Genetic Studies in Scottish Brown Trout (*Salmo trutta* L.)

by

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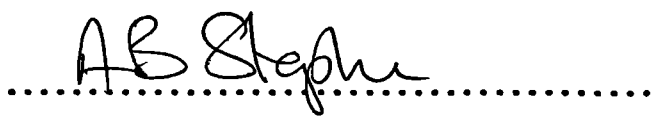
To my Mother and Father

Declaration

I hereby declare that this thesis has been composed by myself and is the result of my own investigations. It has neither been accepted nor is being submitted for any other degrees. All the sources of information have been duly acknowledged.



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Abstract

The Scottish brown trout (Salmo trutta L.) is identified as an important resource which requires responsible and continual management. This study was divided into two parts; an electrophoretic survey of wild trout populations in Scotland, and a quantitative assessment of the genetic component to growth rate in various stocks, grown under hatchery and farm conditions.

Sixty wild populations were sampled by various methods. All fish were typed using brain, eye, heart, liver and muscle tissue and starch gel electrophoresis for thirty four enzyme loci, thirteen of which were found to be polymorphic. Gene diversity analysis was conducted on the data collected, 33% of the diversity being attributed to differences between populations, much of the variation was thought to be due to founder effects. Evidence is presented to support a hypothesis that the trout in Scotland are derived from two main post glacial invasion stocks. Future management strategies for wild stocks of Scottish brown trout are discussed.

Growth trials were conducted at Howietoun fish farm in order to calculate heritability estimates for growth rate. Hierarchical and factoral crossing schemes were employed, using broodstock from three stocks. Heritability estimates for growth rate were found to be high and it was concluded, significant genetic gains could be achieved if growth rate was the only trait of commercial interest and truncated mass selection was adopted.

Attempts were made to investigate the relationship between heterozygosity and growth rate in the hatchery populations. It was concluded that more data were required to make a meaningful assessment, but from this study little evidence exists for a positive correlation between heterozygosity and growth rate.

Correlations between early life cycle stages and subsequent growth are discussed.

CHAPTER 1

1. Introduction

What we know as the science of genetics is meant to explain two apparently antithetical observations - that organisms resemble their parents and differ from their parents. That is genetics deals with both the problem of heredity and the problem of variation (Lewontin, 1974). The existence of an all encompassing theory concerning evolutionary and population genetics can only be possible once enough emphasis is attached to the concept of variation. Mendel recognised the importance of variation amongst offspring of the various breeding experiments he conducted and instead of taking an average description of those variations as being representative derived his all important laws from the very existence of variation.

Mendelism and Darwinism both regard the fact of variation and its nature as central and essential to their laws and theories. It is not surprising, therefore, that the study of genetically determined variation within and between species should be the starting point of modern day population and evolutionary investigations (Lewontin, 1974).

The genotypic distribution in a population is subject to a complex array of different factors that act separately and together to increase, decrease or stabalise the amount of variation (Lewontin, 1974).

Since the acceptance by geneticists that morphological/phenotypic variation did exist, the arguments concerning how this variation is maintained have raged ever since. Everyone concerned with scientific studies of evolution recognised that natural selection, a revolutionary concept developed by Darwin (1859), was the underlying cause and maintainer of variation.

It is the role of natural selection that genticists cannot agree on.

When Darwin formulated his theory the mechanism of inheritance and the nature of heritable variations were unknown and this prevented him from being fully confident of the role of natural selection.

By the end of the 19th century Mendelian principles had evolved and following the important contributions made by Hardy (1908) and Weinberg (1908), the consequences of Mendelian inheritance were worked out by Fisher, Haldane and Wright. All three produced various mathematical models and developed the stochastic theory of population genetics. In 1930 Fisher published "Genetical Theory of Natural Selection", in 1931 Wright published "Evolution in Mendelian Populations" and in 1932 Haldane published "The Causes of Evolution". These together represent the culmination of the classical population genetics whereby the synthesis of Darwinism and Mendelism was fully achieved. The orthodox view was that the rate and direction of evolution was almost exclusively determined by natural selection, with mutation, migration and random drift

playing no substantial roles.

In the USA the theory became known as the 'Synthetic Theory' of evolution.

Fisher is thought by Kimura (1983) to be responsible for the stagnation of innovative thought concerning the theory of evolution, especially in England where panselectionism has dominated for decades (Kimura, 1983). Throughout Fisher's writings he constantly minimises the role played by random drift in evolution and this Kimura suggests has discouraged English geneticists from pursuing the topic.

Fisher (1922) purported to show that overdominant alleles were actively maintained in a population by natural selection. Selection favoured the heterozygote and was a condition of stable equilibrium, and both alleles would continue in the stock. This hypothesis was to have profound influence on the later thinking of population and evolutionary geneticists.

Wright (1932) developed a theory of evolution that was later called "the Shifting Balance Theory". This disagreed with Fisher's view that migration and random drift play little or no role in the process of evolution. Wright's theory consisted of three phases, and concluded that a large subdivided population structure is most favourable for rapid evolutionary progress, throughout the shifting balance process.

The three phases were

1. Random Drift - extensive gene frequency drift occurs in local populations due to accidents of sampling or to fluctuations in the coefficients measuring various evolutionary processes.

2. Mass Selection - by chance a local population may cross one of the innumerable 'two factor saddles' in the surface of fitness values, leading to rapid genetic change in the local population.

3. Inter-population Selection - a population which came to a new fitness peak superior to surrounding populations will expand through inter-population selection.

Wright was misunderstood by following generations of geneticists. Many thought he was attributing undue importance to the process of random drift, compared to the process of natural selection. He was criticised by Fisher, and a fierce debate arose, and continues to this day.

The issue is whether random drift has an important role to play in evolution. Fisher and his followers were convinced that if a population is made up of many individuals the chance effect due to random sampling of gametes is negligible. Fisher (1953) also regarded the existence of substantial neutral mutants in a population to be impossible because he thought for most mutant alleles the product of the population size and the selection coefficient was

unlikely to be restricted to the near zero in the course of evolution. Kimura (1983) could not find any compelling evidence to suggest the shifting balance theory was correct.

Upon the foundation constructed by Fisher, Haldane and Wright as well as by Muller (1929), who in the early 1920's had discovered the fundamental nature of gene mutation, various studies of natural populations were conducted by Dobzhansky (1937). Dobzhansky worked on natural and captive populations of Drosophila pseudoobscura and investigated chromosome polymorphisms, especially inversion polymorphisms. Statistical analysis of his results indicated that inversion heterozygotes had higher fitness values than inversion homozygotes (Wright and Dobzhansky, 1946). Thus heterosis as it was called led Dobzhansky to hold the view that overdominance of heterozygote advantage at individual gene loci was prevalent in other natural populations.

Dobzhansky's view coincided with that of Lerner (1954) who was putting forward his theory of genetic homeostasis. According to Lerner, Mendelian populations are possessed of self-equilibrating properties tending to retain a genetic composition that produces maximum mean fitness. He claimed that the most likely mechanism for this is heterozygote advantage or heterosis, and for the normal development of the individual an obligate level of heterozygosity is needed. Kimura (1983) quotes Lerner "not only gene contents, but homozygosity as such must be considered to play a role in inbreeding degeneration."

Lerner (1954) emphasized the importance of epistatic interaction in fitness being influenced by Wright's concept of evolution as an irregular shifting state of balance. This school of thinking attached a paramount importance to the existence of heterozygosity as the "adaptive norm".

Dobzhansky (1955) condensed the thoughts of the current day geneticists and divided them into two schools of thought. The two hypotheses were called the classical and balance hypotheses.

Dobzhansky (1955) was regarded by Lerner as the direct protagonist for the balance hypothesis which held that the adaptive norm is an array of genotypes, heterozygous for a number of alleles. Homozygotes for these alleles occur in normal outbred populations only in a minority of individuals and they are inferior to heterozygotes in fitness. Natural selection plays a large role in maintaining heterozygosity and selection pressure favours the development of series of multiple alleles at many loci.

The classical hypothesis by contrast recognised heterozygosity as of minor importance. Although homozygosity in the wild population was considered the norm, heterozygosity was thought to have four main sources (Dobzhansky, 1955):

1. Deleterious mutants which are eliminated by natural selection in a certain number of generations.

2. Adaptively neutral mutants

3. Adaptive polymorphism maintained by the diversity of the environments.

4. The rare "good" mutants, in the process of spreading through the population.

Despite shaky evidence (Kimura, 1983), Dobzhansky has a tremendous influence on subsequent opinions among population geneticists particularly in the United States.

Some opposed the claim that overdominance or heterozygote supremacy (Muller, 1958) was playing a pre-dominant role in maintaining genetic variability, let alone the claim as made by Lerner (1954) that heterozygosity per se tends to be beneficial.

By the early 1960's, Dobzhansky and his school, along with paleontological studies of Simpson (1953), the ecological genetic studies of Ford (1964) and his followers, and the specialism theory of Ernst Mayr (1963) were combined to give the synthetic theory and the selectionists what to them seemed like a watertight case. A consensus was reached, that every biological character can be interpreted in the light of adaptive evolution by natural selection, and that almost no mutant genes were selectively neutral.

Up until the mid-1960's conventional studies of evolution were

conducted at the phenotypic level, and there was no way of unambiguously connecting the theory of population genetics with the concept of gene frequencies. The advent of molecular genetics removed these limitations. Two developments quickly followed. Firstly, it became possible through studies of amino acids sequences of proteins among related organisms to estimate the evolutionary rates of amino acid substitutions (Zuckerkandl and Pauling, 1965). This enabled an estimate of the evolutionary rates of nucleotide substitutions inside genes to be made. Secondly, the development of electrophoretic techniques enabled enzyme variability among individuals to be identified, and these studies have disclosed a wealth of polymorphic variants at the enzyme level in many organisms (Harris, 1966; Lewontin and Hubby, 1966).

The picture of evolutionary change that actually emerged from the molecular studies was ambiguous. Kimura (1968a) thought the evidence was quite incompatible with the expectations of selectionists, neo Darwinism and the synthetic theory of genetic evolutionary thinking.

Many population geneticists attempted to explain the polymorphism encountered in terms of overdominant selection with overdominant gene action (King, 1967). They thought such a high degree of polymorphism could not be maintained without some kind of balancing selection. Kimura (1968b) attempted to explain the findings by a different theory "the neutral mutation theory" and later (Kimura, 1969) the "neutral mutation-random drift hypothesis". Strong

support for these ideas came from King and Jukes (1969) who controversially coined the phrase "non-Darwinian evolution" to illustrate the differences from the neo-Darwinian-selectionist school of thought.

Unlike the traditional synthetic (neo-Darwinian view) the neutral theory claimed that the great majority of evolutionary mutant substitutions are not caused by positive Darwinian selection, but by random fixation of selectively neutral or nearly neutral mutants. The theory also asserts that much of the intraspecific genetic variability at the molecular level, such as identified in the form of protein polymorphism by electrophoresis, is selectively neutral or nearly so, and maintained in the species by the balance between mutational input and random extinction or fixation of alleles (Kimura, 1983).

The neutral theory is accompanied by a well developed mathematical theory and it attempts to treat quantitatively numerous problems of molecular evolution and polymorphism from the standpoint of population genetics.

Since 1970 the Neutralist v Selectionist argument has been continued to the present day. For recent reviews of the Neutralist argument see Kimura (1979, 1982, 1983) and Nei (1983). For reviews of the ongoing Selectionist v Neutralist argument see Crow (1972, 1981), Lewontin (1974) and Nei (1983).

A brief description of the mathematical and conceptual ideas relating

to the neutral evolution by random genetic drift is given below (from Kimura, 1983).

1. The probability that a selectively neutral mutant eventually spreads through the whole population is equal to its initial frequency. In a population of N diploid individuals, if a mutant allele is represented only once at the moment of appearance, the probability of its eventual fixation is 1/(2N).

2. The rate of decrease of the heterozygosity by random drift is 1/2(Ne) per generation, where Ne is the effective population size. Usually Ne is considerably smaller than N.

3. If a new allele is produced at a locus with the rate V per generation, then the average length of time between consecutive substitutions at alleles in the population is 1/V generations.

4. For each mutant allele destined to reach fixation it takes on average 4Ne generations from its first appearance until fixation, where Ne is the effective size of the population.

5. If the assumption is made that every mutation is unique and leads to a new allele (ie not pre-existing) then the expected frequency of homozygotes under mutation-random drift equilibrium is Ho = 1/(4NeV + 1) where V is the mutation rate, and Ne is

the effective population size. The reciprocal of $1/(4NeV + 1)$ is called the effective number of alleles (ne) so that $ne = 4NeV + 1$. The average heterozygosity of equilibrium ie 1 - Ho is then $He = \frac{4NeV}{4NeV + 1}$ (Kimura and Crow, 1964).

6. Consider the process by which molecular mutants are substituted one after another. Let K be the rate of evolution in terms of mutation substitutions. This is defined as the long term average of the number of molecular mutants that are substituted at a given locus or site in the species, per unit of time. If consideration is restricted to selectively neutral mutations only then K = V, where V is the mutation rate per unit time. In other words, the rate of evolution in terms of mutant substitutions in a population is equal to the mutation rate per gamete and is independent of population size. This remarkable property is only valid for neutral alleles. If the mutant substitution is due to positive Darwinian selection acting as definitely advantageous mutants, the corresponding formula for the rate of evolution is $K = 4NeSV$ where S is the selective advantage of the mutant alleles, and V is the mutation rate for such advantageous alleles. In this case the rate of evolution depends on the effective population size (Ne) and on selective advantage (S) as well as the rate (V) at which mutants having such selective advantage are produced in each generation. But if the mutant alleles are nearly neutral such that their selective advantage or disadvantage (S) is much smaller than

1/(2Ne) equation K = V holds approximately.

The neutral mutation - random drift hypothesis has in recent years been reinforced by two main discoveries.

1. The rate of molecular evolution is thought to be constant. The balance - selectionist hypothesis predicts that molecular evolution is not constant and is different for different organisms. For each protein the rate of evolution in terms of amino acid substitutions is approximately constant per amino acid site per year for various lineages. Evidence to support this finding comes from work done by Kimura (1979, 1982, 1983 and references within), which indicates that haemoglobin evolution has continued at the same rate in a variety of widely separated species, including what has been regarded as a living fossil - the Port Jackson shark.

   This apparent constancy of amino acid substitution rate in evolution was termed "a molecular evolutionary clock" by Zuckerkandl and Pauling (1965).

   Higher rates of evolution have been obtained, notably by Goodman et al., (1974, 1975) working on globin evolution. Goodman et al., (1975) claims this disproves the Neutral theory. Kimura (1981) reported that the reason the apparent evolutionary rates were so high was that assignment of geological dates to duplication events in the early history of globin evolution

were wrong.

The rate constancy assumption is obviously an important argument in the formation of the Neutral mutation hypothesis. Li and Tanimura (1987) review recent DNA sequence data and suggest this rate of constancy is not constant at all. Rates at nucleotide substitutions in rodents are estimated to be 4-10 times higher than those in higher primates and 2-4 times higher than those in artiodactyls.

Li and Tanimura (1987) go on to say that just because the rate of constancy concept has been violated, or apparently violated, this should not be taken as evidence against the neutral mutation hypothesis. Li and Tanimura (1987) point out "a serious criticism of the rate-constancy argument has been that the approximate constancy seen in protein sequence data is in terms of chronological time rather than generation number, but mutation rates in different organisms are more nearly comparable when measured in generations, than in absolute time units". They sum up by arguing the discovery that the rate constancy theory is not fundamentally correct, and that the rate of nucleotide substitutions is higher in short-lived organisms than in long-lived organisms, and this is actually more in line with the neutral mutation hypothesis, than if the rates were equal for all organisms.

2. The molecules or parts of molecules that are subject to less functional constraint evolve faster (in terms of mutant substitutions) than those that are subject to stronger constraint. This may explain some of the selectionist criticisms of the neutralist theory that rate of molecular evolution is constant. Kimura (1983) summarizes the evidence that suggests there is selective constraint affecting neutral substitutions. The fastest evolutionary rate for proteins is observed in fibrinopeptides, they become separated from fibrinogen during blood clotting and have little known function. Thus suggesting the weaker the functional constraint, the higher the evolutionary rate of mutant substitutions.

This theory is supported by work conducted on insulin. Insulin is formed from proinsulin made up of three units of peptides. peptide A and peptide B go to form insulin itself, whereas peptide C has no known function. Kimura (1982) worked out that peptide C evolved at at least 6 times the rate of peptides A and B. Haemoglobins which perform a vital function in carrying oxygen in the blood are under much more selective constraint than fibrinopeptides and thus have a much reduced evolutionary rate. Cytochrome C interacts with cytochrome oxidase and reductase and there is more functional constraint on cytochrome C than in haemoglobins. Thus cytochrome C has a lower evolutionary rate than haemoglobins (Kimura 1983). The theory has been expanded to nucleotide changes. It has been shown that substitutions at a codon's third position constitutes 70% of the

random nucleotide substitutions (known as synonymous as they seem not to lead to functional amino acid changes). Kimura (1983) has estimated from evidence derived from work done by Grunstein et al., (1976) who worked on histone H_4 messenger RNA sequences of two related sea urchins that the rate of nucleotide substitution per year at the third position of the codon was very high $(3.7 \pm 1.4) \times 10^{-9}$. These observations along with a systematic examination of synonymous nucleotide substitutions for various animals was explained once again by the fact that the weaker the functional constraint the higher the rate of evolutionary change.

Another observation concerning evolutionary rate is of importance in the neutralist-selectionist argument. There is evidence for the rapid evolution of pseudogenes. A pseudogene is defined as a region of DNA that shows definite homology with a known functional gene but has lost ability to produce a functional product due to mutational changes. Kimura (1983) maintains these genes have been liberated from the constraints of negative selection, and mutate much faster than their functional counterparts. He also observes that "unlike the 'conservative' mode of change that characterises the evolution of many normal genes, base substitutions at the first and second positions of codons in the pseudogenes occur just as frequently as they do at the third position".

Li et al (1981) performed a statistical analysis on the evolutionary rates of pseudogenes in a human globin pseudogene and established

that the average substitution rate per site for the pseudogenes during their non-functional periods is 4.6×10^{-9}, one of the highest rates of nucleotide substitutions so far estimated.

Although the estimate of substitutions rate is high, it is not as high as the neutral theory would suspect if there was no constraint acting at all (Kimura, 1983). The inference is that there must be some functional constant limiting the rate of substitution. This constraint was found to be the "non-random" usage of synonymous codons. Even though the number of synonymous codons can be as many as six, coding for a single amino acid, the availability of tRNA seems to be the limiting factor (Grantham et al.,1981). This leads to the hypothesis that the preferential codon usage represents the optimum state at which the population of synonymous codons matches the cognate tRNA available in the cell.

Clarke (1975) voiced considerable opposition to the whole idea of the neutral mutation - random drift theory. He put forward arguments in favour of the balance theory. He describes heterozygosity advantage at length, citing the enzyme alcohol dehydrogenase as a prime example, where heterozygotes for the gene producing the enzyme have a fitness advantage.

Frequency-dependant selection is a concept quoted by selectionsists to support the role of natural selection in balancing wild populations of organisms. The idea of frequency-dependant selection involves the concept that any advantage or disadvantage conferred on a variant

is dependant on the frequency of the variant (Clarke, 1975). Some of the evidence cited by Clarke included the idea that birds hunting prey by sight would maintain colour polymorphism in their prey populations, and mammals hunting by smell should maintain olfactory polymorphisms. He also cited work done on Drosophila melanogaster. In wild populations this species has two varieties which can be electrophoretically distinguished. It was found when populations of the two variants were kept together, each variant survived better when it was rare rather than when it was common. In these experiments no predators are involved in the frequency dependant selection. The inference concerning natural populations is they are better off polymorphic so that both variants survive better and exploit their habitat more efficiently than a genetically uniform population. The argument the neutralists raise to explain this evident polymorphism is that natural selection acts not on the alcohol dehydrogenase but on the products of some other gene on the same chromosome.

Clarke (1975) claims that the neutralist argument has been refuted by direct biochemical studies of the proteins. He claimed to show that they "differ not only in electrophoretic mobility but also in other ways more likely to be of significance in the economy of the organism."

Clarke (1975) claims the case of the classical and neutralist views of variation is weak. "It has been demonstrated that most natural populations of plants and animals are genetically heterogeneous. Moreover, there is strong evidence that the diveristy of forms

exists because natural selection favours it, that is because the variants themselves affect the survival and reproduction of the individual carrying them."

Kimura (1983) ends a discourse on the Neutral theory by saying "classical evolutionary studies have shown beyond doubt that positive Darwinian Selection is the major cause of evolutionary change at the phenotypic level, that is at the level of form and function." He goes on to sum up, that mutation and random genetic drift are the forces driving evolutionary change at the molecular level.

But why should positive natural selection be so prevalent at the phenotypic level and yet random fixation of selectively neutral or nearly neutral alleles prevails at the molecular level.

One answer is "stabilizing" selection, which eliminates phenotypically extreme individuals and preserves those that are near the population mean (Haldane, 1959). Various studies have been performed that support this theory (Parkin, 1979 for a review).

Kimura (1983) observes that as most phenotypic characteristics are determined genetically by a large number of loci in a genome each locus has a very small effect on the eventual phenotype. So the intensity of natural selection involved at each of the relevant loci is very small. Each mutation Kimura (1983) suggests will be neutral or slightly deleterious and mutant substitutions are mainly controlled by random drift.

Milkman (1982) suggested the idea that neutral molecular evolution is an inevitable process under stabilising phenotypic selection when a very large number of nucleotide sites are involved. He called it a "unified selection theory".

Nei (1983) reviewing the evolutionary arguments between the classical and balance theories, and summarizes, "it is very difficult to study a corresponding mathematical model and derive any testable predictions analagous to these for neutral mutations. One common feature of these hypotheses (balance and derivitives of the balance hypothesis) is that genetic variability is actively maintained by selection and leads to heterozygosity higher than the neutral expectations."

Livshits and Kobyliansky (1985) compare the schools of thought and conclude that neither satisfactorily explain completely the mass of collected data. They suggest the main cause of discrepancy in theories is that the variability of each locus is considered independently in both the approaches. They suggest the genome should not be regarded as an assortment of independent genes and that different loci influence the variability of other loci.

In the last 5 years many authors have produced results relating heterozygosity and fitness, measured by different morphological and meristic parameters. This information in theory should help settle the argument between the neutralists and the selectionists. The evidence so far accumulated seems to have added fuel to the

fires of controversy.

Mitton and Grant (1984) in a paper summarizing the association between protein heterozygosity growth rate and developmental homeostasis state, "Our interpretation of the literature leads us to believe that roughly 70-80% of the effects of growth and developmental stability can be attributed to heterozygosity per se, about 15-20% to the effects of specific gene combinations, and the remainder to as yet unidentified causes".

They also state that "the observation that heterozygosity strongly influence vigour and stability has been generally accepted in the applied literature for many years". Their premise that these observations are generally accepted is palpably untrue. There is much debate about the findings relating to heterozygosity but many of the results do seem to give support to the selectionists' theory of evolution.

Positive correlations between growth rate and individual heterozygosity were found by Singh and Zouros (1978) and Zouros et al.,(1980), working with the American oyster, Crassostrea virginica. Bivalves have been studied quite thoroughly since then and other such correlations have been recorded (Koehn and Gaffney, 1984; Diehl and Koehn, 1985). Koehn worked with the mussel Mytilus edulis but only found a positive correlation between heterozygosity and growth in the early stages of life. No such correlation was found once the mussels matured (Koehn and Gaffney, 1984). Mulinia lateralis

also exhibited positive correlations between heterozygosity and growth rate (Garton, et al.,1984) as did Macoma balthica (Green et al., 1983) and similar results were reported by Fujio (1982) for the Pacific oysters, Crassostrea gigas.

Although marine bivalves have recieved a lot of attention, many other animals have also been studied. Foetal growth rates were studied in the white tailed deer, Odocoileus virginianus with regard to individual heterozygosity and a positive correlation was found (Cothran et al., 1983). Earlier, Bottini et al.,(1979) had found a similar correlation in man. Positive correlations have been found in the salamander, Ambystoma tigrinum (Pierce and Mitton, 1982) and King (1985) found a correlation between multi-locus heterozygosity and length in the herring, Clupea harengus. Fundulus heteroclitus, the killifish has been extensively studied and genotype-phenotype-fitness correlations have been shown to exist (Place and Powers, 1979; Dimichele and Powers, 1982a, 1982b). Bruce and Ayala (1978) also showed a positive correlation between morphological variance and enzyme heterozygosity in the monarch butterfly Danaus plexippus. Fleischer et al., (1983) demonstrated a correlation between allozyme heterozygosity and morphological variation in the house sparrow, Passer domesticus.

The idea of heterozygosity being related to increased growth rate and fitness is not confined to animals. Mitton and Grant (1980) found such a relationship in Populus tremuloides, the quaking aspen.

Thus there seems to be a substantial amount of evidence in favour of heterozygosity being linked with superior phenotypic characteristics. But there are many workers who have found no correlation between heterozygosity and growth or fitness.

McAndrew et al., (1986) gives many examples including studies conducted on wild populations of marine bivalves. Foltz and Zouros (1984) working on Placopecten magellanicus and Beaumont et al., (1985) working on Pecten maximus found no correlation. Foltz and Chatry (1986) also showed no evidence to support heterozygosity correlated to growth in Crassostrea virginica. Beaumont et al., (1983) conducted a similar survey of captive populations of the mussel Mytilus edulis in the laboratory and found no correlation.

Similar findings were cited by McAndrew et al., (1986) for various forest tree species, Pinus rigida (Ledig et al, 1983), Pinus ponderosa and Pinus contorta (Knowles and Mitton, 1980; Knowles and Grant, 1981; Mitton et al., 1981; Grant et al., 1982; Mitton, 1983). Handford (1980) working on a warm blooded vertebrate, the songbird Zonotrichia capensis also found no correlation.

McAndrew et al., (1982) compared heterozygosity and the variability of caudal, anal and dorsal fin rays in one of the largest studies of any vertebrate, the plaice Pleuronectes platessa. But despite thorough analyses of the data, no hint of any relationship between heterozygosity and morphological variability was found. Thus there seems to be no agreement among informed geneticists concerning

the effects of heterozygosity.

Meffe (1987) in an article concerned with conserving fish genomes, suggests there is a need for urgent research in order to gain a better understanding of the role genetic heterozygosity plays in individual fitness of fishes. Meffe (1987) points out, that not everyone agrees with the fact that individuals displaying a high degree of heterozygosity are at an advantage. This is a selectionist argument and assumes that allelic variance is subject to natural selection and that different genotypes have different fitnesses in a given environment. This is of course opposed to the neutralist hypothesis (Kimura, 1968a; Kimura and Ohta, 1971) which affirms that variation at a locus is selectively neutral and that selection merely screens out grossly deleterious mutants.

Applying genetic theory to conservation of genetic resources, Meffe (1987) suggests a conservative approach to conservation genetics. "The neutralist approach suggests the loss of genetic variability in small populations, resulting in fixation of loci, would not be harmful. If alleles are indeed selectively neutral, then this would be true. However, if this is not the case, potential loss of a population or species could result. Alternatively, the selectionist approach, if wrong, would only result in unnecessary conservation of genetic diversity that is neither beneficial nor harmful to the organisms involved". Hence the benefit of the

conservative approach. Ryman (1983) agrees but adds that there is a manifest need to investigate further the relationship between heterozygosity and increased fitness.

Ryman (1983) concerned with stock identification and utilization in breeding and enhancement programmes, asks two major questions that need to be answered namely:

1. Is there a correlation between the level of genetic variability of biochemical loci (eg as measured by average heterozygosity) on one hand and that of phenotypic characters (eg as measured by heritability) on the other?

2. Is there a relation between the amount of allelic differentiation of biochemical loci and that of loci controlling the expression of phenotypic characters?

There is a fundamental difference between the estimates of genetic differentiation measured by using electrophoresis and other biochemical investigative techniques on the one hand and measures expressed in terms of heritability for certain phenotypic characters on the other.

Allozyme data provide estimates of the absolute magnitude of genetic variation and permit the assessment of the amount of genetic differentiation between populations.

Heritabilities express the relative importance of additive and non-additive genetic factors for the variation of phenotypic traits such as growth rate, survival and disease susceptibility.

Allendorf and Utter (1979) proposed an association between average heterozygosity and heritability for morphological characters. They too, highlighted the urgent requirement for more in depth study on the subject.

In the last 20 years a substantial amount of information has built up relating to the population genetics and heritability estimations for an array of animals and plants. Since Harris (1966) and Lewontin and Hubby (1966) pioneered the technique of gel electrophoresis using different species of Drosophila many geneticists have used dozens of different organisms to identify evolutionary relationships between and within species. Reviews on electrophoretic variability and interpretation of the results have been published (Lewontin, 1974; Powell, 1975; Selander, 1976; Allendorf and Utter, 1979; Nevo, 1978).

In the last 10 years the extensive use of electrophoresis has lead to much information being accumulated concerning the structure of salmonid fish populations (Ryman, 1983).

The identification of individual stocks of fish and the consequent implications concerned with conservation of genetic resources has been highlighted (Allendorf and Phelps, 1981a; Ryman and Stahl, 1981;

Gjedrem, 1981; Altukhov, 1981; Ihssen et al., 1981; Guyomard et al., 1984).

Most of the salmonids have been studied and in each species, electrophoresis has shown up population structuring unknown until the introduction of biochemical techniques.

Evidence has accumulated indicating genetically distinct populations existing in the char, Salvelinus alpinus (Child, 1977), the Atlantic salmon Salmo salar (Child et al., 1976; Payne and Cross, 1977; Thorpe and Mitchell, 1981; Stahl, 1981, 1983), the brook trout, Salvelinus fontinalis (Eckroat, 1973), the lake trout, Salvelinus namaycush (Dehring et al., 1981; Brown et al., 1981), the cutthroat trout, Salmo clarki (Allendorf and Utter, 1976; Gyllensten et al., 1985), the rainbow trout Salmo gairdneri (Allendorf, 1975; Allendorf and Phelps, 1981b) the sockeye salmon, Oncorhyncus nerka (Grant et al., 1980) and the brown trout, Salmo trutta (Allendorf et al., 1976, 1977; Ryman et al., 1979; Taggart et al., 1981; Ferguson and Mason, 1981; Ryman, 1981, 1983; Jonsson, 1982; Krieg and Guyomard, 1983; Guyomard and Krieg, 1983; Ferguson and Fleming, 1983; Gyllensten, 1984).

Ryman (1983) points out that there are considerable genetic differences within the salmonid species on a micro as well as a macro-geographical scale. He also establishes there are significant differences between the variability patterns of different species. A considerably larger portion of the total gene diversity (Chakraborty et al., 1982) is found within populations in the Atlantic salmon

and the rainbow trout as compared to the brown trout Salmo trutta for example.

In the case of the brown trout Salmo trutta there is evidence to suggest distinct genetic populations reproduce and live sympatically in the same water body (Ryman et al.,1979; Ferguson and Mason 1981).

Electrophoretic evidence has also been used to estimate the amount of inbreeding present in populations of hatchery brown trout stocks (Vuorinen, 1984). Genetic tags have been developed in the form of naturally rare alleles, and used to mark hatchery brown trout, in order to evaluate the success of artificial stocking programmes (Taggart and Ferguson, 1984).

It has been claimed that some of the observed phenotypic differences found within salmonid species may be directly related to the biochemical variation observed. These claims have been made in brown trout (Ryman et al., 1979; Ferguson and Mason., 1981), Atlantic salmon (Riddel et al., 1981; Heggberget et al., 1986) and in rainbow trout (Northcote and Kelso, 1981; McKay et al.,1984; 1986).

It is generally acknowledged that a thorough understanding of the genetic variability patterns constitutes a requisite for an efficient and effective management of natural and cultured fish populations (Allendorf and Utter, 1979; Wilkins, 1981; Ryman and Stahl, 1981; Gjedrem, 1983; Ryman, 1983).

To be able to utilize genetic variability in the future it is suggested the first step that should be taken is to conserve what exists already (Soule and Wilcox, 1980; Frankel and Soule, 1981; Ryman, 1981 ; Allendorf and Phelps, 1981; Soule, 1985; Meffé, 1987).

Running concurrently with the research carried out using biochemical techniques to assess population structure and to identify sub-populations, is work directed at identifying and utilizing phenotypically important characteristics, desirable in the fish culture industry. Genetic variation for some of the most commercially important traits in some of the most commercially important species have been identified. This side of genetics is referred to as quantitative genetics and the theory of animal breeding has only recently been applied to fish culture, and is not yet widely practised (Kinghorn, 1983; Gjedrem, 1983). Research in quantitative genetics of fish is mostly restricted to salmonids (North America, Norway and France) and carp species (Israel and USSR).

In order to choose the most appropriate method of selection in animal genetics the heritability of the trait in which one is interested has to be quantified. The higher the heritability, the more successful individual broodstock selection will be for the trait in question. The lower the heritability, the lower the success of individual selection and the adoption of an alternative strategy for selection is necessary such as family selection (Falconer, 1981).

The trait most important to aquaculturists is growth rate. Maximising this trait ultimately leads to more turnover of a saleable end product and thus increased profits.

Wohlfarth et al.,(1975) reported differences in growth rate between strains of carp and heritabilities of various growth rate paramaters ranged from 0.1 (Kirpichnikov, 1972) to 0.48 (Nagy et al.,1980). Similar reports suggesting inter-strain variation were reported for rainbow trout. Heritabilities for growth differ between sire and dam and age of the fish - the lowest estimates tend to be for sire heritabilities ranging from less than 0.1 to 0.37 (Aulstad et al.,1972; Refstie, 1980; Gunnes and Gjedrem, 1981); the highest estimates tend to be for dam heritabilities at a young age with values of 1.0 or more (Gall and Gross, 1978; Klupp, 1979). Work has also been conducted on Atlantic salmon with similar results - heritabilities for growth rate ranging from 0.1 to 0.84 (Naevdal et al.,1975, 1976; Refstie and Steine, 1978; Gjerde and Gjedrem, 1984). Channel catfish Ictalurus punctalus is another popular species for growth rate heritability studies. Estimates range from 0.12 to 0.81 (Reagan et al., 1976; El-Ibiarty and Joyce, 1978; Bondari, 1980).

Other organisms studied include mosquitofish Gambusia affinis (Busack and Gall, 1983; Stearns, 1984), Coho salmon Oncorhynchus kistuch (Iwamoto et al.,1982), brook trout Salvelinus fontinalis (Robison and Luempert, 1984).

The heritability of other traits have been investigated. Kinghorn (1983) reported the heritability of food conversion for rainbow

trout was 0.41. Gjedrem and Aulstad (1974) report the differences in resistence to vibrio disease of salmon parr was 0.07 for females and 0.12 for males. Genetic variation in carotenoid deposition in salmonids is reported by Torrissen and Naevdal (1984).

Blanc et al., (1979) report on the heritability estimates for the number of pyloric caecae for brown trout and rainbow trout (heritability = 0.53). Blanc and Toulange (1981) investigated brown trout alevins' swimming performance and found a heritability of 0.3. Blanc et al., (1982) report work conducted on spot pattern in brown trout and concluded the heritability for this trait to be 0.4.

Gjedrem (1983) and Kinghorn (1983) give extensive reviews on the subject of heritabilities and quantitative genetics in the breeding of fish and shellfish.

To obtain the best results in a selection experiment, the population of the organism under consideration should preferably show good performance for the character of interest, eg growth rate. It is emphasized, however, that a population that is characterised by a fast growth rate does not necessarily harbour a high level of genetic variation for that character (Ryman, 1983). The situation can be likened to a hatchery stock which has been kept under the same conditions for many generations. The growth rate may be excellent but the heritability for growth rate may be low, leading to poor results from individual selection in future generations.

What is ideally required is a population with (a) a good growth rate to start with and (b) a high heritability for that trait. The problem when choosing wild broodstock is that it is impossible to tell the heritability for a trait from phenotypic characters, and methods of estimating heritability are tedious, expensive and time consuming (Ryman, 1983).

It has been suggested that instead of heritability, heterozygosity could be measured by means of electrophoresis. This is making, once again, one large assumption, that heritability and heterozygosity are positively correlated. This again raises the controversy about the importance of heterozygosity.

Ryman (1983) argues that since some of the traits which are of interest to the fish breeders may not be under strong selective pressure, in the wild natural conditions, the variability within the stock may be greater leading to possibly high heritabilities for these traits. But can one identify the possibility of high heritability by assuming it is correlated to electrophoretic variation?

In the absence of empirical data, a lot of population geneticists are assuming the existence of such a relationship and are selecting populations for crossbreeding and suitability for aquaculture purposes on the basis of genetic divergence as estimated from biochemical loci. Thus using Meffe's (1987) conservative model for genetic conservation, and assuming the balance theory could be correct.

The present study was established to shed light on the following questions asked about brown trout in Scotland.

1. What is the heritability estimates for brown trout under hatchery conditions for the main commercially important phenotypic character - growth rate?

2. Is there a difference between sire and dam heritabilities for growth rate and other parameters?

3. Do heritability estimates vary from a farmed stock and a wild stock?

4. Is high growth rate and high heritabilities, or slow growth rate and low heritabilities correlated to electrophoretically detectable heterozygosity or homozygosity?

5. How are the wild populations of brown trout in Scotland genetically distributed and does electrophoretic data used to 'classify' variation help in future management of the resource?

6. Is any electrophoretically detectable variation found correlated with other parameters?

CHAPTER 2

2. The Biology of the Brown Trout (Salmo trutta L.)

2.1. Distribution

The brown trout has been studied extensively throughout its natural range, and where it has been introduced elsewhere in the world. It is one of the most widely spread species of fish in the world, having been introduced primarily as a sporting species.

The brown trout introductions have extended self sustaining populations to every continent except Antarctica. For a detailed account of the introductions carried out during the last one hundred years see MacCrimmon and Marshall (1968) and MacCrimmon et al.,(1970).

Figures 2.1 and 2.2 illustrate the world wide distribution of the brown trout and its native range respectively.

2.2. Taxonomy

The brown trout has been extensively studied for hundreds of years due mainly to the interest generated by its sporting and eating qualities. Trout from different rivers and lakes show a remarkable diversity in such features as size and colour, age at maturation, longevity and in maximum weight attained. This has lead in the past to different names being attached to trout originated from different areas. Some workers actually referred to these different forms on the species level.

Linnaeus in 1758 first distinguished three races of trout in Sweden, Salmo trutta, the lake trout, Salmo eriox the sea trout and Salmo

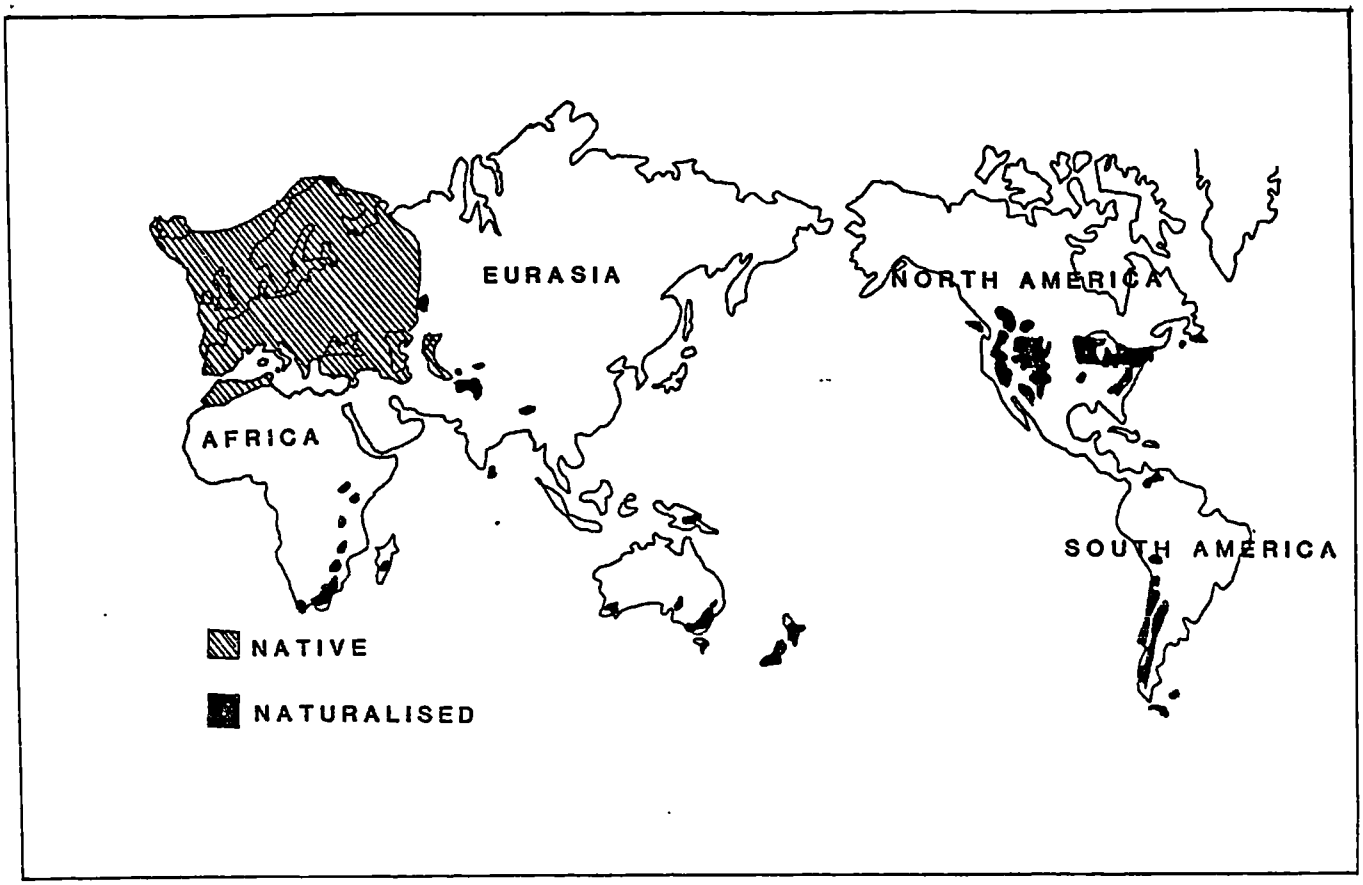


FIG 2.1 WORLD DISTRIBUTION OF SALMO TRUTTA

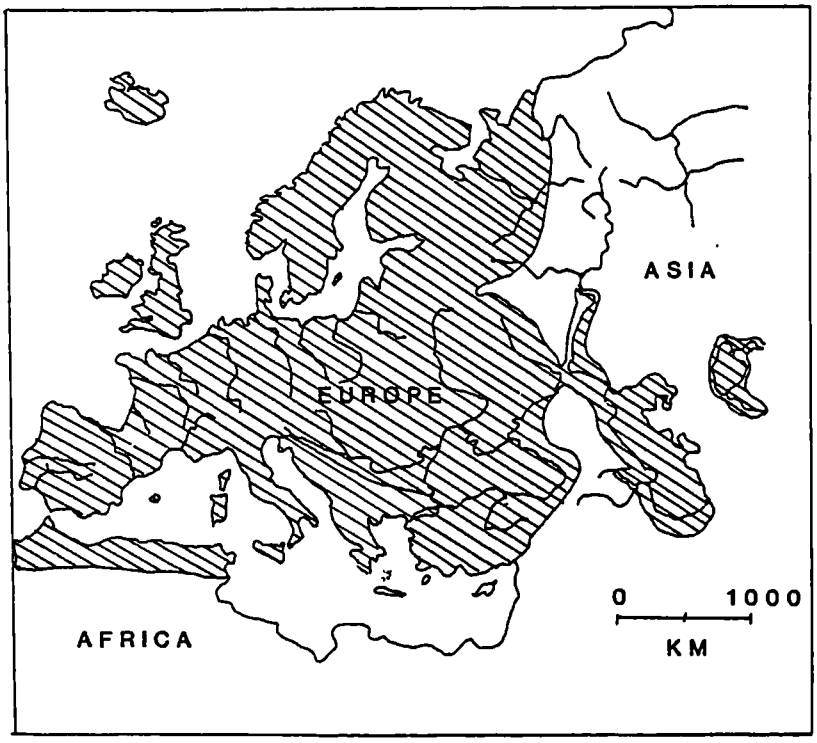


FIG 2.2 NATIVE DISTRIBUTION OF SALMO TRUTTA

fario the brook trout (not to be confused with the modern day American Salvelinus fontinalis). In 1866 Günther published his catalogue of fishes of the British Museum, Vol 6, in which he classified the British trout into ten different species.

Salmo trutta	Sea or salmon trout
S. cambricus	Sewin or Western sea trout
S. branchypoma	Eastern sea trout
S. gallivensis	Galway sea trout
S. orcadensis	Orkney sea trout
S. fario	River trout
S. ferox	Great lake trout
S. stomachicus	Gillaroo
S. nigripinnis	Welsh black finned trout
S. levenensis	Loch Leven trout

Regan (1911) also listed many specific and common names given to various subspecies of trout in his introduction.

Common trout	- (S. fario, gairnardi, cornubiensis)
English salmon trout	- (S. trutta, eriox, cambricus, albus, phinoc, branchypoms)
Golden estuarine trout	- (S. estuarius, orcadensis, gallivensis)
Great black lake trout	- (S. ferox, nigripinnis)
Gillaroo	- (S. stomachicus)
Loch Leven trout	- (S. caecifer, levenensis)

Berg (1932) divided the European trout into what he called six subspecies in which all British variations were classed together. The six divisions are as follows:

Salmo trutta trutta - Northern and Western Europe (including Britain)

Salmo trutta labrax - Black Sea and its tributaries

Salmo trutta caspius - Caspian Sea and its tributaries

Salmo trutta aralensis - Sea of Aral and the River Oxus

Salmo trutta macrostigma - Mediterranean region

Salmo trutta carpione - Large trout of Lake Gorda in Italy

The problem of brown trout taxonomy was further complicated by the exchange of brown trout stocks among European countries, such as the transfer of German brown trout to England in 1884, and to Italy in 1885 (Pavesi, 1887).

Modern fishery biologists now regard the brown trout as one polytypic species, Salmo trutta L. (Regan, 1911; Trewavas, 1953). As biochemical techniques have been introduced to brown trout population studies it has become apparent that many of the old, so called species, subspecies or strains, are actually genetically distinct. Ferguson and Mason (1981) found the 'gillaroo', 'sonaghan' and 'ferox' (local names given to morphologically distinct types of trout) all living sympatrically in Lough Melvin, Northern Ireland, were significantly different from each other electrophoretically and maintained individual populations by spawning in different parts of the lough.

Where stocking of trout has taken place, MacCrimmon and Marshall, (1968) are convinced most populations of brown trout now resident in hatcheries and natural waters throughout the world emanated from three sources: the sea run specimens of the European trout, European trout permanently resident in freshwater and the trout from Loch Leven and other waters of Scotland and Northern England.

2.3. Origin of recognised variation in Salmo trutta

A problem encountered by those working with Salmo trutta has been to characterise and distinguish between forms especially between migratory sea trout and non-migratory brown trout. Did the brown trout develop from the sea trout or did the sea trout evolve from the brown trout? In the three genera, Oncorhynchus, Salvelinus, and Salmo which make up the subfamily, Salmonini, there are no exclusively marine species. This fact has been widely cited and regarded as being a significant indication of ancestral origin (Fahy, 1985). Freshwater species and genera have forms which can survive and indeed thrive at sea, but none of the salmonini can complete their life cycle unless they return to freshwater to spawn, laying their eggs in suitable gravel not found in marine environments. This suggests that salt water was secondarily invaded.

Salmonid migration to the sea enables sufficient growth to occur to facilitate effective spawning. The similar purpose of a descent by lake trout from the nursery burn to richer feeding in the lake environment is often overlooked. Thus the biology of lake and sea trout are very similar.

But what makes Salmo trutta sometimes smolt and run to sea in the first place? Fahy (1985) points out that the existence of the smolt has been regarded as strong evidence supporting a marine ancestry, otherwise how could the smolt develop the ability to anticipate circumstances which the juvenile of an entirely freshwater species could not know.

But as brown trout, not silvered-up in any way, can be introduced directly into salt water without detriment, smoltification can be regarded as a secondary development in trout rather than a vestige of a marine ancestry (Fahy, 1985).

Having suggested the sea trout are a consequence of a secondary invasion of the sea by a freshwater species; one has to explain how the various populations, races and strains of the species are distributed throughout the native range.

The last great ecological and climatic event in the earth's history which not only affected the salmonid population of Europe but all living creatures, was the ice age of the Quaternary period. Its duration was approximately 900,000 years and consisted of nine major advances of ice that can be recognised by geologists in Britain and Ireland. Each of these intervals consisted of periodic extensions of the ice sheets from the North, as the earth's surface and air temperature dropped. Atmospheric warming caused the ice to retreat periodically.

When the ice was at its maximum southerly latitude it covered most of Britain and Ireland. Due to the vast nature of the ice sheets, the sea level at the time was lower than it is today. Rivers draining the western part of Britain and south and east of Ireland were thought to join and flow south by way of what has been called the Celtic River basin. Fahy (1985) illustrates with a map, the proposed extent of the ice. Everything to the north and west in Britain and Ireland was inpenetrable to fish as the lochs and rivers as we know them did not exist under the blanket of ice.

When the ice withdrew the land rebounded and rose as did the sea level, which did so more quickly. The ice was thought not to have withdrawn in one episode but by a series of retreats. Thus fish previously isolated, trapped in European refuges during the ice bound periods, once more could migrate and invade rivers and lochs no longer under ice in Northern Britain. Different refuges may have been isolated for many thousands of years between the glacial periods, giving rise to separate populations evolving independently. Thus different strains of salmonid species were thought to reinvade British waters from the sea, and probably at different times as different refuges became once more connected to the main marine environment.

The climate continued to warm and this is one explanation for the loss of anadromy. The salmonids followed the retreating ice sheet and as the environment became kinder and the lochs and rivers warmed,

food became more abundant, and migration to richer areas was no longer necessary to complete their life cycle.

Recent electrophoretic work on Coregomus (Ferguson et al, 1978) and Salvelinus (Ferguson, 1981; Andersson et al, 1983) has shown that the non-migratory whitefish and charr in Irish and British waters are very closely related to the whitefish and charr in Alaska and Scandinavia, which regularly migrate to sea, but are presumably living in a "harsher" freshwater environment.

Fahy (1985) mentions that the Mediterranean is fringed by resident brown trout populations which must have been established there during cooler times but whose members do not migrate to sea at all at present.

Thus the last 100,000 years has been a period of potential for great diversification for Salmo trutta as the ice sheets melted and proffered more freshwater for colonisation (Fahy, 1985).

2.4 Life cycle

Brown trout (Salmo trutta) normally spawn in fresh running water in the autumn of the year. In the British Isles, brown trout may become ripe and spawn between October and February; they are never found in breeding condition outside this period (Frost and Brown, 1967). Spawning usually occurs in moving water and trout living in lakes migrate into the feeder streams. Those living in rivers tend to move upstream prior to spawning.

The actual time of migration is determined by two sets of factors, the physiological developmental state of the ripening gonads and the environment in which the trout is living.

The spawning migration is associated with a rise in water level as well as a drop in temperature. Stuart (1957) found that migration always occurred when the stream into which the trout were to migrate had dropped to 6-7°C, and it was the first time it had fallen to that level.

The female (hen) lays her eggs in a redd constructed in suitable gravel. The redds are found in quickly moving water of moderate depth. The width of the stream is irrelevant and stones upto 7cm are utilized in the redd (Stuart, 1957).

The eggs laid by the female are fertilised by milt from the male. The rate of development depends on the temperature, being faster at higher temperatures (Dahl, 1918-1919). It is reported a high proportion of eggs hatch successfully at temperatures between 3°C and 12°C. Above and below these temperatures mortality increases (Frost and Brown, 1967).

After hatching the alevins spend upto six weeks hidden in the gravel of the river bed, utilizing the food reserve of their yolk sac. They start feeding once the majority of the yolk sac has been used. The fry which start feeding earlier obviously acquire an initial advantage in size over others which start later and they typically

maintain this greater size if all the fry are left together in one group (Frost and Brown, 1967; Campbell, 1971). It is postulated that the larger fry hold better feeding territories and so grow faster. As the year progresses the disparity in length between the smallest and largest fry increases.

Mortality during early life is enormous (upto 95%). Once the fry are established the mortality rate decreases and drops to 25% for the period from fry to yearling trout (Le Cren, 1961).

When the adult trout live in lakes or lochs rather than in river environments, and spawn in streams, the fry live and feed in the streams. Those that grow fastest, may move down into the lake when one year old (0^+), the rest at two years old (1^+) (Ball and Jones, 1961; Campbell, 1971; Thorpe, 1974).

Growth is faster in the lake and those trout that drop back first tend to have a size advantage over siblings that remain in the streams for longer. Where spawning is limited and streams are small, all the fry will drop back during their first summer (Frost and Brown, 1967).

Sea trout tend to move out of the lake or river and migrate to sea as one, two or three year olds (Le Cren, 1984). Their appearance changes from the parr marked young salmonid to that of a silvery smolt. The sea trout spend varying lengths of time at sea, where

they grow much faster than their relatives, resident in freshwater. They return to the rivers to spawn and are known by a variety of coloquial names: peel, phinnock, sewin, white trout, being some of the commonest.

Male trout (cocks) tend to mature a year earlier than the females (hens) in the same population and two years old is the earliest that cocks normally mature. Although Campbell (1971) mentioned a mature one year old male that was only 7.9cm in length, but this is an exception rather than a rule.

In productive environments where there is plenty of suitable spawning gravel available, individual trout tend to spawn every year, once they are mature, but in some lakes which have little or no spawning available the fish may spawn every other year, giving enough time for them to recover fully from the previous spawning (Stuart, 1957). Campbell (1971) points out, some old trout, which may or may not be of considerable size, may be immature for some reason, and others which may appear to be 'resting' for a year between spawnings have in fact finished spawning and will not become gravid again during their lives. Some populations of sea trout tend to be multiple spawners (Le Cren, 1984) and the same holds true for the larger non-migratory trout. Southern (1932) reported a twelve year old trout which had spawned eight times in consecutive years. Campbell (1979) cites examples of the large piscivorous 'ferox' trout not maturing until 5 to 8 years old, by which time they have attained a size, which makes them hydrodynamically efficient at catching other fish,

mainly Salvelinus alpinus. Campbell (1979) also reports other workers (Kennedy and Fitzmaurice, 1971; Wojho, 1961) have found large trout (> 55cm) with atrophied or "resting" gonads, although the fish they studied had matured and spawned before.

According to Frost and Brown (1967) the fate of most wild trout, which live past the parr stage is to be preyed upon by some predator. Campbell (1971) suggests that a large proportion of each year class of trout do not survive in Scottish lochs. It is difficult to estimate mortality in these wild populations, as the trout may drop back into the lochs and die and decompose undetected. The larger individuals which have not matured early, have escaped the physiological stress and disease and the physical hazards of spawning that the smaller individuals in the population have had to endure (Campbell, 1979). The larger trout, use deeper and therefore safer spawning grounds and when they drop back to the loch to recover, shoals of young charr (Salvelinus alpinus) are an abundant food supply aiding recovery. If the larger trout do not turn to a piscivorous diet, they will become weak and die of starvation. Campbell (1979) calculated one 15cm charr is equivalent in weight to 4,500 12mm chironomid larvae. As trout grow older they are more likely to become heavily infectd by parasites, thus they may become progressively debilitated.

There is considerable evidence to suggest brown trout possess an accurate 'homing instinct' which is expressed by their return to spawn again and again in a particular stream, which is very probably

that in which the fish was hatched and lived as a fry (Stuart 1953, 1957). Allendorf et al.,(1976) and Ferguson and Mason (1981) working in Scandinavia and Ireland respectively found sympatric populations living in the same lakes, separated by accurate homing instincts to separate spawning areas.

Thus the brown trout exhibits great plasticity in every part of its life cycle. Not only does its physical appearance vary greatly, so does its growth rate, diet, feeding behaviour, age at maturation, place and time of spawning and its longevity.

The question asked by most informed anglers and fishery biologists is how much of this variation is genetically controlled and how much is influenced by the environment in which the trout lives?

Until recently the scientific investigations that have been conducted have mainly encompassed ecological aspects of the brown trout's life history. Much work has centred in the English lake district (Allen, 1938; Swynnerton and Worthington, 1939; Frost and Smyly, 1952; Frost, 1945) and in Wales (Ball and Jones, 1960, 1961; Graham and Jones, 1962). For a review of the work carried out up until the mid 1960's see Frost and Brown (1967).

The genetic aspect of trout biology has always been of interest. Dahl (1918-1919) conducted extensive trials with different populations of brown trout in Germany and produced some results that suggested genetic as well as environmental effects influenced growth rate.

Davis (1934) stated that experiments with brown and rainbow trout showed rapidly growing strains could be obtained by selective breeding. Donaldson and Olsen (1957) agreed and concluded that dramatic improvement could be attained using generations of selective breeding in rainbow trout.

Alm (1959) demonstrated experimentally that late maturity was hereditary in Swedish trout by carrying out controlled rearing experiments. A more thorough review of current genetic research concerning population structure of salmonids is given in the chapter on electrophoretic examination of wild Scottish brown trout.

2.5 The Growing Importance of Brown Trout

The brown trout used to be an important source of protein in areas of Scotland and were extensively trapped and netted (St John, 1878; Brookes, pers comm). The angling potential of the lochs and rivers was not publicised until the late eighteenth century and early nineteenth century. Campbell (1971) lists a number of sportsmen, tourists and naturalists who wrote about the sports fishery potential and highlighted the tourist potential in Scotland. (Thornton, 1804; Pennant, 1769; St John, 1878; Stoddart, 1866).

Due to the development of the angling resource and initially because of a need for food, trout were stocked randomly into many lochs to attempt to improve quality and yield. Unfortunately, little if any record has been kept of these introductions, and even less information is available on their success (Campbell, 1971).

In 1971 a government white paper on game angling in Scotland (HMSO, 1971) stated that there existed a great need to make more waters available to the resident population and to visitors to Scotland. In order to bring about this development, a complete reorganisation of angling was suggested, including the evolution of a new body, the Scottish Angler's Trust (SAT) and also the development of area boards (Hails, 1978).

The proposals of the white paper were based on the findings of the Hunter committee which produced its main report on Scottish salmon and trout fisheries in 1965 (HMSO, 1965). Although the committee sought evidence from a very large number of organisations and individuals the information was largely of a qualitative rather than quantitative nature (Hails, 1978). In 1976, the Freshwater and Salmon Fisheries (Scotland) Act was passed bringing about the legislation necessary for statutory protection of trout waters and the provision of financial assistance towards organisations developing trout fisheries.

Since 1976 some areas have reorganised the trout angling and applied for and been granted protection orders under the legislation brought about by the act. However the vast majority of the trout populations and the angling have received little attention and are still managed as they have been for the best part of the last 100 years, namely through hotels, estates and angling clubs.

In contrast to the apparently apathetic nature of governmental interest in recreational fisheries in Britain, other countries have recognised

their extensive value and potential and have acted accordingly. In Scandinavia where recreational fisheries are affected by acidification large government run research programmes are on going to identify ways in which to aid the situation. Brown trout have been collected from over 200 populations and kept as an egg and sperm bank for future innovative management of the fisheries (Gjedrem, 1981).

A great deal of controversy is present in the literature on acceptable methods for estimating the value of fisheries. Crutchfield (1962) contended that the value of a commercial fishery equals the market value of the fish. This cannot be applied to recreational fisheries because values other than the harvest are involved. A few of the methods to valuate recreational fisheries include, unit day value, gross expenditure, replacement cost, income multiplier, property values, willingness to pay and travel cost (Weithman and Haas, 1982).

Barber (1976) produced figures avaiable in the USA for individual states for numbers of fish caught and the number of anglers fishing. For example in Oregon alone in 1972, 14 million salmonids were caught by 5.5 million anglers at an average of 2.6/day. In the USA there is a coordinated programme of fisheries management run by the US Fish and Wildlife Service whose aim is in "assisting in meeting the public demand for recreational fishing while maintaining the nation's fishes and their habitat at a level and in a condition that will ensure their survival".

The detailed aims of the government and state funded service are as follows:

1. To maintain existing sport fishery populations at the level required to meet public demand to the maximum feasible extent.

2. To increase fish opportunities by restoring destroyed or depleted fisheries.

3. To create greater fishing opportunities by up-grading existing fish populations, developing fisheries in new waters and introducing new species.

4. To protect the nation's fisheries by limiting the introduction or distribution of diseased fish and their attendant pathogens.

5. To control the distribution and populations of exotic species.

6. To carry out the responsibilities established by treaties and other commitments of the federal government, to maintain or restore fish resources.

The cost of such operations is jointly shared between federal and state finances (Barber, 1976). Twelve large research laboratories and over 500 state and federal hatcheries are financed to cope with the growing demand for recreational fisheries not necessarily with salmonids as the only quary. Burrows et al, (1974) estimated that

the hatcheries produced over 600 million fish of fingerling size or larger/year and > 1000 million fry mainly walleye, striped bass and northern pike, but also salmonids.

The situation in Scotland is far from being as organised. The relevant bodies that may be expected to have some idea of how many trout are caught by how many anglers have virtually no data to work on and there is no coordinated government encouragement to improve the situation. Although the Department of Agriculture and Fisheries for Scotland (DAFS) is charged with the responsibility of analysing and monitoring all types of fisheries, simple statistics vital for the effective evaluation of resource utilization at present and in the future are not available. The Scottish Tourist Board conducted a Leisure Survey of tourists visiting Scotland in 1981 and of 4.1 million tourists 10% said they fished, but there was no mention of what type of fishing this entailed. In 1983 a report by a countryside sports consortium estimated 350,000 Scots fished. The Scottish Sports Council in 1986 conducted a limited study which showed more males between the ages of 13-24 fished than females of the same age!

No figures are available for the number of trout caught every year in Scotland. Various 'guestimates' have been voiced but nothing has appeared in print. A recent figure of between 300-500 tons (Walker pers comm) has been suggested for the total weight of trout taken by anglers in Scotland annually.

As the Scottish economy is becoming more and more reliant on non-manufacturing industries, the tourist industry is playing a larger and larger role in the economy of many rural areas, and as recreational fishing is a major tourist attraction, Scotland and in fact the UK as a whole could profit from adopting many methods currently in use in the USA in fisheries management. Barber (1976) includes the following strategies:

1. That public money is best spent in providing angling for the general public by acquiring and running public waters.

2. Non-endemic species can provide valuable fisheries, and important subjects for fish farming without jeopardising native species or fisheries.

3. The investment of public money in supporting and creating fisheries can bring considerable incomes to communities local to the fisheries.

4. Waters supporting heavy angling pressure need careful management.

5. Given the assurance that their money is spent on supporting and improving fisheries, anglers are prepared to pay $4-6 or £2-3/year (1976 prices) for a local licence and an additional 11% tax on fishing tackle and bait.

6. The success of stocking operations and therefore hatchery programmes depends upon many factors, not just the numbers of

fish released. Some of these factors are: species, size, strain or genetic make up and health of the released fish, method and time of release and nature of water being stocked.

7. The spending of public money on research develops the expertise to advise the public and allow the successful commercial exploitation of new ideas in fish culture and fisheries management.

8. Cost benefit studies are an important aspect of management programmes.

To sum up, the vast majority of Scottish brown trout fishing represents a large unexploited poorly managed resource, with considerable scope for future development.

The aims of promoting angling and increasing tourism and thus wealth to local economies, rely on well managed fisheries. One of the most important prerequisites for a successful conservation and management programme is the accurate identification and characterisation of the genetic resources available (Taggart, 1981; Ryman, 1981; Thorpe and Mitchell, 1981; Ihssen et al., 1981; Altukhov, 1981). The identification of intraspecific variation is concerned with estimating the distribution of genetic variation within the species concerned.

Ryman (1981) working with brown trout in Sweden, found

1. The genetic structure of naturally occurring populations was

more complicated than previously thought.

2. Genetically differentiated subpopulations exist within extremely small geographical areas.

3. The genetic differentiation appeared to be coupled with ecological and morphological variation.

Ryman (1981) continues "The genetic pattern observed in waters affected by human perturbations indicate that the disturbances have drastically altered the distribution of genetic variation in these areas and that the genetic characteristics of previously existing subpopulations have most likely been lost. There is also strong evidence showing the current hatchery stocking procedures may frequently change the genetic composition of the stock (genetic resource) they were intended to preserve".

Taggart and Ferguson (1986) assessing the effects of stocking Lough Erne in Northern Ireland, by the use of an electrophoretic investigation found evidence that the hatchery stock, characterised by low frequencies of the LDH_5-105 variant allele, had interbred with the native Lough Erne trout characterised by a high frequency of the same allele. Taggart and Ferguson (1986) end their paper by looking to the future and suggesting a separate hatchery should be established and maintained for both introductions and as a gene bank, on Lough Erne, as the only practical method of "reconciling

the conservation of a unique gene pool with increasing angling pressures and the resultant need for supplementary stocking". This scenario of course can be expanded to many other areas throughout the brown trout's range.

In Scotland very little genetic research has been carried out on the brown trout. Niall Campbell and Andy Walker have kept various strains of brown trout in captivity and introduced them into fishless waters but little quantitative results on the performance of these trout is available. Campbell has produced a number of papers comparing growth of trout and gave reasons for the differences in many highland lochs (Campbell, 1957, 1961, 1963, 1971, 1979). Campbell (1971) states "It would appear that whatever is the genetical pattern controlling growth and life span in different stocks of trout, these are for practical purposes completely masked, within extreme limits, by the immediate environmental conditions".

Campbell (1967a, 1967b) also gave methods by which the highland trout populations could be improved.

In recent years the Department of Agriculture and Fisheries for Scotland, that used to conduct extensive brown trout research at Pitlochry, now concentrates its research on salmon and to a lesser extent sea trout. The brown trout has been overlooked although to many it is a vital and sustainable resource which is under utilized.

Ferguson and Fleming (1983) have conducted a minimal electrophoretic

survey of some Scottish lochs and rivers (9 locations). Fleming and Ferguson (unpublished) in 1981 conducted an electrophoretic survey of the three main spawning burns of Loch Leven.

The electrophoretic part of the present study is designed to establish a baseline of information concerning the intraspecific genetic variation of the brown trout found in Scotland. It is envisaged that this information will be made available to aid more rational trout management strategies in the future.

CHAPTER 3

3. Quantitative Study of Brown Trout at Howietoun Fish Farm

3.1. Introduction

When one is interested in the cultivation of a species of organism which is of economic importance, whether it is bred for food or for some other reason, one of the main biological traits that is often vital to the success or failure of the programme is the growth rate of the species involved. Rapid growth speeds up the turnover of production and frequently larger animals attain a higher price per unit of weight compared to smaller ones (Gjerde, 1986).

Rapid growth rate is a problem in some types of meat production, where a correlated response in mature weight causes an increase in the cost of maintaining broodstock, but this factor is negligible in fish and shellfish production (Gjerde, 1986).

The present project is concerned with the brown trout which has limited nutritional value in the countries where it is native or has been introduced, but forms the basis of many recreational fisheries of great socio-economic value (MacCrimmon and Marshall, 1968), and many are sustained by supplemental stocking programmes. This is the situation in Scotland.

In common with other organisms, which are produced commercially a knowledge of the brown trout's genetic makeup is a prerequisite for a sound and rational breeding and management programme (Ryman, 1981; Rasmuson, 1981), both in the wild and in cultured systems. Gall (1972) stated "we must make every effort to learn and understand

both the biology and genetics of the organism before we attempt to tamper with the essential but perishable resource, genetic variability".

Smith and Chesser (1981) in a paper concerned with the rationale for conserving genetic variation in fish gene pools, point out that genetic resources are being eliminated at an alarming rate. They introduce a useful analogy connected with the gene bank concept. "The genetic make-up of populations is the currency within the gene bank of resources, that make man's long-term well-being and survival possible. Conservation of these genetic resources is inherent to the gene bank concept and analagous to a savings account in an actual bank. Our account in the gene bank not only allows certain future activities that would otherwise be impossible, but also pays dividends to future generations".

Gjedrem (1981) in a paper concerned with conserving brown trout populations in Norway, which are under great threat due to acidification, found certain strains of trout were much less susceptible to acidification than others. Gjedrem (1981) suggested the conservation of existing strains as these represent a resource of genetic diversity for potential use in future breeding programmes.

Smith and Chesser (1981) concluded there were five important points in their rationale for conserving genetic variation in fish and they were as follows:

1. Modern techniques, such as electrophoresis, demonstrate the existence of a large amount of genetic variation within and between fish populations.

2. There is evidence that some of this genetic variation permits the adaptation of fish to local environmental conditions.

3. The overall level of genetic variability itself may be adaptive in ways that are partially independent of single-locus effects. This is highly controversial and will be dealt with later.

4. Because of the known and potential adaptive values of genetic variability conservation efforts should be directed toward maintaining existing levels of genetic variability in natural populations.

5. Selective breeding programmes, while resulting in the emphasis of certain characteristics, such as growth rate, often result in a reduction of the level of genetic variability and should be applied to natural populations only with extreme care.

This chapter is concerned with assessing the genetic variability for growth rate in populations of brown trout derived from both hatchery stock and wild strains, kept in cultured situations. This is facilitated by measuring the heritability of the trait in question.

Later chapters will deal with electrophoretic analyses of the trout grown under artificial conditions and compare them with wild stocks obtained elsewhere in Scotland. Comparisons will also be possible, between genetic variability for growth rate and genetic variability identified by electrophoresis.

3.1.2. Advantages and Disadvantages of Using Fish for Genetic Work

Skjervold (1976) and Wilkins (1981) both highlight various traits that make fish easier to genetically "manipulate and improve" than other organisms. The traits involved are as follows:

1. The genetic variability of fish in general, in the form of heterozygosity of individual loci is higher than in most other vertebrates studied.

2. The majority of fish species used in aquaculture today are mostly taken directly from wild stocks and thus their genomes are unaltered by intensive artificial selection procedures, to which most domesticated livestock have been subjected.

3. External fertilization and high fecundity generally in fish species make it possible to raise many more siblings and therefore selection studies can theoretically be done much more intensively than with other livestock, such as sheep or cattle.

4. The sex determination mechanism is much more plastic in some

fishes, which allows production of monosex, gynogenetic and androgenetic populations leading to the production of inbred lines in fewer generations.

5. Intergeneric and interspecific hybrids are very often viable and fertile in fishes, which allows the possibilities of obtaining "tailor made" stocks through the combination of several commercially important characteristics from different species.

There are disadvantages to using fish species when it come to genetic improvement procedures. Amongst them the following are the most important:

1. Many species of fish including temperate species such as salmon and trout exhibit long generation times, which slows up genetic research and development.

2. At present there is considered to be a lower level of knowledge concerning the technology of fish farming than other types of livestock production. This is due to the relatively short history associated with the farmed production of fish species, with the notable exception of the Common Carp (Cyprinus carpio) which has been farmed for food for over 2,000 years by the Chinese, and intensively bred in Europe for over 1,000 years.

3. Fish in a population generally develop a hierarchy which interferes with the experimental design this is more accute in some

species than in others.

4. Young fish and even larger non-mature individuals show a lack of visible markers associated with their sex or other commercially important characters.

5. The final and obvious disadvantage is that, fish require a sufficiently clean and plentiful water supply. Temperate species such as salmon and trout require well oxygenated, cool clean water, and if a large genetic research programme is envisaged a large amount is also required, to allow different populations, families or crosses to be held separately.

3.1.3. The brown trout as a species used in genetic research

The brown trout according to Frost and Brown (1967) are not convenient animals for genetic experiments because they breed only once a year and take at least two years and usually longer, before they attain sexual maturity. Frost and Brown (1967) also highlight the problems of obtaining enough cool, pure water, and the space required for rearing large numbers of families of trout. They conclude that in most circumstances genetical experimentation is impracticable and/or prohibitively expensive.

Due to the obvious problems highlighted in rearing sufficient trout, and the lack of financial support to overcome them, it is not surprising that until recently little quantitative genetic research has been completed. An exception to this situation involves the Norwegian

Institute of Animal Genetics and Breeding station at Sunndalsora, which was built in 1971-1972 in response to the demand for research in the different fields of breeding, nutrition, management and pathology, of the greatly expanding Norwegian fish farming industry. Sunndalsora described by Gjedrem and Aulstad (1974) consists of over 250 $2m^2$ tanks, 140 $1m^2$ tnaks, 36 circular concrete ponds 10m in diameter, and the capacity to keep over 600 batches of eggs separate in the hatchery. This development reflects the foresight and importance that the Norwegians attached to the developing fish farming industry 18 years ago. It is a lesson that should be learned in Britain, as fish farming in the west and north of Scotland is now one of the major employers, and virtually no coordinated genetic research or even applied instruction takes place, in the industry.

Kinghorn (1983) in an overview of genetical fish research states "Animal breeding theory has only recently been applied to fish culture, and is not yet widely practiced. Research in quantitative genetics in fish is mostly restricted to salmonids (North America, Norway and France) and carp species (Israel and USSR). Commerical interest in breeding programmes is overshadowed by continuing efforts to develop optimum husbandry techniques and few companies practice anything other than mass selection". This is in great contrast to the situation in the agriculture industry where intense genetic selection of many kinds has been widely practised for many years.

Gjedrem (1983) identified one reason for a lack of genetic breeding programmes in aquaculture and that is "the education of researchers,

advisory personnel and fish farmers. Education in fish biology involves little attention to selective breeding and quantitative genetics". This is surprising because of the economic importance which aquaculture has reached in many countries, and as Gjedrem (1983) points out "Fish and shellfish seem to be little different from farm animals and plants in response to selection and hybridization effects". Bye and Ponniah (1983) point to the fact that the aquaculture industry is young, and aquatic organisms have more complicated life cycles than land animals as reasons for the lack of genetic improvement.

Most farmed aquatic animals with the exception of carp and some salmonid species are genetically indistinguishable from the wild populations from which they were captured. This of course leaves considerable scope for genetic improvement and for the application of specific genetic manipulations which will significantly improve the productivity of aquaculture in general.

Wilkins (1981) discusses the selection of strains in farming, and Kinghorn (1983) and Gjedrem (1981) suggest that much emphasis should be placed on the need to make contemporary comparisons between stocks for commercially important traits under commercial conditions.

3.1.4. The Concept of Selection

The fundamental concept of selection is that like begets like. Bye and Ponniah (1983) state "there is always some variation within a group of individuals from which preferred progenitors can be

selected to derive the domesticated line of animals or plants towards that combination of characteristics which are considered desirable".

The raw material for a geneticist or fish farmer to work upon and thus 'improve' a species is the variation naturally present in the productive traits of the individuals in a population. No genetic gain can be achieved where there is little or no genetic variation within the trait under consideration, or in which the observed variation is primarily caused by the environment.

Any observable or measurable characteristic of an individual organism is a product of both the genetic constitution of the individual and the environment in which it lives. The relative contributions of genotype and environment vary considerably depending on the particular characteristic under consideration and the particular conditions under which measurements of the trout were taken.

3.1.5. The Concept of Heritability

With controlled experiments it is possible to estimate the genetic and environmental components of the variation of the character/ trait in question, and calculate its heritability (h^2). Heritability expresses the proportion of the total variance that is attributable to the average effects of genes, and this is what determines the degree of resemblance between relatives.

If heritability is high and close to 1.0, most of the variation

for a trait is heritable and selection for the character will be very effective. If environmental factors have caused most of the variation, the heritability value will be low and if h^2 is zero, no genetic gain can be obtained by selection. Strictly there are two different types of heritability.

Kirpichnikov (1981) defines heritability in a narrow sense and a broad sense. In the broad sense the word heritability (ha^2) is equal to the ratio between the genotype and phenotypic variance:

$$ha^2 = \frac{\sigma^2 G}{\sigma^2 p}$$

and the ratio $\sigma^2 G/\sigma^2 p$ expresses the extent to which individuals phenotypes are determined by their genotypes. In other words, the degree to which the appearance of an individual is a direct consequence of its genetic constitution.

It is regarded as more important for the animal or fish breeder, however to define the fraction of the additive genetic variation, or to express the extent to which phenotypes are determined by the genes transmitted from the parents (Kirpichnikov, 1981). This is known as the heritability in the narrow sense, and it is this measure that most geneticists and animal breeders refer to when considering heritability. It is expressed thus

$$h^2 = \frac{\sigma^2 A}{\sigma^2 p}$$

The different variance (σ^2) components are thus very important in analysing the mechanisms underlying the observed variation, in particular for the separation of two of its main components: the genotypic and the environmental components. Such separation can be achieved by the use of various analyses of variance (ANOVAS), which present the variance as a sum of its components.

The essence of quantitative genetics involves the separation of variation and the intepretation of the results obtained from different forms of Anova.

Determination of the contribution of the hereditary variation in the total variation of a given trait is associated with many difficult practical problems. If the environmental variance was equal to zero, and all the individuals within the population under consideration grew up and lived under identical conditions, then the genotype variation would be measurable as the variation observed within the population. In practice, however, it is impossible to make the living conditions of each individual identical even within a single family. The situation is more acute when dealing with fish, than with farm animals. Several methods are used in fish breeding to determine heritability and they are reviewed by Kirpichnikov (1981), and can be split into four main categories.

1. Determination of realised heritability on the basis of selection effectiveness (response).

2. Determination of heritability from the regression between parents and offspring.

3. Determination of heritability from the correlation between the values of a trait in close relatives.

4. Determination of heritability from the expression of the variance of phenotypic variation using variance analysis.

In methods (1), (2) and (3), parental values and numbers of generations are required to obtain meaningful estimates of hertibalities.

Using method (4), it is necessary to obtain simultaneously a sufficient number of related offspring from parents representing a population of fishes. The offspring are obtained either by diallele crosses or on the basis of the so called hierarchic complex (Kirpichnikov, 1981).

The hierarchical design has been used frequently in fish breeding. The external fertilisation of eggs in most fish species coupled with the usual high fecundity of the females, facilitates the simultaneous fertilization of a large number of crosses.

Once heritability has been determined for the trait under consideration, the correct method of selection should be employed. Selection can be based on a single desirable trait such as growth rate or a combination of traits.

There are prerequisites before a selection programme can be established, and up until recently many of the following were not properly evaluated, leading to failure or at least imprecise results, derived from inappropriate selection procedures.

The prerequisites are

1. The breeding goal, i.e. the trait under selection has to be defined as specifically as possible.

2. The entire life cycle of the animal should be under one's control.

3. It should be possible to hold and individually evaluate a number of generations in more or less identical rearing systems.

4. The individuals within a tested population should be identified by means of external tags or distinct biochemical genetic markers. This last point creates many problems for the research facilities let alone for the technically untrained fish farmer.

5. For the traits one has selected, their relative economic value should be established.

6. For the selected traits, the phenotypic variances and the heritabilities should be known. Again this is a great problem facing research institutes and fish farmers alike. As already mentioned large facilities are required and extensive breeding programmes

initiated to yield worthwhile results.

Gjedrem (1983) stressed the point that selection in general should be based on traits taken close to the time of marketing the animals. Problems that can arise are exemplified by the following. Selection for fast growth rate in the freshwater stage (pre smolt) of Atlantic salmon may not lead to fast growth rate in salt water or to large size eventually. But if one is a smolt producer and is relying on rapid turnover of stock, fast growth rate and heritability estimates derived from the pre smolt stage would be all important. If on the other hand one is a salmon farmer producing large adults, the production of fast growing early maturing adults (grilse) may be a positive disadvantage. One requires estimates of genetic and phenotypic variance and heritability estimates, for the strain of fish one is culturing, at the time at which one wants to sell ones produce to the market.

It has been shown that investment in selection programmes if carried out thoroughly and effectively may give rise to very high returns, considering the initial capital investment. Hill,(1971) and Gjedrem (1983) see no reason to believe that fish and shellfish are exceptions in this respect.

When it comes to deciding on a particular method of selection appropriate to ones stock and the trait involved, reference to the figures 3.1 and 3.2 reproduced from Falconer (1960) makes the options quite clear.

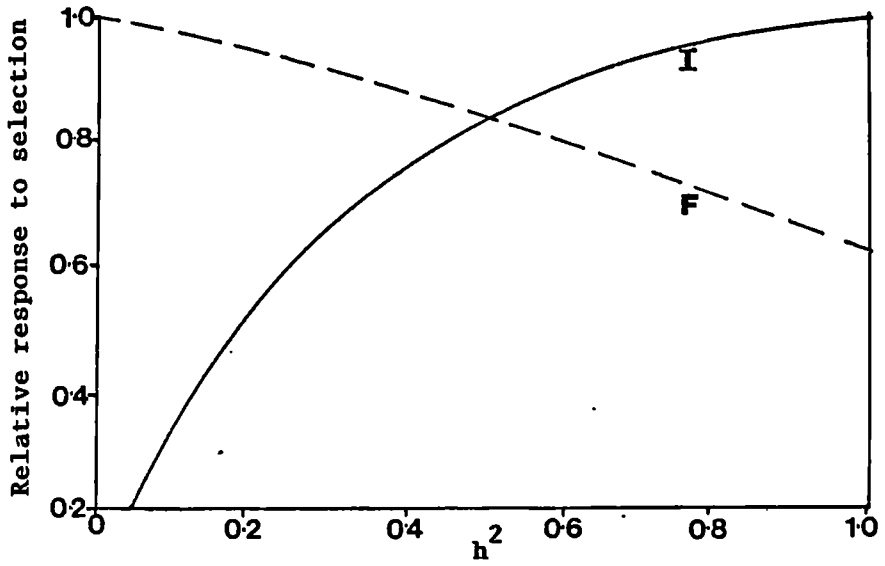


Figure 3.1 Relative merit of fullsib family (F) selection compared with individual (I) selection. Number per family is infinite and there is no variance due to common environment (Falconer, 1960).

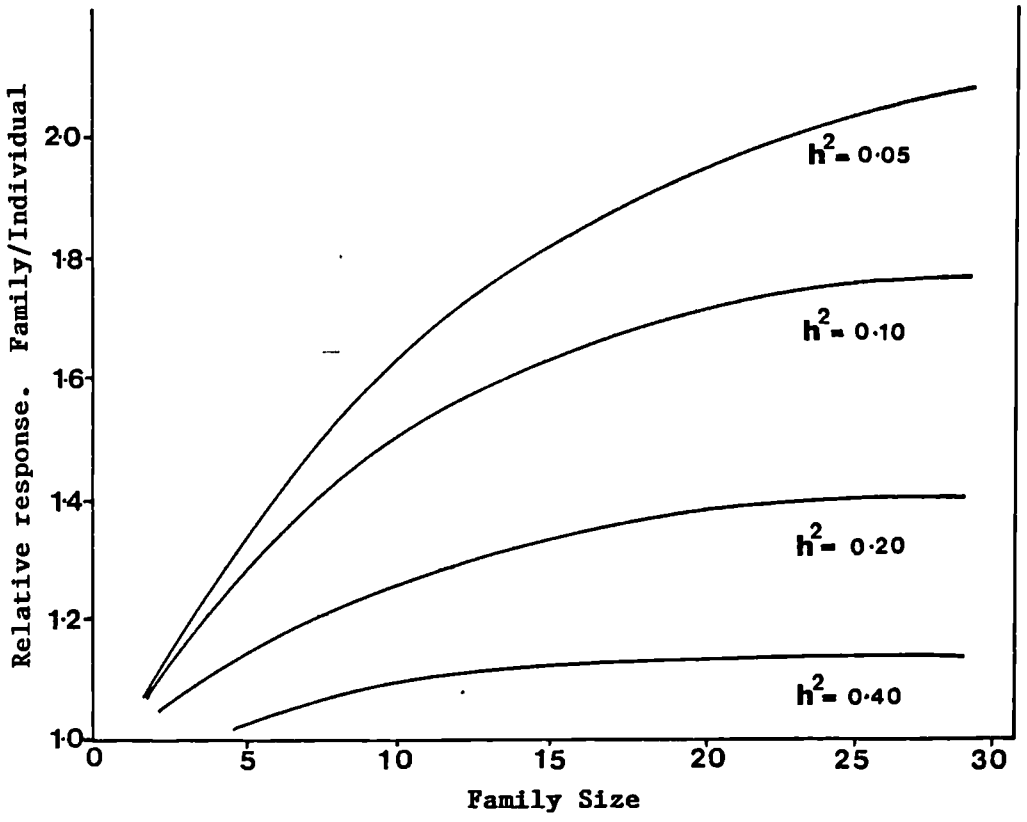


Figure 3.2 Response expected under family selection relative to that for individual selection, plotted against family size. It is considered that there is no variance due to common environment (Falconer, 1960).

Falconer (1960) and Gjedrem (1983) draw the following conclusions as far as selection procedures are concerned.

(a) The combination of individual and family selection is always most efficient (Figure 3.1).

(b) When the estimated heritability is approximately 0.5 then both family and inidvidual selection have the same efficiency. But when the heritability is lower, family selection is more efficient, and when h^2 is more than 0.5, individual selection (mass selection) is more efficienct than family selection (Figure 3.1).

(c) Whenever the heritability estimated is below 0.4, the efficiency of family selection compared with individual selection increases markedly as the number of families increase (Figure3.2).

Gjedrem (1983) states "with fish and shellfish, selection should be based on a combination of individual and family merit. Individual selection alone is only of interest when growth rate is the only trait of economic importance and is highly heritable".

Historically, mass selection has been the main method used in fish breeding but, in general, success had been limited for production characteristics. This is primarily because the fundamental requirements for selection have not been known or applied.

There are problems associated with mass selection and these include the one illustrated earlier with Atlantic salmon. Selection for high fingerling size in fishes in general may not improve the overall growth rate to marketable size, or the selection of marketable size may not improve the growth rate at fingerling size. This is because growth at different ages is influenced by different factors and heritability for size often increases by 2 to 3 times after the fingerling stage (Bye and Ponniah, 1983).

Other problems when selecting fish individually include inadvertant selection of aggressive individuals which turn out not to be optimum converters of food, and the ever present problem in broodstock husbandry, that of inbreeding, which limit the scope of selection.

Mass selection can be more effective if unrelated populations are used to start the breed, thus producing a heterogeneous gene pool from which to select (Kirpichnikov, 1981).

Family selection requires multiple crossings between selected parents, comparative evaluation of the progeny and selection of progeny from the best families for further raising. Falconer (1960) states that environmental variation should be kept to a minimum to minimize induced interfamiliar variability.

3.1.6. Concept of Genetic Gain

Knowledge of heritability for the trait under consideration gives

one an estimate of genetic variability but it does not inform the researcher or fish farmer of the potential gain that can accrue per generation or per year to the trait, once selection procedures are initiated.

Genetic gain can be calculated by using the following formula (Falconer, 1960) $G = \frac{i.\ h^2.\ \sigma p}{L}$

where i = selection

h^2 = heritability

σp = phenotypic standard deviation

L = generation time

The selection differential is a measurement of the intensity of selection, and can be predicted in advance provided that two conditions are satisfied. The first is that the values of the character being selected are normally distributed and secondly that selection is by truncation. Falconer (1960) defines truncation selection as "individuals are chosen strictly in order of merit as judged by their phenotypic values, no individual being selected that is less good than any of those rejected".

If these conditions are satisfied the selection differential depends on the proportion of the population included in the selected group. Gjedrem (1975) points out that because of the high fecundity exhibited by salmonids, only a small fraction of the population are required as broodstock, and this leads to a very high selection differential.

Under the majority of farmed trout and salmon conditions, the proportion of selected broodstock never rises above 1% and the corresponding i value is 2.66 (Truncated Normal Distribution tables).

Thus genetic gain depends on the size of the heritability and the phenotypic standard deviation and the proportion of individuals used as broodstock.

3.1.7. Research Undertaken

Heritability estimates are invaluable, if calculated correctly in order to predict response to selection, plan breeding programmes and estimate breeding values (Gjerde, 1986).

Very many determinations of heritability have been made for a great variety of characters in animals and plants. Cunningham (1983) summarises the extent of the selection work carried out in animal breeding research. Many hundreds of heritability estimates for a great number of traits have been determined and selection based on these estimates have taken place in the cattle, pig, sheep and poultry industries, for decades. An annual rate of improvement in the order of 1% for most traits appears relatively frequently in farm animal species (Cunningham, 1983). Falconer (1981) gives examples of heritability estimates for various types of animals, ranging from Drosophila sp. to man and recognises that heritabilities cannot easily be calculated with any great precision, and that the majority of estimates have large standard errors. A small number

of sires used in each experiment is often cited as reason for highly variable heritability estimates between years (Gunnes and Gjedrem, 1981) and also the reason for large standard errors (El-Ibiary and Joyce, 1978; Klupp, 1979; Refstie, 1980; Busack and Gall, 1983).

Falconer (1981) also alludes to the connection between the magnitude of the heritability estimate and the nature of the character under consideration; "on the whole the characters with the lowest heritabilities are those most closely connected with reproductive fitness while characters with the highest heritabilities are those that might be judged on biological grounds to be the least important as determinants of natural fitness. Falconer (1981) gives figures to illustrate this point, showing that body weight for cattle, pigs, poultry, mice, man and Drosophila have high heritabilities (h^2 between 0.35 and 0.65). Characters connected with reproductive fitness, such as litter size, or egg production have low heritabilities (h^2 between 0.05 and 0.2).

Only recently have heritabilities concerning traits in fish populations been studied. Extensive and thorough accounts of the research carried out in the field of quantitative fish genetics in the last decade are given by Kirpichnikov (1981), Gjedrem (1983), Kinghorn (1983) and Gjerde (1986).

A summary of heritability estimates derived from fish and shellfish species is given in Table 3.1, an extended version of Gjedrem's

Table 3.1 lists phenotypic and genetic parameters for quantitative traits in fish and shellfish. Basically the table consists of the one given by Gjedrem (1983) with some later additions. X = mean; σ = standard deviation; CV = coefficient of variation; SE = standard error. Heritability (h^2) as estimated from (S) sire, and (D) dam and (F) family component of variance.

Species/Trait	X	σ	CV	$h^2 \pm$ SE	$h^2F \pm$ SE $h^2D \pm$ SE	No. of families/ No. of observations	Authors
Rainbow trout							
150 day weight (g)	13.6	4.12	30	0.09 ± 0.10	0.06D ± 0.09	14S; 28D	Aulstad et al (1972)
150 day length (cm)	9.8	1.14	12	0.16 ± 0.14	0.06D ± 0.09	14S; 28D	Aulstad et al (1972)
280 day weight (g)	13.6	4.37	33	0.29 ± 0.20	0.01D ± 0.05	14S; 28D	Aulstad et al (1972)
280 day length (cm)	10.1	1.26	12	0.37 ± 0.23	0.03D ± 0.04	14S; 28D	Aulstad et al (1972)
180 day weight (g)					0.48F	8F	Moller & Naevdal (1973)
2 year weight (kg)	1.1	0.26	23		0.21F ± 0.05	39F	Gall (1975)
300 day weight (g)	51.0	16.0	32		0.13F	19F	Chevassus (1976)
Deadeyed eggs				0.15 ± 0.04	0.27D ± 0.04	143D	Kanis et al (1976)
Dead alevins				0.14 ± 0.03	0.06D ± 0.01	143D	Kanis et al (1976)
147 day weight (g)				0.06		Realised h^2	Kincaid et al (1977)
175 day weight (g)	7.0	2.41	34		0.60F ± 0.10	54F	Gall & Gross (1978a)
150 day weight (g)	2.8	0.82	29		0.32F ± 0.08	46F	Gall & Gross (1978a)
610 day weight (kg)	0.8	0.12	15		0.74F ± 0.10	54F	Gall & Gross (1978a)
630 day weight (kg)	0.6	0.13	22		1.04F ± 0.12	46F	Gall & Gross (1978a)
2 year weight (kg)			17		0.50F	126F	Gall & Gross (1978b)
2 year weight (kg)			20		0.31F	126F	Gall & Gross (1978b)
Survival of eyed eggs			20		0.23F	126F	Gall & Gross (1978b)
125 day weight (g)					1.06F ± 0.49	6F	Klupp (1979)
334 day weight (kg)					0.82F ± 0.38	6F	Klupp (1979)
140 day weight (g)	29.7	16.6	56	0.06	1.04D	34S; 41D	Refstie (1980)
140 day length (cm)	12.5	2.4	19	0.20	0.63D	34S; 41D	Refstie (1980)
2½ year weight (kg)	3.0	0.9	30	0.17	0.24D	34S; 115D	Gunnes & Gjedrem (1981)

continued . . .

Table 3.1continued

Species/Trait	X	σ	CV	$h^2 \pm SE$	$h^2F \pm SE$ $h^2D \pm SE$	No. of families/ No. of observations	Authors
Rainbow trout continued							
2½ year length (cm)	56.3	5.6	10	0.23	0.20D	58S; 115D	Gunnes & Gjedrem (1981)
Growth rate (%/day)	1.63	0.31	19	0.26 ± 0.12		34S	Kinghorn (1981)
Food consumed (%/day)	1.37	0.27	20	0.41 ± 0.13		34S	Kinghorn (1981)
Net food conversion	1.46	0.08	6	0.31 ± 0.11		34S	Kinghorn (1981)
Egg size				0.02 ± 0.16	0.65 ± 0.20	51S;124D(717obs)	Haus (1984)
Egg volume				0.44 ± 0.20	0.34 ± 0.15	51S;124D(717obs)	Haus (1984)
Egg number				0.33 ± 0.20	0.50 ± 0.17	51S;124D(717obs)	Haus (1984)
Weight 2.5 yr (1)				0.38 ± 0.22		17 factoral (2x2) (747obs)	McKay et al (1986)
2.5 yr (2)				0.37 ± 0.21		17 factoral (2x2) (747 obs)	Mckay et al (1986)
4 years old				0.27 ± 0.20		17 factoral (2x2) (699 obs)	McKay et al (1986)
Fork length 2.5 yr (1)				0.33 ± 0.20		17 factoral (2x2) (747 obs)	McKay et al (1986)
2.5 yr (2)				0.32 ± 0.21		17 factoral (2x2) (747 obs)	McKay et al (1986)
4 yr old				0.25 ± 0.20		17 factoral (2x2) (699 obs)	McKay et al (1986)
Condition factor 2.5 yr (1)				0.66 ± 0.27		17 factoral (2x2) (747 obs)	McKay et al (1986)
2.5 yr (2)				0.52 ± 0.24		17 factoral (2x2) (747 obs)	McKay et al (1986)
4 yr old				0.48 ± 0.26		17 factoral (2x2) (699 obs)	McKay et al (1986)

continued . . .

Table 3.1 continued

Species/Trait	X	O	CV	$h^2 \pm$ SE	$h^2F \pm$ SE $h^2D \pm$ SE	No. of families/ No. of observations	Authors
Rainbow trout continued							
Gain in fork length at 2.5 yrs				0.23 ± 0.16		17 factoral (2x2) (747 obs)	McKay et al (1986)
2.5 - 4 yrs				0.00 ± 0.10		17 factoral (2x2) (641 obs)	McKay et al (1986)
Instantaneous growth rate (weight) at 2.5 yrs				0.29 ± 0.14		17 factoral (2x2) (747 obs)	McKay et al (1986)
2.5 - 4 yrs				0.10 ± 0.13		17 factoral (2x2) (641 obs)	McKay et al (1986)
Age at sexual maturity				0.21 ± 0.14		17 factoral (2x2) (641 obs)	McKay et al (1986)
Fat %	9.71	1.00	10	0.47 ± 0.34		135	Kinghorn (1981)
Oxygen consumption	3.73	0.68	18	0.51 ± 0.12		34S	Kinghorn (1981)
2½ year weight (kg)	2.8	0.7	26	0.17 ± 0.11	0.39D ± 0.11	56S; 108D	Gjerde & Gjedrem (1984)
2½ year gutted wt (kg)	2.3	0.6	26	0.19 ± 0.12	0.40D ± 0.11	56S; 108D	Gjerde & Gjedrem (1984)
2½ year length (cm)	54.6	4.6	8	0.11 ± 0.11	0.44D ± 0.12	56S; 108D	Gjerde & Gjedrem (1984)
Dressing percent	82.3	4.5	6	0.01 ± 0.05	0.14D ± 0.07	56S; 108D	Gjerde & Gjedrem (1984)
Carcass quality score	3.6	0.7	20	0.14 ± 0.06	0.07D ± 0.05	56S; 108D	Gjerde & Gjedrem (1984)
Flesh colour, score	3.4	0.8	23	0.06 ± 0.08	0.28D ± 0.09	56S; 108D	Gjerde & Gjedrem (1984)
Age at maturity				0.18 ± 0.07	0.09D ± 0.05	56S; 108D	Gjerde & Gjedrem (1984)
Atlantic salmon							
Tolerance to vibriosis				0.12 ± 0.05	0.07D ± 0.05	42S; 140D	Gjedrem & Aulstad (1974)
180 day weight (g)				0.15 ± 1.00	0.21D ± 1.00		Naevdal et al (1975)

continued . . .

