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Quantitative genetics of body size and reproduction in rainbow trout (*Oncorhynchus mykiss*)

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Quantitative Genetics of Body Size and Reproduction

in Rainbow Trout (*Oncorhynchus mykiss*)

By

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Graduated (Northwestern Agriculture University) 1977
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Quantitative Genetics of Body Size and Reproduction in Rainbow Trout (Oncorhynchus mykiss)

ABSTRACT

Body size and developmental characters of 13 traits were measured with samples taken every two mo starting at 4 mo of age and continuing through their first complete reproduction cycle. The overall total sample size was 2570 progeny from 102 families sired by 46 males of two broodstocks. Three different diets, a standard commercial formulation and two with different crude protein and digestible energy levels, were fed to subgroups of fish of each stock from 12 to 24 mo of age. The standard commercial diet was fed to all fish during the first year.

Significant stock, sire and dam effects were observed for body weight, carcass weight, fork length, liver weight, gonad weight, oocyte diameter, condition factor, gonadosomatic index, and hepatosomatic index ($P<0.05$) for samples taken at most ages. Progeny of the RTH stock were lighter and shorter than those of the RTL stock before 18 mo of age, and exceeded RTL progeny thereafter. At 22 mo, the advantage of RTL over RTH was 208.71 gm in body weight and 2.07 cm in fork length. This response at the fixed age influenced by the fact that RTH females were 14 days younger at maturity (722 days vs 736 days) than RTL. Since there was little difference between stocks for post-spawning weight, difference observed at 22 mo reflected maturation. From individually freeze-branded fish, the RTH stock demonstrated a higher relative growth

rate than RTL from 14 to 20 mo of age, and lower growth rate than RTL as the fish approached sex maturity.

Diet effects were not significant on body weight, carcass weight and fork length until 22 mo. From samples at 22 and 24 mo, fish on diet H were significantly heavier and longer than fish on diet L in both stocks. Fish on diet C were intermediate. Diet significantly affected liver weight at 16, 18, 22 and 24 mo of age. Heavier liver weight was consistently observed from fish on diet H than diet L in both stocks. Larger oocyte diameters were also observed from fish on diet H than diet L at 14 and 16 mo in both stocks. Significant diet effect on volume of eggs retained at artificial spawning was also observed with the high protein-high energy diet causing on increase in retained eggs.

Estimated heritability for body size based on sire components ranged from 0.22±.20 to 0.39±.23 during first year. Observed heritability for liver weight and gonad weight varied from 0.20±.20 to 0.25±.20 while oocyte diameter had a heritability of about 0.35±.60. Heritabilities for plasma concentration alkali-labile protein phosphorus (ALPP) (0.71±.38 to 0.87±.52) and volume of eggs retained (0.34±.22) were also reported for the first time.

Data from a sample of 223 mature, artificially spawned 2-year-old rainbow trout females showed that eggs retained mainly in ovaries at the time of artificial spawning might reduce spawned egg volume by 15% . The data also suggested that sire had very significant effect on the volume of eggs their daughters retained at spawning.

DEDICATION

To my parents.

It is their love, understanding and support that

make my part of this work possible.

ACKNOWLEDGEMENT

I wish to express my special thanks to Dr. Graham Gall for his objective and patient guidance during the course of this study. I also sincerely thank Dr. Thomas Famula and Dr. Serge Doroshov for their critical suggestions and help during the analyses and the preparation of the final draft of this work.

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Table of Contents

List of Tables and Appendices

List of Figures

INTRODUCTION

Quantitative genetics and animal breeding theory have well-known history of tremendous success in the genetic improvement in the livestock and poultry industries. The correct identification of genetic and environmental factors, precise quantification of the possible impacts of these factors on economically important traits, and a clear understanding of the interrelationships among these traits were critical to the practical programs of genetic improvement. The present investigation was designed to explore the growth, development, and reproduction of rainbow trout, and to assess genetic and environmental effects on economic traits and the associations among the traits during one complete life cycle.

Two genetically diverse rainbow trout broodstocks were employed in the study. One originated from the Hot Creek State Hatchery, California Department of Fish and Game, the other from Mount Lassen Trout Farm, Red Bluff, California. Data collection was started at 4 months of age, and continued at two month intervals until 25 months of age, at which time all experimental fish had been either sampled or spawned.

At one year of age, three different diets containing different level of crude protein and digestible energy were

fed to a sample of fish from the two broodstocks. Meanwhile, about one-third of the fish were individually marked at one year by freeze-branding; these fish were used to examine individual growth rate.

Traits measured included body weight, carcass weight, fork length, relative growth rate, liver weight, gonad weight, oocyte diameter, alkali-labile protein phosphorus, pre-spawning weight, post-spawning weight, spawned egg volume, and retained eggs. Specific questions asked were:

1. Do stock, sex, sire and dam have any effect on performance? If so, at what age?

2. Does diet have any effect on performance and if so, what are the biological and genetic responses?

3. What is the nature of phenotypic associations among the traits, particularly the correlations of the body size and growth with developmental traits such as liver weight, gonad weight, oocyte diameter, and ALPP?

The presentation of results is organized into four distinct chapters addressing: overall performance during the first year of life; growth and sexual development during the second year of life; an analysis of ALPP as a indicator character for sexual development; and an analysis of body size, genetic effects on retained eggs; Chapter V provides a summary of all results.

Chapter 1.

A Genetic Analysis of Body Size, Carcass Weight and Developmental Characters Through One Year of Age for Hatchery Reared Rainbow Trout (Oncorhynchus mykiss)

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ABSTRACT

Data was obtained on body weight, carcass weight, fork length, liver weight, gonad weight, and oocyte diameter for two stocks of rainbow trout from 4 months to 12 months of age.

Multivariate analysis indicated that stocks, sire and dam had a significant influence on body weight, carcass weight, fork length, liver weight, gonad weight, oocyte diameter, condition factor, gonadosomatic index, and hepatosomatic index ($P < 0.05$) at most ages from 4 to 12 months.

However, from 6 to 12 mo, RTL was significantly larger than RTH in body weight, carcass weight, and fork length at all the ages except for fork length and carcass weight at 12 months of age ($P < 0.05$). By 12 mo, RTL had reached average body weight, carcass weight, and fork length of 221.1±5.08 g, 193.0±4,55 gm and 25,6±19 cm while RTH was 203.9±5.98 g, 180.3±5.35 g, and 25.4 cm respectively. It appeared that sex did not have an important influence on any of the three body size traits within the developmental period of 4 to 12 mo ($P > 0.05$).

Estimates of heritability for body size based on sire

components varied from 0.22 ± 0.20 to 0.39 ± 0.23. The heritability of liver weight and gonad weight appear to be between 0.20 ± 0.20 and 0.25 ± 0.20 while that for oocyte diameter was near 0.35 ± 0.60.

Genetic correlations between measures of body size were high and positive within ages (between age estimates could not be obtained) as were estimates of environmental correlations. At 12 months of age, liver weight appeared to be genetically correlated with body size but not environmentally. Estimates of genetic correlations for gonad weight and oocyte diameter were small and inconsistent.

INTRODUCTION

Studies of quantitative genetics of rainbow trout began in the early nineteen seventies (Kinghorn, 1983; Gall and Huang, 1988). To improve applications, effort should be placed on contemporary comparisons of stocks (Wilkins, 1981), to reveal variability at various physiological stages at which selection of some sort might take place, to estimate phenotypic and genetic parameters for populations which may possibly have different origins, and to understand economically important traits in order to design efficient genetic improvement programs. A number of researchers have concluded that large phenotypic and genetic variation exists for many economically important traits of rainbow trout (Gjedrem, 1975, 1983; Gall, 1983; Gall and Huang, 1988; Iwamoto et al., 1986; Kinghorn, 1983; Morkramer et al., 1985; Linder et al., 1983).

Klupp (1979) using full-sib data published estimates of heritability for body weight of rainbow trout at 68, 125, 184 and 334 d of age to be 0.73, 1.06, 0.74 and 0.82, respectively. Gall and Gross (1978) also using full-sib data, reported estimates of heritability for body weight at 120 and 271 d of age as 0.58 and 0.52 for two year spawning stocks. Refstie (1980) and Aulstad et al. (1972) reported

heritabilities of 0.06, 0.09 and 0.29 for body weight at 140, 150 and 280 d of age, respectively, based on half-sib data for a number of age 3 spawning populations while Chevassus (1976) estimated heritabilities for weight of rainbow trout fingerlings, which ranged from 0.05 to 0.18. For body length in rainbow trout, Refstie (1980) reported a heritability estimate of 0.20 for 140 d of age while Aulstad et al. (1972) reported estimates of 0.16 and 0.37 for 150 and 280 d of age, respectively.

Naevadal et al. (1979) observed phenotypic correlations between average body length at 6 and 12 mo of age to be 0.81 and 0.91 for two successive year-classes of fish. Springate and Bromage (1985), in a study on relationship of water hardened egg size and fry weight from 8 to 16 wk post-fertilization at two-week intervals, showed that there was a positive phenotypic correlation between the two traits at the time of first feeding (8 wk), but it decreased to zero by 4 weeks after the first feeding. Genetic correlation estimates between body weight and body length in rainbow trout are consistently close to unity at various ages as reviewed by Kinghorn (1983).

The present report represents the results of the first year of a comprehensive 2 year study on growth and development

of rainbow trout. Fixed effects of stock and sex as well as random genetic effects of sire and dam on body size (body weight, fork length and conditional factor), carcass weight and sex related, developmental characters (liver weight, gonad weight, oocyte diameter, hepatosomatic index and gonadosomatic index) were examined at two-month intervals from 4 months to 12 months of age.

MATERIALS AND METHODS

Experimental Animals:

Random samples of two stocks of rainbow trout (Oncorhynchus mykiss, formerly Salmo gairdneri) were obtained and designated as RTH and RTL. All RTH parental fish were spawned and eggs fertilized on September 13, 1984 at the California State Hatchery at Hot Creek and taken to the UC Davis Animal Science Department hatchery for incubation. The RTL parental fish were spawned on November 8, 1984 at the UC Davis fish hatchery where the eggs were immediately fertilized and placed into incubation trays. For both stocks, approximately 500 eggs per full-sib family were incubated in drip incubators at an average water temperature of 13 °C. Ages of fish were all defined as time from date of fertilization.

After hatching, the fry were moved to nursery troughs with a water flow of 11.5 l/min; average temperatures were 12.4 and 10.9 °C for RTH and RTL stocks, respectively due to season of transfer. At 4 mo of age, 55 random fish per full-sib family were moved outdoors to six round fiber-glass tanks (1.22 m diameter x 71 cm water depth) with water flows of 26 l/min; the average water temperature was 12.2 and 13.1 °C for RTH and RTL stocks, respectively. When the RTL fish were 7 mo and RTH fish 8 mo old, they were moved to circular fiber-glass tanks (3.66 m diameter x 66 cm) where they remained for the

duration of the experiment. Water temperature in these tanks fluctuated from 10.7 °C in the winter to 15.9 °C in the summer. The average water temperature was about 14 °C and the water flow was 55 l/min.

It is important to note that the progeny of each dam for a given sire were reared in separate tanks so that all sires had progeny within each tank and stock. This design did require that dam effects be confounded with tank effects. The fish were fed a standard starter diet followed by a commercial grower diet containing approximately 49% protein and 3214 Kcal/kg digestible energy. Fish were fed by hand four times a day and fed to satiation to maintain maximum growth during the first year; feeding rates were adjusted bi-weekly.

Data Collection:

Random samples of progeny were taken every 2 months, starting at 4 mo of age. The intention was to sample three fish per full-sib family at each sampling period. However, this balanced sampling scheme was not always realized due to limited numbers of fish in some families caused by mortalities during the experiment.

At each sampling time, fish were euthanized with tricaine methanesulfonate (MS 222) immediately bagged, and put on ice

for transport to the laboratory. Body weight (BW) and fork length (FL) were recorded at all ages beginning at 4 mo. Starting at 6 mo, each fish was dissected after BW and FL measurements were obtained, the contents of the coelomic cavity (stomach, liver, intestinal tract and mesentera) removed and carcass weight (CW) and liver weight (LW) recorded. Gonad weight (GW) was first obtained at 8 mo of age. BW and CW were recorded to the nearest 0.01 g before 10 mo of age and to the nearest 0.1 g at 10 and 12 mo. LW and GW were recorded to the nearest 0.001 g and FL to the 0.1 cm. Condition factor (CF), gonadosomatic (GSI) and hepatosomatic index (HSI) were defined as: $CF=100(BW/FL^3)$, $GSI=100(GW/CW)$ and $HSI=100(LW/CW)$.

After weighing, ovaries were preserved in 10% formalin and slides prepared following standard histological procedures. Oocyte diameter (DIAM) were measured from the slides by tracing the perimeter of cells possessing a visible nucleus, using a light microscope with a image-analysis tablet linked to a microcomputer; observations were recorded to the nearest 0.0001 mm. Oocyte diameter for ovaries of females sampled at 4 mo were obtained using the same equipment, but with slides prepared from a cross section of whole fish.

Data Analysis

A total of 1491 progeny were available for only 20 sires and 43 dams from the RTH stock and 26 sires and 59 dams from the RTL stock (Table 1). Due to the unbalanced nature of the data set, no unique analysis of variance model could be constructed. Therefore, both multivariate and univariate analyses of variance were carried out in an effort to achieve the best approximation of effects of factors in the model. Multivariate analysis of variance was also used to obtain estimates of partial correlation coefficients for all the traits of interest.

First, a saturated statistical model was fitted. None of the interaction terms were statistically significant (P>15% to P>20%) and thus, could be eliminated from the model. Consequently, the model used for the analysis was

$$Y_{ijklf} = \mu + T_i + S(T)_{ij} + D(TS)_{ijk} + X_l + e_{ijklf}$$

where Y_{ijklf} was the f^{th} observation value on an individual fish of the l^{th} sex class, from the k^{th} dam and j^{th} sire of the i^{th} stock. On the right hand side of the model, μ was a constant common to all the observations, T was the fixed genetic effect imposed by stocks, S the random genetic effect of sires nested within stocks, and D a random genetic effect of dams nested

within stocks and sires; X was a fixed effect imposed by sex of fish, and e was a random residual term.

The statistical analyses were accomplished using the SAS GLM procedure (Freund et al, 1986). The multivariate analysis of variance was done for two cases: one included all the traits (left side of Appendix Tables 1-4), and the other excluded oocyte diameter (right side of Appendix Tables 1-4) due to the fact that oocyte diameter was a sex limited trait. Four test statistics for were used, each being a different function of the two matrices, E and H (Freund et al, 1986) defined as

$$E = M'(Y'Y-b'(X'X)b)M$$

$$H = M'(k'b)'(k'(X'X)^{-k})^{-1}(k'b)M$$

where M is a pxp identity matrix and k may be either a coefficient matrix or a vector for the estimable functions used to construct type III tests, X is the matrix of classification variables, Y the matrix of observations of all response variables, and b is the vector of least square solutions $(X'X)^{-X}'Y$. The four statistics were defined as: Wilk's Lambda (WL) = det(E)/det(H+E); Pillai's Trace (PT) = trace$(H(H+E)^{-1})$; Hotelling Lawley Trace (HLT) = trace$(H^{-1}H)$; Roy's Maximum Root (RMR) = lambda, the largest eigenvalue.

To examine the phenotypic associations between traits, partial correlations coefficients were derived from the error sum of squares and sum of products of the model. The estimates were obtained from a matrix defined as:

$$S = (e'e)/(n-r) = (Y-Xb)'(Y-Xb)/(n-r)$$

where e is the vector of residuals, n is the number of observations, X, Y and b are the same as defined earlier and r is the rank of the X matrix. The partial correlation coefficients were obtained by scaling the S matrix to unit diagonal and represented the phenotypic associations between response variables after correction for all the independent variables in the model (SAS/STAT User's Guide, Edition 6.03, 1988, pp601-615).

For univariate analyses of variance, proper type III sums of squares were chosen to construct F tests (Freund et al, 1986, pp104) each factor in the model at each sampling time. The expectations of the type III mean squares for the univariate model are given in Appendix Table 5. The coefficients of determination (R^2) for the univariate analyses are given in Appendix Table 6.

Genetic correlations, environmental correlations and

heritabilities were estimated by Harvey's Mixed Model Least-squares and Maximum Likelihood Computer Program package (Harvey, 1987).

RESULTS

Multivariate Analysis

The results of multivariate analyses of variance showed that stock had a very significant simultaneous effect on body weight, carcass weight, fork length, liver weight, gonad weight, oocyte diameter, conditional factor, gonadosomatic index and hepatosomatic index ($P<0.01$) at all ages from 4 to 12 months (Appendix Table 1). The results without oocyte diameter led to the same conclusion for all the samples except at 6 mo, when the null hypothesis of no overall stock effect was accepted ($P>0.05$). This difference in results at 6 mo was probably due to the loss of the numerator degrees of freedom when oocyte diameter was left out. A test of sex effect could be carried out only for the case without oocyte diameter (Appendix Table 2) and was found not to be a significant factor until the fish reached the age of 8 months. From 8 to 12 months, the simultaneous effect of sex was very significant for all traits ($P<0.02$).

The simultaneous influence of sire within stock was not clear (Appendix Table 3). The Roy's Maximum Root (RMR) statistic showed that overall sire effect within stocks, either with or without oocyte diameter, was very significant ($P<0.01$) for all samples from 4 to 12 months of age. However,

the other three statistics showed it was not significant for samples at 6, 8 and 12 mo ($P>0.05$). At 4 months of age in the test with oocyte diameter, both WL and PT indicated overall sire effect to be marginally significant ($0.03<P<0.05$) whereas HLT was not significant ($P>0.05$). At 10 months of age for the test with oocyte diameter, both WL and PT suggested a significant overall sire effect ($P>0.05$) whereas HLT did not. In the test without oocyte diameter, all four statistics showed that sires within stocks did imposed a very significant overall influence on body weight, fork length and conditional factor at 4 months of age and on body weight, carcass weight, fork length, liver weight, gonad weight, condition factor, GSI, and HSI at 10 months of age.

All four of the multivariate test statistics showed that dams had a very significant overall effect on all the traits simultaneously for the case without oocyte diameter (Appendix Table 4). When oocyte diameter was included in the analysis, the results were not consistent at 4 and 12 months of age. At 4 mo, the PT statistic was not significant ($P< 0.05$) whereas at 12 mo the RMR statistic was the only one of four which indicated an overall dam influence on the response variables.

Means and Coefficients of Variation

The least square means are presented in Table 2 for the

two stocks and sexes. With regard to body size, RTH was significantly heavier (2.63 g) and longer (5.92 cm) than RTL (2.09 g; 5.62 cm) at 4 mo of age ($P<0.01$). However, from 6 to 12 mo, RTL was significantly larger than RTH in body weight, carcass weight and fork length at all the ages except fork length and carcass weight at 12 months of age ($P>0.05$). By 12 mo, RTL had reached average body weight, carcass weight and fork length of 221.1±5.08 g, 193.0±4.55 g and 25.7±.19 cm while RTH was 203.9±5.98 g, 180.3±5.35 g and 25.4 cm, respectively. It appeared that sex did not have an important influence on any of the three body size traits within the developmental period of 4 to 12 mo of age ($P>0.05$).

Condition factor for RTH was significantly higher than that of RTL at 4 months of age but this had disappeared by 6 mo and the condition of RTH became significantly lower than that of RTL at 8 and 12 mo of age ($P < 0.01$). This may indicate that there was a change of relative weight gain between the two stocks during the first year development. Sex of fish had no apparent effect on conditions factor through 12 months of age.

For the sexual-maturity-related group of traits, the average liver weight of RTL was heavier than that of RTH from 6 through 12 months of age, as was observed for body size

($P>0.05$). Sex did not impose a significant influence on liver weight through 12 mo of age. At 12 mo, the average liver weights were 1.89±.06 g for RTH and 2.13±.05 g for RTL.

The average gonad weight for the two stocks was not statistically different through 12 mo of age but average ovary weight was slightly heavier than testis weight from 8 through 12 mo of age although the difference between sexes at 12 mo of age were not significant ($P>0.05$). At the end of their first year development, male fish had an average testis weight of 0.127±.034 g, while female fish had ovaries averaging 0.217±.036 g.

No consistent differences between stocks were observed for average oocyte diameter over the 12 month period. Statistical examination suggested that the average oocyte diameter for RTH females was significantly larger than that of RTL females by 0.006 mm at 4 mo, but smaller than RTL by 0.023 mm at 6 mo, and again larger than RTL by 0.024 mm at 10 mo. By the end of the first year, the average oocyte diameters were 0.262±.0045 mm and 0.251±.0048 for RTH and RTL, respectively.

