THE UNIVERSITY OF MANITOBA

GROWTH, HETEROSIS, HETEROZYGOSITY AND EFFECTS OF TEMPERATURE AND COMPETITION IN PURE AND HYBRID STRAINS OF RAINBOW TROUT (*Salmo gairdneri* Richardson)

by

© Cleophas Charles Barasa Wangila

A Thesis
submitted to the Faculty of Graduate Studies
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ABSTRACT

Assessment of growth rate in strains and inter-strain hybrids of rainbow trout (Salmo gairdneri Richardson) showed significant temperature and genotype differences. The same assessment over short periods ranging from 14 to 98 days revealed inconsistencies in the ranking of strains. This led to the recommendation that, for a meaningful growth assessment, observations should span economically important phases set in a production system.

A summary of weight variability in a strain over a weight range was devised by the use of a variance-mean weight regression relationship. From the equation was constructed a relative variance index or "RVI" which was used to compare variability among strains and between families within strains.

The differences in growth rates were complimented with significant differences in the distribution of allelic frequencies at 7 out of 10 genetic loci analyzed electrophoretically. Crossbreeding among rainbow trout strains has possible economic value as heterosis for growth among hybrids was estimated at about 7%. Heterozygote advantage was found to be more

pronounced at the sub-optimal temperature of 7°C than at 15°C indicating that failure to consistently demonstrate associations between heterozygosity and growth/weight or variance in morphological characters by some workers could partly be due to environmental factors.

It was also shown that experimental designs which use mixing of strains in the same tanks without taking into account effects of the strains on each other could lead to erroneous conclusions.

To my mother and late father who gave up everything for my education.

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GENERAL INTRODUCTION

Fish culture literally means the growing of fish under some management system. It is not surprising, therefore, to find that nearly all workers in this area are in one way or another concerned with some aspect of growth assessment of fish species, strains of a species or even families of a given strain. The growth rate of any organism under culture and the possibilities of its manipulation in a management system have a direct bearing on the success or failure of a production facility. Research on the possibilities of manipulating growth through husbandry practices have recently been conducted with respect to genotype x environment interactions.

A long history of natural and artificial selection of trout has established significant differences between populations (Ayles, 1975; Kanis et al., 1976; Gall and Gross, 1978; Reinitz et al., 1978; Ayles and Baker, 1983) some of which have earned recognition as distinct "races" or strains.

Growth rate among strains of brook trout (Salvelinus fontinalis Mitchell) was shown to differ (Green, 1952; Vincent, 1960; Flick and Webster, 1967

& 1976). Reisnbichler and McIntyre (1977) observed differences in growth rate between juvenile hatchery and wild steelhead trout (Salmo gairdneri) while Reinitz et al. (1978) found a differential growth performance in four strains of rainbow trout reared under standard conditions. Sadler et al. (1986) found significant growth differences in lake trout, Salmo namaycush and rainbow trout strains but not in brook trout strains. Such findings indicate that there are always possibilities of choosing a desirable strain based on superiority in growth performance provided that proper screening of the available strains is performed.

It is well known that fish are poikilotherms and thus their metabolism is directly influenced by ambient temperature. Low growth rates are generally associated with low temperatures while fast growth rates with high non-lethal temperatures (Sadler et al., 1986). Genotype by temperature interactions once elucidated can be used to exploit the growth-temperature dynamics in the choice of species, strain or even family for a particular temperature. Genotype by environment interactions have been evaluated in various rainbow trout strains (Klupp et al., 1978; Ayles, 1975; Uraiwan, 1982; McKay et al.,1984).

Kinghorn (1983) while reviewing quantitative genetics in fish stated that there was insufficient evidence that crossing species or strains within species is of any potential commercial value. Refstie and Gjedrem (1975), however, reported heterosis in 11 month weight of crosses between charr, brown trout and salmon but unlike Blanc and Chevassus (1979), did not observe heterosis when strains within the various salmonid species were crossed. Contrary to this, Gall (1975) recorded heterotic effects on body weight at 2 years for progeny from a cross between two strains of rainbow trout. On the other hand, Gjerde (1981) reported lack of significant heterosis for slaughter weight in crosses of five Atlantic salmon strains. Edwards and Gjedrem, (1979) found that an inter-strain cross of brown trout had higher survival at the alevin stage in acid water than either of the pure strains and attributed this to heterosis.

Hybrid vigour or heterosis is usually expected when lines with sufficiently different gene frequencies (preferably in-bred lines) are crossed (Falconer, 1981). That races/strains are isolated, as far as gene flow is concerned, is an indication that they differ in gene frequencies at a number of gene loci. Failure to demonstrate heterosis may only mean that the trait

assessed is not influenced appreciably by the alleles by which two populations differ. Both academic and applied questions can be asked on the subject of heterosis. Heterosis has usually been achieved by crossing highly inbred lines (Falconer, 1981) on the assumption that such inbred lines would be homozygous at many loci and crossing them would create highly heterozygous progeny. In separate reviews, Mitton and Grant (1984) and Zouros and Foltz (1987) recalled that heterosis was first coined by Shull (1914) to describe hybrid vigour without having to involve heterozygosity as a mechanism that led to "heterozygosis" another term used at the time to mean hybrid vigour. Today heterosis is still used as a synonym to heterozygosity (Zouros and Foltz, 1987). With the advent of gel electrophoresis, protein heterozygosity can now be assessed directly at various gene loci to test the connection between protein heterozygosity and hybrid vigour as observed in progeny from crosses between lines that differ in gene frequencies.

This thesis had five main objectives: 1. to compare three analytical methods in the assessment of growth performance in three strains of hatchery reared rainbow trout (Salmo gairdneri Richardson) together with their inter-strain crosses, 2. to investigate

heterotic effects in specific growth rate due to inter-strain crosses in rainbow trout, 3. to characterize three strains of rainbow trout using isozymes, 4. to investigate the relationship between the degree of heterozygosity at electrophoretically determined genetic loci and weight or group growth performance in rainbow trout and, 5. to examine effects of interactions between and among rainbow trout strains reared together in the same tank.

Chapter 1

Growth assessment in hatchery-reared pure and hybrid strains of rainbow trout (*Salmo gairdneri* Richardson).

ABSTRACT

Significant differences in specific growth rate were found among strains of rainbow trout (Salmo gairdneri Richardson) together with their hybrids. Specific growth rates ranged from 0.94% to 1.18% day^{-1} among fish reared at 7°C and 2.29% to 2.68% day^{-1} among those reared at 15°C. The temperature effect was significant and fish grew about 2.5 times faster at 15°C than at 7°C. Strains as well as families within strains re-ranked in growth performance indicating that some genotypes performed better at specific temperatures. This re-ranking was manifested into significant strain, or family within strain interactions with temperature. Variance component analysis revealed that variation in specific growth rate due to families within strains was higher than that due to strains.

Analysis for growth differences over segments of data yielded inconsistent results. This strengthens the fact that for experimental results to have useful application in fish production, studies should span periods of economic importance. A summary of all the data using a multivariate approach showed significant strain differences.

The relationship between specific growth rate and fish size (weight) was influenced by temperature and genotype. Heterogeneity among the regression slopes for strains (genotype) as well as interaction with temperature invalidated the use of analysis of covariance to adjust for initial fish size differences. Use of the slope in the relationship to compare specific growth rates in fish, as proposed by Jobling (1983), was questioned.

The slope in the length-weight relationship was readily estimated using the ratio of the coefficient of variation of weight to that of length with very little loss of accuracy. The were no significant differences among genotypes and between temperatures for this relationship. Fulton's Condition Factor was also nonsignificantly different among genotypes as well as between temperatures.

Records of fish weight from initial mean weight of 3.0 g to 100.0 g for 14 weeks showed that $\log_e$ variance increased approximately as the square of $\log_e$ mean weight in both pure and inter-strain crosses. The rates of increase were higher at 7°C than at 15°C. Log_e variance-$\log_e$ mean weight relationship equations were:

at (7°C) $\log_e V = -2.946 + 2.128 \log_e W$

at (15°C) $\log_e V = -2.841 + 2.111 \log_e W$

The genetic characteristic of variance in the strains and full-sib families within strains was demonstrated using a "relative variance index" which was defined as the ratio of observed variance to expected variance. The expected variance was predicted from the linear regression equation from pooled data for the relevant level of comparison. This index may have application in aquaculture for fish selection for low variance since it could be used to rank strains and families of fish by their variance in weight.

Food conversion efficiency was significantly different among strains, ranging from 18.8% in the pure Manx strain to 33.2% in a hybrid between the Mount Lassen and Tagwerker strains. Growth rate was positively correlated with food conversion efficiency, but negatively to the relative variance index, which indicated that selection for low variability in fish size, condition factor and high conversion efficiency would not conflict with selection for fast growth.

INTRODUCTION

The importance of assessment of growth in fish in aquaculture is evidenced by the large number of published works on the subject (e.g. Brett and Shelbourn, 1973; Stauffer, 1973; Elliot, 1975; Jobling, 1983; McKay et al., 1984, 1985, 1986; etc.). When the existing literature is examined closely, however, it is evident that analyses of growth in fish are not standardized; the common feature is that means of either weight or length or both at the beginning and end of the study period are recorded, in order to calculate the specific growth rate (G) after Brown (1957) and Ricker (1958) and then analyze G for differences. The time intervals for growth are often brief: anywhere from 14 to 42 days with the rationalization that fish are in an exponential growth phase. In fish production, however, the aquaculturist is more interested in assessing performance over the production phase rather than over some arbitrarily chosen time interval which, although it might refine computational accuracy, may not necessarily translate into economic objectives.

While some workers have used an analysis of covariance (ANCOVA) to adjust for differences in

initial mean weights among fish groups in which G is compared (e.g. Jobling, 1983), others have compared G as calculated despite differences in initial sizes among fish groups (e.g. Klupp et al., 1984; Mckay et al., 1985, 1986; Sadler et al., 1986). Since growth performance is a trait of great economic importance it is important that, statistically, methods are plausible and comparable. The fact that different workers employ different methods in assessing growth performance makes it difficult to compare results.

The primary reason for analyzing growth performance among genotypes is to decide on which genotype and at what temperature, ration etc. a given strain, or family should be reared. Consequently, decisions made using any of the analyses should be consistent or else only the more reliable approaches should be used. For these reasons, the present data is analyzed using analysis of variance (ANOVA), analysis of covariance (ANCOVA), multivariate analysis of variance (MANOVA) and repeated-measures analysis to check for consistency in conclusions.

The remainder of the chapter examines four additional aspects of growth: 1. a measure of total variability in fish using an index, 2. an examination of length-weight relationship, 3. an assessment of

Fulton's condition factor and 4. an evaluation of gross food conversion efficiency.

Variability in fish weight is important because fish culture production systems are usually geared towards producing fish of a fairly uniform size. It is known that genetic variability and variation due to environment, generate fish size heterogeneity that requires physical grading to select a uniform product (Gunnes, 1976; Jobling, 1983). The occurrence of large size differences in a stock of fish can cause considerable economic loss in terms of facilities and sorting time. This important problem led Yamagishi (1969) to wonder whether researchers studying fish growth by comparing mean sizes should instead be studying variation in fish sizes.

While several researchers have sought an explanation for size variability as observed in fish (Brown, 1946; Aulstad et. al., 1972; Purdom, 1974; Jobling and Wandsvik, 1982; Koebele, 1985), the relationship between weight and variance in stocks of fish under culture conditions has not yet been examined. Purdom (1974) used the coefficient of variance (the ratio of variance in length to the square

of the mean length) to show that social hierarchy is the source of variation in length in flatfish.

In animals and plants, variance of body size increases with increasing mean weight (Wright, 1968; Falconer, 1981). Empirical relationships between variance (especially the standard deviation) and the mean have ordinarily been investigated only in the light of scale transformations (Rasmussen, 1933; Wright, 1968; Falconer, 1981). For example, it is known that in biological growth the variance of the natural logarithm ($\log_e$) of weight is approximately proportional to the square of the weight (Causton, 1969). In this study genotypic as well as temperature influences in the relationship between variance and mean weight were examined in rainbow trout for possible application in selection programs. The predictive aspect of the $\log_e$ linear regression equation may be used by fish culturists to forecast variance for a given mean weight target. Once the variance is forecast, statistics such as coefficient of variation and standard deviation can be determined for planning purposes. From the variance-mean weight regression equation as well, an index termed "relative variance index" for comparing variance in strains, inter-strain crosses, hybrids and families is proposed as a ranking

standard based on performance of genotypic groups of fish with respect to variance for a given mean weight.

MATERIALS AND METHODS

General

Three strains of rainbow trout (S gairdneri Richardson) with four inter-strain crosses were studied at the Rockwood Hatchery of the Government of Canada's Department of Fisheries and Oceans Freshwater Institute, Manitoba, Canada (Ayles et al., 1980). Table 1 shows the number of families spawned and used in the growth studies. Each of the families was set up by artificially spawning a different pair of randomly selected parents from a brood of ripe individuals. Families 1 and 4 in the Mount Lassen strain (LAS) were half-sibs as they shared a common female. Family 1 in the TAGMAN cross had a common Tagwerker (TAG) male with family 4 of the TAGLAS cross while family 4 of the TAGMAN cross shared a male with family 4 of the TAGLAS cross. The origin of the Manx (MAN) and Mount Lassen strains was described by Uraiwan (1982) and the latter further described by Baker (1983). The Tagwerker strain was originally obtained from the Tagwerker Fish Farm, Ontario, Canada, where it is said to have been selected for early spawning and fast growth (Papst, pers. comm.).

Table 1. Strains[1] and inter-strain crosses[2] studied, their origin, number of families and years at the Rockwood Hatchery.

STRAIN/CROSS	FAMILIES		ORIGIN	YEARS
	# SPAWNED	# SURVIVED		
Manx	5	4	Isle of Man	12
Mount Lassen	5	4	Mount Lassen Farm, Kamloops, B.C., Canada.	8
Tagwerker	5	3	Tagwerker Farm Ontario, Canada.	5
MAN X LAS	2	2	-	-
LAS X MAN	2	2	-	-
TAG X LAS	5	4	-	-
TAG X MAN	5	2	-	-

1. Throughout this work the following abbreviations will be used for names of the strains: Manx (MAN), Mount Lassen (LAS), Tagwerker (TAG).
2. Inter-strain crosses will be referred to as strains except in cases where it would create ambiguity. In all cases the male parent will be given first for inter-strain crosses e.g. TAGLAS would refer to a male Tagwerker crossed with a female Mount Lassen.

Fertilized eggs from each pair were separately incubated in plastic jars at 7°C. On hatching the larvae were transferred into labelled, 60 litre tanks also maintained at 7°C. They were fed ad lib on finely ground trout pellets (Martin's Feed Mills, Elmira, Ontario) on onset of swim-up.

Randomization

At a mean weight of about 2 g, fish were randomly selected from each family, individually weighed and placed one at a time in 4 aerated water buckets numbered 1 to 4 so that each bucket finally held 75 fish of a known weight. Two of the fish groups were each placed in a 0.6 x 0.6 m, 60 litre tank at 7°C and the other two placed in similar tanks in which water temperature was gradually raised to 15°C in the ensuing week. Families were randomized over tanks to ensure that replicates of each family appeared in the top and bottom tanks equally. In all, there were 36 tanks at 7°C and 42 at 15°C carrying a total of 5,750 fish.

Feeding

All fish were fed to satiation three times a day on a commercial trout feed (Martin's Fish Meal, Elmira,

Ontario). Food was offered by hand and satiation point assumed when voluntary intake of food by fish virtually ceased. A record of the amount of food fed per tank was kept. Feed for fish in a tank was kept in a marked plastic container with a tight fitting lid to prevent it from getting wet. At sampling periods, the containers were filled with an equal amount of feed, approximately 50% in excess of standard ration tables of Hilton and Slinger (1981).

Sampling

A random sample of 40 fish from each of the 78 tanks was taken and each fish weighed individually every 28 days alternating with batch weight records when fish were weighed together, counted and the mean weight determined. This sampling regime resulted in individual and batch weight records alternating bi-weekly. The same dip net was used for sampling individual fish from each tank. At the beginning and end of the study period all fish in the tank were individually weighed. In all cases fish were wiped with a damp cloth to remove excess water before weighing on the electronic Mettler Balances that were used through out the 98 day study period. Fish were not fed on sampling days.

Data Analysis

Assignment of fish within families within strains to tanks within temperatures fits a nested design. Fish were repeatedly weighed over an extended period to assess growth performance not only for one period of 98 days employing least square methods in univariate analysis of variance (Harvey, 1978), but also between censuses within strains and families within strains using multivariate repeated measures analysis. Because there were small differences between initial mean weights among various classes at the beginning of each growth period, the method of analysis of covariance was used to correct for these differences (Jobling, 1983). The assessment of growth is therefore divided into three sections each employing one of the methods. In some cases where ranks were used for strains or families within strains, Spearman's Rank Correlation Coefficient was employed to estimate the repeatability of ranks at both temperatures. Because results obtained from individual weight data were not different from those from batch weight data only the former were reported.

Regardless of the method, however, the specific growth rate, "G" was calculated as:

$$G = \frac{(\log_e W_2 - \log_e W_1)}{(t_2 - t_1)} \times 100 \quad [1]$$ (Brown, 1957).

where, $\log_e$ = natural logarithms

W_2 = weight at time 2

W_1 = weight at time 1

$(t_2 - t_1)$ = growth period in days

Least square method for univariate analysis of variance

The statistical model used to assess specific growth rate of the strains and families within strains at one temperature on log transformed data was:

$$Y_{ijk} = \mu + S_i + F(S)_{j(i)} + t(SF)_{k(ij)} + e_{ijk} \quad [2]$$

where Y_{ijk} = $\log_e$ G for fish in the k^{th} tank containing the j^{th} family from the i^{th} strain/cross

μ = overall mean

S_i = Strain/cross effect (i= 1 to 7)

$F(S)_{j(i)}$ = family within strain/cross effect (j=1 to 2, 3, 4)

$t(SF)_{k(ij)}$ = tank effect (k=1 to 2)

e_{ijk} = error

Strain/cross effect was considered fixed while the rest of the effects were considered random. Family within strain/cross effect was used as error term for testing for no strain effect, and tank effect as error term for testing no family within strain/cross effect.

Model [2] was extended to test for temperature effect, and becomes:

$$Y_{ijkl} = \mu + T_i + S_j + F(S)_{k(j)} + TS_{ij} + TF(S)_{ik(j)} + t(TFS)_{l(ijk)} + e_{ijkl} \qquad [3]$$

where Y_{ijkl} = $\log_e$ G recorded for fish of the k^{th} family from the j^{th} strain/cross reared at the i^{th} temperature in the l^{th} tank

μ = overall mean

T_i = temperature effect (i= 1 to 2)

S_j = strain/cross effect (j= 1 to 7)

$F(S)_{k(j)}$ = family within strain effect

TS_{ij} = temperature by strain interaction

$TF(S)_{ik(j)}$ = temperature by family within strain interaction

$t(TFS)_{l(ijk)}$ = tank effect

e_{ijkl} = error

Temperature and Strain effects were considered fixed while the rest of the effects were considered random.

The approximate error term for temperature effect was temperature by strain interaction.

SAS (1985) components of variance (method=TYPE1) was used to obtain expected mean squares as a guide for choosing the correct error terms to test for the effects of interest in the respective models. Variance components were estimated for strains and families within strains at each of the temperatures and for both temperatures again using the SAS (1985) components of variance procedure. Pairwise comparisons of least square means of specific growth rate (using LSD option of SAS, 1985, which controls for comparisonwise error rate i.e. type 1 error) for families within strains and among strains were conducted separately for each temperature.

Analysis of covariance

Analysis of covariance was used with initial mean weight as a covariate. The relationship between specific growth rate and fish size was linearized by $\log_e$ transformation (Brett and Shelbourn, 1975; Elliot, 1975; Jobling, 1983a). Tests of heterogeneity of slopes among strains and families within strains between the two temperatures were carried out using the

procedures described by Freund et al., (1986). Intercepts which represented adjusted specific growth rates were compared where appropriate using the same procedure. The statistical model was:

$$Y_{ijk} = \mu + S_i + F(S)_{j(i)} + t(SF)_{k(ij)} + b_1W_{ijk} + b_{2i}W_{ijk} + b_{3j(i)}W_{ijk} + b_{4k(ij)}W_{ijk} + e_{ijk} \quad [4]$$

where Y_{ijk} = $\log_e$ specific growth rate for fish in the k^{th} tank containing the j^{th} family of the i^{th} strain/hybrid
($i=1$ to 7; $j=1$ to 2, 3, 4; $k=1$ to 2);

μ = overall mean $\log_e$ specific growth rate

S_i = the i^{th} strain/hybrid

$F(S)_{j(i)}$ = the j^{th} family within the i^{th} strain/hybrid: used as error term for testing the hypothesis of no S_i effect

$t(SF)_{k(ij)}$ = k^{th} tank effect: used as error term for testing the hypothesis of no family within strain/hybrid effect

b_1W_{ijk} = regression relationship between specific growth rate and weight

$b_{2i}W_{ijk}$ = regression slopes for the relationship among strains/hybrid

$b_{3j(i)}W_{ijk}$ = regression slopes for the relationship among families within strains/hybrid

$b_{4k(ij)}W_{ijk}$ = regression slopes for the relationship among the j^{th} families of the i^{th} strain/hybrid contained in the k^{th} tank

e_{ijk} = error term

which, on inclusion of temperature effect, became :

$$Y_{ijkl} = \mu + S_i + F(S)_{j(i)} + T_l + ST_{il} + TF(S)_{lj(i)} + t(SFT)_{k(ijl)} + b_1W_{ijkl} + b_{2i}W_{ijkl} + b_{3j(i)}W_{ijkl} + b_{4k(ijl)}W_{ijkl} + b_{5l}W_{ijkl} + b_{6il}W_{ijkl} + b_{7lj(i)}W_{ijkl} + e_{ijkl} \quad [5]$$

where,

Y_{ijkl} = $\log_e$ specific growth rate for fish reared at the l^{th} temperature in the k^{th} tank holding the j^{th} family of the i^{th} strain/hybrid (i=1 to 7; j=1 to 3,4; k=1 to 2; l=1 to 2;)

μ = overall mean $\log_e$ specific growth rate

S_i = the i^{th} strain/cross effect

$F(S)_{j(i)}$ = the j^{th} family within the i^{th} strain/cross: used as error term for testing the hypothesis of no S_i effect

T_l = l^{th} temperature effect

ST_{il} = Strain/cross by temperature interaction

$TF(S)_{1j(i)}$ = temperature by family within strain/cross interaction

b_1W_{ijkl} = regression relationship between specific growth rate and weight

$b_{2i}W_{ijkl}$ = regression slopes for the relationship among strains/hybrid

$b_{3j(i)}W_{ijkl}$ = regression slopes for the relationship among families within strains/hybrid

$b_{4k(ijl)}W_{ijkl}$= regression slopes for the relationship among the j^{th} families of the i^{th} strain/hybrid contained in the k^{th} tank at the l^{th} temperature

$b_{5l}W_{ijkl}$ = regression slopes for the relationship between temperatures

$b_{6il}W_{ijkl}$ = regression slopes for the relationship among strains/hybrid across temperatures

$b_{7lj(i)}W_{ijkl}$ = regression slopes for the relationship among families within strains/hybrid across temperatures

e_{ijkl} = error term

Multivariate Repeated-Measures Analysis

Multivariate repeated-measures analysis of variance (Freund et al., 1986) was used to test whether there were any differences in specific growth

rate within strains and families within strains over the study period. This test was provided by the within subject analysis. The trend was assessed from a multivariate analysis considering the specific growth rate for period one (G1) through the fourth period (G4) as different variables measured on the same unit and examining the correlation matrices. The general model for this analysis was:

$$[Y] = [X][B] + [U] \qquad [6]$$

where [Y] = a matrix of specific growth rates (G1 G2 G3 G4) calculated for 14, 28, 28, and 28 days, respectively

[X] = design matrix of the classes: temperature, strain, line(strain) and tank(temperature, strain, line)

[B] = matrix of effect coefficients

[U] = matrix of random errors

This procedure also provided tests for overall effects of strains and families within strains at separate temperatures by omitting temperature in the [X] matrix.

Other statistics

Length-weight relationship

The length-weight relationship was determined for each of the strains and families using the standard formula :

$$\log_e W = a + b \log_e L \qquad \text{(Le Cren, 1951)} \qquad [7]$$

Where W = the weight of fish in grams

L = the fork length of fish in centimeters

a = intercept

b = the slope

The slopes were compared using the test of heterogeneity of slopes in the ANCOVA package as described by (Freund et al. 1986).

Objects which vary in size but maintain constant shapes, have all linear measures perfectly correlated, with equal coefficients of variation, so that volume would be related to length by its cube (Schmallhausen, 1927). This means that the slope in [7] can be estimated by the use of the coefficient of variation in weight and length or standard deviation and the mean as shown in the expressions:

$$b' = CV_W/CV_l \qquad [8]$$

Where

b' = the length-weight relationship coefficient

CVw = the coefficient of variation in weight

CVl = the coefficient of variation in length

On rearranging [8] the expression becomes:

$$b' = D_w \cdot Y_l / Y_w \cdot D_l \qquad [9]$$

where D_w = standard deviation of weight

Y_w = corresponding mean weight

D_l = standard deviation of length

Y_l = corresponding mean length

b' = length-weight relationship coefficient

Condition factor

Condition factor was calculated according to the formula:

$$K = \frac{W \times (1.0 \times 10^{-5})}{L^3} \qquad [10]$$ (Ricker, 1973)

Where W = weight of fish in grams

L = fork length of fish in millimeters.

The condition factor was then analyzed in ANOVA (Freund et al. 1986) for any differences between temperatures, strains, and families within strains.

Variance-mean weight relationship

Assuming that variance follows the well known metabolic-fish size relationship that has given rise to many other empirical relationships such as that between specific growth rate and fish size (Winberg, 1956; Brett and Groves, 1979; Jobling, 1983), the relationship of variance with the mean weight may be expressed as $V = a\ W^{b}$, where V is the observed variance, a is a constant, W mean weight and b an exponent. A logarithmic transformation of this equation gives model 11 below below:

$$\log_e V = a + b\log_e W \qquad [11]$$

where V = the variance in weight

a = intercept

b = regression coefficient (slope)

W = mean weight of fish

The parameters a (=intercept) and b (=slope) were estimated from the regression of the natural logarithms

of variance on the natural logarithms of the respective mean weights obtained from each family of fish in each tank. Fish were individually weighed at monthly intervals to estimate the mean and the variance. Model 11 was applied on a hierarchical basis, i.e. for strain/hybrid comparisons all data for strains/hybrid were pooled while for family comparisons the regression was established using pooled data from the families within the respective strains/hybrid. A test of possible nonlinearity in the regression relationship was conducted by the examination of residuals (Neter et al., 1985).

Again the test of heterogeneity of slopes was applied using ANCOVA (Freund et al., 1986). An index termed " Relative Variance Index" or "RVI" was devised to rate relative performance of strains and families within strains. It was defined as:

$$RVI = \frac{\text{observed variance}}{\text{expected variance}} \qquad [12]$$

Where expected variance was obtained from [11] above and observed variance was calculated from raw data.

Gross food conversion efficiency

Gross food conversion efficiency (FCE) was estimated by dividing the amount of food fed by weight gain in fish. Dry weights used in this calculation were obtained by oven-drying finely ground samples of feed and fish at 110°C and 130°C respectively (Tabachek, pers. comm). From dry weights, factors for converting wet into dry weights were determined. The gross food conversion efficiencies obtained for each strain/family were then compared in an ANOVA (Freund et al. 1986) using the least square models described in [2 and 3]. In these models all the terms remain the same except that the observations were FCEs in a tank containing family within a particular strain (and temperature for model 2).