Table 31 continued

Species/Trait	$\bar{X}$	O	CV	$h^2 \pm SE$	$h^2F \pm SE$ $h^2D \pm SE$	No. of families/ No. of observations	Authors
Atlantic salmon continued							
2 year length (cm)				0.27 ± 0.36	0.07D ± 0.36 D		Naevdal et al (1975)
Dead eyed eggs				0.05 ± 0.04	0.62D ± 0.05	36lD	Kanis et al (1976)
Dead fry				0.02 ± 0.01	0.11D ± 0.02	144D	Kanis et al (1976)
3½ year weight (kg)				0.34	0.34D		Naevdal et al (1976)
3½ year length (cm)				0.76	0.84D		Naevdal et al (1976)
Age at maturity				1.00	0.67D		Naevdal et al (1976)
190 day weight (g)	7.8	5.9	76	0.08	0.15D	28S; 29D	Refstie & Steine (1978)
190 day length (cm)	8.2	1.9	23	0.12	0.17D	28S; 29D	Refstie & Steine (1978)
3½ year weight (kg)	5.6	1.6	28	0.31	0.31D	107S; 214D	Gunnes & Gjedrem (1978)
3½ year length (cm)	78.0	6.9	9	0.28	0.30D	107S; 214D	Gunnes & Gjedrem (1978)
3½ year gutted wt (kg)	5.0	1.2	25	0.44 ± 0.11	0.54D ± 0.08	105S; 248D	Gjerde & Gjedrem (1984)
3½ yr weight (kg)	5.7	1.4	25	0.44 ± 0.11	0.53D ± 0.07	105S; 248D	Gjerde & Gjedrem (1984)
3½ year length (cm)	80.0	5.7	7	0.35 ± 0.10	0.60D ± 0.08	105S; 248D	Gjerde & Gjedrem (1984)
Dressing percent	90	3.1	4	0.03 ± 0.02	0.02D ± 0.02	105S; 248D	Gjerde & Gjedrem (1984)
Carcass quality score	3.8	0.7	19	0.16 ± 0.05	0.14D ± 0.03	105S; 248D	Gjerde & Gjedrem (1984)
Flesh colour score	3.6	0.6	16	0.01 ± 0.03	0.17D ± 0.04	105S; 248D	Gjerde & Gjedrem (1984)
Age at maturity				0.42 ± 0.08	0.15D ± 0.03	105S; 248D	Gjerde & Gjedrem (1984)
Egg size				0.42 ± 0.16		91S; 218D(854obs)	Halseth (1984)
Egg volume				0.40 ± 0.17		91S; 218D(854obs)	Halseth (1984)
Egg number				0.37 ± 0.16		91S; 218D(854obs)	Halseth (1984)
Weight 12 wks (gm)	0.7			0.35 ± 0.29	0.44 ± 0.14	43S; 86D	Bailey & Loudenslager (1986)
Weight 6 months (gm)	8.0			0.09 ± 0.20	0.28 ± 0.10	43S; 86D	Bailey & Loudenslager (1986)
Length 6 months (cm)	6.3			0.10 ± 0.17	0.23 ± 0.08	43S; 86D	Bailey & Loudenslager (1986)
Length 12 wks (cm)	3.4			0.79 ± 0.31	0.90 ± 0.24	64S; 154D	Bailey & Loudenslager (1986)
Weight 12 wks (gm)	0.4			0.89 ± 0.32	0.93 ± 0.10	64S; 154D	Bailey & Loudenslager (1986)
Length 6 month (cm)	7.1			0.57 ± 0.28	0.77 ± 0.14	64S; 154D	Bailey & Loudenslager (1986)

continued . . .

Table 3.1 continued

Species/Trait	X	O	CV	$h^2 \pm$ SE	h^2 $\pm$ SE $h^2D \pm$ SE	No. of families/ No. of observations	Authors
Atlantic salmon continued							
Weight 6 month (g)	4.3			0.40 ± 0.26	0.76 ± 0.13	64S; 154D	Bailey & Loudenslager (1986)
Length 15 month (cm)	14.4			0.73 ± 0.32	0.75 ± 0.16	64S; 154D	Bailey & Loudenslager (1986)
Weight 15 month (g)	34.2			0.67 ± 0.32	0.75 ± 0.16	64S; 154D	Bailey & Loudenslager (1986)
Pacific salmon							
Tolerance to IHN				0.32			McIntyre & Amend (1978)
Coho salmon							
(1977)							
Wt 57 days post swim up	1.82	0.64	35	0.61 ± 0.31	0.65D ± 0.21	20S; 40D	Iwamoto et al (1982)
Wt 84 days post swim up	3.81	1.73	30	0.38 ± 0.25	0.67D ± 0.22	20S; 40D	Iwamoto et al (1982)
Wt 141 days after swim up	14.49	9.32	64	0.25 ± 0.22	0.67D ± 0.22	20S; 40D	Iwamoto et al (1982)
(1978)							
Wt 66 days post swim up	2.83	0.94	33	0.36 ± 0.21	0.49D ± 0.16	20S; 40D	Iwamoto et al (1982)
Wt 94 days post swim up	6.08	2.11	35	0.47 ± 0.22	0.37D ± 0.13	20S; 40D	Iwamoto et al (1982)
Wt 137 days post swim up	17.60	9.92	58	0.26 ± 0.20	0.590 ± 0.12	20S; 40D	Iwamoto et al (1982)
(1977)							
Lnt 84 days post swim up	6.60	0.62	10	0.30 ± 0.24	0.72D ± 0.22	20S; 40D	Iwamoto et al (1982)
Lnt 141 days post swim up	10.2	1.82	18	0.22 ± 0.21	0.68D ± 0.22	20S; 40D	Iwamoto et al (1982)
(1978)							
Lnt 94 days post swim up	7.6	0.87	11	0.56 ± 0.25	0.37D ± 0.21	20S; 40D	Iwamoto et al (1982)
Lnt 937 days post swim up	10.6	2.12	19	0.37 ± 0.31	0.50D ± 0.16	20S; 40D	Iwamoto et al (1982)

continued . . .

Table 31 continued

Species/Trait	X	0	CV	$h^2 \pm SE$	$h^2F \pm SE$ $h^2D \pm SE$	No. of families/ No. of observations	Authors
Brook trout							
Fingerling weight 144 days	0.03	0.016		0.08 ± 0.13	0.34D ± 0.19	4s x 4d replicated 8 times	Robison & Leumpert III (1984)
Juvenile weight 243 days	9.56	2.45		0.60 ± 0.27	0.37D ± 0.22	4s x 4d replicated 8 times	Robison & Luempert III (1984)
Survival through sac-fry				0.15 - 0.20		4s x 4d replicated 8 times	Robison & Luempert III (1984)
After sac-fry				0.00		4s x 4d replicated 8 times	Robison & Luempert III, (1984)
Carp							
Weight of fingerlings				0.10 ± 0.20			
Body weight				0.25			Smisek (1979)
Dry matter				0.15 ± 0.18			Smisek (1979)
Fat content				0.14 ± 0.15			Smisek (1979)
N in dry matter				0.15 ± 0.17			Smisek (1979)
4 month weight (g)	71	17	23		0.48F	9F	Nagy et al (1980)
4 month weight in lab (g)					0.12F	9F	Nagy et al (1980)
Tolerance to Hypoxia	179	50	28		0.51F	9F	Nagy et al (1980)
Weight gain (g)	366	81	22	0.47		17 offspring/ parents	Brody et al (1981)

continued . . .

Table 31 continued

Species/Trait	X	O	CV	$h^2 \pm SE$	$h^2F \pm SE$ $h^2D \pm SE$	No. of families/ No of observations	Authors
Catfish							
5 month weight (g)				0.61 ± 0.35		17S; 34D	Reagan et al (1976)
5 month length (cm)				0.12 ± 0.30		17S; 34D	Reagan et al (1976)
15 month weight (g)				0.75 ± 0.53		17S; 34D	Reagan et al (1976)
15 month length (cm)				0.67 ± 0.57		17S; 34D	Reagan et al (1976)
48 week weight (g)	237	56	24	0.52 ± 0.42	0.51D ± 0.37	13S; 26D	El-Ibiary, Joyce (1978)
48 week length (cm)	31	2.3	7	0.81 ± 0.46	0.25D ± 0.27	13S; 26D	El-Ibiary, Joyce (1978)
Dressing %	69	1.5	2	0.00 ± 0.20	0.470 ± 0.36	13S; 26D	El-Ibiary, Joyce (1978)
Lipids %	42	3.8	9	0.08 ± 0.25	0.50D ± 0.32	13S; 26D	El-Ibiary, Joyce (1978)
48 week weight (g)	328	90	27	0.27 ± 0.37	0.67D ± 0.41	13S; 26D	El-Ibiary, Joyce (1978)
48 week length (cm)	33	2.6	8	0.37 ± 0.39	0.60D ± 0.38	13S; 26D	El-Ibiary, Joyce (1978)
Dressing %	68	1.2	2	0.00 ± 0.27	0.84D ± 0.44	13S; 26D	El-Ibiary, Joyce (1978)
Lipids %	43	3.1	7	0.00 ± 0.23	0.65D ± 0.37	13S; 26D	El-Ibiary, Joyce (1978)
42 week weight (g)	53	2.4	46				Bondari (1980)
60 week weight (g) (1975)	375	11.2	30				Bondari (1980)
150 day weight (g) (1976)				0.28 ± 0.85		10S; 20D	Reagan (1980)
150 day weight (g) (1977)				0.0		10S; 20D	Reagan (1980)
150 day weight (g)				0.81 ± 0.95		10S; 20D	Reagan (1980)
18 months weight (g)				0.41 ± 0.69		10S; 20D	Reagan (1980)
Fat %				0.61 ± 0.78		10S; 20D	Reagan (1980)
Tilapia							
90 day weight (g)	19	5.1	27	0.04 ± 0.06	0.04D ± 0.08	16S; 32D	Tave and Smitherman (1980)

continued . . .

Table 31 continued

Species/Trait	X	0	CV	$h^2 \pm SE$	$h^2F \pm SE$ $h^2D \pm SE$	No. of families/ No. of observations	Authors
Tilapia continued							
90 day length (cm)	99	0.8	8	0.06 ± 0.06	0.02D ± 0.07	16S; 32D	Tave & Smitherman (1980)
42 week weight (g)	187	52	28				Bondari (1980)
42 week weight (g)	116	37	32				Bondari (1980)
60 week weight (g)	676	148	22				Bondari (1980)
60 week weight (g)	363	75	21				Bondari (1980)
T. mossambica (growth)				0.10	0.16	Realised h^2	Thien (1971)
T. aurea (growth)				0.23 ± 0.05		Realised h^2	Bondari et al (1983)
Blue mussel							
16 day length				0.16	0.29F	6S; 36F	Innes and Haley (1977)
Growth rate				0.12	0.62F		Newkirk (1980)
Length of larvae (mm)	169	8	5	0.43			Newkirk (1980)
Length of larvae (mm)	189.8	39.2	20.6	0.11 ± 0.02		1265 obs.	Mallet et al (1986)
Juvenile length (mm)	8.2	2.9	35.2	0.62 ± 0.06		2007 obs.	Mallet et al (1986)
Adult - Env 1 (mm)	24.3	7.5	30.9	0.92 ± 0.27		1931 obs.	Mallet et al (1986)
Adult - Env 2 (mm)	29.8	5.8	19.5	0.22 ± 0.07		1789 obs.	Mallet et al (1986)
Survival larval	24.0	3.1	12.9	0.00		120 obs.	Mallet et al (1986)
Adult - Env 1	81.0	17.5	21.6	0.15 ± 0.44		99 obs.	Mallet et al (1986)
Adult - Env 2	68.3	17.8	26.1	0.0		87 obs.	Mallet et al (1986)
Oysters							
18 month weight (g)	30				0.33F ± 0.19	11F	Lannan (1972)
18 month meat weight (g)	63				0.37F ± 0.06	11F	Lannan (1972)
Larval survival %	35				0.31F ± 0.06	11F	Lannan (1972)

continued . . .

Table 3.1 continued

Species/Trait	X	O	CV	h^2 + SE	$h^2F \pm SE$ $h^2D \pm SE$	No. of families/ No. of observations	Authors
Oysters continued							
Setting success %	45				0.09F ± 0.08	11F	Lannan (1972)
Larval growth				0.24			Longwell (1976)
6 day growth				0.33	0.43F	8S	Newkirk et al (1977)
16 day growth				0.50	0.60F	8S	Newkirk et al (1977)
7 day length				0.44 ± 0.21		8S	Losee (1978)
6 week length				0.50 ± 0.30		5S	Losee (1978)
Shell convexity				0.32		Realised h^2	Wada (1984)
Shell convexity				0.467		Realised h^2 30 parents/gen	Wada (1986)
Lobster							
100 day weight (g)					0.33F	12F	Hedgecock et al (1976)
90 day weight (g)					0.38F	9F	Hedgecock & Nelson (1978)

(1983) table II. Only estimates derived from experiments using 5 or more sires or 5 or more full sib groups are included. Gjedrem (1983) considered estimates based on less than these numbers to be of little value. He expressed a wish to set the limit much higher, but concluded that very few estimates would be left.

It appears that fish along with other animals exhibit a similar phenomena as described by Falconer (1981), some of the lowest heritabilities in Table 3.1 are calculated for egg production traits. The heritabilities shown for growth (Table 3.1), are on averge much lower than growth rate heritabilities found in most domesticated animals. Kirpichnikov (1981) suggests that the low heritability of weight and size among fishes is closely related to fertility, time of maturity and viability representing the main components of the breeding values or 'fitness' of an individual, and thus should have low heritabilities.

Heritability estimates have been calculated for a range of economically important traits, and the following section lists these traits and the authors who have published work on the subject.

1. Growth rate of various stages of the fishes life-cycle

Growth rate in rainbow trout (Aulstad et al., 1972; Moller and Naevdal, 1973; Gall, 1975; Kincaid et al., 1977; Gall and Gross, 1978a; Gall and Gross, 1978b; Klupp, 1979; Refstie, 1980; Gunnes and Gjedrem, 1981; Kinghorn, 1981; Gjerde and Gjedrem, 1984; McKay et al., 1986).

Growth rate in Atlantic Salmon (Naevdal et al., 1975; Naevdal et al., 1976; Refstie and Steine, 1978; Gunnes and Gjedrem, 1978; Gjerde and Gjedrem, 1984; Bailey and Loudenslager, 1986).

Growth rate in Carp (Kirpichnikov, 1972; Smisek, 1979; Nagy et al., 1980; Brody et al., 1981).

Growth rate in Channel Catfish (Reagan et al., 1976; El-Ibiary and Joyce, 1978; Bondari, 1980; Bondari, 1984)

Growth rate in Tilapia (Thien, 1971; Tave and Smitherman, 1980; Bondari, 1980; Bondari et al., 1983).

Growth rate in Blue Mussel (Innes and Haley, 1977; Newkirk, 1980; Mallet et al., 1986).

Growth rate in Oysters (Lannan, 1972; Longwell, 1976; Newkirk et al., 1977; Losee, 1978).

Growth rate in Lobster (Hedgecock et al., 1976; Hedgecock and Nelson, 1978).

Growth rate in Brook Trout (Robison and Luempert III, 1984).

Growth rate in Pacific Salmon (Iwamoto et al., 1982).

2. Carcass traits, including dressing percent, percentage lipid, belly thickness, flesh colour

Rainbow trout (Gjerde and Gjedrem, 1984; Kinghorn, 1981; McKay et al., 1986).

Atlantic salmon (Gjerde and Gjedrem, 1984).

Carp (Smisek, 1979).

Catfish (El-Ibiary and Joyce, 1978).

3. Egg size, egg volume, egg number

Rainbow trout (Gall and Gross, 1978; Haus, 1984).

Atlantic salmon (Halseth, 1984).

4. Food Conversion efficiency

Rainbow trout (Kinghorn, 1981; 1983).

5. Survival of eggs, alevins, fry

Rainbow trout (Kanis et al., 1976; Gall and Gross, 1978b).

Splake hybrids (Ayles, 1974)

Atlantic salmon (Kanis et al., 1976).

Brook trout (Robinson and Luempert III, 1984).

Blue mussel (Mallet et al., 1986).

Oyster (Lannan, 1972).

6. Condition Factor

Rainbow trout (McKay et al., 1986)

7. Resistance to disease

Atlantic salmon resistance to vibriosis (Gjedrem and Aulstad, 1974).

Pacific salmon. INH tolerance (McIntyre and Amend, 1978).

Splake (Salvelinus fontinalis x S. namaycush) resistance to 'blue sac diseases' in alevin stage (Ayles, 1974).

8. Tolerance of adverse conditions

Hypoxia in Carp (Nagy et al., 1980).

Acid water tolerance in brown trout (Gjedrem, 1976; Edwards and Gjedrem, 1979).

9. Age at Maturity

Rainbow trout (Gjerde and Gjedrem, 1984; McKay et al., 1986).

Atlantic salmon (Naevdal et al., 1976; Gjerde and Gjedrem, 1984)

10. Oxygen consumption

Rainbow trout (Kinghorn, 1981)

11. Shell convexity

Oysters (Wada, 1984; 1986).

3.1.8. Quantitative work carried out using Brown trout

The number of heritability estimates found for various traits relating to brown trout are few, in marked contrast to the number of electrophoretic examinations that have been reported for the species. One of the only investigations found involved more than 250 strains of brown trout, that showed significant genetic variation in tolerance to acid water both between strains and between families within strains (Gjedrem, 1976; Edwards and Gjedrem, 1979). The heritability estimates ranged from 0.09 to 0.27. This has lead to a selective breeding programme of brown trout strains for stocking in acid rivers. The future success of the project is not yet known (Gjedrem, 1981).

Years before quantitative methodology in genetics became established Dahl (1919) showed that brown trout from different waters grew at different rates and maintained the observed differences when eggs from different populations were grown on artificially. Alm (1949) investigated the inheritance of differences between naturally occurring variations of brown trout in Sweden, and demonstrated genetic differences between his "fario" and "lacustris" strains

for age at maturity and fin colour. Alm (1949) also postulated that maturity is a function of growth rate, and that trout that grew fastest matured earliest. These were valuable works in their day, but judged on modern day methodology they are not statistically very valuable.

3.1.9. Response to Selection

Gjerde (1986) illustrates various realised responses to selection for growth rate in fish and shellfish in his table (1). The fish species that have been used in selection experiments are given below:

1. Carp

Common carp have been farmed for thousands of years and have adapted well to pond environments. Kirpichnikov (1972) reported improved growth rate and resistance to disease in selected carp breeds in the Soviet Union. Moav and Wohlfarth (1976) working in Israel attempted to select for fast growth and slow growth, using mass selection for the traits upto an age of 7 months. The selection was practiced in earth ponds for five generations. Moav and Wohlfarth (1976) reported selection for slow growth rate yielded a strong response for the first three generations, while high growth rate groups showed no response to selection. They suggested that selection for fast growth rate had reached a plateau and the variation existing within the population was not large enough to warrant simple mass selection. However family selection over two subsequent generations showed significant increase in growth rate. This illustrates the

need to choose the correct selection procedure, in order to maximise the gain per generation that the available variability will allow.

2. Salmonids

Lewis (1944) selected rainbow trout for fast growth rate and size of eggs at 2 years of age, and reported large gains in both parameters, but common to many of the first experiments in selection, no control lines were used, with which the results could be compared. Donaldson and Olson (1957) and Donaldson (1970) in a long term, often referred to, selection experiment reported remarkable progress for selecting all manner of traits in rainbow trout. These traits included growth rate and egg production. However in this experiment, the reported selection responses are confounded by changes in management techniques, feeding regimes and upgraded facilities, as well as the fact that control populations were not maintained. Kincaid et al, (1977) reported 5% gain per year for weight of rainbow trout at 147 days post fertilisation and Gjerde (1986) reported gains of 7% per year for growth rate of Atlantic salmon at 190 day weight and a 3.6% gain in body weight per year at 2 years of age (salmon kept in sea cages) when compared to wild control lines. Gjedrem (1981) reported that selection programmes were being carried out in Norway in some wild populations of brown trout to improve growth rate, disease resistance and acid tolerance, but effectiveness of these activities is yet unkown.

3. Channel Catfish

Bondari (1980) and Reagan (1980) reported very high response to

selection for fast growth rate. Bondari (1980) reported a 33% increase and Reagan (1980) reported a 59g increase per generation at a 90 day weight.

4. Oysters

Haley et al., (1975) reported that mass selection of adult oysters gave an apparent strong reponse to selection for growth rate. But as the environment was considered extremely variable, a combination of family and mass selection was suggested to achieve maximum response. Newkirk (1980) indicated a 10-20% per generation gain in growth rate was a reasonable expectation.

The realised responses quoted by Gjerde (1986) are very high compared to what has been reported in species of farm animals. Cunningham (1983) reported an annual rate of improvement of the order of 1% for most traits in farm animals, while fish and shellfish give figures 5 to 10 times greater, probably correlated with the 'wild type' genome encountered within fish and shellfish populations which have yet to be domesticated.

Although relatively few selection programmes have been initiated or at least reported, many authors have suggested such trials would be beneficial, their views based on the heritability estimates and genetic correlations they found. Iwamoto et al., (1982) suggest that the growth of Coho salmon in the wild could be greatly enhanced by mass selection due to the high calculated heritability for this trait. Robison and Luempert III (1984) working with Salvelinus

fontinalis found high heritabilities for juvenile weight (h^2 = 0.6) and large variation within the population (CV = 26%) and suggested that mass selection would be an effective means of significantly increasing juvenile weight. Similar conclusions have been made by Gjerde (1984) working with age of sexual maturity in Atlantic salmon, Bondari (1984) working on body weight in channel catfish, Wada (1986) working with shell growth rate in Japanese pearl oysters (Pinctada facata mortensii), and Busack and Gall (1983) working on growth rate, and fecundity of the mosquitofish. There are plans to introduce the mosquitofish which preys on mosquito larvae, into areas with a mosquito problem. The fish used in pilot projects so far have been from totally wild stock and the variability for the various traits observed is very great. Stearns (1984) working also with mosquitofish on a more academic approach, postulates that the mosquitofish has not been under much selective pressure, exhibits high heritabilities for growth rate, because the trait is not under strong selection pressure, thus agreeing with Falconer (1981) and Kirpichnikov (1981).

Hulata et al., (1986) warns that mass selection for rapid growth in a strain of O. niloticus is not a promising method of improvement, unless genetic variation is increased in the basic population, and measures are taken to avoid inbreeding. Tave and Smitherman (1980) and Kincaid (1976) also highlight the problem of utilization of a too narrow genetic base when estimating heritabilities and initiating selection experiments.

Hulata et al., (1986) point out that mass selection is also a difficult method to use in tilapia because of non-synchronous spawning.

McKay et al., (1986) also voice warnings concerning mass selection for juvenile size in salmonids, without regard to physiological status. McKay et al (1986) postulate that it may lead to some improvement (genetic growth) but alterations in the population distribution with respect to physiological status from generation to generation may reduce the rate of improvement. This type of selection may result in earlier maturing fish with poorer performance in later life. McKay et al., (1986) point out that because smolting and maturation are threshold traits, small changes in the environment or the genetic make up of the population may lead to relatively large shifts in physiological status. Such environmentally induced shifts have been reported for Atlantic salmon (Naevdal, 1983; Saunders et al., 1983). It is clear that more information on the relationships between size, growth, maturity and smolting in salmonids is required, before advice on genetic management and selective procedures can be given (McKay et al., 1986).

Gall and Gross (1978) recognise that many of the estimates of heritability, particularly those made from a full-sib family structure are biased upwards and they suggest waiting for results from selective programmes before obtaining realised heritabilities. However, as stated by Gjedrem (1975) there seems to be sufficient evidence to suggest that heritability for growth is high enough to obtain significant genetic gains from selection, especially if family

selection is employed (Gall and Gross, 1978).

3.1.10. Aims of this study and involvement of sponsors

It was felt the stock of brown trout, which had been kept at Howietoun fish farm, part of Stirling University's aquaculture facility, might be somewhat inbred. The history of the farm dates back to the late 1870's when Sir James Maitland founded the establishment. The farm was originally stocked with brown trout from Loch Leven, then known as Salmo levenensis. Since then there have been introductions from other local sources, and from populations of trout outwith the area (locations unknown). The recent strategies concerned with broodstock management, prior to the University taking over the farm in 1979 were unknown.

It was felt a project was required to evaluate the effectiveness of the previous broodstock management, and to advise whether or not fresh genetical input from other stocks of trout was desirable to maintain the genetic variability of the stock, and thus the potential for improvement via various selection strategies.

Two approaches were envisaged; firstly a quantitative breeding programme was initiated and secondly an electrophoretic investigation was performed. Both these approaches were designed to establish the current genetical make up of the brown trout at Howietoun fish farm, and other wild brown trout populations in Scotland, and answer the following questions in particular:

1. What was the state of genetic variability within the captive stock at Howietoun, for the most economically important trait, namely growth rate?

2. What was the estimated heritability for growth rate in the Howietoun stock compared with a wild stock?

3. From calculated heritabilities, what potential genetic gain exist within the Howietoun farm stock?

4. Was the genetic variability within the Howietoun stock typical of brown trout populations?

Prior to the commencement of the present project, an experimental interstrain cross was performed between brown trout from Howietoun and brown trout from Loch Leven. The cross was carried out in the autumn of 1980. The resultant progeny were known as "Ballantine trout" in recognition of the financial support the whisky company, George Ballantine and Sons, had given to the University of Stirling and Howietoun fish farm in particular.

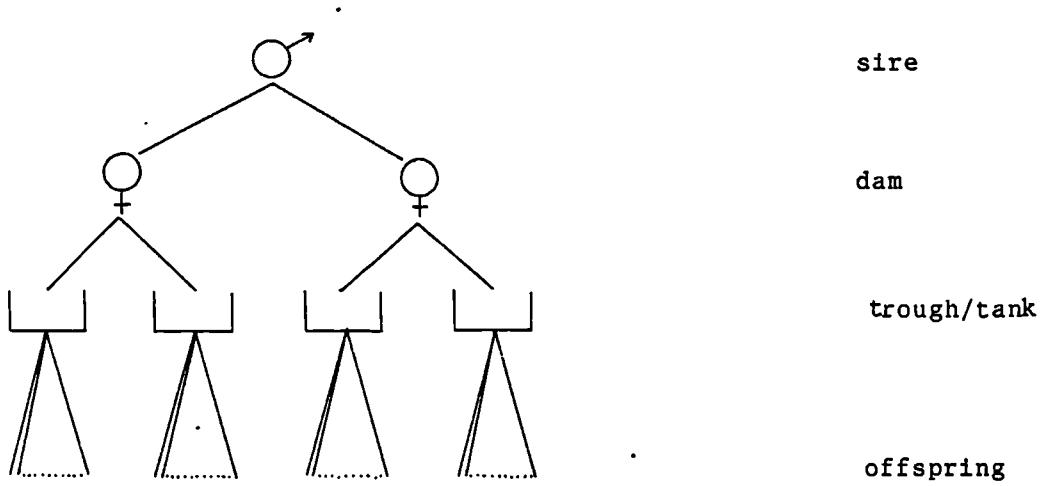
This cross or strain was widely reported to be "very vigorous" and to grow "exceptionally well", exhibiting "hybrid vigour or heterosis". Another aspect of the present work was to investigate this claim, and to establish whether the "Ballantine trout" cross was worth repeating. The present work was funded by George Ballantine and Sons as an extension to the Ballantine trout concept and as

a more indepth study of the genetic variability and importance of Scottish brown trout.

3.2 Materials and Methods

3.2.1. Broodstock used

In October 1982, 18 female and 9 male Howietoun trout were used in a hierarchical style cross. Each female having her eggs split into two by volume, to give replicates and were fertilised by one male. Each male fertilising the eggs from 2 females, see diagram below.



All the female broodstock were 3 year old first spawners having not been used previously on the farm. It was intended that the males should also be 3 year olds but, due to a shortage, two 2 year olds were used. Table 3.2 gives a list of the broodstock used in 1982.

In November 1983, Loch Leven trout, electrofished from the North Quiech spawning burn were stripped at the holding facility at Loch Leven, using separate containers to collect spawn from each broodfish. Table 3.3 gives a list of the broodstock used. Scales were taken from each fish, so that parental age could be established. It would have been better to use trout of all the same age to minimise variation

in the size of the eggs, but due to the nature of the wild stock, this was not possible. Only 6 females and 3 males were successfully stripped on the 26th November 1983 and the remaining 12 females and 6 males were stripped on 29th November 1983.

Once the eggs and milt were back at Howietoun hatchery the eggs from two females were crossed with the milt from one male and each batch of eggs split by volume to give replicate treatments. Thus setting up a hierarchical style cross.

During stripping operation in 1982 and 1983, all the fish were stripped by the same individual (self) to cut down on potential variation in mortalities due to variations in individual stripping techniques.

In 1984 broodstock from three localities were used. Two males and two females from Howietoun, two males and two females from Loch Leven's North Quiech feeder burn and two males and two females from the 'Nashua' strain of brown trout kept at Faskally, Freshwater Fisheries Laboratory in Pitlochry, were used in a diallele cross.

Each male being crossed with each female and vice versa. Table 3.4 lists the broodstock used, and Figure 3.3 illustrates the cross carried out. The eggs derived from one female had to be split into six equal portions before the milt from each male was added. This necessitated using as large females as possible to obtain a large number of eggs. The Nashua females were not as large and did not

Table 3.2

List of broodstock used 17/11/82 for hierarchical crosses

Stock: Howietoun

♀	Tag No.	Length (cms)	Age (winters +)	State of maturity *	♂	Tag No.	Length (cms)	Age (winters +)
1	1618	38.5	2++	2	1	1620	37.4	2++ †
2	1640	40.5	2++	3				
3	1607	34.8	2++	2	2	1216	39.5	2++
4	1608	37.5	2++	2				
5	1668	38.2	2++	2	3	1648	36.9	2++
6	1617	38.9	2++	2				
7	1634	40.5	2++	2	4	1621	39.6	2++
8	1624	37.6	2++	2				
9	1217	40.6	2++	2	5	1638	35.8	2++
10	1218	37.5	2++	2				
11	1641	38.3	2++	2	6	0591	38.0	2++
12	1219	35.0	2++	2				
13	1645	36.5	2++	1	7	1647	31.7	2++
14	1603	38.2	2++	3				
15	1220	37.4	2++	3	8	1221	26.0	1++
16	1622	36.8	2++	3				
17	1646	39.5	++	2	9	1222	23.8	1++
18	1623	38.5	2++	2				

* 'under-ripe - 1
'ripe' - 2
'over-ripe - 3

† moribund male (sperm motile)

Table 3.3

List of broodstock used 26/11/83 and 29/11/83

Stock: Loch Leven trout

Date Crossed	♀	Length (cms)	Age (winters +)	State of maturity	♂	Length	Age (winters +)	State of sperm
26/11	1	42.0	3++	2	1	47.0	3++	✓
26/11	2	51.0	4++	2				
26/11	3	45.0	3++	2	2	57.0	4++	✓
26/11	4	47.5	4++	3				
26/11	5	38.0	3++	2	3	55.5	4++	✓
26/11	6	42.0	3++	2				
26/11	7	37.0	3++	2	4	61.0	5++	✓
26/11	8	41.5	4++	2				
29/11	9	44.0	3++	2	5	49.0	4++	✓
29/11	10	47.0	3++	3				
29/11	11	52.5	4++	2	6	47.0	4++	✓
29/11	12	45.5	4++	2				
29/11	13	45.0	3++	2	7	50.0	4 or 5	✓
29/11	14	51.5	5++	2				
29/11	15	48.0	5++	2	8	46.0	5++	✓
29/11	16	44.5	4++	2				
29/11	17	43.5	3++	2	9	47.0	4++	✓
29/11	18	42.0	4++	dead				

State of maturity

1 - under-ripe
2 - ripe
3 - over-ripe

7 and 8, 4 } S Quiech

Rest N Quiech

Table 3.4

List of broodstock used 7/11/84

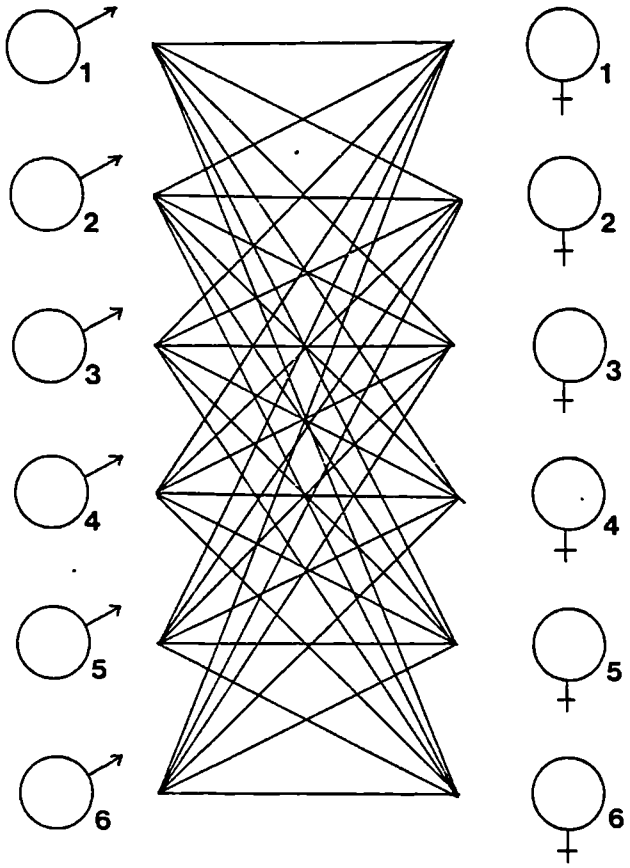
Stock: Howietoun 5,6 - and

Leven 3,4 - and

Nashua 1,2 - and

Date crossed	♀	Length (cms)	Age	State of maturity	♂	Length (cms)	Age	State of sperm
7/11/84	1	40.5	3++	3	1	42.5	3++	✓
7/11/84	2	47.0	3++	3	2	48.0	3++	✓
7/11/84	3	48	5++	2	3	53.0	5++	frozen
7/11/84	4	47.5	5++	2	4	44.5	3++	✓
7/11/84	5	60.0	5++	2	5	43.0	3++	✓
7/11/84	6	55.0	5++	2	6	45.0	4++	✓

Figure 3.3
Diagram illustrating the Factoral cross



Factoral cross performed, producing 36 different combinations.

produce as many eggs as did the Howietoun and Leven dams. Once the Nashua eggs had been split into six the number per batch was below 500 and thus the egg numbers from the other dams had to be reduced to a comparable level before the first feeders were introduced to the tanks. Thus the initial density of first feeders in 1985 was fewer than in 1983 or 1984.

Due to the nature of the 6 x 6 diallele cross, which produced 36 different male, female combinations, the trial was carried out with no duplicates.

3.2.2. Brief history of the broodstock sources

1. Howietoun trout have existed as such since about 1880 when the farm was founded. The trout originally came from the Loch Leven strain and was then crossed with a variety of local populations. The stock has had trout added to it over the last one hundred years. But prior to the University taking over in 1979, the broodstock management may have been suspect.

2. Loch Leven trout, formally known as Salmo levenensis is renowned worldwide for its fast growth rate, sporting qualities and excellent eating.

3. The Nashua strain of brown trout were imported as eggs and milt by Department of Agriculture and Fisheries for Scotland (DAFS) in Pitlochry from North America. There a strain of brown trout which has become known as the Nashua strain has been developed over a period of 50 or 60 years. The fish came originally from

Europe. DAFS had kept these fish in a restricted system of concrete tanks for four generations, and they have shown remarkable growth rate (Walker, pers. comm.), although their appearance is not as desirable as, for example, the Loch Leven trout. They tend to be short, heavy and virtually unspotted. It was thought the fish used as broodstock in this study had a high probability of being closely related to one another, being all of the same age class, and derived from a limited number of broodstock four generations back.

3.2.3. Stripping and fertilization procedure

Eggs from each female were stripped into separate clean dry containers and the quantity of eggs divided by volume and placed in other labelled containers.

Milt from the males was stripped into glass viles and equal volumes mixed with the different batches of eggs, using a syringe. The milt was mixed with the eggs and left for 10 minutes. They were then washed using clean burn water and left to harden for 3 hours. Each batch of eggs was then placed into prepared numbered egg trays at random.

For eggs and milt travelling from Loch Leven or from Pitlochry, they were kept separate and fertilization was not initiated until back at Howietoun. The time between stripping and fertilization was between two and three hours. Excellent fertilization rates

were achieved as long as the containers into which eggs were stripped were clean and dry.

3.2.4. Egg incubation and hatchery

The system in which the eggs were incubated is illustrated in Figure 3.4 and Plate 1. The trays in which the eggs were kept were made of perforated aluminium. A constant even flow of water was maintained, occasionally checked by adding a small quantity of malachite green to the inflow, to chart the passage of the water.

The water supply was gravity fed from a header tank five feet above the top of the system, and the flow was increased as the eggs hatched and alevins emerged. The depth of water covering the eggs was controlled by means of standpipes positioned at the bottom of each trough.

Each egg tray was so designed to let water pass under the front of the tray and up through perforations in the base, over the eggs and then out of the rear side of the tray.

The eggs were not counted into the system but an accurate count of mortalities was recorded daily. Dead eggs were removed using a pipette and bulb picker.

Silt in the water caused problems in 1982-1983, partially covering eggs and later causing gill problems with the smaller alevins (Richards, pers. comm.). In 1983-1984 and 1984-1985 Armitages polymer filter

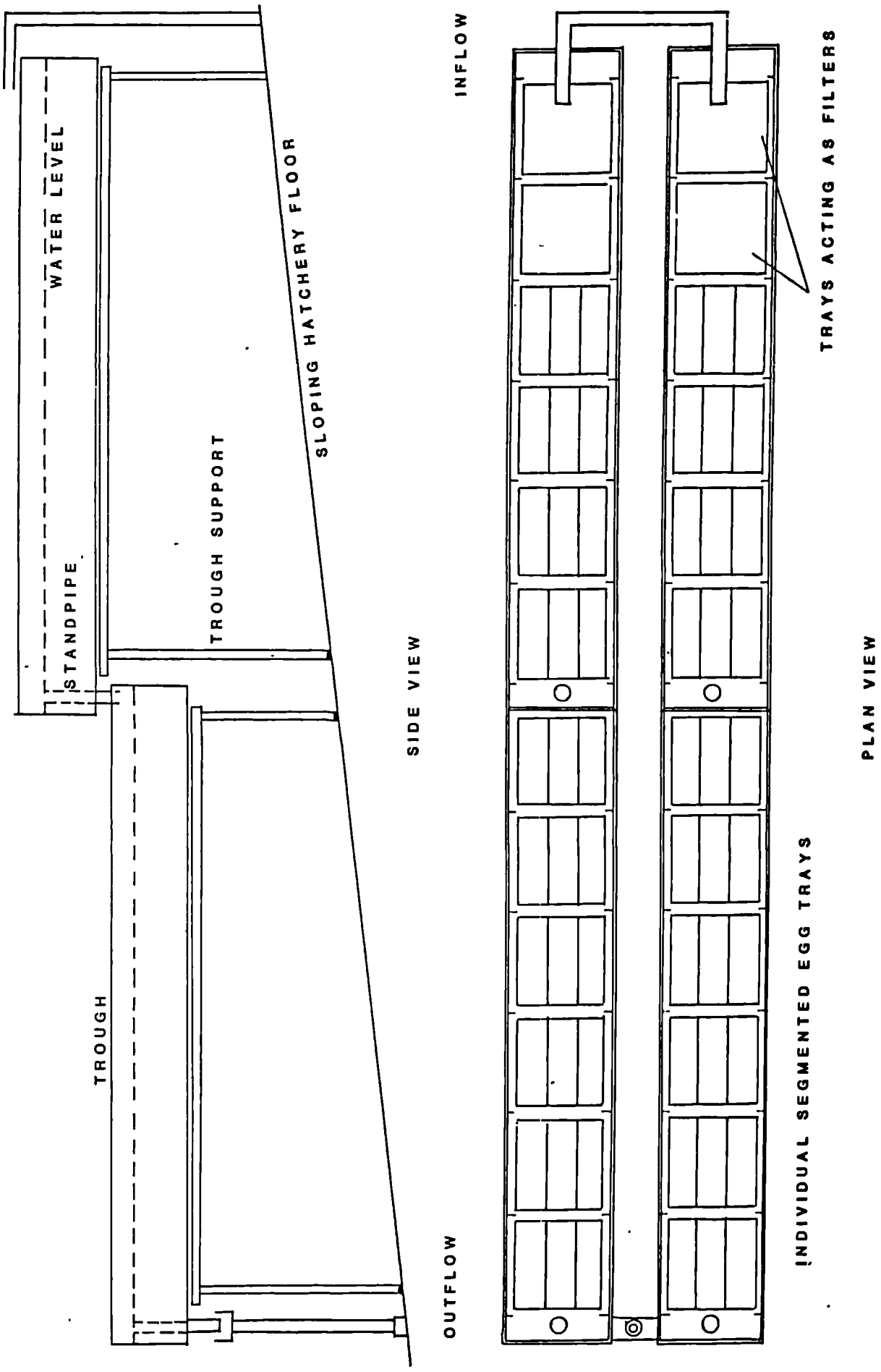


FIGURE 3.4 SHOWING HATCHING TROUGHS

Plate 1 Photograph showing segmented egg trays

Plate 1

wool was used in the inflow trays (see Figure 3.4) which acted as an effective filter. The wool was washed or replaced once a week or more often if necessary. Silt that penetrated beyond the filters, was carefully removed from the system by syphoning water from beneath the trays. The troughs were thoroughly cleaned once the eggs had reached the eyed stage and could withstand movement.

The eggs were kept in the dark by covering the troughs with hardboard sheets, and later black polythene sheeting, weighted on either side to keep it in position.

Once the eggs were eyed, they were shocked (between 40 and 50 days afer fertilization). This procedure entailed syphoning each set of eggs into a bucket, washing them, and counting them back into the same (now washed) segregated tray. Shocking enabled one to identify eggs which had not been fertilised or were not developing normally. Such eggs turned opaque and white in colour and were easily removed.

Eggs were counted effectively and quickly using a small sheet of perspex countersunk with exactly 200 egg-sized depressions. By keeping accurate daily counts of mortalities it was possible to calculate exactly how many eggs were laid down.

So as to achieve the same density of fish in the tank system later in the experiment it was thought the most accurate method of attaining this, was to reduce the egg number to around 500 before they hatched.

This was done in 1983, only two days before the alevins started hatching, and it was thought that the disturbance caused by the reduction in numbers, may have speeded up hatching to a limited extent. But as the disturbance was caused to all the batches, even those not being reduced were counted, it was thought any resulting changes in developmental rates were insignificant. Reduction in egg numbers took place in 1984 but due to the smaller batch sizes in 1985 as a consequence of the diallele crossing procedure, numbers were not reduced to the same extent.

Estimates of proportions of the different batches that had successfully hatched were recorded daily along with the first and last day successful hatching took place. The alevins were kept in the dark and the troughs kept clean by syphoning waste egg shells and extraneous detritus daily. Malformed or dead alevins were removed and recorded and preserved in 70% alcohol.

In 1983, due to unforseen circumstances the tank system was not in working order until 11th March. Some of the fry in the trays were at the swim up feeding stage a week before this and first feeding by hand commenced on the 9th March 1983. In 1984 and 1985 feeding was not attempted until the fry were moved to the tank system.

In 1983 the tank system consisted of 30 tanks and therefore a number of batches had to be excluded from the experiment from then onwards. Fry from females 1-12, and 17 and 18 were placed into the tanks,

along with fry from female 15. The latter were used to fill up the system rather than to partake in the heritability experiment, due to not having enough tanks for the progeny of female 16, and because the severe mortality suffered up to first feeding reduced numbers below an acceptable level.

In 1984 and 1985 fry from all the parental combinations at the fertilization stage were transferred to the tank system. Although in 1985 numbers were much reduced and the initial stocking was of 200 fry per tank. Some of the batches were below this but it was felt that at this low density any differences in growth caused by a density effect were minimal.

Tables 3.5, 3.6 and 3.7 list the dates and days after fertilization that various event occurred in 1982-83, 1983-84 and 1984-85.

Table 3.5 Howietoun stock

Event	Date	Time from fertilisation (days)
Eggs laid down	17.11.82	0
Eggs shocked	6.1.83	50
Eggs thinned out to 150	18.1.83	62
Eggs started to hatch	19.1.83	63
Eggs finished hatching	29.1.83	73
First feeding started	9.3.83	112
Into tanks	11.3.83	114
1st accurate individual weighing	27.6.83	222
2nd accurate individual weighing	29.9.83	326
3rd accurate individual weighing	10.5.84	549

Table 3.6 Leven stocks

Event	Date	Days from	
Date laid down	26.11.83 and 29.11.83	0	(-3)
Eggs shocked and counted	9.1 84	44	41
Eggs reduced to 520	18.1.84	53	50
Eggs started to hatch	2.2.84	68	65
Finished hatching	12.2.84	78	75
First fed	13.3.84	107	104
Into tanks	13.3.84	107	104
1st accurate measurement	3.7.84	219	216
2nd accurate measurement	4.10.84	312	309
3rd accurate measurement	10.5.85	530	527

Table 3.7 Mixed stock Diallele Cross or Factoral Cross

Event	Date	Days from
Date laid down	7.11.84	0
Eggs shocked	17.12.84	40
Eggs started to hatch	7.1.85	61
Finished hatching	19.1.85	73
First fed	20.2.85	105
Into tanks	27.2.85	112
1st accurate measurement	11.6.85	216
2nd accurate measurement	2.9.85	299

3.2.5. The tank system

Due to the sloping nature of the floor in the one hundred year old hatchery at Howietoun, arranging the 1 metre tanks to obtain even flow created a problem. Columns of thermolite blocks and concrete wedges were constructed to enable the timber on which the tanks rested to be at the same level. This meant the gravity fed ring main could supply water at a constant, similar pressure to each tank.

The tanks at the bottom of the system were considerably higher off the hatchery floor than those at the top (see Figures 3.5, 3.6 and Plate 2). The water supply was piped within the hatchery floor from the header tank at the top end of the building. The effective head of water being approximately five feet. The water supply was spring and burn fed, and shuttering and valve systems enabled water to be channelled either from the spring or the burn or from both. The supply of water was controlled to suit the needs of the commercial side of operations at the hatchery.

The ring main supplying the tanks was designed to run round the whole system attached to the timber on which the tanks stood. Two inch piping was used for the main. The individual tanks were supplied by half inch pipe and the flows controlled by half inch taps (see Figure 3.5). Initially the flows were found to be insufficient with the half inch pipe feeding directly into the tanks, so was reduced three times to produce sufficient current. The diameter

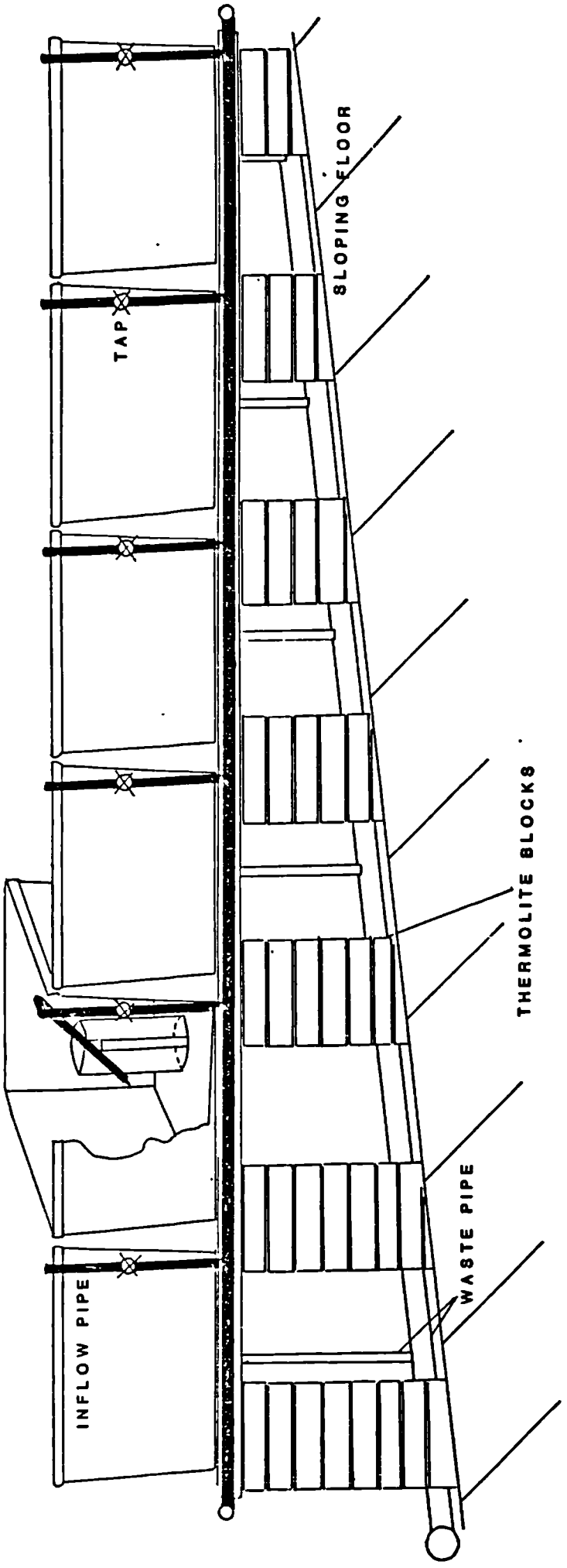


FIGURE 3.5 SHOWING SIDE VIEW OF THE TANK SYSTEM

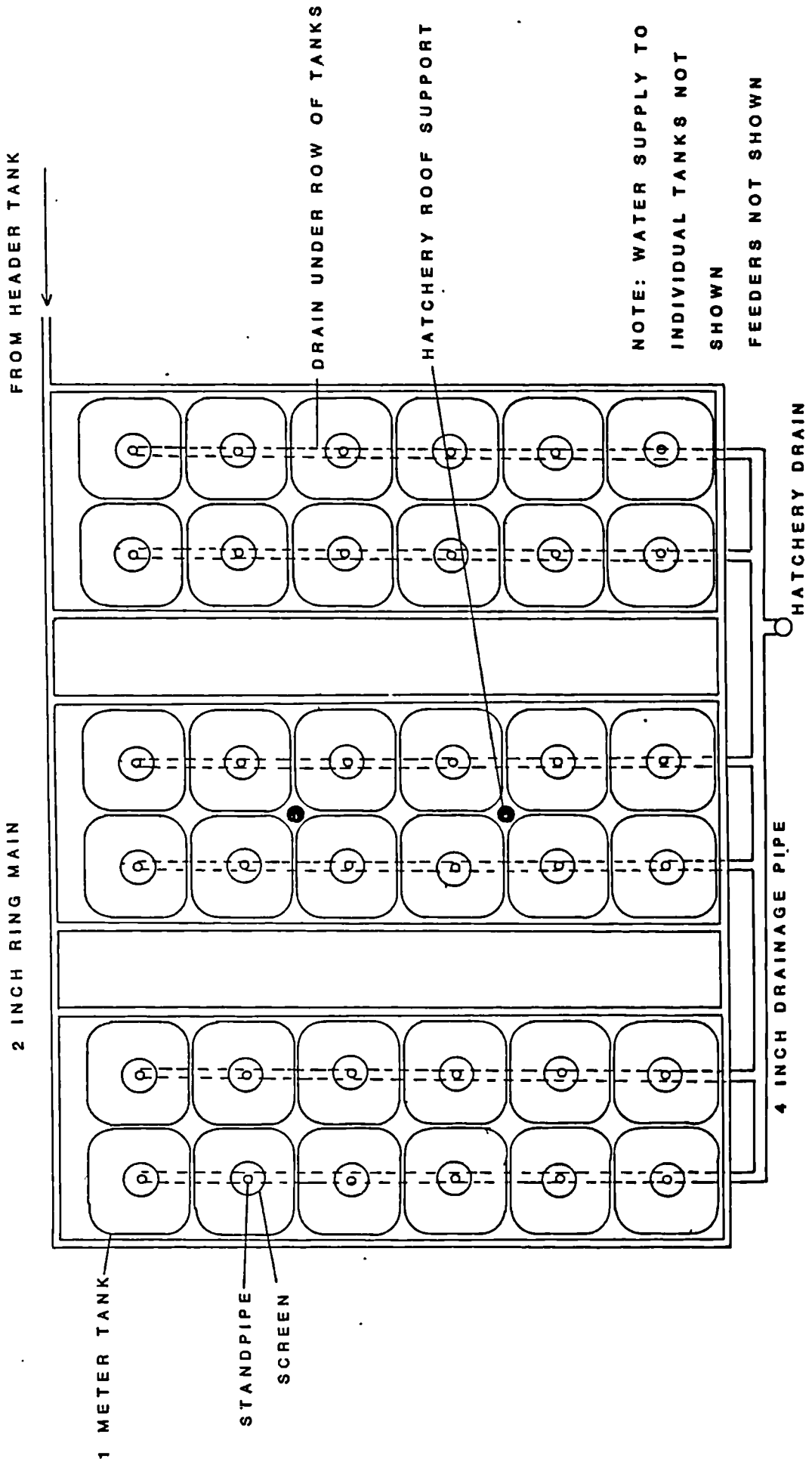


FIGURE 3.6 PLAN VIEW OF TANK SYSTEM

Plate 2 Photograph of lm tank system

Plate 2

of the reduced inflow pipes was 6mm.

The maximum delivery of water to each tank was 10 litres/minute. But at the first feeding stage, the supply was reduced to allow the young trout to maintain position on the bottom of the tank (1 litre/minute).

The fibreglass tanks (1 metre square with rounded corners) were made by Stirling training workshop. The system was built in 1983, ready for the first feeders of that year. Due to limited initial finance, 30 tanks were installed but the system was extended a year later to 36 tanks with the addition of an extra row.

The depth of water in the tanks varied. At the fry stage a water depth of 15 cm was sustained by means of standpipes placed in the central well of each tank. Once the trout were growing well and the effective density had increased, new standpipes were introduced raising the depth of water to 25 cm.

The screens in the centre of the tanks, surrounding the standpipes were also changed during each growing season. In 1983 the first screens were made of punched zinc with holes of 1.5 mm in diameter. These were found not to be satisfactory, because after approximately three months of cleaning, they started to disintegrate. The possible toxicity of the zinc screens was tested by leaving a sample of fry in still water with sections of the material for 48 hours. No apparent damage was observed in the fry. The tanks run on a flow through

system and the fish would not have been in contact with water that had been in contact with the screens for more than a few minutes.

Once the trout started to grow and the size of food increased the small size of screen slots or holes became a problem as food and waste built up around each screen encouraging ectoparasites and Saprolegnia fungus to thrive. New screens were required but due to expense and logistical problems of securing larger screens while the tanks still contained trout, a system of 12 cm (diameter) plastic pipes, drilled with 5 mm holes and placed over the standpipes was devised. These pipes, obviously taller than the standpipes, were not secured to the base of the tank and simply rested in the central sump.

Great care was required when the tanks were cleaned and the standpipes removed every morning. Minimal losses occurred due to accidental escapement via the standpipe during cleaning.

The trout were fed by means of Danish clockwork belt feeders (Dansk Ørredfoder a.s. Brande). The belt which once extended to its maximum length wound up the clockwork mechanism, pulled the belt back again over a 12 hour period. The feeders, although bulky, were positioned on each tank using dexian strips allowing food to fall onto the water surface in the same position in each tank, namely where the inflow pipe enters the tank.

Once the trout reached approximately 9 months old, it was decided they had become too large to be over-wintered in the 1 metre tanks, the maximum biomass in the tanks reached 5kg/tank. Each tank contained $0.25m^3$ of water so the stocking density was $20kg/m^3$. Besides, no fish were kept in the hatchery during the winter as the water was required for egg incubation. In accordance to commercial practice the trout were transferred to earthen ponds, which measured 33 metres long, 5 metres wide and had a maximum depth of 1.6 metres. In 1983 all the fish (7,428) were placed in Pond 28. In 1984 half the fish were stocked into Pond 29 and half into Pond 30. The duplicate sets were placed in the two different ponds. 4,338 averaging 11.26 gms were stocked in Pond 29 and 4,370 averaging 11.68 gms were stocked in Pond 30.

To follow the progress of the trout studied in the 1 metre tanks it was decided to mark individual trout and attempt to follow their growth over the following winter and spring period.

Various methods of marking the fish were considered. But the only practical way of achieving any success with the equipment available was to use the panjetting technique. This involves injection of a dye, in this instance Alcian blue, into the dermis of the fish. Alcian blue is indelible and remains visible for up to two years (Johnstone, 1981).

Individual marking of the trout was attempted by panjetting fins

and individual fin rays, but this turned out to be impracticable due to the small size of the fish, and the inaccuracy and inconsistency of the panjet itself. Body panjetting fish with individually recognisable marks was considered but not enough readable combinations of spots could be accurately applied to the fish. As an alternative a proportion of fish from each tank (20%) were panjetted with a batch mark. Fifty fish from each tank population of 250 individuals was marked in 1983 and 1984. The same fish as had been accurately weighed and lengthed.

The trout were transferred to the ponds on 28th September 1983 and on 10th October 1984, which were completely enclosed by 4 inch netting to prevent predation from piscivorous birds. The trout were fed at approximately 1% total biomass daily by a twice daily hand feed in the usual manner adopted by the commercial side of the Howietoun operation. The food consisted of Ewos Baker pellets No. 4 and No. 5 mixed.

The trout were netted out of the ponds during the following May using a seine net, with the help of the fish farm staff, so the operation had to fit in with the commercial running of the farm, and could not be conducted at a comparable time each year.

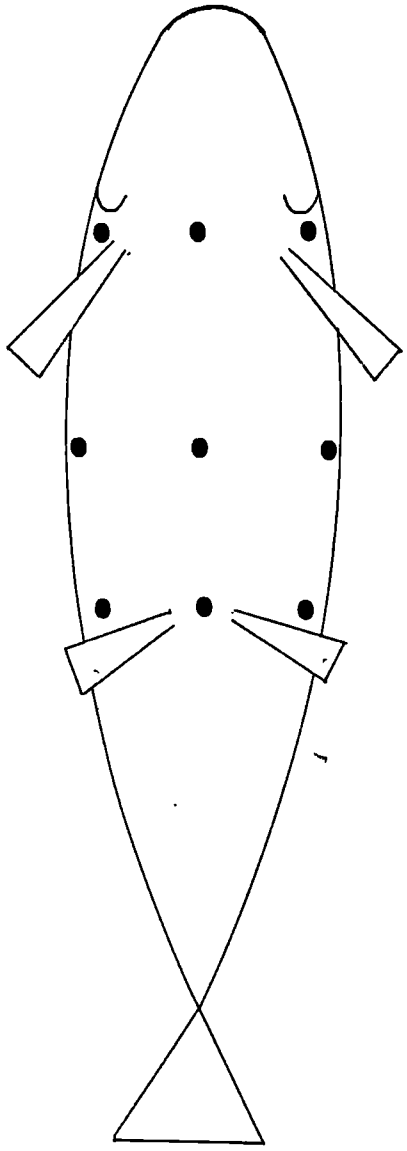
The fish, once netted out, were anaesthetised and separated into panjetted and non-panjetted fish. The panjetted individuals were then identified using key cards, and the weights and lengths recorded

for the appropriate batch. Figure 3.7 illustrates the panjet spot locations used for identifying the different populations. The weights were recorded using a battery operated digital balance which recorded weights to the nearest gram. Fork lengths were taken using a measuring board. Benzocaine solution was the anaesthetic employed.

3.2.6 Husbandry

As mentioned, the trout were introduced to the system each year, when the fish were coming on to the first feeding, swim-up stage. Attempts were made to introduce the same number of trout to each tank so that the initial density was identical. The number of eggs was equalised at or near stocking to alleviate problems of individually counting and handling the alevin first feeder, which was not advised (Robertson, pers. comm.). An average weight was obtained for each tank by wet weighing each batch of fry as they were introduced. The fish were fed by means of the previously described clockwork belt feeders. Every morning once each tank had been partially drained and cleaned using a soft bristled brush, food was added to the centre of the belt. The amount varied but was always between 5 and 10% of the biomass of trout present in the tanks, this effectively fed

Figure 3.7



Spot locations for identifying batches of fish. Only 2 spots maximum were used for any one batch mark.

Ventral view of trout

the trout to excess every day. Due to the damp atmosphere in the hatchery the very small particles of the fry and fingerling diets tended to stick to the belt. Excess food was thus added to make sure enough food was made available for trout.

The feeders worked well with only a few giving cause for concern. Certain batches of fish effectively received slightly less food than others, due to the inefficiency of the feeders on those particular tanks. This will be discussed later in the results section.

In 1983 the food consisted of a mixture of three major trout feeds namely BP, Fulmer and Ewos-Baker. It was mixed in equal proportions and fed identically in all tanks. In 1984 and 1985 only Ewos-Baker was used.

The size of food given varied during the year. In 1983 the fry diet initially seemed too large and the first feeders were having difficulty taking the particles. A sample of the diet was ground down even finer using an electric grinding mill. The fry took the resulting finer particles more readily, but more food stuck to the feeder belt. The process of reducing the initial food size particles was repeated in 1984 and 1985.

As the fry grew into fingerlings and then into parr the food particle size was altered according to the recommended BP food chart for trout (BP Publication, 1982). Due to the unequal average weights of the trout in different tanks, the food particle size was always

adjusted to the tank involved. By September every year the food size varied from No. 3 to No. 4 (Ewos Baker).

Monthly batch weights were taken to estimate the biomass in each tank and thus enable the correct amount of food to be calculated. The timing of these weighings was not crucial as food was being fed in excess.

Accurate weights and lengths were taken for estimating heritability of growth rates, in 1983, 1984 and 1985 at approximately similar stages of development, at 5 months and 9 months after hatching. The dates when accurate weighing and lengthing were taken was not crucial because heritability estimates are only relevant for the experiment under consideration, and cannot strictly be compared (Kirpichnikov, 1981).

In 1983 and 1984, fifty fish from each tank were selected at random and anaesthetised (Benzocaine) and weighed and lengthed individually. The fish were wet weighed to the nearest tenth of a gram using a Mettler 400 balance, and measured at 5 months old with a micrometer to the nearest half a millimeter and at 9 months old using a conventional measuring board. The lengths recorded were all fork lengths. In 1985 only thirty fish from each tank were measured because of the smaller number of trout in each tank.

The first accurate weights and lengths were measured at the end of June and the beginning of July in 1983 and 1984 respectively.

But even after increasing water levels and flow rates, the stocking density was thought to be potentially limiting in the future, so each population was reduced to 250 fish per tank at the same time as data from fish was recorded.

The spare fish were incorporated into the commercially farmed stock (1983 - 7,000; 1984 - 8,023).

A close watch was kept everyday for evidence of disease. Mortalities were accurately recorded and examined for ectoparasites and possible gill damage, using conventional skin scrapes and gill preparations. Temperature was also recorded daily using a maximum and minimum thermometer placed in one of the tanks. If the temperature rose above 17°C feed was not given.

Gill damage was evident in 1983 when the alevin and first feeding stages showed increased mortalities especially amonng the smaller fish. The damage was caused by increased silt load and was a direct result of three dirty spates that were experienced. The hatchery had no filtering or settling system, and the silt could not be avoided.

The young fish were treated with Roccal - to clean off mucus, detritus, bacteria and food from the gills. Roccal was applied using a bath treatment for ¾ hour at 1ppm.

Each year as the water temperature increased in late spring, or early summer, the trout stopped feeding and started 'flashing'. Each time Costia were identified from skin and gill scrapes, along with Schyphidia and Trochodina. These protozoan parasites were successfully treated with a bath solution of 40% Formaldehyde at a rate of 1:5000. Two or three treatments spread over a 3 week period cleared the problem each year.

In July 1983, Ichthyophthirius was identified when the fish stopped feeding and started flashing. This was successfully treated by cleaning the system thoroughly and giving a bath treatment of Formaldehyde and Malachite green together. the former at a concentration of 200ppm and the latter at 1ppm, for a period of approximately ¾ of an hour.

In 1983 numbers of fry died due to never coming on to feed. The problem, producing fish known as pinheads, was thought to be more accute amongst the smallest fry, which indicated the yolk sac may have been completely utilized earlier than the yolk sac of the larger fry, and by the time food was given, the smaller individuals were effectively too weak to take the food. This was investigated in 1984 when yolk sac utilization was monitored in three different sizes of alevins. Small alevins come from small eggs, so a sample of what were regarded as "small", "medium" and "large" eggs were kept separate to test the rate at which the yolk sac was utilized in the resultant alevins. The growth of the fry was monitored using 10 individuals every week. The length was measured and their

total wet weight was recorded (dried briefly before weighing). Each alevin's yolk sac was removed and weighed to the nearest milligram. The alevins were not fed at all and the trial continued until the yolk sac was thoroughly utilized.

In August in 1983 and 1984 fish were observed jumping in the tanks, so to prevent the resultant possible problems this would create certain procedures were adopted. The inflow pipes were lowered to the bottom of the tanks, to prevent surface disturbance which was thought to be encouraging the trout to jump. Black polythene was stretched over half of each tank to give the fish some cover. This alleviated the problem of fish jumping but effectively increased the density of fish in the tanks as more trout tended to maintain position beneath the polythene. Exactly the same conditions prevailed in each tank so partially covering the tanks did not significantly contribute to variation in average size of the fish between populations.

3.2.7. Analysis of Data

The estimation of heritability from half-sib and full-sib analysis. Using the hierarchical design, a number of males (sires) were each crossed with two females (dams). (For details of parental broodstock, see materials and methods section). The progeny from each female were split into two, yielding 2 tanks per female. Fifty offspring were measure from each tank. The individuals measured thus formed a population of half-sibs and full-sib families. The statistical model used in the hierarchical design scheme assumes that the individuals

were chosen at random from the reference population and that the inbreeding coefficient was zero. Statistical model (Becker, 1975) balanced design.

$$Y_{ijkl} = U + \quad i + Bij + t_{ijk} + eijkl$$

where Y_{ijkl} is the record of the ith progeny in the km tank, of the j-th dam, mated to the i th sire; U is the common mean; i = effect of the i th sire; Bij is the effect of the j-th dam mated to the i th sire, tijk is the effect of the kth tank in which are progeny from the j th dam mated to the i th sire; and eijkl = the uncontrolled environmental effect and genetic deviation attributable to the individuals.

The analyses of variance was divided into observational components attributable to differences between the progeny of different males (the between-sire component), to differences between the progeny of female mated to the same male (between dam, within sire component) and to differences between the progeny in tanks derived from the same female (between tanks, within dam component) and to differences between individual offspring (within-progeny component).

Table 3.8 illustrates the form of the analysis.

Table 3.8

Source	df	Mean square	(Expected) Composition of mean square
Between sire	s-1	Ms_s	$= \sigma^2 w + K\sigma^2 a + aK\sigma^2 d + dak\sigma^2 s$
Between dams (within sires)	s(d-1)	Ms_d	$= \sigma^2 w + K\sigma^2 a + aK\sigma^2 d$
Between tanks (within dams)	sd(a-1)	Ms_a	$= \sigma^2 w + K\sigma^2 a$
Progeny within tanks	sda(k-1)	Ms_w	$= \sigma^2 w$

where s = number of sires

d = number of dams per sire

a = number of tanks per dam

k = number of fish measured per tank

The mean square for 'within progeny' is equal to the within-progeny variance component $\sigma^2 w$ but the other mean squares are not equal to the appropriate variance component. Table 3.8 shows the composition of the mean squares in terms of the observational components of variance. The variance components are thus estimated using the following equations.

Sire component $(\sigma^2 s) = (Mss - Msd)/dak$

Dam component $(\sigma^2 d) = (Msd - Msa)/ak$

Tank component $(\sigma^2 a) = Msa - Msw/k$

Within tank component $(\sigma^2 w) = Msw$

The estimate of total phenotypic variance is given by the sum of observational components

$$\sigma^2 T = \sigma^2 s + \sigma^2 d + \sigma^2 a + \sigma^2 w$$

The components of $\sigma^2 d$ and $\sigma^2 s$ are estimates of genetic variances. The progeny of a dam being full-sibs and the progeny of a sire, within dam effects removed, being half-sibs (Becker, 1975). Each of the variances σ^2_s and $\sigma^2 d$ contains one quarter of the additive genetic variation of the parents (Kirpicknikov, 1981). Heritability estimates can be derived from the variance components thus

Sire heritability $$h^2 s = \frac{4\,\sigma^2 s}{\sigma^2 s + \sigma^2 d + \sigma^2 w} \quad \text{or} \quad \frac{4\,\sigma^2 s}{\sigma^2 T}$$

Dam heritability $$h^2_d = \frac{4\,\sigma^2 d}{\sigma^2 T}$$

Sire + Dam heritability $$h^2_{s+d} = \frac{2(\sigma^2 + \sigma^2 d)}{\sigma^2 T}$$

To determine standard errors for the heritability estimates obtained, the following procedure devised by Anderson and Bancroft (1952) was employed. They showed that a satisfactory approximate of standard error can be calculated, provided the degrees of freedom associated with the numerater mean squares are moderately large. In this study the degrees of freedom are regarded as very large indeed and thus

this method is acceptable.

The standard errors then are:

a) when $h^2s = \frac{4\sigma^2 s}{\sigma^2 s + \sigma^2 d + \sigma^2 w} = \frac{4\sigma^2 s}{\sigma^2 p}$

$$\sigma(h^2 s) \simeq \frac{4A}{\sigma^2 p}$$

where $A = \sqrt{\frac{2}{K^2_2}\left(\frac{Mss^2}{ns+2} + \frac{Msd^2}{nd+2}\right)}$

A = S.E. of variance

b) when $h^2_d = \frac{4\sigma^2 d}{\sigma^2 p}$

$$\sigma(h^2 d) \simeq \frac{4B}{\sigma^2 p}$$

where $B = \sqrt{\frac{2}{K^2_3}\left(\frac{Ms^2 d}{nd+2} + \frac{Msa^2}{na+2}\right)}$

B = S.E. of variance

c) when $h^2 = \frac{2(\sigma s^2 + \sigma^2 d)}{\sigma^2 p}$

$\sigma(h^2)$ $\frac{2(A^2 + B^2 + 2C)}{\sigma^2 p}$

where $C = -\frac{K1}{K2}\left(B^2 - 2\frac{(Msw)^2}{NwK^2_3}\right)$

C = S.E. of variance

ns, nd and nw are degrees of freedom associated with the sires, dams and offspring sums of squares respectively

K1 - number of dams/sire

K2 - number of progeny/sire

K3 - number of progeny/dam

When the factoral design was employed the following statistical model was used (Becker, 1975).

$Yijk = \mu + \alpha i + Bj + (\alpha B)ij + eijk$

where Yijk is the observation of the k-th individual from a mating of the i-th sire with the j-th dam; μ is the common mean; αi is the effect of the i-th sire; Bj is the effect of the j-th dam; $(\alpha B)ij$ is the interaction of the i-th sire mated to the j-th dam; eijk is the environmental and remainder of the genetic deviations.

When the Factoral crossing design was employed the calculation of the expected mean squares and variances was different.

Source	df	Mean square	Composition of expected mean square
Sire	s - 1	Mss	$\sigma^2 w + K\sigma^2_{sd} + KS\sigma^2_s$
Dam	d - 1	Msd	$\sigma^2 w + K\sigma^2_{sd} + Kd\sigma^2 d$
Sire/dam interaction	(s-1)(d-1)	Mssd	$\sigma^2 w + K\sigma^2_{sd}$
Progeny	sd(k-1)	Msw	$\sigma^2 w$

where s = number of males

d = number of females

k = number of progeny/tank

The various components were calculated as follows

Sire component $\sigma^2 s$ = MSs-MSsd/sk

Dam component $\sigma^2 d$ = MSd - MSsd/dk

Sire Dam interaction σ^2_{sd} = MSsd - MSw/k

Progeny component $\sigma^2 w$ = MSw

Total phenotypic component $\sigma^2 T = \sigma^2 s + \sigma^2 d + \sigma^2 sd + \sigma^2 w$

The heritability estimates were calculated in the same manner as for the hierarchical design model, e.g.

$$h^2 s = \frac{4\,\sigma^2 s}{\sigma^2 T}$$

$$h^2d = \frac{4\,\sigma^2d}{\sigma^2T}$$

$$h^2sd = \frac{2\,(\sigma^2s + \sigma^2d)}{\sigma^2T}$$

Standard errors were also based on the method employed by Anderson and Bancroft (1952).

3.3 Results

3.3.1. Results using hierarchical breeding schemes

The results are organised as follows. For each accurate weight or length taken for the years 1982-1983 and 1983-1984 a table is presented, laying out the simple statistics derived from the measurements. The tables include the following information: minimum, maximum, mean, standard error, variance, standard deviation, range, kurtosis and skewness, along with an indication of the relevant tank number and from which female the progeny were derived.

Following each simple statistical table there is an analysis of variance table along with the resulting heritability estimates for that particular weight or length at that particular time, along with the appropriate standard errors.

All the analyses give high heritabilities (which will be discussed later) but due to the nature of the test there is virtually no other statistical information directly available concerning differences

observed between tanks, dams or sires.

The Fisher's F test can be used to identify significant levels in the anova, and is calculated by dividing the mean square for one level in the anova table by the mean square of the level below. The figures obtained are then compared to 1 tail F table and by using the correct degrees of freedom, listed in each anova table, one can identify which levels of the anova contribute significantly to the variation observed.

The F values obtained for each level are listed at the right hand side of the anova tables along with an indication whether the value is significant or not.

When a significant F value has been calculated one still knows nothing about the particular level in the anova table, for example one does not know which tanks or which females have given rise to significantly different sized progeny.

Duncan's multiple range test (1955) was devised to identify whether a set of mean values were significantly different from one another. The test is performed using mean square values obtained from analyses of variance tables. One only uses the test, when the level in the anova in which one is interested, is significant and this is established by use of the F-test statistic already described. At the bottom of each anova table, the relevant mean, overall standard deviation, and coefficient of variation are given.

Coefficient of variation (CV) is a useful parameter for judging the magnitude of variation. It expresses the ratio of phenotypic standard deviation to the mean of the trait in question ($CV = (\sigma p/\bar{X})100$). The CV enables one to compare the sizes of variances of different trials.

Following each Anova table is a graph illustrating the means of the particular weight or length ranked in order, along with the relevant standard deviation. Along each x-axis are the tank numbers and the relevant female number. Above the graph is a series of lines representing visually the results from a Duncan's multiple range test (1955) performed on the data set. The tanks which have a common line under-ruling them are not significantly different from one another.

To illustrate the variation in growth between the trout populations in the 1 metre tank system, Figure 3.20 shows the tanks with the largest and smallest mean weights with their respective duplicates, from the Howietoun and Leven trials. Figures 3.21 and 3.27 illustrate the growth of the same populations after they have been transferred to the earth ponds.

Table 3.9 Listing statistics for each tank population at weight (1) in the Howietoun trial

♀ No.	Tank No.	Minimum (gms)	Maximum (gms)	Mean (gms)	S.E. Mean	Variance	S.D.	Range (gms)	Kurtosis	Skewness
1	20	1.32	2.36	1.83	0.032	0.051	0.226	1.04	0.300	0.156
1	29	1.21	2.32	1.74	0.035	0.062	0.249	1.11	0.255	0.062
2	1	1.35	2.33	1.90	0.037	0.068	0.260	0.98	0.571	0.086
2	12	1.11	2.55	1.94	0.046	0.084	0.253	1.44	1.661	0.503
3	2	1.33	2.66	1.90	0.041	0.084	0.290	1.33	0.083	0.408
3	30	1.31	2.54	1.63	0.034	0.059	0.242	1.23	3.078	1.426
4	13	0.84	2.45	1.70	0.046	0.107	0.327	1.61	0.243	0.211
4	21	0.79	1.84	1.43	0.029	0.042	0.204	1.05	0.730	0.508
5	3	1.16	2.52	1.69	0.042	0.090	0.300	1.36	0.027	0.403
5	14	1.20	1.87	1.48	0.020	0.021	0.144	0.67	0.171	0.513
6	4	1.12	2.70	1.89	0.042	0.089	0.298	1.58	0.868	0.102
6	22	0.96	1.91	1.35	0.027	0.035	0.186	0.95	0.929	0.570
7	15	0.94	1.87	1.49	0.026	0.033	0.182	0.93	0.523	0.083
7	23	0.54	1.88	1.29	0.038	0.072	0.267	1.34	0.677	0.510
8	5	1.80	3.06	2.36	0.045	0.101	0.318	1.26	0.614	0.343
8	16	1.46	2.39	1.83	0.031	0.049	0.221	0.93	0.437	0.322
9	6	1.24	2.74	2.25	0.044	0.099	0.314	1.50	1.293	0.950
9	24	1.11	2.56	1.90	0.037	0.068	0.261	1.45	1.398	0.046
10	17	1.28	2.15	1.66	0.026	0.033	0.181	0.87	0.219	0.227
10	25	0.87	1.83	1.43	0.029	0.041	0.203	0.66	0.275	0.539
11	7	1.55	2.74	2.02	0.039	0.077	0.278	1.19	0.009	0.556
11	18	1.34	2.45	1.76	0.036	0.064	0.254	1.11	0.354	0.575
12	8	1.06	2.42	1.89	0.040	0.081	0.285	1.36	0.800	0.418
12	26	1.24	2.02	1.61	0.024	0.029	0.170	0.78	0.041	0.190
13	10	0.64	1.96	1.25	0.035	0.061	0.247	1.32	0.654	0.244
13	19	0.71	1.77	1.16	0.028	0.40	0.200	1.06	1.037	0.430
14	11	0.80	1.77	1.26	0.032	0.050	0.223	0.97	0.028	0.102
14	28	0.69	1.82	1.20	0.036	0.065	0.255	1.13	0.216	0.141

Table 3.10 Heritability estimation

Experiment: Howietoun Weight 1

Sources of Variation	Degrees of Freedom	Sum of Squares	(ss%)	Mean Squares	F	5%
Sire	6	57.505	(26.75)	9.584	1.529	NS
Sire. Dam (Within Sires)	7	43.876	(20.41)	6.268	3.060	Sig
Sire. Dam. Tank (Between tanks within dams)	14	28.677	(13.34)	2.148	33.03	HS
Sire. Dam. Tank. Individual (Within tank)	1372	84.908	(39.50)	0.062		
TOTAL	1399	214.967	(100.00)			

Heritability Estimate:	$h^2s = 0.411$	$h^2d = 1.052$	$h^2(s+d) = 0.720$
Standard Error of Heritability	$SE(h^2s) = 0.700$	$SE(h^2d) = 0.758$	$SE\ h^2(s+d) = 0.729$

Mean 1.67 gms
S.E. 0.25
CV% 14.9%

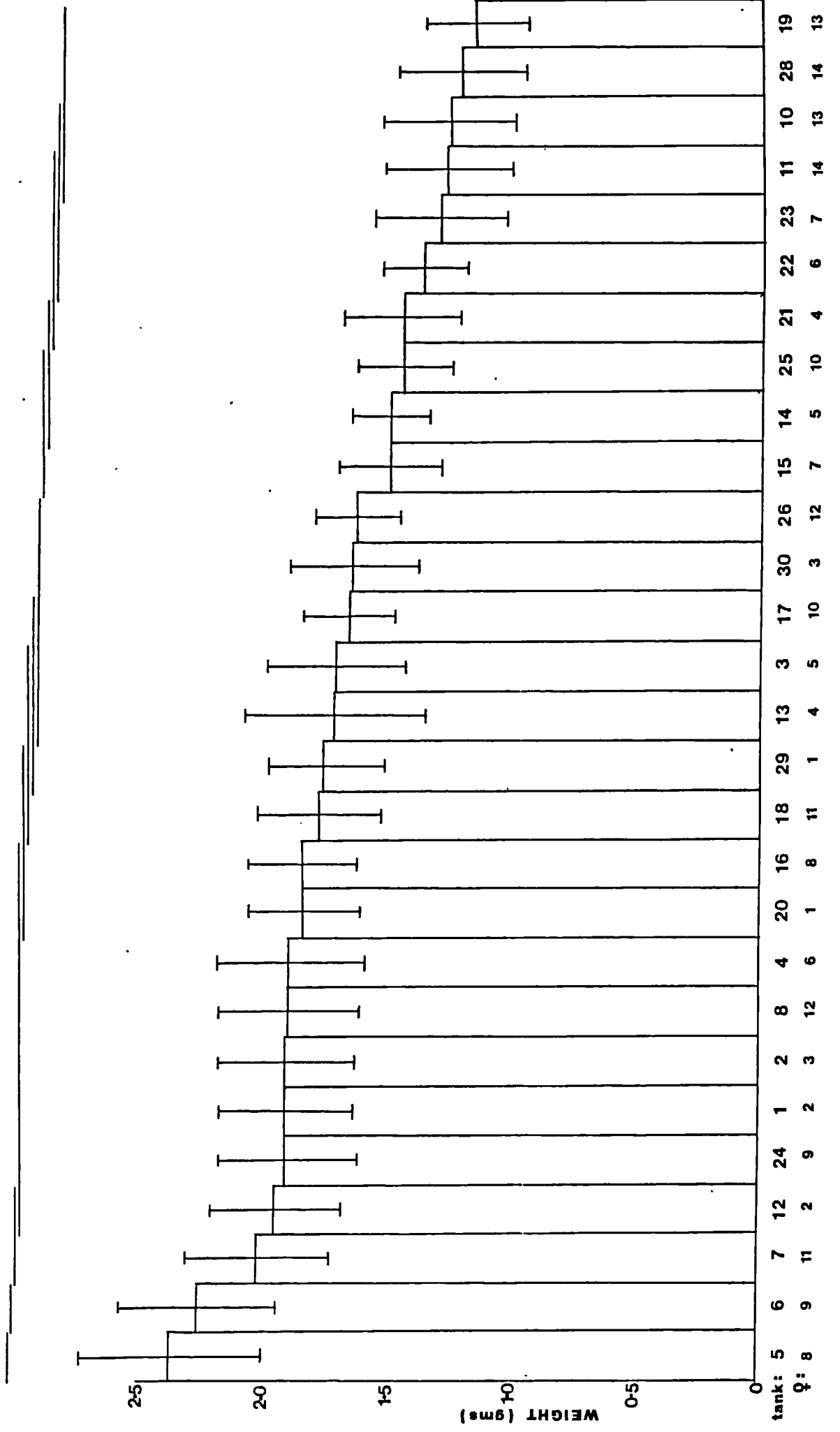


Figure 3.8 Histogram of ranked means for each tank population of weight (1) in the Howietoun trial, with standard deviations.

Table 3.11 Listing statistics for each tank population of weight (2) in the Howietoun trial

♀ No.	Tank No.	Minimum (gms)	Maximum (gms)	Mean (gms)	S.E. Mean	Variance	S.D.	Range (gms)	Kurtosis	Skewness
1	20	7.00	30.90	18.96	0.847	35.87	5.98	23.9	- 0.488	0.048
1	29	7.10	30.70	17.58	0.711	25.52	5.02	23.6	0.146	0.083
2	1	4.20	24.70	12.81	0.613	18.80	4.33	20.5	0.535	0.350
2	12	6.70	22.30	14.06	0.620	19.20	4.38	15.6	- 1.025	0.030
3	2	5.90	20.60	11.51	0.488	11.88	3.44	14.7	0.699	0.810
3	30	6.00	19.20	12.00	0.391	7.66	2.77	13.2	0.226	0.285
4	13	6.40	21.40	12.73	0.478	11.42	3.38	15.0	- 0.249	0.345
4	21	5.20	20.80	11.05	0.508	12.91	3.59	15.6	0.860	0.962
5	3	4.50	20.00	9.10	0.443	9.80	3.13	15.5	2.977	1.473
5	14	5.10	22.00	9.88	0.584	17.02	4.12	16.9	0.671	1.086
6	4	3.90	17.50	8.70	0.463	10.70	3.27	13.6	1.017	1.134
6	22	3.80	24.10	10.26	0.658	21.64	4.65	20.3	1.423	1.164
7	15	6.60	32.50	15.18	0.707	24.96	4.99	25.9	1.827	0.915
7	23	4.80	22.50	11.93	0.534	14.27	3.78	17.7	0.344	0.484
8	5	8.80	30.10	17.54	0.723	26.11	5.11	22.3	- 0.203	0.424
8	16	7.50	37.70	20.08	0.837	35.00	5.92	30.2	1.335	0.546
9	6	6.90	29.90	17.91	0.783	30.66	5.54	23.0	- 0.320	0.166
9	24	11.60	25.10	18.67	0.461	10.62	3.26	13.5	- 0.123	- 0.209
10	17	8.80	30.20	14.94	0.639	20.41	4.52	21.4	2.522	1.498
10	25	8.50	28.30	16.01	0.511	13.04	3.61	19.8	1.768	0.635
11	7	10.50	26.50	15.89	0.408	10.94	3.31	16.0	0.836	0.723
11	18	8.00	24.00	16.40	0.498	12.42	3.52	15.2	- 0.568	0.138
12	8	12.50	30.80	21.04	0.651	21.20	4.61	18.3	- 0.911	0.056
12	26	7.70	33.80	19.76	0.758	28.74	5.36	26.1	0.320	0.246
13	10	3.20	20.90	11.20	0.551	15.15	3.89	17.7	- 0.070	0.274
13	19	4.40	18.10	10.91	0.530	14.05	3.75	13.7	- 0.707	0.359
14	11	6.00	22.80	13.15	0.626	19.62	4.43	16.8	- 0.561	0.374
14	28	4.10	25.60	13.83	0.756	28.56	5.34	21.5	- 0.674	- 0.011

Table 3.12 Heritability estimation

Experiment: Howietoun Weight (2)

Sources of Variation	Degrees of Freedom	Sum of Squares	(SS%)	Mean Squares	F	5%
Sire	6	12357.77	(28.64)	2059.63	3.469	NS
Sire. Dam	7	4156.46	(9.63)	593.78	10.853	SIG
Sire. Dam. Tank	14	7165.94	(1.78)	54.71	2.902	SIG
Sire. Dam. Tank. Individual	1372	24867.42	(59.59)	18.85		
TOTAL	1399	43147.60	(100.00)	30.84		

Heritability Estimate: $h^2s = 0.907$ $h^2d = 0.668$ $h^2s+d = 0.787$

Standard Error of Heritability: $SE(h^2s) = 0.661$ $SE(h^2d) = 0.347$ $SE(h^2s+d) = 0.504$

Mean = 14.4 gms
S.E. = 4.34
CV = 30.2%

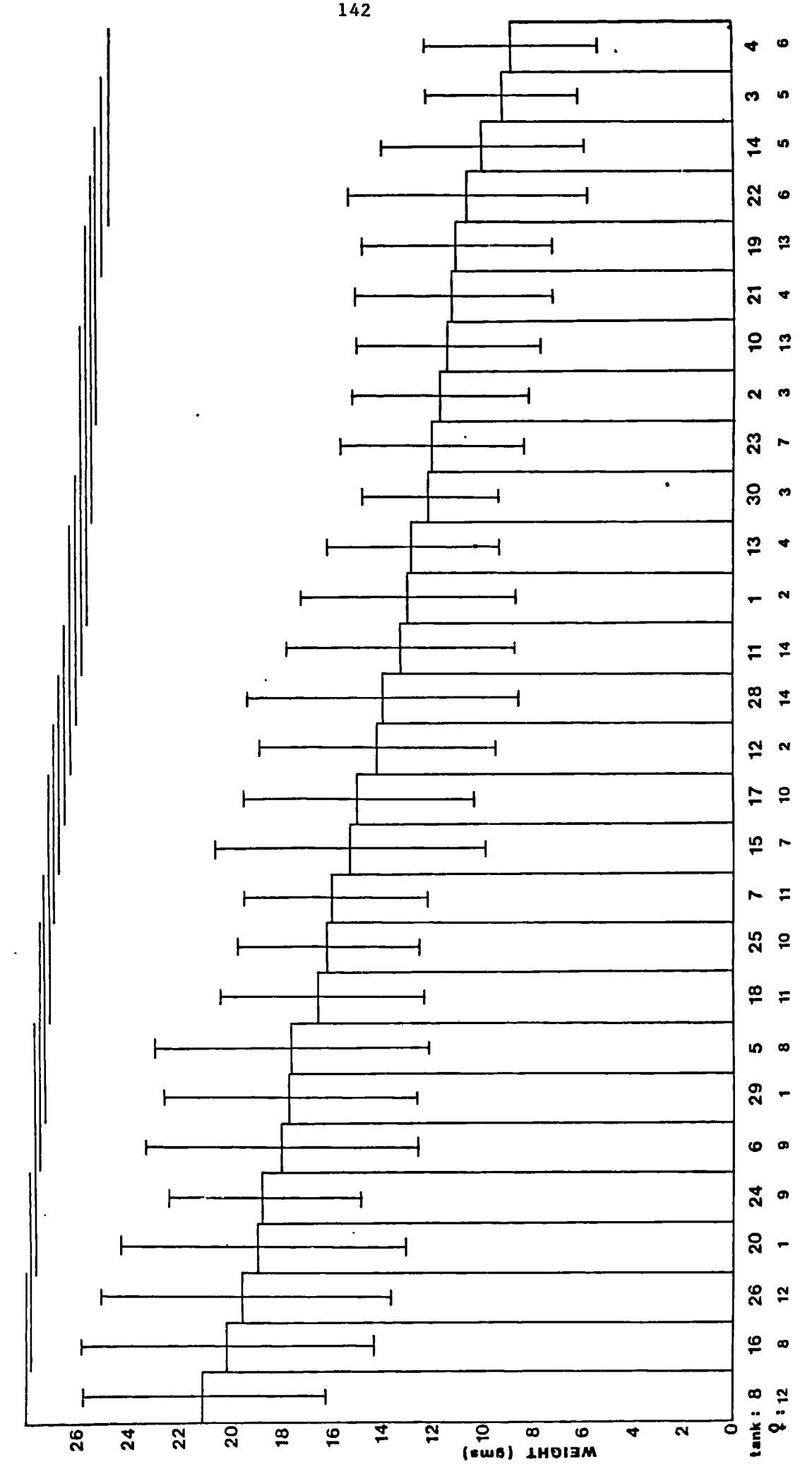


Figure 39 Histogram of ranked means for each tank population of weight (2) in the Howietoun trial, with standard deviations.

Table 3.13 Listing statistics for each tank population at weight (3) in the Howietoun trial

♀ No.	Tank No.	Minimum (gms)	Maximum (gms)	Mean (gms)	S.E. Mean	Variance	S.D.	Range (gms)	Kurtosis	Skewness
1	20	10.0	82.0	54.0	2.967	264.0	16.3	72	0.910	- 0.792
1	29	20.0	92.0	58.4	2.814	237.2	15.4	72	0.734	- 0.452
2	1	10.0	78.0	50.0	3.319	330.5	18.2	68	- 0.291	- 0.669
2	12	15.0	84.0	51.9	2.983	266.9	16.3	69	- 0.252	- 0.243
3	2	20.0	74.0	50.2	2.929	257.3	16.0	54	- 0.658	- 0.529
3	30	22.0	78.0	48.0	2.862	245.8	15.7	56	- 0.846	0.136
4	3	12.0	82.0	45.9	3.246	316.0	17.8	70	- 0.579	0.108
4	21	12.0	102.0	46.2	3.519	371.6	19.3	90	1.041	0.885
5	3	12.0	68.0	29.1	2.306	159.5	12.6	56	1.599	1.146
5	4	10.0	80.0	35.1	3.722	415.6	20.4	70	- 0.497	0.680
6	4	8.0	100.0	35.7	4.529	615.5	24.8	92	- 0.110	0.835
6	22	10.0	100.0	42.9	4.416	584.9	24.2	90	- 0.170	0.769
7	15	16.0	104.0	59.0	3.268	320.5	17.9	88	0.947	- 0.201
7	23	12.0	85.0	45.8	3.430	353.0	18.8	73	- 0.598	0.138
8	5	22.0	84.0	55.8	3.339	334.4	18.2	62	- 1.001	- 0.253
8	16	22.0	102.0	72.1	3.499	367.2	19.1	80	0.531	- 0.950
9	6	10.0	90.0	49.5	4.200	529.2	23.0	80	- 1.117	- 0.298
9	24	22.0	100.0	56.6	3.134	294.7	17.2	78	0.660	- 0.134
10	17	16.0	100.0	44.6	3.854	445.5	21.1	84	0.772	0.778
10	25	18.0	94.0	56.3	3.196	306.4	17.5	76	- 0.006	- 0.291
11	7	16.0	82.0	59.9	2.590	201.3	14.2	66	1.651	- 0.998
11	18	22.0	78.0	55.8	2.632	207.8	14.4	56	0.061	- 0.828
12	8	30.0	100.0	69.0	3.538	375.6	19.3	70	- 0.498	- 0.524
12	26	12.0	84.0	60.7	3.433	353.4	18.8	72	0.681	- 1.037
13	10	18.0	66.0	42.5	2.397	172.3	13.1	48	- 0.593	- 0.404
13	19	6.0	70.0	42.0	3.124	292.7	17.1	64	- 0.740	- 0.391
14	11	14.0	72.0	46.7	3.023	276.0	16.6	58	- 0.757	- 0.408
14	28	12.0	100.0	52.8	3.719	414.9	20.4	88	0.209	- 0.095

Table 3.14 Heritability estimation

Experiment: Howietoun Weight 3

Sources of Variation	Degrees of Freedom	Sum of Squares	(ss%)	Mean Squares	F	5%
Sire	6	52350.0	(15.15)	8725.0	6.067	SIG
Sire. Dam	7	10060.0	(2.91)	1438.0	1.543	NS
Sire. Dam. Tank	14	13050.0	(3.78)	931.8	2.802	SIG
Sire.Dam. Tank. Individual	812	270000.0	(78.16)	332.5		
TOTAL	839	345500.0	(100.00)	411.7		

Heritability Estimate: $h^2s = 0.864$ $h^2d = 0.080$ $h^2s+d = 0.164$

Standard Error of Heritability: $SE(h^2s) = 0.349$ $SE(h^2d) = 0.119$ $SE(h^2s+d) = 0.234$

Mean = 5.05gms
S.E. = 48.2
CV = 36.1%

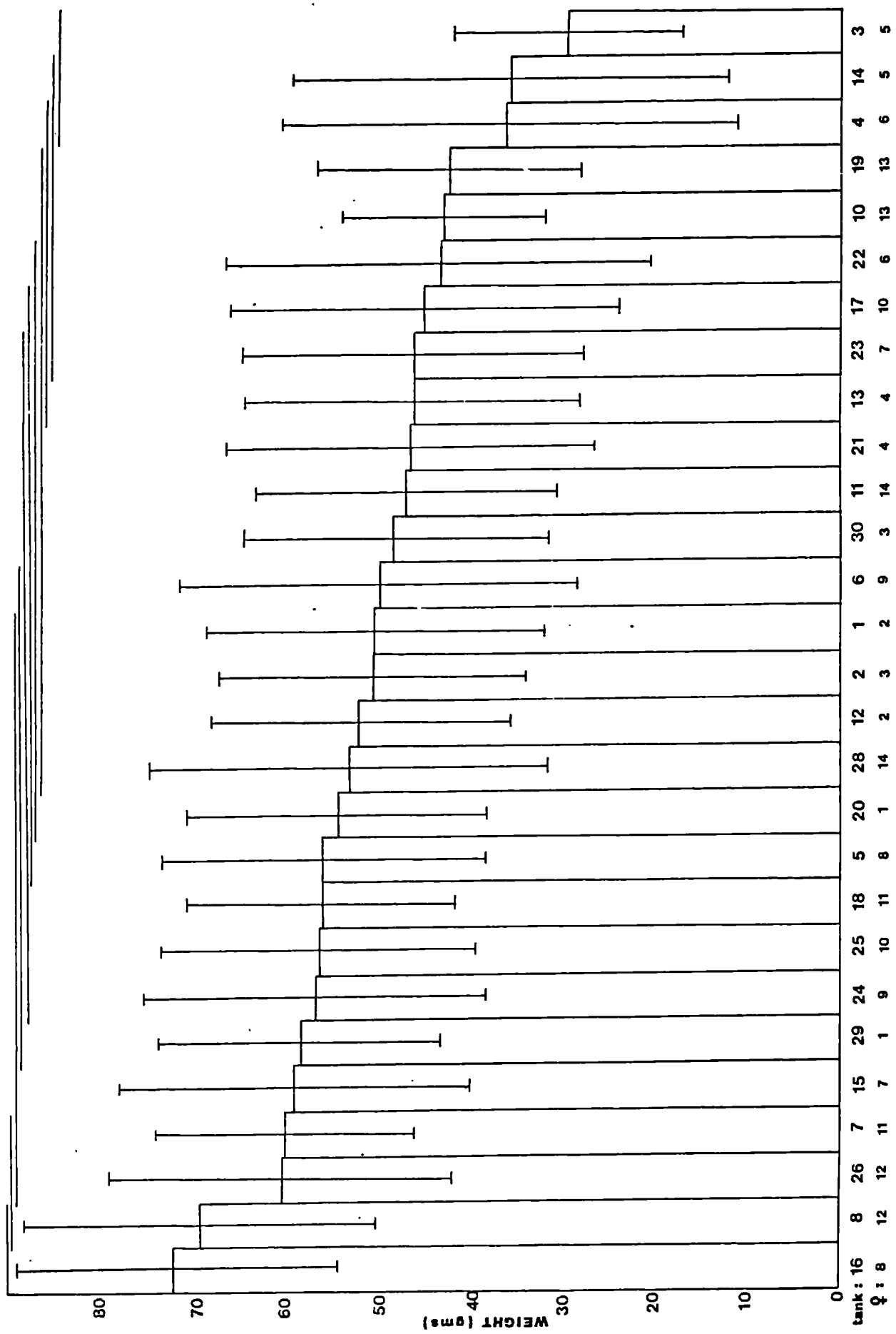


Figure 3.10 Histograms of ranked means for each tank population of weight (3) in the Howietoun trial with standard deviations.

