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Molecular and quantitative analyses of genetic variation in farmed Atlantic salmon

A thesis submitted to the University of Dublin for the degree of Doctor of Philosophy

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September 2002

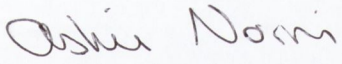
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Ashie Norris



September 2002

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Abbreviations

ASM	Allele sharing method
Bp	base pairs
BLUP	Best linear unbiased prediction
D_A	Nei's standard genetic distance
d.f.	Degrees of freedom
GSI	Gonadosomatic index
HWE	Hardy Weinburg equilibrium
IAM	Infinite Allele Model of neutral mutation
N_e	Effective population size
NIR	Near infrared reflectance
NQC	Norwegian quality cut
PCR	Polymerase chain reaction
REML	Restricted maximum likelihood
S0/S1	Smolts transferred before/after one year of age
SMM	Stepwise Mutation Model of neutral mutation

Summary

Genetic diversity between three farmed and four wild populations and within six 6 year classes of one farmed population of Atlantic salmon were analysed using 15 microsatellite markers. High levels of polymorphism were observed over all populations with average heterozygosity at 0.70. Two farmed salmon populations showed less genetic variability than wild salmon in terms of allelic diversity and heterozygosity. Between farmed populations significant differences were observed in expected heterozygosity suggesting that more intensive breeding practices may have resulted in a further erosion of genetic variability. Phylogenetic analysis using either populations or individuals as nodes show a clustering of populations into two groups, farmed and wild. This suggests that founder effects and subsequent selection have had more effect on the genetic differentiation between these strains than geographical separation.

This study also demonstrates how these same microsatellite markers can be used to determine both parentage and relatedness in a mixed aquaculture situation in the absence of physical tags and/or pedigree information. Using a number of different datasets, parentage was assigned to offspring with varying degrees of accuracy depending on number and variability of the microsatellite markers. Three different suites of microsatellite loci (eight, fifteen and the eight most variable) were used to discriminate between related and unrelated individuals in a situation where no pedigree information is known and among groups of related individuals (full-sibs, half-sibs and cousins). The degree of accuracy of the discrimination depended on the number and variability of the loci and on the classification of unrelated in the analysis.

The assignment of offspring to parents using microsatellite markers was applied to a large scale breeding program where parentage was assigned for over 8000 offspring reared in a mixed aquaculture environment. This pedigree data was combined with performance data from live and harvested fish to estimate genetic parameters under a number of different models and methodologies for body weight and body composition, maturation and quality traits. The importance of defining the most appropriate model for the estimation of both genetic parameters and breeding values is demonstrated.

The additive genetic component of each trait and the genetic correlation between traits is discussed with an emphasis on flesh quality traits which have a particular importance for the aquaculture industry. The components of variation are shown to be highly dependant on sex, body weight and maturation status for most traits. Further analysis of these parameters at different stages of the life cycle and for different year classes of fish will help explain the complex relationships between growth, maturity and flesh quality traits and will improve the accuracy of selection of breeding individuals based on BLUP breeding values.

Chapter 1

General Introduction

1-1 *Production of Atlantic salmon in Aquaculture*

Aquaculture, with output growing at 11% per annum, is the world's fastest growing food sector. The role of aquaculture in the production of Atlantic salmon has increased dramatically in the past 15 years. Between 1984 and 2000 the global production of farmed Atlantic salmon increased 30 fold from 30,000 tonnes in 1984 to 797,642 tonnes in 1999 (FAO 2000). The majority of this growth, particularly in the past ten years, has come from Chile where production has climbed rapidly, since salmon aquaculture was started in the early 1980's, to approximately 167,000 tonnes in 2000 (Compendium of the Aquaculture and Fisheries of Chile) making Chile the second largest producer of Atlantic salmon in the world. In Ireland the production of Atlantic salmon in aquaculture has grown since the start of the industry in the early 1980s to over 18,000 tonnes in 1999 valued at over £46 million (Department of the Marine and Natural Resources - Annual Report 1999). The Irish government identified aquaculture as an industry for potential growth in the CIRCA report in 2000 and outlined a development strategy (CIRCA 2000). CIRCA identified a "window of opportunity" in Ireland for significant growth in the years ahead. It set ambitious targets and predicts an annual production of 70,000 tonnes for the Irish industry by 2015 – providing a critical mass of 35,000 tonnes can be reached within the next 2-3 years.

Increased production has been, and will continue to be, dependant on the granting of new licences for freshwater and seawater production. It will also require continued efficiency of production. Past achievements in improving efficiency have been remarkable. For example, in Norway, in the past 30 years the average time to harvest for Atlantic salmon has been reduced from an average of 3.5 years to an average of 2.5 years. During that same time, feed

conversion ratio (kg of feed to produce 1kg of salmon) decreased from 4.5 to 1.2 and antibiotic use fell from 800g per ton to 3g per ton produced (Talbot et al. 1999).

While productivity of farmed Atlantic salmon has increased over the past 30 years, as evidenced by the statistics shown above, relatively little emphasis has been placed on selective breeding by the major producers until recently. At present, only approximately 1% of aquaculture production is based on genetically improved stocks (Gjedrem 1997) compared to mammal and poultry production where genetic selection techniques have increased production between 2 and 3 fold (ICLARM 1991) since 1940.

1-1-a Marine Harvest Ireland.

Marine Harvest Ireland is located on the Fanad peninsula in Donegal, North West Ireland. The company started in 1979 as "Fanad Fisheries" and harvested its first Atlantic salmon in 1982. It is now a fully owned subsidiary of Nutreco Holding N. V. (www.Nutreco.com) an international public company (Euronext stock exchange, Amsterdam) with leading positions in the high-quality animal and fish feed industries and in fish farming. Marine Harvest Ireland is a totally integrated farming operation with all aspects of farming from breeding to processing being carried out within the company. The company produced approximately 5,000 tonnes of salmon in 2000 from six sea rearing facilities in Mulroy Bay and Lough Swilly in County Donegal. The same year approximately 25 million eyed eggs and 2.5 million smolts were produced in the company's three freshwater facilities of which 70-80% are destined for sale both within Europe and to South America (Chile).

1-2 *Lifecycle of Atlantic salmon in aquaculture.*

The production cycle of Atlantic salmon in aquaculture begins when the broodstock fish are stripped of ova and semen and eggs are fertilised in November and December (Figure 1-1). The ova are incubated until the eyed egg stage, that is when the eyes of the embryo of the fish become visible. After approximately 500 day degrees (number of days X mean temperature of water during the incubation period in ^{o}C) the embryos will hatch from the egg. When the yolk sac has been absorbed the alevins will begin to feed for themselves. For the production of earlier smolts (S0 smolts), the water at incubation is heated above ambient temperature, thereby speeding up the development process. Alevins are transferred to freshwater tanks for first feeding and as they develop into fry will remain in tanks for the remainder of the freshwater rearing period or be transferred to lakes.

Smoltification involves changes in morphology and physiology which prepare the fish for the marine environment (Wedermeyer et al. 1980; Hoar 1988). In farmed salmon this process naturally occurs mostly in the spring, approximately one year after hatching, and the smolts are referred to as 1+ smolts or S1s. In recent years, however, there has been an increase in the production of earlier, out of season, smolts (S0s) for transfer to the sea in the autumn. At the present time S0 smolts contribute about 30% of total smolt production in European Atlantic salmon production. To produce S0 smolts in autumn, growth must be accelerated using heated water and fish must receive the appropriate photoperiod cues (Saunders et al. 1990; Gaignon 1992). The transfer of smolts to sea at two periods in the year results in a more prolonged period for which to harvest fish throughout the year.

After being transferred to sea fish spend 2 winters at sea before being harvested at an average weight of 4.5Kg (Figure 1-1). A proportion of fish (grilse) will reach sexual maturation before harvest, after only one winter at sea. The onset of sexual maturation in fish is

accompanied by a deterioration of flesh quality, slow growth and higher mortality (Gjerde 1984; Naevdal 1983). Early maturation, or grilsing, is therefore a major problem for the Atlantic salmon farming industry. In 1999 grilsing accounted for 32% of total production by Scottish fish farms (SOAEFD 1996). Grilse rates are determined in part by environmental conditions, in particular temperature and photoperiod (Taranger 1993; Taranger et al. 1998) and genetic factors (Naevdal 1983; Wild et al. 1994). The percentage of fish maturing at the one-sea-winter stage can be reduced by providing continuous additional light in the sea cages (Krakenes et al. 1991; Hansen et al. 1992; Taranger et al. 1995; Oppedal et al. 1997).

Fish which are not harvested after two sea-winters will eventually become sexually mature as two seawinter or three seawinter fish when they are transferred to broodstock holding tanks in freshwater or brackish water. It is common practice to breed two-sea-winter broodstock (2SW) with three-sea-winter broodstock (3SW) in order to avoid full-sib mating in the absence of pedigree information.

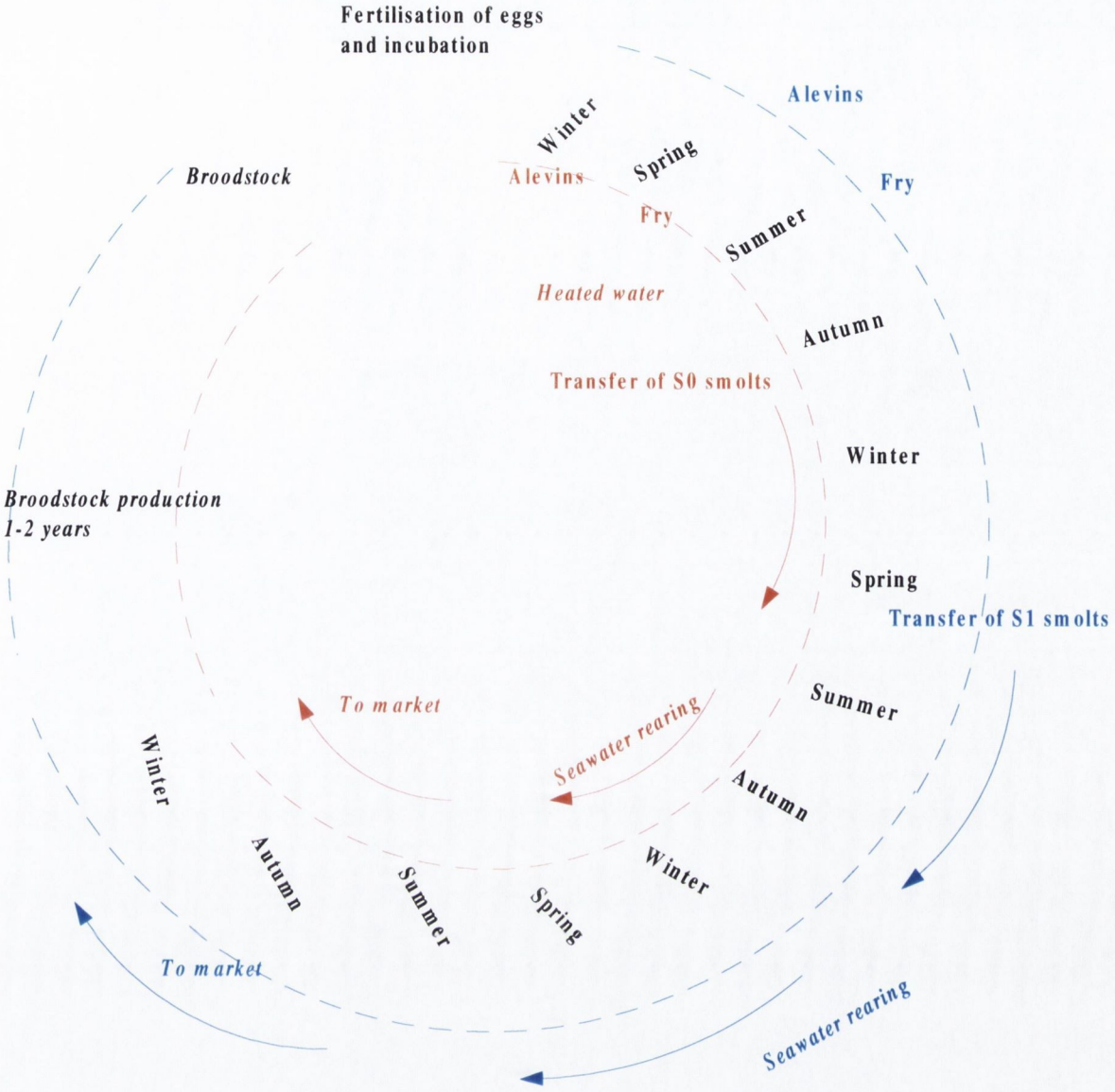


Figure 1-1 Life cycle of salmon in aquaculture.

Blue markings and text represent the S1, or traditional lifecycle and red markings and text represent the shortened, S0, lifecycle. S0 smolts are transferred to sea in the autumn whereas S1 smolts are transferred in the following spring. All production fish spend two winters at sea before being harvested. Broodstock are used for breeding after either three or four winters at sea.

1-3 Breeding in aquaculture

The application of quantitative genetics theory to the selection of animal and plant species in agriculture has yielded considerable gains in productivity and quality over the past 50 years. (McMillan et al. 1990; ICLARM 1991). The application of the same technology to aquaculture species offers a substantial opportunity for increased production efficiency, health, product quality and ultimately profitability in aquacultural enterprises. In fact, fish species possess a number of attributes which confer advantages over mammal and poultry species and which will accelerate genetic progress.

The most important prerequisite for genetic improvement through selection is additive genetic variation between individuals for the traits of interest. It is this variation that makes genetic improvement by selection possible. High levels of additive genetic variation have been reported for farmed fish stocks suggesting that selection for economically important traits should be effective (Gjedrem 1983; Gjerde 1986). Genetic variability at the molecular level is, not surprisingly, also extremely high in salmonids, in particular, as evidenced from DNA marker studies (Estoup et al. 1993; Brooker et al. 1994; Wright and Bentzen 1994; McConnell 1995; Wright and Bentzen 1994; McConnell 1995; Morris et al. 1996; Coughlan 1998; DeWoody and Avise 2000). In addition, in contrast to other farmed animals, most aquaculture species have the advantage of high reproductive capacity. For example, in Atlantic salmon the average number of eggs produced in a single spawning is approximately 6000 (own data). This high fecundity facilitates high selection intensities, as only relatively few broodstock are required for the next generation production of offspring and the large number of full and half

sibs which are available for measurement of traits increases the accuracy of breeding value[1] estimation. High selection intensities, combined with moderate to high levels of genetic variation for most traits have been shown to result in 5-20% increases in body weight per generation (body weight only) in fish (Gjedrem 1983; Gjerde 1986; Villanueva et al. 1996; Gjedrem 1997). The facility for external and artificial fertilisation also allows for the design of more efficient breeding programs in aquaculture than are available in agriculture.