The hepatosomatic indices for RTH were significantly larger than that for RTL at 8 mo and smaller at 12 mo ($P<0.05$). No significant differences between sexes were found

during the first year (P>0.05). The gonadosomatic indices for RTH and RTL fish were very similar with a difference between the stocks observed only at 10 months of age (P<0.05). However, but GSI for females was consistently larger than for males by 0.051, 0.069 and 0.054 at 8, 10 and 12 months of age, respectively (P<0.05).

The coefficients of variation (Table 3) indicated that variability was similar for the two stocks for all the traits at almost all the ages from 4 to 12 mo. Secondly, coefficients of variation tended to decrease as the age of fish increased from 4 to 12 mo for all the traits except gonad weight and gonadosomatic index; variation for the latter two traits tended to increase as age increased from 8 to 12 mo.

The coefficients of variation for body weight ranged from 52.9% in RTH and 50.1% in RTL at 4 months of age to 24.4% in RTH and 23.0% in RTL at 12 months of age. For carcass weight, they ranged from 69.5% and 41.3% at 6 months of age to 24.3% and 23.5% at 12 months of age for RTH and RTL, respectively. Fork length showed less variability both within age or over the series of ages, ranging from 19.3% and 18.6% at 4 mo of age to 8.1% and 7.8% at 12 mo for RTH and RTL, respectively.

Variability for liver weight and hepatosomatic index was

inconsistent for stocks, sexes and ages although the coefficient of variation for liver weight stabilized at about 30% by 10 mo. The coefficients of variation for oocyte diameter fanged from a high of only 19.8% to 13.6%. Variation for gonad weight for RTH were increased from 41.5% at 8 mo to 59.6% at 12 mo of age, while that for RTL increased from 48.1% at 8 mo to 64.4% at 12 mo. However, the sexes showed similar values at 8 and 12 mo of age (25% to 39%) but high values at 10 mo; males was 49.6% and females were 85.9%.

Parameter Estimates

The estimated partial, phenotypic correlation coefficients between all the traits are given in Table 4 month of age. The correlations indicated high associations between body weight, carcass weight, fork length, and condition factor. The coefficients were consistently close to unity, ranging from 0.75 to 0.99 and were statistically different from zero ($P<0.01$). Among the traits indicative of sexual development, liver weight was not significantly correlated with oocyte diameters ($P>0.05$) until the fish reached 12 months of age. Liver weight and gonad weight were positively correlated with HSI and GSI respectively, at all ages, while a negative correlation was observed with HSI and GSI with gonad weight and liver weight, respectively, at 8 months of age only; otherwise correlation was zero. Gonad weight and

oocyte diameters were not significantly correlated ($P>0.05$) up to 10 months of age, but positive correlation coefficient ($P<0.01$) was observed at 12 months of age.

Body weight was closely associated with liver weight and gonad weight after 6 months of age. The observed correlation coefficients for body weight and liver weight ranged from 0.63 at 10 months of age to 0.86 at 8 months of age, 0.15 at 12 months of age ($P<0.05$) to 0.66 at 8 months of age for body weight and gonad weight. Body weight was negatively correlated with HSI at 6 months of age, (-0.18; $P<0.01$), and with both HSI and GSI at 8 months of age (−0.26 and -0.32; $P<0.01$). This relationship, however, was lost after 8 months of age ($P>0.05$). No consistent relationship was observed between body weight and oocyte diameters over the time of samplings although very significant correlation coefficients, were observed at 6 and 12 months of age. Correlations between carcass weight and fork length and measures of sexual development were similar to those observed for body weight.

Very significant correlation coefficients of 0.80, 0.58 and 0.76 were observed between condition factor and liver weight at 8, 10 and 12 months of age, respectively. Condition factor was also positively correlated with gonad weight from 8 months (0.56; $P<0.01$) to 12 months (0.17; $P < 0.05$) of age.

Condition factor was negatively correlated with HSI at 6 months of age, and HSI and GSI at 8 months of age ($P<0.05$) and was positively and significantly correlated with oocyte diameter at 6 and 12 months of age.

Estimates of heritability were inconsistent across ages due to the modest number of sires and dams used in the experiment and due to some environmental factor, probably fish density which disrupted growth. Estimates based on sire components of variance for each stock ranged from 0.01 ± .20 at 8 months to 0.50 ± .52 at 10 months for RTH while those for RTL were 0.49 ± .33 at 6 months and 0.07 ± .22 at 12 months of age. To improve statistical reliability, final estimates were derived from sums of squares pooled over stocks (Table 5).

Sire component estimates of heritability for body size (BW, CW and FL) varied from 0.22 ± .20 to 0.39 ± .23 except at 8 and 10 months of age; estimated were small at 10 months and zero at 8 months due to negative size variance component estimates. It is noteworthy that estimates for 8 and 10 months of age based on dam variance components were exceptionally high. This contrast between sire and dam component estimates suggests a strong environmental effect common to full-sibs. In fact, the fish were transferred from 1.2 m diameter tanks to 3.55 m diameter tanks at 7 and 8

months of age to reduce rearing density. Since full-sib family effects were confounded with tank effects, the heritability estimates suggest that density became limiting and that heritability estimates for 8 and 10 month samplings probably are not reliable.

The heritability estimates based on sire components of variance can be summarized for data obtained at 4, 6 and 12 months of age (Table 5). Estimates for body weight and carcass weight were about 0.25 ± .20 while the heritability of fork length appeared to be near 0.35 ± .25. Estimated for liver weight and gonad weight indicated a heritability of around 0.20 ± .20 to 0.25 ± .25 although estimated for liver weight were smaller than this at younger ages. The heritability of oocyte diameter appeared to be high with estimates of about 0.35 ± .6.

Estimates of genetic and environmental correlation for data obtained at 8 and 10 months of age must be considered unreliable for the reasons given earlier for heritability estimates. However, the data for 4, 6, and 12 months offer some general estimates worthy of note. Genetic and environmental correlations between the three estimates of body size (BW, CW, FL) were all positive and large.

There was no apparent genetic association between liver weight and body size at 6 months of age but there were strong positive estimates of the environmental correlations. However, at 12 months of age there was a strong positive genetic correlation, about 0.80, between liver weight and body size as well as a strong environmental correlation. Estimates of correlations between gonad weight and body size were not available for the younger ages and were very small and inconsistent at 12 months. The genetic correlation between body size and oocyte diameter at 12 months appeared to approach 0.50 while the environmental correlations were zero.

DISCUSSION

Coefficients of determination (R^2) indicated that the model used for the univariate analysis of variance of the data set was adequate (Appendix Table 6). The R^2 values ranged from 0.33 (for HSI at 6 months of age) to a maximum value of 0.92 (for oocyte diameter at 10 months of age). Twenty-four out of 38 of the R^2 values were greater than 0.50.

Both multivariate and univariate analyses of variance showed that the two stocks differed for most traits studied. This result is consistent with the reports of some early studies (Gjedrem, 1975, 1983; Gall, 1983; Gall and Gross, 1978; Iwamoto et al., 1986; Kinghorn, 1983; Morkramer et al., 1985; Linder et al., 1983). The differences between the two stocks of rainbow trout may be a reflection of different initial genetic structures of the founding broodstocks or two stocks may have responded to different natural and artificial selection pressures. One known difference is that the fish at Hot Creek Hatchery (Busack et al 1980) have been maintained under selection in water at a relatively high temperature of 12.5°C year round while the fish at Mt. Lassen Trout Farms experience seasonal fluctuations.

The fact that RTH exceeded RTL in body size means at 4

months of age was probably due to the high average water temperature experienced by RTH during hatching. However, RTL stock exceeded RTH in body size and liver weight after 4 months of age suggesting that fast body weight gain was accompanied by fast liver development. This was confirmed by the observed partial correlation coefficients and genetic correlations of liver weight with body weight, carcass weight and fork length. Gonadosomatic index did not show any change from 8 to 12 months of age, indicating that the gonadal development was not very active. However, a few precocious males were observed at 12 months of age in the RTH stock.

The observed coefficients of variation of body weight of 52.9% and 50.1% for RTH and RTL at 4 months and 24.4% and 23.0% for RTH and RTL at 12 months of age, respectively, were in good agreement with the coefficients of variation of 55.8% for nursery weight and 23.0% for yearling weight in rainbow trout reported by Gall and Huang (1988).

The estimates of heritability of body weight, carcass weight, fork length and gonad weight based on sire component of variance were similar to those reported by other workers and the estimates based on dam components were, in general exceptionally large due to the intentional confounding of tank effects with full sib families. It is interesting that the

estimates of heritability for oocyte diameter based on sire components of variance were similar within ages indicating that the strong common environment confounding was not important at this early stage of female development.

The estimated genetic correlation of near unity between body weight and fork length at all ages agreed very well with the report by Refstie (1980). Overall, body weight appeared very highly and positively correlated with carcass weight and fork length, and these traits were positively correlated with liver weight, gonad weight and oocyte diameter both phenotypically and genetically.

Observed phenotypic and genetic correlations between the three body size traits and traits related to stage of sexual development, gonad weight and oocyte diameter, indicated positive associations. This result might suggest that large fish have large gonads with larger oocyte diameters, and thus are sexually more advanced at one year of age than small fish.

Table 1. Sampling distribution by stock, month and sex

	RTH			RTL			
Month	Males	Females	Combined	Males	Females	Combined	Total
4	75	54	129	118	59	177	306
6	75	54	129	100	77	177	306
8	75	79	154	92	84	176	330
10	31	43	74	104	71	175	249
12	60	63	123	103	74	177	300
Total	316	293	609	517	365	882	1491

Table 2. Least square means of body weight, fork length, carcass weight, condition factor, liver weight, gonad weight, oocyte diameter, HSI and GSI at 4 to 12 mo of age in rainbow trout[1]

	4 MONTH	6 MONTH	8 MONTH	10 MONTH	12 MONTH
Body Weight (g)					
RTH	2.63±.15**	12.5±.75*	39.4±1.67*	107.8±4.9*	203.9±5.9*
RTL	2.09±.14	15.0±.65	48.2±1.45	124.1±3.3	221.1±5.1
MALE	2.34±.12	13.9±.60	43.4±1.41	116.6±3.6	217.3±4.9
FEMALE	2.38±.13	13.7±.64	44.2±1.41	115.3±3.6	207.8±5.1
Fork Length (cm)					
RTH	5.9±.15**	9.9±.18*	14.7±.20*	20.3±.3*	25.4±.2
RTL	5.6±.13	10.6±.16	15.5±.17	21.2±.2	25.7±.2
MALE	5.8±.11	10.3±.15	15.1±.17	20.7±.2	25.7±.2
FEMAL	5.8±.12	10.2±.16	15.1±.17	20.8±.2	25.4±.2
Carcass Weight (g)					
RTH		11.0±.72*	35.1±1.49*	93.5±4.3*	180.3±5.3
RTL		13.1±.62	41.8±1.30	105.7±2.9	193.0±4.5
MALE		12.2±.63	38.1±1.25	100.2±3.1	191.0±4.4
FEMALE		12.0±.68	38.8±1.26	99.0±3.1	182.3±4.5
Condition (g/cm^3)					
RTH	1.13±.02**	1.22±.01	1.17±.13**	1.25±.02	1.22±.01**
RTL	1.04±.01	1.19±.01	1.25±.01	1.27±.01	1.28±.01
MALE	1.09±.01	1.20±.01	1.21±.01	1.27±.01	1.25±.01
FEMALE	1.08±.01	1.21±.01	1.21±.01	1.25±.01	1.25±.01
Liver Weight (g)					
RTH		0.18±.04	0.56±.02*	1.16±.06**	1.89±.06**
RTL		0.28±.04	0.60±.02	1.41±.04	2.13±.05
MALE		0.26±.04	0.58±.02	1.30±.05	2.07±.05
FEMALE		0.20±.05	0.58±.02	1.27±.05	1.96±.05
Gonads Weight (g)					
RTH			0.03±.002	0.09±.009	0.20±.036
RTL			0.03±.002	0.08±.007	0.14±.031
MALE			0.02±.002*	0.05±.008*	0.13±.034
FEMALE			0.04±.002	0.12±.008	0.22±.036
Oocyte Diameter (mm)					
RTH	.056±.001*	.098±.002*	.156±.003	.227±.002*	.262±.004
RTL	.050±.001	.121±.002	.161±.002	.203±.002	.251±.005

Table 2. Continued

	4 MONTH	6 MONTH	8 MONTH	10 MONTH	12 MONTH
Hepatosomatic Index					
RTH		1.59±1.40	1.62±.04*	1.26±.10	1.05±.02*
RTL		3.30±1.20	1.46±.03	1.37±.06	1.11±.02
MALE		3.40±1.37	1.56±.03	1.36±.08	1.08±.02
FEMALE		1.50±1.52	1.52±.03	1.27±.08	1.08±.02
Gonadsomatic Index					
RTH			0.08±.004	0.10±.010*	0.11±.018
RTL			0.08±.003	0.08±.006	0.08±.015
MALE			0.06±.005**	0.05±.008**	0.06±.017*
FEMALE			0.11±.004	0.12±.008	0.12±.018

1. * indicates stocks (or sexes) were sognificantly different at P<0.05 (** P<0.01).

Table 3. Coefficients of variations (%) of body weight, fork length carcass weight, condition factor, liver weight, gonad weight, oocyte diameter, HSI and GSI from 4 to 12 months of age for rainbow trout

	4 MONTH	6 MONTH	8 MONTH	10 MONTH	12 MONTH
Body Weight (g)					
RTH	52.9	41.9	38.5	24.8	24.4
RTL	50.1	41.0	31.8	27.7	23.0
MALE	52.9	42.3	33.6	29.4	22.1
FEMALE	53.8	42.7	38.5	25.6	26.0
Fork Length (cm)					
RTH	19.3	15.4	14.1	9.1	8.1
RTL	18.6	14.2	11.3	9.5	7.8
MALE	19.2	15.7	12.2	10.5	7.2
FEMALE	18.9	14.3	13.6	8.3	8.7
Carcass Weight (g)					
RTH		69.5	38.8	24.9	24.3
RTL		41.3	32.8	28.1	23.5
MALE		59.6	33.7	29.8	22.2
FEMALE		43.8	39.0	25.5	26.1
Condition (g/cm^3)					
RTH	7.7	6.1	8.9	7.1	6.8
RTL	11.6	10.1	8.5	7.3	8.9
MALE	11.6	8.0	8.8	7.0	8.1
FEMALE	9.5	9.6	9.7	7.7	9.1
Liver Weight (g)					
RTH		72.9	40.8	22.4	25.4
RTL		51.5	34.5	36.2	26.7
MALE		30.5	36.8	37.8	27.0
FEMALE		32.2	38.2	30.8	26.8
Gonad Weight (g)					
RTH			41.5	49.1	59.6
RTL			48.1	58.6	64.4
MALE			38.9	49.6	25.1
FEMALE			36.8	85.9	35.7
Oocyte Diameters (mm)					
RTH	18.4	9.4	15.6	7.2	13.5
RTL	19.8	17.5	14.1	10.0	13.6

Table 3. Continued

	4 MONTH	6 MONTH	8 MONTH	10 MONTH	12 MONTH
Hepatosomatic Index					
RTH		66.5	19.4	11.4	15.1
RTL		47.0	18.0	62.9	16.3
MALE		14.5	20.6	71.1	18.1
FEMALE		13.6	18.0	15.2	13.9
Gonadsomatic Index					
RTH			40.0	50.9	59.4
RTL			50.3	51.8	60.8
MALE			34.7	46.0	22.3
FEMALE			32.4	92.4	25.9

Table 4. Coefficients of partial correlations for body weight (BW), carcass weight (CW), fork length (FL), condition factor (CF), liver weight (LW), gonad weight (GW), oocyte diameter (DIAM) and hepatosomatic index (HSI) in rainbow trout

	BW	CW	FL	CF	LW	GW	DIAM	HSI
4 Months								
FL	.97**							
CF	.94**		.93**					
DIAM	-.05		-.04	-.09				
6 Months								
CW	.80**							
FL	.96**	.76**						
CF	.93**	.75**	.88**					
LW	.01	.02	-.03	.02				
DIAM	.37**	.38**	.41**	.37**		.07		
HSI	-.18**	-.13	-.22**	-.18**		.90**		-.01
8 Months								
CW	.99**							
FL	.96**	.95**						
CF	.91**	.90**	.81**					
LW	.86**	.85**	.84**		.80**			
GW	.66**	.66**	.66**	.56**		.54**		
DIAM	-.13	-.12	-.08	-.27	-.20	.07		
HSI	-.26**	-.29**	-.24*	-.20*	.24*	-.21*	-.16	
GSI	-.32**	-.32**	-.31**	-.40**	-.33**	.39**	.34*	.02
10 Months								
CW	.99**							
FL	.95**	.95**						
CF	.91**	.90**	.81**					
LW	.63**	.62**	.56**		.58**			
GW	.17*	.16*	.13	.23**		.07		
DIAM	.11	.09	.13	.05	.26	-.07		
HSI	-.14	-.15	-.19*	-.13	.61**	-.06	.36*	
GSI	-.07	-.08	-.11	-.01	-.10	.96**	-.10	-.05
12 Months								
CW	.99**							
FL	.95**	.95**						
CF	.90**	.89**	.75**					
LW	.83**	.81**	.80**	.76**				
GW	.15*	.14*	.12	.17*		.14		
DIAM	.46**	.46**	.37*	.46**		.41**	.54**	
HSI	-.01	-.04	.01	.03	.52**	.03	.04	
GSI	.06	.05	.04	.09	.07	.99**	.26	.04

** P<0.01, * P<0.05.

Table 5. Heritabilities, genetic and environmental correlations for body weight (BW), carcass weight (CW), fork length (FL), liver weight (LW), gonad weight (GW) and oocyte diameter (DIAM) in rainbow trout. Dash indicates estimates were not available due to negative variance component estimates, blanks indicate no data[1]

Trait	BW	CW	FL	LW	GW	DIAM
4 Months						
BW	**0.30±.21**		0.97			0.47
	0.75±.26		0.99			--
FL	0.97±.03		**0.31±.21**			0.55
	0.98±.01		**0.86±.25**			-0.53
DIAM	-0.35±.62		-0.44±.62			**0.30±.60**
6 Months						
BW	**0.46±.24**	0.80	0.97	0.01		
	0.39±.25	0.84	0.96	--		
CW	--	**0.25±.20**	0.75	0.02		
	0.82±.19	**0.28±.25**	0.77	--		
FL	0.96±.02	--	**0.39±.23**	-0.04		
	0.98±.05	0.95±.19	**0.27±.25**	--		
LW	0.75±.82	0.75±.95	0.76±.88	**0.08±.17**		
	--	--	--	--		
DIAM	--	--	--	--		--
	-0.01±.45	-0.03±.49	-0.30±.54	--		**0.94±.48**
8 Months						
BW	--	--	--	--	--	--
	0.71±.25	0.92	0.85	0.87	0.27	-0.14
CW	--	--	--	--	--	--
	0.99±.01	**0.73±.25**	0.85	0.91	0.29	-0.12
FL	--	--	--	--	--	--
	0.99±.03	1.00±.02	**0.61±.24**	0.91	0.30	0.06
LW	--	--	--	--	--	--
	0.80±.09	0.74±.11	0.68±.14	**0.71±.25**	-0.05	-0.36
GW	--	--	--	--	--	--
	0.79±.23	0.78±.23	0.77±.23	0.79±.24	**0.60±.38**	-0.69
DIAM	--	--	--	--	--	--
	0.65±1.2	0.65±1.1	0.37±1.1	--	0.93±1.4	**0.24±.55**
10 Months						
BW	**0.09±.20**	0.99	0.97	0.67	0.22	0.90
	0.79±.29	0.99	0.93	--	0.54	--
CW	0.99±.01	**0.09±.20**	0.97	0.67	0.20	0.94
	0.99±.00	**0.79±.29**	0.94	--	0.51	--
FL	0.68±.46	0.72±.40	**0.15±.21**	0.64	0.18	0.86
	0.99±.02	0.99±.02	**0.64±.29**	--	0.42	-0.99
LW	1.00±.45	0.90±.48	0.19±.89	**0.13±.21**	0.20	--
	0.88±.13	0.88±.14	0.84±.19	**0.54±.29**	--	-1.00
GW	0.59±.80	0.69±.84	0.44±.62	0.09±.74	**0.25±.23**	--
	--	--	--	--	--	-0.85
DIAM	-0.39±.41	-0.38±.39	-0.55±.60	--	--	0.79±.56
	--	--	--	0.60±.37	--	**0.89±.53**

Table 5. Continued

Trait	BW	CW	FL	LW	GW	DIAM
12 Months						
BW	**0.22±.20**	0.99	--	0.85	--	0.52
	0.54±.26	0.99	0.94	0.81	0.13	--
CW	1.00±.00	**0.24±.20**	--	0.83	--	0.53
	0.99±.00	**0.53±.26**	0.93	0.79	0.14	
FL	0.88±.07	--	--	--	--	--
	0.95±.05	0.91±.06	**0.72±.26**	0.85	0.21	0.15
LW	0.83±.16	0.79±.17	--	**0.24±.20**	--	0.39
	0.94±.10	0.93±.11	0.75±.17	**0.36±.25**	0.09	--
GW	--	--	--	--	--	--
	0.09±.57	0.06±.58	-0.14±.50	0.16±.71	**0.21±.25**	0.65
DIAM	0.01±.82	-0.01±.80	--	0.41±1.0	--	**0.41±.59**
	--	--	0.87±1.1	--	0.02±1.2	**0.44±.70**

[1] the upper figures are estimates based on sire components; the lower figures are that based on dam components; the values on the main diagonal are the estimates of heritability; the values above the diagonal are environmental correlations, those below the diagonal are genetic correlations.