Table 3.15 Listing statistics for each tank population at length (l) in the Howietoun trial

♀ No.	Tank No.	Minimum (cms)	Maximum (cms)	Mean (cms)	SE Mean	Variance	SD	Range (cms)	Kurtosis	Skewness
1	20	5.03	6.00	5.49	0.031	0.047	0.217	0.970	- 0.307	0.008
1	29	5.00	5.81	5.38	0.031	0.048	0.216	0.810	- 0.489	0.048
2	1	4.91	5.87	5.41	0.034	0.058	0.241	0.960	- 0.682	0.058
2	12	4.73	5.95	5.51	0.032	0.051	0.227	1.220	1.652	0.051
3	2	4.75	5.61	5.21	0.025	0.032	0.178	01860	0.171	0.032
3	30	4.95	5.60	5.23	0.021	0.021	0.145	0.650	0.054	0.021
4	13	4.44	5.54	5.13	0.037	0.067	0.259	1.100	0.431	0.067
4	21	4.25	5.40	5.03	0.033	0.054	0.232	1.150	1.178	0.054
5	3	4.30	5.60	5.11	0.034	0.059	0.243	1.300	2.589	0.059
5	14	4.36	5.68	5.28	0.030	0.045	0.212	1.320	4.628	0.045
6	4	4.62	5.78	5.17	0.030	0.046	0.215	1.160	1.087	0.046
6	22	4.50	5.38	4.97	0.026	0.035	0.187	0.880	0.099	0.035
7	15	4.55	5.60	5.14	0.031	0.047	0.218	1.050	- 0.059	0.047
7	23	3.90	5.90	4.94	0.049	0.121	0.348	2.000	1.472	0.121
8	5	5.12	6.10	5.51	0.031	0.048	0.220	0.980	- 0.102	0.048
8	16	5.10	5.95	5.46	0.030	0.044	0.211	0.850	- 0.586	0.044
9	6	4.58	6.04	5.52	0.037	0.069	0.263	1.460	2.109	0.069
9	24	4.40	5.95	5.52	0.037	0.068	0.261	1.550	6.458	0.068
10	17	5.00	5.92	5.46	0.028	0.040	0.199	0.920	- 0.155	0.040
10	25	4.52	5.50	5.16	0.027	0.037	0.192	0.980	1.754	0.037
11	7	5.00	6.00	5.41	0.029	0.043	0.208	1.000	0.586	0.043
11	18	5.06	6.10	5.49	0.031	0.047	0.218	1.040	0.495	0.047
12	8	4.35	5.70	5.34	0.035	0.060	0.246	1.350	6.733	0.060
12	26	4.95	5.81	5.27	0.025	0.037	0.176	0.860	0.508	0.031
13	10	3.95	5.32	4.84	0.039	0.077	0.277	1.370	0.965	0.077
13	19	4.17	5.50	4.82	0.035	0.063	0.250	1.330	0.557	0.063
14	11	4.08	5.30	4.87	0.041	0.085	0.292	1.320	0.416	0.085
14	28	4.01	5.45	4.82	0.043	0.093	0.305	1.440	0.209	0.093

Table 3.16 Heritability estimation

Experiment: Howietoun Length 1

Sources of Variation	Degrees of Freedom	Sum of Squares	(ss%)	Mean Squares	F	5%
Sires	6	55.407	36.69	9.23	4.524	SIG
Dams (Between dams within sires)	7	14.589	9.66	2.04	5.10	SIG
Tanks (Between tanks within dams)	14	5.688	3.77	0.40	8.00	SIG
Within Tanks Individual	1372	75.336	49.88	0.05		
TOTAL	1399	151.022	100.00	0.107		

Heritability Estimate: $h^2 = 1.24$ $h^2d = 0.583$ $h\ s+d^2 = 0.914$

Standard Error of Heritability: $SE(h^2s) = 0.847$ $SE(h^2d) = 0.381$ $SE(h\ s+d^2) = 0.614$

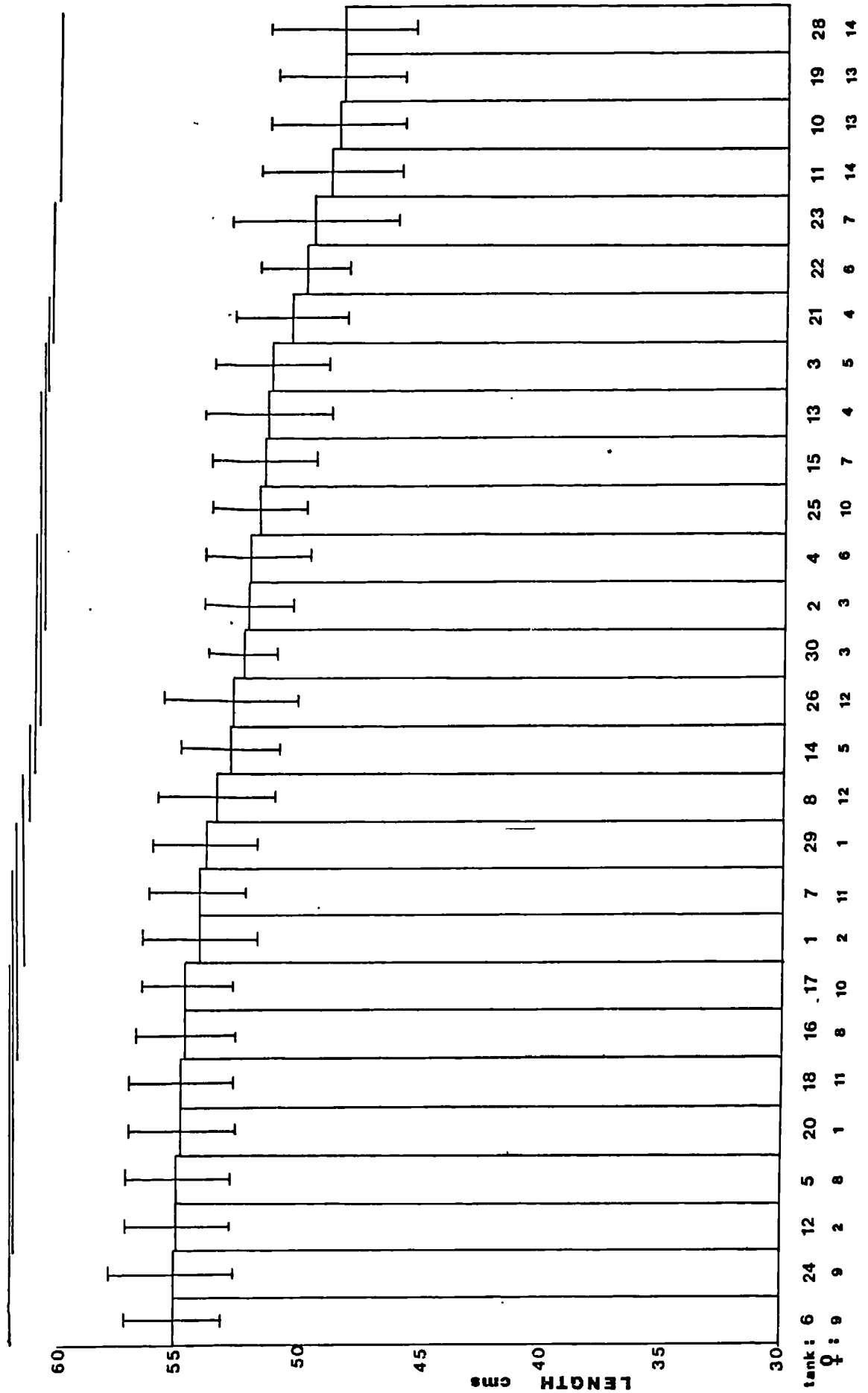


Figure 3.11 Histogram of ranked means for each tank population length (1) in the Howietoun trial, with standard deviations.

Table 317 Listing statistics for each tank population at length (2) in the Howietoun trial

♀ No.	Tank No.	Minimum (cms)	Maximum (cms)	Mean (cms)	SE Mean	Variance	SD	Range (cms)	Kurtosis	Skewness
1	20	8.55	13.65	11.41	0.166	1.376	1.173	5.10	0.021	- 0.501
1	29	8.40	13.22	11.27	0.143	1.017	1.009	4.82	0.557	- 0.645
2	1	7.21	12.50	10.05	0.167	1.389	1.178	5.29	0.146	- 0.399
2	12	8.00	12.00	10.29	0.151	1.135	1.065	4.00	- 0.781	- 0.401
3	2	7.85	11.60	9.71	0.125	0.787	0.887	3.75	- 0.040	0.235
3	30	8.20	11.70	9.94	0.106	0.564	0.751	3.50	0.033	0.393
4	13	8.00	11.41	9.84	0.116	0.669	0.818	3.41	- 0.431	- 0.090
4	21	7.70	11.51	9.55	0.125	0.786	0.887	3.81	0.042	0.330
5	3	7.21	11.90	9.24	0.133	0.886	0.941	4.69	0.823	0.621
5	14	7.62	12.05	9.44	0.156	1.212	1.101	4.43	- 0.287	0.558
6	4	6.92	11.46	8.99	0.150	1.130	1.063	4.54	- 0.008	0.516
6	22	7.22	12.20	9.31	0.176	1.549	1.245	4.98	- 0.357	0.506
7	15	8.20	13.65	10.65	0.153	1.175	1.084	5.45	0.501	0.136
7	23	7.72	12.35	9.98	0.140	0.980	0.990	4.63	0.173	- 0.126
8	5	9.05	13.43	11.03	0.147	1.077	1.038	4.38	- 0.405	0.039
8	16	8.56	14.20	11.56	0.156	1.224	1.106	5.64	0.541	- 0.231
9	6	8.30	13.41	11.24	0.160	1.273	1.128	5.11	0.410	- 0.510
9	24	9.92	12.51	11.44	0.090	0.409	0.639	2.59	- 0.086	- 0.580
10	17	9.40	13.30	10.73	0.121	0.733	0.856	3.90	1.086	0.884
10	25	9.42	13.20	11.00	0.113	0.644	0.802	3.78	0.190	0.102
11	7	9.61	12.90	11.05	0.100	0.496	0.704	3.29	0.219	0.299
11	18	7.50	12.30	10.86	0.125	0.786	0.887	4.80	2.857	- 1.156
12	8	9.83	13.12	11.70	0.111	0.617	0.786	3.29	- 0.662	- 0.182
12	26	8.35	13.31	11.54	0.146	1.069	1.034	4.96	0.967	- 0.526
13	10	6.32	11.82	9.56	0.157	1.229	1.109	5.50	0.521	- 0.545
13	19	7.00	11.50	9.57	0.148	1.092	1.045	4.50	- 0.508	- 0.270
14	11	8.00	12.50	10.26	0.152	1.154	1.074	4.50	- 0.501	- 0.093
14	28	7.02	12.80	10.45	0.195	1.899	1.378	5.78	- 0.245	- 0.594

Table 3.18

Experiment: Howietoun Length 2

Sources of Variation	Degrees of Freedom		Sum of Squares	(ss%)	Mean Squares	F	5%
Sire	6	(s - 1)	700.56	(30.37)	116.761	4.439	SIG
Sire. Dam (Between dams/sires)	7	(s(d-1))	184.12	(7.98)	26.302	11.366	SIG
Sire. Dam. Tank (Between tanks within dams)	14	(sd(a-1))	32.29	(1.40)	2.314	2.246	SIG
Sire. Dam. Tank. Individuals (Progeny)	1372	(sda(k-1))	1389.50	(60.24)	1.013		
TOTAL	1399		2366.58	(100.00)	1.649		

Heritability Estimate: $h^2s = 1.045$ $hd^2 = 0.553$ $hs+d^2 = 0.799$

Standard Error of Heritability: $SEhs^2 = 0.689$ $SEhd^2 = 0.287$ $SE(h^2s+d) = 0.488$

Mean = 10.4 cms
S.E. = 1.0
CV = 9.7%

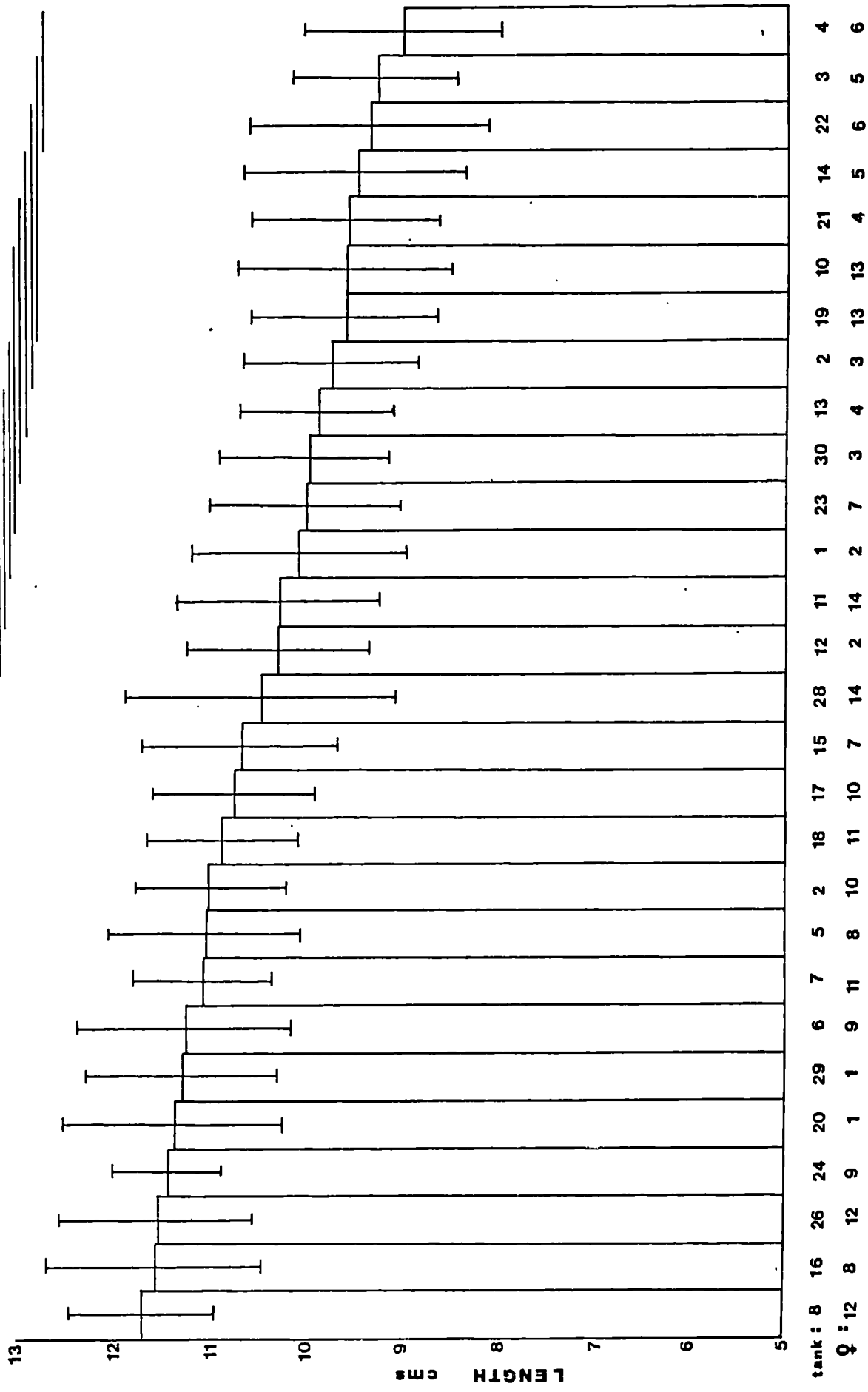


Figure 3.12 Histogram of ranked means for each tank population at length (2) in the Howietoun trial with standard deviations.

Table 3.19 Listing statistics for each tank population at length (3) in the Howietoun trial

♀ No.	Tank No.	Minimum (cms)	Maximum (cms)	Mean (cms)	SE Mean	Variance	SD	Range (cms)	Kurtosis	Skewness
1	20	9.4	19.3	16.79	0.380	4.34	2.08	9.9	4.67	- 1.961
1	29	12.5	19.4	17.27	0.289	2.51	1.58	6.9	2.48	- 1.542
2	1	9.8	18.6	15.92	0.431	5.56	2.36	8.8	0.591	- 1.137
2	12	11.8	18.7	16.27	0.341	3.48	1.87	6.9	- 0.111	- 0.787
3	2	11.3	18.6	15.81	0.367	4.05	2.01	7.3	0.003	- 0.841
3	30	12.6	18.5	15.65	0.332	3.31	1.82	5.9	- 1.140	- 0.076
4	13	11.8	18.4	15.45	0.342	3.51	1.87	6.6	- 0.797	- 0.244
4	21	9.3	19.1	15.16	0.423	5.38	2.32	9.8	- 0.107	- 0.367
5	3	10.1	18.3	13.45	0.361	3.91	1.98	8.2	0.013	0.321
5	14	9.0	18.6	14.09	0.463	6.43	2.54	9.6	- 0.852	- 0.028
6	4	7.9	20.5	13.60	0.647	12.56	3.54	12.6	- 1.003	0.127
6	22	9.0	19.5	14.40	0.542	8.80	2.97	10.5	- 1.014	- 0.059
7	15	11.1	20.3	16.97	0.348	3.63	1.91	9.2	2.404	- 1.242
7	23	10.4	18.9	15.54	0.430	5.54	2.35	8.5	- 0.499	- 0.526
8	5	13.3	19.5	16.80	0.335	3.37	1.84	6.2	- 0.903	- 0.499
8	16	9.6	20.5	17.96	0.391	4.58	2.14	10.9	7.250	- 2.343
9	6	9.2	19.3	15.75	0.540	8.74	2.97	10.1	- 0.668	- 0.755
9	24	12.4	19.7	16.68	0.333	3.33	1.83	7.3	0.942	- 1.081
10	17	11.3	20.2	15.57	0.436	5.69	2.39	8.9	- 0.720	- 0.231
10	25	12.0	19.4	16.88	0.347	3.62	1.90	7.4	0.417	- 1.048
11	7	11.2	19.1	17.32	0.270	2.18	1.48	7.9	9.613	- 2.623
14	18	12.8	19.0	16.98	0.281	2.38	1.54	6.2	0.787	- 1.158
12	8	13.4	20.2	17.98	0.344	3.55	1.88	6.8	0.205	- 0.991
12	26	10.6	20.6	16.25	0.418	5.25	2.29	10.0	2.636	- 1.541
13	10	11.3	17.5	15.58	0.314	2.96	1.72	6.2	0.703	- 1.311
13	19	9.3	18.4	14.72	0.447	6.00	2.45	9.1o	- 0.716	- 0.656
14	11	10.5	18.5	15.83	0.446	5.97	2.44	8.0	- 0.267	- 0.934
14	28	11.5	20.0	16.47	0.383	4.41	2.10	8.5	0.797	- 1.050

Table 3.20 Heritability estimation

Experiment: Howietoun Length 3

Sources of Variation	Degrees of Freedom	Sum of Squares	(SS%)	Mean Squares	F	5%
Sire	6	931.6	(18.28)	155.3	10.09	SIG
Sire. Dam (Between dams within sires)	7	107.7	(2.11)	15.39	1.547	NS
Sire. Dam Tank (Between tanks within dams)	14	139.2	(2.73)	9.943	2.061	SIG
Sire. Dam. Tank. Individual (Between progeny within tanks)	812	3917.0	(76.87)	4.824		
TOTAL	839	5096.0	(100.00)	6.074		

Heritability Estimate:	$h^2s = 0.746$	$h^2d = 0.058$	$h^2s+d = 0.402$
Standard Error of Heritability:	$SE(h^2s) = 0.416$	$SE(h^2d) = 0.012$	$SE(h^2s+d) = 0.214$

Mean = 16.0 cms
S.E. = 2.2
CV = 13.7%

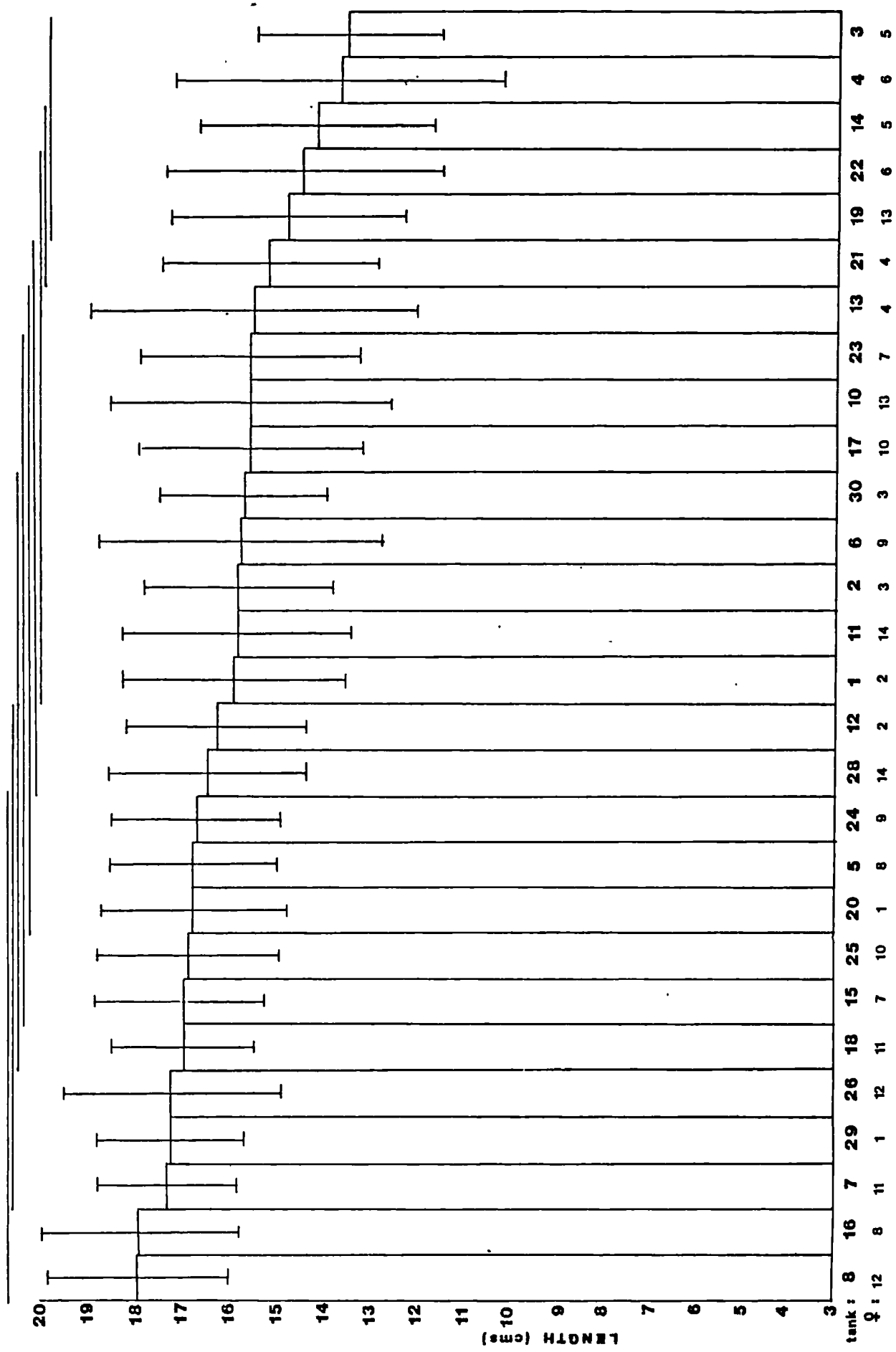


Figure 3.13 Histogram of ranked means for each tank population at length (3) in the Howietoun trial with standard deviations.

Table 3.21 Listing statistics for each tank population at weight (1) in the Leven trout trial

♀ No.	Tank No.	Minimum (gms)	Maximum (gms)	Mean (gms)	SE Mean	Variance	SD	Range (gms)	Kurtosis	Skewness
1	24	1.50	3.25	2.18	0.058	0.166	0.407	1.75	0.297	0.606
1	35	1.69	3.17	2.30	0.048	0.113	0.337	1.48	0.235	0.366
2	1	1.04	2.49	1.67	0.051	0.130	0.360	1.45	0.424	0.134
2	13	1.21	2.74	1.94	0.054	0.145	0.381	1.53	0.474	0.293
3	2	1.05	2.07	1.49	0.034	0.060	0.244	1.02	0.401	0.265
3	36	0.96	2.78	1.66	0.051	0.131	0.362	1.82	1.106	0.732
4	14	1.00	2.41	1.59	0.037	0.070	0.264	1.41	1.613	0.594
4	25	0.97	2.31	1.40	0.046	0.106	0.325	1.34	0.232	0.858
5	3	0.75	2.38	1.58	0.049	0.122	0.349	1.63	0.003	0.116
5	15	0.55	2.34	1.47	0.056	0.158	0.398	1.79	0.242	0.190
6	7	1.06	2.48	1.72	0.047	0.110	0.331	1.42	0.391	0.173
6	26	0.73	2.53	1.61	0.053	0.140	0.375	1.80	0.098	0.240
7	16	1.02	2.20	1.60	0.038	0.072	0.269	1.18	0.691	0.117
7	27	0.98	2.38	1.62	0.046	0.108	0.328	1.40	0.472	0.180
8	5	0.73	2.75	1.41	0.051	0.132	0.363	2.02	3.713	1.366
8	17	0.70	1.97	1.48	0.040	0.080	0.283	1.27	0.046	0.281
9	6	0.60	2.18	1.45	0.047	0.111	0.333	1.58	0.020	0.163
9	28	0.47	2.26	1.37	0.058	0.166	0.407	1.79	0.351	0.123
10	18	0.35	1.60	1.00	0.033	0.055	0.235	1.25	1.339	0.493
10	29	0.35	1.45	0.96	0.034	0.056	0.237	1.10	0.175	0.287
11	4	0.54	1.66	1.05	0.054	0.057	0.239	1.12	0.322	0.136
11	19	0.53	1.54	1.02	0.029	0.043	0.207	1.01	0.010	0.118

continued . . .

Table 3.21 continued

♀ No.	Tank NO.	Minimum (gms)	Maximum (gms)	Mean (gms)	SE Mean	Variance	SD	Range (gms)	Kurtosis	Skewness
12	8	0.98	1.83	1.34	0.030	0.045	0.212	0.85	0.599	0.079
12	30	0.25	1.64	o.17	0.035	0.061	0.248	1.39	2.585	-0.876
13	20	0.68	1.95	1.25	0.040	0.081	0.284	1.27	- 0.035	0.345
13	31	0.63	4.68	1.35	0.083	0.348	0.590	4.05	20.774	3.873
14	9	1.03	2.60	1.77	0.052	0.137	0.370	1.57	- 0.180	0.304
14	21	1.25	3.21	1.97	0.061	0.188	0.434	1.96	0.379	0.770
15	10	1.02	1.96	1.38	0.030	0.046	0.215	0.94	- 0.038	0.559
15	32	0.87	2.30	1.65	0.051	0.129	0.359	1.43	- 0.477	-0.324
16	22	0.59	1.80	1.14	0.039	0.077	0.278	1.21	- 0.620	0.155
16	33	0.89	1.94	1.41	0.035	0.061	0.247	1.05	- 0.177	0.250
17	11	0.92	1.97	1.27	0.032	0.051	0.226	1.05	0.395	0.508
17	23	0.93	2.03	1.57	0.034	0.058	0.241	1.10	0.083	-0.260
18	12	1.10	2.36	1.67	0.037	0.068	0.260	1.26	0.563	0.243
18	34	1.03	2.37	1.71	0.038	0.074	0.271	1.34	0.305	0.309

Table 3.22 Heritability estimation

Experiment: Loch Leven Weight (1)

Sources of Variation	Degrees of Freedom	Sum of Squares	(SS%)	Mean Squares	F	5%
Sire. Dam	8	104.60	(30.07)	13.080	2.52	NS
Sire. Dam	9	46.69	(13.42)	5.187	7.374	SIG
Sire. Dam. Tank	18	12.66	(3.64)	0.7034	6.744	SIG
Sire. Dam. Tank. Individual	1764	184.00	(52.87)	0.1043		
TOTAL	1799	348.00	(100.00)	0.1934		

Heritability Estimate:	$hs^2 = 0.787$	$hd^2 = 0.893$	$hs^2+d = 0.840$
Standard Error of Heritability:	$SE(hs^2) = 0.622$	$SE(hd^2) = 0.443$	$SE(hs+d^2) = 0.532$

Mean = 1.51 gms
S.E. = 0.305
CV = 20.2%

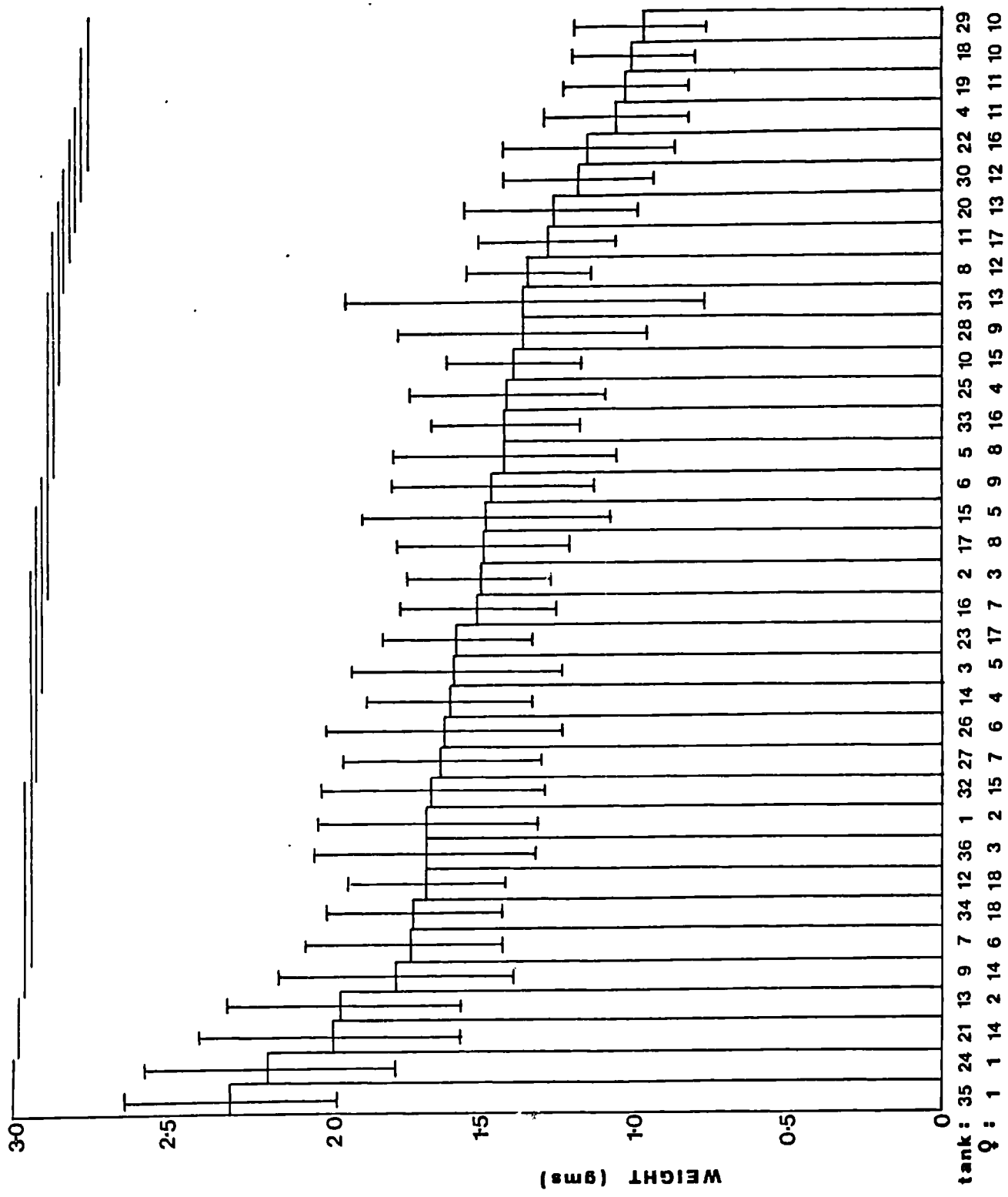


Figure 3.14 Histogram of ranked means for each tank population at weight (1) for the Leven trout trial with standard deviations.

Table 3.23 Listing statistics for each tank population at weight (2) in the Leven trout trial.

♀ No.	Tank No.	Minimum (gms)	Maximum (gms)	Mean (gms)	SE Mean	Variance	SD	Range (gms)	Kurtosis	Skewness
1	24	7.09	28.66	18.55	0.745	27.75	5.27	21.57	- 0.474	- 0.245
1	35	10.28	27.96	20.03	0.578	16.70	4.09	17.68	- 0.564	- 0.149
2	1	5.10	25.45	14.38	0.659	21.70	4.66	20.35	- 0.507	0.029
2	13	6.95	27.08	16.88	0.666	22.20	4.71	20.13	- 0.473	0.114
3	2	3.66	24.23	11.34	0.684	23.37	4.83	20.57	0.042	0.760
3	36	4.91	30.52	14.69	0.783	30.67	5.54	25.61	- 0.026	0.346
4	14	5.01	26.75	12.16	0.840	35.30	5.94	21.74	- 0.432	0.698
4	25	4.25	30.82	10.60	0.833	34.70	5.89	26.57	3.027	1.606
5	3	4.05	23.54	12.77	0.663	21.96	4.69	19.49	- 0.183	0.432
5	15	4.90	25.95	12.40	0.651	21.22	4.61	21.05	0.276	0.716
6	7	4.07	17.20	11.36	0.422	8.90	2.98	13.13	- 0.379	- 0.186
6	26	4.22	25.27	11.86	0.560	15.69	3.96	21.05	1.432	0.662
7	16	4.65	23.43	11.54	0.585	17.09	4.13	18.78	0.694	0.825
7	27	4.03	18.20	9.58	0.480	11.54	3.40	14.17	- 0.367	0.356
8	5	1.71	22.11	8.68	0.683	23.34	4.83	20.40	1.022	1.181
8	17	4.05	26.93	11.81	0.828	34.28	5.86	22.88	- 0.020	0.876
9	6	5.17	27.75	13.50	0.828	34.27	5.85	22.58	- 0.939	0.374
9	28	2.75	22.61	12.00	0.810	32.77	5.73	19.86	- 1.332	0.048
10	18	2.09	17.61	7.23	0.479	11.46	3.39	15.52	0.293	0.707
10	29	2.48	13.50	6.65	0.405	8.20	2.86	11.02	- 0.177	0.681
11	4	2.10	21.06	6.54	0.510	13.03	3.61	18.96	8.106	2.632
11	19	2.16	10.98	5.22	0.319	5.08	2.25	8.82	0.378	1.004

continued . . .

Table 3.23 continued

♀ No.	Tank No.	Minimum (gms)	Maximum (gms)	Mean (gms)	SE mean	Variance	SD	Range (gms)	Kurtosis	Skewness
12	8	3.90	20.82	9.02	0.488	11.91	3.45	16.92	1.730	1.216
12	30	2.61	25.15	8.83	0.608	18.48	4.30	22.54	4.505	1.811
13	20	3.56	25.43	12.79	0.824	33.92	5.82	21.87	- 0.607	0.470
13	31	2.97	21.36	12.38	0.687	23.61	4.86	18.39	- 0.502	0.229
14	9	6.62	34.20	14.92	0.754	28.45	5.33	27.58	2.391	1.007
14	21	5.50	30.98	16.07	0.871	37.95	6.16	25.48	- 0.002	0.749
15	32	3.96	19.50	8.43	0.443	9.81	3.13	15.54	1.902	1.207
16	22	3.24	23.64	8.20	0.617	19.04	4.36	20.40	2.403	1.536
16	33	3.54	24.61	9.38	0.646	20.84	4.57	21.07	2.292	1.533
17	11	3.35	18.51	9.46	0.513	13.18	3.63	15.16	- 0.023	0.595
17	23	2.71	21.73	10.13	0.612	18.70	4.33	19.02	0.481	0.760
18	12	3.95	35.32	11.83	0.806	32.46	5.70	31.37	4.534	1.580
18	34	5.18	25.40	12.19	0.670	22.42	4.74	20.22	0.757	0.961

Table 3.24 Heritability estimation

Experiment: Loch Leven Weight (2)

Sources of Variation	Degrees of Freedom	Sum of Squares	(SS%)	Mean Squares	F	5%
Sire	8	14570.0	(25.49)	1821.0	4.438	SIG
Sire. Dam	9	3693.0	(6.46)	410.3	6.679	SIG
Sire. Dam. Tank	18	1106.0	(1.94)	61.43	2.867	SIG
Sire. Dam. Tank. Individual	1764	37780.0	(66.11)	21.42		
TOTAL	1799	57140.0	(100.00)	3.176		

Heritability Estimate: $h^2s = 0.861$ $h^2d = 0.426$ $h^2s+d = 0.643$

Standard Error of Heritability: $SE(h^2s) = 0.508$ $SE(h^2d) = 0.215$ $SE(h^2s+d) = 0.361$

Mean = 11.43gms
S.E. = 4.51
CV = 39.4%

Figure 3.15 Histogram of ranked means for each tank population at weight (2) in the Leven trout trial with standard deviations.

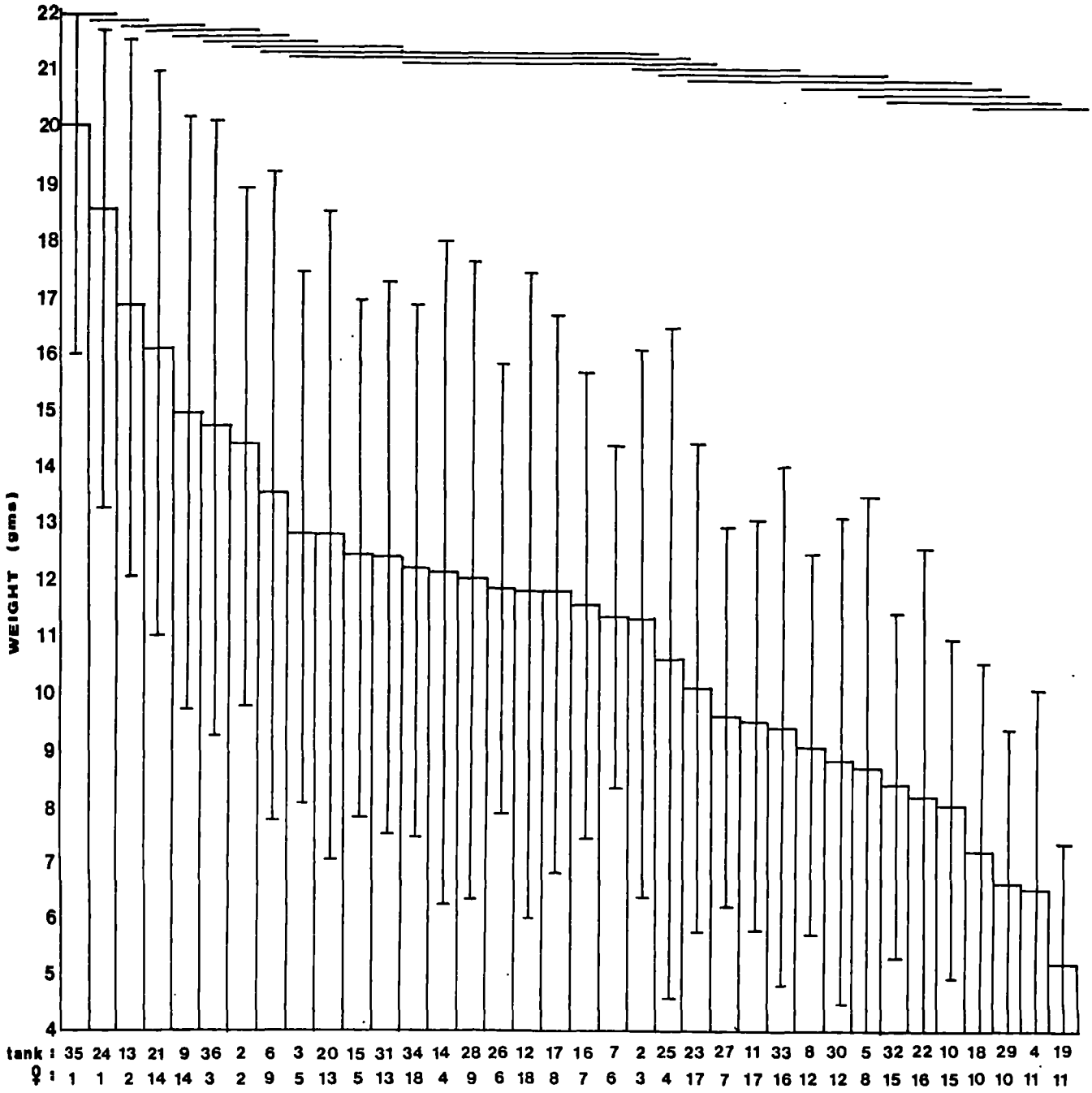


Table 3.25 Listing statistics for each tank population at weight (3) in the Leven trout trial

♀ No.	Tank No.	Minimum (gms)	Maximum (gms)	Mean (gms)	SE Mean	Variance	SD	Range (gms)	Kurtosis	Skewness
1	24	27.0	90.0	64.3	3.13	293.2	17.1	63	- 0.242	- 0.665
1	35	50.0	102.0	69.8	2.38	170.1	13.0	52	0.355	0.592
2	1	16.0	87.0	56.1	2.94	260.1	16.1	71	0.633	- 0.417
2	13	44.0	94.0	63.4	2.34	164.6	12.8	50	- 0.527	0.354
3	2	16.0	71.0	45.2	3.04	277.2	16.6	55	- 1.081	- 0.131
3	36	15.0	102.0	53.3	3.52	371.3	19.3	87	0.601	0.059
4	14	13.0	91.0	46.3	4.58	628.4	25.1	78	- 1.414	0.264
4	25	10.0	103.0	46.1	4.91	723.4	26.9	93	- 0.915	0.219
5	3	8.0	72.0	43.2	3.06	280.7	16.8	64	- 0.227	- 0.426
5	15	13.0	82.0	48.8	3.20	307.3	17.5	69	- 0.577	0.042
6	7	26.0	70.0	47.3	1.57	74.0	8.6	44	1.172	- 0.052
6	26	11.0	72.0	40.3	2.32	161.6	12.7	61	1.385	0.071
7	16	25.0	90.0	54.6	2.94	259.1	16.1	65	- 0.137	0.334
7	27	14.0	78.0	41.5	2.86	246.1	15.7	64	- 0.241	0.469
8	5	8.0	89.0	38.8	4.22	534.1	23.1	81	0.362	0.840
8	17	22.0	98.0	54.7	3.96	470.5	21.7	76	- 0.732	0.461
9	6	31.0	98.0	65.0	3.29	325.5	18.0	67	- 0.617	- 0.420
9	28	15.0	86.0	60.9	2.54	193.3	13.9	71	3.112	- 1.204
10	18	12.0	81.0	37.0	3.21	308.6	17.6	69	- 0.545	0.388
10	29	8.0	52.0	26.7	2.45	180.0	13.4	44	- 0.985	0.423
11	4	7.0	59.0	24.4	2.61	204.7	14.3	52	- 0.194	0.869
11	19	7.0	43.0	19.1	1.67	83.1	9.1	36	0.113	0.812

continued . . .

Table 3.25 continued

♀ No.	Tank No.	Minimum (gms)	Maximum (gms)	mean (gms)	SE Mean	Variance	SD	Range (gms)	Kurtosis	Skewness
12	8	11.0	76.0	42.6	2.97	263.8	16.2	65	- 0.573	0.141
12	30	9.0	82.0	36.7	3.53	372.8	19.3	73	- 0.472	0.394
13	20	18.0	92.0	52.9	3.78	428.6	20.7	74	- 0.670	0.046
13	31	9.0	76.0	46.2	2.84	241.1	15.5	67	0.150	0.010
14	9	24.0	95.0	56.8	3.16	300.1	17.3	71	- 0.262	0.082
14	21	17.0	96.0	57.7	3.61	390.9	19.8	79	- 0.227	- 0.259
15	10	4.0	74.0	33.9	3.19	305.2	17.5	70	- 0.084	0.692
15	32	12.0	61.0	31.9	2.33	162.7	12.7	49	0.027	0.793
16	22	12.0	86.0	38.2	3.63	395.2	19.9	74	- 0.258	0.818
16	33	11.0	86.0	36.4	3.16	299.1	17.3	75	1.375	1.057
17	11	23.0	64.0	42.0	2.37	166.5	12.9	41	- 1.137	0.339
17	23	19.0	72.0	35.4	2.36	167.3	12.9	53	1.184	1.021
18	12	17.0	120.0	52.1	4.21	531.3	23.0	103	0.989	0.773
18	34	11.0	78.0	42.0	3.16	299.5	17.3	67	- 0.406	0.309

Table 3.26 Heritability estimation

Experiment: Loch Leven Weight (3)

Sources of Variation	Degrees of Freedom	Sum of Squares	(SS%)	Mean Squares	F	5%
Sire	8	87330.0	(18.88)	10920.0	2.175	NS
Sire. Dam	9	45180.0	(9.77)	5020.0	5.765	SIG
Sire. Dam. Tank	18	15670.0	(3.39)	870.7	2.891	SIG
Sire. Dam. Tank. Individual	1044	314400.0	(67.97)	301.1		
TOTAL	1079	462600.0	(100.00)	428.7		

Heritability Estimate:	$h^2s = 0.448$	$h^2d = 0.631$	$h^2s+d = 0.540$
Standard Error of Heritability:	$SE(h^2s) = 0.405$	$SE(h^2d) = 0.328$	$SE(h^2s+d) = 0.367$

Mean = 45.88gms
S.E. = 17.7
CV = 38.6%

Figure 3.14 Histogram of ranked means for each tank population at weight (3) in the Leven trout trial with standard deviations.

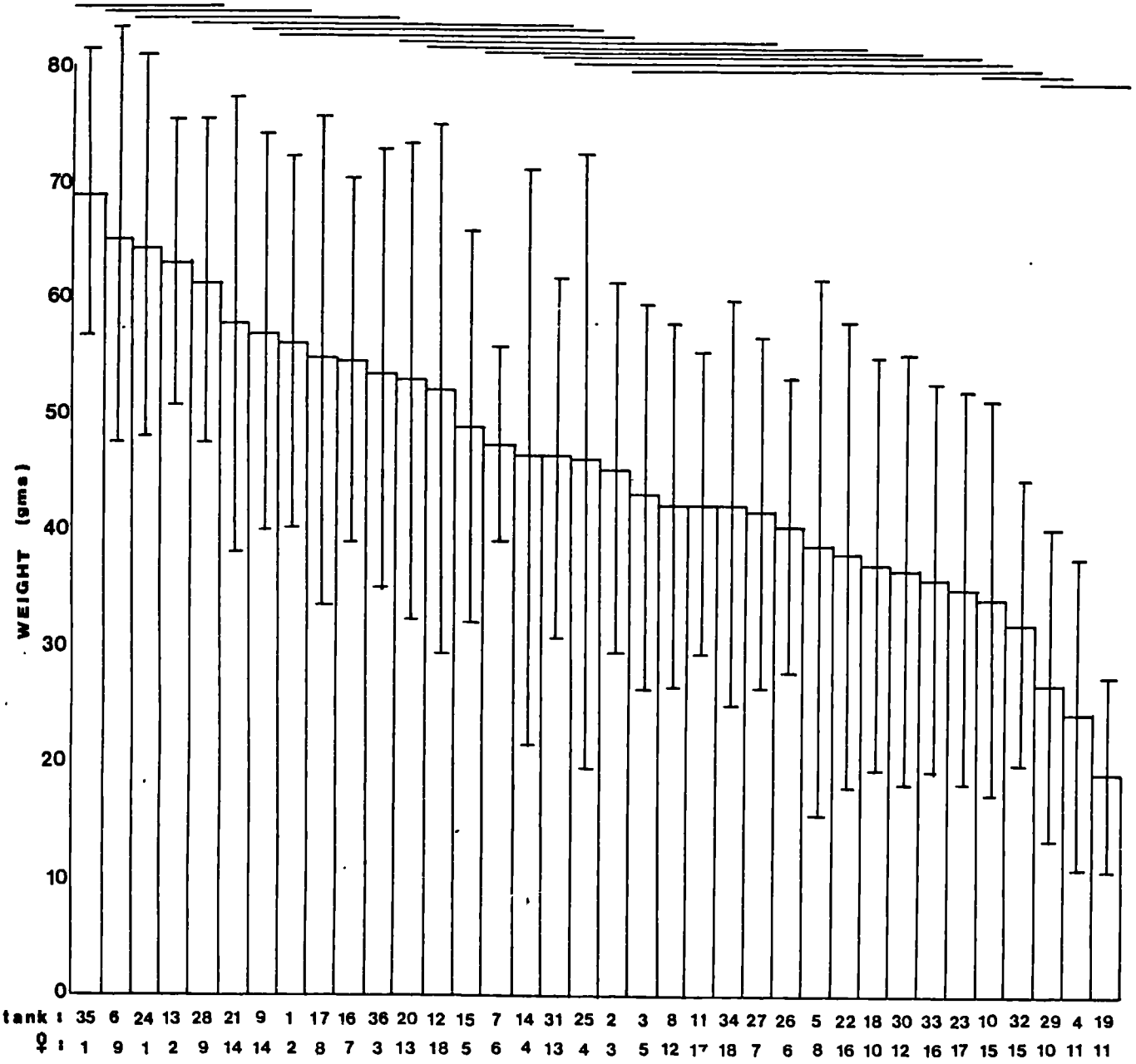


Table 3.27 Listing statistics for each tank population at length (l) in the Leven trout trial

♀ No.	Tank No.	Minimum (cms)	Maximum (cms)	Mean (cms)	SE Mean	Variance	SD	Range (cms)	Kurtosis	Skewness
1	24	5.15	6.45	5.75	0.045	0.100	0.316	1.30	- 0.293	0.052
1	35	5.30	6.45	5.86	0.035	0.061	0.248	1.15	0.112	- 0.276
2	1	4.50	6.00	5.25	0.049	0.120	0.346	1.50	- 0.253	- 0.133
2	13	4.95	6.20	5.57	0.042	0.088	0.297	1.25	- 0.486	- 0.033
3	2	4.60	5.80	5.25	0.038	0.072	0.269	1.20	- 0.465	- 0.143
3	36	4.60	6.30	5.40	0.050	0.123	0.351	1.70	0.273	0.327
4	14	4.70	6.00	5.30	0.035	0.063	0.250	1.30	1.402	0.353
4	25	4.50	6.00	5.11	0.050	0.125	0.353	1.50	- 0.305	0.610
5	3	4.10	5.85	5.20	0.053	0.140	0.374	1.75	0.684	- 0.751
5	15	3.85	5.90	5.06	0.062	0.195	0.442	2.05	0.503	- 0.714
6	7	4.80	6.15	5.48	0.043	0.094	0.307	1.35	- 0.502	- 0.178
6	26	4.30	6.00	5.33	0.058	0.166	0.407	1.70	0.175	0.732
7	16	4.70	5.90	5.31	0.038	0.073	0.270	1.20	- 0.370	- 0.099
7	27	4.50	5.80	5.29	0.047	0.112	0.334	1.30	- 0.386	- 0.513
8	5	4.25	6.15	5.17	0.049	0.118	0.344	1.90	1.472	0.238
8	17	3.80	5.60	5.17	0.049	0.121	0.347	1.80	3.444	- 1.274
9	6	4.05	5.80	5.16	0.051	0.128	0.358	1.75	0.959	- 0.694
9	28	3.50	5.90	5.02	0.070	0.243	0.493	2.40	0.701	- 0.789
10	18	3.20	5.30	4.65	0.053	0.139	0.373	2.10	1.500	0.238
10	29	3.20	5.20	4.58	0.054	0.146	0.382	2.00	3.400	- 0.952
11	4	2.90	5.20	4.62	0.083	0.348	0.590	2.30	0.959	- 0.702
11	19	3.80	5.25	4.69	0.041	0.085	0.291	1.45	- 0.502	- 0.178

continued . . .

Table 3.27 continued

♀ No.	Tank No.	Minimum (cms)	Maximum (cms)	Mean (cms)	SE Mean	Variance	SD	Range (cms)	Kurtosis	Skewness
12	8	4.70	5.60	5.12	0.034	0.059	0.242	0.90	- 0.475	0.148
12	30	4.40	5.30	4.94	0.035	0.063	0.250	0.90	0.503	- 0.702
13	20	4.20	5.55	4.94	0.041	0.083	0.288	1.35	3.200	- 1.000
13	31	3.80	5.60	4.97	0.055	0.153	0.391	1.80	1.222	- 0.003
14	9	4.60	6.20	5.60	0.048	0.114	0.338	1.60	- 0.416	0.085
14	21	5.00	6.70	5.74	0.052	0.137	0.370	1.70	0.209	0.093
15	10	4.60	5.70	5.12	0.034	0.059	0.242	1.10	- 0.155	0.040
15	32	4.20	5.90	5.30	0.056	0.156	0.395	1.70	- 0.307	0.009
16	22	3.75	5.30	4.68	0.052	0.135	0.367	1.55	4.628	- 0.123
16	33	4.45	5.70	5.09	0.037	0.069	0.263	1.25	1.287	0.050
17	11	4.40	5.65	4.95	0.035	0.060	0.245	1.25	0.092	0.044
17	23	4.5	5.70	5.24	0.036	0.063	0.251	1.20	0.832	- 1.022
18	12	4.75	6.15	5.41	0.038	0.071	0.267	1.40	0.586	0.043
18	34	4.60	6.00	5.39	0.038	0.038	0.271	1.40	0.549	- 0.500

Table 3.28

Experiment: Loch Leven Length (1)

Sources of Variation	Degrees of Freedom	Sum of Squares	(SS%)	Mean Squares	F	5%
Sire	8	90.84	(24.83)	11.360	1.550	NS
Sire. Dam	9	65.94	(18.02)	7.327	9.29	SIG
Sire. Dam. Tank	18	14.19	(3.88)	0.7884	7.135	SIG
Sire. Dam. Tank. Individual	1764	194.90	(53.27)	0.1105		
TOTAL	1799	365.90	(100.00)	0.2034		

Heritability Estimate:	$h^2s = 0.384$	$h^2d = 1.243$	$h^2s+d = 0.814$
Standard Error of Heritability:	$SE(h^2s) = 0.567$	$SE(h^2d) = 0.596$	$SE(h^2s+d) = 0.582$

Mean = 5.19 cms
S.E. = 0.32
CV = 6.2%

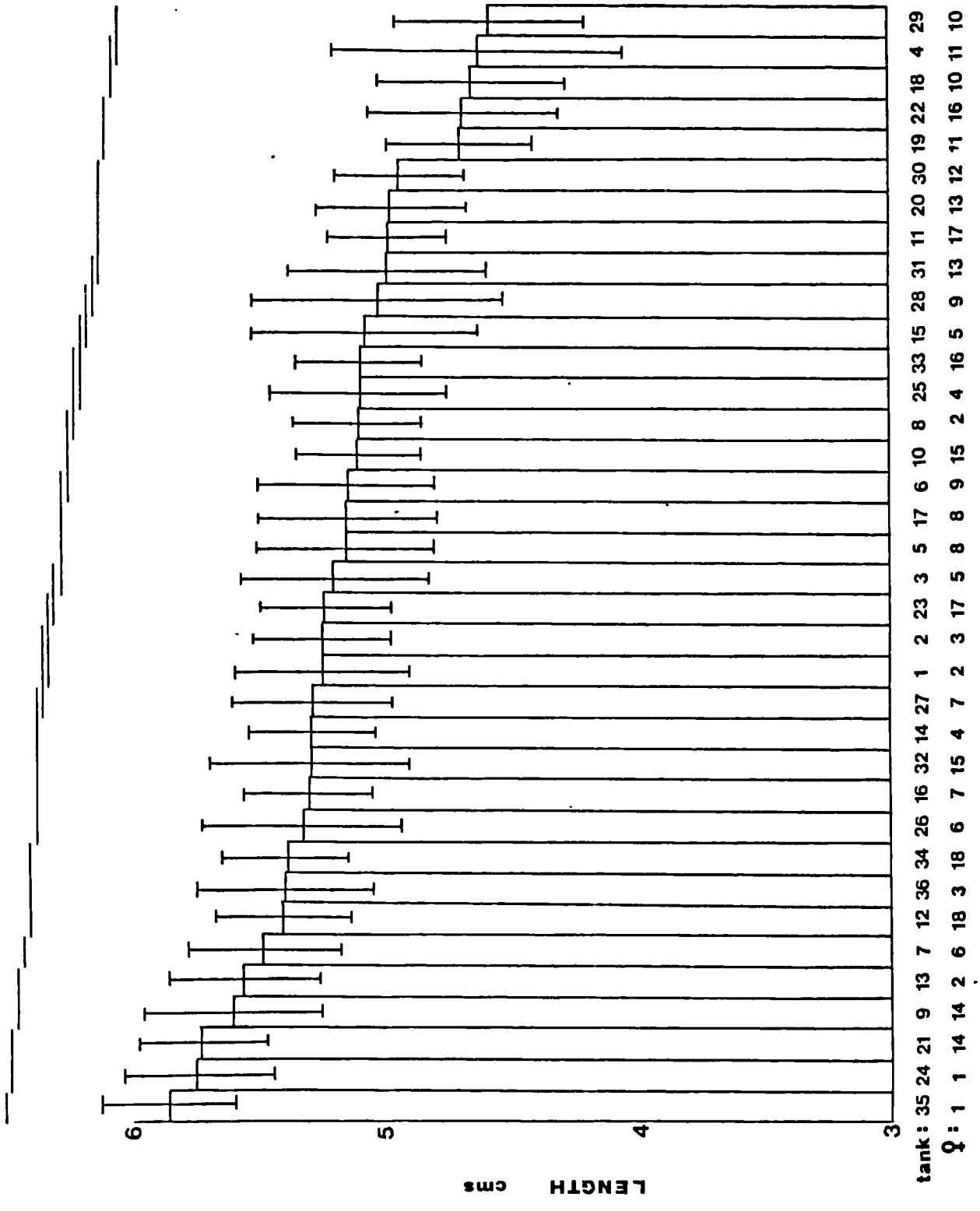


Figure 3.17 Histogram of ranked means for each trout population at length (l) in the Leven trout trial with standard deviations.

Table 3.29 Listing statistics as for each tank population at length (2) int he Leven trout trial

♀ No.	Tank No.	Minimum (cms)	Maximum (cms)	Mean (cms)	SE Mean	Variance	SD	Range (cms)	Kurtosis	Skewness
1	24	8.60	13.20	11.45	0.152	1.162	1.078	4.6	0.183	- 0.801
1	35	9.60	13.10	11.77	0.110	0.610	0.781	3.5	- 0.171	- 0.578
2	1	7.70	12.30	10.46	0.145	1.053	1.026	4.6	0.377	- 0.694
2	13	8.40	12.80	11.01	0.140	0.983	0.991	4.4	- 0.284	- 0.328
3	2	7.00	12.80	9.85	0.186	1.721	1.312	5.8	- 0.301	0.072
3	36	7.95	18.50	10.83	0.235	2.761	1.662	10.6	8.154	1.749
4	14	7.65	13.10	9.89	0.210	2.210	1.487	5.5	- 0.965	0.285
4	25	7.30	13.80	9.49	0.220	2.429	1.559	6.5	0.287	0.723
5	3	7.30	12.30	10.05	0.165	1.366	1.169	5.0	0.o42	- 0.284
5	15	7.70	12.80	10.04	0.159	1.264	1.124	5.1	- 0.386	0.228
6	7	7.30	11.40	10.00	1.125	0.778	0.882	4.1	0.555	- 0.847
6	26	7.30	12.95	10.05	0.152	1.156	1.075	5.7	0.618	- 0.157
7	16	7.70	12.65	10.01	0.161	1.299	1.140	5.0	- 0.104	0.204
7	27	7.10	11.70	9.39	0.154	1.184	1.088	4.6	- 0.505	- 0.191
8	5	5.60	12.50	8.94	0.215	2.321	1.524	6.9	- 0.229	0.242
8	17	7.45	13.05	9.91	0.207	2.150	1.466	5.6	- 0.705	0.296
9	6	7.80	13.15	10.38	0.207	2.137	1.462	5.4	- 1.125	- 0.005
9	28	6.50	12.50	9.92	0.238	2.844	1.686	6.0	- 1.222	- 0.321
10	18	6.15	11.60	8.56	0.183	1.669	1.292	5.5	- 0.667	- 0.016
10	29	6.30	10.60	8.37	0.165	1.366	1.169	4.3	- 0.892	0.038
11	4	5.90	11.95	8.30	0.164	1.337	1.156	8.1	2.384	1.149
11	19	6.05	10.00	7.84	0.142	1.010	1.005	4.0	- 0.348	0.460
12	8	7.20	11.80	9.22	0.139	0.964	0.982	4.6	- 0.087	0.466
12	30	6.40	12.70	9.14	0.174	1.520	1.233	6.3	0.915	0.592

continued . . .

Table 3.29 continued

♀ No.	Tank No.	Minimum (cms)	Maximum (cms)	Mean (cms)	SE Mean	Variance	SD	Range (cms)	Kurtosis	Skewness
13	20	7.00	13.00	10.28	0.211	2.224	1.491	6.0	- 0.689	-0.145
13	31	6.65	12.40	10.17	0.194	1.875	1.369	5.8	0.222	-0.575
14	9	8.50	14.10	10.95	0.171	1.463	1.210	5.6	0.186	0.019
14	21	8.20	14.00	11.20	0.182	1.654	1.286	5.8	- 0.202	0.208
15	10	7.15	11.20	8.79	0.128	0.816	0.903	4.1	0.317	0.785
15	32	7.00	11.50	8.79	0.14o	0.978	0.989	4.5	- 0.011	0.532
16	22	6.55	12.50	8.76	0.188	1.761	1.327	6.0	0.035	0.737
16	33	7.10	12.50	9.18	0.170	1.442	1.201	5.4	0.499	0.856
17	11	6.75	11.45	9.28	0.155	1.206	1.098	4.7	- 0.129	-0.185
17	23	6.50	12.00	9.43	0.174	1.509	1.228	5.5	- 0.189	0.010
18	12	7.00	14.30	10.01	0.198	1.956	1.399	7.3	0.582	0.494
18	34	7.90	12.70	10.15	0.168	1.407	1.186	4.8	- 0.436	0.265

Table 3.30 Heritability estimation

Experiment: Loch Leven Length (2)

Sources of Variation	Degrees of Freedom	Sum of Squares	(SS%)	Mean Squares	F	5%
Sire	8	10470.0	(24.99)	130.9	3.565	SIG
Sire. Dam	9	330.4	(7.89)	36.71	7.383	SIG
Sire. Dam. Tank	18	89.50	(2.14)	4.972	3.222	SIG
Sire. Dam. Tank. Individual	1764	2722.0	(64.98)	1.543		
TOTAL	1799	4189.0	(100.00)	2.328		

Heritability Estimate:	$h^2s = 0.785$	$h^2d = 0.528$	$h^2s+d = 0.657$
Standard Error of Heritability:	$SE(h^2s = 0.505$	$SE(h^2d) = 0.262$	$SE(h^2s+d) = 0.383$

Mean = 9.77cm
S.E. = 1.21
CV = 12.4%

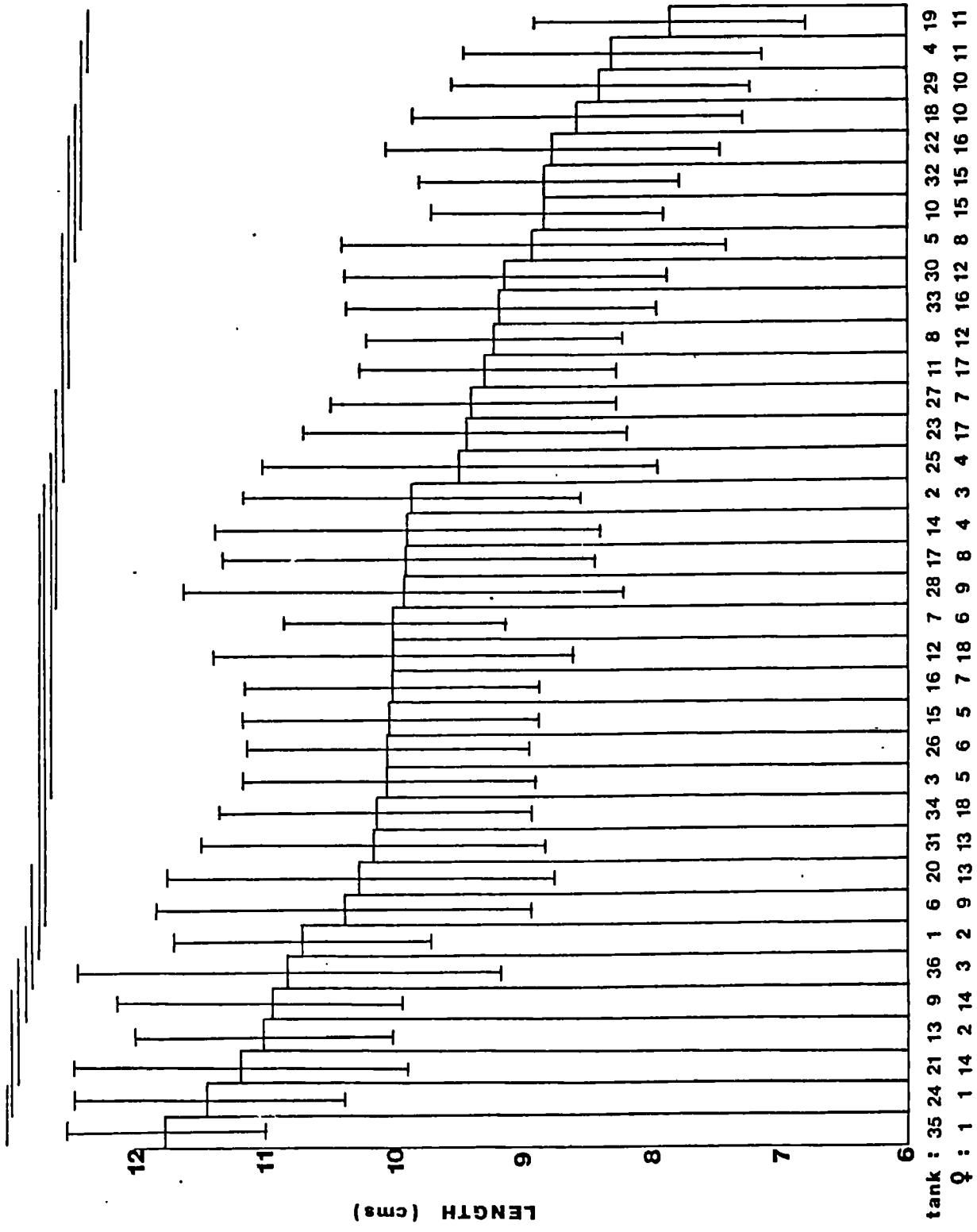


Figure 3.18 Histogram of ranked means for each tank population of length (2) in the Leven trout trial with standard deviations

Table 3.31 Listing statistics for each tank population at length (3) in the Leven trout trial

♀ No.	Tank No.	Minimum (cms)	Maximum (cms)	Mean (cms)	SE Mean	Variance	SD	Range (cms)	Kurtosis	Skewness
1	24	13.5	20.1	18.1	0.324	3.146	1.77	6.6	0.746	-1.172
1	35	16.8	20.6	18.6	0.177	0.944	0.97	3.8	-0.387	0.080
2	1	11.8	19.2	17.1	0.312	2.914	1.71	7.4	2.859	-1.451
2	13	15.9	19.6	17.7	0.194	1.129	1.06	3.7	-1.209	-0.046
3	2	12.2	19.3	16.2	0.370	4.113	2.03	7.1	-0.560	-0.588
3	36	11.7	20.8	17.1	0.384	4.430	2.11	9.1	0.936	-1.000
4	14	11.1	20.4	15.8	0.538	8.696	2.95	9.3	-1.386	-0.083
4	25	10.3	20.2	15.8	0.569	9.712	3.12	9.9	-1.107	-0.525
5	3	9.5	19.2	15.7	0.435	5.666	2.38	9.7	0.960	-1.155
5	15	10.8	19.4	16.4	0.374	4.190	2.05	8.6	0.258	-0.783
6	7	14.0	18.2	16.8	0.189	1.072	1.04	4.2	0.236	-0.765
6	26	10.7	19.4	15.9	0.342	3.509	1.87	8.7	2.579	-1.143
7	16	13.5	20.3	17.2	0.294	2.601	1.61	6.8	-0.189	-0.228
7	27	11.2	19.3	15.5	0.341	3.494	1.87	8.1	-0.177	-0.171
8	5	9.6	19.3	14.6	0.479	6.893	2.63	9.7	-0.727	0.121
8	17	13.2	20.6	16.9	0.383	4.397	2.10	7.4	-1.061	0.113
9	6	14.5	20.8	18.1	0.309	2.861	1.69	6.3	-0.262	-0.750
9	28	11.6	20.2	17.9	0.288	2.484	1.58	8.6	8.204	-2.200
10	18	10.5	19.4	15.1	0.435	5.669	2.38	8.9	-0.990	-0.368
10	29	7.0	17.2	13.5	0.479	6.887	2.62	10.2	-0.235	-0.563
11	4	8.7	17.6	12.9	0.422	5.355	2.31	8.9	-0.741	0.274
11	19	8.5	19.4	12.3	0.405	4.911	2.22	10.9	2.532	1.195
12	8	10.3	19.3	15.7	0.370	4.116	2.03	9.0	0.408	-0.614
12	30	9.8	19.6	14.9	0.495	7.345	2.71	9.8	-0.763	-0.438

continued . . .

Table 3.31 continued

♀ No.	Tank No.	Minimum (cms)	Maximum (cms)	Mean (cms)	SE mean	Variance	SE	Range (cms)	Kurtosis	Skewness
13	20	12.1	20.3	16.9	0.411	5.080	2.25	8.2	- 0.306	- 0.647
13	31	9.8	19.5	16.3	0.357	3.824	1.96	9.7	3.072	- 1.195
14	9	13.1	20.7	17.5	0.342	3.514	1.88	7.6	- 0.081	- 0.556
14	21	12.0	20.6	17.5	0.389	4.529	2.13	8.6	0.796	- 0.924
15	10	10.4	18.2	14.4	0.376	4.251	2.06	7.8	- 0.753	0.072
15	32	10.7	17.6	14.2	0.321	3.094	1.76	6.98	- 0.491	0.301
16	22	10.4	19.3	14.8	0.442	5.851	2.42	8.9	0.720	0.162
16	33	10.3	19.4	14.8	0.394	4.661	2.16	9.1	- 0.008	- 0.101
17	11	13.1	18.6	15.8	0.284	2.421	1.56	5.5	- 1.143	0.138
17	23	12.6	18.6	14.9	0.302	2.736	1.65	6.0	0.362	0.346
18	12	11.9	22.2	16.8	0.449	6.060	2.46	10.3	- 0.512	- 0.108
18	34	10.7	19.4	15.8	0.420	5.287	2.30	8.7	- 0.379	- 0.406

Table 3.32 Heritability estimation

Experiment: Loch Leven Length (3)

Sources of Variation	Degrees of Freedom	Sum of Squares	(SS%)	Mean Squares	F	5%
Sire	8	1324.0	(19.18)	165.5	1.998	NS
Sire. Dam	9	745.3	(10.79)	82.8	5.79	SIG
Sire. Dam. Tank	18	257.2	(3.72)	14.3	3.26	SIG
Sire. Dam. Tank. Individual	1044	4577.0	(66.30)	4.385		
TOTAL	1079	6904.0	(100.00)	6.399		

Heritability Estimate:	$h^2s = 0.421$	$h^2d = 0.697$	$h^2s+d = 0.559$
Standard Error of Heritability:	$SE(h^2s) = 0.417$	$SE(h^2d) = 0.362$	$SE(h^2s+d) = 0.390$

Mean = 15.98 cm
SE = 2.05
CV = 12.8%

Figure 3.19 showing histogram of ranked means for each tank population at length (3) in the Leven trout trial, with standard deviations.

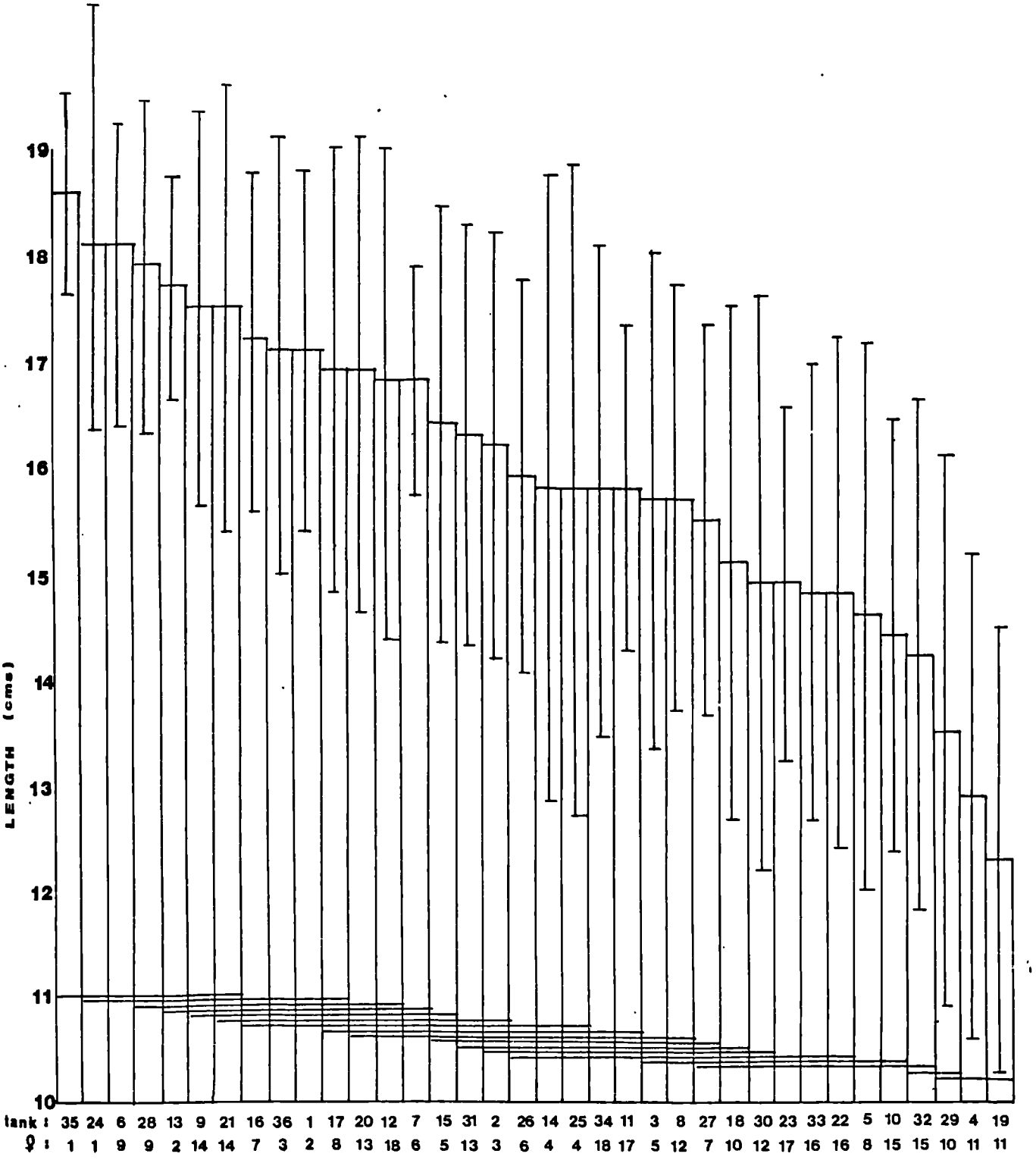


Table 3.33 The heritability estimates derived from the hierarchical design experiments in 1982-1983 and 1983-84. It also gives other relevant information pertaining to the particular parameter in question, namely the mean, standard deviation and coefficient of variation.

Parameter	$\bar{X}$	σ	CV	$h^2_s \pm$ SE	$h^2 d \pm$ SE	No. of sires and dams used + No. of total observations used
Howietoun						
Trial length (1)	5.23	0.23	4.5	1.24 ± 0.847	0.583 ± 0.381	7 , 14 1400 obs
Trial length (2)	10.40	1.01	9.7	1.04 ± 0.690	0.553 ± 0.287	7 , 14 1400 obs
Trial length (3)	16.0	2.20	13.7	0.746 ± 0.416	0.058 ± 0.012	7 , 14 840 obs
Trial weight (1)	1.67	0.25	14.9	0.411 ± 0.700	1.052 ± 0.758	7 , 14 1400 obs
Trial weight (2)	14.40	4.34	30.2	0.907 ± 0.661	0.668 ± 0.347	7 , 14 1400 obs
Trial weight (3)	50.50	18.23	36.1	0.864 ± 0.349	0.080 ± 0.119	7 , 14 840 obs
Leven						
Trial length (1)	5.19	0.32	6.2	0.384 ± 0.567	1.243 ± 0.596	9 , 18 1800 obs
Trial length (2)	9.77	1.21	12.4	0.785 ± 0.505	0.528 ± 0.262	9 , 18 1800 obs
Trial length (3)	15.98	2.05	12.8	0.421 ± 0.417	0.697 ± 0.362	9 , 18 1140 obs
Trial weight (1)	1.51	0.30	20.2	0.787 ± 0.622	0.893 ± 0.443	9 , 18 1800 obs
Trial weight (2)	11.43	4.51	39.4	0.861 ± 0.508	0.426 ± 0.215	9 , 18 1800 obs
Trial weight (3)	45.88	17.1	38.6	0.448 ± 0.405	0.631 ± 0.328	9 , 18 1140 obs

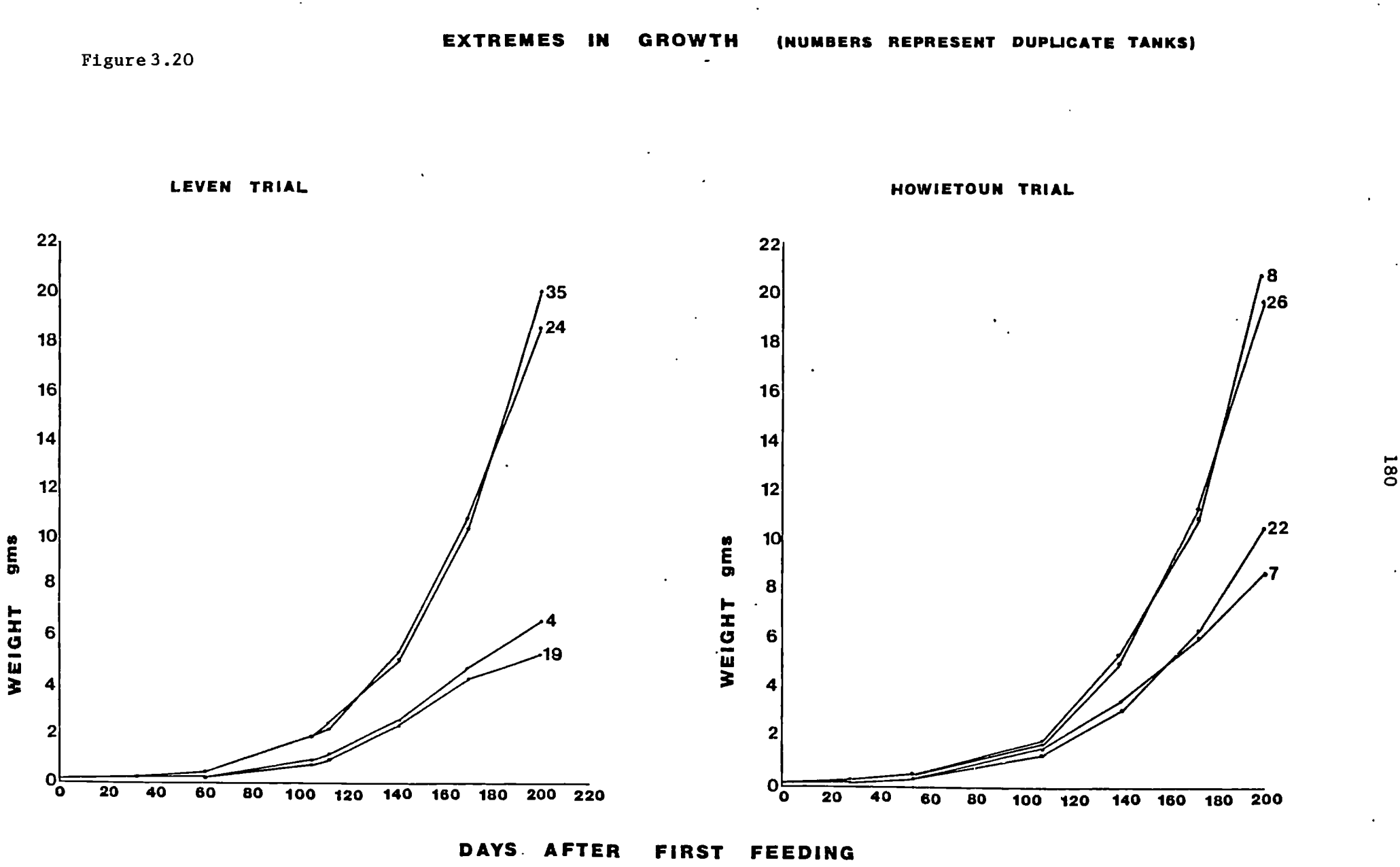
Length in cms
Weight in gms

$\bar{X}$ = mean; σ = standard deviation; CV = coefficient of variation;

$h^2 s \pm$ SE = heritability (sire component) estimate ± standard error

$h^2 d \pm$ SE = heritability (dam component) estimate ± standard error

Figure 3.20


EXTREMES IN GROWTH (NUMBERS REPRESENT DUPLICATE TANKS)
LEVEN TRIAL
HOWIETOUN TRIAL
WEIGHT gms
DAYS AFTER FIRST FEEDING
35
24
4
19
8
26
22
7
0
2
4
6
8
10
12
14
16
18
20
22
0
20
40
60
80
100
120
140
160
180
200
220

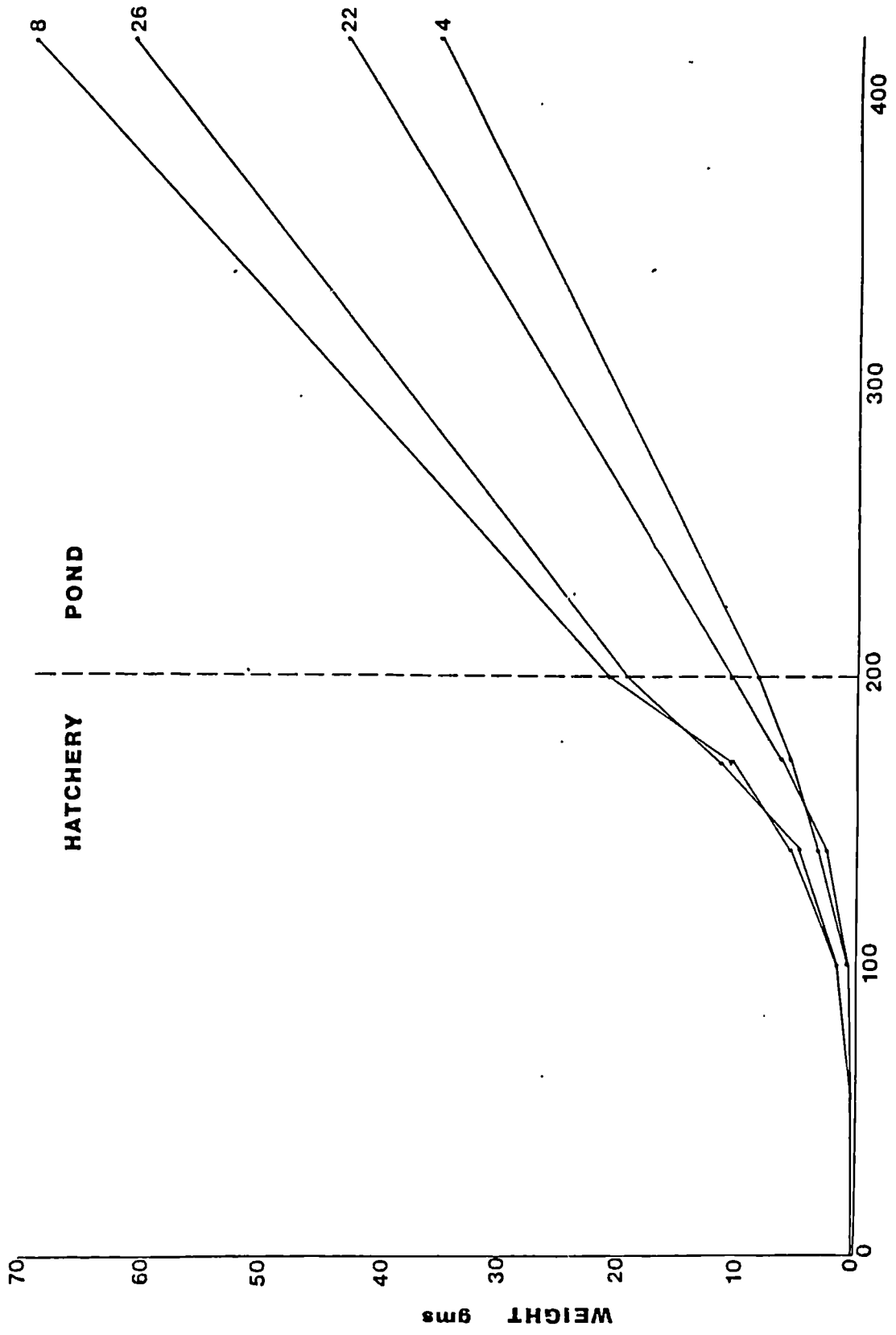

HOWIETOUN TRIAL GROWTH EXTREMES TO 420 DAYS AFTER FIRST FEEDING
HATCHERY
POND
8
26
22
4
WEIGHT gms
0
10
20
30
40
50
60
70
0
100
200
300
400
DAYS AFTER FIRST FEEDING COMMENCED

Figure 3.21

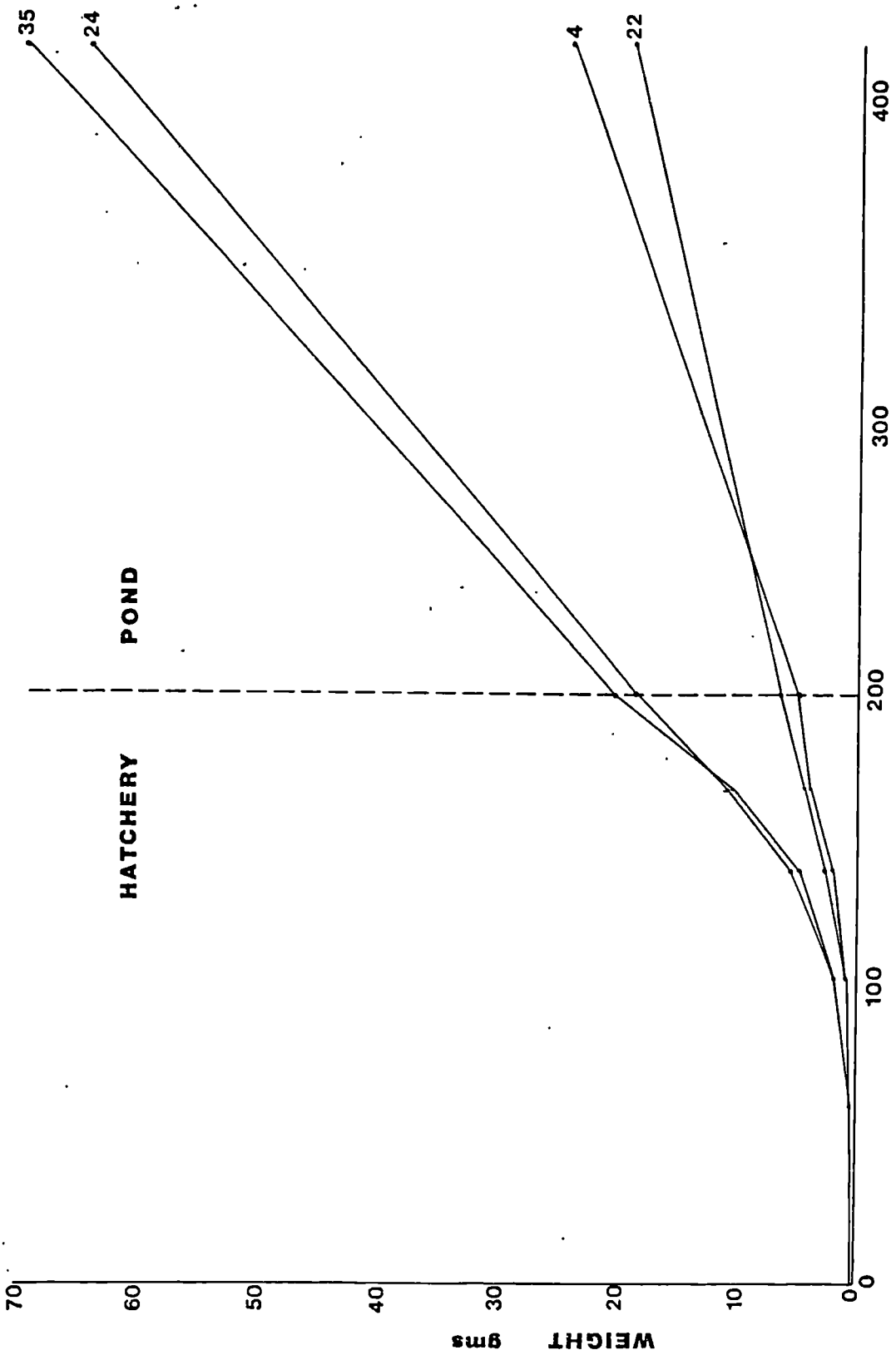


Figure 3.22 LEVEN TRIAL GROWTH EXTREMES TO 420 DAYS AFTER FIRST FEEDING

There is doubt whether one can use Duncan's multiple range test on final weights at the end of a growth trial if the initial weights were statistically different. This appears to be the case in the growth trials in this study. Another way of obtaining a converient measurement to compare growth rates is to use specific growth rate (SGR). This is a statistic which expresses growth as a percentage weight gain per day during the trial.

$$SGR = \frac{\text{Loge } (Wt_2 - Wt_1)}{t_2 - t_1} \times 100$$

where Wt_2 and Wt_1 are the weights at time t_2 and t_1 respectively.

SGRs can only be calculated when the slope of the growing curve is linear or approximately linear throughout the period t_1 to t_2.

The periods between the 1st and 2nd accurate weighings in each of the three trials conducted conformed to this requisite. The growth during that period being linear.

Once the specific growth rates were calculated for each tank over the three years, Duncan's (1955) multiple range test was employed on the resulting data. The resultant estimates of significance between SGRs are illustrated in Figure 3.23 for the first two trials and in Figure 3.32 for the third 'factoral' trial.

Like heritability there is little point in comparing specific growth

Figure 3.23 Illustrating significant differences between SGRs of progeny from different females

Howietoun Trial

♀ Number	SGR	Duncan's
12	3.23	
12	3.19	
8	3.19	
1	3.14	
1	3.12	
9	3.10	
9	3.02	
8	3.00	
11	2.95	
10	2.95	
11	2.89	
7	2.87	
10	2.84	
14	2.79	
2	2.74	
14	2.72	
4	2.64	
2	2.63	
7	2.60	
3	2.57	
13	2.52	
13	2.51	
4	2.49	
3	2.48	
6	2.45	
5	2.34	
5	2.20	
6	2.11	

Leven Trial

♀ Number	SGR	Duncan's
1	2.95	
1	2.87	
2	2.77	
14	2.73	
14	2.64	
3	2.64	
2	2.61	
9	2.56	
13	2.52	
13	2.48	
5	2.48	
5	2.46	
9	2.44	
18	2.43	
4	2.43	
8	2.40	
18	2.38	
6	2.37	
7	2.36	
3	2.35	
6	2.33	
4	2.29	
17	2.25	
17	2.16	
16	2.14	
7	2.12	
12	2.11	
12	2.09	
8	2.04	
16	2.03	
15	1.98	
15	1.94	
10	1.87	
10	1.79	
11	1.75	
11	1.49	

rates from year to year or from trial to trial, because different environmental conditions will prevail each year giving rise to possibly different specific growth rates as well as different heritability estimates.

It was felt that an assessment of the mean weights and lengths derived from the duplicate pairs of tanks would give information concerning the magnitude of the tank effect.

To this end t-tests were performed on the pairs of means. The t test used is given below.

t-test

$$d = \frac{\text{difference between means}}{\text{SE of that difference}} = \frac{X_1 - X_2}{\sqrt{\left(\frac{S_1^2}{N_1} + \frac{S_2^2}{N_2}\right)}}$$

Where S_1^2 and S_2^2 are the variances of the populations/tanks

N_1 and N_2 are the number of individuals measured in each population/tank

X_1 and X_2 are the means of the populations being compared.

Table 3.34 lists the t-tests performed on the means of weights and lengths of 50 individuals taken from each tank and the respective duplicate tank. There were a considerable number of duplicate pairs

Table 3.34 Listing the results of t tests perfomred on weights and lengths of corresponding duplicate tanks in the Howietoun loch trials and Loch Leven trials

HOWIETOUN TRIAL

Tank No. and Duplicate		Weight (1)		Weight (2)		Weight (3)		Length (1)		Length (2)		Length (3)	
		d	Sig	d	Sig	d	Sig	d	Sig	d	Sig	d	Sig
20	29	1.70	NS	1.26	NS	0.51	NS	2.20	SIG	0.64	NS	0.40	NS
		df98		df98		df48		df98		df98		df48	
1	12	0.75	NS	1.39	NS	0.38	NS	2.00	SIG	1.07	NS	0.70	NS
2	30	5.09	SIG	0.78	NS	0.56	NS	0.61	NS	1.77	NS	1.08	NS
13	21	5.00	SIG	2.41	SIG	0.08	NS	2.00	SIG	1.70	NS	0.06	NS
3	14	4.68	SIG	1.07	NS	0.80	NS	2.44	SIG	1.03	NS	0.64	NS
4	22	10.91	SIG	1.94	NS	0.54	NS	5.00	SIG	1.44	NS	0.65	NS
15	23	4.22	SIG	3.66	SIG	2.45	SIG	3.33	SIG	3.19	SIG	4.13	SIG
5	16	9.04	SIG	2.23	SIG	3.09	SIG	1.16	NS	2.47	SIG	2.06	SIG
6	24	6.03	SIG	0.85	NS	1.09	NS	0.00	NS	1.09	NS	1.36	NS
17	25	6.32	SIG	1.32	NS	2.31	NS	7.75	SIG	1.63	NS	2.59	SIG
7	18	4.91	SIG	0.76	NS	1.15	NS	1.86	SIG	1.19	NS	0.96	NS
8	26	6.00	SIG	1.07	NS	0.86	NS	1.63	NS	0.87	NS	0.69	NS
10	19	2.00	SIG	0.58	NS	0.20	NS	0.38	NS	0.05	NS	0.53	NS
11	28	1.25	NS	0.69	NS	0.33	NS	0.83	NS	0.77	NS	0.11	NS

Table 3.34 continued

LEVEN TRIAL

Tank No. and Duplicate		Weight (1) d	Sig	Weight (2) d	Sig	Weight (3) d	Sig	Length (1) d	Sig	Length (2) d	Sig	Length (3) d	Sig
24	35	1.71 df98	SIG	1.54	NS	1.34	NS	2.20	SIG	1.70	NS	1.59	NS
1	13	3.61	SIG	2.91	SIG	2.38	SIG	5.33	SIG	2.73	SIG	2.40	SIG
2	36	2.83	SIG	3.22	SIG	1.43	NS	2.33	SIG	3.34	SIG	1.55	NS
14	25	3.22	SIG	1.31	NS	0.82	NS	3.80	SIG	1.39	NS	0.55	NS
3	15	1.47	NS	1.39	NS	0.09	NS	1.92	NS	0.04	NS	0.17	NS
7	26	1.69	NS	0.45	NS	2.68	SIG	2.28	SIG	0.20	NS	2.41	SIG
16	27	0.20	NS	2.75	SIG	2.62	SIG	0.33	NS	2.74	SIG	3.20	SIG
5	17	1.07	NS	2.91	SIG	0.72	NS	0.00	NS	3.18	SIG	4.01	SIG
6	28	1.08	NS	1.26	NS	0.49	NS	1.54	NS	1.43	NS	0.42	NS
18	29	0.085	NS	0.91	NS	3.29	NS	0.95	NS	0.73	NS	2.98	SIG
4	19	0.671	NS	2.16	SIG	1.76	NS	0.76	NS	1.79	NS	0.77	NS
8	30	3.81	SIG	0.26	NS	1.10	NS	3.40	SIG	0.31	NS	1.25	NS
20	31	0.63	NS	0.39	NS	1.83	NS	0.43	NS	0.38	NS	1.68	NS
9	21	2.19	SIG	1.00	NS	0.10	NS	2.00	NS	1.00	NS	0.25	NS
10	32	4.75	SIG	0.71	NS	0.77	NS	2.79	SIG	0.00	NS	0.40	NS
22	33	5.47	SIG	1.24	NS	0.73	NS	6.72	SIG	1.69	NS	0.80	NS
11	23	6.45	SIG	0.83	NS	1.44	NS	5.80	SIG	0.68	NS	1.08	NS
12	34	0.75	NS	0.34	NS	1.62	NS	0.38	NS	0.43	NS	1.40	NS

that were significantly different from each other (P = 0.05) at the first accurate measurement, especially in the Howietoun trial but it appeared that by the second accurate measurement the differences between the duplicate tanks were small, with few pairs of tanks being significantly different from one another.