Although a number of commercial Atlantic salmon producers have, recently, begun genetic selection programs, most of the published literature on genetic parameters and genetic gain/response in Atlantic salmon breeding has come from two national breeding programs. The first was started as the Norwegian selection program for Atlantic salmon in the early 1970's (Gjedrem 1991) and run by the Institute of Aquaculture Research, AKVAFORSK. In 1992, AKVAFORSK transferred the breeding activity to a separate unit, organised under a new mother company, Norwegian Salmon Breeding (Norsk Lakseavl, NLA). The second is the genetic research program started in 1984 (Friars et al. 1995) at the North American Salmon Research Centre in New Brunswick, Canada (no longer running). Both these programs have generated valuable knowledge regarding genetic variation in Atlantic salmon, genetic-environment interactions and potentials for genetic gain. In the past 5 years a number of commercial breeding programs for Atlantic salmon have started in Norway, Scotland, Iceland and Chile. However, little or no published information regarding genetic parameters has come from these enterprises since their inception. The data in this study come from a breeding program started in 1998 in Marine Harvest Ireland.

[1] breeding value of an individual can be defined as in terms of the sum of the average effects of the genes of an individual, the summation being made over the pair of alleles at each locus and over all loci or the mean value of the progeny of an individual [Falconer, 1989 #167]

The first step in a breeding program is the definition of the breeding goal or objectives. The breeding goal can be defined as a linear function of traits, which are the main target of the improvement program, and economic values for those traits. The traits to be included in the breeding goal must be of economic importance, be possible to measure or judge and the genetic variation of the trait must be greater than zero. An understanding of environmental and genetic parameters, and their magnitude, influencing performance traits is vital when deciding on a breeding goal and to some extent will determine which traits to measure and include in the breeding goal. In salmonids, there have been a number of investigations on genetic and phenotypic parameters in economically important traits. These studies are outlined in as they pertain to three groups of economically important traits; body size and body composition, sexual maturity and flesh quality.

1-3-a Breeding structure

Individual selection, or mass selection where selection decisions are based on the performance of the individual alone, is still the most widely practiced selection method in fish and shellfish in aquaculture. In situations where heritability is high, individual selection may be more efficient than selection using information from relatives (Gjerde et al. 1996). However, mass selection can only be used to select for traits which can be recorded on the individuals themselves. Flesh quality and body composition traits are not measurable on live fish with any degree of accuracy (Douirin et al. 1998). By applying family based selection, flesh quality traits, disease resistance and early maturity can all be included in the breeding goal. Utilising the performance of relatives in this way increases the accuracy of the estimation of breeding values. Also, a knowledge of pedigrees will allow inbreeding rates to be monitored and controlled.

Family based selection methods include selection between families, within-family selection, progeny testing and combined selection. As discussed by Gjedrem (1983), progeny testing is of little interest in fish and shellfish mainly because it increases the generation interval and because of low survival of broodstock in many species. Within-family selection (selects the best individuals from each family and discards all others) has low efficiency compared with all other selection methods (Gall and Huang 1988; Falconer 1989) where lower efficiency for within-family selection was reported compared with between-family selection when common environment is of moderate magnitude. Family selection (selects the top proportion of families and discards all other families) is of particular interest in fish and shellfish because of their high fecundity; both full- and half-sibs can be used.

Generally, a combination of individual and family selection will be more efficient than using only one of them (Falconer 1989). Combined selection is a method to combine the benefits of both family and individual selection by including information obtained on the individual itself and on all relatives past and present in the population. The statistical procedure known as BLUP (properties of which are described in chapters four and five) is now the most commonly used method to estimated breeding values combining all available information on the individual and relatives.

1-3-b Identification of fish in aquaculture

Identification of fish is necessary for maintaining pedigree records and it is essential when family or progeny testing are applied. Even non family based selection strategies, such as mass selection, can result in increases in inbreeding in the absence of pedigree information (Bijma et al. 2000). Before individual marking methods for fish were available a combination of fin clipping and freeze branding allowed for identification of fish to family of origin. The

subsequent development of electronic PIT (passive integrated transponders) tags which are inserted into the body cavity of juvenile fish allowed for individual as well as family identification of fish in a breeding program. Individual identification has allowed breeding programs to advance from family selection where entire families and accepted or rejected based on family breeding values to animal model BLUP selection where selection is based on an individuals own estimated breeding value (EBV).

Individual tagging of progeny from family groups means that family groups, normally half sib groups, must be isolated and reared together in costly multi-tank facilities until they are large enough to be tagged. Not only is this approach space and labour intensive, but it also introduces environmental effects common to full-sib groups which are confounded with the genetic effects under study (Herbinger et al. 1999b; Rye and Mao 1998). Environmental effects common to groups reared together include food availability, competition, water quality and tank density. Ideally in a testing scheme applied to breeding programs environmental effects common to full sibs should be avoided from as early a stage as possible thereby avoiding the problem of confounding environmental and genetic effects. Even after careful environmental standardisation, 'tank effects' of unknown nature are often observed to cause growth rate variation among tanks (Chevassus 1982; Bagley et al. 1994). In an experiment designed to assess the impact of the confounding of environmental tank effect with genetic family effect, Herbinger et al. (1999a), examined early growth performance of Atlantic salmon full-sib families reared in single family tanks versus in mixed family tanks. Families in mixed family tanks were identified using four microsatellite markers. Although there was a strong correlation of family survival rates in the mixed tanks and the single family tanks, family growth performance in the single tank environment was poorly correlated with family growth performance in the mixed tank environment. These results suggest that differences in

early growth performance reflect environmental differences among tanks rather than genetic differences among families. When family groups are reared in single tanks these differences in performance may lead to inflated estimates of genetic variance and heritability, as well as greatly reducing the effectiveness of family selection.

A number of studies have estimated the magnitude of the full-sib family effect in salmonids which includes maternal , non-additive genetic and well as common environmental effects (Su et al. 1996; Martinez et al. 1999). These studies demonstrated significant variance components for this effect and that common environmental effects and non-additive genetic effects are more important sources of variability than maternal genetic effects. Ignoring these effects will result in inflated estimates of additive genetic variance by allocating dominance and common environmental to the additive component of variance. In the investigation into (co)variance components of performance traits described here all fish were mixed shortly after hatching and therefore no environmental effects common to family will be present. Non-additive genetic effects could not be estimated as performance data on only one generation of fish are available at the present time.

Genetic profiles from DNA markers would allow retrospective assignment of individuals to family groups even after different progeny groups had been reared communally after hatching. Parentage assignment using genetic profiles has already been suggested for use in aquaculture situations in Atlantic salmon (Wright and Bentzen 1994; Doyle and Herbinger 1995). The feasibility of this approach has been assessed in communally reared rainbow trout, where 91% of fish were traced to one or two parental couples using 4 microsatellite loci (Herbinger et al. 1995) and in Atlantic salmon where between 80 and 99% of offspring were assigned to one set of parents in a number of different crosses using 4 microsatellite loci by O'Reilly et al.

(1998) and by Norris et al. (2000), where 95% of offspring were assigned to the correct parental pair.

1-4 Application of microsatellite markers to aquaculture

Microsatellite markers represent the most widely applicable DNA technology for the investigation of population differentiation. Microsatellites are short stretches (tens to hundreds of base pairs) of DNA composed of di-, tri-, or tetranucleotide repeats arrayed in tandem. They have been shown to be highly polymorphic in teleost fish, with as many as 52 alleles observed at one locus in Atlantic salmon (Estoup et al. 1993; Brooker et al. 1994); (Wright and Bentzen 1994; McConnell 1995; Wright and Bentzen 1994; McConnell 1995; Morris et al. 1996; Coughlan 1998). This variability makes for suitability in a variety of applications in fisheries and aquaculture, particularly where genetic differentiation between populations may be limited. Potential applications in aquaculture include monitoring changes in genetic variation over time, as a consequence of different breeding strategies (Butler and Cross 1996; Cross and King 1983; Davidson et al. 2000; Hansen et al. 2000; Luikart 1998; O'Reilly et al. 1996; O'Reilly et al. 1998), parentage assignment in a mixed aquaculture environment (Bernatchez and Duchesne 2000; Norris et al. 2000), and estimation of relatedness between potential breeding pairs (Blouin et al. 1996).

Studies using protein, DNA markers (Stahl 1983; McConnell et al. 1995; Stone et al. 1997) and variation of quantitative traits (Taylor 1991) have demonstrated genetic differentiation between different river populations of Atlantic salmon. This difference has generally been attributed to the homing of wild Atlantic salmon resulting in river populations which are geographically restricted and thus represent genetically different populations. Differences that

exist between wild salmon stocks may be the reflection of local adaptation or may be a result of genetic drift resulting from low population sizes.

The increase in farming of Atlantic salmon in the past few decades has led to an increase in the escape of farmed salmon into the natural environment. Escape of farmed fish into natural river populations may threaten the wild populations genetically by reproducing with them. The long term impact of such escapes will be dependent on both the reproductive success of the escaped fish and on the nature and extent of the genetic differences between the escaped and native populations. Most farmed populations have been selected for high performance in certain quantitative traits, such as growth and age at sexual maturation. Intensive breeding practices combined with the effect of founding populations from small number of originators may result in a reduction in genetic variation in farmed fish relative to their wild ancestors. In general, the greater the adaptive differentiation between wild and farmed fish, the larger the expected decrease in survival among the hybrid offspring.

Using microsatellite DNA markers, the performance and reproductive success of wild and farmed salmon can be compared in a mixed natural environment (McGinnity et al. 1997; Taggart et al. 2001) and the genetic consequences of escapes of farmed fish can be examined on the local environment from the resulting microsatellite genotypes of hybrids (Clifford et al. 1998).

In breeding programs where a limited number of full sibs per family are available for selection, as is the case when juvenile fish are tagged, the high fecundity of fish, which allows for high selection intensities, cannot be fully exploited. In a situation where all families are reared separately and later tagged, it is normal for cost and logistics reasons to tag

approximately 100-200 fish per family. In the case of a DNA tagging system, thousands of full sibs per family can be mixed and included in the population for selection. Mass selection can then be carried out for the most important traits before family selection on breeding value is applied. In this way higher selection intensities can be applied compared to a situation where only a small number of fish are available for selection (Gjerde et al. 1996; Villanueva et al. 1996).

The study presented here attempts to quantify and compare levels of genetic variation, with the use of microsatellite DNA markers between and within farmed and wild strains of Atlantic salmon. The application of these same markers for the purposes of identifying parentage and relatedness in farmed Atlantic salmon is also investigated. In a mixed aquaculture situation, in the absence of specialised facilities for the rearing of family groups, a family-based breeding program was initiated using DNA markers to identify parentage. After three years, a proportion of these fish were harvested and measured for body size, body composition, sexual maturity and a number of important quality traits. Genetic and phenotypic parameters and breeding values were estimated from these data and are presented in this study under a number of different experimental designs and methodologies.

Chapter 2

Genetic variation between and among wild and farmed Atlantic salmon populations.

2-1 Introduction

Aquaculture practices may inadvertently decrease the genetic variability present in farmed stocks by breeding related individuals or by the use of small numbers of parents as broodstock. Some selective breeding programs breed from a small number of 'superior' families that may be related. Other farms operate a mass selection approach with high selection intensities. Unless pedigree records are being maintained, there is a probability of selecting related individuals as parents and thereby increasing inbreeding.

The effects of inbreeding in salmonids include moderate to high inbreeding depression of survival and growth traits (Gjerde et al. 1983; Kincaid 1983; Su et al. 1996). Inbreeding will also result in a decrease in genetic variability which will limit the potential for future genetic gain from artificial selection. As more intensive breeding practices are put in place care must be taken to avoid loss of genetic variation and accumulation of inbreeding within farmed stocks.

Most studies of genetic variation in wild and farmed strains of Atlantic salmon have utilised protein electrophoresis. A number of these have demonstrated a significant difference between allele frequencies of farmed and wild Atlantic salmon and a reduction in genetic variation in the reared strains (Cross and Ni Challanain 1991; Verspoor 1988; Wilson et al. 1995). In contrast, other studies with protein polymorphisms have shown no reduction in genetic variation in farmed strains (McElligott et al. 1987; Youngson et al. 1991; Cross et al. 1993; Mjolnerod et al. 1997). This lack of concordance may in part be attributable to the low potential for discrimination using protein polymorphisms.

In recent years mitochondria and nuclear DNA techniques have been found to be more powerful in analysing population structure in salmonid strains (McConnell 1995; Tessier et al. 1995; Nielsen et al. 1996; O'Reilly et al. 1996; Sanchez et al. 1996; McConnell et al. 1997; Mjolnerod et al. 1997; Clifford et al. 1998). Several authors have compared populations using DNA methods in parallel with protein polymorphisms, and populations which exhibit little or no differentiation using the latter have shown significant differences using DNA techniques (Sanchez et al. 1996; Mjolnerod et al. 1997; Estoup et al. 1998; Reilly et al. 1999).

A preliminary investigation into levels of genetic variation in farmed and wild populations of Turbot showed that while there was a considerable loss of rare alleles in one farmed population, there was no significant difference in overall heterozygosity between farmed and wild strains (Coughlan 1998). While a number of studies have investigated genetic differentiation between wild populations of Atlantic salmon, as revealed by microsatellite markers, (McConnell et al. 1995; Tessier et al. 1995; Sanchez et al. 1996; McConnell et al. 1997) this is the first study in which microsatellites are employed to differentiate within and between farmed and wild strains of Atlantic salmon.

This chapter analyses variability at 15 microsatellite loci within and between three populations of farmed salmon of Norwegian origin and four wild populations of European Atlantic salmon. The hypotheses being tested were that; (i) farmed salmon differ from wild salmon in terms of genetic variability and, (ii) genetic differences exist between farmed strains as a result of breeding practices and between wild strains from different locations. It was also investigated whether a perceptible change in allele frequencies was detectable over time within one farmed strain.

2-2 *Materials and methods*

2-2-a Fish Samples

Wild juvenile salmon from the rivers Numedalslagen (1996, Norway), Mulkear and Burrishoole (1996, Ireland) were sampled by electrofishing. Samples from the rivers Burrishoole and Corrib (1994, Ireland) were taken from fertilised eggs exported to Denmark as part of a Danish river restocking program. Three farmed populations were sampled, which for the purposes of this study were called Irish farmed (174 individuals), Norwegian farmed I (originally 30 smolts from 1996 year class were sampled, this group were genotyped at 15 loci and used for the comparison with other farmed and wild salmon. Two years later 320 broodstock of mixed year classes 1995 and 1996 were sampled and genotyped at 6 microsatellite loci, this group were compared to the other year classes of Irish farmed salmon) and Norwegian farmed II (26 individuals). The accepted nomenclature in salmon farming is for fish originating from the same breeding season to be given the classification of the year in which they are transferred to sea. Therefore, fish originating from broodstock in 1995 might be transferred to sea in 1997 and henceforth called 97G fish.