Overall assessment

A multivariate analysis of variance was used to assess specific growth rate, condition factor, relative variance index and food conversion efficiency in strains and families for overall comparisons. The partial correlation matrix involving these traits was also examined for relationships. To pictorially examine trends in the relationships regression lines were plotted.

RESULTS

Least square univariate analysis of variance

The natural log transformation in formula (1) resulted in a linear relationship of weight with time in days depicting that over the study period fish were still in an exponential growth phase (Figs. 1 & 2). Examination of residual plots did not reveal any departure from the linear relationship.

Analysis of variance of specific growth rate revealed significant differences between strains (Table 2) at each of the temperatures. Families within strains were significantly different only at 7°C. For the model including the temperature effect, temperature was found significant ($P<0.0001$) as well as temperature by strain interaction ($P<0.001$).

Table 3 shows least square mean specific growth rates of strains and families within strains at 7°C and 15°C. The overall means ranged from 0.94% per day to 1.18% per day and from 2.29% per day to 2.68% per day at 7°C and 15°C respectively. Strains re-ranked in growth performance between the temperatures (Table 3). While the LAS strain ranked first at both temperatures,

Fig. 1. Log_e mean weight plot over growth period in days for strains reared at 7°C (each data point represents mean weights pooled over families within strains).

Fig. 2. Log_e mean weight plot over growth period in days for strains reared at 15°C (each data point represents mean weights pooled over families within strains).

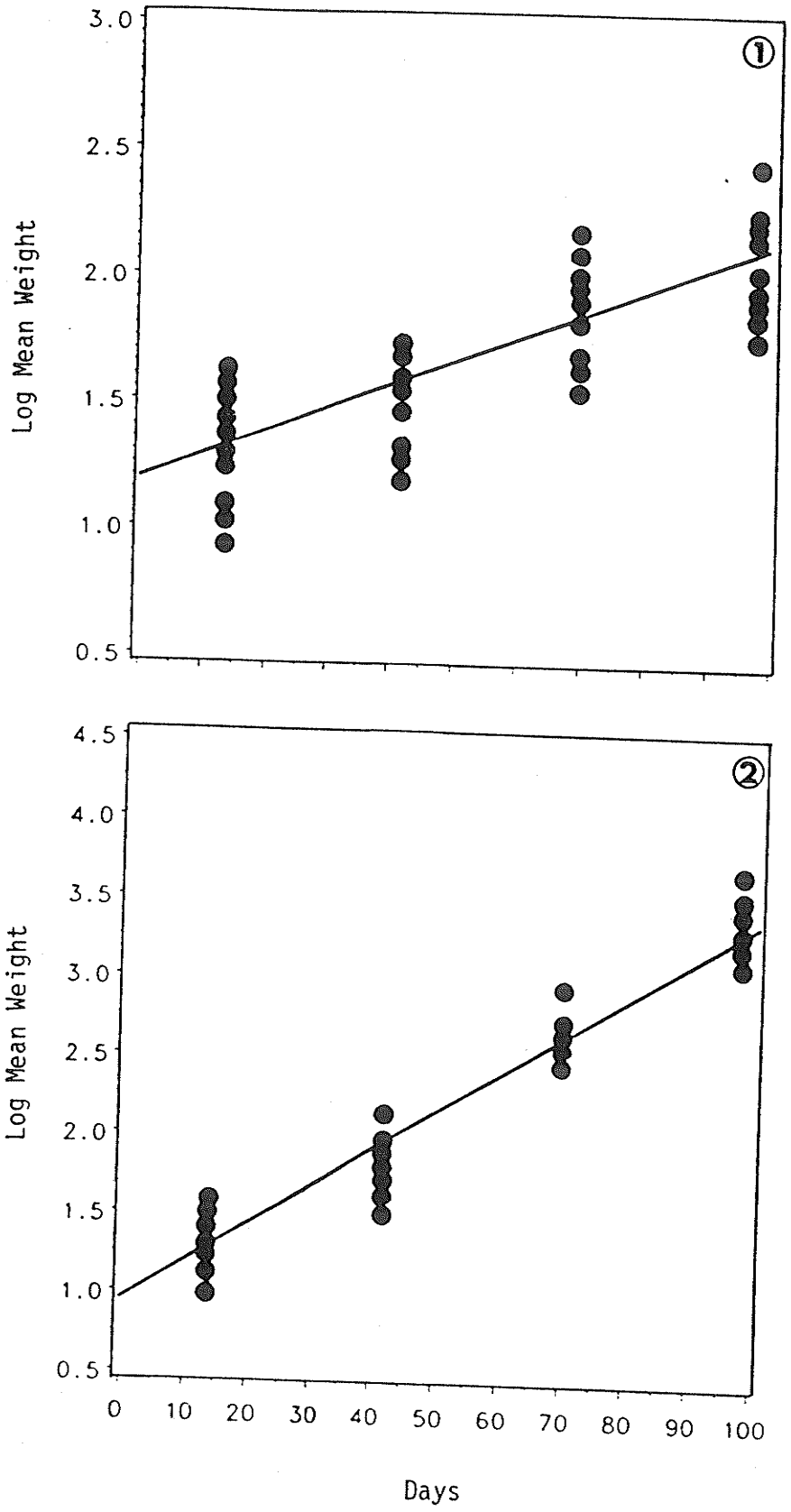

①
Log Mean Weight
3.0
2.5
2.0
1.5
1.0
0.5
②
Log Mean Weight
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
10
20
30
40
50
60
70
80
90
100
Days

Table 2. Least square analysis of variance for specific growth rate using models [2], [3], [4] and [5].

	Remarks					
Effect	model[2]		model[3]	model[4]		model [5]
	7°C	15°C		7°C	15°C	
T	-	-	***	-	-	***
S	*	*	**	*	ns	**
F(S)	*	ns	*	ns	*	*
TS	-	-	**	-	-	**
TF(S)	-	-	**	-	-	**
BWXStr	-	-	-	***	ns	**
BWXF(S)	-	-	-	***	ns	ns
BWXTXS	-	-	-	-	-	***
BWXTXF(S)	-	-	-	-	-	***
Tanks	ns	ns	ns	ns	ns	ns

ns- nonsignificant at P<0.05

* Significant at P<0.05

** significant at P<0.001

*** significant at P<0.0001

Table 3. Least square means of specific growth rates (G) of strains, inter-strain crosses, their families together with ranks and the ratio of G at 15°C to G at 7°C.

		7°C		15°C		
STRAIN	FAMILY	G	RANK	G	RANK	G15/G7
LAS	1	1.11	4	2.44	4	2.2
	2	1.19	2	2.77	2	2.3
	3	1.12	3	2.95	1	2.6
	4	1.32	1	2.58	3	2.0
OVERALL		1.18	1	2.68	1	2.3
MAN	1	0.88	3	2.30	3	2.6
	2	1.17	1	2.66	1	2.3
	3	0.97	2	2.42	2	2.5
	4	-	-	2.37	-	-
OVERALL		1.00	5	2.44	4	2.4
TAG	1	0.99	2	2.50	1	2.5
	2	-	-	2.61	-	-
	3	1.27	1	2.39	2	1.9
OVERALL		1.13	3	2.50	2	2.2
TAGLAS	1	1.01	4	2.48	3	2.5
	2	1.20	2	2.56	1	2.2
	3	1.15	3	2.36	4	2.1
	4	1.33	1	2.55	2	1.9
OVERALL		1.17	2	2.49	3	2.1
TAGMAN	1	-	-	2.48	1	-
	4	0.95	-	2.34	2	2.5
OVERALL		0.95	6	2.41	5	2.5
LASMAN	1	0.88	2	2.25	2	2.6
	2	1.14	1	2.40	1	2.1
OVERALL		1.01	4	2.33	6	2.3
MANLAS	1	0.84	2	2.24	2	2.7
	2	1.04	1	2.33	1	2.2
OVERALL		0.94	7	2.29	7	2.4

the TAG strain moved from rank 3 at 7°C to rank 2 at 15°C exchanging positions with the TAGLAS cross. These two, however, were not much different in specific growth rate at 15°C: 2.50% per day; for TAG compared to 2.49% per day; in the TAGLAS cross.

Families within strains also re-ranked between the two temperatures except in the MAN strain where the three families maintained their ranks. The MAN strain showed highest improvement in specific growth rate among the pure strains at the higher temperature as evidenced by the ratio of the rate at 15°C to that at 7°C. The top three performers at 7°C (Table 4), LAS, TAGLAS and TAG were not significantly different from each other although they were, as a block, significantly different from all the rest ($P<0.01$). At 15°C (Table 5), the LAS strain was significantly different from all the rest ($P<0.05$). Table 6 shows relevant pairwise comparisons of least square means of specific growth rate for families within strains. Worthy of note is, families, significantly different in specific growth rate at one of the temperatures, were not necessarily so at the other. In the TAG strain only family 1 and 3 were nonsignificantly different at 15°C. Partitioning components of variance showed that variation due to families within strains

was greater than that due to strains at either temperature (Table 7).

Separate univariate analyses of variance on each of the growth rates for the four periods showed inconsistency in differences both at strain and family levels. While at 7°C, strains were not significantly different in growth rate for the first 14 days, differences were significant in the next 28 days ($P<0.05$) and nonsignificant at periods three and four ($p>0.05$). Families within strains were significantly different at periods 1, 2 and 4 ($P<0.05$) but nonsignificant at period 3 at 7°C. Strains were significantly different at period 3 ($P<0.05$) but nonsignificant for the rest of the periods at 15°C. Families within strains at this temperature were significantly different ($P<0.05$) for periods 1 and 2 only. As shown in Table 8, ranks of least square means of the specific growth rate for strains and their hybrids reared at either temperature were not static. However, the LAS strain did not drop below second rank at both temperatures except in the last 28 days when it was ranked in the fourth position at 7°C. LAS was first overall while the TAGLAS cross exchanged second and third position with TAG at 7°C and 15°C respectively. It was interesting to note that MANLAS and LASMAN crosses seemed to move together in rank at

Table 4. Pairwise comparisons of least square means of specific growth rates of strains reared at 7°C.

	LAS	TAGLAS	TAG	LASMAN	MAN	TAGMAN	MANLAS
LAS	1.18	ns	ns	*	*	*	*
TAGLAS		1.17	ns	*	*	*	*
TAG			1.13	*	*	*	*
LASMAN				1.01	ns	ns	ns
MAN					1.00	ns	ns
TAGMAN						0.95	ns
MANLAS							0.94

* $P<0.05$

ns nonsignificant at $P=0.05$

Table 5. Pairwise comparisons of least square means of specific growth rate for rainbow trout strains and inter-strain crosses raised at 15°C.

	LAS	TAG	TAGLAS	MAN	TAGMAN	LASMAN	MANLAS
LAS	2.68	*	*	*	*	*	*
TAG		2.50	ns	ns	*	*	*
TAGLAS			2.49	ns	ns	*	*
MAN				2.44	ns	*	*
TAGMAN					2.41	ns	*
LASMAN						2.33	ns
MANLAS							2.29

* $P<0.05$

ns nonsignificant at $P=0.05$

Table 6. Pairwise comparisons of least square means of specific growth rate in families within strains at 7°C and 15°C.

	FAMILIES											
	1 & 2		1 & 3		1 & 4		2 & 3		2 & 4		3 & 4	
TEMP.	7	15	7	15	7	15	7	15	7	15	7	15
LAS	ns	*	ns	*	*	ns	ns	ns	*	ns	*	*
MAN	*	*	ns	*	-	ns	*	ns	-	*	-	*
TAG	-	*	*	ns	-	-	-	*	-	-	-	-
MANLAS	*	*	-	-	-	-	-	-	-	-	-	-
LASMAN	*	*	-	-	-	-	-	-	-	-	-	-
TAGMAN	-	-	-	-	-	ns	-	-	-	-	-	-
TAGLAS	*	ns	*	ns	*	ns	ns	*	*	ns	*	*

* $P<0.05$

ns .. nonsignificant at $P=0.05$

Table 7. Variance component estimates and their % proportion to total variance in growth due to strains and families within strains raised at 7°C and 15°C.

	7°C			15°C		
DAYS	STRAIN	FAM[1](STRAIN)	ERROR	STRAIN	FAM(STRAIN)	ERROR
G1	0.0041	0.0425	0.0683	0.0413	0.2254	0.0930
	15.1	37.0	47.9	11.5	62.7	25.9
G2	0.0387	0.0255	0.0252	0.0002	0.0657	0.0672
	43.3	28.5	28.1	0.1	49.4	50.5
G3	0.0100	0.0192	0.0390	0.0115	0	0.0510
	14.7	28.2	57.1	18.5	0	81.5
G4	0	0.0383	0.0185	0.0056	0	0.0346
	0	67.4	32.6	13.9	0	86.1
GF	0.0037	0.0167	0.0048	0.0103	0.0184	0.0041
	14.8	66.1	19.2	31.5	56.1	12.5

[1] family

Table 8. Ranks of least square means of specific growth rates for rainbow trout strains and their hybrids reared at 7°C and 15°C.

	7°C					15°C				
	G1	G2	G3	G4	GF	G1	G2	G3	G4	GF
LAS	2	2	2	4	1	1	1	2	2	1
MAN	3	5	6	3	5	3	2	5	7	4
TAG	7	1	3	5	3	5	4	1	6	2
LASMAN	5	6	4	2	4	6	7	6	4	6
MANLAS	6	7	5	6	7	7	6	7	3	7
TAGMAN	1	3	7	7	6	2	5	3	5	5
TAGLAS	4	4	1	1	2	4	3	4	1	3

G1 to G4 = growth rates for 14, 28, 28, and 28 days respectively
GF = overall growth rate for 98 days.

either temperature except when they separated to ranks 2 and 6 respectively in the fourth period at 7°C: otherwise they ranked 5, 6; 6, 7; 4, 5 at 7°C and 6, 7; 7, 6; 6, 7; and 4, 3 at 15°C.

Analysis of covariance

Analysis of covariance (Table 2) showed significant differences between strains at the lower temperature but nonsignificant at 15°C while families within strains were found significantly different at 15°C but not at 7°C ($P<0.05$). Table 9 shows adjusted least squares means of specific growth rate for each of the strains as well as families at 7°C and 15°C. Reranking of strains between the two temperatures was observed (Table 9). For instance the MAN strain occupying fourth position when reared at 7°C dropped to fifth rank at 15°C while the TAGMAN cross moved from 7th rank at 7°C to fourth at 15°C. Examination of ranks at family level within strains also showed shifts between temperatures (Table 9). For example family 1 of the TAGLAS cross moved from fourth rank with an adjusted specific growth of 1.08% per day at 7°C to second position with an adjusted specific growth rate of 2.54% per day at 15°C. Pairwise comparisons of adjusted least squares means for strains and families

are presented in Tables 10, 11 and 12 respectively. Mount Lassen, Tagwerker and TAGMAN were not significantly different in specific growth rate while they were all significantly different from MANLAS, LASMAN and MAN ($P<0.05$). All the families within the MAN strain and the LASMAN cross compared amongst themselves were nonsignificantly different at both temperatures whereas families of the rest of the strains compared within the
strains showed some significant differences (Table 12). The ratio of specific growth rate at 15°C to specific growth rate at 7°C was comparable to that already described under the least square analysis of variance section.

A test of heterogeneity of slopes performed at 7°C for the strain effect in the regression portion of the analysis of covariance model [4] revealed a significant W*strain interaction ($P<0.0001$) (Table 2). The interaction W*strain was however nonsignificant at 15°C indicating homogeneity of slopes. Homogeneity of slopes was demonstrated for families within strains at both temperatures. Model [5] that included the temperature effect showed a strong W*strain*temperature interaction ($P<0.0001$) i.e. a high heterogeneity of slopes. Assessment for differences

Table 9. Adjusted least square means of specific growth rate for strains, inter-strain crosses their families together with their ranks and the ratio G at 15°C to G at 7°C.

		7°C		15°C		
STRAIN	FAMILY	G	RANK	G	RANK	G15/G7
LAS	1	1.15	2	2.45	4	2.1
	2	1.12	3	2.64	2	2.4
	3	0.90	4	2.80	1	3.1
	4	1.37	1	2.54	3	1.9
OVERALL		1.20	1	2.70	1	2.3
MAN	1	0.82	3	2.13	3	2.6
	2	0.91	2	2.34	2	2.6
	3	0.98	1	2.49	1	2.5
	4	-	-	2.35	-	-
OVERALL		1.01	4	2.39	5	2.4
TAG	1	1.02	2	2.52	1	2.5
	2	-	-	2.92	-	-
	3	1.30	1	2.45	2	1.9
OVERALL		1.10	3	2.48	3	2.3
TAGLAS	1	1.08	4	2.54	2	2.4
	2	1.18	2	2.70	1	2.3
	3	1.12	3	2.39	4	2.1
	4	1.34	1	2.50	3	1.9
OVERALL		1.16	2	2.49	2	2.1
TAGMAN	1	-	-	2.55	1	-
	4	0.93	-	2.35	2	2.1
OVERALL		0.93	-	2.42	4	2.6
LASMAN	1	0.87	2	2.22	2	2.6
	2	1.14	1	2.54	1	2.2
OVERALL		0.99	5	2.33	6	2.4
MANLAS	1	0.88	2	2.25	1	2.6
	2	1.02	1	2.21	2	2.2
OVERALL		0.94	6	2.29	7	2.4

Table 10. Pairwise comparisons of adjusted least square means of specific growth rates for strains and inter-strain crosses reared at 7°C.

	LAS	TAGLAS	TAG	MAN	LASMAN	MANLAS	TAGMAN
LAS	1.20	ns	ns	ns	*	*	*
TAGLAS		1.16	ns	ns	*	*	*
TAG			1.10	ns	*	*	*
MAN				1.01	ns	ns	*
LASMAN					0.99	ns	ns
MANLAS						0.94	ns
TAGMAN							0.93

* significant at $P<0.05$

Table 11. Pairwise comparisons of adjusted least square means of specific growth rate for strains and inter-strain crosses of rainbow trout reared at 15°C.

	LAS	TAGLAS	TAG	TAGMAN	MAN	LASMAN	MANLAS
LAS	2.70	ns	ns	ns	*	*	*
TAG		2.49	ns	ns	*	*	*
TAGMAN			2.48	ns	*	*	*
TAGMAN				2.42	ns	ns	*
MAN					2.39	ns	ns
LASMAN						2.33	ns
MANLAS							2.29

* significant $P<0.05$

using pooled data showed significant strain differences ($P<0.001$) while families within strains were nonsignificant ($p>0.05$).

The regression of log specific growth rate on mean size (weight) was used to test for the significance of the relationship between the two variables. Table (13) shows the values of the slope b, standard error (SE), R^2s and intercept for each of the strains and families. It can be seen that at 7^oC the slopes were more varied ranging from -1.37 ± 0.27 in the TAGMAN cross reared at 7^oC to -0.01 ± 0.13 in the TAGLAS cross also reared at 7^oC. At 7^oC the relationship was associated with very low R^2s and in some cases the slopes were not significantly different from zero. On the other hand at 15^oC the slopes were all significantly different from zero and had high R^2s. All the TAGLAS families and Tagwerker families had nonsignificant slopes at 7^oC. Regression lines at each of the temperatures for the strains are shown in Figs. 3 and 4. The slopes at 7^oC were highly heterogeneous thus pictorially confirming the significance of the test of heterogeneity of slopes performed above. The slopes at 15^oC were more homogeneous (Fig. 4).

Table 12. Pairwise comparisons of least square means of adjusted specific growth rates of families within strains at 7°C and 15°C.

	FAMILIES											
	1 & 2		1 & 3		1 & 4		2 & 3		2 & 4		3 & 4	
TEMP.	7	15	7	15	7	15	7	15	7	15	7	15
LAS	ns	ns	*	*	ns	ns	ns	ns	*	ns	*	ns
MAN	ns	ns	ns	ns	-	ns	ns	ns	-	ns	-	ns
TAG	-	ns	*	ns	-	-	-	ns	-	-	-	-
MANLAS	ns	ns	-	-	-	-	-	-	-	-	-	-
LASMAN	*	ns	-	-	-	-	-	-	-	-	-	-
TAGMAN	-	-	-	-	-	ns	-	-	-	-	-	-
TAGLAS	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns

Table 13. Intercepts and slope for the specific growth rate-fish size relationship: $Log_e\ G = a + bLog_e\ W$ for rainbow trout reared at 7°C and 15°C.

		7°C				15°C			
STRAIN	FAM	a ± SE	b ± SE		R»	a ± SE	b ± SE		R»
LAS	1	1.16±0.38	-0.60±0.21	*	57	1.72±0.12	-0.36±0.05	**	89
	2	0.65±0.28	-0.31±0.19	NS	32	1.77±0.10	-0.35±0.05	**	91
	3	1.18±0.31	-0.74±0.21	*	67	1.97±0.06	-0.41±0.03	***	98
	4	1.33±0.19	-0.59±0.10	**	40	1.82±0.12	-0.38±0.06	**	91
OVERALL		0.89±0.16	-0.44±0.01	***	40	1.83±0.05	-0.38±0.02	***	91
MAN	1	0.19±0.96	-0.21±0.61	NS	2	1.79±0.32	-0.45±0.14	*	65
	2	1.19±0.41	-0.75±0.51	NS	51	1.80±0.14	-0.41±0.06	***	88
	3	0.59±0.39	-0.36±0.23	NS	29	1.86±0.15	-0.41±0.06	***	88
	4	-	-	-	-	1.70±0.20	-0.37±0.08	**	76
OVERALL		0.82±0.30	-0.53±0.19	*	25	1.79±0.10	-0.41±0.04	***	76
TAG	1	1.11±0.44	-0.64±0.24	*	55	1.64±0.17	-0.31±0.07	*	77
	2	-	-	-	-	2.00±0.18	-0.40±0.07	**	86
	3	0.51±0.39	-0.14±0.19	NS	8	1.55±0.24	-0.28±0.10	*	60
OVERALL		0.53±0.38	-0.23±0.20	NS	9	1.72±0.12	-0.33±0.05	***	71
MANLAS	1	0.50±0.79	-0.36±0.42	NS	11	1.32±0.14	-0.22±0.06	*	71
	2	0.29±0.72	-0.16±0.45	NS	2	1.61±0.09	-0.36±0.04	***	93
OVERALL		0.57±0.45	-0.37±0.26	NS	13	1.48±0.09	-0.29±0.04	***	82
LASMAN	1	0.66±0.79	-0.47±0.45	NS	15	1.53±0.26	-0.32±0.11	*	59
	2	0.07±0.76	-0.04±0.41	NS	0	1.85±0.09	-0.40±0.04	***	95
OVERALL		0.08±0.57	-0.05±0.31	NS	0	1.68±0.14	-0.35±0.06	***	73
TAGMAN	1	-	-	-	-	1.79±0.14	-0.37±0.06	***	88
	4	2.44±0.50	-1.37±0.27	*	81	1.74±0.20	-0.38±0.08	*	78
OVERALL		-	-	-	-	1.76±0.12	-0.37±0.05	***	81
TAGLAS	1	0.81±0.72	-0.43±0.39	NS	17	1.71±0.16	-0.34±0.07	**	81
	2	0.15±0.24	-0.01±0.13	NS	0	1.77±0.09	-0.34±0.04	***	93
	3	0.10±0.32	-0.01±0.16	NS	0	1.43±0.22	-0.24±0.09	*	57
	4	0.50±0.42	-0.12±0.24	NS	4	1.69±0.10	-0.34±0.04	**	91
OVERALL		0.42±0.21	-0.15±0.11	NS	5	1.65±0.07	-0.31±0.03	***	79
ALL GROUPS		0.62±0.12	-0.32±0.07	***	13	1.72±0.04	-0.35±0.02	***	76

* P<0.05, ** P<0.001, *** P<0.0001

Multivariate and repeated-measures analysis

Use of multivariate analysis of variance showed that strains as well as families within strains were significantly different in growth rate at both temperatures (P=0.0001). Examination of the residual correlation matrix showed that growth at period one was most closely correlated with that at period two. On the other hand the correlation with growth rate at period one diminished into a significant but negative correlation with that at the fourth period.

A repeated-measures analysis showed a significant time effect (P<0.0001). Temperature by time, strain by time interactions were also significant (P<0.0001) while family within strain by time interaction was nonsignificant. Tests of hypotheses for the between strain effects showed temperature, strain and families within strains all significantly different (P=0.0001). The within subjects hypotheses of no differences in growth rate among strains over time and temperature were significant (P=0.0001) while families within strains were nonsignificant. The differences in patterns of decline in growth were apparent (Figs 5 and 6) when growth rates were plotted over time interval for fish reared at $7^{o}C$ and $15^{o}C$ respectively.

Fig.3. Regression of $\log_e$ specific growth rate on $\log_e$ mean weight for rainbow trout strains reared at 7°C.

LAS ——————

MAN - - - - - - - - - -

TAG — — — — — — —

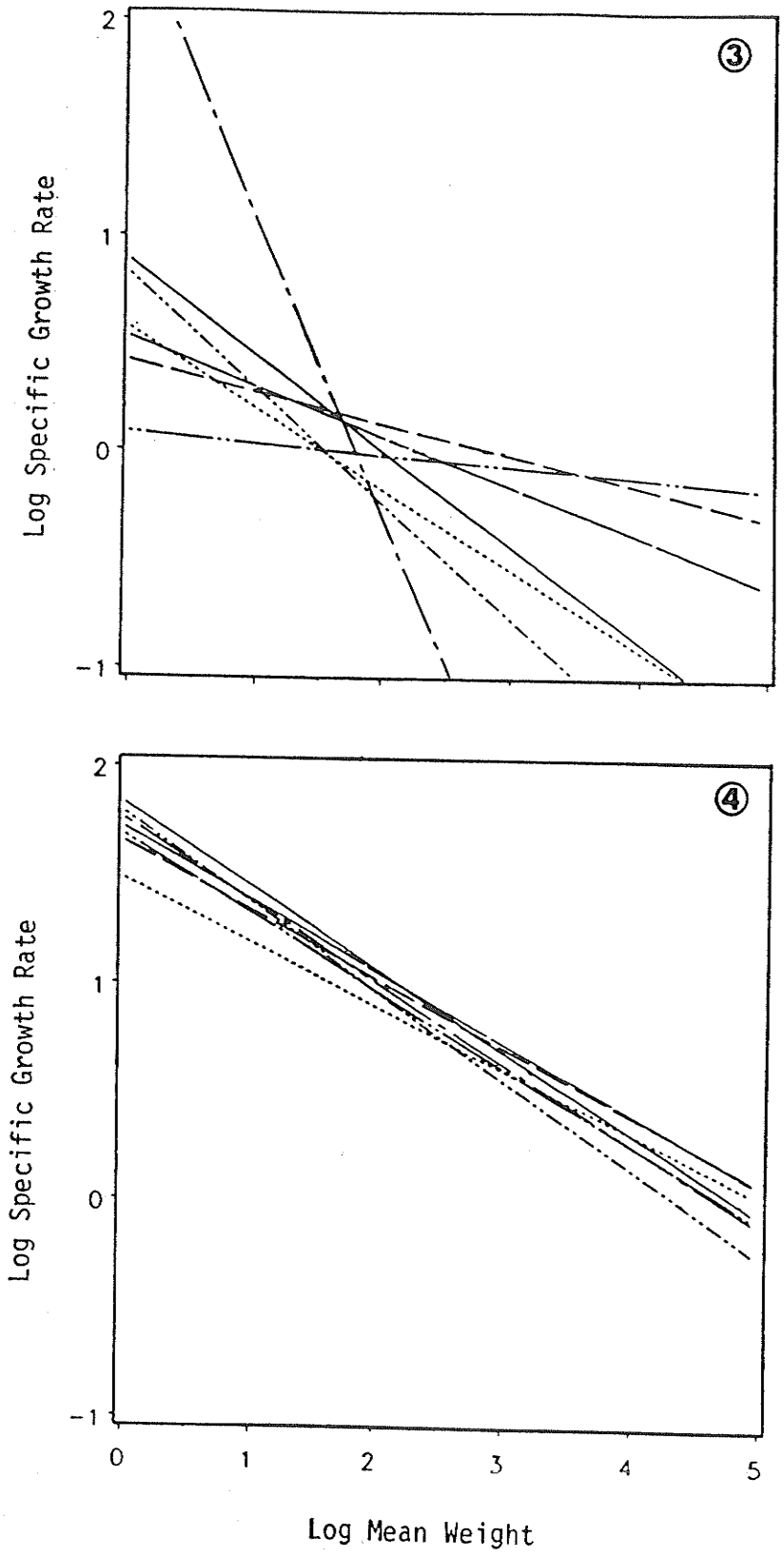
LASMAN — — — —

MANLAS ——— — ——— —

TAGMAN — ——— — ———

TAGLAS ——— ———

Fig.4. Regression of $\log_e$ specific growth rate on $\log_e$ mean weight for rainbow trout strains reared at 15°C. (Lines same as in Fig.3)


③
④
Log Specific Growth Rate
Log Specific Growth Rate
2
1
0
−1
2
1
0
−1
0
1
2
3
4
5
Log Mean Weight

Specific growth rate analysis summary

Conclusions made from the three methods of assessment of growth were compared (Table 14). Only the MANOVA showed that strains and families within strains were significantly different at each of the temperatures.

