	40.84	-6.6	-6.7
Offspring	OO	271.43	-32.3	-40.1
Sex				
F		23.13	0	0
M		0	0	0

Table 6.5. Summary statistics of adjusted EBVs.

Trait	Group	N	Mean	SD	Min	Max
2-year weight (g)	Parent females	209	-26.55	473.68	-575.82	1083.03
	Parent males	199	2.10	464.60	-533.78	1065.38
	Offspring females	292	-3.50	227.41	-507.46	560.93
	Offspring males	275	-18.43	219.06	-483.10	542.45
3-year female spawn date (d)	Parent females	209	14.54	32.39	-61.79	90.61
	Parent males	199	12.86	29.64	-39.96	70.36
	Offspring females	292	-2.94	24.70	-51.64	63.14
	Offspring males	275	-0.47	22.47	-40.93	53.49
4-year female spawn date (d)	Parent females	209	3.01	37.63	-81.13	86.17
	Parent males	199	0.98	35.54	-65.51	70.46
	Offspring females	292	1.35	27.59	-60.41	74.31
	Offspring males	275	4.49	25.01	-49.84	63.05

6.4. Discussion and Conclusions

6.4.1. Heritability estimates

The heritability estimate of 0.49 for 2-year old weight is higher than other published estimates for this age, which range from 0.17 to 0.38 (see Gjerde and Gjedrem, 1984; McKay et al., 1986; Crandell and Gall, 1993A). Traits related to skeletal dimensions such as mature body weight tend to be highly heritable, with estimates over 0.4 (Bourdon, 1997). The higher estimate from this study may have been partly due to the high genetic and phenotypic diversity of the crossed population.

Published heritability estimates for spawn dates are higher, ranging from 0.5 to 0.9 (see Siitonen and Gall, 1989; Sadler et al., 1992; Su et al., 1997) which are similar to the estimates of 0.66 and 0.46 found in this study. Traits with heritabilities above 0.4, such as found in this study are considered highly heritable (Bourdon, 1997). The high heritability estimates mean that an individual's performance is probably a good indicator

of its breeding value for weight and spawning date, so the accuracy of selection should be good and genetic change from selection should be rapid (Bourdon, 1997). This is supported by the high correlations of individuals' EBV and performance for all three traits.

6.4.2. Correlations

Weight and spawning date had negative phenotypic correlations, meaning that females who had high 2-year old weights tended to have early spawn dates, while those with low 2-year old weights tended to have late spawn dates. Other published phenotypic correlations between weight and spawning time have been low and positive: Su et al. (1997) reported a phenotypic correlation of 0.16 between post-spawning weight and spawn date, and Huang and Gall (1990) reported a phenotypic correlation of 0.12 between post-spawning weight and spawning age. Different population structures and environmental conditions may have contributed to the different correlations found in the current study compared with those cited. The cited studies obtained their results from separate selected lines of rainbow trout that were raised in warmer water and matured earlier than the fish in this project.

Genetic correlations between 2- year old weight and spawn dates were close to 0, meaning that there is no relationship between an animal's additive genetic value for weight and its additive genetic value for spawn date. This suggests that the genes controlling growth are not the same, or linked with those controlling spawn date. The few published genetic correlations of spawning time with body weight have been high and positive: 0.726 between post-spawning weight and spawn date (Su et al., 1997); 0.45

between 25-month weight and spawning age, and 0.48 and 0.41 between post-spawning weight and spawning age (Huang and Gall, 1990).

There was a substantial negative environmental correlation between 2-year old weight and spawn date. Environmental correlation is a measure of the strength of the relationship between environmental effects on one trait and environmental effects on another trait (Bourdon, 1997). Environmental effects that caused high 2-year old weights were associated with ones that caused early spawn dates, and those that caused lower 2-year old weights were associated with ones causing later spawn dates. Similar to this study, Su et al. (1997) reported an environmental correlation of -0.36 between post-spawning weight and spawn date, but Huang and Gall (1990) reported a very low environmental correlation of 0.07 between post-spawning weight and spawning age.