Appendix 1. Values of four multivariate statistics used along with the F ratio, probability and degrees of freedom to detect stock effects on body weight, carcass weight, fork length, liver weight, gonad weight, condition factor, gonadosomatic index and hepatosomatic index. Data on gonad weight and gonadosomatic index were not available at 6 months so estimates were limited to body weight, fork length and condition factor[1]

	With Diam			Without Diam		
Statistic	Value	F	P(df)	Value	F	P(df)
4 Month						
WL	.678	4.38	.005(4,37)	.525	12.6	.000(3,42)
PT	.321	4.38	.005(4,37)	.475	12.6	.000(3,42)
HLT	.474	4.38	.005(4,37)	.903	12.6	.000(3,42)
RMR	.474	4.38	.005(4,37)	.903	12.6	.000(3,42)
6 Month						
WL	0.17	25.9	.0001(7,38)	.739	2.28	.055(6,39)
PT	0.83	25.9	.0001(7,38)	.260	2.28	.055(6,39)
HLT	4.77	25.9	.0001(7,38)	.351	2.28	.055(6,39)
RMR	4.77	25.9	.0001(7,38)	.351	2.28	.055(6,39)
8 Month						
WL	0.46	3.98	.002(9,31)	.254	12.8	.000(8,35)
PT	0.53	3.98	.002(9,31)	.746	12.8	.000(8,35)
HLT	1.15	3.98	.002(9,31)	2.93	12.8	.000(8,35)
RMR	1.15	3.98	.002(9,31)	2.93	12.8	.000(8,35)
10 Month						
WL	0.35	6.18	.0001(9,30)	0.47	4.73	.001(8,33)
PT	0.65	6.18	.0001(9,30)	0.53	4.73	.001(8,33)
HLT	1.85	6.18	.0001(9,30)	1.15	4.73	.001(8,33)
RMR	1.85	6.18	.0001(9,30)	1.15	4.73	.001(8,33)
12 Month						
WL	.544	3.16	.007(9,34)	.553	3.74	.003(8,37)
PT	.455	3.16	.007(9,34)	.447	3.74	.003(8,37)
HLT	.835	3.16	.007(9,34)	.809	3.74	.003(8,37)
RMR	.835	3.16	.007(9,34)	.809	3.74	.003(8,37)

[1]. WL = Wilk's Lambda,
PT = Pillai's Trace,
HLT = Hotelling Lawley,
RMR = Roy's Maximum Root.

Appendix 2. Values of four multivariate statistics used along with the F ratio, probability and degrees of freedom to detect sex effects on body weight, carcass weight, fork length, liver weight, gonad weight, condition factor, gonadosomatic index and hepatosomatic index. Data on gonad weight and gonadosomatic index were not available at 6 months so estimates were limited to body weight, fork length and condition factor [1]

Statistic	Value	F	P(df)
4 Month			
WL	0.994	.432	.731(3,201)
PT	0.006	.432	.731(3,201)
	0.006	.432	.731(3,201)
RMR	0.006	.432	.731(3,201)
6 Month			
WL	0.988	.402	.877(6,198)
PT	0.012	.402	.877(6,198)
HLT	0.012	.402	.877(6,198)
RMR	0.012	.402	.877(6,198)
8 Month			
WL	0.473	13.3	.000(8,96)
PT	0.527	13.3	.000(8,96)
HLT	0.11	13.3	.000(8,96)
RMR	1.11	13.3	.000(8,96)

Appendix Table 2. Continued

Statistic	Value	F	P(df)
10 Month			
WL	0.71	7.86	.000(8,156)
PT	0.29	7.86	.000(8,156)
HLT	0.40	7.86	.000(8,156)
RMR	0.40	7.86	.000(8,156)
12 Month			
WL	0.91	2.47	.014(8,191)
PT	0.09	2.47	.014(8,191)
HLT	0.10	2.47	.014(8,191)
RMR	0.10	2.47	.014(8,191)

[1] See Appendix 1.

Appendix 3. Values of four multivariate statistics used along with the F ratio, probability and degrees of freedom to detect sire within stock effects on body weight, carcass weight, fork length, liver weight, gonad weight, condition factor, gonadosomatic index and hepatosomatic index. Data on gonad weight and gonadosomatic index were not available at 6 months so estimates were limited to body weight, fork length and condition factor [1]

	With Diam			Without Diam		
Statistic	Value	F	P(df)	Value	F	P(df)
4 Month						
WL	0.02	1.35	.043(160,118)	0.08	1.64	.001(132,163)
PT	2.53	1.37	.032(160,128)	1.70	1.66	.001(132,168)
HLT	7.66	1.32	.061(160,110)	4.05	1.62	.002(132,158)
RMR	2.84	2.27	.009(40,32)	1.84	2.35	.001(44 ,56)
6 Month						
WL	.003	0.91	.79(308,214)	0.02	1.16	.111(264,312)
PT	3.59	0.84	.92(308,245)	2.86	1.16	.098(264,336)
HLT	11.3	1.01	.49(308,191)	6.11	1.14	.132(264,296)
RMR	5.30	4.22	.0001(44,35)	1.75	2.23	.002(44,56)
8 Month						
WL	.001	0.79	.975(351,251)	0.01	0.85	.932(336,333)
PT	4.43	0.84	.938(351,306)	3.45	0.85	.940(336,376)
HLT	10.8	0.75	.992(351,218)	7.44	0.85	.932(336,306)
RMR	3.12	2.72	.002(39,34)	1.91	2.14	.006(42,47)
10 Month						
WL	.0001	1.21	.059(342,233)	0.00	1.36	.004(320,293)
PT	5.16	1.13	.132(342,288)	4.48	1.34	.004(320,336)
HLT	19.8	1.29	.024(342,200)	13.1	1.36	.005(320,266)
RMR	6.60	5.56	.000(38,32)	3.84	4.03	.000(40,42)
12 Month						
WL	.0003	1.00	.510(378,244)	0.01	0.93	.764(352,396)
PT	5.06	1.01	.459(378,297)	3.37	0.91	.822(352,440)
HLT	15.7	0.96	.619(378,209)	7.22	0.95	.692(352,370)
RMR	4.27	3.35	.0003(42,33)	2.26	2.83	.000(44,55)

[1] See Appendix 1.

Appendix 4. Values of four multivariate statistics used along with the F ratio, probability and degrees of freedom to detect dam within sire and stock effects on body weight, carcass weight, fork length, liver weight, gonad weight, condition factor, gonadosomatic index and hepatosomatic index. Data on gonad weight and gonadosomatic index were not available at 6 months so estimates were limited to body weight, fork length and condition factor [1]

	With Diam			Without Diam		
Statistic	Value	F	P(df)	Value	F	P(df)
4 Month						
WL	0.04	1.40	.026(128,138)	0.24	2.17	.000(168,604)
PT	2.03	1.20	.143(128,148)	1.09	2.08	.000(168,609)
HLT	6.70	1.70	.001(228,130)	1.89	2.26	.000(168,599)
RMR	4.50	5.20	.000(32,37)	1.09	3.95	.000(56,203)
6 Month						
WL	.006	1.32	.012(245,295)	0.15	1.32	.001(336,1191)
PT	3.29	1.19	.071(245,329)	1.59	1.31	.001(336,1218)
HLT	8.88	1.42	.002(245,275)	2.29	1.34	.000(336,1178)
RMR	2.94	3.95	.0001(35,47)	0.67	2.42	.000(56,203)
8 Month						
WL	.0001	2.02	.0001(306,354)	0.01	1.77	.000(376,776)
PT	5.13	1.79	.0001(306,414)	3.43	1.65	.000(376,824)
HLT	19.2	2.27	.0001(306,326)	7.79	1.95	.000(376,754)
RMR	6.52	8.82	.0001(34,46)	3.02	6.63	.000(47,103)
10 Mmonth						
WL	.0008	1.39	.002(288,317)	0.11	1.21	.011(336,1246)
PT	4.35	1.23	.031(288,378)	1.87	1.19	.020(336,1304)
HLT	14.7	1.64	.000(288,290)	2.70	1.24	.006(336,1234)
RMR	5.95	7.81	.000(32,42)	0.73	2.84	.000(42,163)
12 Month						
WL	.001	1.12	.166(297,301)	0.06	1.47	.000(440,1532)
PT	4.17	1.04	.337(297,360)	2.29	1.44	.000(440,1584)
HLT	11.8	1.21	.058(297,272)	3.51	1.51	.000(440,1514)
RMR	4.27	5.18	.0001(33,40)	1.01	3.65	.000(55,198)

[1] See Appendix 1

Appendix 5. Type III Expected Mean Squares (E(MS))

Source	E(MS)
Stock(T)	$\sigma^2_e + k_4\sigma^2_{D:TS} + K_5\sigma^2_{S:T} + kK^2_T$
Sire(S)/T	$\sigma^2_e + k_2\sigma^2_{D:TS} + K_3\sigma^2_{S:T}$
Dam(D)/S/T	$\sigma^2_e + k_1\sigma^2_{D:TS}$
Sex(X)	$\sigma^2_e + kK^2_X$
Residual(e)	σ^2_e

Appendix Table 6. Coefficients of determination (R^2) for the models used for analysis of variance of growth and reproduction traits in raibow trout [1]

Trait	4 Month	6 Month	8 Month	10 Month	12 Month
BW	.59	.53	.51	.58	.51
CW	--	.44	.51	.57	.50
FL	.63	.49	.45	.52	.48
LW	--	.35	.46	.51	.48
GW	--	--	.66	.49	.34
DIAM	.74	.84	.58	.92	.75
CF	.61	.52	.56	.64	.65
HSI	--	.33	.71	.36	.43
GSI	--	--	.76	.46	.35

[1] See Table 4 for abbreviations. Dash indicated data not available.

Chapter 2.

Stock, Diet, Sire Effects and Canonical Correlation Analyses for Body Size, Carcass Weight, Growth Rate and Developmental Characters During Second Year of the First Reproductive Cycle in Female Rainbow Trout

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Abstract

A total of 1079 female rainbow trout from two broodstocks were measured for body size, growth rate and developmental characters for the period of second year of the first reproductive cycle. All fish were either sampled at two-month intervals or spawned. Three diets, with different protein and digestible energy levels, were used to feed the fish starting at 12 month of age.

The data set was split into three subsets: one included body size, carcass weight, fork length, liver and gonad weight, and oocyte diameters; another contained data on relative growth rate; and the third contained of data on pre- and post-spawning weight and egg volume. Three different statistical models were employed in the analyses of testing effects of stock, diet, sire and age at spawning on the traits. A sire dam mixed model was used to estimate variance components, from which heritabilities of the traits were estimated from age at 14 to 24 month.

Canonical correlation analysis was carried out to identify and quantify the associations between one group of body weight, carcass weight, fork length, pre-spawning weight, post-spawning weight, and the other group of liver weight,

gonad weight, ALPP, oocyte diameter, relative growth rate and egg volume. Observed positively high canonical correlation between the body size group and its opposite group of developmental characters in subset A, B and C suggested that body weight, carcass weight, fork length, relative growth rate, pre-spawning weight and post spawning weight were strongly associated with liver weight, gonad weight, ALPP, oocyte diameter and egg volume.

INTRODUCTION

Body size, carcass weight, and growth rate are very important economic traits, whereas gonad weight, alkali-labile protein phosphorus level as an indication of exogenous vitellogensis, oocyte diameter and egg volume per spawner are critical for reproductive potential in fish. As early as in the 1970's, Gall and Gross (1978_b) experimentally investigated growth of domesticated rainbow trout and reported that the growth rate was nearly exponential at young and nearly linear at older age. Morkramer et al (1985) concluded that the final weight and carcass characteristics of rainbow trout were highly controlled genetically after they examined samples of 17 different populations of European rainbow trout.

Estimates of heritability for body weight of trout have been actively reported, ranging from 0.09 for 150 day weight (Aulstad et al, 1972) to 0.82 for 334 day weight (Klupp, 1979). Most other reports for body weight within 3 years of age falls between a range of 0.10 to 0.25 (Kinghorn, 1983). Studies concerning the association between growth and reproduction traits are reported in fish. Springate and Bromage (1985) observed a positive correlation between the sizes of water-hardened eggs and the subsequent fry in rainbow trout. Kamilov (1986) reported a positive correlation between

growth rate and gonad development in silver carp.

The relative importance of nutritional effects on the growth of fish have also been studied. Roley (1983) found that for maximum pre-spawning growth in rainbow trout, a diet should contain about 37 to 47% of dietary protein and 2.8 kcal/g metabolizable energy. Watanabe et al (1984) reported that growth rate was high in broodstock receiving diet containing 36% protein and 18% lipid, whereas the growth of fish kept on diet containing 28% protein decreased 60 days after the feeding. Khon et al (1984) also observed higher growth rate and larger eggs in rainbow trout fed diets with higher protein and fat content. Washburn (1987) in a study using similar diets concluded that fish on low protein diet (30-32%) produces high hatchability eggs, whereas high protein diet greatly reduces egg quality.

The present paper examines stock, diet and sire effects on growth and reproductive development, and attempts to reveal the high-dimensional relationship between a set of growth traits and another of developmental characters through canonical correlation analyses in rainbow trout.

MATERIALS AND METHODS

Fish and Feeding:

The two stocks of rainbow trout used in this study were the Hot Creek (RTH) and the Mount Lassen (RTL) stocks, both normal two-year maturing stocks. The mating, initial feeding, fish marking procedures, and sampling scheme were given by Zhang and Gall (1991_a) and by Zhang et al (1991_b).

At the beginning of the second year (12 mo of age), three full sib families of each sire of both stocks were randomly assigned to three different diets. The three diets were high-protein high-energy diet (diet H), low-protein low-energy diet (diet L) and a control diet (diet C), the standard commercial trout diet all fish had been fed during the first year of the experiment. The diets H, L and C contained 63.5%, 37.5%, 48.7% of crude protein and 3381, 2792 and 3214 kcal/kg of digestible energy, respectively. The formulation of the diets was reported by Zhang et al (1990).

Samples were consecutively taken at two-month intervals from 14 to 24 mo of age. However, fish that individually marked by freeze-branding were only sampled at 16, 20 and 24 mo. Due to a shortage of fish, no fish were sampled for RTH on diet C at 22 mo. Starting at 22 mo of age, all females were

checked for ripeness and spawned on a weekly basis. A total of 1079 females from 101 full sib families produced by 46 sires either were sampled or spawned (Table 1).

Traits and Measurements:

Sampled fish were euthanized with tricaine methanesulfonate (MS 222), and bled from the caudal vein with a heparinized vacuntainer. Each blood sample was analysed for alkali-labile protein phosphorus (ALPP, Zhang et al, 1990). Body weight (BW) and carcass weight (CW) of the sampled fish were then individually measured to the nearest 0.1 g, fork length (FL) was recorded to the nearest o.1 cm, liver weight (LW) and gonad weight (GW) to the nearest 0.01 g. Whole ovaries were then preserved in 10% formalin to prepare slides for histological analysis. When the follicles reached 1 mm in diameter or greater (at or after 18 months of age), fresh oocytes were removed from the fish before placeing the remaining ovarian tissue in formalin. For spawned fish, a sample of oocytes was retained for measurement. Histological slides of the preserved ovaries were prepared following standard histology procedures. Fresh oocyte diameter (OD) was measured directly, to the nearest 0.05 mm, using a dissecting scope and camera lucida by tracing the perimeters of cells with an image-analysis tablet linked to a microcomputer. A total of 24 fresh oocytes were measured for each fish. For the

preserved tissues, oocyte diameters were measured under a light microscope to the nearest 0.01 mm by tracing the perimeters of cells possessing a visible nucleus with the image-analysis tablet. The number of cells measured ranged from 4 to 40 depending on the number of observable cells available.

Oocyte diameters of 19 fish were measured in both ways, freshly and histologically. The data were used to serve the purpose of conversion of histological measurements to fresh measurement equivalents for a consistent analysis. The conversion was done by multiplying each histological measurement with a constant factor of 1.313684, which was a ratio of the mean of freshly measured oocytes to the mean of histologically measured oocyte of the same 19 fish.

Relative growth rate (RGR) was calculated for fish marked individually by freeze-branding; RGR was defined as $[(W_t - W_{t0})/W_{t0}]*100$ where W_t and W_{t0} were the body weights of the same individual at time t and t0, respectively. The interval between time t and t0 was a constant of two mo. Body weight of ripe fish was taken before and after spawning, recorded, as pre-spawning body weight (PREWT) and post-spawning body weight (POSTWT), to the nearest 0.1 g. Total volume of stripped eggs (EGGVOL) from each spawned fish was measured with a graduated

cylinder, to the nearest 0.5 ml. Age at spawning (WEEK) for spawned fish was recorded as the first week, or second week etc, during which the females were checked out as ready to spawn. The first week of spawning for RTL was not the same first week for RTH since there was a two mo gap between RTH and RTL at date of spawning.

Data Analysis:

The entire data set was split into three subsets according to the measurements recorded. The subsets are called A, B and C for simplicity. Subset A contained fish with complete records for body weight, carcass weight, fork length, liver weight, gonad weight and oocyte diameter. Subset B was the data set of relative growth rates, and subset C contained measurements of pre-spawning weight, post-spawning weight and egg volume from spawned females. The statistical models used in the analyses were:

$$Y_{ijkl} = \mu + T_i + D_j + S_{ik} + SD_{ijk} + e_{ijkl} \quad (A)$$

$$Y_{ijkl} = \mu + T_i + D_j + S_{ik} + e_{ijkl} \quad (B)$$

$$Y_{ijkl} = \mu + W_i + D_j + S_k + e_{ijkl} \quad (C)$$

for subset A, B and C, respectively, where Y_{ijkl} was the l^{th} fish (observation) on the j^{th} diet from k^{th} sire of the i^{th} stock (or week spawned). μ was a constant, T was the stock effect, D the diet effect, S the sire influence, SD was the sire by diet interaction, and W was the week effect.

Univariate analyses of variance were employed to test the significance of stock, diet, sire, week effects and the interaction between sire and diet within stock. Stock effect in both model (A) and (B) were tested by using the type III mean square of sire within stock as proper denominator in the F test, while mean square of the sire by diet interaction was used as denominator in the F tests for diet and sire effects in model (A). The interaction in model (A) and all other terms in (B) and (C) were tested against their corresponding residual mean squares. Since the spawning dates (weeks in model C) of the two stocks were different at all sampling times, the data subset C was analysed for each stock separately, using model C.

Heritabilities of all traits for each sampling age were estimated with a sire dam mixed model, in which stock and diet for the data subsets A and B, and week and diet for subset C were treated as fixed effects. The computation was accomplished with the "programs to estimate variance components for individual animal models by restricted maximum likelihood (REML)" method, which followed a derivative-free algorithm suggested by Graser et al (1987). Specified convergence criterion was $1x10^{-20}$, along with a guessed starting value of 0.3 for heritability and a maximum of 200 simplex iterates. Convergence was achieved between 140 to 170

rounds of iteration.