Other statistics

Length-weight relationship

Table 15 illustrates values of the intercept , a and the slope, b in equation [7] for the length-weight relationship in strains and families raised at 7°C and 15°C. Overall values of b for strains ranged from 2.76 to 3.25. A test of heterogeneity of slopes showed there were significant differences ($P<0.05$) among strains and families within strains at either temperature. Table 15 also shows an approximation of the slopes b' derived from equations [8] and [9]. Values of b and b' were not significantly different although b' was slightly smaller in some cases.

Condition factor

Fulton's condition factor for each of the strain and families at both temperatures is presented in Table 16. An analysis of variance revealed that the overall condition for fish raised at 7°C was significantly different from that for fish at 15°C with the latter being better. It ranged from 1.23 in MAN to 1.32 in LAS at 7°C and from 1.30 in TAGLAS to 1.39 in LASMAN at 15°C. Strains were not significantly different in condition factor. Rank repeats between the temperatures among families was about 70% and Spearman's rank correlation between the temperatures was significant ($r_s=0.95$, $P<0.05$). With data pooled over strains none of the strains retained the same rank at both temperatures (Table 16). Spearman's rank correlation was nonsignificant for strains at both temperatures.

Variance-mean weight relationship

The natural logarithm of variance was found to vary approximately as the square of the natural logarithm of mean weight. Values of a and b for the regression equation [11] are presented in Table 17. The value of the coefficient b (slope) was consistently lower at the higher temperature for each of the strains. The overall values for strains varied

Fig 5. Specific growth rates (± sd) plotted over time intervals for rainbow trout strains reared at 7°C. 1. LAS 2. MAN 3. TAG 4. TAGLAS 5. LASMAN 6. MANTAG

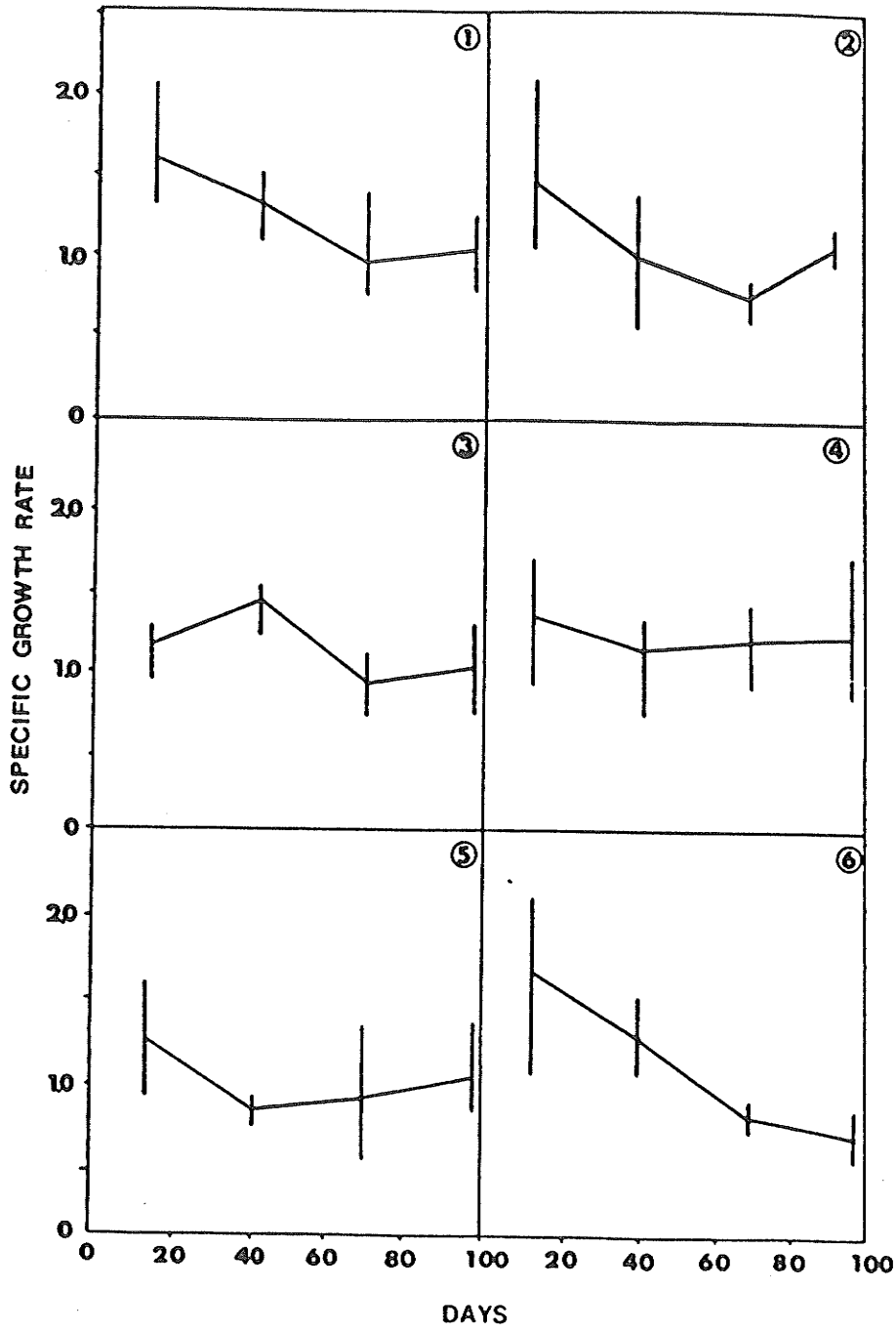

①
②
③
④
⑤
⑥
20
10
0
SPECIFIC GROWTH RATE
0
20
40
60
80
100
DAYS

Fig 6. Specific growth rates(± sd) plotted over time intervals for rainbow trout strains reared at 15°C. 1. LAS 2. MAN 3. TAG 4. TAGLAS 5. LASMAN 6. MANTAG

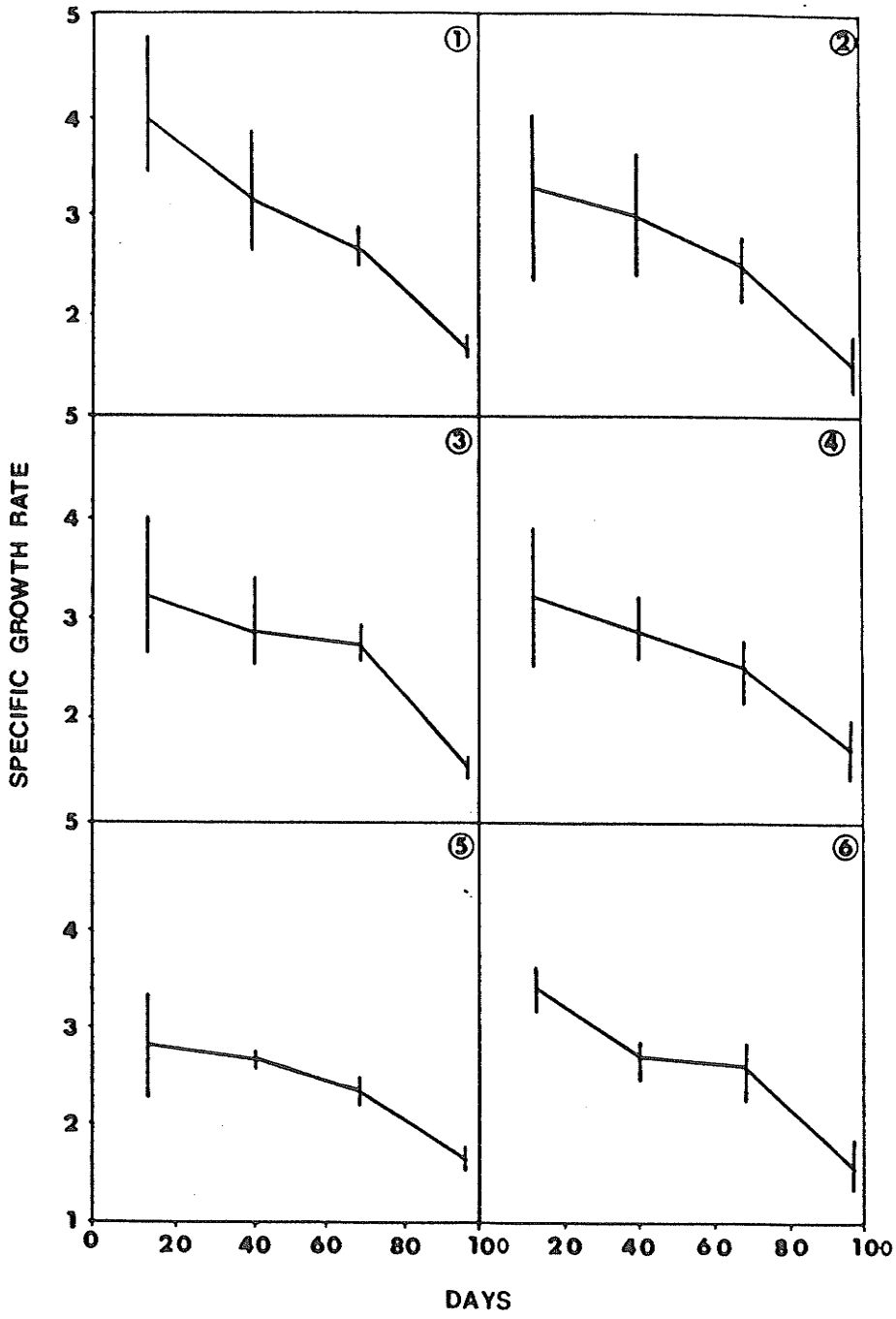

SPECIFIC GROWTH RATE
DAYS
①
②
③
④
⑤
⑥
5
4
3
2
1
0
20
40
60
80
100

Table 14. Summary of specific growth rate (G) assessment for rainbow trout strains and families within strains at 7°C and 15°C.

	ANOVA		ANCOVA		MANOVA	
	7°C	15°C	7°C	15°C	7°C	15°C
STRAIN	*	*	*	ns	*	*
FAM(STR)	*	ns	ns	*	*	*

* significant $P<0.05$.

ns nonsignificant.

from 2.13±0.13 to 2.42±0.16 and from 2.02±0.05 to 2.11±0.04 among the pure strains reared at 7°C and 15°C respectively. The range among the inter-strain crosses was from 2.29±0.11 in the TAGLAS cross to 2.93±0.14 in the MANLAS cross at 7°C while at 15°C the TAGLAS cross again had the smallest slope of 1.96±0.08 with the LASMAN and MANLAS crosses having the highest slope of 2.27±0.10.

The relative variance indices (RVI) for pooled data at 98 days revealed strain and family characteristics (Table 18). Ranks in magnitude of RVI's for families within the LAS strain and the MANLAS and LASMAN crosses remained the same at both temperatures while those for families within the TAG and MAN strains and the TAGLAS cross changed slightly e.g. TAGLAS family 2 with RVI = 1.26 ranked third at 7°C moved to first rank with RVI = 0.49 at 15°C and MAN families 1 and 2 exchanged positions 2 and 3 between the temperatures. The TAG families 1 and 3 different at the lower temperature had equal RVI values at the higher temperature. Among pure strains (Table 18), LAS had lowest RVI's at both temperatures (RVI = 1.12 at 7°C and RVI = 0.87 at 15°C) followed by TAG with RVI = 1.60 at 7°C and RVI = 1.06 at 15°C and third by MAN with RVI = 1.89 at 7°C and RVI = 1.25 at 15°C. The

inter-strain crosses MANLAS and TAGLAS exchanged ranks between temperatures with the former being overall best at 7°C and the latter at 15°C.

Food conversion efficiency

Analysis of variance showed significant differences in the gross food conversion efficiency (FCE) among families within strains at 7°C ($P<0.05$) but nonsignificant at 15°C while strains were significantly different at 15°C ($P<0.05$). Table 19 shows that the TAGLAS cross had the highest overall FCE (21.8%) and the MAN strain the lowest FCE (13.5%) at the lower temperature. At the higher temperature, the TAGLAS cross ,again, had the highest FCE (33.3%) closely followed by the TAG and LAS strains with FCE=31.7% and 31.2% (Table 19) respectively. Pairwise comparisons of least square means of FCE for fish reared at 7°C revealed that TAGLAS, TAG, LASMAN and LAS were nonsignificantly different (Tables 20). MANLAS, TAGMAN and MAN were also nonsignificantly different at 7°C but all significantly different from TAGLAS and TAG ($p<0.05$). Differences in FCE among TAGLAS, TAG and LAS were nonsignificant at 15°C (Table 21).

Overall assessment

Evaluation of specific growth rate, condition factor, food conversion efficiency and relative variance index as traits in a multivariate analysis of variance showed significant differences among strains ($P<0.05$) at each temperature. The same analysis showed that overall differences among families within strains were significant ($P<0.05$). Table 22 shows the residual correlation matrix for specific growth rate, condition factor, food conversion efficiency and relative variance index for data at 7°C. The correlation coefficient ($r=0.477$) between specific growth rate and relative variance index was significant ($P<0.05$). The correlation between growth rate and the condition factor at 15°C (Table 23) was also significant ($r=0.427$, $P<0.05$) and the trends can be seen in the regression lines in Fig. 7. High FCE and high condition factor were associated with high specific growth rates while fast growers tended to have lower values of RVI. The regression slopes of FCE on specific growth rate showed a strong temperature interaction as opposed to parallel slopes for condition factor on specific growth rates.

Table 15. Regression coefficients, estimated coefficient b' and intercepts for the length-weight relationship, $\log_e W = a + b \log_e L$ for rainbow trout reared at 7°C and 15°C.

		7°C			15°C		
STRAIN	FAM	a±SE	b±SE	b'	a±SE	b±SE	b'
LAS	1	-4.59±0.10	3.11±0.05	3.07	-4.16±0.16	2.95±0.06	3.04
	2	-4.15±0.09	2.91±0.04	2.81	-4.23±0.14	2.98±0.05	2.83
	3	-4.35±0.08	2.99±0.04	2.74	-4.19±0.20	2.97±0.07	2.97
	4	-4.09±0.14	2.91±0.06	2.92	-4.01±0.25	2.90±0.09	3.05
OVERALL		-4.50±0.05	3.08±0.02	2.95	-4.21±0.09	2.97±0.03	2.99
MAN	1	-4.48±0.08	3.01±0.04	2.91	-3.96±0.16	2.85±0.06	2.49
	2	-4.32±0.09	2.97±0.04	2.94	-4.17±0.17	2.96±0.06	2.80
	3	-4.48±0.08	3.05±0.04	2.86	-4.42±0.15	3.04±0.05	2.95
	4	-	-		-4.00±0.13	2.89±0.05	2.74
OVERALL		-4.40±0.05	3.00±0.02	2.89	-4.08±0.08	2.91±0.03	2.69
TAG	1	-3.93±0.09	2.80±0.04	2.89	-4.00±0.13	2.86±0.05	2.82
	2	-	-		-4.30±0.17	2.99±0.06	2.59
	3	-3.97±0.09	2.86±0.04	2.70	-3.44±0.17	2.68±0.06	2.70
OVERALL		-4.19±0.07	2.93±0.03	2.98	-3.99±0.10	2.88±0.04	2.78
MANLAS	1	-4.25±0.10	2.97±0.05	2.96	-4.38±0.15	3.05±0.06	2.98
	2	-4.51±0.08	3.06±0.04	2.96	-4.96±0.11	3.25±0.04	2.90
OVERALL		-4.72±0.08	3.15±0.04	2.98	-4.47±0.14	3.06±0.05	2.96
LASMAN	1	-4.74±0.10	3.13±0.04	2.99	-4.91±0.10	3.21±0.04	3.02
	2	-4.42±0.10	3.03±0.04	2.88	-4.01±0.22	2.90±0.08	2.94
OVERALL		-4.48±0.08	3.06±0.03	3.13	-4.92±0.09	3.25±0.03	3.06
TAGMAN	1	-	-		-3.98±0.15	2.88±0.06	2.54
	4	-4.21±0.08	2.93±0.03	2.89	-4.50±0.11	3.06±0.04	2.92
OVERALL					-4.27±0.09	2.98±0.04	2.74
TAGLAS	1	-4.15±0.07	2.94±0.03	2.73	-3.82±0.14	2.84±0.05	2.69
	2	-4.49±0.08	3.05±0.04	2.71	-4.31±0.15	2.98±0.05	2.85
	3	-4.44±0.10	3.00±0.04	2.60	-4.19±0.11	2.92±0.04	2.67
	4	-4.68±0.13	3.12±0.05	3.02	-4.61±0.28	3.10±0.10	3.09
OVERALL		-4.04±0.06	2.86±0.02	2.66	-3.68±0.10	2.76±0.04	2.78

Table 16. Condition factor (K) for strains and families reared at 7°C and 15°C.

		7°C		15°C	
STRAIN	FAM	K	RANK	K	RANK
LAS	1	1.31	2	1.35	4
	2	1.30	3	1.38	3
	3	1.28	4	1.42	1
	4	1.38	1	1.39	2
OVERALL		1.32	1	1.38	2
TAG	1	1.27	2	1.27	2
	2	-	-	1.34	-
	3	1.35	1	1.36	1
OVERALL		1.31	2	1.32	5
LASMAN	1	1.35	1	1.44	1
	2	1.26	2	1.35	2
OVERALL		1.30	3	1.39	1
TAGMAN	1	-	-	1.34	1
	4	1.27	-	1.30	2
OVERALL		1.27	4	1.32	6
TAGLAS	1	1.40	1	1.43	1
	2	1.26	2	1.26	3
	3	1.19	4	1.20	4
	4	1.24	3	1.31	2
OVERALL		1.27	5	1.30	7
MANLAS		1.18	2	1.29	2
	2	1.31	1	1.41	1
OVERALL		1.25	6	1.35	3
MAN	1	1.17	3	1.30	3
	2	1.26	1	1.40	1
	3	1.25	2	1.34	2
	4	-	-	1.36	-
OVERALL		1.23	7	1.35	4

Table 17. Intercepts and slopes for the variance-mean weight relationship, equation [1k] for rainbow trout strains raised at 7°C and 15°C.

		7°C			15°C		
STRAIN	FAM	a±SE	b±SE	R»	a±SE	b±SE	R»
MAN	1	-2.80±0.32	2.50±0.19	0.96	-2.84±0.31	2.26±0.12	0.98
	2	-2.98±0.23	2.74±0.15	0.98	-2.29±0.23	2.07±0.09	0.99
	3	-2.82±0.33	2.36±0.18	0.96	-2.55±0.20	2.13±0.07	0.99
	4	-	-	-	-2.42±0.22	1.93±0.08	0.97
overall		-2.64±0.22	2.39±0.13	0.92	-2.48±0.16	2.08±0.06	0.97
LAS	1	-3.82±0.26	2.51±0.13	0.98	-3.06±0.15	2.14±0.06	0.99
	2	-3.24±0.35	2.33±0.21	0.94	-2.72±0.20	2.08±0.07	0.99
	3	-2.72±0.31	2.22±0.19	0.94	-2.65±0.17	2.13±0.06	0.99
	4	-3.40±0.46	2.23±0.24	0.92	-2.98±0.21	2.11±0.08	0.99
overall		-2.95±0.23	2.13±0.13	0.88	-2.84±0.11	2.11±0.04	0.99
TAG	1	-3.17±0.54	2.33±0.28	0.90	-2.82±0.19	2.06±0.07	0.99
	2	-	-	-	-2.43±0.14	2.03±0.05	1.00
	3	-2.97±0.39	2.36±0.18	0.96	-2.14±0.18	1.93±0.06	0.99
overall		-3.23±0.33	2.42±0.17	0.93	-2.51±0.13	2.02±0.05	0.99
LASMAN	1	-4.44±0.34	3.00±0.18	0.97	-3.37±0.26	2.31±0.10	0.99
	2	-3.84±0.38	2.80±0.19	0.96	-2.62±0.21	2.19±0.07	0.99
overall		-4.22±0.28	2.93±0.14	0.96	-3.03±0.23	2.27±0.08	0.98
MANLAS	1	-4.30±0.76	2.64±0.38	0.86	-3.98±0.25	2.38±0.09	0.99
	2	-3.73±0.36	2.60±0.21	0.95	-3.16±0.31	2.25±0.12	0.98
overall		-3.59±0.44	2.39±0.24	0.85	-3.46±0.27	2.27±0.10	0.97
TAGMAN	1	-	-	-	-2.30±0.20	2.04±0.07	0.99
	2	-3.42±0.29	2.61±0.14	0.98	-2.83±0.17	2.25±0.06	0.99
overall		-		-	-2.55±0.14	2.13±0.05	0.99
TAGLAS	1	-3.28±0.34	2.62±0.17	0.97	-2.45±0.16	2.16±0.06	0.99
	2	-3.23±0.23	2.50±0.12	0.98	-2.25±0.23	1.83±0.08	0.98
	3	-2.83±0.24	2.10±0.11	0.98	-3.00±0.25	1.96±0.09	0.98
	4	-3.06±0.26	2.34±0.13	0.98	-2.40±0.21	1.92±0.08	0.99
overall		-2.93±0.22	2.29±0.11	0.92	-2.50±0.22	1.96±0.08	0.94
All STRAINS		-3.02±0.14	2.31±0.07	0.85	-2.70±0.08	2.09±0.03	0.96
POOLED TEMPS		-2.66±0.06	2.09±0.03	0.95			

Table 18. Mean weights (W), observed variance (OVAR), expected variance (EVAR) and relative variance index (RVI) among strains/families of rainbow trout reared at 7°C and 15°C (N=sample size).

STRAIN	FAM	W(N)		OVAR		EVAR		RVI	
		7°C	15°C	7°C	15°C	7°C	15°C	7°C	15°C
LAS	1	11.7(149)	42.5(149)	12.0	141.6	14.3	170.5	0.84	0.83
	2	9.4(148)	44.1(150)	7.6	156.3	8.6	183.6	0.88	0.85
	3	8.3(149)	48.8(150)	7.6	229.4	6.5	227.5	1.16	1.01
	4	12.9(148)	44.2(148)	10.4	120.6	18.1	184.5	0.58	0.65
OVERALL		10.5(594)	44.9(597)	12.7	166.9	11.4	191.0	1.12	0.87
MAN	1	8.5(149)	37.6(148)	13.0	208.7	6.8	132.0	1.90	1.58
	2	7.9(148)	36.4(130)	12.9	169.0	5.9	123.4	2.21	1.37
	3	10.1(147)	43.1(142)	14.3	216.0	10.2	174.9	1.39	1.23
	4	-	43.0(146)	-	128.3	-	174.0	-	0.74
OVERALL		8.8(444)	40.1(566)	14.2	188.7	7.5	150.7	1.89	1.25
TAG	1	11.2(150)	48.5(148)	14.3	193.0	13.1	224.2	1.09	0.86
	2	-	59.5(115)	-	417.2	-	344.3	-	1.21
	3	16.8(147)	51.9(145)	39.8	222.2	33.2	258.0	1.20	0.86
OVERALL		14.0(297)	52.8(408)	34.8	285.1	21.7	267.9	1.60	1.06
MANLAS	1	10.9(147)	42.7(125)	8.1	126.6	12.2	171.7	0.66	0.74
	2	9.7(149)	34.5(147)	9.2	130.6	9.4	110.0	0.99	1.19
OVERALL		10.3(296)	38.3(272)	9.0	145.0	10.7	136.6	0.84	1.06
LASMAN	1	9.9(146)	40.1(139)	11.1	169.0	9.7	150.3	1.14	1.12
	2	13.4(149)	46.5(143)	27.8	284.4	19.6	205.3	1.42	1.39
OVERALL		11.6(295)	43.3(282)	22.5	237.1	14.2	177.1	1.58	1.34
TAGMAN	1	-	47.1(139)	-	253.1	-	210.7	-	1.20
	4	10.8(149)	41.8(147)	19.4	260.5	12.0	164.3	1.62	1.59
OVERALL		-	44.4(286)	-	263.0	-	186.1	-	1.41
TAGLAS	1	11.9(150)	48.3(146)	26.5	356.3	15.1	222.2	1.76	1.60
	2	13.2(149)	52.1(149)	24.0	126.5	19.1	260.6	1.26	0.49
	3	15.4(150)	48.7(147)	18.3	112.7	27.5	226.0	0.67	0.50
	4	13.5(150)	43.6(149)	20.3	117.2	20.3	179.2	1.00	0.65
OVERALL		13.5(599)	48.1(591)	23.8	185.9	20.2	221.0	1.18	0.84

Table 19. Gross food conversion efficiencies (FCE) for strains and families reared at 7°C and 15°C.

		7°C		15°C		
STRAIN	FAMILY	FCE	RANK	FCE	RANK	FCE15:FCE7
LAS	1	20.5	1	30.6	3	1.5
	2	17.5	3	30.0	4	1.7
	3	13.9	4	33.4	1	2.4
	4	19.5	2	30.9	2	1.6
OVERALL		17.8	4	31.2	3	1.8
MAN	1	13.2	2	26.6	2	2.0
	2	12.2	3	22.7	3	1.9
	3	15.2	1	29.1	1	1.9
	4	-	-	27.5	-	-
OVERALL		13.5	7	26.5	6	2.0
TAG	1	15.4	2	31.3	2	2.0
	2	-	-	31.7	-	-
	3	27.6	1	32.2	1	1.2
OVERALL		21.5	2	31.7	2	
LASMAN	1	14.3	2	27.4	2	1.9
	2	23.0	1	28.5	1	1.2
OVERALL		18.7	3	28.0	5	1.5
MANLAS	1	16.1	2	25.9	1	1.6
	2	16.7	1	25.2	2	1.5
OVERALL		16.4	5	25.5	7	1.6
TAGMAN	1	-	-	29.2	1	-
	4	14.9	-	27.5	2	1.8
OVERALL		14.9	6	28.4	4	1.9
TAGLAS	1	19.5	4	33.3	2	1.7
	2	20.5	3	35.6	1	1.7
	3	27.0	1	32.3	3	1.2
	4	23.7	2	32.1	4	1.4
OVERALL		21.8	1	33.3	1	1.5

Table 20. Pairwise comparisons of least square means of FCE among rainbow trout strains reared at 7°C.

