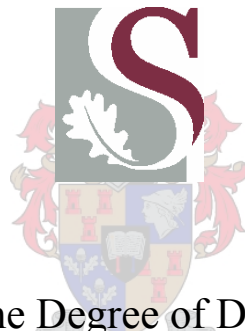# Genetic improvement of growth rate in rainbow trout (*Oncorhynchus mykiss*)

By

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DECLARATION

I, the undersigned, herby declare that the work contained in this dissertation is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signature: ....

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ABSTRACT

A breeding programme aimed at the genetic improvement of growth rate of rainbow trout was initiated in 1988 by the Department of Genetics, University of Stellenbosch, in collaboration with the local trout producer's organisations. The first phase of the breeding programme included the collection, evaluation and selection of the best available genetic material from 13 different genetic groups (nine local and four overseas) to make up two separate base populations as odd and even year-groups. This was done to establishment a base population with high genetic merit and variation at the onset of the breeding programme. Statistically significant and commercially valuable genetic differences in terms of weight and length gain were detected between the various hatchery groups.

The next two generations of the breeding program included a series of single and double crosses in order to increase the levels of genetic variation in the base populations, and to investigate possible heterosis and specific and general combining ability among the crosses. Significant levels of heterosis (6.7% to 9.6%) and general combining ability was found for weight and length gain during consecutive growth stages. No evidence was found for specific combining ability among the crosses. The crossing of selected offspring from the original genetic groups followed by the application of intensive multi-stage selection for growth rate within progeny groups has led to the establishment of second and third generation parental populations with higher levels of genetic variation and improved individual genetic merit with regard to growth rate. The exploitation of non-additive genetic variation within the base populations through crossbreeding and heterosis during the early stages of the selection programme was delayed in favour of the utilization of additive genetic variance through a procedure of multi-stage selection that incorporated high intensities of selection within and between family groups.

The estimation of genetic parameters during the fourth generation on the basis of a hierarchical half-sib family structure confirmed the presence of high levels of additive genetic variation within the respective populations/year-groups. High heritability values in the range of 0.40 to 0.53 were recorded for body weight and length at 150 days. Genetic correlations between the traits were also high, in the range of 0.74 to 0.82. The cumulative realized response of 50% in body length for the EVEN year-group after six generations of selection (8.3% per generation), and the 33% for the ODD year-group after five generations of selection (6.6% per generation) confirms the efficiency of the multi-stage selection procedure to exploit the available additive genetic variation for growth rate within the respective populations.

The programme is still ongoing, entering its 7th generation in 2004 and is supplying about 50-60% of commercial material through direct supplies of broodstock, ova and fingerlings and indirect supplies via multiplier stations (commercial hatcheries). The programme was the first of its kind in relation to aquaculture species in the Southern African region, and has since initiated the introduction of programmes of genetic improvement in three other indigenous species, namely tilapia (*Oreochromis mossambicus*), African catfish (*Clarias gariepinus*) and abalone (*Haliotis midae*).

OPSOMMING

'n Teelprogram gerig op die verbetering van groeitempo in reënboogforel is in 1988 ingestel onder toesig van die Departement Genetika aan die Universiteit van Stellenbosch, in sameweking met die plaaslike forelprodusenteverenigings. Die eerste fase van die teelprogram behels die versameling, evalasie en seleksie van die beste beskikbare genetiese materiaal vanuit, 13 verskillende genetiese groepe (nege plaaslike en vier van oorsee) om twee basispopulasies te ontwikkel in elk van die gelyke en ongelyke jaargange. Die doel daarvan was om 'n basispopulasie met hoë genetiese meriete en variasie te ontwikkel met die aanvang van die teelprogram gerig op genetiese verbetering, deur middel van seleksie. Statisties betekenisvolle en ekonomies belangrike genetiese verskille in massa- en lengtetoename is aangetref, tussen die onderskeie genetiese groepe.

Die daaropvolgende twee generasies binne die teelprogram behels die uitvoering van 'n reeks enkel- en dubbelkruisings ten einde 'n verdere toename in genetiese variasie in die basispopulasies te bewerkstellig, sowel as om die voorkoms van heterose en algemene, sowel as spesifieke kombinerings-vermoë tussen die kruisings te bepaal. Betekenisvolle vlakke van heterose (6.7% tot 9.6%) sowel as algemene kombineringsvermoë, is aangetref ten opsigte van massa- en lengtetoename in opeenvolgende groeifases. Daar kon geen aanduiding van betekenisvolle, spesifieke kombineringsvermoë gevind word nie. Die kruising van geselekteerde nageslag vanuit die oorspronklike genetiese groepe, gevolg deur 'n multi-fase seleksiemetode vir groeitempo binne nageslaggroepe, het bygedra tot die ontwikkeling van 'n tweede en derde generasie broeipopulasie wat beskik oor hoër vlakke van genetiese variasie en verbeterde individuele meriete ten opsigte van groeitempo. Die benutting van nie-additatiewe genetiese variasie binne die basispopulasies deur middel van kruisteling en heterose tydens die vroeë stadium van die teelprogram is uitgestel ten gunste van die benutting van additatiewe genetiese variasie deur middel van 'n multi-fase seleksiemetode, wat berus het op die toepassing van hoë vlakke van seleksie-intensteit binne en tussen familiegroepe.

Die beraming van genetiese parameters tydens die vierde generasie het die voorkoms van hoe vlakke van additatiewe variasie binne die onderskeie jaargroepe bevestig. Hoë oorerflikhede van 0.40 tot 0.53 is beraam vir ligaamsmassa en -lengte op die ouderdom van 150 dae. Genetiese korrelasies tussen die kenmerke was ook hoog met waardes van 0.74 tot 0.82. Die saamgestelde gerealiseerde seleksierespons van 50% vir liggaamslengte vir die "EVEN"-jaargroep na afloop van ses generasies van seleksie (8.3% per generasie) en die 33% van die "ODD"-jaargroep na afloop van vyf generasies van seleksie (6.6% per generasie) het die doeltreffendheid van die multi-fase seleksiemetode bevestig ten opsigte van die benutting van die additatiewe variasie vir groeitempo binne die onderskeie basispopulasies/jaargroepe.

Die teelprogram duur steeds voort en sal die 7de generasie in 2004 bereik. Die program voorsien nagenoeg 50-60% van die kommersiele materiaal vanuit direkte voorsiening van teelmateriaal, eiers en vingerlinge asook die indirekte voorsiening via kommersiële teelstasies. Die teelprogram was die eerste van sy soort met betrekking tot akwakultuurspesies in Suider Afrika en het bygedra tot die implimentering van programme van genetiese verbetering in drie inheemse spesies, naamlik die tilapia (*Oreochromis mossambicus*), die baber (*Clarias gariepinus*) en die perlemoen (*Haliotis midae*).

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THESIS OUTLINE

Title: Genetic improvement of growth rate in rainbow trout (*Oncorhynchus mykiss)*

1. Rational

Global population growth is leading to an increasing demand for fisheries products. Natural fisheries have reached upper sustainable limits (95-100 million ton/yr) and increased aquaculture production is needed to meet global demand. FAO statistics for 2001 reported a total harvest of 91.3 million ton for global fisheries with a contribution of 37.5 million ton from aquaculture, which is expected to increase over 60 million ton by the year 2025 (FAO 2002). Increasing demand for aquaculture production and global trade is bringing about increasing pressure to improve efficiency of production systems. Significant improvements have been achieved through enhanced management, nutrition, disease control and water quality management. The contribution of genetics is, however, limited with about 1% of production based on genetically improved stocks (Gjedrem, 2000). This is in stark contrast to agricultural production based on highly bred plant and livestock strains and breeds. The genetic productivity of domesticated populations of mammals and birds is often 3-5 times higher than their wild progenitors (Bentsen and Gjerde, 1994) and substantial progress has been made during the last 50-60 years through the application of modern animal breeding theory.

The aquaculture industry in Southern Africa currently has to rely mainly on the use of genetically undomesticated indigenous species such as tilapia (*Oreochromis mossambicus, O. niloticus*), catfish (*Clarias gariepinus*), shrimp (*Penaes monodon*), eel (*Anguilla mossambicus*), abalone (*Haliotis midae*) together with the introduction of exotic species such as trout (*Oncorhynchus mykiss*), salmon (*Salmo salar*) and oyster (*Grassostria gigas*), which are generally not genetically well adapted to local environmental conditions. Undomesticated genotypes do not convert available food resources in an efficient way, they tend not to thrive in captivity and live constantly under stressed conditions. The development of the aquaculture industry in Southern Africa is characterized by the absence of government assistance in terms of policy, research and development. The genetic improvement of indigenous and exotic species is therefore primarily the responsibility of the respective sectors of industry in which they are utilized.

Rainbow trout is the oldest commercial aquaculture sector in Southern Africa with the introduction of the species from the Northern hemisphere as a sport fishery during the 1890s expanding to commercial production of food fish during the 1970s. The industry was at the time characterized by the complete absence of genetic management systems and concerns were raised about the genetic quality of the South African gene pool and improvement thereof. The concept of genetic improvement of aquaculture species, salmonids in particular, through the application of modern animal breeding technology was strongly promoted by various authors during the late 1970s and 1980s such as Gjedrem (1975, 1985), Kincaid et al. (1977),Kincaid (1983), Gall and Gross (1978) and Gjerde (1986).

Against this background a breeding programme aimed at the genetic improvement of growth rate of rainbow trout was initiated in 1988 by the author in conjunction with the Department of Genetics at the University of Stellenbosch, in collaboration with the local trout producer's organisations. It was set as a

priority at the time to start with the selection programme with immediate effect. Other parallel initiatives included a series of workshops, publications and papers to improve the awareness and understanding of the role of genetic management systems within the sector. The programme is still ongoing, entering its 7th generation in 2004 and is supplying about 50-60% of commercial material through direct supplies of broodstock, ova and fingerlings and indirect supplies via multiplier stations (commercial hatcheries). The programme was the first of its kind in the Southern African region, and has since initiated the introduction of programmes of genetic improvement in three other indigenous species, namely tilapia (*Oreochromis mossambicus*), African catfish (*Clarias gariepinus*) and abalone (*Haliotis midae*).

2. Objectives

The objective of the breeding programme was to attempt to improve the growth rate of rainbow trout under local conditions in order to improve their overall production efficiency and profitability and to overcome the limitations associated with high summer temperatures (>24°C) characteristic of most parts of the region. Improvement in growth rate would enable producers to complete the production cycle for salmon trout before the onset of the second summer, thereby avoiding the high risk and low productivity associated with production of large size fish under these conditions. This implies the development of specific genotypes or strains capable completion of a production cycle up to a body weight of at least 1.2 kg within a period of 16-18 months from fertilization.

3. Breeding programme

The breeding programme constructed was based on a conventional approach adapted to the specific circumstances that prevailed at the time, with reference to available resources (genetic and financial) and limited facilities. The breeding programme is made up of the following key elements:

- **formation of a base population**

  The formation of a base population and procedures to generate maximum possible genetic variation within the base population in view of future selection, is discussed in detail in Chapters 1, 2 and 3.

- **definition of the breeding goal**

  The breeding goal was defined as that of improved growth as discussed in Chapter 1. Other traits of economic importance under consideration were temperature tolerance and feed conversion. It was decided that the issue of temperature tolerance would be best addressed through selection for improved growth, in order to shorten the production cycle and avoid exposure to a second period of adverse summer conditions. The selection method also incorporated temperature tolerance in an indirect manner in as far as that the one stage of growth evaluation coincided with the period of adverse summer conditions.

  Favourable and moderate to strong genetic correlations between growth rate (i.e. body weight and length) and feed conversion efficiency are found in farm animals, frequently ranging from -0.80 to -0.95, as reported by Anderson (1977), Vangen (1984) and Crawford (1990). Gjoen et al. (1993)

estimated a genetic correlation of -0.78 between growth rate and feed conversion in rainbow trout. These values indicate that significant correlated response could be expected in relation to these traits. Growth rate were chosen as the preferred trait as it is easier to measure and expresses considerably more genetic variation than feed conversion efficiency, an approach also recommended by Gjedrem (2000) and Henryon et al. (2002).

- **choice of selection and breeding method**

  A multi-stage selection method was implemented that incorporated a combination of selection within and between groups, in order to conduct the programme within the constraints of limited facilities and resources whilst achieving optimal intensities of selection. The facilities at the Jonkershoek Fisheries Research Station can accommodate a maximum of 30-32 families, well below the recommended testing capacity of at least 200 per year class (Gjedrem, 1992). The limitation on the number of families is mainly due to the unavailability of suitable family identification systems during the early growth stages which then require separate rearing facilities. It was then decided to introduce a multi-stage selection method that would allow for maximum selection intensity within families, in an effort to compensate for the limited number of families. The multi-stage selection method is discussed in detail in Chapters 1, 2 and 3. A range of breeding methods ranging from mass spawning to full-sib and half-sib mating systems were used as discussed throughout the thesis.

- **selection of broodstock**

  Broodstock was selected according to the multi-stage selection method on the basis of various growth criteria (i.e. body width, body length, average daily length gain) at different stages, as discussed throughout the thesis.

- **measuring of response**

  Response to selection was measured in terms of average daily length gain with the use of a control population, as discussed in Chapters 3 and 4.

- **commercialisation of the breeding programme**

  Commercialisation of the breeding programme is done in collaboration with local producer organisations, which is ensuring the efficient distribution and application of improved genotypes originating from the programme. The programme is ongoing, entering its 7th generation in 2004 and is supplying about 60-65% of commercial material through direct supplies of broodstock, ova and fingerlings and indirect supplying via multiplier stations (commercial hatcheries).

4. Thesis outline

The thesis reports mainly on the implementation and stepwise progress of the above mentioned breeding programme. The thesis is structured and presented in the form of four key chapters, with each chapter presented in scientific publication format that report accordingly on distinct phases (generations) of the breeding programme under the headings of:

Title: Genetic improvement of growth rate in rainbow trout (*Oncorhynchus mykiss*)

The scientific publication format adopted inevitably leads to some duplication of references in the chapter reference lists and to some repetition in chapter introductions and descriptions of materials and methods. However, the latter has been minimized as far as possible by cross-references to chapters and chapter sections in the thesis text.

Chapter 1: Comparison of genetic resources and the formation of base populations

Chapter 1 reports in detail on the formation of a synthetic base population with a broad genetic base for future genetic improvement through selection. The establishment of a base population with high levels of genetic variation is widely recommended (e.g. Gall, 1990; Refstie, 1990; Gjedrem, 1992 and Gjoen and Bentsen, 1997) as a first and important step in the implementation of genetic improvement programmes. All available genetic sources, both local and foreign, were sampled but access to foreign sources was limited due to the political climate which prevailed at the time. The formation of the base populations included the implementation of a multi-stage selection method that incorporated the comparative evaluation of growth rate between genetic groups and selection of the best individuals within genetic groups. The absence of a suitable family structure precluded the estimation of genetic parameters during this stage.

Chapter 2: Evaluation of first generation offspring from single crosses

Chapter 2 reports on the evaluation of growth rate of the first generation offspring from diallel crosses between genetic groups. The aim of the crossing of genetic groups was to generate the maximum possible genetic variation within the base populations in view of future selection. An analysis for possible effects of heterosis and specific and general combining ability among the crosses are included in this chapter. A multi-stage selection method was implemented that incorporate the comparative evaluation of growth rate between genetic groups and selection of the best individuals within genetic groups. A mass spawning technique was used as breeding method that prevented the formation of a suitable family structure required for the estimation of genetic parameters.

Chapter 3: Evaluation of second generation offspring from double crosses

Chapter 3 reports on the evaluation of the rate of growth of second generation offspring from the double crossing of genetic groups from each of two base populations. The objective of a second generation of outcrossing (double crosses) was to further increase the levels of variation within the base populations. A

similar multi-stage selection method was implemented that incorporate the comparative evaluation of growth rate between genetic groups and selection of the best individuals within genetic groups. A full-sib mating system was used during this generation that precluded its use for the estimation of genetic parameters due to non-additive genetic, maternal and or tank effects.

Chapter 4: Estimation of genetic and phenotypic parameters and comparative evaluation of fourth, fifth and sixth generation progeny groups

Chapter 4 reports on the estimation of genetic and phenotypic parameters and the evaluation of the rate of growth of rainbow trout over consecutive generations of multi-stage selection. A hierarchical mating scheme was used in generation four that presented a suitable half-sib family structure for the reliable estimation of genetic and phenotypic parameters. A similar multi-stage selection method was implemented throughout these generations that incorporate a combination of selection within and between families. A comparative evaluation of progeny groups were conducted against a control group/population that form the basis for the estimation of a realised selection response in each of the generations. In spite of the hierarchical mating scheme and subsequent family structure selection of individual fish could not be conducted on the basis of BLUP estimates of breeding values due to the lack of a suitable method of identification of individuals. The use of various physical tagging methods suitable for the identification of large numbers of individuals, such as Carlinge and spaghetti tags (McAllister et al., 1992) as well as Passive Integrated Transponder (PIT) tags (Moore, 1992), was precluded on the basis of costs or availability. A group/family identification systems based on an ink tattoo method described by Bridcut (1993) was used during the final stage of growth evaluation in each generation as well as for the pedigree identification of selected broodstock.

Chapter 5: Conclusions

The thesis is concluded with a final chapter (Chapter 5) on overall conclusions and recommendations.

5. References

Andersen BB. 1977. Genetic studies concerning growth rate in cattle, body development and feed efficiency. Report no 488. National Institute of Animal Science, Copenhagen.

Bentsen HB and Gjerde B, 1994. Design of fish breeding programmes. Proceedings of the 5th Congress on Genetics Applied to Livestock Production. Volume 19: 353-359.

Bridcut EE. 1993. A coded Alcian blue marking technique for the identification of individual brown trout, *S. trutta*: An evaluation of its use in fish biology. Biology and Environment: Proceedings of the Royal Irish Acadamy. Vol. 93B, 2, 107-110.

Crawford RD. 1990. Poultry breeding and genetics. Elsevier, Amsterdam

Gall GAE. 1990. Basis for evaluating breeding plans. Aquaculture, 85: 125-142.

Gall GAE and Gross SJ. 1978. Genetic studies of growth in domesticated rainbow trout. Aquaculture, 13: 225-234.

Gjedrem T. 1975. Possibilities for genetic gain in salmonids. Aquaculture, 6: 23-29.

Gjedrem T. 1985. Improvement of productivity through breeding schemes. Geo Journal, 10 (3): 233-241.

Gjedrem T. 1992. Breeding plans for rainbow trout. Aquaculture, 100: 73-83.

Gjedrem T. 2000. Genetic improvement of cold-water fish species. Aquaculture Research, 31 (1): 25-33.

Gjerde B. 1986. Growth and reproduction in fish and shellfish. Aquaculture, 57: 37-55.

Gjoen HM, Storebakken T, Autreng E and Refstie T. 1993. Genotypes and nutrient utilization. In: Fish nutrition practice, Biarritz, France 24-27 June 1993. Ed. INRA, Paris (Les Coloques no 61): 19-26

Gjoen HM and Bentsen HB. 1997. Past, present, and future of genetic improvement in salmon aquaculture. ICES Journal of Marine Science, 54 (6): 1009-1014.

FAO. 2002. The State of World Fisheries and Aquaculture (SOFIA): 2002. Biennial report on fisheries and aquaculture globally. Ed. Wijkström, U.; Gumy, R.; Grainger, R. Fisheries Department, FAO, 2002. http://www.fao.org/WAICENT/FAOINFO/FISHERY/FISHERY.HTM.

Henryon M, Jokumsen A, Berg P, Lund I, Pedersen PB, Olesen NJ and Slierendrecht WJ. 2002. Genetic variation for growth rate, feed conversion efficiency, and disease resistance exists within a farmed population of rainbow trout. Aquaculture, 209 (1-4): 59-76.

Kincaid HL, Bridges WR and von Limbach B. 1977. Three generations of selection for growth rate in fall-spawning rainbow trout. Transactions of the American Fisheries Society, 106: 621-628.

Kincaid HL. 1983. Results from six generations of selection for accelerated growth rate in a rainbow trout population. The future of aquaculture in North America, p. 26-27. Fish Culture Section, American Fisheries Society.

McAllister KW, McAllister PE, Simon RC and Werner JK. 1992. Performance of nine external tags on hatchery-reared rainbow trout. Transactions of the American Fisheries Society, 121: 317-340.

Moore A. 1992. Passive integrated transponder tagging of channel catfish. Progressive Fish Culture, 54:125-127.

Refstie T. 1990. Application of Breeding Schemes. Aquaculture, 85: 163-169.

Vangen O. 1984. Future breeding programme in pigs in a situation with artificial insemination. Aktelt Fra Statens Fagtjeneste for Landbruket, 1: 300-306.

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CHAPTER 1:   COMPARISONS OF GENETIC RESOURCES AND THE FORMATION OF BASE POPULATIONS

1. Abstract

This chapter reports comparable growth rates of 13 different genetic groups of rainbow trout (nine local and four overseas) over a period of 380 days. This was in preparation of the establishment of a base population with high genetic merit and variation to be followed by an ongoing breeding programme aimed at genetic improvement of growth rate. Significant genetic differences for growth rate, in terms of both weight and length gain, were found between hatchery groups. Significant group x stage interactions were found over consecutive stages of growth signifying the importance of the genetic development of strains under local conditions. Water temperature was the main factor of environmental variation over the three stages of evaluation. The extent of the interactions, however, would not seem to justify the development of temperature specific strains. Positive correlations between growth criteria over consecutive stages of evaluation indicate that multi-stage selection procedures can be implemented in order to obtain maximum intensities of selection within genetic groups. The results emphasize the importance of intensive evaluation of available genetic resources in order to establish base populations with high genetic merit and genetic variability at the onset of programmes aimed at genetic improvement through selective breeding.

Keywords: *Oncorhynchus mykiss*; Selection; Heterogeneity, Growth rate; Correlations, GroupxStage interactions

2. Introduction

The industry of aquaculture in South Africa has relied mainly on species and technology of countries of the Northern Hemisphere. Although local environmental conditions differ vastly from those of the countries of origin, little has been done to improve the adaptation of foreign species to South African conditions. Rainbow trout was first introduced into South Africa from Britain during the 1890s for the purpose of sport fishing. Commercial production of portion sized trout (280–350g) started in the 1960s, with a further focus on the production of a larger trout (>1200g) early in 1980. At that time the local industry was experiencing problems with the rearing of the larger sized trout in the warm summer months, when water temperatures often exceed 26°C, and the need for strains with a higher level of temperature tolerance was identified as a priority by the industry.

Conventional farm animal production and breeding research has long relied on modern breeding theory based on Mendelian and quantitative genetic principles. However, no application has been attempted in South Africa with regard to the genetic improvement of introduced aquaculture species, in spite of progress in defining genetic parameters in several publications. Even in global terms it is estimated that only about 1% of aquaculture production is based on genetically improved fish and shellfish (Gjedrem, 2000). The response to selection in aquatic species seems to be very good compared to that with terrestrial farm animals. This is mainly attributed to higher genetic variance in fish (CV = 20-35%) compared to farm animals (CV =

7-10%) (Gjedrem, 1998), the high fecundity of aquatic organisms that allows for higher selection intensity than in farm animals and the domestication and selection of fish is still at an early stage.

For salmonids, a high degree of phenotypic variation has been reported for most traits of economic importance (Gjedrem, 1983; Gjerde, 1986). Heritability estimates range from low values for traits such as disease resistance (Gjedrem and Aulstad, 1974; McIntyre and Amend, 1978; Withler and Evelyn, 1990; Fjalestad et al., 1993; Eide et al., 1994; Stromsheim et al., 1994; Fevolden et al., 2002) and feed conversion (Kinghorn, 1981, 1983b), to medium to high values for developmental traits such as rate of growth, age of sexual maturity, condition factor and fecundity (Gall, 1975; Gall and Gross, 1978b; Gall and Huang, 1988b; Gall et al., 1988; Gjerde, 1986; Gjerde and Gjedrem, 1984; Nilsson, 1994; Henryon et al., 2002), as well as for various other body and quality traits (Aulstad, et al., 1972; Gall and Gross, 1978a,b; Gunnes and Gjedrem, 1978, 1981; Gall and Huang, 1988a; Gjerde and Gjedrem, 1984; Iwamoto et al., 1986; Elvingson and Johansson, 1993; Choe and Yamazaki, 1998; Myers, et al., 2001; Vandeputte, et al., 2002a; Kause, et al., 2003). The high reproductive capability of trout provides opportunities for relatively much higher selection intensities in comparison with many other farm animals and the short generation interval of two years, as experienced for rainbow trout in South Africa, is also a positive factor with regard to response to selection. .

The introduction of modern breeding procedures into aquaculture in order to improve productivity has been recommended by Gjedrem (1975, 1983, 1985, 1992, 1998, 2000), Kinghorn (1983a), Refstie (1990), Gall (1990), Gjoen and Bentsen (1997), Knibb (2000), Hulata (2001) and Fjalestad et al. (2003). Initial reports on genetic response to selection in salmonids have been largely positive, although still limited in duration with regard to the number of generations, and often also deficient in experimental design such as the maintenance of reliable genetically stable control populations (Kincaid et al., 1977; Hersberger and Iwamoto, 1984; Gjerde, 1986; Siitonen and Gall, 1989; Hersberger et al., 1990; Gjoen and Bentsen, 1997; Gjedrem, 2002). Improvements in body weight through selection in salmonids have been reported by Gjedrem (1979, 200), Kincaid (1983) and Hershberger et al. (1990), Ehlinger (1977) and Fjalestad et al. (1993). Fevolden et al. (2002) reported increased disease resistance with selective breeding programmes.

After consultations with industry, the Division of Aquaculture at the University of Stellenbosch introduced a breeding programme for rainbow trout during 1988. The aim of the programme was to attempt to improve growth rate under local conditions in order to overcome the limitations associated with high summer temperatures (above 24°C). Improvement in growth rate would enable producers to complete the production cycle for salmon trout before the onset of the second summer, thereby avoiding the high risk and low productivity associated with production of large trout under these conditions. This implies the completion of a production cycle up to a body weight of at least 1.2 kg within a period of 16 months after fertilization, which reduces the need for selection for high temperature tolerance previously identified as a priority.

This report presents the results of the first phase of the breeding programme which included the collection, evaluation and selection of the best available genetic material to make up two separate base populations in consecutive years. The use of superior strains is a first step in applying genetic principles to improved aquaculture performance. Identification of strain variation is also important in terms of other

genetic enhancement approaches, such as intraspecific crossbreeding, interspecific hybridization, sex control and genetic engineering. Differences in performance between populations, strains and genetic groups of salmonids in traits ranging from growth, body traits to disease resistance have been well documented by Refstie et al. (1978), Kinghorn (1983b), Morkramer et al. (1985), Iwamoto et al. (1986), Smith et al. (1988), Refstie (1990), Hershberger (1992), Elvingson and Johansson (1993), Jonasson (1993), Okamoto et al. (1993), Erythorsdottir et al. (1994), Withler and Beacham (1994), Hedrick et al. (2003) and Overturf et al. (2003). The term genetic group refers here to fish collected from different hatcheries, often with no reliable information on previous genetic history. Differences in performance between strains has also been reported in a variety of other species such as gilthead seabream (Knibb et al., 1997), tilapia (Basiao et al., 1996; Bentsen et al., 1998), carp (Gjerde et al., 2002; Vandeputte et al., 2002b), stripped bass (Jacobs et al., 1999) and channel catfish (Wolters and Johnson, 1995) for traits such as growth, survival and disease resistance. Results from these investigations have emphasized the importance of the evaluation of available genetic resources to establish base populations of high genetic merit and genetic variability.

3.   Materials and Methods

3.1. The 1988 Local collection

A sample of approximately 10 000 eyed ova was collected from each of eight commercial hatcheries in South Africa during June, 1988. Each of the participating hatcheries was requested to ensure that at least 16 randomly selected females and eight males contributed to the sample, in order to obtain as wide a representation of the genetic variation within the hatchery as possible. All of the participating hatcheries had used a mating design whereby eggs and milt of up to four females and two males are pooled prior to fertilization, but exact records of the numbers and ratios of males and females were not available. Samples were made up of different batches of pooled egg and milt in order to meet the minimum requirement of at least 16 females and eight males.

Source hatcheries were representative of the industry in S.A., consisting of four hatcheries from the Northern Highlands region, two from the Eastern Highlands region and two from the Western Coastal region. Source hatcheries were to remain anonymous by prior agreement, except for their regional identification, viz. Northern (N1, N2, N3, and N4); Eastern (E1, E2) and Western (W1, W2). A sample from a "wild" strain from the river on which the hatchery W1 is situated was also included (WW). These nine hatchery-groups established in 1988 are hereafter referred to collectively as the year group EVEN 88.

The periods of operation of the source hatcheries varied from five to over one hundred years. Records of introductions, breeding systems and population sizes were generally not available, but foreign introductions are known to have taken place on a regular basis and included importations from Denmark, Scotland, Ireland, Italy and North America. Since 1973 all foreign imports were from certified disease free sources. A free exchange of material also took place between these hatcheries, particularly within regions. None of the source hatcheries had conducted any prior artificial selection programmes nor managed breeding systems to

prevent inbreeding. At the stage of sampling, two hatcheries had obtained disease free certification, implying effective closures of these populations for a period of three years prior to the sampling.

The procedure of sampling aimed to achieve the widest possible range of genetic variability between and within the genetic groups used in the study. Electrophoretic examination of polymorphic enzymes obtained from the base population, by Van der Bank et al., (1992), indicates some success in achieving this aim. Twenty polymorphic loci were examined and heterozygosity values ranged from 0.0922 to 0.1208, indicating the presence of moderate to high genetic variability in the base population. These values are substantially higher than the average heterozigosity of 0.059 reported by Hersberger (1992) on a study of 38 populations of rainbow trout from North America and well above the average value of 0.051 for 51 fish species reported by Nevo (1978).

3.2. The 1988 Overseas collection

Trout ova are imported annually during December and January from Northern Hemisphere countries in order to ensure a year round supply of market size fish. Availability and price have been the criteria for importation rather than the respective production performance of importations under local conditions. Samples of approximately 10 000 eyed ova were obtained for this study from batches of commercial imports during December 1988. Suppliers in 1988 were limited to four hatcheries, one each from North America (NA), Denmark (D), Scotland (S) and the Isle of Man (I-M). Hatcheries were again anonymous by agreement, excepting the country of origin and, again, no quantitative evaluations of production performance under local conditions had been undertaken. These four hatchery-groups are referred to as the year group OVERSEAS 88.