Some of the differences can be explained by 2 distinct problems.

(a) Faulty feeders, and

(b) human disturbance.

During the Howietoun trial the automatic feeder on tank 23 was defective and for a period of over six weeks consistently gave less food than required. During the Leven trial the same situation occurred with the feeder on tank 27. This explains the significant difference found between tanks 15 and 23 in the Howietoun trial and tank 16 and 27 in the Leven trial. While the Howietoun trial was in progress human interference was kept to a minimum but during the Leven trial due to circumstances beyond my control, the tanks adjacent to the entrance of the hatchery were disturbed frequently by visitors to the farm. The fish stopped feeding for periods after disturbance and this is the reason thought to be responsible for the significant differences between tanks pairs 1 and 12 and 2 and 36. Both tanks 1 and 2 are adjacent to the hatchery entrance, and both these tanks had mean weights and lengths that were significantly less than their corresponding duplicate tank.

Apart from these problems it was shown that the tank effect on variation was not as significant as had been first thought. It was decided

on the basis of these figures that the factoral trial planned for 1985 could go ahead, with no duplicate tanks.

The t-tests performed on the fish once they had been in the earth ponds over winter gave interesting results. Slightly more significant differences occurred between duplicate populations of fish, but the situation regarding the majority of duplicate pairs remained similar to that found at the second accurate measurement (28 out of 32 pairs remained the same).

3.3.2 Results using Factoral breeding scheme

The results for the 1985 diallele cross growth trial are presented in the same form as for years 1982-83 and 1983-84. A statistical table illustrates each accurate measurement, followed by a reproduction of the relevant Anova table with the calculated growth heritability estimates. Two sets of graphs then illustrate the difference in growth partially attributable to each male broodstock and each female broodstock. The mean values for the particular parameter are ranked in order with their appropriate standard deviations for each female crossed with the 6 different males, and each male crossed with the 5 different females.

Above the sets of histograms are a group of lines representing the results of Duncan's multiple range test. This indicates that there are considerably significant differences between progeny of the same male crossed with the 5 different females and between the same female crossed with the 6 different males for each of the accurate measurements taken.

Table 3.35 Listing statistics for each tank population at weight (1) in the factoral trial 1985

Tank No.	Minimum (gms)	Maximum (gms)	Mean (gms)	SE Mean	Variance	SD	Range (gms)	Kurtosis	Skewness
1	1.32	1.89	1.54	0.023	0.016	0.127	0.57	0.594	0.674
2	1.15	1.90	1.47	0.029	0.025	0.158	0.75	0.700	0.271
3	0.66	1.10	0.88	0.023	0.016	0.125	0.44	- 0.886	0.022
4	0.70	1.50	1.13	0.031	0.029	0.171	0.80	0.688	0.036
5	1.29	1.92	1.56	0.031	0.028	0.169	0.63	- 0.262	0.611
6	0.95	1.78	1.35	0.032	0.030	0.174	0.83	0.743	0.037
7	1.35	2.02	1.64	0.035	0.036	0.190	0.68	- 0.809	0.299
8	1.24	2.12	1.70	0.038	0.042	0.206	0.88	- 0.066	0.011
9	0.58	o.92	1.17	0.045	0.062	0.248	1.34	2.277	0.567
10	0.91	1.57	1.19	0.031	0.029	0.172	0.66	- 0.784	0.102
11	1.16	1.96	1.59	0.034	0.034	0.185	0.78	0.617	-0.565
12	0.71	1.85	1.36	0.041	0.050	0.223	1.14	1.875	- 0.559
13	1.30	2.45	1.84	0.047	0.066	0.257	1.15	0.102	0.075
14	1.19	2.49	1.85	0.049	0.073	0.270	1.30	0.360	0.088
15	1.12	1.97	1.51	0.035	0.036	0.190	0.85	0.673	0.464
16	1.26	1.87	1.47	0.025	0.018	0.134	0.61	2.224	1.293
17	1.73	2.60	2.03	0.038	0.044	0.209	0.87	0.361	0.622
18	1.34	2.15	1.77	0.040	0.049	0.221	0.81	-0.852	-0.070
19	1.05	1.69	1.33	0.032	0.031	0.176	0.64	-0.731	0.185
20	1.08	1.77	1.39	0.031	0.028	0.168	0.69	-0.446	0.055
21	0.59	1.26	0.96	0.029	0.026	0.161	0.67	-0.162	0.052
22	0.75	1.70	0.99	0.023	0.015	0.124	0.45	- 0.677	-0.065
23	1.04	1.71	1.31	0.030	0.027	0.165	0.67	- 0.152	0.404
24	0.81	1.52	1.13	0.030	0.028	0.167	0.71	- 0.175	0.177
25	1.10	1.77	1.38	0.034	0.035	0.187	0.67	- 0.182	0.663
26	1.00	1.66	1.36	0.033	0.032	0.180	0.66	- 0.571	0.072
27	0.74	1.86	1.08	0.040	0.049	0.221	1.12	4.095	1.236
28	0.78	1.35	1.10	0.021	0.013	0.112	0.57	1.410	-0.370
29	1.14	2.03	1.51	0.042	0.052	0.228	0.89	- 0.416	0.553
30	0.84	1.75	1.32	0.040	0.049	0.220	0.91	- 0.536	0.093

Tabe 3.36 Heritability estimation

Experiment: Factoral Weight (1)

Sources of Variation	Degrees of Freedome	Sum of Squares	(SS%)	Mean Squares	F	5%
Sire	5	32.0609	(32.10)	6.4122		
Dam	4	33.5679	(33.61)	8.3920		
Sire x Dam interaction	20	3.2710	(3.28)	0.1635		
Random	870	30.9721	(31.01)	0.0356		
TOTAL	899	99.8719	(100.00)			

Heritability Estimate: $h^2s = 1.31$ $h^2d = 1.44$ $h^2sd = 1.39$

Standard Error of Heritability: $SE(h^2s) = 0.600$ $SE(h^2d) = 1.00$ $SE(h^2sd) = 0.803$

Mean = 1.39 gms
S.E. = 0.333
CV = 24%

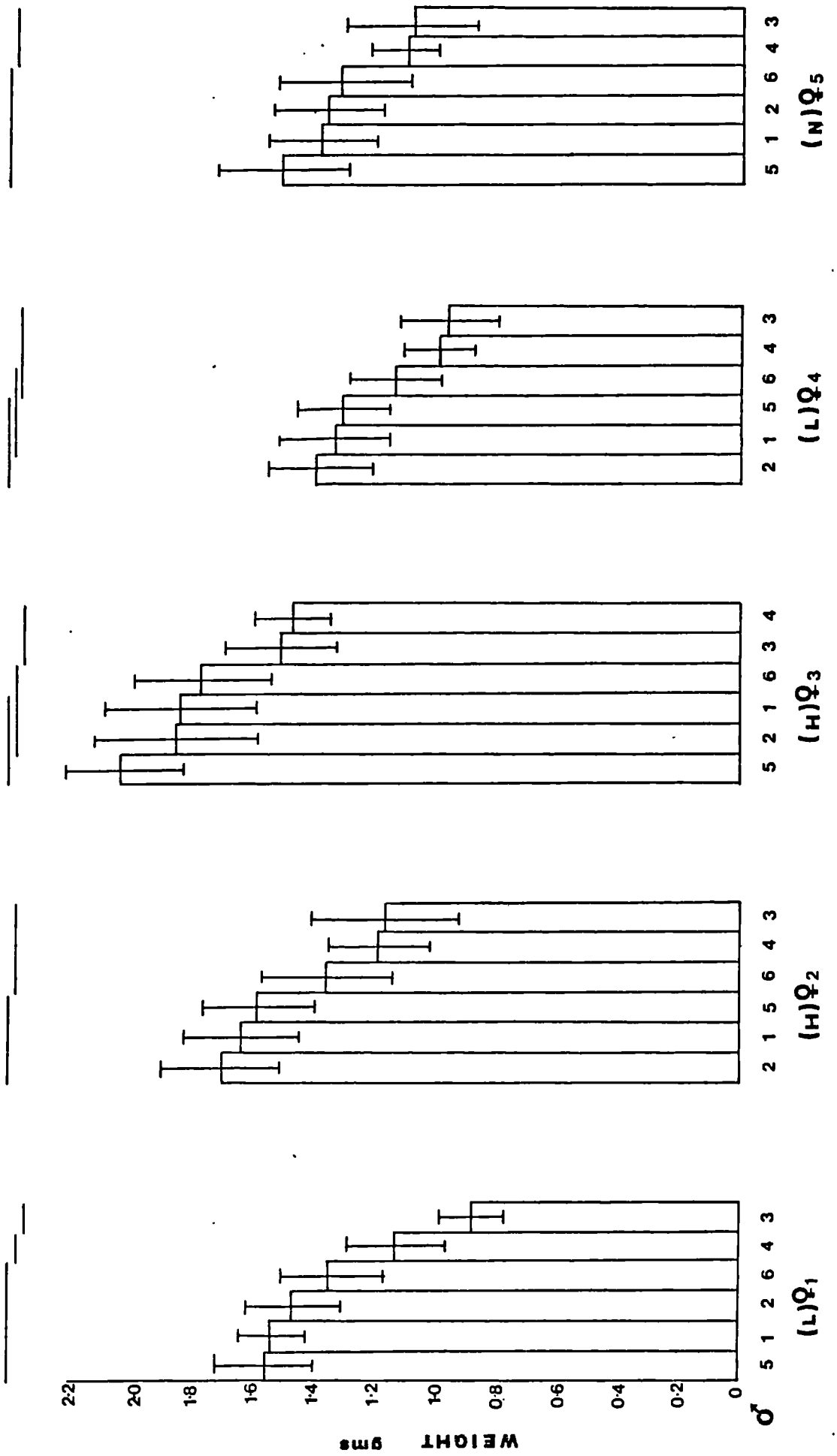


Figure 3.24 Histograms of ranked means attributable to each dam for weight (1) in the factoral trial 1985

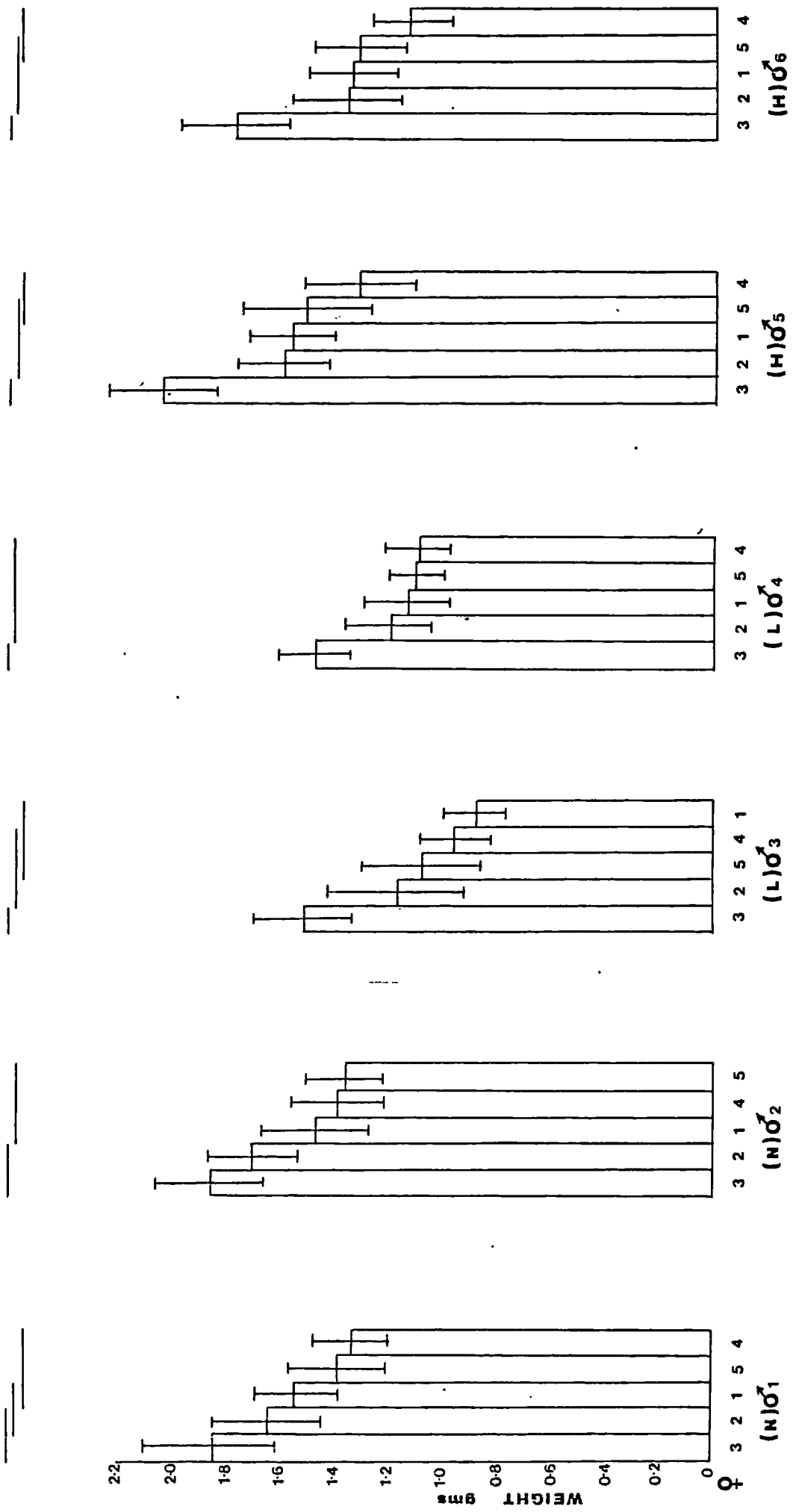


Figure3.25 showing histograms of ranked means attributable to each sire for weight (1) in the factoral trial in 1985

Table 3.37 listing statistics for each tank population at weight (2) in the factoral trial 1985

Tank No		Minimum (gms)	Maximum (gms)	Mean (gms)	SE Mean	Variance	SD	Range (gms)	Kurtosis	Skewness
7	1	8.00	11.0	9.11	0.124	0.463	0.068	3.0	0.928	0.586
8	2	6.80	18.8	11.00	0.539	8.703	2.95	12.0	0.492	0.788
9	3	3.90	11.9	5.92	0.337	3.412	1.85	8.0	3.661	1.732
10	4	4.80	11.3	7.95	0.314	2.965	1.72	6.5	-0.613	0.472
11	5	5.40	15.0	8.23	0.372	4.148	2.04	9.6	3.400	1.646
12	6	4.60	13.1	7.92	0.352	3.740	1.93	8.5	0.835	0.685
13	7	7.10	22.6	12.19	0.592	10.497	3.24	15.5	2.540	1.145
14	8	5.10	18.6	12.75	0.541	8.785	2.96	13.5	0.405	-0.319
15	9	3.70	16.3	7.89	0.453	6.166	2.48	12.6	3.353	1.265
16	10	5.50	14.3	9.26	0.352	3.712	1.93	8.8	0.526	0.554
17	11	3.80	15.3	10.59	0.447	5.890	2.45	11.5	1.341	-0.814
18	12	4.70	15.7	9.91	0.526	8.285	2.88	11.0	-0.512	0.188
19	13	9.70	23.0	13.45	0.582	10.147	3.18	13.3	1.565	1.230
20	14	9.30	24.9	15.05	0.611	11.188	3.35	15.6	1.738	0.613
21	15	5.90	17.0	9.54	0.533	8.516	2.92	11.1	0.494	1.036
22	16	6.00	16.0	10.04	0.461	6.387	2.53	10.0	0.158	0.552
23	17	7.50	18.2	12.07	0.442	5.857	2.42	10.7	0.912	0.809
24	18	7.70	21.0	12.46	0.569	9.704	3.11	13.3	0.596	0.728
25	19	4.70	11.3	8.00	0.341	3.491	1.87	6.6	-1.053	0.107
26	20	4.70	16.0	9.69	0.475	6.776	2.60	11.3	0.386	0.162
27	21	3.00	13.0	6.05	0.383	4.400	2.09	10.0	3.231	1.512
28	22	4.20	10.0	7.10	0.290	2.529	1.59	5.8	-0.513	0.249
29	23	5.10	13.8	9.14	0.472	6.696	2.59	8.7	-1.181	0.17
30	24	3.20	12.9	7.56	0.440	5.814	2.41	9.7	-0.260	0.174
31	25	6.60	14.8	11.11	0.408	5.003	2.24	8.2	-0.883	-0.041
32	26	9.40	18.0	13.19	0.364	3.968	1.99	8.6	-0.215	0.351
33	27	6.10	14.7	8.75	0.348	3.631	1.91	8.6	2.322	1.380
34	28	7.20	14.3	9.42	0.337	3.402	1.84	7.1	2.185	1.522
35	29	5.80	18.3	11.48	0.561	9.426	3.07	12.5	-0.300	0.497
36	30	6.50	17.2	10.31	0.474	6.750	2.59	10.7	-0.014	0.551

Figure 3.38 Heritability estimation

Experiment: Factoral Weight 2

Sources of Variation	Degrees of Freedom	Sum of Squares	(SS%)	Mean Squares	F	5%
Sire	5	2008.38	(20.88)	401.68		
Dam	4	2177.62	(22.65)	544.40		
Sire x Dam interaction	20	193.48	(2.01)	9.67		
Random	870	5235.94	(54.45)	6.02		
TOTAL	899	9615.42	(100.00)			

Heritability Estimate: $h^2s = 0.89$ $h^2d = 1.01$ $h^2s+d = 0.95$

Standard Error of Heritability: $SE(h^2s) = 0.406$ $SE(h^2d) = 0.713$ $SE(h^2sd) = 0.560$

Mean = 9.91 gms
S.E. = 3.27
CV = 33%

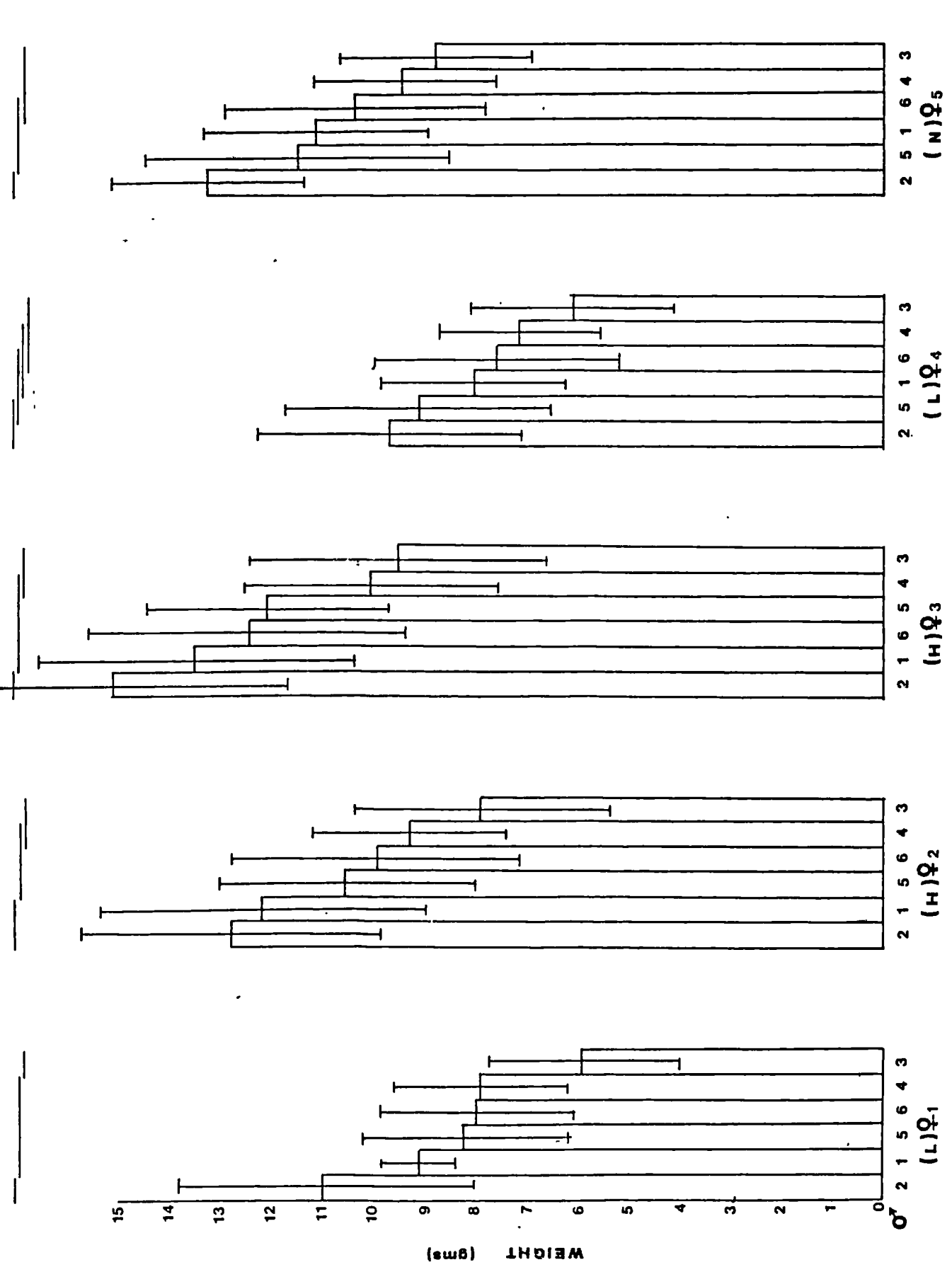


Figure 3.26 Histogram of ranked means attributable to each for weight (2) in the factoral trial 1985

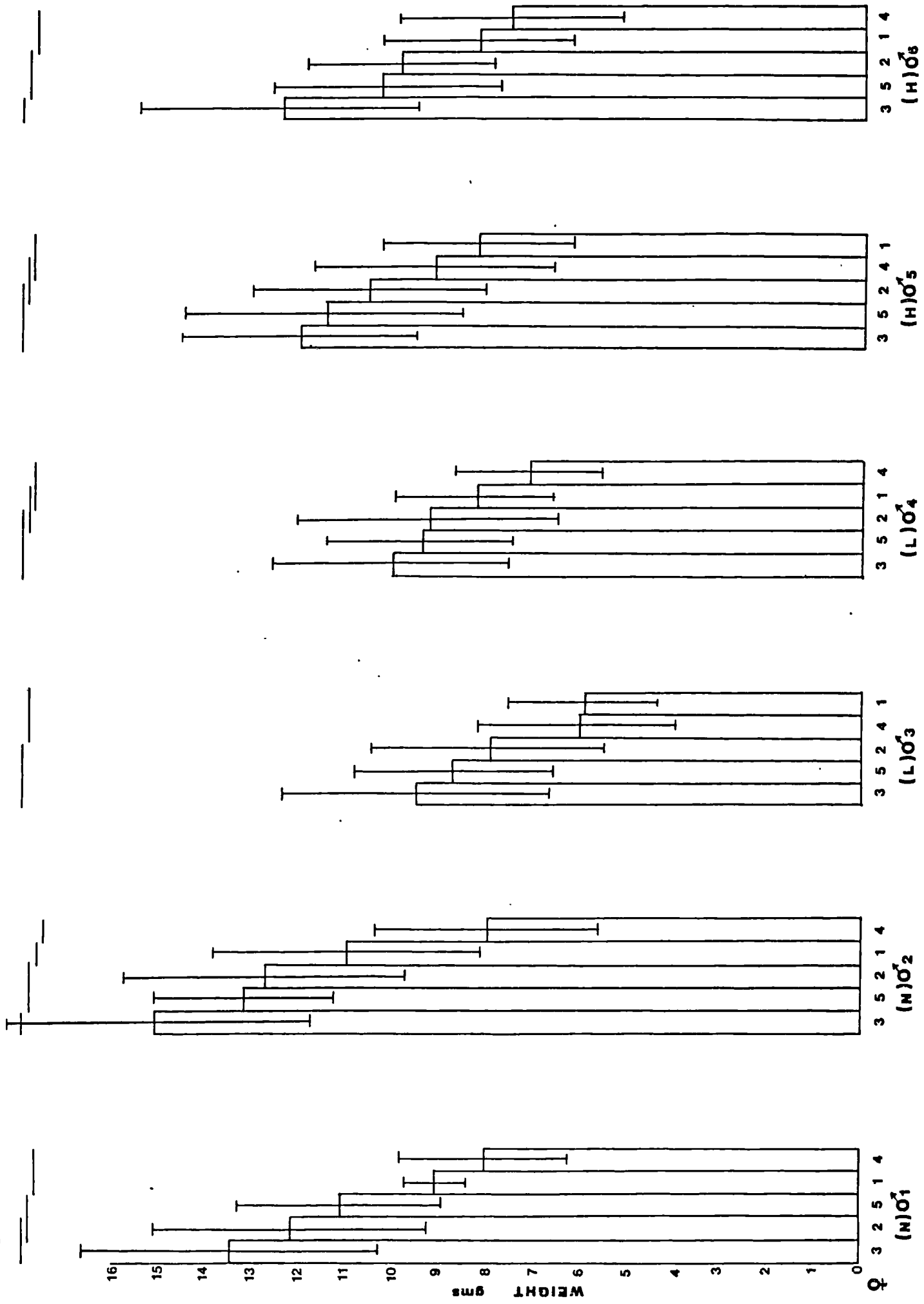


Figure 3.27 Histograms of ranked means attributable to each site for weight (2) in the factoral trial (1985)

Table 3.39 Listing statistics for each tank population at length (1) in the factoral trial 1985

Tank No		Minimum (cms)	Maximum (cms)	Mean (cms)	SE Mean	Variance	SD	Range (cms)	Kurtosis	Skewness
7	1	5.00	5.50	5.29	0.021	0.014	0.118	0.50	0.023	- 0.196
8	2	4.70	5.45	5.15	0.029	0.026	0.161	0.75	1.318	0.678
9	3	4.00	4.75	4.40	0.035	0.038	0.194	0.75	- 0.681	- 0.046
10	4	4.00	5.15	4.76	0.039	0.045	0.212	1.15	4.912	- 1.224
11	5	5.00	5.65	5.28	0.028	0.024	0.155	0.65	0.783	0.664
12	6	4.70	5.25	5.02	0.029	0.025	0.158	0.55	- 0.842	- 0.508
13	7	5.00	5.80	5.39	0.033	0.033	0.181	0.80	0.234	0.198
14	8	4.80	5.95	5.40	0.040	0.048	0.270	1.15	1.856	- 0.108
15	9	4.00	5.15	4.73	0.045	0.060	0.244	1.15	1.385	- 0.527
16	10	4.40	5.40	4.85	0.037	0.041	0.201	1.00	1.263	0.326
17	11	4.80	5.70	5.34	0.036	0.039	0.197	0.90	1.008	- 0.518
18	12	4.30	5.60	5.07	0.049	0.071	0.266	1.30	2.207	- 1.096
19	13	4.95	6.00	5.56	0.042	0.052	0.227	1.05	0.423	- 0.616
20	14	5.15	6.00	5.52	0.039	0.045	0.213	0.85	- 0.508	0.195
21	15	4.85	5.50	5.17	0.032	0.030	0.174	0.65	- 0.907	- 0.010
22	16	4.80	5.45	5.08	0.030	0.026	0.162	0.65	0.446	0.394
23	17	5.40	6.15	5.69	0.030	0.027	0.163	0.75	1.484	1.061
24	18	5.15	5.80	5.47	0.036	0.038	0.195	0.65	- 0.977	0.033
25	19	4.70	5.40	5.02	0.036	0.038	0.195	0.70	- 0.613	- 0.256
26	20	4.75	5.70	5.06	0.038	0.043	0.207	0.95	1.887	1.025
27	21	3.85	4.90	4.51	0.044	0.058	0.241	1.05	0.636	0.709
28	22	4.25	4.80	4.54	0.027	0.021	0.146	0.55	- 0.143	- 0.453
29	23	4.65	5.45	5.03	0.033	0.034	0.183	0.80	- 0.042	0.079
30	24	4.30	5.70	4.75	0.038	0.043	0.207	0.90	- 0.125	- 0.105
31	25	4.60	5.30	4.99	0.036	0.040	0.200	0.70	0.879	0.016
32	26	4.55	5.30	4.94	0.036	0.038	0.195	0.75	- 0.778	- 0.159
33	27	4.10	5.00	4.61	0.043	0.054	0.233	0.90	- 0.099	- 0.606
34	28	4.20	5.00	4.64	0.027	0.022	0.148	0.80	2.364	- 0.736
35	29	4.50	5.65	5.15	0.044	0.057	0.239	1.15	1.320	- 0.182
36	30	4.20	5.40	4.90	0.046	0.084	0.252	1.20	2.096	- 0.993

Table 3.40 Heritability estimation

Experiment: Factoral Length (1)

Sources of Variation	Degrees of Freedome	Sum of Squares	(SS%)	Mean Squares	F	5%
Sire	5	50.4184	(38.50)	10.0837		
Dam	4	41.1374	(31.45)	10.2843		
Sire. Dam. Interaction	20	4.7077	(3.60)	0.2354		
Random	870	34.5496	(26.41)	0.0397		
TOTAL	899	130.8131	(100.00)			

Heritability Estimate:	$h^2s = 1.557$	$h^2d = 1.336$	$h^2s+d = 1.447$
Standard Error of Heritability:	$SE(h^2s) = 1.684$	$SE(h^2d) = 1.498$	$SE(h^2sd) = 1.589$

Mean = 5.04 cm
S.E. = 0.38
CV = 7.5%

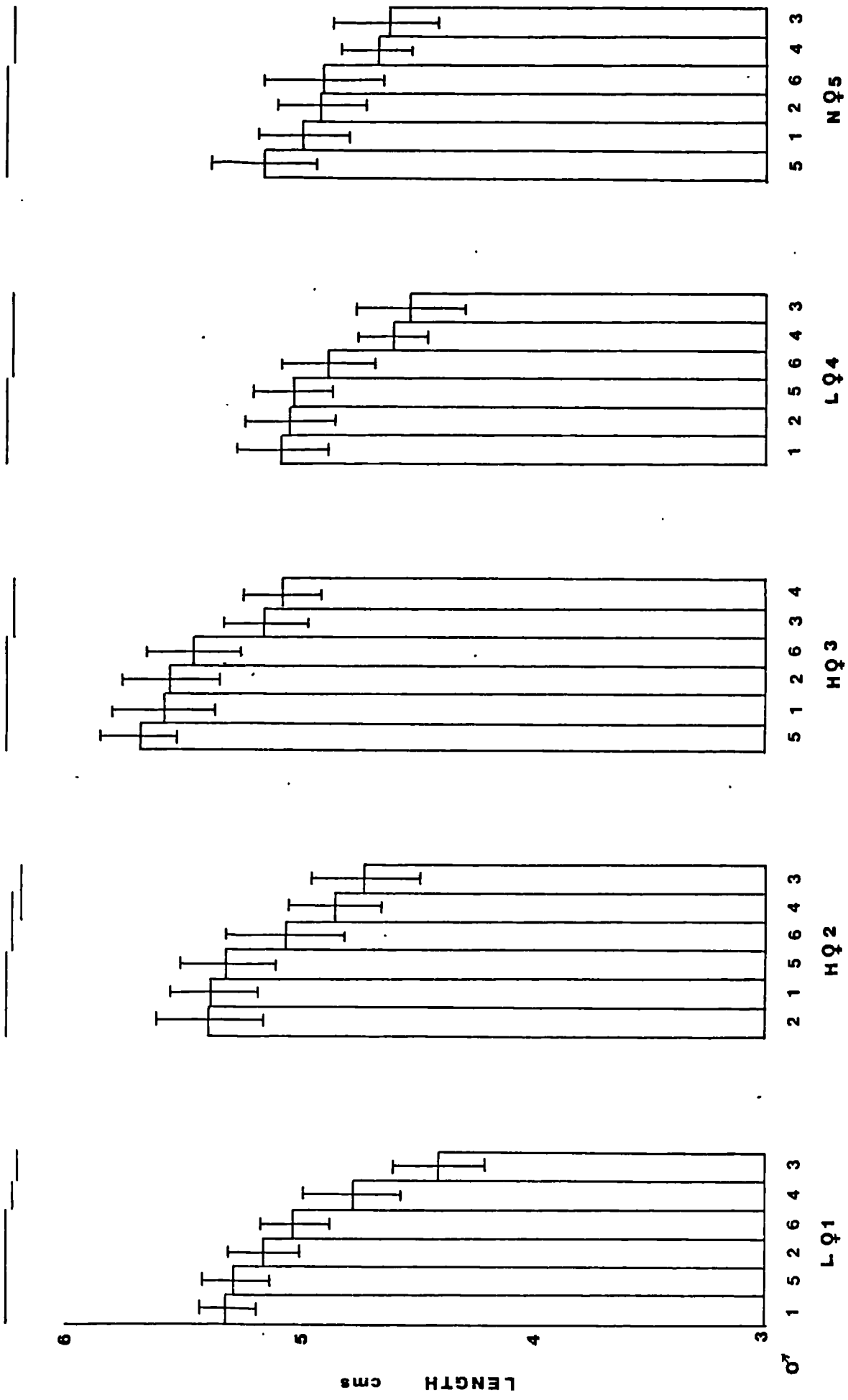


Figure 3.28 histograms of ranked means attributable to each dam for length (1) in the factoral trial 1985

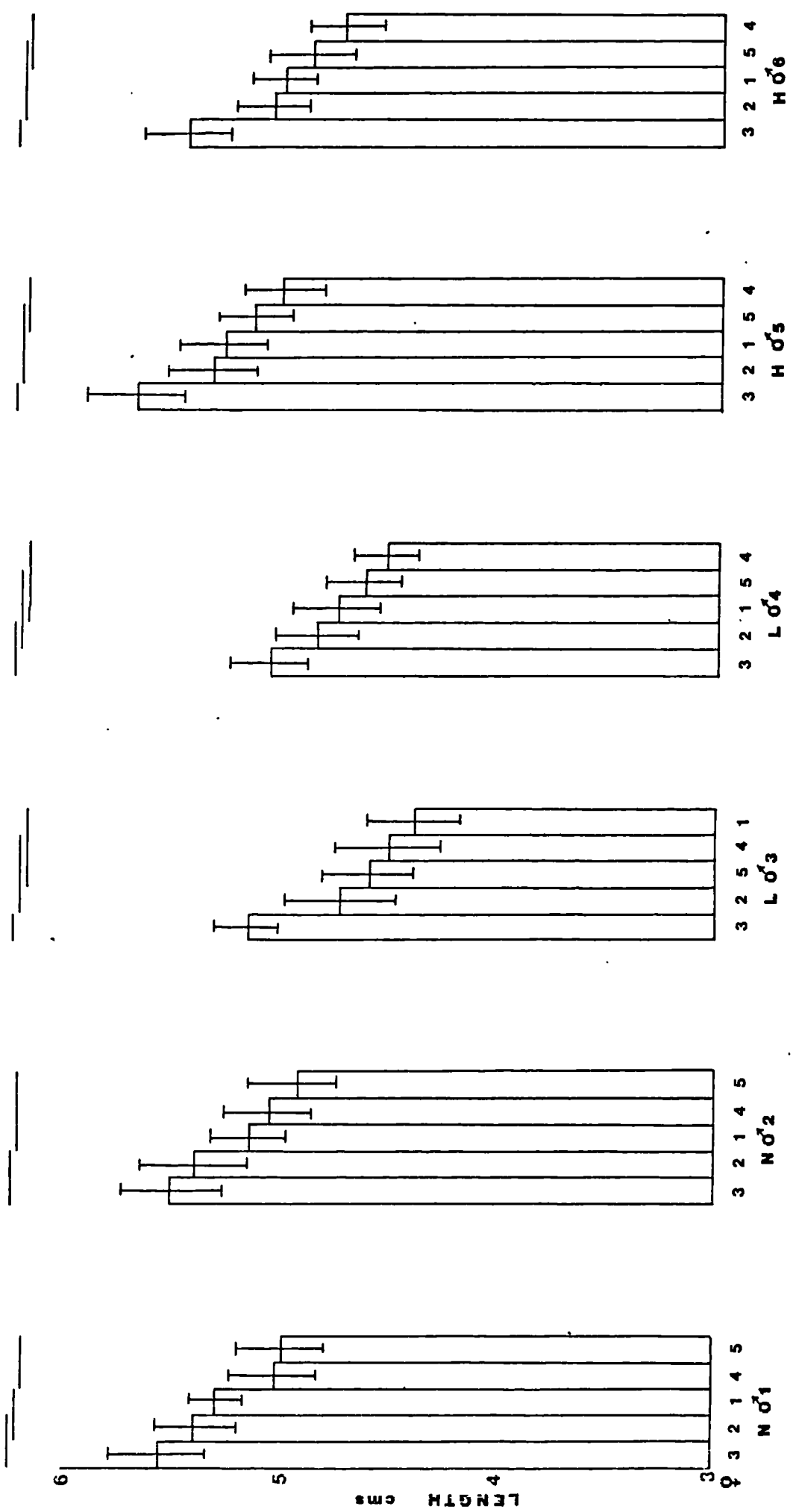


Figure 3.29 histograms of ranked means attributable to each sire for length (1) for the factoral trial 1985

Table 3.41 listing statistics for each tank population at length (2) in the factoral trial 1985

Tank No		Minimum (cms)	Maximum (cms)	Mean (cms)	SE mean	Variance	SD	Range (cms)	Kurtosis	Skewness
7	1	8.00	11.00	9.13	0.123	0.454	0.674	3.0	1.007	0.523
8	2	8.30	10.80	9.49	0.124	0.460	0.678	2.5	- 0.498	0.187
9	3	7.00	9.60	7.93	0.117	0.413	0.642	2.6	1.335	0.986
10	4	7.60	9.70	8.75	0.100	0.300	0.548	2.1	- 0.573	0.288
11	5	8.30	10.70	9.05	0.108	0.347	0.589	2.4	0.962	0.987
12	6	7.60	10.40	8.89	0.112	0.375	0.612	2.8	0.593	0.156
13	7	8.60	11.90	9.97	0.134	0.537	0.733	3.3	0.822	0.497
14	8	7.70	11.20	9.98	0.133	0.532	0.730	3.5	2.658	- 1.293
15	9	6.80	10.80	8.69	0.151	0.682	0.826	4.0	0.609	0.144
16	10	8.00	10.40	9.22	0.104	0.325	0.570	2.4	- 0.134	0.159
17	11	5.00	10.90	9.48	0.220	1.457	1.207	5.9	6.742	- 2.436
10	12	7.60	11.10	9.38	0.152	0.693	0.832	3.5	- 0.017	- 0.186
19	13	9.40	12.20	10.31	0.127	0.484	0.695	2.8	0.598	0.992
20	14	9.20	12.10	10.58	0.121	0.437	0.661	2.9	0.464	- 0.263
21	15	7.30	11.80	9.14	0.159	0.756	0.870	4.3	1.392	0.742
22	16	8.20	11.00	9.37	0.131	0.514	0.717	2.8	- 0.003	0.426
23	17	8.80	11.50	10.17	0.120	0.429	0.655	2.7	- 0.136	0.170
24	18	8.80	12.00	10.20	0.136	0.552	0.743	3.2	0.149	0.336
25	19	7.60	9.90	8.30	0.115	0.398	0.631	2.3	- 0.882	- 0.081
26	20	7.20	10.80	9.23	0.142	0.604	0.777	3.6	0.999	- 0.634
27	21	6.40	10.10	7.98	0.146	0.637	0.798	3.7	0.811	0.598
28	22	7.40	9.50	8.44	0.105	0.336	0.580	2.1	- 0.477	- 0.013
29	23	7.70	10.80	9.28	0.155	0.719	0.848	3.1	- 0.872	- 0.015
30	24	3.30	10.20	8.42	0.234	1.647	1.283	6.9	8.034	- 2.295
31	25	8.20	10.50	9.62	0.107	0.340	0.583	2.3	- 0.316	- 0.431
32	26	9.20	11.90	10.18	0.111	0.371	0.609	2.7	1.216	0.969
33	27	7.90	10.50	8.86	0.103	0.321	0.567	2.6	1.454	1.065
34	28	8.40	10.40	9.15	0.092	0.255	0.505	2.0	1.283	1.112
35	29	4.50	11.70	9.65	0.238	1.692	1.301	7.2	7.802	- 2.041
36	30	8.30	11.30	9.48	0.127	0.481	0.994	3.0	0.148	0.318

Table 3.42 Heritability estimation

Experiment: Factoral Length (2)

Sources of Variation	Degrees of Freedom	Sum of Squares	(SS%)	Mean Squares	F	5%
Sire	5	179.263	(20.45)	35.853		
Dam	4	191.783	(21.88)	47.946		
Sire x Dam interaction	20	17.226	(1.97)	0.861		
Random	870	488.138	(55.69)	0.561		
TOTAL	899	876.409	(100.00)			

Heritability Estimate:	$h^2s = 0.88$	$h^2d = 0.98$	$h^2sd = 0.93$
Standard Error of Heritability:	$SE(h^2s) = 0.40$	$SE(h^2d) = 0.69$	$SE(h^2sd) = 0.54$

Mean = 9.30 cm
S.E. = 0.987
CV = 10.6%

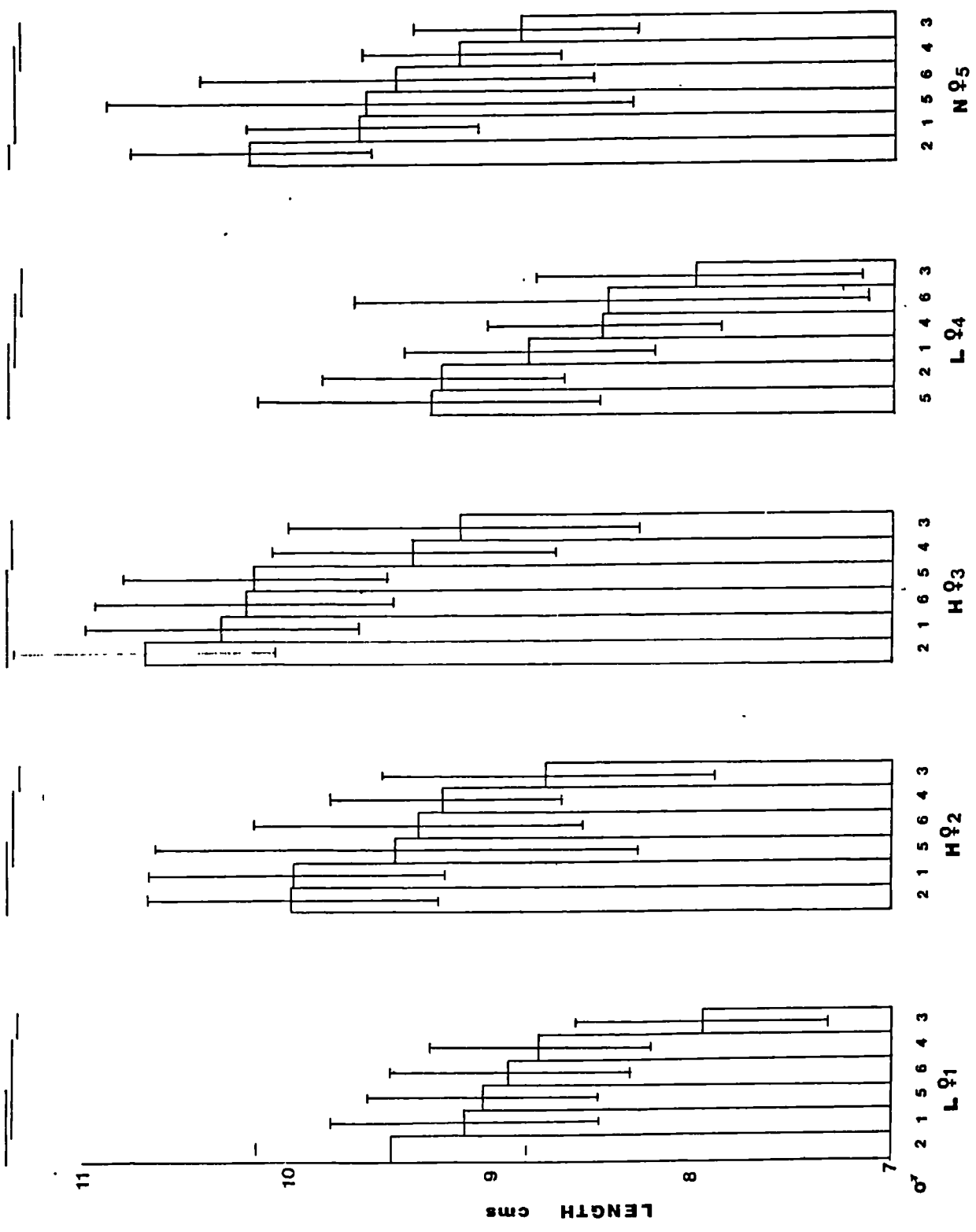


Figure 3.30 Histograms of ranked means attributable to each dam for length (2) in the factoral trial 1985

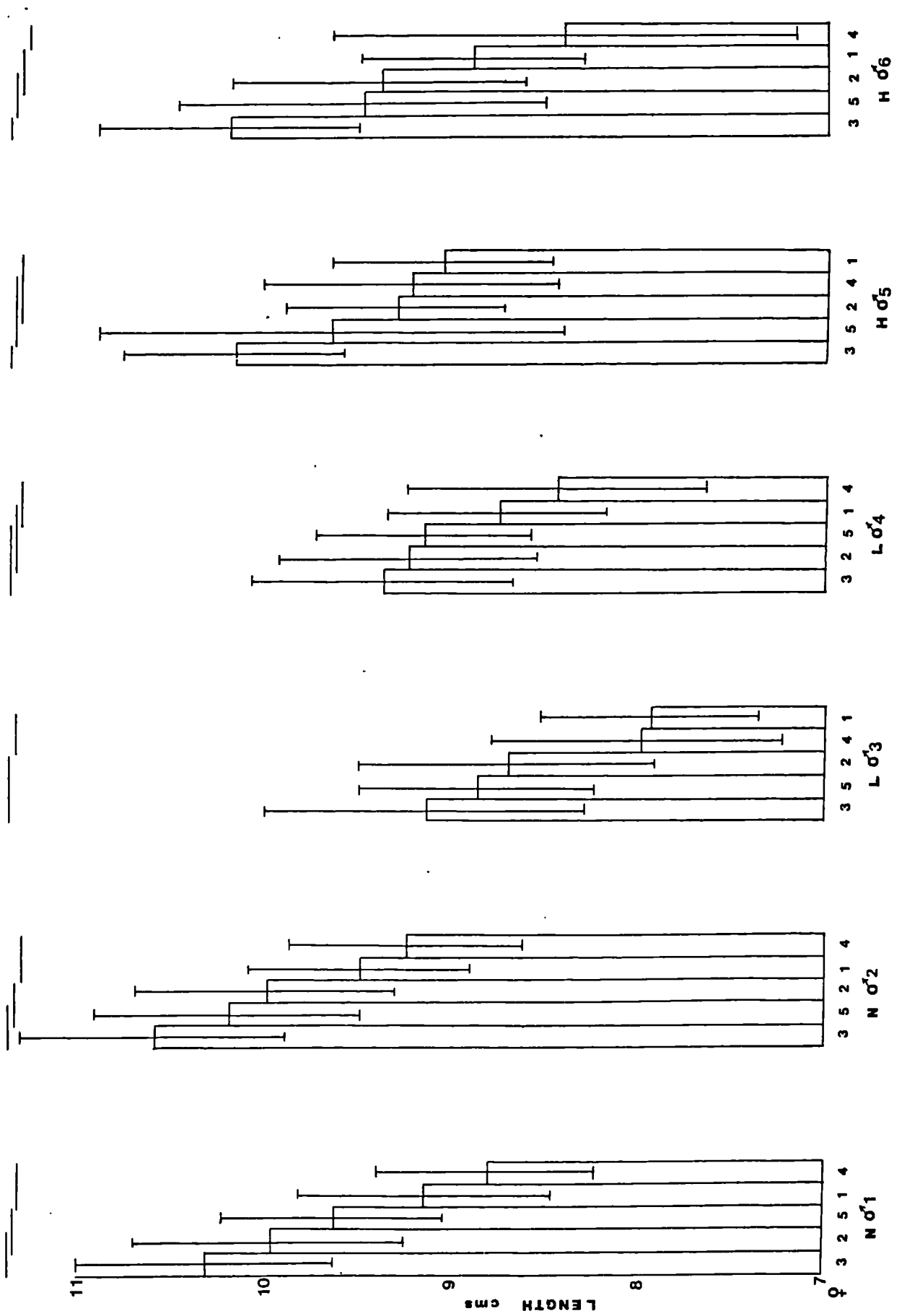


Figure 3.31 Histograms of ranked means attributable to each sire for length (2) in the factoral trial 1985

Table 3.43 The heritability estimates obtained for growth rate using the factoral crossing design in 1985. Also shown are statistics of importance relating to the particular parameter, namely the mean, overall standard deviation and coefficient of variation.

Parameter	X	σ	CV	$h^2s \pm SE$	$h^2d \pm SE$	Number of sires and dams used , + number of observations
Factoral cross						
Length (1)	5.04	0.38	7.5	1.56 ± 1.13	1.34 ± 1.50	6 5 , 900 obs
Length (2)	9.30	0.99	10.6	0.88 ± 0.40	0.98 ± 0.69	6 5 , 900 obs
Weight (1)	1.39	0.33	24.0	1.31 ± 0.60	1.44 ± 1.00	6 5 , 900 obs
Weight (2)	9.91	3.27	33.0	0.89 ± 0.41	1.01 ± 0.71	6 5 , 900 obs

Weight in gms
Length in cms

$\bar{X}$ = mean; σ = standard deviation; CV = coefficient of variation;

$h^2s \pm SE$ = heritability (sire component) $\pm$ standard error

$h^2d \pm SE$ = heritability (dam component) $\pm$ standard error

Figure 3.32 Illustrating differences between the SGRs of progeny from different factoral crosses

Factoral Trial

♂ Number	♀ Number	SGRs	Duncan's
2	3	3.15	
2	5	3.01	
1	3	2.99	
2	2	2.93	
6	3	2.89	
1	2	2.87	
5	3	2.81	
5	5	2.80	
1	5	2.78	
2	1	2.74	
5	2	2.68	
6	5	2.68	
6	2	2.62	
4	3	2.59	
2	4	2.58	
4	5	2.58	
4	2	2.55	
3	3	2.54	
5	4	2.51	
3	5	2.48	
1	1	2.47	
4	1	2.34	
3	2	2.32	
5	1	2.31	
1	4	2.31	
6	1	2.30	
6	4	2.27	
4	4	2.21	
3	4	1.99	
3	1	1.97	

Specific Growth Rates were calculated using the first accurate weight as Wt_1 and the second accurate weight as Wt_2. The results are given in Figure 3.32 and illustrated by Figures 3.33 and 3.34 . Figure 3.33 shows the SGRs ranked for each dam. The lines above both sets of histograms represent the significance lines from Duncan's multiple range test (1955).

From Figure 3.34 it can be seen that the SGRs calculated for sire two are much larger than for sires three and four. Sires one, five and six exhibit not dissimilar SGRs and intermediate between two and three and four. From Figure 3.33 it can be seen that the SGRs calculated for dam three are larger than for dams one and four. Dams two and five exhibit not dissimilar SGRs intermediate between dams three and one and four.

Figure 3.35 illustrates the ranked weights partially attributable to each broodstock, derived from figures available from the anova undertaken using data from the factoral cross.

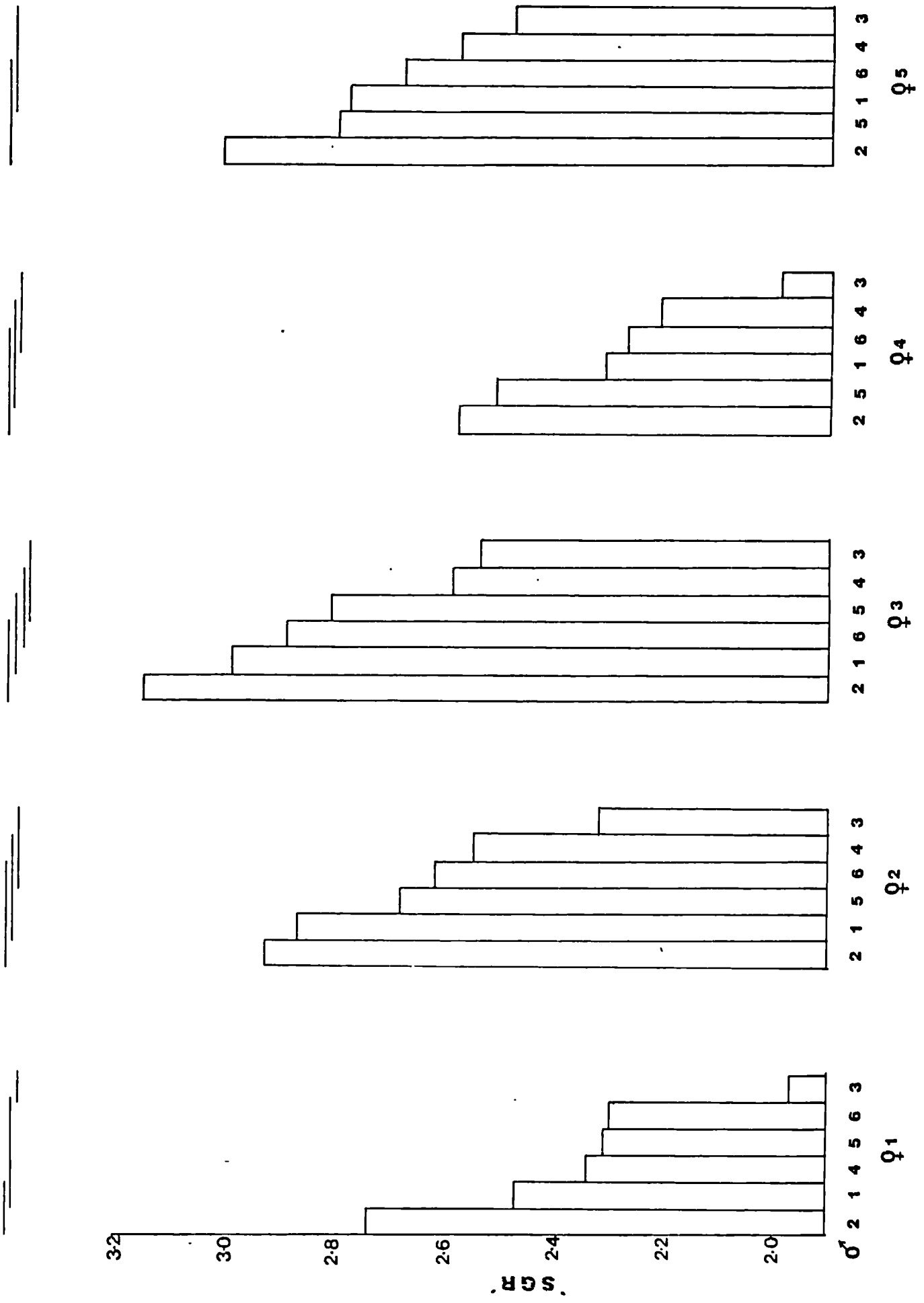


Figure 3.33 Histograms of ranked SGRs attributable to each dam in the factoral trial 1985

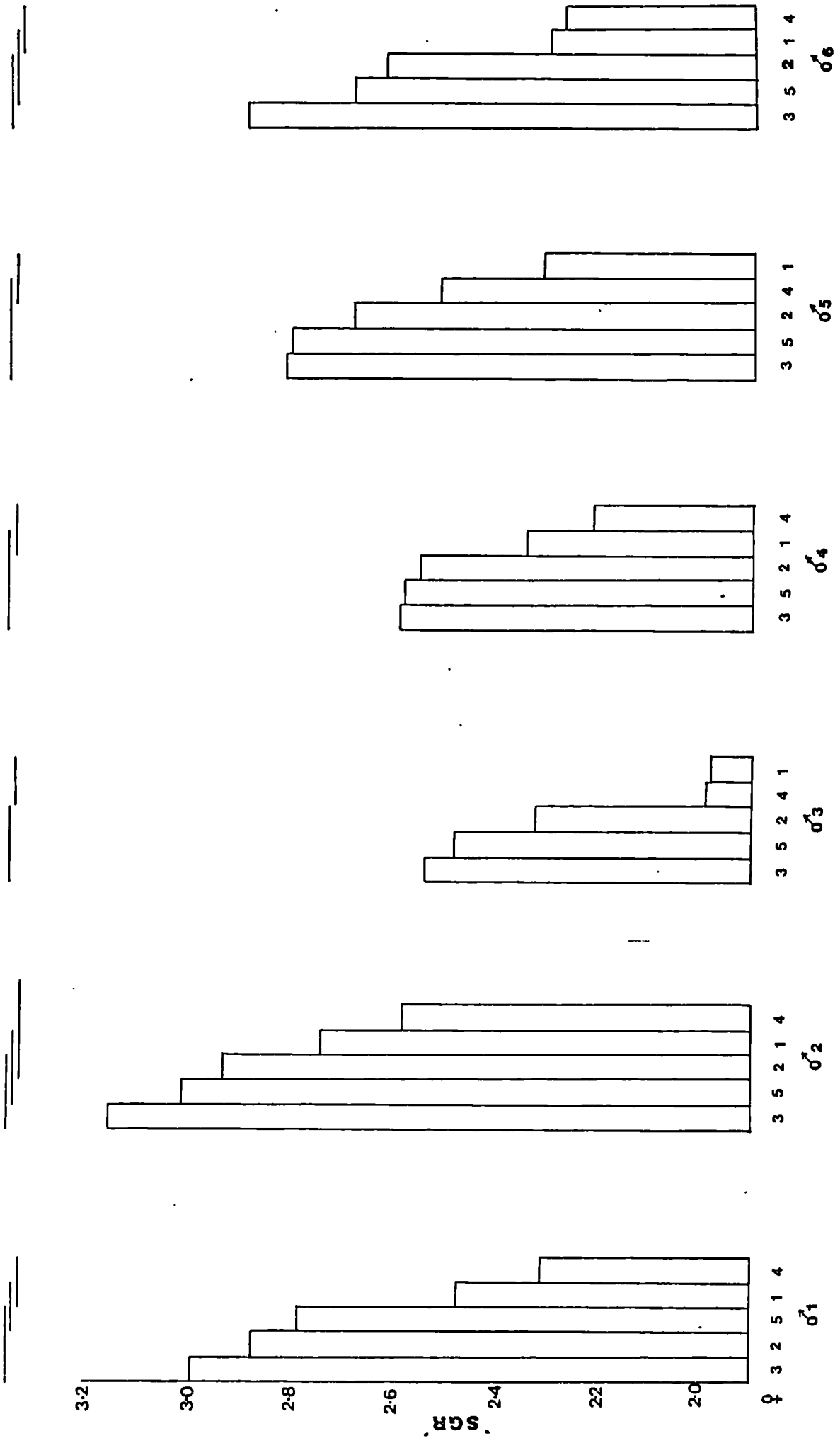
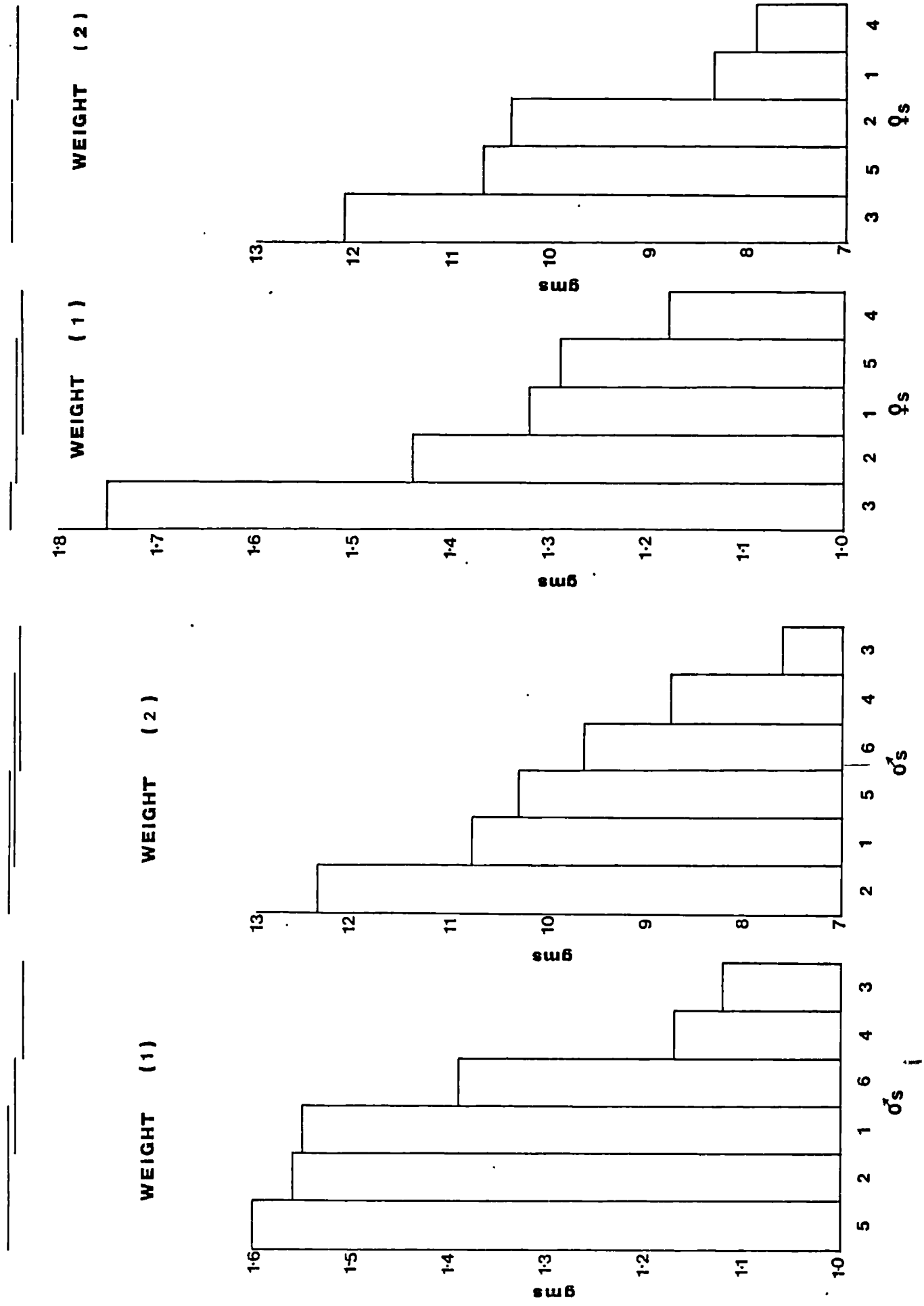


Figure 3.34 Histograms of ranked SGRs attributable to each sire in the factoral trial 1985

Figure 3.35 RANKED WEIGHTS PARTIALLY ATTRIBUTED TO EACH BROODSTOCK



3.3.3 Summary of results

1. The heritability estimates for growth rate using the hierarchical breeding schemes were exeptionally high. (Table 3.33)

2. The standard errors on the heritability estimates are high.

3. The female heritability components for weight and length in the Howietoun trial became less pronounced, especially at measurement (3).

4. The male heritability component for weight in the Howietoun trial became more pronounced as the trial progressed.

5. The male heritability component for length in the Howietoun trial remained high throughout the trial.

6. The female heritability component for weight in the Leven trout trial dropped from 0.89 to 0.63, as the trial progressed.

7. The male heritability component for weight in the Leven trout trial dropped from 0.78 to 0.45 as the trial progressed.

8. The female heritability component for length in the Leven trial dropped from 1.24 to 0.52 as the trial progressed and then again to 0.70 at measurement (3). The figure of 1.24 obtained for the first accurate length taken is one of the largest heritability estimates found.

9. The male heritability component for length on the Leven trout trial was lower than the corresponding female component figures calculated. The heritability started at 0.38 rose to 0.78 at the second accurate measurement and declined again to 0.42 at the third.

10. The heritability estimates for growth rate using the factoral breeding scheme were even higher than those derived from the hierarchical breeding scheme.

11. The standard errors were also very large

12. From the 16 pairs of heritability estimates (factoral and hierarchical included) the dam heritability components were larger than the sire heritability component on 8 occasions, and the sire heritability components were larger than the dam heritability components on 8 other occasions.

13. The highest dam heritabilities were obtained at the beginning of each trial.

14. The highest sire heritability estimates were obtained also at the beginning of the trials, but the sire components remained consistently high to the end of each trial.

15. Coefficient of variation was much larger for weights than for lengths.

16. Coefficients of variation increased as the trials progressed.

This was consistently the case, whether weight or length was being considered.

17. According to Duncan's (1955) range test there were significant differences between the means of many of the tanks of fish studied during the three yearly trials. These differences were apparent at the first accurate measurements in each year and highly significant differences were observed throughout the trials.

If one compares the results for the first two trials obtained from calculating specific growth rates (Figure 3.23) and the histograms illustrating the final weights in each trial (Figures 3.10, 3.16) it is apparent that the tanks and females exhibiting the best and worst SGRs correspond with the largest and smallest final mean weights. The Duncan's (1955) multiple range test gives similar statistical results whether SGRs or final weights are used.

18. The SGR values for fish in the sets of duplicate tanks are much less significantly different than the mean final weights of the fish. The Duncan's multiple range tests indicate for the first two trials that only one duplicate pair of tanks out of thirty two exhibited significantly different SGR values (Figure 3.23) that being female number 6 in the Howietoun trial, tank numbers 4 and 22. There were no duplicate tanks in trial three, each tank being effectively a separate experiment.

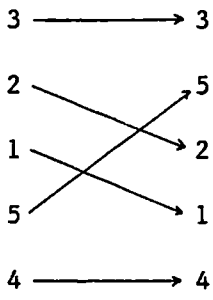
19. The t-tests performed on mean weights and lengths from duplicate pairs of tanks showed significant differences between each member of a duplicate pair more frequently at the beginning of the first two trials, but the differences became less as the trials progressed (Table 3.34). At 5% significance level (P = 0.05) 75% of the duplicate pairs showed no significant difference between their mean weights after being kept in the tanks for over six months. Over 80% of the duplicate pairs showed no significant difference between their mean lengths, after being kept in the tanks for the same period. Because of this high level of uniformity between the duplicates, it was felt that the factoral breeding scheme could take place without duplicates.

20. SGRs calculated for the third trial based on weights taken at the first accurate measurement and the second accurate measurement reflect the same situation as was found in the first two trials. The fish which had the largest final weights (Figure 3.26, 3.27) came from tanks that exhibited the highest SGRs (Figure 3.32) and fish which had the lowest final weights came from tanks that exhibited the lowest SGRs.

21. The ranked order of weight, changed as the trial progressed, reflecting the differences in the SGRs attributable to each broodstock.

The ranked order of weight attributable to each broodstock changed as follows:

♂



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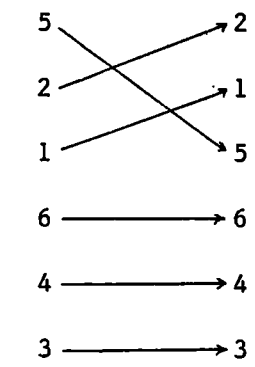


Where 1 = Leven (1)
2 = Howietoun (1)
3 = Howietoun (2)
4 = Leven (2)
5 = Nashua (2)

1 Nashua (1)
2 Nashua (2)
3 Leven (1)
4 Leven (2)
5 Howietoun (1)
6 Howietoun (2)

3.4 Discussion

3.4.1. Discussion of heritability estimates

The heritabilities found for growth rate in this study are higher than most other studies of salmonids. From Table 3.33, listing the estimates for heritabilities calculated from the hierarchical design scheme and from Table 3.43 listing the estimates of heritability calculated from the factoral design scheme, sire estimates range from $h^2_s = 0.38 \pm 0.56$ to 1.56 ± 1.13, and dam estimates range from $h^2_d = 0.08 \pm 0.12$ to 1.44 ± 1.00.

Gjedrem (1983) calculated average heritability estimates for growth rate in a number of fish species, based on published sire heritability for body weight of juveniles he reports as being rather low for Atlantic salmon ($h^2_s = 0.08$), rainbow trout ($h^2_s = 0.12$) and carp ($h^2_s = 0.15$), but higher in channel catfish ($h^2_s = 0.42$) and oysters ($h^2_s = 0.36$). Heritability for body weight of adults, Gjedrem (1983) reports as being higher than for juvenile fish (rainbow trout $h^2_s = 0.17$, Atlantic salmon $h^2_s = 0.36$, carp $h^2_s = 0.36$ and channel catfish $h^2_s = 0.49$).

Gjedrem (1983) found body length showed varying heritabilities from low to medium in large Atlantic salmon (average $h^2 = 0.41$) and medium in oysters ($h^2 = 0.47$) to high in channel catfish ($h^2 = 0.6$).

The majority of heritability estimates calculated in this study are larger than Gjedrem's estimates for juvenile salmonids. Although Gjedrem (1983) reviewed the literature, estimates calculated since

1983 will obviously not be included. Bailey and Loudenslager (1986) using different stocks of Atlantic salmon in New Brunswick produced estimates of heritability for growth which were larger than those found in the literature by Gjedrem (1983). Summary of their results appears in Table 3.1 in the Introduction. h^2_s ranged from 0.73 to 0.79 for length and from 0.67 to 0.89 for weight. The h^2_d values were even larger. Iwamoto et al., (1982) working with Coho salmon also produced heritability estimates for growth larger than normally reported for salmonids (see Table 3.1 in the Introduction). The sire heritabilities for weight dropped from 0.61 $\pm$ 0.31 to 0.25 $\pm$ 0.22 in their first experiment while the dam heritability for weight remained constant (h^2_d 0.65 $\pm$ 0.21 to 0.67 $\pm$ 0.22). The heritability for length followed the same pattern. These results suggest a moderate to high heritability for growth rate especially when weight is the parameter measured. They also suggest a strong maternal effect but this will be discussed later.

Robison and Leumpert III (1984) working with brook trout also produced a large heritability estimate for growth (weight at 243 days, h^2_s = 0.60 $\pm$ 0.27 and h^2_d = 0.37 $\pm$ 0.22). Therefore recent estimates for juvenile salmonid heritability for growth rate tend to suggest that the genetic variability for this trait is greater than previously thought, at least for the species involved, and that because of this, there is excellent potential for future genetic gains. These findings are more in agreement with the estimates calculated in

this study, although they are still on the whole higher, especially those derived from the factoral mating scheme. The broodstock for this trial were taken from three different "strains" of brown trout, and thus the potential variation for production traits such as growth rate will be higher than if one took broodstock from a single strain as was done for the Howietoun and Loch Leven trials. It is therefore not surprising to find the heritability estimates are higher. It should be pointed out that very small numbers of broodstock were used. By the nature of the factoral design, originally 36 (6 x 6) different crosses were produced, and as 36 tanks was the total extent of the research facility it was impossible to use any more broodfish.

Another reason for the exceptionally high heritability estimates derived from the 'factoral design' trial is the problem of level of domestication in each separate strain of trout used. Domestication causes genetic changes in behaviour, morphology and physiology by eliminating genotypes which are unsuited to hatchery environments (Doyle, 1983). It was noted during this study that progeny derived from Loch Leven brown trout stock were much easier to scare in the 1 metre tanks, and it took longer for the fish to settle down and feed once the tanks had been cleaned, than it did for progeny derived from the Howietoun stock. This became even more obvious in 1985 in the factoral cross, when progeny derived from pure Leven x Leven stock were shown to be far more 'tank shy' than fish derived from either the Howietoun or Nashua stocks, both with long histories of domestication. This 'tank shyness' will obviously

effect the growth rates adversely and increase variability for the trait thus increasing the heritability estimate for it.

3.4.2. Trends in heritability estimates as the fish grow

In contrast to the findings of Gjedrem (1983) the heritability estimates for weight and length in this study, tended to decrease as the age of the fish increased (see Tables 3.33 and 3.43) but in agreement with Iwamoto et al., (1982) and McKay et al., (1986) who also recorded a decrease in the heritability estimate for growth as the fish grew older.

Bailey and Loudenslager (1986) recorded large values for growth rate heritability (using weight) at 12 weeks old in Atlantic salmon ($h^2_s = 0.89 \pm 0.32$) which decreased at six months ($h^2_s = 0.40 \pm 0.26$) but increased again at 15 months ($h^2_s = 0.67 \pm 0.32$).

3.4.3. Explanation for high heritability estimates for growth rates

Where both sire and dam heritabilities are available for growth rate in Table 3.1 in the Introduction, there are 8 estimates for sire component which are higher than estimates based on dam components, 10 estimates are approximately equal, and 35 dam estimates that are higher than the corresponding sire component. From the summary of results, in this study, it can be seen that 8 estimates of sire heritability are higher than the corresponding dam component and 8 estimates of dam heritability are higher than the corresponding sire component.

It may be concluded that there is some non-additive genetic variance or maternal and common environment variance contributing to the growth rate in the fish populations studied (Gjedrem, 1983).

The dam estimates may be biased upwards by non-additive genetic variances, including common environment variance, maternal genetic effects and covariance between maternal genetic and additive genetic effects (Kirpichnikov, 1981; Gjerde, 1986).

The environmental effects in the present study are dealt with to a certain extent by the tank effect in the analysis of variance at least for the hierarchical design trials. This reduces the dam heritability and variance component because when calculating variance for the dam, one subtracts mean squares for the tanks effect from mean square for dams. This still leaves h^2_d surprisingly high.

Maternal effects in salmonids cannot be easily disregarded because of the large amount of yolk deposited by the female in each egg, which sustain the nutritional requirements of the embryo until well after hatching (Iwamoto et al., 1982). It is unclear as to how these effects persist in various species, although Iwamoto et al., (1982) indicate that maternal effects may be present up to 90 days post fertilisation, in coho salmon, studies with rainbow trout indicate maternal effects related to egg size are similarly important and may be long lasting (Gall, 1974; Kincaid, 1972). Chapter 5 is concerned with identifying correlated traits for growth and survival in this study, which include maternal effects.

Robison and Luempert III (1984) working with brook trout, indicate that non-additive genetic variance was of considerable importance for all developmental stages except fertilization, and high heritability for fingerling weight estimated from the dam component may be explained by maternal effects. Fingerlings were weighed 144 days post fertilization but only 35 days after first feeding, so that 76% of their life, they were dependant on the yolk exclusively for nutrients (Robison and Luempert III, 1984).

The conclusion from these workers is that the closer the estimate of heritability for growth rate is to fertilization or first feeding, the more likely maternal effects will be significant, boosting the heritability estimate for the dam component higher than it should be. Gjedrem (1983) therefore suggests that sire components of heritability estimates are usually more reliable.

Of the six heritabilities in this study measured at the first accurate weighing which represented in the first year 108 days after first feeding, in the second year 112 days after first feeding and in the third year 104 days after first feeding, four dam heritability estimates were larger than the corresponding sire heritability estimate, indicating a maternal effect was probably still present.

But as measurement (1) did not take place until the trout had been in the 1m tanks for over 100 days, maternal effects may well have become less. Further extensive work is required at an earlier age to elucidate the extent of maternal and non-additive effects.

The hierarchical complex, according to Kirpichnikov (1981) does not allow the variance resulting from the interaction of genotypes of sires and dams to be singled out. This variation is an integral part of the σ^2_s and σ^2_d terms. This causes decreased precision of heritability determination using the breeding schemes.

A more unusual aspect of the heritability estimates in this study is the high h^2_s values recorded which on 8 occasions were higher than the corresponding h^2_d estimate.

The reason why $h^2_s > h^2_d$ is that $\sigma^2_s > \sigma^2_d$, which means that CoV half-sib > CoV full-sib - CoV half-sib (Falconer, 1981) i.e. resemblance of half-sibs within sires is much greater than resemblance of full-sibs within dams. There are two explanations for this. The first is that there are for some reason larger genetic differences between sires than dams.

The second is that there is big variations within dams of a compensatory kind (perhaps due to competition for food). But the high variation within the dams is not reflected in a variation between dams within sires.

As only a very few broodstock have been used in the trials, it is possible by chance that a couple of pairs of females with similar characteristics (such as producing small eggs) have been each crossed with one male artificially accentuating the role of the male especially in influencing the growth of offspring at the beginning of each

trial. If this occurred, the heritabilities based on sire components will be artificially high. A better design would be to use far more sires and cross each with at least three females to minimize this effect. Kirpichnikov (1981), Gunnes and Gjedrem (1981) and Gjedrem (1983) all make reference to the problems of using too few sires in heritability experiments. Falconer (1981) regards the main cause of errors as being associated with the technical problems related to growing of a large number of different offspring. Kirpichnikov (1981) regards high growth rate heritabilities as an exception rather than the rule, and blames such estimates on "methodological inadequacy" which has led to a very high variance between "different batches". Kirpichnikov (1981) cites a number of experts in mathematical genetics who have pointed out that bias in heritability is unacceptably high. He cites authors (Nikora and Vasilyeva, 1976) who recommend that only regression coefficients, the parent-offspring correlation, and correlations between sibs and half-sibs within each class should be calculated.

The high estimates revealed in this study reflect so called methodological inadequacies, in so far as there was not enough space in the hatchery to conduct trials that were more representative and where more broodstock could have been used. This is a common fault in many trials undertaken (Gjedrem, 1983), but is an insurmountable problem considering the restrictions involved. Kirpichnikov (1981) observes that although many heritability estimates are inprecise for a number of reasons, they do "in many cases give an unequivocal picture of the level of genetic heterogeneity within a population,

at least with respect to the additive variation, used in mass selection of fishes."

The heritability estimates in this study are probably biased due to

1. Lack of broodstock numbers, especially sires.

2. Domestication of Howietoun and Nashua strains compared to the wild Leven strain.

3. Husbandry - especially preferential feeding of larger individuals once the fish were placed in the ponds.

4. Sampling errors.

The results still indicate a high level of genetic variation for growth rate in the stocks studied.

3.4.4. Differences in heritability from year to year

All the heritabilities for growth were found to be high, but varied from year to year. Bailey and Loudenslager's (1986) heritability results derived from work carried out with Atlantic salmon illustrates that the heritability estimates for the same traits can be markedly variable when they are determined in different years and in different populations, despite the efforts to standardise environmental conditions. Bailey and Loudenslager (1986) explain such differences as being due "in part to husbandry effects, levels of domestication,

differences among stocks and/or sampling".

Falconer (1981), Kirpichnikov (1981) and Gjedrem (1983) warn that it is not valid to compare heritabilities when the fish have been grown in different trials with different sets of environmental conditions. Even within the same research facility it is strictly not valid to compare the results of heritability trials. In this study the environmental conditions were kept as near identical as possible, but the water temperatures in the 3 successive years varied dramatically, altering feeding regimes, flow rates, and thus growth rates. See Figure 3.36, which graphically represents daily maximum water temperatures experienced during the three yearly trials. It can be seen that from the end of June onwards the water temperatures varied considerably. 1985 was an exceptionally cool, wet summer, unlike 1983, when the Stirling area experienced unusually warm, dry conditions. Therefore valid comparisons can be made between growth of trout in tanks in the same year but not between years.

3.4.5 Standard Errors

In common with many reported heritabilities (Gjedrem, 1983), the standard errors in this study are large. Falconer (1981) points out that the standard errors associated with heritabilities are normally large, being caused by the design of the experimental layout rather than any significant characteristic of the populations studied. Becker (1975) observes that standard errors are always

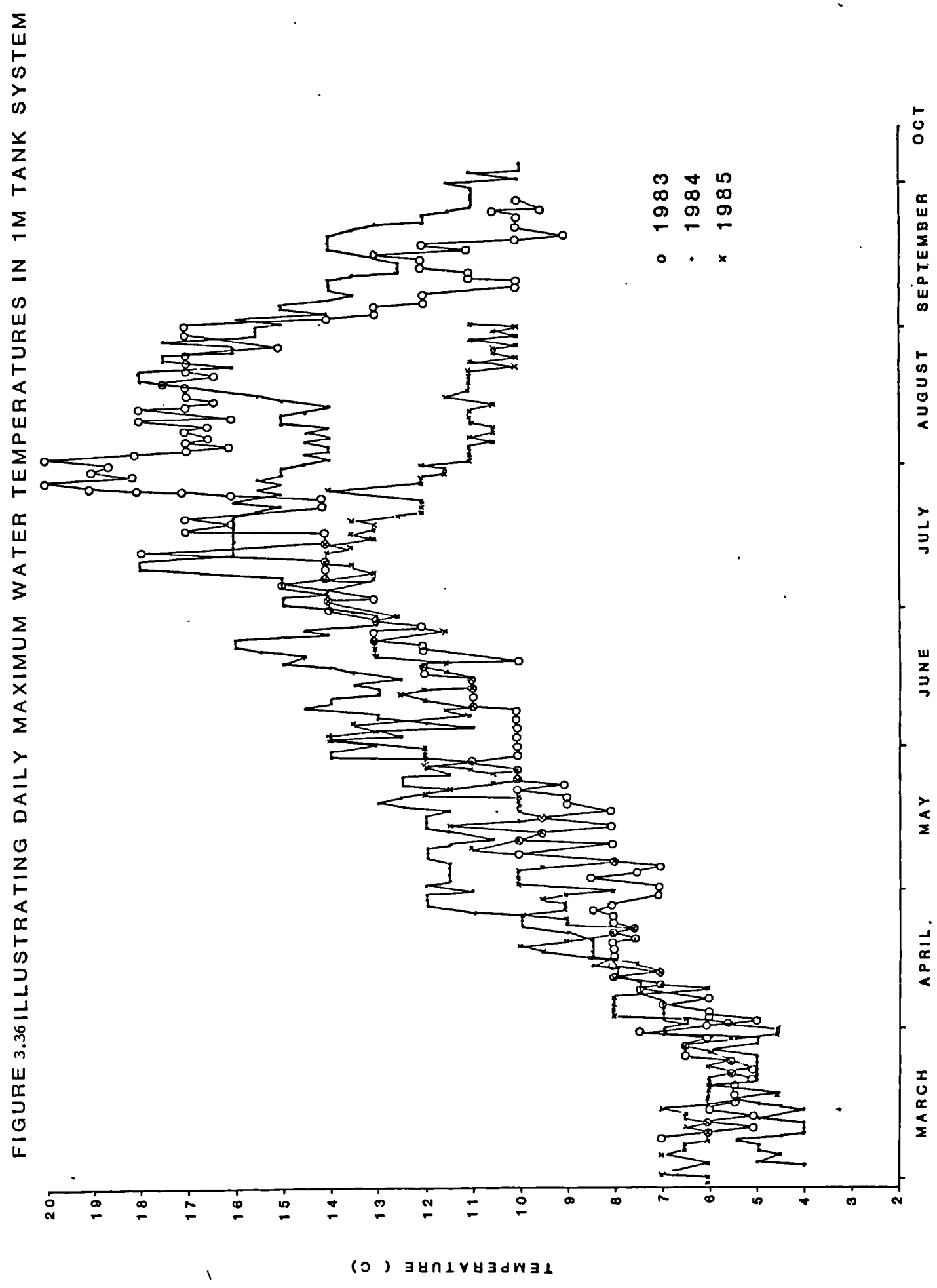


FIGURE 3.36 ILLUSTRATING DAILY MAXIMUM WATER TEMPERATURES IN 1M TANK SYSTEM

high when small numbers of sires and dams are employed in heritability trials. This agrees with recent observations made by authors involved in heritability estimations, who found very large standard errors when using small number of sires (El-Ibiary and Joyce, 1978; Klupp, 1979; Refstie, 1980; Busack and Gall, 1983). Gjedrem (1983) states that "heritability estimates based on less than five sires or five full-sib groups are considered to be of little value".

These comments are justified, but one has to accept vast problems with estimating quantitative genetic traits (Kirpichnikov, 1981) in fish strains or populations as already mentioned. The associated large standard errors are an inevitable consequence of heritability estimation trials conducted in the manner shown in the study (Hill, pers comm). One has to obtain as much information from the data collected, even though it may be limited.

3.4.6. Coefficient of Variation (CV)

The coefficient of variation enables one to compare the size of variances of different traits and different species. Gjedrem (1975) compared CVs from different traits with data from farm animals. The size of CVs for growth rate in cattle, sheep and pigs varied from 7 to 17%. In this study the CVs are very high for body weight for all stages studied. CVs range from 14.9% to 39.4%. This is in general agreement with the coefficients of variations calculated by Gjedrem (1983) using previously published data from different species. CVs for weight ranged from 22% in adult rainbow trout to 78% for juvenile Atlantic salmon. Gjedrem (1983) noted that

CVs for body weight tended to be higher for young fish compared to older fish. In this study, the exact opposite is true. In each trial CVs increased as the fish became larger and older. For the Howietoun trial, CVS for weight started at 14.9% and increased to 36.1%. For the Loch Leven trout trial, CVs for weight started at 20.2% and increased to 38.6% and for the factoral design trial the CVs for weight rose from 24% to 36%. The probable reason for the first two increases is that at the beginning of each trial the environment was actually under more precise control; stocking densities, feed rates and water conditions were all similar. Once the fish have grown to a certain extent they were transported from the 1 metre tanks and placed in earth ponds. Densities changed, and the effective amount of food available to the smaller fish, whether they were sibs, half-sibs or completely unrelated, dropped due to competition with the larger individuals. The size of the pellets fed to the trout in the ponds tended to discriminate against the smaller fish. The trout were fed according to the farm's commercial practice of feeding with pellets suitable for the larger individuals in the pond population. The food consisted of commerical rainbow trout or salmon pellets, a diet not necessarily correct for brown trout.

In 1985, the trial ended before fish were transported to the earth ponds so the increase in CV cannot be connected with the reasons given above. In 1985 the number of fish per tank was not standardised effectively when they were introduced to the tank system at the first feeding stage. In 1983 and 1984 the large numbers of eggs

originally laid down left scope for mortalities and enabled identical stocking densities to be introduced. But in 1985 because of reduced numbers of eggs per batch laid down under the factoral breeding scheme, and subsequent mortalities in some batches, stocking densities could not be standardised. This could account for the CV increasing from weight (1) to weight (2).

Another reason for large CVs is connected with the concept of natural hierarchies being set up in fish populations. Kirpichnikov (1981) cites examples, where if the largest fishes, known as shooters, are removed from a population, and intensive competition for food continues, other individuals rapidly occupy their place. He calls them "random winners of the food competition". These successful fish only exhibit minor genetic differences from other members of the community (Kirpichnikov, 1981). This situation both in the tanks and earth ponds probably led to an increase in CVs for weight, although feeding regimes were designed, in the tanks at least, to feed the trout in excess, which should have partially eliminated the problems of shooters.

Coefficient of variation for body length in fish is quite low according to Gjedrem (1983) who gives estimates of between 9% - 23% as average for rainbow trout and Atlantic salmon. The CVs for length in the three trials in this study ranged from 4.5% to 13.7%. But again they rose in each trial as the fish grew older (see Tables 3.33 and 3.43). The reasons for this are the same as for the increase in CV for weight.

There was evidence to suggest that shooters were present in some populations, not only by visually observing the fish in the tanks and when sorting the pond grown trout, but in the form of skewness estimations for each population which are given in the results section. Skewness is a measure of how near the weights and lengths of a given population of fish equates to a normal distribution. Most of the distributions equated well to the normal distribution but some tanks contained shooters exhibited by a tail to the right in the distribution pattern. Histograms for all the populations are available on request.

It is reported (Falconer, 1981) that heritability estimates are only valid when one is dealing with normally distributed populations. It was thought that the shooters evident in some populations might upset the heritability estimations. So Howietoun weight (2) and Loch Leven length (3) data sets were taken as examples. The five largest individuals (10%) of each tank recorded were removed from the analysis of variance. This made very little difference to the heritability estimates except to increase them very slightly. It was therefore felt no action needed to be taken, and the data collected could be analysed with no alterations to adjust for the shooters.

3.4.7. Examination of experimental methodology

At the beginning of the trials, it was decided to use 20% of the population in each tank, selected at random, as the sample from which lengths and weights would be estimated. It was felt that the means derived from such a sample would be accurate and take

Table 3.44 Heritability estimates for length calculated using different numbers of individuals

Parameter	X		CV	$h^2_s \pm$ SE	$h^2_D \pm$ SE	Number of individuals measured per tank and expressed as %
Howietoun						
Trial length (1)	5.23	0.23	4.5	1.24 ± 0.847	0.583 ± 0.381	50 fish 20%
	5.22	0.23	4.4	1.23 ± 0.828	0.614 ± 0.372	40 fish 16%
	5.22	0.23	4.5	1.142 ± 0.805	0.657 ± 0.406	30 fish 12%
	5.23	0.245	4.7	1.178 ± 0.790	0.460 ± 0.356	20 fish 8%
	5.21	0.235	4.5	1.678 ± 0.938	0.098 ± 0.215	10 fish 4%
Trial length (2)	10.40	1.01	9.7	1.04 ± 0.690	0.553 ± 0.287	50 fish 20%
	10.37	1.02	9.8	1.06 ± 0.699	0.543 ± 0.289	40 fish 16%
	10.35	1.04	10.0	1.072 ± 0.686	0.464 ± 0.264	30 fish 12%
	10.31	0.986	9.6	1.23 ± 0.793	0.572 ± 0.311	20 fish 8%
	10.19	0.923	9.1	1.52 ± 0.934	0.455 ± 0.313	10 fish 4%
Trial length (3)	16.00	2.20	13.7	0.746 ± 0.416	0.058 ± 0.012	30 fish 12%
	16.01	2.19	13.7	0.955 ± 0.523	0.040 ± 0.096	20 fish 8%
	16.23	2.14	13.2	1.01 ± 0.905	0.133 ± 0.204	10 fish 4%

Table 3.45 Heritability estimates for weight calculated using different numbers of individuals.

Parameter	X		CV	$h^2_s \pm$ SE	$h^2D \pm$ SE	Number of Individuals measured per tank and expressed as %
Howietoun						
Trial weight (1)	1.67	0.25	14.9	0.411 ± 0.700	1.052 ± 0.758	50 fish 20%
	1.66	0.25	14.8	0.380 ± 0.700	1.020 ± 0.775	40 fish 16%
	1.66	0.24	14.7	0.261 ± 0.695	1.126 ± 0.843	30 fish 12%
	1.67	0.25	14.8	0.305 ± 0.697	1.037 ± 0.824	20 fish 8%
	1.65	0.23	14.1o	0.713 ± 0.759	0.586 ± 0.517	10 fish 4%
Trial weight (2)	14.40	4.34	30.2	0.907 ± 0.661	0.668 ± 0.347	50 fish 20%
	14.21	4.37	30.8	0.907 ± 0.659	0.639 ± 0.345	40 fish 16%
	14.13	4.41	31.2	0.973 ± 0.661	0.512 ± 0.300	30 fish 12%
	13.95	4.24	30.4	1.051 ± 0.732	0.644 ± 0.353	20 fish 8%
	13.48	4.06	30.1	1.32 ± 0.860	0.535 ± 0.351	10 fish 4%
Trial weight (3)	50.50	18.23	36.1	0.864 ± 0.349	0.080 ± 0.119	30 fish 12%
	49.94	18.06	36.2	0.692 ± 0.416	0.060 ± 0.141	20 fish 8%
	51.00	17.77	34.2	0.621 ± 0.415	0.020 ± 0.212	10 fish 4%

into account the 'shooters' if they existed. Once the populations in years 1 and 2 had been reduced to 250 fish per tank, this meant 50 trout per tank were weighed and lengthed accurately for heritability estimation. In year three, 30 fish were taken from tank populations of approximately 200 individuals (15%).

On reflection, the time to weigh and length fish, seems to have been excessive. The coefficient of variation, standard deviation, means and estimates of heritability do not change greatly as one reduces the number of fish one uses from each tank. Tables 3.44 and 3.45, illustrate the Howietoun trial heritabilities and associated statistics, giving a range of numbers of fish measured per tank, and used in the analysis of variance. The heritabilities start to differ once one reduces to 20 or 30 fish per tank, representing 8 to 12% of each population. It is therefore recommended in future work that experimental calculations of heritability estimates be made to ascertain the appropriate number of individuals that should be measured for each population, that will give one a valid result without excessive time measuring individuals. Thirty fish per population is recommended as a minimum number for such work carried out in future dealing with similar population sizes.

3.4.8. Potential Genetic Gain

Taking all the drawbacks and criticisms of heritability estimation into consideration, there seems no point in conducting such trials without coming to a conclusion about possible genetic gains that

could accrue from future selection policies.

Selection experiments with real-life populations were initially perceived as ways of proving theoretical population genetics. But their importance to the science of quantitative genetics has derived in far greater measure from their failure than their successes. Fredeen (1986) states, "By providing insight into the limitations of theory, the failures have encouraged biometricians to seek ways to improve both the specifics and the generalities of the theoretical framework and to sharpen the tools for statistical analyses of data".

If selection for growth rate in the Howietoun fish farm trout populations was by truncation and growth rate was the only trait being selected for, according to the high heritabilities calculated, genetic gains would vary between 3.6% and 33%. Refer to Table 3.46.

The genetic gains were calculated using the formula given by Falconer (1981). The selection differential was taken to be 2.66 (Gjedrem, 1975) because the Howietoun turnover of brown trout is between 40 and 50,000 per year, of which approximately 400 are selected as broodstock (1% of the population). The standard deviation used were those found while estimating heritabilities. It was assumed that the generation time for brown trout was three years.

Table 3.46 Genetic gain ($G = \frac{ih^2\sigma p}{L}$)

PARAMETER		GAIN PER YEAR	% GAIN PER YEAR
Howietoun	wt (1)	0.16 gms	9.6
Trial	wt (2)	3.04 gms	21.1
	wt (3)	2.60 gms	5.1
	len (1)	0.19 cm	3.6
	len (2)	0.72 cm	6.9
	len (3)	2.34 cm	14.6
Leven	wt (1)	0.22 gms	14.6
Trial	wt (2)	2.56 gms	22.4
	wt (3)	8.47 gms	18.5
	len (1)	0.69 cm	13.3
	len (2)	0.71 cm	7.1
	len (3)	1.02 cm	6.4
Factoral	wt (1)	0.46 gms	33.1
Trial	wt (2)	3.11 gms	31.4
	len (1)	0.55 cm	10.9
	len (2)	0.85 cm	9.1

The heritability estimates are regarded as large and probably biased heavily upwards, but there still appears to be plenty of scope to select brown trout at Howietoun for fast growth rate. If the only trait of interest is growth rate, then mass selection would be advised. But the farm uses other traits, such as spot pattern and body shape (Semple, pers. comm.) when selecting broodstock. If this policy is continued then family selection would yield better results than straight forward individual selection.

Some of the theoretical genetic gains calculated from the available heritability estimates are large compared to gains recorded by other authors. Refer to Table 1 in Gjerde's (1986) paper where he gives a list of genetic gains recorded in fish ranging from 1.7% to 11.5% gain per year. He also mentions that these estimates are 5 to 10 times those found for farm animals. Although no genetic gains were calculated, the authors of recent papers giving high heritability estimates for growth rate in juvenile salmonids (Iwamoto et al., 1982; Robison and Luempert III, 1984; Bailey and Loudenslager, 1986) all conclude by speculating that selection for the traits studied, would yield substantial genetic gains in the future.