The phenotypic correlation of 0.82 between spawn date at 3 years old and spawn date at 4 years old is essentially an estimate of the repeatability of spawn date and is close to the repeatability estimates of 0.72 and 0.83 found in Chapter 5. This estimate indicates that females tend to spawn at the same times within the season from year to year. These values are higher than the repeatability estimate of 0.5 found by Sadler et al. (1992). The high positive genetic correlation of 0.92 indicates that animals tend to have the same breeding values for spawn dates from year to year. Genetically, correlations between traits are mainly due to pleiotropy, where the same genes are controlling two traits (Falconer, 1981) so it is probable that spawn date at 3 years and at 4 years are controlled by the same genes.

6.4.3. Recommended improvement program

Based on the results of this study, the following recommendations can be made as to the most efficient breeding scheme to develop a synthetic strain of rainbow trout with fast growth and a delayed spawning season. The high heritabilities of growth and spawning date predict that selection would be an effective way of improving these traits.

The negative phenotypic correlation between weight and spawn date implies that simultaneous phenotypic selection for high weight and late spawn date may not be effective because there will not be many animals with high performances for both traits that could be used as parents. It would be very difficult to do intense selection while maintaining population size and keeping inbreeding levels low. In addition, males can not be selected based on their performance for spawn date since this is a sex-limited trait. Selection intensity would have to be low, and so genetic progress would be slow. Phenotypic selection might be improved by selecting males based on quantitative trait loci (QTL), such as been found by Sakamoto et al. (1999), which would allow higher selection intensities.

Since there is zero genetic correlation between weight and spawn date, simultaneous selection on EBVs for these traits would be an effective and efficient method of improving both weight and spawn date. There should be more potential parents with good EBVs for weight and spawn date, and selection can be made among males as well.

APPENDIX 6.1. GENETIC PARAMETERS ANALYSIS OF VARIANCE TABLES

Table 6A.1. Model 6.2 MANOVA for 2-year old weight performance and EBV.

Source	df	Performance			EBV		
		MS	F	Pr>F	MS	F	Pr>F
Model	23	4510352.1	85.92	<0.0001	4490211.8	308.71	<0.0001
Sex	1	51385.0	0.98	0.3227	8543.5	0.59	0.4436
Strain	11	9369564.1	178.49	<0.0001	9357864.0	643.37	<0.0001
Sex×Strain	11	51943.7	0.99	0.4539	19110.8	1.31	0.2110
Error	949	52494.3			14545.1		
Total	972						

Table 6A.2. Model 6.3 MANOVA for 3-year old spawn date performance and EBV.

Source	df	Performance			EBV		
		MS	F	Pr>F	MS	F	Pr>F
Strain	11	20626.66	43.28	<0.0001	21326.66	99.56	<0.0001
Error	385	476.61			214.22		
Total	396						

Table 6A.3. Model 6.3 MANOVA for 4-year old spawn date performance and EBV.

Source	df	Performance			EBV		
		MS	F	Pr>F	MS	F	Pr>F
Strain	11	29892.23	35.70	<0.0001	29278.05	139.18	<0.0001
Error	373	837.42			210.36		
Total	384						