3.3. The 1989 Local collection

The collection of local genetic material was repeated in June 1989 following the same procedures as in the previous year. The same nine hatchery-groups were included and the same nomenclature was used, except that variation in the age of entry of participating genetic groups was reduced to be within 7 days of each other, as opposed to 14 days in EVEN 88. These nine hatchery-groups established in 1989 are referred to as the year group ODD 89. Participating hatcheries were again requested to ensure that at least 16 females and eight males contribute to the sample through the use of a mating design whereby eggs and milt were pooled prior to fertilization.

3.4. Growth rate evaluation

Spawning and incubation up to the eyed-egg stage were carried out at the hatchery of origin from which random samples of eyed ova were obtained as described in sections 3.1, 3.2 and 3.3. All samples of eyed ova as received were then hatched and reared in separate containers under standard conditions at the Jonkershoek Fisheries Research Station near Stellenbosch in the Western Cape Province for an initial period of three months during which no evaluation was conducted. The comparative evaluation of genetic groups was also conducted at this station. The evaluation period was divided into three distinct stages with duration as summarized in Table 1.1. These stages were largely determined by practical considerations with regard to the capacity of facilities available and to local seasonal conditions.

The age of fish and the duration of the growth stages were standardized for local and overseas groups (Table 1.1), though not according to the same time of year due to the out of season nature of ova from the Northern Hemisphere. For local groups, Stage I coincided with a period in spring (October-November) with an average water temperature of 16.3°C, Stage II coincided with a period in summer (December–March) with an average water temperature of 22.4°C and Stage III with a period in autumn continuing through to winter (April–June) with an average water temperature of 13.7°C. For overseas groups, Stage I coincided with a period in autumn (April-May) with an average water temperature of 16.5°C, Stage II coincided with a period in winter (June–September) with an average water temperature of 12.3°C,,and Stage III with a period in spring through to summer (October-December) with an average water temperature of 19.7°C.

Throughout all stages of evaluation, rearing conditions such as numbers per pond, densities, flow rates, type of feed, feeding levels and methods were standardized over all groups and replications, according to commercial standards. A standard range of commercial trout feed, supplied by WPK Aquafeeds Pty Ltd, with an approximate composition of 40% crude protein, 16% crude fat, 6% crude fibre, 3% calcium and 0.7% phosphorous, was used during all stages of evaluation.

A hand feeding method was used with feeding levels being standardized according to a feed table on the basis of water temperature and size of fish. The standardized application of commercial production conditions is regarded as very important during evaluation and selection in order to minimize environmental variation and possible genotype-environment interactions, i.e., selection should be practiced under conditions as similar as possible to those under which the progeny are expected to be produced (Gjedrem, 1992). Groups were kept in separate tanks through all stages of evaluation and there were three randomized replicate tanks for each hatchery-group. Tanks were sampled every 14-21 days in order to record average weight and fork length, and this information formed the basis for the adjustment and standardization of rearing conditions. Tanks were circular PVC of 10 cubic meters in volume and one meter in depth. Water temperature, oxygen levels, daily mortality and feed consumption were monitored at each stage.

Of the original 10 000 ova, a total of between 6 000 to 8 000 fingerlings per group, was available at the beginning of Stage I. From the available fingerlings a total of approximately 6 000 per group, 2000 per replicate (see Table 1.1), was randomly selected for inclusion in the growth trial. Reduction in the number of fish per tank from one stage to the next was done through selection of equal proportions between replicates and groups, rather than through random sampling in an attempt to secure the best available genetic material from the hatchery groups for the establishment of the base population groups.

Reduction in the number of fish from Stage I to II was by means of a box grader for body width in order to handle the high initial numbers. Reduction from stage II to III was by means of selection based on individual body weight. Final selection at the end of Stage III was also based on the basis of individual body weight. Numbers and proportions selected at each stage and for each hatchery group were standardized as far as practically possible (Table 1.1). Justification for this procedure of multi-stage selection (Allan et al., 1993) was based on the high genetic correlation between body traits of rainbow trout at different ages, such as weight, width and length as reported by Gall and Huang (1988a, 1988b), Crandell and Gall (1993a, b), Elvingson and Johansson (1993), Winkelman and Peterson (1994b), Myers et al. (2001) and Su et al. (2002).

3.5. Measurements and experimental design

Rate of growth was measured as Average Daily Weight Gain in grams per day (ADWG) and Average Daily Length Gain in mm per day (ADLG) per replicate. This was done to account for variation in the initial age, weight and length between hatchery groups as reported in Table 1.1, which would have caused a biased comparison on the basis of average body weight in g at the end of a stage or body weight gain in g during a particular stage. ADWG and ADLG are defined as:

ADWG = (Final – Initial average body weight of replicate) / Duration of stage in days
where
Average body weight of replicate = Total body weight of replicate / Total number of fish of replicate

ADLG = (Final– Initial average body length of replicate) / Duration of stage in days
where
Average body length of replicate = Total body length of replicate / Total number of fish of replicate

For Stage I the total body weight for each replication was determined by means of a dry weighing method that involves the weighing of all fish in mass. No individual weights were recorded. For Stage I the total number of fish per replicate was determined by means of random sampling of 300 fish ($\approx$15%) of which the average weight was calculated. The total number of fish per replicate was then estimated by dividing the total body weight of the replicate by the average body weight of the sample. The average body weight per replicate was then determined by dividing the total body weight by the total number of fish. During stages II and III a random sample of 60 ($\approx$15%) and 25 fish ($\approx$15%) per replicate were taken respectively to determine the average weight.

For Stage I the average body length for each replication was calculated by means of the fork lengths of a random sample of 300 fish ($\approx$15%). During stages II and III the total body length (fork length) per replicate was determined through the summation of the fork lengths of all the fish per replicate. The total number of fish per replicate used was the same as in the case of body weight, i.e., an estimated total number for Stage I. Random samples of 60 ($\approx$15%) and 25 fish ($\approx$15%) were taken during stages II and III to determine the average length for each replicate. Measurements of weight and length were made to the nearest whole g and mm and were conducted by the same person at all stages. Typing of sex was not possible during the evaluation trial since signs of sexual maturation up until the age of 12 months were not identifiable.

The experiments were laid out as Complete Randomized Block Designs with 9 treatments as fixed effects and three randomized replications for each of EVEN 88 and ODD 89, and with four treatments as fixed effects and three randomized replications in the case of OVERSEAS 88. The treatment design for each of EVEN 88 and ODD 89 was a 9x3 Factorial with factors nine families (E1, E2, N1, N2, N3, N4, W1, W2, WW) and three growth stages (I, II and III). The treatment design for OVERSEAS 88 was a 4x3 Factorial with factors four families (NA, D, S and IM) and three growth stages (I = 64 days, II = 89 days and III = 81 days). Data were analyzed by the SAS General Linear Model Procedure (SAS Institute Inc., 1996) and

included an analysis of correlations (phenotypic) between traits, both within and between growth stages in order to quantify further the effects of the multi-stage selection practiced. Student's t-LSD (Least Significant Difference) was calculated at the 5% level to compare treatment means.

Correlations between the means for ADWG and ADLG of the founder populations, over the three stages of evaluation, and two consecutive generations (EVEN 88 and ODD 89) for 9 local hatchery-groups were calculated by means of multivariate analyses of variance (MANOVA), using the model:

Trait 1, 2, 3, 4, 5, 6 = ADWGI, ADLGI, ADWGII, ADLGII, ADWGIII, ADLGIII
= Group + Year + Group x Year + Error

The correlation between traits is taken as the correlation among error terms (Residual Sum Squares and Products) averaged over the two years.

Some fish from the Isle of Man group (approx. 4%), expressed symptoms related to a genetic disorder of dwarfism that manifested during the evaluation period. The affected fish appeared completely stunted in length, and were therefore easily identifiable. Data of fish from the I-M group with symptoms of the disorder were not included in their analysis.

3.6. Composition of EVEN 88, ODD 89 and OVERSEAS 88 base populations

At the conclusion of Stage III of the evaluation, the two highest ranking males and females in each replicate tank of each hatchery-group were selected as founder fish to form the EVEN 88, ODD 89 and OVERSEAS 88 base populations. Selection at this stage was based on individual body weight. The Isle of Man group was excluded from OVERSEAS 88 base populations because of the dwarfism mentioned earlier. Each of the selected individuals received an identification mark according to its hatchery-group by the tattooing method. A Panjet apparatus with Alcian Blue ink was used to apply the marking on the ventral area according to a method described by Bridcut (1993).

4. Results

The test for non-normality of the data was done on the basis of error terms for each of the traits by means of the Shapiro-Wilk test (Shapiro and Wilk, 1965). There was significant evidence for non-normality of error terms in the case of ADWG in all year-groups as presented in Table 1.2. Non-normality was due to kurtosis rather than skewness, for example in the case of ADWG in EVEN 88 the value for kurtosis was 10.1282 and that for skewness was 0.6939. It was therefore decided to continue with the interpretation of the analysis (Glass, 1972). The more significant evidence for non-normality observed for ADWG in year-group EVEN 88 (P<0.01) is possibly ascribable to the larger variation in initial age of the hatchery-groups within this year-group (Table 1.1). The variation in age was reduced by more stringent standardization of initial age in ODD 89 and OVERSEAS 88. Skewness of the distribution for both ADWG and ADLG increased

progressively from stage to stage over all year-groups, as might be expected due to the selection applied at the end of each stage.

The ANOVA of ADWG and ADLG over three consecutive growth stages (Table 1.2) indicates significant differences (P<0.01) between hatchery groups for both these traits in all year groups. The results of an ANOVA of ADWG and ADLG per stage (Table 1.3) has confirmed these findings and showed highly significant differences (P<0.01) among hatchery groups for all year-groups and stages, with the exception of ADWG in Stage I of EVEN 88 (P=0.397) and ODD 89 (P=0.078). The observed differences in ADWG and ADLG present convincing evidence of significant genetic differences between the hatchery-groups. This was a most promising result in view of the aim of establishing genetically heterogeneous base populations upon which the programme of recurrent selection for growth rate was to be implemented. The ANOVA of ADWG and ADLG over three consecutive growth stages (Table 1.2) also indicates significant hatchery group x stage interactions (P<0.01) in all year groups. The extent of the hatchery group x stage interactions can also be observed in the Least Square Means for ADWG and ADLG and the associated changes in rank order, of all hatchery groups in consecutive stages of evaluation as presented in Table 1.4. The groups with the best ADWG and ADLG within a Stage (a-range, SAS), based on the t-LSD values (P=5%) is also indicated in Table 1.4.

Hatchery groups from the Western Coastal Region generally performed poorly with regard to ADWG and ADLG during all stages in both years in comparison with other regions. Later inquiries have pointed to small founder population numbers and occasional bottlenecks which may have led to inbreeding within this regional group. Van der Bank et al. (1992) reported lower average heterozygosity values for Western Cape populations in comparison with populations from the Northern and Eastern regions.

The replicate mean, standard error and coefficient of variation (CV) for weight and length at the end of each growth stage, for the three year groups are presented in Table 1.5. The data from Tables 1.1 and 1.4 indicate that the comparisons of the hatchery and year groups were conducted during similar stages of the life cycle with regard to age, duration of stages, average weight and length. The observed CV of ADWG (11-14%) and ADLG (6-7%) among groups for Stage I, prior to selection, was lower than the range of 23 to 28% for body weight and 7 to 10% for fork-length within populations, reported by Gall and Gross (1978a), Gall and Huang (1988b), Gjerde and Gjedrem (1984), Elvingson and Johansson (1993) and Henryon et al. (2002). This observation is supported by reports from Hershberger (1992) and from Gjoen and Bentsen (1997) which indicates that the majority of genetic variance in salmonids is often located within populations, rather than between populations. Gjoen and Bentsen (1997) reported that more than 90% of additive genetic variance in Norwegian populations of Atlantic salmon has been found within, as apposed to between populations. Hershberger (1992) reported findings of a survey of genetic variability in 38 natural populations of rainbow trout spread across the states of Idaho, Oregon and Washington in the USA, and concluded that only 8% of the total genetic variation was attributable to differences between populations and 92% to variation within populations. A report by Erythorsdottir et al. (1994) ascribed 27% of the total variation to variation between strains, during a comparison of body weight among 13 strains of arctic char. Overturf et al. (2003) reported a reduction in genetic variation between commercial populations of rainbow trout in comparison to non-

commercial populations. The lower observed CVs for ADWG and ADLG can therefore be ascribe to the fact that the values refer to variation among groups, rather than within populations, as well as the fact that most of the genetic groups included in this study can be considered as commercial populations.

The correlations between means for ADWG and ADLG of 9 local hatchery groups of rainbow trout over three stages of evaluation over two consecutive generations are presented in Table 1.6. All correlations between traits, within and over stages, were positive and significantly different from zero ($P<0.05$). The correlations declined progressively from one stage to another. The results indicate high values between traits within stages and medium high values between traits in consecutive and alternate stages (I-III). These values and tendencies correspond to those reported by Winkelman and Peterson (1994b), for body weight and length in Chinook salmon, body weight of masu salmon reported by Choe and Yamazaki (1998), and for body traits in rainbow trout (Elvingson and Johansson, 1993; Grandell and Gall, 1993a, 1993b; Su et al., 2002; Henryon et al., 2002). The influence of the selection procedure on the observed phenotypic correlations should also not be overlooked. Multi-stage selection for body weight as practiced in this investigation is expected to lead to an increase in the observed values, above the values expected with random sampling procedures.

Table 1.5 indicates a steady decline in the CV of replicate ADWG and ADLG from one stage to another in all year groups, and reflects the effect of selection based on body weight at the end of each stage. In view of the phenotypic correlations between body weight and length, a correlated reduction of the CV for ADLG was to be expected, as has also been reported in other studies on additive and phenotypic correlations between body traits of rainbow trout (Elvingson and Johansson, 1993; Gjerde, 1986; Gjerde and Schaeffer, 1989; Jonasson, 1993; Winkelman and Peterson, 1994b; Myers et al., 2001). The reduction of the CV of ADLG was, however, less profound than for ADWG. Correlations between these traits are of particular importance given the nature of the multi-stage selection procedure and the use of these traits as criteria for selection during future generations.

5. Discussion

A major objective of this study was the collection and evaluation of available genetic resources, and the establishment of base populations through a process of multi-stage selection, in order to establish the widest possible range of genetic variation with a view to future genetic improvement through selection. The establishment of a base population with high levels of genetic variation is widely recommended (Kinghorn, 1983a; Gall, 1990; Refstie, 1990; Gjedrem, 1992, 1998 and Gjoen and Bentsen, 1997) as a first and important step in the implementation of genetic improvement programmes. All available genetic sources, both local and foreign, were sampled but access to foreign sources was limited due to the political climate which prevailed at the time. However, in spite of these limitations and through the application of recommended procedures with regard to collection, evaluation and selection of genetic resources available (Refstie, 1990; Gjedrem, 1992), a unique set of base populations was established (EVEN 88, OVERSEAS 88 and ODD 89) with high levels of genetic variability as indicated by the electrophoretic analysis of polymorphic enzyme loci by Van der Bank, et al., (1992) referred to above. The heterozygosity values of the

base populations ranging from 9-12% are higher than others reported by Danzman et al. (1989) for six hatchery strains of rainbow trout ranging from 4-8%.

The differences in observed CVs for ADWG (7-12%) and ADLG (2-4%) within hatchery-groups (Table 1.5) provide further evidence of high levels of variation in the base populations. Although the observed CVs were lower than those reported for body weight (23 to 28%) and for body length (7 to 10%) by Gall and Gross (1978a), Gall and Huang (1988b), Gjerde and Gjedrem (1984), Elvingson and Johansson (1993) and Henryon et al. (2002), it should be noted that these reports refer to CVs within populations. Reports by Hershberger (1992), Erythorsdottir et al. (1994) and Gjoen and Bentsen (1997) provide evidence that most of the genetic variance in salmonids (some 90%) tends to be located within populations rather than between populations. The observed variation for ADWG and ADLG between hatchery-groups can therefore be interpreted as high in comparison to other reports.

This investigation has confirmed the presence of statistically significant and potentially commercially valuable genetic differences in the growth rate of different genetic groups from local and overseas hatcheries. Differences in growth rate among local groups amounts to a total of some 60 days in the time required to reach market size (>1200g), and to some 80 days between overseas groups. These results further stress the necessity for the quantification of genetic qualities of different commercial groups in order to facilitate informed decisions by producers regarding the purchase of stock and to determine the standards of competitors. A lack of awareness of such differences and absence of such information indicate that decisions relating to purchase of stock in the past were based on factors such as health status, unit costs, availability, historical links and logistic convenience rather than genetic potential and economic productivity.

The detection of significant group x stage interactions for ADWG and ADLG was not unexpected given the diverse genetic background of participating hatchery groups and the duration of the evaluation process which covered a wide spectrum of the normal growth process as well as diverse temperature conditions between stages. This has important practical implications for future selection procedures in that selection between groups or strains should be delayed until the final stage of selection in order to identify temperature tolerant strains, i.e., strains that grow well through all stages of evaluation.

The detection of group x stage interactions may also be seen as indicative of the presence of genotype-environment interactions. Water temperature was the main factor of environmental variation over the three stages of evaluation during this study. Significant genotype-temperature interactions associated with temperature and expressed as a change in the rank order of families of rainbow trout for growth rate has been reported by McKay et al. (1984) who suggested selection for temperature specific strains, or the use of an index of performance for selection under varying conditions in such cases. On the level of strains, Wangila and Dick (1988) reported significant genotype-environment interaction between two strains of rainbow trout for growth at different temperature regimes. Okamoto et al. (1993) further reported a small magnitude of strain x environment interaction during the comparison of 13 strains of Arctic Charr in terms of growth in two environments with a distinctive temperature range. However, interactions often seems to be proportional of nature, not resulting in extensive re-ranking of breeding candidates from one test environment to another as reported by Bentsen and Gjerde (1994). Gunnes and Gjedrem (1978, 1981) reports on the results of

extensive experiments on genotype-environment interaction in Atlantic salmon and rainbow trout that estimates of interaction variance accounts for only 1.2-3.7% of the total phenotypic variance. Winkelman and Peterson (1994a) detected no genotype-environment interaction on the strain level when comparing three strains of chinook salmon for weight and length in a salt and seawater environment. Reports by Beacham (1990), Heath et al. (1993), Bagley et al. (1994) and Wild et al. (1994) confirm the presence of significant genotype-environment interaction in salmonid species between families within populations for traits such as growth, weight, length and sexual maturation under a variety of environmental conditions such as fresh and seawater, stocking densities, temperatures and photoperiods. Hanke et al. (1989) on the other hand found no indication of genotype-environment interaction between families of Atlantic salmon for growth under different photoperiod conditions, whilst Glover et al. (2001) report no genotype-environment interaction between families of sea trout at high and low feeding levels.

In general, genotype-environment interactions tend to obscure genetic differences between hatchery groups, and have important implications in the design of selection programmes. This is of particular importance with regard to southern African conditions which are characterized by warm summer and cold winter temperatures, associated with all of the major production areas. In this instance, the observed changes in rank order were of a minor nature and would not seem to justify selection for temperature specific strains nor the use of an index of performance under varying conditions as recommended by McKay et al. (1984). The findings of this study as well as the other reports referred to indicate that genotype-environment interaction should be assessed on the basis of the specific population, traits and environments under consideration, when decisions are made in terms of the design of selection programmes.

Persistence of positive correlations for ADWG and ADLG over the various stages of evaluation (Table 1.6) is of particular importance given the nature of the multi-stage selection procedure which implies selection at the end of each stage. High overall selection intensity as in this study (i $\approx$ 3σ_P), can only be achieved if high initial numbers per group can be introduced. This in turn requires the implementation of multi-stage selection in order to meet the constraints of facilities by means of a consecutive reduction in numbers during the growth cycle. The presence of high positive correlations between the selection criteria over consecutive stages is required to ensure a good cumulative response during multi-stage selection (Allan et al., 1993). The results indicate high values for correlations between traits within stages and medium to high values between traits in consecutive (I-II, II-III) and alternate stages (I-III). The correlations declined progressively between stages. The possible influence of the selection procedure on observed phenotypic correlations should not be overlooked. Multi-stage selection for body weight as practiced in this investigation is expected to lead to an increase in the observed values, above the values expected with random sampling procedures.

Values and tendencies as reported for this study are in line with those reported by Pohar (1992), Elvingson and Johansson (1993), Grandell and Gall (1993a, b), Winkelman and Peterson (1994b), Gjerde at al. (1994), Choe and Yamazaki (1998), Henryon et al. (2002) and Su et al. (2002). Winkelman and Peterson (1994b) reported genetic correlations between body weight and length of first and second year traits in the range of 0.74 to 0.80. Phenotypic correlations between body weight of masu salmon at the ages of four and

eight months have been reported by Choe et al. (1998) to be in a similar range of 0.45 to 0.63 as recorded during the present study. Grandell and Gall (1993a,b) reported higher levels of genetic and phenotypic correlations between body weights of rainbow trout over seven ages, ranging respectively from 0.19 to 0.95 and 0.29 to 0.94. Genetic and phenotypic correlations were consistently high (0.74 to 0.95) between contiguous ages and decreased as the difference in ages increased. Su et al. (2002) reported similarly high values for genetic correlations between body weight at contiguous ages, ranging from 0.57 to 0.93, again decreasing with increasing intervals between ages. Henryon et al. (2002) reported genetic correlations ranging from 0.55 to 0.99 between body weight and length at contiguous ages. These results indicate that selection for growth rate at any stage would result in a correlated response of up to 90% for growth rate at other stages (e.g. Winkelman and Peterson, 1994a,b), similarly that a correlated response can be expected through indirect selection based on different growth traits at earlier ages. Gjerde et al. (1994) also conclude that selection on the basis of growth traits in a breeding programme may take place within a period of several months around marketing size.

The reduction in the coefficient of variation for ADWG from stage I to III as presented in Table 1.5 is evidence of effective application of the multi-stage selection for improved rate of growth on the basis of body width during stage I, and on individual body weight during stages II and III. The correlated reduction in the CV of ADLG is expected in view of the correlations between these traits (Table 1.6) and between body weight and length as reported by Elvingson and Johansson (1993), Winkelman and Peterson (1994b) and Grandell and Gall (1993a, 1993b).

The aim of the breeding programme as stated was to improve the rate of growth of rainbow trout in order to shorten the production cycle for large size trout (>1200g), ideally to a period of less than sixteen months from fertilization and should also include the maintenance of a salmon like body conformation, which is desirable for further processing of the product. The current selection procedure was effective in the identification of superior individuals on the basis of body width and body weight. The procedure was based on multi-stage selection that included selection on the basis of individual body width at the end of Stage I followed by selection on the basis of individual body weight at the end of stages II and III. Observations on the body conformation of the 12 best individuals from each group, selected on the basis of body weight at the end of Stage III, raised some concerns. The average condition factor (CF = [(ungutted weight, g) / (fork-length, cm)^3]x100) gave a value of 2.1±0.17 for the selected individuals compared to the required market standard of 1.6-1.8 for salmon trout. McKay et al. (1986), Gjerde and Schaeffer (1989), Nilson (1992), Sylven and Elvingson (1992), Elvingson and Johansson (1993), Gjedrem (1997) and Kause et al. (2002, 2003) have all reported positive genetic correlations (0.36-0.56) between body weight and CF for rainbow trout at various stages of production. Kause et al. (2003) refers specifically to the tendency for fast growing fish to become rotund. Morkhamer et al. (1985) refer to a negative relationship between growth rate and carcass traits in rainbow trout. Gjedrem (1997, 2000) also refers to the positive genetic correlation (r_G=0.30) between body weight and fat content which suggest that fat content is expected to increase with selection for body weight. Selection on the basis of body weight could therefore lead to an unfavourable indirect increase in condition factor while a more salmon- like body conformation is preferred by the market. The

combination of body weight and length observations into a condition factor would seem to offer no benefit for use as a criterium for selection to improve the rate of growth. Heritability estimates for CF in salmonids appear to be low (Gunnes et al., 1978; Refstie, 1978) mainly because of the reduction of variance for CF when compared to the variation estimates based on separate measurements of weight and length. The criteria of selection to be used will therefore have to be reconsidered during future generations of selection for improved rate of growth.

The inclusion of the large number of 6 000 individuals per genetic group at the first stage of evaluation facilitated the application of high selection intensities during each stage of evaluation and formed part of a cumulative multi-stage selection procedure over the period of 12 months. This ensured that individuals of high genetic quality from each of the hatchery groups could be included in the respective base populations. Results of further crossbreeding of genetic groups followed by the high intensity multi-stage selection procedure will be reported in what follows.
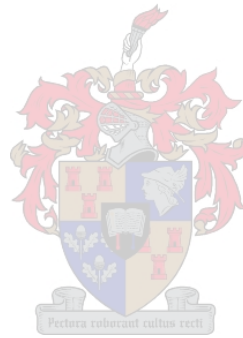
6. References

Allan JS, Randall JH and Brink D. 1993. Predicting the efficiency of multi-stage selection in the breeding of rainbow trout. South African Journal of Animal Science: 23: 1-3.

Aulstad D, Gjedrem T and Skjervold H. 1972. Genetic and environmental sources of variation in length and weight of rainbow trout. Journal of Fisheries Research Board of Canada, 29: 237-241.

Bagley MJ, Bentley B and Gall GAE. 1994. A genetic evaluation of the influence of stocking density on the early growth of rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 121 (4): 313-326.

Basiao ZU, Doyle RW and Arago AL. 1996. A statistical power analysis of the 'internal reference' technique for comparing growth and growth depensation of tilapia strains. Journal of Fish Biology, 49 (2): 277-286.

Beacham TD. 1990. A genetic analysis of meristic and morphometric variation in chum salmon (*Oncorhynchus keta*) at three different temperatures. Canadian Journal of Zoology, 68 (2): 225-229

Beacham TD and Evelyn TPT. 1992. Genetic-variation in disease resistance and growth of Chinook, Coho, and Chum Salmon with respect to Vibriosis, Furunculosis, and Bacterial Kidney-Disease. Transaction of the American Fisheries Society, 121 (4): 456-485.

Bentsen HB and Gjerde B, 1994. Design of fish breeding programmes. Proceedings of the 5th Congress on Genetics Applied to Livestock Production. Volume 19: 353-359.

Bentsen HB, Eknath AE, Palada-de Vera MS, Danting JC, Bolivar HL, Reyes RA, Dionisio EE, Longalong FM, Circa AV, Tayamen MM and Gjerde B. 1998. Genetic improvement of farmed tilapias: growth performance in a complete diallel cross experiment with eight strains of *Oreochromis niloticus*. Aquaculture, 160 (1-2): 145-173.

Bridcut EE, 1993. A coded aclcian blue marking technique for the identification of individual brown trout, *S. trutta*.: An evaluation of its use in fish biology. Biology and Environment: Proceedings of the Royal Irish Academy. Vol. 93B, 2: 107-110.

Choe MK and Yamazaki F. 1998. Estimation of heritability of growth traits, and phenotypic and genetic correlations in juvenile masu salmon *Oncorhynchus masou*. Japanese Society of Fisheries Science, 64:, 6, 903-908.

Crandell PA and Gall GAE. 1993a. The genetics of age and weight at sexual maturity based on individually tagged rainbow-trout (*Oncorhynchus mykiss*). Aquaculture, 117 (1-2): 77-93.

Crandell PA and Gall GAE. 1993b. The genetics of body-weight and its effect on early maturity based on individually tagged rainbow-trout (*Oncorhynchus mykiss*). Aquaculture, 206 (1-2): 1-17.

Danzman RG, Ferguson MM and Allendorf FW. 1989. Genetic variability and components of fitness in hatchery strains of rainbow trout. Journal of Fisheries Biology, 35: 313-319.

Eide DM, Linder RD, Stromsheim A, Fjalestad K, Larsen HJS and Roed KH. 1994. Genetic variation in antibody-response to Diphtheria Toxoid in Atlantic Salmon and Rainbow-Trout. Aquaculture, 127 (2-3): 103-113.

Elvingson P and Johansson K. 1993. Genetic and environmental components of variation in body traits of rainbow trout in relation to age. Aquaculture, 118: 191-204.

Ehlinger NF.   1977. Selective breeding of trout for resistance to furunculosis. New York Fish Game Journal. 24: 25-36.

Erythorsdottir E, Petursdottir T and Svavarsson E.   1994. Comparison of strains of Arctic charr (*Salvelinus alpinus*) in Iceland for body weight and age at sexual maturity. Proceedings, 5th World Congress on Genetics Applied to Livestock Production, University of Guelph, Guelph, Ontario, Canada, 7-12 August 1994. Volume 19. Selection and quantitative genetics; growth; reproduction; lactation; fish; fiber; meat; p. 387-390.

Falconer DS and Mackay TFC. 1996. Introduction to Quantitative Genetics. Fourth Edition. Longman Scientific & Technical, England, 464 pp.

Fevolden SE, Roed KH and Fjalestad K.   2002. A combined salt and confinement stress enhances mortality in rainbow trout (*Oncorhynchus mykiss*) selected for high stress responsiveness. Aquaculture, 205 (1-2): 61-75.

Fjalestad KT, Gjedrem T and Gjerde B. 1993. Genetic-improvement of disease resistance in fish - an overview. Aquaculture, 111 (1-4): 65-68.

Fjalestad KT, Moen T and Gomez-Raya L. 2003. Prospects for genetic technology in salmon breeding programmes. Aquaculture Research, 34 (5): 397-406.

Gall GAE. 1975. Genetics of reproduction in domesticated rainbow trout. Journal of Animal. Science., 40: 19-28

Gall GAE and Gross SJ. 1978a. Genetic studies of growth in domesticated rainbow trout. Aquaculture, 13: 225-234.

Gall GAE and Gross SJ. 1978b. A genetic analysis of the performance of three rainbow trout broodstocks. Aquaculture, 15 (2): 113-127.

Gall GAE, Baltodano J and Huang N.   1988. Heritability of age at spawning for rainbow trout. Aquaculture, 68: 93-102.

Gall GAE and Huang N. 1988a. Heritability and selection schemes for rainbow trout: body weight. Aquaculture, 73: 43-56.

Gall GA. and Huang N. 1988b. Heritability and selection schemes for rainbow trout: Female reproductive performance. Aquaculture, 73: 57-66.

Gall GAE. 1990. Basis for evaluating breeding plans. Aquaculture, 85: 125-142.

Gjedrem T and Aulstad D. 1974. Selection experiments with salmon: I. Differences in resistance to vibrio disease of salmon parr (*Salmo salar*). Aquaculture, 3: 51-59.