	TAGLAS	TAG	LASMAN	LAS	MANLAS	TAGMAN	MAN
TAGLAS	21.8	ns	ns	ns	*	*	*
TAG		21.5	ns	ns	*	*	*
LASMAN			18.7	ns	ns	ns	*
LAS				17.8	ns	ns	ns
MANLAS					16.4	ns	ns
TAGMAN						14.9	ns
MAN							13.5

(* significant at $P<0.05$)

Table 21. Pairwise comparisons of least square means of FCE among strains of rainbow trout reared at 15°C .

	TAGLAS	TAG	LAS	TAGMAN	MANLAS	MAN	LASMAN
TAGLAS	33.3	ns	ns	*	*	*	*
TAG		31.7	ns	ns	*	*	*
LAS			31.2	ns	ns	*	*
TAGMAN				28.4	ns	ns	ns
MANLAS					28.0	ns	ns
MAN						26.5	ns
LASMAN							25.5

(*=significant difference at P<0.05); ns=nonsignificant)

Table 22. Residual correlation matrix for specific growth rate (GROWTH), condition factor (CONFACT), food conversion (FCE) and relative variance index (RVI) for rainbow trout strains raised at 7°C.

	GROWTH	CONFACT	FCE	RVI
GROWTH	1.000	0.002	0.088	-0.477*
CONFACT		1.000	0.374	-0.300
FCE			1.000	-0.100
RVI				1.000

* significant $P<0.05$.

Table 23. Residual correlation matrix for specific growth rate (GROWTH), condition factor (CONFACT), food conversion (FCE) and relative variance index (RVI) for rainbow trout strains raised at 15°C.

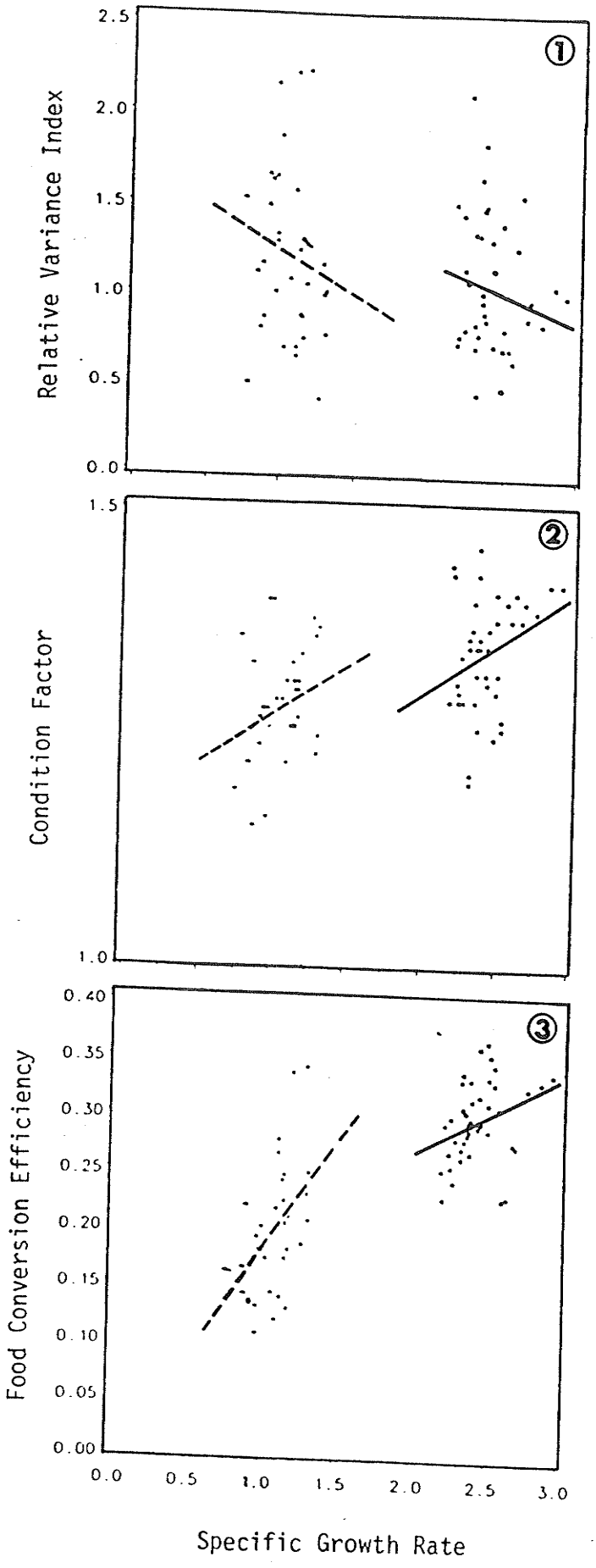
	GROWTH	CONFACT	FCE	RVI
GROWTH	1.000	0.427*	-0.072	-0.170
CONFACT		1.000	-0.317	0.112
FCE			1.000	0.110
RVI				1.000

* significant $P<0.05$.

Fig. 7. Regression slopes of the relationship between specific growth rate and: (1) relative variance index, (2) condition factor and (3) FCE.

7C - - - - - - - -

15C ————————


①
Relative Variance Index
2.5
2.0
1.5
1.0
0.5
0.0
②
Condition Factor
1.5
1.0
③
Food Conversion Efficiency
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Specific Growth Rate

DISCUSSION

It was evident from the summary of results that the multivariate approach was reliable since it tested overall performance using all the data points during the study period. However, this method has the disadvantage that it does not reveal details such as least square means for appropriate pairwise comparisons. The univariate least squares analyses should be used for these purposes but Freund et al. (1986) pointed out that when overall effects are nonsignificant in the MANOVA there is little point in conducting univariate analyses. For experimental designs where there are no confounding factors such as age and initial weight differences the univariate least square analysis of variance is appropriate. Analysis of covariance, using Jobling (1983a) approach would become handy in cases where there were differences in weight but only when these differences were not directly attributed to genotypic differences. Crow (pers. com.) pointed out that initial differences in fish weight when age was controlled for are results in themselves and should not be adjusted.

Univariate least square analysis of variance

Significant differences among strains reared at 7°C indicated that at this temperature, choice of a better growing strain was possible. At 7°C the LAS and TAG strains together with their inter-strain cross TAGLAS grew faster than all the other strains. Reranking of strains between temperatures confirmed Sadler's et al. (1986) and Mckay's et al. (1986) recommendation that testing of fish for growth performance should be conducted in the environment in which the fish were likely to be grown.

Families within strains were significantly different in some cases indicating differential growth performance among families of a given strain. Based on this observation it can be seen that selection at the family level could be of value. For instance family 4 of the LAS strain grew about 16% faster than family 1 while family 4 of the TAGLAS cross grew about 25% faster than family 1. Examination of variance components showed that 69.7% variation was due to families within strains, the magnitude of which further indicated that selection at family level could be possible as suggested by Mckay et al. (1986).

The ratio of growth at the higher temperature to that of the lower temperature may be of use when there

is a choice between temperatures and strains . Good growers at the lower temperature will have low ratios while high ratios would be matched with those strains that show improvements in rank at the higher temperature.

The analysis for separate sampling periods showed that one could get different results depending on which period was considered. This observation raised questions as to the reliability of results when growth studies are conducted for periods as short as 14 days or even for arbitrary durations. This indicated that studies to evaluate a number of strains should not only be carried out in the environment fish are likely to be grown (McKay et al., 1986) but they should be conducted for a period covering the time frame of the production system.

Analysis of covariance

Conclusions when specific growth rate differences were analyzed with initial weight as a covariate were different from that discussed in the preceding analysis of variance section. While significant differences among strains were observed at both temperatures in the analysis of variance, differences among strains were shown significant only at $7^{o}C$ in the analysis of

covariance. Families within strain were significantly different only at 15°C whereas the reverse was the case in the analysis of variance where they were significantly different only at 7°C. Ranks for families within strains shifted slightly in comparison with those recorded in the analysis of variance although those ranked best retained their positions. The ratio of specific growth rate at 15°C to that at 7°C retained the same magnitude, leading to the same decisions in terms of improvement in growth rate from the lower to the higher temperature. For example, among the pure strains the MAN strain was first out as one with the highest gain in growth rate at 15°C compared with the growth rate at 7°C. Interpretations of the results using this analysis, however, depend on whether the assumptions underlying the validity of analysis of covariance hold. Use of the standard model of the analysis of covariance for comparison of adjusted means assumes, among other conditions, that the regression slopes involving the covariate and the classes were homogeneous (Snedecor and Cochran, 1980). Sadler et al. (1986) analyzed differences in specific growth rate in the salmonids, rainbow trout, brook trout, splake and lake trout with different initial sizes ranging from 1.85 g to 3.25 g without the use of analysis of covariance to correct for the initial

differences in size. Fish in the present study ranged from a mean weight of 2.5 g to 4.8 g in initial sizes among strains and families. Jobling (1983a), however, recommended the use of the specific growth rate-fish size relationship as a means of overcoming differences in fish size in order to compare growth rates. According to the assumptions inherent in the analysis of covariance it means that the intercepts in the regression relationship (defined as specific growth rates for unit weight fish, Jobling, 1983a) could only be meaningfully compared in different species, strains, environments, etc. only after establishing that the slopes were homogeneous. The test of the assumption of homogeneity of slopes, however, showed a significant interaction in the W*STRAIN term at 7°C ($p<0.05$) while there was no significant interaction at 15°C in the same term. With the temperature effect the term W*STRAIN*TEMPERATURE was highly significant ($p<0.0001$) indicating heterogeneity of slopes among strains between temperatures. In simpler terms this means that there were genetic and environmental components in the specific growth rate-fish size relationship, i.e., the slopes were not universal among genotypes and temperatures; hence use of analysis of covariance was inappropriate here. In addition there was very little association between specific growth rate and fish size

as exemplified by nonsignificant slopes and R^2s that were as low as zero. Lack of a significant interaction in the term W*STRAIN at 15°C means that slopes were homogeneous and indicated that use of analysis of covariance would be appropriate as long as other assumptions were satisfied. The same comparison would however be misleading at 7°C. Pooling the data at both temperatures and applying ANCOVA would also yield misleading results for intercept (growth rate) comparisons since the W*STRAIN*TEMPERATURE was significant.

While comparing the slope of -0.34 for coho salmon, calculated by Stauffer (1973) with Elliot's (1975) values on brown trout, Brett (1979) concluded that there could be a wide range of possible slopes. This was supported by the present study which demonstrated that in fact the relationship was influenced by genotype as well as temperature. Jobling's (1983a) generalization of this relationship for all fish species was not supported by this thesis.

Repeated-measures multivariate analysis

Wishart (1938) used weekly weight gain in pigs to analyze for growth, pointing out that such an approach

picked up additional information contained in the data as opposed to an analysis which considers only two points. Cole and Grizzle (1966) discussed the application of multivariate analysis to repeated-measures experiments. Kokoska and Lucinda (1987) compared statistical techniques for analysis of growth curves and recommended the multivariate approach as it did not require homogeneity of variance and covariance. Freund et al.(1986) suggested that the multivariate analysis was more accurate and while univariate analysis of variance was more sensitive it was too liberal. The multivariate methods used here revealed that overall effects due to strains, families within strain and temperature were all significant. Based on the fact that these methods utilized all the data the significance of these effects was considered real and so conclusions arrived at take precedence over those made in the univariate analysis of variance and covariance. There was no ambiguity as to which period should be used to make conclusions as experienced in the univariate analyses where periods 1, 2, 3 and 4 were considered separately. In the repeated-measures analysis the time effect was significant. This reflected the behaviour of the variable under study i.e., that growth rate decreased over time in all classes and that the rate of decrease was not uniform

among classes between intervals. The time*temperature, time*strain interactions were significant indicating different responses over time for strains and at different temperatures. The time*families(strain) was nonsignificant showing that within a strain the responses over time for families tended to be similar. This was expected as families should be genetically closer to each other than strains.

Other statistics

Length-weight relationship

Allometric growth in organisms has been represented by the expression $Y=aX^b$; in which Y is a measurement of one part of an organism and X, a measurement of another part on the same organism (Jalicouer, 1975). The main interest in the use of this expression in fisheries has been to establish how length and weight vary in relation to each other so that one can be converted into the other depending on which one was found convenient to measure. Constant slopes have been reported for the length-weight relationship as lying between 2 and 4 (Le Cren, 1951; Bagenal and Tesch, 1968; Ricker, 1975). There have been considerable controversies over whether it is

correct to use the allometric relationship in its standard statistical form as a bivariate model (Ricker, 1973). Ricker (1973) proposed the use of a geometric mean functional regression which Jalicouer (1975) criticized, pointing out that in Ricker's (1973) approach, the slope was estimated by the ratio of the standard deviations which are known to be highly variable. Schmallhausen (1927) quoted by Yablokov (1974) showed that objects which vary in size but maintain constant shapes, have all linear measures perfectly correlated, with equal coefficients of variation, so that volume would be related to length by its cube. Following this, a simple (non-mathematical application) but logical estimate of the slope should be possible using ratios of the coefficients of variation. Wright (1968) pointed out that coefficients of variation are fairly stable. In fact, as shown here the slopes obtained from the standard regression model were comparable to the values approximated by the ratio. Where one needs to estimate this relationship with a simple hand calculator all that are required are the standard deviations and the means for length and weight of fish.

Condition factor

The condition factor estimates the degree of "fatness" or "well being" of the fish (Le Cren, 1951; Tesch and Bagenal, 1968; Ricker, 1975) in a given habitat. Mckay et al. (1986) used it as one of the traits while studying rainbow trout reared in tanks. In this thesis condition factors were quite stable as shown by the unchanging ranks between temperatures and confirmed by the significant rank correlation. Condition factors for fish reared at 15°C were slightly higher than those for fish at 7°C although differences were nonsigificant. Under hatchery conditions where fish are on artificial feed, the importance of this trait is not really apparent unless it affected the shape of fish to the point where their marketability would be affected. As found here, fast growth was positively associated with condition factor, suggesting that fast growing fish would also be those in "good condition".

Variance-mean weight relationship

While several researchers have sought an explanation for size variability in fish (Brown, 1946; Aulstad et al., 1972; Purdom, 1974; Jobling and Wandsvik, 1982; Koebele, 1985) the relationship between weight and variance in stocks of fish under culture conditions has not been examined. In animals and

plants, variance of body size increases with increasing mean (Wright, 1968; Falconer, 1981). Empirical relationships between variance (especially standard deviation) and the mean have ordinarily been investigated only in light of scale transformations (Rasmussen, 1933; Wright, 1968; Falconer, 1981). This relationship was developed in accordance with the standard allometric expression discussed under the length-weight relationship above. The regression coefficient in the variance-mean weight relationship was shown to be a constant lying around 2 with very little variation. This confirms Causton's (1969) observation that the variance of $\log_e$ weight was proportional to the square of the weight in biological growth. Consistently lower values of the slope at the higher temperature indicated overall decreased variance build-up when rainbow trout were reared at 15°C. This temperature was reported to be optimal for rainbow trout (Brett, 1979). The high rate of variance increase at the lower temperature appears to be a genetic fitness response to a challenging environment where only those individuals fit to perform well at 7°C grow fast, thus leaving behind the unfit leading to an increase in variance. This interpretation disagrees with Purdom (1974) who suggested that variance was largely environmental caused primarily by social

hierarchical patterns in a fish tank. Interestingly, in my study, variances between replicates were comparable. The question then was how social hierarchies could be so precisely duplicated in different tanks. It appears that there was a substantial genetic effect in the variability of a given family of fish. This was in general agreement with the report that variability was inherent in a family (Wright, 1968). The relative variance index (RVI) proposed here was constructed along the same line the relative condition factor was constructed by Le Cren (1951). This approach in analyzing variability in fish provides a summary of variance for a growth period unlike the coefficient of variation (CV) which is determined only as a point statistic without taking into account the previous variation. It could be used in selection when one of the goals in a selection program was low variability in fish size. Families of fish with RVI's near or less than 1 would be desirable as they would be associated with low variability in size. For example, families 4, 1, 2 and 3 would be given appropriate weighting in that order at 7°C in a multi-trait selection program where the relative variance index was considered. The predictive aspect of the variance-mean weight regression equation also allows an aquaculturist to forecast variance for a

given mean weight target. Estimation of the coefficient of variation, if desired, would be straight forward once variance was estimated.

Food conversion efficiency

Food conversion efficiency studies in experiments involving large numbers of fish present at best a crude estimate of the true picture as it is always difficult to ascertain the exact amount of food actually consumed. This is a recurring problem regardless of the method of feeding. Assuming, however, that errors in deciding satiation points and errors due to uneaten food were random over the tanks and hence fish groups, the FCEs reported here can be considered as fair estimates which can legitimately be used to evaluate the different strains and families under study.

At 7°C families within strains were significantly different in FCE which suggested that at this temperature family selection for better performers could be of economic importance. Family ranks between temperatures were remarkably stable in the MAN, TAG strains and the MANLAS cross indicating no temperature by genotype interactions among these families. This was in agreement with Elliot (1976) who found that

change in conversion efficiency was a matter of scale. Overall performance of strains in FCE was significantly different only at 15°C indicating that decisions on the type of strain to rear would best be made at this temperature.

Overall assessment

Gjedrem (1983) considered growth rate, food conversion efficiency, resistance to disease, meat quality and age at maturation as traits of high economic importance. Other workers, e.g. Mckay et al. (1986) included traits such as condition factor. "Gregariousness" or high density tolerance, size variability and colour are also important traits (Dick, pers. comm) and should be studied along with other traits. In this study, overall differences in specific growth rate, condition factor, food conversion efficiency and relative variance index were significant among strains and families within strains in the MANOVA. This indicated, if these traits are considered together, that the strains and families were separable. Furthermore, high FCE and high condition factor were positively correlated to specific growth rate while low RVI's were associated with high specific growth rates

implying the feasibility of selecting for these traits along with specific growth rate without any antagonism.

Chapter 2

Genetic effects in a diallelic cross of two strains of rainbow trout (*Salmo gairdneri* Richardson).

ABSTRACT

Crossbreeding between two strains of rainbow trout resulted in about 7% heterosis for growth rate in the hybrids. Variance component analysis showed that dams accounted for 20.3% to 65.8% variability in growth while sires accounted for none. Maternal effects were significant, but showed a diminishing trend over the study period. Additive genetic effects were nonsignificant. The Mount Lassen strain was better in maternal ability so that source of dam was important. Interactions between sires and dams were significant with dam source being important.

Heritability for weight based on half-sib sire component of variance was estimated at 0.020 ± 0.001 and 0.269 ± 0.016 based on the dam component. Heritability for growth rate was 0.27 ± 0.04 based on the sire component and 0.88 ± 0.35 based on the dam component.

Variability in weight and Fulton's condition factor were lower in the hybrids. Maternal effects on variability in weight were mildly important, apparently through egg size, which was found correlated with fish size in the early growth phase. Additive effects were very low and nonsignificant, implying that direct

selection for variability as a trait would be very difficult. Low size variability among hybrids as well as hybrid vigour indicated that crossbreeding of rainbow trout strains could be advantageous. Although it was found that hybrids had generally a low condition factor, an argument was advanced as to the real importance of condition as a trait.

INTRODUCTION

In livestock production it has long been recognized that breed differences are an important source of genetic improvement in the efficiency of food production for human populations. As pointed out by Dickerson (1969) such improvement has been achieved through breed grading, heterosis from systematic cross breeding and development of new breeds. For a realization of similar improvements in fish culture, the procedures employed in classical livestock genetics definitely need closer attention from workers interested in fish husbandry.

Existence of different strains of rainbow trout is an indication of genetic diversity which in its self would form a basis for the application of the various procedures available for breed enhancement.

For selection to be possible, traits of interest should be expressed additively i.e. alleles at different loci add a fixed amount to the phenotype (Falconer, 1981). Maternal effects are a form of environmental effect that influences the performance of progeny usually in the early stages of life. These effects must always be considered in any breeding

design in order to improve the precision of the estimation of additive genetic effects for a given trait. Maternal effects have been reported to be important in early life in fish (Refstie, 1980). Another important factor in selection gain is heritability, defined as a ratio of additive variance to total phenotypic variance (Falconer, 1981). It is basically a measure of the degree to which variance in the distribution of a phenotype is caused by the aditive action of genes. Because heritability determines the degree of resemblance among relatives, it is an invaluable statistic in breeding programs (Falconer, 1981). Heterosis was defined by Falconer (1981) as "hybrid vigour", complementary to the phenomenon of inbreeding depression. Heterosis is expected when populations that differ in gene frequency are crossed. Crossbreeding has routinely been used in other livestock for heterotic gain (Dickerson, 1969).

Several workers have examined salmonid breeding genetics (Aulstad et al., 1972; Refstie, 1980; McKay et al., 1984, 1986; Beacham and Murray, 1988). In this study, growth rate, condition factor and relative variance index for progeny from a diallel cross were examined to estimate genetic effects such as strain

direct, strain maternal, and heterosis. Knowledge of the relative importance of additive direct genetic effects and dominance effects as well heritabilities for traits of economic importance is important for laying strategies in selection programs. Heritability for weight and growth at 21 day intervals based on sires and dams was also estimated.

MATERIALS AND METHODS

Mating design

Two diallel crosses were made by artificially spawning four sires and four dams of the Mount Lassen and Manx strains of rainbow trout (Salmo gairdneri Richardson). Two sires and two dams from each strain were used so that in each of the two diallels, designated DI and DII, there were sixteen half-sib families i.e. four pure Mount Lassen, four pure Manx and eight crosses including reciprocals. These strains are the same strains described in chapter I.

Care of fish

The resulting 32 half-sib families were treated uniformly from the time of artificial spawning throughout the study period. Fertilized eggs were incubated in jars with a constant flow-through of water at 7°C. One hundred and sixty fertilized eggs were taken from each dam, preserved in Stokers solution and later individually weighed on an electronic Mettler balance and diameters measured on a binocular Zeiss dissecting microscope fitted with an ocular micrometer. During the study period fish were fed according to

standard ration tables (Hilton and Slinger, 1981) on Ontario Martin's Trout Pellets.

Randomization and data collection

At an average weight of 1.0 g, two lots of 60 fish randomly selected from each family were each reared in a 60-litre tank containing water at 12°C. All the 60 fish in each of the 64 tanks were individually weighed every 21 days for 105 days. Because the second diallel cross was made a week after the first one, sampling of the two groups was staggered so that fish in the second diallel were always weighed a week later.

Data analysis

Specific growth rate, condition factor and relative variance index as calculated in chapter 1 equations 1, 10, and 12 respectively were the traits of interest. Comparisons between the classifications i.e. strain as source of sire effect, strain as source of dam effect, sire within strain effect, dam within strain effect and various interaction effects were examined using the statistical model below:

$$Y_{ijklm} = \mu + M_i + F_j + S(M)_{k(i)} + D(F)_{l(j)} + SD(MF)_{kl(ij)} + MF_{ij} + t(MFSD)_{m(ijkl)} + BW_{ijklm} + e_{ijklm} \quad [13]$$

where Y_{ijklm} = observation on fish from the mating between the k^{th} sire from the i^{th} strain of sire and the l^{th} dam of the j^{th} strain of dam reared in the m^{th} tank

μ = overall mean

M_i = Strain of sire effect - fixed (i=1 to 2)

F_j = Strain of dam effect - fixed (j= 1 to 2)

$S(M)_{k(i)}$ = sire within strain effect - random (k=1 to 2)

$D(F)_{l(j)}$ = dam within strain effect - random (l=1 to 2)

$SD(MF)_{kl(ij)}$ = sire within strain by dam within strain interaction

MF_{ij} = strain of sire by strain of dam interaction

$t(MFSD)_{m(ijkl)}$ = tank effect - random (m= 1 to 32)

BW_{ijklm} = covariance term for adjusting for initial mean weight (W) differences which were a result of differential

survival of larvae and hence different densities

e_{ijklm} = error - random and NID

Genetic effects

The least square analysis of variance model appropriate for testing for the significance of strain direct genetic effects, maternal effects and heterotic effects was:

$$Y_{ij} = \mu + L_i + SD(L)_{j(i)} + BWi_j + ei_j \qquad [14]$$

where

Yi_j = observation on the j^{th} sire by k^{th} dam full sib family

μ = overall mean

L_i = strain/cross effect

$SD(L)_{j(i)}$ = sire by dam interaction (i.e. full sib family in strain/cross)

BWi_j = covariance for adjusting for differences in initial mean weight (W).

e_{ij} = error

In the genetic effects model [14], the main classifying factors were the family and the kind of

cross i.e. pure or hybrid. In this regard the model could be extended to include both diallels as replicates, allowing the testing of overall genetic effects. The extended model was:

$$Y_{ijk} = \mu + R_i + L_j + RL_{ij} + SD(RL)_{k(ij)} + BW_{ijk} + e_{ijk} \quad [15]$$

Where Y_{ijk} = observation on the k^{th} full sib family in the j^{th} strain/cross

μ = overall mean

R_i = replicate effect

L_j = strain/cross effect

RL_{ij} = replicate by strain/cross interaction

$SD(RL)_{k(ij)}$ = sire by dam interaction (i.e. full sib family in replicate in strain/cross)

BW_{ijk} = covariate

e_{ijk} = error

Tests for strain direct genetic effects, maternal genetic effects and heterosis

Using an argument similar to the one advanced by workers such as Dickerson (1969), Gregory et al., (1978) and Eisen et al., (1983), the average growth

performances (G) for the LAS and MAN strains was represented by the linear models:

$G_L = \mu + D_L + M_L$ and $G_M = \mu + D_M + M_M$ respectively. The performance of the cross-breds, LASMAN and the reciprocals, MANLAS could be represented by the linear models:

$$G_{LM} = \mu + 0.5 (D_L + D_M) + M_M + h_{LM}$$

and

$$G_{ML} = \mu + 0.5 (D_L + D_M) + M_L + h_{ML} ,$$

respectively.

The symbols in these models are: u = the mean of the population of the two pure strains; D_L and D_M = the direct genetic effects of the LAS and MAN strains respectively; M_L and M_M = maternal genetic effects due to LAS dams and MAN dams respectively; and h_{LM} and h_{ML} = heterosis for growth of the cross-breds : expected to be equal to each other.

Based on the linear models above, contrasts were constructed to test strain direct genetic effects, maternal genetic effects and heterosis. These contrasts can be shown to have the expected values:

$(D_L) - (D_M)$, $M_L - M_M$ and h_{LM} which were directly obtainable from models [14] and [15].

Heritability

Heritabilities were estimated on half-sib families for weight and specific growth rate following the method described for diallel crosses by Becker (1984) and as applied in fish by Kirpichnikov (1981). The relative influence of the sexes on heritability estimates was examined by calculating heritability for 21 day intervals through the study period. The model from which heritabilities were estimated was:

$$Yij = \mu + Si + Dj + SDij + eij \qquad [16]$$

where Yij= observation on progeny of the ith sire mated with the jth dam.

μ = overall mean

Si= ith sire (i=1 to 4)

Dj= jth dam (j=1 to 4)

SDij= sire by dam interaction effect

eij = error

all effects considered random

This model assumes negligible effects due to sire strain and dam strain.

RESULTS

As can be seen from Table 24, analysis of variance showed that the sire strain effect on specific growth rate was nonsignificant in both diallels while the dam strain effect was nonsignificant in the first diallel but significant in the second diallel ($F=26.37$, $P<0.05$). The sire within sire strain effect was nonsignificant in both diallels while the dam within dam strain effect was significant only in the first diallel ($F=12.60$, $P<0.01$). The interactions sire strain*dam strain and sire strain*dam(dam strain) were significant ($F=5.47$, $P<0.05$; $F=8.01$ $P<0.01$ respectively) only in the first diallel. Table 25 gives the least square means of specific growth rate for pure-breds and crosses between the two strains. Families from dam LAS2 in the first diallel had a higher overall mean than those from dam LAS1 ($p<0.05$). The MAN2 dam progeny had the highest growth rate in comparison with all the families from the rest of the dams in the first diallel. Means for all sires were nonsignificantly different. There were no significant differences in means for any grouping in the second diallel except for the dam strain classification. Progeny from families of LAS as a source of dams had an overall growth rate of 2.48 ± 0.07 which was

Table 24. Least square analysis of variance for specific growth for various classifications in the two diallels.