APPENDIX 6.2. GENETIC PARAMETERS FIGURES

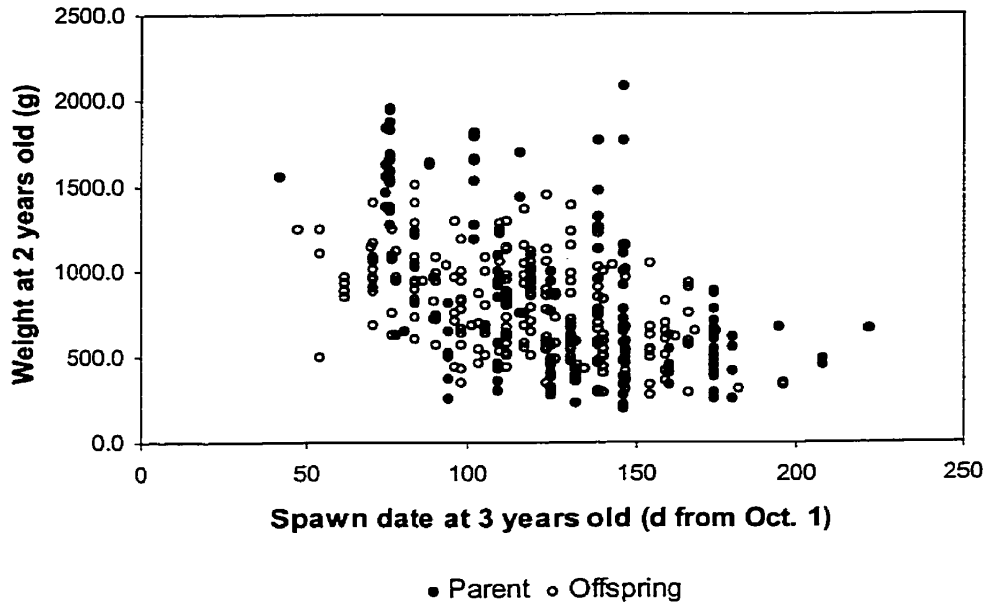

Figure 6. 1. Relationship of female phenotypes for weight at 2 years old and spawn date at 3 years old in parent and offspring generations.

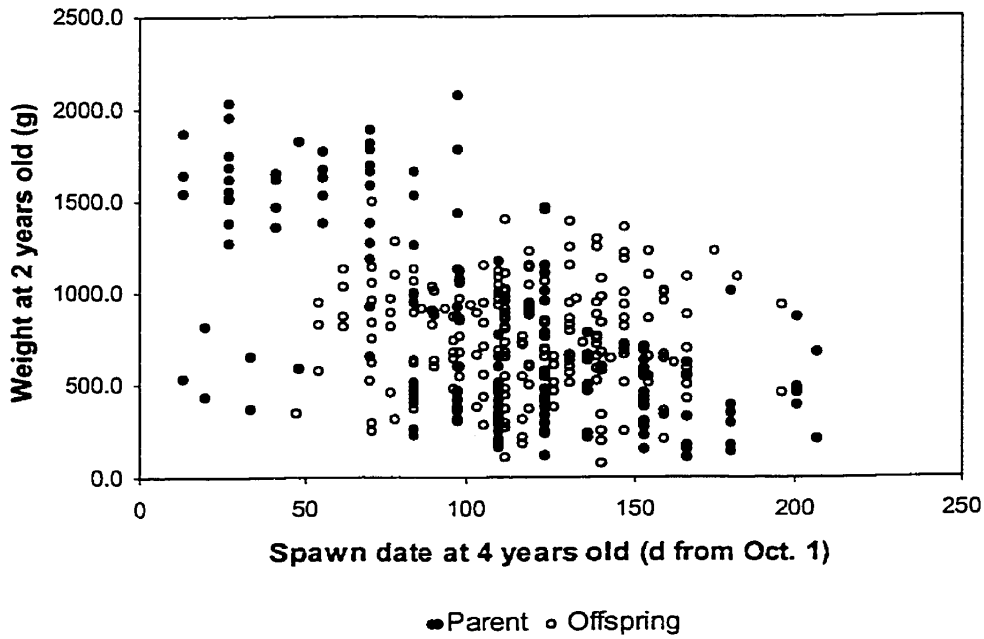

Figure 6. 2. Relationship of female phenotypes for weight at 2 years old and spawn date at 4 years old in parent and offspring generations.