Gjedrem T. 1975. Possibilities for genetic gain in salmonids. Aquaculture, 6: 23-29.

Gjedrem T.   1979. Selection for growth rate and domestication in Atlantic salmon. Z. Tierz. Zuechtungsbiology, 96: 56-59.

Gjedrem T. 1983. Genetic variation in quantitative traits and selective breeding in fish and shellfish. Aquaculture, 33: 51-72.

Gjedrem T. 1985. Improvement of productivity through breeding schemes. Geo Journal, 10 (3): 233-241.

Gjedrem T. 1992. Breeding plans for rainbow trout. Aquaculture, 100: 73-83.

Gjedrem T. 1997. Flesh quality improvement in fish through breeding. Aquaculture International, 5: 197-206.

Gjedrem T. 1998. Developments in fish breeding and genetics. Acta Agriculturae Scandinavica, Section A-Animal Science: 19-26.

Gjedrem T. 2000. Genetic improvement of cold-water fish species. Aquaculture Research, 31 (1): 25-33.

Gjedrem T. 1992. Breeding plans for rainbow trout. Aquaculture, 100: 73-83.

Gjerde B and Gjedrem T. 1984. Estimates of phenotypic and genetic parameters for carcass traits in Atlantic salmon and rainbow trout. Aquaculture, 36: 97-110.

Gjerde B. 1986. Growth and reproduction in fish and shellfish. Aquaculture, 57: 37-55.

Gjerde B and Schaeffer LR. 1989. Body traits in rainbow trout. II. Estimates of heritabilities and of phenotypic and genetic correlations. Aquaculture, 80: 25-44.

Gjerde B, Simainer H and Refstie T. 1994. Estimates of genetic and phenotypic parameters for body weight, growth rate and sexual maturity in Atlantic salmon. Livestock Production Science, 38: 133-143.

Gjerde B, Reddy PVGK, Mahapatra KD, Saha JN, Jana RK, Meher PK, Sahoo M, Lenka S, Govindassamy P and Rye M. 2002. Growth and survival in two complete diallele crosses with five stocks of Rohu carp (*Labeo rohita*). Aquaculture, 209 (1-4): 103-115.

Gjoen HM and Bentsen HB. 1997. Past, present, and future of genetic improvement in salmon aquaculture. ICES Journal of Marine Science, 54 (6): 1009-1014.

Glover KA, Taggart JB, Skaala O and Teale AJ. 2001. Comparative performance of juvenile sea trout families in high and low feeding environments. Journal of Fish Biology, 59 (1); 105-115.

Grandell PA and Gall GAE. 1993a. The genetics of body weight and its effect on early maturity based on individually tagged rainbow trout (*Oncorhynchuss mykiss*). Aquaculture, 117 (1-2): 77-93.

Grandell PA and Gall GAE. 1993b. The genetics of age and weight at sexual maturity based on individually tagged rainbow trout (*Oncorhynchuss mykiss*). Aquaculture, 117 (1-2): 95-105.

Gunnes K and Gjedrem T. 1978. Selection experiments with salmon: IV. Growth of Atlantic Salmon during two years in the sea. Aquaculture, 15: 19-33.

Gunnes K and Gjedrem T. 1981. A genetic analysis of body weight and length in rainbow trout reared in sea water for 18 months. Aquaculture, 24: 161-174.

Hanke AR, Friars GW, Saunders RL and Terhune JM. 1989. Family x photoperiod interaction on growth in juvenile Atlantic salmon, *Salmo salar*. Genome, 32 (6): 1105-1112.

Heath DD, Bernier NJ, Heath JW and Iwama GK. 1993. Genetic, environmental, and interaction effects on growth and stress response of chinook salmon (*Oncorhynchus tshawytscha*) fry. Canadian Journal of Fisheries and Aquatic Sciences, 50 (2): 435-442.

Hedrick RP, McDowell TS, Marty GD, Fosgate GT, Mukkatira K, Myklebust K and El-Matbouli M. 2003. Susceptibility of two strains of rainbow trout (one with suspected resistance to whirling disease) to *Myxobolus cerebralis* infection. Diseases of Aquatic Organisms, 55 (1): 37-44.

Henryon M, Jokumsen A, Berg P, Lund I, Pedersen PB, Olesen NJ and Slierendrecht WJ. 2002. Genetic variation for growth rate, feed conversion efficiency, and disease resistance exists within a farmed population of rainbow trout. Aquaculture, 209 (1-4): 59-76.

Hersberger WK and Iwamoto RN. 1984. Systematic genetic selection and breeding in salmonid culture and enhancement programmes. In. C.J. Sinderman (Editor). Proceedings of the 11th US-Japan Meetings on Aquaculture, Salmon Enhancement, Tokyo, Japan, 1019 October, 1982. NOAA Tech. Rep. NMFS 27, pp. 29-32.

Hersberger WK, Myers JM, Iwamoto RN, Mcauley WC and Saxton AM, 1990. Genetic changes in the growth of coho salmon in marine net-pens, produced by ten years of selection. Aquaculture, 85: 187-197.

Hershberger WK. 1992. Genetic variability in rainbow trout populations. Aquaculture, 100: 51-71.

Hulata G. 2001. Genetic manipulations in aquaculture: a review of stock improvement by classical and modern technologies. Genetica, 111 (1-3): 155-173.

Iwamoto RN, Myers JM and Hersberger WK. 1986. Genotype-Environment Interaction for Growth of Rainbow Trout, *Salmo gairdneri*. Aquaculture, 57: 153-161.

Jacobs JM, Lindell S, Van Heukelem W, Hallerman EM and Harrell RM. 1999. Strain evaluation of striped bass (*Morone saxatilis*) under controlled conditions. Aquaculture, 173 (1-4): 171-177.

Jonasson J. 1993. Selection experiments in salmon ranching; 1. Genetic and environmental sources of variation in survival and growth in fresh-water. Aquaculture, 109 (3-4): 225-236.

Kause A, Ritola O, Paananen T, Mantysaari E and Eskelinen U. 2002. Coupling body weight and its composition: a quantitative genetic analysis in rainbow trout. Aquaculture, 211 (1-4): 65-79.

Kause A, Ritola O, Paananen T, Eskelinen U and Mantysaari E. 2003. Big and beautiful? Quantitative genetic parameters for appearance of large rainbow trout. Journal of Fish Biology, 62(3): 610-622.

Kincaid HL, Bridges WR and von Limbach B. 1977. Three generations of selection for growth rate in fall-spawning rainbow trout. Transactions of the American Fisheries Society, 106: 621-628.

Kincaid HL. 1983. Results from six generations of selection for accelerated growth rate in a rainbow trout population. The future of aquaculture in North America, p. 26-27. Fish Culture Section, American Fisheries Society.

Kinghorn BR. 1981. Quantitative genetics in fish breeding. Ph.D. Thesis University of Edinburgh.

Kinghorn BR. 1983a. A review of quantitative genetics in fish breeding. Aquaculture, 31: 283-304.

Kinghorn BR. 1983b. Genetic variation in food conversion efficiency and growth in rainbow trout. Aquaculture, 32: 141-155.

Knibb W, Gorshkova G and Gorshkov S. 1997. Growth in strains of gilthead seabream *Sparus aurata L.* Israeli Journal of Aquaculture-Bamidgeh, 49 (2): 43-56 JUN 1997

Knibb W. 2000. Genetic improvement of marine fish - which method for industry? Aquaculture Research, 31: 11-23.

McIntyre ID and Amend DF. 1974. Heritability of tolerance for infectious hematopoictic necrosis in sockeye salmon. Transaction of the American Fisheries Society, 107: 305-308.

McKay LR, Friars GW and Ihssen PE. 1984. Genotype x Temperature interactions for growth rate of rainbow trout. Aquaculture, 41: 134 -140.

McKay LR, Ihssen PE and Friars GW. 1986. Genetic Parameters of growth in rainbow trout, *Salmo gairdneri*, as a function of age and maturity. Aquaculture, 58: 241-254.

Morkramer S, Horstgen Schwark and Lanholz HJ. 1985. Evaluation of different European rainbow trout populations under intensive production conditions. Aquaculture, 44: 303-320.

Myers JM, Hershberger WK, Saxton AM and Iwamoto RN. 2001. Estimates of genetic and phenotypic parameters for length and weight of marine net-pen reared coho salmon (*Oncorhynchus kisutch Walbaum*). Aquaculture Research, 32 (4): 277-285.

Nevo E. 1978. Genetic variation in natural populations: patterns and theory. Theoretical Population Biology, 13: 121-177.

Nilsson J. 1992. Genetic-parameters of growth and sexual maturity in arctic char (*Salvelinus-Alpinus*). Aquaculture, 106 (1): 9-19.

Nilsson J. 1994. Genetics of growth of juvenile arctic char. Transactions of the American Fisheries Society, 123 (3): 430-434.

Okamoto N, Tayaman T, Kawanobe M, Fujiki N, Yasuda Y and Sano T. 1993. Resistance of a rainbow trout strain to infectious pancreatic necrosis. Aquaculture, 117: 71-76.

Olesen I, Gjedrem T, Bentsen HB, Gjerde B and Rye M. 2003. Breeding programmes for sustainable aquaculture. Journal of Applied Aquaculture, 13: 179-204.

Overturf K, Casten MT, LaPatra SL, Rexroad C and Hardy RW. 2003. Comparison of growth performance, immunological response and genetic diversity of five strains of rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 217 (1-4): 93-106.

Pohar J. 1992. Estimates of heritabilities and genetic correlations for consecutive body weights of rainbow trout reared in fresh and sea water. Aquaculture, 100: 103.

Refstie T and Torstein A. 1978. Selection Experiments with Salmon III. Genetic and environmental sources of variation in length and weight of Atlantic Salmon in the freshwater phase. Aquaculture, 14: 221-234.

Refstie T. 1990. Application of Breeding Schemes. Aquaculture, 85: 163-169.

SAS Institute Inc., 1986-1996. SAS User's Guide: Statistics. Version 6.12 Edition. SAS Ins. Inc., P.O. Box. 10066, NC 27605, USA. 956pp.

Shapiro SS and Wilk MB. 1965. An analysis of variance test for normality (complete samples). Biometrika, 52, 591-611.

Siitonen L and Gall GAE. 1989. Response to selection for early spawn date in rainbow trout. Aquaculture, 78: 153-161.

Smith RR, Kincaid HL, Regenstein JM and Rumsey GL. 1988. Growth, carcass composition, and taste of rainbow trout of different strains fed diets containing primarily plant or animal protein. Aquaculture, 10: 309-321.

Stromsheim A, Eide DM, Hofgaard PO, Larsen HJS, Refstie T and Roed KH. 1994. Genetic-variation in the humoral immune-response against *vibrio-salmonicida* and in antibody titer against *vibrio-anguillarum* and total igm in atlantic salmon (*Salmo-Salar*). Veterinary Immunology and Immunopathology, 44 (1): 85-95.

Su GS, Liljedahl LE and Gall GAE. 2002. Genetic correlations between body weight at different ages and with reproductive traits in rainbow trout. Aquaculture, 213 (1-4): 85-94.

Sylven S and Elvingson P. 1992. Evaluation of rainbow trout (*Oncorhynchus mykiss)* strains for body weight, length and age at maturity in different Swedish production systems. Aquaculture, 104: 37-50.

Van der Bank FH, Swart MKJ and Ferreira JT. 1992. Genetic variation in nine rainbow trout (*Oncorhynchus mykiss*) populations in South Africa. Comparative Biochemical Physiology, 103B: 495-499.

Vandeputte M, Quillet E and Chevassus B, 2002a. Early development and survival in brown trout (*Salmo trutta fario L.*): indirect effects of selection for growth rate and estimation of genetic parameters. Aquaculture, 204 (3-4): 435-445.

Vandeputte M, Peignon E, Vallod D, Haffray P, Komen J and Chevassus, B. 2002b. Comparison of growth performances of three French strains of common carp (*Cyprinus carpio*) using hemi-isogenic scaly carp as internal control. Aquaculture, 205 (1-2): 19-36.

Wangila BCC and Dick TA. 1988. Influence of genotype and temperature on the relationship between specific growth rate and size of rainbow trout. Transactions of the American Fisheries Society, 117 (6): 560-564.

Wild V, Simianer H, Gjoen HM and Gjerde B. 1994. Genetic parameters and genotype x environment interaction for early sexual maturity in Atlantic salmon (*Salmo salar*). Aquaculture, 128 (1-2): 51-65.

Winkelman AM and Peterson RG. 1994a. Heritabilities, dominance variation, common environmental effects and genotype by environment interactions for weight and length in chinook salmon. Aquaculture, 125 (1-2): 17-30.

Winkelman A and Peterson RG. 1994b. Genetic parameters (heritabilities, dominance ratios and genetic correlations) for body weight and length of chinook salmon after 9 and 22 months of saltwater rearing. Aquaculture, 125: 31-36.

Withler RE and Evelyn TPT. 1990. Genetic variation in resistance to Bacterial Kidney Disease within and between two strains of Coho salmon from British Colombia. Transaction of the American Fisheries Society, 119: 1003-1009.

Withler RE and Beacham TD. 1994. Genetic variation in body weight and flesh colour of the coho salmon (*Oncorhynchus kisutch*) in British Columbia. Aquaculture 119: 135 –148.

Wolters WR and Johnson MR. 1995. Analysis of a diallel cross to estimate effects of crossing on resistance to enteric septicemia in channel catfish, *Ictalurus punctatus*. Aquaculture, 137 (1-4): 263-269.

Table 1.1: The stages and method of evaluation of growth rate in rainbow trout (*Oncorhynchus mykiss*). Proportion selected = proportion selected for next stage; S = random samples of 15% per replicate for measurement.

Stage	Age at start (Days from fertilization) ± s.d.	Duration (Days)	Number per Replicate ± s.d.	Proportion selected (%) ± s.d.	Measurements			
					Average Weight (g)		Average Length (mm)	
					Initial	Final	Initial	Final
EVEN 88								
I	120±5.6	66	2089±90.8		S	S	S	S
II	195±5.6	94	430±19.3	21.0±0.9	S	S	S	S
III	298±5.6	89	160±7.8	7.7±0.4	S	S	S	S
ODD 89								
I	120±2.6	69	2102±18.4		S	S	S	S
II	199±2.6	97	420±3.8	20.0±0.2	S	S	S	S
III	291±2.6	84	120±1.1	5.7±0.1	S	S	S	S
OVERSEAS 89								
I	120±4.5	64	2087±48.0		S	S	S	S
II	188±4.5	89	420±9.9	20.1±.0.5	S	S	S	S
III	273±4.5	81	120±2.8	5.8±0.1	S	S	S	S

s.d. = standard deviation

Table 1.2: Analysis of Variance of Average Daily Weight Gain and Average Daily Length Gain of 9 local and 4 overseas hatchery populations of rainbow trout over three consecutive stages of evaluation during 1988 and 1989.

		Average Daily Weight Gain			Average Daily Length Gain	
EVEN 88						
Source	d.f.	Mean Square	P	d.f.	Mean Square	P
Group	8	0.0799	<0.01	8	0.0248	<0.01
Growth Stage	2	96.5619	<0.01	2	0.6633	<0.01
Group x Stage	16	0.0358	<0.01	16	0.0042	<0.01
Error	54	0.0117		54	0.0013	
Total Corrected	80			80		
Non-normality			<0.01			0.8413
ODD 89						
Source	d.f.	Mean Square	P	d.f.	Mean Square	P
Group	8	0.1077	<0.01	8	0.0424	<0.01
Growth Stage	2	100.9159	<0.01	2	0.6758	<0.01
Group x Stage	16	0.0601	<0.01	16	0.0047	<0.01
Error	54	0.0054		54	0.0016	
Total Corrected	80			80		
Non-normality			0.0389			0.8760
OVERSEAS 88						
Source	d.f.	Mean Square	P	d.f.	Mean Square	P
Group	3	0.8101	<0.01	3	0.0674	<0.01
Growth Stage	2	35.0448	<0.01	2	0.2845	<0.01
Group x Stage	6	0.3580	<0.01	6	0.0037	<0.01
Error	24	0.0072		24	0.0013	
Total Corrected	35			35		
Non-normality			0.0362			0.1979

Table 1.3: Analysis of Variance of Average Daily Weight Gain and Average Daily Length Gain of 9 local and 4 overseas hatchery populations of rainbow trout for each of three separate evaluation stages (I, II, III) during 1988 and 1989. MS = Mean Square.

		Average Daily weight gain						Average Daily Lenght Gain					
Stage		I		II		III		I		II		III	
EVEN 88													
Source	d.f.	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P
Group	8	0.0015	0.397	0.0141	<0.01	0.1360	<0.01	0.0093	<0.01	0.0052	<0.01	0.0186	<0.01
Error	18	0.0014		0.0021		0.0318		0.0020		0.0004		0.0015	
Total	26												
ODD 89													
Source	d.f.	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P
Group	8	0.0019	0.078	0.0139	<0.01	0.2121	<0.01	0.0160	<0.01	0.0085	<0.01	<0.01	<0.01
Error	18	0.0009		0.0017		0.0135		0.0016		0.0006		0.0027	
Total	26												
OVERSEAS 88													
Source	d.f.	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P
Group	3	0.0037	<0.01	0.0943	<0.01	1.4282	<0.01	0.0093	<0.01	0.0266	<0.01	0.0388	<0.01
Error	8	0.0003		0.0014		0.0120		0.0004		0.0005		0.0031	
Total	11												

Table 1.4: Least Squares Means of Average Daily Weight Gain and Average Daily Length Gain of 9 local and 4 overseas genetic groups of rainbow trout during the three evaluation stages (I,II,II) during 1988 and 1989.

Year group	Mean Square ADWG (g/day)			Mean Square ADLG (mm/day)		
	I	II	III	I	II	III
EVEN 88						
E 1	0.301*	0.595*	3.944	1.099*	0.825*	1.098*
E 2	0.290*	0.914*	3.864	0.989	0.743	1.115*
N 1	0.306*	0.942*	3.623	1.060*	0.757	0.985
N 2	0.332*	1.086*	4.034*	1.068*	0.843*	1.140*
N 3	0.318*	0.977*	4.154*	1.064*	0.780	1.132*
N 4	0.316*	1.027*	4.036*	1.113*	0.807*	1.081
W 1	0.285*	0.985*	3.959	1.033*	0.791*	1.061
W 2	0.267*	0.993*	3.798	0.970	0.759	0.949
W Wild	0.261*	0.844	3.489	0.980	0.716	0.936
t-LSD (P=5%)	0.1770			0.0584		
ODD 89						
E 1	0.303*	0.987*	4.263*	1.067*	0.814*	1.158*
E 2	0.276*	1.033*	4.017	1.001	0.796*	1.163*
N 1	0.288*	0.949	4.111	1.057*	0.754	1.067
N 2	0.318*	1.020*	3.921	1.116*	0.818*	1.103*
N 3	0.310*	1.104*	3.975	1.070*	0.834*	1.097*
N 4	0.317*	1.008*	4.192*	1.114*	0.781*	1.088
W 1	0.260*	0.914	3.829	0.927	0.712	0.981
W 2	0.271*	0.904	3.471	0.953	0.683	0.889
W Wild	0.250*	0.908	3.580	0.945	0.721	0.945
t-LSD (P=5%)	0.1198			0.0661		
OVERSEAS 88						
D	0.245*	1.361	3.927	0.842*	1.046*	1.202*
I-M	0.189	1.018	2.571	0.710	0.837	0.942
N-A	0.271*	1.420*	4.105*	0.796*	0.945	1.145*
S	0.252*	1.263	3.701	0.763*	0.862	1.054
t-LSD (P=5%)	0.0590			0.0590		

Local groups: E = Eastern Highlands, N = Northern Highlands, W = Western Coastal Region.
Overseas groups: D = Denmark, I-M = Isle of Man, N-A = North America, S = Scotland.
* Groups with the best ADWG and ADLG within a stage, i.e. the a-range based on t-LSD (P=0.05)

Table 1.5: The average age, weight (g), length (mm), with standard error and coefficient of variation (%) at the end of each growth stage, for the three year-groups of rainbow trout EVEN 88, ODD 89 and OVERSEAS 88. Age = days from fertilization.

	Stage I			Stage II			Stage III		
	Age	Weight	Length	Age	Weight	Length	Age	Weight	Length
EVEN 88	186	21±0.5 12.6%	116±1.5 6.8%	289	119±2.1 9.2%	197±2.1 5.4%	387	478±5.0 5.5%	298±2.7 4.7%
ODD 89	189	22±0.5 11.3%	121±1.5 6.4%	296	144±2.2 8.0%	218±2.2 5.1%	375	520±7.2 7.2%	318±3.1 5.1%
OVERS EAS 88	184	24±1.0 14.2%	127±2.2 5.9%	277	152±4.2 9.6%	228±3.5 5.3%	354	410±8.0 6.8%	306±4.3 4.9%
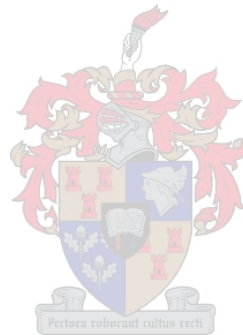
Table 1.6: Correlations between means for ADWG and ADLG of 9 local hatchery-groups of rainbow trout over three stages of evaluation during two consecutive generations. (d.f. = 36).

	ADWG			ADLG		
	Stage I	Stage II	Stage III	Stage I	Stage II	Stage III
ADWG I						
ADWG II	0.531**					
ADWG III	0.425**	0.507**				
ADLG I	0.648**	0.404*	0.341*			
ADLG II	0.395*	0.691**	0.468**	0.576**		
ADLG III	0.335*	0.324*	0.715**	0.464**	0.566**	

* = P<0.05 ** P<0.01
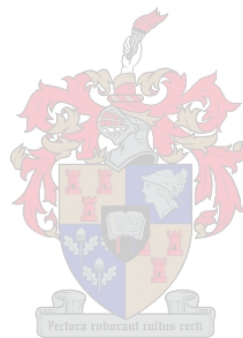
CHAPTER 2:   EVALUATION OF FIRST GENERATION OFFSPRING FROM SINGLE CROSSES

1. Abstract

This paper reports on the evaluation of the rate of growth of first generation offspring from the crossing of six genetic groups from each of two base populations in consecutive years, as part of an ongoing breeding programme for improved rate of growth. The crosses were conducted in order to increase the levels of genetic variation in the base populations in view of future selection, as well as to investigate possible heterosis and specific and general combining ability among the crosses. Significant heterosis was found for weight and length gain during consecutive growth stages. No evidence was found for specific combining ability among the crosses. Significant levels of general combining ability for weight and length gain were found in consecutive growth stages. Selective crossing of groups therefore holds little advantage for improvement in weight and length gain, while weight and length is favoured rather by the use of specific parental groups (e.g. Eastern-Danish, Danish-North American combinations). The crossing of selected offspring from the original genetic groups followed by the application of intensive multi-stage selection for growth rate within progeny groups has led to the establishment of second generation parental populations with higher levels of genetic variation and improved individual genetic merit with regard to growth rate.

Keywords: *Oncorhynchus mykiss*, Crossbreeding, Heterosis, Combining ability, Growth rate, Selection

2. Introduction

Rainbow trout was first introduced into South Africa during the 1890s although commercial production of portion size trout (280–350g) only started in earnest during the 1960s, with further emphasis being placed on the production of large "salmon trout" (>1200g) since the early 1980s. The aim of the current breeding programme is the improvement in growth rate of rainbow trout to enable local producers to complete the production cycle for salmon trout before the onset of the second summer (age 16-18 months). Conditions tend to be unfavourable for the production of trout in most parts of South Africa during summer when water temperatures often rise above 24°C. High risks and low productivity, particularly for the production of larger size trout, are associated with these conditions.

Further introductions of rainbow trout into South Africa occurred at regular intervals since the early 1980s, though from a limited number of sources in the Northern Hemisphere. The lack of genetic improvement programmes and genetic management procedures, together with possible founder effects, have raised concern with regard to the genetic quality of the South African gene pool of rainbow trout. The first objective of the breeding programme was therefore the collection, evaluation and selection of the best genetic material available from local and overseas sources in order to establish a base population with high genetic merit and variation, as recommended by Refstie (1990) and Gjedrem (1992). A detailed description of the procedures and results of the first phase of the breeding programme has been reported in Chapter one, and has led to the establishment of two distinct base populations, EVEN 88 and ODD 89, in consecutive years during 1988 and 1989.

This chapter reports on the evaluation of growth rate of the first generation offspring of crosses involving six genetic groups of rainbow trout from the base populations, EVEN 88 and ODD 89, during multi-stage selection within groups for improved rate of growth. The aim of the crossing of genetic groups from the respective base populations was to generate the maximum possible genetic variation within the base populations in view of future selection, as well as to investigate possible heterosis and specific and general combining ability among the crosses. The genetic merit of the various groups have been established as part of the comparison of genetic resources and the formation of base populations referred to in Chapter 1.

Intra- an interspecific crossbreeding is common practice in various aquaculture species, in particular with carp (Wohlfarth, 1993; Hulata, 1995; Bakos and Gorda, 1995), catfish (Smitherman and Dunham, 1985; Dunham, 1987) and tilapia (Wohlfarth, 1994; Bentsen et al., 1998). Intraspecific crossbreeding (crossing of different strains) may increase growth rate but heterosis (differences between offspring and parents) may not occur in every case. Levels of heterosis for growth in salmonids in the range of 7- 55% has been reported by Gall (1969, 1975), Gall and Gross (1978), Kincaid (1981), Ayles and Baker (1983), Gjerde (1988), and Wangila and Dick (1996) on the basis of crossing of strains and populations.

Reports by Gjerde and Refstie (1984), Gjerde (1988), Gjedrem (1992) and Horstgen-Schwark et al. (1996) emphasize that current evidence still seems to be inconclusive with regard to the beneficial application of crossbreeding and the utilization of heterosis in order to obtain genetic improvement in growth of rainbow trout. Frairs et al. (1979), Nilsson (1993) and Dunham (1996) reported no significant heterosis for growth rate in respective strain crosses of Atlantic salmon, Arctic char and Chum salmon. McKay and McMillan (1997) reported little evidence of heterosis in growth, maturation and spawning in crosses of three rainbow trout strains, whilst Zamora Balbuena et al. (1999a,b) reported heterosis for growth rate in crosses between two lines of rainbow trout with no heterosis for fertility and viability. Johnson et al. (1999) also reported no evidence of heterosis in disease resistance between wild and domesticated strains of Chinook salmon. Gjedrem (1992) has concluded that crossbreeding between strains and inbred lines of rainbow trout in general does not offer significant advantages as a breeding method, although exceptions may occur. In general, the investigation of heterosis and specific and general combining ability among crosses of strains and populations appears to be well justified. Crossbreeding also remains an important mechanism to increase the level of genetic variation in populations at the onset of breeding programmes aimed at genetic improvement through selection.

3. Material and methods

3.1. Base populations

The origin of the base populations EVEN 88, ODD 89 and OVERSEAS 88 and the contributing genetic groups were described in Chapter 1. Briefly, the base population EVEN 88 was established in 1988 through the collection of 10 000 ova from each of nine local and four overseas hatcheries. These 13 hatchery-groups were evaluated on the basis of growth rate over three consecutive stages, I, II and III of duration 60, 90 and 90 days respectively. Multi-stage selection was conducted within hatchery-groups on the basis of individual body width at the end of Stage I, and on the basis of individual body weight at the end of Stages II and III.

The base population, EVEN 88, was constituted through selection of the 12 best individuals from each of 12 hatchery-groups at the end of growth stage III. This procedure attempted to ensure a base population with the highest possible genetic merit and widest possible genetic diversity as recommended by Refstie (1990), Gjedrem (1992) and Olesen et al. (2003) with regard to the implementation of genetic improvement programmes.

The founder fish selected from the local hatchery-groups through the process of multi-stage selection, to form the base population EVEN 88, were proportionally combined into four regional groups according to geographic areas or origin, viz.; the Northern group (N) combining the four hatchery-groups from the Northern Highlands Region of South Africa; the Eastern group (E) combining two hatchery-groups from the Eastern Highlands Region; the Western group (W) combining three hatchery-groups from the Western Coastal Region. The grouping of the local hatchery groups according to their region of origin seemed advisable on the evidence of Van der Bank, et al., (1992) who found closer genetic relationships among genetic groups within regions, than between regions. The founder fish selected from overseas groups were maintained in their original groups, viz. one group from each of North America (A), Denmark (D) and Scotland (S) to form the base population OVERSEAS 88.

The collection of 10 000 ova from each of the 9 local hatcheries was repeated during 1989 and the base population ODD 89 was established in a similar, way, also consisting of selected founder fish from the regional Northern (N), Eastern (E) and Western (W) groups.

3.2. Diallel crosses: 1990 and 1991

During the 1990 season the founder fish selected from the base population EVEN 88 were crossed according to a diallel mating pattern. Eggs and sperm collected on the same day from six females and six males from each group were pooled before fertilization. Founder fish from the base population OVERSEAS 88 from the Northern Hemisphere were between 18 and 20 months of age at the time. Many fish were found to be still sexually immature, particularly females, thus limiting the planned sample size. Six mature females were available from the North American group, four from the Danish group and no females from the Scottish group. The Scottish group was therefore excluded as a female parental group. Pooled eggs and sperm were divided into six batches and the pooled sperm from each group was used to fertilize pooled eggs from the same group and from all other groups. The outcome was an incomplete set of 21 progeny groups (Table 2.1) established during 1990, referred to as the EVEN 90 year-group, as first generation offspring from crosses between the EVEN 88 and OVERSEAS 88 base populations.

The same procedures were followed in a diallel cross during 1991 with six females and six males selected to represent each group from the base population ODD 89, together with the same overseas groups as in 1990. An incomplete set of 32 progeny groups, referred to as the ODD 91 year-group (Table 2.1), was established from these crosses of 1991. The improvement in the number of progeny groups of the ODD 91 year-group in comparison to the EVEN 90 year-group can be attributed to improved levels of sexual maturity in groups originating from the Northern Hemisphere, coinciding with the higher average age (30 to 32 months) of these groups at the time.