Characters within each of the three subsets A, B and C were then divided into two groups. One was notated as body size group (V), and the other as developmental trait group (U). For subset A, body size group included characters of body weight, carcass weight and fork length, while the other group contained liver weight, gonad weight, ALPP and oocyte diameter data. For subset B, body size group included relative growth rate at 14 to 24 mo of age, body weight, carcass weight and fork length, the developmental group, liver weight, gonad weight and oocyte diameter. The body size group of subset C had only pre-spawning weight and post-spawning weight, their opposite group contained oocyte diameter and spawned egg volume. Canonical correlation analyses were carried out seeking to identify and quantify the associations between the two groups of characters (variables). Canonical correlations measure the strength of association between two groups of variables (Johnson, 1988). Let

$U = a'X^{(1)}$, and $V = b'X^{(2)}$

where $X^{(1)'} = (X_1 , X_2 , X_3 , \dots , X_p)$, p traits in group $X^{(1)'}$

and $X^{(2)'} = (X_{p+1} , X_{p+2} , X_{p+3} , \dots , X_{p+q})$, q traits in group $X^{(2)'}$. Canonical correlation seeks pairs of coefficient vectors a and b such that

$$\text{CORR}(U,V) = \frac{a'\Sigma_{12}b}{\sqrt{a'\Sigma_{11}a}\,\sqrt{b'\Sigma_{22}b}}$$

is maximized. The correlations between the pairs of canonical variables of U for one character group and V for the other character group are independent, the interpretation of one pair does not affect the other. The significance of the canonical correlation coefficients were all tested based on Rao's approximation to the distribution of the likelihood ratio (Rao 1973).

RESULTS

Stock differences:

The means and the standard errors of the means of body weight, carcass weight, fork length, relative growth rate, liver weight, gonad weight and oocyte diameters are given in Table 2. Analysis of variance showed that RTH was significantly different from RTL in both body weight and carcass weight at 16, 20 and 22 mo of age. It was interesting to notice that RTH was lighter in both body weight and carcass weight at 16 mo, but became significantly heavier than RTL by 20 and 22 mo. A similar trend was also observed in liver weight. Analysis of variance also showed that the relative growth rate of RTH was consistently, significantly higher than that of RTL until 22 mo of age. The averages of relative growth rate at two mo intervals, from 14 to 24 mo, were 58.47±1.27, 40.32±1.03, 47.07±1.45, 34.48±.96, 26.64±1.81 and 17.98±2.80%/2 mo for RTH, and 45.74±.78, 34.91±.64, 31.88±.74, 20.68±.69, 33.15±.83 and 28.87±1.39%/2 mo for RTL, respectively. These means also showed that the relative growth rate for RTH decreased as the fish approached maturation, while growth rate was maintained throughout for RTL. The means and standard errors for pre-spawning weight, post-spawning weight and egg volume are given in table 3.

Diet effects:

Diet effects were not significant on body weight, carcass weight and fork length until 22 mo. From both samples of 22 and 24 mo, fish on diet H were significantly heavier and longer than fish on diet L in both stocks. Fish on diet C were intermediate at 22 and 24 mo in RTL, and at 24 mo in RTH (diet C treatment was missing at 22 mo in RTH). Diet significantly affected liver weight at 16, 18, 22 and 24 mo of age. Heavier livers were consistently measured from fish on diet H than that from fish on diet L in both stocks. Larger oocyte diameters were also observed from fish on diet H than those from fish on diet L at 14 and 16 mo in both stocks. This difference, however, became obscure after 16 mo of age. Fish on diet H also showed significantly higher relative growth rate, heavier pre-spawning weight, post-spawning weight and larger spawned egg volume than fish on diet L in RTL. RTH females on diet H showed slightly higher relative growth rate than fish on diet L, and no significant difference in pre-spawning weight, post-spawning weight and total egg volume. The averages and standard errors for body size, carcass weight, liver and gonad weight, and oocyte diameters are given in Appendix 1, for relative growth rate in Appendix 2, and for pre- and post-spawning weight, egg volume, in Appendix 3 by diets.

Sire influence and sire by diet interaction:

Analysis of variance suggested that sire influence on body weight, and carcass weight became significant after 22 mo. Sire influence was significant on fork length at 22 and 24 mo, and very significant on the relative growth rate at 14, 16, 18 and 22 mo. Very significant sire influence on pre-spawning weight, post-spawning weight and egg volume was also detected in both stocks. The effect of sire by diet interaction was significant on body weight and carcass weight in the first three samples of 14, 16, 18 mo and the last sample at 24 mo. Examination of progeny average body weight by sire and diet (Appendix 4) showed this may be a typical case of genotype by environmental interaction. In RTH, for instance, the averages of body weight at 16 and 24 mo of age of progeny group of the sire 11 were heavier that those of sire 7 by 52.27 g and 355.38 g on diet H, but lighter by 126.8 g and 33.7 g on diet L, respectively. In RTL, the averages of progeny body weight of sire 44 were heavier than the averages of progeny body weight of sire 55 by 109 to 132.9 g on diet H while lighter by 322.7 to 564.97 g on diet L at 16 and 24 mo of age, respectively. The effect of the interaction on fork length was also significant at 14, 16 and 18 mo of age.

Phenotypic variation:

Table 4 gives the coefficients of variation for all the

characters in subset A. The coefficients showed that relative growth rate and gonad weight of both stock possessed the highest variability among all the characters ranging from 24% to 80%. The coefficients of variation for RGR indicated a different trend between the two stocks. RTH showed much higher variation than RTL from 22 to 24 mo of age. Body weight and carcass weight showed higher phenotypic variability than fork length. All the coefficients of variation increased as the age of fish increased except for body weight in RTL after 18 mo of age. The coefficient of variation for pre-spawning weight, post-spawning weight and spawned egg volume ranged from 19 to 38% in RTH, and 23 to 30% in RTL.

Phenotypic variances of oocyte diameter are listed in Table 5 along with the estimates of their heritabilities for each sample and for fish spawned. First, the phenotypic variation of oocyte diameters increased with age. Second, spawned fish showed lower variation than the samples at 18 mo and thereafter, indicating that fish at the same physiological stage are less variable in their oocyte diameters than fish on the same chronological stage.

Estimates of heritability:

Estimated heritabilities of oocyte diameter based on sire components (h^2_s) were low, ranging from 0.12 to 0.13 except for

RTL 18 mo, and moderate based on dam components (h^2_d), which ranged from 0.18 to 0.39 (Table 5). The average estimates of heritability for body weight, carcass weight, fork length, relative growth rate, liver weight and gonad weight in RTL were near zero based on sire components, ranging from 0.03 to 0.18, and moderately high based on dam components, ranging from 0.10 to 0.34 (Table 6). The large differences between the estimates based on sire and that based dam suggested there might exist either dominance or maternal effects or both on the body size and developmental characters. The estimates of heritability for RTH and for pre-spawning, post-spawning and egg volume in both stocks were poor and near to zero due to limited number of sires and dams.

Canonical correlation analyses:

The partial, phenotypic correlations between all traits in subset A were moderately high, ranged from r=0.72 between body weight and gonad weight at 14 mo to r=0.95 between body weight and liver weight at 20 mo. The partial correlation for subset B were moderate, ranging from 0.06 to 0.83. The between group correlations in subset C were low. The estimated largest correlation coefficient was r=0.44 between pre-spawning weight and egg volume in RTH and r=0.73, in RTL.

The first canonical correlations were 0.85±.07 to

0.96±.01 in subset A, which appeared to be substantially larger than any of the between group partial correlation within each sample. The first canonical correlations were 0.91±.02 to 0.96±.01 in subset B, and 0.70±.05 to 0.83±.02 in subset C, which all were larger than any of the between group correlations in their subset. All the first canonical correlations were very significantly different from zero (Table 7). The positively high canonical correlation between the body size group and its opposite group of developmental characters in subset A, B and C suggested that body weight, carcass weight, fork length, relative growth rate, pre-spawning weight and post spawning weight are strongly associated with liver weight, gonad weight, ALPP, oocyte diameter and egg volume.

Table 8 gives the standardized coefficients for the first canonical variable of body size group and developmental group in subset A and B. The coefficients show that the first canonical variable extracted out of the body size group in subset A was a weighted difference of body weight and carcass weight, with more emphasis on body weight except for 22 mo. Since the canonical structure matrices showed positive correlation between carcass weight and its first canonical variable for all samples, carcass weight served as a suppressor variable with 22 mo as an exception. In the

developmental group, liver weight appeared as the major positive contributor to their first canonical variable. The relative importance of the other characters in this group is rather obscure with poor consistency from age to age. The standardized coefficients for subset B showed similar trends as to subset A.

Canonical redundancy analysis showed that the first pair of canonical variables is a adequately good overall predictor of the opposite group of variables. The proportion of variance of the body size group explained by the developmental group being 68%, 73%, 84%, 87%, 31% and 31% for subset A at 14 to 24 mo of age, and 45%, 39% and 13% for subset B at 16, 20 and 24 mo, respectively. The proportion of variance of the developmental group explained by the body size group was 37% to 51% in subset A, and 50% to 55% in subset B (Table 9). The proportion of variance that could be explained by the opposite group of variables in subset B was low.

Squared multiple correlations (Table 10) indicated that the first canonical variable of the developmental group in both subset A and subset B has some good predictive power for the original variable of body weight (0.77 to 0.91) and fork length (0.52 to 0.81) through 20 mo of age. The predictive power is good for carcass weight until 22 mo of age, but poor

for the relative growth rate at any stage of age. The first canonical variable of the body size group also has good predictive power for liver weight, gonad weight and fairly good for oocyte diameters in female rainbow trout.

DISCUSSION

The relative growth rate in RTH was consistently, significantly higher than that of RTL until 22 mo of age, at which time it became relatively slow and stayed below that of RTL. This could be due to the fact that RTH females matured earlier than RTL. Based on limited number of observations on age at maturation during this study, a total of 118 RTH females ovulated at age of 722.4±1.4 days. In comparison, a total of 277 RTL females ovulated at 736.0±0.9 days, which gave a significant 13.6 day difference in age at maturity between the two stocks ($P<0.01$). Earlier initiation of the ovarian cycle (vitellogenesis) may lead to decrease in growth rate.

The estimates of heritability for body weight based on sire components were very near to zero between 14 to 20 mo of age, but the estimates based on dam components for the same age period were relatively high compared to the most of the estimates reviewed by Kinghorn (1983). This might suggest that trout at this period expresses low additive genetic variation. This also indicated that maternal effect or dominant effect might substantially contribute to observed genetic variation.

Data from this study also showed disagreement to some

extent with the literature reviewed earlier that 37 to 40% of dietary protein and 2.8 kcal/g metabolizable energy meet the requirement for maximum growth in rainbow trout (Roley, 1983). The diet H used in this study contained about 64% of protein and 3381 kcal/kg digestible energy, while diet L and diet C contained about 38 to 49% of protein and 2792 to 3214 kcal/kg digestible energy, respectively. The body weight, carcass weight and fork length of fish fed diet H were significantly higher than fish fed diet L or diet C in both stocks by 24 mo of age. Oocyte diameter, however, agreed well with Roley's report, that is, it was not affected by dietary protein level near spawning.

Table 1. Numbers of sires, dams, and fish examined in RTH and RTL stocks of rainbow trout at different ages

	RTH			RTL		
Month	Sires	Dams	Fish	Sires	Dams	Fish
BW, CW, FL, LW and GW:						
14	13	17	26	23	45	60
16	17	21	30	25	48	78
18	16	22	32	23	42	68
20	19	36	54	26	52	76
22	9	13	21	25	43	75
24	16	25	48	26	53	116
Subtotal	20	42	211	26	59	473
Relative growth rate:						
14	20	39	204	26	59	368
16	20	39	194	26	59	358
18	19	35	163	26	57	252
20	19	35	166	26	58	242
22	15	23	111	25	47	180
24	15	24	49	25	47	139
Subtotal	20	39	204	26	59	368
Prewt, postwt and eggvol:						
	17	31	108	26	53	234
Age at spawning:						
	17	31	118	26	56	277
Gradtotal:	20	42	329	26	59	1079

Table 2. Averages of BW, CW, FL, RGR, LW GW and OD in rainbow trout

Traits	Age (mo) 14	16	18	20	22	24
RTH:						
BW	299.76±11.12	391.77±19.66	667.01±28.43	886.96±31.61	1143.90±58.66	1174.36±54.35
CW	263.83± 9.26	353.35±17.45	585.83±24.58	787.38±28.08	989.50±59.95	928.63±42.83
FL	28.34± 0.33	31.33± 0.55	36.08± 0.54	39.21± 0.49	41.95± 0.81	41.81± 0.74
RGR	58.47± 1.27	40.32± 1.03	47.07± 1.45	34.48± 0.96	26.64± 1.81	17.98± 2.80
LW	0.43± 0.02	3.78± 0.19	7.78± 0.44	9.30± 0.42	16.05± 1.37	18.58± 1.46
GW	0.51± 0.01	0.77± 0.06	2.25± 0.19	7.63± 0.56	43.95± 5.78	164.37±11.89
DIAM	0.51± 0.01	0.65± 0.04	1.04± 0.07	1.40± 0.09	2.85± 0.05	4.38± 0.21
RTL:						
BW	313.84±11.39	451.09±10.51	596.31±24.95	697.47±22.66	935.19±31.53	1176.12±32.80
CW	280.81±10.15	399.92± 9.37	523.97±21.69	620.39±20.07	799.12±26.28	942.58±25.92
FL	28.63± 0.35	32.17± 0.25	34.76± 0.45	36.56± 0.41	39.88± 0.63	42.88± 0.51
RGR	45.74± 0.78	34.91± 0.64	31.88± 0.74	20.68± 0.70	33.15± 0.83	28.87± 1.39
LW	3.05± 0.13	4.54± 0.13	6.81± 0.30	6.78± 0.23	12.64± 0.52	14.67± 0.49
GW	0.36± 0.01	0.69± 0.03	1.88± 0.15	5.03± 0.33	46.70± 3.59	157.33± 7.72
DIAM	0.46± 0.04	0.71± 0.01	1.09± 0.03	1.39± 0.13	3.12± 0.17	4.74± 0.41

66

Table 3. Averages of pre-spawning weight (Prewt), post-spawning weight (Postwt) and spawned egg volume (Eggvol)

RTH:	Prewt	Postwt	eggvol
Diet H	1341.07±34.17	1099.97±28.77	218.35±12.00
Diet C	1199.44±86.35	972.89±72.16	185.67±24.89
Diet L	1169.09±29.12	950.71±24.03	190.29±9.73
RTL:			
Diet H	1382.46±28.69	1139.19±24.74	233.03±6.81
Diet C	1191.45±28.01	994.83±23.33	202.48±7.43
Diet L	1050.87±29.43	855.25±24.51	185.12±5.75

Table 4. Coefficients of variation for BW, CW, FL, RGR, LW, GW and OD in rainbow trout

Traits	Age (mo) 14	16	18	20	22	24
RTH:						
BW	18.9	27.5	24.1	26.2	23.5	32.1
CW	17.9	27.1	23.7	26.2	27.9	31.9
FL	5.9	9.6	8.4	9.3	8.9	12.3
RGR	30.9	35.5	39.3	36.0	71.7	80.1
LW	24.2	27.7	31.9	33.0	39.1	54.6
GW	27.7	41.4	48.2	53.2	60.2	50.1
DIAM	14.7	20.0	20.7	18.5	24.4	16.8
RTL:						
BW	28.1	20.6	34.5	28.3	29.2	30.0
CW	28.0	20.7	34.1	28.2	28.5	29.6
FL	9.4	6.9	10.8	9.8	13.7	12.7
RGR	32.8	34.9	36.7	52.4	33.7	56.7
LW	32.3	25.7	36.9	29.7	35.4	36.2
GW	30.4	31.9	66.3	58.1	66.7	52.9
DIAM	19.0	18.0	28.8	29.4	32.1	26.0

Table 5. Phenotypic variances and heritabilities of average oocyte diameters in Rainbow trout at different ages

	RTH Stock			RTL Stock		
Month	σ^2_p	h^2_s	h^2_d	σ^2_p	h^2_s	h^2_d
14	.005	0.13	0.39	.008	0.12	0.24
16	.022	0.13	0.39	.018	0.13	0.18
18	.063	0.13	0.39	.100	0.06	0.39
20	.085	0.13	0.18	.185	0.13	0.18
22	.536	0.13	0.18	1.11	0.13	0.18
24	.784	0.12	0.24	1.88	0.13	0.18
Spawned	.058	0.13	0.18	.091	0.13	0.18
Average	.222	0.13	0.28	.485	0.12	0.22

Table 6. Estimated heritabilities for body Size, carcass weight, growth rate and developmental traits in rainbow trout in RTL stock

	BW		CW		FL		RGR		LW		GW	
Month	h^2_s	h^2_d	h^2_s	h^2_d	h^2_s	h^2_d	h^2_s	h^2_d	h^2_s	h^2_d	h^2_s	h^2_d
14	.00	.59	.00	.59	.66	.60	.08	.05	.06	.21	.02	.00
16	.05	.27	.07	.26	.23	.13	.11	.01	.12	.24	.06	.00
18	.00	.24	.00	.24	.00	.08	.00	.26	.00	.42	.00	.40
20	.00	.18	.00	.20	.00	.15	.00	.15	.00	.11	.00	.21
22	.16	.38	.24	.28	.00	.14	.27	.01	.00	.32	.00	.42
24	.22	.37	.17	.39	.21	.15	.00	.10	.00	.30	.23	.01
Average	.07	.34	.08	.33	.18	.21	.08	.10	.03	.27	.05	.17

Table 7. Canonical correlation coefficients between the first two Pairs of canonical variables

	Age (mo)					
	14	16	18	20	22	24
Subset A:						
1	.94±.01**	.94±.02**	.96±.01**	.96±.01**	.85±.07**	.96±.01**
2.	.53±.08**	.42±.08**	.36±.09*	.43±.07**	.49±.09**	.54±.06**
Subset B:						
1		.91±.02**		.94±.01**		.96±.01**
2		.28±.09		.29±.09		.62±.06**
Subset C:		RTH:				RTL:
1		.70±.05**				.83±.02**
2		.15±.10				.03±.07

Note: ** P<0.01.

Table 8. Standardized canonical variate coefficients for body size and developmental traits in rainbow trout

	Age at Sampling (mo)					
	14	16	18	20	22	24
Subset A:			$\hat{V}_1$			
BW	5.21	3.04	4.90	2.53	0.17	4.04
CW	-3.87	-1.99	-4.20	-1.46	0.92	-3.47
FL	-0.47	-0.09	0.35	-0.07	-0.01	0.09
			$\hat{U}_1$			
LW	0.70	0.66	0.81	0.91	0.95	0.26
GW	0.26	0.31	0.16	-0.00	-0.16	1.06
ALPP	0.41	0.13	-0.10	0.14	-0.21	0.04
DIAM	0.28	0.12	0.13	0.01	0.37	-0.14
Subset B:			$\hat{V}_1$			
RGR14		-0.12		-0.03		-0.05
RGR16		0.04		-0.03		-0.03
RGR18				-0.11		0.05
RGR20				0.00		0.04
RGR22						-0.04
RGR24						-0.02
BW		3.83		2.08		4.21
CW		-2.77		-0.90		-3.73
FL		-0.11		-0.16		0.10
			$\hat{U}_1$			
LW		0.70		0.89		0.25
GW		0.32		0.17		0.96
DIAM		0.12		-0.00		-0.02

Table 9. Canonical redundancy analysis of body size and developmental Traits in rainbow trout

	Standardized variance of body size measurements explained by: Their own canonical variables						The other canonical variables					
	Age at Sampling (mo)						Age at Sampling (mo)					
	14	16	18	20	22	24	14	16	18	20	22	24
Subset A:												
1[a]	.77	.83	.92	.95	.43	.34	.68	.73	.84	.87	.31	.31
2	.19	.06	.05	.04	.19	.50	.05	.01	.01	.01	.05	.14
3	.04	.11	.03	.01	.38	.16	.01	.00	.00	.00	.01	.00
Subset B:												
1		.55		.43		.14		.45		.39		.13
2		.08		.03		.17		.01		.00		.06
3		.08		.08		.11		.00		.01		.02
Standardized variance of developmental traits explained by:												
Subset A:												
1	.42	.54	.55	.54	.66	.41	.37	.48	.51	.50	.48	.38
2	.29	.08	.08	.15	.14	.19	.08	.02	.01	.03	.03	.06
3	.13	.17	.14	.15	.08	.25	.02	.00	.00	.00	.00	.00
Subset B:												
1		.67		.57		.55		.55		.51		.50
2		.08		.35		.24		.01		.03		.09
3		.25		.07		.21		.01		.01		.03

[a] The number 1, 2 and 3 refers to the first, second and third canonical variables.