Weight would seem to respond more to selection than length in the present study. The highest percentage gain per year calculated for length was 14.6% in the Howietoun stock trial. The highest percentage gain per year for weight was at the first accurate weight in the factoral trial with 33.1%. This reflects the great variation

in size of the fish due to using three different stocks of trout in the experiment.

The highest levels of genetic gain in the other two trials were both at the second accurate measurement, while the trout were still in the 1 metre tanks in the autumn of their first year. The Howietoun trial yielded a 21.7% gain per year, while the Leven trout trial yielded a 22.4% gain per year. This is about double the largest gain per year recorded by Gjerde (1986) but these estimates are based on heritabilities close to 1.0 which are very probably over-estimates.

Freeden (1986) warns that characteristics deemed to be important for economic or other reasons, in a population may not be deemed important in the future. "Since 'economic' merit is a composite of many different productivity traits, and since perceptions of the relative importance of these component traits will differ among breeders and will be subject to change over time, the definition of total genetic merit, for any domestic species will be both variable and dynamic."

CHAPTER 4

Chapter 4 Electrophoretic Analysis of Wild Scottish Brown Trout

4.1 Introduction

There are three basic reasons for the long term conservation of our genetic resources, according to Smith and Chesser (1981). Firstly, diversity or variability is aesthetically pleasing to the human eye in most environments. Secondly, there is often local pride in populations or species that are characteristic of an area, and people often become disturbed when a local form of an animal is threatened by extinction. Finally, and biologically most importantly, it is generally agreed by ecologists, geneticists and evolutionary biologists that species diversity and genetic variability are necessary for the long term maintenance of stable, complex ecosystems and species themselves. Maintaining genetic variability is important because of its potential use under a variety of different environmental conditions that exist at present or may exist in the future. The conservation of genetic variability in general has been dealt with very widely (Lewontin, 1974; Powell, 1975; Nevo, 1978; Altukhov, 1981).

Genetic variation between populations of a species permits adaptation to various environments of a wide geographical range, whereas variation amongst individuals within a single population, provides for functional diversity within a similar environment (Allendorf and Phelps, 1981b; Smith and Chesser, 1981; Falconer, 1981).

An efficient use of the amount and distribution of genetic variability within the species considered including salmonids (Allendorf and

Utter, 1975; Hedgecock et al., 1976; Ihssen et al., 1981; Altukhov, 1981; Allendorf and Phelps, 1981a; Ryman and Stahl, 1981; Gjedrem, 1981; Guyomard et al., 1984), is vital.

4.1.2 Variation within salmonids with emphasis on brown trout

It has been intuitively recognised for a long time that salmonids seem to be divided into distinct subspecies, strains or morphs. The differences between these being characterised by variety in general appearance, morphology, aspects of their ecology and behaviour. In 1866 Günther said of the salmo genus "we know of no other group of fishes which offer so many difficulties to the ichthyologists with regard to the distribution of the species". Günther went on to describe ten different species of trout from the British Isles alone. The Reverend Haughton (1879) illustrated Günther's species and allocated specific morphological, ecological and behavioural characteristics to each fish, giving details of previously noted recordings of such fish.

Since Victorian times the brown trout has become known as a single variable species. Regan (1911) grouped or lumped all Günther's "species" together along with continental trout varieties. Trewavas (1953) and Frost and Brown (1967) agreed with his conclusions, that the brown trout was just a very variable species. Whether the variations within the brown trout necessitate re-arrangement of taxonomic nomenclature is at this point academic, the important fact is that great variation does exist.

Ferguson and Mason (1981) while discussing the morphological evidence of the existence of three separate types of trout in Lough Melvin, Northern Ireland state, "in spite of much discussion on the subject the basic question still remains, are these types (of trout) simply ecophenotypes of the same stock or do they represent reproductively isolated and genetically distinct forms of the brown trout". This question can be extrapolated to every loch, or river containing apparently dissimilar types of trout. Do the differences represent differences in the trout's genome? The present electrophoretic investigation of Scottish brown trout was designed to answer this question.

4.1.3 Resource identification

It is now commonly recognised at least by geneticists if not by all fisheries biologists that a pre-requisite for any comprehensive management programme either of wild or hatchery stocks of salmonids, is the identification of the available resource (Ryman, 1981; Allendorf and Phelps, 1981**a**,**b**) Genetic diveristy, ultimately determines the characteristics of the resource and its relative magnitude can be assessed by detailed phenotypic measurements that include morphological, karyotypic, and electrophoretic variation (Smith and Chesser, 1981). Morphological differences, such as size, pigmentation, and skeletal dimensions have long been used in studies of geographic variation and taxonomic investigations (Sneath and Sokal, 1973). Many phenotypic traits however are polygenically inherited and have low heritabilities and are primarily determined by environmental conditions, and do not represent genetic differences (Falconer, 1981). Work carried

out in 1952 by Taning using an anadromous form of Danish brown trout, showed that by using cold and heat shocks on young stages produced individuals with the same number of vertebrae as found in natural stocks of brown trout from Scandinavia to the Mediterranean.

Fisheries biologists have long been interested in differences between salmonid populations. Showing the genetic basis of such differences however has presented problems. The characteristics of most interest, varying between populations, have been growth rate, colouration, age at maturity and various morphometric and meristic counts. All of these will be affected by environment and intuitively most are thought to be under polygenic control (Allendorf and Phelps, 1981).

Dahl (1918) recognised the possibility that trout grown in waters different from the ones they were spawned in retained characteristics of their parents, but it wasn't until the work of Alm (1949, 1959) in Sweden, that hereditary traits were shown to pass from one generation of brown trout to the next. In his classic experiments using trout from a river and a lake, Alm showed genetic influence was present on growth rate, age of maturity and fin coloration, by keeping the two different stocks separate under hatchery conditions for three generations.

Since then other workers have used morphological and meristic characteristics only, to answer the question 'are there any genetic differences' and Ryman (1983) cites some (Richter, 1972; Saunders, 1981; Thorpe and Mitchell, 1981) the majority of whose evidence for the existence

of genetic differentiation he claims is circumstancial.

Allendorf and Phelps (1981b) highlight the problem of describing genetic relationships among the populations within a species. "Say we have three stocks, two fast-growing and one slow-growing. Can we infer that the two fast growing stocks are relatively genetically similar to each other? No. Growth rate is a polygenic character determined by many loci. Many different combinations of alleles of individual loci, may yield the same phenotype (e.g. fast growth rate)."

Methods of unequivocally demonstrating genetic variation by estimating allelic frequencies at many individual genetic loci are required (Allendorf and Phelps, 1981b; Ferguson, 1980).

Gel electrophoresis of enzymes provides one such method. The technique of gel electrophoresis used by Hubby (1966) and Lewontin and Hubby (1966) has given geneticists and population biologists a tool to measure the extent of molecular variation in natural populations of both animals and plants.

Lewontin (1974) extols the advantages of electrophoretic detection of genetic variation. The major advantages of electrophoresis being the direct relationship between protein variants and allelic differences at individual genetic loci (Lewontin, 1974; Dobzansky et al., 1977; Nei, 1977; Allendorf and Phelps, 1981b)

4.1.4 The rationale behind electrophoresis

Electrophoresis is based on the principle that enzymes differing in net charge and molecular weight will travel through a gel matrix with a current applied across it at different speeds and when separated will form bands which can be visualized using appropriate histochemical stains (Ferguson, 1980; Thorpe, 1982).

Enzymes are made up of polypeptide chains (i.e. proteins) which in turn are made up of a sequence of amino acids. The amino acids are coded for by the sequence of DNA nucleotides comprising the structural gene. Many changes in the sequence of bases on the DNA are reflected by changes in the amino acid sequence. Different amino acids have different charges associated with them, therefore many of the changes in amino acid sequence will affect the mobility of the enzyme created in the electric field. The products of individual loci can thus be identified in a mixture of proteins derived from various tissues within an individual specimen by histochemically staining specifically for the enzyme under consideration, thus visualising polymorphism.

Electrophoresis does have its limitations, as each amino acid substitution does not necessarily change the charge of the enzyme one is studying. Maruyama and Kimura (1978) estimated that only about 25% of all mutations were detectable by electrophoresis. This estimate agreeing with others (King and Wilson, 1975; Lewontin, 1974).

A more serious problem with electrophoresis when using it as a tool in systematic investigations highlighted by Thorpe (1982) is that substitution rates may vary between loci. Sarich (1977) has proposed that protein loci can be split into 'fast' and 'slow' groups, which differ in substitution rates by about an order of magnitude. If this is so, calculations of genetic distance may be largely affected by the proportion of the fast and slow loci used to generate the D value (Sarich, 1977).

Unfortunately there are also cases where non-genetic variation can complicate the interpretation of electrophoretic evidence and non-genetic variation must be eliminated prior to concluding that a particular variant has a simple genetic basis (Allendorf and Phelps, 1981a). Non-genetic variation may result from:

1. Developmental changes in gene expression (Shaklee, et al., 1974).

2. Changes reflecting environmental differences, such as temperature, salinity or disease (Amend and Smith, 1974).

3. Changes caused by dissection or extraction procedures (Allendorf and Phelps, 1981a).

4. Changes resulting from conditions or length of storage (Allendorf and Phelps, 1981a).

4.1.5 The salmonid tetraploid event

The potential non-genetic variation highlighted above must be specifically ruled out when analysing zymograms of salmonids because of an ancient tetraploid event (Ohno, 1970) that resulted in many additional loci for most enzymes that have been studied (Allendorf and Utter, 1975; 1976; Allendorf et al., 1975; Engel, et al., 1975; May et al., 1979; May et al., 1980; Taggart et al., 1981; Ryman, 1983).

Difficulties in interpretation arise when polypeptides coded by different loci form active enzymes with identical electrophoretic mobilities (Allendorf and Phelps, 1981b).

Inheritance studies are required to demonstrate the mode of inheritance (disomic or tetrasomic) for such duplicate systems. In addition gene frequencies cannot be accurately estimated for the individual loci involved in such duplicated sytems. Polymorphism for some duplicated loci result in variation that can be ascribed to a particular locus but in which the heterozygotes cannot be positively identified, so that the electrophoretic variation must be treated as a simple recessive trait. Taggart and Ferguson (1984) working on the previously reported polymorphisms identified in brown trout (Taggart et al., 1981) confirmed the genetic basis for the variation and the inheritance mode to be disomic. This is in agreement with previous inheritance trials carried out with other salmonids (Allendorf and Utter, 1973; Allendorf et al., 1975; May et al., 1975; May et al., 1979; Stoneking et al., 1979).

Taking these disadvantages into account, the level of genetic polymorphism at the protein level found in many natural populations in the last two decades is very high and sheds much light on many aspects of theoretical and practical biological problems.

4.1.6 Theory of evolution in the light of electrophoretically detectable variation

Kimura (1968a) and King and Jukes (1969) encouraged by the obvious amount of polymorphism at the protein level that had been uncovered and attributed to a genetic basis, formulated the neutral theory, in which evolution occurs mainly by random fixation of neutral or nearly neutral mutations. This was an extension of the classical theory maintained by Muller (1950) who proposed that natural selection plays a less important role than mutation and its chief role is to preserve useful mutations and eliminate unfit genotypes (purifying selection); the creative role is given to mutation (Nei, 1983).

In sharp contrast (see main Introduction) is the Balance Theory of Evolution (Dobzansky, 1955, 1970) who gives the natural selection a creative role in evolution and where mutation is not discounted but is of minor importance.

With the advent of electrophoretic techniques a large amount of evidence has accumulated on both polymorphism and long-term evolution at the molecular-level, which has given new insight into the mechanism of evolution.

Nei (1983) argues that mutation plays a much more important role in evolution than many evolutionists believe. He pays special attention to the consistent explanation of polymorphism and long-term evolution. Nei (1983) cites Kimura and Ohta (1971) who observe that currently detected polymorphisms are merely a "snapshot picture of long-term evolution" and any theory purporting to explain current day polymorphism must also explain long-term evolution as well.

Nei (1983) postulates an extension to the neo-classical theory of Morgan and Muller. In Nei's view the new form of neo-classicism can be characterised as follows:

1. At the nucleotide level many mutations are deleterious but a substantial proportion of them are neutral or nearly neutral. Only a small proportion of mutations are advantageous, and that is sufficient for adaptive evolution.

2. Natural selection is primarily a process to save beneficial mutations or eliminate unfit genotypes.

3. New mutations spread through the population either by selection or by genetic drift but a large proportion of them are eliminated by chance.

4. Populations do not necessarily have the genetic variability needed for new adaptation, though the variability of the molecular level is usually very large. When there is not enough genetic

variability needed, the population stays unchanged until new mutations occur or the population becomes extinct.

It is often said that genetic polymorphism is beneficial to the population, because in the presence of genetic variability the population can adapt easily to new environments (Dobzansky, 1970). Thus any mechanism that increases genetic variability is advantageous and is selected for. Nei (1983) disagrees with this, and suggests "genetic variability of a population at present is simply a product of evolution in the past". The variability present in a particular population may be useful in future generations but it may be completely irrelevant, leading to the suggestion that genetic variability is not "stored for future use".

Natural selection in Nei's opinion (1983) is a consequence of the existence of two or more functionally different genotypes in the same environment, and the functional efficiency of a genotype is determined by the genes possessed by the inidvidual. Therefore the most important process of adaptive evolution for those adhering to the neo-classical theory of evolution, is the creation of better (functionally more efficient) genotypes by mutation (including nucleotide substituion and gene duplication) in a particular environment.

Ferguson (1980) notes that during electrophoretic screening of many species over the past 20 years, many loci have been shown to be polymorphic and individuals within a population may be heterozygous at a substantial proportion of its loci. Ferguson (1980) remarks

that this is in agreement with the balancing model of evolution, but he also notes that "the maintenance of the high degree of polymorphism by various forms of balancing selection, however, has not been proven".

Nei (1983) reviewing the evidence accumulated for polymorphism either at protein level or DNA level concludes that

1. The extent of protein polymorphism is nearly equal to or lower than the level expected under the equilibrium theory of neutral mutations. The differences between the observed and expected levels he explains by the bottleneck effect or by diversity reducing selection.

2. The patterns of distribution of allele frequencies, single locus heterozygosity, genetic distance, and so forth are in rough agreement with the expectations from the neutral theory but are not consistent with those from several methods of balancing selection.

3. Functionally important parts of genes are generally less polymorphic than unimportant parts and evolve more slowly.

4. A large amount of genetic variation may be generated by mutation alone. Nei (1983) gives examples of immunoglobulins and the influenza haemagglutinin.

Not only has electrophoresis lead to arguments concerning the role of mutation and selection in evolution but it has allowed substantial evidence to build up which has helped work on systematics.

4.1.7 The molecular clock

In its simplest form, the molecular clock hypothesis predicts that amino acid substitutions in protein molecules is an approximately regular but random process, and that consequently the number of substitutions occurring between homologous proteins may be related to evolutionary time (Thorpe, 1982). Although the suggestion of random substitution is a consequence of the concept of selective neutrality, the existence of a molecular clock does not depend upon the validity of the neutral hypothesis (Thorpe, 1982).

The molecular clock theory seems to be under pressure when there appears evidence of extremely rapid speciation which does not fit in with the idea of a uniform rate of molecular evolution. Thorpe (1982) calls the mechanisms suggested for speciation and to accommodate the molecular clock concept "highly speculative". In these mechanisms it is proposed that the availability of unexploited niches has resulted in strong selective pressure for speciation and morphological adaptation. These have therefore occurred with great rapidity while the short time has permitted little biochemical evolution.

The level of genetic differentiation between two species or populations over a range of enzyme loci detectable by electrophoresis, may be reduced to a single figure using one of several measures of genetic

similarity or genetic identity, (measures of similarity) or of genetic distance (measures of dissimilarity) (Kimura and Ohta, 1971; Nei, 1975).

Thorpe (1982) reviews the use of such measures and concludes that those of Rogers and Nei are the only ones extensively used. Nei's genetic distance, D, is claimed to estimate the number of substitutions per locus and to be linearly porportional to evolutionary time, assuming the concept of the molecular clock to be correct. Nei's measure is the only measure for which methods for the estimation of sampling and other errors are available.

For taxonomic use it is desirable to be able to place standard deviations on figures for genetic distance (Thorpe, 1982), so that one can estimate whether different figures calculated are significant or not.

Most methods of calculating genetic distances and similarities are based on the assumption that populations are routinely outbreeding and in Hardy-Weinberg equilibrium, these problems seriously reduce the usefulness of Nei's genetic distance for systematic work (Thorpe, 1982).

The literature as Thorpe (1982) points out, concerning the use and construction of dendrograms by various methods from identity or similarity values, is growing rapidly. The large error values associated with the majority of I values (Nei, 1972) means that

for many studies of congeneric species, few, if any I values will differ significantly. Thorpe (1982) concludes that "frequently the data could not refute the hypothesis that all the species diverged simultaneously from one common ancestor. Nevertheless I or D values are often quoted to three significant figures and taxonomic arguments made using differences substantially smaller than the errors".

4.1.8 Species, strain or population identification

The need to conserve unique gene pools has recently been receiving more attention (Hedgecock et al., 1976; Ryman and Stahl, 1981; Smith and Chesser, 1981; Allendorf and Phelps, 1981a; Rasmuson 1981; Gjedrem, 1981; Altukhou, 1981; Ihssen et al, 1981; Ryman, 1983; Ferguson and Fleming, 1983; Guyomard et al., 1984).

The use of electrophoresis has proven a useful tool for delineation of population structure in different species of salmonids and results have shown that the population structure in many to be much more complex than had previously been acknowledged (Ryman, 1983).

Existence of genetically distinct populations have been documented and appropriate management techniques advised for many salmonid species. The following list is not comprehensive but gives an indication of the amount of work conducted recently, since electrophoresis became a widely used technique, in the field of salmonid population genetics.

Kornfield et al., (1981) and Child (1977, 1984) identified separate populations of Salvelinus alpinus, while Andersson et al., (1983) found 10 Swedish populations of the same species had the same amount of electrophoretically detectable genetic variation as other populations of Arctic char found in Ireland (Ferguson, 1981) and North America, but the populations in Sweden showed a high degree of similarity indicating they were derived from a relatively recent common ancestor.

Brown et al., (1981) and Dehring et al., (1981) identified discrete populations of Salvelinus namaycush and Allendorf and Utter (1976) and Gyllensten et al., (1985) worked with electrophoretic variation in Salmo clarkii.

Atlantic salmon, (Salmo salar) populations have been identified electrophoretically by Stahl (1981), Ryman and Stahl (1981), Stahl et al., (1983) and Heggberget et al., (1986) in Scandinavia, while Child et al., (1976), Payne and Cross (1977), Child (1980) and Cross and Ward (1980) conducted similar work in the British Isles. Different British stocks of salmon were found to be characterised by different transferrins (Child, et al., 1976) and Atlantic salmon from North America could be distinguished from European salmon by allele differences using liver AAT (Payne and Cross, 1977). Heggberget et al., (1986) report electrophoretic differences in stocks within the same river in Norway and correlate the differences to the differences in smoltification time of salmon from high up the river and from individuals growth in the river's lower stretches.

Wild rainbow trout (Salmo gairdneri) populations have been identified electrophoretically by Allendorf (1975), Allendorf and Utter (1979) and Allendorf and Phelps (1981b) in the Western United States.

Electrophoretic investigations with populations of sockeye salmon (Oncorhynchus nerka) and chum salmon (Oncorhynchus keta) have been conducted by Altukhov (1981) in various locations including Lake Azabash where spring and summer spawning chum salmon subpopulations were identified.

Whitefish have also been studied, using morphology and electrophoretic techniques. Ihssen (1981) investigated 5 allopatric stocks of Coregonus clupeaformis in the Great Lakes region in Canada and Coregonus pollan Thompson have been investigated electrophoretically in Ireland and compared with holarctic coregoninae from Alaska, Finland and Sweden (Ferguson et al., 1978). The Irish pollan C. pollan and the Alaskan C. autumnalis gave identical electrophoretic patterns for all proteins suggesting they were conspecific and separated only since the last glaciation, whereas C. peled, C. albula, and the 'C. lavaretus' complex gave unique patterns for a number of proteins.

Brown trout populations have been extensively studied in recent years, mainly in Ireland and Scandinavia (Allendorf et al., 1976; Allendorf et al., 1977; Ryman et al., 1979; May et al., 1979b; Ferguson, 1980; Ryman, 1981; 1983; Taggart et al., 1981; Ferguson and Mason,

1981; Jonsson, 1982; Ferguson and Fleming, 1983; Krieg and Guyomard, 1983; Guyomard and Krieg, 1983; Gyllensten, 1984).

These investigations have shown dramatic differences in population structuring in a number of locations. Sympatric populations of brown trout have been identified living within the same water bodies, and reproducing in isolation (Ferguson and Mason, 1981; Ferguson and Fleming, 1983; Ryman et al., 1979; Allendorf et al., 1976).

Detection of sympatrically reproducing populuations is not confined to brown trout. Child (1984) confirmed genetic isolation of two temperally distinct spawning populations of char (Salvelinus alpinus L.) in Windermere in Cumbria, Northern England, while Heggberget et al., (1986) produced evidence to support a theory that different stocks of Atlantic salmon were spawning in the same Norwegian river (Alta).

The obviously large amount of genetic variation in salmonid populations is perpetuated by the very strong behavioural trait to return by homing instinct to the individual's natal river, thus increasing the chance of stocks becoming isolated (Ferguson, 1980).

It is thus quite clear that there are considerable genetic differences within most salmonid species on a micro as well as a macro-geographical scale. Ryman (1983) points out that "there is a lack of quantitative estimates of the magnitude and the relative importance of these differences at various levels of organisation e.g. between rivers,

between lakes within drainages, between ecological or taxonomic forms, etc". This means that informed decision making by authorities or individuals responsible for both conservation of genetic resources and efficient use of existing genetic variation is virtually impossible. It is therefore regarded as necessary (Ryman, 1983) to identify levels of gene diversity for different species before further activities likely to damage populations take place.

4.1.9 Gene diversity analysis

The gene diversity analysis follows the logic of Nei (1972, 1975). Within each particular population subunit the gene diversity at a single locus is defined as

$h = 1 - \Sigma x^2_i$ where x_i denotes the frequency of the ith allele. The average gene diversity of a particular population (Hs) is the average of h over all loci.

The total gene diveristy is divided into two components

$Ht = Hs + D_{st}$

representing the average gene diversity within population (Hs) and the gene diversity due to differences between populations (DST). The D_{st} component can be further split into components like

$H_T = H_S + D_{si} + D_{ij} \ldots\ldots + D_{kt}$

where the different D_{ij} terms correspond to different levels in a hierarchal population structure. The relative importance of the various components is expressed by dividing each component by H_T and can be expressed in percentage terms by multiplying by 100.

Table 4.1 lists some calculated gene diversities for various salmonid species. Ryman (1983) cautions the use of such calculated diversities unless enough loci are used in the investigation. He illustrates that within each species there are considerable differences between the variability patterns of single loci, and as there are no particular loci that can be considered typical for the species, a large number of loci (including monomorphic ones) are necessary to provide an accurate picture of the average variability pattern (Lewontin, 1974; Nei, 1975).

There are striking differences betweeen the different salmonid species with regard to the distribution of gene diversity (Ryman, 1983; Gyllensten, 1985).

Ryman (1983) concludes that:

1. The rainbow trout constitutes the extreme with regard to the absolute amount of gene diveristy. In rainbow trout the average diversity within population is larger than the total gene diversity in any other species. In Table 4.1 the chum salmon and coho salmon both have larger absolute diversity figures listed but they were calculated using just polymorphic loci - so they are not strictly comparable.

2. Sockeye salmon and brown trout represent two extremes when considering relative distribution of genetic variation, with 95% and 65% of the total gene diversity found within populations respectively.

Table 4.1 Gene diversity for salmonid species

Species	Author	Absolute Gene Diversity			Relative Gene diversity (%)				
		Total (HT)	Within Pops. (HS)	Within Pops.	Level 1	Level 2	Level 3	Level 4	Level 5
Atlantic salmon (Salmo salar)	1	0.034	0.026	78.6	Between pops. within rivers	Between rivers within drainage	Between landlocked and andromous forms within drainage	Between drainage groups (Atlantic/Baltic)	
					2.8	6.1	0.2	12.3	
		SE 0.019	0.015	6.6	0.8	2.1	0.1	7.3	
Brown trout (Salmo trutta)	1	0.040	0.025	63.3	Between years in pops.	Between pops. in areas	Between areas within migratory form	Between migratory form within drainages	Between drainages
					0.03	13.4	15.3	0.48	7.5
		SE 0.016	0.010	4.2	0.01	2.7	2.0	0.22	1.7
Rainbow trout (Salmo gairdneri)	1	0.069	0.058	85.0	Between pops. within years	Between areas within forms	Between coastal and interior forms		
					5.3	2.4	7.3		
		SE 0.031	0.026	3.8	1.4	0.2	4.0		
Sockeye salmon (Oncorhyncus nerka)	1	0.046	0.044	94.2	Between years within pops.	Between localities within river	Between rivers		
					0.2	3.1	2.5		
		SE 0.026	0.025	0.9	0.1	0.2	0.8		
Chum salmon (Oncorhyncus keta)	2	0.213*	0.208	97.8	Between localities	Between groups			
					0.8	1.4			
		SE 0.053							
Coho salmon (Oncorhynchus kisutch)	2	0.108*	0.105	97.1	Between localities	Between groups			
		SE 0.045			0.8	2.1			
Arctic char (Salvelinus alpinus Sweden)	2	0.010	0.007	75.6	Between localities	Between groups			
		SE 0.008			19.9	4.5			
Arctic char (N. America)	2	0.049	0.022	46.7	Between localities	Between groups			
					53.3				
		SE 0.028							
Lake trout (Salvelinus namaycush)	2	0.027	0.026	94.5	Between localities	Between groups			
					5.5				
		SE 0.014							

* Only polymorphic loci scored. Authors (1) Ryman (1983) and refs therein; (2) Gyllensten (1985) and refs therein

Work extended by Gyllensten (1985) shows that other Oncorhynchus species namely coho and chum salmon also exhibit a very high percentage (>97%) of their gene diversity within populations. Gyllensten (1985) also quotes Arctic char genetic diversity measurements (see Table 4.1) and concludes that North American char populations sampled showed an even lower within population density percentage than Ryman (1983) found for brown trout.

3. Compared to humans (Ryman, 1983) salmonids show a remarkably high fraction of the total gene diversity between populations within a species. Ryman (1983) calculated that 90% of the total variation in humans was found within populations, while 10% was equally distributed between major racial groups and between populations within these groups.

4. Comparing the two most different Salmo species, i.e. the brown trout and the rainbow trout, it was noted that the smaller fraction of genetic differences observed between populations in the rainbow trout is not compensated by the larger total gene diversity found for this species (see Table 4.1). In absolute terms, the genetic differences among brown trout populations (0.015) are larger than the corresponding figure for rainbow trout populations (0.011).

4.1.10 Heterozygosity

Another way of characterising genetic diversity in populations of organisms is to calculate the heterozygosity, which is normally

expressed as the mean frequency of heterozygotes per locus ($\bar{H}_L$). Heterozygosity can also be expressed as the mean frequency of heterozygous loci per individual (H_I). The values of $\bar{H}_L$ and $\bar{H}_I$ are the same, but their standard errors are different (Ferguson, 1980). Table 4.2 gives a list of heterozygosities calculated for salmonids and also for comparison a range of other organisms, including invertebrates and man. Ferguson (1980) advises caution when comparing heterozygosities between species and suggests that the values should not be taken as definitive statements of the amount of variability, merely as indications. Problems arise when comparing different experiments and experimenters, who may use different electrophoretic techniques, buffer systems, and may well be testing different enzymes and different numbers of loci.

Allendorf and Utter (1979) highlight the question of number of loci used when calculating heterozygosities. They point out that Nei and Roychoudhury (1974) when outlining the statistical procedures appropriate for estimating the variance of heterozygosity measures, emphasised the importance of examining as many loci as possible.

Allendorf and Utter (1979) also highlight another serious problem when estimating heterozygosities, that being the type of loci used. Are the loci one uses in electrophoretic examinations representing the state of heterozygosity in the rest of the genome? However Allendorf and Utter (1979) conclude that it is a reasonable assumption that the amount of variation of isozyme loci reflect the relative

Table 4.2 Average heterozygosity in salmonids compared with other organisms.

Species	Common name	No. of Populations	H	Range of H	Author
Oncorhynchus					
O. gorbuscha	Pink salmon	6	0.039	0.032-0.047	1
O. keta	Chum salmon	5	0.045	0.043-0.048	1
O. kisutch	Coho salmon	10	0.015	0.000-0.025	1
O. nerka	Sockeye salmon	10	0.018	0.008-0.024	1
O. tshawytcha	Chinook salmon	10	0.035	0.024-0.052	1
Salvelinus					
S. alpinus	Arctic char	9	0.007	0.000-0.024	2
S. namaycush	Lake trout	3	0.015		3
Salmo					
S. apache	Apache trout	1	0.000		1
S. clarkii	Cutthroat trout				
	(Coastal form)	6	0.063	0.022-0.077	1
	(Interior form)	2	0.023	0.021-0.025	1
S. gairdneri	Rainbow trout	41	0.060	0.020-0.098	1
S. gairdneri	Rainbow trout		0.059±0.013		4
S. salar	Atlantic salmon	2	0.024	0.020-0.028	1
S. salar	Atlantic salmon	18	0.025	0.015-0.035	5
S. salar	Atlantic salmon	6	0.028	0.018-0.029	6
S. trutta	Brown trout	38	0.025	0.000-0.053	7
S. trutta	Brown trout	116	0.038	0.000-0.062	8
Invertebrates	47 species		0.06-0.31		9
Fish	18 species		0.03-0.12		9
Amphibians	16 species		0.02-0.14		9
Reptiles	9 species		0.05		9
Birds	7 species		0.04-0.17		9
Mammals	29 species		0.01-0.09		9
Homo sapiens	Man		0.07		9
Fish	51 species		0.051		10
Lewontin's average for man, mouse, drosophila and horse-shoe crabs			0.061-0.184		11

1 Allendorf and Utter (1979)
2 Andersson et al. (1983)
3 Dehring et al. (1981)
4 Allendorf and Phelps (1981)
5 Stahl (1983)
6 Ryman and Stahl (1981)
7 Ryman (1983)
8 Ferguson and Fleming (1983)
9 Ferguson (1980) and refs therein
10 Nevo (1978) and refs therein
11 Lewontin (1974) and refs therein

amounts of genetic variation found at other loci in the genome, because the processes affecting the amount of genetic variation act uniformly on the genome. Allendorf and **Utter** (1979) also advise not restricting examination of variation to one major set of isozyme loci and, "if one's goal is to estimate heterozygosity in a population using isozyme data, one must strive to examine a large number and wide range of isozyme loci."

Heterozygosity estimates can be compared more reliably between populations of the same species, in contrast to comparing heterozygosity estimates of different species. The inclusion of an extra loci in the calculation with the same species tends to make little difference even if it is polymorphic because it is likely to be polymorphic for all or at least some of the populations examined, whereas a loci that is polymorphic for one species is not necessarily polymorphic for another, especially if it is distantly related (Allendorf and **Utter** , 1979).

4.1.11 Linkage disequilibrium

The theory of linkage disequilibrium was established at about the same time that electrophoresis was introduced into population genetics. The theory predicts that non-additive fitness interactions between different loci would result in an excess of certain gametic types and in a deficit of others, provided the loci are linked (Wright, et al., 1980).

Models analysing linkage disequilibrium in a two locus situation

have been developed (Lewontin and Kojima, 1960).

The existence or non-existence of linkage disequilibrium is important because different theories associated with the maintenance of electrophoretic variation in natural populations predict different linkage results.

The neutralist or classical or neo-classical protagonists suggest that electrophoretic variation in natural populations is isoallelic in respect to fitness, and is maintained by a balance between mutation producing new variation and loss by random processes. Protagonists of the selectionist school of thought, suggest that molecular variation is maintained by deterministic processes such as balancing selection of frequency dependant selection.

These two different views for the maintenance of polymorphic variation predict different levels of linkage disequilibrium. Selectionists predict strong linkage with disequilibrium between closely linked loci, while the neutralist/mutationalists predict at best, weak linkage due to random genetic drift. Therefore if proof of linkage disequilibrium could be found this would greatly strengthen the model predicted by Franklin and Lewontin (1970) and the selectionist theory in general. For Salmonidae the phenomenon of linkage and its interpretation is complicated by their tetraploid origin (Ohno, 1970). For a review on linkage associations studied in salmonidae see Wright et al., (1983). Wright et al., (1983) also identify what is known as pseudo linkage, which is the phenomenon in which

non parental progeny types significantly exceed parental types. It has never been observed for similar crosses involving doubly heterozygous females, and is often characterised by progeny of back crosses involving males heterozygous for certain loci (Taggart and Ferguson, 1984). This phenomenon is more prevalent in genomes from two diverse sources (e.g. inter-specific hybrids) and always involves duplicate loci. Pseudo linkage has been detected for both Salmo and Salvelinus (May et al., 1980; Wright et al., 1980). Taggart and Ferguson (1984) state that "these forms of aberrant segregation are considered to reflect a degree of "residual tetrasomy" (May et al., 1979b) within the salmonid genome.

Linkage involving brown trout has been reported to be restricted to the following loci; $AAT_{1,2}$ with MDH_2 (Taggart and Ferguson, 1984).

$G3P_{-1}$ (same as $G3PDH_2$) with MDH_1 (unpublished - reference Wright et al., (1983).

IDH-3 with ME_{-2} (unpublished - reference Wright et al., 1983).

DIA with CK_{-2} (unpublished - reference Wright et al., 1983).

Taggart and Ferguson (1984) also suggest the following show non random association. $AAT_{-1,2}$ with CK_{-2}, $MDH_{-3,4}$ with PGI_{-2} and DIA with PGI_{-2}. All other pairwise examinations of brown trout loci were found to be in random association.

The AAT_1, AAT_2 - MDH_2 linkage grouping has been extensively investigated for other salmonids (Allendorf and Utter, 1976; May et al., 1980; Wright et al., 1980). Wright et al. (1983) have developed a chromosomal model to state the occurrence of both classical linkage and pseudo linkage among $sAAT_{-(1,2)}$, $MDH_{-(1,2)}$ and $G3p_{-1}$ ($G3PDH_{-2}$) with cytological observations.

They propose an ancestral fusion of a non-homologous acrocentric chromosome with the $G3P_{-2}$ locus, to one of a pair of homologous acrocentrics which has a distantly situated sAAT locus and a proximately situated sMDH locus.

These studies were performed using hatchery electrophoretically identifiable individuals. Information on linkage concerning wild trout populations will be of limited use but it was planned to identify any aberrant associations of loci.

4.1.12 Applications of electrophoretic results on salmonid management

Apart from purely identifying strains of different species that can be used in future programmes of fisheries development, electrophoresis has other useful applications.

4.1.12.1 Hatchery stock assessment

Electrophoresis can be used to check on hatchery stocks to ensure that inbreeding is not taking place, thus reducing the genetic variability of the stock and causing problems in future generations. Cross and King (1983) recorded erosion of genetic variability,

as measured by mean heterozygosity and mean number of alleles over six previously polymorphic loci, in two Irish hatchery populations of Atlantic salmon. Cross and King (1983) argue that the observed genetic changes were caused by founder effects and genetic drift rather than selection by some aspects of the artificial rearing regime. The differences that Cross and King (1983) observed between the wild stocks from which the hatchery stock were derived, and the hatchery stock itself are as great as between natural populations from Irish rivers. The importance of using adequate numbers of parents in hatchery situations is thus evident. Stahl (1983) working with Swedish Atlantic salmon stocks recorded reduced electrophoretic variation within hatchery stocks which represented significantly lower amounts of genetic variability than displayed by natural populations. Stahl (1983) also found that hatchery stocks appear to be genetically more similar to one another than what is typical for natural populations.

Similar reductions in genetic variability have been found by Ryman and Stahl (1980, 1981) and Vuorinen (1984) working with Scandinavian hatchery brown trout stocks and by Allendorf and Phelps (1980) who were studying hatchery populations of cutthroat trout (Salmo clarkii).

In all cases the reduction in genetic variability is associated with the use of too few broodstock. The inbreeding coefficient (ΔF) expressed as $1/2Ne$, where Ne (effective number of parents) $= \frac{4 N♂ N♀}{N♂ + N♀}$ Ryman and Stahl (1980) increases most rapidly when

the sex ratio varies greatly from equality. In most fish farms this occurs when large numbers of females are fertilised with a small number of males. This model also assumes that there is no artificial selection proceeding in the population concerned. Allendorf and Utter (1979) refer to heterozygosity in the selected strain of rainbow trout kept at University of Washington, as measured electrophoretically to be three times lower than in wild populations. Allendorf and Phelps (1980) also mention that decrease in genetic variability at loci which confer disease resistance, may lead to an increase in susceptibility to disease, which will confound the effect of inbreeding depression (Kincaid, 1976).

It is interesting to note that not all hatchery stocks exhibit electrophoretically detectable reduced variability. Various authors working with different species have found higher levels of variability expressed as heterozygosities in hatchery stocks compared to the corresponding wild populations. Thompson (1985) observed higher levels of heterozygosity in rainbow trout strains than those reported for wild populations, as did Busack et al (1979). Guyomard and Krieg (1983) observed high levels of heterozygosity in hatchery brown trout stocks in France. Further, these hatchery strains seemed to be more heterozygous than populations in phylogenetically closely related species of salmonids. See Guyomard and Krieg's table 7 (1983).

Two explanations are offered by Guyomard and Krieg to explain the unexpectedly high values obtained.

1. The brown trout is a highly polymorphic species in some parts of its geographical range.

2. The hatchery strains are the result of mixing differentiated populations. Such a trend has been demonstrated in rainbow trout (Allendorf and Utter, 1979; Busack et al., 1979).

4.1.12.2 Introductions to the wild of hatchery stocks

Determining the effects of planting of hatchery fish on native salmonids of the same species is a major concern to fishery management biologists (Allendorf and Utter, 1979). Fish and salmonids in particular have been stocked or introduced into virgin waters and waters already inhabited by the same species of fish for over a hundred years, and only recently have workers become aware of dangers inherent in introducing "foreign genes" into discrete gene pools. Even today most hatchery and restocking establishments in Britain have little or no idea about the genetic constitution of either the fish to be stocked or the fish in the water body which are to receive the stocked fish. This is true of establishments who are just starting up in business and those highly respected who have been operating for many years. This is because, above all, the first consideration is a financial one.

Although native fish may be more adapted to a particular environment than hatchery fish there are three main potential dangers from which wild fish can suffer (Allendorf and Utter, 1979).

1. Competition for spawning and rearing grounds resulting from large hatchery releases.

2. Possible earlier hatching of progeny of hatchery fish resulting in a competitive advantage, and

3. Hybridization of native and hatchery fish resulting in disruption of adaptive gene pools.

Recent work has shown that fears about loss of wild stocks are justified. Fraser (1981) showed that when stocking with brook trout hybrid, wild x hatchery performed better than hatchery stock but both grew and survived worse than the wild populations already present.

Reisenbichler and McIntyre (1977) highlighted the problem in the United States, using electrophoretic markers to distinguish between hatchery x hatchery, hatchery x wild, and wild x wild individuals of a summer steelhead trout population. The markers involved Lactate deyhydrogenase genotypes and individuals were stocked into natural streams at the eyed egg or unfed swim-up fry stage. The wild x wild fish had the highest survival and the hatchery x wild fish had the highest growth rates when significant differences were found. It was postulated that when the hatchery fish interbred with the wild fish a lower number of smolts was produced.

Kruger and Menzel (1979) working with brook trout in Wisconsin using transferrin and Lactate dehydrogenase ($Ldh\text{-}B_2$) systems showed that interbreeding between wild and hatchery fish did not occur, rather

the decreasing wild type alleles were explained by alteration of selective pressures induced by ecological interactions between the two stocks.

Taggart and Ferguson (1986) investigating the stocking of brown trout into Lough Erne and the Macnean system in Northern Ireland, identified by Ferguson and Fleming (1983), as the most genetically distinct group among 116 British and Irish populations examined, concluded that the aforementioned loughs have suffered extensive introgression with the hatchery stock. Taggart and Ferguson (1986) regard the native trout of the system as a unique genetic resource and identify a clear threat from the current stocking of young fish and eggs to the inflowing streams.

Alternative stocking policies were suggested if stocking is required in future. These include stocking with 1^+ and 2^+ hatchery stock direct to the lough, which reduces the likelyhood of the resultant maturing adults from successfully spawning due to not being imprinted on a natural stream (O'Grady, 1984). Alternatively sterile brown trout are suggested as a stocking alternative to conserve the gene pool (Taggart and Ferguson 1986), but it is thought that this will not be cost effective.

4.1.12.3 Electrophoresis as an aid to development of genetic tags

Supplemental stocking programmes require careful and indepth evaluation, and with the already mentioned increased awareness of the complex genetic constitution of salmonid populations, the need to develop

more sophisticated monitoring techniques is vital. The identification of introduced fish still remains a major obstacle in the assessment of supplemental stocking programmes (Taggart and Ferguson, 1984).

The idea of a genetic allelic tag has many advantages over such methods as conventional tagging or panjetting. Taggart and Ferguson (1984) list 4 of them

1. They are permanent and stable and can be detected from the eyed ova stage onwards;

2. they do not affect the fitness or behaviour of the fish;

3. the fish require no special handling prior to release;

4. genetic markers can be passed on to subsequent generations in a predictable fashion, enabling the contribution of stocked fish to future generations to be monitored.

Allendorf and Utter (1979) point out two potential pitfalls that must be kept in mind when producing a population of individuals with a distinct allelic marker. The first one conflicts with point (2) above.

1. The variant form of enzyme chosen could have a selective disadvantage contrasted to the common form of the enzyme, and thus

conclusions drawn from the selected stock pertaining to the parent stock would be biased. Controlled tests are advised (Allendorf and Utter, 1979) to make sure the allele chosen will not infer a disadvantage on the hatchery stock.

2. The other problem involves potential inbreeding when setting up one's allelically marked hatchery stock. Allendorf and Utter recommend at least six males should be used in the first generation assuming 100 females are used. In subsequent generations 50 or more of each sex should be used to reduce the chances of inbreeding.

Both of these potential sources of genetic weakness must be anticipated before genetically tagged populations are set up.

Utter et al. (1976) and Allendorf and Utter (1979) used AGPD A^1 allele present in the Washougal hatchery population at a level of 0.15 to set up a steelhead strain which was fixed for this allele.

Taggart and Ferguson (1984) identified the variant allele PGI_{-3} (110) in brown trout stocks in Ireland and found it to be present in very few populations and at low frequencies, although it occured in three hatchery stocks. Taggart and Ferguson (1984) selectively bred individuals heterozygous for the PGI_{-3} variant allele and produced a stock of trout which are fixed for PGI_{-3} (110). The other major advantage of this tag is that it is expressed strongly in adipose fin tissue, permitting simple biopsying not only of potential

broodstock but of individuals in populations stocked with this tagged stock. Taggart and Ferguson (1984) also report that from population survey data and from monitoring experimental progeny the (PGI_{-3} (110) variant confers no selective disadvantage on individuals either in homozygous or heterozygous state.

Thus electrophoresis has been used imaginatively in the last decade to aid salmonid management in both wild and hatchery populations.

One type of trout which exist in Scottish waters and on which little quantitative work has been conducted is the 'ferox', Gunther's Salmo ferox.

4.1.13 The 'Ferox' problem

The ferox, described by Berkenhout (1789) and by Jardine and Selby (1835) as its name implies, is supposed to be a formidable fish both in size and habits. The Reverend Houghton (1879) maintains that "next to the pike, it is perhaps the most ferocious of freshwater inhabitants of our lakes and rivers". The ferox have been known to be closely associated with Arctic char (Salvelinus alpinus L.) which tend to grow slowly and shoal together. This makes them easy prey for a large predator, such as the trout (Mills, 1971; Campbell, 1971, 1979).

Hardie (1940) stressed the angling interest in these large predators and described methods of catching them as did many before him

(Thornton,1804; St John, 1878; Malloch, 1910; Mackenzie, 1924).

Campbell (1974) concluded from an in depth study of the Scottish ferox that the occurrence of such fish was governed by 3 factors

(a) oligotrophic waters

(b) the presence of char

(c) a large loch (over 100 ha in extent)

but he did not exclude the possibility that there was a genetic influence on the propensity of a trout to be or become a ferox.

Fishing specifically for them has declined over the years. From the earlier experience of Thornton (1804) and other contemporary anglers it might appear that large trout were then much more plentiful and more easily caught by trolling and bait fishing, than more recently. On the other hand Campbell (1971, 1979) points out that most modern anglers are not able to afford these time-consuming methods, and knowledgable boatmen and guides are much rarer.

As for all aspects of the trout, the ferox's behaviour, physiology and appearance, the relative importance of environmental and genetic components are still uncertain.

Ferguson and Mason (1981) confirmed that there were three sympatric populations of trout living in Lough Melvin. Anglers for centuries had identified the fish caught by their appearance. The ferox being distinguished by their overall dull brown/green coloration with

little spotting, their disproportionately long head and wide mouth containing many large teeth. The other phenotypes in the lough, the gillaroo and sonaghan differ markedly in appearance (Ferguson and Mason, 1981).

Electrophoretic evidence suggests the phenotypes are indeed reproductively sympatric, the ferox being characterised by a significantly higher allele frequency of the LDH_5 105 variant than the gillaroo or sonaghan. It is also suggested that this allele could be correlated with ferox type growth elsewhere in Ireland (Ferguson and Mason, 1981) as it is found where specimen brown trout occur (Lough Macnean and Lough Erne) but the allele is absent or at least at very low frequencies in most Irish populations (Ferguson and Mason, 1981; Ferguson and Fleming, 1983).

During this project it was hoped to collect ferox from various waters and add to the electrophoretic data, to hopefully enable further conclusions to be drawn concerning the origins of the fish.

4.1.14 Aims of electrophoretic survey of brown trout in Scotland

1. To investigate and identify enzyme polymorphisms in wild populations.

2. To determine heterozygosity values and the extent of genetic diversity.

3. To determine the distribution of that genetic diversity.

4. To produce estimates of genetic distance and similarities between stocks so as to determine approximate times of divergence.

5. To identify pristine stocks for future management use.

6. To identify any allelic marker(s) to distinguish major divisions in the trout populations (special reference to 'Ferox' trout).

7. To identify any allelic marker(s) to distinguish stocked trout or markers that could be used in the future as genetic tags.

4.2 Materials and Methods

4.2.1 Wild populations of brown trout in Scotland

Table 4.3 lists the lochs which were sampled in this survey, along with the appropriate grid reference. The number of fish taken at each site is also given as is the code by which the site can be referred to later in the results and discussion sections.

Figure 4.1 illustrates the distribution of sampling sites throughout Scotland. Figures 4.2 and 4.3 give a more detailed analysis of the position of sampling sites in Perthshire and north west Scotland respectively. The numbers given on the maps, after the loch names refer to the code in Table 4·3.

Population No. 55 refers to a collection of 12 "Ferox" trout from 11 different lochs delivered to the University by anglers answering an advert for large brown trout information placed in the angling press.

Table 4.4 lists the number of ferox reported caught during the period of this study. Unfortunately due to logistic problems and length of time in storage after capture, or complete absence of the carcass only 12 fish were used successfully for electrophoretic analyses. These fish are marked * in the table. The list is included to illustrate the wide range of lochs producing so called ferox trout and the actual number being caught.

The number of large trout reported to myself during this study is an underestimate of the number taken altogether. Reasons for this

Table 4.3 Locations, grid references for the lochs samples in electrophoretic survey

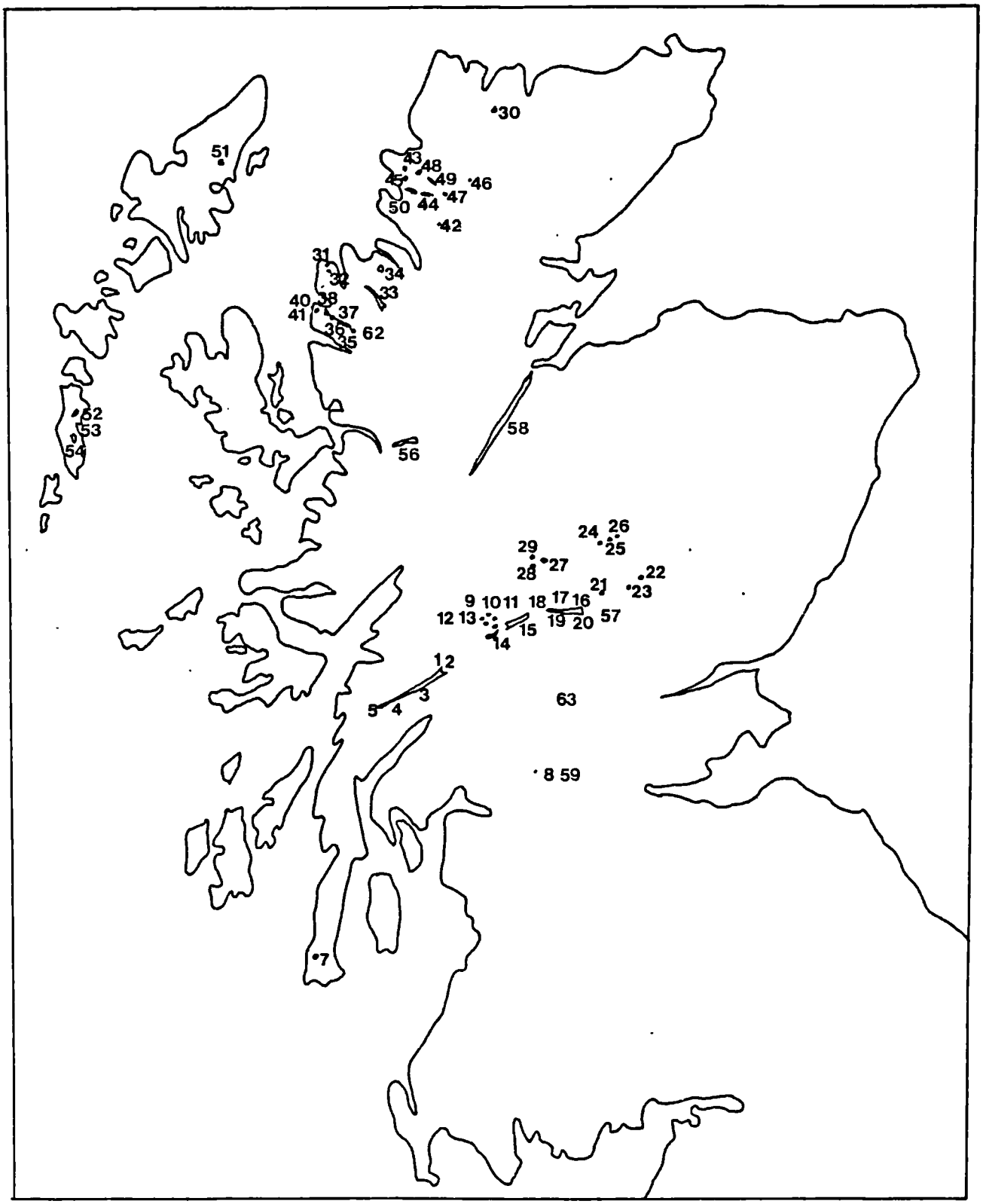
Location	Grid Reference	No. of fish taken	Code No.
Loch Awe	Sheets 50-55	33	
Burn (1) East of Mollie		4	1
(2) Mollie		3	2
(3) Allt Ferna		10	3
(4) Blar Gmour		9	4
(5) Inverliever		5	5
(6) North of Inverliever		2	6
Tangy Loch	68/692280	27	7
Howietoun Fish Farm stock	57/785884	82	8
Rannoch Moor Pool 1	41/308537	14	9
Pool 2	41/310534	15	10
Pool 3	41/314530	10	11
Pool 4	41/317517	10	12
Pool 5	41/332516	16	13
Loch Ba, Rannoch Moor	41/330510	6	14
Loch Laidon, Rannoch Moor	41/360520	34	15
Loch Rannoch			
Burn (1) Annat	42/635592	70	16
(2) Allt Na Cardiach	42/589585	40	17
(3) Allt Chomraidh	42/500567	21	18
(4) Finnat	42/514568	50	19
(5) Carie Burn	42/618572	18	20
Loch Fincastle	43/870626	15	21
Loch Vatigan	43/975694	43	22
Loch Moraig	43/907667	12	23
Loch an Duin	42/725800	16	24
Loch Brodain	42/744830	44	25
Loch an Tseilach	42/756857	13	26
Loch Pattack	42/540790	36	27
Loch a Bhealaich Bheithe	42/512725	40	28
Loch an Sgoir	42/490750	50	29
Loch na Creige Riabhaich	9/430505	18	30
Loch nan Eun	19/772912	6	31
Loch an Draing	19/755902	14	32
Loch Fionn	19/950785	40	33
Loch A'Mhadaidh Mor	19/966866	37	34
Loch A'Bhealiach	19/870640	43	35
Loch Gaineamhach	19/834670	50	36+37
Loch Horrisdale	19/797705	50	38+39
Loch Badachro	19/785728	14	40
Loch Clair	19/773717	30	41
Loch an Ealachan	15/175090	22	42

continued . . .

Table 4.3 continued

Location	Grid Reference	No. of fish taken	Code No.
Loch Crocach (Lochinver)	15/105275	26	43
Loch Veyatie	15/190130	60	44
Loch Druim Suardalain	15/098217	97	45
Loch Gillaroo	15/276194	6	46
Loch Awe	15/245154	13	47
Loch Beannach	15/140265	20	48
Loch Assynt	15/200250	41	49
Fionn Loch	15/130176	18	50
Loch Beag A'Chocair (Lewis)	8/342346	10	51
N. Uist (1) Unknown	Sheet 22	10	52
(2) Unknown	Sheet 22	6	53
(3) Unknown	Sheet 22	9	54
Ferox (11 different lochs)	See separate table	12	55
Loch Quoich	33/020020	20	56
Loch Rannoch	42/600580	6	57
Loch Ness	Sheets 24-26	24	58
Howietoun Fish Farm stock	57/785884	36	59
Loch Crocach x Nashua strain	N.A.	10	60
Nashua strain (DAFS, Piltochry)	N.A.	17	61
Loch a' Ghobhainn	19/8555455	42	62
River Earn (sea trout)	53/640240	10	63

Figure 4.1



MAP OF SCOTLAND SHOWING THE DISTRIBUTION OF LOCATIONS SAMPLED IN ELECTROPHORETIC SURVEY

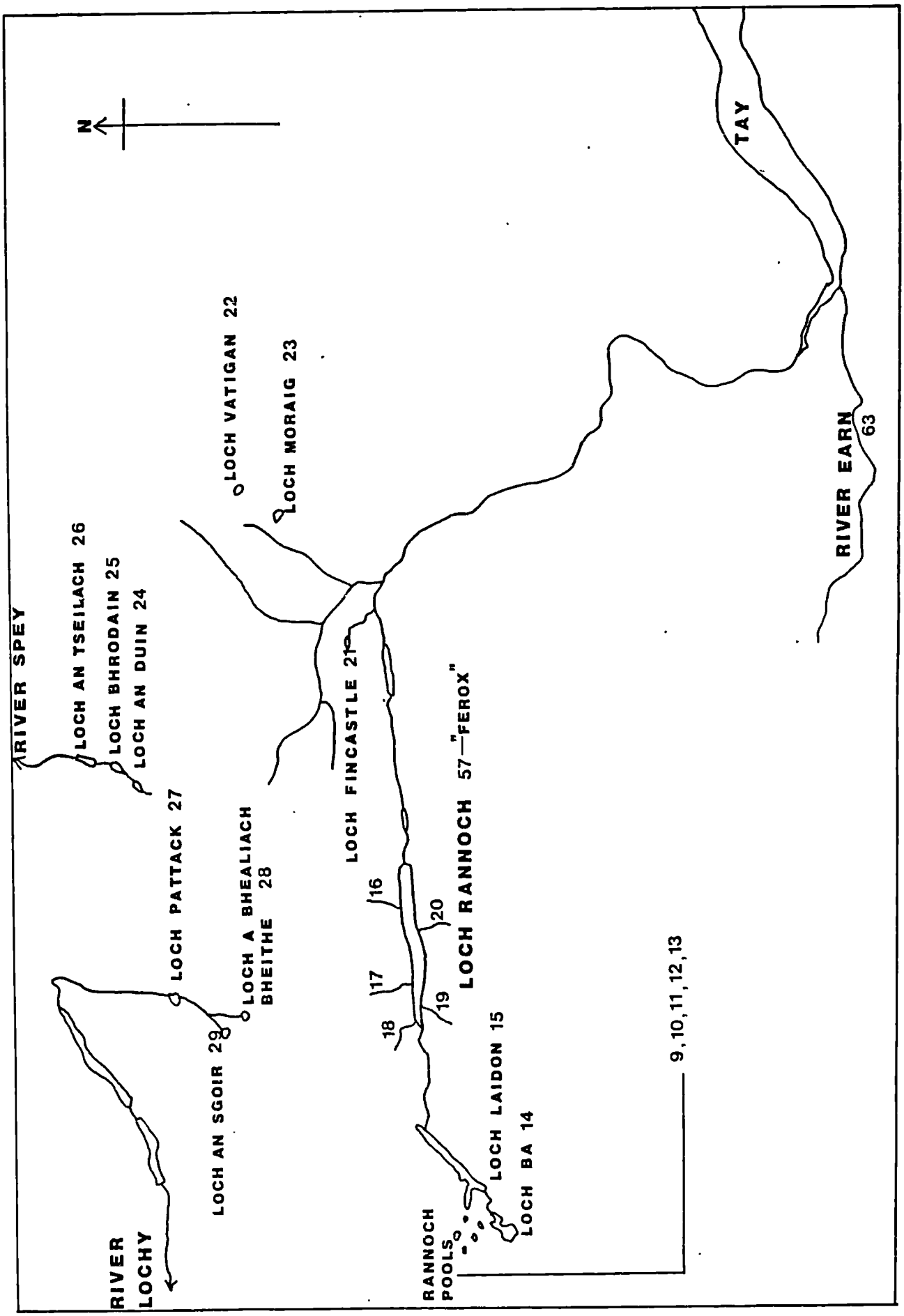


FIGURE 4.2 MAP ILLUSTRATING SAMPLING SITES IN PERTHSHIRE, SCOTLAND

FIGURE 4.3 MAP ILLUSTRATING SAMPLING SITES IN N.W. SCOTLAND

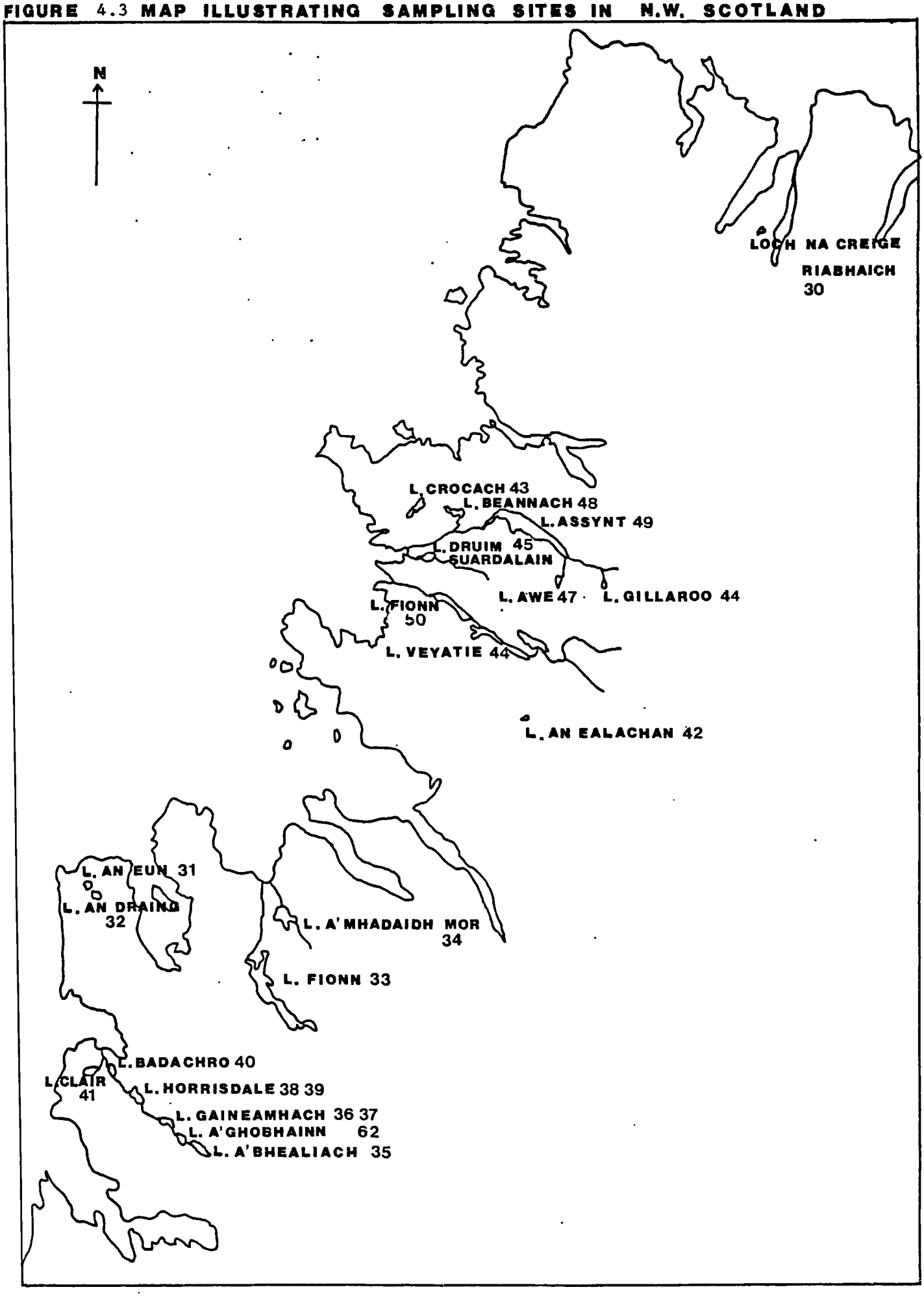


Table 4.4 Lists in alphabetical order the ferox trout reported by the angling public during this project.

Location	Date	Weight lbs	gms	Length	Age	Grid reference	How caught
Loch in Ardnamurchan	April 84	4lbs	1,800		8+	-	Fly
Loch Arkaig	4.6.85	11lbs	4,950		9+	34/160890	Dead trout
Loch Arkaig	16.6.84	10½lbs	4,725		11+	34/160890	Trolling
Loch Assynt	14.5.86	6lb 10 oz	2,998		8+	15/200250	Trolling
Loch Awe	October 84	9lbs	3.600		6/7	Sheets 50-55	Barrage/trap
Loch Awe	October 84	22½lbs	10,125		9+	Sheets 50-55	Barrage/trap
Loch Bheannaich Uig	May 86	11lb 2 oz	5,006		8++	13/056333	Minnow
Loch Crocach*	August 84	8½lbs	3,825		6++		Minnow
Loch Earn*	26.5.83	9lb 4 oz	4,162		9++	51/640240	Worm
Loch Erich*	84	5lb 10 oz	2,666	55.0	8+	42/500640	Trolling
Fionn Loch*	July 83	7½lbs	3,375		8+	19/950785	Sunk fly
Fionn Loch*	July 83	8½lbs	3,825		9+	19/950785	Sunk fly
Fionn Loch	84	8lb	3,690	61.0	11+	19/950785	Trolling
Fada*	84	8½lbs	2,640	50.0	8	-	Trolling
Fionn Loch (Sutherland)	86	2lbs	900	40.0	7	15/130176	Fly
River Gaur	14.4.83	4lbs 8 oz	2,025		9+	42/450570	Worm
Loch Laggan	August 85			58.4	9	41/374809	Trolling
Loch Laggan	August 85			64.8	12	41/374809	Trolling
Loch Laggan	August 85			67.8	11	41/374809	Trolling
Loch Laggan	August 85			55.5	9	41/374809	Trolling
Loch Laggan	August 85			63.5	8	41/374809	Trolling
Loch Laggan		8lbs 7 oz	3,800		8+	41/374809	Trolling
Loch Laggan	83	6lb 12 oz	3,040		11+	41/374809	Trolling
Lanarkshire Mill Loch	85	4lbs	1,800		6	-	Minnow
Lanarkshire	17.8.85	6lb 5 oz	2,870		8	-	Fly
Loch Leathan (Skye)	24.5.83	4lb 8 oz	2,025		6	23/505515	Fly
Lasson Loch	4.8.84	8lb 7 oz	3,800		7+	-	Minnow

continued . . .

Table 4.4 continued

Location	Date	Weight lbs	gms	Length	Age	Grid reference	How caught
Loch Leven*	12.7.84	41b 10 oz	2,085		5+	58/150010	Fly
Loch Leven	2.9.83	41b 3 oz	1,900		5+	58/150010	Fly
Loch Leven	23.8.83	4½lbs	2,025		4+	58/150010	Fly
Loch Lomond	April 83	61bs	2,700		8	Sheet 56	Toby
Loch Lomond	25.3.83	41b 12 oz	2,140		6+	Sheet 56	Trolling
Loch Morar	14.4.83	51bs	2,250		8+	40/690920	Trolling
Loch Morar	84	71b 2 oz	3,206		10+	40/690920	Trolling
Loch Mullordoch	14.6.84	31b 12 oz	1,688		8+	25/190305	Trolling
Loch Quoich	17.9.82	161bs	7,200		8+	33/020020	Trolling
Loch Quoich	6.6.85	121bs	5,400		7+	33/020020	Trolling
Loch Quoich	6.6.85	16½lbs	7,425		7+	33/020020	Trolling
Loch Quoich	16.9.85	141bs	6,300		8+	33/020020	Trolling
Loch Quoich	28.5.85	81b 11 oz	3,900		11+	33/020020	Trolling
Loch Ness	October 85	61b	2,700	60.0		Sheets 24-26	Net
Loch Ness	October 85	61b 8 oz	2,925	61.5		Sheets 24-26	Net
Loch Ness	October 85	9 1b 8 oz	4,275	74.1		Sheets 24-26	Net
Loch Ness	October 85	71bs	3,150	63.2		Sheets 24-26	Net
Loch Ness	October 85	41bs	1,800	49.0		Sheets 24-26	Net
Loch Ness	October 85	21b 6 oz	1,070	40.8		Sheets 24-26	Net
Loch Ness	October 85	31bs	1,350	48.5		Sheets 24-26	Net
Loch Rannoch	7.12.82	3½lbs	1,575		8+	42/600580	Netted
Loch Rannoch*	83	51b 12 oz	2,590		12+	42/600580	Trolling
Loch Rannoch	18.5.85	101b 8 oz	4,725	75.0	11+	42/600580	Trolling
River Spey*	November 84	141bs	6,300		13+	-	Worm
Loch Strakey*	27.4.83	61bs 1 oz	2,730		8+	-	
Loch Shin	10.6.83	51bs 7oz	2,450		7+	16/500160	Toby
Loch Tay	May 83	61bs	2,700		5+	Sheet 51	Trolling
River Tay	May 83	61b 10 oz	3,100		10+	Sheet 51	Worm

continued . . .

Table 4.4 continued

Location	Date	Weight lbs	gms	Length	Age	Grid reference	How caught
River Tummel*	84	4½lbs	2,025		7+	52/820595	Spinning
River Tweed*	September 83	3lb 1 oz	1,395		5+	-	Fly
Loch Veyatie	4.9.86	1lb 10 oz	725	40.1		15/190130	Net
Loch Veyatie	4.9.86	2lbs	890	43.2		15/190130	Net
Loch Veyatie	4.9.86	2lb 7 oz	1,100	44.2		15/190130	Net
Loch Veyatie	4.9.86	5lb 4 oz	2,350	48.8		15/190130	Net
Loch Veyatie	4.9.86	2lb 1 oz	920	41.2		15/190130	Net
Loch Veyatie	4.9.86	2lb 1 oz	930	42.5		15/190130	Net
Loch Veyatie	4.9.86	3lb 3 oz	1,500	48.5		15/190130	Net
Loch Veyatie	4.9.86	8lbs	3,600	64.0		15/190130	Net
Loch Veyatie	4.9.86	2lb 11oz	1,220	50.5		15/190130	Net
Loch Veyatie	4.9.86	1lb 11 oz	780	37.7		15/190130	Net
IRELAND							
Lough Corrib		4lb 2 oz	1,860		5+	-	Trawling
Lough Corrib	June 82	12lbs	5,400		8+	-	Trawling
Lough Garve	June 82	5lb 7 oz	2,420		6+	-	Trawling
Lough Erne Lower	16.4.83	6lb 1 oz	2,740		6+	-	Trawling

* refers to fish used for electrophoretic analysis

include:

1. Not every angler catching a large trout had read the adverts in the press, requesting information.

2. Not every angler catching a large trout would be willing to participate in an exercise which meant revealing details of their catches.

4.2.2 Electrophoretic techniques

4.2.2.1 Sample preparation

Small pieces of muscle, and liver and the whole heart, eyes and brain were dissected from each trout and placed in five separate coded reaction vials. This procedure was conducted as soon after capture as possible. The viles were then either kept on ice until they could be placed in the deep freeze at -40°C, or placed directly into the freezer. It was the aim at all times to rinse the tissues in distilled water before placing them in the reaction viles, clean washed scalpel blades were used for each dissection to reduce the likelyhood of contamination. Originally, the samples were moistened with 25 µl of distilled water (using a sigma micro-pipette) and homogenised using a rotating glass rod and a small quantity of acid washed sand in each vile.

The sphere of the eye was punctured when the samples were removed from the fish to ensure that the retinal fluid would come into contact with the filter paper when thawing took place.

The samples were then centrifuged at 500 g for 10 minutes. The homogenate was then absorbed onto 3mm x 7mm rectangles of Whatman No. 1 filter paper. Later in the study it was found that similar or better results could be obtained by missing out the homogenisation and centrifugation of each sample and instead rely simply on freeze-thaw action to break down the cell walls and release the enzyme source. Thus the filter paper rectangles were place on the frozen tissue within the reaction vile and left to defrost while the gels were prepared for the appropriate electrophoretic run.

4.2.2.2 Electrophoresis

The technique was that of horizontal starch gel electrophoresis as described by Beckman and Johnson (1964) and Harris and Hopkinson (1976). The starch gel was made up to 12½% (Connaught) starch in the appropriate buffer system (Table 4.5). The starch suspension was heated in a Buchner flask with a hand held continuous swirling action. The gel was degassed using a vacuum pump before being poured into Shandon starch gel formers (18cm x 9cm x 0.6cm) supported on a clean glass plate placed on top and allowed to cool. Gels were normally prepared the same day as the electrophoretic run.

The gels were sliced vertically and parallel to the long axis, normally 3cm for one edge, and the samples were applied to the cut edge of the large slice. The exception to this was when the enzyme G-3PDH was being stained for and AM Buffer was being used. The gel was then cut 4.5cm from the edge as G-3PDH runs cathodally when AM

Table 4.5 Showing formulae of buffers used in the electrophoretic assays

Buffer		pH	Voltage	Polymorphic Enzymes Studied	Source
TCB TRIS/CITRIC/ BORIC	Electrode: 0.3M Boric Acid 0.1M Lithium Hydroxide (196 g Boric Acid, 42 g Lithium Hyrdroxide in 10 litres Gel: 0.076M Tris 0.005M Citric Acid 0.015M Boric Acid 0.005M Lithium Hydroxide (92 g Tris (Hydroxymethyl Methylamine 10.5 g Citric Acid in 10 litres + 530 mls electrode buffer)	8.6 8.6	14v/cm for 4-5 hours	LDH G3PDH PGI	Taggart et al (1981)
AM	Electrode: 0.04M Citric Acid (84.06 g Citric Acid adjusted to pH 6.1 with N-(3-Aminopropyl) - Morpholine made upto 10 litres Gel: 0.002M Citric Acid (Dilute electrode buffer 1:20) Check pH and adjust as necessary	6.1 6.1	16v/cm for 4-5 hours except for Enzymes AAT and IDH 11v/cm for 4-5 hours	MDH G3PDH IDH DIA AAT	

Buffer with a pH of 6.1 is used. Each run consisted of between twenty and forty samples absorbed onto Whatman filter paper.

The two pieces of the gel were then placed back together and a perspex spacer inserted between the former and the gel to ensure the sample slit did not open up during the run because of shrinkage.

The gel was then placed in a shandon horizontal electrophoresis bath and with the appropriate electrode buffer, and lint wicks were applied as electrodes to ensure an even current through the gel. A polythene sheet was placed on the gel to prevent water loss during the electrophoretic process. The baths were placed in a refrigerator which standardised the run temperature at 4°C. Power was applied using constant current or voltage from Heathkit power packs. The length of the run varied depending on the enzymes under examination, but on average, was 5 hours. Four gels were normally run simultaneously.

Staining was carried out using standard histochemical techniques (Brewer, 1970; Harris and Hopkinson, 1976; Ferguson, 1984). (See Table 4.6).

Table 4.6 Stain formulae for the enzymes (polymorphic) studied in brown trout

Enzyme	Formula		Conditions	Source
AAT	Tris	300 mg	Incubate gel with staining mixture for 15 minutes before adding the fast blue stain	Harris and Hopkinson (1976)
	L.-aspartic acid	65 mg		
	-ketoglutanic acid	20 mg		
	Pyroxidol-s-phosphate	10 mg		
	Poly vinyl polypyrolidone in distilled water	10 mg 30 mls		
	Fast Blue RR in distilled water	25 mg in 10 mls		
DIA	NADH	10 mg	+ 25 mls 0.025M Tris/HCl pH 8.5	Harris and Hopkinson (1976)
	2,6-Dichlorophenol-indophenol in distilled water	1		
	MTT (dissolved in distilled water)	75 mg	Filter 2,6; Dichloro phenol indophenol then add to Tris/HCl with NADH/MTT + agar overlay	
G3PDH	DL Glycerophosphate	1 gm	+ 30 mls 0.1M Tris/HCl + agar overlay	Ferguson (1984)
	NAD	10 mg		
	MTT	5 mg		
	PMS	2 mg		
IDH	Sodium Isocitrate (D.L. Isocitric acid)	35 mg	+ 25 mls 0.1M Tris/HCl	Harris and Hopkinson (1976)
	NADP	10 mg	Add $MgCl_2$ $6H_2O$ just before mixing with Tris/HCl + agar overlay	
	MTT	5 mg		
	PMS	2 mg		
	$MgCl_2$ $6H_2O$	50 mg		

continued . . .

Table 4.6 continued

Enzyme	Formula		Conditions	Source
LDH	Sodium Lactate Solution	0.2 ml	+ 25 mls 0.1M Tris/HCl	Brewer(1970)
	NAD	10 mg		
	MTT	5 mg	+ agar overlay	
	PMS	1 mg		
MDH	D.L. Malic acid	60 mg	+ 30 ml 0.1M	Harris and Hopkinson (1976)
	NAD	15 mg	HCl/Tris	
	MTT	7 mg		
	PMS	2 mg	+ agar overlay	
PGI	Fructose-6-phosphate	20 mg	+ 30 ml 0.1M	
	NADP	4 mg	Tris/HCl	Brewer (1970)
	MTT	5 mg	Add $MgCl_2$ $6h_2O$ just	
	$MgCl_2$ $6h_2O$	20 mg	before adding	
	Glucose-6-phosphate		Tris/HCl and then add	
	dehydrogenase	10-20yl	G3PDH + agar overlay	
Staining buffer	5 litres 0.1M Tris/HCl 60.5 g tris in 1500 ml distilled water. pH brought to 7.4 by adding Analar Conc. HCl. Made up to 5 litres.			

Table 4.7 Polymorphic loci. Occurrence and distribution in Scottish trout populations

Locus	Variant Alleles	Tissues Screened	No. of Pops. Examined	No. Pops with Variant Allele	Maximum Frequency	Previously Reported Variant Alleles (and Ref)*
$_{s}AAT_{-1,2}$	140	M	59 †	43	1.00	119(1) 140(7) 130(7)
$_{s}AAT_{-1,2}$	45	M	59	9	0.19	45(7)
$_{s}AAT_{-4}$	74	L	59	44	0.83	74(7) 65(8)
DIA	90	E	59	14	0.12	90(2) 90(7)
$G\text{-}3PDH_{-2}$	50	M	59	43	1.00	8(3) 50(1) 50(7) 50(8)
$_{s}IDH_{-1}$	160	B	59	50	0.77	160(7) 180(8)
$_{s}IDH_{-2}$	130	B	59	6	0.07	130(7)
LDH_{-1}	Null	M	59	15	0.38	240(1) (4) Null(7)
LDH_{-5}	105	E	59	50	1.00	105(5) 105(7)
$_{s}MDH_{-2}$	152	H	59	52	1.00	152(6) 152(7) 200(8)
$_{s}MDH_{-3,4}$	125	H	59	41	0.70	125(8) 125(7) 10(8)
PGI_{-2}	135	M	59	10	0.19	135(7)
PGI_{-3}	110	M	59	7	0.10	110(4) 110(7) 103(8)

* (1) Allendorf et al. (1976)
(2) May (1980)
(3) Engel et al. (1971)
(4) Stahl (1981)
(5) Allendorf et al. (1977)
(6) May et al. (1979a)
(7) Taggart et al. (1981)
(8) Guyamard and Krieg (1983)

† 59 includes Howietoun fish farm population

Following most authors when dealing with salmonids recently the nomenclature of enzyme variants used here is an extension of the system proposed by Allendorf and Utter (1979) and followed by Taggart et al. (1981). Each protein or enzyme is abbreviated and where applicable the prefix "m" or "s" is used to denote the mitochondrial or supernatant form of the enzyme respectively. Hyphenated numerals designate multiple loci which are numbered in order of increasing electrophoretic mobility from the cathodal end of the gel. Variants at a locus are referred to in parenthesis by the mobility of the homomeric band relative to a standard allele which is designated 100. Where variation occurs and cannot be attributed to either locus of a duplicate pair a comma is used to separate the loci (e.g. $MDH_{-3,4}$ AAT-1,2)

4.2.2.3 Polymorphisms

The polymorphisms described below (see Table 4.7) all have a firm genetic basis, verified by extensive inheritance studies (Taggart and Ferguson, 1984). which also confirmed that inheritance was disomic and not tetrasomic.

Breeding studies have also verified the genetic basis of similar polymorphisms in rainbow trout and other salmonids (Utter et al., 1973; Allendorf et al., 1975; Clayton et al., 1975; May et al., 1978b).

It must be pointed out that the loci number estimates may well be on the low side, since a single invariant band may represent more than one locus producing electrophoretically identical products.

In alphabetical order there follows a description of the polymorphisms studied.

1. Enzyme: Aspartate Aminotransferase. Variant allele: $_{s}AAT_{-1,2}$ (140)
This polymorphism was identified using muscle tissue

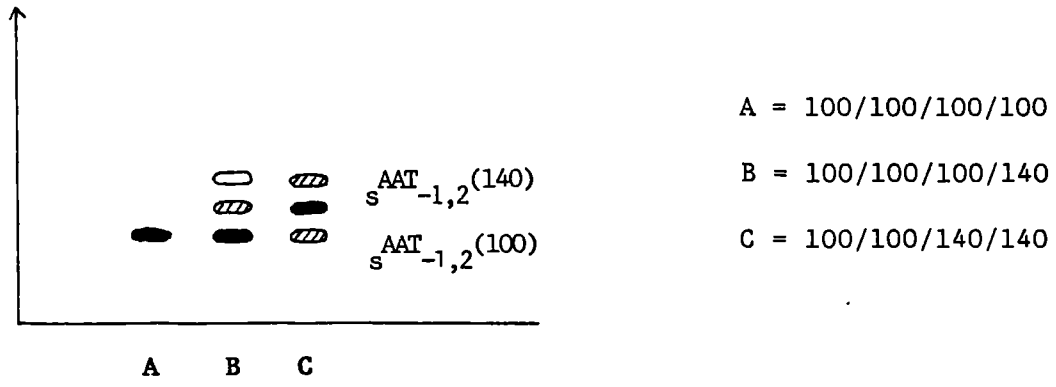
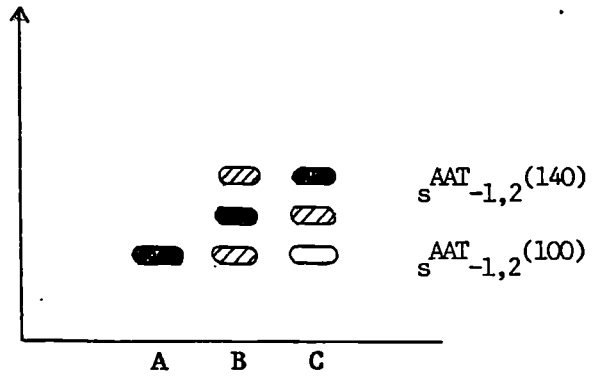


Figure represents zymograms of $AAT_{1,2}$ polymorphism observed in

this study. AAT is a dimeric enzyme coded for by 4 loci, and as can be seen from the staining pattern of the heterozygotes 9:6:1 the first locus is a duplicated one. The homozygote for $AAT_{1,2}(140)$ being a three banded zymogram with a staining intensity of 1:2:1. Taggart et al (1981) reviewed the work conducted on AAT on brown trout and other salmonids and concluded that the absence of heterozygotes with a staining intensity of 1:6:9 or the presence of a single banded faster homozygote in populations with the $_{s}AAT_{-1,2}$ (140) allele present at a frequency of 0.62 indicated that the polymorphism was restricted to one of the duplicate pair of loci. In this study two populations did show this 1:6:9 heterozygote phenotype but at very low levels but no faster homozygotes were identified.



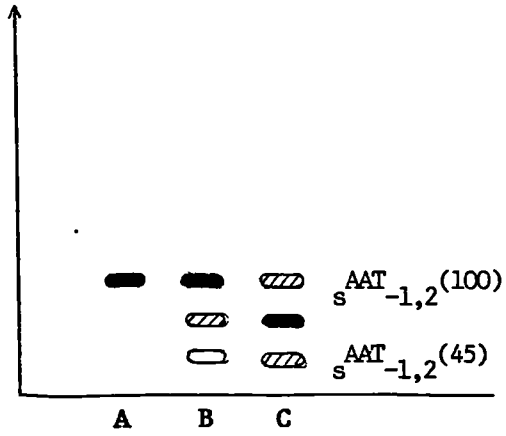
A = 100/100/100/100

B = 100/100/140/140

C = 100/140/140/140

So although this indicates both loci are polymorphic as far as calculating allele frequencies are concerned it has been assumed in this study that only one locus is effectively polymorphic.

Variant allele: $_{s}AAT_{1,2}$ (45) A few populations also exhibited this less common polymorphism, involving a three banded 9:6:1 pattern with a variant allele $_{s}AAT_{-1,2}$ (45). Only two homozygotes for $AAT_{-1,2}$ (45) were found in this study and the polymorphism never occurred



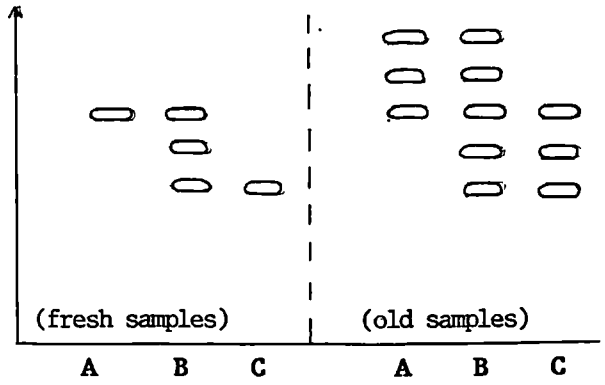
A = 100/100/100/100

B = 100/100/100/45

C = 100/100/45/45

with the polymorphism involving $s^{AAT}_{-1,2}$ (140) in the same individual. To calculate allele frequencies and Hardy- Weinberg the $s^{AAT}_{-1,2}$ (45) polymorphism was treated as a separate locus.

Variant allele: $AAT_{-4}(74)$ This was a common poymorphism found in liver tissue, and in agreement with Taggart et al. (1981) conformed to a simple polymorphism if the samples were stored for less than a month before being examined. After this point in agreement with Taggart et al (1981) artefact bands appeared and instead of single banded homozygotes and three banded heterozygotes one had three banded homozygotes and five banded heterozygtoes.



A = 100/100

B = 100/74

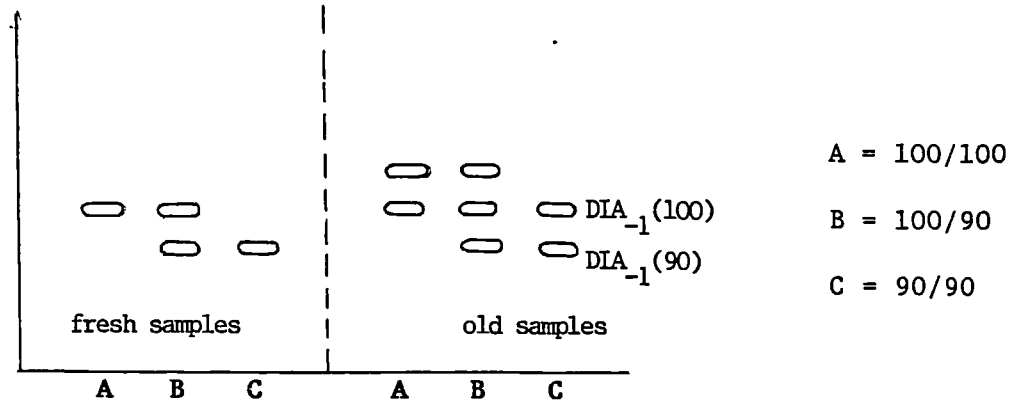
C = 74/74

Taggart and Ferguson (1984) have confirmed by inheritance trials

that these are indeed artefacts created by prolonged storage.

Enzyme: Diaphorase Variant allele : DIA_{-1} (90)

This monomeric enzyme is presumed to be coded for by a single locus in many salmonids (Taggart et al., 1981 and references therein). Liver extracts showed a single band of common mobility in most individuals. A few double banded phenotypes representing the heterozygote DIA_{-1} (100/90) were observed and even fewer homozygotes. Brain tissues showed a similar polymorphism and this tissue was used in preference in this study as it tended to retain activity longer under the storage conditions. If the samples were stored for too long (more than 3 months) an artefact band appeared (see diagram).



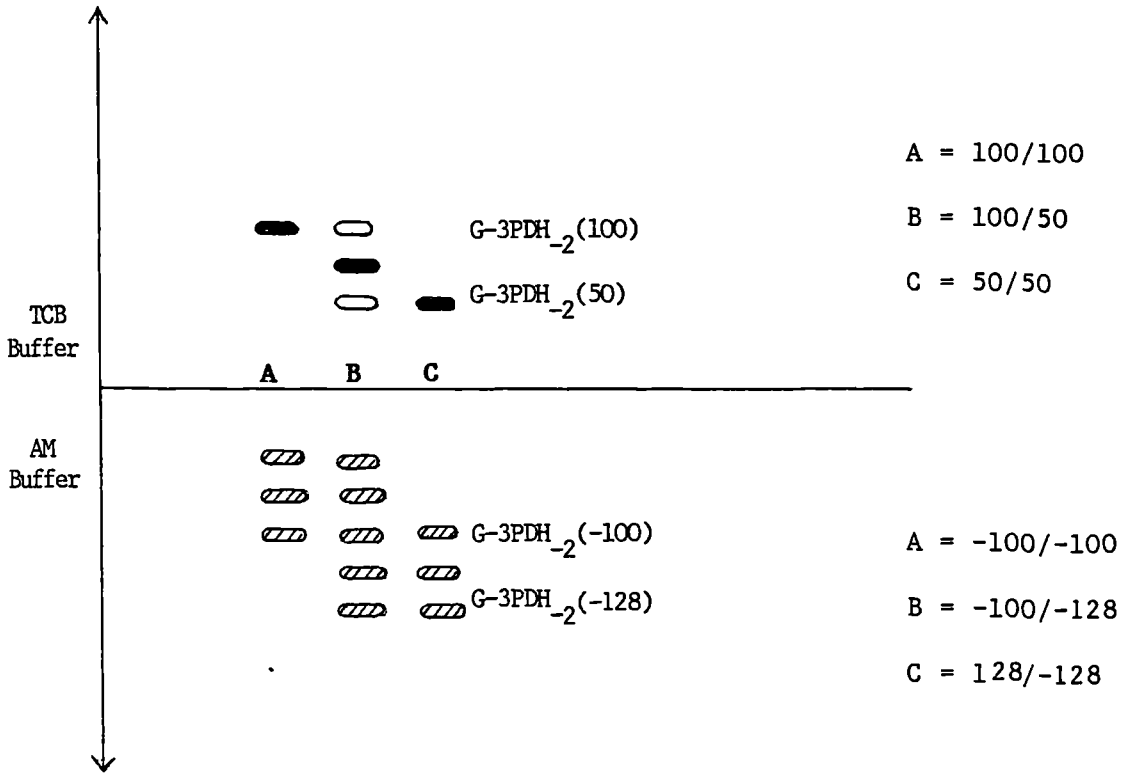
This artefact band was confirmed by Taggart and Ferguson (1984) during their inheritance examination of Irish brown trout. They also showed that this artefact had the same 'mobility' as a polymorphism they named $DIA_{-1}(120)$. In this study DIA_{-1} (90) was the only polymorphism positively identified and used in subsequent calculations.

Enzyme: Glycerol-3-Phosphate Dehyrdogenase Variant Allele: $G3PDH_2(50)$

The patterns exhibited by this dimeric enzyme are explained by Taggart et al. (1981), by postulating the existence of three loci, which

agrees with previous authors working with brown trout (Engel et al., 1971; Allendorf et al., 1977). Only G-3PDH-2 expressed in muscle tissue exhibited polymorphism in this study. Using the TCB buffer system, extracts exhibited two single banded phenotypes and one three banded phenotype suggesting a polymorphism for two co-dominant alleles and the 1:2:1 staining of the heterozygote indicated the polymorphism was the product of a single locus.

G-3PDH did not always stain up well and reading the pattern from the TCB gels became unpredictable. As noted by Taggart et al., (1981) G-3PDH also runs on gels made up with AM buffer, but migrates cathodally rather than anodally. The resultant pattern was equivalent to the polymorphism observed using the TCB buffer system except the relative mobilities were different, and the homozygotes were represented by three banded phenotypes, and the heterozygote by five banded phenotypes (see diagrams below)



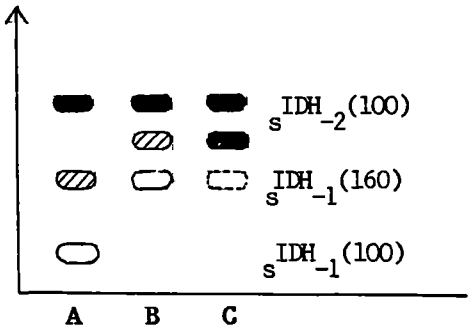
In all cases in this study where possible G-3PDH was examined using both AM and TCB buffers, and where they were both examined the typing of phenotypes was in agreement.

Enzyme: Isocitrate Dehydrogenase (NADP Dependant)

Variant Allele: IDH-1 (160)

Taggart et al. (1981). point out the problems of typing IDH patterns of polymorphism. The resolution of IDH zymograms was unpredictable, which Taggart et al. (1981) attribute to the storage liability of salmonid IDH. In this study at least to begin with, IDH tended to run slightly cathodally or stay very near the gel origin. this was put down to slight variations in pH of the gel mixture. Brain tissue, as long as it was stored at -40°C for less than 6 months gave readable zymograms of this dimeric enzyme. As Taggart et al. (1981) suggest, running the enzyme with a reduced field voltage (11v/cm) gave better results than with a higher field voltage.

The diallelic polymorphism involving a variant allele $_{s}IDH_{-1}$ (160) as tentatively proposed by Taggart et al. (1981) and confirmed by Taggart and Ferguson (1984) was a common polymorphism identified in Scottish brown trout. (See diagram).



A = $_{s}IDH_{-1}$ (100/100) $_{s}IDH_{-2}$(100/100)

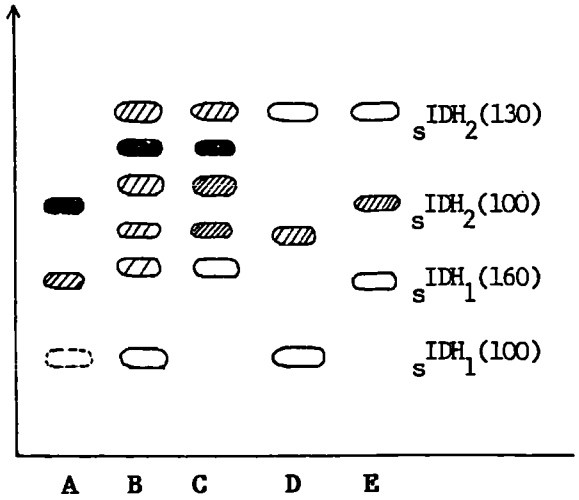
B = $_{s}IDH_{-1}$ (100/160) $_{s}IDH_{-2}$(100/100)

C = $_{s}IDH_{-1}$ (160/160) $_{s}IDH_{-2}$(100/100)

The homodimer being coincident with the $_{s}IDH_{-1/2}$ heterodimeric isozyme.

Variant allele $IDH_{-2}(130)$

This polymorphism also tentatively suggested by Taggart et al. (1981) and confirmed to have a genetic basis by Taggart and Ferguson (1984) was only observed in a few populations.



A = $_sIDH_{-1}(100/100)$ $_sIDH_{-2}(100/100)$
B = $_sIDH_{-1}(100/100)$ $_sIDH_{-2}(100/130)$
C = $_2IDH_{-1}(100/160)$ $_sIDH_{-2}(100/130)$
D = $_sIDH_{-1}(100/100)$ $_sIDH_{-2}(130/130)$
E = $_sIDH_{-1}(160/160)$ $_sIDH_{-2}(130/130)$

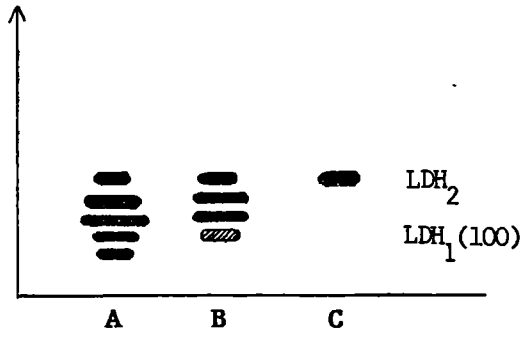
Only A, B, C and D phenotypes were observed in this study.

Enzyme: Lactate Dehydrogenase Variant allele: LDH_{-1} (ϕ)

There is general agreement (Wright et al., 1975; Bailey et al., 1976; Taggart et al., 1981) that in salmonids LDH is coded for by five loci. LDH_{-1} and LDH_{-2} are predominantly expressed in muscle. LDH_{-3} is expressed in the heart, LDH_{-4} is expressed in the liver and and LDH_{-5} in the eye. As in Taggart et al. (1981), this study concentrated on LDH_{-1} and LDH_{-5} both exhibiting polymorphism.

The muscle extracts examined, exhibited for the most part the same five banded phenotype recorded by Taggart et al. (1981) composed of two homotetrameric and three intermediate heterotetrameric products of the two loci LDH_{-1} and LDH_{-2}. Some individuals showed differential

staining with more heavily stained bands occurring at or near LDH_{-2}. In the homozygote of this variation LDH_{-1} was missing altogether. This polymorphism which Allendorf et al. (1976) designated as $LDH_{-1}(240)$ was later thought to be $LDH_{-1}(\emptyset)$ by Taggart et al. (1981) and Stahl (1980) and according to Taggart and Ferguson (1984) was confirmed by inheritance trials by Henry (1984).



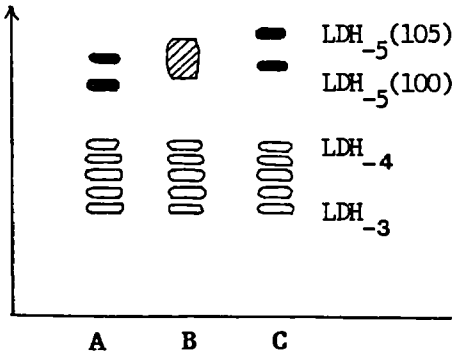
A = $LDH_{-1}(100/100)$

B = $LDH_{-1}(100/\emptyset)$

C = $LDH_{-1}(\emptyset/\emptyset)$

Variant allele: LDH_{-5} (105)

This polymorphism was common in Scottish brown trout stocks and conforms with the variant allele found by Allendorf et al. (1977) and Taggart et al. (1981). The variant allele $LDH_{-5}(105)$ was found only in eye tissue. The $LDH_{-5}(105/105)$ homozygote gave an identical banding pattern to that of the $LDH_{-5}(100/100)$ homozygote but of faster mobility.