3.3. Growth rate evaluation

Ova from all progeny groups of EVEN 90 and ODD 91 were hatched, and the fish were reared and evaluated in separate containers under standardized conditions. The evaluation period was again divided into three stages as for the evaluation of the previous EVEN 88 and ODD 89 as described in Chapter 1 and summarized in Table 2.2. The three stages of evaluation were determined by practical considerations with regard to the capacity of facilities available and seasonal periods. Growth stages I, II and III were characterized by distinct differences in average water temperature due to seasonal variation. The average water temperatures during Stages I, II and III were 15.9, 22.8 and 13.5°C respectively. Rearing prior to Stage I was done in rectangular cement troughs. Throughout all stages of evaluation, rearing conditions such as numbers per pond, densities, flow rates, type of feed, feeding levels and methods were standardized over all groups, according to commercial standards. A standard range of commercial trout feed, supplied by WPK Aquafeeds Pty Ltd, with an approximate composition of 40% crude protein, 16% crude fat, 6% crude fiber, 3% calcium and 0.7% phosphorous, was used during all stages of evaluation in both years. A hand-feeding method was used with feeding levels being standardized according to a feed table on the basis of water temperature and size of fish. Groups were kept separately in randomly allocated tanks through all stages of evaluation. Tanks were circular PVC of 10 cubic meters in volume and one meter in depth. Water temperature, oxygen levels, mortality and feed consumption were recorded on a daily basis during each stage. Tanks were sampled every 14-21 days to record the average weight and length, and this information formed the basis for the adjustment and standardization of rearing conditions. The standardized application of commercial production conditions was regarded as very important during evaluation and selection in order to minimize environmental variation and possible genotype-environment interactions, and to simulate practical conditions under which progeny are expected to be reared for commercial production as far as possible as has been advocated as desirable practice in such programmes by Gjedrem (1992).

Reduction in the number from Stage I (approx 42 000) to Stage II was by means of a box grader for body width. Reduction from Stage II to Stage III was by means of selection based on individual body length. Body length was used as an alternative criterion of selection for the EVEN 90 and ODD 91 groups after observing extremely high levels of the condition-factor (CF) in fish selected on the basis of individual body weight from the EVEN 88 and ODD 89 base populations, as referred to in Chapter 1 and further supported by published reports on the positive genetic correlation between body weight and CF by Gjerde and Schaeffer (1989), Sylven and Elvingson (1992) and Elvingson and Johansson (1993). Selection on the basis of body weight could therefore lead to an unfavorable indirect increase in CF while the market prefers a more salmon-like body conformation. The combination of body weight and length observations into a condition factor offers little benefit with regard to it's use as a criterion for selection to improve the rate of growth due to the low heritability estimates reported in salmonids. The low heritability of CF in salmonids seems to be due to the reduction of variance estimates of CF compared to the variances of the separate measurements of weight and length as reported by Gunnes and Gjedrem (1978) and Refstie and Torstein (1978).

At the end of Stage III the six best fish were selected from each progeny group on the basis of individual body length to be used as parents of the EVEN 92 and ODD 93 year-groups. Numbers of males of females

were not recorded at this stage due to the absence of visible external sexual dimorphism at the age of one year.

3.4. Definition of traits

Traits measured as criteria of growth rate in progeny groups were Average Daily Weight Gain (ADWG) in grams per day and Average Daily Length Gain (ADLG) in mm per day. Methods of calculation of ADWG and ADLG during the various stages of evaluation were as described in Chapter 1, Section 3.5.

3.5. Statistical models and analysis of variance

Data obtained from the EVEN 90 and ODD 91 groups were analyzed as a combined data set by means of the generalized least square procedure (SAS Institute Inc., 1995a) because of the absence of replications within year-groups and the repetitive nature of the foundation of the two year-groups.

The nature of this analysis is based on a sequential analysis of sums of squares or sequential analysis of variance, which entails a Sum of Squares of Type I (SAS Institute Inc., 1995b; see page 109, Chapter 9). The linear models described by Randall (1976), based on work of Harvey (1960) for the analysis of diallel cross data were used to analyze the data with regard to heterosis, general combining ability (GCA), specific combining ability (SCA) and parental effects. In this approach, separate models are constructed for crosses, reciprocals, "selfs" and heterosis as follows:

For crosses (C)

$$Y_{ijk} = \mu_C + g_i + h_j + s_{ij} + e_{ijk} \tag{1}$$

where g_i is the general combining ability (GCA) of the ith group as first parent, h_j is the GCA of the jth group as second parent and s_{ij} is the specific combining ability (SCA) of the ith group (male) and the jth group (female).

For reciprocal crosses (R)

$$Y_{jik} = \mu_R + g_j + h_i + t_{ji} + e_{ijk} \tag{2}$$

where t_{ji} is the same as for s_{ij} except that the parental roles of the groups are reversed.

For selfs (S)

$$Y_{ijk} = \mu_S + g_i + g_i + s_{ii} + e_{iik} \tag{3}$$

For heterosis

$$Y_{ijk} = \mu + a_i + a_j + b_i - b_j + v_{ij} + w_{ij} + e_{ijk} \tag{4}$$

where $a_i = \frac{1}{2}(g_i + h_j)$ = average contribution of the ith group, averaged over parents

$b_i = \frac{1}{2}(g_i - h_j)$ = half the difference in parental contribution,

$v_{ij} = \frac{1}{2}(s_{ij} + t_{ji}) = v_{ji}$ = average joint contribution, averaged over reciprocals

$w_{ij} = \frac{1}{2}(s_{ij} - t_{ji}) = w_{ji}$ = half the difference of joint contributions.

The prototype table for the analysis of variance with sources of variance, sums of squares and degrees of freedom is given in Table 2.3.

4. Results

4.1. Crosses: 1990 and 1991

The LS means of ADWG and ADLG of each cross in each growth stage, for the EVEN 90 and ODD 91 groups are presented in Tables 2.4, 2.5, 2.6 and 2.7 where diagonal data are for selfs (S), above diagonal for crosses (C) and off diagonal for reciprocals (R). The male and female group averages refer to the average of the common parental group as male (vertical) or female (horizontal). The overall group-average refers to the overall average of a common parental group. The averages of selfs (S), combined crosses (C+R), crosses (C) and reciprocals (R) are also given in these tables.

The regional (Northern, Eastern, Western) and overseas-groups (American, Danish, Scottish) show minor shifts in rank order for ADWG (Tables 2.4 and 2.6) and ADLG (Tables 2.5 and 2.7) over all growth stages within years. They further display a relationship in the rank order for ADWG and ADLG within stages over years (Tables 2.4, 2.5, 2.6 and 2.7). The Western group shows consistently inferior results on the basis of group-averages, while the Eastern and Danish groups were consistently superior over all stages and years, for both weight and length gain. These results to some extend correspond to those achieved in the previous generation as reported in Chapter one, Table 1.2). The average of the combined crosses (C+R) in terms of ADWG and ADLG were also consistently higher than that of the self's (S), whilst the averages of the reciprocals (R) were consistently higher than that of the crosses (C).

4.2. Analysis of Variance

The Analysis of Variance (ANOVA) for ADWG and ADLG over the EVEN 90 and ODD 91 year-groups for each of the growth stages I, II and II are presented in Tables 2.8, 2.9, and 2.10. With regard to specific combining ability (SCA), the line "differential SCA" provides for the detection of possible differences SCA between crosses and reciprocals and is not statistically significant in any case for both ADWG and ADLG over all stages. The line "common SCA" provides for the detection of possible differences in SCAs without distinguishing between crosses and reciprocals and is also not significant in any case over all stages.

With regard to general combining ability (GCA), the lines "differential GCA" and "general GCA" have the same meaning as in the analysis of SCA. The evidence from Stage I (Table 2.8) for differences between crosses and reciprocals is weak for ADWG (P = 0.5379) though nearly convincing for ADLG (P = 0.0655). "Common GCA" in Stage 1 is not significant in the case of ADWG (P = 0.1063) but highly significant in in the case of ADLG (P = 0.0002). The parental groups therefore appear to contribute equally to the crosses in the case of ADWG but not in the case of ADLG. This evidence is further supported by observations of the "Overall Group Averages" for ADWG (Tables 2.4, 2.6) and ADLG (Tables 2.5, 2.7) in this stage.

A similar pattern is apparent in Stage II results (Table 2.9) for "differential GCA" in the case of ADWG (P = 0.2605) and ADLG (P = 0.0057). "Common GCA" was highly significant for both ADWG (P = 0.0097) and ADLG (P = 0.0003). With regard to Stages I and II, ADWG would therefore seem not to gain significant advantage from the use of specific parental groups, while ADLG may well be favoured by the use of specific

parental groups such as the Eastern Group in EVEN 90 (Table 2.5) and the Danish Group in ODD 91 (Table 2.7). Results for Stage III (Table 2.10) provide strong evidence for differential GCAs for selfs and crosses with regard to both ADWG (P = 0.0402) and ADLG (P = 0.0286). With regard to Stage III, it thus appears that the use of specific parental groups as indicated will benefit both ADWG and ADLG.

The line "Heterosis" in the ANOVA tests for significant deviation of the means of crosses from the means of parental groups (selfs), per definition of heterosis. In Stage I (Table 2.8) there is no evidence for heterosis for ADWG (P = 0.1274), and strong evidence for heterosis in ADLG (P = 0.0014), and is evident as well in the data on the averages of the selfs and crosses in Tables 2.5 and 2.7. This result repeats itself for Stage II (Table 2.9) (P = 0.0235 for ADLG) with some indications of heterosis in ADWG (P = 0.0721) as well. On the other hand, heterosis in Stage III (Table 2.10) is highly significant for both ADWG (P = 0.0002) and ADLG (P = 0.0001).

5. Discussion

The main objective of the crossing of genetic groups from the respective base populations was to increase the amount of genetic variation in the respective populations to allow for further selection for improved rate of growth. This has been achieved, particularly in the case of the ODD 91 year-group in which an almost complete diallel set of crosses was obtained. An intensive multi-stage selection procedure, based on individual performance within groups was applied in all stages of evaluation in the EVEN 90 and ODD 91 year-groups (Table 2.2). At the end of Stage III in the respective years, the six best fish were selected from each progeny group on the basis of individual body length to be used as parents of the EVEN 92 and ODD 93 year-groups. This procedure has assured the formation of the EVEN 90 and ODD 91 parental populations that presumably contained more genetic variation and were of higher individual genetic merit than the EVEN 88 and ODD 89 base populations. Provision was made for the maintenance of a sufficient effective population size (Ne = ±150) in each of these populations to ensure low levels of inbreeding, in the range of $\Delta F = 0.003$ (Falconer and MacKay, 1996). The actual population size and therefore the levels of inbreeding is however hard to quantify as the possibility exist that some of the selected fish may have originated from the same family.

The use of individual length as criterion for selection within groups at the end of Stages II and III, as opposed to individual weight that was used during the previous generation (see Discussion, Chapter one), rendered a favourable response. The average condition factor (CF = [(ungutted weight, g) / (fork-length, cm)^3]x100)) of the six best individuals selected from each progeny group at the end of Stage III were respectively 2.02±0.11 for EVEN 90 and 1.92±0.15 for ODD 91, as compared to the previous values of 2.17±0.18 for EVEN 88 and 2.14±0.15 for ODD 89. These values were, however, still higher than the preferred market requirements of 1.6-1.8 and emphasis should therefore be concentrated on body length as criterion of selection for improved rate of growth during future generations.

A second objective of the crossing of genetic groups from the respective base populations was to evaluate the occurrence of heterosis and specific and general combining ability among the crosses. The results of the ANOVA confirm that there is no convincing evidence of specific combining ability, differential or common,

for these traits among any of the crosses, with regard to Stages I, II and III. No specific combination or crosses is therefore expected to yield significantly better performance in ADWG or ADLG. In terms of general combining ability, the observed significant differential GCA is limited to ADLG in Stages I and II, with a progressive change to include both ADWG and ADLG in Stage III. The conclusion can therefore be drawn that both weight and length gain are to be favoured by the use of specific parental groups, particularly toward the later growth stages.

The presence of heterosis is demonstrated in the generally higher average values for weight and length gain of the combined crosses (C+R) in comparison to the selfs (S) as confirmed by the significance levels for heterosis in the ANOVA tables of all three stages. Heterosis also seems to become more prominent during the later stages as indicated by the average values in Tables 2.4 to 2.7 and the significance levels in Tables 2.8 to 2.10. This result contrasts the results reported by Klupp (1979) in which case heterosis for growth appears to diminish with age, finally to become negative at the age over 200 days. Ayles and Baker (1983) also reported significant heterosis for growth in rainbow trout, though only for some crosses as in the present study. Results of several other studies by Gall (1975), Gall and Gross (1978), Webster and Flick (1981), Kincaid (1981), Ayles and Baker (1983), Iwamoto, et al. (1986), Horstgen-Schwark, et al. (1996), Gjerde (1988) and Wangila and Dick (1996) indicate the presence of significant but variable levels of heterosis for growth in salmonids, and trout in particular. In general, current evidence on heterosis in rainbow trout is inconclusive as justification of the use of systematic crossbreeding programmes for the improvement of growth rate, as opposed to programmes of improvement through recurrent selection. Varying levels of heterosis have been reported by Gjerde and Refstie (1984), Gjerde (1988), Gjedrem (1992), Horstgen-Schwark et al. (1996), McKay and McMillan (1997) and Zamora Balbuena et al. (1999a,b). The cost and time involved in the development and test crossing of specific lines is a further factor to be considered, as noted by Gjerde (1988). However, crossbreeding remains an essential means for the creation of genetic variation, essential to fish breeding programmes (Gjedrem, 1992), as well as to the upgrading of inferior populations and the elimination of inbreeding. The combination of specific traits through systematic crossbreeding strategies also holds future promise, as specialized lines are being developed through specific selection programmes.

Although significant general combining ability and heterosis (9.6% and 6.7% for ADWG and ADLG respectively) were detected in the crosses over the three consecutive growth stages in the present study, the immediate exploitation thereof was restrained by the early stage of the selection programme which then focused rather on the exploitation of genetic variation through selection within the newly formed cross populations.

6. References

Ayles GB and Baker RF. 1983. Genetic differences in growth and survival between strains and hybrids of rainbow trout stocked in lakes in the Canadian prairies. Aquaculture, 33: 269-280.

Bakos J and Gorda S.   1995. Genetic improvement of common carp strains using intraspecific hybridization. Aquaculture, 129: 183-186.

Bentsen HB, Eknath AE, Palada-de Vera MS, Danting JC, Bolivar HL, Reyes RA, Dionisio EE, Longalong FM, Circa AV, Tayamen MM and Gjerde B. 1998. Genetic improvement of farmed tilapias: growth performance in a complete diallel cross experiment with eight strains of *Oreochromis niloticus*. Aquaculture, 160 (1-2): 145-173.

Dunham RA.   1987. American catfish breeding programmes. Selection, hybridization and genetic engineering in aquaculture. European Inland Fisheries Advisory Commission (EIFAC) Symposium. Bordeaux, 1986

Dunham RA.   1996. Contribution of genetically improved aquatic organisms to global food security. International Conference on Sustainable Contribution of Fisheries to Food Security. Government of Japan and FAO, Rome, 150 pp.

Elvingson P and Johansson K. 1993. Genetic and environmental components of variation in body traits of rainbow trout in relation to age. Aquaculture, 118: 191-204.

Falconer DS and Mackay TFC.   1996. Introduction to Quantitative Genetics. Fourth Edition. Longman Scientific & Technical, England, 464 pp.

Frairs GW, Bailey JK and Saunders RL.   1979. Considerations of a method of analyzing diallel crosses of Atlantic salmon. Canadian Journal of Genetics and Cytology, 21: 121-128.

Gall GAE.   1969. Quantitative inheritance and environmental response of rainbow trout. In O.W. Neuhaus & J.E. Halver, eds. Fish research, New York, Academic Press.

Gall GAE.   1975. Genetics of reproduction in domesticated rainbow trout. Journal of Animal Science, 40: 19-28

Gall GAE and Gross SJ. 1978. Genetic studies of growth in domesticated rainbow trout. Aquaculture, 13: 225-234.

Gjedrem T. 1992. Breeding plans for rainbow trout. Aquaculture, 100: 73-83.

Gjerde B. 1988. Complete diallel cross between six inbred groups of rainbow trout. Aquaculture, 75: 71-87.

Gjerde B and Gjedrem T.   1984. Estimates of phenotypic and genetic parameters for carcass traits in Atlantic salmon and rainbow trout. Aquaculture, 36: 97-110.

Gjerde B and RefstieT. 1984. Livestock Production Sciences, 11: 207-226.

Gjerde B and Schaeffer LR.   1989. Body traits in rainbow trout. II. Estimates of heritabilities and of phenotypic and genetic correlations. Aquaculture, 80: 25-44.

Gunnes K and Gjedrem T.   1978. Selection experiments with salmon: IV. Growth of Atlantic salmon during two years in the sea. Aquaculture, 15: 19-33.

Harvey WR. 1960. Least Square analysis of data with unequal subclass numbers. USDA ARS Publication number 20-8.

Horstgen-Schwark G, Frick, H and Lanholz HJ. 1996. The effect of strain crossing on the production performance of rainbow trout. Aquaculture, 57: 14-152.

Hulata G. 1995. A review of genetic improvement of the common carp (*Cyprinus carpio L.*) and other hybrids by crossbreeding, hybridization and selection. Aquaculture, 129: 143-155.

Iwamoto RN, Myers JM and Hersberger WK. 1986. Genotype-Environment Interaction for Growth of Rainbow Trout, *Salmo gairdneri*. Aquaculture, 57: 153-161.

Johnson R, Bryden C and Heath DD. 1999. Can indicators of disease resistance predict performance in pure and hybrid strains of wild and domestic chinook salmon? Bulletin of the Aquaculture Association of Canada. 1999 4:31-33.

Kincaid HL. 1981. Trout salmon registry. FWS/NFC-L/81-1, U.S. Fish and Wildlife Service, Kearneysville, WV.

Klupp R. 1979. Genetic variance for growth in rainbow trout. Aquaculture, 18: 123-134.

McKay LR and McMillan I. 1997 Growth, maturation and spawning times in crosses of three strains of rainbow trout. Bulletin of the Aquaculture Association of Canada, 2: 15-17.

Nilsson J. 1993. Arctic charr strain crosses: effects on growth and sexual maturity. Journal of Fish Biology, 43 (2): 163-171.

Olesen I, Gjedrem T, Bentsen HB, Gjerde B and Rye M. 2003. Breeding programmes for sustainable aquaculture. Journal of Applied Aquaculture, 13: 179-204.

Randall JH. 1976. The Diallel Cross. M.S. Thesis. Department of Plant Breeding and Biometry, Cornell University, pp. 125.

Refstie T and Torstein A. 1978. Selection Experiments with Salmon III. Genetic and environmental sources of variation in length and weight of Atlantic salmon in the freshwater phase. Aquaculture, 14: 221-234.

Refstie T. 1990. Application of Breeding Schemes. Aquaculture, 85: 163-169.

SAS Institute Inc. 1986-1995. SAS User's Guide: Statistics. Version 6.2 Edition. SAS Ins. Inc., P.O. Box. 10066, NC 27605, USA. 956pp.

Smitherman RO and Dunham RA. 1985. Genetics and breeding. In C.S. Tucker, ed. Channel catfish culture, p. 283-316. Amsterdam, Elsevier Scientific Publishing.

Sylven S and Elvingson P. 1992. Evaluation of rainbow trout (*Oncorhynchus mykiss*) strains for body weight, length and age at maturity in different Swedish production systems. Aquaculture, 104: 37-50.

Van der Bank FH, Swart MKJ and Ferreira JT. 1992. Genetic variation in nine rainbow trout (*Oncorhynchus mykiss*) populations in South Africa. Comparative Biochemical Physiology, 103B: 495-499.

Wangila BCC and Dick TA. 1996. Genetic effects and growth performance in pure and hybrids strains of rainbow trout, *Oncorhynchus mykiss*. Aquaculture Research, 27: 35-41.

Webster DA and Flick WA. 1981. Performance of indigenous, exotic, and hybrid strains of brook trout (*Salvelinus fontinalis*) in waters of the Adirondack Mountains, New York. Canadian Journal of Fisheries and Aquatic Sciences, 38: 1701-1707.

Wohlfarth G. 1993. Heterosis for growth rate in common carp. Aquaculture, 113: 31-46.

Wohlfarth GW. 1994. The unexploited potential of tilapia hybrids in aquaculture. Aquaculture and Fisheries Management, 25: 781-788.

Zamora Balbuena G, Vasquez Pelaez CG, Berruecos Villalobos JM and Soto Ruiz L. 1999a. Valuation of some genetic effects of rainbow trout (*Oncorhynchus mykiss*) from a complete diallelic cross of two lines. I. Fertility and viability. Veterinaria Mexico, 30 (3): 231-234.

Zamora Balbuena G, Vasquez Pelaez CG, Berruecos Villalobos J M and Soto Ruiz L. 1999b. Valuation of some genetic effects of rainbow trout (*Oncorhynchus mykiss*) from a complete diallelic cross of 2 lines. II. Growth and production. Veterinaria Mexico, 30 (3): 235-241.
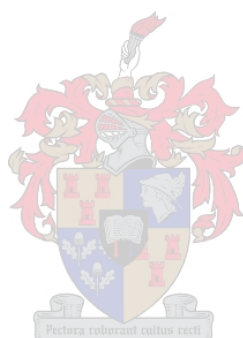
Table 2.1: Progeny groups of rainbow trout obtained from diallel crosses of genetic groups from the EVEN 88 and OVERSEAS 88, and ODD 89 and OVERSEAS 88 base populations to constitute respectively the EVEN 90 and ODD 91 year-groups. C = completed crosses, * = uncompleted crosses. See text for regions of origin.

EVEN 90

Females	Males					
	Eastern	Northern	Western	N. America	Denmark	Scotland
Eastern	C	C	C	*	C	*
Northern	C	C	C	*	*	C
Western	C	C	C	C	*	*
N. America	C	C	C	C	C	*
Denmark	C	C	C	*	*	C
Scotland	*	*	*	*	*	*

ODD 91

Females	Males					
	Eastern	Northern	Western	N. American	Denmark	Scotland
Eastern	C	C	C	*	C	C
Northern	C	C	C	C	C	C
Western	C	C	C	C	C	C
N. America	C	C	C	C	C	C
Denmark	C	C	*	C	*	C
Scotland	C	C	C	C	*	C

Table 2.2: Stages and method of evaluation of growth rate in rainbow trout (*Oncorhynchus mykiss*). A = all fish measured, S = random sample of 15% measured per group.

Stage	Duration (Days)	Age at start (Days)	Approximate		Measurements			
			Number per group	Starting weight (g)	Average Weight (g)		Average Length (mm)	
					Initial	Final	Initial	Final
I	60	120	2000 - 2100	18	S	S	S	S
II	90	190	400	150	S	S	S	S
III	90	290	120	500	A	A	A	A

Table 2.3: Prototype ANOVA table as applied to the separate analyses of variance of the data. See text for explanation of symbols and abbreviations.

Source	Degrees of Freedom	Expected Means Squares (Type I SS)
Heterosis	1	$\sigma^2 + f(w,v,b,a,h)$
Common GCA	5	$\sigma^2 + f(w,v,b.a)$
Differential GCA	5	$\sigma^2 + f(w,v,b)$
Common SCA	14	$\sigma^2 + f(w,v)$
Differential SCA	8	$\sigma^2 + f(w)$
Error	18	σ^2
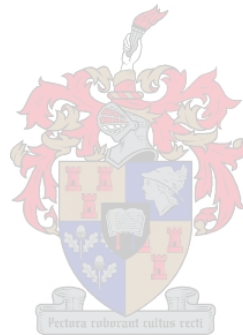
Table 2.4: Average daily weight gain (ADWG) of progeny groups from the EVEN 90 year-group during three consecutive growth stages. The progeny groups were obtained from the crossing of genetic groups from the EVEN 88 and Overseas 88 base populations. (Female parents are listed horizontally and male parents vertically.)

	Regional groups			Overseas groups			Female Group Average (s.e.)
	Eastern	Northern	Western	N. Am.	Denmark	Scotland	
Stage I							
Eastern	0.323	0.263	0.208	-	0.260	-	0.264 (0.024)
Northern	0.252	0.191	0.220	-	-	0.234	0.224 (0.013)
Western	0.244	0.233	0.183	0.171	-	-	0.208 (0.018)
N. America	0.273	0.192	0.218	0.248	0.210	-	0.228 (0.014)
Denmark	0.319	0.347	0.208	-	-	0.237	0.278 (0.033)
Scotland	-	-	-	-	-	-	
Male Group	0.282	0.245	0.207	0.210	0.235	0.236	
Average (s.e.)	(0.017)	(0.029)	(0.007)	(0.038)	(0.025)	(0.002)	
Overall Group	0.268	0.242	0.211	0.219	0.264	0.236	
Average (s.e.)	(0.014)	(0.018)	(0.009)	(0.015)	(0.033)	(0.002)	

Average: Selfs 0.236 (0.032)   Average: Combined Crosses (C+R): 0.241 (0.011)   Average: Crosses (C) 0.225 (0.011)   Average: Reciprocals (R) 0.254 (0.017)

	Eastern	Northern	Western	N. Am.	Denmark	Scotland	Female Group Average (s.e.)
Stage II							
Eastern	1.052	0.997	0.690	-	0.727	-	0.867 (0.092)
Northern	1.107	0.884	1.012	-	-	1.069	1.018 (0.049)
Western	0.991	0.856	0.731	0.731	-	-	0.827 (0.062)
N. America	1.087	0.953	0.792	0.888	0.813	-	0.907 (0.053)
Denmark	1.129	1.218	0.722	-	-	0.990	1.015 (0.108)
Scotland	-	-	-	-	-	-	
Male Group	1.073	0.982	0.789	0.810	0.770	1.030	
Average (s.e.)	(0.024)	(0.064)	(0.058)	(0.079)	(0.043)	(0.039)	
Overall Group	0.973	1.012	0.816	0.877	0.933	1.030	
Average (s.e.)	(0.057)	(0.042)	(0.044)	(0.052)	(0.108)	(0.039)	

Average: Selfs: 0.889 (0.066)   Average: Combined Crosses (C+R): 0.934 (0.040)   Average: Crosses (C): 0.879 (0.054)   Average: Reciprocals (R): 0.984 (0.056)

Table 2.4   continued

<u>Stage III</u>

Eastern	3.885	3.853	3.408	-	4.598	-	3.936 (0.246)
Northern	3.990	2.996	3.216	-	-	3.958	3.540 (0.255)
Western	3.845	3.077	3.026	3.555	-	-	3.376 (0.197)
N. America	4.448	3.597	3.705	3.962	3.520	-	3.846 (0.168)
Denmark	4.448	4.749	3.764	-	-	3.851	4.203 (0.237)
Scotland	-	-	-	-	-	-	
Male Group Average (s.e.)	4.123 (0.135)	3.654 (0.317)	3.424 (0.141)	3.759 (0.204)	4.059 (0.539)	3.905 (0.053)	
Overall Group Average (s.e.)	4.059 (0.135)	3.680 (0.207)	3.450 (0.112)	3.798 (0.145)	4.155 (0.237)	3.905 (0.053)	

Average: Selfs:	Average: Combined Crosses (C+R):	Average: Crosses (C):	Average: Reciprocals (R):
3.467 (0.264)	3.858 (0.115)	3.745 (0.151)	3.958 (0.172)

Table 2.5: Average daily length gain (ADLG) of progeny groups from the EVEN 90 year-group during three consecutive growth stages. The progeny groups were obtained from the crossing of genetic groups from the EVEN 88 and Overseas 88 base populations. (Female parents are listed horizontally and male parents vertically.)

Stage	Regional Groups			Overseas -groups			Female Group
Stage I	Eastern	Northern	Western	N. Am.	Denmark	Scotland	Average (s.e.)
Eastern	1.037	0.995	0.927	-	1.113	-	1.018 (0.039)
Northern	1.036	0.911	0.966	-	-	0.970	0.971 (0.026)
Western	0.986	0.954	0.875	0.946	-	-	0.940 (0.023)
N. America	0.973	0.943	0.943	0.967	0.977	-	0.961 (0.007)
Denmark	1.144	1.071	0.929	-	-	0.952	1.024 (0.051)
Scotland	-	-	-	-	-	-	
Male Group	1.035	0.975	0.928	0.957	1.045	0.961	
Average (s.e.)	(0.030)	(0.028)	(0.015)	(0.011)	(0.068)	(0.009)	
Overall Group	1.026	0.981	0.941	0.958	1.031	0.961	
Average (s.e.)	(0.024)	(0.018)	(0.012)	(0.006)	(0.051)	(0.009)	

Average: Self's: 0.948 (0.035)   Average: Combined Crosses (C+R): 0.990 (0.016)   Average: Crosses (C): 0.981 (0.020)   Average: Reciprocals (R): 0.998 (0.024)

Stage	Eastern	Northern	Western	N. Am.	Denmark	Scotland	Female Group Average (s.e.)
Stage II							
Eastern	0.936	0.892	0.702	-	0.926	-	0.864 (0.055)
Northern	1.023	0.846	0.811	-	-	0.959	0.910 (0.049)
Western	0.917	0.789	0.744	0.858	-	-	0.827 (0.038)
N. America	0.862	0.913	0.754	0.878	0.803	-	0.842 (0.028)
Denmark	0.980	0.883	0.738	-	-	0.771	0.843 (0.055)
Scotland	-	-	-	-	-	-	
Male Group	0.944	0.865	0.750	0.868	0.865	0.865	
Average (s.e.)	(0.027)	(0.022)	(0.018)	(0.010)	(0.061)	(0.094)	
Overall Group	0.905	0.890	0.789	0.845	0.850	0.865	
Average (s.e.)	(0.032)	(0.027)	(0.025)	(0.023)	(0.055)	(0.094)	

Average: Self's: 0.851 (0.040)   Average: Combined Crosses (C+R): 0.858 (0.022)   Average: Crosses (C): 0.840 (0.030)   Average: Reciprocals (R): 0.873 (0.033)

Table 2.5	continued						

Stage III

Eastern	1.123	1.164	1.125	-	1.313	-	1.181 (0.045)
Northern	1.189	0.932	0.997	-	-	1.175	1.073 (0.064)
Western	1.227	0.987	0.956	1.134	-	-	1.076 (0.064)
N. America	1.261	1.102	1.111	1.146	1.151	-	1.154 (0.028)
Denmark	1.292	1.213	1.120	-	-	1.123	1.187 (0.041)
Scotland	-	-	-	-	-	-	
Male Group	1.218	1.080	1.062	1.140	1.232	1.149	
Average (s.e.)	(0.029)	(0.053)	(0.035)	(0.006)	(0.081)	(0.026(	
Overall Group	1.212	1.095	1.082	1.151	1.202	1.149	
Average (s.e.)	(0.026)	(0.038)	(0.033)	(0.023)	(0.041)	(0.026)	

| Average: Self's:	Average: Combined Crosses	Average: Crosses (C):	Average: Reciprocals (R):
1.039 (0.055)	(C+R):	1.148 (0.031)	1.167 (0.032)
	1.158 (0.022)		

Table 2.6: Average daily weight gain (ADWG) of progeny groups from the ODD 91 year-group during three consecutive growth stages. The progeny groups were obtained from the crossing of genetic groups from the ODD 89 and Overseas 88 base populations. (Female parents are listed horizontally and male parents vertically.)