73

Table 10. Squared multiple correlations (R^2) between the original variables and the first canonical variable of their opposite group of variables in rainbow trout

	Age at Sampling (mo)					
	14	16	18	20	22	24
Subset A:				$\hat{U}_1$		
BW	.77	.85	.87	.91	.21	.46
CW	.73	.82	.85	.91	.71	.27
FL	.53	.52	.80	.81	.00	.21
				$\hat{V}_1$		
LW	.54	.73	.89	.91	.68	.06
GW	.48	.66	.58	.48	.54	.66
ALPP	.02	.17	.09	.37	.17	.13
OD	.46	.35	.45	.23	.52	.46
Subset B:				$\hat{U}_1$		
RGR14		.04		.03		.01
RGR16		.07		.07		.01
RGR18				.05		.16
RGR20				.02		.05
RGR22						.05
RGR24						.05
BW		.79		.88		.39
CW		.77		.86		.22
FL		.59		.79		.23
				$\hat{V}_1$		
LW		.73		.87		.10
GW		.57		.45		.86
OD		.35		.20		.55

Appendix 1. Means and standard errors of body, carcass weight (BW, CW), fork length (FL), liver, gonad weight (LW, GW) and oocyte diameter (Diam) traits by stock, diet and age

Mo.	BW	CW	FL	LW	GW	Diam
RTH	Diet C:					
16	402.94±18.12	363.62±17.79	31.98±0.49	3.79±0.28	0.78±0.08	0.6810±0.037
18	695.40±69.01	608.80±56.27	36.05±1.68	7.05±0.77	1.86±0.35	0.9916±0.082
20	910.98±62.29	813.72±57.41	40.23±0.81	8.98±0.68	6.63±0.89	1.3118±0.055
24	1204.0±172.94	956.10±151.1	41.28±2.23	23.42±4.86	136.08±36.7	4.2708±0.268
	Diet H:					
14	334.53±15.30	292.54±12.49	29.29±0.41	3.23±0.18	0.47±0.04	0.5186±0.030
16	462.90±20.66	415.53±18.41	33.08±0.48	4.42±0.23	0.87±0.10	0.6719±0.045
18	758.27±36.03	668.38±31.05	37.68±0.62	9.42±0.64	2.63±0.25	1.1204±0.060
20	965.38±46.53	856.68±40.94	40.23±0.66	10.66±0.60	8.97±1.03	1.5027±0.070
22	1282.31±67.61	1119.17±56.65	43.95±0.78	18.53±1.89	47.66±8.97	2.8870±0.197
24	1323.32±79.70	1055.52±64.76	44.12±0.99	22.40±2.72	180.10±12.7	4.6276±0.122
	Diet L:					
14	278.03±12.87	245.88±10.86	27.74±0.41	3.47±0.24	0.40±0.02	0.5031±0.014
16	310.06±29.74	281.72±26.59	29.17±0.97	3.09±0.28	0.65±0.09	0.5967±0.041
18	591.48±39.74	518.18±33.97	34.89±0.79	6.72±0.56	2.06±0.32	1.0201±0.070
20	769.42±49.86	679.81±43.03	37.16±0.94	7.79±0.73	6.71±0.75	1.3987±0.059
22	991.69±74.56	838.46±90.89	39.75±1.15	13.33±1.68	39.88±7.34	2.8240±0.234
24	1045.33±74.26	818.31±54.72	39.98±1.06	14.58±1.32	155.97±20.5	4.2523±0.227
RTL	Diet C:					
14	316.07±22.80	281.67±20.20	28.71±0.66	2.85±0.25	0.39±0.03	0.5012±0.019
16	461.16±18.33	406.43±16.47	32.20±0.40	4.34±0.20	0.69±0.05	0.7071±0.022
18	603.99±39.18	532.66±33.96	35.06±0.62	6.25±0.39	1.92±0.18	1.0570±0.052
20	694.46±33.97	616.83±30.41	36.75±0.53	6.42±0.33	5.89±0.55	1.5421±0.067
22	929.23±47.54	783.55±40.03	39.70±0.68	12.06±0.79	53.88±7.50	3.3203±0.235
24	1172.11±52.04	938.37±40.71	42.39±0.70	13.39±0.69	166.82±14.6	4.8086±0.205
	Diet H:					
14	313.45±14.07	282.16±12.67	28.85±0.46	3.19±0.16	0.33±0.02	0.4296±0.016
16	460.28±17.91	408.15±16.19	31.91±0.41	5.21±0.23	0.64±0.03	0.7312±0.028
18	604.33±44.25	530.12±38.50	34.75±0.91	7.84±0.59	1.73±0.25	1.1175±0.069
20	741.86±37.29	657.90±33.12	37.40±0.65	7.16±0.40	5.05±0.58	1.3436±0.079
22	1066.33±62.33	916.13±51.82	42.68±1.64	14.17±1.08	48.52±5.16	3.0531±0.219
24	1387.41±52.83	1107.37±43.84	45.98±1.02	17.71±0.87	192.43±10.7	5.0342±0.134
	Diet L:					
14	310.99±22.31	277.51±20.00	28.21±0.71	3.16±0.25	0.37±0.02	0.4411±0.024
16	433.89±18.38	386.81±16.20	32.38±0.50	4.15±0.21	0.74±0.04	0.7025±0.026
18	574.62±48.72	502.49±42.48	34.25±0.98	6.69±0.65	1.99±0.42	1.0775±0.082
20	644.55±46.71	576.86±41.22	35.26±0.95	6.74±0.48	3.97±0.56	1.3017±0.097
22	825.22±45.98	711.19±37.44	37.58±0.62	11.87±0.79	37.91±5.31	2.9914±0.152
24	978.63±45.95	789.52±34.97	40.36±0.61	12.89±0.76	115.61±11.9	4.2654±0.259

Appendix 2. Average relative growth rate by diet

Diet	RGR14	RGR16	RGR18	RGR20	RGR22	RGR24
RTH:						
C	54.32±2.4	36.97±2.0	50.07±5.2	25.86±2.3	21.29±2.3	41.41±6.0
H	64.69±1.9	46.12±2.3	47.02±4.3	36.36±1.9	36.36±1.1	11.14±4.6
L	53.07±2.8	36.60±2.2	44.14±2.8	34.74±2.1	23.12±2.7	21.41±4.0
RTL:						
C	45.62±1.7	38.08±1.6	28.39±1.7	19.54±1.2	33.38±1.9	31.75±2.6
H	53.51±1.5	35.78±1.5	36.42±1.2	21.61±1.3	40.64±1.6	34.78±3.5
L	36.54±1.3	30.26±1.0	30.46±1.8	19.58±1.7	24.77±2.3	21.73±1.6

Appendix 3. Averages of pre- and post-spawning weight (Prewt, Postwt) and egg volume (Eggvol) by stock and diet in Rainbow trout

Age	Prewt	Postwt	Eggvol
RTH	Diet C:		
712	1265.5±119	1079.0±123	86.0±11.0
719	987.0	829.0	165.0
726	1212.8±122	961.5±100	222.3±23.1
	Diet H:		
691	1248.5±95.5	1033.5±88.5	164.0±31.0
698	1215.5±43.5	1033.0± 7.0	160.5±52.5
705	1272.4±74.1	1043.4±62.7	201.9±17.5
712	1406.5±28.5	1161.5±13.5	233.5±45.5
719	1404.4±77.0	1162.1±72.3	241.4±16.4
726	1324.5±90.4	1068.9±75.9	222.3±24.5
733	1231.0	1034.0	100.0
740	1482.8±59.2	1221.4±40.6	240.0±54.8
747	1316.7±137	1068.3±98.4	256.7±64.4
	Diet L:		
691	1277.0±331	878.0±132	232.7±107
698	1021.0±122	858.5±107	144.8±19.0
705	1131.8±50.0	943.8±46.0	185.3±11.4
712	1247.4±85.5	1046.3±79.4	185.0±10.5
719	1117.5±70.0	929.9±62.6	173.8±17.1
726	1150.5±63.6	921.8±48.6	181.3±17.6
733	1220.7±70.7	987.9±66.5	214.6±25.4
740	1171.3±101	941.7±83.2	205.7±50.7
747	1385.0±55.0	1124.0±56.0	253.0±21.0

Appendix 3 (Cont.). Averages of pre- and post-spawning weight (Prewt, Postwt) and egg volume (Eggvol) by stock and diet in Rainbow trout

Age	Prewt	Postwt	Eggvol
RTL Diet C:			
700	1101.3±69.8	930.8±53.7	183.0±26.5
707	1021.0±44.7	853.0±38.4	159.7± 9.4
714	1037.7±55.5	875.0±47.4	159.5±15.9
721	1178.0±65.2	983.5±49.1	197.5±19.2
728	1215.4±52.0	995.8±43.6	219.7±16.7
735	1234.3±74.6	1048.6±62.5	207.0±18.3
748	1197.8±189	1000.6±162	190.2±22.2
756	1237.6±65.5	1031.7±57.9	212.3±16.7
763	1268.5±244	1075.0±204	209.0±41.0
Diet H:			
700	1387.0±106	1160.0±93.0	235.0±30.0
707	1364.5±86.5	1098.5±82.5	232.5±41.5
714	1231.3±105	1001.7±96.5	169.3±22.8
721	1390.5±70.6	1134.3±62.6	242.0±20.6
728	1358.3±63.9	1050.6±54.5	268.1±23.2
735	1478.6±58.0	1240.9±52.3	244.7±12.9
742	1359.7±69.6	1123.8±55.6	225.0±17.6
748	1347.8±83.6	1121.3±68.0	215.8±18.6
756	1262.9±84.8	1034.3±73.9	234.4±16.5
763	1774.0	1509.0	213.0
Diet L:			
700	944.3±56.5	772.3±35.9	162.7± 5.8
707	954.7±201	760.7±154	188.0±45.7
714	1086.7±34.1	870.7±39.8	201.7± 3.8
721	990.9±76.7	816.3±64.1	168.9±12.1
728	1172.9±68.4	935.1±55.7	220.5±15.0
735	948.2±73.1	764.0±62.3	164.4±13.8
742	1103.8±42.1	923.0±36.4	179.8±12.2
748	1116.6±74.7	919.9±63.2	192.1±14.4
756	1132.9±109	939.4±92.1	192.7±15.4
763	889.5±35.5	727.0±27.0	158.5± 7.5

Appendix 4. Rank of sire groups based on average body weight (BW) of their progeny groups on different diet (D) at 14, 16, 18 and 24 month (mo) of age showing sire by diet interactions

Mo.	D	Sire	BW	Mo.	D	Sire	BW	Mo.	D	Sire	BW
RTL:											
14	H	53	209.00	14	C	40	148.70	14	L	54	101.80
14	H	67	260.90	14	C	61	206.25	14	L	44	269.50
14	H	39	277.70	14	C	47	225.47	14	L	42	270.30
14	H	44	289.60	14	C	44	278.20	14	L	31	285.20
14	H	49	294.70	14	C	67	295.00	14	L	39	288.80
14	H	54	330.57	14	C	55	304.50	14	L	51	306.20
14	H	41	332.60	14	C	51	307.50	14	L	53	315.10
14	H	45	339.50	14	C	50	321.20	14	L	40	376.20
14	H	55	341.20	14	C	42	343.20	14	L	50	388.20
14	H	37	341.50	14	C	53	344.10	14	L	47	391.30
14	H	61	362.30	14	C	45	371.30	14	L	55	393.10
14	H	47	376.75	14	C	49	384.15	14	L	63	433.20
14	H	51	437.00	14	C	63	481.00				
				14	C	41	520.00				
				14	C	37	582.90				
16	H	55	295.30	16	C	48	304.90	16	L	48	249.00
16	H	43	375.30	16	C	67	330.40	16	L	44	338.40
16	H	39	391.30	16	C	40	382.20	16	L	43	343.30
16	H	49	392.90	16	C	53	386.50	16	L	54	375.20
16	H	47	393.40	16	C	61	388.80	16	L	39	377.50
16	H	44	404.30	16	C	49	431.65	16	L	50	396.35
16	H	67	411.50	16	C	50	453.20	16	L	38	407.70
16	H	61	432.80	16	C	38	463.50	16	L	47	427.17
16	H	40	462.00	16	C	41	507.75	16	L	53	441.90
16	H	45	476.20	16	C	42	508.13	16	L	42	471.45
16	H	41	476.65	16	C	62	515.70	16	L	51	496.35
16	H	51	521.10	16	C	55	523.15	16	L	40	576.40
16	H	62	524.80	16	C	45	550.80	16	L	55	661.10
16	H	54	583.40	16	C	51	602.20				
16	H	53	593.30								

Appendix 4 (Cont.). Rank of sire groups based on average body weight (BW) of their progeny groups on different diet (D) at 14, 16, 18 and 24 month (mo) of age showing sire by diet interactions

Mo.	D	Sire	BW	Mo.	D	Sire	BW	Mo.	D	Sire	BW
18	H	53	383.60	18	C	51	129.20	18	L	42	347.90
18	H	45	451.15	18	C	40	383.30	18	L	43	405.95
18	H	55	543.30	18	C	66	386.00	18	L	39	498.05
18	H	49	593.90	18	C	48	534.60	18	L	63	504.40
18	H	43	639.10	18	C	53	539.95	18	L	40	613.30
18	H	67	652.40	18	C	55	583.60	18	L	66	655.10
18	H	63	660.30	18	C	38	587.75	18	L	51	663.65
18	H	48	750.50	18	C	47	626.80	18	L	55	694.10
18	H	39	752.40	18	C	45	634.90	18	L	38	762.40
18	H	37	830.00	18	C	67	642.00	18	L	47	931.50
18	H	51	974.00	18	C	49	653.10				
				18	C	42	711.95				
				18	C	63	1136.30				
24	H	55	1017.00	24	C	66	775.60	24	L	43	483.10
24	H	54	1018.63	24	C	45	788.15	24	L	38	663.00
24	H	47	1019.85	24	C	44	811.07	24	L	48	678.37
24	H	43	1055.95	24	C	67	884.43	24	L	67	746.47
24	H	44	1149.90	24	C	40	938.60	24	L	44	780.63
24	H	45	1194.60	24	C	50	1020.10	24	L	33	814.80
24	H	49	1213.45	24	C	38	1159.60	24	L	40	926.50
24	H	41	1280.55	24	C	61	1185.43	24	L	47	967.00
24	H	67	1324.17	24	C	53	1224.93	24	L	54	1004.00
24	H	61	1347.83	24	C	42	1264.50	24	L	39	1037.37
24	H	33	1437.60	24	C	47	1399.80	24	L	53	1124.40
24	H	39	1485.80	24	C	49	1417.33	24	L	42	1143.60
24	H	48	1690.70	24	C	51	1484.20	24	L	63	1147.60
24	H	51	1697.35	24	C	41	1558.80	24	L	66	1164.87
24	H	40	1713.60	24	C	62	1763.00	24	L	55	1345.60
24	H	62	1818.83					24	L	50	1371.70
24	H	63	2084.00					24	L	51	1404.40
RTH:											
14	H	11	274.45					14	L	4	205.50
14	H	4	303.00					14	L	11	240.63
14	H	1	360.87					14	L	1	292.85
14	H	8	372.30					14	L	8	301.60
16	H	7	479.73					16	L	11	197.20
16	H	11	532.00					16	L	7	324.00
16	H	1	601.30					16	L	1	330.80
18	H	1	761.00					18	L	4	415.20
18	H	7	807.60					18	L	3	518.47
18	H	3	813.00					18	L	1	561.75
18	H	4	900.40					18	L	17	680.50
								18	L	7	739.30
24	H	7	727.90					24	L	11	795.93
24	H	11	1083.28					24	L	7	829.63
24	H	4	1178.90					24	L	9	1014.35
24	H	9	1191.65					24	L	8	1069.47
24	H	8	1435.20					24	L	4	1265.10
24	H	3	1548.00					24	L	3	1410.37

Chapter 3.

Variation in Plasma Alkali-labile Protein Phosphorus Level and Its Relation to Body, Liver and Gonad Weight in Rainbow Trout (*Oncorhynchus mykiss*)

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ABSTRACT

Plasma concentration of alkali-labile protein phosphorus (ALPP) for 887 male and 877 female rainbow trout from two stocks (RTH and RTL) was determined at two month intervals from 12 to 24 months of age. The fish came from 46 paternal half-sib families consisting of 101 full-sib families reared on three different diets. Overall average plasma ALPP values (mean, sd) for females were 6.02±.27, 6.47±1.5, 7.12±1.4, 12.18±.61, 19.56±1.9, 59.67±12.1 and 152.42±3.3 μg/ml at 12, 14, 16, 18, 20, 22 and 24 mo with a peak value of 400.00 μg/ml at maturity. The minimum level of plasma ALPP accompanying final ovarian maturation appeared to be about 90 μg/ml. Average ALPP for males was 6.95±.21 μg/ml and remained relatively constant throughout the experimental period.

Significant stock and diet effects were observed in some sampling intervals; however, neither factor produced consistent large differences in ALPP level. RTL males exhibited slightly higher plasma ALPP levels than RTH males, as did the RTL females, at early sampling times (14, 16 mo), but RTH female ALPP levels exceeded RTL females by 20 mo of age. It seems that fish fed a high protein and energy diet exhibited higher ALPP levels. Significant stock by diet interactions also were observed, although interpretation of the effect was complicated by age or physiological stage of

the fish.

High average coefficients of variation, 36.2% for males and 44.6% for females, were observed. The variation in males remained relatively constant, but increased in females with increase of age.

Genetics effects, as estimated by sire variation, on the plasma ALPP level of females were significant. Estimates of heritability based on sire components of variance ranged from 0.71±.38 to 0.87±.52 for the period from 12 to 16 mo of age. However, estimates were zero during the period of exogenous vitellogen, from 18 to 24 mo. Significant estimates of partial phenotypic correlations of ALPP with female body weight, carcass weight, fork length, liver weight, and gonad weight ranged from 0.32 to 0.60, 0.32 to 0.59, 0.30 to 0.59, 0.33 to 0.60 and 0.32 to 0.77, respectively. A significant phenotypic correlation between ALPP level and relative growth rate was observed also. Genetic correlations of ALPP level were estimated to be 0.34±.31 with body weight and 0.41±.30 with carcass weight at 22 mo of age. The data suggested that fish with higher plasma ALPP level before their sexual maturation were also heavier and longer, with larger livers and gonads.

INTRODUCTION

Vitellogenin (Vg) and plasma protein phosphorus levels in fish have been the subject of numerous investigations. Since Vg is the dominant protein phosphorus in oviparous animals, its concentration can be estimated from protein phosphorus levels in the serum (Wallace and Jared, 1968; Emmerson and Petersen, 1976; Whitehead et al., 1983; Craik and Harvey, 1984). Norberg and Haux (1988) reported that plasma Vg concentration measured by radioimmunoassay (RIA) and as alkali-labile protein phosphorus (ALPP) were very highly correlated (r=0.999) in brown trout.

Whitehead and Bromage (1978), in a study with two-year-old immature male and female rainbow trout, examined seasonal changes of plasma ALPP under a simulated natural photoperiod. They observed a level of 25±7 μg/ml for males and females from April to July, and a maximum level of 400±60 μg/ml for females just prior to spawning in November. No significant change was found in males throughout the cycle. They concluded that photoperiod could be the major factor in the environmental control of reproduction in rainbow trout.

Bohemen et al. (1981) examined the annual changes of alkali-labile acid-dissolvable Vg-bound phosphates by monthly sampling of three-year-old female rainbow trout from March to

February and found that the level of the Vg-bound phosphates varied from 5.2 μmol/ml in May to 11.3 μmol/ml in December. DeVlaming et al (1984) reported that serum protein-bound phosphorus, oocyte size, and serum estradiol levels increased in parallel from September through November in the teleostean (Leptocottus armatus) fish. Sumpter et al. (1984) in a study with two-year-old male and female rainbow trout, observed that "the first marked sign of reproductive activity was evident by the increase in the level of blood vitellogenin which occurred between January and February, 12 mo before ovulation".

There has been little research on nutritional effects or genetic variation of the plasma Vg and its relationship with body size in rainbow trout. In this study, an attempted was made to determine stock and diet effects on plasma ALPP levels, and to examine phenotypic and genetic variability in rainbow trout during the second year of the first reproductive cycle. Estimates were also obtained concerning associations of plasma ALPP level with body, liver, and gonad weight.