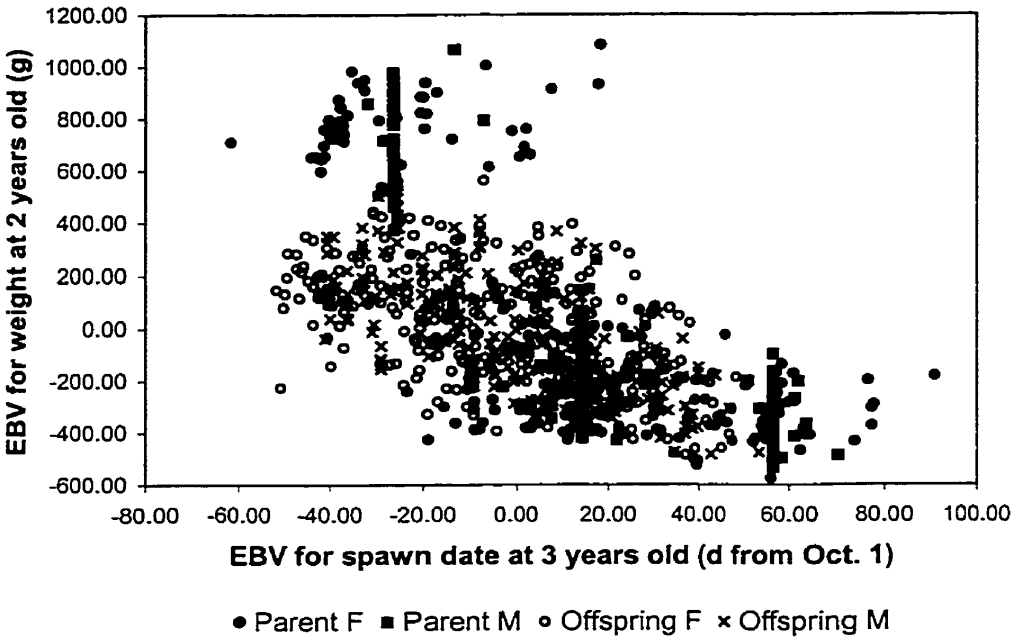

Figure 6. 3. Relationship of EBVs for weight at 2 years old and spawn date at 3 years old in females (F) and males (M) from parent and offspring generations.

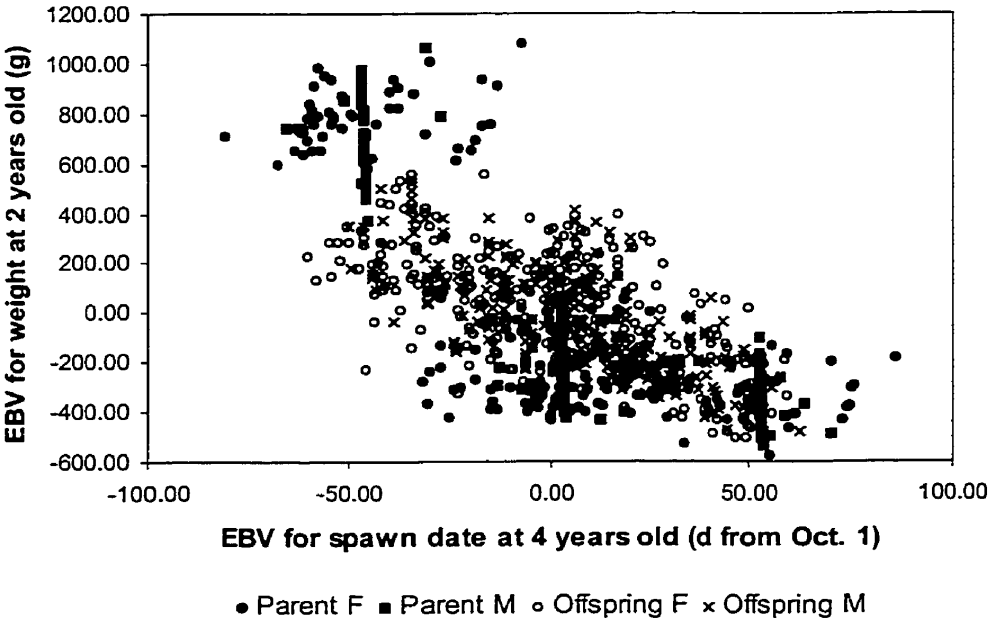

Figure 6. 4. Relationship of EBVs for weight at 2 years old and spawn date at 4 years old in females (F) and males (M) from parent and offspring generations.