	DI		DII	
SOURCE	DF	F VALUE	DF	F VALUE
Sire strain	1	3.81	1	0.30
Dam strain	1	0.33	1	26.37*
Sire(sire strain)	2	0.25	2	0.59
Dam(dam strain)	2	12.60**	2	0.17
Sire strain x dam strain interaction	1	5.47*	1	2.80
Sire strain x dam within dam strain interaction	2	8.01**	2	0.04
Dam strain x sire within sire strain interaction	2	0.19	2	1.58
Sire x dam within sire strain by dam strain	4	2.77[a]	4	0.32
Tank (sire strain x dam strain x sire x dam)	16	0.30	15	0.35
Initial weight	1	19.57**	1	204.41
Error	121		117	

* P<0.05
** P<0.01
a P<0.1

Table 25. Least square means of specific growth rate for pure bred and crosses of the Mount Lassen (LAS) and the Manx (MAN) strain of rainbow trout in two diallels I & II.

	DIALLEL I				DIALLEL II			
DAM	LAS1	LAS2	MAN1	MAN2	LAS3	LAS4	MAN3	MAN4
SIRE								
LAS1	1.57	2.18	2.05	2.37	-	-	-	-
LAS2	1.57	2.17	2.03	2.26	2.24	2.40	2.33	2.37
MAN1	2.07	2.24	2.15	2.12	2.52	2.75	2.09	2.20
MAN2	2.20	2.14	2.05	2.26	2.49	2.74	2.35	2.31
LAS3[a]	-	-	-	-	2.36	2.34	1.95	2.26

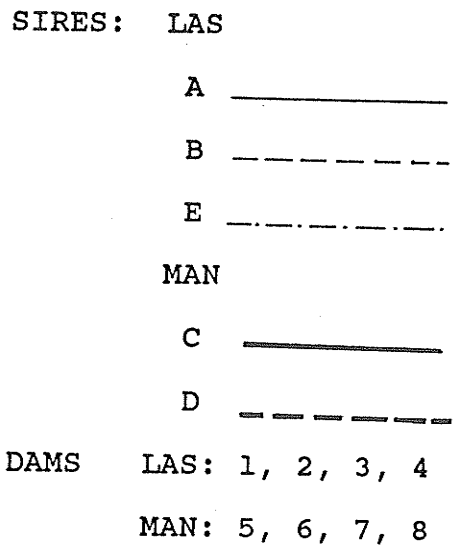
a..Mount Lassen sire that replaced LAS1 which was in poor condition during spawning for the second diallel

significantly different from those of MAN as a source of dams with a growth rate of 2.23 ± 0.05. Examination of components of variance showed that variability in growth in the first diallel was zero due to sires, 20.3% due to dams, 30% due to sire by dam interactions and 43.6% due to error. In the second diallel variability due to sires was again zero, 65.8% due to dams, zero due to sire by dam interactions and 33.7% due to error. Figs 8 and 9 show graphical presentations of progeny performance in growth rate for individual parental combinations and strain combinations. As can be seen in Fig.8, the differences in performance in the first diallel for pure LAS progeny appeared to be due to dam-sire combinations rather than due to interactions. There was a definite interaction among the parental combinations in the second diallel for the pure LAS. Meanwhile there were interactions among individual parental combinations in the MAN strain in both diallels. Cross-breds showed interactions in both diallels except for the MAN sires mated with LAS dams in the second diallel where differences were due to the contributions of individual parents without any apparent interactions. In the first diallel MAN5 X MAN1,MAN2 had a parallel relationship with LAS2 X MAN1,MAN2 while MAN6 X MAN1,MAN2 and LAS1 X MAN1, MAN2 showed a similar

relationship. Fig 10 summarizes the between strain relationships in which it is demonstrated that interactions occurred in both diallels. All these interactions led to heterosis running at 8% in the first diallel and 6% in the second diallel. Mount Lassen dams when crossed with Manx sires resulted into higher heterotic gain than the reciprocal crosses.

There were no significant differences in egg size in the first diallel between the LAS dams while for the MAN dams egg sizes were significantly different ($P<0.05$). In the second diallel egg size differences were significant ($P<0.05$) for the LAS dam while the differences were nonsignificant for the MAN dams. Table 26 shows correlations for egg diameter(DE), egg weight (WE) and weight one (W1) through weight six (W6) for diallel I. As expected there was a high significant correlation between DE and WE ($r=0.88$, $P<0.001$). Egg diameter and fish weight (W1 through W6) were positively correlated although all nonsignificant in the first diallel. Egg diameter was again nonsignificantly correlated with W1 through W6 with the exception of W5 ($r=0.09$, $P<0.05$) in the second diallel. Egg weight and fish weight showed a diminishing correlation for records W1 through W6 with r dropping from 0.12 for W2 ($P=0.04$) to $r=0.06$ ($P<0.15$) for W6 in

Fig. 8. Graphical representation of interactions between specific sexes the two rainbow trout strains using specific growth rate as the response in each of the diallels.



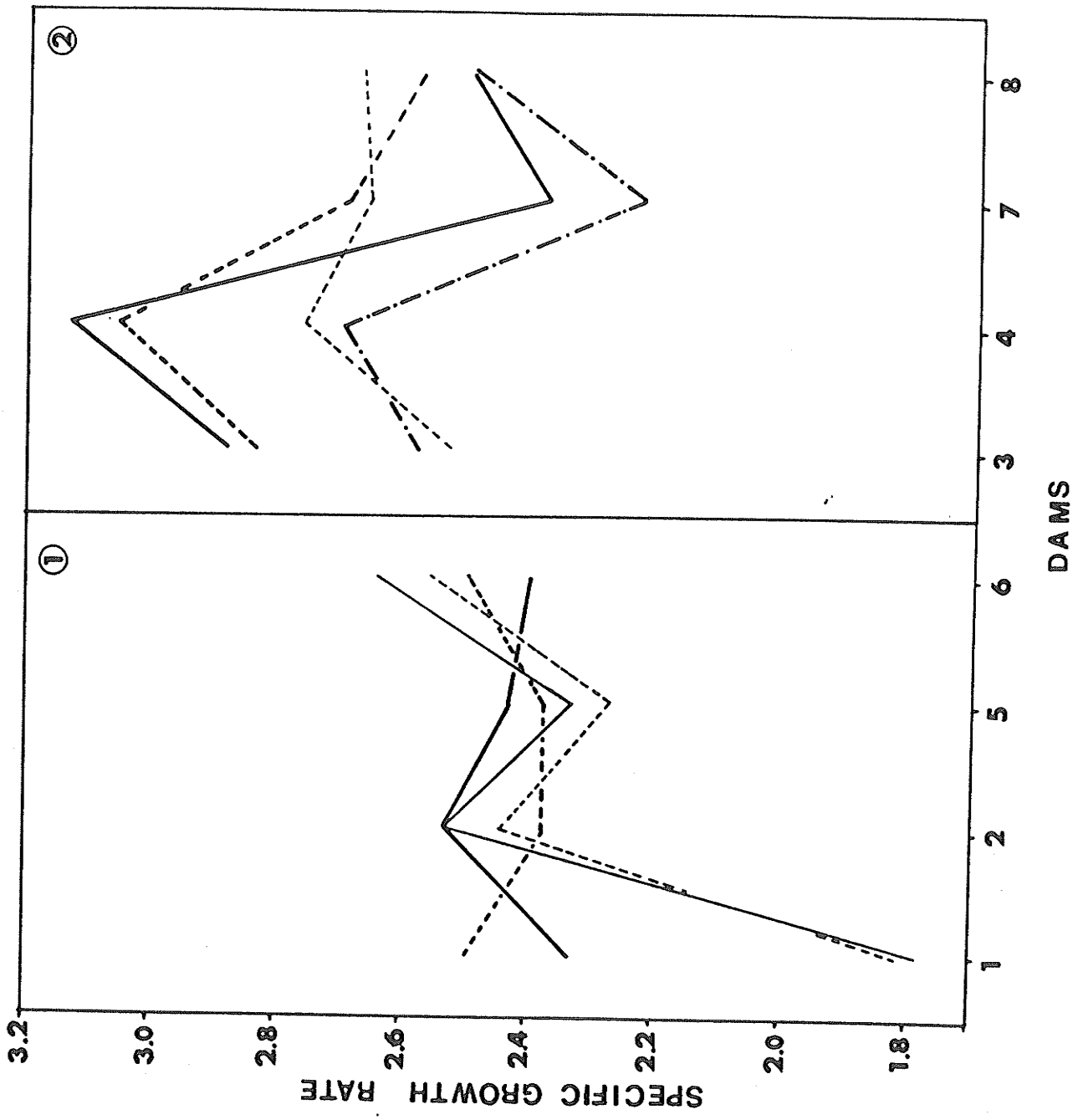
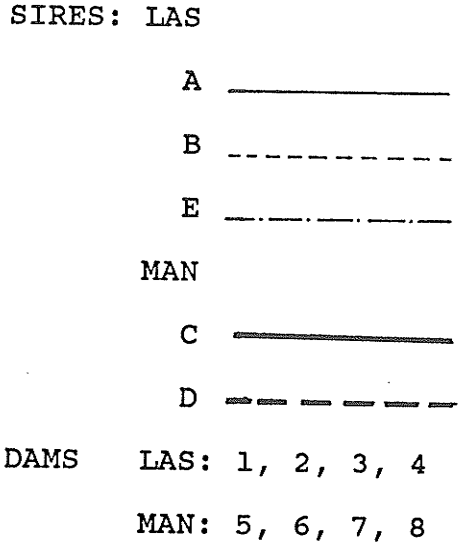

①
②
SPECIFIC GROWTH RATE
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1
2
3
4
5
6
7
8
DAMS

Fig 9. Mean deviations of specific growth rate for specific crosses of rainbow trout strains in each of the diallels.



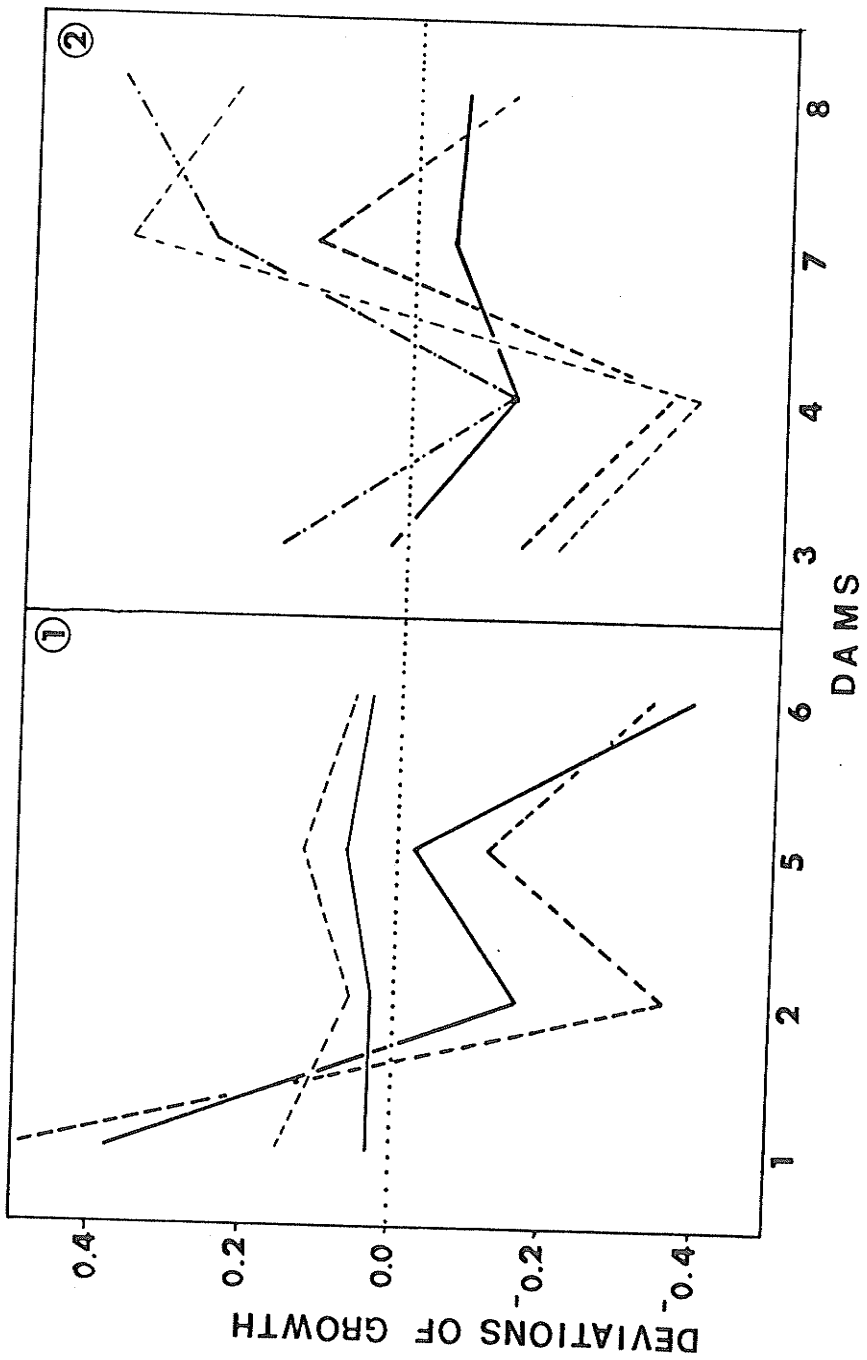

DEVIATIONS OF GROWTH
0.4
0.2
0.0
-0.2
-0.4
1
2
5
6
DAMS
3
4
7
8
①
②

Fig 10. Graphical presentation of interactions between the two rainbow trout strains (1 & 2) using specific growth rate as the response in each of the diallels and for the two dialles pooled (3).

SIRES: LAS _ _ _ _ _ _ _ _

MAN _____

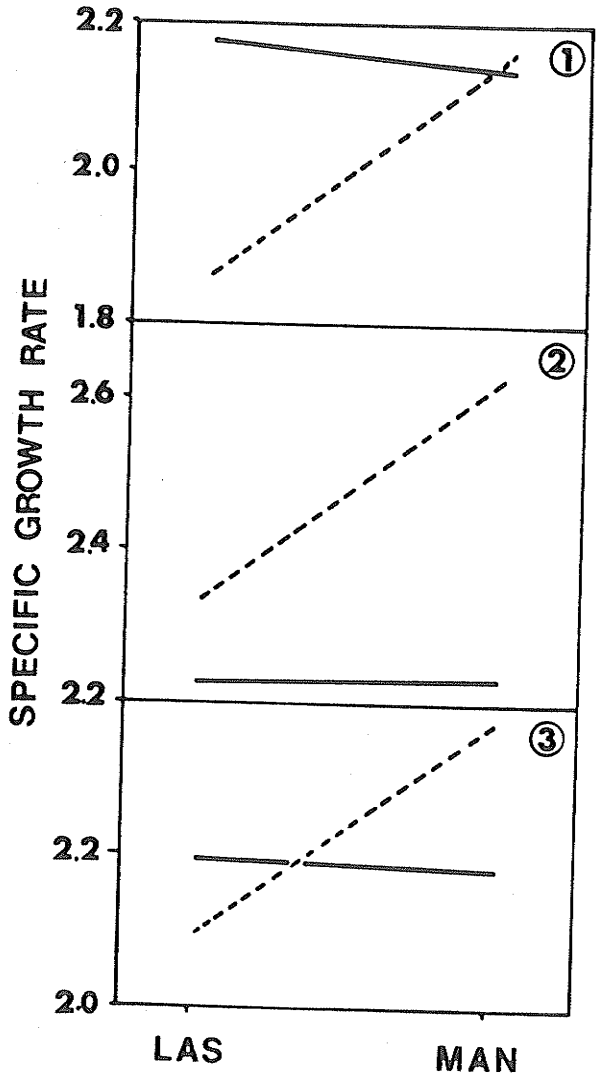

SPECIFIC GROWTH RATE
2.2
2.0
1.8
2.6
2.4
2.2
2.2
2.0
①
②
③
LAS
MAN

Table 26. Pearson's correlations[1] for egg diameter (DE), egg weight (WE) and fish weight (W1--W6) for the six sampling periods for diallels one (DI) and two (DII).

	DE	WE	W1	W2	W3	W4	W5	W6
DI								
DE	1.00	0.87	0.02	0.03	0.05	0.06	0.07	0.01
		(0.0001)	(0.66)	(0.47)	(0.21)	(0.17)	(0.09)	(0.78)
WE		1.00	0.03	0.07	0.06	0.06	0.06	- 0.00
			(0.43)	(0.11)	(0.17)	(0.14)	(0.18)	(0.95)
DII								
DE	1.00	0.90	-0.00	0.01	-0.06	0.03	0.09	-0.04
		(0.0001)	(0.98)	(0.77)	(0.17)	(0.52)	(0.04)	(0.34)
WE		1.00	0.03	0.05	-0.07	0.07	0.05	-0.01
			(0.54)	(0.29)	(0.11)	(0.13)	(0.26)	(0.89)

1. Values in parenthesis are levels of significance of correlations.

the first diallel. The same trend was observed for the correlation of egg weight with fish weight in the second diallel when r=0.05 (P=0.29) for W2 dropped to 0.03 (P=0.56) for W6. There was no indication that egg size had influence on the overall specific growth rate when ranks for egg diameter and egg weight were compared with those of specific growth rates averaged for dams.

Table 27 shows least square analysis of variance for strain direct, maternal direct effects and heterosis for growth rate, in diallels I and II as well as for pooled data from both diallels. All the genetic effects on growth were nonsignificant in the first diallel although heterosis was marginally significant ($P<0.1$). In the second diallel, however, strain direct and maternal direct effects due to both strains for growth were significant ($p<0.05$) again with heterosis marginally significant at $p<0.10$. Both diallels pooled showed maternal effects and heterosis significant ($P<0.05$). Breed differences were significant in the second diallel ($P<0.05$) and for pooled data ($P<0.05$).

Examination of genetic effects on RVI (Table 28) showed only a weak maternal influence in Diallel I

($F=4.07$ $P<0.1$) and for pooled data ($F=3.29$ $P<0.1$) with all the other effects nonsignificant.

While strain direct effects were marginally significant for condition factor in the first diallel ($P<0.1$), they were nonsignificant in diallel II (Table 29). Maternal direct effects on condition factor in the second diallel were also significant ($P<0.001$) although they were nonsignificant in diallel I. Overall strain direct (additive) effects on condition factor were nonsignificant. Heterosis for condition factor was negative but nonsignificant in both diallels although significant overall ($P<0.05$).

Heritability based on the dam component of variance, initially high, assumed a somewhat downward trend through the growth period. In instances where heritabilities due to sires were negative they were assumed to be zero and are reported thus in Table 30, in which heritabilities for growth rate and weight calculated at 21 day intervals are presented. Heritability for weight due to sires was 0.021 ± 0.001 and 0.269 ± 0.016 due to dams. Heritabilities for growth based on sires ranged from 0.16 to 0.37 with a mean of 0.27 ± 0.04 while the range based on dams was 0.18 to 2.11 with a mean of 0.88 ± 0.35 in the first diallel.

Heritabilities for growth rate over the 105 days was 0.44 and 0.85 based on sire and dam components of variance respectively for the first diallel. The overall mean for sires in the second diallel was 0.67 ± 0.32 with a range of 0.08 to 1.85 while for dams the overall mean was 1.23 ± 0.17 with a range of 0.84 to 1.73. Heritability calculated for the growth rate over 105 days was 0.02 for sires and 2.65 for dams.

Table 27. Least square analysis of variance for genetic effects on specific growth rate in the separate diallels and pooled data.

	DI		DII		OVERALL	
Source	df	F	df	F	df	F
Replicate	-	-	-	-	1	22.29***
Strain	3	1.82	3	4.47*	3	3.63*
Rep X Str	-	-	-	-	3	2.67
Str X dam(Str)	12	0.50	12	1.03	-	-
Str X dam(Str x Rep)	-	-	-	-	24	0.49
Strain direct	1	1.02	1	1.78	1	1.74
Maternal	1	0.02	1	9.09*	1	5.22*
Heterosis	1	3.20[a]	1	3.60[a]	1	5.58*

* significant at P<0.05

*** significant at P<0.0001

a significant at P<0.1

DI... first diallelic cross; DII... second diallelic cross

Table 28. Least square analysis of variance for genetic effects in the separate diallels and pooled data for RVI.

	DI		DII		OVERALL	
Source	df	F	df	F	df	F
Replicate	-	-	-	-	1	0.05
Strain	3	2.08	3	1.10	3	1.38
Rep X Str	-	-	-	-	3	1.54
Strain direct	1	2.59	1	0.18	1	0.41
Maternal	1	4.07[a]	1	0.55	1	3.29[a]
Heterosis	1	2.09	1	0.93	1	0.01

a significant at $P<0.1$

DI... first diallelic cross; DII... second diallelic cross

Table 29. Least square analysis of variance for genetic effects in the separate diallels and pooled data for condition factor.

	DI		DII		OVERALL	
Source	df	F	df	F	df	F
Replicate	-	-	-	-	1	5.95*
Strain	3	2.18	3	8.37**	3	4.57*
Rep X Str	-	-	-	-	3	3.48*
Strain direct	1	4.21[a]	1	0.01	1	2.77
Maternal	1	2.24	1	11.88**	1	0.39
Heterosis	1	2.32	1	2.32	1	4.44*

* significant at P<0.05

** significant at P<0.001

a significant at P<0.1

DI... first diallelic cross; DII... second diallelic cross

Table 30. Heritabilities based on sire and dam components of variance for weight and growth rate at 21-day intervals for both diallels.

DAYS	0	21	42	63	84	105
WEIGHT						
DI						
SIRE	0	0.02	0.08	0.06	0.08	0.004
DAM	0.25	0.26	0.24	0.22	0.30	0.20
DII						
SIRE	0.001	0	0.01	0	0	0
DAM	0.33	0.37	0.33	0.21	0.23	0.27
GROWTH						
DI						
SIRE	-	0.31	0.16	0.37	0.20	0.30
DAM	-	1.15	2.11	0.43	0.18	0.52
DII						
SIRE	-	0.12	1.85	0.08	0.69	0.59
DAM	-	0.86	1.25	1.73	0.84	1.45

DISCUSSION

Although the diallel crosses involved only two strains, a few important observations were evident. It was shown that a strain as a source of sires was probably unimportant in the growth performance of progeny in the first 205 days after hatching as indicated by the nonsignificant sire strain effect in both trials. It appeared, however, that a strain as source of dams was important in the second diallel whereas in the first experiment it had no significant effect. This difference in the dam strain effect may be explained by the fact that a different set of dams was used in the second experiment. Dams within strain effect was significant in the first diallel, indicating that dam selection may lead to enhanced early growth of progeny.

It has been documented that early growth rate differences among progeny may be correlated to egg size (Gall, 1974). Correlations between egg size and fish weight diminished with time in the present experiments and indirectly supports this view. However, when growth rate was examined over 105 days, there was no evidence that dams with larger eggs had progeny with superior growth. This was further illustrated by the

fact that while egg sizes were significantly different in the second diallel for the two LAS dams growth rates for progeny of these two dams were not significantly different.

Heterosis or hybrid vigour (Falconer, 1981) is usually expected in a cross involving highly inbred lines or lines that sufficiently differ in gene frequencies at loci for the trait under study (Eisen, 1980). Graphical presentations of interactions indicated specific combining ability were greater when LAS dams were crossed with MAN sires. Heterosis for growth which averaged 7% for both diallels was quite encouraging. Heterosis for weight has been reported in inter-strain crosses in rainbow trout (Gall, 1975) and tolerance to acid water in inter-strain crosses in brown trout (Edward and Gjedrem, 1979). The only report of heterosis for growth rate in fish has been for inter-strain crosses in carp (Wolharth et al., 1975). The results presented here suggest that inter-strain crosses in rainbow trout may result in heterotic effects on growth performance, an event that gives weight to consideration of the use of inter-strain crosses for growth improvement.

Use of the genetic linear model to evaluate strain, maternal ability and heterosis gave comparable

results in strain ranking for growth e.g. the LAS strain as dam source was better than the MAN strain when dam strain in the ANOVA was interpreted as "maternal ability" with respect to strain. Maternal ability for low variance in weight of progeny was again attributed to the LAS strain. On the contrary, maternal ability for good condition for the LAS dams was only slightly better than that of the MAN dams in the first experiment but poorer in the second diallel. To get an overall picture of this a larger experiment involving more trials would have to be conducted.

A decline in condition among hybrids was indicated in both diallels, an observation already discussed in Chapter 1 where it was shown that fast growth was negatively correlated with condition factor. It appears that among hybrids that grew faster than pure strains length was always slightly greater proportionally so that condition factor which is a ratio of weight to length was smaller.

Russell (1979) reported greater phenotypic variance in inbred than in line-cross populations for weaning, post-weaning and mature weights in beef cattle (quoted by Hohenboken, 1985). Leamy (1982) also reported more phenotypic variability in 14 skeletal and

body size traits in inbred mice. In rabbits variability was 11% smaller in cross-breds than in straight-breds (Lukefahr et al., 1983). From these reports Hohenboken (1985) concluded that single generation breed crossings could lead to uniformity among progeny. In this study, low relative variance indices were observed for hybrids in the second diallel and in the pooled data. This supports Hohenboken's (1985) conclusions and suggests for rainbow trout also, that crossbreeding would lower weight variability. The nonsignificant additive effects for RVI, however, suggested that selection for variance would be difficult. In fact, Hohenboken (1985) pointed out that although it is possible to select for variability it would be tedious and costly.

Aulstad et al. (1970) reported a heritability for weight in rainbow trout based on sire as 0.17 after 150 days rearing, increasing to 0.32 at 280 days. Linder et al. (1983) and McKay et al. (1986) reported heritabilities of 0.20 to 0.38 based on sire component of variance also in rainbow trout. The range of heritabilities for weight and growth reported in this study based on the sire component of variance was in agreement with these authors. Heritabilities based on the dam component of variance is usually in excess of

1.00 because of environmental, maternal and non-additive effects (Refstie, 1980). Refstie (1980), Refstie and Stein (1978) and Naevdal et al., (1975) all reported higher heritabilities based on dam components of variance than those based on the sire variance component in various salmonids. The same trend was observed in this study. Heritabilities for weight based on the dam component declined slightly with time reminiscent of the drop in correlation between egg size and subsequent fish weights. The downward trend in heritability based on the dams was probably a manifestation of the diminishing correlation between egg size and weight discussed above. Chevassus (1976) reported a maternal effect in early growth of rainbow trout which disappeared after two months. This seems to be supported by my observations for heritability based on the dam and the egg size and fish size correlations. However, it should be pointed out that these observations failed to account for the increase in heritability for weight in the Pacific salmon as reported by Beacham and Murray (1988).

Chapter 3

Electrophoretic characterization of three hatchery-reared strains of rainbow trout (Salmo gairdneri Richardson).

ABSTRACT

Seven out of eleven loci showed significant differences in allele frequency distribution among three strains of rainbow trout (Salmo gairdneri Richardson). Idh-3,4 locus was fixed in two of the strains while the Sod-1 locus was absent in one of the strains and in a frequency of 0.03 in the other compared to 0.14 in the third strain. Sod-1 was proposed as marker for isolating the strains.