MATERIALS AND METHODS

Fish rearing

Fish used in the study originated from two stocks, Hot Creek Stock (RTH) and Mount Lassen stock (RTL) that normally mature at two years of age. Details of the stocks and husbandry procedures, and the sampling scheme were given by Zhang and Gall (1991). Initial feeding took place in partitioned hatchery troughs where full-sib groups could be identified until they were marked with a combination of fin clips and move to 1.2 m outdoor circular tanks. When the fish were 8 mo (RTH) and 7 mo (RTL) of age, they were moved to 3.6 m diameter tanks with a water depth of 66 cm, where they remained to the end of the experimental period. The water temperature fluctuated from 10.7 °C in the winter to 15.9 °C in the summer and averaged about 14 °C. The water flow was 55 l/min. All fish were maintained under natural photoperiod.

During the first year, all fish were fed a standard commercial trout diet, which contained approximately 49% protein and 3214 kcal/kg of digestible energy. Starting at 12 mo of age, the fish in each stock were divided into three groups. One group continued to receive the commercial diet which served as a control (diet C), one group was fed a prepared high-protein high-energy diet (diet H) and one was fed a low-protein low-energy diet (diet L). Diet H contained

63.5% crude protein and 3381 kcal/kg of digestible energy while diet L contained 37.5% crude protein and 2792 kcal/kg of digestible energy (Appendix 1). All diets were supplemented with a standard double vitamin package.

Sampling

Samples were taken from 101 full-sib families consisting of 42 families from RTH sired by 20 males, and 59 families from RTL sired by 26 males. Sampling was at two mo intervals starting at 12 mo of age. Three fish were randomly sampled from most families of both stocks; less than three fish per family was obtained at some sampling dates due to limited numbers of fish in some families. A total of 1758 fish were sampled and consisted of 881 males and 877 females. A sample of 380 females that were individually marked by freeze-branding when the fish were 12 mo of age were included in the analysis. All freeze-branded fish were weighed at each two mo interval until sampled.

After 22 mo of sampling, a total of 307 females (RTH, 103; RTL, 204) matured and were spawned during the period before the last sampling at 24 mo. The spawned females accounted for 52.6% and 46.7% of the total fish remaining and 62.8% and 54.8% of the remaining females, for RTH and RTL, respectively. These spawned fish could not be included in the study since ALPP records were not available. The females

sampled at 24 mo were unspawned fish sampled from each of the families.

Sampled fish were euthanized with tricaine methanesulfonate (MS 222). A blood sample was taken from the caudal vein with a heparinized vacuntainer and centrifuged at 2500 rpm for 5 min. The plasma was removed from the vacuntainer, placed in an 8 ml Wheaton sample bottle and refrigerated at -20 °C for later ALPP analysis. Body weight and carcass weight of each fish were measured to the nearest 0.1 g, liver weight and gonad weight to the nearest 0.01 g, and fork length was recorded to the nearest 0.1 cm. Relative growth rate (RGR) was calculated for the individually freeze-branded fish at 16, 20 and 24 mo of age; RGR was defined as $(W_t-W_{t0})/W_{t0}$ where W_t and W_{t0} were the body weights of the same individual at time t and t0.

Plasma ALPP level was determined using a modification of the procedure described by deVlaming et al. (1984). One ml of 24% trichloroacetic acid (TCA) was added to 200 µl of plasma and centrifuged at 800 rpm for 20 min at 4 °C. The pellet was washed with 1 ml of a 3:1 mixture of ethanol-ether followed by a wash with 1 ml of diethylether. The pellet was resuspended in 0.25 ml of 1.5 N NaOH and the tubes were placed on a dry heating block at 102 °C for 15 min. After cooling, the mixture was neutralized with 0.25 ml of 1.5 N HCl, the

protein precipitated with 0.5 ml of 24% TCA, and centrifuged. The supernatant was collected and the phosphorus content determined with a Technicon Auto Analyzer. Duplicate determinations were done for each fish and the average value used for data analysis.

Data Analysis

Data for males and females were analyzed separately since a preliminary analysis showed differential changes in plasma ALPP levels for males and females over time. A full model was fitted first to describe the data, examine main effects, and possible interactions among all combinations of the main effects. After non-significant interaction terms were deleted, the final statistical model used in the analyses was:

$$Y_{ijkl} = \mu + T_i + S(T)_{ij} + D_k + TD_{ik} + e_{ijkl}$$

where Y_{ijkl} was the observation of ALPP in the univariate analysis, or the observations on ALPP, body weight, carcass weight, fork length, liver weight and gonad weight in the multivariate analysis, for the l^{th} fish on a k^{th} diet from the j^{th} sire group of the i^{th} stock. The μ was a constant, T was the fixed genetic effect of stock, S(T) the random genetic effect of sires within stock, D the fixed diet effect, TD the interaction between stocks and diets, and e was a random residual. Univariate analyses were used to test for main effects and a stock by diet interaction for each trait individually. A multivariate analysis was employed to examine

the phenotypic associations between plasma ALPP and the other traits.

Phenotypic analyses were performed with the SAS GLM procedure (Freund et al, 1986). The type IV mean squares were used in the analysis of variance for data with missing cells. Proper mean squares were chosen as the denominators for F tests of the fixed and random effects based on expectations of the mean squares. Stock effects were tested against the mean square for sires within stock, while other factors were tested against the residual mean square. When a significant interaction between stock and diet was present, the tests for stock effect and diet effect were only approximations. In these cases, the test was for the significance of main effect (stock or diet) plus some linear function of the interaction between stock and diet. Significance of effects was tested minimally at $P<0.05$.

Transformation to natural log was used to linearize the pattern of plasma ALPP. Partial phenotypic correlations between transformed plasma ALPP level and the other traits studied were estimated with the multivariate GLM procedure described by Freund et al (1986). The correlations represented estimates of phenotypic relationships after correction for the effects of sire and diet within stock.

Heritabilities and genetic correlations were estimated

based on sire components of variance and covariance following the procedures described by Harvey (1987). In an effort to increase sample size and number of sires, data for the two stocks were pooled.

RESULTS

The sample sizes, means, standard deviations and coefficients of variation for ALPP are given in Table I by sex, stock, diet and age at sampling. The overall means of ALPP at 12, 14, 16, 18, 20, 22 and 24 mo of age were 6.12±.17, 6.55±1.5, 6.82±.63, 8.22±.52 7.52±.71, 7.39±.26 and 6.07±.82 μg/ml plasma for males, and 6.02±.27, 6.47±1.5, 7.12±1.4, 12.18±.61, 19.56±1.9, 59.67±12.1 and 152.42±3.3 μg/ml plasma for females, respectively. The plasma ALPP level for males was maintained at around 6.96±.30 μg/ml. In contrast, females maintained about the same levels of ALPP as males through 16 mo of age, after which plasma ALPP levels increased dramatically, reaching a peak level sometime before spawning (24 mo).

Stock and Diet Effects: The interpretation of male data was complicated by the presence of random chance stock and diet differences at 12 mo, the time feeding of the diets was initiated. This probably resulted in residual effects later in the experiment. Stock effect on plasma ALPP was small but analysis of variance revealed differences between the two stocks at 14, 16, 22 and 24 mo of age for males, and 14, 16 and 20 mo of age in females (Table II). The ALPP levels for RTL males were significantly higher than those of RTH males for fish sampled at 14, 16, 22 and 24 mo. The same

relationship held in females at 14 and 16 mo, but at 20 mo of age, plasma ALPP levels for RTH females exceeded RTL females ($P<0.05$).

Overall diet effect was significant at 16, 18, 22 and 24 mo of age for males but only at 18 mo of age for females. Further tests within stock using orthogonal contrasts of diets H and L against diet C showed that only the ALPP level of RTL males for diet H (8.81±.44 μg/ml) was significantly different from the ALPP level of males on diet C (6.75±.22 μg/ml) at 16 mo of age ($P<0.01$). This might suggest that diet H did impart a relatively strong influence on fish to express higher levels of plasma ALPP compared to the other two diets, even if the main dietary effect was minor.

A stock by diet interaction effect on plasma ALPP level was significant at 14 and 18 mo of age for males, and 14, 18 and 24 mo of age for females (Fig 1 and 2). However, the interaction effect was not sufficient enough to alter the rank order of ALPP level of stocks fed the three diets until the fish were 18 mo of age. Graph (A) of Fig 1 illustrates the nature of the interaction for both males and females at 14 mo of age; the extreme sex difference on diet L was not evident for diet H. A stock by diet effect was clearly demonstrated at 18 mo (Graph C, Fig 1). The interaction for males was due to the relative size of differences in ALPP levels between the

two stocks over all three diets, but the interaction for females was due to changes in ranking of the two stocks.

The first column of mean squares listed in Table II shows significant differences for diets and diet by stock interaction effects for males at 12 mo of age. These were due to random sampling errors committed during the process of assigning fish to diet treatment groups at 12 mo of age.

Phenotypic variation: The phenotypic variability in level of plasma ALPP was examined using coefficients of variation (Table I). The average coefficients of variation were 30.0 and 37.4 for males, and 44.2 and 45.0 for females of RTH and RTL, respectively. The RTL stock showed higher variability than RTH, and females of both stocks showed greater variability than males. In addition, for both stocks the coefficient of variation for females averaged over all three diets indicated a trend of increasing variability in plasma ALPP level with increasing age through 22 mo. Males maintained relatively constant levels of variability throughout the experimental period, although the coefficient of variability tended to be high at about 20 mo.

ALPP pattern of females: The level of plasma ALPP over the course of 12 months appeared to subdivide conveniently into three periods (Fig. 2). It was clear that from 12 to 16 mo of age, the ALPP level on all three diets was relatively

constant at around 5 μg/ml in RTH and 7 μg/ml in RTL (this will be referred to as the constant period). Plasma ALPP began to rise after 16 mo for both stocks on all diets and continued a slow increase through 20 mo (period of slow increase). The increase over this period resulted in a significant difference between 20 mo average values of 17.6±1.7 and 21.5±2.9 μg/ml for RTL and RTH, respectively.

A dramatic increase in plasma ALPP took place between 20 and 24 mo (period of rapid increase). However, the responses of the two stocks to the three diets were not consistent. For RTL, the increase during this four month period was nearly linear for all three diets, but slower for fish fed diet L (116.2±14.1 at 24 mo) than those fed diets C and H (165.5±17.1 at 24 mo). In contrast, females of the RTH stock showed a dramatic increase to 197.0±29.5 μg/ml at 24 mo when fed diet C while those fed diet H responded slowly and linearly to reach a final ALPP level of only 89.3±15.0 μg/ml. RTH females fed diet L showed little increase from 20 to 22 mo, but a very rapid increase from 22 to 24 mo to a value of 180.5±22.5 μg/ml. Consequently, the lowest plasma ALPP level was observed for RTH female fed diet H while the highest level was expressed by RTH females fed diet C and L. The stock by diet interaction was, again, evident from the fact that for RTL, the lowest plasma ALPP level was observed for female fed diet L.

ALPP level and maturation of females: As presented in the previous section, plasma ALPP level was observed to be constantly increasing for females undergoing maturation. By 22 mo of age, the average plasma ALPP levels were 47.5±8.4 μg/ml for RTH and 71.8±8.8 μg/ml for RTL, but none of the females of either stock had matured. The first mature females (defined as those with strippable eggs) were observed 7 and 8 days after the 22 mo sampling. Although most females were spawned before sampling at 24 mo of age, a total of 37 females (6 RTH, 31 RTL) were found in ovulatory condition during the process of their dissection at 24 mo. The average ALPP levels of these females were 260.0±29.2 μg/ml for RTH and 192.8±13.4 μg/ml for RTL, and ranged from 90.0 to 400.0 μg/ml. The data indicated that the minimum level of plasma ALPP during final ovarian maturation was about 90 μg/ml.

Phenotypic correlations: There were no significant phenotypic associations between ALPP and body weight, carcass weight, fork length, liver weight, and gonad weight at 12 and 14 mo of age for either stock (Table III). Significant partial phenotypic correlation coefficients of ALPP with body weight, carcass weight and fork length were observed at 16, 18 and 20 mo of age (range 0.29-0.71) in both stocks, and with body weight and carcass weight at 22 mo for RTL females (0.59) but not RTH females. ALPP level and fork length also were significantly correlated (0.41) at 24 mo of age for RTH

females while ALPP level and liver weight were significantly correlated (range 0.37-0.54) at 18 and 20 mo for females of both stocks and at 22 and 24 mo (range 0.64-0.35) for RTL females. ALPP level and gonad weight were highly and significantly correlated from 16 mo through 24 mo of age for RTL stock (range 0.40-0.74), but only at 18 and 20 mo for RTH females (0.75-0.61).

In addition, it was observed that plasma ALPP for females at 16 mo was significantly correlated with body weight at 12 and 14 mo, and plasma ALPP at 20 mo was significantly correlated with body weight at 14, 16 and 18 mo; significant correlation coefficients ranged from 0.19 to 0.34. No association was found between ALPP at 24 mo and body weight at any previous age. Low, positive correlations between ALPP level and relative growth rate of females were found at 20 and 24 mo; the coefficients were 0.32 ($P<0.01$) at 20 mo and 0.18 ($P<0.05$) at 24 mo. The correlation coefficient of ALPP with relative growth rate at 16 mo of age was positive, but not significant. This positive relationship may suggest that the fish exhibiting relatively higher ALPP levels were growing faster before maturity.

Heritability and genetic association with carcass weight: The contribution of sires within stocks to the total variation of plasma ALPP level for females was significant at

12, 14, 16 and 22 mo of age (Table II). The estimated heritabilities of plasma ALPP level based on sire component of variance for pooled samples of both stocks, ranged from 0.71±.38 to 0.87±.52 for samples of 12 through 16 mo (Table IV). The estimates at 18, 20 and 24 mo were poor. Causes responsible for the poor estimates of heritability from data obtained during the last four sampling periods are uncertain, but possibly the physiological and/or the external environmental effects became so large that they over-shadowed the additive genetic variation.

To demonstrate sire effect on plasma ALPP level and the genetic association between ALPP and carcass weight graphically, unweighted means of plasma ALPP and carcass weight were computed for specific sire groups based on the rank of the unweighted mean of carcass weight of daughters at each sampling. The unweighted means of ALPP level for daughters of the 3 sires with the lowest and highest carcass weights was plotted along with the unweighted means for (Fig. 3). The large difference in ALPP and carcass weight response for the two sire groups suggested that there is a genetic factor governing the level of plasma ALPP in rainbow trout females. The data in Fig 3 also showed that the changes of plasma ALPP level and carcass weight were very closely synchronized from 16 to 22 mo of age indicating a potential genetic correlation between ALPP and carcass weight. Estimates

of genetic correlations were inconsistent and had very large standard errors due to the small number of sires with progeny at each sampling. Nevertheless, estimates of positive genetic correlation coefficients of 0.34±.31 and 0.41±.30 between plasma ALPP levels and carcass weight and body weight, respectively, were obtained from RTL data at 22 mo of age.

DISCUSSION

Plasma ALPP level directly indicates the concentration of serum vitellogenin because of phosphorylation of serine moieties in the vitellogenin molecule (Mommsen and Walsh, 1988). Vitellogenin is a product of genes expressed in the female trout liver in response to ovarian estrogen stimulation (Chen, 1983; Valotaire et al., 1984; Maitre et al., 1985). It is then transported via the blood stream to the ovary where it is sequestered, specifically by the vitellogenic oocyte. A large amount of vitellogenin is required in oviparous animals (Whitehead and Bromage, 1978) and although, there is still a lack of information about what controls the concentration of serum vitellogenin and its reduction after ovulation, factors such as environmental conditions, neuroendocrine regulation, stage of gonadal development and, perhaps, nutritional state are, in general, believed to be important from the physiological point of view.

The changes of plasma ALPP of the two stocks over time were inconsistent. This may be related to further complications about the detected stock by diet interaction effect on ALPP levels, to differences in rates of reproductive development of the fish or differences of age or month at which the stocks reached sexual maturity. In addition, it is not too difficult to image that large differences of serum

vitellogenin concentration among individuals in the early and late stages of the reproductive cycle contributed to the poor estimates of phenotypic correlations and heritabilities.

Whitehead and Bromage (1978) observed an average level of serum protein phosphorus in a stock spawning at two years of 25 μg/ml from April to July for both males and females, and an average of 400 μg/ml for females prior to spawning. It is difficult to compare the results of the present study with the observations reported for the period of April to July because of likely differences in age of fish in the two studies. The peak value of 400 μg/ml for females prior to spawning, however, was more than twice the peak value observed for maturing females at 24 mo of age in the present study, but agreed exactly with the observed maximum ALPP level among the ovulating females. Also, the increase of plasma ALPP levels for females in the present study was very similar to the increase from March to November reported by Riazi (1988). The ALPP levels of males also agreed well with Whitehead and Bromage (1978) observation, in that it remained almost constant throughout the experiment period of 12 to 24 mo of age. Under normal conditions, vitellogenin genes in fish are not expressed during the early reproductive development (Mommsen and Walsh, 1988).

Although both stocks (RTH and RTL) spawned first at age 2, there was a two month seasonal difference between the

spawning time of RTH (September) and RTL (November). The plasma ALPP level for females of the two stocks at the same season, say in July, were very different. While RTH females in July (22 mo) reached an average level that ranged from 39.2 to 55.9 μg/ml among diet groups, RTL females (20 mo) ranged from only 13.9 to 19.8 μg/ml. However, The average ALPP level for the two stocks was very similar at same age. For instance, at 20 months of age, the average ALPP level of RTH females ranged from 18.9 to 22.6 μg/ml (May), which was very close to the average level of RTL females at 20 months of age (July). This suggests that parental spawning season and, as a consequence of it, the age of the progeny at a critical season, would be one of the most important factors affecting plasma ALPP level within each cycle of reproduction. Monitoring seasonal profiles of plasma ALPP without considering the age of fish could easily create confusion or even be misleading.

The large coefficients of variation and the estimated high heritabilities indicated that plasma ALPP profiles have considerable phenotypic and genetic variability. Therefore, selection to alter the plasma ALPP level of trout at a particular season should be effective. Successful selection for season of spawning may involved selection of females with high plasma ALPP levels since such selection must operate on differences in age at vitellogenesis and age at sexual

maturity (Siitonen and Gall, 1990).

Correlation analysis showed that level of plasma ALPP was also positively and significantly associated with body weight, carcass weight, fork length, liver weight and gonad weight about 9 mo before spawning. These consistently positive phenotypic correlation coefficients indicate that fish with above average levels of plasma ALPP before sexual maturation were also heavier (in both body weight and carcass weight) and longer, with larger livers and more advanced gonadal development.

Table I. Plasma ALPP concentrations (μg/ml) in two stocks of Rainbow trout fed a low protein, low energy diet (L), a commercial diet (C) or a high protein, high energy diet (H).

Age (Month)	Male N	Male Mean	Male SD	Male CV%	Female N	Female Mean	Female SD	Female CV%
RTH on L Diet:								
12	16	6.21	2.13	34.2	26	5.83	1.57	27.0
14	17	4.23	1.88	44.4	16	4.28	1.43	33.3
16	22	5.91	1.19	20.2	19	5.39	1.98	36.7
18	16	7.87	5.85	74.3	17	9.53	4.48	47.0
20	22	8.07	2.33	28.8	18	21.67	14.31	66.1
22	11	6.55	1.97	30.1	9	39.17	30.31	77.4
24	8	4.63	0.92	19.8	24	180.46	110.44	61.2
RTH on C Diet:								
12	23	5.56	1.28	23.0	15	5.43	1.10	20.2
14	3	4.83	0.29	6.0	0	na[1]	na	na
16	21	6.11	1.86	30.5	13	5.75	1.22	21.2
18	4	8.50	2.89	34.0	4	11.50	4.65	40.5
20	14	8.91	3.42	38.4	13	20.18	12.45	61.7
22	0	na	na	na	0	na	na	na
24	5	5.80	0.84	14.4	4	197.00	59.09	29.9
RTH on H Diet:								
12	19	6.08	2.06	33.9	23	6.00	1.48	24.7
14	20	5.95	1.52	25.6	10	5.65	1.31	23.2
16	19	6.54	1.33	20.3	22	6.02	1.81	30.1
18	17	9.85	3.79	38.4	12	17.33	9.35	53.9
20	17	7.71	2.95	38.3	24	22.63	9.71	42.9
22	7	7.71	0.95	12.3	8	55.87	41.11	73.6
24	11	5.32	1.82	34.2	17	89.29	61.86	69.7
RTL on L Diet:								
12	29	6.38	2.11	33.1	28	5.86	1.69	28.9
14	22	8.86	2.66	30.0	19	8.84	2.31	26.2
16	29	6.79	2.38	35.1	28	8.63	2.45	28.4
18	27	7.61	2.75	36.1	22	11.28	5.64	49.9
20	34	5.37	3.05	56.9	22	13.93	7.85	56.4
22	16	7.94	5.57	70.1	27	54.96	30.10	54.8
24	11	5.73	2.10	36.7	41	116.21	90.95	78.3

Table I (continued).