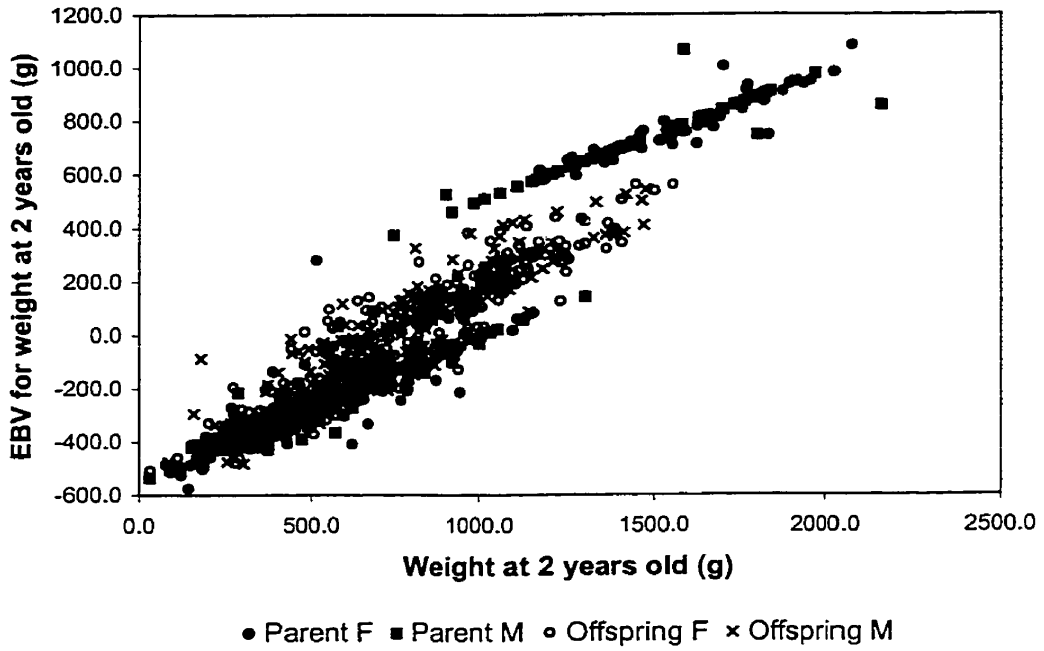

Figure 6. 5. Relationship of 2-year old weight EBV and performances in females (F) and males (M) from parent and offspring generations.

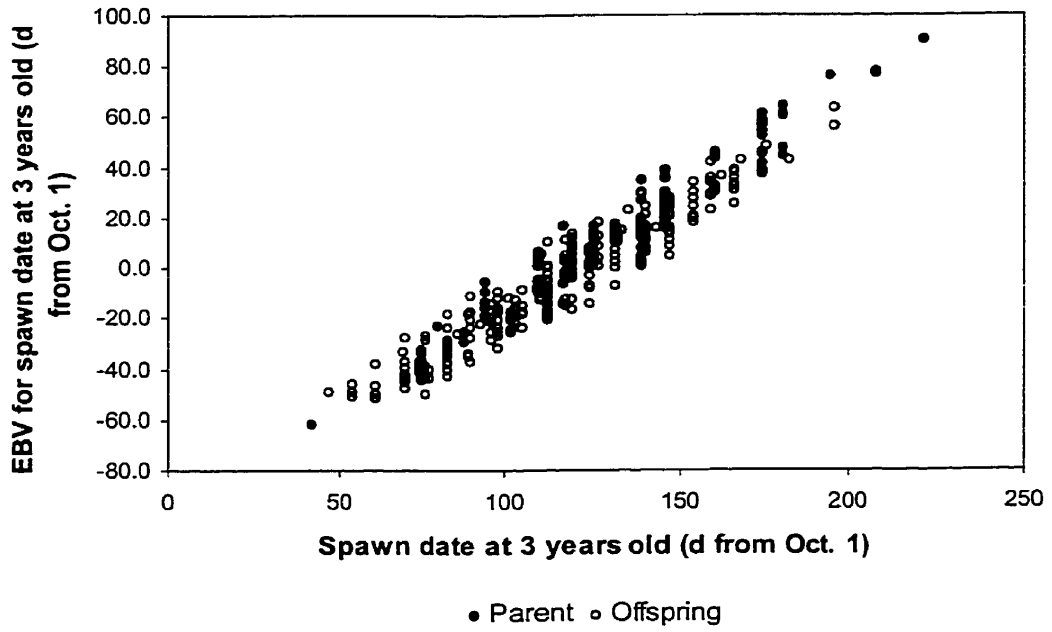

Figure 6. 6. Relationship of female EBVs and performances for spawn date at 3 years old in parent and offspring generations.