A = $LDH_{-5}(100/100)$

B = $LDH_{-5}(100/105)$

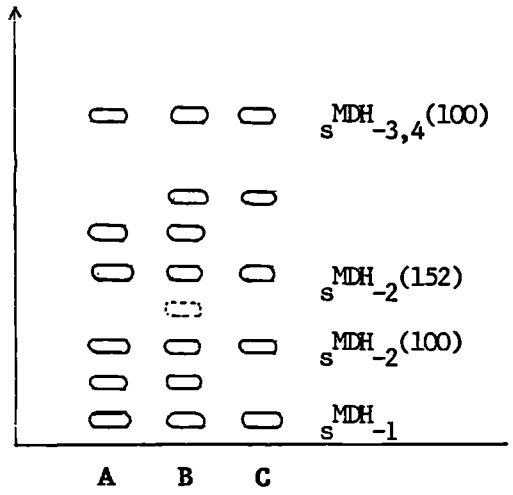
C = $LDH_{-5}(105/105)$

The heterozygote which is expected to segregate into 5 distinct bands (Taggart and Ferguson, 1986) was only evident in this study as a diffuse blurr of intermediate

mobility between LDH_{-5}(105/105) and LDH_{-5}(100/100) homozygotes, agreeing with Taggart et al. (1981). Although little difference was evident between the mobilities of two homozygotes it is relatively easy to distinguish between the two if reference samples were applied to each gel run for comparison.

Enzyme: Malate Dehydrogenase Variant allele MDH_{-2}(152)

MDH is a dimeric enzyme. Work has been carried out by a number of authors on MDH in various salmonid species. Taggart et al. (1981) detail work by Bailey et al. (1970), Allendorf et al. (1977) who investigated brown trout populations in Sweden, May et al. (1979) verified the existence of two variant alleles, MDH_{-1}(Ø) and MDH_{-2}(152) in North-American stocks. Taggart et al. (1981) also cite Allendorf et al. (1977) who identified two other variant alleles MDH_{-3}(80) and MDH_{-4}(125). MDH_{-3} and MDH_{-4} are actually a duplicated locus and the designation of the two variant alleles to MDH_{-3} and MDH_{-4} were purely arbitrary. However all workers agree that 4 loci are coding for $_{s}MDH$ in brown trout as well as other salmonids (Taggart et al., 1981). In this study MDH was examined using muscle, liver, heart, brain and eye but only heart tissue was used to screen populations as this gave the clearest representation of all 4 loci. $_{s}MDH_{2}$ was found to be commonly polymorphic with wild Scottish brown trout populations, and the variant allele was denoted $_{s}MDH_{-2}$(152) in accordance with May et al. (1979a) and Taggart et al. (1981). Polymorphism was also identified at the duplicated locus $_{s}MDH_{-3,4}$. It was thought that there was at least one variant allele slower



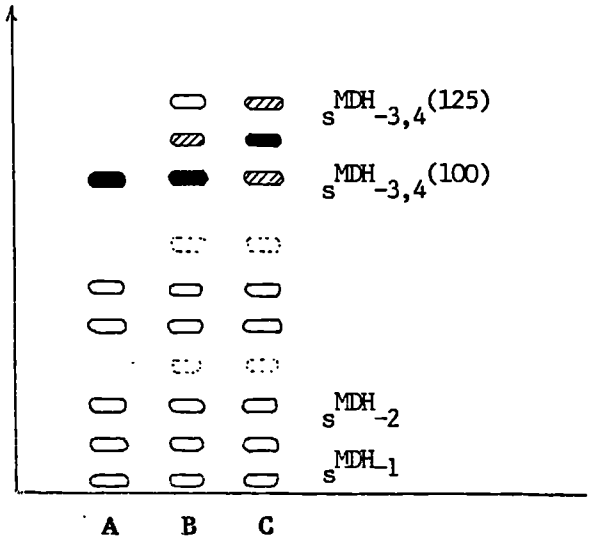
A = $_{s}MDH_{-2}(100/100)$

B = $_{s}MDH_{-2}(100/152)$

C = $_{s}MDH_{-2}(152/152)$

than that of $_{s}MDH_{3,4}(100)$ locus, but due to inconsistent staining and artefact problems, possibly due to length of storage these alleles were not used in population studies. Taggart et al. (1981) identified $_{s}MDH_{-3,4}(85)$ and $_{s}MDH_{-3,4}(75)$ variant alleles which were thought to be similar to the ones found in this study. As already mentioned Allendorf et al. (1977) noted the presence of $MDH_{-3}(80)$. Taggart and Ferguson (1984) have verified the presence of $_{s}MDH_{-3,4}(85)$ and (75) using breeding studies.

Another polymorphism was observed and was easier to identify, which was the $_{s}MDH_{-3,4}(125)$ allele. Unfortunately it was impossible to tell which locus was polymorphic but as only 9:6:1 and 1:2:1 staining patterns were observed it was concluded from the populations studied that only one locus exhibited polymorphism



A = $_{s}MDH_{-3,4}(100/100/100/100)$

B = $_{s}MDH_{-3,4}(100/100/100/125)$

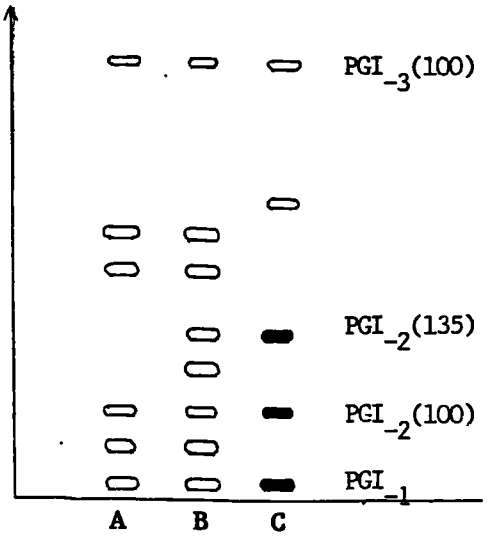
C = $_{s}MDH_{-3,4}(100/100/125/125)$

This is in agreement with Taggart et al. (1981) analysis and equivalent to $MDH_{-4}(125)$ reported by Allendorf et al. (1977). This study did not identify Taggart's $MDH_{-3,4}(135)$ variant allele.

Enzyme: Phosphoglucose isomerase Variant allele PGI-2 (135)

PGI is a dimeric enzyme and in brown trout there appear to be three loci coding for it. PGI_{-1} and PGI_{-2} are expressed most fully in muscle tissue and PGI_{-3} appears to be expressed most fully in eye and brain. Taggart et al. (1981) found in accordance with Avise and Kitto (1973) and Allendorf et al. (1977) that the most common zymogram for PGI in brown trout consisted of a six banded phenotype representing the random association of the products of three loci. This contradicts West German workers who found a common 3 banded zymogram which they interpreted as the expression of just 2 loci (Engel et al., 1975, 1977). This pattern coincides with a pattern reported by Taggart et al. (1981) which is the result of polymorphism at PGI_{-2} with a variant allele $PGI_{-2}(65)$ which in its homozygous form has the same mobility as PGI_{-1} and thus produces a three band effect. Taggart et al. (1981) suggests that $PGI_{-2}(65)$ could be fixed in the German population, and also report an additional three variant alleles segregating at PGI_{-2}, making a total of five (100, 135, 130, 122, 65).

In this study, only $PGI_{-2}(135)$ variant was positively identified



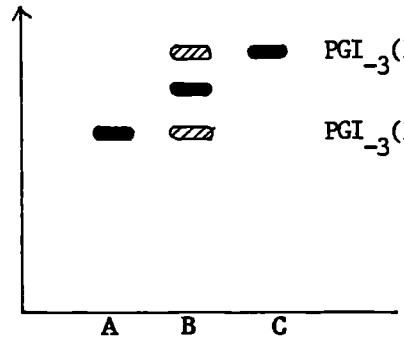
A = PGI_{-2}(100/1000

B = PGI_{-2}(100/135)

C = PGI_{-2}(135/135)

Variant allele PGI_{-3} (110)

Another variant allele was identified and used for screening although like the PGI_{-2}(135) it was a rare allele. It coincided with Taggart et al. (1981)'s PGI_{-3}(110). The three banded heterozygotes exhibited an approximate 1:2:1 staining intensity ratio and was therefore concluded to be typical of a polymorphism at a single locus for a dimeric enzyme.



A = PGI_{-3}(100/100)

B = PGI_{-3}(100/110)

C = PGI_{-3}(110/110)

Failure to identify CK polymorphism

It is noted that although the same variant allele has been identified for CK_{-1} by Taggart et al. (1981) and Allendorf et al. (1976) and termed CK_{-1}(115), this study did not obtain sufficiently good resolution to use the enzyme polymorphism to screen the different populations under consideration. The same procedures and staining mixtures

were used as recommended by Taggart et al. (1981) but CK did not resolve sufficiently at any stage in this study. The reasons for the failure to obtain sufficiently clear zymograms could be many. As Taggart et al. (1981) state, "even within a single technique such as starch gel electrophoresis resolution is determined by a number of interacting factors including make and batch of starch, gel concentration, buffer composition, pH and ionic strength, purity of buffer chemicals, and temperature, duration and field strength of the electrophoretic run".

Taggart et al. (1981) report difficulty in obtaining adequate resolution for AAT, as did Allendorf et al. (1977), whereas in this study AAT especially derived from muscle tissue stained up very clearly and was made more readable when a bad gel was obtained by placing the gel overnight in fixing solution.

Monomorphic loci also screened

AAT_{-3}	$G3PDH_{-1}$	ME_{-1}	PGM_{-1}
ADH_{-1}	$G-3PDH_{-3}$	ME_{-2}	PGM_{-2}
EST_{-1}	LDH_{-2}	ME_{-3}	SDH_{-1}
EST_{-2}	LDH_{-3}	MDH_{-1}	SDH_{-2}
EST_{-3}	LDH_{-4}	PGI_{-1}	SOD_{-1}
			SOD_{-2}

Total 21 monomorphic loci.

4.2.3 Analysis of Electrophoretic data

1. Calculation of allelic frequencies

Allelic frequencies calculated using the formula

$$\frac{2Ho + He}{2N}$$

Where Ho = number of homozygotes for the allele

He = number of heterozygotes for that allele

N = number of individuals examined

2. Hardy-Weinberg equilibrium

If a population is in Hardy-Weinberg equilibrium then the frequencies of the genotypes will be in the ratio of p^2 2pq and q^2 for a two allele polymorphism where p is the frequency of the allele A and q is the frequency of allele B.

Expected frequencies were calculated for all the alleles and for all the populations and the G-test was used to test for significant differences between the observed and expected values. The G-test was used in preference to the x^2 test for goodnes of fit because small numbers were involved in a lot of the populations and Sokal and Rohlf (1969) recommend the use of the G-test in these circumstances.

Ferguson (1980) points out that the calculation of degrees of freedom when examining genotype values has been wrongly calculated in the past. The 2 allele 3 genotype case typical of this study has one degree of freedom, not two, because the number of genotypes is less than N-1, and degrees of freedom are calculated by using the formula $\frac{1}{2}(n^2-n)$.

3. Heterozygosity

This is calculated per locus as $H = 1 - \Sigma x_i^2$ where x_i is the frequency of the ith allele at that locus.

The mean heterozygosity $\bar{H}L$ was calculated for all populations, and is the sum of HL over all loci divided by the total number of loci examined. All monomorphic loci are included in this calculation. Thus the more monomorphic loci included the smaller the value of $\bar{H}L$

4. Inter population heterogeneity of genotypic frequencies

Inter population heterogeneity in genotypic frequencies was tested using contingency tables and analysed for dissimilarities by Nass x^2 (Nass, 1959). The Nass Chi square is a very robust test and is little affected by zero of low expectations, the calculations are long and laborious and a computer programme written by Dr D. Skibinski and adapted by Dr. B. J. McAndrew was used in this survey.

5. Genetic identity and distance

As mentioned in the introduction there are various ways of calculating genetic distance/identity but in this study Nei's coefficient of genetic identity is the only one used.

Nei's coefficient $I = \dfrac{xi\ yi}{\sqrt{\Sigma x_i^2 \Sigma y_i^2}}$

Where xi and yi are the frequencies of the ith allele in the populations x and y.

This equation refers to the genetic identity between two populations, groups or species using one locus.

The main genetic identity ($\bar{I}$) which is much more meaningful and covers all loci studied including the monomorphic ones, and is most conveniently calculated as $\bar{I} = \frac{I \times y}{\sqrt{(Ix\ Iy)}}$ (Ferguson, 1980)

Where Ixy, Ix and Iy are the means, over all loci of Σ xiyi, Σ xi^2 and Σ yi^2 respectively.

In this study due to the large amount of calculation involved, using 34 loci (including 21 monomorphic) and approximately 60 populations the Nei's coefficient of identities was calculated using a Fortran computer program slightly modified from one used by McAndrew (1984).

From the genetic identities, genetic distances (D) can be computed $D = -\ln I$ McAndrew's program also computes genetic distances and standard errors.

The end product of these calculations is in the form of a matrix, and it is very difficult to fully interpret the data without transferring it into a pictoral representation.

Dendograms were constructed from Nei's distance matrix using unweighted pair-group arithmetic average (UPGMA) cluster analysis. (See Ferguson (1980) for worked example) and by using the cluster facility on

the SPSSX (statistical package for social scientists) package available on the VAX at Stirling University.

6. Gene diversity analysis

Ryman (1983) advises breaking down the gene diversity identified by electrophoresis into its component parts to enhance understanding of the nature of the diversity. To this end the allele frequency data was reorganised and used in a computer program capable of handling 5 levels of hierarchy. The program NEGST was obtained from Nils Ryman, but written by Chakraborty who used it to analyse brown trout data (Chakraborty et al., 1982). The gene diversity of the total population, H_T is decomposed into components, D_{1T} (between sub populations at first or highest level of subdivision) D_{21} (between sub populations of level (2) within each sub population) D_{32} (between sub populations of level (3) within each sub population of level (2)) D_{43} (between sub populations of level (4) within each sub population of level 3), D_{54} (between sub populations of level 5 within each sub population of level 4) and finally H_5 (between individuals within each sub population of level 5) to get

$H_T = H_5 + D_{54} + D_{43} + D_{32} + D_{21} + D_{1T}$

H_5 = within populations

D_{54} = between locations within lochs

D_{43} = between lochs within drainages

D_{32} = between drainages within areas

D_{21} = between areas within East/West divide (major drainage)

D_{1T} = between East/West divide

7. Linkage disequilibrium

To estimate whether one locus was linked to another 3 x 3 contingency tables were constructed using the genotypes of the two loci. To test significance for linkage disequilibrium, the x^2 test was used when all the cells of the table contained 5 individuals or more. When this was reduced below 5, Nass x^2 was used. Nass (1959) developed the test to cope with nil and small expectations in contingency tables. The calculations were completed using a computer program written by Dr. D. Ski binski and modified by Dr. B. McAndrew.

4.3 Results

4.3.1. Allele frequencies and heterozygosity

Table 4.8 lists the locations in Scotland sampled in this survey with accompanying map reference, year of sampling and the number of fish examined at each site. The first column numbers, on the left hand side of the table refer to the number codes given to each location, which can be found on the dendrograms later in the results and on the maps in the materials and methods. The rest of the table is comprised of the allele frequencies calculated for each polymorphic locus. The right hand side of the table gives the proportion of polymorphic loci (P:99% criterion) and the estimate of average heterozygosity (H). Both P and H are based on 34 loci.

The number of heterozygotes scored per individual was recorded for each of the locations examined electrophoretically, and the results for each location are presented in Table 4.9 A summary of the total heterozygosity of all wild brown trout examined is given in Table 4.10.

Table 4.8 Allele frequencies for all wild populations of brown trout examined. Figures in the table represent the proportion of the '100' allele at each polymorphic locus. H = Heterozygosity of each population and P = number of polymorphic loci for each population. (H and P estimates based on 34 loci.)

	Location	Map Ref.	Drainage River	Year of Sampling	No. of Fish	$AAT_{1,2}$ 100	$AAT_{1,2}$ 100	AAT_{4} 100	DIA 100	G3PDH 2 100	IDH 1 100	IDH 2 100	LDH_{1} 100	LDH_{5} 100	MDH_{2} 100	$MDH_{3,4}$ 100	PGI 2 100	PGI 3 100	P (%)	H (%)
	Loch Awe	Sheets 50		1983	33	0.90	1	0.71	1	0.82	0.86	1	0.97	0.53	0.62	0.91	1	0.95	26.5	7.1
(1)	East of Mollie	-55		1983	4	1	1	0.50	1	1	0.88	1	1	0.75	0.62	1	1	1	11.8	4.8
(2)	Mollie Burn			1983	3	1	1	0.67	1	1	0.83	1	1	0.50	0.67	0.67	1	1	14.7	6.2
(3)	Blar Ghour		1	1983	10	0.80	1	0.60	1	0.65	0.90	1	0.90	0.40	0.50	0.85	1	0.90	26.5	8.9
(4)	Unnamed burn			1983	9	0.83	1	0.89	1	0.72	0.83	1	1	0.39	0.55	0.94	1	1	20.6	6.6
(5)	Allt Ferna			1983	5	1	1	0.80	1	1	0.90	1	1	0.80	0.80	1	1	0.90	14.7	3.9
(6)	Inverliever			1983	2	1	1	0.75	1	1	0.75	1	1	1	1	1	1	1	5.9	2.2
(7)	Tangy Loch	68/692280	2	1983	47	0.65	1	0.86	1	0.89	1	1	0.96	0.94	0.78	1	1	0.97	20.6	4.3
(8)	Howietoun	57/785884	-	1984	82	0.76	0.91	0.84	0.97	0.88	0.85	0.91	0.99	0.96	0.81	0.99	0.98	0.92	38.2	6.4
(9)	Rannoch Moor	41/308537	3	1984	14	0.86	1	0.71	0.89	0.64	0.82	1	1	0.04	0.32	1	1	1	20.6	6.3
(10)	Pool B	41/310534	3	1984	15	0.87	1	0.43	0.93	0.87	0.93	1	1	0.23	0.37	1	1	1	20.6	6.0
(11)	Pool C	41/314530	3	1984	10	0.65	1	0.65	0.90	0.60	0.85	1	1	0.40	0.25	1	1	1	20.6	7.9
(12)	Pool D	41/317517	3	1984	10	0.80	1	0.85	0.70	0.70	0.85	1	1	0.45	0.50	1	1	1	20.6	7.9
(13)	Pool E	41/332516	3	1984	16	0.75	1	0.66	0.94	0.75	0.59	1	1	0.03	0.19	1	1	1	20.6	6.4
(14)	Loch Ba	41/330510	3	1984	6	1	1	0.83	1	0.92	0.42	1	1	0.17	0.92	1	1	1	14.7	4.0
(15)	Loch Laidon	41/360520	3	1984	34	0.94	1	0.93	0.94	0.71	0.93	0.99	0.93	0.29	0.41	1	1	1	26.5	5.7
(16)	Rannoch Stream 1	42/635592	3	1984	70	0.93	0.99	0.89	1	0.72	0.76	1	1	0.88	0.41	1	1	1	20.6	5.3
(17)	Stream 2	42/589585	3	1984	40	0.96	0.93	0.89	1	0.76	0.71	1	0.95	0.82	0.43	1	1	1	23.5	6.1
(18)	Stream 3	42/500567	3	1984	21	0.86	1	0.74	1	0.83	0.88	1	1	0.48	0.45	1	1	1	17.6	6.2
(19)	Stream 4	42/514568	3	1984	50	0.88	0.98	0.72	1	0.66	0.88	0.99	0.98	0.53	0.44	0.99	1	1	29.4	7.1
(20)	Stream 5	42/618572	3	1984	18	0.83	1	0.86	1	0.72	0.89	1	1	0.78	0.58	1	1	1	17.6	5.0
(21)	Loch Fincastle	43/975694	3	1983	15	0.70	1	0.93	0.93	0.87	0.67	0.93	0.97	0.93	0.63	0.80	1	1	29.4	7.3
(22)	Loch Vatigan	43/975692	3	1984	43	0.73	0.98	1	1	1	0.67	1	1	0.03	0.20	1	1	1	14.7	3.7
(23)	Loch Moraig	43/907667	3	1984	12	0.38	1	0.71	1	1	0.79	1	0.79	0.13	0.54	1	1	0.92	20.6	7.1
(24)	Loch an Duin	42/725800	4	1985	16	0.78	1	0.97	1	0.81	0.59	1	0.94	0.00	0.44	1	1	1	17.6	5.3
(25)	Bhrodain	42/744830	4	1985	44	0.84	1	1	1	0.86	0.65	1	0.98	0.47	0.51	0.97	1	1	20.6	6.1
(26)	Loch an Tseilach	42/756857	4	1985	13	0.69	1	1	1	0.92	0.73	1	1	0.65	0.58	0.92	1	1	17.6	6.1
(27)	Loch Pattack	42/540790	4	1984	36	1	1	1	1	0.24	0.90	1	1	0.14	0.57	1	1	1	11.8	3.7
(28)	L. A Bhealiaich Bheithe	42/512725	4	1984	40	0.98	1	1	1	0.01	0.95	1	1	0.00	0.55	1	1	1	11.8	1.9
(29)	Loch an Sgour	42/490750	4	1984	50	1	1	1	1	0.00	0.23	1	0.90	0.00	1	1	1	1	5.9	1.6
(30)	Loch na Criege Riabhaich	9/430505	5	1984	18	0.00	1	1	1	1	1	1	1	0.00	1	1	1	1	0.0	0.0
(31)	Loch nan Eun	19/772912	6	1984	6	0.67	1	1	1	0.83	0.58	1	1	0.92	0.67	1	1	1	14.7	4.6
(32)	Loch an Draing	19/775902	6	1984	14	0.82	1	0.96	1	0.68	0.68	1	1	0.89	0.86	1	1	1	17.6	5.0
(33)	Loch Fionn (W.R.)	19/950785	7	1985	40	0.36	1	0.39	1	0.81	0.86	1	1	0.69	0.75	0.98	0.81	1	23.5	7.8
(34)	L. A' Mha Daidh Mor	19/966866	8	1985	37	1	1	0.51	1	1	0.90	1	0.62	1.00	0.41	0.30	1	1	14.7	6.1
(35)	L. A'Bhealiach	19/870640	9	1984	43	1	1	0.21	1	1	0.46	1	0.99	0.04	1	1	1	1	11.8	2.7
(36)	L. Gaineamnach	19/834670	9	1984	50	0.99	1	0.20	1	1	0.41	1	1	0.08	1	1	1	1	11.8	2.8
(37)	L. Gaineamnach		9	1985	51	0.81	1	0.17	0.97	0.99	0.61	1	1	0.00	1	1	1	1	14.7	3.4
(38)	L. Morrisdale	19/797705	9	1984	50	0.96	1	0.56	0.90	0.99	0.90	1	1	0.11	0.82	0.93	1	1	23.5	4.6
(39)	L. Morrisdale		9	1985	41	1	1	0.39	0.96	0.87	0.94	1	1	0.04	0.71	0.88	1	1	20.6	4.8

continued . . .

Table 4.8 continued

Location	Map Ref.	Drainage River	Year of Sampling	No. of Fish	AAT_{12} 100	AAT_{12} 100	AAT_{4} 100	DIA 100	$G3PDH_{-2}$ 100	IDH_{-1} 100	IDH_{-2} 100	LDH_{1} 100	LDH_{5} 100	MDH_{2} 100	MDH_{34} 100	PGI_{-2} 100	PGI_{-3} 100	P (%)	H (%)
(40) L. Badachro	19/785728	9	1984	14	0.82	0.89	0.61	0.89	1	1	1	1	0.11	0.61	1	1	0.93	20.6	5.8
(41) Loch Clair	19/773717	9	1984	30	0.95	0.92	0.85	0.88	0.83	1	1	1	0.17	0.80	1	0.90	1	23.5	5.2
(42) L. An Easlachan	15/175090	10	1985	22	1	1	0.98	1	0.98	0.68	1	1	1	0.00	1	1	1	8.8	1.5
(43) L. Crocach	15/105275	10	1985	26	1	0.81	0.98	1	1	0.98	0.94	1	1	0.94	1	1	1	14.7	1.8
(44) Loch Veyatie	15/190130	12	1985	60	0.96	1	0.94	1	0.98	0.72	1	1	0.83	0.48	1	0.66	1	20.6	5.5
(45) L. Druim Suardalain	15/098217	13	1985	97	0.83	0.99	0.89	1	0.85	0.69	1	1	0.91	0.72	1	0.95	1	23.5	5.4
(46) Loch 'Gillaroo'	15/276194	14	1986	6	0.92	1	0.67	1	0.92	1	1	1	1	0.92	1	1	1	11.8	2.6
(47) Loch Awe	15/245154	14	1986	13	0.96	1	1	1	0.54	1	1	1	1	0.85	0.96	1	1	11.8	2.7
(48) Loch Beannach	15/140265	14	1986	20	1	1	0.93	1	1	1	1	1	0.90	0.88	1	1	1	8.8	1.6
(49) Loch Assynt	15/200250	14	1986	41	0.85	1	0.77	1	0.80	0.90	1	1	0.95	0.54	0.92	1	1	20.6	5.4
(50) Fionn Loch	15/130176	12	1986	18	0.97	1	1	1	1	0.83	1	1	0.72	0.28	1	0.75	1	14.7	6.5
(51) L. Beag A' Chocair	8/342346	15	1985	10	0.90	1	0.70	1	0.80	0.95	1	1	1	0.80	1	0.85	1	17.6	4.7
(52) N. Uist (A) Unknown	Sheet 22		1983	10	0.95	1	1	1	0.80	1	1	1	1	0.90	1	0.95	1	11.8	2.0
(53) N. Uist (B) Unknown	Sheet 22		1983	6	1	1	0.75	1	0.92	0.83	1	1	0.83	0.67	0.92	0.92	1	20.6	5.4
(54) N. Uist (C) Unknown	Sheet 22		1983	9	0.56	1	0.67	1	0.72	0.78	1	1	0.94	0.33	1	1	1	17.6	6.6
(55) Ferox different lochs			1983-1985	12	0.71	1	1.00	1	0.83	0.88	1	1	0.54	0.92	0.96	1	1	17.6	4.8
(56) Loch Quoich	33/020020	16	1985	20	0.54	1	0.73	1	0.85	0.70	1	1	0.98	0.45	0.95	1	1	20.6	6.5
(57) Loch Rannoch	42/600580	3	1985	6	0.80	1	1	1	6.75	0.75	1	1	1	0.83	1	1	1	11.8	3.1
(58) Loch Ness	Sheets 24-26	17	1985	24	0.92	1	0.96	1	0.85	0.90	1	0.96	0.83	0.60	1	1	1	20.6	4.4
(59) Howietoun (Pond 14)	57/785884	-	1984	36	0.83	0.93	0.96	0.97	0.86	0.94	0.99	0.97	0.99	0.71	0.99	0.99	0.96	38.2	4.5
(60) Crocach x Nashua		-	1984	10	0.25	1	1	1	1	0.85	1	1	0.50	0.65	1	1	1	8.8	4.6
(61) Nashua x Nashua		-	1984	17	0.56	1	1	0.79	1	0.91	1	1	0.11	1	1	1	1	14.7	4.9
(62) L. A'Ghobhainn	19/870640	9	1985	42	0.95	1	0.36	1	0.98	0.41	1	1	0.11	1	1	1	1	14.7	3.7
(63) River Earn (S.T.)		3	1984	10	0.56	1	0.95	1	1	0.80	1	1	1	0.95	1	1	1	11.8	2.9
(64) Leven		18	1984	360	0.83	1	0.76	1	0.92	0.87	1	0.99	1	0.75	0.96	1	1	20.6	4.4

Table 4.9 Listing heterozygote frequencies/individual for all the populations examined

Location code	1 - 6		7		8 + 59		9		10		11		12		13		14		15		16		17		18		19		20	
No. of hets/ individual	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
0	2	6.1	5	18.5	15	11.7	3	21.4	0	0.0	0	0.0	0	0.0	2	12.5	2	33.3	8	23.5	7	10.0	4	10.0	1	4.8	5	10.0	2	11.1
1	9	27.0	12	44.4	30	23.4	4	28.6	5	33.3	2	20.0	1	10.0	4	25.0	2	33.3	9	26.5	21	29.6	11	27.5	7	33.3	14	28.0	7	38.9
2	11	33.0	7	25.9	40	31.2	3	21.4	4	26.7	4	40.0	1	10.0	5	31.2	1	16.7	8	23.5	28	40.0	17	42.5	6	28.6	13	26.0	6	33.3
3	6	18.2	3	11.2	28	21.9	2	14.3	4	26.7	3	30.0	5	50.0	3	18.8	1	16.7	7	20.6	11	15.7	6	15.0	6	28.6	12	24.0	1	5.5
4	3	9.1	0	0	5	3.9	2	14.3	1	6.6	1	10.0	2	20.0	2	12.5	0	0	2	5.9	1	1.4	1	2.5	1	4.8	5	10.0	2	11.1
5	2	6.6	0	0	0	0	0	0	1	6.6	0	0	1	10.0	0	0	0	0	1	2.9	1	1.4	1	2.5	0	0	1	2.0	0	0
6	0	0.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1.4	0	0	0	0	0	0	0	0

Location code	21		22		23		24		25		26		27		28		29		30		31		32		33		34		35	
No. of hets/ individual	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
0	0	0	9	20.9	0	0	3	18	5	11.4	0	0	2	5.6	14	35.0	26	52.0	18	100	0	0	1	7.1	2	5.0	2	4.6	10	23.2
1	2	13.3	23	53.5	2	16.6	6	37	12	27.3	6	46.1	14	38.9	23	57.5	20	40.0	0	0	2	33.3	6	42.8	8	20.0	8	21.6	22	51.2
2	3	20.0	9	20.9	6	50.0	3	18	11	25.0	3	23.1	17	47.2	3	7.5	4	8.0	0	0	1	16.7	4	28.6	16	40.0	16	43.2	11	25.6
3	6	40.0	2	4.7	2	16.6	3	18	12	27.3	3	23.1	3	8.3	0	0	0	0	0	0	2	33.3	1	7.1	10	25.0	10	27.0	0	0
4	3	20.0	0	0	1	8.3	1	6	4	9.1	1	7.7	0	0	0	0	0	0	0	0	1	16.7	2	14.2	1	2.5	1	2.7	0	0
5	1	6.7	0	0	1	8.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Location code	36 + 37		38 + 39		40		41		42		43		44		45		46		47		48		49		50		51		52+53+54	
No. of hets/ individual	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
0	41	40.6	10	10.9	1	7.1	5	16.7	8	36.4	13	50.0	6	10.0	13	13.4	2	33.3	3	23.1	9	45.0	7	17.0	1	5.6	1	10.0	5	20.0
1	43	42.6	30	33.0	2	14.2	8	26.7	13	59.1	10	38.5	24	40.0	30	30.9	3	50.0	5	38.5	9	45.0	10	24.4	9	50.0	2	20.0	8	32.0
2	13	12.9	38	41.8	7	50.0	11	36.7	1	4.5	3	11.5	22	36.7	33	34.0	1	16.7	4	30.8	2	10.0	15	36.6	5	27.8	4	40.0	7	28.0
3	3	2.9	10	10.9	4	28.4	5	16.7	0	0	0	0	6	10.0	17	17.5	0	0	1	7.7	0	0	7	17.0	3	16.7	3	30.0	4	16.0
4	1	0.9	3	3.4	0	0	1	3.3	0	0	0	0	2	3.3	4	4.1	0	0	0	0	0	0	2	4.8	0	0	0	0	1	4.0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

continued . . .

Table 4.9 continued

Location code	55		56		57		58		60		61		62		63	
No. of hets/ individual	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
0	3	25.0	4	20.0	2	33.3	4	16.7	0	0	0	0	7	16.7	4	40.0
1	5	417	6	30.0	2	33.3	9	37.5	1	10.0	5	29.4	22	52.4	2	20.0
2	2	16.7	6	30.0	2	33.3	9	37.5	4	40.0	10	58.8	12	28.6	3	30.0
3	0	0	3	15.0	0	0	2	8.3	4	40.0	1	5.9	1	5.0	1	10.0
4	1	8.3	0	0	0	0	0	0	1	10.0	1	5.9	0	0	0	0
5	1	8.3	1	5.0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 4.10 Individual heterozygosity for wild trout in Scotland

No. of hets per individual	No. of fish (all lochs)	%	Representing individual heterozygosity (inc. monomorphics)
0	299	18.2	0.00
1	560	34.1	0.029
2	480	29.2	0.059
3	229	13.9	0.088
4	63	3.8	0.118
5	12	0.7	0.147
6	1	0.1	0.176
TOTALS	**1644**	**100**	**0.045**

Tables containing the results of individual tests to calculate whether each population was in Hardy-Weinberg equilibrium for each of the polymorphic loci studied, are regarded as too large to include in this volume, but are available on request. The tables are constructed giving the observed and expected genotypes for each location along with the appropriate allele frequency. The differences between the observed and expected values was tested using the G-test and the results appear along with the associated significance level.

4.3.2 Gene diversity analysis

Table 4.11 and 4.12 represent the results of the gene diversity analysis using 58 wild populations. Populations 8 and 59 were left out because the Howietoun fish farm stock does not truly represent a wild population. Population 55 was not used because the 'ferox' trout which made it up came from different locations. Populations 60 and 61 were not included because of their artificial nature. (60 being a hatchery strain and 61 a cross between the hatchery strain (60) and a wild population (30)).

The analyses used was that described in the materials and methods section.

4.3.3 Genetic distances and identities

Figure 4.4 is a graphical representation of the genetic relationship of the population of trout studied. This includes all 63 populations including hatchery ones. The dendrogram was constructed as explained in the materials and methods. The tables listing Nei's genetic distance and the standard errors of the distances calculated are too large to inlcude, but are available if required.

Parts of the whole table are reproduced to illustrate genetic relationships within small areas.

4.3.3.1 Illustration of genetic diversity within small geogrphic areas

1. Two areas have been selected to illustrate the use of Nei's genetic distance and the subsequently constructed dendogram. The Badachro system comprises 6 lochs situated in the North-west of Scotland adjacent to the sea. Figure 4.5 and Table 4.13 relate to this system.

Table 4.11 Absolute gene diversities. Scottish brown trout

Locus	Within Populations (H5)	Between locations within lochs (D54)	Between lochs within separate drainages (D43)	Between drainages within areas (D32)	Between areas within east/west watershed (D-21)	Between east/west watershed (D-1T)	Total (HT)
$AAT_{1,2}$ (140)	0.1893	0.0030	0.0204	0.0130	0.0360	0.0020	0.2631
$AAT_{-1,2}$(45)	0.0155	0.0001	0.0015	0.0002	0.0001	0.0000	0.0174
AAT_{-4} (74)	0.2454	0.0058	0.0296	0.0219	0.0399	0.0048	0.3476
DIA_{-1} (90)	0.0355	0.0001	0.0037	0.0004	0.0004	0.0003	0.0405
$G3PDH_{-2}$ (50)	0.2029	0.0054	0.0172	0.0046	0.0642	0.0002	0.2945
IDH_{-1}(160)	0.2669	0.0024	0.0386	0.0086	0.0099	0.0009	0.3274
IDH_{-2} (130)	0.0049	0.0000	0.0003	0.0000	0.0000	0.0000	0.0052
LDH_{-1} (0)	0.0312	0.0004	0.0017	0.0045	0.0003	0.0000	0.0382
LDH_{-5}(105)	0.2063	0.0158	0.0695	0.0491	0.1538	0.0044	0.4991
MDH_{-2}(152)	0.3419	0.0106	0.0457	0.0322	0.0118	0.0205	0.4629
$MDH_{-3,4}$ (125)	0.452	0.0030	0.0021	0.0148	0.0015	0.0004	0.0672
PGI_{-2}(135)	0.0328	0.0002	0.0004	0.0058	0.001	0.0004	0.0405
PGI_{-3}(110)	0.0119	0.0005	0.0004	0.000	0.0002	0.0000	0.0130
Mean and SE including monomorphic loci:							
Mean	0.048	0.001	0.007	0.005	0.009	0.001	0.071
SE	0.016	0.0005	0.003	0.002	0.005	0.0006	0.025
Mean and SE excluding monomorphic loci:							
Mean	0.125	0.004	0.018	0.012	0.025	0.003	0.186
SE	0.033	0.001	0.006	0.004	0.012	0.002	0.051

Table 4.12 Relative gene diversity expressed in % terms

Locus	Within Populations	Between locations within lochs	Between lochs within separate drainages	Between drainages within areas	Between areas within east/ west watershed	Between east/west watershed	Total
$AAT_{-1,2}(140)$	71.95	1.09	7.78	4.77	13.77	0.62	1.0000
$AAT_{-1,2}(45)$	88.92	0.73	8.72	0.89	0.66	0.06	1.0000
$AAT_{-4}(74)$	70.58	1.67	8.52	6.32	11.50	1.38	1.0000
$DIA_{-1}(90)$	87.73	0.44	9.03	1.04	0.89	0.84	1.0000
$G3PDH_{-2}(50)$	68.89	1.84	5.84	1.55	21.78	0.07	1.0000
$IDH_{-1}(160)$	81.52	0.73	11.79	2.64	3.01	0.29	1.0000
$IDH_{-2}(130)$	94.44	0.05	4.95	0.26	0.21	0.06	1.0000
$LDH_{-1}(0)$	81.72	0.94	4.59	11.89	0.79	0.05	1.0000
$LDH_{-5}(105)$	41.34	3.16	13.93	9.84	30.83	0.89	1.0000
$MDH_{-2}(152)$	73.88	2.28	9.88	6.97	2.55	4.42	1.0000
$MDH_{-3,4}(125)$	67.24	4.50	3.26	22.01	2.33	0.64	1.0000
PGI_{-2} (135)	80.34	0.42	0.93	14.34	2.74	1.21	1.0000
$PGI_{-3}(110)$	92.13	3.53	2.73	0.07	1.47	0.06	1.0000
Mean and SE excluding monomorphic loci:							
Mean	67.43 %	1.95 %	9.57	6.41	13.23	1.40	1.000
SE	6.15	0.34	1.15	1.22	4.73	0.66	

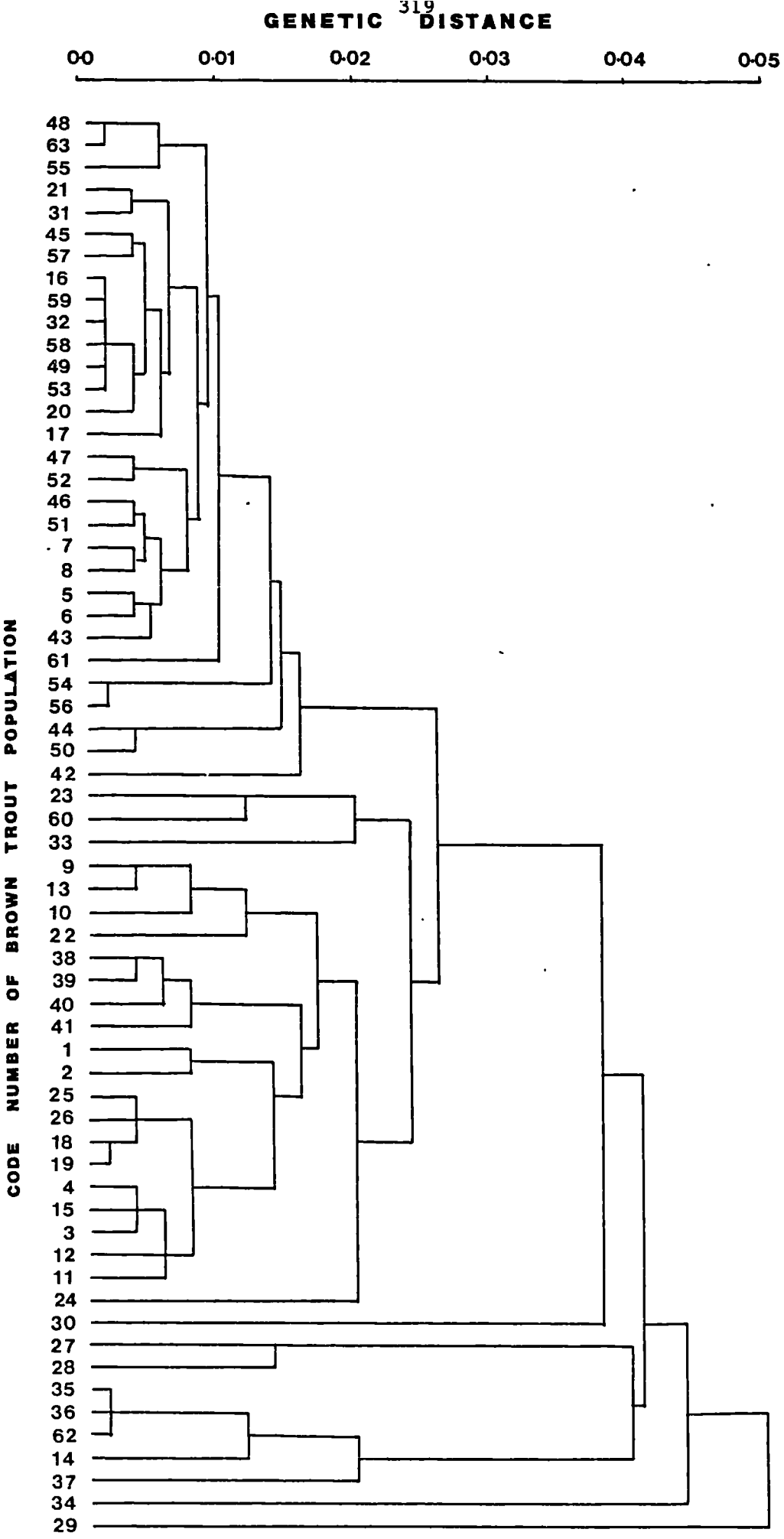


Figure 4.4 Dendrogram for brown trout constructed from the allele frequencies in Table 4.8. Designation of locations is explained in Table 4.3

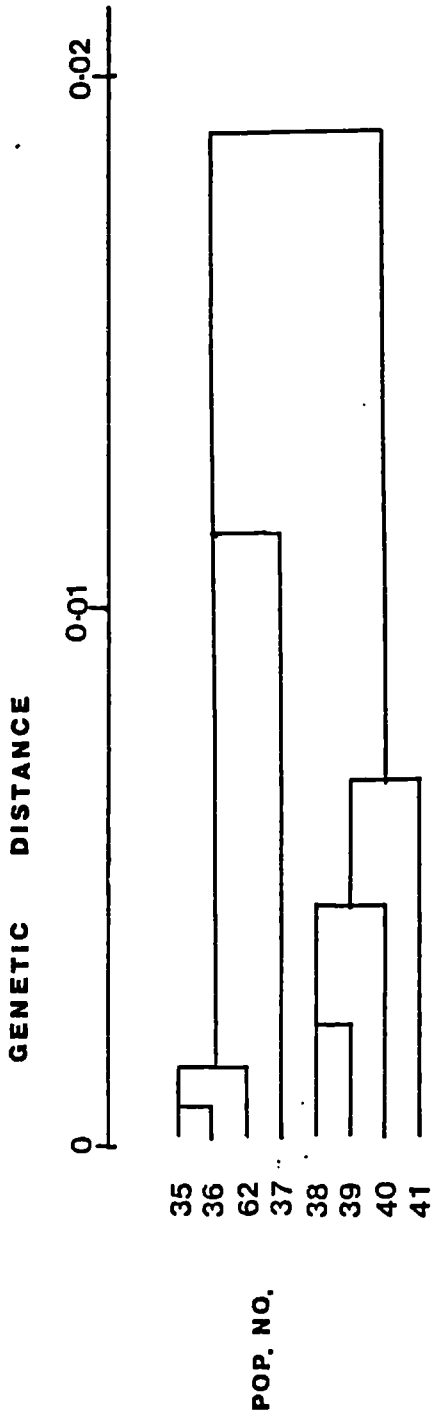


FIGURE : 4.5 DENDROGRAM ILLUSTRATING THE GENETIC RELATIONSHIP OF BROWN TROUT IN THE BADACHRO AREA

Table 4.13 Listing Nei's genetic distance (below the diagonal with corresponding standard errors (above the diagonal) for populations of trout in the Badachro system.

	35	36	37	38	39	40	41	62
35		0.000	0.014	0.007	0.008	0.011	0.016	0.001
36	0.000		0.013	0.009	0.009	0.013	0.017	0.001
37	0.016	0.015		0.014	0.015	0.016	0.020	0.013
38	0.011	0.013	0.022		0.001	0.002	0.003	0.008
39	0.012	0.013	0.024	0.002		0.002	0.007	0.009
40	0.210	0.022	0.029	0.003	0.005		0.002	0.012
41	0.025	0.027	0.034	0.005	0.00(	0.005		0.022
62	0.001	0.001	0.015	0.010	0.012	0.019	0.021	

Rows and column numbers refer to codes for populations given in materials and methods

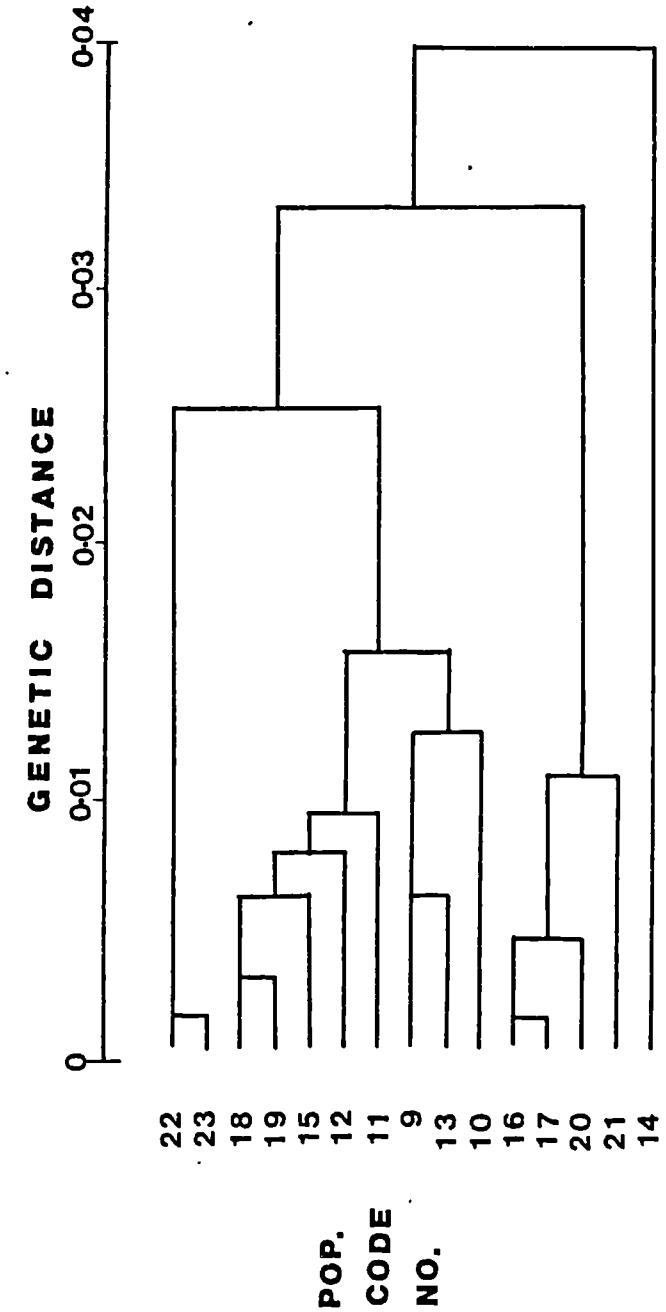


FIGURE: 4.6 DENDROGRAM ILLUSTRATING THE GENETIC RELATIONSHIPS OF BROWN TROUT IN THE LOCH RANNOCH AREA

Table 4.13 Listing Nei's genetic distances (below the diagonal) with corresponding standard errors (above the diagonal) for lochs in the Rannoch area of Perthshire.

	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
9		0.003	0.004	0.006	0.002	0.013	0.003	0.023	0.020	0.006	0.008	0.018	0.026	0.005	0.009
10	0.006		0.003	0.006	0.004	0.014	0.008	0.015	0.013	0.004	0.004	0.011	0.018	0.010	0.008
11	0.006	0.007		0.003	0.005	0.016	0.004	0.008	0.007	0.003	0.002	0.002	0.006	0.011	0.007
12	0.008	0.011	0.006		0.007	0.009	0.002	0.006	0.005	0.003	0.003	0.004	0.008	0.007	0.008
13	0.003	0.008	0.008	0.014		0.017	0.005	0.023	0.020	0.007	0.009	0.019	0.027	0.004	0.006
14	0.021	0.023	0.030	0.019	0.022		0.012	0.018	0.016	0.010	0.011	0.014	0.019	0.017	0.014
15	0.005	0.009	0.007	0.004	0.011	0.019		0.011	0.009	0.002	0.002	0.008	0.014	0.004	0.010
16	0.024	0.022	0.013	0.010	0.028	0.029	0.012		0.000	0.005	0.004	0.001	0.003	0.023	0.021
17	0.022	0.020	0.013	0.009	0.026	0.025	0.011	0.000		0.004	0.003	0.001	0.003	0.020	0.019
18	0.008	0.005	0.005	0.004	0.012	0.018	0.003	0.007	0.006		0.001	0.003	0.007	0.007	0.009
19	0.009	0.007	0.004	0.004	0.014	0.021	0.004	0.005	0.005	0.001		0.002	0.006	0.009	0.010
20	0.021	0.018	0.011	0.007	0.027	0.024	0.009	0.002	0.003	0.004	0.003		0.002	0.019	0.015
21	0.035	0.030	0.021	0.014	0.037	0.028	0.021	0.006	0.006	0.012	0.013	0.005		0.027	0.021
22	0.090	0.016	0.015	0.016	0.006	0.022	0.010	0.028	0.025	0.013	0.018	0.027	0.034		0.014
23	0.015	0.014	0.015	0.017	0.014	0.023	0.017	0.033	0.031	0.014	0.019	0.025	0.030	0.012	

The numbered columns and rows represent the codes for the lochs/location studied in the Rannoch area.

The Rannoch area situated in the centre of Scotland comprises many lochs and lochans, here 15 different populations are considered. Figure 4.6 and Table 4.14 relate to this system.

2. The second way of illustrating genetic diversity involves construction of allele frequency pie charts adjacent to a map of the lochs or locations which the pie charts purport to represent. In conjunction with the map, are a series of contingency tables representing the genotypes of the individuals scored in each location, from which the allele frequencies were calculated. The contingency tables are examined for levels of significance using Nass x^2 thus giving a quantitative analysis of the levels of difference between locations.

Allele frequencies can be tested for significance but as they are calculated using the genotypes it was felt that there was no need to transform the data any more than necessary. Figure 4.7 and Table 4.15 illustrate the situation in the Badachro system. (See map).

Figure 4.8 and Tables 4.16 illustrate the situation found on Rannoch Moor.

Figure 4.9 and Table 4.17 illustrate the situation found at Loch Rannoch.

Figure 4.10 and Tables 4.18 illustrate the situation found at Loch Pattack and associated lochs.

Figures 4.11 and Tables 4.19 illustrate the situation found on the

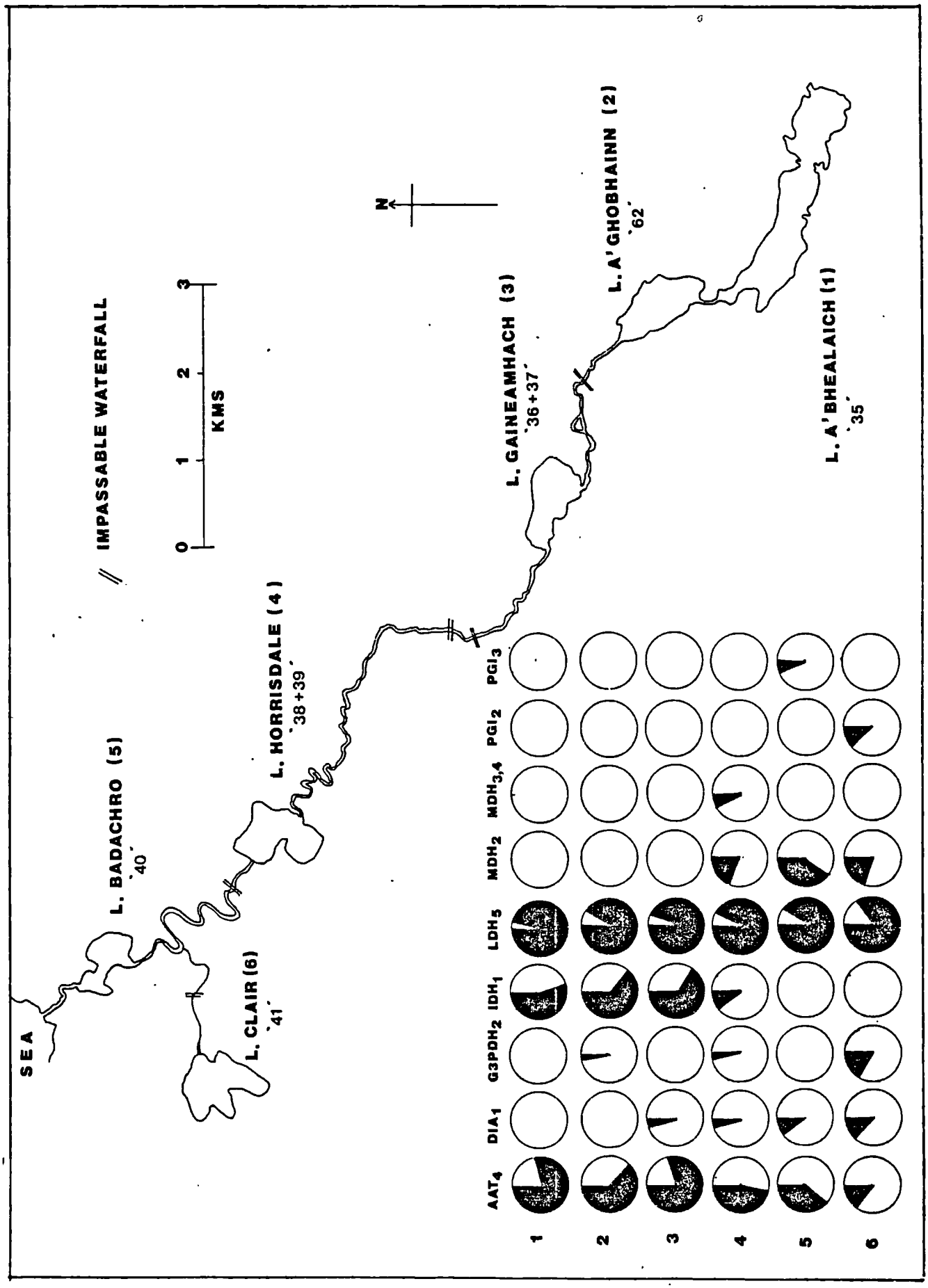


Figure 4.7 The Badachro system showing lochs sampled and allele frequencies of polymorphic loci

Tables 4.15 Illustrating genotypes of individual fish used to calculate allele frequencies illustrated in Figure 4.7 (Badachro System)

$AAT_{1\ 2}$ (140)			
Genotype Location	100/100	100/140	140/140
1	43	0	0
2	40	2	0
3	84	14	3
4	87	4	0
5	10	3	1
6	27	3	0
Nass x^2 = 21.58 df = 9.10 Sign P<0.05			

$AAT_{1,2}$(45)			
Genotype Location	100/100	100/45	45/45
1	43	0	0
2	42	0	0
3	01	0	0
4	91	0	0
5	11	3	0
6	25	5	0
Nass x^2 = 48 df = 4.61 Sign P< 0.001			

AAT_4(74)			
Genotype Location	100/100	100/74	74/74
1	2	14	27
2	4	22	16
3	8	21	72
4	19	39	23
5	5	7	2
6	21	9	0
Nass x^2 = 114.73 df 10.30 Sign P< 0.001			

DIA_1(90)			
Genotype Location	100/100	100/90	90/90
1	43	0	0
2	42	0	0
3	98	3	0
4	78	13	0
5	11	3	0
6	21	8	1
Nass x^2 = 20.00 df = 6.52 Sign P<0.01			

$G3PDH_2$(50)			
Genotype Location	100/100	100/50	50/50
1	43	0	0
2	41	0	1
3	100	1	0
4	81	8	2
5	14	0	0
6	22	6	2
Nass x^2 = 32.99 df = 9.19 Sign P<0.001			

IDH_{-1}(160)			
Genotype Location	100/100	100/160	160/160
1	7	26	10
2	7	20	15
3	33	37	31
4	78	11	2
5	14	0	0
6	30	0	0
Nass x^2 = 150.65 df = 10.29 Sign P<0.001			

Table 4.15 continued

LDH_{-5}(105)			
Genotype Location	100/100	100/105	105/105
	1	2	43
	2	5	35
3	2	4	96
4	1	12	78
5	0	3	11
6	3	4	23
Nass χ^2 = 17.60 df = 9.72 NS (at P =0.05)			

MDH_{-2}(152)			
Genotype Location	100/100	100/152	152/152
1	43	0	0
2	42	0	0
3	101	0	0
4	51	38	2
5	5	7	2
6	20	8	2
Nass χ^2 = 109.76 df = 9.54 Sign P<0.001			

MDH_{-34}(125)			
Gentoype Location	100/100	100/125	125/125
1	43	0	0
2	42	0	0
3	101	0	0
4	74	17	0
5	14	0	0
6	30	0	0
Nass χ^2 = 44.30 df = 4.89 Sign P<0.001			

PGI_{-2}(135)			
Genotype Location	100/100	100/135	135/135
1	43	0	0
2	42	0	0
3	101	0	0
4	91	0	0
5	14	0	0
6	24	6	0
Nass χ^2 = 52.48 df = 4.44 Sign P<0.001			

PGI_{-3}(110)			
Genotype Location	100/100	100/110	110/110
1	43	0	0
2	42	0	0
3	01	0	0
4	91	0	0
5	12	2	0
6	30	0	0
Nass χ^2 = / df = Sign P /			

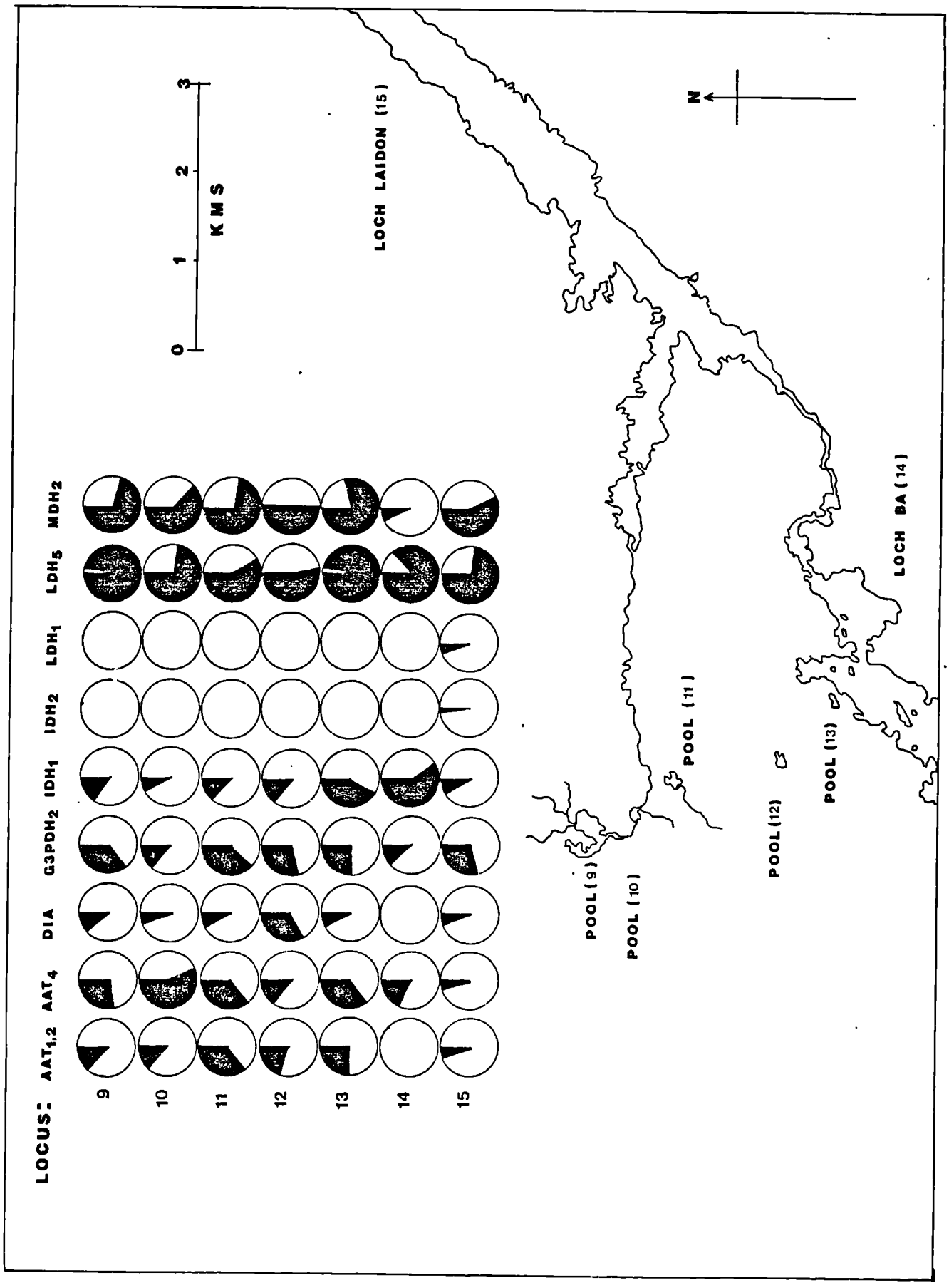


Figure 4.8 The Rannoch Moor area showing locations sampled and allele frequencies of polymorphic loci

Tables 4.16 Listing gentoypes of individual fish used to calculate allele frequencies illustrated in Figure 4.8 (Rannoch Moor)

$AAT_{1,2}$(140)

Genotype Location	100/100	100/140	140/140
9	10	4	0
10	11	4	0
11	5	3	2
12	7	2	1
13	10	4	2
14	6	0	0
15	31	2	1

Nass x^2 = 18.214 df = 12.93 NS (P = 0.05)

AAT_{-4}(74)

Genotype Location	100/100	100/74	74/74
9	7	6	1
10	2	9	4
11	5	3	2
12	7	3	0
13	7	7	2
14	4	2	0
15	29	5	0

Nass x^2 = 32.568 df = 13.05 Sign P<0.01

DIA_{-1}(90)

Genotype Location	100/100	100/90	90/90
9	12	1	1
10	13	2	0
11	8	2	0
12	4	6	0
13	14	2	0
14	6	0	0
15	30	4	0

Nass x^2 = 22.674 df = 11.75 Sign P<0.05

$G\text{-}3PDH_2$(50)

Genotype Location	100/100	100/50	50/50
9	6	6	2
10	11	4	0
11	4	4	2
12	4	6	0
13	9	6	1
14	5	1	0
15	18	12	4

Nass x^2 = 11.822 df = 13.06 NS (P = 0.05)

IDH_{-1}(160)

Genotype Location	100/100	100/160	160/160
9	10	3	1
10	13	2	0
11	8	1	1
12	7	3	0
13	6	7	3
14	1	3	2
15	30	3	1

Nass x^2 = 30.02 df = 13.01 Sign P<0.01

IDH_{-2}(130)

Genotype Location	100/100	100/130	130/130
9	14	0	0
10	15	0	0
11	10	0	0
12	10	0	0
13	16	0	0
14	6	0	0
15	33	1	0

Nass x^2 = 1.836 df = 5.27 NS (P = 0.05)

Table 4.16 continued

LDH_{-1}(Ø)

Genotype Location	100/100	100/Ø	Ø/Ø
9	14	0	0
10	15	0	0
11	10	0	0
12	10	0	0
13	16	0	0
14	6	0	0
15	29	5	0

Nass χ^2 = 11.325 df = 6.26 NS (P = 0.05)

LDH_{-5}(105)

Genotype Location	100/100	100/105	105/105
9	0	1	13
10	2	3	10
11	2	4	4
12	2	5	3
13	0	1	15
14	1	0	5
15	6	8	20

Nass χ^2 = 24.209 df = 13.08 Sign P<0.05

MDH_2(152)

Genotype Location	100/100	100/152	152/152
9	3	3	8
10	1	9	5
11	0	5	5
12	0	6	2
13	1	4	11
14	5	1	0
15	6	16	12

Nass χ^2 = 35.977 df = 13.14 Sign P<0.001

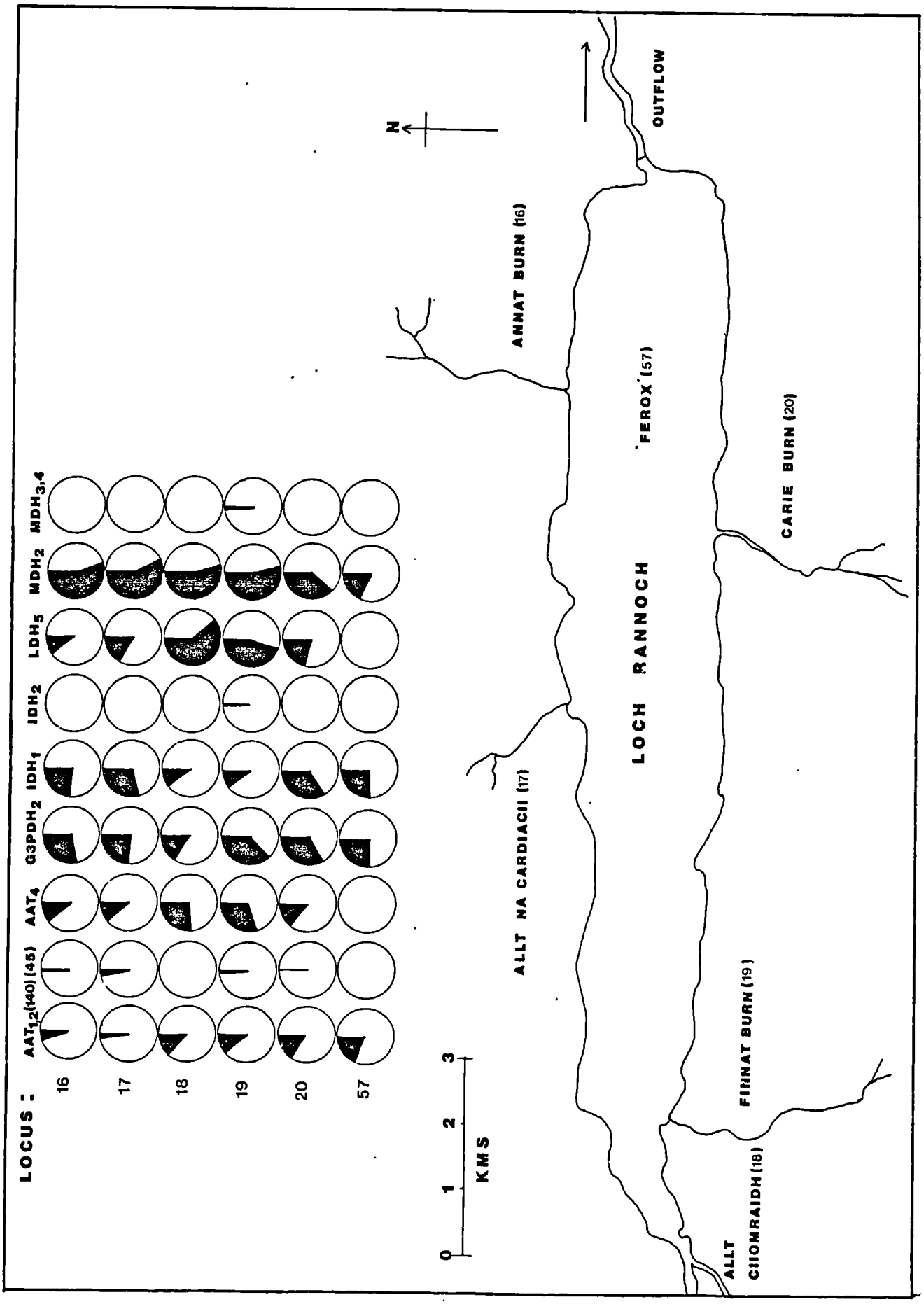


Figure 4.9 Loch Rannoch showing locations sampled and allele frequencies of polymorphic loci

Tables 4.17 Listing genotypes of individudal fish used to calculate allele frequencies illustrated in Figure 4.9 (Loch Rannoch)

$AAT_{1,2}$(140)

Gentoype Location	100/100	100/140	140/140
16	61	8	1
17	37	3	0
18	16	4	1
19	39	10	1
20	13	4	1
57	6	0	0

Nass x^2 = 7.517 df = 8.23 NS (P = 0.05)

$AAT_{1,2}$(45)

Genotype Location	100/100	100/45	45/45
16	68	2	0
17	34	6	0
18	21	0	0
19	48	2	0
20	18	0	0
57	6	0	0

Nass x^2 = 7.322 df = 4.68 NS (P = 0.05)

AAT_4 (74)

Genotype Location	100/100	100/74	74/74
15	57	11	2
17	33	5	2
18	11	9	1
19	27	18	5
20	13	5	0
57	6	0	0

Nass x^2 = 21.28 df = 9.67 Sign P<0.05

$G\text{-}3PDH_2$(50)

Genotype Location	100/100	100/50	50/50
16	34	33	3
17	25	11	4
18	14	7	0
19	23	20	7
20	10	6	2
57	3	3	0

Nass x^2 = 11.822 df =13.06 NS (P = 0.05)

IDH_{-1}(160)

Genotype Location	100/100	100/160	160/160
16	44	18	8
17	20	17	3
18	17	3	1
19	40	8	2
20	15	2	1
57	4	1	1

Nass x^2 = 30.02 df = 13.00 Sign P<0.01

IDH_{-2}(130)

Genotype Location	100/100	100/130	130/130
16	70	0	0
17	40	0	0
18	21	0	0
19	49	1	0
20	18	0	0
57	6	0	0

Nass $\bar{x}^2$ = 1.836 df = 5.24 NS (P = 0.05)

Table 4.17 continued

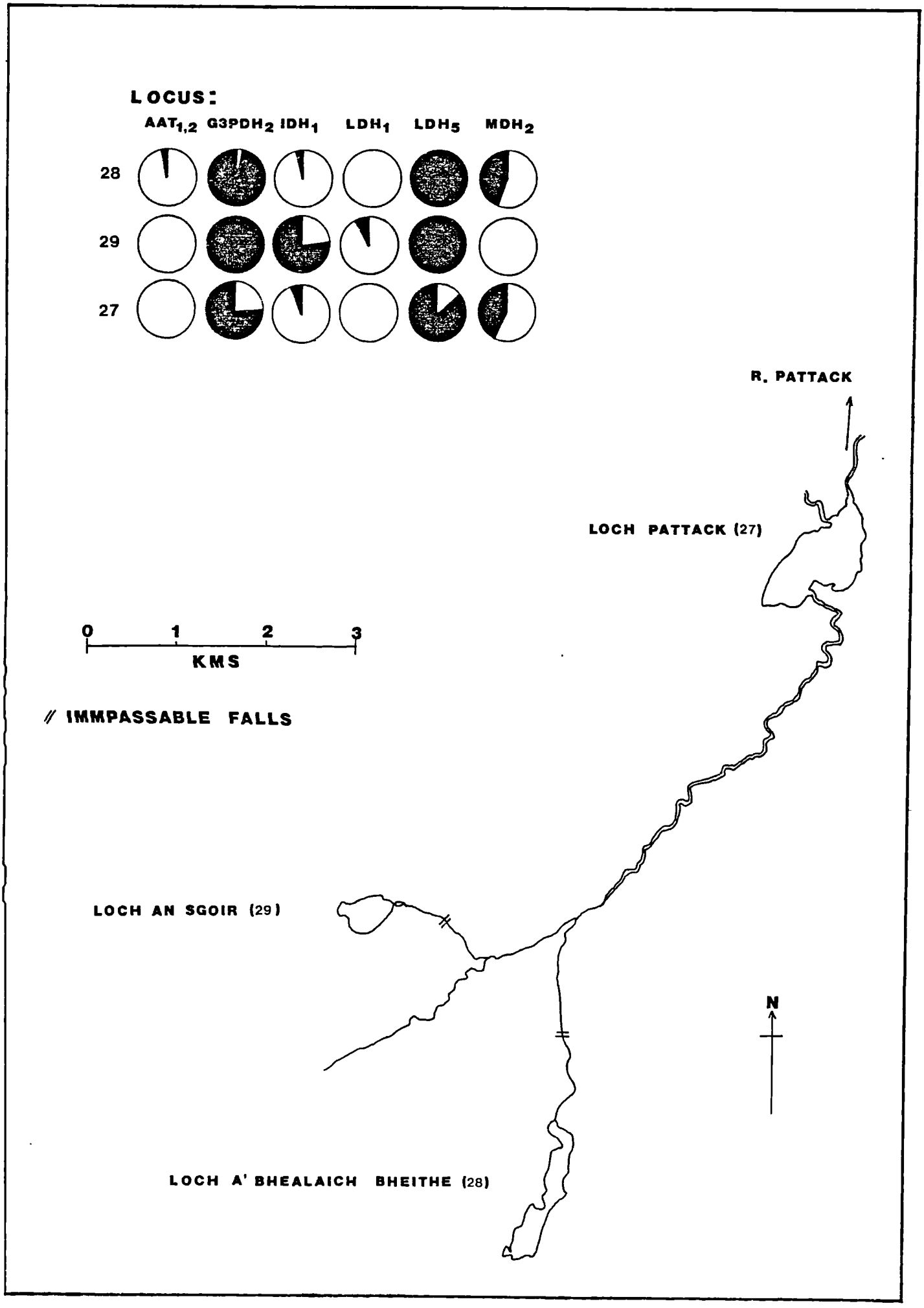
LDH_{-1}(0)			
Genotype Location	100/100	100/0	0/0
16	70	0	0
17	37	2	1
18	21	0	0
19	48	2	0
20	18	0	0
57	6	0	0
Nass x^2 = 11.325 df = 6.26 NS (P = 0.05)			

LDH_{-5}(105)			
Genotype Location	100/100	100/105	105/105
16	54	15	1
17	29	8	3
18	7	5	8
19	17	19	14
20	11	6	1
57	6	0	0
Nass x^2 = 24.21 df = 13.08 Sign P<0.05			

MDH_{-2}(152)			
Genotype Location	100/100	100/152	152/152
16	13	31	26
17	7	20	13
18	3	13	5
19	13	18	19
20	7	7	4
57	4	2	0
Nass x^2 = 35.98 df = 13.14 P<0.001			

MDH_{34}(125)			
Genotype Location	100/100	100/125	125/125
16	70	0	0
17	40	0	0
18	21	0	0
19	49	1	0
20	18	0	0
57	6	0	0
Nass x^2 = 1.836 df = 5.27 NS (P = 0.05)			

Figure 4.10 Loch Pattack system showing locations sampled and allele frequencies of polymorphic loci



Tables 4.18 Listing genotypes of individual fish used to calculate allele frequencies illustrated in Figure 4.10 - (Pattack System)

$AAT_{1,2}(140)$

Genotype Location	100/100	100/140	140/140
28	42	2	0
29	50	0	0
27	36	0	0

Nass x^2 = 7.52 df = 3.82 NS (P = 0.05)

$G\text{-}3PDH_2(50)$

Genotype Location	100/100	100/50	50/50
28	0	1	39
29	0	0	50
27	0	17	19

Nass x^2 = 46.67 df = 2.102 Sign P<0.001

$IDH_{-1}(160)$

Genotype Location	100/100	100/160	160/160
28	36	4	0
29	4	15	31
27	29	7	0

Nass x^2 = 88.58 df = 4.18 Sign P<0.001

$LDH_{-2}(0)$

Genotype Location	100/100	100/0	0/0
28	40	0	0
29	40	10	0
27	36	0	0

Nass x^2 = 18.01 df = 2.19 Sign P<0.001

$LDH_{-5}(105)$

Genotype Location	100/100	100/105	105/105
28	0	0	40
29	0	0	50
27	0	10	26

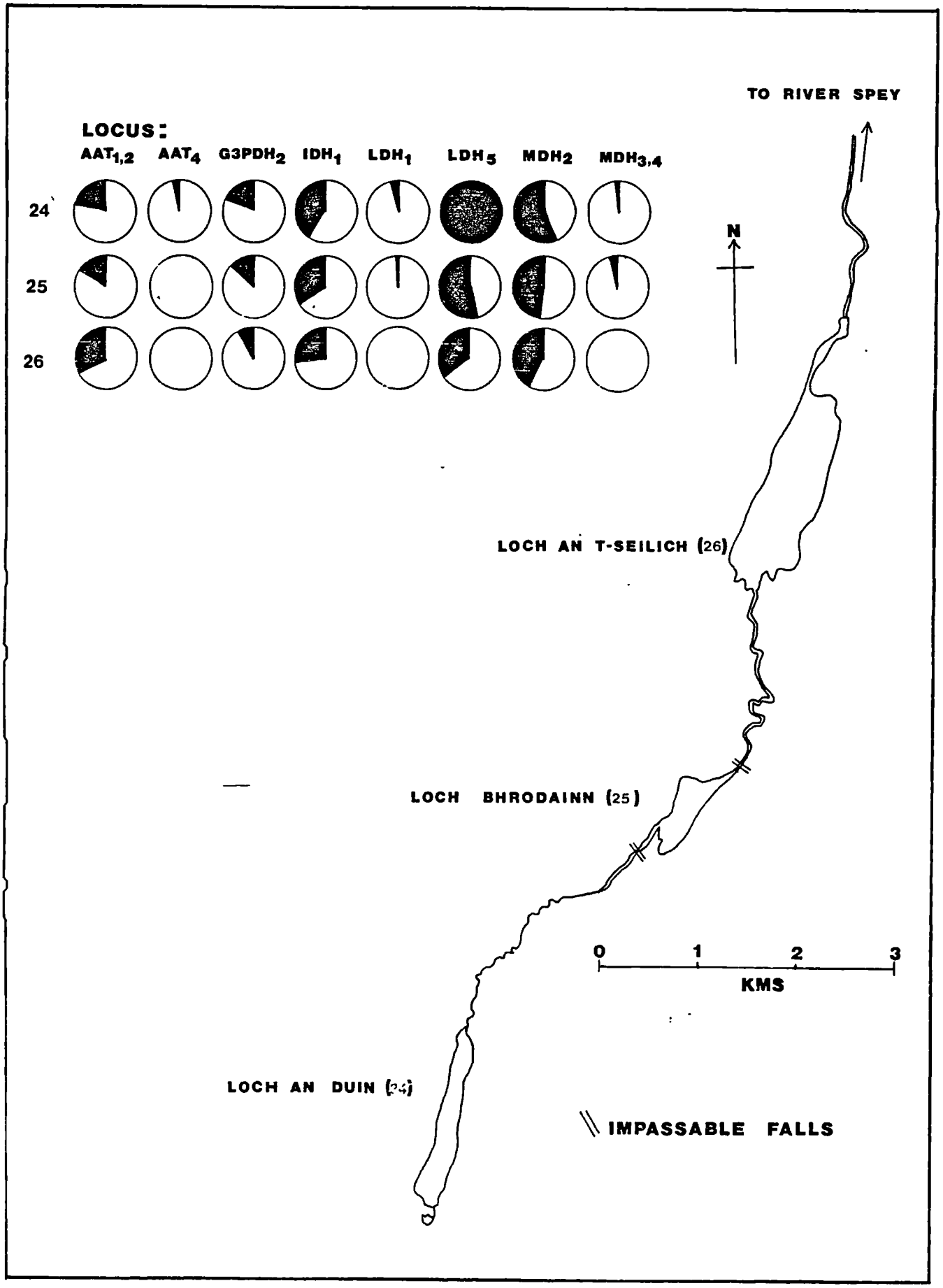
Nass x^2 = 29.63 df = 2.20 Sign P<0.001

$MDH_{-2}(152)$

Genotype Location	100/100	100/152	152/152
28	11	22	7
29	50	0	0
27	9	23	4

Nass x^2 = 70.75 df = 4.22 Sign P<0.001

Figure 4.11 The Loch an Duin system showing locations sampled and allele frequencies of polymorphic loci



Tables 4.19 Listing gentoypes of indviduals fish used to calculate allele frequencies illustrated in Figure 4.11 (An Duin System)

$AAT_{1,2}(140)$

Genotype Location	100/100	100/140	140/140
24	10	5	1
25	30	14	0
26	7	4	2
Nass x^2 = 0.919 df = 4.42 NS (P = 0.05)			

AAT_{-4} (74)

Genotype Location	100/100	100/74	74/74
24	15	1	0
25	43	1	0
26	13	0	0
Nass x^2 = 1.347 df = 2.39 NS (P = 0.05)			

$G3PDH_2(50)$

Genotype Location	100/100	100/50	50/50
24	10	6	0
25	33	10	1
26	11	2	0
Nass x^2 = 3.30 df = 4.42 NS (P = 0.05)			

$IDH_{-1}(160)$

Genotype Location	100/100	100/160	160/160
24	7	5	4
25	20	17	7
26	6	7	0
Nass x^2 = 4.21 df = 4.275 NS (P = 0.05)			

LDH_{-1} (Ø)

Genotype Location	100/100	100/Ø	Ø/Ø
24	14	2	0
25	42	2	0
26	13	0	0
Nass x^2 = 2.57 df = 2.22 NS (P = 0.05)			

$LDH_{-5}(105)$

Genotype Location	100/100	100/105	105/105
24	0	0	16
25	12	17	15
26	5	7	1
Nass x^2 = 30.589 df = 4.264 Sign. P<0.001			

$MDH_{-2}(152)$

Genotype Location	100/100	100/152	152/152
24	4	6	6
25	11	23	10
26	5	5	3
Nass x^2 = 2.646 df = 4.26 NS (P = 0.05)			

$MDH_{-34}(125)$

Genotype Location	100/100	100/125	125/125
24	16	0	0
25	41	3	0
26	12	0	1
Nass x^2 = 8.38 df = 5.10 NS (P = 0.05)			

western edge of the Cairngorms in the Loch an Duin drainage system.

All numbers in brackets, next to the loch names correspond to those given in the materials and methods, except in the Badachro examples. The equivalent numbers in this area are given in parentheses, because there was more than one coded population examined in Loch Horrisdale and Gaineamhach. It was felt that it would be less confusing to re-number the lochs 1-6 in this case.

4.3.4 The LDH_{-5} gentoype distribution

Previous work carried out by Ferguson and Mason (1981) and Ferguson and Fleming (1983) suggested that the variant allele LDH_{-5} (105) may be associated with an ancestral form of brown trout in the British Isles and also with the so called 'Ferox' trout. Figure 4.12 represents the distribution of LDH_5 alleles in Scotland found in this project.

Table 4.20 lists the coefficients (Nonparametric-Spearman) between all the polymorphic enzyme loci studied and various parameters associated with the locality of the loch in which the trout were caught, and with the trout themselves.

4.3.5 The 'Ferox' analysis

Table 4.21 gives genotypes and allele frequencies of trout classified as ferox, taken from 14 different localities. The localities are listed below the table.

Table 4.22 represents a set of 3 x 2 contingency tables using each of the polymorphic loci correlated to whether or not the fish was classified as a ferox.

4.3.6. Linkage disequilibrium

Table 4.23 gives the results of each loci tested for linkag dis-equilibrium with each other loci for all individuals screened in

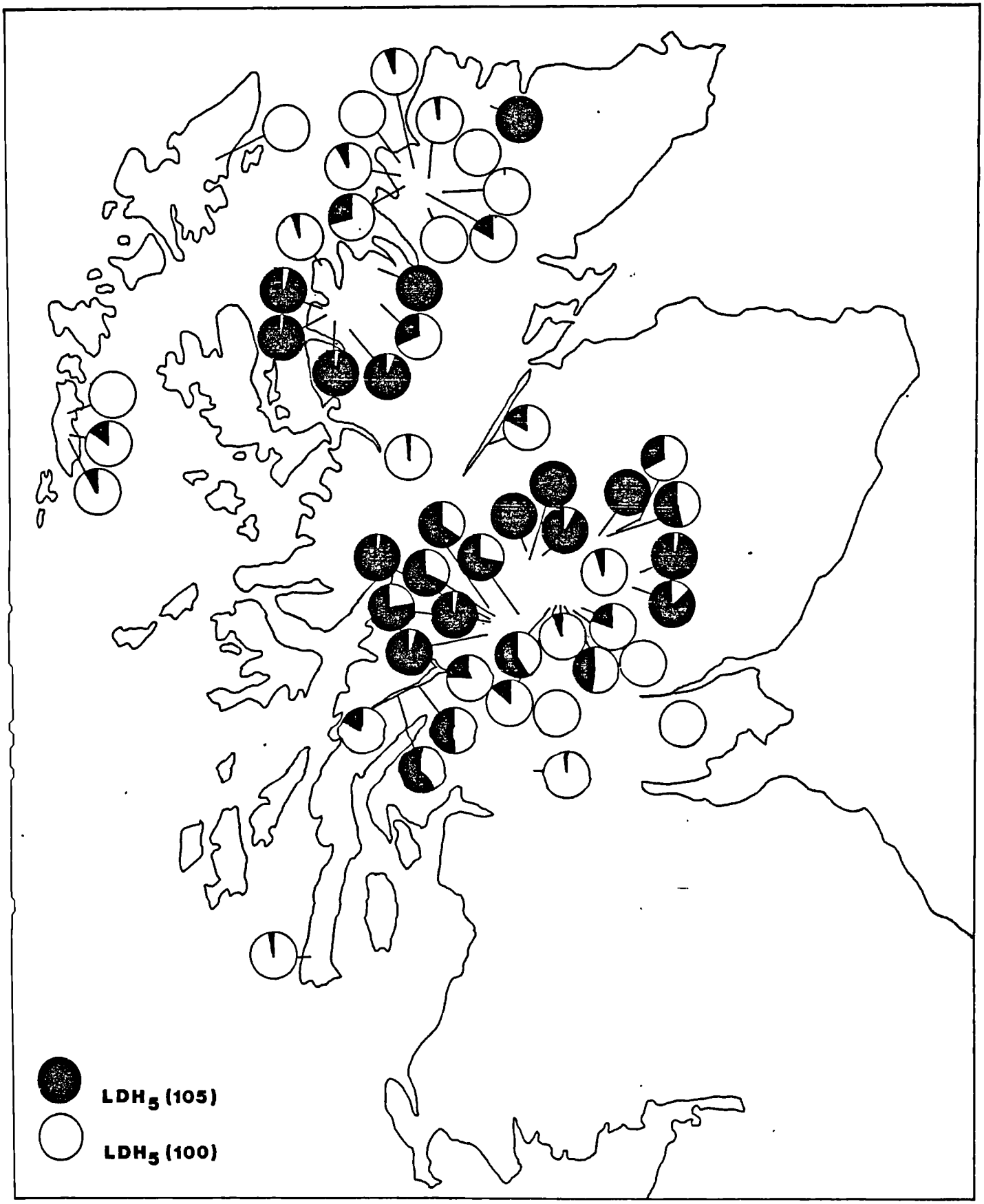


FIG: 4.12 MAP OF SCOTLAND SHOWING THE DISTRIBUTION OF LDH_5 ALLELES

Table 4.20Non parametric (Spearman) correlation coefficients with levels of significance below each figure

Parameter / Variant Allele	Above/below Impassable falls	Height above sea level	Distance from the sea	Water stocked/ not stocked	Sex	Length	Ferox	Age
$AAT_{-1,2}(140)$	-0.122 0.00	-0.048 0.026	-0.046 0.032	-0.168 0.000	0.024 0.164	0.094 0.000	-0.019 0.211	-0.016 0.266
$AAT_{-1,2}(45)$	-0.137 0.000	-0.100 0.000	-0.091 0.000	-0.080 0.001	0.063 0.005	-0.058 0.010	-0.041 0.050	-0.123 0.000
$AAT_{-4}(74)$	0.158 0.000	0.050 0.021	-0.058 0.010	0.146 0.000	-0.064 0.005	-0.004 0.444	0.116 0.000	0.011 0.336
$DIA_{-1}(90)$	0.077 0.001	-0.058 0.009	-0.027 0.136	-0.027 0.132	0.006 0.404	-0.008 0.368	0.046 0.032	-0.002 0.471
$G3PDH_{-2}(50)$	-0.016 0.264	0.225 0.000	0.258 0.000	0.036 0.068	0.078 0.001	-0.136 0.000	0.007 0.390	-0.004 0.436
$IDH_{-1}(160)$	0.192 0.000	0.240 0.000	0.132 0.000	0.021 0.192	-0.055 0.013	0.039 0.056	-0.010 0.330	0.070 0.002
$IDH_{-2}(130)$	-0.085 0.000	-0.047 0.028	-0.052 0.017	-0.158 0.000	0.045 0.035	-0.025 0.160	0.026 0.149	-0.080 0.001
$LDH_{-1}(Ø)$	0.083 0.000	0.071 0.002	0.028 0.129	0.01 0.317	0.006 0.400	0.024 0.163	0.017 0.241	0.035 0.078

continued . . .

Table 4.20 continued

Parameter / Variant Allele	Above/below Impassable falls	Height above sea level	Distance from the sea	Water stocked/ not stocked	Sex	Length	Ferox	Age
$LDH_{-5}(105)$	0.587 0.000	0.499 0.000	0.312 0.000	0.101 0.000	-0.138 0.000	0.054 0.015	0.089 0.000	0.194 0.000
$MDH_{-2}(152)$	0.001 0.484	0.059 0.008	0.259 0.000	0.126 0.000	0.131 0.000	- 0.095 0.000	- 0.020 0.210	-0.077 0.001
$MDH_{-3,4}(125)$	0.104 0.000	-0.075 0.001	-0.098 0.000	0.129 0.000	-0.031 0.102	0.044 0.039	-0.009 0.353	0.054 0.014
$PGI_{-2}(135)$	0.028 0.129	-0.142 0.000	-0.120 0.000	0.156 0.000	-0.060 0.035	0.075 0.150	0.037 0.097	0.068 0.000
$PGI_{-3}(110)$	-0.120 0.000	-0.099 0.000	-0.096 0.000	-0.142 0.000	0.045 0.035	-0.026 0.150	0.032 0.097	-0.098 0.000

All figures calculated using 1644 brown trout from many different locations.

Table 4.21 Genotypes and allele frequencies
Ferox. Total number included in electrophoretic examination = 86

Allele	(OBS) HOM 100/100	(EXP)	(OBS) HET	(EXP)	(OBS) HOM Variant	(EXP)	100	Variant	G	Significance
$AAT_{1,2}(140)$	63	(60.2)	18	(23.2)	5	(2.6)	0.84	0.16	3.122	NS
$AAT_{1,2}(45)$	86	(86)	0	(0)	0	(0)	1	0	-	-
$AAT_{-4}(75)$	77	(74)	6	(11.2)	3	(0.8)	0.93	0.07	6.566	P 0.05
$DIA_{1}(90)$	86	(86)	0	(0)	0	(0)	1	0	-	-
$G3PDH_{-2}(50)$	61	(61.9)	24	(22.4)	1	(1.5)	0.85	0.15	-7.660	P 0.01
$IDH_{-1}(160)$	52	(50.7)	28	(31.0)	6	(4.3)	0.77	0.23	0.984	NS
$IDH_{-2}(130)$	86	(86)	0	(0)	0	(0)	1	0	-	-
$LDH_{-1}(0)$	84	(84.3)	2	(1.7)	0	(0)	0.99	0.01	0.458	NS
$LDH_{5}(105)$	52	(45.6)	21	(34.4)	13	(6.0)	0.73	0.27	12.988	P 0.00L
$MDH_{-2}(152)$	37	(33.5)	34	(40.4)	15	(12.1)	0.63	0.37	-6.832	P 0.01
$MDH_{-34}(125)$	81	(79.9)	4	(6.0)	1	(3.1)	0.97	0.03	3.670	NS
$PGI_{-2}(135)$	85	(84.95)	1	(1.0)	0	(0.05)	0.99	0.01	0.059	NS
$PGI_{-3}(110)$	86	(86)	0	(0)	0	(0)	1	0	-	-

NS = not significant at P = 0.05

Number of ferox	Location	Code	Number of ferox	Location	Code
1	Peat Pool (5)	13	6	Loch Rannoch	57
23	Loch Brodain	25	24	Loch Ness	58
13	Loch An Tseiliach	26	1	Loch Ericht	55
3	Loch Fionn (Wester Ross)	33	1	Loch Earn	55
3	Loch Veyatie	44	1	Loch Fada	55
2	Loch Assynt	49	1	Loch Lomond	55
6	Loch Quoich	56	1	Loch Corcach	55

Table 4.22 The following represent 3 x 2 contingency tables using each of the polymorphic loci correlated to whether the fish was classified as a ferox or not. The figures in brackets represent expected values.

AAT_{12}(140)	Ferox		Not ferox	
100/100	63	(66.4)	1204	(1200.6)
100/140	18	(13.7)	243	(247.3)
140/130	5	(6.0)	109	(108.4)
x^2 = 1.79	df = 2		NS	

AAT_{12}(45	Ferox		Not ferox	
100/100	86	(83.4)	1508	(1510.6)
100/45	0	(2.5)	47	(44.5)
45/45	0	(0.1)	2	(1.9)
x^2 = 2.88	df = 2		NS	

AAT_4(74)	Ferox		Not ferox	
100/100	77	(57.6)	1024	(1043.4)
100/74	6	(17.5)	328	
74/74	3	(10.9)	205	(197.1)
x^2 = 20.8	df = 2		P<0.01	

DIA_1(90)	Ferox		Not ferox	
100/100	86	(82.8)	1495	(1498.2)
100/90	0	(3.1)	60	(56.9)
90/90	0	(6.1)	2	(1.9)
x^2 = 3.6	df = 2		NS	

G-3PDH$_{-2}$(50)	Ferox		Not ferox	
100/100	61	(61.6)	1115	(1114.4)
100/50	24	(16.6)	293	(300.4)
50/50	1	(7.9)	149	(142.1)
x^2 = 9.8	df = 2		NS	

IDH_{-1}(160)	Ferox		Not ferox	
100/100	52	(55.7)	1012	(1008.3)
100/160	28	(21.9)	391	(397.1)
160/160	6	(8.4)	154	(151.6)
x^2 = 2.74	df = 2		NS	

IDH_{-2}(130)	Ferox		Not ferox	
100/100	86	(85.0)	1537	(1538.0)
100/130	0	(0.9)	18	(17.1)
130/130	0	(0.1)	2	(1.9)
x^2 = 1.12	df = 2		NS	

LDH_{-1}(Ø)	Ferox		Not ferox	
100/100	84	(82.8)	1497	(1498.2)
100/Ø	2	(2.8)	51	(50.2)
Ø/Ø	0	(0.5)	9	(8.5)
x^2 = 0.75	df = 2		NS	

LDH_{-5}(105)	Ferox		Not ferox	
100/100	52	(40.6)	723	(734.4)
100/105	21	(12.0)	208	(217.0)
105/105	13	(33.4)	626	(605.6)
x^2 = 23.7	df = 2		P<0.001	

MDH_{-2}(152)	Ferox		Not ferox	
100/100	37	(40.7)	741	(737.3)
100/152	34	(30.8)	554	(557.2)
152/152	15	(14.5)	262	(262.5)
x^2 = 0.73	df = 2		NS	

MDH_{34}(125)	Ferox		Not ferox	
100/100	81	(81.4)	1480	(1497.3)
100/125	4	(3.2)	57	(57.8)
125/125	1	(1.1)	20	(19.9)
x^2 = 0.23	df = 2		NS	

PGI_{-2}(135)	Ferox		Not ferox	
100/100	85	(82.1)	1484	(1486.9)
100/135	1	(3.1)	58	(55.9)
135/135	0	(0.8)	15	(14.2)
x^2 = 2.42	df = 2		NS	

PGI_{-3} (110)	Ferox		Not ferox	
100/100	86	(84.4)	1526	(1527.6)
100/135	0	(1.5)	29	(27.5)
135/135	0	(0.1)	2	(1.9)
x^2 = 1.75	df = 2		NS	

Table 4.23 Showing the results of each loci tested for linkage disequilibrium with each other loci, for all the individuals screened electrophoretically. See text for details

	$AAT_{-1,2}$ 140	$AAT_{-1,2}$ 45	AAT_{-4}	DIA_{-1}	$G3PDH_{-2}$	IDH_{-1}	IDH_{-2}	LDH_{-1}	LDH_{-5}	MDH_{-2}	$MDH_{-3,4}$	PGI_{-2}	PGI_{-3}
$AAT_{-1,2}$ 140		NS	NS	NS	Sig	NS	NS	NS	SG	NS	NS	Sig	NS
$AAT_{-1,2}$ 45	1.059 = 2.535		NS	NS	NS	NS	Sig	NS	Sig	NS	NS	NS	Sig
AAT_{-4}	3.127 · df:4	5.665 V=3.703		NS	Sig	Sig	NS	NS	Sig	Sig	Sig	NS	NS
DIA_{-1}	4.635 V=2.543	0.187 df:4	8.876 V=3.706		NS	NS	NS	Ns	Sig	NS	NS	NS	NS
$G3PDH_{-2}$	27.012 df:4	4.367 V=3.099	71.508 df:4	2.000 V=3.106		Sig	NS	Sig	Sig	Sig	Sig	Sig	NS
IDH_{-1}	8.265 df:4	7.358 V=3.407	22.361 df:4	8.508 V=3.412	40.672 df:4		NS	NS	Sig	Sig	Sig	NS	NS
IDH_{-2}	1.552 V=2.474	24.637 df:4	3.828 V=3.684	2.975 df:4	1.246 V=3.054	9.303 V=3.374		NS	Sig	NS	NS	NS	NS
LDH_{-1}	5.738 V=3.522	0.539 df:4	9.398 df:4	0.919 df:4	14.635 V=3.749	4.732 V=3.849	0.490 df:4		NS	NS	Sig	NS	NS
LDH_{-5}	18.622 df:4	15.790 V=4.325	112.895 df:4	20.069 V=4.322	122.874 df:4	53.485 df:4	13.695 V=4.348	5.024 df:4		Sig	Sig	sig	NS
MDH_{-2}	4.486 df:4	7.274 V=4.600	63.448 df:4	3.285 V=4.653	17.892 df:4	35.815 df:4	3.048 V=4.710	8.633 · V=4.150	60.369 df:1		Sig	NS	NS
$MDH_{-3,4}$	6.807 V=3.770	0.378 V=0.571	17.675 df:4	0.654 V=0.575	11.021 V=3.886	13.478 V=3.935	0.143 V=0.540	111.871 V=1.617	80.228 V=4044	39.664 V=4.075		NS	NS
PGI_{-2}	10.823 V=3.692	0.596 df:4	5.145 V=3.963	0.685 df:4	13.554 V=3.844	1.807 V=3.909	0.954 df:4	1.036 V=1.366	23.790 df:4	8.144 V=4.087	1.096 V=2.116		NS
PGI_{-3}	6.731 V=2.511	16.104 df:4	7.828 V=3.696	3.867 df:4	2.174 V=3.082	0.916 V=3.395	9.228 df:4	5.568 df:4	6.807 V=4.834	8.827 V=4.679	0.0092 V=0.558	1.489 df:4	

P = 0.05 where degrees of freedom (df) refers to χ^2 where (V) refers to Nass χ^2

Table 424 Showing the results of each loci tested for linkage disequilibrium with each other loci, for all the individuals at location 8.