	Male				Female			
Month	N	Mean	SD	CV%	N	Mean	SD	CV%
RTL on C Diet:								
12	35	6.09	2.43	39.9	25	6.12	2.51	40.9
14	37	8.24	2.95	35.8	23	7.91	1.41	17.8
16	34	6.75	1.26	18.7	26	8.33	2.23	26.8
18	29	7.05	2.06	29.3	31	10.98	4.49	40.9
20	35	7.87	4.22	53.7	26	19.85	8.18	41.2
22	26	5.85	2.85	48.7	28	76.09	46.73	61.4
24	14	7.43	2.62	35.3	37	164.93	112.49	68.2
RTL on H Diet:								
12	36	6.39	2.28	35.7	24	6.87	2.13	31.0
14	30	7.20	2.68	37.2	25	7.16	2.46	34.3
16	35	8.81	2.59	29.4	25	8.62	2.60	30.1
18	33	8.45	2.72	32.2	20	12.43	4.12	33.1
20	30	7.18	4.38	60.9	28	19.11	9.63	50.4
22	24	9.19	1.05	11.4	31	84.42	73.58	87.2
24	15	7.50	1.52	20.3	39	166.11	98.27	59.2

[1]na: data not available.

Table II. The effects of stock, sire, diet and stock by diet interaction on plasma ALPP levels for rainbow trout males and females at different ages.

	Age at Sampling											
	Month 14		Month 16		Month 18		Month 20		Month 22		Month 24	
Source	df	MS	df	MS	df	MS	df	MS	df	MS	df	MS
Males:												
Stock(T)	1	189**	1	33*	1	18	1	41	1	52*	1	25*
Sire/T	38	10**	41	5	39	21**	43	16	31	7	32	5
Diet(D)	2	3	2	13*	2	61**	2	15	2	34*	2	11*
T x D	1	47**	2	1	2	20*	2	16	1	2	2	1
Residual	85	5	113	3	81	6	103	12	48	11	26	2
Females:												
Stock(T)	1	75**	1	209**	1	51	1	513*	1	1767	1	12
Sire/T	35	5*	43	6*	38	30	43	95	32	4946**	39	11684
Diet(D)	2	2	2	1	2	181**	2	72	2	3938	2	9744
T x D	1	25**	2	1	2	185**	2	106	1	294	1	62005**
Residual	53	3	84	4	62	29	82	109	66	1603	117	8977

*: P<0.05, **: P<0.01.

Table III. Phenotypic correlations of the natural logarithm of plasma ALPP level with body weight (BW),carcass weight (CW), fork length (FL), liver weight (LW) and gonad weight (GW) for females from two stocks of rainbow trout.

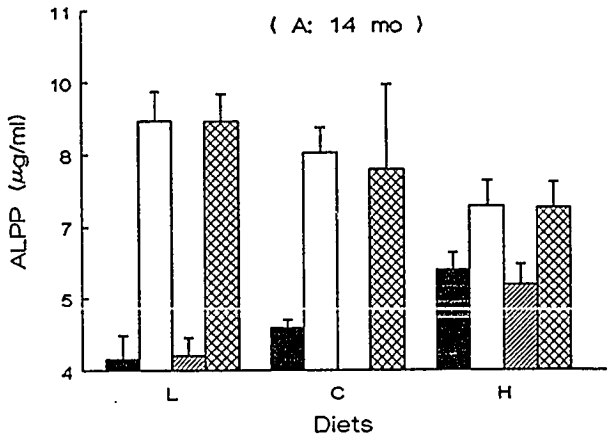
Month	Stock	BW	CW	FL	LW	GW
12	RTH	-0.16	-0.16	-0.13	-0.15	-0.13
	RTL	0.15	0.16	0.13	0.11	-0.03
14	RTH	0.42	0.47	0.52	-0.17	-0.28
	RTL	0.29	0.29	0.27	-0.09	0.26
16	RTH	0.45**	0.45**	0.57**	0.13	0.28
	RTL	0.42**	0.41**	0.29*	0.21	0.40**
18	RTH	0.69**	0.70**	0.71**	0.69**	0.75**
	RTL	0.38**	0.35**	0.31*	0.39**	0.49**
20	RTH	0.66**	0.65**	0.55**	0.58**	0.61**
	RTL	0.57**	0.56**	0.61**	0.44**	0.74**
22	RTH	-0.25	0.07	-0.28	0.26	-0.04
	RTL	0.59**	0.59**	0.24	0.64**	0.58**
24	RTH	0.28	0.30	0.41*	0.00	0.16
	RTL	0.13	-0.01	0.15	0.35**	0.64**

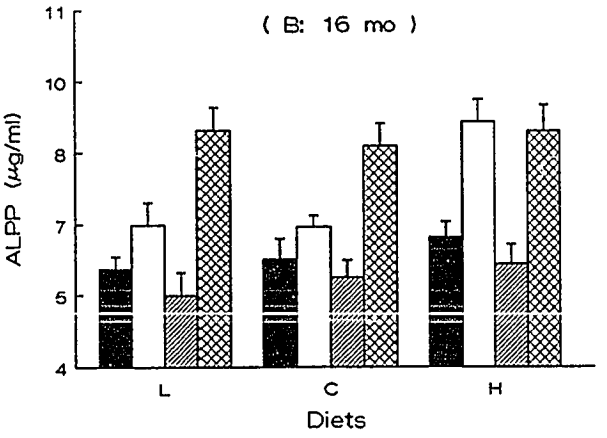
*: $P<0.05$, **: $P<0.01$.

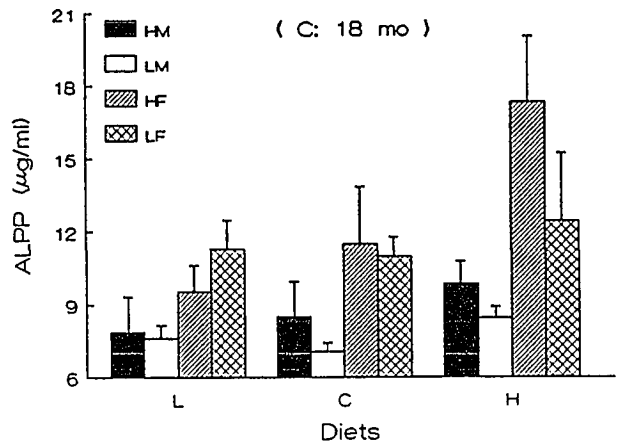
Table IV. Heritabilities of plasma ALPP level for female rainbow trout

Month	h^2
12	0.71±.38
14	0.87±.52
16	0.71±.40
18	0.02±.46
20	0.00±.00
22	1.66±.43
24	0.00±.00

Fig 1. Stock by diet interaction effects on plasma ALPP level. (A): For males and females at 14 mo, the stocks differed greatly when fed diet L, but the relative differences were dramatically smaller on diet H. (B): The interaction was present. (C): At 18 mo, average plasma ALPP level of RTH females was lower than that of RTL females on diet L, were similar to RTL on diet C, and were reversed in rank on diet H. Average level for males was inconsistent over the three diets. (D): Average ALPP level of RTH females was higher than RTL on both diet L and C, but lower on diet H. HM = RTH males, LM = RTL males, HF = RTH females and LF = RTL females.







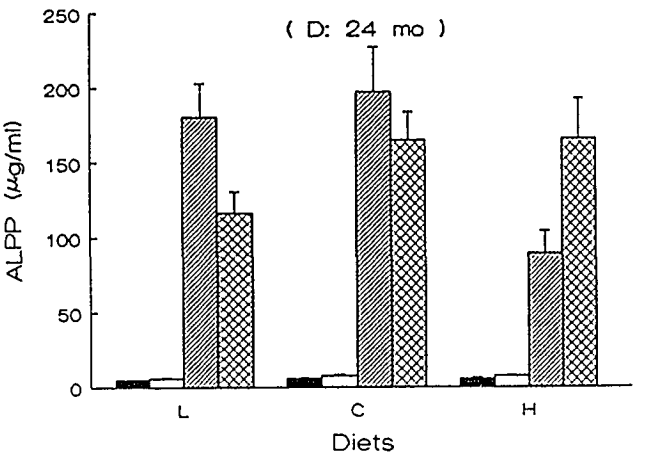
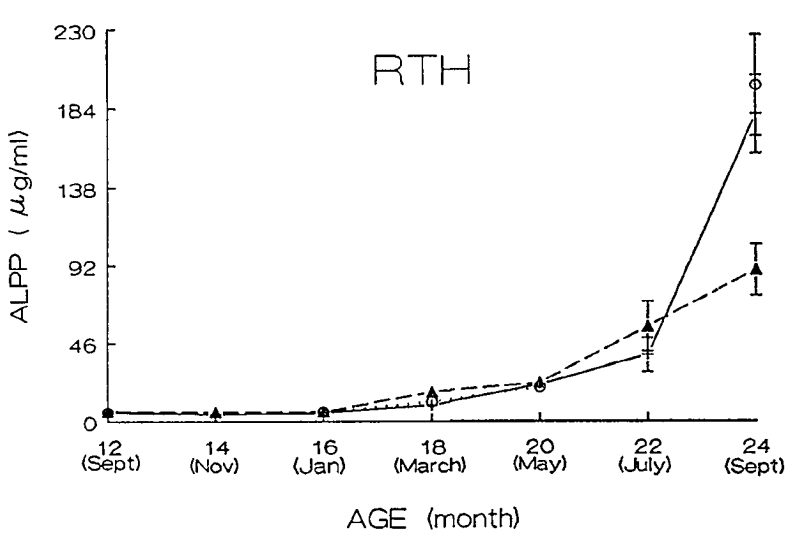


Fig 2. Average plasma ALPP levels of female rainbow trout demonstrating the changing patterns over the time period of 12 to 24 months of age. Data were not available for diet C at 14 and 22 mo for RTH. Diet L -+-; Diet C ··o··; Diet H - ▾ - .



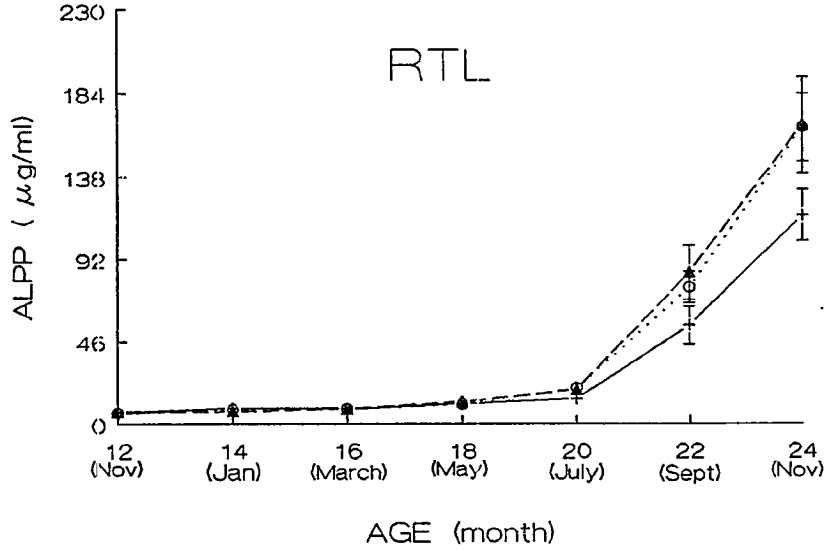
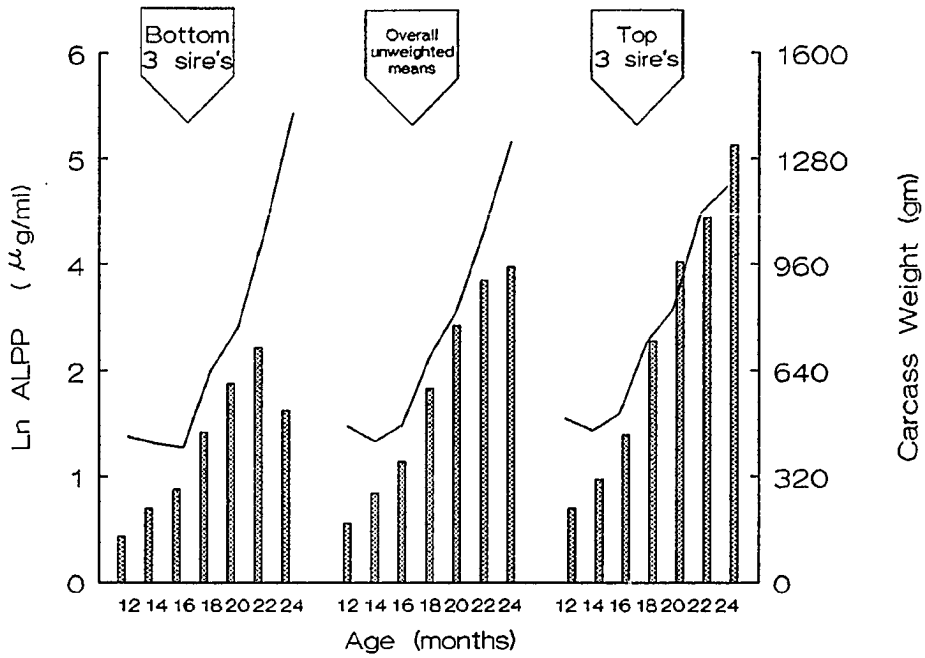
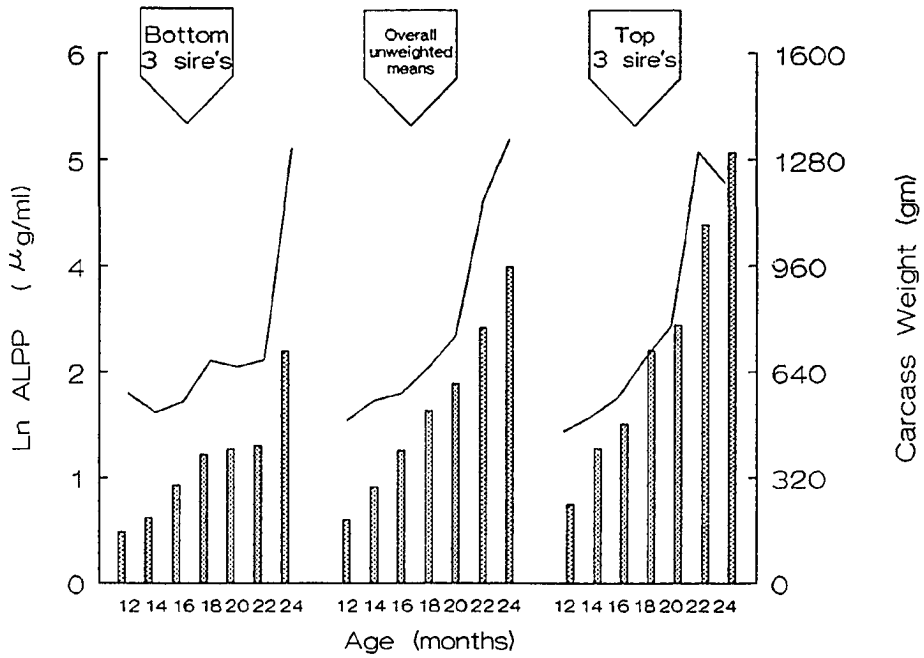


Fig 3. Unweighted means of ALPP and carcass weight of daughter for all sires; the three sires with daughters of lowest carcass weight (bottom 3 sires) and the three sires with daughters of highest carcass weight (top 3 sires) for RTH (Top graph) and RTL (Bottom graph) over the time period of 12 to 24 mo. The progeny group of the top three sires, those with a rapid increase in carcass weight, showed a rapid early increase in ALPP level, while the progeny group of the bottom three sires showed a slower increase in ALPP level. Lines represent plasma ALPP level, and the histograms are carcass weight.





Appendix 1.

Formulation of the diets

Ingredients	Formulation L	H	C
Anchovy Meal	9.8	35.0	
Herring Meal	11.8	35.0	
Soybean Meal	9.8	20.0	
Meat & Bone Meal	9.8	---	closed
Brewers Yeast	2.9	3.0	formula
Durabond	2.5	2.5	diet
Dicalcium Phosphate	2.0	---	
White Flour	45.0	0.7	
Fish Oil	5.9	3.2	
Vitamin Premix	0.25	0.25	
Trace Mineral Premix	0.09	0.10	
Choline Chloride	0.20	0.20	
Ascorbic Acid	0.06	0.06	
	Proximate Composition (% as fed)[1]		
Moisture	10.0	8.6	9.9
Lipid	10.7	10.8	13.5
Protein	37.5	63.5	48.7
Ash	9.9	12.3	10.9
Carbohydrate	29.6	2.5	14.7
Crude fibre	2.3	2.3	2.3
DE (Kcal/kg)[2]	2792	3381	3214

1. Proximate composition of diets was determined by the AOAC method (1984).

2. Digestible energy of diets was estimated using 3.9, 8 and 1.6 Kcal for each gram of dietary protein, lipid, and carbohydrate as suggested by Phillips (1972).

Chapter 4.

Effect of sire, diet and week of spawning on volume of eggs retained by artificially spawned rainbow trout

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ABSTRACT

Based on the performance of 223 mature, artificially spawned, 2-year-old rainbow trout females, consisting of progeny from 26 sires and 52 dams, the average volume of eggs retained in the ovary and the body cavity (35.9 ml) represented 14.7% of the average of 244.2 ml of eggs spawned per female. Although not specifically measured, it was observed that the majority of the retained eggs were held in the ovary. The variability among females was very large; the standard deviation was 18.7 ml with a coefficient of variation of 52.1%. In terms of number of eggs, an average of 357.4 eggs (14.5%) out of a total of 2453.9 were retained by the female. Diet had a significant effect on eggs retained. Fish reared on a high protein, high energy diet retained an average of 41.5 ml which was significantly higher than the 29.4 ml retained by females reared on a low protein, low energy diet. The volume of eggs retained by females reared on a commercial control diet (intermediate levels of protein and energy) was intermediate (36.5 ml). Differences in volume of eggs retained among weeks of the spawning season were not significant. The distribution of genetic effects for volume of eggs retained was evaluated by estimating breeding values for daughters from each sire, using data corrected for diet effects.

The estimated breeding values ranged from a low of 23.9 ml to a high of 62.2 ml. The average values for paternal half sib groups ranged from 26.8 to 45.4 ml, with a mean of 35.5 and standard deviation of 5.1 ml. The estimated heritability of volume of eggs retained was 0.34 + 0.22.

INTRODUCTION

During the last decade, considerable research has involved the spawning performance of rainbow trout. Some of the experimental evidence showed that the spawning season of non- interbreeding stocks may vary from August through April (Busack and Gall, 1980) and season of spawning can be altered by water temperature (Kaya, 1977) and light (Elliott et al, 1984). Photoperiod regimes such as 18 h light : 6 h dark and long days early in the year followed by short days regimes have been shown to advance spawning time from 6 weeks to 6 months (Scott et al, 1984; Bromage et al, 1983, 1984; Elliott et al, 1984), while constant short days or short days followed by constant long days are effective in delaying spawning time by up to 4 months (Bromage et al, 1983; 1984; Bourlier and Billard, 1984). Changing spawning season with altered photoperiod can affect spermatogenesis, follicle diameter, egg quality and fecundity. In addition, diet can alter egg production performance of fish (Harris, 1984; Shimma et al, 1978) and available spawning space in natural conditions might be another factor that affects the overall spawning success of rainbow trout from egg deposition to fry emergence (Hayes, 1987).

Overall egg production will depend also on the extent that ovulated eggs in well developed ovaries are released at

the very moment of stripping. Although no reports appear to exist, it can be speculated that some eggs will be retained in the ovaries of rainbow trout after artificial spawning. Additional eggs may be left in the abdominal cavity as a result of ineffective stripping. The present study was undertaken to quantify the retained egg mass and attempt to identify genetic and environmental factors which might determine the quantity of eggs retained by artificially spawned, hatchery-reared rainbow trout.

MATERIALS AND METHODS

Experimental Protocol

A total of 223 rainbow trout females from a commercial cooperator stock were spawned at 2 years of age. The females were the progeny of 26 sires and 52 dams. Initial feeding took place in partitioned hatchery troughs where full-sib groups could be identified until they were marked with a combination of fin clips and moved to 1.2 m circular tanks. At seven months of age, all fish were placed in six 3.66 m circular tanks where they remained until spawned. Males and females were reared together up to the time they were mature and sex could be determined. Water temperature ranged from 10.7oC in the winter to 15.9oC in the summer. The average water temperature was of 14oC and the water flow to the 3.66 m tanks was 55 l/min. The fish were maintained under natural photoperiod throughout the experiment.