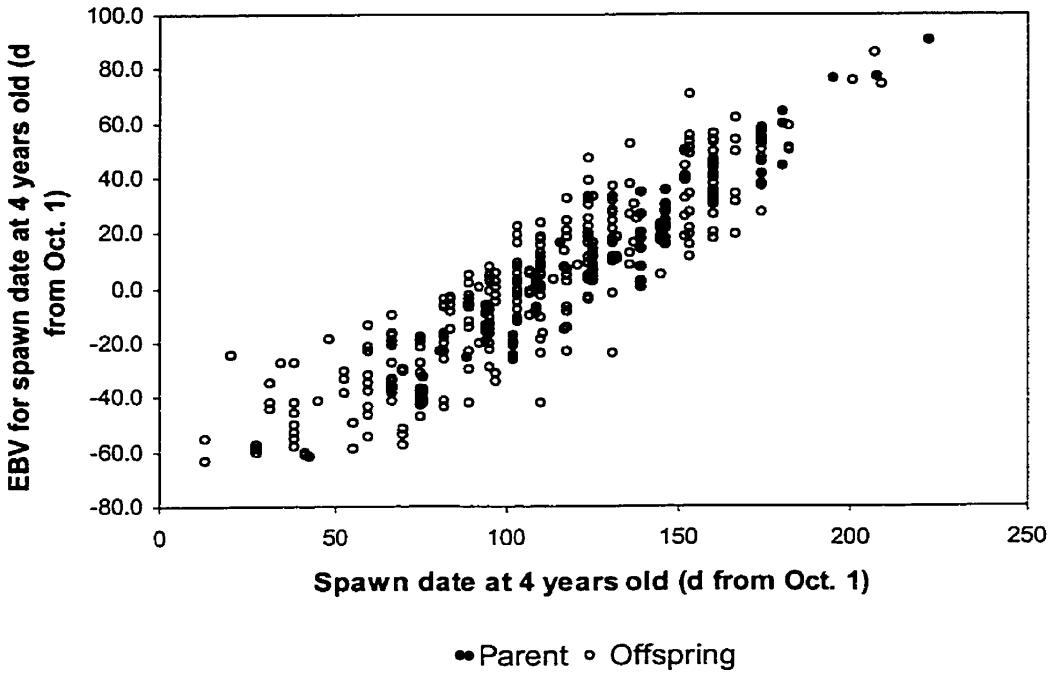

Figure 6. 7. Relationship of female EBVs and performances for spawn date at 4 years old in parent and offspring generations.

CHAPTER 7. CONCLUSIONS

To conclude, the major results of this thesis are summarised here in relation to the objectives stated in the introduction. Recommendations are also made for future research.

1. Determine the effects of time of fertilisation on growth and spawning time.

No general fertilisation week effect on growth or spawning time could be found using this data set. Any overall time effect could not be separated from dam strain effects. The experiment was not designed to detect time of fertilisation effects, so this was not unexpected. Though the effects of fertilisation time were not included in the strain comparison analyses, it should be remembered that there may still be effects and interpretation of the results should keep that in mind.

There are several ways a researcher could further examine fertilisation time effects. Females of a strain could be photoperiod-manipulated to spawn over a wider time period and differences among offspring could be tested. Hatching may also be delayed by decreasing incubator water temperature, so progeny with the same parents could be hatched over a range of times and those effects could be studied.