In the Irish farmed population, salmon were sampled from 6 year classes (IF-93-98G). The 1997 year class was comprised of fish which had been imported into Ireland from the Norwegian I population as ova (IF-97G_ N) and Irish farmed fish from 1997 year class (IF-97G). Some year classes were sampled as juvenile fish and later as broodstock. The progeny of the first two year classes (IF-93G and IF-94G) were also sampled as broodstock. All samples from these year classes were genotyped at a minimum of seven microsatellite loci.

The oldest farmed population (Norwegian farmed I) was constituted in the late 1960's three wild Norwegian populations from the west of Norway; Vossovassdraget, Mauranger and Hordaland Aroy and formed the basis for most early farmed stock in Norway. Ova from this population were imported into Ireland between 1982 and 1986 to create an Irish farmed population. Since 1986 the breeding system in Ireland has been closed and stock derived from this program now constitute the majority of Irish farmed salmon. A second farmed sample, Norwegian farmed II, represents the main proportion of current farmed salmon in Norway and was created in 1971 from Atlantic salmon sampled from 41 rivers in Norway (including Numedalslagen) (Gjedrem et al., 1991). Since 1971 this strain has been through 7 generations of selection to maximise genetic gain for economic traits as part of the Norwegian national breeding program. Unlike the other farmed populations in this study, the Norwegian farmed II population maintains a limited number of families in each generation (~200) and approximately 20% of families are used to provide parents of each succeeding generation. There is no information available for either of these farmed strains on the numbers of founder parents from each wild strain used to make the farmed populations. Subsequent investigations into the genetic origin of the Norwegian farmed II have shown that by the late 1980's only a small number of the original 41 wild strains dominate the genetic make-up of the farmed strain strain. This is likely to be the result of selection and variation in fertility success in the founder parents.

2-2-b Microsatellite analysis

Genetic variation was assayed in all the above populations using 15 microsatellite loci. Details of PCR conditions and references for all microsatellite loci are given in **Table** 2-1. One population, Norwegian farmed II, failed to amplify at three loci due to poor sampled DNA quality. PCR primers for loci SSa141, SSa13, SSa132, SSa12, SSa129 and SSa85 were

designed from published sequences, while all other primers were synthesised according to referenced author (see Table 2-1). Within the farmed populations all individuals were genotyped at either 6 or 7 microsatellite loci. Ten fin tissue samples from North American Atlantic salmon were used to standardise allele sizes from this study with published sizes from the same microsatellites in a Canadian study (O'Reilly et al. 1996).

DNA was extracted from fin and muscle tissue samples by boiling samples in 500 μl solution A (200mM NaOH) and then adding 500 μl solution B (200mM HCL, 100mM Tris-HCL, pH 8.5). PCRs were performed in 5 μl reaction volume containing 0.55 μl Taq polymerase buffer (100mM Tris-HCL [pH 9.0], $MgCl_2$ concentrations as given in Table 2-1, 1% Triton X-100 , 500mM Kcl), 0.3mM primer A, 0.3mM primer B, 200 μl of each dGTP, dTTP, dATP, 20 μl dCTP, 0.5 μC α^{32} PdCTP (3,000 Ci/mmol), 0.5 μl units Taq polymerase. Temperature cycling conditions were as follows: one denaturation at 94°c for 3 min; 38 cycles of 45s at 94 °c; 45s at annealing temperatures given in Table 2-1; 45s at 72 °c and a final extension at 72 °c for 4 min. PCR products were electrophoresed in 6% acrylamide and autoradiographed.

Microsatellite locus	$MgCl_2$ mM	Annealing Temp. (^{0}c)	Allele size range (bp)	Reference/GenBank accession number
SSa141	1.5	60	176-272	GenBank U58905
SSa13[a]	2.0	58	123-205	GenBank U58903
SSa132	1.5	55	190-210	GenBank U58901
SSa12[a]	1.5	55	176-192	GenBank U58900
SSa129	1.5	54	220-226	GenBank U58899
SSa197[a]	1.5	59	163-259	O'Reilly *et al.*, 1996
SSa171[a]	2.0	58	198-252	O'Reilly *et al.*, 1996
SSa202[a]	2.0	55	236-292	O'Reilly *et al.*, 1996
SSa85[a]	2.0	57	144-194	O'Reilly *et al.*, 1996
SLOSL456	2.0	55	171-177	Slettan *et al.*, 1997
SLOSL444	1.5	56	108-212	Slettan *et al.*, 1996
SLOSL439	1.5	58	125-217	Slettan *et al.*, 1996
SLOSL438	1.5	55	116-152	Slettan *et al.*, 1996
F43[a]	1.5	56	103-143	Sanchez *et al.*, 1996
20.19	1.5	56	96-102	Sanchez *et al.*, 1996

[a] Microsatellite loci used to genotype all year classes of Irish farmed salmon.

Table 2-1 Details of the Atlantic salmon microsatellites used in this study.

2-2-c Statistical analysis

Expected heterozygosities for all loci and populations were calculated according to (Nei 1978). As there was considerable variation in the sample size of the different populations used in this study, which would affect estimates of average number of alleles (Nei 1987), a resampling procedure was used to standardise sample sizes for all population-locus combinations in a procedure described by (Park 2001) using the Excel microsatellite toolkit software package. Because one population (Norwegian farmed II) was genotyped over only 12 loci, the estimation of average number of alleles was done over only 12 loci for the comparison of farmed and wild populations. In order to compare levels of genetic variation across all farmed year classes and stocks, the resampling procedure was performed over seven loci.

Deviations from Hardy-Weinburg equilibrium, genotypic disequilibrium and population differentiation were all analysed using version 3 of GENEPOP (Raymond and Rousset 1995). Significance of deviations across loci and populations were estimated according to (Fisher 1922). Fst values across populations (Weir and Cockerham 1984) and population pairwise F_{ST} (Slatkin 1995) were calculated using Fstat (Goudet 1995). F_{ST} significance values for each locus were determined by bootstrapping over samples, and significance values over all loci were calculated by jacknifing over loci, using procedures outlined in (Weir 1990). R_{ST} values (Slatkin 1995) were calculated using the program RstCalc (Goodman 1997) which also calculates significance of Rho values across all loci by permutation tests. The distributions of the expected heterozygosities for each locus and population under both the IAM (Infinite Allele Model) and the SMM (Stepwise Mutation Model) were calculated using BOTTLENECK (Cornuet and Luikart 1997).

D_A distances between populations were estimated according to (Nei et al. 1983) and phylogenetic trees were constructed using the neighbor joining (NJ) method from matrices of DA distances (Saitou and Nei 1987). Neighbor joining trees for both D_A distances and F_{ST} pairwise distances were constructed using the Neighbor and Drawgram/Drawtree subroutines in PHYLIP (Felsenstein 1994). For the purposes of comparing farmed and wild populations, three year classes (1993, 1994 and 1995) were combined to represent the Irish farmed population. Farmed strains, Irish farmed and Norwegian farmed I were also divided into year classes and distinct groups based on origin for the purposes of detecting differences between populations and over time. A C^{++} program, SWAMP (Garret Taylor, Genetics Department, Trinity College, Dublin) was used to calculate pairwise distances between individuals based on the proportion of shared alleles between individuals (Bowcock et al. 1994).

2-3 Results

2-3-a Single locus statistics.

High levels of polymorphism were observed for all populations of Atlantic salmon studied and within year classes of farmed salmon (Table 2-2 and Table 2-3 and graphically illustrated in Figure 2-1 and Figure 2-2). Out of 15 microsatellites, 14 were polymorphic in all populations and 9 of these exhibited overall heterozygosity levels greater than 80%. SSOSL444 was the most variable locus with 46 alleles and heterozygosity of 95%. 12 loci are dinucleotide repeats and three (SSa197, SSa171 and SSa202) are tetranucleotide repeats. The tetranucleotide repeats exhibited high levels of polymorphism in all populations but were not as variable as the more variable dinucleotide loci. Allele size range at individual loci varied between 6bp and 118bp. Long repeat stretches and high levels of polymorphism at microsatellite loci in salmonid fishes have been reported in previous studies (Slettan et al. 1993) (Estoup et al. 1998) (McConnell et al. 1995) (Morris et al. 1996).

Hardy-Weinburg disequilibrium was observed at a number of loci and in several populations (Table 2-2). Two farmed and one wild population showed significant deviations when probabilities were pooled across all loci (Raymond and Rousset 1995). The Norwegian farmed II population also gave a significant deviation across loci, perhaps a result of the intensive selection programme using a small population size. One wild population, Burrishoole, comprised of two different year classes, displayed Hardy-Weinburg equilibrium deviations averaged over all loci. Once separated into 1994 and 1996 classes only one

subpopulation, 1996, displayed HWE deviations. This may be due to a small sample size or structuring within the sample.

Within the farmed populations, three year classes showed significant deviations from HWE across all loci; IF-97_N, IF-96 and NF-I (Table 2-3). This deviation may be the result of selection practices in those particular year classes. The IF-97_N year class was imported from the NF-I population and has many similar characteristics across loci in terms of variability and allele frequency. A number of loci showed significant deviations from HWE across all populations, mostly as a result of excess of homozygotes, possibly indicating the presence of null alleles although no repeat genotyping was performed to clarify this hypothesis. This disequilibrium could also be the result of the effects of selection acting on genes associated to these loci. Linkage disequilibrium was assessed for all possible combinations of loci condensed across populations. The results for each pairwise locus-by-locus comparison (105) are presented in Table 2-4. Significant disequilibrium ($p<0.05$) was observed between 5 pairs of loci out of 105 possible combinations. With a 5% significance threshold, approximately five significant results would be expected to emerge from a total of 105 tests by chance alone.

Population		Locus																Standard
n		SSa141	SSa13	SSa132	SSa12	SSa129	F43	SSa197	SSa171	SSa202	SLOSL456	SLOSL444	20.19	SLOSL439	SLOSL438	SSa85	Average#	error
Irish farmed	H_o	0.81	0.73	0.40	0.65	0.15	0.55	0.83	0.86	0.74	0.27	0.84	0.41	0.85	0.75	0.77	**0.64**	0.013
180	H_e	0.91*	0.78	0.42	0.73*	0.15	0.61	0.87*	0.84	0.75	0.26	0.92*	0.41	0.89*	0.77	0.88*	**0.68****	0.014
	a	25	13	5	8	3	6	17	13	10	2	29	4	22	8	14	**4.67**	0.083
Norwegian	H_o	0.93	0.42	0.56	0.65	0.04	0.52	0.54	0.80	0.92	0.41	0.85	0.37	0.84	0.52	0.52	**0.59**	0.034
farmed I	H_e	0.89	0.59	0.51	0.65	0.04	0.52	0.75	0.81	0.76	0.49	0.82	0.34	0.80	0.50	0.80*	**0.62**	0.032
30	a	18	4	3	5	2	3	9	7	8	2	14	3	9	7	9	**3.98**	0.054
Norwegian	H_o	----------	0.52	0.40	0.53	0.11	0.53	0.63	0.59	0.35	0.04	0.57	0.32	0.33	----------	----------	**0.41**	0.026
farmed II	H_e	----------	0.80*	0.46	0.62	0.10	0.65	0.86	0.83	0.55*	0.04	0.87*	0.33	0.75	----------	----------	**0.57****	0.039
26	a	----------	9	3	7	2	6	10	8	8	2	17	4	8	----------	----------	**3.88**	0.064
Numedal-	H_o	0.90	0.80	0.54	0.64	0.20	0.36	0.81	0.84	0.87	0.00	0.90	0.50	0.88	0.80	0.87	**0.66**	0.029
slagen	H_e	0.94	0.80	0.52	0.59	0.18	0.44	0.92	0.83	0.83	0.00	0.93	0.43	0.87	0.72	0.88	**0.66**	0.029
49	a	28	10	4	6	3	5	18	9	10	1	26	4	18	10	15	**4.74**	0.093
Mulkear	H_o	0.90	0.84	0.73	0.41	0.11	0.77	0.63	0.85	0.70	0.00	0.87	0.58	0.73	0.86	0.86	**0.66**	0.028
48	H_e	0.95	0.89	0.65	0.44	0.12	0.65	0.88*	0.87	0.87	0.00	0.88	0.69	0.87*	0.84	0.90	**0.70**	0.030
	a	25	17	5	4	3	5	11	11	12	1	27	5	20	10	17	**4.91**	0.094
Corrib	H_o	0.93	0.76	0.50	0.68	0.11	0.73	0.87	0.91	0.85	0.00	0.93	0.64	0.68	0.58	0.93	**0.68**	0.041
28	H_e	0.86	0.79	0.50	0.81*	0.10	0.68	0.86	0.85	0.79	0.00	0.91	0.63	0.84*	0.49	0.89	**0.69**	0.040
	a	15	8	3	8	3	5	10	9	7	1	17	4	12	4	15	**4.43**	0.075
Burrishoole	H_o	0.89	0.82	0.62	0.66	0.03	0.68	0.59	0.74	0.86	0.00	0.88	0.68	0.73	0.94	0.85	**0.67**	0.028
50	H_e	0.94	0.86	0.57	0.66	0.03	0.57	0.81*	0.82	0.81	0.00	0.83	0.59	0.81*	0.81	0.86	**0.66****	0.028
	a	27	10	3	6	2	6	15	8	10	1	25	4	17	8	15	**4.30**	0.077
Total	H_o	0.86	0.73	0.50	0.61	0.12	0.58	0.74	0.82	0.77	0.14	0.84	0.48	0.79	0.76	0.80	0.64	
	H_e	0.95#	0.84	0.52	0.69	0.12	0.61	0.90#	0.86	0.84	0.16	0.95#	0.50	0.91#	0.80	0.90	0.70	
	a	38	23	7	8	4	11	22	14	15	2	46	6	34	14	23		

Table 2-2 Summary statistics for genetic variation at 15 microsatellite loci surveyed in 7 poulations of Atlantic salmon.
H_o= Observed heterozygosity. H_e=Expected heterozygosity ((Nei 1978)) a=Number of alleles.
* Loci showing significant (p<0.01) deviations from Hardy Weinberg Equilibrium
** loci/populations showing significant (p<0.01) deviations from Hardy Weinberg Equilibrium across all populations/loci
# Average number of alleles across 12 loci estimated by resampling 100 replicates of the smallest from each population/locus combination.