Stage	Regional Groups			Overseas -groups			Female Group
Stage I	Eastern	Northern	Western	N. Am.	Denmark	Scotland	Average (s.e.)
Eastern	0.202	0.298	0.264	-	0.328	0.310	0.280 (0.022)
Northern	0.303	0.250	0.190	0.325	0.313	0.282	0.277 (0.020)
Western	0.210	0.259	0.241	0.316	0.293	0.318	0.273 (0.018)
N. America	0.305	0.278	0.190	0.285	0.343	0.308	0.285 (0.021)
Denmark	0.324	0.253	-	0.266	-	0.271	0.279 (0.016)
Scotland	0.226	0.197	0.292	0.298	-	0.208	0.244 (0.021)
Male Group	0.262	0.256	0.235	0.298	0.319	0.283	
Average (s.e.)	(0.022	(0.014	(0.020	(0.011	(0.011	(0.017	
Overall Group Average (s.e.)	0.277 (0.015)	0.268 (0.013)	0.257 (0.015)	0.291 (0.013)	0.299 (0.033)	0.271 (0.014)	

Average: Self's:	Average: Combined Crosses (C+R):	Average: Crosses (C):	Average: Reciprocals (R):
0.237 (0.015)	0.280 (0.008)	0.297 (0.010)	0.262 (0.012)

Stage II							
Eastern	0.888	1.006	0.753	-	0.989	1.084	0.944 (0.057)
Northern	1.182	0.874	0.717	1.149	1.162	0.926	1.002 (0.078)
Western	0.702	0.864	0.885	0.945	1.028	0.962	0.898 (0.046)
N. America	1.246	1.012	0.701	1.038	1.192	1.032	1.037 (0.078)
Denmark	1.250	0.735	-	0.934	-	0.828	0.937 (0.112)
Scotland	0.849	0.861	0.834	1.063	-	0.699	0.861 (0.058)
Male Group	1.020	0.892	0.778	1.026	1.093	0.922	
Average (s.e.)	(0.096)	(0.042)	(0.035)	(0.040)	(0.050)	(0.057)	
Overall Group Average (s.e.)	0.995 (0.062)	0.953 (0.049)	0.839 (0.037)	1.031 (0.049)	1.015 (0.117)	0.914 (0.039)	

Average: Self's:	Average: Combined Crosses (C+R):	Average: Crosses (C):	Average: Reciprocals (R):
0.887 (0.054)	0.963 (0.032)	0.984 (0.039)	0.941 (0.054)

Table 2.6	continued						
Stage III							
Eastern	3.372	3.477	2.757	-	4.442	4.442	3.698 (0.328)
Northern	3.904	3.311	3.322	3.968	4.642	4.437	3.931 (0.225)
Western	3.825	2.936	3.332	3.859	3.912	3.790	3.609 (0.160)
N. America	4.122	3.760	3.394	3.897	5.138	4.508	4.137 (0.251)
Denmark	5.182	3.520	-	3.731	-	3.457	3.973 (0.407)
Scotland	3.909	3.414	3.478	4.364	-	3.473	3.728 (0.182)
Male Group	4.052	3.403	3.257	3.964	4.534	4.018	
Average (s.e.)	(0.248)	(0.112)	(0.128)	(0.107)	(0.254)	(0.205)	
Overall Group	3.943	3.699	3.461	4.074	4.253	3.927	
Average (s.e.)	(0.212)	(0.153)	(0.125)	(0.155)	(0.477)	(0.148)	

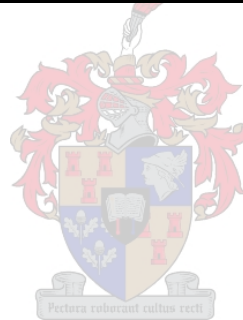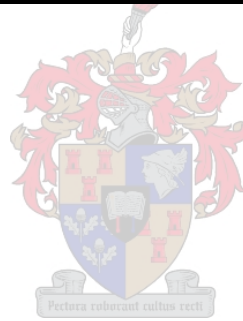| Average: Self's:	Average: Combined Crosses (C+R):	Average: Crosses (C):	Average: Reciprocals (R):
3.477 (0.109)	3.914 (0.114))	4.011 (0.169)	3.811 (0.152)
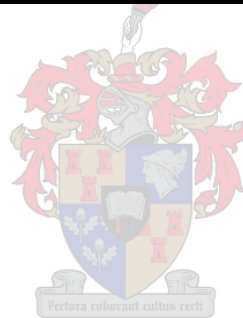
Table 2.7: Average daily length gain (ADLG) of progeny groups from the ODD 91 year-group during three consecutive growth stages. The progeny groups were obtained from the crossing of genetic groups from the ODD 89 and Overseas 88 base populations. (Female parents are listed horizontally and male parents vertically.)

Stage	Regional Groups			Overseas -groups			Female Group Average (s.e.)
Stage I	Eastern	Northern	Western	N. Am.	Denmark	Scotland	
Eastern	0.879	0.972	0.934	-	1.093	1.098	0.995 (0.044)
Northern	1.063	0.970	0.897	1.020	1.065	1.043	1.010 (0.027)
Western	1.021	0.966	0.931	1.005	1.011	1.016	0.992 (0.015)
N. America	1.052	0.988	0.910	0.975	1.205	1.070	1.033 (0.042)
Denmark	1.200	0.979	-	0.990	-	0.998	1.042 (0.053)
Scotland	1.031	0.959	1.008	1.016	-	0.961	0.995 (0.015)
Male Group	1.041	0.972	0.936	1.001	1.094	1.031	
Average (s.e.)	(0.042	(0.004	(0.019	(0.008	(0.041	(0.020	
Overall Group Average (s.e.)	1.034 (0.029)	0.993 (0.015)	0.970 (0.015)	1.023 (0.024)	1.068 (0.113)	1.020 (0.014)	

Average: Self's: 0.943 (0.018)   Average: Combined Crosses (C+R): 1.023 (0.014)   Average: Crosses (C): 1.031 (0.020)   Average: Reciprocals (R): 1.014 (0.019)

	Eastern	Northern	Western	N. Am.	Denmark	Scotland	
Stage II							
Eastern	0.793	0.780	0.729	-	0.930	0.932	0.833 (0.041)
Northern	0.855	0.817	0.774	0.907	0.992	0.938	0.881 (0.033)
Western	0.831	0.780	0.807	0.796	0.852	0.827	0.816 (0.011)
N. America	1.034	0.865	0.791	0.841	0.964	0.931	0.904 (0.036)
Denmark	1.023	0.807	-	0.851	-	0.802	0.871 (0.052)
Scotland	0.863	0.802	0.731	0.851	-	0.749	0.799 (0.026)
Male Group	0.900	0.809	0.766	0.849	0.935	0.863	
Average (s.e.)	(0.042)	(0.013)	(0.016)	(0.018)	(0.030)	(0.033)	
Overall Group Average (s.e.)	0.877 (0.032)	0.847 (0.022)	0.792 (0.013)	0.883 (0.024)	0.903 (0.097)	0.843 (0.024)	

Average: Self's: 0.801 (0.015)   Average: Combined Crosses (C+R): 0.861 (0.016)   Average: Crosses (C): 0.868 (0.022)   Average: Reciprocals (R): 0.853 (0.024)

Table 2.7	continued						
Stage III							
Eastern	0.997	0.956	0.907	-	1.152	1.283	1.059 (0.069)
Northern	1.136	1.025	1.107	1.134	1.244	1.299	1.158 (0.040)
Western	1.228	0.923	1.067	1.131	1.130	1.181	1.110 (0.043)
N. America	1.142	1.122	1.128	1.099	1.260	1.251	1.167 (0.029)
Denmark	1.309	1.140	-	1.091	-	1.112	1.163 (0.050)
Scotland	1.203	1.129	1.038	1.117	-	1.103	1.118 (0.026)
Male Group	1.169	1.049	1.049	1.114	1.197	1.205	
Average (s.e.)	(0.043)	(0.039)	(0.039)	(0.009)	(0.33)	(0.035)	
Overall Group	1.131	1.110	1.084	1.148	1.180	1.172	
Average (s.e.)	(0.043)	(0.033)	(0.033)	(0.019)	(0.125)	(0.027)	

Average: Self's:	Average: Combined Crosses (C+R):	Average: Crosses (C):	Average: Reciprocals (R):
1.058 (0.021)	1.143 (0.020)	1.153 (0.031)	1.131 (0.025)
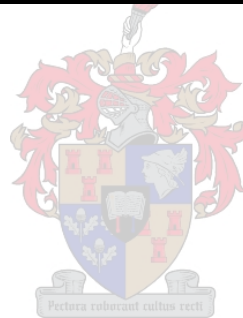
Table 2.8: ANOVA Tables for average daily weight gain (ADWG) and average daily length gain (ADLG)
during Stage I for the year-groups EVEN 90 and ODD 91. (GCA = general combining ability,
SCA = specific combining ability)

Source	df	Type I SS	Mean Square	Significance Levels
Table 2.8a: ANOVA for ADWG: Sums and differences				
Year	1	0.0143	0.0143	0.0148
Heterosis	1	0.0050	0.0050	0.1274
Common GCA	5	0.0212	0.0042	0.1063
Differential GCA	5	0.0068	0.0014	0.6379
Common SCA	14	0.0271	0.0019	0.5065
Differential SCA	8	0.0106	0.0013	0.7084
Error	18	0.0355	0.0020	
Table 2.8b: ANOVA for ADLG: Sums and differences				
Year	1	0.0103	0.0103	0.0336
Heterosis	1	0.0279	0.0279	0.0014
Common GCA	5	0.0866	0.0173	0.0002
Differential GCA	5	0.0248	0.0050	0.0655
Common SCA	14	0.0548	0.0039	0.0825
Differential SCA	8	0.0242	0.0030	0.2077
Error	18	0.0351	0.0020	

Table 2.9: ANOVA Tables for average daily weight gain (ADWG) and average daily length gain (ADLG) during Stage II over year-groups EVEN 90 and ODD 91. (GCA = general combining ability, SCA = specific combining ability)

Source	df	Type I SS	Mean Square	Significance Levels
Table 2.9a: ANOVA for ADWG: Sums and differences				
Year	1	0.0073	0.0073	0.5483
Heterosis	1	0.0713	0.0713	0.0721
Common GCA	5	0.4181	0.0836	0.0097
Differential GCA	5	0.1399	0.0280	0.2605
Common SCA	14	0.1628	0.0116	0.8357
Differential SCA	8	0.1797	0.0225	0.3793
Error	18	0.3517	0.0195	
Table 2.9b: ANOVA for ADLG: Sums and differences				
Year	1	0.0003	0.0003	0.7593
Heterosis	1	0.0202	0.0202	0.0235
Common GCA	5	0.1387	0.0277	0.0003
Differential GCA	5	0.0796	0.0159	0.0057
Common SCA	14	0.0469	0.0033	0.4816
Differential SCA	8	0.0097	0.0012	0.9255
Error	18	0.0595	0.0033	

Table 2.10: ANOVA Tables for average daily weight gain (ADWG) and average daily length gain (ADLG) during Stage III over year-groups EVEN 90 and ODD 91.

Source	df	Type I SS	Mean Square	Significance Levels
Table 2.10a: ANOVA for ADWG: Sums and differences				
Year	1	0.0499	0.0499	0.4651
Heterosis	1	1.9418	1.9418	0.0002
Common GCA	5	6.7665	1.3533	0.0001
Differential GCA	5	1.3255	0.2651	0.0402
Common SCA	14	2.4751	0.1768	0.0875
Differential SCA	8	0.9125	0.1141	0.3161
Error	18	1.6115	0.0895	
Table 2.10b: ANOVA for ADLG: Sums and differences				
Year	1	0.0004	0.0004	0.7435
Heterosis	1	0.0975	0.0975	0.0001
Common GCA	5	0.1854	0.0371	0.0001
Differential GCA	5	0.0626	0.0125	0.0286
Common SCA	14	0.0924	0.0066	0.1395
Differential SCA	8	0.0170	0.0021	0.8022
Error	18	0.0692	0.0038	
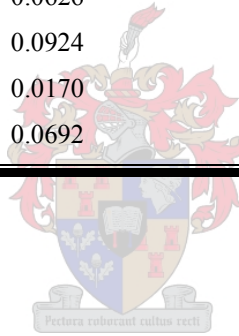
CHAPTER 3:   EVALUATION OF SECOND GENERATION OFFSPRING FROM DOUBLE CROSSESS

1. Abstract

This chapter reports on the evaluation of the rate of growth of second generation offspring from the double crossing of genetic groups from each of two base populations, as part of an ongoing breeding programme for improved rate of growth in rainbow trout. A second generation of outcrossing has further increased the levels of variation within the base populations. The variation within and between families was utilized by means of a multi-stage combined selection procedure that has resulted in an average realized response of 3.5% per year in body length at age 12 months after two generations of multi-stage selection. Correlations between growth parameters at different stages of evaluation indicate that between family selection should be delayed until the last stage of evaluation, as close to the age at marketing as possible, while selection within families could continue throughout all stages of the multi-stage selection procedure.

Keywords: *Oncorhynchus mykiss*, Multi-stage, Selection, Crossing, Growth rate; Correlations

2. Introduction

The aquaculture industry in Southern Africa currently has to rely mainly on the use of genetically undomesticated local species such as tilapia (*Oreochromis mossambicus*), catfish (*Clarias gariepinus*), abalone (*Haliotis midae*) and the introduction of foreign species such as trout (*Oncorhynchus mykiss*), salmon (*Salmo salar*) and oyster (*Grassostria gigas*), which are generally not genetically well adapted to local environmental conditions. The biological productivity of these species under local conditions, in terms of growth rate, feed conversion, survival and yield will, however, have to be improved in order to ensure competitiveness on local and international markets. The development of the aquaculture industry in South Africa is characterized by the absence of government assistance in terms of policy, research and development. The genetic improvement of indigenous and exotic species is therefore primarily the responsibility of the respective sectors of industry in which they are utilized.

Rainbow trout (*O. mykiss*) was first introduced into South Africa during the 1890s from the United Kingdom and was followed later by regular introductions from various sources in the Northern hemisphere. With initial interest being mainly in angling and sport fisheries, no emphasis was placed on genetic improvement and proper genetic management procedures. This situation prevailed throughout the 1970s, when commercial trout farming started to emerge, and into the mid 1980s when concerns were first raised about the genetic quality of the South African gene pool of rainbow trout and the quality of genetic management practices within the industry.

A programme aimed at the genetic improvement of the growth rate of rainbow trout was launched during 1988 by the Division of Aquaculture at the University of Stellenbosch in cooperation with the local producer's organization. The primary objective of the breeding programme was a comparative evaluation of

the growth rate of all available genetic resources, both local and imported, to be followed by the establishment of a base population with the best possible genetic merit and genetic variability for future breeding and selection. This approach of optimal utilization of available genetic resources through a process of selective breeding in order to improve productivity in aquaculture has been advocated by various authors as early as Gjedrem (1975), through Kinghorn (1983), Gall (1990), Refstie (1990) and Gjedrem (1992), Gjoen and Bentsen (1997), Gjedrem (2000) and Knibb (2000), Hulata (2001) to Fjalestad et al. (2003). Chapter one reports on the comparison of the available genetic resources and the formation of the base populations. The results confirmed the presence of significant genetic variation in growth rate between genetic groups, as well as genotype-environment interactions during consecutive stages of growth evaluation associated with variability in water temperature. The nature of correlations between growth criteria over consecutive stages of evaluation suggested that multi-stage selection procedures should be effective in procuring maximum intensities of selection within genetic groups. The efficiency of the procedure of multi-stage selection in the breeding of trout had been confirmed by Allan et al. (1993).

A further objective of the programme was to increase the genetic variation for selection purposes within each of the two base populations through crossing of genetic groups, and to investigate the occurrence of heterosis, general and specific combining ability among such crosses. Various authors have reported on the occurrence of heterosis for growth in salmonids through the intraspecific crossing of strains and populations, although its application is far less common than for species such as carp reported by Wohlfarth (1993), Bakos and Gorda (1995), Hulata (1995) and catfish reported by Smitherman and Dunham (1985) and Dunham (1987). Reports by Gall (1969), Gall and Gross (1978), Kincaid (1981), Ayles and Baker (1983), Wangila and Dick (1996) on the presence of heterosis in growth rate between crosses of domesticated and wild strains of rainbow trout present evidence of heterosis in the range from seven to 55 percent.

Other reports by Gjerde and Refstie (1984), Gjerde (1988), Gjedrem (1992) and Horstgen-Schwark et al. (1996) however present inconclusive evidence with regard to the beneficial application of crossbreeding and the utilization of heterosis in order to obtain genetic improvement in growth of rainbow trout. Nilsson (1993) and Dunham (1996) also reported no significant heterosis for growth rate in respective strain crosses of Chum salmon and Arctic char. McKay and McMillan (1997) reported little evidence of heterosis in growth, maturation and spawning in crosses of three rainbow trout strains, whilst Zamora Balbuena et al. (1999a, b) reported heterosis for growth rate in crosses between two lines of rainbow trout with no heterosis in terms of fertility and viability. Johnson et al. (1999) also reported no evidence of heterosis in disease resistance between wild and domesticated strains of Chinook salmon. Gjedrem (1992) concludes that crossbreeding between strains and inbred lines of rainbow trout in general does not offer significant advantages as a breeding method, although exceptions does occur which justify the evaluation of the presence of heterosis and specific and general combining ability among the crosses of strains and populations. Crossbreeding also remains an important mechanism to increase the levels of additive genetic variation in populations, particularly at the onset of breeding programmes aimed at genetic improvement through selection.

Chapter two reported on the results obtained from the crosses between genetic groups from the respective base populations, which provide evidence of significant levels of heterosis and general combining ability, in

the range of six to ten percent, for weight and length gain during consecutive growth stages. No evidence of specific combining ability could be found among the crosses. It was decided to continue with the procedures for creating maximum possible levels of genetic variation within the base populations through further outcrossing, and to attempt to exploit this variation through stringent multi-stage selection for growth rate within and between groups. This chapter reports on the evaluation of second generation offspring derived from the double crossing of genetic groups originating from the base populations.

3.   Material and methods

3.1.   Formation of the base populations

The formation of the base populations, EVEN 88, OVERSEAS 88 and ODD 89, through a process of sampling, evaluation and selection from various available genetic groups was described in Chapter one. During 1988 and 1989 the founder fish from nine local hatcheries were proportionally combined into four regional groups according to geographic areas of origin, viz.; the Northern group (N) combining the four hatchery-groups from the Northern Highlands Region of South Africa; the Eastern group (E) combining two hatchery-groups from the Eastern Highlands Region; the Western group (W) combining three hatchery-groups from the Western Coastal Region, to form the EVEN 88 and ODD 89 base populations. The founder fish from overseas groups were maintained in their original groups, viz., one from each of North America (A), Denmark (D) and Scotland (S) to form the base population OVERSEAS 88.

3.2.   Diallel crosses: 1990 and 1991

The EVEN 90 and ODD 91 year-groups were obtained through the conduction of a series of diallel crosses of genetic groups from the base populations EVEN 88, OVERSEAS 88 and ODD 89 as described in Chapter two. A total of 21 and 31 single crossed progeny groups were obtained from the respective EVEN 90 and ODD 91 year-groups. The six highest ranking fish were then selected from each of these progeny groups on the basis of body length, according to the procedure as described in Chapter two. Selected individuals received a pedigree identification mark by means of Alcian Blue ink according to the method described by Bridcut (1993).

3.3.   Double crosses: 1992 and 1993

A set of double crosses was then conducted to increase the levels of genetic variation in the respective base populations. Selected individuals from the EVEN 90 year-group were individually mated during the winter of 1992 to produce the EVEN 92 year-group, consisting of 25 double-crossed, full-sib families and four groups of "selfs", as indicated in Table 3.4. These progeny groups were included in the growth evaluation phase and multi-stage selection procedures of 1992. Not all the possible combinations of crosses could be completed for incorporation in the growth evaluation phase due to a lack of facilities. Matings were carried out once per week over a three-week period in an attempt to standardize the age of the full-sib progeny groups. Mature males and females were selected on a random basis from the respective genetic groups for inclusion in the cross breeding system. Matings were also conducted between fish from the same region as

"selfs", for example, the crosses EExEE or AAxAA. In the case of "selfs", sperm and ova were collected from at least three males and three females and pooled before fertilization. These parental fish were, however, selected from the same genetic groups of the EVEN 90 year-group (e.g., EE or AA), to which six unrelated males and females had originally contributed. Contributions from related matings could therefore not be excluded amongst the "selfs" of the EVEN 92 year-group. An excess of double-crossed, full-sib family groups was prepared, of which 25 family groups were identified and selected for inclusion in the growth trials on the basis of survival rates at age 90 days from fertilization.

Fish selected from the ODD 91 year-group were mated in similar fashion during the winter of 1993 to produce the ODD 93 year-group, also consisting of 25 double-crossed, full-sib families and four groups of "selfs" as indicated in Table 3.5.

3.4. Control population

The importance of genetically stable control populations as a reliable reference to monitor genetic improvement in parallel populations under selection has been referred to by many authors such as Kincaid et al. (1977), Hersberger and Iwamoto (1984), Fredeen (1986), Gjerde (1986), Siitonen and Gall (1989), Hersberger et al. (1990) and Gall et al. (1993). A commercial hatchery in the Northern Highlands region of South Africa has stood up to most of the standard requirements for use as a genetic control population such as a large effective population size (Ne>2000), no introduction of foreign genetic material as a requirement to protect its disease free certification, mating according to a rotational breeding system in order to avoid inbreeding and the absence of any designed breeding procedures aimed at internal genetic improvement. An agreement was reached whereby 10 000 eyed ova were supplied by the hatchery on an annual basis for the purpose of inclusion as a control group in the breeding programme. Ova were randomly collected from 10 or more batches of eggs, with eight females and three males contributing to a batch, in order to ensure consistent representation of the control population over years. This source hatchery had in fact also contributed to the formation of both base populations EVEN 88 and ODD 89 and was originally evaluated under the group name of Northern 3 (N3) as reported in Chapter one.

3.5. Growth rate evaluation

Ova from all progeny groups of the EVEN 92 and ODD 93 year-groups were hatched, reared and evaluated in separate containers under standardized conditions at the Jonkershoek Fisheries Research Station near Stellenbosch in the Western Cape Province. The evaluation period was divided into three distinct stages as in the previous years, described in Table 3.1. The three stages of evaluation were determined by practical considerations with regard to the capacity of facilities available and seasonal periods. Growth stages I, II and III were characterized by distinct differences in average water temperature due to seasonal variation. The average water temperature during Stage I was 15.4°C; 21.9°C during Stage II and 14.5°C during Stage III of 1992. Rearing prior to Stage I was done in rectangular cement troughs. Throughout all stages of evaluation, rearing conditions such as numbers per pond, densities, flow rates, type of feed, feeding levels and methods were standardized over all groups, according to commercial standards. A standard range of commercial trout

feed, supplied by WPK Aquafeeds Pty Ltd, with an approximate composition of 40 percent crude protein, 16 percent crude fat, 6 percent crude fiber, 3 percent calcium and 0.7 percent phosphorous, was used during all stages of evaluation in both years. A hand feeding method was used with feeding levels being standardized according to a feed table on the basis of water temperature and size of fish. Groups were kept separately in randomly allocated tanks through all stages of evaluation. Tanks were circular PVC of 10 cubic meters in volume and one meter in depth. Water temperature, oxygen levels, mortality and feed consumption were monitored on a daily basis during each stage. Tanks were sampled every 14-21 days to record average weight and length, and this information formed the basis for the adjustment and standardization of rearing conditions. The standardized application of commercial production conditions is regarded as very important during evaluation and selection in order to minimize environmental variation and possible genotype-environment interactions as emphasized by Refstie (1990) and Falconer and Mackay (1996). Selection should therefore be practiced under conditions similar to those under which the commercial progeny are expected to be produced as recommended by Gjedrem (1992).

A standard number of 2000 fish per family was introduced into Stage I to ensure a high and standardized intensity of selection for all families over all three stages. Reduction in the number of fish from Stage I to II to approximately 500 fish per family was done by means of a box grader on the basis of body width as a practical manner to handle the high initial number (±60 000) of fish. Reduction in the number of fish from Stage II to III to approximately 150 fish per family was done by means of selection based on individual body length. The control group was treated in a similar way to the family groups through all stages of evaluation, including the procedures of reduction from stage to stage. The basis of the choice of body length as the criterium for selection for improved growth rate was as explained in Chapter two (section 3.3).

During stage III the family-groups were evaluated in a common pond environment, according to an incomplete balanced block design, in order to reduce the influence of pond effects during the final stage of evaluation of growth rate. All fish therefore received a family identification mark before the onset of Stage III using the Alcian Blue ink tattooing method as describe by Bridcut (1993). The duration of Stage III for EVEN 92 had to be limited to 70 days because of limitations on pond carrying capacity. The entry numbers of fish for Stage III in ODD 93 were therefore reduced to 120 fish per family to make allowance for the continuation of this stage for the duration of 90 days.

At the end of Stage III the ten best families were identified on the basis of the LS Mean for average daily length gain during stage III. Ten families were selected to contribute to the next generation in order to minimize possible loss of genetic variation between families as is often the case with high intensities of selection between families such as reported by Saxton (1983), and to minimize inbreeding recommended by Gjedrem (1992, 1998). Bentsen and Olesen (2002) recommended that for mass selection programmes, a minimum of 50 breeding pairs should be selected and not less than 30-50 progeny per pair should be tested in order to keep the inbreeding rate at below one percent per generation. From the ten best families the ten best fish were then selected on the basis of individual body length, providing a total of 100 fish to be used as parents of the next generations, the EVEN 94 and ODD 95 year-groups. No counts on the numbers of males

and females could be recorded at this stage due to the absence of externally visual sexual dimorphism at the age of one year. A normal 1:1 sex ratio could be expected among the selected individuals as no evidence could be found in literature of differential sex effects with regard to growth traits of rainbow trout, including weight and length, at the age of one year.

3.6. Definition of traits and experimental design

Traits measured as criteria of growth rate in progeny groups were Average Daily Weight Gain (ADWG) in grams per day and Average Daily Length Gain (ADLG) in mm per day as described in Section 3.5, Chapter one for previous years.

The absence of replication in Stages I and II due to restraints on facilities available precluded any further meaningful analysis of variance structure between and within families but Stage III data were analyzed by the SAS General Linear Model Procedure (SAS Institute Inc., 1996) in accordance with the incomplete balanced blocks as experimental design and expressing ADWG and ADLG as family LS Means. Each block was made up of an unique combination of six families represented by 25 randomly allocated individuals per family, with each family being represented in a total of six blocks. The number of individuals was reduced to 20 per family for the ODD 93 year-group to allow for the extension of the Stage III growth period to a total of 90 days. Further analysis included the calculation of standard phenotypic correlations between traits as well as the Spearman rank correlation coefficient (Snedecor and Cochran, 1980) within and between stages in order to investigate the effects on the multi-stage selection procedure.

4. Results

There were no significant deviations from a normal distribution for the data of either of the two year-groups. The analysis of variance reveals highly significant differences in the growth rates ($P<0.0001$) of families for both the EVEN 92 and ODD 93 year-groups as reflected in Tables 3.2 and 3.3. This was expected in view of the significant differences observed between genetic groups in the previous generations, as reported in Chapters one and two, followed by the further series of double crosses to establish the EVEN 92 and ODD 93 year-groups. Tables 3.2 and 3.3 also reveal the significance of tank effects ($P=0.077$; $P<0.0001$) on growth rate despite efforts to standardize environmental conditions over tanks (see section 3.5). This observation was an important consideration in delaying selection between families to a stage when evaluation could be conducted in a common environment.

Randomization of families in a common environment, as is usually advised for family selection, was prohibited during Stages I and II by practical limitations such as it not being possible to identify the large numbers of groups (n=30), each consisting of a large number of fish (±2000), of small initial size of two to three grams. Replication during the first two stages to remove bias due to tank effects would have led to a pro rata reduction in the number of families that could be evaluated due to the limited number of 31 ponds that were available. This would in turn reduce the intensity of selection between families. Growth rates of families obtained from Stages I and II are therefore reported as single replication values in Tables 3.4 and 3.5. The limited number of 30 families that could be accommodated in the present study is somewhat of a

weakness when compared to other programmes such as the Norwegian national and commercial breeding programmes for rainbow trout and Atlantic salmon in which numbers of families ranging from 120 up to 400 could be accommodated, as reported by Refstie (1990) and Gjedrem (1992, 1998, 2000) but was unavoidable under local circumstances.

In order to achieve optimal usage of the limited facilities available in the present study, selection between families was postponed to the final stage of evaluation (Stage III) during which families could be identified and replicated in a common environment according to the incomplete balanced block design. The smaller number of fish per family (n=120-150) included at the start of Stage III allowed for the identification of all fish according to family groups and a common pond environment could then be employed. The incomplete balanced block was use as experimental design whereby tank effects could largely be eliminated through the calculation of family performance as LS Mean values as indicated in Tables 3.4 and 3.5. Table 3.6 gives the Spearman rank order correlation coefficients (r_s) between the growth parameters ADWG and ADLG over stages I, II and III as calculated for the year-groups EVEN 92 and ODD 93and provides information on the repeatability of and differences between the growth performances of families in consecutive growth stages.

4. Discussion

The objective of the procedure of second generation outcrossing of the original genetic groups was to attempt to maximize the genetic variation within the base populations for subsequent exploitation through multi-stage selection within and between families, in order to improve genetic merit in growth rate. Van der Bank et al. (1992) reported an average heterozygosity value of 0.110±0.009 for the EVEN 88 base population, based on allozyme analysis. Swart (1998) subsequently reported an increase in average heterozygosity to 0.138±0.017 for the EVEN 92year-group, i.e., after two generations of outcrossing. Results of the experiments reported here indicate that an increase in genetic variation within the base populations was achieved through the outcrossing of the original genetic groups. Further evidence for an increase in variation is observable in the coefficients of variation (CV) for the respective traits, ADLG and ADWG, over the two generations of outcrossing. The CV of ADLG and ADWG increased respectively from five and nine percent in the founder population of EVEN 88, to eight and 23 percent in the EVEN 92 population. These observed levels of phenotypic variation compare favourably with those reported for body length (CV=6.9%) and body weight (CV=28%) by Henryon et al. (2002) for rainbow trout. Although these are measures of total phenotypic variation there are no reasons to doubt that the increases are genetic and, as in the case of the increase in average heterozygosity, directly attributable to the two previous generations of outcrossing. In the light of these results on genetic variation generated up to that stage it was decided to discontinue further outcrossing and to proceed with the recurrent selection programme initially envisaged.