2. Compare strains for growth and spawning time performance.

The strains in the populations followed the expected trends for spawning time. For growth rate, however, there were unexpected trends among the strains. Dam strain rankings for growth rates were opposite to rankings for sire strains. These differences may have been due to some seasonality effect on growth rate. Alternatively, the opposing rankings of

parent strains and increased impact of dam strain may have been due to some confounded effect of fertilisation week on growth rate.

3. Determine the types of strain interactions that affected growth and spawning time.

For both growth rate and spawn date, hybrids generally performed intermediate to pure strains; there was little evidence of heterosis. This implied that these traits are, for the large part, additively inherited. This result was supported by the high heritability estimates calculated in Chapter 6.

There were also indications of non-additive genetic effects affecting growth and spawning time. All reciprocal crosses had significantly different growth rates and many had different spawn dates. Reciprocal crosses that had the faster-growing strain as a sire grew faster and with time weighed more than those who had that strain as a dam. There was some evidence of a paternal effect on growth. Further studies are needed to determine if this effect is will continue in the second and future generations. On the other hand, the trend for spawn date, was that hybrids spawned more closely to their dam's strain, which suggested that some maternal effect acted on this trait. Hybrids that had an early-spawning dam strain spawned earlier than hybrids that had the same strain as their sire instead.

4. Determine the effects of sex on growth.

Prior to maturation, males grew faster than females in all strains and crosses. This difference occurred between males and females in general, and between males and females that both matured at 3 years old. Therefore, there appears to be some pre-

maturation growth sexual dimorphism that is not due to the differences in ages of maturation among males and females.

5. Determine effects of age of maturation on growth and spawning time.

Within sexes, there was no difference in growth rate between early-maturing and late-maturing fish, but precocious males were heavier than other males, and early-maturing females were heavier than later maturing females. These results showed that size differences were due to differential growth early in life.

At 4 years old, females who were in their second spawning season spawned earlier than females who were in their first spawning season. Females spawning at 3 years old may have delayed ovulation by some weeks within the reproductive season due to having less time for growth and development of gametes. Females spawning at 4 years old have had an additional year of growth and development, so their date of ovulation is more likely to equal a genetically-determined spawning date.

6. Calculate the repeatability of spawning time.

Repeatability estimates of spawn date were high; therefore females that spawned early in the season at 3 years old also tended to spawn early in the season at 4 years old. Similarly, animals that spawned late in the season at 3 years old also tended to spawn late in the season at 4 years old. Though the correlation between 3-year and 4-year spawn dates was high, the entire reproductive season occurred earlier when the populations were 4 years old. As explained above, 4-year old females have had more time for growth and development, and so may have been able to ovulate earlier in the year than they did at 3 years old.

7. Estimate genetic parameters related to growth and spawning time.

Two-year old weight and spawning date at 3 and 4 years old were all highly heritable. Two-year old weight and spawning date had negative phenotypic correlations, but genetic correlations between them were close to 0. There was a substantial negative environmental correlation between 2-year old weight and spawn date. The phenotypic correlation between spawn date at 3 years old and spawn date at 4 years old was close to the repeatability estimated in Chapter 5. There was a high positive genetic correlation between spawn dates at 3 and 4 years old.

8. Determine the most efficient breeding scheme to develop a synthetic strain of rainbow trout with fast growth and a delayed spawning season.

The patterns of inheritance found in Chapters 4 and 5 and the genetic parameters calculated in Chapter 6 indicated that growth and spawning time could be improved with the appropriate breeding program. The additive pattern of inheritance, and high heritabilities predict that selection would be an effective way of improving these traits.

The negative phenotypic correlation between weight and spawn date implied that simultaneous phenotypic selection for high weight and late spawn date may not be effective. But since there was zero genetic correlation between weight and spawn date, simultaneous selection on EBVs for these traits would be an effective and efficient method of improving both weight and spawn date.

Phenotypic and EBV-based selection has been done for weight at 2 years old and female spawn date. Future studies will examine the progeny of these matings to evaluate the genetic progress made by each of these selection methods.

A third trait that would benefit the Ontario aquaculture industry is reduced incidence of precocious maturation. Future studies will examine the strains and diallel crosses for rates of maturation and evaluate methods of including this trait into the breeding program.

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