Population/ year class	number sampled	H_e	H_o	SE of H_e	Ave. no. of alleles	SE
IF-93G	31	0.774	0.784	0.05	6.577	0.076
IF-94G	36	0.794	0.791	0.04	7.186	0.087
IF-95G	178	0.751	0.771	0.04	5.956	0.065
IF-96G	324	0.748	0.758	0.05	6.603	0.099
IF-97G	66	0.700	0.730	0.09	6.446	0.087
IF-97G_N	143	0.735	0.718	0.05	6.371	0.093
IF-98G	36	0.779	0.799	0.04	6.759	0.074
NF-I	342	0.724	0.755	0.05	5.811	0.090

Table 2-3 Summary statistics for genetic variation at 7 microsatellite loci surveyed in 6 year classes of Irish farmed and one poulation of Norwegian farmed Atlantic salmon.
H_o= Observed heterozygosity. H_e=Expected heterozygosity ((Nei 1978))
Average number of alleles across 12 loci estimated by resampling 100 replicates of the smallest population number (26) from each population/locus combination.

	SSa141	SSa13	SSa132	SSa12	SSa129	SSa197	SSa171	SSa202	SSa85	SLOSL456	SLOSL444	SLOSL439	SLOSL438	F43
SSa141														
SSa13														
SSa132														
SSa12		<0.05												
SSa129														
SSa197	<0.05													
SSa171														
SSa202														
SSa85														
SLOSL456														
SLOSL444				<0.01			<0.05							
SLOSL439														
SLOSL438			<0.05											
F43														
20.19														

Table 2-4 Matrix of all pairwise combinations of loci showing linkage disequilibrium P-values condensed accross populations



Figure 2-1 Average heterozygosity, variance of allele size and average number of alleles for wild and farmed Atlantic salmon populations genotyped at 15 microsatellite loci. IF= Irish farmed, NF I=Norwegian farmed I, NF II=Norwegian farmed II, Expected heterozygosity is shown on the upper axis and average number of alleles across all loci is shown on the lower axis. Variance of allele size is in parenthesis after the population name.

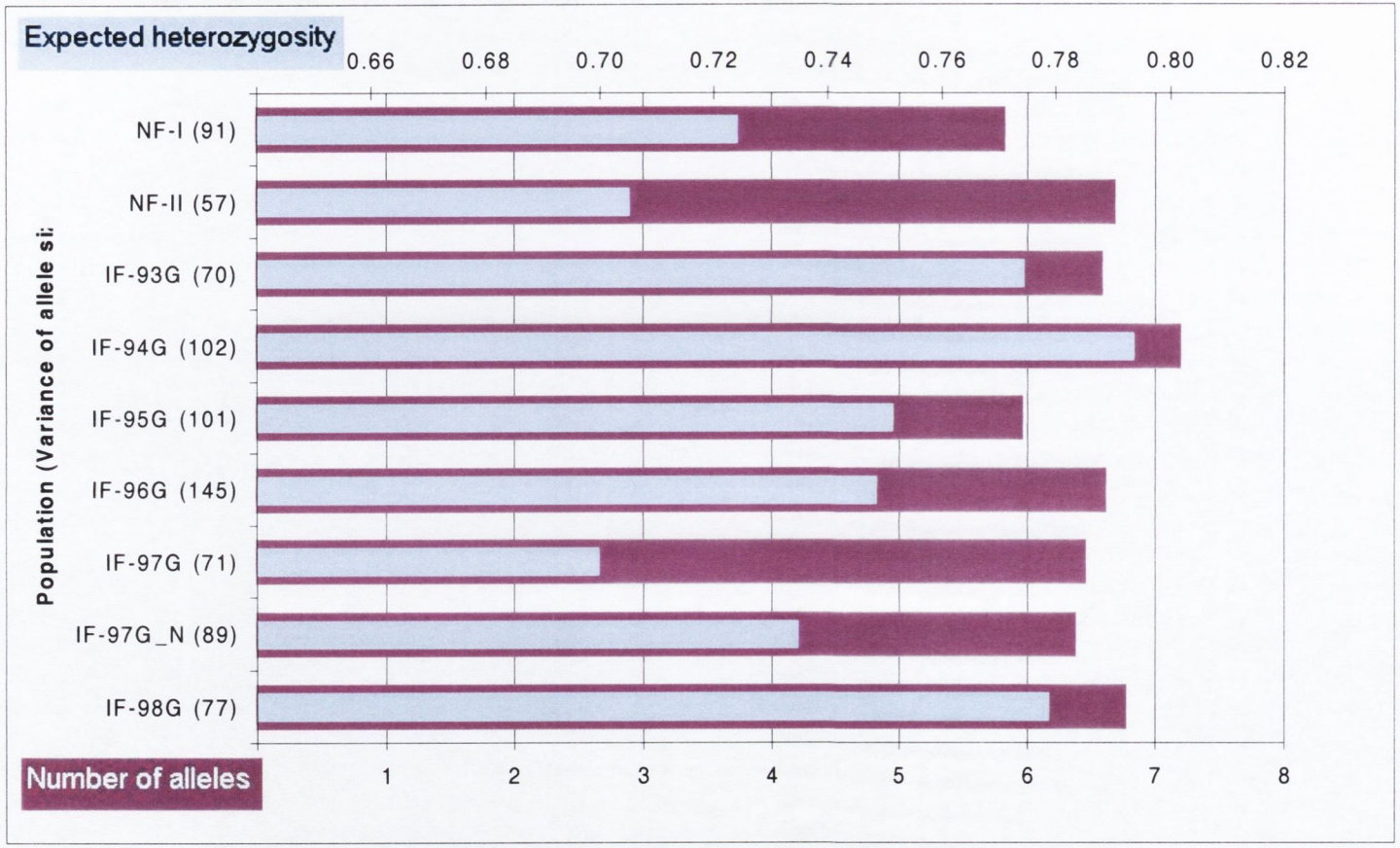


Figure 2-2 Average heterozygosity, variance of allele size and average number of alleles for different year classes farmed Atlantic salmon populations genotyped at 7 microsatellite loci.
IF= Irish farmed (with year class after hyphen), IF-97G_N Irish farmed, '97 year class imported from Norway (Norwegian farmed I), NF I=Norwegian farmed I, NF II=Norwegian farmed II
Expected heterozygosity is shown on the upper axis and average number of alleles across all loci is shown on the lower axis. Variance of allele size is in parenthesis after the population name.

2-3-b Genetic variability of populations.

Observed and expected heterozygosities and their standard errors for all populations over all genotyped loci are shown in Table 2-2 and Table 2-3. Mulkear had the highest heterozygosity of all populations and Norwegian farmed II the least. Levels in Irish and Norwegian farmed I populations do not differ significantly ($P>0.01$) from wild populations, whereas the Norwegian farmed II strain showed a significant reduction in heterozygosity when compared to all strains of wild salmon and to the Irish farmed strain ($P<0.01$). High levels of polymorphism were also observed within year classes of farmed Atlantic salmon (Table 2-3). These populations were genotyped at seven highly variable microsatellite loci and hence the genetic variation is higher than the same populations genotyped at 15 microsatellite loci. There was no clear trend of reduction in genetic variation, in terms of expected heterozygosity, from the 1993 year class to the 1998 year class. The three year classes showing the lowest variability in terms of expected heterozygosity were Irish farmed 1997 (native), Irish farmed 1997 (Norwegian import) and Norwegian farmed I. The lower genetic variation found in Irish farmed 1997 (Norwegian) may reflect the fact that these fish were imported from Norwegian farmed I population which also shows lower variation.

As the average number of alleles per locus is highly sensitive to sample size (Nei 1987), a resampling strategy (Park 2001), with replacement, was used where the minimum sample size was resampled across loci and populations 100 times to give an adjusted average number of alleles for each population. Farmed populations, in general, exhibit fewer alleles when compared with salmon from Numedalslagen, Mulkear or Burrishoole (Table 2-2). Only one farmed population (Irish farmed) has similar, or even higher, average number of alleles when compared to the wild populations. The Mulkear population exhibits the highest number of alleles after resampling across all loci/populations and has significantly ($p<0.01$) more allelic

diversity than all other populations except Numedalslagen. The farmed populations; Norwegian farmed I and Norwegian farmed II exhibit the lowest allelic diversity which is significantly lower then all other populations including Irish farmed.

One explanation for a reduction in allelic diversity in farmed salmon is that these populations went through a population bottleneck when the strains were created from a limited number of founding fish. When a population goes through a bottleneck, many low frequency alleles are eliminated. These rarer alleles contribute little to heterozygosity, and in this situation the heterozygosity measured at a locus (H_e) will exceed the expected heterozygosity computed from the number of alleles sampled assuming mutation-drift equilibrium (H_{eq}) (Watterson 1984; Maruyama and Fuerst 1985; Cornuet and Luikart 1997). The programme BOTTLENECK (Cornuet and Luikart 1997) calculates H_{eq} under two models of mutation: the IAM (Infinite Allele Model) and the SMM (Stepwise Mutation Model). A sign test determines if a significant majority of loci in a population exhibit heterozygosity excess. Table 2-5 shows the ratio of loci showing heterozygosity excess to those showing heterozygosity deficiency under the two models. Three populations, Irish farmed I, Norwegian farmed I and Corrib, show significant ($P<0.05$) heterozygosity excess under the IAM. However, under the more strict single-step SMM, none of these populations exhibited heterozygosity excess. Microsatellite loci are more likely to evolve under a model closer to the SMM (Di Rienzo 1994; Shriver et al. 1993; Valdes et al. 1993). and given that none of the populations showed a significant heterozygosity excess under the SMM, it is insecure to conclude that they do, in fact, represent bottlenecked populations.

Population	IAM		SMM	
	H_e/H_d	Probability *	H_e/H_d	Probability *
Irish farmed	13/2	0.016	2/13	NS
Norwegian farmed I	12/3	0.049	6/9	NS
Norwegian farmed II	5/7	0.232	3/9	NS
Numedalslagen	10/4	0.253	2/12	NS
Mulkear	11/3	0.112	8/6	NS
Corrib	13/1	0.006	7/7	NS
Burrishoole	10/4	0.213	5/9	NS

Table 2-5 Sign tests for heterozygosity excess in over 15 microsatellite loci in 7 populations in Atlantic salmon.
IAM Infinite allele model SMM Step-wise mutation model.
* Probability under the sign test that the population exhibits overall heterozygosity excess over all loci given the model.
H_e/H_d Ratio of number of individuals with a heterozygosity excess to the number with heterozygosity deficiency.

Another measure of genetic variation within a population is the variance of allele length at a locus. **Figure 2-1** and Figure 2-2 graphically illustrate levels of genetic variation between wild and farmed populations (15 microsatellite loci) and within all year classes of farmed populations (7 microsatellite loci) respectively in terms of expected heterozygosities, number of alleles and variance of allele size. Founder effects, small population sizes and artificial selection are likely to result in a decrease in allelic diversity which will be reflected in a corresponding decrease in the variance of allele size. The variance in allele size is lowest for the farmed populations and there is a significant correlation (P=0.003) between number of alleles and the variance in allele size. There were no significant differences in either allele diversity or expected heterozygosity over the 6 year period from which the Irish farmed population was sampled. Hence, in this time period, which approximates to one and a half generations, there is no detectable erosion of genetic variability in the population.

2-3-c Allele Frequencies.

A number of loci showed quite different allele frequency distributions between farmed and wild populations. One microsatellite (SSOSl456) was monomorphic in all wild populations studied but in farmed populations was polymorphic with a second allele at lower frequency. For a number of microsatellites, alleles which exist at high frequencies (up to 0.67) in one or all of the wild populations were not detected in farmed populations (SSa13, SSOSL438, SSOSL444, F43, SSa197, 20.19). There was one private allele for the Irish farmed population at SSa171. At SSa202, an allele displaying a high frequency of between 0.39 and 0.65 in farmed populations showed a frequency of between 0.018 and 0.18 in the four wild populations.

Allele frequency data for the three tetranucleotide microsatellites (SSa197, SSa171, SSa202) from one Canadian wild population, LaHave, were provided by the Marine Gene Probe Laboratory (Department of Biology, Dalhousie University, Halifax, Nova Scotia B3H 4JI). This North American population exhibited widely differing allele frequencies at these loci compared with European wild populations. Figure 2-3 shows allele frequencies for these three loci for the Canadian and four wild European populations combined. The discordant distributions of allele frequencies are indicative of a deep genetic divergence between North American and European Atlantic salmon populations.

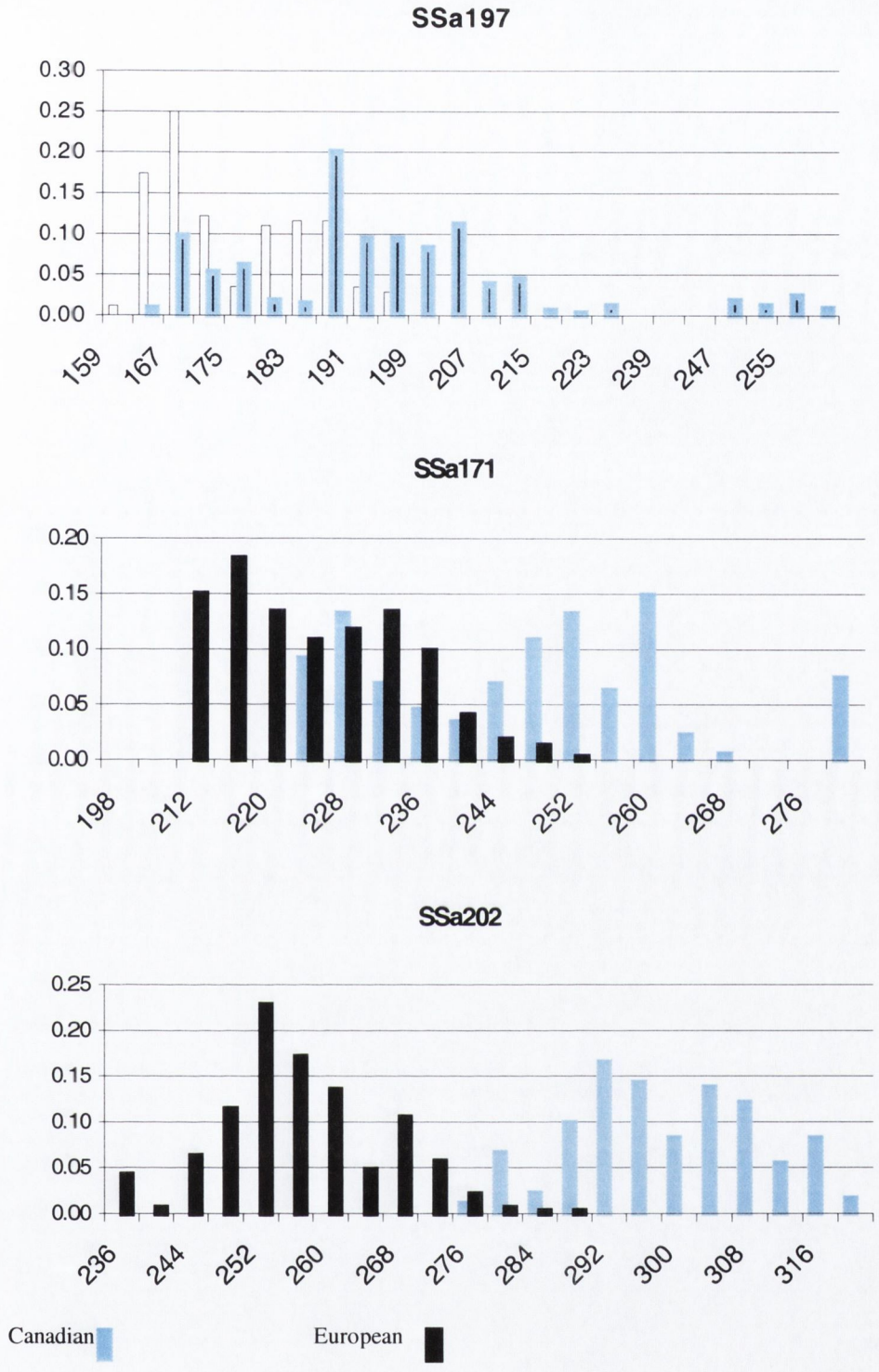


Figure 2-3 Allele frequencies at tetranucleotide loci: SSa197, SSa171 and SSa202 for one Canadian population (LaHave) and combined frequencies for four European wild populations (Numedalslagen, Corrib, Burrishoole and Mulkear).
Allele sizes are on the X axis and frequencies are on the Y axis. The Canadian population exhibits widely different allele frequencies at these loci compared with the European populations.