Similarity indices ranged from 0.973 to 0.988 on the scale of 1.000 among pairwise comparisons of the strains while Nei's genetic distance was 0.012 between Mount Lassen and Manx, 0.025 between Mount Lassen and Tagwerker and 0.027 between Manx and Tagwerker. Although nonsignificant the genetic distance between Manx and Tagwerker was reflected in low survival of progeny from crosses between these two strains. Gene diversity was highest in the Mount Lassen strain followed by Manx and then lastly Tagwerker. These differences in gene diversity were attributed to breeding and management practices used at the hatchery.

Examination of SOD inheritance patterns in a diallel cross indicated that there could be two loci involved for coding for this enzyme in liver contrary to earlier reports. There was evidence of repression of maternal as well as paternal alleles in the model advanced to explain the two-loci inheritance.

INTRODUCTION

Worldwide domestication of fish for aquacultural purposes has created artificial populations whose exact records of origin are often difficult to ascertain. Examples of such uncertainty as to origin of strains of fish found at hatcheries can be seen in works such as Guyormard (1981), Baker (1983) and Uraiwan (1982) in which strains studied could only be traced to the hatchery of origin or at best to a stream as a possible ultimate source.

In the wild, rainbow trout is regarded as a heterogeneous polytypic species that includes geographic forms such as Salmo aquilarium, Salmo smaragdus, Salmo regalis as well as ecologic populations (Guyomard, 1981). As pointed out by Behnke (1972) and reiterated by Taggart et al., (1981), "lumping" taxonomic groups under a single species e.g. Salmo gairdneri Richardson, masks the large variability existing among populations which is in itself a resource on which management strategies can be based.

For successful domestication, identities of strains of fish should be explicit enough to allow the fine manipulation of fish usually practised in animal

husbandry. For instance, a fish breeder planning crossbreeding programs should know at what phylogenetic level he is working. In a crossbreeding program, the breeder should be able to answer the question: Are the crosses inter-strain, inter-species or intra-strain? Given so many "strain names" belonging to fish that are morphologically indistinguishable and with an all encompassing name "rainbow trout" breeders hardly ever know the genetic relationships among the strains held at any one hatchery.

Other aspects compounding the problem of rainbow trout strain identification are selection practices and the fact that strains are bred in isolation at hatcheries, sometimes using small numbers of brood stock, which results in genetic bottlenecks and random drift that only serves to genetically distance strains even where their origin was the same. In this regard, it is not enough to know the origin of the strain alone but as well some measure of genetic variability should be used to describe the strain on hand. Several workers have compared genetic variability in hatchery stocks of various salmonids with those in the wild (Ferguson, 1980; Taggart et al., 1981; Allendorf and Utter, 1976; Ryman et al., 1984).

It has been shown that in cases where slight morphological or ecologic differences could have served to verify strain differences, electrophoretic analyses reveals such strains to be the same genetically (Allendorf et al., 1976). Utter et al. (1974) cited a situation in the Columbia River where electrophoretic data indicated considerable heterogeneity among rainbow trout that were in fact being managed as a single population in some instances. Use of biochemical techniques such as starch gel electrophoresis has become a method readily applicable in investigations for genetic differences among both hatchery and wild populations of fish to solve a variety of problems. For example electrophoretically detectable isozyme variants have been used as genetic markers (Hedgecock et al., 1976; Moav et al., 1976; Allendorf and Utter, 1979; Murphy et al., 1983). Establishment of genetic markers depends on differential occurrence of alleles among fish stocks, either the same alleles at significantly different frequencies or separate fixed alleles among stocks (Taggart et al., 1981). In this study electrophoretic data from three strains of rainbow trout were analyzed to estimate genetic similarity indices, genetic diversity and genetic distances among them. Alleles

found fixed or at low frequencies are proposed as genetic markers for the respective strains.

Allendorf (1975) and Allendorf and Utter (1979) advocated that attempts to genetically interpret electrophoretic data should preferably be supported by breeding experiments. In cases where designation of loci was doubtful their derivation was done by examining progeny in a diallel cross. This approach was illustrated by a detailed investigation into the inheritance of superoxide dismutase in a diallel involving two of the strains of rainbow trout; this design being appropriate for high certainty in the prediction of parental electrophoretic phenotypes without having to sacrifice the parents.

MATERIALS AND METHODS

Six hundred fish representing three strains of hatchery reared rainbow trout (Salmo gairdneri Richardson) were studied. They were a random sample from each of the pure strains at the termination of the growth assessment experiment described in chapter 1. They were then approximately one year old. The samples consisted of 30 fish per family from each of the rearing temperatures, 7°C and 15°C. Parents used in the single pair matings were themselves progeny of mass spawning that involved up to three males used to fertilize a batch of eggs from as many as three females within the same strain. Spawning at the Rockwood Hatchery has been conducted only within strains to ensure their purity (Papst, pers. comm.). The origins of the strains are given on page 15 Chapter 1.

Liver, eye, and muscle tissue samples taken from individual fish were identified by strain, family, individual and temperature at which the fish was reared. On removal from pithed fish, tissues were homogenized in distilled water (tissue volume:water volume approximately equal) using a ground glass tissue grinder in ice. The homogenate was pipetted into labelled centrifuge vials, centrifuged at 15,000 g for

fifteen minutes and stored at -40°C. For the superoxide dismutase inheritance study, 16 fullsib families from the 4 X 4 diallel II described in Chapter 2 were used. After about 200 days from hatching, liver tissues for electrophoretic analysis were obtained from a random sample of 20 individuals from each family in the diallel.

The samples were analyzed for 18 enzyme systems using horizontal starch gel electrophoresis procedures described by Brewer (1970) and Harris and Hopkinson (1976). All gels were made using 13% Connaught's starch by buffer volume. Appendix I gives the list of the enzymes examined, tissues used and remarks on the resolution of band patterns obtained. The staining procedure followed Harris and Hopkinson (1976).

Gels once set were cooled in a refrigerator to about 4°C before use. Samples were introduced into the cold gels by placing into wells four millimetre square Whatman paper no. 3 wicks, soaked with partly thawed samples and blotted to remove excess fluid. The wicks were removed about fifteen minutes after the start of electrophoresis for better band resolution. Electrophoresis was conducted for three and a half to four hours. The power supply was an LKB power pack.

Nomenclature

The nomenclature used followed Allendorf and Utter (1979) and Taggart et al. (1981). The commonest band at a locus was arbitrarily designated 100 and the rest of the bands assigned lower or higher values relative to 100, the more anodal having the higher value and vice versa (Appendix II). Assignment of loci, where in doubt, was decided upon by examination of inheritance patterns among progeny in the diallelic crosses as illustrated by the analysis described for SOD. The duplicated loci Idh-3,4 and Mdh-3,4 were treated as one locus each since allelic products could not be assigned either to the 3rd or to 4th locus without ambiguity.

Data analysis

Allele frequencies for electrophoretically detectable loci were obtained directly through a SAS frequency analysis procedure on digitized data. Where possible chi square contingency tables were used to test for homogeneity in the distribution of alleles among the strains at corresponding loci. In analysis of SOD inheritance patterns, the enzyme was treated as a dimer so that three bands represented heterozygotes while one band represented homozygotes (Harris, 1966).

The commonest allelic isozyme at a locus was arbitrarily numbered 100 (Taggart et al. 1984) and the rest given numbers relative to this so that the slowest migrating allele was numbered 90, the intermediate which was commonest, 100 and the fastest 110. For ease of reference the allelic product numbered 90 was assigned letter B', 100 assigned A (or B in atypical Manx genotypes) and 110 assigned A'. Genotypic and allelic frequencies were calculated as percentages. Deviations from expected Mendelian inheritance were tested using the chi square test.

Gene diversity was estimated according to Nei (1975) as:

$$h = 1 - \Sigma x_i^2 \qquad [17]$$

where x_i denotes the frequency of the i^{th} allele at a locus. The average gene diversity for a strain (H_s) is the mean of h over all loci examined. Total gene diversity (H_T) for all the three strains is the mean of h pooled over the strains.

Gene similarity (I) between any two strains was determined following Nei (1975) and Ayala (1982) as:

$$I = (\Sigma a_i b_i) / \sqrt{\Sigma a_i^2 \Sigma b_i^2} \qquad [18]$$

where a and b are frequencies of alleles at a locus in the strains A and B respectively. Extension of [18] for all loci examined allows an estimate of Nei's genetic distance D between any two strains. The genetic distance, D is the negative natural logarithm of genetic similarity, I.

RESULTS

Table 31 shows allele frequencies at eleven loci scored for seven enzyme systems. Contingency chi-square analyses for the respective loci to test homogeneity in the distribution of allele frequencies are also presented in the table. As can be seen, seven out of the eleven loci showed significant differences in allele frequencies. Significant ($P<0.001$) chi-square values were, in descending order, 154.19 for Sod-1, 85.04 for Idh-3,4, 42.86 for Mdh-3,4, 31.66 for Est-4, 17.95 for Gpi-2, 16.04 for Mdh-2 and 15.07 for Idh-2. The distribution of allele frequencies in the three strains was nonsignificantly different at the Mdh-1, Mpi-1, Mpi-2 and Pgm-2 loci. The Idh-3,4 locus was polymorphic (criterion: frequency of commonest allele not greater than 0.95, Ayala, 1982) only in the Mount Lassen strain with the Tagwerker and Manx strains having a frequency of 1 and 0.99 for allele 100 respectively. The Tagwerker strain did not have Sod-1 (90) while in the Mount Lassen it was at the frequency of only 0.03 compared to a frequency of 0.14 in the Manx strain. There were no 90/90 phenotypes at the Sod-1 in either Mount Lassen or Manx.

Table 32 shows similarity indices and genetic distances for pairwise comparisons among the three strains at eleven loci. Overall similarity indices were 0.988 ± 0.005 between Mount Lassen and Manx strains, 0.975± 0.004 between Mount Lassen and Tagwerker and 0.973 ± 0.069 between Manx and Tagwerker. The Mount Lassen and Manx strain were similar at Mpi-1 and Pgm-2 with a similarity index of 1 just as Mount Lassen was similar to Tagwerker at Mpi-2 and Mdh-1 also with a similarity index of 1 at each of these loci. Manx and Tagwerker had a similarity of 1 at three loci (Idh-3,4, Mdh-1 and Mdh-2). The lowest similarity indices were found at the Sod-1 locus for all the three pairwise comparisons, suggesting that this locus could be of value as a genetic marker. Nei's genetic distances were, in order of increasing magnitude, 0.012, 0.025 and 0.027 for the comparisons Mount Lassen vs Manx, Mount Lassen vs Tagwerker and Manx vs Tagwerker respectively.

Genetic variation in the three strains as estimated by genetic diversity values (=average heterozygosity) at eleven loci is presented in Table 33. Overall, the Mount Lassen strain had the highest gene diversity of 0.4252 ± 0.0486 compared to 0.3904 ± 0.0589 and 0.3668 ± 0.0573 in Manx and Tagwerker.

Table 31. Allelic frequencies for some of the enzyme systems analyzed.

LOCUS	ALLELE	LAS (n=210)	MAN (n=150)	TAG	X^2
	(n=240)				
Gpi-2	95	0.31	0.41	0.40	
	100	0.51	0.46	0.40	
	105	0.18	0.13	0.20	17.95**
Est-4	100	0.43	0.38	0.46	
	105	0.18	0.15	0.05	
	110	0.39	0.47	0.49	31.66**
Idh-2	100	0.54	0.60	0.68	
	90	0.46	0.40	0.32	15.07**
Idh-3,4	100	0.87	0.99	1.00	
	90	0.13	0.01	0.00	85.04**
Sod-1	80	0.03	0.14	0.00	
	100	0.54	0.61	0.36	
	110	0.43	0.25	0.64	154.19**
Mdh-1	80	0.09	0.12	0.10	
	100	0.91	0.88	0.90	2.22
Mdh-2	80	0.21	0.12	0.13	
	100	0.79	0.88	0.87	16.04**
Mdh-3,4	90	0.39	0.29	0.17	
	100	0.61	0.71	0.83	42.86**
Mpi-1	90	0.45	0.46	0.48	
	100	0.55	0.54	0.52	0.67
Mpi-2	90	0.46	0.43	0.46	
	100	0.54	0.57	0.54	0.99
Pgm-2	95	0.13	0.12	0.16	
	100	0.87	0.88	0.84	2.52

These values were not significantly different. In relative terms, Tagwerker had 13% lower diversity than Mount Lassen while Manx was 8.2% less diverse relative to Mount Lassen. In all the strains, Idh-3,4 had the lowest average heterozygosity, culminating in none at all in the Tagwerker strain. Relatively low average heterozygosities were also observed at Mdh-1, Mdh-2 and Pgm-2 in all the strains.

Inheritance of liver Superoxide dismutase (SOD)

Fig 11 shows the electrophoretic patterns and percentages of homozygotes and heterozygotes for SOD among the 16 halfsib families in the diallelic cross of the Mount Lassen and Manx strains of rainbow trout. Table 34 gives the probable electrophoretic genotypes corresponding to the patterns in Fig 11. Among the pure strains, all four Mount Lassen families did not deviate from expected Mendelian ratios based on a one-locus-two-allele inheritance. For instance, the progeny from the mating LAS(2) X LAS(1), assigned the genotype BC (i.e.100/110) revealed that sire LAS(2) had to be of the genotype BB and dam LAS(1) of the genotype CC or vice versa in order for the offspring to be 100% BC. The Manx strain families showed a mixture of products that could not be accounted for by the system

Table 32. Similarity (I) indices and overall genetic distance (D) for pairwise comparisons of the strains at eleven genetic loci.

LOCUS	LAS vs MAN	LAS vs TAG	MAN vs TAG
Gpi-2	0.981	0.973	0.989
Est-4	0.987	0.971	0.983
Idh-2	0.993	0.965	0.989
Idh-3,4	0.990	0.989	1.000
Sod-1	0.947	0.926	0.767
Mdh-1	0.999	1.000	1.000
Mdh-2	0.992	0.994	1.000
Mdh-3,4	0.984	0.933	0.983
Mpi-1	1.000	0.998	0.999
Mpi-2	0.998	1.000	0.998
Pgm-2	1.000	0.972	0.999
Similarity	0.988 (±0.005)	0.975 (±0.008)	0.973 ±0.069
Distance	0.012	0.025	0.027

devised to explain patterns seen in the pure Mount Lassen families. Two of the four Manx families conformed to Mendelian ratios with the genotypes(AB and BB for family MAN(2) X MAN(1), and AB, BB, BC and AC for family MAN(2) X MAN(2). The other two Manx families MAN(1) X MAN(1) and MAN(1) X MAN(2) showed patterns of the genotypes (BC, BB and AB for family MAN(1) X MAN(1) and CC, BC, BB and AB for family MAN(1) X MAN(2). The combination AB could not be explained as neither sire MAN(1) nor the dams MAN(1) and MAN(2) had the allele A.

All the inter-strain crosses including reciprocals produced patterns explainable by a one-locus-three-allele inheritance already described for the pure families of the Mount Lassen strain. For example the cross LAS(1) X MAN(1) produced genotypes BB and BC in the ratio 1:1 which is typical of a cross between a heterozygote and a homozygote. The cross LAS(2) X MAN(1) checked out with the genotype already assigned to sire LAS(2) in the cross LAS(2) X LAS(1).

Table 33. Gene diversity in the three rainbow trout strains based on five loci from some of the enzyme systems scored.

LOCUS	LAS	MAN	TAG
Gpi-2	0.6114	0.6034	0.6400
Est-4	0.6306	0.6122	0.5458
Idh-2	0.4968	0.4800	0.4352
Idh-3,4	0.2262	0.0198	0.0000
Sod-1	0.5226	0.5458	0.4608
Mdh-1	0.1638	0.2112	0.1800
Mdh-2	0.3318	0.2112	0.2262
Mdh-3,4	0.4758	0.4118	0.2822
Mpi-1	0.4950	0.4968	0.4992
Mpi-2	0.4968	0.4902	0.4968
Pgm-2	0.2262	0.2112	0.2688
H_S	0.4252	0.3904	0.3668
	$\pm$0.0486	$\pm$0.0589	$\pm$0.0573

H_T = 0.3941

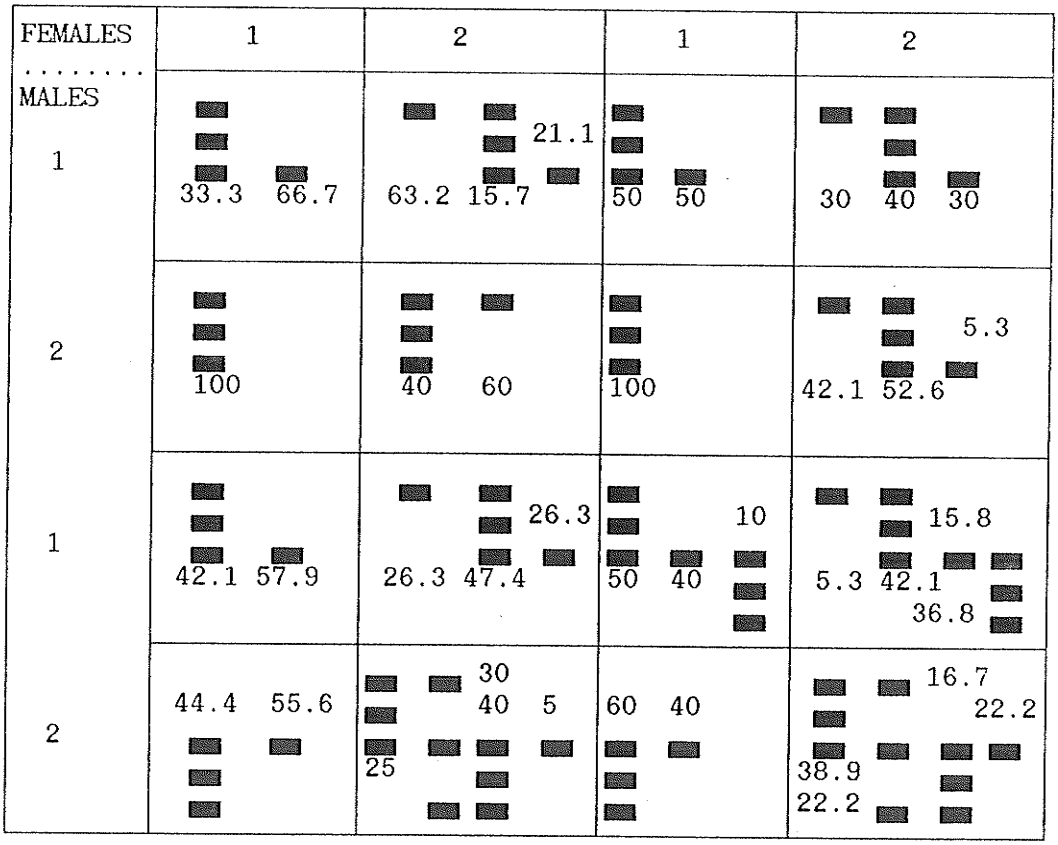
Table 34. Probable superoxide dismutase electrophoretic genotypes for the 4 X 4 diallelic cross of two rainbow trout strains and numbers of individuals for each genotype per family based on a one locus three allele mode of inheritance (symbols adopted from Allendorf et al.,1973).

	LAS		MAN	
FEMALE	1(BB)	2(BC)	1(BB)	2(BC)
MALE				
LAS				
1 (BC)	BC BB 6 12 ns	CC BC BB 11 3 5 ns	BC BB 10 10 ns	CC BC BB 6 8 6 ns
2 (CC)	BC 20 ns	BC CC 8 12 ns	BC 20 ns	CC BC BB 8 9 1 *
MAN				
1 (BC)	BC BB 10 9 ns	CC BC BB 5 9 5 ns	BC BB AB 8 10 2 *	CC BC BB AB 3 8 1 6 *
2 (AB)	AB BB 9 10 ns	BC AC AB BB 4 1 6 9 *	AB BB 12 8 ns	BC AC AB BB 8 4 4 3 *

ns = nonsignificantly different from Mendelian inheritance for a one locus two allele case ($P<0.05$).

* = significant deviation from Mendelian expectation for a one locus two allele case.

Fig 11. Superoxide dismutase electrophoretic phenotypes and phenotypic frequencies (percent) from progeny of a 4 X 4 diallelic cross of two strains of hatchery raised rainbow trout (first 1, 2, are the Mt. Lassen strain and the next 1, 2 Manx).



DISCUSSION

Significant differences in the distribution of alleles at seven of the eleven loci was an indicator of genetic differences between the three strains. Genetic analysis of traits such as growth rate, food conversion efficiency and as well as the knowledge that the Tagwerker strain is an early autumn spawner (Uraiwan, 1982; Baker, 1983; and chapter 1 this thesis) were in agreement with this observation. The absence of Sod-1 (90) in the Tagwerker strain, its presence at a low frequency in the Mount Lassen strain and in the Manx strain at a relatively high frequency could be used as a genetic marker (Taggart and Ferguson, 1984). In this study the Tagwerker strain could be distinguished from both Manx and Mount Lassen strains for lack of the Sod-1 (90) allele while the two could be distinguished by the fact that the frequency of Sod-1 (90) is higher in Manx (over 4 times more common) than in Mount Lassen. SOD has been used to distinguish strains of rainbow trout in the Columbia River by Utter et al. (1974). The fixation of Idh-3,4 in the Tagwerker strain and near fixed condition of Idh-3,4 in the Manx could also be used to distinguish the Mount Lassen strain from the two strains.

The genetic similarity indices at eleven loci showed, on the average, that the Mount Lassen strain was more similar to Manx than to Tagwerker. Manx and Tagwerker strains were most dissimilar. It was interesting to note that although Nei,s genetic distance between Manx and Tagwerker was only 7% greater than that between Mount Lassen and Tagwerker, experience at the hatchery has shown that survivability of crosses between Manx and Tagwerker is very poor at the larval stage (Papst, pers. comm. and chapter 1 this thesis). It appeared that the two strains have some incompatibility which was indicated by genetic distance at the eleven loci and which might be demonstrable in a study involving a larger number of loci. The only autumn spawning strain (A13) found to differ from all the nine other strains studied by Koljonen (1986) in Finland was said to have been imported from the University of Washington. The frequency of Idh-4 (100) allele, reported strain (A13) by Koljonen was 0.963, and could be considered as fixed if the criterion for nonpolymorphism set above was adopted. Furthermore, Sod-1 (69 = my 90?) had a frequency of zero in the A13 strain (Koljonen, 1986). Since the status of these two loci in the Tagwerker strain was similar to that of the A13 strain it is possible that the Tagwerker strain could also have originated from the University of

Washington. Although two loci are not sufficient to make strong conclusions on a trait, it would appear that early autumn spawning has some connection with the fixation of Idh-4 (100) and loss of Sod-1 (90).

It is known that a large number of loci should be analyzed for a balanced estimate of gene diversity in any population (Lewontin, 1974; Nei and Roychoudhury, 1974; Nei, 1975). Within the limits of the present data, however, it appeared that the Tagwerker strain had the least gene diversity of the three strains followed by Manx and Mount Lassen in that order. The low gene diversity, loss of Sod-1(90) allele and fixation of Idh-3,4(100) in the Tagwerker strain was probably a manifestation of canalized breeding that has resulted in loss of genetic variation in the Tagwerker strain. Papst (pers. comm.) has pointed out that the Tagwerker strain had continually been selected for large size, fast growth and early autumn spawning without much regard to separation of breeding lines to avoid inbreeding.

Based on the Rockwood hatchery records, eggs of the Manx strain were obtained from the Manx Trout Hatchery in the Isle of Man in 1976 but there was no information on the number of males and females used. No other fish have been added since and no specific selection program has been applied to them except that

there has not been any planned effort to maintain genetic variation. This practice may partly be responsible for the relatively low gene diversity observed in the Manx strain in comparison to that of the Mount Lassen strain. Although the Mount Lassen strain has been handled exactly like Manx, two lots of fertilized eggs were obtained from the Mount Lassen Trout Farms, one in 1978 and the other in 1980. There has been some inter-breeding between these two groups, apparently leading to the genetic variation observed in the Mount Lassen strain at the loci examined.

Gene diversities found in my study are comparable to those reported by Thompson (1985) for ten strains of rainbow in which H_T was 0.414 at five loci, 0.404 at eight loci and 0.261 at 10 loci. Thompson (1985) concluded that there was no apparent loss of genetic variation in the ten strains. Extremely low values of genetic diversity ranging from 0.072 to 0.140 were reported in ten hatchery strains of rainbow trout in Finland by Koljonen (1986). It is, however, difficult to make direct comparisons as there are probably differences in gene loci designation and scoring protocol of alleles as well as differences in electrophoretic procedures.

Although the conclusions on the characterization of these strains were based on only 21 fullsib families: 4 from Mount Lassen, 4 from Manx and 3 from Tagwerker, the spawning practice used at the Rockwood Hatchery probably qualifies these families to be typical representatives of the populations of these strains at the hatchery. A broad sampling base was ensured by spawning individuals from different broods to avoid brother sister mating.

Superoxide dismutase inheritance

Cederbaum and Yoshida (1970) indicated that in rainbow trout superoxide dismutase was coded for by two separate biochemical loci: one in the blood and the other in the liver, an observation that was refuted in a letter to the editors by Allendorf et al., 1973). Utter et al. (1973), using breeding data, found that SOD was coded for by only one locus in rainbow trout. Allendorf et al. (1977) also found the same mode of coding in brown trout, a species closely related to rainbow trout. Taggart et al. (1981), however, reported that SOD is coded for by two loci. In the diallel cross studied in this thesis, a one-locus three allele system could explain 14 of the 16 matings. In two families of the Manx strain, however, there

appeared patterns that could not fit a one-locus three-allele model. In previous studies of other enzyme systems, aberrant combinations have often been attributed to intragenetic recombinations (Clayton et al., 1975) or to a model that would tolerate both disomic and tetrasomic inheritance (Wolf and Ropers, 1973). The frequency of "extra" genotypes in the pure Manx families MAN(1) X MAN(1) and MAN(1) X MAn(2) was too high to be accounted for by intragenetic recombinations events which are known to be extremely rare (Clayton et al., 1975). There was no evidence for a tetrasomic inheritance pattern either.

Consideration of a second locus (SOD-1) allowed a reasonable fit for all the families. This locus, found coding for a slow migrating allele in the Manx strain is perhaps redundant in the Mount Lassen strain, giving rise to either null alleles or an allele that codes for an enzyme product that is indistinguishable from the BB products in the Mount Lassen.

The mode of inheritance suggested in my investigation indicated that SOD-1 was not expressed in the presence of paternal alleles from the Mount Lassen strain with a reassignment of genotypes as shown in Table 34. Among the reciprocal crosses, only two

families MAN(1) X LAS(1) and MAN(1) X LAS(2) also showed inheritance at the SOD-2 locus. Crosses MAN(2) X LAS(1) and MAN(2) X LAS(2), sired by Manx, expressed only the SOD-1 locus and a mixture of both loci respectively. Suppression through adulthood (9 months) of paternally derived alleles for alcohol dehydrogenase (ADH) was reported in hybrids between quails and chicken by Castro-Sierra and Ohno (1968). Asynchrony of expression of lactate dehydrogenase and alcohol dehydrogenase was observed in brown trout and rainbow trout hybrids (Hitzeroth et al. 1968). Hitzeroth et al. (1968) reported that in the brown trout X rainbow trout hybrids, appearance of paternal ADH alleles was demonstrable 192 days post-hatching. Several workers have reported repression of either the paternal allele or maternal allele for various biochemical alleles in a number of inter-specific crosses of fish (Klose et al., 1969; Goldberg et al, 1969; Whitt et al., 1972; Yamauchi and Goldberg, 1974). In all cases where repression of alleles from either sex is documented, the hybrids in question have been inter-specific. According to Kirpichnikov (1981), a greater probability of anomalies (e.g. allelic suppression) in the inheritance of alleles is expected with a greater evolutionary distance between the parents in a given

Table 35. Probable superoxide dismutase electrophoretic genotypes for the 4 X 4 diallelic cross of two rainbow trout strains and numbers of individuals for each genotype per family based on a two locus three allele mode of inheritance (symbols adopted from Allendorf et al.,1973).