Location: 8 N = 82

	$AAT_{-1,2}$ 140	$AAT_{-1,2}$ 45	AAT_{-4}	DIA_{-1}	$G3PDH_{-2}$	IDH_{-1}	IDH_{-2}	LDH_{-1}	LDH_{-5}	MDH_{-2}	$MDH_{-3,4}$	PGI_{-2}	PGI_{-3}
$AAT_{-1,2}$ 140		NS	NS						NS				NS
$AAT_{-1,2}$ 45	Nass χ^2 3.939 V = 2.82												
AAT_{-4}	Nass χ^2 2.168 V = 2.86												
DIA_{-1}													
$G3PDH_{-2}$									Sig (P 0.05)				NS
IDH_{-1}							NS						
IDH_{-2}						Nass χ^2 0.930 V = 0.73							
LDH_{-1}													
LDH_{-5}	Nass χ^2 2.213				Nass χ^2 7.353					NS			
MDH_{-2}									Nass χ^2 5.934 V = 2.55				
$MDH_{-3,4}$													
PGI_{-2}													
PGI_{-3}	Nass χ^2 3.217 V = 3.39				Nass χ^2 4.555 V = 2.25								

NS = Not significant at P = 0.05

Table 4.23 Showing the results of each loci tested for linkage disequilibrium with each other loci, for all the individuals at location 16.

Location: 16 N = 70

	$AAT_{-1,2}$ 140	$AAT_{-1,2}$ 45	AAT_{-4}	DIA_{-1}	$G3PDH_{-2}$	IDH_{-1}	IDH_{-2}	LDH_{-1}	LDH_{-5}	MDH_{-2}	$MDH_{-3,4}$	PGI_{-2}	PGI_{-3}
$AAT_{-1,2}$ 140					NS	NS							
$AAT_{-1,2}$ 45													
AAT_{-4}					NS	NS							
DIA_{-1}													
$G3PDH_{-2}$	Nass χ^2 1.345 V = 1.414		Nass χ^2 1.897 V = 2.26						NS				
IDH_{-1}	Nass ^{2} 2.846 V = 3.58		Nass ^{2} 6.391 V = 3.95										
IDH_{-2}													
LDH_{-1}													
LDH_{-5}				Nass χ^2 1.137 V = 1.47						NS			
MDH_{-2}									Nass χ^2 2.131 V = 6.31				
$MDH_{-3,4}$													
PGI_{-2}													
PGI_{-3}													

Level of significane (P=0.05) NS = Not significant

Table 4.26 Showing the results of each loci tested for linkage disequilibrium with each other loci, for all the individuals at location 42.

Location: 42 N = 60

	$AAT_{-1,2}$ 140	$AAT_{-1,2}$ 45	AAT_{-4}	DIA_{-1}	$G3PDH_{-2}$	IDH_{-1}	IDH_{-2}	LDH_{-1}	LDH_{-5}	MDH_{-2}	$MDH_{-3,4}$	PGI_{-2}	PGI_{-3}
$AAT_{-1,2}$ 140						NS			Sig (P 0.01)			NS	
$AAT_{-1,2}$ 45													
AAT_{-4}													
DIA_{-1}													
$G3PDH_{-2}$						NS			NS				
IDH_{-1}	$Nass^2$ 2.324 V = 2.68				$Nass^2$ 3.867 V = 1.28							NS	
IDH_{-2}													
LDH_{-1}													
LDH_{-5}	$Nass^2$ 13.232 V = 2.06				$Nass^2$ 4.223 V = 1.20					NS			
MDH_{-2}									$Nass^2$ 5.907 V = 4.59				
$MDH_{-3,4}$													
PGI_{-2}	$Nass^2$ 8.657 V = 6.79					$Nass^2$ 5.253 V = 4.44							
PGI_{-3}													

NS = Not significant at P = 0.05

Table 427 Showing the results of each loci tested for linkage disequilibrium with each other loci, for all the individuals at location 43.

Location: 43 N = 97

	$AAT_{-1,2}$ 140	$AAT_{-1,2}$ 45	AAT_{-4}	DIA_{-1}	$G3PDH_{-2}$	IDH_{-1}	IDH_{-2}	LDH_{-1}	LDH_{-5}	MDH_{-2}	$MDH_{-3,4}$	PGI_{-2}	PGI_{-3}
$AAT_{-1,2}$ 140			NS			NS			NS	NS			
$AAT_{-1,2}$ 45													
AAT_{-4}	$Nass^2$ 3.798 V = 3.29												
DIA_{-1}													
$G3PDH_{-2}$						Sig (P 0.05)							
IDH_{-1}	$Nass^2$ 9.391 V = 4.19				$Nass^2$ 10.243 V = 414								
IDH_{-2}													
LDH_{-1}													
LDH_{-5}	$Nass^2$ 4.627 V = 3.22												
MDH_{-2}	$Nass^2$ 4.088 V = 3.99												
$MDH_{-3,4}$													
PGI_{-2}													
PGI_{-3}													

NS = Not significant at P = 0.05

the electrophoretic survey using 3 x 3 contingency tables. Where each cell of the table contained more than 5 individuals the test was used for goodness-of-fit, while when the cell counts dropped below 5 (many contained 0) the Nass x^2 test was used. Below the diagonal are the values of either x^2 or Nass x^2 with the accompanying degrees of freedom (df for x^2 and v for Nass x^2). Above the diagonal are the associated estimates of significance. The next four tables (4.24, 25, 26 and 27) give the same information as Table 4.23 except the results in each table represent just one location screened electrophoretically. These tables are full of blanks. Each blank represents a non-significant x^2 or Nass x^2 test, the only cells which contain anything are those that showed the largest Nass x^2 values and were thus likely to give information concerning linkage disequilibrium.

4.4 Discussion

4.4.1 Protein polymorphism

The identification of enzyme polymorphisms was conducted successfully using gel electrophoresis although some commonly reported polymorphisms for brown trout such as at CK_{-1} were not identified due to poor resolution. Taggart et al. (1981) comment on the differences in resolution between laboratories and identified many possible causes.

The enzymes examined in this study represented at least 34 loci of which 13 (38%) were polymorphic. All have been shown to be disomically inherited (Ferguson and Taggart, 1984). The proportion of loci polymorphic in individual wild populations ranged from 0 to 29.4%, while the hatchery population of Howietoun fish farm gave

a figure of 38%. The percentages calculated for the wild trout populations are in broad agreement with Ferguson and Fleming's (1983) estimates, who found the proportion of loci polymorphic in natural populations in Ireland and Britain ranged from 0 to 21%, using 60 loci of which 22 were found to be polymorphic. Ryman (1983) found the proportion of polymorphic loci for wild populations of trout in Sweden, ranged from 0 to 17.1% using 35 loci of which 9 were found to be polymorphic (P: 99% criterion).

The figures calculated in this study are likely to be slightly higher than would be expected if no previous knowledge of brown trout enzyme polymorphisms was assumed. The enzymes examined in this study included the ones reported to be polymorphic for brown trout in Scandinavia and Ireland.

4.4.2 Heterozygosity

The mean heterozygosity for natural populations in this study was 4.5% (range 0.0% - 8.9%). The same figure was derived either from observed heterozygosity (Table 4.9) or expected heterozygosity (Table4.8). Allendorf and Phelps (1981) gave an expected heterozygosity figure of 5.9% for rainbow trout and Stahl (1981) gave an expected heterozygosity figure of 2.3% for Atlantic salmon. Fleming and Ferguson (1983) quote a mean figure of 3.8% for the British and Irish brown trout populations they studied (range 0.0 - 6.2%).

Ryman (1983) gives expected heterozygosities for a number of Swedish brown trout populations which range from 0.0% to 5.3%. Thus the

figure calculated for this study is in broad agreement with the work carried out with other salmonids (see Table 4.2 and Table 24 in Kirpichinikov, 1981). Nevo (1978) produced an overall average heterozygosity value for 57 species of fish of 5.1%. Most salmonids are slightly below this figure apart from rainbow trout (Allendorf and Utter, 1979; Allendorf and Phelps, 1981b).

The Howietoun fish farm heterozygosity value was 6.4%, above average. The relatively high heterozygosity exhibited by the Howietoun fish farm brown trout stock is similar to the situation found by Thompson (1985) and Busack et al. (1979), working with rainbow trout and by Guyomard and Krieg (1983) who worked with brown trout. They all found high levels of heterozygosity in captive populations and Guyomard and Krieg (1983) put forward two possible explanations. The first was that the hatchery fish were derived from a naturally more diverse population and secondly that due to crossing of various wild populations the hatchery fish had become electrophoretically were heteozygous. The second explanation seems more feasible and explains the relatively high heterozygosity found in the Howietoun stock.

These results are in direct contrast to other hatchery stocks which have been found to be much less variable than closely neighbouring wild stocks. Cross and King (1983) and Stahl (1983) working with Atlantic salmon found variant alleles present in wild populations had disappeared in the hatchery strains. The same situation was evident in two hatchery populations of brown trout (Ryman and Stahl,

1980; 1981; Vuorinen,1984), as well as in a population of cutthroat trout Salmo clarkii (Allendorf and Phelps, 1980). The explanation for these depressions in genetic variability put forward by most of the authors is

1. that of genetic drift and the problem of not using a sufficient number of wild broodstock initially when the hatchery populations were formed, so that the full quantity of variation exhibited by the wild populations was not represented;

2. that once populations were set up too few broodstock were used in subsequent generations.

The value of expected heterozygosity is dependent on the number of loci examined overall, not only on the polymorphic systems, and thus requires a certain amount of interpretation when trying to compare results obtained by different workers.

An interesting point to note in this study was that the populations examined that showed very low heterozygosity values or even values of zero, are all isolated in small high lochs (locations 28, 29, 30, 42). It is thought that the populations in these lochs could be limited in number, or may well have only been represented by a few individuals when the lochs were first colonised. Thus substantial amounts of random drift may have taken place (Lewontin, 1976; Cook, 1976; Falconer, 1981) and what is known as the founder effect may have occurred.

Loch (30), Loch na Creige Riabhaich is the only location studied with trout fixed for the variant $AAT_{1,2}$(140) allele. They were also fixed for the variant LDH_5(105) allele, and was the only population to have a heterozygosity value of 0.0.

The trout in loch (30) are unusually coloured being conspicuously green, with many hundreds of very small black spots all over their flanks and opercula. Their bellies are yellow with grey lateral patches, and every fish caught looked similar. The spawning available to the trout is very limited, and although the loch was visited three times at different months of the year, fry or young trout were never found in the feeder burn even when electrofished. The trout grew well probably due to lack of competition for food (3 year olds reaching 30 cm in length). It was decided to attempt to catch some of these fish and breed them under artificial conditions to study their growth characteristics, subsequent development, age at maturity and longevity. Eighteen trout were brought back alive in October 1984 and were kept at DAFS Pitlochry. They were used by Andy Walker in various interstrain cross breeding experiments. Unfortunately the two mature females captured died before spawning but males were crossed with other strains, including the Nashua fast growing American hatchery strain. The resulting crosses did not grow as fast as the pure Nashua crosses but the F_1 hybrids were very heavily spotted in contrast to the practically unspotted Nashua strain. Electrophoretic examination of the cross was performed and results appear in Table 4.8 (Population 60). Not surprisingly Population (60) exhibits heterozygote excess, indicating two different

populations are involved. (Discussed more fully later). Little quantitative work has been carried out but the F_1 hybrids have been stocked into virgin lochs and their performance is being monitored.

Of the 13 variant alleles found in this survey, the $MDH_{-2}(152)$, $G3PDH_{-2}(50)$, $AAT_{-1,2}(140)$, $IDH_{-1}(160)$ and $LDH_{-5}(105)$ alleles were found in most populations (see Table 4.7).

It has been pointed out by Ferguson and Fleming (1983) that $MDH_{-2}(152)$ and $G\text{-}3PDH_{-2}(50)$ have been found to be polymorphic in most of the species of salmonids studied. The $MDH_{-2}(100/100)$ allozyme of brown trout has the same electrophoretic mobility as the common allozyme in Atlantic salmon and rainbow trout (Ferguson and Fleming, 1983). They also point out that Atlantic salmon have an MDH_{-2} variant with the same mobility as the $MDH_{-2}(152)$ variant in brown trout. The same situation is true for $G\text{-}3PDH_{-2}$ allozymes with brown trout and Atlantic salmon being similar. Ferguson and Fleming (1983) conclude, due to the same ancestral alleles in various species being highly polymorphic, that this is maintained by selection.

4.4.3 Agreement and deviations from Hardy-Weinberg equilibrium

Although Table 4.8 illustrates the calculated allele frequencies and heterozygosities, it does not give any information regarding genotype frequency and whether they conform with the Hardy-Weinberg law.

The Hardy-Weinberg law applies to populations in equilibrium only. In general, five forces can be considered as causing populations to deviate from equilibrium (Ferguson, 1980).

1. Mating choice. The Hardy-Weinberg law assumes that the population under consideration is panmictic

2. Mutation. In theory mutation can bring about changes in allele frequencies but as this process takes place at a sufficiently low rate, for practical population studies it can be ignored (Ferguson, 1980).

3. Migration. When individuals from one population enter another allele frequency changes may be expected. Differential migration is known as gene flow.

4. Genetic Drift. This is a random process which mostly effects small populations. If there are limited numbers of individuals within a breeding population fluctuations in allele frequencies can occur from generation to generation, and this is called genetic drift. In small populations allele frequencies drift with time and alleles may be lost from the population and thus the smaller the population the quicker the decrease in genetic variability. A specific type of drift is the founder effect, already discussed for population (30), which is where founder individuals of a new population are few in number and represent only a limited part of the variation present in a parental population (Ferguson, 1980).

5. Natural Selection. If an allele in a population gives the individuals which possess it an advantage over breeding success, then the individuals with it will out compete the individuals without it, thus allele frequencies altered by natural selection and influenced by a directional process, unlike point (1)-(4).

One of the commonest reasons for producing deviations from Hardy-Weinberg expectations when dealing with wild populations, is the treatment of two fully or partially isolated populations characterised by different allele frequencies as a single panmictic population. This can show up a significant deficit in heterozygotes.

If on the other hand, one has disassortative mating (pairing of unlike individuals) the results can be an excess of heterozygotes. Both scenarios have been identified in brown trout population studies. Ryman et al. (1979) showed in Lake Bunnersjoarna in Sweden that there were 2 reproductively isolated populations of brown trout living in the same water body. The Hardy-Weinberg law when applied to LDH_{-1} genotypes for all the fish concerned, identified a complete lack of heterozygotes, indicating the population was not a single panmictic one. In fact the two populations were characterised by being fixed for the two different LDH_{-1} alleles (100 and 240).

Ferguson and Mason (1981) identified three separately breeding populations of brown trout in Lough Melvin, Northern Ireland, by showing that when all individuals were treated together there was highly significant heterozygote deficiency at the LDH_{-5} and PGI_{-2} loci, yet

when the three phenotypes were dealt with separately the populations all conformed to Hardy-Weinberg expectations.

Ryman (1981) also used deviations from Hardy-Weinberg law to illustrate the effect of introducing brown trout to a water. Ryman (1981) showed that there was a highly significant heterozygote excess when dealing with the locus AGP_{-2} in the River Skelleftealva stocked from hatcheries. This illustrates that crosses between genetically distinct populations may be expected to result in an excess of heterozygotes.

4.4.4 Discussion of reasons for the deviations from Hardy-Weinberg equilibrium (Listed in Table 4.28)

Table 4.28 lists the relevant enzyme, the number of fish used for electrophoretic screening, the genotypes recorded and the level of significance by which the observed genotypes disagree from the expected genotypes assuming the populations are in Hardy-Weinberg equilibrium. The number of tests that did not confrom (P = 0.05) to Hardy-Weinberg expectations represented 4% of the tests calculated. Therefore the vast majority are in agreement with Hardy-Weinberg equilibrium.

1. Heterozygote Excess

As indicated, this phenomenon is usually caused by disassortative matings. It is explained in wild populatons by stocking of non-endemic strains of trout leading to cross matings between the endemic and non-endemic strains. The populations exhibiting heterozygote excess were:

Table 4.28 Summarizing enzymes and populations not conforming with expected Hardy-Weinberg equilibria.

Enzyme	Population	No. of fish	Significance P	HOM	HET	HOM	Description
$AAT_{-1,2}$ (140)	22	43	0.01	27 (23.2)	9(16.8)	7(3.0)	Heterozygote deficiency
	33	40	0.001	12(5.2)	5(18.4)	23(16.4)	Heterozygote deficiency
	45	97	0.01	70 (66.0)	20(28.1)	7(2.9)	Heterozygote deficiency
	59	36	0.05	27(24.8)	6(10.1)	3(1.1)	Heterozygote deficiency
AAT_{4}(74)	7	47	0.001	39(34.8)	3(11.3)	5(0.9)	Heterozygote deficiency
	23	12	0.05	6(6)	5(4.9)	1(1.1)	Due to small sample size
	36	50	0.01	6(2.0)	8(16.0)	36(32.0)	Heterozygote deficiency
G-3PDH$_{-2}$(50)	27	36	0.05	0(2.2)	17(13.0)	19(20.8)	Het. exess
	32	14	0.05	5(6.4)	9(6.2)	0(1.4)	Het. excess
	47	13	0.05	2(3.8)	10(6.5)	1(2.7)	Het. excess
IDH_{-1} (160)	16	70	0.05	44(39.9)	18(25.9)	8(4.2)	Het. deficiency
	31	6	0.05	1(2.0)	5(2.9)	0(1.1)	Het excess
	37	51	0.01	24(18.9)	14(24.5)	13(7.6)	Het. deficiency
	50	18	0.05	14(12.4)	2(5.0)	2(0.6)	Het. deficiency
	53	6	0.05	5(4.1)	0(1.7)	1(0.2)	Het. deficiency
LDH_{-5}(105)	1-6	80	0.01	15(9.2)	9(16.5)	13(7.3)	Het. deficiency
	8	82	0.01	77(75.4)	3(6.5)	2(0.1)	Het. deficiency
	14	6	0.05	1(0.2)	0(1.7)	5(4.1)	Het. deficiency
	15	34	0.05	6(3.1)	8(13.9)	20(17.0)	Het. deficiency
	18	21	0.05	7(4.8)	5(10.5)	8(5.9)	Het. deficiency
	35	43	0.05	1(0.1)	2(3.4)	43(39.5)	Het. deficiency
	36	50	0.05	2(0.5)	4(7.5)	44(42.0)	Het. deficiency
	41	30	0.05	3(0.9)	4(8.4)	23(20.7)	Het. deficiency
	44	60	0.01	45(41.4)	10(16.8)	5(1.8)	Het. deficiency
	45	97	0.05	83(80.5)	11(15.5)	3(1.0)	Het. deficiency
	55	12	0.01	6(3.5)	1(6.0)	5(2.5)	Het. deficiency
	58	24	0.01	19(16.6)	2(6.7)	3(0.7)	Het. deficiency
	60	10	0.001	0(2.5)	10(5.0)	0(2.5)	Het. excess
	62	42	0.05	2(0.4)	5(8.0)	35(33.6)	Het. deficiency
MDH_{-2}(152)	8	82	0.01	50(53.3)	32(25.4)	0(3.3)	Het. excess
	21	15	0.01	4(6.2)	11(6.9)	0(1.9)	Het. excess
	34	37	0.01	2(5.9)	26(17.8)	9(13.3)	Het excess
	50	18	0.05	0(1.4)	10(7.2)	8(9.4)	Het. excess
$MDH_{-3,4}$(125)	26	13	0.05	12(11.1)	0(1.8)	1(0.1)	Het. deficiency
PGI_{-2}(135)	44	60	0.05	30(25.8)	19(27)	11(7.2)	Het. deficiency

(Expected genotypes in brackets)

(8) - Howietoun farm stock for MDH_{-2}

(21) - Loch Fincastle for MDH_{-2}

(27) - Loch Pattack for $G3PDH_{-2}$

(31) - Loch Nan Eun for IDH_{-1}

(32) - Loch An Draing for $G3PDH_{-2}$

(34) - Loch A'Mhadaidh Mor for MDH_{-2}

(47) - Loch Awe (Sutherland) for $G\text{-}3PDH_{-2}$

(50) - Loch Fionn (Sutherland) for IDH_{-1}

(60) - Loch Na Criege Riabhaich ♂s x Nashua strain ♀ s for MDH_{-2} and LDH_{-5}

It may be expected to identify a certain amount of heterozygote excess in hatchery stock, especially as in the case of the Howietoun stock (8), when wild trout are periodically included in the broodstock.

The excess of heterozygotes in population (60) is also easily explained as it was derived from a cross between male trout from Loch na Creige Riabhaich (30) and females from the Nashua strain of American brown trout kept at DAFS, Faskally, Pitlochry.

Lochs (21) and (27) both have been stocked using non-endemic trout (Walker pers. comm., Campbell pers. comm.), and therefore the heterozygote excesses can be explained.

Populations (31) and (32) contained only 6 and 14 individual trout respectively and thus more samples would have to be collected before anything statistically valid, regarding heterozygote excess could be commented on.

Loch A'Mhadaidh Mor (34) is located on an estate where recreational fishing is carried out but no records of any stocking of the loch are available. Loch (34) is located above a 15 metre waterfall and yet is producing smolts, three were taken in the outflow of the loch in June 1984. Whether transplantation of fish has occurred between lochs is uncertain, but it would explain the heterozygote excess found.

Lochs Awe (47) and Fionn (50) in Sutherland, are in an area where recreational fishing has taken place for over 100 years, and before this trout were a major source of protein for local inhabitants. The lochs in this area have been extensively managed or mismanaged with frequent transfer of fish from one to another (Morrison pers. comm.) and no record of such introductions has been kept. Loch Awe (47) in particular is very accessible and heavily fished and it is thought likely that transfer of fish to the loch from elsewhere is the reason for heterozygote excess. Of course any of these differences have shown up using small sample sizes, and confirmation of heterozygote excesses would require more extensive screening of larger samples.

2. Heterozygote Deficiency

As indicated the phenomenon of heterozygote deficiency in wild populations is usually associated with the sampling of a non-panmictic population, i.e. individuals used for electrophoretic screening are assumed to be from one normal breeding populations, when in fact they may originate from more than one breeding population.

In table 4.28 there are 25 estimates of heterozygote deficiency representing 21 different locations, 8 of which have less than 30 individuals represented. The majority of the heterozygote deficiencies are significant at $P = 0.05$ but 11 are significant at $P < 0.01$.

It should not be surprising to find sympatric populations living in many lochs, as once reproductive isolation is achieved it is maintained by the innate tendency of brown trout to spawn in their natural streams or rivers (Ferguson and Fleming, 1983).

Lochs sampled in this study which are large and have many inflow burns suitable for spawning, and which exhibit heterozygote deficiency at various loci include:

(1) - (6) Loch Awe (Argyll)
(14) Loch Ba
(15) Loch Laidon
(16) - (20) Loch Rannoch
(33) Fionn Loch (Wester Ross)
(35) Loch a'Bhealiach
(44) Loch Veyatie
(50) Loch Fionn (Sutherland)
(58) Loch Ness

Due to low numbers of samples, conclusive evidence of sympatric populations in these lochs is not available, but the suggestion is that this could be a widespread phenomenon and requires much

more work to provide more information, so that a sensible and rational approach can be made concerning the future management of the large lochs in Scotland.

From Table 4.28 one can see that 13 of the 26 heterozygote deficiencies identified were found for the LDH_5 locus. This leads on to a section discussing the distribution of LDH_5 100 and 105 alleles in Scotland.

4.4.5 The LDH_{-5} alleles as markers of invasion stocks

Ferguson and Fleming (1983) identify the LDH_{-5} (105) allele as being of particular interest in its geographic distribution. In Ireland and a few locations sampled in Britain they found 60 populations out of 116 which showed the LDH_5 (105) polymorphism. But only 8 populations were identified with the variant allele frequency in excess of 0.20.

Ferguson and Fleming (1983) point out that the LDH_{-5} (105/105) allozyme has the same electrophoretic mobility as the LDH_{-5} (100/100) common allozyme in other salmonids, including rainbow trout, Atlantic salmon, Arctic charr and all the Pacific salmons.

They postulate that the LDH_{-5}(105) brown trout allele is in fact the ancestral allele for LDH_{-5} found in salmonids and the so called LDH_{-5}(100) allele is the variant which has occurred through mutation since the brown trout evolved from the salmonid lineage. Ferguson and Fleming (1983) noticed that the populations characterised by high frequencies at the LDH_{-5}(105) allele were found above impassable

falls isolated from modern day migratory trout, and postulated that in immediate post-glacial times, rivers and lakes in Britain and Ireland were colonised by migratory brown trout which were fixed for the LDH_{-5} (105) ancestral allele. In more recent times, they suggested "migratory" brown trout, which were characterised by the LDH_{-5}(100) allele, and which were possibly of more southern origin colonised those areas of freshwater to which they had access and replaced the "ancestral type". Ferguson and Fleming (1983) conclude on the subject of LDH_{-5}, that "more evidence from other remote brown trout populations is required to complete the picture".

In this study 84% of the populations screened exhibited the LDH_{-5}(105) polymorphism and 38 out of 63 populations showed a frequency of >0.20. In fact 21 populations exhibited a frequency of > 0.70 with 8 populations fixed or almost fixed (> 0.96) for LDH_{-5}(105). Figure 4.12 gives the distribution of LDH_{-5} alleles studied.

This is in contrast with the situation found by Ferguson and Fleming (1983) in Ireland and by Ryman (1983) in Sweden. Many of the populations surveyed in this study are found many kilometres inland and at considerable height above sea level, many being situated above impassable falls.

When the polymorphic loci used for screening in this study were cross correlated against various parameters relating to the loch from which the fish were caught or parameters relating to the fish themselves the largest correlation coefficients found were for LDH_{-5}

against height above sea level, distance from the sea, and whether the loch from which thefish were caught was situated above an impassable fall (Table 4.20). Of course in Scotland the three parameters are all correlated because the further one travels inland the higher the land masses and the more likely one is to find lochs situated above impassable falls. The above correlations tend to lend support for the theory that the LDH_5(105) allele is associated with a primary invasion stock of trout and the LDH_5(100) allele is characteristic of a secondary invasion stock.

Payne et al. (1971) proposed the existence of two races of Atlantic salmon in Britain and Ireland on evidence based on transferrin allelic frequencies. They name the two races "Boreal" and "Celtic". The Boreal was proposed to have been isolated in a North Sea refuge during the last period of the ice age and subsequently colonised the North of Britain and West of Ireland when the ice melted. Meanwhile the Celtic race which was not an isolated one remained in the non-glaciated region to the South, colonised the South of Ireland and the South West of Britain. Ferguson and Fleming (1983) postulated a similar scenario for the brown trout in the British Isles.

One problem associated with the scenario of two invasion stocks, is that impassable falls at the present time, may not have been impassable obstacles when the secondary invasion stock arrived to colonise the rivers and lochs.

Some lochs studied in the North West of Scotland illustrate this

point. Loch a'Mhadaidh Mor (34), Loch Fionn (Sutherland) (50) and Loch Veyatie (44) all above now substantial impassable obstacles, (the Kirkaig falls above which Fionn and Veyatie are situated, being 20 metres high) have high $LDH_5(100)$ allele frequencies. In fact the trout from Loch (34), from the data available are apparently fixed for the $LDH_5(100)$ allele. An interesting observation which has already been mentioned is that three sea trout smolts were caught in the outflow to the loch above the impassable falls in June 1984, indicating the population although isolated for some time (impassable falls being 10 metres in height) is still producing sea running individuals while it would be impossible for those individuals ever to return to their natal spawning burn. This piece of evidence lends more support to the hypothesis that the $LDH_5(100)$ allele is associated with sea running Salmo trutta characteristic of the secondary invasion stock.

In contrast to this situation is Loch Badachro (40) situated less than 1.5 kilometres from the sea. A substantial run of salmon ascend a steep gorge-like section between the sea and the loch and spawn in extensive spawning areas above the loch and below Loch Horrisdale (38). They can get no further up the system due to impassable falls (see Figure 4.7). Although the last section of the river has a steep gradient it is probably impassable for small sea trout at present. But it may have been passable in the recent geological past and would be passable to larger individuals at present. The frequency of the $LDH_5(105)$ allele which is fixed or almost fixed in the upper lochs of this system (Lochs 62, 35, 36, 38, 41) is still at a very

high frequency in Loch Badachro (0.89) whereas intuitively one would have expected a higher frequency of the LDH_{-5} (100) allele. When the angling register from the Sheildaig Lodge Hotel (runs the recreational fishing in the area) was examined, no sea trout have been recorded from Loch Badachro, and only very occasionally are any seen (pers. comm.).

In the Badachro system, the post glacial invasion of trout characterised by the LDH_{-5}(105) allele must have taken place. The system then must have become impassable to the later stock characterised by the LDH_{-5}(100) allele. In recent geological times the impassable falls below Loch Badachro, must have become less severe and allowed passage of large salmonids capable of running the system. Salmon must have been able to ascend while the sea trout could not. The salmon has now filled the available spawning niche and due to the number running the system, the sea trout which now could ascend from the sea lose out through competition. The innate accuracy of sea trout spawning migration (Ferguson and Fleming, 1983) also explains the lack of sea trout running the Badachro system, as sea trout ascending to spawn in non-natal rivers are rare. The above scenario explains the high frequency of the LDH_{-5} (105) allele so close to the sea, in a loch allowing the passage of present day migratory salmonids.

A more thorough examination of sea trout populations is required to confirm the theory that the migratory habit seems to be restricted to populations exhibiting a high frequency of the LDH_{-5} (100) allele.

The heterozygote deficiencies identified in 13 locations for the LDH_5 locus can be explained either by the fact that the two postulated invasion stocks have set up separate spawning populations within each of the lochs studied, giving rise to non-panmictic spawning populations, or that trout have been stocked from waters characterised by one of the alleles into lochs characterised by trout with the other allele and the introduced stock has reproduced separately from the endemic trout stock causing non-panmictic populations to be sampled. In Campbell's (1971, 1979) opinion most waters in Scotland have at one time or another been stocked to satisfy first food requirements and later recreational needs. If the Loch Leven trout, formally known as Salmo levenensis were used for restocking programmes, (which was common in Victorian times (Maitland, 1887) and in the first few decades of this century), because of their reputation as being fast-growing, hard fighting and fine eating, then because the Leven trout is characterisitc of the secondary invasion stock, deviations from Hardy-Weinberg equilibrium in the form of heterozygote deficits may be expected if these trout breed true in the lochs to which they were introduced.

4.4.6 Ferox

Another controversy mentioned in the introduction to this chapter relates to the existence of the ferox trout, formally known as Salmo ferox, massive piscivorous individuals that are highly prized by anglers.

It has been postulated (Ferguson and Fleming, 1983; Ferguson and Mason, 1981) that the ferox may be associated with the LDH_{-5} (105) allele. Populations of ferox have been shown in Ireland to have high LDH_{-5}(105) allele frequencies. It has also been suggested that trout populations with a high LDH_{-5}(105) frequency have a higher growth potential under suitable conditions than do those with high LDH_{-5} (100) allele frequencies (Ferguson and Fleming, 1983).

Unfortunately due to the problems associated with collection and storage of these large trout, the number examined in this study using electrophoresis was limited. Many fish of this type were reported by anglers but by the time the fish or parts of the fish had been transported to the laboratory, the enzymes of interest in the various tissues were usually denatured. However during sampling of lochs by myself, what have been classified as "ferox" were caught and used in electrophoretic screening.

Table 4.21 gives a list of all the "ferox" grouped together, so the deviations from Hardy-Weinberg equilibrium are understandable as many different populations of trout have been sampled to give these results. It can be seen that LDH_{-5} genotypes do not conform to Hardy-Weinberg equilibrium, and are most significantly different from expectations. These genotypes show a heterozygote deficiency, but as so many lochs are represented this is not surprising, as obviously a non-panmictic breeding population has been sampled. The overall LDH_{-5}(105) allele frequency of 0.27 is comparatively

high when compared to the Irish trout populations examined by Ferguson and Fleming (1983) but is low compared to populations examined in Scotland in this study.

Various lochs of interest have been studied to attempt to answer some of the questions posed by the ferox problem.

1. Loch Rannoch which is reputed to hold large ferox type trout, and which produces fish of over 3 kg every season, was sampled. But only 6 individual ferox were taken and the LDH_5 (100/100) genotype was recorded for each of them. Although very small numbers of trout were included this may indicate that the ferox in Loch Rannoch live sympatrically with the other trout, but many spawn separately. Large specimens are caught frequently at the beginning of each angling season in the outflow of the loch (Walker, pers. comm.). Using Nass $\mathbf{x}^2$ on genotypes for populations 16, 17, 18, 19, 20 and 57 the LDH_5 genotypes showed significant differences from expectations ($P < 0.05$). See Table 4.17.

2. The ferox caught from Loch Brodainn on the other hand were characterised by the LDH_5(105) allele.

3. Loch Quoich was visited because it currently holds the official British rod caught brown trout record, at just under 20lbs, and is renowned for its large trout. Twenty trout were taken from the loch in this study of which six were classified as

ferox because they were caught on dead bait charr or trolled lures. The frequency of the LDH_{-5}(105) allele was 0.02 for the twenty fish caught. Five large ferox (3900 to 7425 gms) were reported to me, with their scales during this study, but as the fish were returned alive to the water no chance of enzyme analysis was possible. The growth rate exhibited by these fish was remarkable. The three largest fish were all 7^{++} years of age according to their scales, a phenomenal growth rate, the fastest recorded in Scotland in this study, apart from that exhibited by fish captured in the Loch Awe barrage in October 1984 (see Table 4.4). The largest fish at 10.13 kgs (22½lbs) was only 9^{+} years old. Again this fish was smoked before enzyme analysis could be performed!

Loch Quoich which used to be much smaller was dammed by the Hydroboard. Before damming, the loch used to be connected to Loch Garry and the River Garry with no impassable falls impeding migration of salmonids. The loch according to experienced anglers was typical of many highland lochs producing many small trout, and few over the 450 gm size (1lb). Since the loch has been dammed the topography and nature of the loch has changed drastically, and is typical of most hydrodams in the highlands of Scotland. Due to the large compensation level in Loch Quoich, the water level can drop quickly (more than 15 metres). This, with the subsequent wave action, has effectively destroyed most of the littoral zone, and thus has reduced feeding for trout drastically. On the other hand, Arctic charr (Salvelinus alpinus)

that predominantly feed on zooplankton, do not rely on the littoral zone, and in fact thrive in such conditions. Loch Quoich has a very large population of charr and what appears to be a small population of trout. The logical reason for the existence of large piscivorous trout in this environment is that the trout that do survive in the harsh environment and reach a certain size (25-30 cms) have a large food supply, in the form of young charr that shoal together, making it easier for predators to take individuals. Campbell (1979) identifies a length of about 30-35 cm when the growth rate of ferox in Scotland suddenly increases dramatically, indicating they become hydrodynamically suitable to feed on larger fast moving food items, which prior to that size they were not able to take.

Many lochs in Scotland contain these type of trout, many of them are severely affected by the drastic fluctuations in water level associated with hydro developments. These include Lochs Shin, Monar, Mallardoch, Cluanie, Laggan, Blackwater Reservoir, Quoich, Ericht, Garry (Drummochtar) Errochty, Shira, Glascarnoch and to a lesser extent lochs Rannoch, Tummel, Garry (Lochaber) and Earn.

The ferox type life style therefore may be a prerequisite for trout that lives past a certain size in these types of environment. Most of the brown trout population never exceed 30 cm (Campbell, 1979).

Ferox or large piscivorous trout are also recorded in lochs unaffected by hydro developments and fluctuations in water level. Lochs such as Sionascaig, Fionn (Sutherland), Fionn (Wester Ross), Veyatie, Awe, Cama and Lomond, Killin, Laidon, Assynt, Morar, Ness, Tay. These lochs all contain large populations of charr except Loch Lomond which contains the powan (Coregonus lavaretus L.) in large numbers.

What ever the reason for the switch of ferox from feeding on invertebrates to taking fish, the nutritional advantage of a piscivorous diet is enormous. Campbell (1979) estimated one 15 cm long charr is equivalent in weight to 4,500 x 12 mm chironomid larvae. Campbell (1979) also calculated on an average conversion rate of 7:1, "a ferox increasing in length from 35 to 45 cm during a single growing season and not maturing, would have to ingest approximately 4,000 gm of prey flesh, the equivalent of about 100 charr, 15 cm long. Assuming that a ferox ingests at one feed 15% of its body weight of charr about 1/3 of its own length, then at 35 cm (c 510g) it would take 4 charr about 12 cm long and at 45 cm (c 1075g) 4 charr 15 cm long at a time". Campbell (1979) thus showed that even when the ferox population in a loch is at very low density, they depend on a considerable charr population.

Campbell (1979) compared evidence from Scottish waters with those in Norway and Iceland and suggests that the numbers of ferox in a loch may be directly related to the abundance of

charr and that the predators are not very efficient at catching their prey and rely on weaker members of a shoal of charr. Thus where charr populations are small the likelihood of finding large ferox populations is small. But in lochs such as Loch Quoich where charr thrive the number of ferox is likely to be much greater.

The ferox studied by Campbell (1979) were by and large long lived individuals which reached their age and size he suggests by not maturing at the same time as other individuals within a population and not mating every year once they have first spawned. Campbell (1979) states that "longevity is the fundamental property required for the production of ferox. Length of life in the salmonidae is the result of a complex relationship between maturity and rate of growth: how these factors interact as to what extent heredity play a part has yet to be ascertained and much contradicting evidence exists". It requires a detailed examination of these fish grown under controlled conditions to ascertain certain key facts such as age at maturation, growth rate, food preference and behavioural traits at spawning.

Summary of ferox data

1. Ferox feed on Arctic charr and their growth and number seems proportional to size of the charr populations in each loch. They tend to be long lived and mature late (Campbell, 1979).

2. Lochs not containing ferox prior to damming, now contain very large fast growing piscivorous trout, which are utilizing the expanded char populations which have benefitted from the hydro developments.

3. Ferox are no more associated with LDH_{-5}(105) allele in Scotland than any other trout according to this study. But so little data is available through this project further extensive collection of data is required to identify any electrophoretic markers within the ferox trout in Scotland.

4. Certain evidence tends to suggest some populations of ferox may live sympatrically in lochs with other strains of trout (Rannoch) and spawn separately. The evidence is very tenuous.

If the last point is valid, it can be explained in that trout return to their natal spawning streams, and even if the so called ferox did so in each loch at the same time as the other small trout, reproductive isolation could conceivably be explained by assortative matings. Most salmonids spawn with fish of their own size.

If relatively small numbers of individual ferox were involved one could also envisage founder effects occurring and subsequent genetic drift, explaining how populations of ferox could become genetically distinct as far as electrophoretically detectable enzymatic variation is concerned within each loch.

4.4.7 Genetic variation in Scottish brown trout

A great deal of genetic variation in the Scottish brown trout appears to be due to variation among populations. The large amount of divergence between closely located populations is well illustrated by the dendrogram (Figure 4.4) (UPGMA Sneath and Sokal 1973) based on the pairwise genetic distance values (Nei, 1975), constructed from the allele frequencies calculated for this study. There is an apparent lack of correspondence between geographic area and genetic distance as measured from the electrophoretic loci. For example populations 16 and 17 and widely separated by the dendrogam from populations 18 and 19 but all are derived from the same loch (Rannoch). Similarly populations 48 and 63 which are very remote geographically form a close cluster in the dendrogram. Of course, this does not imply that these two populations are genetically very similar over the major fraction of the genome; they are most likely quite different. This agrees with Ryman's (1983) dendrogram representing 35 populations of Swedish brown trout. Both Ryman's estimate of maximum genetic distance and the one calculated in this study are approximately equal (almost 0.05) and yet both studies show most of the locations exhibit genetic distances at < 0.02 and most are below 0.01.

Nei (1972) estimated that by multiplying the genetic distance by 5×10^6 years one could estimate the time when the populations under consideration diverged and became reproductively separate. More recently Nei's time of divergence has been questioned and Gorman et al. (1976) who proposed multiplying the genetic distance estimates

by 18 x 10^6 years. Thus by using both conversion figures with the genetic distance estimates in this study, two different ranges of estimates of time of divergence are calculated.

Genetic Distance	Time of Divergence (yrs)	
	Nei	Gorman et al.
0.01	50,000	180,000
0.02	100,000	360,000
0.03	150,000	540,000
0.04	200,000	720,000
0.05	250,000	900,000

It must be remembered that the method of producing dendrograms has its limitations as mentioned in the introduction. The figures for genetic distance in this study are small and the standard errors associated with these estimates are comparatively large and reduce the significance of the distance estimates. Other problems with the dendrogam technique include:

1. The fact that electrophoresis as mentioned only detects approximately 27% of codon changes and thus will always underestimate differences between populations (King and Wilson, 1975).

2. Differences in number of individuals and number of loci examined leads to variation in estimate of genetic distance. More

information will be available by increasing the number of loci rather than the number of individuals in the species or population, and by increasing the number of loci one will decrease the size of the standard error and lead to a more accurate interpretation (Thorpe, 1982).

3. Sarich (1977) also pointed out that independently derived sets of similarities for distances tend to differ which is usually a result of using different loci.

The other assumption one has to make when using the UPGMA method of producing dendrograms is that the rate of evolution for the different proteins one is studying is the same (Thorpe, 1982).

Taking all drawbacks and assumptions into consideration the dendrogram produced in this study for the wild population of brown trout studied in Scotland shows similar variation as a similar study by Ryman (1983). The dendrogram divergence pattern is that expected to be observed for selectively neutral or nearly neutral loci among populations characterized by a very restricted amount of gene flow even between closely located populations.

In Ryman's (1983) dendrogram, the population which has the highest genetic distance compared to the next pairing is D1. This population amongst other allele frequencies is characterised by being fixed for the $LDH_5(105)$ allele. This is similar to the situation found

in this study. Population 29 is the population exhibiting the greatest genetic distance compared to the next pairing and is also fixed for the LDH_5(105) allele. Other populations in the dendrogram situated adjacent to population 29 are fixed or nearly fixed for the LDH_5(105) allele. (Populations 37, 14, 62, , 36, 35, 28, 27, 30).

Genetic variability has been demonstrated but as Ryman (1983) pointed out, it is essential to determine the distribution of genetic variation and thus Table 4.11 and 4.12 illustrate the results of the gene diversity analysis performed (Chakraborty et al., 1982).

The table of relative gene diversities (Table 4.12) gives a within population gene diversity of 67.4% which agrees very well with Ryman's estimate of 63.3% calculated using 38 Swedish samples (35 locations).

Ryman (1983) thus calculated that a very high proportion (37%) of the total gene diversity was distributed between populations. He also showed considerable differentiation on a micro geographical scale with difference between populations within areas (13.4%) being approximately of the same magnitude as that between areas (15.3%) whereas the difference between major drainages was much smaller (7.5%). The results in this study are in broad agreement, approximately 33% of the total gene diversity is distributed between populations. Almost 10% of the diversity is attributed to populations within the same drainage areas, while only 1.4% of the gene diveristy is attributed to the major East/West drainage divide.

These results when compared to other salmonids give a situation with the Atlantic salmon exhibiting a more similar gene diversity to that of the brown trout than that of rainbow trout, or any of the Oncorhynchus species (Table 4.1 in the introduction). The only value higher than brown trout for gene diversity distributed between populations comes from one study cited by Gyllensten (1985) on Salvelinus alpinus where 53.3% of the relative gene diversity was attributed to between localities. Another study also cited for the same species gives a reduced value attributable to diveristy between localities (24.4%).

In Ryman's (1983) study, it was shown that there were considerable differences in the distribution of genetic variability between closely related species. This is very important when attempting to utilize wild populations in research and fish culture. Ryman (1983) gives an example, "in the rainbow trout that exhibits an absolutely high level of genetic variation with a comparatively small fraction of the total gene diversity distributed among populations, the result of a directional selection programme is expected to be successful within any "typical" population. In contrast, the success of a breeding programme in Atlantic salmon or brown trout should be much more dependent on the access to multiple stocks for utilization of the between population component of genetic variation".

Because of the large genetic variability shown to exist between geographically closely related populations of brown trout in this study and others, the genetic basis of a breeding programme may be considerably increased through the inclusion of geographically

closely related stocks. Local breeding programmes will become significantly more successful as it implies that novel genetic variation can be found among populations that have been adapted to the same or very similar climatic and other environmental factors (Ryman, 1983).

The importance of the inclusion of as many loci as possible when calculating gene diversity was stressed by Ryman (1983) who showed that there were considerable differences exhibited by different loci as far as their distribution of gene diversity was concerned. Ryman's (1983) within population relative gene diversities ranged from 36.2% (LDH_{-1}) to 91.5 (SOD).

In this study approximately the same total number of loci were used but 13 polymorphic loci were screened instead of 9 in Ryman's (1983) survey. In this study the within population relative gene diversities for individual loci ranged from 41.3% (LDH_{-5}) to 94.4% (IDH_{-2}). (See Tables 4.11and 4.12).

4.4.8 Illustrations of genetic heterozygosity amongst populations of brown trout in restricted geographic areas

Ryman (1981) illustrated distribution of genetic variation in natural populations of brown trout in Sweden by including data on the source area of the River Fjallsjöälven in Jantland, Northern Sweden, and showed "remarkable genetic heterogeneity" on a micro geographical scale. He concluded that the gene frequency differences reflected the existence of several completely or partially reproductively

isolated gene pools within a very restricted geographical area. Ryman (1981) also showed marked genetic heterogeneity among sampling sites in the Lake Lulejaure area. Taggart (1981) also recorded highly significant genetic heterogeneity among populations within restricted geographical areas.

In this study similar levels of genetic heterogeneity have been identified and illustrated at 5 different areas in Scotland. Each of the five selected areas is treated in the same manner with a map of the locations involved along with pie-charts representing allele frequencies of the polymorphic enzymes identified. This gives a visual impression of the micro geographical variation but the allele frequencies are obviously derived from different numbers of individual fish per location, and therefore the significance of allele frequency differences is often not possible to judge from the visual interpretation of the data. The contingency tables accompanying each map gives levels of significance between the observed genotypes at each location.

1. The Badachro system. Using Nass χ^2 goodness-of-fit test for all six lochs in the system all the polymorphic loci except LDH_5 showed significant differnces between the populations.

   From Figure 4.7 one strain of trout appears to be confined to the first three lochs in the upper part of the system while the lower three are different. Large allele frequency differences exist for AAT_{-4}, IDH_{-1}, and MDH_{-2}. Three variant alleles ($MDH_{3,4}(125)$,

$PGI_{-2}(135)$, $PGI_{-3}(100)$ appear in only one of the lochs surveyed at the bottom of the system, each in a separate loch. The genotype differences are significant but the samples sizes in Lochs Clair and Badachro are small so that variant alleles at low frequencies which may have been present, may not have shown up in the electrophoretic screening.

If one also studies the dendrogram in Figure 4.5 and the Table 4.13 containing Nei's genetic distances from which the dendrogram was constructed, for the Badachro system, they support the argument that the populations samples are derived from two separate strains of trout, although the genetic distances separating the populations is small and the associated standard errors are comparatively large.

Population 37 which was taken from the same loch as 36, from the same area, shows a larger genetic distance value than would be expected (see Table 4.13 and Figure 4.5) illustrating the problems of attaching too much significance to variations manifesting themselves as small differences in genetic distance estimates. Such differences may have a strictly genetic component, but may also be due to aberent sampling errors.

2. Rannoch Moor. Once again there appears to be significant substantial heterogeneity in this small area. From Figure 4.8 the allele frequency pie-charts indicate considerable variation between locations. Due to small sample sizes (Populations

9 - 14 $\leqslant$ 16 individuals) some of the allelic variation is not significant. Tables 4.16 listing the genotypes and Nass x^2 significance tests identify five loci showing significant differences. AAT_4($P < 0.01$), DIA_{-1}($P < 0.05$), IDH_{-1}($P < 0.01$), LDH_{-5}($P<0.05$) and MDH_{-2}($P< 0.001$).

Loch Laidon (15) at the bottom of the system was the only location to show variation at IDH_{-2} and LDH_{-1} but as the sample size of this population was larger than for the other locations sampled this was not significant.

Population (13) which was taken from a very small isolated pool on the moor seems to be genetically distinct from the trout occupying Loch Ba (14), although the distance separating these two locations is less than 200 metres. The sample size from Loch Ba is very small (6) so that the differences found require to be validated. The trout themselves were conspicuously different in appearance.

The spawning populations in locations (11), (12) and (13) are all small, restricted by the physical size of the peat pools they live in. The spawning areas are also severely limited giving rise to conditions where genetic drift and the founder effect are likely to have played a significant role.

3. Loch Rannoch. From Figure 4.9 illustrating the sampling sites on Loch Rannoch, with accompanying allele frequency pie-charts, and the genotype Tables 4.17 it can be seen that according to Nass χ^2 test there are significant differences between populations at 4 different loci, (AAT_{-4}($P < 0.05$), IDH_{-1}($P < 0.01$), LDH_{-5}($P < 0.05$), MDH_{-2}($P < 0.001$). This indicates that isolation due to reproductive separation between streams has set up separate genetically distinguishable populations. In population (19) two variant alleles (IDH_{-2} (130), $MDH_{-3,4}$(125)) were identified at very low frequencies but were not significant. Population (57) represents 6 ferox trout netted in the loch, and variant alleles were present at all the highly polymorphic loci except LDH_{-5} and AAT_{-4}. To conclude anything from this would be a mistake owing to the very small sample size involved. More work is required to ascertain whether the ferox represents a separate spawning population of brown trout in Loch Rannoch.

Another piece of evidence to suggest there is at least one spawning population of trout in Loch Rannoch is the deviation from the Hardy-Weinberg expectation of LDH_5 genotypes grouped together for the whole of Loch Rannoch. There appears to be a definite heterozygote deficit ($G = 19.52$ $df = 1$ $P<0.001$).

Areas (2) and (3) are also represented by the dendrogram (Figure 4.6) and the Table 4.14giving Nei's genetic distances and standard errors. Populations (21) ⟶ (23) are also included as they are situated in the same major drainage system. The genetic

distances exhibited are surprisingly high within a small area. Again too much interpretation of the dendrogram is not valid as for example population (14) only represents six individual fish. Although the larger populations may give more information, there seems to be a definite dichotomy between populations 18, 19, 15, 12, 11, 9, 13 and 10 and populations 16, 17, 20, and 21. Once again this illustrates the amount of genetic diversity present in small geographical areas.

4. Pattack system. This area illustrated in Figure 4.10 with genotypes analysed in Table 4.18 for significance between locations is interesting as Lochs (28) and (29) are both situated above 700 metres (2,100 feet), and are less than 3 kms apart. Despite this, considerable differences are evident at allele frequency and gentoype frequencies. According to the Nass x^2 test five loci show highly significant differences ($P < 0.001$) in genotypic distribution between the three lochs. The loci being G-3PDH$_{-2}$, IDH$_{-1}$, LDH$_{-1}$, LDH$_{-5}$, and MDH$_{-2}$.

Populations (28) and (27) are similar compared to population (29) except the proportion of G3PDH$_{-2}$(100) and LDH$_{-5}$(100) alleles is greater in (27) than (28). This could be associated with the fact that the loch has been stocked (Walker, pers. comm.). The differences are still remarkable considering the small geographic area involved. .

5. In this system on the edge of the Cairngorm mountains there

appears to be less genetic heterogeneity. If one studies Figure 4.11 and the genotype Tables 4.19 one can see that only LDH_{-5} shows any significnt differnce in interpopulation heterogeneity. (Nass χ^2 = 30.59 $P < 0.001$). The fact that LDH_{-5}(105) allele is fixed in population (24) probably means that the sample represents an isolated ancestral population, above impassable falls. Populations (25) and (26) have been influenced by the secondary invasion stock of trout characterized by the LDH_{-5}(100) allele, but are probably still receiving an input of the LDH_{-5}(105) allele due to one way immigration downstream from loch (24) creating gene flow.

4.4.9 Pristine populations

Pristine populations have often been claimed to be of use in future fishery management policies (Ryman, 1981; Ferguson and Fleming, 1983). They can be identified by electrophoretic screening but require extensive quantitative growth trials under a variety of environmental conditions before one can say whether they will be of use for stocking or reintroductions. The populations fixed for LDH_{-5}(105) in this study seem likely to be as pristine as one is likely to find in the highly perturbated waters of Scotland. The least heterozygous population of fish, fixed for LDH_{-5} came from Loch na Criege Riabhaich (30) in the Crocach complex adjacent to Loch Hope in Sutherland. Other lochs regarded as pristine include Loch an Duin (24), Loch a'Bhealiach Bheithe (28), Loch an Sgcir (29), Loch a Bhealiach (35).

4.4.10 The use of allelic markers to distinguish stocked trout or markers that could be used in future as genetic tags

As mentioned in the introduction, the success of most stocking or enhancement programmes involving salmonids, including brown trout, is uncertain because of no means of successfully monitoring the progress of individual or stocks of fish. Taggart and Ferguson (1984) list four advantages of using genetic allelic tags over conventional tags (see introduction) and conclude that certain variant alleles are ideal for the purpose. Taggart and Ferguson (1984) used PGI_3 as a tag as it appeared in their screening of hatchery populations and rarely in the wild.

It is suggested that once sufficient initial electrophoretic screening of appropriate lochs in Scotland has taken place, a variety of tags could be used. If one was intending to stock one of the high isolated lochs with trout for whatever reason, the LDH_5 (100) allele could be used as a genetic tag. It is not advised to adopt such a strategy unless it is felt absolutely necessary, but it might shed light on how, or if, the strain of trout characterized by LDH_5(105) allele is outcompeted by the LDH_5(100) allele stock.

In contrast, if lowland waters characterized by the LDH_5(100) allele stock is to be stocked, a population fixed for LDH_5(105) could be used. The Howietoun fish farm stock has the variant LDH_5(105) allele present and in theory, stocks fixed for LDH_5(105) and LDH_5(100) could be produced.

Other alleles suitable for such tags include PGI_{-3} as explained by Taggart and Feguson (1984). The Howietoun fish farm stock also contains PGI_{-3} at low levels and by selective breeding a stock fixed for $PGI_{-3}(110)$ could be produced. Interestingly the American hatchery strain of trout kept at DAFS in Pitlochry also has the $PGI_{-3}(110)$ variant present, although it is rare in the wild populations of trout in Scotland. Another advantage pointed out by Taggart and Ferguson (1984) is that PGI_{-3} is expressed well in the adipose fin of brown trout and is thus easily screened for, without killing the individuals within the population.

Other likely candidate variant alleles at a very low frequecny in wild populations but present in the hatchery population which are reliable for mass screening purposes are $IDH_{-2}(130)$, $PGI_{-2}(135)$. The other variant alleles are too common in wild populations to make any effective use as genetic tags.

It should be emphasised that before genetic tagging is undertaken, the relative performance of trout characterised by the homozygotes for the particular variant allele should be ascertained, to ensure no selective disadvantage is associated with the genotype.

4.4.11 Discussion of linkage disequilibrium

From Table 4.23 it appears that there are many different non-random associations between loci but there are other ways of generating linkage disequilibrium which do not reflect a genetic basis. These can be summarised as follows:

1. Sampling error (Ohta and Kimura, 1969).

2. Random drift in small populations (Hill and Robertson, 1968; Ohła and Kimura, 1969).

3. The collection of animals from heterogenous populations.

4. Pooling of data from different populations (Nei and Li, 1973)

All four points can be applied to the results in Table 4.23 Some of the populations studied represented limited numbers of individuals which, as already mentioned, probably has lead to random drift. Some of the populations exhibited heterozygote deficiencies indicating heterogenous populations had been sampled, and as all the individuals in the survey of wild trout were included in the examination of joint segregation of loci, 63 populations have been pooled.

It was then decided to treat the four largest populations ($n \geq 60$) electrophoretically screened separately. This showed that there was no joint segregation of loci identified in population (16) and only three other significant segregations appeared for populations (8), (42) and (43).

These were G-3PDH$_{-2}$ with LDH$_{-5}$	- Pop (8)	$P<0.05$
AAT$_{-1,2}$ with LDH$_{-5}$	- Pop (42)	$P<0.01$
G-3PDH$_{-2}$ with IDH$_{-1}$	- Pop (43)	$P<0.05$

The sample numbers are regarded as so small that these results require verification.

It is therefore felt that the present population data collected in this study is not sufficient to enable conclusions to be drawn regarding joint segregation and possible linkage disequilibrium among the polymorphic loci identified.

4.4.12 Conclusions and Recommendations

Electrophoretically determined protein variants form useful markers for the delimitation of brown trout populations. They are particularly useful in the identification of gentically distinct sympatric populations (Ferguson and Fleming, 1983).

Ryman (1981) in his summary of the conference on fish gene pools recommended the following to conserve the genetic resources represented by wild trout populations:

1. The identification of the genetic resource. The gathering of data on the diversity of existing populations over a wide geographical range and environmental conditions is essential, to establish the extent of differentiation among populations. It is stressed that not only electrophoretic information is required but information pertaining to such traits as the ecology, physiology, disease susceptibility and behaviour of the different populations is required.

2. The maintenance of natural ecosystems. Maintaining the environment can contribute greatly to the maintenance of endangered populations.

3. The maintenance of genetic variability. "Efforts should be directed to conserve the most divergent and genetically variable forms as they are most likely to provide material for the best management of our natural resources and future use of these resources in aquaculture". The brown trout shows so much genetic variability between very close geographic populations and it is stressed that management programmes are required to conserve this variability. The obvious problems in this country connected with these objectives are

   (a) financial constraint, and

   (b) conflict with other requirements in society.

   If important populations cannot be saved in the wild, then techniques such as storage of sperm and eggs need to be developed to enable the genetic resource to be saved.

4. Careful exploitation of wild populations. Trout fisheries should be managed in such a way as not to alter the genetic characteristics of each population through selective fishing procedures.

5. Careful husbandry of cultured populations. Care should be taken to avoid problems inherent in maintaining brown trout stocks, by applying existing knowledge of population genetics. Electrophoretic, as well as quantitative monitoring of cultured populations, should be initiated to monitor possible inbreeding effects.

6. Careful stocking of hatchery trout into natural populations. Care should be taken when releasing fish to avoid damage to local genetically differentiated populations due to either direct adverse genetic interactions or by competition set up between the introduced fish and the endemic stock.

7. Research and management. Funds are required for both basic and applied research to provide for the continued existence of the brown trout as a biological resource for future generations. Ryman (1981) states "Because of the complex and widespread problems involved, research and management should be pursued on an inter-disciplinary and international basis".

8. Education and dissemination of information. Mechanisms must be made available by which all the interested and relevant organisations pursuing trout management should have the latest existing knowledge readily available, to ensure sensible practical applications.

Gjedrem (1981) indicates that large numbers of valuable trout populations have been and are continuously being lost as a result of habitat destruction and unwise management programmes. The continuously increasing exploitation of land and water resources calls for immediate implementation of the above strategy.

Ryman's summary encapsulates the problems encountered in Scottish brown trout populations, and his recommendations concerning the

future are also relevant.

The Scottish populations of brown trout are genetically very diverse with much of the variation apparent within small geographic areas, very similar to the situation found in Scandinavia.

This study represents an extremely brief examination of the trout populations present in Scotland, and the gathering of further extensive data is an urgent requirement. I am in full agreement with Ryman who points out the need, not only to identify sub populations or strains electrophoretically, but to subsequently study quantitatively, triats of those populations likely to be useful for future management of the resource.

It is realised that this exercise is likely to be exceptionally expensive and unless drastic changes occur in the decision making processes relating to fishery resources in this country it will remain merely a concept.

Ryman's second recommendation, concerning the preservation of natural ecosystems is a much more realistic goal and as he points out, it can contribute greatly to the preservation of endangered populations. But a prerequisite required for the maintenance of the correct environment is a thorough knowledge of the resource one is trying to protect. This, once again, leads to the problem of identifying genetically endangered populations and the obvious method available at present is electrophoretic screening.

The brown trout populations in Scotland seem to be different from those in Ireland and Scandinavia, because they have been extensively perturbed by various human activities. It is therefore a priority to identify genetically pristine populations before any more are lost due to artificial introductions, water extraction, hydro development, other land use changes and increase in acidification.

Where artificial stocking is required, it is also suggested that small hatcheries be constructed which would supply eggs, fry, parr or adult trout to waters within each area of Scotland. The broodstock used, should be derived from local stocks, thus eliminating the damage to local genetically differentiated populations due to introductions from outwith the area.

This of course would only be feasible once a co-ordinated approach was taken to the management of trout stocks in Scotland. These hatcheries should be funded by government, but would only be feasible if angling as such was reorganised in line with salmonid angling in USA and Canada. This strategy would have the benefit of creating employment.

The above is probably an unlikely scenario and thus alternative provisions should be made for the well being of the Scottish trout stocks. Farms such as Howietoun should take a lead. They should not stock lochs which have not received previous introductions without detailed investigations. A set of different breeding lines could

be established so that the farm endeavours to stock waters with similar trout to those inhabiting them. The ancestral stock identified by the LDH_{-5}(105) allele should be collected and bred.

Careful husbandry of such populations is essential to minimise possible inbreeding effects. Electrophoresis as well as quantitative monitoring of the traits of the cultured populations is recommended.

This type of approach, which would replace the "chuck it and chance it" philosophy to salmonid stocking so prevalent at the moment in Scotland, would require a more highly trained workforce. The stocking of non-endemic salmonids, including brown trout into waters with no prior examination of the existing stock and no planned investigative back up procedure, is an inditement of fishery management.

One reason for this apparent lack of concern about the subsequent effects of stocking is financial. If monetary considerations were not such a restraint then a more enlightened philosophy to management might be forthcoming.

Government funds are required for both basic and applied research to provide the continued existence and sensible exploitation of the brown trout as a biological resource for future generations in Scotland and elsewhere. In conjunction with this funding and extensive network for the rapid dissemination of information is required. Useful research had been conducted by various organisations

in Scotland in the past, including DAFS, and little of this is made widely available to those that need to be informed.

CHAPTER 5

5 Early stage of the hatchery trout life cycle and subsequent heterozygosity and growth relationships

5.1 Aims of Chapter 5

In the previous chapters I have dealt exclusively with either heritability estimation for growth rate or electrophoretic variability in brown trout.

In this chapter there is an attempt to:

1. Link heterozygosity (calculated from an electrophoretic survey of the populations used in the growth/heritability trials), with early growth rate and other parameters measured during the young stages of life. The aim of this investigation was to identify any relationship between heterozygosity and growth rate, which would lead to conclusions relating to the ongoing neutralist/selectionist argument of the theory of molecular variation and evolution.

2. Link various parameters and statistics concerned with early life stages in the hatchery stocks (some of which are without genetic origin) with the subsequent growth rate of trout. The aim of this exercise was to show that if growth rate is taken in total isolation as the parameter of interest when calculating heritabilities and recommending subsequent selection procedures, false estimates could be derived if non-genetic parameters are not also considered.

5.2 Introduction. Factors affecting growth and survival of young trout

Environmental as well as genetic factors may affect salmonid egg survival, hatching time, and development of alevins, and the growth and survival of fry (Beacham et al., 1985). The time of emergence and the size of the resulting fry at this stage, may influence subsequent survival and growth. The size of alevins and fry are also influenced by the size of the eggs from which they hatch, with larger alevins and fry generally developing from larger eggs. Thus size of alevins may significantly affect the subsequent growth and survival of the fingerlings and older fish.

This part of the study is included so as to identify any correlation between growth and early life cycle parameters, and to attempt to determine how much the environmental factors that influence the early stages of the life cycle, are correlated with the eventual size of the trout studied. Various factors may affect subsequent heritability estimates determined for growth rate. The following traits are thought to be relevant, when considering later growth, and survival:

5.2.1 Egg size

Gall (1974) studied the relationship between fecundity, age and size of fish in natural rainbow trout populations, and showed that in general larger and older females produced greater numbers of larger eggs than smaller and/or younger individuals. Similar results have been obtained for hatchery-reared stocks of rainbow trout (Buss nad McCreary, 1960). Thorpe et al. (1984) gives extensive

references for work carried out on Atlantic salmon. Many authors have recorded that Atlantic salmon egg size increased with parental size. Egg size and parental age are apparently less clearly related according to Thorpe et al (1984) who cites Aulstad and Gjedrem (1973) who studied 16 differenct salmon stocks and found no correlation. Springate and Bromage (1984) and Springate et al. (1984) state that larger older rainbow trout have higher total fecundities and produce larger eggs than smaller, younger fish.

Bagenal (1969) noted that eggs of brown trout varied in size and number even from parent fish of the same length, weight and stock, but that the eggs produced by one individual female tend to be more uniform in size.

Significant differences in egg size have been reported in all salmonid species and these differences are believed to be mainly of genetic origin (Gall and Cross, 1978a and b).

Gjerde (1986) gives a resumé of the heritability estimates available for egg size, number and volume for rainbow trout and Atlantic salmon, and comes to the conclusion that the data gives strong evidence of significant additive genetic variation for the above traits.

5.2.2. Time of stripping

This parameter is often ignored when dealing with subsequent mortalities at various stages of young fishes life histories.

Ovulated eggs of oviparous teleosts become overripe if retained in the body cavity and these eggs show a progressive reduction in viability (Mollah and Tan, 1983 and references within).

The reason for the decrease in viability is due to morphological changes which are characterised by the aggregation and fusion of oil droplets, and the migration of cortical alveoli to the animal pole (Nomura et al., 1974).

Craik and Harvey (1984) working with rainbow trout found hatching percentage declined sharply within 18 days after ovulation from over 90% to near 0% and they conclude that the time of stripping of the eggs in relation to the date of ovulation is a much more significant parameter in determining "egg quality" than any of the chemical and physical aspects of egg composition which they investigated.

Springate et al. (1984) indicated that maxmimum egg and fry survivals are achieved if eggs from rainbow trout are stripped 4-6 days post-ovulation. Survival of the developmental stages were closely correlated with fertilisation percentage. Low fertilization was followed by reduced success at each subsequent developmental stage.

Springate et al. (1984) conclude by suggesting that "determination of percentage fertilization is proposed as a management tool to predict subsequent egg and fry performance".

5.2.3. Egg size and subsequent developmments

Various authors have studied the development of alevins and fry and correlated these parameters with initial egg size. Bagenal (1969) working with brown trout showed that at constant temperature, large fry derived from large eggs survived longer without food than small fry from small eggs. Beacham et al. (1985) working with chum salmon (Oncorhynchus keta) and Coho salmon (Oncorhynchus kisutch) found fry derived from large eggs had greater tissue weight at exogenous yolk absorption than those derived from small eggs. Bagenal (1969) also cited Blaxter and Hempel (1963, 1966) who showed that larger herring eggs conferred an advantage on the larger larvae produced, as they survived longer without exogenous food.

Springate and Bromage (1985) found in rainbow trout a significant correlation between egg and fry size at hatching but this correlation was lost 4 weeks after the time of first feeding. Gall (1974) reported similar results but the positive correlation between egg size and growth was extended upto 75 days and 4 months respectively. Reagan and Conley (1977) working with channel catfish reported a correlation lasting one month between egg size and initial growth.

Springate and Bromage (1985) also reported no significant correlation between initial egg size and survival rates at the eyed stage, hatching and swim up and as 3 month fed fry.

Craik and Harvey (1984) working with rainbow trout found significant positive correlations ($P < 0.05$) between percentage of hatched eggs (alevins) surviving to first feeding and each of the following:-

egg weight (dry and wet) and absolute levels in the egg of bound lipid, precipitable protein and protein phosphorus.

It is obvious that there is great variation in salmonid egg size, but what advantages are there in natural populations, for individuals producing large or small eggs, and thus large or small alevins?

It has been shown that small eggs give rise to small alevins and these alevins are reported to use up their yolk food reserve before larger alevins hatched at the same time. It has been postulated that small alevins would thus emerge from the gravel and take up feeding positions before the larger fry, and thus gain an advantage. Bagenal (1969) dispelled this theory for brown trout in the wild "the survival of trout is significantly greater in the fry derived from large eggs than those from small eggs. If therefore, there should be a mutation to produce a higher fecundity at the expense of egg size, it is unlikely that this would spread through the population. It is more likely that the mutation would be eliminated fairly rapidly by the competition of the resulting smaller fry with other larger ones derived from few larger eggs".

The same constraints on selection are obviously not the same in hatchery conditions, where because small eggs give rise to viable healthy fry, if husbandry practices are sufficiently good, there will be little selection against small eggs unless the fish farmer especially selects for larger eggs.

5.2.4 Malformities at hatching

Aulstad and Kittelsen (1971) conclude, following an experiment involved with inbreeding rainbow trout, that body curvature is at least partly heritable, occurring in the inbred line, but not in the control in their experiment. They also postulate that the factor (or factors) that cause the observed deformity may also cause higher mortality of eggs and of fry not showing deformity. Aulstad et al. (1972), Kincaid (1976a, b) and Gjerde et al. (1983) have shown highly significant inbreeding depression for survival of eyed eggs, alevins and fry. Kincaid (1976b) also reported a moderate inbreeding depression for growth of fry whereas Moav (1976) reported a large inbreeding depression for growth in carp (Cyprinus carpio)

Kincaid (1976a, b) showed that at an inbreeding intensity of one generation of full sib matings, the level of inbreeding had no effect on egg hatchability, but it significantly increased the frequency of crippled malformed fry by 37.6%, and significantly decreased fry survival at 147 days by 14.7% and growth rate at 147 days by 6%. When two generations of full sib matings was used as the inbreeding intensity, the percentage values increased to 191.5%, 29.7% and 13.4% respectively.

Kincaid (1976b) concluded with remarks directed at personnel maintaining broodfish on farms. He recommended they adopt breeding approaches that will minimise potential future inbreeding problems. Kincaid (1976b) notes the current approaches used to avoid inbreeding

fall into 4 categories:

1. Use of large random-mating populations

2. Rotational line crossing

3. Periodic introduction of unrelated stocks

4. Use of hybrid populations.

Heritability estimates for early life stages may be inaccurate because of factors that may tend to make the estimates biased, especially if only a limited number of broodstock are used.

The dam component is expected to be higher than the sire component, because it includes in addition to the additive genetic variance, maternal effects, and non additive genetic variance. The maternal effects would consist of the variation in egg size, and quality of the yolk sac, together with a possible tray/tank effect, which includes differences in density and other environmental effects (Kanis et al., 1976). Among the environmental effects is the ripeness at stripping which has been shown can alter subsequent performance drastically.

Sires stripped badly (contaminated by water and/or faeces), or the condition of the sire at stripping can also have serious consequences as far as fertilization and subsequent mortalities is concerned.

To determine the relationships between the various factors described, the following studies were undertaken, in addition to the measurements of growth described in chapter 3.

1. Measurement of egg size

2. Monitoring of mortality from fertilization through to the fry stage

3. Monitoring of malformities within each population

4. Measurement of rate at which different sized alevins developing from different sized eggs, utilize their yolk sac

5. Estimation of time of hatching and length of emergence period.

5.2.5 Introduction to the relationship between heterozygosity and growth rate

In recent years quite a number of papers have been published (see main introduction) examining the relationship between growth rate and heterozygosity. A variety of plants and animals have been investigated, and the majority of these studies indicated the existence of a significant positive correlation (Mitton and Grant, 1984).

The variety of organisms originally studied was limited with different species of bivalves yielding positive correlations between multi-locus heterozygosity and growth rate. Singh and Zouros (1978), Zouros *et al.* (1980) and Singh (1982) concentrated on the American oyster, *Crassostrea virginica*, while Garton *et al.* (1984) found the same correlation in *Mulinia lateralis*, and Green *et al.* (1983

worked with Macoma balthica. Koehn and Gaffney (1984) used Mytilus edulis to show the positive correlation between heterozygosity and growth rate.

Singh and Green (1984) examined excess allozyme homozygosity in marine molluscs and found that

(1) the degree of the excess is dependant on the age and stage of development, being higher in younger rather than older age groups,

(2) the degree of homozygosity had a negative correlation with growth rate and,

(3) the slow growers have a higher post-settlement mortality rate.

Singh and Green (1984) postulate a balancing selectionist model to explain these findings, when the relative fitness of homozygotes and heterozygotes is different during the pelagic larval phase and from stages following settlement.

Singh (1982) suggested that not only was the number of heterozygous loci per individual oyster positively correlated with growth rate, but the variance in weight was lower in heterozygotes. The variance in weight, Singh (1982) claimed also decreased with increase in number of heterozygous loci in a given age group and suggested overdominance in growth rate, to be the most plausible explanation for these observations.

Koehn and Gaffney (1984) along with many others, subsequently using their results with Mytilus edulis which conformed closely with results obtained by Zouros et al (1980) concluded, "the relationship between multiple locus heterozygosity and growth rate is one that is general to a diversity of outbreeding plant and animal populations". Koehn and Gaffney (1986) also state "other studies indicate this relationship is due to to a greater average metabolic efficiency of more heterozygous individuals".

Similar evidence for the link between heterozygosity and growth rate has been presented by authors not working with marine bivalves. Cothran et al. (1983) examined foetal growth rate in the white tailed deer (Odocoileus virginanus) and Bottini et al. (1979) presented similar work conducted on man.

Pierce and Mitton (1982) found 5 out of 7 populations of the salamander (Ambystoma tigrium), exhibited a positive correlation between heterozygosity and length. Mitton and Grant (1980) extended the concept to plants and showed that heterozygosity was positively correlated with growth rate in the quaking aspen (Populus tremuloides)

In contrast to these findings a number of trials have shown no association at all between heterozygosity and growth. No correlations were found between growth rate in mature trees and heterozygosity by Grant et al (1982), Knowles and Mitton (1980), Knowles and Grant (1981), Mitton (1983) and Mitton et al (1981) who worked with the lodgepole pine (Pinus contorta) and the ponderosa pine (Pinus ponderosa).

Similar results were reported from studies using various types of fish. King (1985) found little evidence of correlation between heterozygosity and growth rate in herring (Clupea harengus) and Beacham and Withler (1985) submitted similar results using pink salmon (Oncorhynchus garbuscha).

McAndrew et al. (1982) and (1986) found no correlation between heterozygosity and growth rate and various meristic parameters, in a large survey of Irish sea plaice (Pleuronectes platessa).

Foltz and Zouros (1984) and Beaumont et al (1985) working once again with marine bivalves, Placopecten magellanicus and Pecten maximus, respectively found little or no correlation between heterozygosity and growth rate.

McAndrew et al. (1986) point out that if there is a general phenomenon linking heterozygosity to growth rate, it has "clear theoretical implications for the current neutralist/selectionist controversy and important practical implications for those engaging in animal and plant breeding."

To investigate the relationship between heterozygosity and growth rate in brown trout, an electrophoretic analysis was performed in conjunction with growth assessment using a limited number of individuals from each cross used for the growth rate/heritability trials (chapter 3).

5.3 Materials and methods

The methods by which data were collected on sizes of eggs, alevins and fry and the calculations of percentage mortalities at various stages of development, along with calculations of percentage deformities, are covered in the relevant section of chapter 3. Multiple correlations using different parameters relating to each tank population were performed using the minitab package on the Stirling University computer system.

Malformed trout from the cross between the Nashua ♀ 1 and Nashua ♂ 2 were X-rayed to obtain an accurate image of the common deformity found in many individuals in this cross.

5.3.1. Electrophoretic materials and methods

Ten trout were taken from each of the tanks involved in the Howietoun trial (a total of 20 per cross), before they were transported to the ponds just after the second accurate weighing for heritability estimation took place. These fish were selected from trout which had not been panjetted.

The fish were weighed and lengthed and samples of tissue were dissected and placed in labelled containers as is described in chapter 4. The tissues samples were screened in exactly the same manner as described in chapter 4 for all the loci examined in the wild population.

Ten trout were also taken from each tank in the Leven trial, but here the fish from each duplicate tank were pooled and screened

together. They were taken from the tanks just after the first accurate weighing, when the numbers in each tank were reduced to 250 to minimise the effect of subsequent competition and increasing density. The Leven trout were thus smaller and younger than the Howietoun trout used in these analyses. Instead of taking individual tissues, the fry which averaged less than 2gms in weight were cut into two sections (head and body) and homogenised and centrifuged as described in chapter 4. The enzymes found in the brain and eyes were thus screened for using the anterior portion of each fish and the enzymes found in the liver, muscle and heart, were screened for using the posterior portion of each trout.

Sampling trout from the factoral trial was slightly more complicated, due to the increased mortality associated with female (1) (see table 5.5, 5.6 and 5.7). The numbers representing each cross dropped to levels which were not comparable with the rest of the trial. Heritabilities were thus calculated using 30 tanks and not 36. Ten individual fry were collected from each of the 6 tanks in which female (1) was the dam. These fish were unfortunately not weighed but eletctrophoretic screening was conducted using each whole homogenised fry.

Ten parr were subsequently taken from each of the other 30 tanks just after the second accurate 'heritability' measurement took place in September 1985, and before the fish were stocked into the earth ponds to join the commercial side of the Howietoun operation. These fish were screened electrophoretically in the same manner as the Howietoun stock.

From the gentoypes identified for all the stocks, allele frequencies were calculated and the distribution of genotypes was checked for deviation from Mendelian expectations. This was completed by estimating the parental genotypes from the progeny genotypes (as the adult fish were not screened electrophoretically) and the expected genotype distributions were then calculated from the assumed parental genotypes.

Individual levels of heterozygosity were recorded for each individual, and each stock. The weights and lengths and all the elecrophoretic information relating to each individual was recorded on a computer data file.

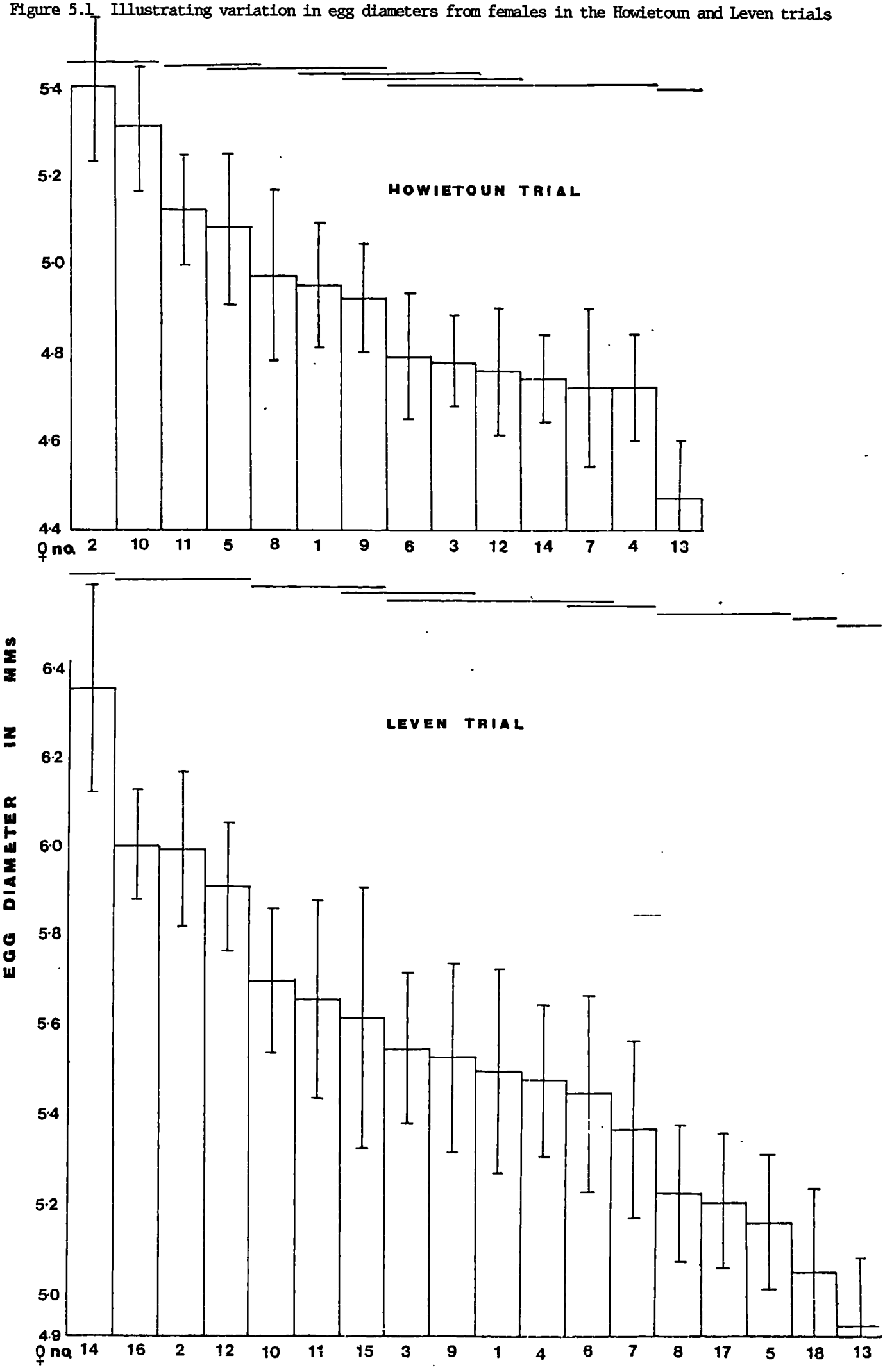
Each trial was dealt with separately and correlations between number of heterozygotes per individual and weight and length were completed (both Pearson and Spearman) using the SPSSX package available on the Stirling University computer system.

5.4 Results

5.4.1 Results of egg size examination and subsequent development

Mean diameters from the eggs of different dams are ranked and graphically represented in Figure 5.1 for the Howietoun and Leven trials. The set of lines above the histograms represent the results of Duncan's (1955) multiple range test, which indicate significant differences do exist between the dams, as far as egg size is concerned. The dam from which the eggs were derived is given below each histogram column.

Figure 5.1 Illustrating variation in egg diameters from females in the Howietoun and Leven trials



As already discussed heritability estimates were not calculated but analyses of variance tables constructed using the egg data are shown in Tables 5.1 and 5.2. The F tests conducted on the results of the one way anovas on egg size for both the Howietoun and Leven trials indicate that the differences exhibited between dams are significant. The F value found for the Howietoun trial is very large indicating considerable variation even within a population of hatchery trout of the same age. The F values found for the Leven trial is even larger. This is not surprising as the dams used for the experiment were not all of the same size or age, giving rise to much larger variation in egg size. Thus maternal effects are apparent as soon as fertilization is complete, and heritability estimates of later growth will be biased if an advantage is subsequently given to the larger eggs and larger fry.

Table 5.1 Anova of egg diameters in Howietoun trial

Howietoun trial	Egg Diameter					
Source of variation	D.F.	SS	SS%	M.S.	F.	
Dam.	13	33.31	67.98	2.562	102.48	highly
Dam. Individual	546	15.69	32.02	0.028		significant
TOTAL	559	49.00	100.00	0.087		

Table 5.2 Anova of egg diameters in Leven trial

Leven trial	Egg Diameter					
Source of variation	D.F.	SS	SS%	M.S.	F.	
Dam.	17	90.41	76.25	5.318	132.95	highly
Dam. Individual	702	28.16	23.75	0.040		significant
TOTAL	719	118.60	100.00	0.165		

Tables 5.3 and 5.4 list the early life cycle parameters measured for the first two trials using Howietoun and Loch Leven broodstock. Table 5.5 lists parameters for the factoral trial in 1984-1985. Tables 5.6, 5.7 and 5.8 give a more detailed assessment of mortalities at fertilisation and shocking and deformities observed in the alevins for the the factoral trial. All the tables referring to the factoral trial contain information concerning the full 6 x 6 factoral cross. Previously in the heritability/growth chapter it was only possible to utilize data from a reduced 5 x 6 version of the cross due to the excess mortalities experienced by progeny derived from female (1).

Heritabilities were not calculated for mortality or survival but analyses of variance were conducted and Tables 5.9, 5.10 and 5.11 give the anova table for various parameters for the three trials undertaken. The right hand column of each set of anova tables consists of the F test values and an indication whether the value is significant or not (P = 0.05).

The Anova results presented in Tables 5.9, 5.10 and 5.11 are an attempt to distinguish between mortalities and deformities caused by or attributable to the influence of the sires or dams. For the Howietoun trial there are 3 highly significant F test values (Table 5.9). The dam influence in the Anovas seem to be contributing towards the majority of the variation between populations for mortality at shocking, mortality at first accurate weight and deformities at hatching. These results are thought possibly to be correlated to the state of "ripeness" of the dams at stripping.

Table 5.3 Howietoun trial. 1982-1983 Egg, mortality, weight data

♂	♀	Tank No.	Egg Size (mms)	Number laid down	MORTALITIES % before shock	% at shock	% total inc. shock	% from hatch to FF *	% from FF to 3 weeks	% from FF to FAW †	Total emerge time (days)	Av. wt. at FF (gms)	% Deformed Fry
1	1	20	4.9	789	0.6	7.0	7.6	2.5	2.2	2.5	6	0.12	0
		29	4.9	809	0.6	3.6	4.3	2.9	2.1	4.2	5	0.12	0
	2	1	5.3	990	0.3	55.7	55.9	2.5	0.0	1.0	2	0.17	0
		12	5.4	980	0.2	50.0	50.1	4.5	0.0	1.1	2	0.16	0
2	3	2	4.7	705	0.0	0.6	1.3	0.0	3.5	3.9	4	0.13	2.0
		30	4.8	780	0.3	0.3	0.8	0.0	0.8	2.0	2	0.12	1.6
	4	13	3.8	939	0.5	1.9	2.4	0.4	2.7	5.3	4	0.13	0.2
		21	4.7	824	1.8	2.3	4.1	0.6	2.2	5.9	5	0.12	0.2
3	5	3	5.0	917	0.5	3.9	4.4	0.6	1.2	2.2	5	0.15	0
		14	5.3	924	0.3	2.1	2.4	0.4	0.8	2.2	2	0.16	0
	6	4	4.9	812	0.8	11.0	11.8	1.2	1.4	5.4	4	0.12	0
		22	4.7	812	1.7	11.4	12.9	1.0	2.6	10.0	6	0.12	0.2
4	7	15	4.7	979	2.4	5.0	8.0	0.8	5.2	12.2	5	0.12	0.2
		23	4.7	982	1.9	6.7	7.9	11.8	5.6	15.3	3	0.11	0.2
	8	5	4.9	772	0.9	2.2	3.1	0.4	0.0	0.8	6	0.15	0
		16	5.1	817	0.7	1.0	1.7	0.4	0.6	1.2	8	0.15	0
5	9	6	4.8	1328	0.5	1.2	1.7	2.7	0.9	2.6	4	0.13	0.2
		24	5.0	1303	0.7	1.0	1.7	0.6	1.2	2.0	6	0.13	0.0
	10	17	5.3	779	0.6	0.0	0.7	0.6	0.8	3.2	5	0.13	0.4
		25	5.3	733	1.7	0.3	1.9	3.3	1.1	3.2	8	0.14	0
6	11	7	5.2	658	1.7	8.8	10.3	0.6	0.8	0.8	7	0.13	0
		18	5.1	636	1.6	11.7	13.1	0.2	0.8	0.8	3	0.14	0.2
	12	8	4.8	720	0.2	0.0	0.3	2.4	2.4	2.8	6	0.12	1.8
		26	4.8	816	1.1	0.2	1.3	2.7	2.4	2.8	6	0.12	2.2
9	17	10	4.5	1016	0.5	1.4	1.7	0.6	6.5	11.6	5	0.11	0
		19	4.5	996	1.2	1.7	2.3	0.8	7.2	12.6	8	0.11	0
	18	11	4.8	884	0.4	2.7	3.7	6.8	1.5	29.7	3	0.11	0.2
		28	5.1	998	0.2	2.3	2.5	1.2	1.6	21.1	3	0.14	0

* FF = First feeding † FAW = First accurate weighing

Table 5.4 Leven trial 1983-84. Egg, mortality, weight data

♂	♀	Tank No.	Egg Size (mms)	Number laid down	MORTALITIES % before shock	% at shock	% total inc. shock	% from hatch to FF*	% from FF to 3 weeks	% from FF to FAW†	Total emerge time (days)	Av. wt. at FF (gms)	% Deformed Fry
1	1	24	5.52	891	0.0	0.3	0.5	0.4	1.8	3.7	5	0.139	0
		35	5.47	1058	0.3	1.0	1.3	0.0	1.0	2.0	5	0.140	0.2
	2	1	6.00	863	3.8	1.0	4.9	0.0	0.4	1.4	7	0.130	0
		13	5.97	971	0.6	1.0	1.6	0.0	0.4	0.8	6	0.135	0
2	3	2	5.60	701	2.6	0.4	3.0	0.4	0.6	1.7	5	0.117	0.4
		36	5.48	719	0.6	0.8	1.4	0.4	0.6	2.9	5	0.127	0
	4	14	5.50	776	0.8	1.0	1.8	0.0	0.6	2.7	5	0.132	0
		25	5.45	795	1.3	1.3	2.5	0.0	1.5	5.4	5	0.125	0
3	5	3	5.14	693	0.0	2.2	2.2	0.4	1.4	14.5	7	0.100	0.4
		15	5.16	727	0.4	1.0	1.4	0.5	0.6	18.2	6	0.114	0.2
	6	7	5.51	912	1.2	2.4	3.6	0.2	0.4	4.8	5	0.116	0.2
		26	5.38	958	0.2	2.1	2.3	0.0	1.5	6.9	5	0.115	0.2
4	7	16	5.40	789	2.7	2.0	4.6	0.0	1.5	5.0	6	0.126	0.2
		27	5.33	760	0.9	2.8	2.5	0.4	3.1	5.6	5	0.136	0.4
	8	5	5.20	774	1.4	1.1	2.5	0.2	0.0	3.3	5	0.127	0.2
		17	5.24	860	1.1	1.4	2.4	0.6	0.3	2.5	5	0.130	0.4
5	9	6	5.56	831	1.0	0.1	1.2	0.0	1.0	3.8	5	0.107	0
		28	5.43	948	0.1	0.2	0.3	0.8	1.0	16.1	5	0.112	0.2
	10	18	5.73	1037	1.2	0.0	0.4	0.2	0.4	4.2	3	0.119	0
		29	5.57	1167	0.3	0.1	1.1	0.0	0.8	7.9	3	0.127	0
6	11	4	5.67	1087	0.1	1.9	2.0	0.4	0.6	6.0	6	0.124	0.4
		19	5.64	1229	0.2	1.4	1.6	0.2	0.6	2.9	4	0.123	0.2
	12	8	5.92	1060	0.0	0.6	0.6	0.4	1.0	2.3	3	0.119	0.2
		30	5.88	1153	0.1	1.1	1.2	0.6	1.2	4.1	2	0.114	0
7	13	20	4.90	1563	0.0	0.5	0.5	0.2	0.8	12.0	4	0.087	0
		31	4.94	1504	0.0	0.5	0.5	0.2	1.2	18.1	6	0.086	0
	14	9	6.39	1036	1.2	2.7	3.9	1.2	0.8	2.7	4	0.130	0.8
		21	6.29	1201	0.4	1.8	2.3	0.6	1.7	3.7	5	0.131	0.6
8	15	10	5.62	1126	0.0	0.4	0.4	0.2	0.2	50.0	6	0.129	0
		32	5.60	1150	0.5	0.4	0.9	0.2	1.2	4.0	4	0.137	0.2
	16	22	5.99	1031	0.5	0.3	0.8	0.8	1.9	6.0	5	0.111	0.2
		33	5.99	960	0.3	0.2	0.5	0.o	0.8	1.0	4	0.119	0
9	17	11	5.21	1219	0.4	0.3	0.7	0.4	0.2	38.0	5	0.102	0
		23	5.19	1250	0.1	0.7	0.8	0.2	0.4	0.8	5	0.097	0
	18	12	5.02	1078	0.4	3.5	3.9	0.6	1.6	2.3	5	0.115	0.4
		34	5.05	1065	0.7	3.8	4.4	0.8	1.0	1.5	6	0.122	0.2

Table 5.5 Showing 1984-85 Egg, mortality, deformity data

♀	♂	Egg Size (mms)	Number laid down	% at laying down	% at shocking	% Total including shocking	% Dead from Day 1 in tanks to 6 weeks	% Deformities (alevins)
1	1	5.28	437	1.6	30.6	42	10.2	19.4
	2		312	1.3	19.6	29	5.0	19.9
	3		347	23.9	64.1	81	7.0	3.4
	4		367	55.0	20.2	71	2.6	9.4
	5		457	37.9	27.8	62	4.5	6.7
	6		346	59.0	25.2	73	4.3.	10.3
2	1	4.99	351	1.7	26.0	32	0.0	24.8
	2		371	11.6	10.9	25	0.0	23.0
	3		366	25.4	45.3	63	0.0	0.7
	4		356	29.2	11.1	44	0.0	0.5
	5		395	13.7	12.9	30	0.7	0.4
	6		377	21.8	15.5	41	0.5	1.4
3	1	5.51	388	0.8	21.0	22	1.0	0.0
	2		404	0.3	17.0	17	0.3	0.9
	3		409	0.o	31.8	32	0.0	0.4
	4		403	0.0	13.4	14	0.3	0.3
	5		427	0.0	18.3	18	0.0	0.0
	6		399	0.0	14.1	15	0.3	0.0
4	1	5.80	481	6.0	6.2	13	0.2	0.7
	2		399	2.0	9.5	12	0.4	0.7
	3		361	3.6	33.3	36	1.4	0.0
	4		361	1.4	17.6	19	0.0	0.0
	5		347	2.6	19.0	22	0.o	0.4
	6		374	3.5	15.5	19	0.o	0.0
5	1	5.55	706	1.1	3.4	6	0.5	2.7
	2		554	0.2	2.5	3	0.6	4.0
	3		592	2.9	22.5	25	0.7	4.2
	4		589	0.2	7.9	9	0.6	3.4
	5		577	0.2	14.4	15	0.4	3.1
	6		638	0.2	25.8	11	0.7	3.8
6	1	6.06	438	0.0	1.4	2	0.o	0.2
	2		570	0.0	1.1	1	0.o	0.0
	3		533	0.6	64.1	65	0.0	0.0
	4		491	0.0	4.1	5	0.0	0.0
	5		533	0.0	9.8	10	0.0	0.4
	6		452	0.0	17.2	16	0.3	0.3

Table 5.6 1985 Trial Mortalities at fertilization/laying down

Mortalities at Laying down	♀1 Nashua		♀2 Nashua		♀3 Leven		♀4 Leven		♀5 Howietoun		♀6 Howietoun		Totals for Each ♀	
♂1	437		351		388		481		706		438		2801	
Nashua	7	1.6%	6	1.7%	3	0.8%	29	6.0%	8	1.1%	0	0.0%	53	1.9%
♂2	312		371		404		399		554		570		2610	
Nashua	4	1.3%	43	11.6%	1	0.3%	8	2.0%	1	0.2%	0	0.0%	57	2.2%
♂3	347		366		409		361		592		533		2608	
Leven	83	23.9%	93	25.4%	0	0.0%	13	3.6%	17	2.9%	3	0.6%	209	7.7%
♂4	367		356		403		361		589		491		2567	
Leven	202	55%	104	29.2%	0	0.0%	5	1.4%	L	0.2%	0	0%	312	12.2%
♂5	457		395		427		347		577		533		2736	
Howietoun	173	37.9%	54	13.7%	0	0.0%	9	2.6%	1	0.2%	0	0%	237	8.7%
♂6	346		377		399		374		638		452		2586	
Howietoun	204	59%	82	21.8%	0	0.0%	13	3.5%	1	0.2%	0	0.0%	300	11.6%
Totals for Each ♂	2266		2216		2430		2323		3656		3017		15908	
	673	29.7%	382	17.2%	4	0.2%	77	3.3%	29	0.8%	3	0.1%	1168	7.3%

Table 5.7 1985 Trial Mortalities at shocking

Mortalities at Shocking	♀1 Nashua	♀2 Nashua	♀3 Leven	♀4 Leven	♀5 Howietoun	♀6 Howietoun	Total for Each ♀
♂1 Nashua	363	335	385	448	683	435	2649
	111 | 30.8%	87 | 26.0%	81 | 21.0%	28 | 6.2%	23 | 3.4%	6 | 1.4%	336 | 12.7%
♂2 Nashua	276	312	403	389	550	570	2500
	54 | 19.6%	34 | 10.9%	69 | 17.1%	37 | 9.5%	14 | 2.5%	6 | 1.1%	214 | 8.6%
♂3 Leven	181	247	409	348	573	526	2284
	116 | 64.1%	112 | 45.3.	130 | 31.8%	116 | 33.3%	129 | 22.5%	337 | 64.1%	940 | 39.4%
♂4 Leven	134	225	402	353	584	489	2187
	27 | 20.2%	25 | 11.1%	54 | 13.4%	62 | 17.6%	46 | 7.9%	20 | 4.1%	234 | 10.7%
♂5 Howietoun	241	318	427	332	571	532	2421
	67 | 27.8%	41 | 12.9%	78 | 18.3%	63 | 19.0%	82 | 14.4%	52 | 9.8%	383 | 15.8%
♂6 Howietoun	123	264	398	360	627	452	2224
	31 | 25.2%	41 | 15.5%	56 | 14.1%	56 | 15.5%	162 | 25.8%	78 | 17.2%	424 | 19.1%
Total for Each ♂	1318	1701	2424	2230	3588	3004	14,265
	406 | 30.8%	340 | 20.0%	468 | 19.3%	362 | 16.2%	456 | 12.7%	499 | 16.6%	2531 | 17.7%

Table 5.8 Alevin deformities in the factoral cross

Deformities (Alevins)	♀1 Nashua	♀2 Nashua	♀3 Leven	♀4 Leven	♀5 Howietoun	♀6 Howietoun	Total for Each ♀
1 Nashua ♂	41 / 19.4	59 / 24.8	0 / 0.0	3 / 0.7	17 / 2.7	1 / 0.2	121 / 5.3%
2 Nashua ♂	39 / 19.9	64 / 23.0	3 / 0.9	3 / 0.7	21 / 4.0	0 / 0.0	130 / 5.7%
3 Leven ♂	2 / 3.4	1 / 0.7	1 / 0.4	0 / 0.0	22 / 4.2	0 / 0.0	26 / 1.9%
4 Leven ♂	8 / 9.4	1 / 0.5	1 / 0.3	0 / 0.0	18 / 3.4	0 / 0.0	28 / 1.4%
5 Howietoun ♂	10 / 6.7	1 / 0.4	0 / 0.0	1 / 0.4	15 / 3.1	2 / 0.4	29 / 1.4%
6 Howietoun ♂	8 / 10.3	3 / 1.4	0 / 0.0	3 / 1.0	21 / 3.8	1 / 0.3	36 / 1.9%
Total for Each ♂	108 / 11.8	129 / 7	5 / 0.3	10 / 0.4	114 / 5.8	4 / 0.3	370 / 3.1%

Deformities include Blue sacs

Table 5.9 Howietoun trial 1982-83

Parameter: Mortality at laying down

Source of Variance	DF	SS	SS%	MS	F (Sig/NS)
Sire	6	2.75	24.6	0.458	0.642 (NS)
Sire. Dam	7	4.99	44.5	0.713	2.88 (NS)
Sire. Dam. Tank	14	3.46	30.9	0.247	
Total	27	11.12	100.0	0.415	

Parameter: Mortality at shocking

Source of Variance	DF	SS	SS%	MS	F (Sig/NS)
Sire	6	2322.0	47.7	387.1	1.09 (NS)
Sire. Dam	7	2478.0	50.9	354.0	75.3 (Sig)
Sire. Dam. Tank	14	66.21	1.4	4.7	
Total	27	4867.0	100.0	180.3	

Parameter: Mortality from hatching to first feeding

Source of Variance	DF	SS	SS%	MS	F (Sig/NS)
Sire	6	31.4	18.8	5.24	0.71 (NS)
Sire. Dam	7	51.7	30.9	7.38	1.23 (NS)
Sire. Dam. Tank	14	84.3	50.4	6.02	
Total	27	167.4	100.0	6.20	

Parameter: Mortality at first accurate wt.