2-3-d Genetic differentiation.

All estimates of relative genetic differentiation (pairwise F_{ST}) (Slatkin 1995) between populations were significant (Table 2-6). The largest distance between populations, using both D_A and F_{ST} distances, is between Norwegian farmed salmon I and the three Irish wild populations, indicating that these two groups appear to be genetically most divergent. Farmed populations compared to farmed, and wild populations compared to wild, are genetically most similar. Estimates of population structure were obtained using R_{ST} (Slatkin 1995) and F_{ST} (Weir and Cockerham 1984) are shown in Table 2-7. R_{ST} values were similar but generally higher (in all but three loci) than Fst values. This agrees with (Slatkin 1995) who demonstrated that F_{ST} will tend to underestimate the true level of genetic differentiation when applied to microsatellite data. Both measures are significant ($p<0.01$) over all loci except for one locus, SSa129. Higher F_{ST} and R_{ST} values were observed for microsatellites in which alleles unique to farmed/wild salmon were observed at high frequency. Population differentiation was assessed using pairwise comparisons according to Raymond and Rousset (1995), and each combination displayed significant differentiation ($p<0.001$).

Population	Irish farmed	N-F I	N-F II	Numeda-lslagen	Mulkear	Corrib	Burrishoole
Irish farmed		0.108	0.190	0.125	0.178	0.258	.0211
Norwegian farmed I	0.030		0.185	0.197	0.262	0.331	0.274
Norwegian farmed II	0.035	0.080		0.059	0.073	0.075	0.074
Numedalslagen	0.037	0.072	0.160		0.156	0.224	0.172
Mulkear	0.049	0.096	0.190	0.033		0.206	0.187
Corrib	0.050	0.099	0.212	0.053	0.052		0.220
Burrishoole	0.066	0.119	0.185	0.046	0.043	0.052	

Table 2-6 Pairwise genetic differentiation (F_{st}) , below diagonal, and pairwise D_A distances, above diagonal, between 7 populations detected by 12 microsatellite loci.

Locus	Fst	Rst
SSa141	0.03*	0.066
SSa13	0.042*	0.182
SSa132	0.056*	0.058
SSa12	0.031*	-0.008
SSa129	0.009	0.024
F43	0.026*	0.059
SSa197	0.056*	0.049
SSa171	0.033*	0.087
SSa202	0.108*	0.061
SSOSL456	0.125*	#
SSOSL438	0.096*	0.353
SSOSL444	0.063*	0.073
SSa85	0.025*	0.092
20.19	0.017*	0.026
SSOSL439	0.014*	0.071
overall	0.057*	0.085*

* = $P < 0.001$
# = not estimable in RstCalc (Goodman 1997) due to the fact that this locus is monomorphic in most populations.

Table 2-7 Overall and per locus Fst and Rst values for 7 populations of European Atlantic salmon.

2-3-e Phylogenetic analysis.

D_A distances between populations were estimated according to (Nei et al. 1983) using data from the 12 loci common to each population, and a phylogenetic tree was constructed by using the neighbor joining method. A phylogenetic tree was also constructed using pairwise F_{ST} distances and this tree gave the same pattern of clustering. D_A distances between year classes within farmed populations were also estimated using data from the 7 microsatellite loci, and a phylogenetic tree was constructed by using the neighbor joining method. The phylogeny shown in Figure 2-4 demonstrates separate clustering of farmed and Irish wild populations, with the Norwegian wild population outside both of these groups. This clustering, however, depends on the rooting of the tree and a different rooting could result in the placement of the Norwegian farmed II population outside this farmed group. The Irish

farmed and Norwegian farmed I populations group together as would be expected given their common ancestry.

The phylogeny of farmed year classes also shows significant bootstrap values. There are three major splits all supported by bootstrap values of greater than 80% (Figure 2-6). The first grouping if for Irish farmed year classes 1993, 1994 and 1998. Broodstock from year classes 1993 and 1994 would have been the parents (2SW and 3SW) of the year class 1998 and this may explain the grouping of these year classes. The grouping of the two 1997 year classes (IR-97_N) with the Norwegian farmed I population can be explained by the fact that the IR-97_N group was imported as eyed eggs from the Norwegian farmed I population.

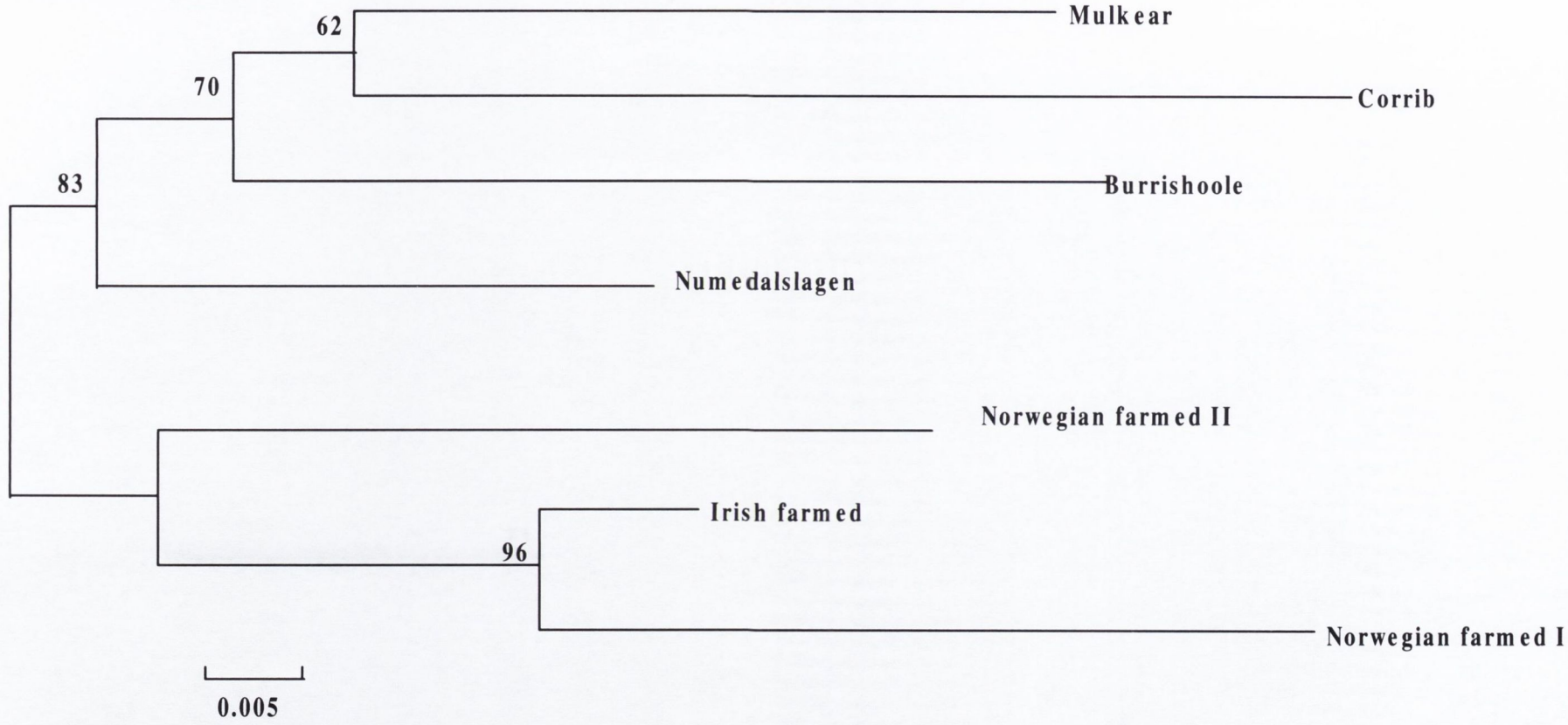


Figure 2-4 Neighbor joining tree with DA distances for seven populations (three farmed and four wild) using 12 microsatellite loci.
The scale represents the distance between branches and bootstrap values are shown beside each node. Bootstrap values represent the percent of all replicates consistent with each branch point.

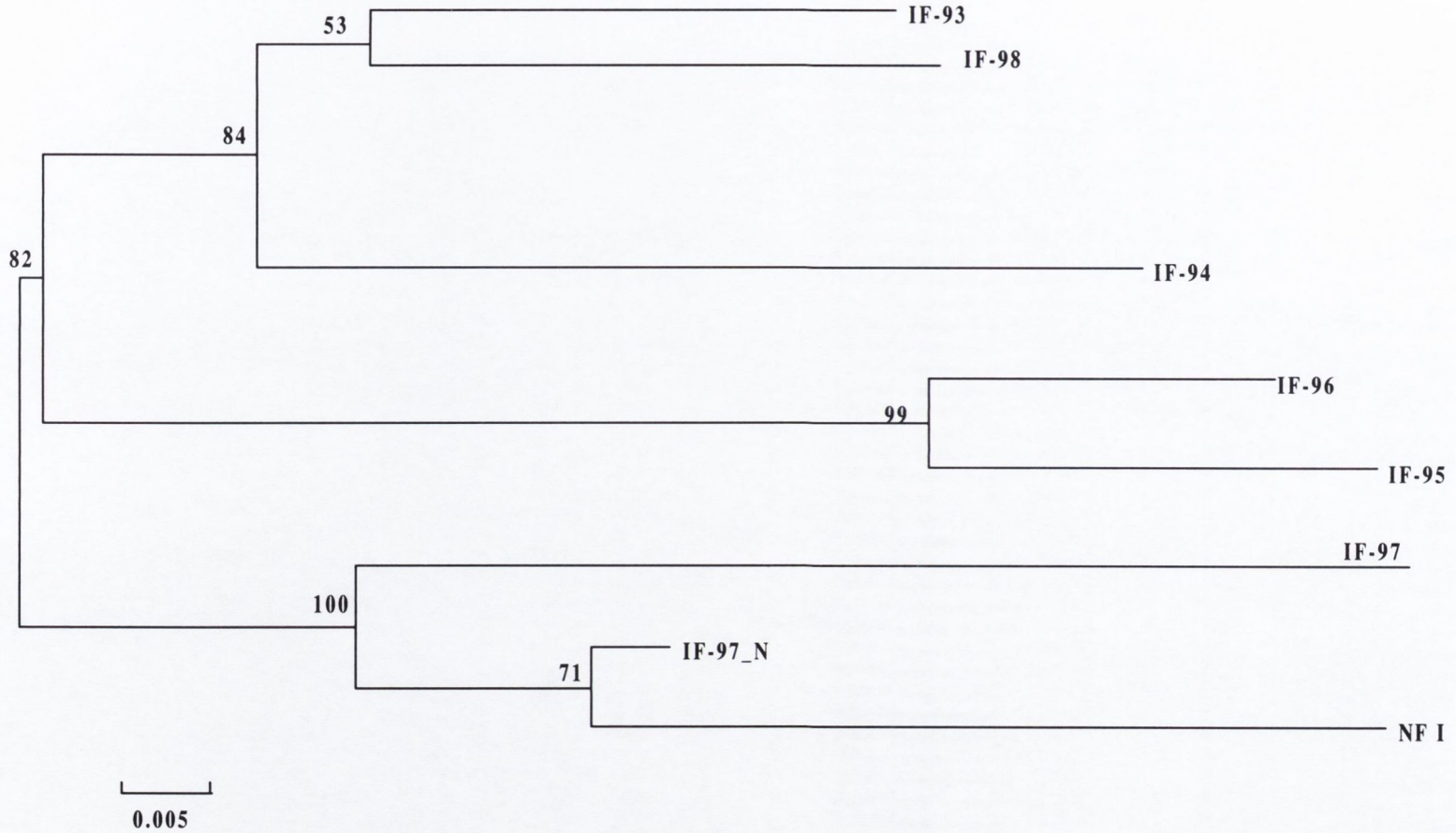


Figure 2-5 Neighbor joining tree with DA distances for 6 year classes of Irish farmed population and two samplings of Norwegian farmed I using 7 microsatellite loci.

The scale represents the distance between branches and bootstrap values are shown beside each node. Bootstrap values represent the percent of all replicates consistent with each branch point.

IF= Irish farmed (with year class after hyphen), IF-97G_N Irish farmed, '97 year class imported from Norway (Norwegian farmed I), NF I=Norwegian farmed I, NF II=Norwegian farmed II

The allele sharing method developed by (Bowcock et al. 1994), generates distances between individuals based on the proportion of shared alleles. These distances were used to construct the neighbor joining dendrogram shown in Figure 2-6. 208 samples from 4 wild populations (Numadalslagen, Mulkear, Corrib and Burrishoole) and 2 farmed populations (Irish farmed and Norwegian farmed I) were used in the analysis. Irish and Norwegian farmed I populations are shown as one strain (farmed salmon of Norwegian origin) and the three Irish populations are also shown as one group. This dendrogram (Figure 2-6) revealed that most clusters either contained individuals of farmed origin, or of wild origin and where there was mixing between the two it was mostly of farmed and Numadalslagen fish. This is hardly surprising given the Norwegian origin of all farmed fish in this study.