The three test diets (Appendix 1) differing in both crude protein and digestible energy level were used to provide an environmental factor. One was a low protein, low energy diet (L), locally formulated to contain about 37.5% crude protein and 2792 Kcal/kg digestible energy (DE). The second diet was a high protein, high energy diet (H) formulated with 35% herring meal and 20% soybean meal plus other standard ingredients. It contained a minimum of 63.5% crude protein and

3381 Kcal/kg DE. The final diet was a commercial trout broodstock feed of the type currently in wide use on trout farms, and was used in this experiment as a control diet (C). It contained about 48.7% crude protein and 3214 Kcal/kg DE. All diets contained a standard double vitamin package.

All fish were fed a standard commercial trout feed for the first year. At one year, full-sib families within sires were assigned to at least two of the three test diets where they remained through spawning. Of the total of 223, 80 females sired by 18 males were raised on the C diet, 74 females sired by 19 males were raised on the H diet, and 69 females sired by 15 males were raised on the L diet. Fish were fed by hand four times per day and feeding rate was adjusted bi-weekly in an effort to maintain maximum growth by feeding to satiation.

All the fish were checked for ripeness and spawned on a weekly basis by one experienced technician. After stripping, the quantity of stripped eggs were measured in a graduated cylinder to the nearest 1.0 ml and recorded as spawned egg volume. A sample of 30 ml of stripped eggs per female was taken and counted. Each fish was then individually dissected. There were very small portions of released eggs lying in the body cavity, and unovulated eggs in the ovaries. All eggs not released were cleaned out, immediately measured in a graduated

cylinder and recorded as retained egg volume. Spawned egg number and retained egg number were estimated for each female from egg volume and the number of eggs in the 30 ml subsample of spawned eggs. Total egg volume and number were calculated for each female as the sum of eggs spawned and retained.

Data Analysis

The statistical model employed in the analysis was a reduced model with all the possible interaction terms deleted since none of the interactions was statistically significant. The model was

$$Y_{ijkl} = \mu + D_i + W_j + S_k + e_{ijkl}$$

where Yijkl was the observation on the lth female the rom kth sire, spawned in the jth week of the spawning season, and raised on the ith diet; u was a constant; Di was the fixed effect of the ith diet, i= 1, 2, 3; Wj was the fixed effect of the jth week of spawning, j= 1, 2, ..., 9; Sk was the random effect of the kth sire, k= 1, 2, ..., 26; and eijkl was the random error of the lth observation, l= 1, 2, ..., nijk.

The fixed effects were not estimatable due to the unbalanced nature of the data. However, one of the possible solutions of the fixed effects as obtained using Harvey's (1975) least squares method of estimating least squares (LS)

constants. The difference between the LS constants for different diets and different weeks yielded best linear unbiased estimates (BLUE) for diet and week since

$$D_i - D_{i'} = \mu+D_i+W_j+S_k - (\mu+D_{i'}+W_j+S_k)$$
$$W_j - W_{j'} = \mu+D_i+W_j+S_k - (\mu+D_i+W_{j'}+S_k)$$

are estimatable and μ is the overall LS constant, Di and Wj are the LS constants for diet and week effects, respectively and the Sk are the sire effects. A sum to zero constraint was imposed on the solutions of the LS constants for both diets and weeks. The standard errors for the BLUE's were computed as follows, where diets (Di) can be replaced with weeks (Wi).

$$\sigma_{(Di-Di')} = \left[g^{ii} + g^{i'i'} - 2g^{ii'} \right]\sigma_e^2$$

where g^{ii}, $g^{i'i'}$ and $g^{ii'}$ are the corresponding elements of the inverse of the coefficient matrix and σ_e^2 is the estimated error component of variance. F-tests were used to assess level of significance. F-values for the tests concerning differences between fixed effects of diets and weeks, which tested the likelihood that all corresponding BLUEs were zero, were calculated as

$$F(H) = (k'b^o)'[k'Gk]-1(k'b^o)/(r\sigma_e^2)$$

where k is the contrast matrix; b^0 is the vector of solutions of the estimates involved in the test; G is the generalized inverse of the coefficient matrix, and r is the rank of the contrast matrix.

Components of variance attributable to sire groups (σ_s^2) and error (σ_e^2), after adjustment for diet and week effects, were estimated by Harvey's (1970) indirect method. The ndirect method is featured by the procedure of absorption of the equations for the constant u and the set of random effects in a mixed model and is preferred over direct methods for computational convenience in applications where the number of the random effect classes is large (Henderson, 1953). Heritability of retained egg volume was estimated as:

$$h^2 = \frac{4\ \sigma^2_s}{\sigma^2_s + \sigma^2_e}$$

The standard error of the estimate of heritability was computed with a modified formula of Swiger et al (1964);

$$\sigma^2_{h^2} = \frac{1}{a^2_{ii'}} \left\{ \frac{2(n.-1)(1-t)^2\ [1+(k-1)t]^2}{k^2\,(n.-s)(s-1)} \right\}$$

where

$$k = \frac{1}{s-1}\ (n.- \frac{1}{n.}\ \Sigma n^2_i\ )$$

$$t = \frac{\sigma^2_s}{\sigma^2_s + \sigma^2_e} = \text{intraclass correlation coefficient,}$$

$a_{ii'}$ = additive genetic relationship among the female progenies within each sire group, n. = total number of observations, s = number of sires, and n_i = number of female progeny of the i^{th} sire.

A similar mixed model of

$$Y_{ijk} = \mu + D_i + W_j + A_k + e_{ijk}$$

was used to estimate the breeding values of female progeny, where all terms are the same as before except Ak, which is the breeding value of each female. Estimates of breeding values of females, based on sire, dam and sib effects, were obtained by the best linear unbiased prediction technique (BLUP, Henderson 1972), in which the procedure leads to the correction of observations for all fixed effects with the best linear unbiased estimates of the fixed effects. The mixed model equations were solved following the procedures described by Misztal and Gianola (1987).

RESULTS

The means and standard deviations of spawned, retained and total egg volume and egg number are given in Table 1. The overall mean and standard deviation of retained egg volume were 35.9 ml and 18.7 ml, respectively, which resulted in a high coefficient of variation of 52.1%. The average retained egg volume accounted for 14.7% of the average total egg volume of 244.2±4.7 and ranged from a minimum of 1.2% to a maximum of 53.2%; average retained egg volume accounted for 17.3% of the average stripped egg volume, ranging from 1.4% to 62.4%. In terms of egg number, the average number of eggs retained (357.4±11.7) was 14.5% of the average total egg number (2453.9±44.7); the number of eggs retained was 17.1% of the average number of eggs stripped.

The distribution of retained egg volume deviated from normality (Table 2) with a distinct skew toward high values. The retained egg volume for 95% of the fish was within the interval of 3 and 70 ml; the remaining 4.9% fell in a range from 71 to 130 ml.

Analysis of variance showed that diet was a very significant environmental factor affecting the quantity of eggs retained (Table 3). Week in which the fish ripened and spawned did not play a significant role in determining

retained egg volume. Sire, a genetic factor, also had a significant effect on the quantity of eggs retained.

The means, standard deviations, coefficients of variation, and solutions of the least square constants for each diet and each week are given in Table 4. Females raised on the H diet had an observed average retained egg volume of 41.5±2.3 ml while those raised on the C diet had an average retained egg volume of 36.5±2.1 ml. Females raised on the L diet had the lowest observed average retained egg volume of 29.4±1.7 ml. The best linear unbiased estimates of the difference between the effect of the L and C diets was 4.3±3.5 ml, which was not significantly different from zero ($P > 0.05$) but the difference between the H and C diets of 13.3±4.0 ml was highly significant ($P > 0.01$).

The best linear unbiased estimates of differences between week effects were not statistically different ($P > 0.05$) except for those between week 3 and week 4, and week 6 and week 8.

The distribution of genetic variation for retained egg volume was demonstrated by calculating average progeny breeding values for each sire (Table 5), corrected for diet and week effects. The estimated breeding values of all individual daughters varied from a low of 23.9 ml to a high

of 62.2 ml. The range of the estimated individual breeding values of daughters within sires varied from 32.2 to 33.2 ml (cv of 1.6%) as a minimum and from 38.3 to 62.2 (cv of 17.4%) as a maximum. The average daughter breeding value for all sire groups ranged from 26.8 to 45.4 ml with a sire group mean of 35.5 and a between sire group standard deviation of 5.1 ml.

The coefficient of variation of the estimated average daughter breeding values among the sire groups was 14.4% compared to 9.7% for estimated average daughter breeding values within sire groups. Of the 26 coefficients of variation of average progeny breeding values within sires, 21 or 80.8% were below 14.4%, indicating generally larger genetic variation between sire groups than within sire groups.

Based on diet and week corrected variance estimates, the sire component of variance was 27.65 ml2. This yielded an estimated heritability of 0.34±0.22 for retained egg volume for hatchery raised, artificially spawned, 2-year-old rainbow trout females.

DISCUSSION

Although not recorded separately, it was noted that the released, unspawned eggs in the abdominal cavity were only a small proportion of the total volume of retained eggs and the quantity from fish to fish was almost constant. The major part of the retained eggs consisted of an unovulated egg mass in the ovaries, and varied greatly from on fish to another. The variability in volume of retained eggs observed was, therefore, mainly attributable to the unovulated eggs.

In addition to two obvious environmental factors, accuracy of determining ripeness and skill of the spawner, the data showed that dietary crude protein and energy content are also important environmental factors impacting on the quantity of eggs retained by the female after artificial spawning. The results of this experiment suggest that, at least in the range of 30% to 58% crude protein along with an average of 2640 to 3232 Kcal/kg of digestible energy, females on a high protein high energy diet retain a significantly larger number of eggs than females fed a low protein, low energy diet. Further research is needed to determine the optimum dietary content of crude protein that would be sufficient for maximum development while having a minimum effect on retained egg volume at spawning.

Roley (1983) reported that dietary protein level did not affect the absolute number of eggs spawned relative to egg size in two feeding trials with rainbow trout. This is equivalent to saying that protein level of the diet did not effect spawned egg volume since egg volume is a function of egg number and egg size. If this was the case then the relationship between dietary protein level and production might be pictured hypothetically as high protein level enabling females to produce a higher total egg volume but more of eggs are retained compared to females on a low protein diet which would produce a lower total egg volume but also retain a lower egg volume. Consequently, the balance of the two factors could result in no significant differences in the number of eggs spawned by females on high protein and low protein diets.

The genotype of the female also plays an important role in determining the quantity of eggs retained by rainbow trout. In efforts toward genetic improvement, selection to reduce retained egg volume should be effective since the heritability of the trait appears to be quite high, although the observed standard error of the estimate was large. Since retained egg volume is a maternal character that requires slaughtering of females to quantify the trait, it is a difficult trait to evaluate in a selection program. One possible approach would be to base selection on estimated breeding values of males

using data from the first spawning of surplus daughters. It will also be important to assess the correlation between female performance at first and second spawning since most commercial egg production is obtained from 3-year and older females.

It is possible that high-protein high energy diet resulted in larger egg volume and longer ovulation time. If ovulation time was longer, and fish on all diets were stripped with same interval of time, the probability of partial ovulation in high-protein high-energy diet would be lighter compared to other diets.

Alternative explanation is that diet H had caused physiological disturbance during the pre-ovulatory period, due to a high level of absorbed nutrients in blood. Typically, salmonid fish do not eat or eat little during pre-spawning period in the wild, and show degenerative changes in their gut, and reduced absorptive capacity of the gut (Buddington, Doroshov and Gall, 1987).

Table 1. Mean and standard deviation (SD) for egg volume and egg number for 223 matured rainbow trout females spawned by stripping at two years of age.

	Egg volume (ml)		Egg number (#)	
Fraction	mean	SD	mean	SD
Spawned	208.3	63.4	2096.4	615.9
Retained	35.9	18.7	357.4	175.1
Total	244.2	70.6	2453.8	667.2

Table 2. Distribution of retained egg volume for 2 year old rainbow trout females after artificial stripping.

Retained eggs (ml)	Frequencies		Cumulative %
	Number	%	
0— 10	4	1.8	1.8
11— 20	38	17.0	18.8
21— 30	51	22.9	41.7
31— 40	65	29.1	70.9
41— 50	29	13.0	83.9
51— 60	18	8.1	91.9
61— 70	7	3.1	95.1
71— 80	3	1.3	96.4
81— 90	3	1.3	97.8
91—100	3	1.3	99.1
101—110	0	0.0	99.1
111—120	1	0.4	99.6
121—130	1	0.4	100.0

Table 3. Mean squares for volume of eggs retained (ml) for female rainbow trout artificially spawned at 2 years of age. The sire variance component was 27.65.

Source	d.f.	Mean square	F
Diet	2	2646.39	5.96**
Week/D	8	444.13	1.33
Sire/D/W	25	515.94	1.72*
Residual	187	299.50	

* $P < 0.05$
** $P < 0.01$

Table 4. Means, standard deviations (SD), coefficients of variation (CV), and estimated least squares (LS) constants for diets and weeks for rainbow trout females artificially spawned at 2 years of age.

	Number females	Mean (ml)	SD (ml)	CV (ml)	LS constant (ml)
Diet[1]					
L	69	29.4	14.1	48.0	-7.27+2.0
C	80	36.5	19.2	52.6	-3.01+2.1
H	74	41.5	20.2	48.7	10.28+2.4
Week					
1	8	24.4	8.4	34.6	-2.81+6.2
2	10	40.1	21.1	52.6	6.50+5.6
3	30	30.4	12.6	41.4	-3.25+3.3
4	44	40.5	20.6	50.9	6.87+3.1
5	54	35.7	20.5	57.5	-0.00+2.8
6	22	38.9	16.6	42.8	-3.44+4.0
7	25	34.8	19.7	56.6	-2.25+3.9
8	25	34.5	14.8	42.9	-2.87+3.7
9	5	42.6	31.9	74.9	1.25+7.8

1 L = Low protein, low energy diet; H = high protein, high energy diet; C = standard commercial broodstock diet.

Table 5. Estimated average progeny, breeding values (BV) for retained egg volume, and the corresponding coefficients of variation (CV) for each sire in a hatchery raised stock of rainbow trout spawned at 2 years of age.

Sires	Number daughters	Daughter BV (ml)	CV%
Sire 54	14	26.8	8.3
Sire 48	6	27.9	9.2
Sire 44	6	28.5	5.5
Sire 37	3	28.6	2.2
Sire 62	8	30.3	14.1
Sire 43	3	30.4	3.5
Sire 49	7	30.5	10.2
Sire 31	3	32.6	1.6
Sire 66	12	33.4	14.8
Sire 40	15	34.2	7.3
Sire 67	12	34.9	16.2
Sire 53	13	35.0	8.7
Sire 33	4	35.1	8.7
Sire 63	2	35.1	4.1
Sire 42	12	36.0	5.5
Sire 47	9	36.1	9.1
Sire 41	9	37.7	13.8
Sire 51	11	38.9	9.9
Sire 50	6	39.1	9.1
Sire 39	18	39.9	16.5
Sire 52	4	40.1	6.6
Sire 38	12	40.5	6.4
Sire 34	7	40.8	16.5
Sire 55	12	42.5	13.9
Sire 45	8	43.1	12.0
Sire 61	7	45.4	17.4
Average		35.5	9.7

Appendix 1.

Formulation of the diets

Ingredients	Formulation L	H	C
Anchovy Meal	9.8	35.0	
Herring Meal	11.8	35.0	
Soybean Meal	9.8	20.0	
Meat & Bone Meal	9.8	--	
Brewers Yeast	2.9	3.0	closed
Durabond	2.5	2.5	formula
Dicalcium Phosphate	2.0	--	diet
White Flour	45.0	0.7	
Fish Oil	5.9	3.2	
Vitamin Premix	0.25	0.25	
Trace Mineral Premix	0.09	0.10	
Choline Chloride	0.20	0.20	
Ascorbic Acid	0.06	0.06	
Proximate Composition (% as fed)1			
Moisture	10.0	8.6	9.9
Lipid	10.7	10.8	13.5
Protein	37.5	63.5	48.7
Ash	9.9	12.3	10.9
Carbohydrate	29.6	2.5	14.7
Crude fibre	2.3	2.3	2.3
Digestible energy (Kcal/kg)2	2792	3381	3214

1. Proximate composition of diets was determined by the AOAC method (1984).

2. Digestible energy of diets was estimated using 3.9, 8, and 1.6 Kcal for each gram of dietary protein, lipid, and carbohydrate as suggested by Phillips (1972).

CHAPTER V. SUMMARY AND CONCLUSIONS

Data collected during the first year of experiment showed that stock, sire and dam had significant influences on body size characters (body weight, carcass weight and fork length) and developmental traits (liver weight, gonad weight, oocyte diameter, condition factor, gonadosomatic index, and hepatosomatic index) at most stages of development from 4 to 12 months of age. By end of the first year, RTH fish reached an average body weight, carcass weight, and fork length of 221.1±5.08 gm, 193.0±4.55 gm and 25.6±19 cm while RTH was 203.9±5.98 g, 180.3±5.35 g, and 25.4±17 cm, respectively. It appeared that RTH fish exhibited slower overall growth during the first 12 months compared to RTL.

Additive genetic variance was detected for the body size characters, and estimated heritabilities based on sire components varied from 0.22±0.20 to 0.39±0.23. The heritability of liver weight and gonad weight appeared to be between 0.20±0.20 and 0.25±0.20 while that for oocyte diameter was near 0.35±0.60. The first year data also showed that body size characters were highly genetically correlated during this time. Genetic correlations between body weight and liver weight were also observed.

The analyses of the second year of the experiment focused on female reproduction. Again stock effects on body weight, carcass weight were significant. Average of relative growth rate showed very significant differences between the two stocks: RTH was the faster growing broodstock from 14 to 20 months of age. Although the average body weight of RTH was not heavier than RTL until 18 months of age, by age of 22 months, RTH females reached an average of 1143.9±58.6 g body weight whereas RTL females had an average of 935.2±31.5 gm, showing an average difference of 208.7 gm between the two stocks.

Diet effect on body size characters was not significant until 22 months of age, but there was significant effect of diet on relative growth rate from 14 to 18 months. The data showed that fish of both stocks on the high_protein, high_energy diet had larger body size and heavier liver at 22 and 24 months of age. Significant sire by diet interaction was also observed for body weight and fork length.

Canonical correlation analyses demonstrated that the canonical variable extracted from a body size group of traits consisting of body weight, carcass weight and fork length was highly correlated with the canonical variable of a developmental group of traits consisting of liver weight, gonad weight, alkali-labile protein phosphorus (ALPP) and

oocyte diameter. These results indicated high dimensional relationship between the two groups of traits. Canonical redundancy analysis showed that the canonical variable of the body size group could be used as one good predictor of the characters in the developmental group.

The data from the second year showed that RTH females matured earlier than RTL. Since fast growth rate was also observed in RTH females, this might suggest that growth rate and age at sex maturity are negatively correlated, that is, early maturing fish grow fast.

ALPP concentrations of 887 male and 877 female rainbow trout from the two stocks was determined at two month intervals from 12 to 24 months of age. Average ALPP level for males was as 6.95±.21 μg/ml and remained relatively constant throughout the experimental period. Females maintained the same level of ALPP as males until 16 month and then it increased as the fish approach of maturation. A peak value of 400.00 μg/ml was observed for females at maturity. The minimum level of plasma ALPP accompanying final ovarian maturation appeared to be about 90 μg/ml. Significant stock and diet effects were observed at some sampling intervals; it appeared that fish fed a high protein and energy diet exhibited higher ALPP levels. Genetics effects, as estimated from sire variation, on the plasma ALPP level of females were significant. Estimates of

heritability ranged from 0.71±.38 to 0.87±.52 for the period from 12 to 16 mo of age. Correlation analysis suggested that fish with higher plasma ALPP levels before sexual maturity were heavier and longer, with larger livers and gonads. This information is important since it suggests strong genetic correlation of vitellogenin synthesis by trout liver, not reported by previous investigators.

Diet and genetic effect on the volume of eggs retained in fish ovaries after artificial spawning were investigated for the first time. Significant diet effect on eggs retained was observed with fish reared on a high_protein, high_energy diet retaining more eggs. Significant differences were also observed among paternal half-sib groups. An estimated heritability of 0.34±0.22 based on sire component was obtained for the volume of eggs retained. Thus, the ovulatory response and the efficiency of egg production by stripping are determined by the environmental (diet) and genetic components.

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