Source of Variance	DF	SS	SS%	MS	F (Sig/NS)
Sire.	6	844.7	65.6	140.8	2.55 (NS)
Sire. Dam.	7	386.8	30.0	55.2	13.66 (Sig)
Sire. Dam. Tank	14	56.5	4.4	4.04	
Total	27	1288.0	100.0	47.7	

Parameter: Deformities after hatching

Source of Variance	DF	SS	SS%	MS	F (Sig/NS)
Sire	6	139.0	44.1	23.1	0.97 (NS)
Sire. Dam	7	165.8	52.6	23.7	31.6 (Sig)
Sire. Dam. Tank	14	10.5	3.3	0.75	
Total	27	315.3	100.0	11.68	

Table 5.10 Leven trial 1983-84

Parameter: Mortality at laying down

Source of Variation	DF	SS	SS%	MS	F (Sig/NS)
Sire	8	7.96	32.3	0.994	1.49 (NS)
Sire. Dam	9	5.97	24.3	0.663	0.07 (NS)
Sire. Dam. Tank	18	10.68	43.4	0.594	
Total	35	24.61	100.0	0.703	

Parameter: Mortality at shocking

Source of Variation	DF	SS	SS%	MS	F (Sig/NS)
Sire	8	15.53	45.6	1.94	1.08 (NS)
Sire. Dam	9	16.14	47.4	1.79	13.76 (Sig)
Sire. Dam. Tank	18	2.40	7.0	0.13	
Total	35	34.08	100.0	0.97	

Parameter: From hatching to first feeding

Source of Variation	DF	SS	SS%	MS	F (Sig/NS)
Sire	8	0.75	17.8	0.095	0.49 (NS)
Sire. Dam	9	1.74	40.8	0.192	1.98 (NS)
Sire. Dam. Tank	18	1.76	41.4	0.097	
Total	35	4.25	100.0	0.122	

Parameter: Mortality at first accurate wt.

Source of Variation	DF	SS	SS%	MS	F (Sig/NS)
Sire	8	640.5	17.5	80.06	0.63 (NS)
Sire. Dam	9	1138.0	31.1	126.40	1.21 (NS)
Sire. Dam. Tank	18	1886.0	51.4	104.8	
Total	35	3665.0	100.0	104.7	

Parameter: Deformities after hatching

Source of Variation	DF	SS	SS%	MS	F (Sig/NS)
Sire	8	9.56	27.9	1.19	0.62 (NS)
Sire. Dam	9	17.25	50.3	1.91	4.65 (Sig)
Sire. Dam. Tank	18	7.50	21.8	0.41	
Total	35	34.31	100.0	0.98	

Table 5.11 'Factoral' trial 1984-85

Parameter: Mortality at laying down

Source of Variance	DF	SS	SS%	MS	F (Sig/NS)
Dam	5	4539	54.9	908	7.97 (Sig)
Sire	5	871	10.5	174	1.53 (NS)
Error	25	2861	34.6	114	
Total	35	8271	100.0		

Parameter: Mortality at shocking

Source of Variance	DF	SS	SS%	MS	F (Sig/NS)
Dam	5	1212.5	16.0	242.5	3.17 (NS)
Sire	5	4468.6	58.8	893.7	11.68 (Sig)
Error	25	1913.4	25.2	76.5	
Total	35	7594.4	100.0		

Parameter: Mortality from first feeding to first accurate wt.

Source of Variance	DF	SS	SS%	MS	F (Sig/NS)
Dam	5	141.7	78.9	28.3	23.6 (Sig)
Sire	5	7.3	4.1	1.5	1.3 (NS)
Error	25	30.7	17.0	1.2	
Total	35	179.7	100.0		

Parameter: Deformities after hatching

Source of Variance	DF	SS	SS%	MS	F (Sig/NS)
Dam	5	707·3	42.7	141.5	5.4 (Sig)
Sire	5	288.1	17.4	57.6	2.2 (NS)
Error	25	659.4	39.9	26.4	
Total	35	1654.8	100.0		

Parameter: Emergence time

Source of Variance	DF	SS	SS%	MS	F (Sig/NS)
Dam	5	99.5	93.0	19.9	99.5 (Sig)
Sire	5	2.1	2.0	0.4	2.0 (NS)
Error	25	5.4	5.0	0.2	
Total	35	107.0	100.0		

In the Leven trial (Table 5.10) a similar result for the mortality at shocking parameter, was obtained although it was not as highly significant as for the Howietoun trial. The Leven dams were all much more uniform in their readiness to be artificially stripped than the Howietoun fish in the first trial.

A significant F value was also obtained for deformities after hatching for the dam component in the anova. The value was not highly significant but still the deformities found appeared to be caused by the dam component rather than the sire.

The Factoral trial showed several interesting results. For 4 out of the 5 parameters analysed, the dam component showed a significant contribution to the variation exhibited. The dam component was significant for mortalities at fertilization, and for the period between first feeding and the first accurate weighing. The dam component was also significant when the deformities after hatching are considered. These results are interpreted as showing that the state of ripeness of the Nashua females was important in contributing to subsequent mortalities and deformities. See also Tables 5.6, 5.7 and 5.8 which show that the Nashua dams are responsible for higher than average mortalities at fertilization and shocking, and deformities after hatching. The variation in emergence time during hatching was also significantly affected by the dam component (Table 5.10).

Interestingly the sire component for mortality at shocking was

also significant. Indicating that the quality of milt at fertilization is important and can lead to significant differences in survival up to and including the shocking period. The milt from male (3), one of the Leven sires, used in the factoral trial, was recorded as partially frozen when it arrived at Howietoun (chapter 3). The level of mortality associated with this sire at stocking was 39.4%, much higher than for the other sires used (see Table 5.7).

These results indicate that the state of parental ripeness and handling of gametes can play a large part in subsequent survival at different early stages of growth in the life cycle of the trout.

5.4.2 Alevin Growth

Wet weights of the alevins minus the yolk sac were taken weekly, until the smallest alevins had no visible yolk sac remaining. Specific growth rates (SGRs) were calculated for the four different batches, as was the proportion of yolk sac to overall body weight. This was expressed as a percentage and calculated weekly and Figure 5.2 illustrates the growth of the alevins over 7 weeks and the appropriate SGRs are listed on the right hand side of the graph. Duncan's (1955) multiple range test was conducted and none of the SGRs were significantly different from each other. This was somewhat surprising and was thought to be due to the small number of individuals used at each weighing (10 for each batch) and a larger study would be required to study fully the growth of alevins and yolk sac utilization.

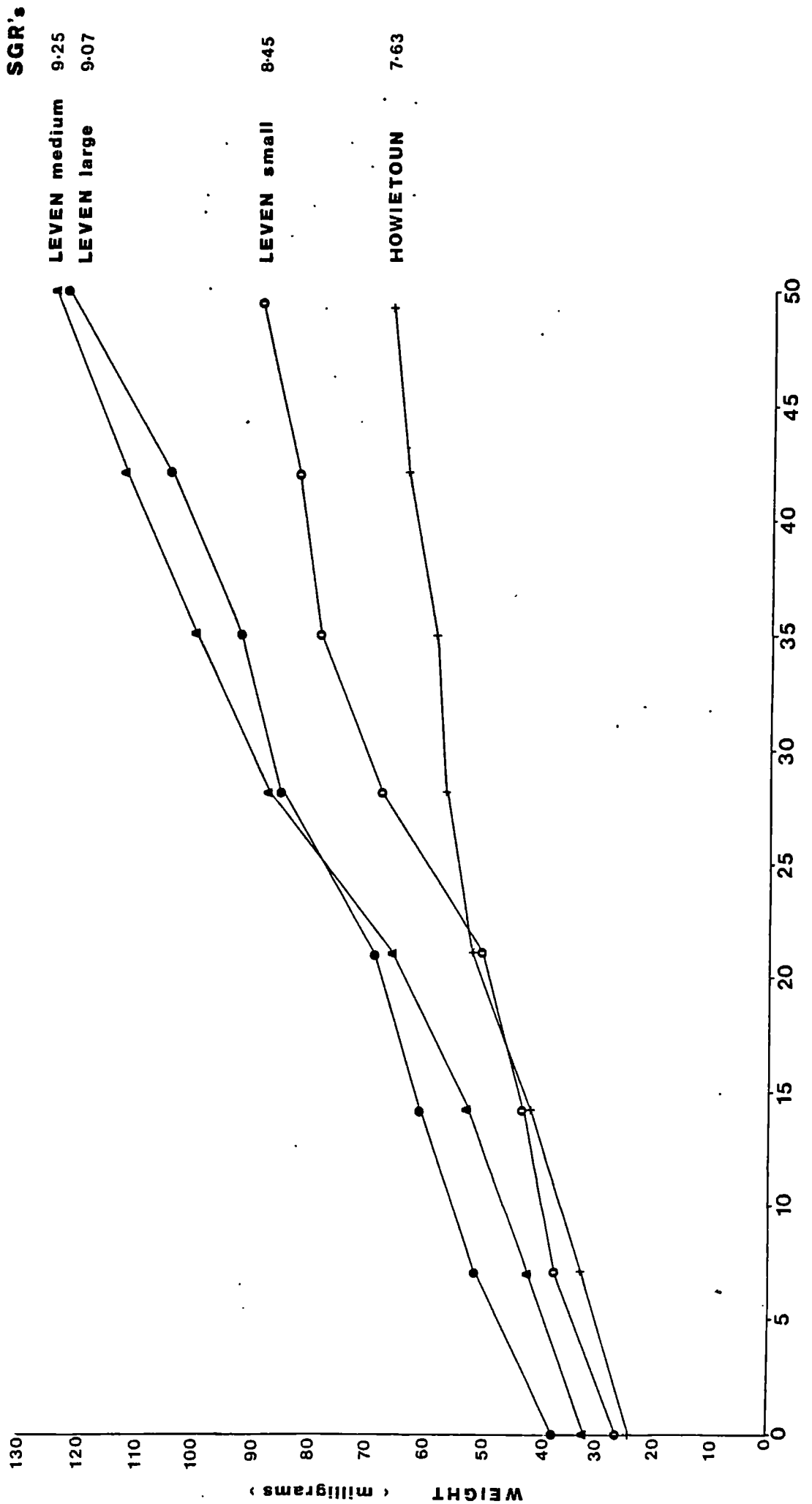


Figure 5.2 Showing alevin growth. Weight (-Yolk Sac) against Time

The apparent difference in the rate of utilization of yolk sac, as demonstrated in Figure 5.3 which shows the Howietoun stock, starting with the third largest % yolk sac to body weight ratio, and ended the trial as the stock with the second largest % yolk sac to body weight ratio. Thus indicating that the yolk sac is being utilized at a differernt rate. This observation can be explained by the fact that the Howietoun population from which the alevins were sampled was part of the commercial side of the farm's operation, and as such, contained progeny from at least five different females. The Leven eggs (small, medium and large) by contrast came from separate individual crosses each involving just one dam. Thus the variation in egg sizes caused by using a mixed batch of alevins from Howietoun stock makes valid comparisons with this population difficult. The Howietoun population was included because it was fertilised and laid down on the same day as the Leven crosses of this study.

Figure 5.4 represents the relationship between the yolk sac weight and the alevin weight (without the yolk sac) for the three Leven populations studied, as the fish grew. This appears to show that the yolk sac utilization rate is similar for different sized alevins, and that the larger fry came from alevins which had larger amounts of yolk sac and started at hatching with heavier body weights.

The reason why the smaller alevins used up their reserves faster than the larger alevins is not that they did so at a different rate but seems to be due to the lack of reserves in the first place.

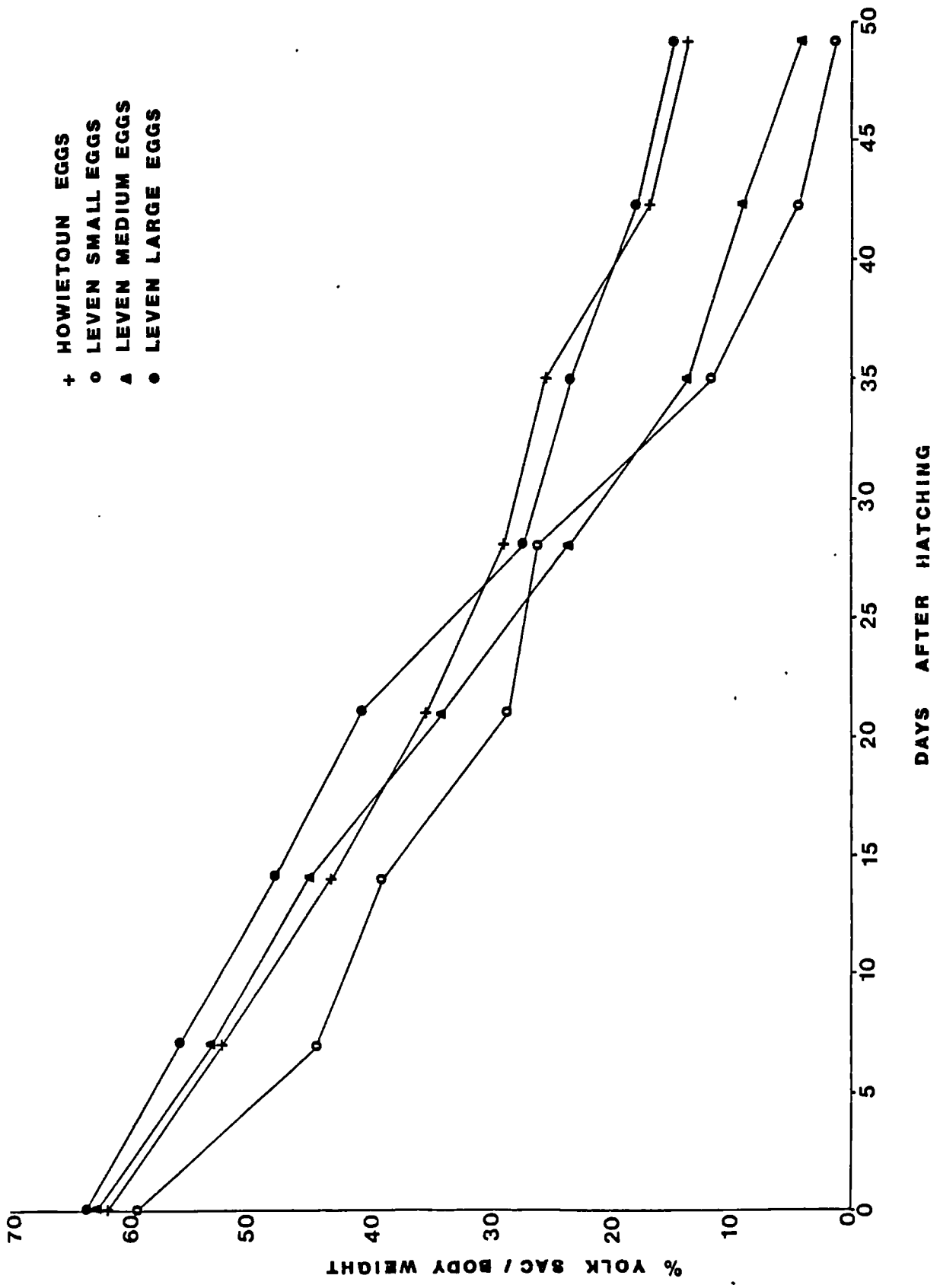
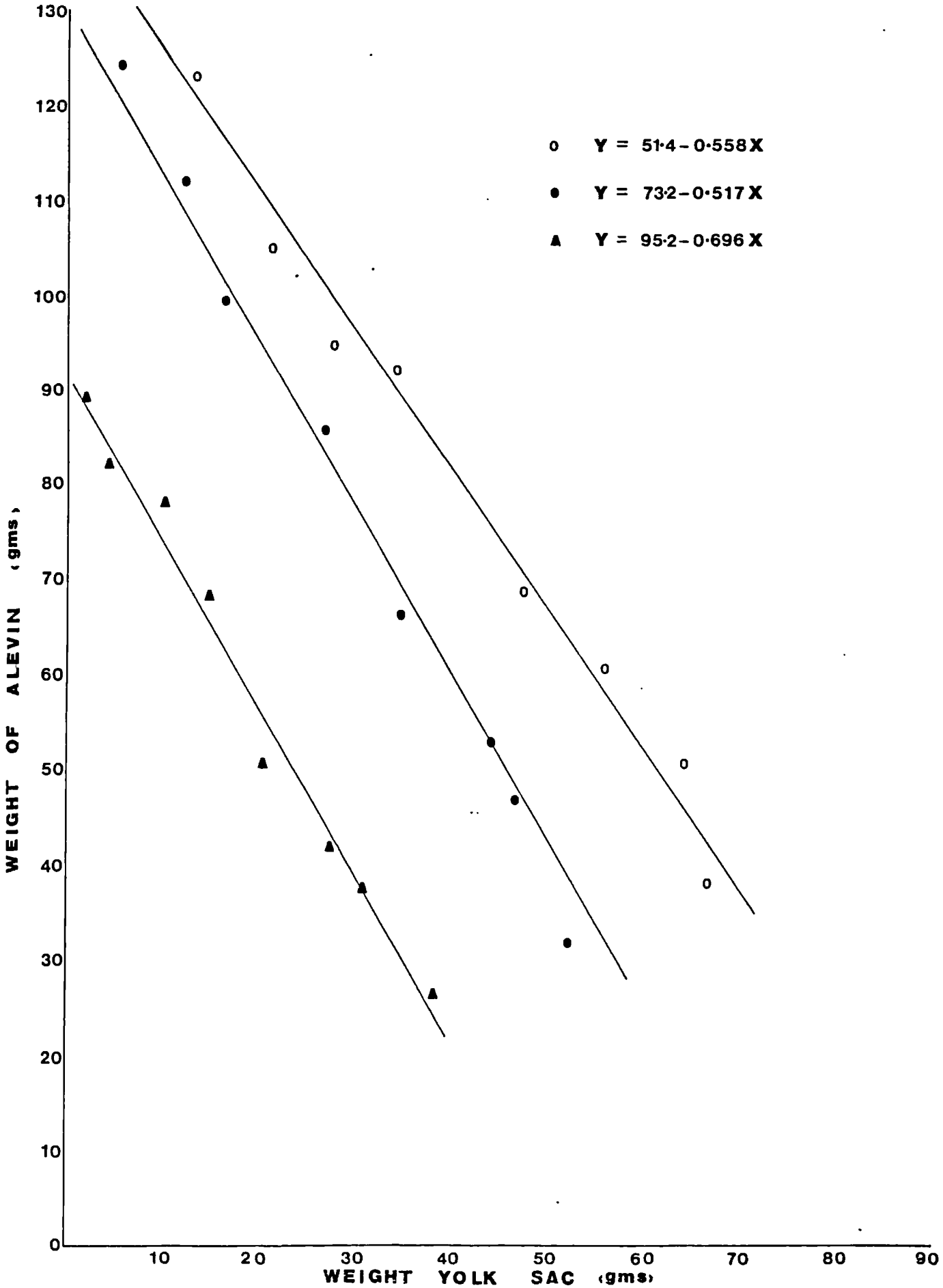


Figure 5.3 Showing the decrease in % yolk sac/body weight relationship with time

Figure 5.4 Graph illustrating relationship between alevin weight and yolk sac weight. The three lines represent the alevins which developed from the small, medium and large Leven eggs



The small alevins not only have higher initial body weights (without the yolk sac) but have proportionally less yolk sac. 59% of the total weight of the small alevins was made up of yolk sac, whilst 62-63% of the medium sized and large alevins was made up of yolk sac, and these batches also had larger initial body weights.

Table 5.12 illustrates the Pearson correlations calculated between various parameters concerned with the growth and survival of trout in the Howietoun trial 1982-1983. 28 tanks were included in the multiple correlation, at P = 0.05 (df =(N-2) r = 0.381.

The columns and rows stand for the following parameters

C_1 - Egg diameter
C_2 - % mortality up to stocking
C_3 - % mortality at stocking
C_4 - % total mortality up to and including shocking
C_5 - % mortality from hatching to first feeding
C_6 - % mortality from first feeding to 8 weeks in tanks
C_7 - % mortality from first feeding to first accurate measurement
C_8 - Total emergence time at hatching
C_9 - Av. wt. at first feeding
C_{10} - First hatch (days from laying down to hatching)
C_{11} - Av. wt. (1)
C_{12} - Av. len. (1)
C_{13} - Av. wt. (2)
C_{14} - Av. len. (2)
C_{15} - Av. wt. (3)
C_{16} - Av. len. (3)
C_{17} - Length of dam
C_{18} - Length of sire
C_{19} - Specific Growth Rate (From wt. (1) - wt. (2))

Table 5.12 Howietoun trial. Multiple correlations between parameters listed on accompanying page

	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}
C_2	-0.205																	
C_3	x 0.478	-0.160																
C_4	x 0.473	-0.120	xxx 0.999															
C_5	-0.093	0.076	0.215	0.215														
C_6	xxx -0.759	0.321	-0.304	-0.297	0.196													
C_7	-0.367	0.092	-0.132	-0.120	xx 0.494	x 0.462												
C_8	-0.197	0.379	x -0.455	x -0.449	-0.323	0.224	-0.158											
C_9	xxx 0.784	-0.307	xx 0.560	xx 0.552	-0.245	xxx -0.673	xx -0.489	-0.250										
C_{10}	0.207	-0.372	x 0.426	x 0.418	0.342	-0.239	-0.131	x -0.874	0.281									
C_{11}	0.287	-0.266	0.186	0.175	-0.223	xx -0.592	xxx -0.706	0.018	x 0.428	-0.022								
C_{12}	xx 0.504	-0.173	0.214	0.206	-0.217	xxx -0.652	xxx -0.782	0.040	x 0.475	-0.037	xxx 0.858							
C_{13}	0.166	-0.008	-0.146	-0.145	0.004	-0.264	-0.305	x 0.405	0.032	x -0.459	x 0.456	xxx 0.632						
C_{14}	0.232	0.017	-0.149	-0.147	0.036	-0.289	-0.272	x 0.382	0.036	x -0.424	x 0.426	xxx 0.625	xxx 0.987					
C_{15}	0.186	0.093	-0.030	-0.022	0.012	-0.226	-0.268	x 0.400	0.069	x -0.477	x 0.384	xx 0.542	xxx 0.923	xxx 0.913				
C_{16}	0.216	0.098	-0.025	-0.017	0.025	-0.218	-0.225	0.377	0.049	x -0.458	0.352	xx 0.533	xxx 0.918	xxx 0.924	xxx 0.983			
C_{17}	0.100	0.246	x 0.438	x 0.446	0.319	0.145	0.226	-0.131	0.115	0.185	-0.066	-0.034	-0.155	-0.130	-0.238	-0.212		
C_{18}	0.180	0.214	0.060	0.067	-0.110	-0.370	xxx -0.705	-0.026	-0.278	-0.106	xx 0.571	xxx 0.607	0.218	0.192	0.265	0.200	-0.252	
C_{19}	0.152	0.040	-0.134	-0.131	-0.052	-0.205	-0.208	x 0.384	-0.012	x -0.437	0.348	xx 0.558	xxx 0.982	xxx 0.983	xxx 0.919	xxx 0.933	-0.114	0.147

Significance x = P<0.05 xx = P<0.01 xxx = P<0.001

Table 5.13 illustrates the correlations calculated between various parameters concerned with the growth and survival of trout during the Leven trout trial in 1983-84. 36 tanks were included in the multiple correlations, at P = 0.06 (df = N-2) , r = 3.025. The columns and rows represent in the same order, the same parameters included for the Howietoun trial. (See Table 5.11 and the accompanying reference sheet).

Table 5.14 illustrates the correlations calculated between various parameters concerned with the growth and survival of trout in the 'factoral' trial (1984-1985). 30 tanks were included in the multiple correlation and at P = 0.05 (df = (N-2)), r = 0.381. The columns and rows represent the following parameters.

C_1 - Egg diameter
C_2 - % mortality at laying down
C_3 - % mortality at shocking
C_4 - % total mortality including shocking
C_5 - % mortality from first feeding to six weeks in tanks
C_6 - % deformities (alevins dead)
C_7 - Total emergence period.(days)
C_8 - Days from laying down to first hatch
C_9 - Av. wt. (1)
C_{10} - Av. len. (1)
C_{11} - Av. wt. (2)
C_{12} - Av. len. (2)
C_{13} - Length of dam
C_{14} - Length of sire
C_{15} - SGR Av. wt. (1) - Av. wt. (2)

The following tables (5.15, 5.16 and 5.17) list the significant correlations found from the multiple correlation tables (5.12. 5.13 and 5.14).

Table 5.13 Leven trial. Multiple correlations of parameters listed on accompanying page

	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}
C_2	0.230																	
C_3	-0.144	0.032																
C_4	0.025	xxx 0.597	xxx 0.756															
C_5	0.027	-0.187	0.268	0.079														
C_6	0.003	-0.514	x 0.342	0.079	0.096													
C_7	-0.248	-0.211	-0.254	x -0.358	0.111	-0.195												
C_8	-0.292	x 0.335	0.276	x 0.390	0.018	-0.011	0.233											
C_9	xx 0.468	0.220	0.168	0.271	-0.080	0.094	-0.286	-0.024										
C_{10}	0.295	x -0.403	-0.203	x -0.400	0.070	0.011	-0.116	-0.926	-0.101									
C_{11}	0.068	0.117	0.325	0.315	-0.002	0.220	-0.251	x 0.363	xx 0.451	x -0.403								
C_{12}	0.102	0.095	x 0.379	x 0.363	-0.008	0.194	-0.251	0.276	xx 0.427	-0.310	xxx 0.973							
C_{13}	0.073	0.131	0.129	0.180	0.059	0.135	-0.200	x 0.361	0.195	xx -0.428	xxx 0.860	xxx 0.817						
C_{14}	0.014	0.135	0.199	0.241	0.102	0.161	-0.192	x 0.345	0.133	x -0.404	xxx 0.846	xxx 0.830	xxx 0.986					
C_{15}	0.011	0.181	0.033	0.150	0.089	0.130	-0.131	0.300	0.118	x -0.417	xxx 0.703	xxx 0.686	xxx 0.896	xxx 0.904				
C_{16}	-0.039	0.155	0.080	0.173	0.108	0.144	-0.120	0.278	0.042	x -0.389	xxx 0.706	xxx 0.708	xxx 0.887	xxx 0.916	xxx 0.983			
C_{17}	xxx 0.630	0.144	-0.205	-0.086	-0.151	0.333	-0.074	-0.195	0.212	0.304	-0.189	-0.156	-0.124	-0.170	-0.224	-0.285		
C_{18}	-0.321	0.142	0.267	0.382	-0.003	0.110	-0.131	0.213	0.112	xx -0.419	0.078	0.136	0.042	0.100	0.166	0.200	-0.304	
C_{19}	-0.030	0.157	0.145	0.208	0.103	0.157	-0.175	x 0.387	0.080	xx -0.454	xxx 0.813	xxx 0.786	xxx 0.976	xxx 0.987	xxx 0.918	xxx 0.923	-0.212	0.127

Significance x = $P < 0.05$ xx = $P < 0.01$ xxx = $P < 0.001$

Table 5.14 Factoral trial. Multiple correlations of parameters listed on accompanying page

	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}
C_2	xxx -0.684													
C_3	-0.101	0.130												
C_4	xx -0.452	xxx 0.624	xxx 0.829											
C_5	-0.186	-0.081	0.073	-0.009										
C_6	xx -0.478	0.054	-0.001	0.068	-0.007									
C_7	-0.087	-0.146	-0.168	-0.268	x 0.395	0.258								
C_8	xxx -0.707	xxx 0.799	0.154	xxx 0.588	-0.224	x 0.421	-0.362							
C_9	xx 0.489	-0.297	x -0.425	xx -0.483	-0.269	-0.033	0.147	-0.195						
C_{10}	xx 0.480	-0.348	x -0.418	xx -0.510	-0.226	-0.043	0.195	-0.268	xxx 0.977					
C_{11}	0.099	-0.020	xx -0.471	x -0.377	-0.194	0.291	0.257	0.120	xxx 0.803	xxx 0.776				
C_{12}	0.110	-0.024	xx -0.487	x -0.394	-0.225	0.265	0.270	0.110	xxx 0.843	xxx 0.824	xxx 0.981			
C_{13}	xxx 0.611	xxx -0.623	-0.187	xx -0.534	-0.145	-0.237	xxx 0.675	xxx -0.820	x 0.433	x 0.460	0.241	0.258		
C_{14}	0.00	0.120	xxx 0.610	xx 0.516	0.262	-0.049	0.003	-0.013	x -0.411	xx -0.462	-0.277	x -0.393	0.00	
C_{15}	-0.00	-0.040	xx -0.466	-0.342	-0.209	0.317	0.268	0.174	xxx 0.748	xxx 0.726	xxx 0.981	xxx 0.964	0.193	-0.315

Significance x = $P < 0.05$ xx = $P < 0.01$ xxx = $P < 0.001$

Table 5.15 Howietoun trial significant correlations

FACTOR	SIGNIFICANT CORRELATIONS POSITIVE	NEGATIVE
Egg size	% mortality at shocking * % mortality total (inc shock)* Av. weight at first feeding*** Length (1)**	% mortality from first feeding to 8 weeks old***
% mortality at shocking	Total % mortality (inc shock)*** Av. weight at first feeding** First hatch (days from fertilization)*	Total emergence time at hatching
% mortality (total) inc. shock	Av. weight at first feeding** First hatch* Length of dam*	Total emergence time*
% mortality from hatching lst feeding	% mortality from first feeding to lst accurate weight**	
% mortality from first feeding 8 weeks	% mortality from first feeding to lst accurate weight*	Av weight at first feeding*** Wt (1)*** length (2)***
% mortality from first feeding lst accurate weight		Av weight at first feeding** Wt (1)*** Wt(2)*** Length of sire***
Total emergence time at hatching	Wt (2)* Length (2)* Weight (3)* SGR*	First hatch (days from fertilization)*
Average weight at first feeding	Wt (1)* Wt (2)*	
First to hatch (days from fertilisation)		Wt (2)* Wt(3)* Length (2)* Length (3)* SGR*
Weight (1)	Wt (2)* Length (2)* Weight (3)* Length (1)*** Length of sire ***	
Length (1)	Length of sire*** SGR*** Wt (2)*** Wt(3)*** Length (2)*** Length (3)**	
Weight (2)	SGR*** Wt(3)*** Length (2)*** Length (3)***	
Length (2)	Wt (3)*** Length (3)*** SGR***	
Weight (3)	Length (3)*** SGR***	
Length (3)	SGR***	

* = P<0.05 ** = P<0.01 *** = P<0.001

Table 5.16 Leven trial significant correlations

FACTOR	SIGNIFICANT CORRELATIONS POSITIVE	NEGATIVE
'Egg size	Length of dam*** Average weight at first feeding**	
% mortality up to shocking	% total at shocking*** Total emergence time/hatching*	First hatching (days from fertilization)*
% mortality at shocking	% mortality total (inc shock)*** % mortality from first feeding* 8 weeks, length (1)*	
% mortality total at shocking	Total emergence time* length (1)*	% mortality from first feeding* 1st accurate wt., first hatch*
Total emergence time at hatching	Wt (1)* Wt (2)* length (2)* SGR*	
Average wt at first feeding	Wt (1)* Wt (2)**	
First hatch (days after fertilization)		Wt (1)* Wt (2)** Wt (3)* Length (2)* Length (3)* SGR** Length of sire**
Wt (1)	Wt (2)*** Wt (3)*** SGR*** Length (1)*** length (2)*** length (3)***	
Length (1)	Length (2)*** Length (3)*** SGR*** Wt (2)*** Wt(3)***	
Weight (2)	Wt (3)*** SGR*** Length (2)*** Length (3)***	
Length (2)	Length (3)*** Wt (3)*** SGR***	
Weight (3)	Length (3)*** SGR***	
Length (3)	SGR***	

Significance of correlation * = $P < 0.05$ ** = $P < 0.01$ *** $P < 0.001$

Table 5.17 Factoral trial significant correlations

FACTOR	SIGNIFICANT CORRELATIONS POSITIVE	NEGATIVE
Egg size	Length of dam*** Wt (1)** Length (1)**	% mortality at fertilization*** % total including shocking** % deformities alevins ** Days from fert to first hatch***
% mortality at fertilisation	% total mortality (inc shocking) *** Days from fertilisation first hatch ***	length of dam ***
% mortality at shocking	% mortality (including shocking) *** length of sire ***	Wt (1)* Wt (2)** length (1)* length (2)** SGR**
% total mortality (including shocking)	Days from fertilization*** to first hatch. Sire length**	Wt (1)** Wt (2)* Length (1)** length (2)* length of dam*
% mortality from first feeding 6 weeks	Total emergence period*	
% alevin deformities	Days from fertilization* to first hatch	
Total emergence period	length of dam***	
Days from fertilization to first hatch		length of dam***
Wt (1)	Length (1)*** Length (2)*** Wt (2)*** Dam length* SGR***	Sire length*
Length (1)	Length (2)*** Wt (2)*** Dam length * SGR***	Sire length**
Wt (2)	Length (2)*** SGR***	
Length (2)	SGR***	Sire length*

Significance of correlation * = $P < 0.05$ ** = $P < 0.01$ *** = $P < 0.001$

5.4.3 The most important points revealed by the multiple correlations conducted

1. Dam length was correlated with egg diameter in the Leven and Factoral trials.

2. Egg diameter was correlated with average weight at first feeding in the Howietoun and Leven trials (average weight at first feeding was not measured in the Factoral trial).

3. Egg diameter was correlated with length (1) in the Howietoun trial and weight (1) and length (1) in the Factoral trial.

4. Egg diameter was negatively correlated with % mortality from first feeding to 8 weeks later in the Howietoun trial and negatively correlated with % mortality at fertilization, % mortality at shocking and % of deformities at the alevin stage, in the factoral trial.

5. The total % mortality at shocking for the Howietoun trial was correlated with the average weight at first feeding. The larger the mortality the larger the average weight, implying differential mortality of small eggs.

6. The total % mortality of shocking for the factoral trial was negatively correlated to the weights and lengths at measurements (1) and (2). The larger the mortality, the smaller the length and weight measurements, indicating differential mortality of the larger eggs.

7. The mortality in troughs and tanks is correlated between different stages. The % mortality at shocking was correlated with % mortality between first feeding and 8 weeks later, in the Leven trial. While in the Howietoun trial, the % mortality from hatching to first feeding is correlated with % mortality from first feeding to the first accurate measurement.

8. Weight (1) and length (1) were highly correlated with weight (2) and length (2) in the Leven and Factoral trials. The Howietoun trial weight (1) was correlated with weight (2) and length (2) but not so highly ($P < 0.05$ rather than $P < 0.001$). Length (1) was highly correlated with weight (2) and length (2) ($P < 0.001$).

9. Weight (2) and length (2) for the Howietoun and Leven trials were highly correlated ($P < 0.001$) with weight (3) and length (3). The factoral trial did not last long enough to obtain weight (3) and length (3).

10. Sire length was positively correlated with weight (1) in the Howietour trial but negatively correlated for weight (1) in both the Leven and factoral trials. Sire length was also negatively correlated for length (1) and (2) in factoral trial.

11. Dam length was positively correlated with weight (1) and length (1) in the factoral trial.

5.4.4. Results of the electrophoretic survey

The genotypes recorded for each polymorphic loci, for each tank population were analysed to establish whether they fitted expected Mendelian inheritance patterns. Each population being the product of one cross. The results of the observed and expected genotypes along with the accompanying G test for significance were presented in a series of large tables. They are too bulky to include in this chapter, but are available on request.

Table 5.18 lists the tank population numbers which showed deviations from Mendelian expectations. 82 populations (28 Howietoun tank populations with 10 fish per tank, 18 Leven populations representing each female used in the broodstock with 20 fish from each, and 36 factoral tank populations with 10 fish per tank) were used and analysed for ten different polymorphic loci so a total of 820 examinations of Mendelian inheritance were completed, with only 6 (< 1%) being found to be significantly different from the expected genotype distribution.

This confirms the genotypes observed for the polymorphic loci examined were segregating in accordance to Mendelian inheritance.

Table 5.18 Listing deviations from Mendelian expectations

Population	Enzyme locus	G value	Significance
38 Leven ♀10 x ♂5 (1984)	$AAT_{-1,2}$	3.852	$P < 0.05$
41 Leven ♀13 x ♂7 (1984)	$AAT_{-1,2}$	3.852	$P < 0.05$
49 Nashua ♀1 x Leven ♂1 (1985)	$AAT_{-1,2}$	5.290	$P < 0.01$
42 Leven ♀14 x ♂7 (1984)	AAT_{-4}	7.122	$P < 0.01$
72 Leven ♀ 2 x Nashua ♂2 (1985)	IDH_{-1}	3.680	$P < 0.05$
32 Leven ♀ 4 x 2 ♂(1984)	$MDH_{-3,4}$	3.854	$P < 0.05$

The number of heterozygotes per individual was examined for each trial and the results of these investigations appear in Table 5.19. It can be seen that the Howietoun and Leven trials show a very similar pattern of heterozygosity classes and accompanying frequencies. The factoral trial also seems to be similar on first inspection, but once this is broken down into its component crosses one can see differences appearing. The Leven x Leven crosses for example show only two classes of heterozygotes per individual, namely 0 and 1. In contrast the crosses between Howietoun and Nashua and Leven and Nashua show heterozygote classes per individual from 0 to 4 or 5.

The average heterozygosity was calculated for each of the crosses in the factoral trial and for the Leven and Howietoun trials separately. The results appear in Table 5.20. The most heterozygous group being progeny derived from the Nashua x Nashua fish, closely followed by the progeny from the crosses between Howietoun and Nashua trout. The lowest heterozygosity value recorded was for the progeny of the Leven fish within the factoral trial.

Accompanying the Tables 5.19 and 5.20 are a series of Figures illustrating the relationship between the number of heterozygotes per individual and the average weight of the individuals within each class.

Figure 5.5 showing the relationship within the Howietoun and Leven trials show no significant correlation between heterozygosity class

Table 5.19 listing distribution of heterozygotes in all three trials and comparing this with the wild trout heterozygote distribution identified in Chapter 4. Based on 34 loci examined

No. of hets per individual	Representing Individual Heterozygosity *	WILD FISH		HOWIETOUN TRIAL 1982-83		LEVEN TRIAL 1983-84		FACTORAL TRIAL 1984-85	
		No. of fish	%	No. of fish	%	No. of fish	%	No. of fish	%
0	0%	299	18.2%	67	23.9%	71	19.7%	113	31.4%
1	2.9%	560	34.1%	121	43.2%	136	37.8%	127	35.3%
2	5.9%	480	29.2%	69	24.6%	97	26.9%	78	21.7%
3	8.8%	229	13.9%	19	6.8%	44	12.2%	40	11.0%
4	11.8%	63	3.8%	4	1.5%	11	3.1%	1	0.3%
5	14.7%	12	0.7%	0	0%	1	0.3%	1	0.3%
6	17.6%	1	0.1%	0	0%	0	0%	0	0%
TOTALS		1644	100%	280	100%	360	100%	360	100%

Factoral trial 1985

No. of hets per individual	Representing Individual Heterozygosity *	LEVEN X LEVEN		NASHUA X NASHUA		HOWIETOUN X HOWIETOUN		LEVEN X HOWIETOUN (xR)	
		No. of fish	%	No. of fish	%	No. of fish	%	No. of fish	%
0	0%	20	50%	4	10%	9	22.5%	38	47.5%
1	2.9%	20	50%	18	45%	13	32.5%	25	31.3%
2	5.9%	0	0%	12	30%	11	27.5%	12	15.0%
3	8.8%	0	0%	6	15%	7	17.5%	5	6.2%
4	11.8%	0	0%	0	0%	0	0%	0	0%
5	14.7%	0	0%	0	0%	0	0%	0	0%
TOTALS		40	100%	40	100%	40	100%	80	100%

LEVEN X NASHUA (xR)		HOWIETOUN X NASHUA (x R)	
No. of fish	%	No. of fish	%
18	22.5%	15	18.8%
37	46.3%	27	33.8%
19	23.8%	24	30.0%
5	6.3%	12	15.0%
1	1.1%	1	1.2%
0	0%	1	1.2%
80	100%	80	100%

xR including reciprocal crosses

Table 5.20 listing levels of heterozygosity recorded for the three different trials with the factoral trial broken down into component crosses.

Trial	Numbers of progeny tested	H^2
Howietoun trial	280 individuals screened	2.84%
Leven trial	360 individuals screened	2.94%
Factoral trial	360 individuals screened	2.60%

Factoral trial (divided)

Leven x Leven	(40 individuals)	0.52%
Nashua x Nashua	(40 individuals)	3.90%
Howietoun x Howietoun	(40 individuals)	3.32%
Leven x Howietoun	(80 individuals)	1.85%
Leven x Nashua	(80 individuals)	2.56%
Howietoun x Nashua	(80 individuals)	3.40%
TOTAL	**360**	**2.60%**

and average weight. Of course the samples, which are given in brackets next to each point on the graphs are small, but this was the extent of the data collected.

In Figure 5.56 which illustrates the same relationship with different crosses of the factoral trial. Due to the fact that only 10 individuals were examined per tank, tanks with the same crosses had to be grouped together to give more sensible numbers from which to work. All three pure crosses, namely Howietoun x Howietoun, Leven x Leven and Nashua x Nashua gave little or no evidence of any correlation between the number of heterozygotes per individual and average weight. The Howietoun x Howietoun trial showing more correlation than the other two pure strain crosses, but the correlation can not be significant due to the large standard deviations associated with the figures plotted on the graph. The numbers of individuals involved are of course very small even when populations are grouped together, which is a questionable method of assessing this relationship between heterozygote class and weight.

Two of the inter strain crosses also show no relationship between heterozygote number and average weight. The Leven x Howietoun and Leven x Nashua both exhibit slight trends but due to the small sample sizes and large standard deviations these are thought to be insignificant.

The Nashua x Howietoun crosses did seem to show some evidence of heterozygote class being correlated with average weight, although again the sample sizes were very small in the larger, heterozygote

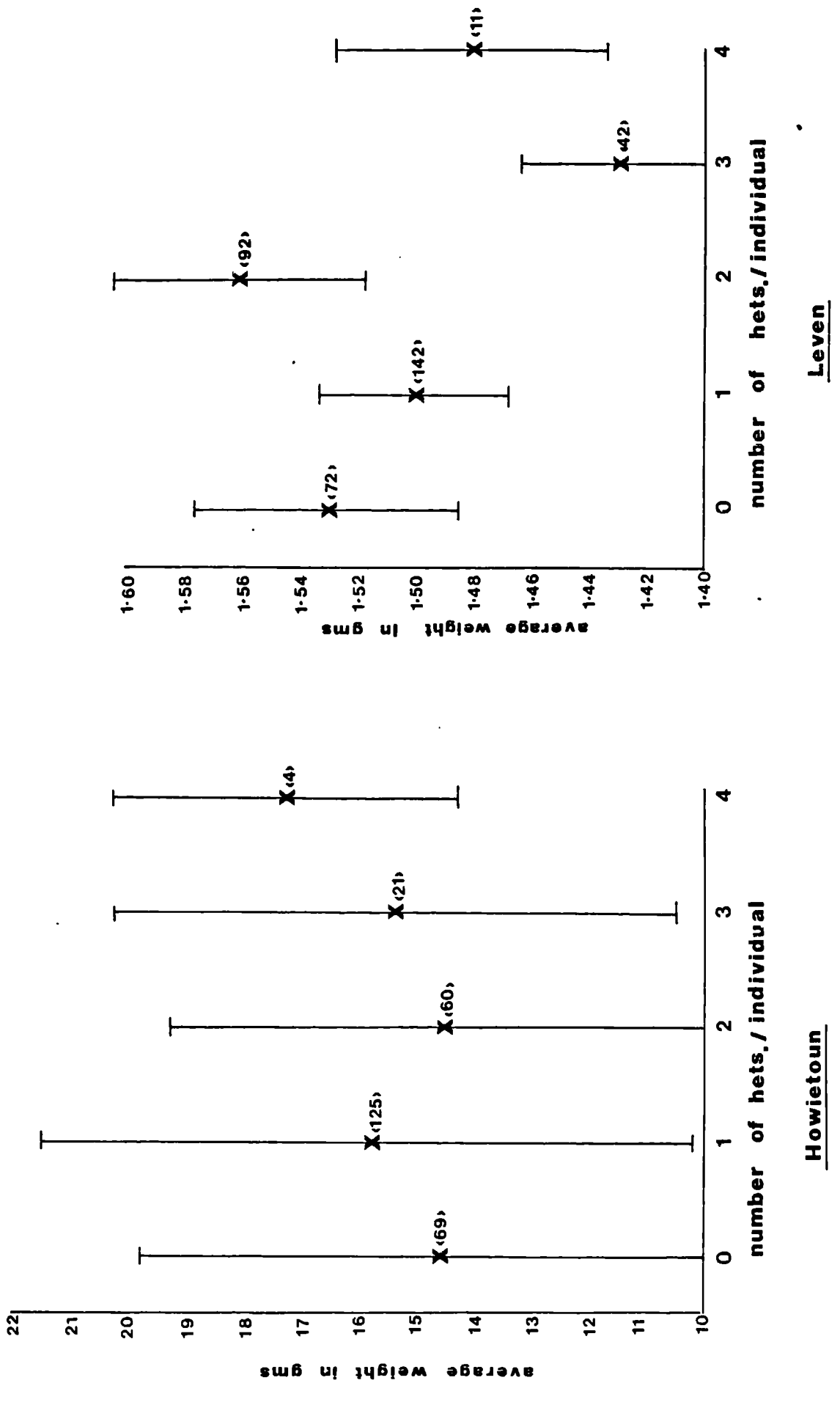


Figure 5.5. Relationship within the Howietoun and Leven trials between heterozygosity class and average weight

Figure 5.6 Graphs showing relationship between heterozygote class and average weight in the factoral trial split into its component crosses

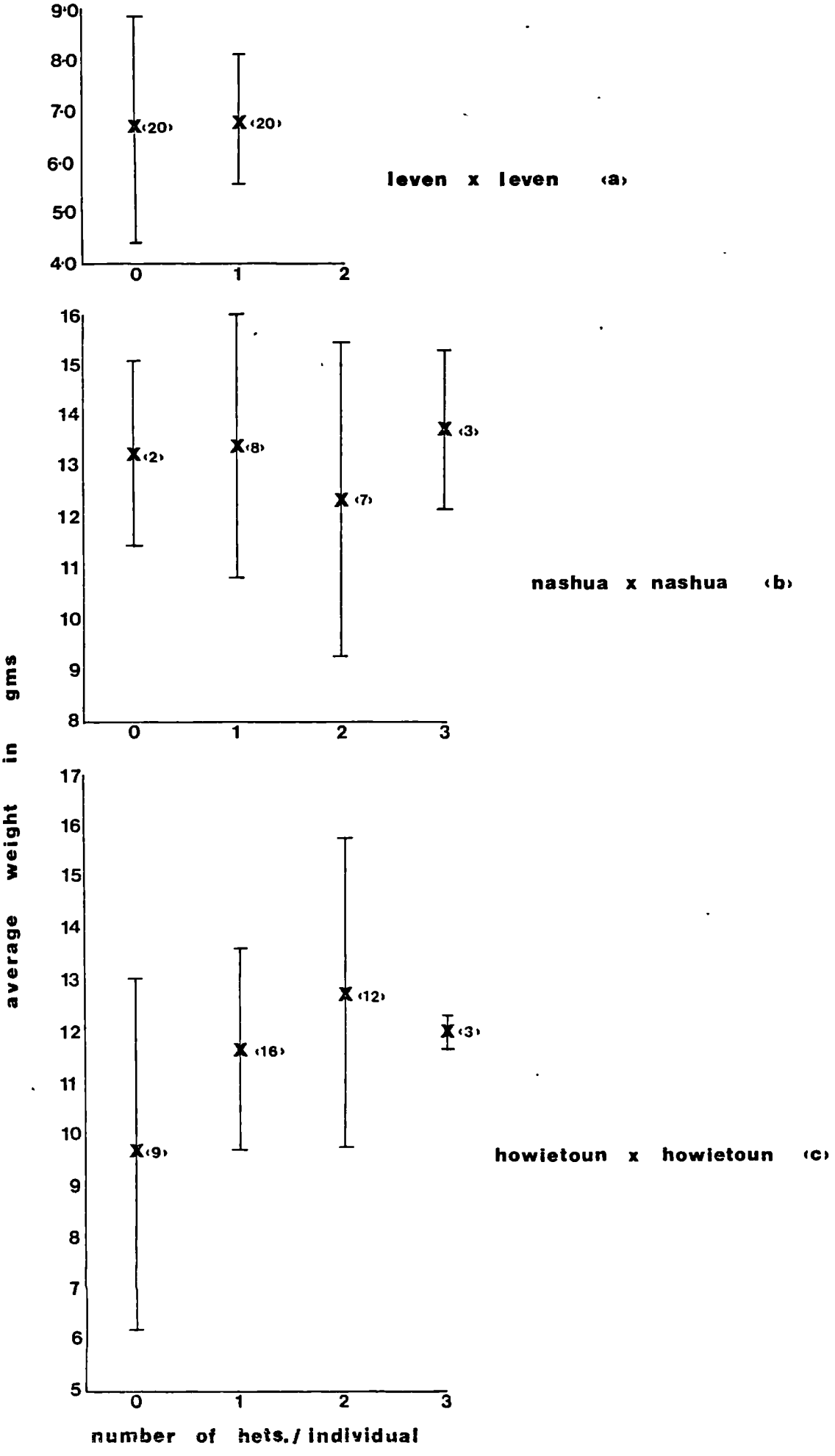
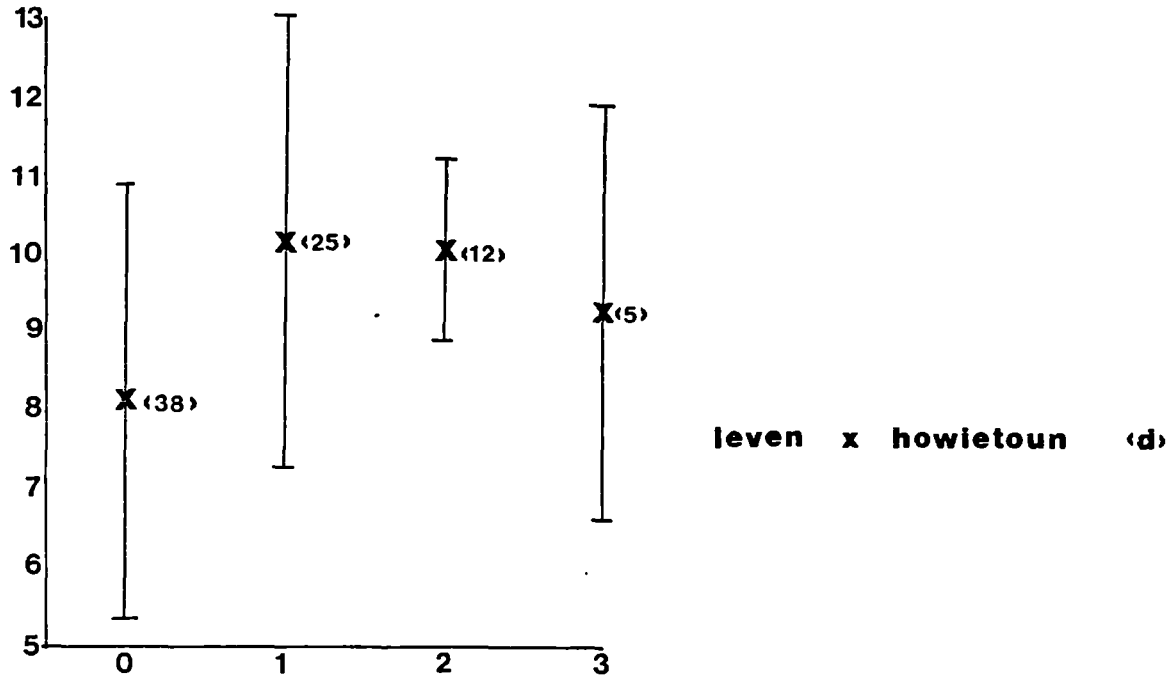


Figure 5.6 continued



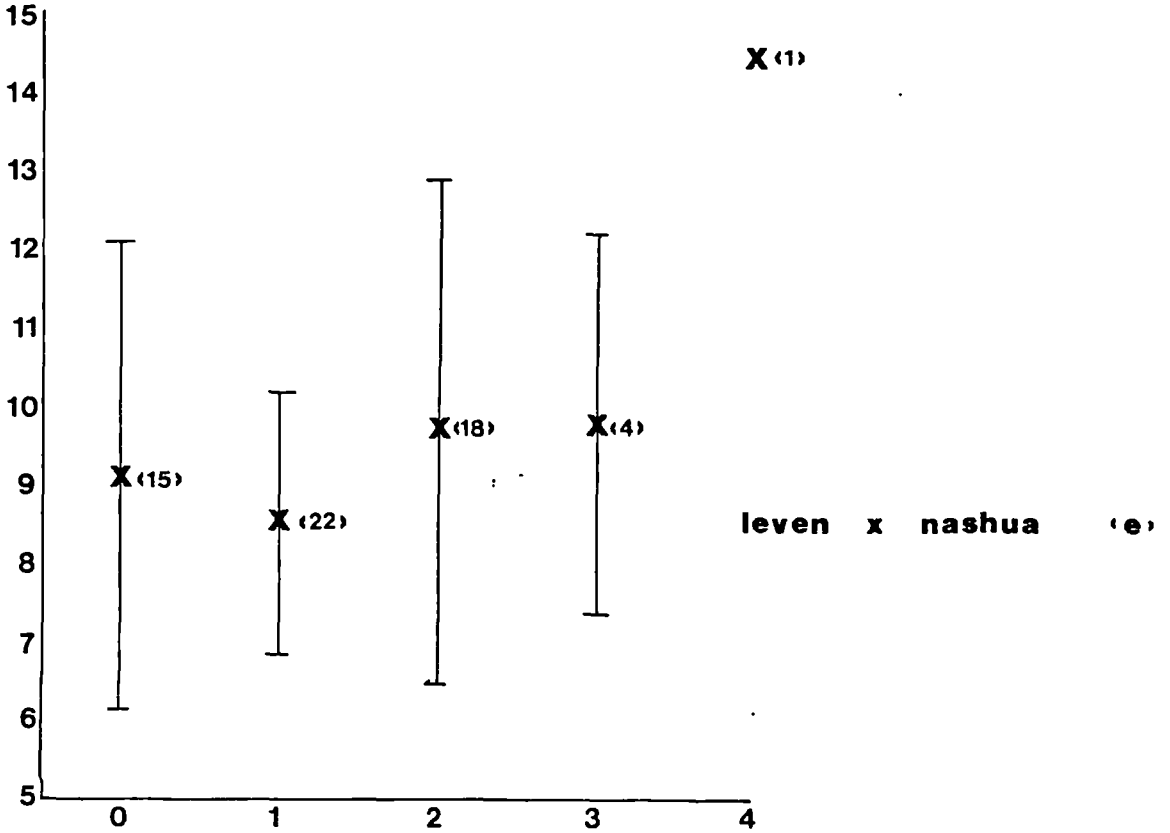
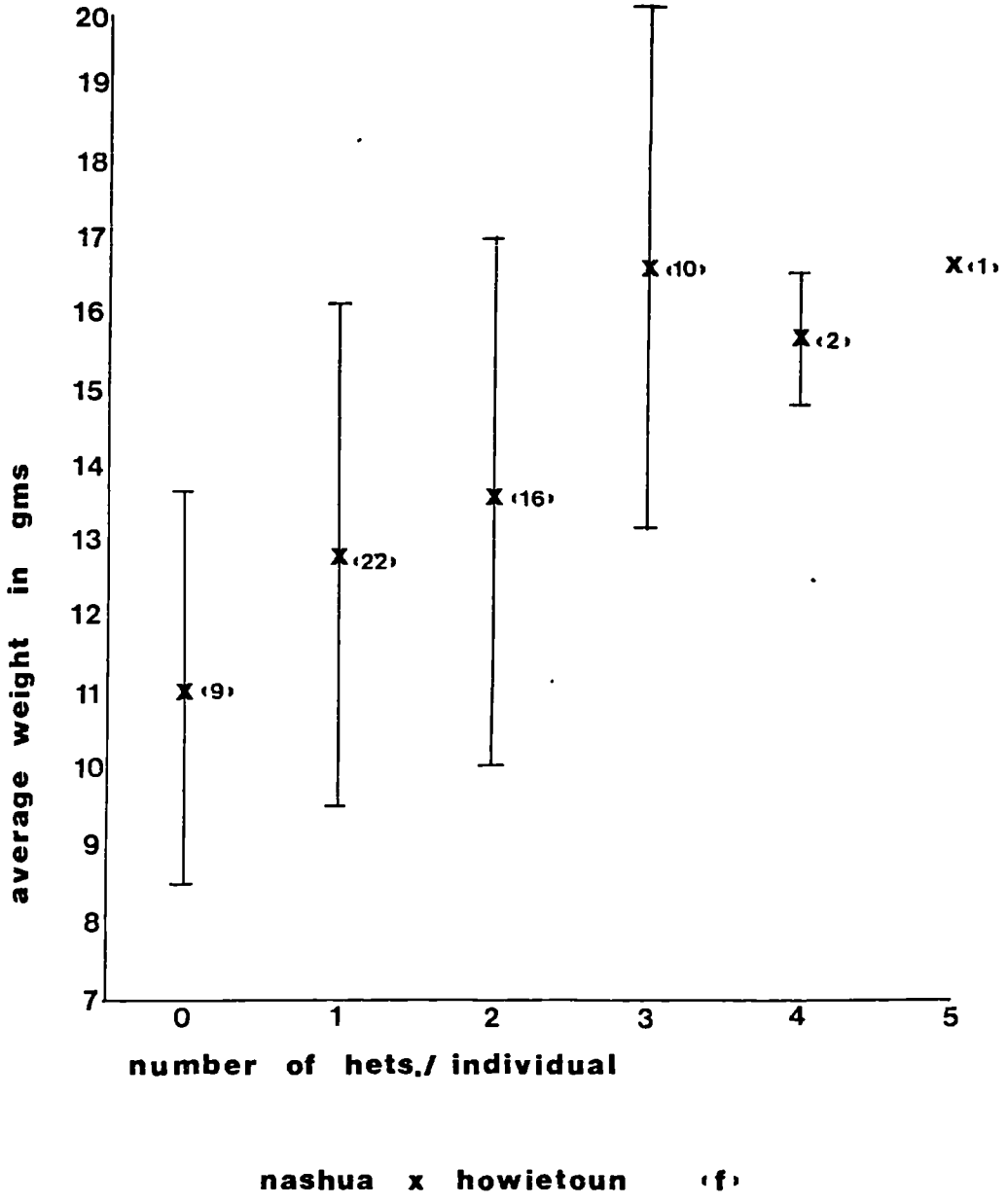


Figure 5.6 continued



number per individual categories. On further examination of Figure 5.6(e) all the heterozygote classes 3, 4 and 5 came from two tanks out of six examined. These two tanks exhibited the highest average weight of fish at the 2nd accurate weighing and thus these tanks should really be treated separately from the other 4. This unfortunately reduces the sample sizes even further and was not considered worthwhile. Thus although a correlation does seem probable in Figure 5.6(e) it is due to the method of exhibiting the data and the population structure of the crosses involved. Both the Howietoun and Nashua strains are both domesticated and to a certain extent inbred lines. Intuitively the crossing of such lines gives rise to very successful progeny especially if grown in the tank environment to which the strains are accustomed. The level of heterozygosity is somewhat irrelevant and is regarded as a consequence of the interaction between the two populations rather than a requisite for increased performances.

The overall correlations between the number of heterozygotes per individual and the weight and length of the fish in each of the three trials is given in Table 5.21 and 5.22 (Spearman and Pearson). The Factoral Trial exhibits a highly significant correlation between the number of heterozygotes per individual and weight and length whereas the trout in the Howietoun and Leven trials show no such correlations.

It is thought the apparent correlation exhibited in the factoral trial is a consequence of the breeding structure of the population rather than representing a true influence of heterozygosity over growth rate.

Table 5.21 giving Pearson coefficients between weight and length and the number of heterozygotes observed per individual

	Number of heterozygotes per individual		
	Howietoun trial (n = 280)	Leven trial (n = 360)	Factoral trial (n = 300)
Weight	0.0267 (P = 0.328) NS	- 0.036 (P = 0.248) NS	0.472 (P = 0.000) Highly
Length	0.0302 (P = 0.307) NS	- 0.0508(P = 0.168) NS	0.477 (P = 0.000)Significant

Table 5.22 giving Spearman correlation coefficients (non-parametric) between weight and length, and number of heterozygotes per individual

	Number of heterozygotes per individual		
	Howietoun trial (n = 280)	Leven trial (n = 360)	Factoral trial (n = 300)
Weight	0.0300 (P = 0.309) NS	- 0.0211 (P = 0.345) NS	0.471 (P = 0.000) Highly
Length	0.0219 (P = 0.358) NS	- 0.0022 (P = 0.484) NS	0.467 (P = 0.000)Significant

n = number of fish used in correlations
NS = not significant P = 0.05

Plates 3 and 4 illustrate X rays taken of fish taken from the tank that contained the cross between Nashua ♀ (1) and Nashua ♂(2). The musculature of the area posterior to the dorsal fin is malformed causing severe kinking of the spinal column.

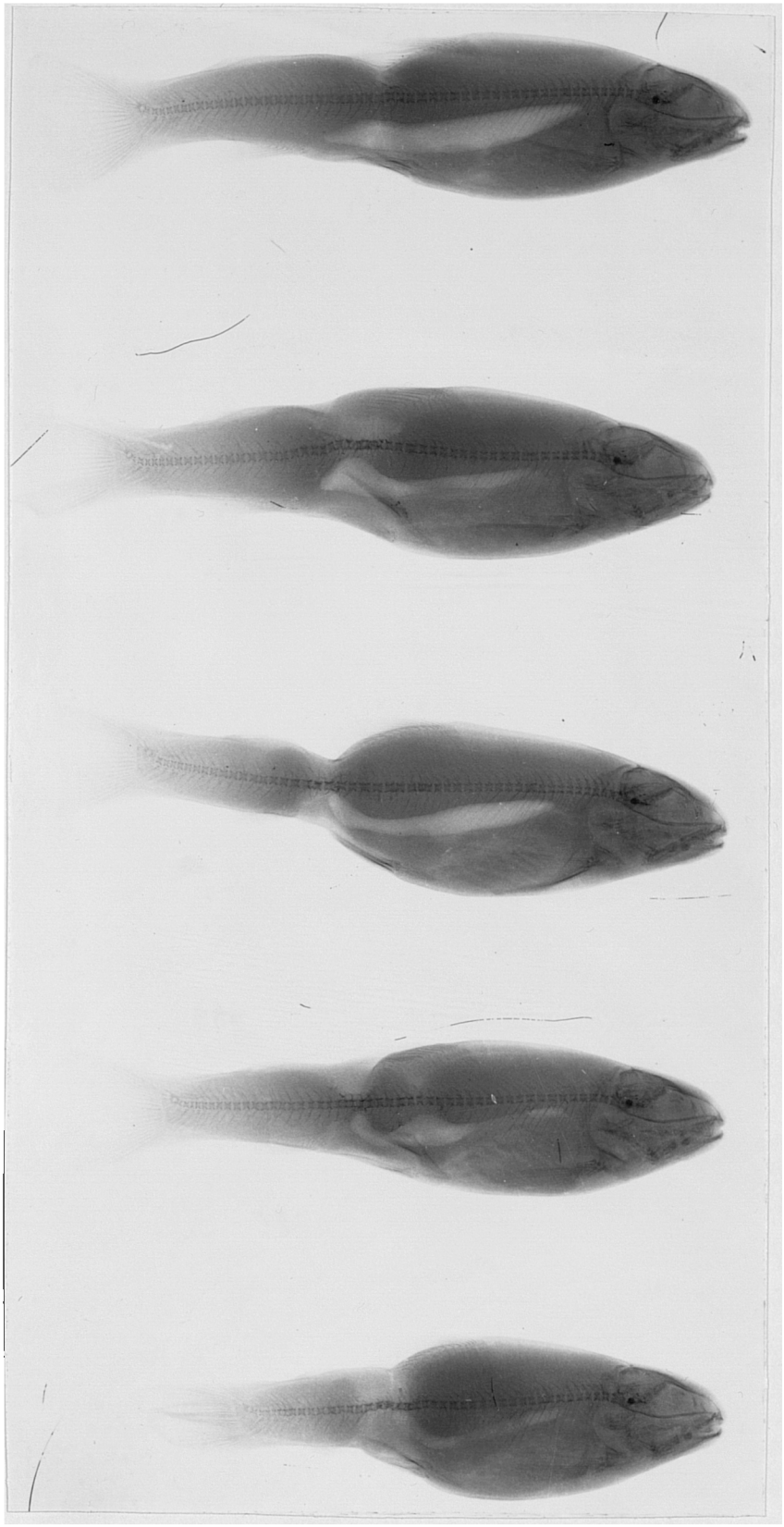
This is included as an example of what was thought to be the result of inbreeding within the Nashua population. This will be commented on in the discussion.

Plate 3 X-rays illustrating dorsal view of malformed parr

Plate 3

Plate 4 X-rays illustrating lateral view of malformed parr

Plate 4



5.5 Discussion

The discussion of the results has been divided into the following categories:

1. The relationship between heterozygosity and growth rate

2. Egg size differences

3. Growth of alevins in relation to egg size and yolk sac utilization

4. Reasons for mortalities and deformities in the three trials

5. Any significant correlations found

6. Conclusions and Recommendations

5.5.1. The relationship between heterozygosity and growth rate

From the data presented in the results section it is apparent that there is no significant correlation between multi-heterozygote classes and growth rate in either of the Howietoun or Leven trials. Table 5.21 and 5.22 give the individual multi-locus heterozygote class correlation coefficient with length and weight both for parametric and non-parametric tests.

From Tables 5.21 and 5.22 it appears there is a highly significant positive correlation between multi-locus heterozygote class and

growth rate in the factoral trial. This relationship is not apparent in Figures 5.5 and 5.6 which show graphically the relationship between number of heterozygotes per individual and weight for each of the various strain crosses in the factoral trial.

How can these results be interpreted?

The Leven and Howietoun trials, showing no correlation between multi-locus heterozygote class and growth rate concur with other work conducted on salmonids (Beacham and Withler, 1985 and Koljonen; 1986) and other fish (McAndrew et al., 1986; and King, 1985).

There are reasons given by some authors to explain why they have not found correlations between heterozygosity and growth rate, which could apply in this study. Beaumont et al. (1983) gave possible reasons why they did not find such correlations in Mytilus edulis.

(i) It is possible that none of the loci investigated was linked directly or indirectly to growth rate. Beaumont et al. (1983) and McAndrew et al (1986) find this solution unlikely however because as they point out, in these studies that have reported a positive relationship and which have partitioned out the effect between loci, virtually all loci analysed, regardless of function, appear to contribute to the general trend (Zouros et al., 1980; Green et al., 1983; Koehn and Gaffney, 1984).

(ii) Beaumont et al. (1983) postulated that if the size range of fish sampled was relatively small and either large individuals or small individuals were missing from the correlation for some reason, the lack of correlation between heterozygosity and growth rate would not be surprising. In this study the problem is not so much the missing out of size groups, but simply the lack of numbers from each tank population.

(iii) Beaumont et al. (1983) point out that the range of heterozygote classes within a single family is small compared to that of a wild population of Mytilus edulis. Because there is a greater range of heterozygote classes per locus in the wild populations, the chances of a link being observed between heterozygosity and growth rate are greater than within a single family. In this study, as each tank's heterozygosity was dependant on the genotype of just two parents and only a limited number of broodstock were used in each trial the likelihood of finding any correlation with heterozygosity and growth rate, if it exists will be limited. Similar heterozygosity/growth rate correlations were attempted using the wild population data (chapter 4) but no populations sampled were large enough to give a reasonable number of individuals of the same age on which to test the correlation.

(iv) Beaumont et al. (1983) also indicate that the link between heterozygosity and growth may be tenuous in some species

and is only detectable during early growth. Mitton and Grant (1984) agree and suggest the reason for such a phenomenon is that young stages of any organism put most of their surplus energy into growth with very little being put into reproduction. So if older fish were used, Mitton and Grant (1984) suggest the relationship may not be apparent. In this study the Howietoun trial was examined for any possible correlation, when the trout were approximately 8 months old (age after hatching). The Leven trial correlations were examined when the fish were 5 months old. At both these stages brown trout are not mature and will not mature for at least another 12 or even 24 months, and therefore the theory that the fish are not putting most of their energy into growth does not fit.

Mitton and Pierce (1980) and McAndrew et al. (1986) point out that heterozygosity when assessed from 5 to 10 loci may not reflect real individual heterozygosity measured across the entire genome, and thus such a general relationship as reported may not be expected.

But how can the phenomenon be explained in those species exhibiting it? In this study, the factoral trial shows a highly significant correlation between individual multi-locus heterozygote class and growth rate, when the individual crosses were analysed separately (Figure 5.6 a, b, c, d and e), this highly significant correlation is not substantiated, thus indicating that the structure of the

whole population may be affecting the correlation results.

Cothran et al. (1983) and McAndrew et al. (1986) suggest one reason for the apparent link between heterozygosity and growth rate is concerned with aberant population structure. McAndrew et al (1986) state, "if the individuals sampled were progeny of random matings between and within two inbred populations, then the more outbred, more heterozygous progeny might well grow faster". This seems to be the explanation in the factoral trial for the positive correlation found. The main problem with this experiment was the way small number of broodstock used from each of the respective stocks. Only 2 males and 2 females from each of these stocks were used.

As two of the stocks, namely Howietoun and Nashua have long hatchery histories, they are more likely to be behaviourally adapted to the tank environment, and will thus grow quicker. The Leven trout progeny were spawned from wild trout which are unlikely to become quickly adapted to the tank environment, and thus will be expected to grow more slowly. This theory was substantiated by observations made during general husbandry duties. The Leven x Leven crosses were far more "tank shy" than the Howietoun or Nashua stocks. The Leven progeny when disturbed swam very erratically and took much longer to return to a settled feeding state than did the other stocks. This factor was difficult to quantify but is thought to have had considerable effects when calculating the correlations between growth rate and heterozygosity.

The Leven broodstock used in the factoral trial must have been very homozygous because the progeny of the Leven x Leven crosses showed a very much lower level of mean heterozygosity (0.52%) than did the other crosses (1.85% - 3.90%) (Refer to Table 5.20). This does not seem completely typical of the Leven stock as the results from the Leven trial (1983-84) indicate the mean level of heterozygosity for the progeny was in fact the same or even a little higher than for the progeny derived from the Howietoun trial (1982-83) (Refer to Table 5.20).

Thus the use of too few broodstock and the fact that the different strains seems to have different characteristics as far as initial growth under artificial conditions are concerned, has led to the apparent correlation between heterozygosity and growth rate in the factoral trial.

It is felt that Koehn and Gaffney's (1984) sweeping statement that "the phenomenon of heterozygosity correlated with growth rate is general to a diversity of plants and animals" seems presumptive. More work is obviously required using a variety of organisms including salmonids, with large enough sample sizes used, to enable sensible conclusions to be drawn.

5.5.2 Discussion of differences in egg sizes

As shown in the introduction Gall and Gross (1978) showed that significant differences in egg size were reported in all salmonid species, and Springate and Bromage (1984) believe that larger older

trout have higher total fecundities and produce larger eggs than smaller younger fish.

There were significant differences found between the sizes of the trout eggs (as measured by diameter) in all the trials. Figures 5.1 a and b illustrate the differences in the Howietoun and Leven trials. The Howietoun broodstock used in the 1982-83 trial were all 3 year old (ranging in length from 35 cm to 40.6 cm) and first time spawners, but still their egg diameters varied from 4.5 mm to 5.6 mm approximately. Thus suggesting there was indeed a genetic component to egg size in brown trout. The Leven eggs ranged in diameter from 4.9 mm to 6.4 mm, a larger range of sizes compared to the Howietoun trial.

The Leven broodstock in the 1983-1984 trial ranged in age from 3 years old to 5 years old and in length from 38 cm to 52.5 cm and so were larger and on average older than the trout in the Howietoun trial. According to Springate and Bromage (1984) the greater difference in size of the eggs is therefore not surprising.

The original intention was to obtain Leven broodstock all of the same age, but due to the impracticalities of arranging to collect and strip 18 females and 9 males all of the same age (preferably 3 year olds similar to those stripped at Howietoun), from a wild population on the same day, it was impossible. Thus the fish stripped were those available and in correct stripping condition.

The eggs used in the factoral trial were stripped from different aged individuals. To obtain a large enough number of eggs to divide into six to allow for the factoral crossing procedure, large females were required. This meant the two Howietoun dams used were five year olds and were 55 and 60 cm in length. The Leven dams ready on the same day were also 5 years old and were both approximately 48 cm in length. The Nashua stock which were available were first time spawners (3 year olds) and approximately 41 cm in length.

Not surprisingly the F values derived from analysis of variance (see Tables 5.1 and 5.2), calculated from egg diameters for all the trials showed that the dam variance is very highly significant.

5.5.3 Egg size and subsequent alevin growth

As shown in the introduction brown trout alevins and fry derived from large eggs are larger than those derived from small eggs (Bagenal, 1969).

The alevin growth in this study which was analysed using different sized eggs indicted that the larger the eggs, the heavier the alevin at the end of exogenous yolk absorption. This agrees with findings by Beacham et al. (1985) who worked with chum and coho salmon.

The rate at which the yolk is used is similar for alevins from different size eggs. The SGRs of the 4 trial populations were different but not significantly so, according to Duncan's multiple range test. (See Figure 5.2).

The decline in the percentage of yolk sac/total body weight was similar for all the alevins, although by the end of the trial it was obvious that the larger alevins hatched from the larger eggs had far more yolk sac reserves remaining. 15% of the total body weight of the larger alevins consisted of yolk sac whereas only 1.5% of the total body weight of the smaller alevins was yolk sac. This confirms the suggestions that

1. large eggs give rise to large fry which give rise to large first feeders and

2. larger alevins use up their food reserves in the form of their yolk sac at the same rate as small alevins, but have more reserves than the smaller alevins.

Robison and Luempert III (1984) working on heritability estimations for fingerling weight in brook trout found that, high dam heritability at 35 days post first feeding was due to maternal effects. They worked out that the closer the measurement of growth to fertilization is, the stronger the maternal effect. This supports the conclusions arrived at in this study.

Therefore it appears it is important to ensure that similar sized eggs are incubated together in a hatchery environment, otherwise there will be disparity in the length of time alevins can be kept before they require external food. If one starts feeding too early in conventional troughs, one runs the risk of smothering the larger alevins not ready to take food and also encourage Saprolegnia fungus

to grow and cause excessive mortalities. If one waits until the larger alevins in a trough are ready to feed it may well be too late for the smaller alevins derived from the smaller eggs to successfully accept external food - and unnecessary mortalities will follow in the form of "pinheads". This explanation accounts for the loss of fry between first feeding and eight weeks later in the Howietoun trial. Due to delays in finishing the tank system first feeding was delayed by about a week. The Tables 5.3 and 5.12 illustrate that the highest mortalities at this stage occurred in tanks derived from small eggs and in fact the correlation between egg size and mortality at this period was -0.759 ($P < 0.001$). Confirming the smaller the egg the larger the mortality. In the Leven trial the fry were stocked into the tanks and first fed before yolk sacs were completely used up and the corresponding correlation was 0.003 (not significant) confirming the observation that no fry were lost due to the pinhead problem.

5.5.4. Reasons for mortalities and deformities in the three trials

One of the factors affecting mortalities at fertilization and upto first feeding is the actual time of stripping. Unless a female salmonid is stripped within a certain time after ovulation it has been shown that the subsequent mortality increases dramatically (Craik and Harvey, 1984; Springate et al., 1984). Likewise if the fish is stripped on the day of ovulation this may be too early and eggs may be damaged by the use of excessive force used to strip the eggs.

There was considerable variation in "ripeness" of the dams involved in the trials especially in the Howietoun trial and the factoral trial. It can be seen from Table 5.9 illustrating the Howietoun trial that the dam effect for mortalities at fertilization, shocking and at the first accurate measurement, was significant. The dam effect is also significant for alevin deformities.

The only significant effects derived from the Leven anova tables (Table 5.10) are the dam effect on mortality at shocking and percentage deformities after hatching. The Nashua dams in particular caused problems. They were "at least 10 days overripe" (Walker, pers. comm.). This overripe state seems to have contributed to large mortalities at fertilization and at subsequent stages therafter (see Tables 5.6 and 5.7). Almost 30% of the eggs stripped from dam (1) were not successfully fertilised and were removed the next day. 17% of dam (2)'s eggs were also unsuccessfully fertilized, while the percentage mortality at fertilization for the Leven and Howietoun dams ranged from 0.1% t 3.3%

The percentage mortality at shocking for the eggs from the two Nashua dams was also higher than for the other dams. Thus overripe females cause great problems in interpreting subsequent growth and survival results.

During the Howietoun and Leven trials some of the sires used were moribund, but had to be used due to limited available broodstock. The sire effect is not significant throughout all the parameters

measured according to the F-tests in Tables 5.9 and 5.10 . Thus indicating the state of the Leven and Howietoun sires did not affect subsequent mortality . In 1985 when eggs and milt from the Nashua and Leven strains were transported to Howietoun fish farm, milt was still motile but obviously not as fresh as the milt stripped from the Howietoun sires. Due to the external temperature on the day of stripping the milt from Leven sire (3) became partially frozen in transit. The sperm were still motile but not as vigorous as compared to the freshly collected Howietoun milt. This may well explain some of the large mortalities experienced when progeny from sire (3) are considered in the early stages of the factoral trial. If one studies Table 5.7 one observes that sire (3) at shocking contributed to large mortalities irrespective of with which female it was crossed. This also explains the highly significant correlation observed between sire length and % mortality at shocking (see Tables 5.13 and 5.16), as the Leven sire in question was 53 cms in length and was larger than any of the other sires used in this experiment (see chapter 3).

It was felt, due to the non genetic factors affecting mortalities that heritability estimates of survival would be misleading and so were not calculated.

5.5.5 Deformities

The number of malformed fry in the first two trials was not significant but rose dramatically in the factoral trial for certain crosses. The Nashua x Nashua crosses all showed higher levels

of malformities than any of the other crosses. The percentage deformity estimates given in Table 5.8 are obviously calculated using the total of surviving alevins and fry. The previous high mortalities left the total of surviving individuals for ♀ 1 at a low number compared with the other crosses. The malformities were only recorded once the individuals had died or become moribund, and included curved spined individuals, two-headed individuals and those suffering from 'blue sac' (Roberts and Shepherd, 1979).

The cross between Nashua ♀ 1 and the Nashua ♂ 2 also exhibited another malformity which did not manifest itself until the surviving fish were much larger. This cross was kept on in a tank but not used in the growth trial because of lack of numbers (< 80). Plates 3 and 4 illustrate the deformity afflicting approximately 30% of the population.

Unfortunately no electrophoretic analysis of these fish was undertaken, but the X rays reveal that the malformity is similar in each individual and consists of an abnormality in the musculature, posterior to the dorsal fin. This had lead to severe stress on the spine of the trout resulting in various levels of vertebral kinking. It appears not to affect the ability of the trout to take food and grow but is a gross deformity none the less.

The electrophoretic screening of the fry from the same tank indicate that the two parents were heterozygous for various loci. For $AAT_{-1,2}$

and PGI_{-3}, both the relevant homozygotes and the heterozygote appeared in the analysis. This indicates the malformity which appeared in approximately 30% of the surviving population may have been the result of a double recessive gene.

Walker (pers. comm.) could not assure me that the Nashua broodstock were not closely related, and it seems very likely that at least dam 1 and sire 2 were closely related.

This highlights the problem of selecting broodstock from a captive population of limited size. The Nashua strain kept at Pitlochry originated from one consignment of eggs brought over from USA (Walker, pers. comm.).

Thus the inclusion of the Nashua strain highlights various points which may confound subsequent growth trials,

1. overripeness of female broodstock

2. possibility of inbreeding effect caused by the use of small number of fish derived from a small breeding unit.

Both (1) and (2) have contributed to increased mortalities and levels of deformities.

5.5.6 Importance of significant correlations identified

From Tables 5.12, 5.13 and 5.14 and Tables 5.15, 15.16 and 5.17 it can be seen that most of the highest correlations ($P < 0.001$) are between the lengths and weights of the fish at each accurate measurement and between each measurement and the next. SGRs are also highly significantly positively correlated with weights and lengths (2) and (3) in the first two trials and weights and lengths (1) and (2) in the factoral tiral (weights and lengths (3) were not taken due to lack of project time).

Egg size is significantly positively correlated in the first two trials with average weight at first feeding. (Not recorded for trial three). ($r = 0.784$ $P < 0.001$, $r = 0.488$ $P < 0.01$).

Significant positive correlation between dam length and egg size is shown in the Leven trial ($r = 0.630$, $P < 0.001$) and the factoral trial ($r = 0.611$ $P < 0.001$), but not in the Howietoun trial ($r = 0.100$ NS). The reason for this is probably because the Howietoun broodstock were all of the same age and approximately the same size, whereas in the Leven and factoral trials, dams from different age groups and different sizes were used and thus the egg sizes are more likely to be significantly different. Thus egg size seems to be correlated with dam age and length, agreeing with Springate and Bromage (1984).

Thus the length of the dam is correlated with the egg size which in turn is positively correlated with the size of the first feeding fry and to some extent with the size of fry upto about 3-4 months old.

The average weights at first feeding in the Howietoun and Leven trials is significantly positively correlated to the weight and length of fry at the first accurate measurement (5 months old). Thus egg size which is influenced by the size of the dam, is positively correlated with the size of first feeding fry. The fry size has a positive effect on 4 month old fingerlings and the size of the fingerlings is positively correlated to the 8 month old parr and the 15 month old trout.

But why should egg size be correlated with average weight at first feeding, thus indicating strong maternal effects, and yet not be correlated with subsequent growth stages? This contradicts the theory that small environmentally or maternally induced advantages conferred on individuals in fish populations are maintained, giving rise to the 'shooters' described earlier.

There are a number of reasons why egg size is not directly related to subsequent growth.

(1) Mortalities. Some tank or trough batches experienced large mortalities and the structure of these populations will change if the deaths are related to size. Thus the average weight of fish in a population increases if differential mortality of the smaller fish occurs. This seems to have occurred in the Howietoun trial, at shocking. The higher the mortality at shocking the higher the average weight at first feeding.

(2) The tank populations were obviously grown independently of each other and thus the egg size was likely to have a relatively small role to play in each population, as far as subsequent growth is concerned, because the size of the individual eggs within each population was much more uniform than was the case between populations (Figure 5.1). If all the eggs and subsequent alevins, fry and trout had been kept together in one large mixed population, then the larger eggs were more likely to confer an advantage over much smaller eggs, and giving rise to the classic shooter scenario. This intuitively was thought, would have happened if the eggs and subsequent growth stages had not been kept separate.

(3) All the growing fish while resident in the tanks were kept at low stocking densities and were fed to excess every day. This also is likely to reduce the chance of shooters appearing in the population and may also be a contributory factor explaining why egg size is not correlated with eventual trout size.

It was interesting that once the fish populations of the first two trials were mixed, as they were transferred from tanks to ponds, any advantage gained in the former was reinforced and strengthened when all the populations were competing in the latter environment. Weight and length (2) were highly correlated to weight and length (3) ($P < 0.001$) for the Leven and Howietoun trials. The advantage of being larger when entering the pond environment was very obvious. From being fed to excess in the tanks, surrounded by fish of a relatively uniform size, the mixed population had to compete for

a much more limited food supply. The trout being fed according to the commercial side of winter operations which consisted of feeding at 1% of the biomass of the pond which is far from feed to excess, especially in milder weather. The size of the trout food also favoured the larger trout. The size of pellets supplied, being determined by the ability of the best fish in the ponds to take the pellets rather than the smallest. Thus the smaller average weight tank populations remained small, and the largest average weight populations grew much better.

5.6 Conclusions

1. Heterozygosity was not correlated with growth rate in the Howietoun and Leven trials.

2. Heterozygosity was apparently correlated with growth rate in the factoral trial, but this was thought to be due to the population structure, and the lack of broodstock used.

3. Dam length was correlated with egg size.

4. Excessive mortalities at fertilization and shocking were connected with the ripeness of the dams in question.

5. Egg size was correlated with size of fry at first feeding.

6. Egg size was not correlated with later growth stages due to differential mortality, and environmental influences caused by husbandry techniques.

7. First feeding fry size was correlated with subsequent growth.

8. Weight and length at the second accurate measurement was highly correlated with weight and length at the third accurate measurement. This effect being compounded by environmental influences associated with the change from tanks to ponds.

If trout are to be grown in tanks to 5 or 6 months old commercially and the same feeding regime adopted from winter feeding, it is strongly suggested that they are graded before being introduced to the ponds. The small trout from the Leven and Howietoun trial at the second accurate measurement did not grow well under pond conditions due to competition with the larger individuals, and due to being fed pelleted food too large.

In the natural environment, large dams producing large eggs will be at a selective advantage as far as progeny survival is concerned, and the level of individual heterozygosity appears to require more investigation before one can identify whether it is a relevant or irrelevant factor, when subsequent growth is considered.

CHAPTER 6

Chapter 6 Summary

1. The electrophoretic evidence relating to the population structure of Scottish brown trout revealed the following:

(a) Average heterozygosity levels ranged from 0-8.9% which is well within the range found for other salmonids (Kirpichnikov, 1981).

(b) The gene diversity analysis conducted agreed with that of Ryman (1983). A large percentage of the gene diversity (33%) was attributed to differences between populations rather than within populations.

(c) Large amounts of genetic diversity exist between populations of brown trout on a micro geographic scale. Evidence is presented, involving a number of locations to show that populations of trout living in the same small drainage area can be genetically diverse and distinct. The major reasons for these differences are attributed to homing behaviour and founder effects.

(d) The LDH_{-5} (105) variant allele appears to be an electrophoretic marker for an ancient, immediately post-glacial, invasion stock of brown trout. The evidence accumulated, positively correlates the occurrence of the allele in stocks, with positions above impassable falls, distance from the sea, and height above sea level. This agrees with the evidence and invasion theory of Ferguson and Fleming (1983).

(e) Dendrograms drawn using Nei's genetic distance and UPGMA cluster analysis, show that the majority of population divergence in the Scottish brown trout occurred in the last 50,000 (Nei, 1972) to 180,000 years (Gorman et al., 1976). The most diverse populations with a genetic distance of 0.05, diverged 900,000 years ago according to the method used by Gorman et al. (1976). This approximately coincides with the beginning of the Quaternary ice age. It must be stressed that Nei's genetic distances calculated in this study have large associated standard errors and the dendrograms derived from the cluster analysis are merely an approximate representation of the genetic relationship between the various stocks analysed, and are not intended to be interpreted any further.

(f) Certain rare alleles identified in only a few populations at a low level are regarded as useful in future genetic tagging schemes. The alleles suggested are PGI_{-2} (135), PGI_{-3} (110), IDH_{-2} (130). LDH_{-5} (105) could also be used in lowland waters characterised by the LDH_{-5} (100) allele, and the LDH_{-5} (100) could be used in the reciprocal situation.

(g) Certain pristine populations of trout have been identified in this study and it is suggested that they whould be anlaysed quantitatively to determine whether they are of potential use in future fishery management strategies. Electrophoresis is a useful tool in identifying wild stocks but heterozygosity levels will not necessarily identify potentially fast growing stocks.

(h) Although it is postulated that sea trout stocks in Scotland are characterised by the LDH_5 (100) allele, this study did not attempt a large survey of migratory Salmo trutta, and it is suggested that such work is required in the future. This electrophoretic survey should include a variety of sea trout stocks including the long lived multi spawning stocks of such systems as Loch Maree and Loch Eilt, on the west coast and the River Tweed in the East.

2. The electrophoretic survey of the Howietoun hatchery stock revealed the level of heterozygosity to be slightly below average, compared to the wild stocks, but the figure for the proportion of polymorphic loci was the largest found in any population in this survey, reflecting the diverse origins of the present broodstock. Many of the loci that did show polymorphisms had low variant allele frequencies. The level of heterozygosity and the proportion of polymorphic loci could be used as an indication of possible reduction in variability and inbreeding in the future.

3. The electrophoretic survey in this study was too broadly based to satisfactorily answer questions associated with heterozygosity and growth rate, and linkage disequilibrium. Some evidence does exist to suggest the level of heterozygosity is correlated with growth rate, in the factoral trial but this was explained by the small number of broodstock used, derived from three different strains, two of which were hatchery based. The wild

populations studied were not large enough to enable year groups of sufficient size to be analysed for potential heterozygosity/ growth rate correlations.

Possible linkage disequilibrium was studied in the wild populations. Some non-random associations were shown to exist, but these were not regarded as being explained by true genetic linkage. The reasons for such apparent disequilibrium was a combination of sampling error; random drift in the wild populations; collecting fish from heterogenous populations, and the pooling of data from many different populations. All have been shown to generate apparent linkage disequilibrium (Nei and Li, 1973). From the limited data there appears to be little evidence for widespread linkage disequilibrium, which agrees with other authors working with brown trout (Taggart and Ferguson, 1984). This tends to lend support to the Neutralist theory of molecular evolution (McAndrew et al., 1986).

4. High heritability estimates were calculated for growth rate at different stages after first feeding for all three trials undertaken. These high estimates were partially due to the way in which the trials were conducted using a small number of broodstock. The small number of sires especially, was considered a problem in estimating accurate heritabilities.

The small number of broodstock also lead to very large standard errors. The coefficients of variations (CVs) increased as each trial progressed for weight and length, with the former giving larger values than the latter. Taking problems of accuracy

into account the large heritability estimates indicate mass selection for growth rate would initially be successful for the stocks studied, and considerable genetic gain could be achieved if growth rate is the only trait of interest.

5. Various husbandry practices were questioned.

(a) Egg sizes need to be uniform to alleviate problems of subsequent pinhead development.

(b) If trout are to be reared in tanks for the first 9 months it is suggested that they should be graded before they are transported to earth ponds. If grading is not performed then the variation in size of the trout stocked into the ponds is exacerbated during the period post introduction leading to very large differences in weight and length between the largest and smallest groups when the fish are examined at 15 months old.

(c) The pond feeding of a population of brown trout characterised by a variety of sizes, with pellets suitable for the larger individuals is questioned. If the fish are not graded, it is suggested a pelleted diet of a size suitable for the smaller individuals within the population should be administered instead of, or along with, larger pellets. This should aid growth of the smaller individuals.

(d) The fry food fed to brown trout first feeders appeared to

be too large for the smaller individuals to accept and the grinding up of first feed diet was successful in helping a successful transition from alevin to healthy fry. It is suggested when dealing with smaller than average brown trout eggs that the grinding of the first feed diet, for the subsequently developing fry would prove beneficial. First time spawners are known to produce smaller eggs and thus smaller fry, and this procedure is suggested for this category.

(e) Careful use of the panjet device, with an extension tube attachment, enables one to panjet fish as small as 5 gms quite accurately using a series of batch marks, without apparent ill-effect, thus extending the potential use of this device in husbandry practice.

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