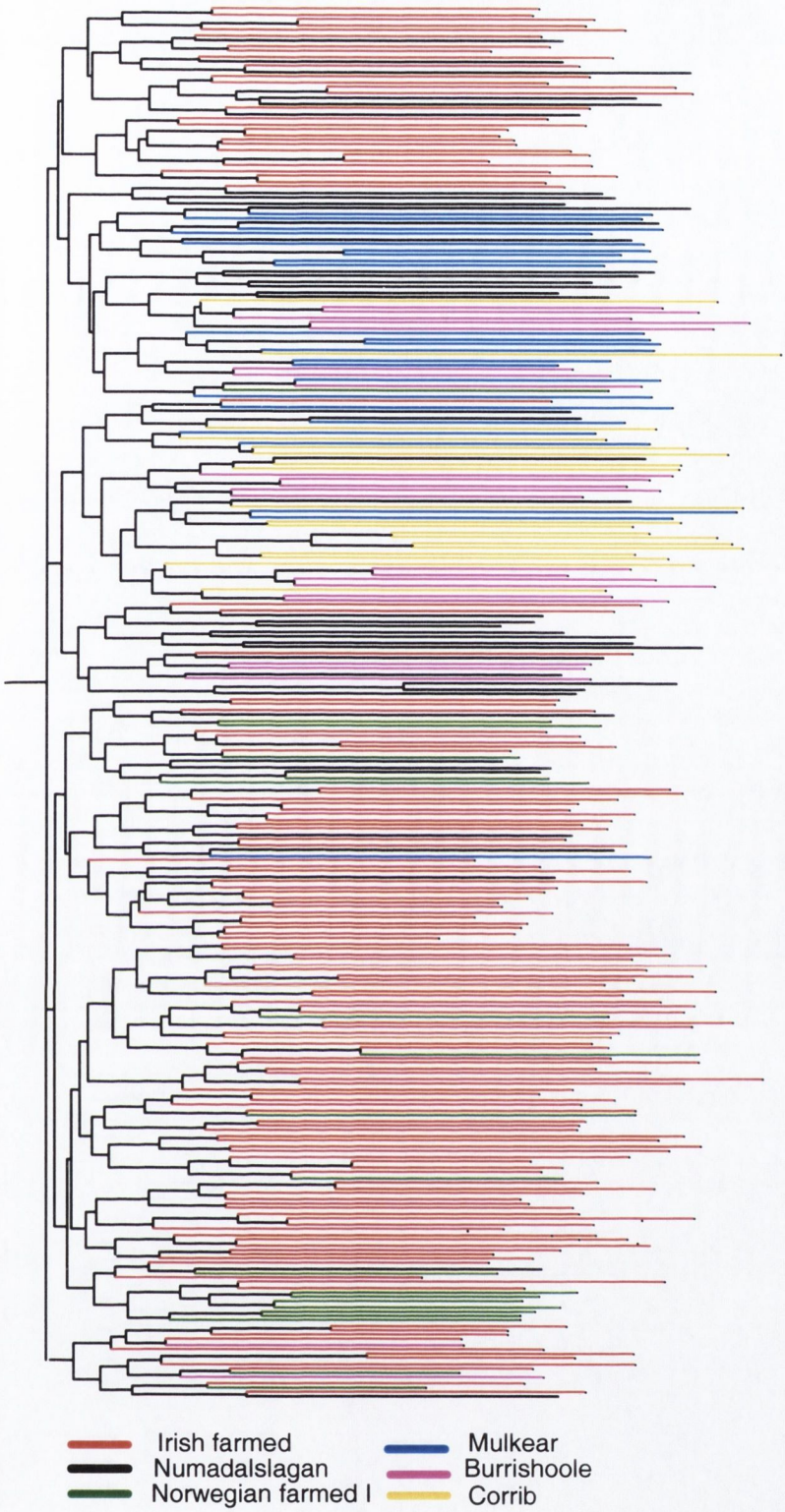


Figure 2-6 Neighbor-joining dendrogram summarising ASM distances for 208 individual Atlantic salmon from farmed and wild populations.
This dendrogram reveals that most clusters contain either individuals of farmed origin, or of wild origin and where there was mixing between the two it was mostly of farmed and Numadalslagen fish. Individuals from wild populations cluster together particularly strongly.

2-4 Discussion.

Farmed and hatchery populations of Atlantic salmon have been reported as having 20-30% lower heterozygosity in a number of studies when examined using polymorphic enzyme loci (Cross and King 1983; Stahl 1983; Verspoor 1988). However, using the same technique other studies have demonstrated no substantial reduction in heterozygosity between wild and reared strains (Crozier and Moffett 1989; Cross et al. 1993; Mjolnerod et al. 1997). DNA markers are more likely to detect small differences between populations due to their higher levels of allelic variation, for example, minisatellite DNA markers have been shown to detect significant differences in both overall heterozygosity and mean number of alleles between wild and farmed strains (Mjolnerod et al. 1997; Clifford et al. 1998). Our study demonstrates differences between some wild and farmed strains in terms of allelic diversity but not in general in overall heterozygosity.

Small effective population sizes and poorly managed breeding programmes are generally blamed for loss of genetic diversity in farmed stocks. Cross et al, (1993), demonstrated that, when measures are taken to avoid inbreeding as part of a breeding programme, genetic variation need not be reduced despite small effective population sizes. This may be achieved by maintaining pedigree information on all individuals in the breeding programme and using this information to arrange matings that will ensure only minimum increases in inbreeding every generation.

The present study demonstrated significant differences between farmed populations in terms of genetic variability. Norwegian farmed II fish displayed the lowest levels of variability of all populations in terms of heterozygosity, allele number and allele size variance. Since their foundation, all strains have undergone breeding programmes to improve commercially

important production traits. Norwegian farmed II population is in its sixth generation of combined family and individual selection and the other two farmed populations are part of mass selection programmes which use individual selection only. Practising family selection, which reduces the effective population size by maintaining a limited number of families, is likely to result in an even more pronounced reduction in genetic variability as evidenced in the Norwegian farmed II population. Although there were some significant differences between year classes of the Irish farmed population, there was no trend in changes in genetic variation, either in terms of average number of alleles or average heterozygosity. This result would suggest that breeding practices over the past one and a half generations are not reducing genetic variation in any systematic manner.

However, all wild populations have higher number of alleles when resampling was performed across all loci and higher heterozygosity when compared to the two populations of Norwegian farmed salmon. Our results, assuming the stepwise mutation model of microsatellite evolution, would not suggest that this was caused by a population bottleneck when the populations were created, but rather, the effects of artificial breeding practices combined with low original founder numbers. The heterozygosity excess evident in the two farmed populations and the wild Corrib population is most likely a result of sampling (only 28 individuals sampled in the Corrib population).

Phylogenetic analysis yielded a clear division between farmed and Irish wild populations. This difference is likely to be a result of the geographical separation of the ancestral strains and the effects of artificial selection on the genetic differentiation of the farmed strain. Irish farmed and Norwegian farmed I exhibit the smallest genetic distance between them for both measures – a reasonable result as the Irish strain was created from the Norwegian strain only

15 years ago and the population has been closed subsequently. The placement of the Norwegian wild population is dependant on the rooting of the tree and this phylogenetic analysis would be greatly helped by the addition of one or more other Norwegian wild populations in order to resolve the best positioning of this group.

Comparing individual genotypes of 208 salmon also shows the clearest differentiation to be between individuals from farmed and wild strains. Despite the common Norwegian origin of farmed strains, there is high genetic differentiation between them and the only Norwegian wild population (Numadalslagen). This illustrates that founder effects and artificial selection have altered the genetic composition of farmed strains relative to wild salmon. . However, not knowing the numbers of founders used to make up the farmed population makes it difficult to intrepret any differences in genetic variation being the result of the founding effect. Farmed fish (of Norwegian origin) group together in phylogenetic analysis. The three Irish wild populations show a clear grouping as populations, and in the dendrogram constructed from individual distance measures, (Figure 2-6).

In conclusion, this study demonstrates the high levels of polymorphism detectable with microsatellite loci even within farmed strains of Atlantic salmon. In an aquaculture situation this technology has the potential to be of great use in monitoring levels of genetic variation within stocks as well as for parentage and relatedness purposes. These results also shoe that allelic diversity is a slightly more sensitive measure of differences in genetic variation between wild and farmed populations than overall heterozygosity, as previously observed in hatchery salmon and farmed rainbow trout (Butler and Cross 1996; Tessier et al. 1998). This is likely to be a result of the loss of low frequency alleles when new populations are created from larger founder ones. Differences in genetic variation observed between farmed stocks may be a result of different breeding regimes and population sizes.

Chapter 3

Parentage and relatedness determination in farmed Atlantic salmon (*Salmo salar*) using microsatellite markers

3-1 Introduction

In selective breeding programs, accuracy of selection is increased by combining information from the breeding individuals and all relatives (Falconer 1989). This is particularly true for traits where the heritability of the trait is low or the trait is not measurable on the breeding individual itself e.g. flesh quality traits, mortality, disease resistance. One of the difficulties in implementing a family based selective breeding program in aquacultural stocks is maintaining pedigree information. Progeny from family groups must be isolated and reared together in costly multi-tank facilities until they are large enough to be tagged. Not only is this approach space and labour intensive, but it also introduces environmental effects common to full-sib groups which are confounded with the genetic effects under study Herbinger et al. (1999b); (Rye and Mao 1998). Environmental effects common to groups reared together include food availability, competition, water quality and tank density. Ideally in a testing scheme applied to breeding programs environmental effects common to full sibs should be avoided from as early a stage as possible thereby avoiding the problem of confounding environmental and genetic effects.

Genetic profiles from DNA markers would allow retrospective assignment of individuals to family groups even after different progeny groups had been reared communally after hatching. Parentage assignment using genetic profiles has already been suggested for use in aquaculture situations in Atlantic salmon (Wright and Bentzen 1994; Doyle and Herbinger 1995). The feasibility of this approach has been assessed in communally reared rainbow trout, where 91% of fish were traced to one or two parental couples using 4 microsatellite loci (Herbinger et al.

1995) and in Atlantic salmon where between 80 and 99% of offspring were assigned to one set of parents in a number of different crosses using 4 microsatellite loci (O'Reilly et al. 1998).

Another difficulty in managing a selective breeding program is loss of genetic variability and increases in inbreeding as a result of the inadvertent mating of related individuals. The effects of inbreeding in salmonids include moderate to high inbreeding depression of survival and growth traits (Gjerde et al. 1983; Kincaid 1983;; Berg et al. 1996). Inbreeding also will result in a decrease in genetic variability, which will limit the potential for genetic gain from artificial selection. Once reliable pedigree information is available, matings can be arranged which minimise inbreeding.

In the absence of pedigree information, as is likely in the first generation of a breeding program, the risk of mating related individuals may lead to inbreeding in families which form the basis of all selection in that population in the future. Estimates of relatedness based on DNA genotyping offer breeding managers of aquacultural stocks a method of avoiding inbreeding and maintaining genetic variation in the absence of pedigree information. Blouin et al. (1996) demonstrated the usefulness of microsatellite markers for discriminating unrelated from related individuals in a wild outbred population of mice using genotypes from 54 captured mice and randomly generated genotypes.

To address these issues, this study was initiated to investigate the feasibility of correctly assigning individuals to family groups using microsatellite data, and to assess the usefulness of these same microsatellites for estimating relatedness between pairs of individuals.

3-2 Materials and methods

3-2-a Fish Samples

The farmed populations used in this study were taken from a salmon farm on the North West coast of Ireland. This Irish farmed population originates from a Norwegian strain which was set up in the late 1960's from a number of wild Norwegian populations. Ova were imported into Ireland between 1982-1986 to create the Irish farmed population and since then the breeding system has been closed.

3-2-b Parentage study

During the breeding season of 1997 tissue samples were taken from 12 broodstock (two males and ten females) which were then mated to create ten full sib and two half sib families. Fertilised ova (16) were taken from each pair mating before the ova were mixed so that the parentage of the offspring was known. These families were mixed and once fry had emerged samples were taken from a further 25 offspring of unknown parentage. 16 individuals were genotyped from each family with the further 25 offspring from the mixed families making a total of 185 offspring for which to assign parentage.

Ten full-sib and two half sib families were therefore available for parentage analysis. However, in a real breeding program parentage for offspring will have to be assigned from a pool of many more parents. In order to simulate this situation, the genotypes from a further 180 salmon from the same population were included in the analysis. DNA from these individuals had already been genotyped at eight microsatellite loci (Norris et al. 1999) (see Table 3-1). These individuals represented 120 pairs of candidate parents (120 females crossed

to 60 males) as a background to the 10 actual pairs of parents. The objective, therefore, was to assign 185 offspring from ten families to their correct parents from within a pool of 110 possible parental pairs (12 real parental genotypes and 180 individuals randomly taken from the same population backround). Furthermore, assignment to correct parent pair was also investigated using a smaller suite of four microsatellite loci (Table 3-1). These microsatellite markers can be amplified together in a multiplex reaction as described by (O'Reilly et al. 1996).

3-2-c Relatedness study

For the relatedness estimations, genetic profiles from all parents and offspring were available at eight microsatellite loci and for the randomly chosen candidate parents at 15 microsatellite loci (see Table 3-1). Additionally, computer simulated genotypes, at 15 microsatellite loci were generated for a further generation of individuals. The mating structure assumed that groups of the actual full-sib progeny were mated to unrelated partners. Thus, their offspring are first cousins. Simulated genotypes were generated using the module PROBMAXG of the program PROBMAX (Danzmann 1997).

Relatedness values between individuals based on actual genotypes at all loci were available for individuals across year classes (could not be full or half-sibs) and individuals within year classes (could be full or half-sibs). For the purposes of this study these two groups will be termed 'unrelated' and 'unknown relatedness' respectively.

3-2-d Microsatellite analysis

Genetic profiles at 15 loci were available for the Irish farmed salmon (Norris et al. 1999) and allele frequency distributions were obtained from these data. Details of PCR conditions, variability in farmed salmon and references for all microsatellite loci are given in Table 3-1.

PCR primers for loci SSa141, SSa13, SSa132, SSa12, SSa129 and SSa85 were designed from published sequences, and all other primers were synthesised according to referenced author (see Table 3-1). Family groups, which included parents and offspring, were assayed at the same eight loci. These loci were chosen for their ease of amplification and the ability to score them easily on autoradiographs of acrylamide gels. For the parentage study two datasets were used based on microsatellite genotype: one containing genotypes of all eight microsatellites used to genotype all parents and progeny and one containing only four microsatellite loci genotypes.