	LAS		MAN	
FEMALE	1(BBQ)	2(BCQ)	1(BBAA)	2(BCAB')
MALE				
LAS				
1 (BCQ)	BC BB 6 12 ns	CC BC BB 11 3 5 ns	BC BB 10 10 ns	CC BC BB 6 8 6 ns
2 (CCQ)	BC 20 ns	BC CC 8 12 ns	BC 20 ns	CC BC BB 8 9 1 *
MAN				
1 (BCAB')	BC BB 10 9 ns	CC BC BB 5 9 5 ns	BC BB AB 8 10 2 *	CC BC BB AB 3 8 1 6 *
2 (ABAB')	AB BB 9 10 ns	BC AC AB BB 4 1 6 9 *	AB BB 12 8 ns	BC AC AB BB 8 4 4 3 *

ns = nonsignificantly different from Mendelian inheritance for a one locus two allele case ($P<0.05$).

* = significant deviation from Mendelian expectation for one a locus two allele case.

Q = null locus in the LAS strain corresponding to A locus in the MAN strain

cross. Repression of an allele is probably less drastic than repression of an entire gene locus. Perhaps rainbow trout is a highly heterogeneous species and geographic isolation and domestication has generated such genetic drift at SOD-1 that there is a partial mismatch at gamete pairing with a probable lack of loci homology. Guyomard (1981) reported genetic homogeneity among four French strains of rainbow trout although there was no data on the exact origin of those strains. Assuming that the Manx strain is part of the rainbow trout genome introduced into Europe some 100 years ago (MacCrimmon, 1971) while the Mount Lassen strain remained in North America and that both strains were subjected to selection and inadvertent bottlenecks, the anomalies observed here could perhaps be accounted for. Conversely I hypothesize that inheritance anomalies may be observed at any level i.e. allelic or locus and that the genetic distance between the parents may increase the probability of gene suppression. With the present information, however, the mechanism that leads to failure of expression of alleles or loci could not be explained.

Chapter 4

Association between growth, heterosis and heterozygosity at enzyme loci in rainbow trout (*Salmo gairdneri* Richardson).

ABSTRACT

A general tendency for heterozygotes to weigh more than homozygotes was found in an analysis of electrophoretic data obtained from 11 loci in hatchery-reared rainbow trout (Salmo gairdneri Richardson). Heterozygotes were heavier more often than homozygotes among fish reared at 7°C than among those reared at 15°C. This was attributed to some kind of selection pressure acting more strongly at the sub-optimal temperature. Out of 13 cases in which homozygotes were heavier than heterozygotes, only one was marginally significant ($P<0.25$). At 7°C 6 cases showed homozygotes and heterozygotes equal in mean weight whereas 29 cases showed heterozygote superiority in mean weight, out of which ten were marginally significant ($P<0.25$). In comparison, there were 21 cases in which homozygotes had greater mean weight among fish reared at 15°C, 4 of which were marginally significant ($P<0.25$). Homozygotes and heterozygotes were equal in mean weight in four cases among fish reared at 15°C. Among 28 cases in which heterozygotes showed a superior mean weight, 9 were significant ($P<0.25$).

At both temperatures, regression analysis showed a significant negative association between homozygosity levels while the same analysis revealed a positive association between mean weight and heterozygosity levels at 9 loci, significant ($P<0.25$) at $7^{o}C$ but nonsignificant at $15^{o}C$.

There was some interaction between temperature with some alleles and association of mean weight with Gpi-2 and Mpi-1,2 but evidence was inconclusive.

Heterosis (hybrid vigour) for growth was supported with a concurrent "heterosis for heterozygosity level" at four loci in a typical diallel analysis which lent further support to a positive association between heterozygosity and growth rate. Heterozygosity was negatively correlated with relative variance index and condition factor.

INTRODUCTION

In plant and animal breeding it has been known for many years that heterozygosity strongly influences vigour in trait performance. In recognition of this phenomenon, the classical approach in tapping hybrid vigour has been: 1. to conduct inbreeding in specific lines for a number of generations so as to create a high level of homozygosity and then, 2. to cross the inbred lines in order to achieve hybrid vigour in the F1 progeny. Because the main interest in crossbreeding was to increase the level of heterozygosity, the resulting vigour was referred to as "heterozygosis" a term which was later renamed "heterosis' by Lush in 1914 (Mitton and Grant, 1984; Zouros and Foltz, 1987).

Two major hypotheses have been advanced to explain heterosis. In the "dominance hypothesis" it is postulated that there are favorable alleles in a population and that such alleles, when in homozygous state at a locus, impart maximum benefit to individuals that possess them. In this hypothesis, the protocol followed in crossbreeding for heterosis requires initial inbreeding of separate lines to segregate out favorable alleles as well as to lose deleterious ones (Zouros and Foltz, 1987). According to this approach,

the crossing of inbred lines is simply a reversal of inbreeding depression (Falconer, 1981) and serves to restore the best combination of the favorable alleles. Heterozygotes in such a scheme will only score between the two homozygotes (Zouros and Foltz, 1987).
The second hypothesis known as "overdominance" gives the heterozygote an advantage over either homozygote. In this hypothesis, heterosis can, theoretically, be explained by one locus (Zouros and Foltz, 1987). This idea is said to have had its origins in the "stimulation hypothesis" of Shull (1914) in which it was postulated that the degree of vigour of an organism is determined by the "dissimilarity" of the gametes from which it was formed (Zouros and Foltz, 1987). The dissimilarity (=heterozygosity) was believed to stimulate increased cell division.

East (1936) postulated a hereditary mechanism in corn involving sets of multiple alleles in which certain of the heterozygous genotypes at a given locus were superior to any of the possible homozygotes. In support of the hypothesis, Crow (1947) concluded that heterosis in excess of 5% of the mean of the random breeding population could not be explained by dominance alone but by other effects such as interaction of non-allelic genes or superiority of heterozygotes to

homozygotes. Heterosis has become so associated with heterozygosity that workers such as Dillard et al. (1981) have freely substituted heterosis with heterozygosity in statistical linear models for estimating heterosis. In cases like Dillard's et al (1981), hybrids have always been assigned a theoretical level of 100% heterozygosity presumably at all loci.

Since protein heterozygosity can be estimated using electrophoretic methods, the two hypotheses have been examined by numerous workers in a wide variety of organisms, ranging from plants to animals. Judging from the findings of many workers, however, the search for associations, either of particular electrophoretic alleles with a given trait (i.e. dominance hypothesis) or level of heterozygosity at enzyme loci with superiority in performance (i.e. overdominance hypothesis), has only created more controversy. As there are numerous reports related to this area of research in many organisms, only a few examples from those concerned with fish will be mentioned here.

Smith and Chesser (1981) reported that in the mosquito fish (Gambusia affinis), length was positively associated with a certain number of polymorphic loci. Similar observations were made by King (1985) in the

herring (Clupea harengus). Taniguch et al. (1981) found significant differences in length and weight among genotypes at 3 loci in the sea bream half-sibling families. Unfortunately, the link between biochemical attributes with such traits has not been fully resolved as there are numerous reports demonstrating nonsignificant associations. For example Beacham and Withler (1985) found no heterozygote superiority for growth rate in the pink salmon (Oncorhynchus gorbuscha) just as Reinitz (1977) could not find any evidence of heterozygote superiority for transferrin in weight gain in the rainbow trout (Salmo gairdneri). Also in rainbow trout (S. gairdneri), Koljonen (1986) failed to find any significant correlations between length/weight with the level of biochemical heterozygosity. Such conflicting findings only suggest that there is need for more research on the topic.

Another hypothesis relating to selection in fish states that heterozygous individuals are well buffered developmentally so that they will exhibit low variance (Allendorf et al., 1986; Leary et al., 1983, 1984, 1985, 1987). This means that heterozygosity levels will be negatively correlated with variability in traits under the influence of the loci examined. The major problem, as for all questions of this nature, is

how big a sample of the genome should be used for a researcher to be certain that loci affecting the traits of interest are included. Such questions cannot directly be answered at the moment except that each attempt to tackle them serves as an addition of new evidence towards further understanding of the problem. Negative correlations between heterozygosity levels with variation in a number of characters have been reported in some organisms. For example, Zouros et al. (1980) found a negative correlation between heterozygosity and variation in the growth rate of the American oyster (Crassostrea virginica) while Mitton (1978) demonstrated similar results for morphological characters in the killifish (Fundulus heteroclitus). Koljonen (1986) found negative correlations between mean heterozygosity with variation in weight and length in ten rainbow trout strains.

In this study, association between heterozygosity with growth rate and weight at ten enzyme loci was investigated in the strains of rainbow trout that were examined for growth performance in Chapter 1. Association of heterosis for growth with heterozygosity was also investigated for fish in diallel II in the second chapter of this thesis in which heterosis was estimated at 8%. The hypothesis tested here is that the estimated significant heterosis for growth should

be supported by higher heterozygosity levels among hybrids in the diallel if there is any association between growth rate and the level of heterozygosity. The relationship between heterozygosity and variation in weight was determined by examining correlations between relative variance index and heterozygosity levels in sixteen full-sib families of a diallel cross.

MATERIAL AND METHODS

Electrophoretic data for heterozygosity levels were obtained on the 21 full-sib families representing the Mount Lassen, Manx and Tagwerker strains from fish already assessed for electrophoretic characterization in Chapter 3 and on the 16 full-sib families from diallel II of the Mount Lassen and Manx strains, also electrophoretically analyzed in Chapter 3. Weight records and growth rates were also obtained from the respective chapters.

Data analysis

Pearson's correlations among weight of fish, homozygosity and heterozygosity frequencies at 10 loci were determined for each locus separately and pooled. Multiple regression analysis was also used to examine the dependence of weight of fish on genotype in the model:

$$Y = a + bX + cX \qquad [19]$$

where Y = observed weight

a = intercept

b = regression coefficient interpreted as a weighting factor due to a homozygous locus

X = genotypic status at a locus (homozygous/heterozygous)

c = regression coefficient interpreted as a weighting factor due to a heterozygous locus

Overdominance would be indicated if the weighting factor c due to heterozygotes was positive and significant.

SAS (1985) R-square procedure was used to examine the relative importance of alleles at each of the 10 loci with respect to fish weight accountability in single allele models. This in effect would test the dominance hypothesis if the weight as a trait were under the influence of the gene loci examined.

The least square model for genetic effects described in Chapter 2 was used to analyze the significance of heterozygosity levels in terms of "heterosis for heterozygosis", maternal effects and strain direct effects on the level of heterozygosity. This exercise, as stated in the objectives, was to

investigate whether the demonstration of significant heterosis for growth rate in diallel II in Chapter 2 would be supported by a corresponding significant "heterosis for heterozygosis". Pearson's correlations between growth rate, relative variance index and condition factor with level of heterozygosity in the 16 families were also determined for the diallel at four enzyme loci.

Significant levels reported for the tests above also included the 75% confidence level as it was not expected that there would be strong associations since the traits used (growth rate and weight) are polygenic.

RESULTS

Mean weights of homozygotes and heterozygotes from seven strains/crosses are given in Tables 36 and 37 for data from fish reared at 7°C and 15°C respectively. A summary of the trends evident in these tables is presented in Table 38. There were 13 cases in which homozygotes were heavier than heterozygotes for the eight loci examined among fish reared at 7°C. Out of the 13 cases only one was marginally significant ($P<0.25$). Six cases showed homozygotes equal in weight to heterozygotes while 29 cases showed heterozygotes weighing heavier, out of which ten were significant ($P<0.25$). On the other hand, there were 21 cases in which homozygotes had greater mean weights among fish reared at 15°C (Table 38). Out of the 21 cases, 4 were significant ($P<0.25$). Four cases had homozygotes equal in weight to heterozygotes. Among the 28 cases in which heterozygotes recorded a higher mean weight, 9 were significant ($P<0.25$).

As presented in Table 39, there was a negative correlation between homozygosity levels at ten loci and weight among fish reared at 7°C ($r=-0.08$, $P=0.0004$, $n=1806$) and at 15°C ($r=-0.08$, $P=0.0017$, $n=1695$). At both temperatures, the correlation between

heterozygosity levels and fish weight was positive ($r=0.09$, $P=0.0002$, $n=1806$ at $7^{o}C$ and $r=0.08$, $P=0.0015$, $n=1695$ at $15^{o}C$).

Regression analysis revealed the model significant with $F = 7.02$ ($P = 0.0009$) and $F = 5.23$ ($P = 0.0054$) at $7^{o}C$ and $15^{o}C$ respectively (Table 39). The test of equality of the regression constants b and c (i.e. weighting due to homozygous and heterozygous condition vz. model 19) revealed they were significantly different at either temperature ($F=14.04$, $P<0.001$ at $7^{o}C$ and $F=10.46$, $P<0.01$ at $15^{o}C$). At both temperatures the constant b was negative although nosignificant while the coefficient c was positive at both temperatures and marginally significantly different from zero ($P<0.25$) at $7^{o}C$ but nonsignificant at $15^{o}C$.

R-square values for single allele regression models using weight as a dependent variable are presented in Table 41. There was some evidence of interaction with temperature at some of the loci. For instance, while Gpi-2 (110) had an R-square of zero at $7^{o}C$, the value increased to 43.07% among fish raised at $15^{o}C$. Meanwhile, at $15^{o}C$ the R-squares for alleles Gpi-2 (100) and (105) dropped from 14.08 and 9.92 to 12.76 and 3.96 respectively. Mdh-1 (100), Mdh-1 (105)

Table 36. Mean weights (to the nearest gram) of homozygotes (M) and heterozygotes (H) for all the strains for fish reared at 7°C.

	LAS		MAN		TAG		LT		LM		ML		MT	
LOCUS	M	H	M	H	M	H	M	H	M	H	M	H	M	H
Idh-3,4	30	28	16	16	22	-	31	31	31	17	28	28	21	21
Mdh-1	27	28	22	18	33a	20a	29	34	24	24	24c	33c	20	19
Mdh-2	29	24	22	17	31	26	30	24	23[b]	43[b]	25	34	-	21
Mdh-3,4	29	27	22	19	31	35	29	31	24	25	24c	34c	21	12
Mpi-1	22	33	10c	24c	-	30	-	27	12a	24a	-	24	-	20
Mpi-2	22	28	20	21	28c	35c	30	31	23	25	16c	34c	13a	22a
Pgm-2	26	28	24	24	20	21	33	31	30	32	-	30	-	27
Sod-1	25a	29a	18	21	28c	35c	29	32	25	26	29	30	16	20
overall	26c	28c	19	20	28	29	30	30	24	27	24a	31a	18	20

pairs of means with the same letter are significantly different

a...P<0.05 b... P<0.10 c... P<0.25

Table 37. Mean weights (to the nearest gram) of homozygotes and heterozygotes for all the strains for fish reared at 15°C.

	LAS		MAN		TAG		LT		LM		ML		MT	
LOCUS	M	H	M	H	M	H	M	H	M	H	M	H	M	H
Idh-3,4	63	71	56	56	67	67	70	58	67b	64b	58	69	59	43
Mdh-1	62a	63a	57	56	79c	116c	75	66	54	59	60	75	65	75
Mdh-2	57	65	57	53	88a	52a	67	72	56	57	66	57	56	72
Mdh-3,4	59	69	50b	62b	89	80	73c	76c	57	53	70	59	58	58
Mpi-1	-	71	83	57	-	93	82b	74b	39a	61a	69	84	-	68
Mpi-2	47c	63c	56	56	77	84	66	71	60	58	73	72	56c	69c
Pgm-2	67	60	54	46	80	61	53	68	69	51	69	79	69	62
Sod-1	58c	64c	53	58	62	86	59	72	64c	54c	70	66	70	59
Overall	59a	66a	58	56	77	80	68	70	58	57	69	70	62	63

pairs of means with the same letter are significantly different

a...P<0.05 b... P<0.10 c... P<0.25

Table 38. Summary of Tables 35 and 36 showing number of strains with homozygotes either heavier than (-1), equal in weight to (0) or lighter than heterozygotes (+1).

	7°C			15°C		
	-1	0	+1	-1	0	+1
LOCUS						
Idh-3,4	2	4	0	2	2	3
Mdh-1	3	1	3	2	0	5
Mdh-2	4	0	2	3	0	4
Mdh-3,4	3	0	4	2	1	4
Mpi-1	0	0	3	2	0	2
Mpi-2	0	0	7	2	1	4
Pgm-2	0	1	4	5	0	2
Sod-1	1	0	6	3	0	4
Total	13	6	29	21	4	28

with Mdh-1 (110) having the highest R-square at the locus. An improvement of R-square from 0.06% at 7°C to 0.49% at 15°C was observed for the hybrid allele Mdh-2 (105) while Mdh-2 (110) R-square dropped from 0.34% at 7°C to 0.05% at 15°C. Mdh-2 (100) had R-squares of zero at both temperatures. Mpi-1 (110) and Mpi-2 (110) also had R-squares of zero at both temperatures. The hybrid allele Sod-1 (105) maintained first rank at both temperatures while Sod-1 (100) and Sod-1 (110) traded second and third position at 15°C. Sod-1 (90) had the lowest R-square at this locus.

Diallel analysis

As evidenced from Table 42, the degree of heterozygosity was significantly different for Glud-1 (P=0.0027) and Gpi-2 (P=0.0161) in the four breeds i.e. pure Mount Lassen, pure Manx, hybrid Mount Lassen X Manx and reciprocal Manx X Mount Lassen but nonsignificant for Idh-3,4 and Sod-1. "Heterosis" for heterozygosity was significant for Glud-1 (P=0.0004) and Gpi-2 (P=0.0035) and again non-significant for Idh-3,4 and Sod-1. Line direct effect was marginally significant at the 25% level for Glud-1 and Gpi-2 but were non-significant for the other loci (P=0.88). At the four loci overall, breed effect and heterosis were

significant. Maternal effects were negligible in all cases. Positive but non-significant correlations were found between growth rate and heterozygosity at Gpi-2, Glud-1, and Idh-3,4 (Table 43). On the other hand, the correlations between the relative variance index and heterozygosity were negative and significant for Glud-1 ($r=-0.41$ $P=0.15$), Gpi-2 ($r=-0.53$ $P=0.12$) and Sod-1 ($r=-0.51$ $P=0.04$) while for Idh-3,4 it was small, non-significant and positive ($r=0.05$). All correlations at the four loci between condition factor and heterozygosity were negative, that for Glud-1 being the only one marginally significant ($r=-0.33$, $P=0.15$).

Table 39. Pearson's correlation coefficients among homozygotes (M), heterozygotes (H) and weight of rainbow trout fish reared at 7°C and 15°C.

	7°C			15°C		
	M	H	WT	M	H	WT
M	1	-0.88[1]	-0.08	1	-0.91	-0.08
		0.0001	0.0004		0.0001	0.0017
H		1	0.09		1	0.08
			0.0002			0.0015
WT			1			1

1. correlation coefficients in the first row of each comparison and probabilities that the coefficients are zero in the second row.

Table 40. Regression analysis (model 19) statistics obtained for 8 loci at each of the temperatures.

SOURCE	DF	MEAN SQUARE	F VALUE	PROB>F
7°C				
Model	2	1050.02	7.02	0.0009
Error	1803	149.60		
Dep. Mean	26.4			
15°C				
Model	2	2825.92	5.23	0.0054
Error	1692	540.15		
Dep. Mean	64.6			

Parameter Estimates

	7°C	15°C
Intercept	26.36±1.14	64.66±2.60
b	-0.84±1.20	-1.73±2.71
c	1.45±1.24 (P<0.25)	2.02±2.74
H_o:b-c = 0	F=14.04 (P<0.001)	F=10.46 (P<0.01)

Table 41. R-squares (%) due to individual alleles at ten loci for fish reared at 7°C and 15°C.

ALLELES			90	95	100	105	110
LOCUS	°C	(n)					
Gpi-2	7	16	-	-	14.08	9.92	0
	15	10	-	-	12.76	3.96	43.07
Idh-3,4	7	109	-	-	0.53	0.16	0
	15	166	-	-	0.03	0	1.87
Mdh-1	7	250	-	-	0	0.06	0.09
	15	187	-	-	0.51	2.26	2.73
Mdh-2	7	267	-	-	0	0.06	0.34
	15	215	-	-	0	0.49	0.05
Mdh-3,4	7	280	-	-	0.01	0.01	0.07
	15	222	-	-	0.24	0	0.33
Mpi-1	7	32	-	-	4.33	-	0
	15	34	-	-	1.43	-	0
Mpi-2	7	363	-	-	3.77	-	0
	15	336	-	-	1.47	-	0
Pgm-2	7	39	-	-	2.13	3.41	0
	15	60	-	-	0.72	0.58	0
Sod-1	7	442	0.16	0.19	0.69	0.88	0.31
	15	447	0.21	0.01	0.69	1.64	1.31

Table 42. Analysis of variance for genetic effects in heterozygosity levels at four electrophoretic loci.

LOCUS		BREED	HETEROSIS	MATERNAL	DIRECT
		Df			
		3	1	1	1
Glud-1	F	8.47	23.44	0.24	1.63
	P	0.0027	0.0004	0.64	0.23
Gpi-2	F	5.16	13.17	0.43	2.05
	P	0.0161	0.0035	0.52	0.18
Idh-2	F	1.27	2.88	0.01	0.00
	P	0.33	0.12	0.45	0.88
Sod-1	F	0.16	0.15	0.26	0.02
	P	0.92	0.71	0.62	0.88
Overall	F	5.24	14.19	0.38	1.47
	P	0.0015	0.0027	0.55	0.25

Table 43. Pearson's correlation coefficients between growth rate (G), relative variance index (RVI), condition factor (K) with level of heterozygosity at four loci.

	Glud-1	Idh-3,4	Gpi-2	Sod-1	OVERALL
G vs H	0.26	0.19	0.29	-0.11	0.10
RVI vs H	-0.41[a]	0.05	-0.53[c]	-0.51[c]	-0.27[c]
K vs H	-0.33[a]	-0.003	-0.26	-0.09	-0.13

a=P<0.25

b=P<0.10

c=P<0.05

DISCUSSION

Although the multiple pairwise statistical tests presented in Tables 36 and 37 and summarized in 38 gave an indication of an association between the level of heterozygosity and weight of fish they may sometimes lead to significance by chance alone and should be interpreted with caution (Zouros and Foltz, 1987). Supporting this association, however, are the correlation and regression analysis which showed that heterozygotes had an overall significant superiority in weight over homozygotes. The interpretation of this comparison was not confounded by age or environment by virtue of the experimental design. In the test by test comparisons there were more than twice the number of comparisons in which heterozygotes had a higher mean weight than that of homozygotes at 7°C (29:13) while at 15°C the comparisons were about equal (28:21). Further to this, comparisons of the weightings b (due to homozygous condition) and c (due to heterozygous condition) revealed higher significance at the lower temperature just as the correlation of 0.08 at both temperatures was significant but much more so at the lower temperature. From these observations, it was implied that heterozygosity was "more important" at the lower temperature. Utter et al. (1974) reported an

excess of heterozygous individuals for PGM and AGPD in deep water collections of Pacific perch, Sebastes alutus, relative to those taken from shallow waters. Utter et al. (1974) interpreted their observations as an indication of selective forces acting on the two loci at greater depths. Powers and Place (1978) found clinal gene frequencies changes in Fundulus heteroclitus correlated with a steep thermal gradient in mean temperature including a correlation between maximum gene diversity at 4 loci and annual temperature fluctuation. Zimmerman et al. (1981) found that heterozygosity levels in fish within 57 km of the cold water discharge of a hydroelectric dam was correlated with fluctuations in water temperature at sampling stations. In support of Levins (1968), Bryant (1974) and Gillespie (1974), Zimmerman et al. (1981) concluded that the degree of genetic polymorphism represented an adaptive response to environmental variability and that increased level of temporal environmental variability should be reflected by increased levels of genic heterogeneity.

Brett and Groves (1979) reported that the optimal temperature for growth of rainbow trout is around 15°C. If 15°C is the optimal temperature, a more frequent tendency of heterozygote advantage among families of

rainbow trout reared at 7°C could be an indirect revelation that heterozygote fitness is more pronounced at the sub-optimal temperature. Although not a safe extension (comparing hatchery with wild habitats), one might speculate that my observations are a corollary to Zimmerman's et al. (1981) and Utter's et al. (1974) observations, i.e. if fish were kept separate for generations at 7°C and 15°C the level of heterozygosity would eventually be higher at the lower temperature as the larger, more fit heterozygotes would get to breed if it was assumed that large size was a fitness trait that bestowed an advantage on individuals during mate acquisition. In cases where workers such as Mitton (1982) have failed to demonstrate an association of heterozygosity with growth consistently (in natural salamander populations) one might conclude that perhaps an unnoticed change in factors contributing to heterozygote advantage have been involved. Therefore only at times when such factors were operative would heterozygote advantage be detectable. This interpretation is based on my observation that heterozygous rainbow trout in my experiment were significantly heavier more often at the sub-optimal temperature of 7°C. This may also explain why the phenomenon is not universal, a situation which has led to conflicting reports.

Based on my results I speculate that selection for large size rainbow trout would improve heterozygosity levels if it was conducted under sub-optimal temperatures. Once selected, such fish would then be grown at a higher temperature for faster growth, with heterozygosity as an added advantage, since, superiority among heterozygous individuals was also significant at 15°C.

The R-squares associated with the alleles in the regression equations were not given much weight in the my biological interpretations. However, it should be noted that while all heterozygote-homozygote comparisons involving the Mpi-1 and Mpi-2 loci showed heterozygotes were always heavier, the R-squares for the alleles Mpi-1 (110) and Mpi-2 (110) were zero at both temperatures. This implied that these alleles played a comparatively small role in determining the weight of individuals in which they were homozygous but in a heterozygous condition they contributed towards overdominance. This could be a chance event.

An indication that there could be a shift in the relative importance of alleles with a change in temperature was observed in the re-ranking of the magnitude of R-squares of for instance, Gpi-1 alleles.

It was conjectured that loci with the least influence on weight of fish could not display such re-ranking in allelic importance (Mdh-1 could be such a locus). Koljonen (1983) thought that the Me-1 locus had some link with growth.

Overall higher heterozygosity levels at four loci among the hybrids in the diallel was indirect evidence that heterozygosity was associated with growth rate since higher heterozygosity corresponded to higher growth rate in the hybrids. Although heterozygosity levels were collectively higher in the pure Manx families than in the pure Mount Lassen ones, growth rate was higher in the latter. Mitton and Grant (1984) pointed out that comparisons of this nature should be restricted to closely related breeding groups. Considering that the two strains, Mount Lassen and Manx are isolated from each other, this observation is not surprising as loci that play a greater role in growth performance in the pure strains might not have been sampled. In Chapter one for instance, it was shown that the Mount Lassen strain was significantly superior to Manx in terms of growth performance.

Relative variance index, a measure of comparative variance over weight range was negatively correlated to

heterozygosity levels and agreed with similar reports by Koljonen (1986) in ten strains of rainbow trout; Leary et al. (1984) in the salmonid species, S gairdneri, S clarki lewisi and Salvelinus fontinalis. Mitton (1978) also reported lower variance in heterozygous fish of the species Fundulus heteroclitus, having found 22 out of 30 tests involving individual enzyme loci had heterozygotes recording lower variances. Further still King (1985) found that 49 out of the 70 comparisons he made between homozygous and heterozygous herring (Clupea harengus) showed low variances associated with heterozygotes. As pointed out earlier, there are as many workers who have found no associations at all between character variance and heterozygosity at enzyme loci (e.g. McAndrew, 1982; Yoshiyama and Sassaman, 1983; Beacham and Withler, 1985a, 1985b; Ryman et al., 1984). These discrepancies could perhaps be explained using the same argument advanced above to explain shifts in weighting of heterozygote advantage with temperature (environment). If environment plays an important role in these associations, conflicting reports on the same species when involving the same character could partly be reconciled. For example, Ryman et al., (1984) found no association between variance in number of vertebrae in the herring (C. harengus) while King (1985) reported a

negative association between variance in number of vertebrae and heterozygosity in another population of the same species.