The analyses of variance (Tables 3.2 and 3.3) provide evidence of significant differences between the growth rates of families in year-groups EVEN 92 and ODD 93. Significant variability between families in rainbow trout populations have also been reported by Morkramer et al. (1985), Gjerde (1986) and Gall and Huang (1988). Hersberger (1992), Gjoen and Bentsen (1997) and Erythorsdottir et al. (1994) also reported

high levels of additive genetic variance within populations (up to 92 percent of the total genetic variation) respectively for rainbow trout, Atlantic salmon and arctic char. A T-test conducted on LS Mean values for ADLG, provided further evidence of significant differences between families. The LSD-values were calculated as 0.088 and 0.089 respectively for the EVEN 92 and ODD 93 year-groups. The T-test confirms in particular that the growth rate of all ten of the selected families differed significantly from the control group in both years. The control group respectively ranked 30th out of 31 and 27th out of 30 in the year-groups EVEN 92 and ODD 93. The performance of the control group as reported in Tables 3.4 and 3.5 must also be viewed in terms of its performance as group N3 in the initial base populations EVEN 88 and ODD 89 (Chapter one, Table 1.4), where above average performance was displayed over all stages in both year-groups. This is seen as significant evidence for positive realized genetic improvement based on the two generations of multi-stage selection.

The average performance of the EVEN 92 family groups indicates some 17 percent superiority in ADLG over the control group, i.e., 8.5 percent improvement per generation or 4.25 percent per year. The average superiority of the 10 best, or selected families from the EVEN 92 year-group was 26 percent. Likewise, the average performance of the ODD 93 family groups indicated some 12 percent superiority in ADLG over the control, representing a 6.0 percent improvement per generation or a 3.0 percent per year. The average superiority of the 10 selected families from the ODD 93 year-group was also 26 percent. These results compare favourably with the response of 2.5% per year in body weight of rainbow trout over six generations of selection reported by Kincaid (1983), 4.3% per year in body weight of rainbow trout at slaughter over two generations between and within families reported by Gjerde (1988), 3.2 to 3.5% per year in seven month weight of coho salmon over 10 generations reported by Hersberger (1992), 10% per generation (2 years per generation) in growth of salmon by Gjoen and Bentsen (1997), 10 to 15% per generation (2 years per generation) in growth of salmonids by Gjedrem (2000), 5% per year in early growth of brown trout over three generations reported by Vandeputte et al. (2002). The genetic improvement in these comparative reports, however, reflect progress in breeding programmes of a much larger magnitudes in terms of resources, the number of families and overall intensity of selection than in the present case.

Another observation was that the "Self's" groups (e.g. AA-AA, DD-DD, EE-EE and WW-WW) displayed growth rates mainly in the bottom half of the rank orders, although being exposed to the same selection pressures since the EVEN 88 and ODD 89 generations. This is perhaps indicative that both additive and non-additive genetic effects were contributing to superior performance of the double-crossed families of year-groups EVEN 92 and ODD 93, as in the case of single-cross family groups reported in Chapter two, Tables 2.6 and 2.7. The one group, WW-WW, in particular was consistently inferior, in correspondence with the performance of the original genetic groups from the western region as reported in Chapter one, Table 1.4.

The observed changes in the rank order of families over the three stages of evaluation is perhaps again suggestive of genotype-environment interactions, although the magnitude was small in comparison to that observed in the EVEN 88 and ODD 89 year-groups, reported in Chapter one, Table 1.4. This might be explainable by the mixing of alleles and allele frequencies among the different genetic groups of the base

populations during the two generations of outcrossing. Genetic differences between families would then be less profound than those between the original groups in the base populations. The differences in average water temperature for Stage I (15.4°C), Stage II (21.9°C and Stage III (14.5°) was probably the main factor of environmental variation over the three stages of evaluation and is suspected as being the main contributor to changes in rank order of families. Significant genotype-temperature interactions associated with temperature and expressed as a change in the rank order of families of rainbow trout for growth rate has been reported by McKay et al. (1984). Reports by Beacham (1990), Heath et al. (1993), Bagley et al. (1994) and Wild et al. (1994) confirm the presence of significant genotype- environment interaction in salmonid species between families within populations for traits such as growth, weight, length and sexual maturation under a variety of environmental conditions such as fresh and seawater, stocking densities, temperatures and photoperiods. In this instance, the observed changes in rank order were of a minor nature and would not seem to justify selection for temperature specific strains nor the use of an index of performance under varying conditions as recommended by McKay et al. (1984). The findings of this study, as well as the other sited reports referred to, indicate that genotype-environment interaction should be assessed on the basis of the specific genotypes, traits and environments under consideration, when decisions are made on the design of selection programmes.

The observed changes in the rank order of families is further quantified by the correlation coefficients presented in Table 3.6 and confirm the presence of significant positive associations between the growth parameters ADWG and ADLG over the various stages in both year-groups, as reported for EVEN 88 and ODD 89 year-groups in Chapter one, Table 1.6. The estimates of correlations between growth parameters at different stages were based on family average and are in a range of 0.31 to 0.86. These values correspond to the values reported by Su et al. (2002) between body weight at contiguous ages, ranging from 0.57 to 0,93, decreasing with increasing interval between ages. As might be expected, the values are considerably higher than those based on individual growth performance, such as the values of 0.20 reported by Aulstad et al. (1972), 0.37 by Mckay et al. (1986), 0.10 to 0.18 by Hersberger et al. (1990), 0.32 to 0.36 by Swift (1991), 0.35 to 0.44 by Elvingson and Johansson (1993) and 0.10 by Withler and Beacham (1994). However, it is doubtful whether these associations can justify implementation of selection between families during the earlier growth stages. For example, of the top 10 families selected on the basis of Stage III ADLG in EVEN 92 and ODD 93, six and seven families respectively maintained a top ten position during the two preceding growth stages. It is therefore recommended that selections between families rather be delayed until the final stages of evaluation, at an age as close as possible to that of marketing, until such time as specific information on genetic correlations and the heritability of the traits at the different stages are available to consider an alternative strategy on the basis of correlated responses. The procedure to delay selection to an age closely related to that at marketing has also been recommended by Gjedrem (1983), Morkramer et al. (1985), Gjerde (1986) and Gall and Huang (1988).

These correlations further also reflect on the effectiveness of the multi-stage selection procedure in combination with the method of outcrossing as a breeding strategy. A multi-stage selection procedure is often required in both animal and plant breeding in order to maximize selection intensity and effectiveness

within the constraints of limited facilities, manpower and funds (Cotterill and James, 1981). Initial selection may cause a loss of genetic variation to an extent that the efficiency of selection during the later stages, as well as overall genetic gain, might be reduced as indicated by Saxton (1983). The efficiency of the current multi-stage selection procedure also needs to be assessed, in particular with regard to relationships between selection criteria at the different stages of selection. The correlation values reported in Tables 1.6 and Table 3.6 indicate reasonably high relationships between the respective traits at the different stages, and are confirmed by the significance levels reflected in Table 3.6. An analysis by Allan et al. (1993) has indicated that the current procedure of introducing a maximum number of individuals per family into Stage I, followed by multi-stage selection during Stages I, II and III on the basis of different selection criteria, offers a meaningful advantage of up to 25 percent over the alternative of a random reduction in the number per family with selection delayed until the end of Stage III.

5. Conclusions

Indications are that the two consecutive generations of outcrossing of genetic groups from the base populations have significantly increased levels of genetic variation within and between families of the EVEN 92 and ODD 93 year-groups, as is also indicated by increased average heterozigosity found by Van der Bank et al. (1992) and by Swart (1998), as well as the coefficients of variation for ADWG and ADLG of families. Two generations of multi-stage selection within and between families have yielded a meaningful realized response of respectively 4.25% and 3.0% per year in body length at age 12 months in the two populations. These results compare favourably with reports of a realized response of 4.3% per year in body weight at slaughter of rainbow trout (Gjerde, 1988), 3.2-3.5% per year in 7-month weight of coho salmon (Hersberger et al., 1990) and 5% per year in early growth of brown trout over three generation (Vandeputte et al., 2002). Multi-stage selection programmes should be carefully structured according to genetic parameters (heritabilities, genetic correlations and selection intensities) of the populations concerned to ensure that the available variation within and between families is not reduced by initial selection to the detriment of the total gain that can be achieved. Based on the positive associations between growth criteria at different stages it is recommended that selection between families be delayed until the later stages closer to the age at marketing, while selection within families could commence throughout all stages of evaluation in order to make optimal use of the high selection intensity that can be achieved in salmonid species.

6. References

Allan JS, Randall JH and Brink D. 1993. Predicting the efficiency of multi-stage selection in the breeding of rainbow trout. South African Journal of Animal Science: 23: 1-3.

Aulstad D, Gjedrem T and Skjervold H. 1972. Genetic and environmental sources of variation in length and weight of rainbow trout. J. Fish. Res. Board Can., 29:237-241.

Ayles GB and Baker RF. 1983. Genetic differences in growth and survival between strains and hybrids of rainbow trout stocked in lakes in the Canadian prairies. Aquaculture, 33: 269-280.

Bagley MJ, Bentley B and Gall GAE. 1994. A genetic evaluation of the influence of stocking density on the early growth of rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 121 (4): 313-326.

Bakos J and Gorda S. 1995. Genetic improvement of common carp strains using intraspecific hybridization. Aquaculture, 129: 183-186.

Beacham TD. 1990. A genetic analysis of meristic and morphometric variation in chum salmon (*Oncorhynchus keta*) at three different temperatures. Canadian Journal of Zoology, 68 (2): 225-229

Bentsen HB and Olesen I. 2002. Designing aquaculture mass selection programmes to avoid high inbreeding rates. Aquaculture, 204 (3-4): 349-359.

Bridcut EE. 1993. A coded Alcian blue marking technique for the identification of individual brown trout, *S. trutta*.: An evaluation of its use in fish biology. Biology and Environment: Proceedings of the Royal Irish Acadamy. Vol. 93B, 2, 107-110.

Cotterill PP and James JW. 1981. Optimising Two-stage independent culling selection in tree and animal breeding. Theoretical and Applied Genetics, 59: 67-72.

Dunham RA. 1987. American catfish breeding programmes. Selection, hybridization and genetic engineering in aquaculture. European Inland Fisheries Advisory Commission (EIFAC) Symposium. Bordeaux, 1986.

Dunham RA. 1996. Contribution of genetically improved aquatic organisms to global food security. International Conference on Sustainable Contribution of Fisheries to Food Security. Government of Japan and FAO, Rome, 150 pp.

Elvingson P and Johansson K. 1993. Genetic and environmental components of variation in body traits of rainbow trout in relation to age. Aquaculture, 118: 191-204.

Erythorsdottir E, Petursdottir T and Svavarsson E. 1994. Comparison of strains of Arctic charr (*Salvelinus alpinus*) in Iceland for body weight and age at sexual maturity. Proceedings, 5th World Congress on Genetics Applied to Livestock Production, University of Guelph, Guelph, Ontario, Canada, 7-12 August 1994. Volume 19. Selection and quantitative genetics; growth; reproduction; lactation; fish; fiber; meat; p. 387-390.

Falconer DS and Mackay TFC. 1996. Introduction to Quantitative genetics. Fourth Edition. Longman Scientific & Technical, England, 464 pp.

Fjalestad KT, Moen T and Gomez-Raya L. 2003. Prospects for genetic technology in salmon breeding programmes. Aquaculture Research, 34 (5): 397-406.

Fredeen H. 1986. Monitoring genetic change. Aquaculture, 57:1-26.

Gall GAE. 1969. Quantitative inheritance and environmental response of rainbow trout. In O.W. Neuhaus & J.E. Halver, eds. Fish research, New York, Academic Press.

Gall GAE. 1990. Basis for evaluating breeding plans. Aquaculture, 85: 125-142.

Gall GAE and Gross SJ. 1978. Genetic studies of growth in domesticated rainbow trout. Aquaculture, 13: 225-234.

Gall GAE and Huang N. 1988. Heritability and selection schemes for rainbow trout: body weight. Aquaculture, 73: 43-56.

Gall GAE, Bakar Y and Famula T. 1993. Estimating genetic change from selection. Aquaculture, 111 (1-4): 75-88.

Gjedrem T. 1975. Possibilities for genetic gain in salmonids. Aquaculture, 6: 23-29.

Gjedrem T. 1983. Genetic variation in quantitative traits and selective breeding in fish and shellfish. Aquaculture, 33: 51-72.

Gjedrem T. 1992. Breeding plans for rainbow trout. Aquaculture, 100: 73-83.

Gjedrem T. 1998. Developments in fish breeding and genetics. ACTA Agriculturae Scandinavica Section A-Animal Science, 19-26.

Gjedrem T. 2000. Genetic improvement of cold-water fish species. Aquaculture Research, 31 (1): 25-33.

Gjerde B and RefstieT. 1984. Livestock Production Sciences, 11: 207-226.

Gjerde B and Gjedrem T. 1984. Estimates of phenotypic and genetic parameters for carcass traits in Atlantic salmon and rainbow trout. Aquaculture, 36: 97-110.

Gjerde B. 1986. Growth and reproduction in fish and shellfish. Aquaculture, 57: 37-55.

Gjerde B. 1988. Complete diallel cross between six inbred groups of rainbow trout. Aquaculture, 75: 71-87.

Gjerde B. 1989. Body traits in rainbow trout II Estimates of heritabilities and phenotypic and genetic correlations. Aquaculture, 80: 25-44.

Gjoen HM and Bentsen HB. 1997. Past, present, and future of genetic improvement in salmon aquaculture. ICES Journal of Marine Science, 54 (6): 1009-1014.

Heath DD, Bernier NJ, Heath JW and Iwama GK. 1993. Genetic, environmental, and interaction effects on growth and stress response of chinook salmon (*Oncorhynchus tshawytscha*) fry. Canadian Journal of Fisheries and Aquatic Sciences, 50 (2): 435-442.

Henryon M, Jokumsen A, Berg P, Lund I, Pedersen PB, Olesen NJ and Slierendreht WJ. 2002. Genetic variation for growth rate, feed conversion efficiency, and disease resistance exists within a farmed population of rainbow trout. Aquaculture, 209 (1-4): 59-76.

Hersberger WK and Iwamoto RN. 1984. Systematic genetic selection and breeding in salmonid culture and enhancement programmes. In. C.J. Sinderman (Editor). Proceedings of the 11th US-Japan Meetings on Aquaculture, Salmon Enhancement, Tokyo, Japan, 1019 October, 1982. NOAA Tech. Rep. NMFS 27, pp. 29-32.

Hersberger WK, Myers JM, Iwamoto RN, Mcauley WC and Saxton AM. 1990. Genetic changes in the growth of coho salmon in marine net-pens, produced by ten years of selection. Aquaculture, 85: 187-197.

Hershberger WK. 1992. Genetic variability in rainbow trout populations. Aquaculture, 100: 51-71.

Horstgen-Schwark G, Fricke H and Lanholz HJ. 1996. The effect of strain crossing on the production performance of rainbow trout. Aquaculture, 57: 14-152.

Hulata G. 1995. A review of genetic improvement of the common carp (*Cyprinus carpio L.*) and other hybrids by crossbreeding, hybridization and selection. Aquaculture, 129: 143-155.

Hulata G. 2001. Genetic manipulations in aquaculture: a review of stock improvement by classical and modern technologies. Genetica, 111 (1-3): 155-173.

Kincaid HL, Bridges WR and von Limbach B. 1977. Three generations of selection for growth rate in fall-spawning rainbow trout. Trans American Fisheries Society, 106: 621-628.

Kincaid HL. 1983. Results from six generations of selection for accelerated growth rate in a rainbow trout population. The future of aquaculture in North America, p. 26-27. Fish Culture Section, American Fisheries Society.

Johnson R, Bryden C and Heath DD. 1999. Can indicators of disease resistance predict performance in pure and hybrid strains of wild and domestic chinook salmon? Bulletin of the Aquaculture Association of Canada. 1999 4:31-33.

Kinghorn BR. 1983. A review of quantitative genetics in fish breeding. Aquaculture, 31: 283-304.

Knibb W. 2000. Genetic improvement of marine fish - which method for industry? Aquaculture Research, 31: 11-23.

McKay LR, Friars GW and Ihssen PE. 1984. GenotypexTemperature interactions for growth rate of rainbow trout. Aquaculture, 41: 134 -140.Refstie, T., 1990. Application of Breeding Schemes. Aquaculture, 85: 163-169.

McKay LR, Ihssen PE and Friars GW. 1986. Genetic Parameters of growth in rainbow trout, Salmo gairdneri, as a function of age and maturity. Aquaculture, 58: 241-254.

McKay LR and McMillan I. 1997. Growth, maturation and spawning times in crosses of three strains of rainbow trout. Bulletin of the Aquaculture Association of Canada, 2: 15-17.

Morkramer S, Horstgen Schwark and Lanholz HJ. 1985. Evaluation of different European rainbow trout populations under intensive production conditions. Aquaculture, 44: 303-320.

Nilsson J. 1993. Arctic charr strain crosses: effects on growth and sexual maturity. Journal of Fish Biology, 43 (2): 163-171.

Refstie T. 1990. Application of Breeding Schemes. Aquaculture, 85: 163-169.

SAS Institute Inc. 1986-1995. SAS User's Guide: Statistics. Version 6.2 Edition. SAS Ins. Inc., P.O. Box. 10066, NC 27605, USA. 956pp.

Saxton A.M. 1983. A comparison of exact and sequential methods in multi-stage index selection. Theoretical and Applied Genetics, 66: 23-28.

Schmidt M. 1985. Produktionsleistung von forellen unterschiedlicher gewichtsklassen unter besonderer berucksichtigung des schlachtkorperwertes. Disseration, Universitat Gottingen, 59 pp.

Siitonen L and Gall GAE. 1989. Response to selection for early spawn date in rainbow trout. Aquaculture, 78: 153-161.

Smitherman RO and Dunham RA. 1985. Genetics and breeding. In C.S. Tucker, ed. Channel catfish culture, p. 283-316. Amsterdam, Elsevier Scientific Publishing.

Snedecor GW and Cochran WG. 1980. Statistical methods. Iowa State University Press, Ames, Iowa, U.S.A. 507 pp.

Su GS, Liljedahl LE and Gal GAE. 2002. Genetic correlations between body weight at different ages with reproductive traits in rainbow trout. Aquaculture, 213(1-4): 85-94

Swart MKJ. 1998. Levels of genetic variation in rainbow trout (*Oncorhynchus mykiss*) after two generation of selection. Proceedings of the Fourth Conference of the Aquaculture Association of Southern Africa; Stellenbosch, South Africa, September 1997.

Swift BD. 1991. Heritabilities and genetic correlations for weight, length and survivability in fresh and salt water of So and S_1 coho salmon (*O. kisutch*). M.Sc. Thesis, University of British Colombia, 51pp.

Vandeputte M, Quillet E and Chevassus B. 2002. Early development and survival in brown trout (*Salmo trutta fario L.*): indirect effects of selection for growth rate and estimation of genetic parameters. Aquaculture, 204 (3-4): 435-445.

Van der Bank FH, Swart MKJ and Ferreira JT. 1992. Genetic variation in nine rainbow trout (*Oncorhynchus mykiss*) populations in South Africa.. Comparative Biochemical Physiology, 103B: 495-499.

Wangila BCC and Dick TA. 1996. Genetic effects and growth performance in pure and hybrids strains of rainbow trout, *Oncorhynchus mykiss*. Aquaculture Research, 27: 35-41.

Wild V, Simianer H, Gjoen HM and Gjerde B. 1994. Genetic parameters and genotype x environment interaction for early sexual maturity in Atlantic salmon (*Salmo salar*). Aquaculture, 128 (1-2): 51-65.

Withler RE and Beacham TD. 1994. Genetic variation in body weight and flesh colour of the coho salmon (*Oncorhynchus kisutch*) in British Columbia. Aquaculture 119: 135 –148.

Wohlfarth G. 1993. Heterosis for growth rate in common carp. Aquaculture, 113: 31-46.

Zamora Balbuena G, Vasquez Pelaez CG, Berruecos Villalobos JM and Soto Ruiz L. 1999a. Valuation of some genetic effects of rainbow trout (*Oncorhynchus mykiss*) from a complete diallelic cross of two lines. I. Fertility and viability. Veterinaria Mexico, 30 (3): 231-234.

Zamora Balbuena G, Vasquez Pelaez CG, Berruecos Villalobos J M and Soto Ruiz L. 1999b. Valuation of some genetic effects of rainbow trout (*Oncorhynchus mykiss*) from a complete diallelic cross of 2 lines. II. Growth and production. Veterinaria Mexico, 30 (3): 235-241.

Table 3.1: Stages of evaluation of growth rate in rainbow trout (*Oncorhynchus mykiss*) for year-groups EVEN 92 and ODD 93. (Number of families: EVEN 92 = 31, ODD 93 = 30; S = fish sampled for measurement, A= all fish per tank measured).

Stage	Duration (Days)	Range at start of growth stage				Measurements			
		Age (days from fertilization) (s.d.)	Avg. number per group (s.d.)	Avg. group weight (g) (s.d)	Percentage selected (s.d.)	Weight (g)		Length (mm)	
						Initial	Final	Initial	Final
EVEN 92									
I	70	120 (7)	2000 (27)	2.3 (0.7)	All included	S	S	S	S
II	80	200 (7)	500 (13)	42.9 (10.7)	25.2 (2.2)	S	S	S	S
III	70	290 (7)	150 (14)	197 (29)	7.8 (0.4)	A	A	A	A
ODD 93									
I	60	120 (7)	2000 (23)	1.8 (0.4)	All included	S	S	S	S
II	90	190 (7)	500 (11)	35.0 (6.2)	25.8 (1.5)	S	S	S	S
III	90	300 (7)	120 (8)	211 (25)	6.1 (0.3)	A	A	A	A
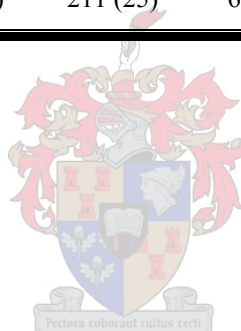
Table 3.2: Analysis of Variance of Average Daily Length Gain (mm/day) of 31 rainbow trout families from the EVEN 92 year-group during stage III of evaluation, over a period 70days.

Source	d.f.	Type I SS	Mean Square	Pr > F
Block (Tank)	30	7 665.317	255.511	0.0005
Family	30	23 341.707	778.057	<0.0001
Error	125	13 546.742	108.374	
Total	185	44 553.766		

Table 3.3: Analysis of Variance of Average Daily Length Gain (mm/day) of 30 rainbow trout families from the ODD 93 year-group during stage III of evaluation, over a period 90 days.

Source	d.f.	Type I SS	Mean Square	Pr > F
Block (Tank)	30	97 064.869	3 235.496	0.0001
Family	29	92 238.615	3180.642	0.0001
Error	120	27 786.708	231.556	
Total	179	217 090.192		

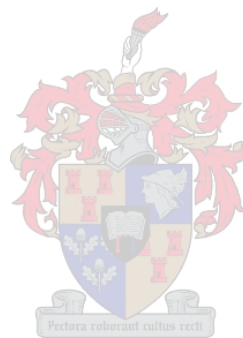**Table 3.4:** The Average Daily Weight Gain (ADWG: g/day) and Average Daily Length Gain (ADLG: mm/day) of rainbow trout families from the EVEN 92 year-group, ranked per stage according to the ADLG value. (Stage I and II ADWG and ADLG are presented as absolute values. Stage III ADWG and ADLG are presented as LSMean values).

	Stage I			Stage II			Stage III	
Crosses	ADWG	ADLG	Crosses	ADWG	ADLG	Crosses	ADWG	ADLG
EW-SD	0.440	1.390	DE-NA	1.048	1.067	DE-NA[*]	5.240	1.351
AW-DE	0.398	1.363	WA-SD	1.383	1.043	ED-SN[*]	4.197	1.328
SN-EA	0.690	1.361	AW-DE	1.200	1.025	EW-SD[*]	4.320	1.296
WA-SN	0.417	1.353	WA-ED	1.561	1.014	DE-SN[*]	4.239	1.290
WN-EA	0.590	1.347	EW-SD	1.261	1.011	WE-ND[*]	5.306	1.281
DE-SN	0.463	1.336	EA-ND	1.255	0.990	WA-SN[*]	4.321	1.266
EA-SD	0.703	1.331	NE-SD	0.760	0.979	EA-ND[*]	3.806	1.256
WE-ND	0.453	1.322	WN-EA	1.546	0.971	NE-SD[*]	3.676	1.252
EA-ND	0.463	1.289	SN-EA	1.762	0.970	SN-EA[*]	4.365	1.251
DE-NA	0.441	1.288	DE-SN	1.541	0.965	AW-DE[*]	3.313	1.250
EN-WD	0.708	1.279	WE-ND	0.990	0.944	EN-WD	4.274	1.245
NE-SD	0.428	1.275	EA-WD	1.177	0.938	WN-EA	4.409	1.233
EE-EE	0.466	1.271	WA-SN	1.118	0.935	WA-ED	3.989	1.231
WA-ED	0.460	1.265	EW-SN	1.138	0.933	EA-WD	3.671	1.224
NA-SD	0.517	1.262	EA-SD	1.504	0.887	EE-EE	3.522	1.220
WE-NA	0.453	1.253	AW-SN	1.281	0.865	WE-NA	2.991	1.195
AW-SN	0.467	1.253	WA-NE	1.096	0.864	WA-SD	3.562	1.194
NA-WD	0.432	1.247	ED-SN	1.114	0.857	EW-SN	3.065	1.169
EW-SN	0.419	1.242	EN-WD	1.360	0.848	EA-SD	3.809	1.167
WA-SD	0.402	1.242	WE-NA	1.100	0.848	AW-SN	3.853	1.160
ED-SN	0.365	1.237	ED-NW	1.164	0.839	AA-AA	3.727	1.155
ED-NW	0.390	1.229	DD-DD	0.746	0.822	NA-WD	3.332	1.153
EA-WD	0.385	1.189	EE-EE	1.255	0.813	DD-DD	2.642	1.146
Control	0.418	1.182	AA- AA	0.998	0.754	ED-NW	3.070	1.142
SN-WD	0.337	1.177	WW-WW	0.827	0.710	SN-WD	2.902	1.139
WW-WW	0.357	1.175	NA-SD	0.923	0.687	WA-NE	3.571	1.136
AA- AA	0.356	1.172	NW-SD	0.704	0.639	NW-SE	3.124	1.074
WA-NE	0.397	1.163	**Control**	0.794	0.630	NA-SD	2.782	1.070
NW-SE	0.385	1.153	NW-SE	0.640	0.617	NW-SD	2.829	1.046
NW-SD	0.395	1.082	NA-WD	0.680	0.594	**Control**	2.926	1.018
DD-DD	0.236	0.930	SN-WD	0.495	0.580	WW-WW	3.361	0.928

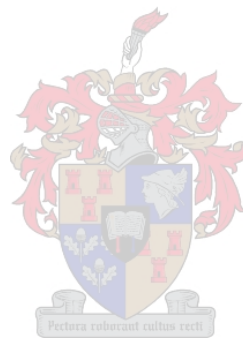- Top 10 families selected on the basis of Stage III ADLG.

Table 3.5: The Average Daily Weight Gain (ADWG: g/day) and Average Daily Length Gain (ADLG: mm/day) of rainbow trout families from the ODD 93 year-group, ranked per stage according to the ADLG value. (Stage I and II ADWG and ADLG are presented as absolute values. Stage III ADWG and ADLG are presented as LSMean values)

	Stage I			Stage II			Stage III	
Crosses	ADWG	ADLG	Crosses	ADWG	ADLG	Crosses	ADWG	ADLG
DN-SE	0.379	1.262	AW-SD	1.255	0.977	DN-EA[*]	4.178	1.244
AW-SD	0.377	1.232	DN-SE	1.165	0.942	DN-SE[*]	4.138	1.236
SD-NE	0.372	1.164	AD-NS	1.239	0.936	AD-EW[*]	3.229	1.216
AD-NS	0.358	1.162	ED-SW	1.069	0.925	SD-NE[*]	4.864	1.213
DA-WS	0.418	1.150	DN-EA	1.001	0.916	AS-WE[*]	3.587	1.189
ED-SW	0.346	1.143	DA-WS	1.249	0.907	AN-EW[*]	3.661	1.186
DE-NS	0.367	1.133	SD-NE	1.210	0.903	DA-WS[*]	4.161	1.159
AW-DE	0.371	1.129	DE-NS	1.193	0.862	DE-NS[*]	4.151	1.150
DN-EA	0.355	1.129	DA-ES	1.117	0.842	ED-SW[*]	3.992	1.149
AW-NE	0.364	1.120	AW-DE	1.047	0.837	EA-SD[*]	3.829	1.124
WN-ES	0.367	1.113	SE-NW	0.933	0.834	AS-ED	4.093	1.110
AS-EN	0.333	1.109	AS-EN	0.732	0.822	AW-SD	4.174	1.083
EA-SD	0.378	1.104	AW-NE	1.141	0.819	SE-NW	4.021	1.081
AN-EW	0.377	1.097	EA-SD	1.427	0.810	AS-EN	3.476	1.080
WA-SN	0.331	1.091	AS-WE	1.001	0.791	AW-DE	3.668	1.080
DN-SA	0.412	1.089	SA-NW	0.996	0.782	EW-NA	3.451	1.065
AD-EW	0.377	1.068	AD-EW	1.090	0.768	SA-NW	3.333	1.058
EW-NA	0.345	1.060	EE-EE	0.878	0.755	AD-NS	4.158	1.049
SE-NW	0.346	1.059	WA-ES	1.010	0.728	AW-NE	3.737	1.043
DE-WN	0.361	1.058	AN-EW	0.873	0.723	DE-WN	3.497	1.033
DA-ES	0.365	1.053	DE-WN	0.886	0.698	WA-SN	2.934	1.030
AS-WE	0.347	1.052	DN-SA	0.888	0.685	DN-SA	3.649	1.028
WA-ES	0.348	1.040	WA-SN	0.751	0.673	WA-ES	3.149	1.022
WE-SA	0.303	1.028	WE-SA	0.768	0.668	DA-ES	3.852	1.017
SA-NW	0.296	1.017	EW-NA	0.974	0.653	WE-SA	3.712	1.001
EE-EE	0.309	1.003	DW-NA	0.903	0.647	EE-EE	3.730	0.983
AS-ED	0.352	0.986	**Control**	0.615	0.642	**Control**	2.829	0.943
DW-NA	0.333	0.980	WN-ES	0.841	0.612	WN-ES	3.336	0.718
Control	0.290	0.972	AS-ED	0.719	0.592	DW-NA	2.999	0.680
WW-WW	0.343	0.955	WW-WW	1.158	0.570	WW-WW	3.284	0.623

* Top 10 families selected on the basis of Stage III ADLG.