Microsatellite locus	$MgCl_2$ mM	Number of alleles	Expected heterozygosity	Annealing Temp.(^{0}C)	Reference/GenBank accession number
SSa141[a]	1.5	25	0.91	60	GenBank U58905
SSa13[a]	2.0	13	0.78	58	GenBank U58903
SSa132	1.5	5	0.42	55	GenBank U58901
SSa12[b]	1.5	8	0.73	55	GenBank U58900
SSa129	1.5	3	0.15	54	GenBank U58899
SSa197[a b c]	1.5	17	0.87	59	O'Reilly *et al.*, 1996
SSa171[a b c]	2.0	13	0.84	58	O'Reilly *et al.*, 1996
SSa202[a b c]	2.0	10	0.75	55	O'Reilly *et al.*, 1996
SSa85[a b c]	2.0	14	0.88	57	O'Reilly *et al.*, 1996
SLOSL456	2.0	2	0.26	55	Slettan *et al.*, 1997
SLOSL444[a]	1.5	29	0.92	56	Slettan *et al.*, 1995
SLOSL439[a b]	1.5	22	0.89	58	Slettan *et al.*, 1995
SLOSL438	1.5	8	0.77	55	Slettan *et al.*, 1995
F43[b]	1.5	6	0.61	56	Sanchez *et al.*, 1996
20.19[b]	1.5	4	0.41	56	Sanchez *et al.*, 1996

[a]: 8 Most variable microsatellite loci
[b] : 8 Microsatellite loci used to genotype all parents and progeny
[c] : 4 Microsatellite loci in smaller parentage assignment dataset.
Table 3-1 Details of the Atlantic salmon microsatellites used in this study.

3-2-e Statistical analysis

Likelihood of pedigree relationships was assessed using a program, *Kinship* version 1.2, developed by (Queller and Goodnight 1989). This program tests the hypothesis of a specified pedigree relationship given the population allele frequencies. After generating a likelihood that the specified relationship is true, *Kinship* estimates a significance level for this likelihood value by generating random pairs of individuals and determining the likelihood value needed to reject the null hypothesis at different significance levels.

The same program was used to generate a relationship statistic r_{xy}, (Queller and Goodnight 1989) which is an unbiased estimate of the true additive genetic relationship between individuals x and y. Both relatedness and parentage statistics use the population allele frequencies (already estimated) to weigh matches between pairs of individuals. Individual pairs were classified as related or unrelated based on a cut-off point of relatedness. To estimate the probability that individuals might be, the frequency of type I errors (related individuals being classified as unrelated) was estimated under different cut-off points of r_{xy}.

3-3 Results

3-3-a Matching offspring to parents.

Based on the output from the *Kinship* program there are a number of different outcomes for each individual: an individual may be assigned to no parent pair, an incorrect parent pair(s), the correct parent pair and no others or more than one pair (including the correct pair). Table 3-2 shows the results of the assignment of offspring to parents under the three different scenarios given above using eight and four microsatellite markers. Initially, 4 individuals had genotypes inconsistent with any parental cross and having confirmed that these were not

scoring errors, were subsequently genotyped a second time. Inconsistent genotypes such as these may occur due to a number of reasons: eggs may be mixed between families in the hatchery or in the laboratory, samples may be inadvertently mixed between wells during DNA extraction and/or PCR, or a mutation may occur in the germ line of the parents or in the developing embryos themselves. Having re-genotyped the samples with inconsistencies, two were confirmed as being mutations (in microsatellite loci *SSa202* and *SSa171*) and the other two were a result of contamination during PCR and were later matched to their correct parents. The two mutations were detected over 320 meioses (160 offspring) and eight loci giving a mutation rate of 7.8×10^{-4} or 1 in 1280. This is twice as high as the mutation rate observed in Atlantic salmon by O'Reilly et al. (1998). However, this difference is unlikely to be significant given the small sample in this study. In the case of the 25 fry, all individuals were unambiguously matched to one set of parents.

Result	Genotypes from:	
	8 Microsatellite loci	4 Microsatellite loci
% of offspring assigned to true parents and no others	95.6%	94.3%
% of offspring assigned to 2 sets of parents (including true parents)	3.3%	4.6%
% of offspring wrongly assigned or assigned to no parental pair	1.1%	1.1%

Table 3-2 Assignment of offspring to parents under three different scenarios. Percentages are given as number of offspring assigned out of a total of 185.

Scoring errors were not a major source of error in this analysis as, with the exception of the 25 mixed fry, all individuals were genotyped alongside their real parents thereby ensuring ease of allele scoring. In a study where parentage was determined for communally reared offspring, O'Reilly et al. (1998) found a scoring error rate of 2-3% per allele scored. However, that study found that 90% of these errors were later detected and only 0.5% would lead to an incorrect assignment of offspring to parent.

Using the data set of 110 potential parent pairs, all offspring, except the 2 individuals mentioned above, were assigned to either one or two parental pairs. Table 3-2 shows the breakdown of allocation of all individuals to parental pairs in this scenario. In total, 95.6% of individuals could be traced uniquely to their real parents out of a possible 130 pairs of parents. A further 3.3% could be narrowed down to two sets of parents. Overall, 98.9% of individuals could be allocated to, at most, two parental pairs and the remainder, as a result of mutations, could not, unless the mutated locus was eliminated from genotype, be matched to any parental pair. The use of only four microsatellite loci to genotype parents and offspring resulted in the percent of offspring correctly assigned to one parent pair reduced to 94.3%.

Allocation to a single pair could presumably be improved further with the replacement of one or two of the less variable markers used in the study by more variable ones. This further discrimination would be required where there are more than 130 sets of potential parents in the family population.

3-3-b Relatedness

A relatedness statistic, r_{xy}, (Queller and Goodnight 1989) was calculated for all parents and offspring which had been genotyped at the 8 loci shown in Table 3-1 and for all other farmed salmon of unknown relatedness which had been genotyped at all 15 loci. The same calculations were performed for individuals whose genotypes had been generated by random simulation. Distributions of r_{xy} for the three datasets mentioned above are shown in Figure 3-1. Using individuals which had been actually genotyped at 8 loci (plot A) results in distributions with higher standard errors for each group than for the other two scenarios. The means and standard errors for r_{xy} for all related groups in plot A are all somewhat lower than would be expected for full-sibs (0.5), half-sibs (0.25) and cousins (0.12). This may be a sampling effect, as the 95% confidence levels for these means are all just above 0.01, or it may be due to the low power of these loci for detecting relatedness. The difference in the standard error of the distributions between plot A and plot B is not observed between plot B and plot C, indicating that the same or similar resolution is being obtained with the 8 most variable microsatellite markers as with 15. The 'unknown' groups here consist of individuals sampled both between and within year classes of farmed salmon and, therefore, could contain full and half-sibs. This explains why the mean of the distribution of this group is significantly ($p<0.01$) higher than zero in all plots. Where genotypes of offspring were generated by simulation from randomly chosen salmon previously genotyped, the relatedness values were all significantly higher ($p<0.01$) than would be expected for full-sibs, half-sibs and cousins (plots B and C). This may be a result of generating offspring from parents which may already have been distantly related.

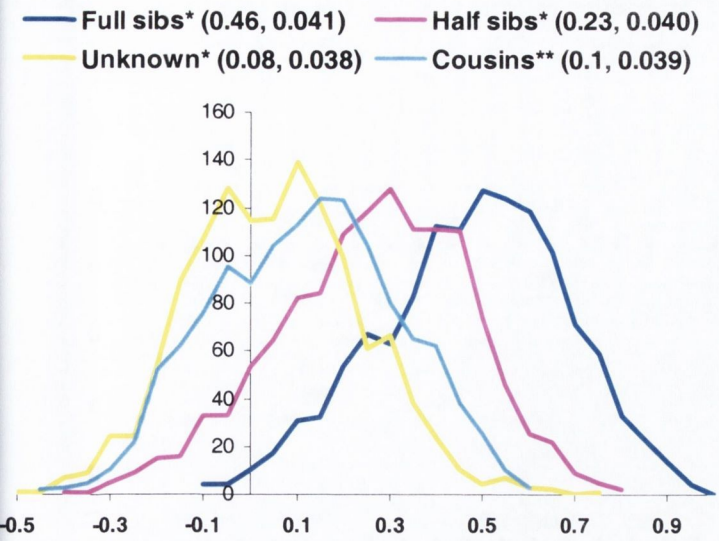
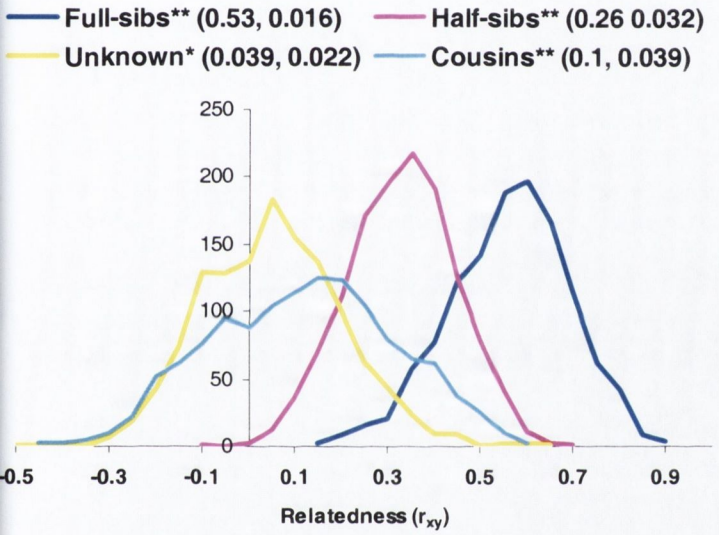


Figure 3-1 Relatedness values for full-sibs, half-sibs, cousins and individuals of unknown relatedness with three different genotype datasets

Plot A: 8 microsatellite loci

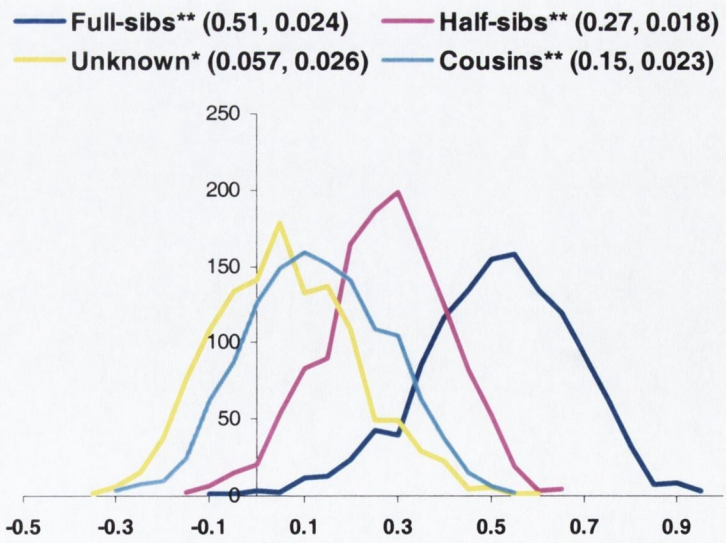


Plot B: 15 microsatellite loci

Means and standard errors of distributions are shown in parenthesis. The 'unknown' group refers to individuals sampled both within and between year classes and, therefore, could contain full-and half-sibs.

* Actual genotypes. ** Randomly generated genotypes.

Plot C: 8 most variable microsatellite loci



In an aquaculture situation, the purpose of estimating relatedness among potential mating pairs is to avoid mating related individuals in the absence of pedigree information. Since broodstock are slaughtered after spawning, no potential parent-offspring pairs will exist, and the related pairs most likely to be found are full-sibs, half-sibs and cousins. The objective of using microsatellite markers is to allow us to classify all potential pairs as either related or unrelated. There are two types of errors in misclassification. A pair may be misclassified as related when they are in fact unrelated (type I error) or a pair may be misclassified as unrelated when they are in fact related (type II error). Type II error is the one we are most concerned with as this error could result in related pairs being mated.

The frequency of both types of errors will depend not only on the number and variability of microsatellite loci used but also on the criteria of relatedness chosen below which pairs will be classified as unrelated. Table 3-3 shows the proportion misclassified as type II errors within each data set using two different values of relatedness as criteria. Using 15 microsatellites greatly improves the misclassification rates.

However, using the 8 most variable loci and the stricter decision criterion discriminates between related and unrelated almost to the same extent. Lowering the criterion improves the classification rates by allowing fewer related pairs to be classified as unrelated. By using 0 instead of the mean of pairs of unknown relatedness results in just over one in ten cousins and one in 100 half-sibs being classified as unrelated (Table 3-3).

Proportion misclassified using mean of distribution of unknown relatedness as cut-off point.

Misclassification type:	Genotypes from: 8 microsatellite loci	15 microsatellite loci	8 most variable microsatellite loci
Full-sibs classified as unrelated	0.05	0.00	0.01
Half-sibs classified as unrelated	0.22	0.01	0.08
Cousins classified as unrelated	0.46	0.26	0.28

Proportion misclassified using 0 relatedness as cut-off point.

Misclassification type:	Genotypes from: 8 microsatellite loci	15 microsatellite loci	8 most variable microsatellite loci
Full-sibs classified as unrelated	0.01	0.00	0.00
Half-sibs classified as unrelated	0.13	0.00	0.01
Cousins classified as unrelated	0.33	0.07	0.08

Table 3-3 Misclassification rates under two different values of relatedness as criteria

The effect of lowering the criterion even further can be seen in Figure 3-2 where the frequency of type II errors (for all related individuals) was estimated using criteria ranging from 0.05 to –0.3 and using the 8 most variable microsatellite loci. As the cut-off point of relatedness is lowered, the frequency of type II errors also decreases. However, using a lower criterion will result in fewer pairs being classified as unrelated and will, therefore, limit the number of pairs available for selection . Figure 3-2 shows the percentage of pairs available for selection when the cut-off point is lowered. For example, using 0 relatedness as the criterion would result in just over 40% of all potential mating pairs being categorised as unrelated, and lowering it further to –0.15 reduces this percentage to 13%. Where relatedness, based on microsatellite markers, is being used to avoid mating related individuals, a balance needs to be

found between lowering the criterion of selection and ensuring that sufficient pairs are available for selection.