One of the main values of electrophoretic studies of this nature lies in the possibility that a certain electromorph highly correlated with a trait of economic importance such as growth rate might be identified. Once such an electromorph is identified then it could be used as a marker for the trait in selection programs. It is obvious that for polygenic traits such as growth, several markers would have to be used in conjunction. My results only indicate that Mpi-1, Mpi-2, Gpi-1, Glud-1 and to a lesser extent, Mdh-2 were somewhat correlated with weight/growth of fish. Furthermore, Koljonen (1986) speculated that Me-1 influenced growth in some way. In the light of the present evidence it would appear that combinations of such markers will be governed by the environment e.g. temperature under which fish have to be cultured. At the allelic level, cases like those seen for Mpi-1 (110) and Mpi-2 (110), if consistent, would indicate that an aquaculturist would gain if spawning involved parents that would not only yield highly heterozygous individuals but individuals heterozygous at the marker loci.

Condition factor is usually employed to estimate "well-being" of fish (Ricker, 1975; Bagenal and Tesch, 1968; Le Cren, 1951). From the formula given in chapter one, the condition factor can be considered as the ratio of fish weight to length. "Good" condition then simply implies that fish are heavier for their length. Growth rate was positively correlated with the condition factor (Chapter 1). The negative correlation between heterozygosity and condition factor was therefore inconsistent with expectations and perhaps on reflection an error in measurement. In chapter 2 it was also observed that hybrids in the diallel crosses depicted a depression in condition although heterosis for growth was recorded. It was not clear as to the actual relationship since condition may also reflect stomach fullness. It was concluded that because of its unpredictability, condition was not a consistent trait that could play a role in fitness.

Chapter 5

Effect of competition on growth among pure and hybrid strains of rainbow trout (Salmo gairdneri Richardson) reared in the same tank.

ABSTRACT

Strains of rainbow trout, Salmo gairdneri, together with their hybrids were found to significantly affect each others growth rates when reared in the same tank. This has implications on experimental designs in which different strains, or even species of fish are usually marked and reared together in order to minimize tank differences. Heterosis for growth among hybrids was depressed when they were reared in combination with other strains.

INTRODUCTION

There are many examples in which different strains of fish (especially salmonids) have been reared in one tank for purposes of reducing tank effects or limiting number of tank requirements in large factorial experimental designs. Although such experimental designs appear sound, one major and often overlooked assumption is the possibility for interactions among or between strains in the tank which could introduce unquantifiable errors in the interpretations of the results. Whereas some workers concerned with tank effects usually mark different strains and group them in the same tanks (e.g. Beacham, 1988; McKay et al., 1986; Sadler et al., 1986), others, wary of unquantifiable interactions among genotypes and effects from marker devices such as tags, study fish in separate tanks (e.g. Uraiwan, 1982; Refstie, 1980). Rearing different strains of rainbow trout in the same tank could be likened to polyculture, a practice ordinarily applied in extensive or semi-intensive culture of fishes like carp species that exploit different trophic levels in ponds (Bardach et al., 1972). In intensive culture, however, individuals of one strain might compete for the only available food more effectively and thus directly suppress the growth

of members of the other. Consequently, if results from two different experiments in which one researcher employed the tank sharing approach and the other reared fish in separate tanks but under comparable conditions were to be meaningfully compared, it should first be demonstrated that inter-strain interactions in a tank were insignificant. It has been documented that fish in a tank compete for food amongst themselves even when they are of the same strain (Jobling, 1983; Jobling and Wandsvik, 1983; Yamagishi, 1962; Yamagishi et al., 1974). The purpose of this experiment was to investigate whether there were significant interactions among four genotypes of rainbow trout strains when reared in groups in one tank. The ultimate aim was to reduce tank requirement when running large factorial designs for examining genotype by environment interactions in case inter-strain interactions were not important.

MATERIALS AND METHODS

Two rainbow trout strains, Mount Lassen and Manx together with their hybrid were hot-wire branded for identification and left to acclimate at 12°C for one week. They were progeny of two Mount Lassen males and two Manx males crossed in a diallel mating design with two females of each strain simultaneously by apportioning sex products. Fertilized eggs were incubated in separate plastic jars at 7°C. On hatching they were transferred into 16 separate 60 litre tanks at the same temperature. At a mean weight of about 20 g the 16 families were combined into four groups comprising pure Mount Lassen, pure Manx, Mount Lassen X Manx cross (LASMAN, male given first) and the reciprocal, Manx X Mount Lassen cross (MANLAS). Three levels of treatment (i.e. degree of tank sharing) were applied as: [1] strains/hybrids reared alone in a tank, [2] strain/hybrid sharing a tank with one other and [3] all strains/hybrids reared in one tank. All fish from each group were randomly assigned to treatments, with each of the 60 litre tanks holding a total of 60 fish either consisting of one group or two or four groups in equal proportions (Table 44). Fish were fed twice a day on Martin's Ontario Fish Meal following a standard ration table (Hilton and Slinger,

1981). The water temperature was maintained at 12°C ± 0.5°C whereas oxygen saturation was never below 80% with a flow-through of about 4 to 5 liters per minute. All fish in a tank were individually weighed every after 21 days by use of a computerized program that utilized an electronic Mettler Balance connected to a Spark Portable Computer, recording both the weight and the brand. The experiment was terminated after a growth period of 63 days and data uploaded through a modem onto the main University Computer for analysis.

Data Analysis

The statistical model for analyzing for overall treatment effects was:

$$Y_{ijk} = \mu + S_i + T_j + t(S\ T)_{k(ij)} + bW_{ijk} + bW_{ijk}S_i + bW_{ijk} + e_{ijk} \qquad [20]$$

where Y_{ijk} = specific growth rate in the k^{th} tank holding the i^{th} strain under the j^{th} treatment

μ = overall mean

S_i = strain effect

Table 44. Rainbow trout strain/hybrid combinations in tanks.

TANK	MT.LASSEN	MANX	LASMAN	MANLAS	TOTAL
1	-	30	30	-	60
2	-	60	-	-	60
3	-	30	30	-	60
4	60	-	-	-	60
5	-	-	-	60	60
6	-	60	-	-	60
7	30	30	-	-	60
8	30	-	-	30	60
9	-	-	30	30	60
10	60	-	-	-	60
11	30	-	-	30	60
12	-	-	-	60	60
13	-	-	60	-	60
14	-	-	30	30	60
15	30	30	-	-	60
16	30	-	30	-	60
17	-	30	-	30	60
18	-	30	-	30	60
19	30	-	30	-	60
20	-	-	60	-	60
21	15	15	15	15	60
22	15	15	15	15	60
23	15	15	15	15	60
24	15	15	15	15	60

T_j = treatment effect

$t(S\ T)_{k(ij)}$ = tank within strain within treatment effect

bW_{ijk} = specific growth rate-weight regression relationship

$bW_{ijk}S_i$ = interaction of the regression of specific growth rate relationship with weight among strains

$bW_{ijk}T_j$ = interaction of the regression of specific growth rate relationship with weight among treatments

e_{ijk} = error

i=1 to 4; j=1 to 3; k=1 to 12 (all effects considered random).

The statistical model used to analyze data within a strain was:

$$Y_{ij} = \mu + T_i + t(T)_{j(i)} + e_{ij} \qquad [21]$$

where Y_{ij} = specific growth rate of fish in the j^{th} tank under the i^{th} treatment

μ = overall mean

T_i = treatment effect

$t(T)_{j(i)}$ = tank within treatment effect

e_{ij} = error

i=1 to 3; j=1 to 2, 4, 6 (all effects considered random).

Appropriate pairwise comparisons were performed within groups under different treatments to detect any changes in performance based either on a particular group as a sharing partner or on the degree of sharing.

RESULTS

As shown in Table 45, overall ANOVA (model 20) indicated that treatment effect wwas significant ($P<0.05$). A pairwise comparison of treatments revealed that competition in a one to one situation (Treatment 2) was significantly different ($P<0.01$) from an all situation (treatment 3).

Specific growth rates in each tank, treatment designation and ANOVA F-tests for the respective strains (Model 21) are given in Table 46. Treatment effects were significant for Manx ($P<0.05$) and LASMAN ($P<0.01$). Fig 12 in which deviations of specific growth rates were plotted against competing groups was used for a more detailed examination of trends in the interactions. Examination of the Mount Lassen profile in Fig 12 showed that this strain had a higher growth rate in competition with each of the other strains than when raised alone, but like all others declined in growth when all strains were in the same tank. The Manx strain had highest growth rate on its own which declined in all competition situations (Fig 12, Manx profile). The hybrid LASMAN showed the greatest decline in growth from 1.39% per day when grown alone to 1.02% per day in competition with all the other

three strains (27% decline). On the other hand, the reciprocal hybrid MANLAS had the lowest overall decline from 1.40% on its own to 1.31% per day in competition with all the strains in the same tank (6% decline). In fact its growth rate in competition with LASMAN was comparable to that depicted in competition with all strains in one tank. Poor competition by other strains against the Mount Lassen strain was evident in all profiles as growth in the respective strains dropped in all the cases they were paired with it.

Heterosis for growth, due to hybrid vigour, estimated as (LASMAN + MANLAS) - (LAS + MAN) was 6% when fish were reared in separate genotypes, 4% in paired groups and 2% in treatment three (all groups together).

Table 45. Analysis of variance for overall treatment effects on specific growth rate (Model 20).

Source	DF	F
Strain	3	0.17
Treatment	2	3.86*
Weight	1	18.49
Weight x Str	3	0.45
Weight x TRT	2	4.72

* significant ($P<0.05$)

Table 46. Specific growth rates in each of the tanks, treatment designation and mean specific growth rates for each group under the respective treatments.

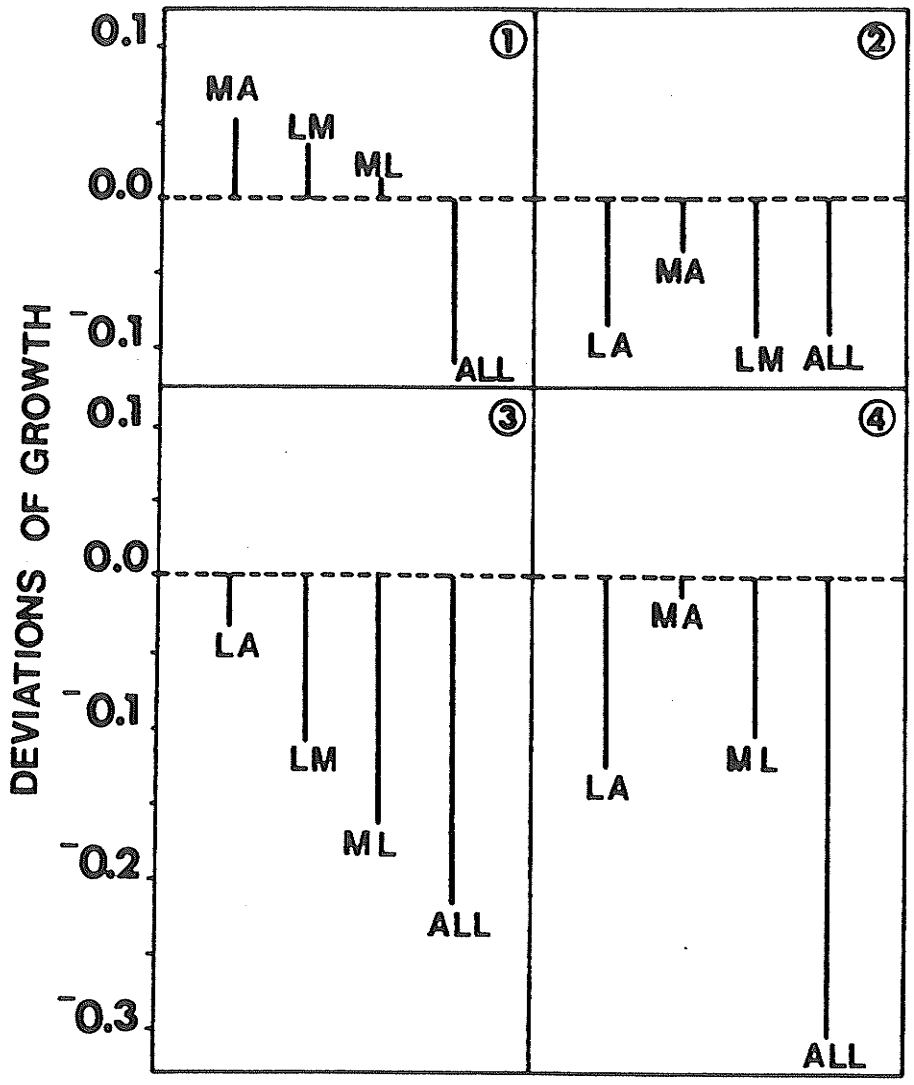
Tank	Mt.Lassen	Manx	LASMAN	MANLAS	TRT
1	-	1.18	1.38	-	2
2	-	1.30	-	-	1
3	-	1.32	1.38	-	1
4	1.29	-	-	-	1
5	-	-	-	1.51	1
6	-	1.40	-	-	1
7	1.33	1.24	-	-	2
8	1.33	-	-	1.35	2
9	-	-	1.26	1.37	2
10	1.25	-	-	-	1
11	1.23	-	-	1.28	2
12	-	-	-	1.28	1
13	-	-	1.39	-	1
14	-	-	1.29	1.24	2
15	1.31	1.39	-	-	2
16	1.29	-	1.16	-	2
17	-	1.13	-	1.37	2
18	-	1.20	-	1.37	2
19	1.30	-	1.37	-	2
20	-	-	1.38	-	1
21	1.51	1.44	1.01	1.83	3
22	1.07	1.29	1.22	1.30	3
23	0.97	0.94	0.89	1.09	3
24	1.09	0.80	0.96	1.00	3
Means					
TRT1	1.27±0.03	1.35±0.07	1.39±0.001	1.40±0.16	
TRT2	1.30±0.04	1.24±0.10	1.31±0.09	1.33±0.06	
TRT3	1.16±0.24	1.12±0.30	1.02±0.14	1.31±0.37	
ANOVA					
F	2.36	2.95*	4.88**	0.59	

*=P<0.05; **=P<0.01

Fig 12. Deviations of specific growth rate (from own mean) among rainbow trout strains/hybrids reared in combination with each other (and all together).

1. Mount Lassen
2. MANLAS3
3. Manx
4. LASMAN

(LA=Mount Lassen, MA=Manx, LM=LASMAN, ML=MANLAS).


DEVIATIONS OF GROWTH
0.1
0.0
-0.1
-0.2
-0.3
①
②
③
④
MA
LM
ML
ALL
LA
MA
LM
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LA
LM
ML
ALL
LA
MA
ML
ALL

DISCUSSION

Normal polyculture as practised in semi-intensive and extensive fish farming, for example by the Chinese in carp species, is usually based on the ecological principle that each species exploits its own food niche in the pond, with virtually no interference from the others stocked in the same pond (Stickner, 1979). A particular carp species would be expected to contend only with intra-species competition for resources. Because food is artificially offered in intensive aquaculture systems, monoculture is usually the rule. Introduction of two or more species/strains in an intensive culture situation could result in competition for resources such as food and space. Competition is used in the context of Milne,s (1961) definition in which "it is the endeavour of two (or more) animals to gain a particular requirement, or to gain the measure each wants from the supply of a requirement, when that supply is not sufficient for both (or all)". In the present experiment it could be assumed that the most critical requirement affecting growth was food since standard ration tables were used.

The results obtained here showed that rainbow trout strains indeed influenced each others growth

performance when reared in the same tank. There was an indication that whereas all the other breeds showed depressed growth, the Mount Lassen strain grew better in combination with each of the others than when it was on its own in a tank. A possible explanation for this was that intra-strain competition for food in this strain outweighed inter-strain competition. In other words competition among Mount Lassen strain individuals was more damaging to growth performance than against individuals from other strains. The Manx strain on the other hand lost out in all combinations just as it had least effect on the growth of each of the other groups. The implication of these observations was that experimental designs in which strains of rainbow trout are tagged and reared together in order to minimize tank effect could give results that include unaccountable interactions. The severity of growth depression increased with the number of groups of fish in a tank for all the groups presumably because in that situation each of the groups was overwhelmed by the other three. Both intra- and inter-species competition have been widely documented by workers in the field of ecology. Beverton (1962) for example attributed limits in larval populations of plaice to intra-specific competition for food. In the present experiment there could have been other less obvious factors that led to

depression of growth observed in the breeds but which could not be identified. The crucial finding, however, was that strains of rainbow trout did influence each others growth rates when reared in the same tank. If food was the major factor it would be expected that treatment effects could have been milder if the investigation was conducted with the use of either excess ration or feed to satiation. This requires another experiment for confirmation.

Hybrid vigour or heterosis in animal production is usually harnessed through crossbreeding (Pirchner, 1983). The trend observed in this study indicated that mixing of hybrids in which heterosis is expected may reduce or wipe out heterosis altogether as observed in the LASMAN hybrid under treatment three. This has the implication that a crossbreeding experiment may fail to demonstrate heterosis for growth if hybrids are reared together with other groups.

Although I have attributed depression in growth rate to competition for food between and among genotypic groups as units, the actual factors leading to these observations may not be that straight forward. Discussion beyond this point would only lead to speculation, however, as the experimental design used

here is limited and cannot be used to isolate any other factors.

GENERAL DISCUSSION

Although numerous studies on genetically related aspects of salmonid culture exist, a closer examination shows that only a few have approached the problem with questions normally asked by classical plant and livestock geneticists. This situation is exemplified by the lack of defined lines/pedigrees at fish hatcheries. The present technology of sperm preservation which has not been fully exploited in fish breeding programs could become important in setting up pedigrees. Presently, almost all genetically related studies have been done on fresh and genetically undefined stocks of fish. My work also suffers from this shortcoming although this information will be of value as the families developed in my study could be used as a base for further investigation.

With genetically defined stocks, methods of evaluation of performance will not only need to be standardized (see Chapter 1) but also will need to be conducted over the relevant production cycles of specific systems. Gjedrem (1983) echoed this concern by recommending that evaluation be conducted close to marketing time.

In my experiments, I examined four traits: growth rate, condition factor, weight variability and food conversion efficiency. McKay et al (1984) examined growth rate and condition in rainbow trout. Gjedrem (1983) recommended growth rate, food conversion efficiency, resistance to disease, meat quality and age at maturation as being of economic importance. Since the weight of a trait varies from region to region, by species and by culture systems, all the factors that influence the weight of a trait should be examined to enable clear identification of breeding goals. In cases where traits have non-antagonistic correlations such as food conversion efficiency and growth, the one easiest to determine should be used (Gjedrem, 1983, also see Chapter 1 this thesis).

There are very few studies in which growth performance has been examined in conjunction with biochemical variation. In those papers where low gene diversity is reported among hatchery reared stocks (e.g. Koljonen, 1986) it is unimportant unless such stocks are intended for supplemental stocking in the wild. Conversely, if hatchery stocks are under a sound breeding program that ensures a broad base, low gene diversity is simply an indication of the success of selection for the desired traits.

Selection experiments require a lot of space for holding different genetic stocks. Gjedrem (1983) discussed existing fish marking methods that would enable the rearing of different groups of fish together in one tank. From my work, it is not so much the merits and demerits of fish marking methods that is of primary importance in experimental work but the interactions between and among genotypes reared together in one tank. In Chapter 5 it was shown that the severity of depression in growth of various genotypes increased with the number of groups sharing a tank.

GENERAL CONCLUSIONS

1. Growth assessment experiments in fish should not only be conducted under the conditions for the intended production system but over a period long enough to cover the growth cycle of interest or conclusions made over brief periods may be of little commercial importance.

2. Data obtained here indicates that :

(i) the rate of increase in the variance of weight in relation to the increase in mean weight of a given brood of fish has a genetic component. The relative

variance index (RVI) proposed can consequently be used to rank lines with respect to this trait for genetic gain.

(ii) growth rate and rate of increase of variance in weight are negatively correlated so that selection for low RVI and fast growth is possible.

3. Heterosis for growth estimated at 6% in crosses between the Mount Lassen and Manx strains was significant, adding to the few reports of heterosis recorded in crosses between strains of rainbow trout.

4. Heterosis was shown to have a positive association with electrophoretically determined mean heterozygosity at 5 genetic loci.

5. Experimental designs that group different strains of rainbow trout in the same tank may yield misleading results if interactions between and among the strains are not taken into account.

6. Heterosis gained in crossbreeding programs may be reduced if crosses expected to show it are reared in a "polyculture situation" with other strains.

7. Inheritance studies indicated that two loci code for

liver superoxide dismutase in rainbow trout, a finding that is contrary to what is believed.

8. Although statistically nonsignificant, the similarity indices between Manx and Tagwerker were smallest just as Nei's genetic distance was greatest between them. Supporting these observations was the fact that crosses between Manx and Tagwerker were not as successful as between these strains and the Mount Lassen strain (only 1 out five crosses survived in numbers large enough to be replicated fully at 7°C and 15°C). This observation may indicate that the genetic distance does not have to be statistically significant in order to influence the outcome of a cross between two genetically different populations.

9. The three strains at the Rockwood Hatchery have not been previously electrophoretically characterized. The Tagwerker strain, with the lowest gene diversity, appears to be the most inbred.

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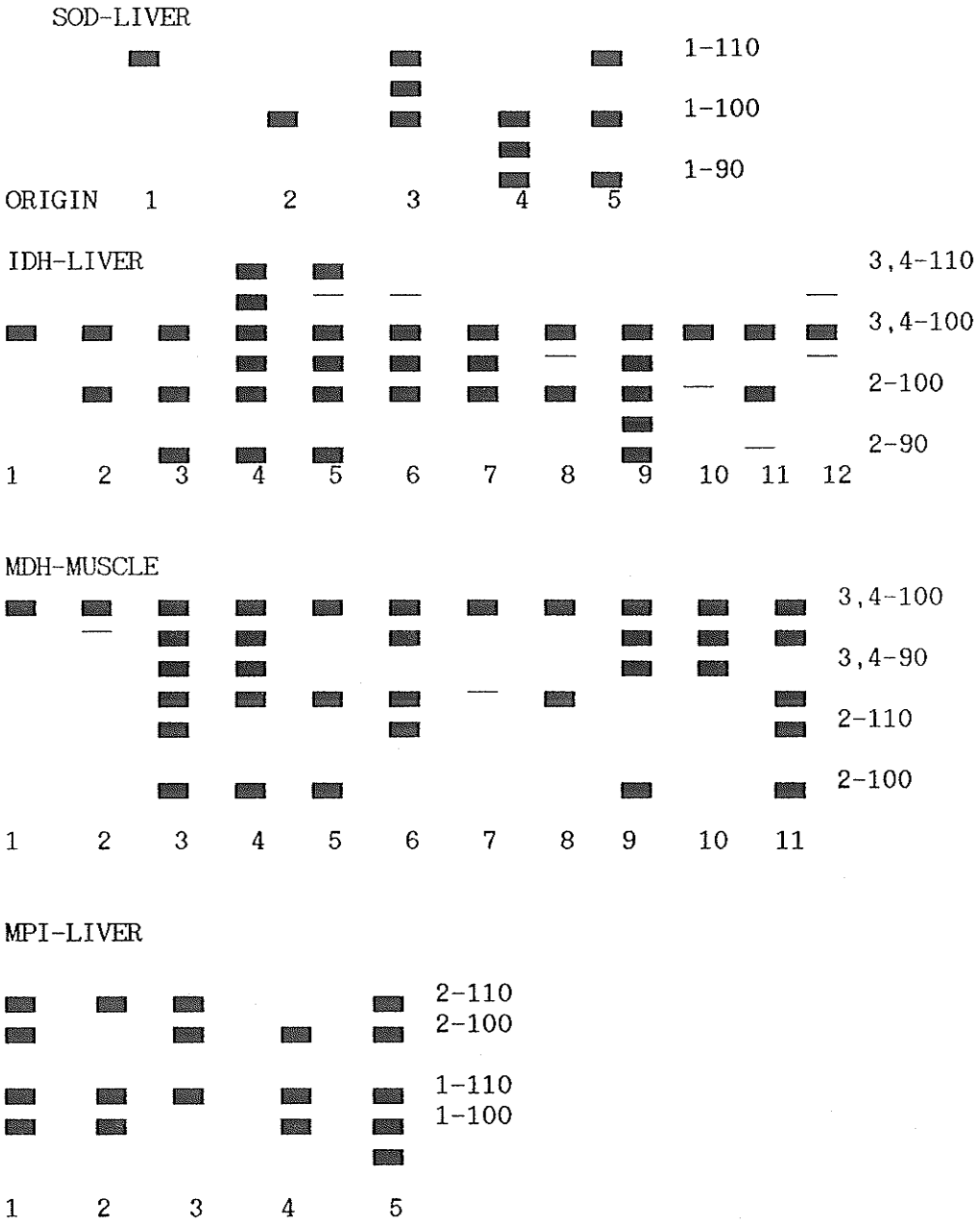
Appendix I. Enzyme systems examined, tissues used and remarks on the quality of resolution.

ENZYME	TISSUE	REMARK
Lactate dehydrogenase (LDH)	l,m,e	gr
Malate dehydrogenase (MDH)	l,m	gr
Mannose phosphate isomerase (MPI)	l	gr
Malate enzyme (ME)	l	gr
Adenylate kinase (AK)	m	pr
Hexokinase (KK)	l	pr
Glucose phosphate isomerase (GPI)	l,m,e	gr
Glucose dehydrogenase (GDH)	l,m	pr
Glutamate dehydrogenase (GLUD)	l	gr
Aldolase (ALD)	l	pr
Isocitrate dehydrogenase (IDH)	l	gr
Creatine kinase (CK)	l	pr
Alcohol dehydrogenase (ADH)	l	pr
Fumarate dehydratase (FH)	l	pr
Phosphoglucomutase (PGM)	l,m	gr
Esterases (ES)	l,m	gr
Peptidases (PEP)	l	pr
Superoxide dismutase (SOD)	l	gr

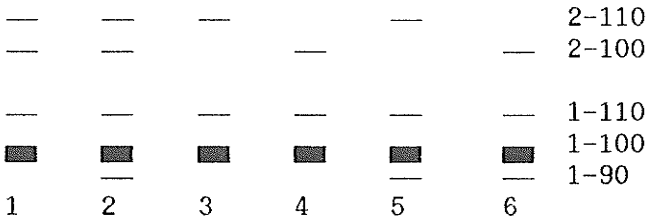
l- liver; m-muscle; e-eye; gr-good resolution; pr-poor resolution

Appendix II. Some of the isozyme patterns and designations of loci and alleles detected in rainbow trout.



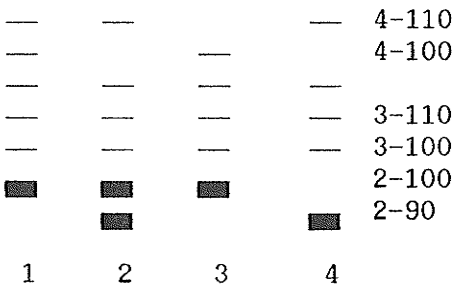
Appendix II cont'd.

PGM-LIVER

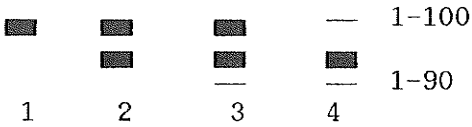


LDH-EYE

ES-LIVER



GLUD-LIVER



GPI-MUSCLE