Table 3.6: The Spearman rank order correlation coefficient for growth parameters Average Daily Weight
Gain (ADWG) and Average Daily Length Gain (ADLG) of rainbow trout, over growth stages
I, II and III of the EVEN 92 and ODD 93 year-groups. (Stage number indicated by numerical
value linked to growth parameter; e.g. ADLG1= Average daily length gain during stage I)

Year	Growth parameters per Stage				
EVEN 92	ADWG1	ADWG2	ADWG3	ADLG1	ADLG2
ADWG2	0.630**				
ADWG3	0.468**	0.581**			
ADLG1	0.567**	0.556**	0.691**		
ADLG2	0.348*	0.657**	0.630**	0.566**	
ADLG3	0.314*	0.489**	0.744**	0.754**	0.782**
ODD 93	ADWG1	ADWG2	ADWG3	ADLG1	ADLG2
ADWG2	0.574**				
ADWG3	0.451**	0.503**			
ADLG1	0.649**	0.568**	0.631**		
ADLG2	0.435*	0.676**	0.731**	0.786**	
ADLG3	0.432*	0.378*	0.858**	0.686**	0.761**

EVEN 92: n=31, *P(5%)= 0.355, **P(1%)= 0.456   ODD 93: n=30, *P(5%)= 0.361, **P(1%)= 0.463
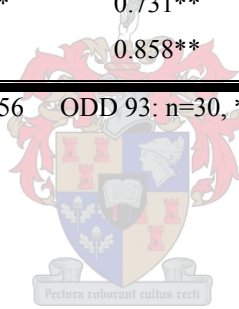
CHAPTER 4:   ESTIMATION OF GENETIC AND PHENOTYPIC PARAMETERS AND COMPARATIVE EVALUATION OF FOURTH, FIFTH AND SIXTH GENERATION PROGENY GROUPS

1. Abstract

This chapter reports on the estimation of genetic and phenotypic parameters and the evaluation of the rate of growth of rainbow trout over consecutive generations of multi-stage selection. The genetic parameters were estimated on the basis of a hierarchical mating scheme in two separate year-groups, with 10 and 15 sires mated to 20 and 30 dams producing 20 and 30 full-sib groups with a total of around 40 000 and 60 000 individuals in the respective populations. High heritability values in the range of 0.40 to 0.53 were recorded for body weight and length at 150 days confirming the presence of high levels of additive genetic variation. The higher heritability estimates based on the dam components of variance gave some non-additive genetic and common environmental effects. Genetic correlations between the traits were also high, in the range of 0.74 to 0.82. A cumulative realized response of 50% for body weight was recorded for the EVEN year-group after six generations of selection equal to an average improvement of 8.3% per generation. A cumulative realized response of 33% was recorded for body length in the ODD year-group after five generations of selection equal to an average improvement of 6.6% per generation.

Keywords: *Oncorhynchus mykiss*, Genetic parameters, Heritability, Growth rate, Multi-stage Selection

2. Introduction

The aquaculture industry in Southern Africa currently has to rely mainly on the use of genetically undomesticated local species such as tilapia (*Oreochromis mossambicus*), catfish (*Clarias gariepens*), abalone (*Haliotis midae*) and the introduction of foreign species such as trout (*Oncorhynchus mykiss*), salmon (*Salmo salar*) and oyster (*Grassostria. gigas*), which are generally not genetically well adapted to local environmental conditions. Undomesticated genotypes do not convert available food resources in an efficient way, they tend not to thrive in captivity and live constantly under stressed conditions.

    This situation is not very dissimilar for other parts of the world, developing countries in particular, where only between 1% and 2% of production is based on the use of genetically improved stocks (Gjedrem, 1997). The biological productivity of these species under local conditions, in terms of growth rate, feed conversion, survival and yield, will however have to be improved in order to ensure competitiveness on local and international markets (Gjedrem, 2000). Substantial progress has been made during the last 50-60 years through the application of modern animal breeding theory and the genetic productivity of domesticated populations of mammals and birds is often 3-5 times higher than their wild progenitors (Bentsen and Gjerde, 1994). The development of the aquaculture industry in South Africa is characterized by the absence of government assistance in terms of policy, research and development so that genetic improvement of indigenous and exotic species is therefore primarily the responsibility of the respective sectors of industry in which they are utilized.

Rainbow trout (*Oncorhynchus mykiss*) was first introduced into South Africa during the 1890s from the United Kingdom and was followed later by regular introductions from various sources in the Northern hemisphere. With initial interest being mainly in angling and sport fisheries, little emphasis was placed on genetic improvement and proper genetic management procedures. This situation prevailed throughout the 1970s, when commercial trout farming started to emerge, and into the mid 1980s when concerns were first raised about the genetic quality of the South African gene pool of rainbow trout and the quality of genetic management practices within the industry. This inferior genetic status was attributed mainly to uncontrolled levels of inbreeding over a long period of time, enhanced by genetic isolation and the small effective population size which prevailed at local hatcheries. Effective population sizes are often too low because of the high reproductive capacity of trout which allows the use of low numbers of broodstock. Inbreeding depression in different traits for cold-water fish has been estimated at 3-6% per 10% increase in inbreeding (Kincaid, 1976a,b; Gjerde at al., 1983). Reduced productivity caused by inbreeding depression has been documented by various authors and is widely reported to be a problem in commercial fish farming (Kincaid, 1976a,b; Gjerde at al., 1983, Eknath and Doyle, 1990). For example, Allendorf (1975) reported a reduction of average heteroziosity of up to 40% in hatchery populations of trout when compared to natural populations.

The development of strains adapted to local conditions was also favoured by local producers because the environmental conditions in Southern Africa under which trout are produced differ substantially from those in the countries of origin. Particularly in terms of a substantially higher annual water temperature profile where temperatures in summer often rise above 24 degrees Celsius for prolonged periods of time. High risks of mortality and low productivity, particularly for the production of larger size salmon trout (>1.2kg) are associated with these conditions.

Against this background a programme aimed at the genetic improvement of the growth rate of rainbow trout was launched during 1988 by the Division of Aquaculture at the University of Stellenbosch in cooperation with local producer's organisations. The first step in the breeding programme was the formation of a synthetic base population with a broad genetic base as recommended by Refstie (1990) and Gjedrem (1992). This was done by the collection and evaluation of available genetic material from local and overseas hatcheries as described in Chapter 1, followed by two generations of outcrossing to increase genetic variability for future breeding and selection, as described in Chapters 2 and 3. The breeding goal was determined as that of improved growth to enable local producers to complete the production cycle for salmon trout (>1.2kg) before the onset of the second summer (age 16-18 months). Phenotypic and genetic parameters were not estimated at the onset of the selection programme due to the absence of a suitable experimental design (i.e., half-sib family structure) during the first three generations. As heritability estimates on a full-sib basis should not be used as values of additive genetic variance because non-additive, maternal effects and other common environmental effects may be included. The programme therefore initially had to rely on estimates on phenotypic and genetic parameters for growth traits in rainbow trout (CV, variation, heritability, correlations) as reported by various authors (e.g. Gjerde and Gjedrem, 1984; McKay et al., 1986; Gall and Huang, 1988a,b; Gjerde

and Shaeffer, 1989; Rye and Refstie, 1995, Hersberger et al., 1990, ; Nilsson 1992, Winkelman and Peterson, 1994) which gave positive indications for prospects of improvement through selection.

A multi-stage selection method was introduced in order to conduct the programme within the constraints of limited facilities whilst achieving optimal intensities of selection (i ≈ $3.3\sigma_p$). The efficiency of the procedure of multi-stage selection in the breeding of trout under the specific conditions of the breeding programme had been confirmed by Allan et al. (1993). The selection method incorporates both within and between family selection and requires the use of different growth related traits as criteria for selection at the various stages of selection. Response was measured with the use of a control population. Commercialisation of the breeding programme was done in collaboration with local producer's organisations which has ensured the efficient distribution and application of improved genotypes originating from the programme. These elements of the breeding programme correspond to the recommendations by Gjedrem (2000) for the genetic improvement of cold-water species.

This chapter reports on the estimation of genetic and phenotypic parameters and the comparative evaluation of growth rate of fourth, fifth and sixth generation progeny groups.

3.   Materials and methods

3.1.   Formation of the base populations

The formation of the base populations, EVEN 88, OVERSEAS 88 and ODD 89, through a process of sampling, evaluation and selection from various available genetic groups was described in Chapter one. Fish from nine local and four overseas hatcheries were introduced for a comparative evaluation of growth rate during which superior individuals from each of the genetic groups were selected according to a multi-stage selection process over a period of twelve months. The selected founder fish from nine local hatcheries were proportionally combined into four regional groups according to geographic areas of origin, viz.; the Northern group (N) combining the four hatchery-groups from the Northern Highlands Region of South Africa; the Eastern group (E) combining two hatchery-groups from the Eastern Highlands Region; the Western group (W) combining three hatchery-groups from the Western Coastal Region, to form the EVEN 88 and ODD 89 base populations. The selected founder fish from overseas groups were maintained in their original groups, viz., one from each of North America (A), Denmark (D) and Scotland (S) to form the base population OVERSEAS 88.

3.2.   Diallel crosses: 1990 and 1991

A series of diallel crosses of genetic groups from the base populations were conducted to establish the EVEN 90 and ODD 91 year-groups according to procedures described in Chapter two. A total of 21 and 31 single cross progeny groups were obtained in the respective year-groups (see Table 2.1). The highest ranking individuals were then selected from each of the progeny groups on the basis of individual growth performance over a 12 month period, according to a multi-stage selection procedure described in Chapter two. Selected individuals received a family pedigree identification mark by means of an Alcian Blue ink tattoo method described by Bridcut (1993).

3.3. Double crosses: 1992 and 1993

Selected individuals from the previous year-groups were used to conduct a set of double crosses on the basis of individual mating to establish the EVEN 92 and ODD 93 year-groups according to procedures described in Chapter three. Each year-group consisted out of 29 double-crossed full-sib families and a control group (see Table 3.4, 3.5). The control group was obtained from a commercial hatchery in the Northern Highlands region of South Africa in accordance to a procedure described in Chapter three. The ten best families from each year-group were identified on the basis of the LSMean average daily length gain at the end of a twelve month multi-stage selection process described in Chapter three. The ten best fish out of an original 2000 were selected from each of the ten best families on the basis of individual body length, to be used as parents of the next generations. Selected individuals received a family pedigree identification mark by means of an Alcian Blue ink tattoo method described by Bridcut (1993).

3.4. Half-sib family groups: 1994 and 1995

The mating system within the programme of genetic improvement has progressed from that of group mating and mass spawning in the first two generations, through individual full-sib mating in the third generation to that of individual half-sib mating in the fourth generation of selection. The EVEN 94 year-group was established during the breeding season of 1994 through the mating of males and females selected from the previous generation (EVEN 92) according to a hierarchical mating scheme with 20 sires mated to 40 dams producing 40 full-sib groups, according to a procedure describe in Chapter three and summarized in the previous section. All matings were conducted by means of artificial spawning and controlled fertilization over a limited period of three weeks in order to minimize age variation during the stages of growth evaluation and selection. Male trout produce sperm throughout the winter breeding season over a period of 10 to 12 weeks. Two male parents were chosen at random from each of the ten selected families to ensure even representation of selected material. Female trout, however, ovulate only once within the breeding season. Female parents were therefore chosen from each of the ten selected families on the basis of ovulation within the designated three week spawning period. Due to a limited number of tanks within the facility only 15 of the available number of 20 half-sib family groups could be assigned to Stage I of the comparative growth phase. The 15 half-sib family groups were selected on the basis of family size, i.e., the number of individuals available at the onset of Stage I (age five months from fertilization), with a minimum requirement of 2 000 fish per family to ensure a high and standardized intensity of selection for all families over all three stages. Some of the experimental groups were lost due to unforeseen circumstances (operational failures) during the course of the growth stages that have lead to a corresponding reduction in the number of half sib and full sib families as reflected in Table 4.2 and Table 4.4.

Fish selected from the ODD 93 year-group were treated in similar fashion during the winter of 1995 to produce the ODD 95 year-group, also consisting of 20 half-sib and 40 full-sib family groups.

3.5. Control population

The selection programme included a standard control population as reference for genetic improvement. The importance of genetically stable control populations as a reliable reference to monitor genetic improvement in parallel populations under selection has been referred to by many authors such as Kincaid et al. (1977), Fredeen (1986) and Hersberger et al. (1990). The limitation of genetically constant control lines in terms of variance caused by random error and genetic drift was emphasised by Gall et al. (1993). The control group was supplied by a commercial hatchery from the Northern Highlands region of South Africa that met most of the standard requirements for use as a genetic control population such as a large effective population size (Ne>2000), no introduction of foreign genetic material as a requirement to protect its disease free certification, mating according to a rotational breeding system in order to avoid inbreeding and the absence of any designed breeding procedures aimed at internal genetic improvement. The hatchery supplied some 10 000 eyed ova on an annual basis for inclusion as a control group in the breeding programme. Ova were randomly collected from 10 or more batches of eggs, with eight females and three males contributing to a batch, in order to ensure consistent representation of the control population over years. A random sample of 2 000 fish were introduced into Stage I to represent the control population during the comparative growth stages.

3.6. Growth rate evaluation

Ova from all progeny groups of the EVEN 94 and ODD 95 year-groups were hatched, reared and evaluated in separate containers under standardized conditions at the Jonkershoek Fisheries Research Station near Stellenbosch in the Western Cape Province. The evaluation period was again divided into three distinct stages as in the previous years, described in Table 4.1. The three stages of evaluation were determined by practical considerations with regard to the capacity of facilities available and seasonal periods. Growth stages I, II and III were characterized by distinct differences in average water temperature due to seasonal variation. The average daily water temperature during Stage I was in the range of 12 to 18°C; for Stage II in the range of 18 to 24°C and 11 to 16°C during Stage III. Rearing prior to Stage I was done in rectangular cement troughs. Throughout all stages of evaluation, rearing conditions such as numbers per pond, densities, flow rates, type of feed, feeding levels and methods were standardized over all groups, according to commercial standards for trout farming. A standard range of commercial trout feed, supplied by WPK Aquafeeds Pty Ltd, with an approximate composition of 40% crude protein, 16% crude fat, 6% crude fiber, 3% calcium and 0.7% phosphorous, was used during all stages of evaluation in both years. A hand feeding method was used with feeding levels being standardized according to a feed table on the basis of water temperature, number and size of fish. Groups were kept separately in randomly allocated tanks through all stages of evaluation leading to Stages III, during which communal housing of groups were introduced. Tanks were circular PVC of 10 cubic meters in volume and one meter in depth. Water temperature, oxygen levels, mortality and feed consumption were monitored on a daily basis during each stage. Tanks were sampled every 14-21 days to record average weight and length, and this information formed the basis for the adjustment and standardization of rearing conditions. The standardized application of commercial production conditions is regarded as

very important during evaluation and selection in order to minimize environmental variation and possible genotype-environment interactions as emphasized by Refstie (1990) and Falconer and Mackay (1996). Even though genotype by environment interactions may be of limited importance in heterogeneous populations across a range of applied farming systems selection should be practiced under conditions similar to those under which the commercial progeny are expected to be produced as recommended by Gjedrem (1992) and Bentsen and Gjerde 1994.

A standard number of 2000 fish per family was introduced into Stage I to ensure a high and standardized intensity of selection ($i \approx 3.3\sigma_p$) for all families over all three stages. Reduction in the number of fish from Stage I to II to approximately 500 fish per family was done by means of a box grader on the basis of body width as a practical manner to handle the high initial number ($\pm 60\ 000$) of fish. Reduction in the number of fish from Stage II to III to approximately 125 fish per family was done by means of selection based on individual body length. The control group was treated in a similar way to the family groups through all stages of evaluation, including the procedures of reduction from stage to stage. The basis of the choice of body length as the criterium for selection for improved growth rate was as explained in Chapter 2 (section 3.3).

The mean performance of non-replicated families could not be used for selection between families during Stages I and II because of confounding effects between the family performance and the environmental effects of the test unit. The significance of such environmental or tank effects has been confirmed during previous generations as reported in Chapter 3 (Table 3.3, 3.4). Gall and Bakar (2002) also identified variation among rearing tanks as a significant fixed effect and demonstrated the potential errors in estimating environmental and genetic effects if performance-tested groups are confounded to separated tanks. Comparisons of and selection between families was therefore delayed until Stage III when a group identification system and a common rearing environment could be introduced. All fish received a family identification mark before the onset of Stage III by means of the Alcian Blue ink tattoo method as describe by Bridcut (1993). During stage III the family-groups were evaluated in a communal tank environment, according to an incomplete balanced block design, in order to reduce the influence of pond effects during the final stage of evaluation of growth rate.

At the end of Stage III the ten top ranking families were selected on the basis of the family LSMean for average daily length gain during stage III to contribute to the next generation in order to minimize possible loss of genetic variation between families as is often the case with high intensities of selection between families such as reported by Saxton (1983), and to minimize inbreeding as recommended by Gjerde et al. (1996), Gjedrem (1998) and Bentsen and Gjerde (1994). The use of a small number of parents can also lead to high variability of response which adds a measure of risk to the breeding programme (Meuwissen and Woolliams, 1994). Gjedrem (2000) recommend that males should be selected from the 10-15 highest ranking families and females from the top 15-20 families, with reference to the Norwegian trout breeding programme that accommodates up to 200 families per generation. Bentsen and Olesen (2002) recommended that for mass selection programmes, a minimum of 50 breeding pairs should be selected and not less than 30-50 progeny per pair should be tested in order to

keep the inbreeding rate at below 1% per generation. The need to restrict the number of selected individuals from each family is even more important in a combined selection programme than under mass selection (Bentsen and Gjerde, 1994). The ten best performing fish were then selected from the 10 top ranked families on the basis of individual body length, providing a total of 100 fish to be used as parents of the next generations, i.e. the EVEN 96 and ODD 97 year-groups. The absence of an effective and affordable individual tagging system prevented the use of BLUP breeding values as a criterion for selection of individuals. No counts on the numbers of males and females could be recorded at this stage due to the absence of externally visual sexual dimorphism at the age of one year. A normal 1:1 sex ratio could, however, are expected among the selected individuals on the basis of results in previous years.

3.7. Full-sib family groups: 1996 to 2001

The selection programme continued for the next three generation, from 1996 to 2001, on the basis of individual full-sib mating followed by a multi-stage selection procedure based on within and between family selection, according to a similar procedure as in previous years described in Section3.5. The EVEN 96, 98 and 2000 year-groups and the ODD 95, 97 and 99 year-groups were created in this manner. Stage I and II are characterised by within family selection on the basis of individual performance, with a combination of within and between family selection being introduced in Stage III. The top ten families were selected on the basis of LSMean average daily length gain (ADLG) at the end of Stage III, from which the top ten individuals were selected on the basis of individual body length. Although both family and individual performance were recorded, the absence of an effective and affordable individual identification system prevented the introduction of selection on the basis of estimated breeding values according to the Best Linear Unbiased Prediction (BLUP) method.

3.8. Definition of traits and experimental design

The estimation of genetic and phenotypic parameters was based on measurements of individual body weight and individual body length at the end of Stage I at an approximate age of 150 days from fertilization. Individual body weight was recorded to the nearest gram by means of a calibrated electronic balance. Individual body length was recorded as fork length to the nearest millimeter by means of a calibrated measuring board. All fish were anesthetized with the use of a benzocaine solution prior to the measurements. Sampling was conducted through the collection of all fish per group in a holding net. From there on sub-samples were taken with a hand-net, from which the required number of individuals were randomly taken for measurements. Stage I data was analyzed according to standard procedures for the analysis of variances (ANOVA) and covariates (ANACOVA) explained by Falconer and Mackay (1996) with the use of the EXEL Microsoft XP software package. No attempt was made to repeat the analysis on data from Stage II and III as the initial normal multivariate distribution was distorted by the consecutive stage of selection which would affect the reliability and complicate the interpretation of results. Selection for one variate may reduce the genetic and phenotypic variances of subsequently measured variates and may also affect the covariances between them.

The comparison of growth rate between families during Stage III was conducted in a similar way as in previous years on the basis of Average Daily Length Gain (ADLG) in mm per day as described in Chapter 1, Section 3.5. Stage III data were analyzed by the SAS General Linear Model Procedure (SAS Institute Inc., 1996) in accordance with the incomplete balanced blocks as experimental design expressing ADLG as family LSMeans. Each block was made up of an unique combination of six families represented by a total 20 randomly allocated individuals per family, with each family being represented in a total of six different blocks (tanks).

3.9. Correction for age effect

The estimation of components of variance and genetic parameters for both year-groups was based on individual body weight and body length at the end of Stage I. Although spawning was limited to a three week period it still caused differences in age between families in both year-groups, i.e., EVEN 94 (152.2 $\pm$ 5.7 days) and ODD 95 (152.4 $\pm$ 4.0 days). The effect of age on individual body weight and length was tested with the use of regression analysis according to the models: body weight = $(\alpha + \beta.\text{Age})$ and body length = $(\alpha + \beta.\text{Age})$. The analysis confirmed a significant linear initial age effect on body weight ($P_{\text{EVEN 94}} \leq 0.001$, $P_{\text{ODD 95}} = 0.011$) and on body length ($P_{\text{EVEN 94}} \leq 0.001$, $P_{\text{ODD 95}} \leq 0.001$) at the end of Stage I (age 150 days) for both year-groups. An age correction was therefore made for individual body weight and length on the basis of the deviation of age from the overall mean and the estimated regression coefficient, β. All further analysis of the components of variance and genetic parameters were conducted on the basis of body weight and length corrected for age effect.

The effect of age on average daily length gain during Stage III was tested in a similar manor for both year-groups. The results indicate that there is no significant linear initial age effect on average daily length gain during Stage III ($P_{\text{EVEN 94}} = 0.173$, $P_{\text{ODD 95}} = 0.317$) in both year-groups and further analysis were therefore conducted on the basis of uncorrected measurements and LSMean values for average daily length gain.

4. Results

The results of an analysis of variance of growth data at the end of Stage I for the EVEN 94 and ODD 95 year-groups are presented in Tables 4.2 and 4.4. The analysis confirm significant differences for mean weight (P=0.099) and length (P=0.095) among sires at the 10% level as well as for mean weight (P$\leq$0.001) and length (P$\leq$0.001) between dams within sires at the 1% level, in the EVEN 94 year-group. Similar results were obtained in the ODD 95 year-group with significant differences in mean weight (P=0.064) and length (P=0.040) among sires and for mean weight (P$\leq$0.001) and length (P$\leq$0.001) between dams within sires.

Estimates of components of variance and genetic and phenotypic parameters for body weight and length at the end Stage I of the EVEN 94 year-group is presented in Table 4.3. The estimates of heritabilities from sire components of variation were 0.48±0.25 for weight and 0.49±0.25 for length. The estimates of heritabilities from dam components of variation were 0.53±0.25 for weight and 0.52±0.25

for length. The genetic correlation between body weight and length was estimated as 0.82±0.21. The relatively high standard errors of some of the estimates such as for heritability is attributed to the limited number (10) of half-sib family groups available and the small number of individuals per family that were sampled (n=16) for the EVEN 94 year-group. A total of 5 half-sib family groups were eliminated from the EVEN 94 year-group on the basis of low survival rates during Stage I due to the outbreak of an infectious disease.

Estimates of components of variance and genetic and phenotypic parameters for body weight and length at the end of Stage I of the ODD 95 year-group is presented in Table 4.5. The estimates of heritabilities from sire components of variation were 0.41±0.16 for weight and 0.49±0.18 for length. The estimates of heritabilities from dam components of variation were 0.52±0.16 for weight and 0.53±0.18 for length. The genetic correlation between body weight and length was estimated as 0.74±0.01. The inclusion of a larger number (15) of half-sib families and the sampling of larger number of fish per family (n=25) improved the degrees of freedom and contributed to a substantial reduction in the standard error of estimates in the ODD 95 year-group (Table 4.5).

The results on an analysis of variance of ADLG for Stage III of the EVEN 94 and ODD 95 year-group is presented in Tables 4.6 and 4.8 and confirm the presence of significant differences between families for both traits in both year-groups (P≤0.001). Tables 4.7 and 4.9 presents a summary of family LSMean values for ADLG over five consecutive generations of selection for the EVEN and ODD year-groups from 1992 to 2000. The realized response is expressed relative to the performance of the control population. Fluctuations in the number of families per year-group were due to unforeseen random events such as technical failure and disease outbreaks that have led to the elimination of some families prior to the Stage III of growth evaluation. The cumulative response in ADLG in the EVEN year-group amounts to 50% after six generations and to 33% in the ODD year-group after five generations of selection.

5. Discussion

5.1. Differences between families

The analysis of variance of growth data confirms the presence of significant differences between families over all stages of growth evaluation for the EVEN 94 (Table 4.2, 4.6) and ODD 95 year-groups (Table 4.4, 4.8). This is an important observation confirming the maintenance of significant levels of variation in spite of the expected reduction in genetic and phenotypic variance in subsequent growth stages due to the multi-stage selection procedure (Allan et al., 1993). Similar results were obtained during the course of generations of EVEN 96 (Table 4.7) to ODD 99 (Table 4.9), with the exception of EVEN 2000 (P=0.138) where no significant differences between family means for ADLG could be detected. This, however, could be a first indication of reduced variation between families after 6 generations of selection and needs to be closely monitored over the following generations. A closed breeding population will always be subjected to long term accumulation of inbreeding and loss of genetic variation. The genetic variability of a population is critical not only in terms of response to selection in each generation, but also

for the long term limits of response. Selection limits on a theoretical basis is however only expected after 20-30 generations (Falconer and Mackay, 1996). Outcrossing between the ODD and EVEN populations remains an integral part of the overall strategy of genetic improvement and will be introduced to re-establish genetic variability within the population and to neutralise accumulated inbreeding as recommended by Bentsen and Gjerde (1994). Strategies for continuous (re)-introduction of genetic variability from outside the breeding nucleus without any adverse performance consequences will be required to alleviate the effect of inbreeding and genetic drift and to ensure the long term genetic progress (Olesen at al., 2003).

The analyses of variance as presented in Tables 4.6 and 4.8 also confirm the presence of significant tank effects (P≤0.001) caused by common environmental effects. This observation necessitates the delay of between family selection until such stage that communal rearing can be accommodated in the experimental design in order to minimize the influence of tanks effects and environmental variance on the accuracy of selection procedures. The period of separate rearing should also be minimised to avoid large environmental correlations of performance within sib families (Bentsen and Gjerde, 1994).

## 5.2. Estimates of the variance components, genetic and phenotypic parameters

Estimates of the genetic and phenotypic components of variance revealed high values for heritabilities of 150 day body weight (0.41 - 0.53) and length (0.49 - 0.53) based on the sire, dam and total components of variance in both year-groups (Table 4.3 and 4.5). The heritability estimates based on sire component of variance (h^2_s) of body weight and length were higher than reported values for growth related traits in salmonids such as 0.09-0.29 (Aulstad et al., 1972), 0.26 (Kinghorn, 1981), 0.21-0.32 (Gjerde and Gjedrem, 1984), 0.10-0.36 (Gjerde et al., 1994) and 0.28 by Vandeputte et al. (2002). The results are, however, in line with other reports of higher sire based heritabilities of growth traits in salmonids with values of 0.38-0.66 reported by Mckay et al. (1986), 0.20-0.52 by Gall and Huang (1988a,b), 0.25-0.62 by Nilsson (1992), 0.28-0.61 by O'Flynn et al. (1992), 0.21-0.53 by Crandell and Gall (1993), 0.38-0.42 by Jonasson (1993), 0.28-0.74 by Elvingson and Johansson (1993), 0.35-0.4 by Choe and Yamazaki (1998), 0.35-0.53 by Henryon et al. (2002) and 0.46-0.61 by Kause et al. (2003). Values of heritability for growth traits is generally expected to decline with age based on reports by McKay et al. (1986), O'Flynn et al. (1992), Crandell and Gall (1993) and Winkelman and Peterson (1994). The high values of heritability as obtained in this study can probably be ascribed to the special effort to increase the levels of genetic variability in the populations through the initial two generations of outcrossing of genetic groups. The high standard error of these estimates must also be taken into consideration, particularly that of the EVEN 94 year-group. The standard error of the estimates for ODD 95 has been substantially reduced with the inclusion of more family groups (15 half-sib families) and larger sample sizes (n=25).

The estimates of heritability in the EVEN 94 year-group based on the dam components of variance (h^2_d) were higher than those calculated from sire components of variance (h^2_s), i.e. for body weight h^2_d = 0.531 *vs* h^2_s = 0.484 and for body length h^2_d = 0.517 *vs* h^2_s = 0.491. The margin of difference for estimates in the ODD 95 year-group increased to values of h^2_d = 0.521 *vs* h^2_s = 0.408 for body weight and h^2_d = 0.528 *vs* h^2_s = 0.506 for body length. These differences are probably an indication of the influence

of non-additive genetic, maternal and/or tank effects on these traits. Gjedrem (1992) has also reported heritability estimates for a series of traits in rainbow trout, with estimates from the dam component being on average 0.13 higher than for the sire component. Similar results of higher estimates of heritability for growth traits from dam components are reported by Naevdal et al. (1975), Refstie and Steine (1978). This is however not a general trend for salmonids species and various researchers reported on estimates of heritability values for growth traits with no significant differences between the sire and dam estimates (Gunnes and Gjedem, 1981; Gjerde and Gjedrem, 1984; Gjerde and Schaeffer, 1989). No effort was made in this study to estimate these effects. There are some reports of significant maternal effects caused by egg size and quality on growth rate of trout up to four months after hatching (Chevassus and Blanc, 1979; Gjerde 1986) weakening with increasing age. Maternal traits though are not likely to be important for salmonids as is often the case for species with a high fecundity and those which do not nurture their offspring. In this instance growth rate was also determined at an age of 4 to 10 months during which maternal effects are expected to weaken to insignificant levels as reported by Chevassus and Blanc (1979) and Gjerde (1986).

High genetic correlations of 0.82 and 0.74 between body weight and length were respectively recorded in both year-groups, and correspond with reported values between growth traits of salmonids, i.e., 0.98 (Jonasson, 1993), 0.98 (Winkelman and Peterson, 1994), 0.95 by Myers et al. (2001) and 0.92-0.99 by Henryon et al. (2002).

The similarity between the genetic parameters as observed over the two year-groups can probably be ascribed to their common genetic origin and parallel experimental procedures during previous generations as outlaid in Chapters 1 to 3. Refstie and Steine (1978) also found that heritability estimates for weight and length of Atlantic salmon to be highly consistent between year classes.
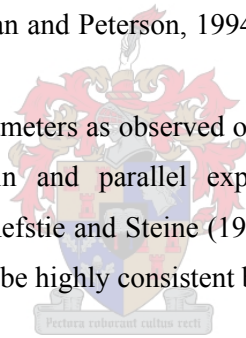
5.3. Selection response

The ranked family LSMean values for ADLG for Stage III of growth evaluation for five consecutive generations from EVEN 92 to EVEN 2000 are presented in Table 4.7 and indicate a stepwise improvement in the overall average of families and the realized response expressed relative to the control. The improvement over the first six generations of selection is fairly consistently in the range of 7% to 10% per generation, i.e. 3.5% to 5% per year. These values compare favourable with the responses to selection in the range of 10-20% per generation that has been achieved in several selection experiments and programmes for growth traits in salmonids species. Kincaid et al. (1977) reported an increase of 67% in body weight of rainbow trout at 147 days after three generations of selection, equal to 15% per generation or 5% per year. Gjerde (1986) reported a genetic gain of 13.0% per generation or 4.3% per year for body weight of rainbow trout at 2.5 years after two generations of selection and an improvement of 14.4% in growth rate of Atlantic salmon after one generation. Hershberger et al. (1990) reported an improvement of 10.1% per generation in growth rate of coho salmon after four generations. Gjedrem (1998) reports on an improvement of 15% per generation in growth rate of Atlantic salmon when compared to wild populations. Gjerde and Korsvoll (1999) reported a cumulated realized response in growth rate of Atlantic salmon of 83.9% after 6 generations, equal to 14% per generation. Vandeputte

et al. (2002) also reported a selection response of 6-12% per generation in growth rate of brown trout after five generations of selection, based on results from the PROSPER programme (Chevassus, unpublished).

The LSD-values in Tables 4.7 and 4.9 indicate that nearly all of the families over all generations differ significantly from the control population, with the exception of the lowest ranking families in each year-group. The selected top ranked families also show a clear statistical distinction on the basis of their LSMean values from the excluded families. The decline in the rate of response in comparison with the previous years, together with the finding of non-significant differences between families (P=0.138) for EVEN 2000, may point to an overall decline in genetic variation within the EVEN year-group. This observation may also be explained by normal variation in selection response over generations and needs to be closely monitored over the following generations in view of possible need for reintroduction of new genetic variance.

The ranked family LSMean values over four consecutive generations from ODD 95 to ODD 99 as presented in Table 4.9 indicates a similar stepwise improvement in the overall average and the realized response. The response to selection of the ODD year-groups is however lower in comparison to the EVEN year-groups, on average about 2% per generation based on the cumulated realized response. The lower level of response in the ODD year-group is somewhat unexpected in view of the equal heritability values and similar experimental procedures that were followed for both year-groups, including that of selection intensities. The ODD year-group in fact had the benefit of slightly higher levels of selection intensity for the between family component of selection, i.e. a larger number of families to choose from. The lower observed response may also be explained by normal variation in selection response over the initial generations.

The results of this investigation provide proof of high levels of additive genetic variation within both populations expressed in terms of heritability estimates. The realised responses, both cumulative and per generation, provide evidence of the efficiency of the current multi-stage selection procedure that compares favourably with reports of progress in other breeding schemes.

6. References

Allan JS, Randall JH and Brink D. 1993. Predicting the efficiency of multi-stage selection in the breeding of rainbow trout. South African Journal of Animal Science: 23: 1-3.

Allendorf FW. 1975. Genetic variability in species poessing extensive gene duplication; genetic interpretation of duplicate loci and examination of genetic variation in populations of rainbow trout. PhD. Thesis, University of Washington, Seattle, WA, 98pp.

Aulstad D, Gjedrem T and Skjervold H. 1972. Genetic and environmental sources of variation in length and weight of rainbow trout. Journal of Fisheries Research Board of Canada, 29: 237-241.

Bentsen HB and Gjerde B, 1994. Design of fish breeding programmes. Proceedings of the 5th Congress on Genetics Applied to Livestock Production. Volume 19: 353-359.

Bentsen HB and Olesen I. 2002. Designing aquaculture mass selection programmes to avoid high inbreeding rates. Aquaculture, 204 (3-4): 349-359.

Bridcut EE. 1993. A coded Alcian blue marking technique for the identification of individual brown trout, *S. trutta*.: An evaluation of its use in fish biology. Biology and Environment: Proceedings of the Royal Irish Acadamy. Vol. 93B, 2, 107-110.

Choe MK and Yamazaki F. 1998. Estimation of heritability of growth traits, and phenotypic and genetic correlations in juvenile masu salmon *Oncorhynchus masou*. Japanese Society of Fisheries Science, 64:, 6, 903-908.

Chevassus B and Blanc. 1979. Genetic analysis of growth performance in salmonids. Communications to the Third European Ichthyological Congress, Warsaw, 1-25 September 1979, 4pp.

Crandell PA and Gall GAE. 1993. The genetics of age and weight at sexual maturity based on individually tagged rainbow-trout (*Oncorhynchus mykiss*). Aquaculture, 117 (1-2): 77-93.

Eknath AE and Doyle RW. 1990. Effective population size and rate of inbreeding in aquaculture of Indian mirror carps. Aquaculture, 85: 293-305

Elvingson P and Johansson K. 1993. Genetic and environmental components of variation in body traits of rainbow trout in relation to age. Aquaculture, 118: 191-204.

Falconer DS and Mackay TFC. 1996. Introduction to Quantitative genetics. Fourth Edition. Longman Scientific & Technical, England, 464 pp.

Fredeen H. 1986. Monitoring genetic change. Aquaculture, 57:1-26.

Gall GAE and Huang N. 1988a. Heritability and selection schemes for rainbow trout: body weight. Aquaculture, 73: 43-56.

Gall GA. and Huang N. 1988b. Heritability and selection schemes for rainbow trout: Female reproductive performance. Aquaculture, 73: 57-66.

Gall GAE, Bakar Y and Famula T. 1993. Estimating genetic change from selection. Aquaculture, 111 (1-4): 75-88.

Gall GAE and Bakar Y. 2002. Application of mixed-model techniques to fish breed improvement: analysis of breeding-value selection to increase 98-day body weight in tilapia. Aquaculture, 212: 93-113

Gjedrem T. 1992. Breeding plans for rainbow trout. Aquaculture, 100: 73-83.

Gjedrem T, 1997. Selective breeding to improve aquaculture production. World Aquaculture, March ed.: 33-45.

Gjedrem T. 1998. Developments in fish breeding and genetics. ACTA Agriculturae Scandinavica Section A-Animal Science, 19-26.

Gjedrem T. 2000. Genetic improvement of cold-water fish species. Aquaculture Research, 31 (1): 25-33.

Gjerde B. 1986. Growth and reproduction in fish and shellfish. Aquaculture, 57: 37-55.

Gjerde B, Gunnes K and Gjedrem T. 1983. Effect of inbreeding on survival and growth of rainbow

Gjerde B and Gjedrem T. 1984. Estimates of phenotypic and genetic parameters for carcass traits in Atlantic salmon and rainbow trout. Aquaculture, 36: 97-110.

Gjerde B and Schaeffer LR. 1989. Body traits in rainbow trout: Estimates of heritability and phenotypic and genetic correlations. Aquaculture, 80: 25-44.

Gjerde B, Simainer H and Refstie T. 1994. Estimates of genetic and phenotypic parameters for body weight, growth rate and sexual maturity in Atlantic salmon. Livestock Production Science, 38: 133-143.

Gjerde B, Gjoen HM and Vilanueva B. 1996. Optimum designs of fish breeding programmes with constrained inbreeding: Mass selection for a normally distributed trait. Livestock Production Science, 47: 59-72.

Gjerde B and Korsvoll SA. 1999. Realized selection differentials for growth rate and early sexual maturity in Atlantic salmon. Abstracts: Aquaculture Europe 99, Trondheim, Norway. European Aquaculture Society, Special Publication no 27: 73-74.

Gunnes K and Gjedrem T. 1981. A genetic analysis of body weight and length in rainbow trout reared in sea water for 18 months. Aquaculture, 24: 161-174.

Henryon M, Jokumsen A, Berg P, Lund I, Pedersen PB, Olesen NJ and Slierendrecht WJ. 2002. Genetic variation for growth rate, feed conversion efficiency, and disease resistance exists within a farmed population of rainbow trout. Aquaculture, 209 (1-4): 59-76.

Hersberger WK, Myers JM, Iwamoto RN, Mcauley WC and Saxton AM, 1990. Genetic changes in the growth of coho salmon in marine net-pens, produced by ten years of selection. Aquaculture, 85: 187-197.

Jonasson J. 1993. Selection experiments in salmon ranching; 1. Genetic and environmental sources of variation in survival and growth in fresh-water. Aquaculture, 109 (3-4): 225-236.

Kause A, Ritola O, Paananen T, Eskelinen U and Mantysaari E. 2003. Big and beautiful? Quantitative genetic parameters for appearance of large rainbow trout. Journal of Fish Biology, 62(3): 610-622.

Kincaid H.L. 1976a. Effects of inbreeding on rainbow trout populations. Transactions of the American Fisheries Society, 105: 273-280.

Kincaid H.L. 1976b. Inbreeding in rainbow trout (Salmo gairdneri). Journal of the Fisheries Research Board of Canada, 33: 2420-2426.

Kincaid HL, Bridges WR and von Limbach B. 1977. Three generations of selection for growth rate in fall-spawning rainbow trout. Transactions of the American Fisheries Society, 106: 621-628.

Kinghorn B.R. 1981. Quantitative genetics in fish breeding. Ph.D. Thesis University of Edinburgh.

Meuwissen THE and Woolliams JA. 1994. Effective size of livestock populations to prevent a decline in fitness. Theoretical and Applied Genetics, 89: 1019-1026

McKay LR, Ihssen PE and Friars GW. 1986. Genetic Parameters of growth in rainbow trout, *Salmo gairdneri*, as a function of age and maturity. Aquaculture, 58: 241-254.

Myers JM, Hershberger WK, Saxton AM and Iwamoto RN. 2001. Estimates of genetic and phenotypic parameters for length and weight of marine net-pen reared coho salmon (*Oncorhynchus kisutch Walbaum*). Aquaculture Research, 32 (4): 277-285.

Naevdal G, Holm M, Moller D and Osthus OD. 1975. Experiments with selective breeding of Atlantic salmon. International Council for Exploration of the Sea, C.M. 1975/M:22.

Nilsson J. 1992. Genetic-parameters of growth and sexual maturity in arctic char (*Salvelinus-Alpinus*). Aquaculture, 106 (1): 9-19.

O'Flynn FM, Frairs GW and Bailey JK. 1992. Development of selection index to improve market value of cultured Atlantic salmon. Genome, 35: 304-310.

Olesen I, Gjedrem T, Bentsen HB, Gjerde B and Rye M. 2003. Breeding programmes for sustainable aquaculture. Journal of Applied Aquaculture, 13: 179-204.

Refstie T. 1990. Application of Breeding Schemes. Aquaculture, 85: 163-169.

Refstie T and Steine TA. 1978. Selection experiments wit salmon. III. Genetic and environmental sources of variation in length and weight of Atlantic salmon in the freshwater phase. Aquaculture, 14: 221-234.

Rye M and Resftie T. 1995. Phenotypic and genetic parameters of body size traits in Atlantic salmon *Salmo salar L*. Aquaculture Research, 26: 875-985.

SAS Institute Inc. 1986-1995. SAS User's Guide: Statistics. Version 6.2 Edition. SAS Ins. Inc., P.O. Box. 10066, NC 27605, USA. 956pp.

Saxton A.M. 1983. A comparison of exact and sequential methods in multi-stage index selection. Theoretical and Applied Genetics, 66: 23-28.

Vandeputte M, Quillet E and Chevassus B, 2002. Early development and survival in brown trout (*Salmo trutta fario L.*): indirect effects of selection for growth rate and estimation of genetic parameters. Aquaculture, 204 (3-4): 435-445.

Winkelman A and Peterson RG. 1994. Genetic parameters (heritabilities, dominance ratios and genetic correlations) for body weight and length of chinook salmon after 9 and 22 months of saltwater rearing. Aquaculture, 125: 31-36.

Table 4.1: Stages of evaluation of growth rate in rainbow trout (*Oncorhynchus mykiss*) for year-groups EVEN 94 and ODD 95. (S = fish sampled for measurement, A= all fish taken for measurement).

Stage	Duration (Days)	Range at start of growth stage				Measurements			
		Age (days from fertilization) (s.d.)	Avg. number per group	Avg. group weight (g) (s.d)	Percentage selected (s.d.)	Weight (g)		Length (mm)	
						Initial	Final	Initial	Final
EVEN 94									
I	60	150 (5)	2000 (18)	2.2 (0.5)	All included	S	S	S	S
II	90	240 (5)	500 (9)	45.3 (11.4)	25.2 (2.3)	S	S	S	S
III	90	330 (5)	120 (5)	218 (22)	6.2 (0.2)	A	A	A	A
ODD 95									
I	60	150 (5)	2000 (24)	2.5 (0.6)	All included	S	S	S	S
II	90	240 (5)	500 (8)	47.2 (8.1)	25.1 (1.3)	S	S	S	S
III	90	330 (5)	120 (6)	211 (27)	6.1 (0.2)	A	A	A	A

Table 4.2: Analysis of Variance of body weight and body length of rainbow trout at the age of 150 days for the
EVEN 94 year-group.

Source		Body Weight			Body Length	
	d.f.	Mean Square	P	d.f.	Mean Square	P
Between sires	9	103.55	0.0999	9	275.56	0.0950
Between dams within sires	10	44.10	0.0001	10	115.08	0.0001
Within dams (Error)	300	11.47		300	30.59	
Total	319			319		

Table 4.3: Estimates of variance components and genetic parameters for body weight and body length of rainbow trout at the age of 150days for the EVEN 94 year-group.

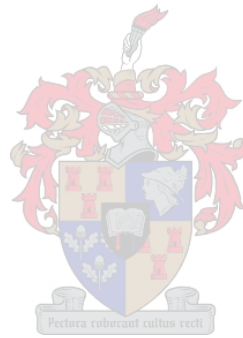| Variance components	Body Weight (g)	Body Length (mm)
Phenotypic variance		
Between sires	1.858	5.015
Dams within sires	2.039	5.281
Within dams	11.472	30.594
Total	15.369	40.889
Additive genetic variance		
Between sires	1.949	5.148
Dams within sires	1.949	5.148
Within dams	3.897	10.295
Total	7.794	20.591
Environmental variance	7.575	20.299
Heritability		
Sire	0.484 ± 0.251	0.491 ± 0.252
Dam	0.531 ± 0.251	0.517 ± 0.252
Total	0.507 ± 0.251	0.504 ± 0.252
Correlation		
Genetic	0.820 ± 0.214	
Environmental	0.049	
Phenotypic	0.371	

Table 4.4: Analysis of Variance of body weight and body length of rainbow trout at the age of 150 days for the ODD 95 year-group.

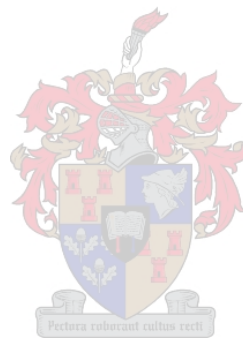| Source		Body Weight			Body Length	
	d.f.	Mean Square	P	d.f.	Mean Square	P
Between sires	14	215.46	0.0637	14	249.20.	0.0404
Between dams within sires	15	94.99	<0.0001	15	97.18	<0.0001
Within dams (Error)	720	18.14		720	17.82	
Total	749			749		
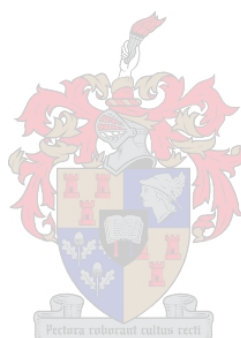
Table 4.5: Estimates of variance components and genetic parameters for body weight and body length of rainbow trout at the age of 150 days for the ODD 95 year-group.

Variance components	Body Weight (g)	Body Length (mm)
Phenotypic variance		
Between sires	2.409	3.040
Dams within sires	3.074	3.175
Within dams	18.137	17.821
Total	23.621	24.036
Additive genetic variance		
Between sires	2.857	3.107
Dams within sires	2.857	3.107
Within dams	5.715	6.215
Total	11.429	12.157
Environmental variance	12.419	11.606
Heritability		
Sire	0.408 ± 0.158	0.506 ± 0.175
Dam	0.521 ± 0.158	0.528 ± 0.175
Total	0.464 ± 0.158	0.517 ± 0.175
Correlation		
Genetic	0.741 ± 0.012	
Environmental	0.086	
Phenotypic	0.222	

Table 4.6: Analysis of Variance of Average Daily Length Gain (mm/day) of 21 rainbow trout families from the EVEN 94 year-group during stage III of evaluation, over a period 90 days.

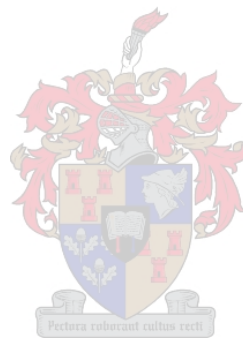| Source	d.f.	Type I SS	Mean Square	Pr > F
Family	20	99 942.964	4 997.148	0.0001
Block (Tank)	20	11 417.201	570.860	0.0002
Error	64	11 218.432	175.288	
Total	104	122 578.598		

Table 4.7: The ranked LSMean values for Average Daily Length Gain (ADLG: mm/day) of rainbow trout families during the Stage III phase of growth evaluation, from the EVEN 92, EVEN 94, EVEN 96, EVEN 98 and EVEN 2000 year-groups. (c = control group, * = selected families)

Family	EVEN 92	EVEN 94	EVEN 96	EVEN 98	EVEN 2000
1	1.351*	1.363*	1.404*	1.425*	1.501*
2	1.328*	1.360*	1.398*	1.349*	1.482*
3	1.296*	1.278*	1.396*	1.336*	1.480*
4	1.290*	1.271*	1.392*	1.335*	1.467*
5	1.281*	1.271*	1.389*	1.333*	1.457*
6	1.266*	1.259*	1.383*	1.326*	1.445*
7	1.256*	1.255*	1.380*	1.318*	1.433*
8	1.252*	1.235*	1.367*	1.308*	1.431*
9	1.251*	1.227*	1.364*	1.304*	1.428**
10	1.250*	1.219*	1.359*	1.301*	1.414*
11	1.245	1.219	1.345	1.299	1.398
12	1.233	1.216	1.289	1.289	1.382
13	1.231	1.211	1.288	1.275	1.371
14	1.224	1.209	1.286	1.243	1.370
15	1.220	1.160	1.260	1.231	1.345
16	1.195	1.150	1.253	1.196	1.311
17	1.194	1.146	1.246	1.194	1.305
18	1.169	1.130	1.236	1.190	1.293
19	1.167	1.114	1.224	1.184	1.246
20	1.160	0.965c	1.208	1.166	1.243
21	1.155	0.925	1.189	1.165	1.222
22	1.153		1.117	1.159	1.185
23	1.146		1.113	1.142	1.168
24	1.142		1.065	1.130	1.164
25	1.139		0.955c	1.127	0.903c
26	1.136			1.111	
27	1.074			1.098	
28	1.070			1.031	
29	1.046			0.981	
30	1.018c			0.964	
31	0.928			0.878c	
Average	1.195	1.211	1.290	1.258	1.356
P (LSMean)	≤0.001	≤0.001	≤0.001	≤0.001	0.138
LSD (P≤0.05)	0.088	0.058	0.094	0.097	0.079
Realised response (%)					
above control	17.4%	25.5%	35.0%	43.3%	50.2%
per generation	8.7%	8.1%	9.5%	8.2%	6.9%
per year	4.3%	4.1%	4.8%	4.1%	3.5%

Table 4.8: Analysis of Variance of Average Daily Length Gain (mm/day) of 31 rainbow trout families from the ODD 95 year-group during stage III of evaluation, over a period 90 days.

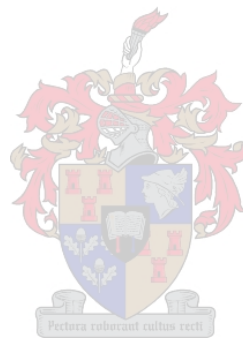| Source	d.f.	Type I SS	Mean Square	Pr > F
Family	30	158 337.597	5 277.920	0.0001
Block (Tank)	30	19 814.834	660.495	0.0026
Error	94	28 734.193	305.683	
Total	185	206 886.624		

Table 4.9: The ranked LSMean values for Average Daily Length Gain (ADLG: mm/day) of rainbow trout families during the Stage III phase of growth evaluation, from the ODD 93, ODD 95, ODD 97 and ODD 99 year-groups. (c = control group, * = selected families)

Family	ODD 93	ODD 95	ODD 97	ODD 99
1	1.244*	1.463*	1.409*	1.521*
2	1.236*	1.426*	1.402*	1.496*
3	1.216*	1.415*	1.360*	1.473*
4	1.213*	1.376*	1.360*	1.417*
5	1.189*	1.350*	1.354*	1.327*
6	1.186*	1.332*	1.328*	1.322*
7	1.159*	1.304*	1.312*	1.310*
8	1.150*	1.293*	1.282*	1.307*
9	1.149*	1.288*	1.267*	1.296*
10	1.124*	1.274*	1.262*	1.238*
11	1.110	1.271	1.251	1.228
12	1.083	1.243	1.220	1.213
13	1.081	1.240	1.218	1.210
14	1.080	1.208	1.214	1.173
15	1.080	1.196	1.203	1.167
16	1.065	1.186	1.161	1.164
17	1.058	1.182	1.149	1.160
18	1.049	1.168	1.148	1.150
19	1.043	1.156	1.142	1.142
20	1.033	1.128	1.140	1.137
21	1.030	1.119	1.137	1.121
22	1.028	1.117	1.132	1.117
23	1.022	1.107	1.116	1.107
24	1.017	1.086	1.103	1.093
25	1.001	1.075	1.087	1.074
26	0.983	1.020	1.019	1.046
27	0.943^c	1.008	1.005	1.042
28	0.718	0.992^c	1.004	1.018
29	0.680	0.976	0.979	0.965
30	0.623	0.965	0.967	0.956
31		0.907	0.947^c	0.903^c
Average	1.057	1.196	1.191	1.200
P (LSMean)	≤0.001	≤0.001	≤0.001	≤0.001
LSD (P≤0.05)	0.089	0.092	0.074	0.171
Realised response (%)				
above control	12.1%	20.6%	25.8%	32.9%
per generation	6.0%	8.5%	5.2%	7.1%
per year	3.0%	4.2%	2.6%	3.6%

CHAPTER 5:   CONCLUSIONS

1.   Background

The first breeding programme on the genetic improvement of fish in the Southern African region was initiated in 1988 by the Department of Genetics at the University of Stellenbosch, in collaboration with South African trout producer's organisations. The objective was to improve the growth rate of rainbow trout under local conditions as a means to improve overall production efficiency and profitability and to overcome the limitations associated with high summer temperatures (>24°C) conducive to most parts of the region. It was set as a priority at the time to start with the selection programme with immediate effect. Other parallel initiatives at the time included a series of workshops, publications and papers to improve the awareness and understanding of the role of genetic management systems within the sector. The programme is still ongoing, entering its 7th generation in 2004 and is supplying about 50-60% of commercial material through direct supplies of broodstock, ova and fingerlings and indirect supplies via multiplier stations (commercial hatcheries). The programme is based on a conventional approach, adapted to the specific circumstances in terms of facilities, resources and environmental conditions. The breeding programme consisted of the following key elements

     a.   formation of a base population

     b.   definition of the breeding goal

     c.   choice of selection criteria

     d.   choice of selection and breeding method

     e.   selection of broodstock

     f.   measuring of response, and

     g.   commercialisation of the breeding programme
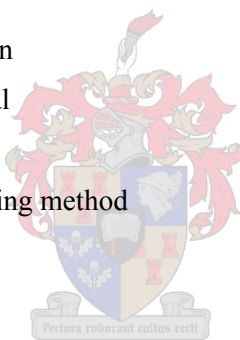
The thesis reports on the implementation and stepwise progress of the above-mentioned breeding programme.

2.   Conclusions

The main conclusions drawn from the results obtained over the first six generations of selection are:

a.   The comparative evaluation of available genetic groups obtained from various local and overseas hatcheries to contribute to the formation of the base populations (Chapter 1) confirmed the presence of statistically significant and commercially valuable genetic differences in terms of weight and length gain between these groups. ***These results emphasize the importance of intensive evaluation of available genetic resources in order to establish a base population with high genetic merit and genetic variability at the onset of a programme aimed at genetic improvement through selective breeding.*** It also confirmed the need to quantify genetic qualities of different commercial groups to ensure informed decisions by producers regarding the purchase of stock and to evaluate the standards of competitors.

b. The procedures for the formation of the base populations as laid out in Chapter 1 resulted in the establishment of a unique set of populations (EVEN 88, OVERSEAS 88 and ODD 89) with high levels of genetic variability. The implementation of a further series of single (Chapter 2) and double crosses (Chapter 3) have increased the genetic variability in these populations (EVEN and ODD year-groups) to the high levels of additive genetic variance, as confirmed by the estimation of genetic parameters (i.e., h^2 = 0.40-0.53) reported in Chapter 4. *The effort that has gone into ensuring high genetic variability within the base populations seemed to be well rewarded by the confirmation of high levels of additive genetic variance (h^2 = 0.40-0.53) and the cumulative realized selection response (33% - 43%) recorded over the first five generations of selection, as reported in Chapter 4.*

c. Significant group x stage interactions were detected during the evaluation of growth rate of genetic groups, signifying the importance of the genetic development of strains under local conditions. Water temperature was identified as the main factor of environmental variation over the various stages of evaluation. *The extent of the interactions were however mainly proportional in nature rather than changes in rank order and would not seem to justify the development of temperature specific strains in view of the extensive and costly changes that is required in terms of management and selection criteria.*

d. The crossing of genetic groups as explained in Chapters 2 and 3, conducted primarily to increase the levels of genetic variation in the base populations, also revealed the presence of significant levels of heterosis and general combining ability for weight and length gain among the crosses. No evidence was found for specific combining ability among the crosses. *In view of the limited scale of heterosis (6.7%-9.6%) observed in the current study, together with conflicting general reports on heterosis for growth in rainbow trout in general, it was decided to focus on the exploitation of genetic variation within the newly formed base populations, rather than the immediate exploitation of heterosis, during the early stages of the selection programme.* Outcrossing between the ODD and EVEN populations remains, however, an integral part of the overall strategy of genetic improvement and will be introduced to re-establish genetic variability within the population, to neutralize accumulated effects of inbreeding and to exploit non-additive genetic effects (heterosis) during future generations. Re-introduction of genetic variability from outside the base populations, without any adverse performance consequences, will also be required to alleviate the effect of inbreeding and genetic drift and to ensure the long term genetic progress.

e. The current method of multi-stage selection relies largely on the within family component of variance to maintain high levels of selection intensity, due to the limited number of 32 families that can be accommodated within the facilities from which 10 families are selected to limit the rate of inbreeding. The inability to implement reliable methods of individual or group identification during the early life stages place a restriction on the number of families than can be accommodated within the available

facilities. *Alternative methods of identification (for example, PIT tags and molecular markers) will have to be considered in an effort to reduce the period of separate rearing of families that affects the accuracy of selection, as well as to increase the number of families than can be accommodated within the available facilities, so as to increase between family selection intensity*. Individual identification methods in particular would allow for the introduction of selection on the basis of BLUP estimated breeding values that will enhance the accuracy of selection and the rate of response.

f. The high initial number of fish per family (±2 000) and the large overall number (±60 000) require the use of different selection criteria at each stage of selection. Selection by means of a calibrated box grader on the basis of body width remains the best way to deal with the large numbers at the end of Stage I. Selection during Stage II and III was initially done on the basis of individually recorded body weight, which has led to a detrimental correlated increase in condition factor (CF). This has necessitated a change to body length as criterium for selection which is maintaining an overall CF on and around the required market standard for salmon trout of 1.6-1.8. CF in itself offers no alternative as criterium for selection to improve the rate of growth due to the low heritability compared to body weight and length. Body weight, however, remains the unit of trade. Phenotypic and genetic correlations between growth parameters at different stages of evaluation were positively moderate to high indicating that selection between families should be best delayed until the final stage of evaluation, as close to the age at marketing as possible, while selection within families could continue throughout all stages of the multi-stage selection procedure. *Selection criteria for improved growth will however have to be evaluated on a continuous basis with regard to the status of genetic parameters such as heritability and correlations within the population, as well as their effect on the correlated traits such as flesh quality and condition factor.*

g. The cumulative realized response of 50% in body length for the EVEN year-group after six generations of selection (8.3% per generation), and the 33% for the ODD year-group after five generations of selection (6.6% per generation) confirms the efficiency of the current multi-stage selection procedure to exploit the available additive genetic variation for growth rate within the respective populations. *Response to selection will have to be evaluated on a continuous basis in terms of the response per generation, the maintenance of genetic variation as well as the genetic stability of the control/reference population with regard to levels of inbreeding, random error and genetic drift.*

h. The extension of the breeding goal to include other traits of economic importance such as quality traits (flesh colour, % yield, fat content, etc.) and disease resistance (survival in challenge tests, specific diseases resistance) will have to be considered during future generations as the initial breeding objective in terms of growth rate is achieved. *Expansion of the breeding goal to include other traits of economic importance will require the development of multi-trait selection indexes for the calculation of breeding values.*