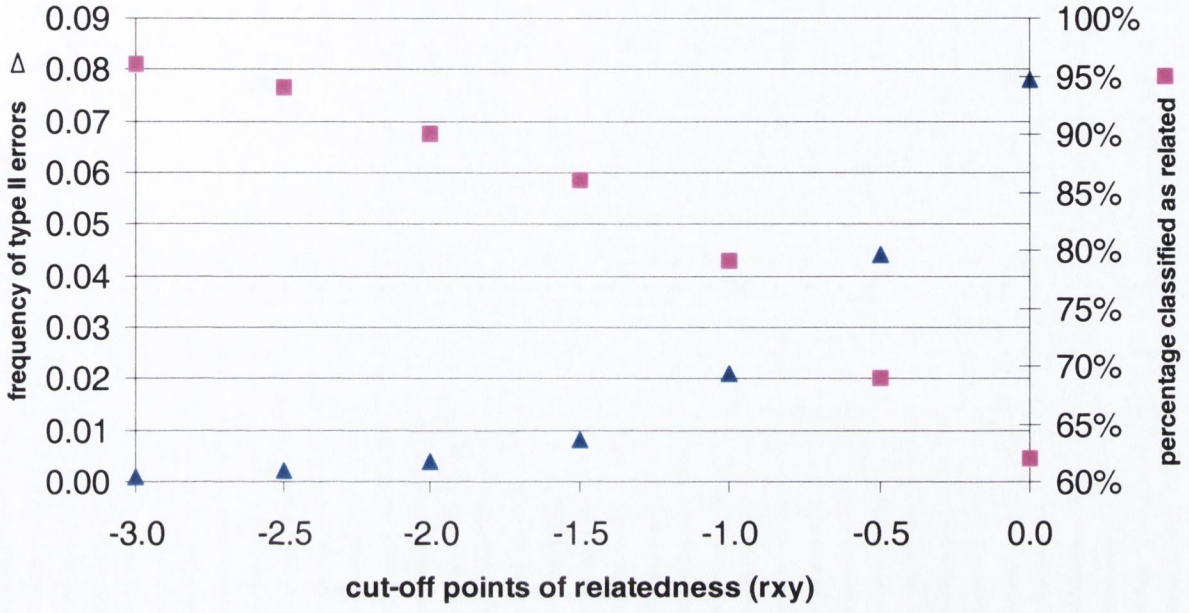


Figure 3-2. Frequency of type II errors (related individuals being classified as unrelated) and percentage of individuals classified as related under relatedness criteria between 0.5 and –3.0.

3-4 Discussion.

This study demonstrates that both parentage and relatedness can be determined in the absence of physical tags and pedigree information in a mixed farmed salmon population. Using the real parent group as well as another 100 possible parents resulted in 95.6% of offspring being unambiguously assigned to one correct set of parents using eight microsatellite loci and 94.3% using four highly variable microsatellite loci. The majority of offspring not assigned to their

true parents and no others, were assigned to two sets of parents, one pair being the real parents. This is a consequence of the factorial mating design where two females are crossed to one male. Where the two females have similar microsatellite profile, it may be impossible to unambiguously assign parentage to the female parent. It should also be considered that after a number of generations of selection, where microsatellite markers have been used to identify parents, the usefullness of any suite of markers will be significantly diminished as the number of alleles in the family population is reduced due to selection. In this case, new markers will have to be found to subsitute some or all of the original suite.

Ideally, however, we would like to achieve the same or similar assignment from a smaller number of microsatellite markers. This is important in order to make genetic profiling a realistic alternative to physical tagging from both a practical and economic point of view. When four highly variable loci (average expected heterozygosity=0.83) were used to generate genotypes of family groups, the correct allocation was reduced by only 1.3% demonstrating that the use of eight, as opposed to four, microsatellite loci, results in very little improvement in assignment to a single pair of parents. The resolution of the offspring assigned to more than one parent pair could be improved by the pre-selection of a particular suite of loci which are capable of differentiating between all the female pairs.

Microsatellite markers were also shown in this study to be capable of discriminating between related and unrelated salmon in a situation where no pedigree information is available. As with the parentage study, using 15 loci gave greatest accuracy but the eight most variable loci result in a decrease in accuracy small enough to be tolerated when balanced with time and money saved in genotyping.

Using genetic profiling for either parentage and/or relatedness purposes will also allow a breeding manager to monitor any changes in genetic variation in each generation which may result from a family based selection program. Chapter two showed significant differences in genetic variation between major farmed salmon producers which could be a result of differences in selection programs between these farms (Norris et al. 1999). Clearly, microsatellite based genetic profiling has been shown here as having the potential to replace traditional procedures for the identification of families in a salmon aquaculture breeding program. This would eliminate the need to rear families separately until they can be physically tagged and also provides a method to minimise increases in inbreeding that may result from a genetic improvement program.

Chapter 4

Analysis of (co)variance components for body size and body composition, sexual maturity and flesh quality traits in Atlantic salmon

4-1 Introduction

4-1-a Quantitative traits of economic importance in Atlantic salmon

Body size and body composition

Body size is still, economically, the most important production trait in Atlantic salmon aquaculture. Salmon are sold by the kilogram and larger fish generally fetch a higher price per kilogram than smaller ones. Aligned with this, high growth rate is positively correlated to feed conversion efficiency and disease resistance (Gjedrem et al. 1991; Rye et al. 1990; Thodesen et al. 2001). Weight at harvest (gutted or ungutted weight), or weight at harvest time, is the most important trait for a selection program as it is on the basis of this weight measurement that fish are sold. Measurements of weight during the freshwater phase of growth may be affected by maternal and/or tank effects, particularly when full-sib groups have been reared together (Refstie and Steine 1978; Gjerde et al. 1994). From the time family groups are mixed there is a lessening of the tank or full-sib family effect. For this reason, there is a general trend for a reduction in heritability estimates for traits measured at increasing age, both at fresh and seawater and earlier estimates of heritability can be considered biased by this rearing effect.

Heritabilities estimated for weight at or close to harvest time for live, gutted and ungutted fish tend to be high to medium (Gjerde and Gjedrem 1984; Standal and Gjerde 1987; Rye and Refstie 1995; Rye and Gjerde 1996; Rye and Mao 1998). Heritabilities estimated from full-sib families (from the dam component of variance) may be biased upwards due to maternal and common environmental effects. Heritabilities estimated from half-sib families (from the sire component of variance), in general, tend to be lower due to the elimination of non-genetic similarities (Gunnes and Gjedrem 1981; Gjerde and Gjedrem 1984; Standal and Gjerde 1987;

Wild et al. 1994; Rye and Refstie 1995). It has been recommended (Gunnes and Gjedrem 1981; Wild et al. 1994) that the sire component of variance gives less biased estimates of heritability of a trait.

At the Salmon Genetic Research Program (SGRP) of the Atlantic Salmon Federation in Canada, Bailey and Loudenslager (1986) investigated the differences in length and weight between two year classes of Atlantic salmon produced from five Canadian stocks. They found no differences among the stocks examined in these traits at 3, 6 or 15 months after hatching. Half-sib heritability estimates for length and weight were low to intermediate in 1978 (0.1-0.4) and intermediate to high in 1979 (0.4-0.9). Heritability estimates were markedly different in the two year classes. Differences among the stocks and among year classes are suggested by the authors as an explanation for such variation.

Gjerde et al. (1994) investigated the factors affecting body weight and growth rate of salmon after 4, 12, 16, 24 and 27 months at sea. Heritabilities for body weight were between 0.10 and 0.32 with lower estimates of heritability found for weight measurements after 2 years at sea. In this study, the authors took account of the full sib effect which expresses the variance caused by factors other than additive genetic variance. The full sib effect was particularly large for body weight at tagging (pre-smolt stage) and growth from presmolts to 5 months at sea. This effect is likely to be accounting for a transitory common environmental effect when fish are reared together before tagging at 10-12 months post-hatch.

The difference between sire and dam estimates of variance for growth in most of the studies (Table 4-1) mentioned above would suggest the presence of non-additive genetic effects and/or environmental effects common to full-sibs. If non-additive effects are important, more

accurate estimates of additive effects may be obtained with non additive effects included in the genetic evaluations. The high fecundity of fish, resulting in high mean non-additive genetic relationships among individuals, will result in higher accuracy of estimating non-additive genetic effects. In order to investigate the magnitude of non additive and common environmental effects for body weight in Atlantic salmon Rye and Mao (1998) analysed growth data from 4 sub-populations over 3 generations of the AKVAFORSK/NLA breeding program. The data were analysed using a series of statistical models of increasing complexity by including non-additive and common environmental effects. Under the most simple additive model heritabilities ranged from 0.22-0.34 similar to previous estimates in Atlantic salmon (Gjedrem 1983; Gjerde 1986; Rye and Refstie 1995). However, by including non-additive and common environmental effects in the model, the estimates of additive genetic variance were reduced by between 50% to 79%. This illustrates that estimates of additive genetic variances from models ignoring non-additive and common environmental effects are likely to be strongly biased upwards due to their strong confounding of these effects with additive genetic effects. In fish populations, in particular, the proportion of full-sib relationships is much higher and full-sib covariances are inflated in the presence of non-additive genetic effects. Better estimates of additive genetic variance and hence heritability will be obtained from models where non-additive and common environmental effects are included.

For processors of Atlantic salmon the final product yield after processing will determine to a high degree their profitability. Farm reared Atlantic salmon typically have a slaughter yield (carcass to body weight ratio) of 90% (Rora et al. 1998). The gutting loss through slaughter is correlated to body shape and weight (Rora et al. 1998). Variations in slaughter yield also have

a genetic origin with 11-23% of the total variance in slaughter yield due to family differences (Gjerde 1996).

The demand for secondary processed salmon is increasing as a result of a increase in value added products (mostly fillets) and ready made meals. In 1999, 15% of the production of Atlantic salmon from Norway was in the form of fillets both fresh and frozen (Statistisk Sentralbyra, Kontali Analyse). In Chile, the second largest producer of Atlantic salmon with the United States as its main market, this figure was 90% (Statistisk Sentralbyra, Kontali Analyse). This demonstrates the difference in market demands between the United States and Europe. The yield for salmon fillets is generally between 60-70% but there is considerable degree of individual variation (Rora et al. 1998). In general, the fillet yield will depend on a number of factors including the type of trim (A, B, C or D explain these) and whether the filleting is being done manually or by machines. Differences in body size and shape are also important determinants of raw fillet yield and since there is substantial genetic variation for both fish size and body shape (Rye and Gjerde 1996) there is likely to be high genetic variation in fillet yield. While there is potential for genetic improvement of fillet yield, effects of selection for this trait should be monitored carefully as selection may result in an undesirable reduction in the size of organs which are important in metabolism and feed digestion (Gjerde and Schaeffer 1989).

Sexual maturity

In farmed Atlantic salmon, age at sexual maturation is an important economic trait. Poor growth, deteriorated flesh quality and increased mortality are all associated with the onset of sexual maturation (Gjerde 1984; Naevdal 1983). It has been shown that the trait of age at sexual maturation is under genetic control with medium to high heritabilities, ranging from

0.16 to 0.48 in these studies (Naevdal et al. 1978; Gjerde and Gjedrem 1984; Gjerde 1989; Glebe and Saunders 1986; Herbinger and Newkirk 1990; Wild et al. 1994; Rye and Refstie 1995). Age at sexual maturation can be measured as the proportion of fish which become mature after a certain period or can be scored at harvest by the gonadosomatic index (weight of both gonads *100/gutted weight of fish). Fish are normally classed as early maturing fish if they become mature after one winter at sea (grilse).

Naevdal (1983) showed clear evidence that age at sexual maturity is a heritable trait in a study which found significant differences in this trait between sib groups and also demonstrated the influence of environmental factors such as light and sea cage on maturity. Gjerde (1984) estimated the heritability of maturation rate at sea, from an offspring-dam regression, to be 0.48. There was also found to be a considerable response to selection using males and females sexually mature at different ages as parents. The observed difference in mean age at sexual maturity between offspring of parents aged 5 and 5 and parents aged 4 and 4 was 1.03 years. This response, along with the high heritability estimate, would indicate that the opportunity to change this trait through selection is considerable.

Similar results for the response to selection for age at sexual maturity were found by (Glebe and Saunders 1986) Their results show that mean parental age at maturity was a factor in maturation among parr (freshwater juveniles) with late maturing parents yielding fewer male parr progeny than grilse/mature parr parents (28% compared to 48%). They found no comparable relationship, at the family level, between the incidence of mature parr and the incidence of grilse in sea cages, suggesting that maturity in fresh water and seawater are independent genetic events. Gjerde and Gjedrem (1984) measured age at sexual maturity

from the carcasses of three year classes of Atlantic salmon. Maturity was initially treated as an all-or-none trait on the basis of gonadal development. The heritability estimates for maturity derived from the sire component of variance and averaged over the three years was 0.39. When heritability was transformed to a continuous scale (on a subjective score of 0-5 based on gonadal development at time of slaughter) the estimates were considerably higher to those based on an all-or-none scale. This difference is greatest in year classes when the heritability is low and when the percentage of immature fish is close to 0 or 100% as has already been documented by Olaussen and Ronningen (1975). This clearly illustrates the importance of taking the incidence level into consideration when comparing heritabilities for all-or-none traits.

The relationship between growth rate and age at maturity was proposed as early as the 1920's when Hubbs (1926) proposed that growth and maturity were expressions of general development rate. Alm (1959) summarised 20 years of experiments with different salmonids and stated that fast growing individuals were in general the first to mature. This work demonstrated that both traits are influenced by genetic and environmental factors. Further research using individually tagged individuals has clearly documented the negative relationship between growth rate and age at first maturation (Thorpe et al. 1983; Naevdal 1983; Skilbrei 1989; Gjerde et al. 1994). These results indicate that while age at maturation is influenced by growth rate in both freshwater and seawater, maturation is more strongly influenced by growth in the earlier freshwater stage. It has been proposed (Skilbrei 1989) that the duration of relatively slow growth before entrance to the sea could influence the time of maturation. It has also been proposed (Duncan and N. 1998; Taranger 1993) that later sexual maturation may be due to an advancement of a postulated 'decision period' as a failure of the fish to reach certain thresholds of developments the previous year as smolts. Age of

maturation is also affected by conditions at sea during the first year after the smolts have been transferred (Saunders et al. 1983; Naevdal 1983) suggesting that environmental conditions in the sea at a certain time during the winter may result in the fish making a physiological decision not to commence sexual maturation that year. Decrease in body weight and increase in gonad weight as sexual maturity is reached is well documented as evidenced by the studies outlined above. However, just prior to the physical manifestation of maturity an increase in body weight has also been demonstrated (Naevdal 1983; Gjerde 1984; Aksnes et al. 1986; Rye and Refstie 1995). Rye and Refstie (1995) recorded body traits at slaughter of $3^1/_2$ year-old tagged salmon. Their results reveal a considerable impact of maturation on body weight of Atlantic salmon (with maturation determined by gonadal inspection at time of slaughter) causing a significant increase in growth rate. The authors stress the need for proper adjustment for these effects when assessing growth traits.

Understanding the magnitude and relationship of (co)variance components of sexual maturation and growth is important for selection programs where both weight and maturation are important economic traits. It is clear that more research is needed to understand more fully the genetic and environmental influences on age at sexual maturation. Results from all the above studies are highly variable, mainly due to the considerable effects of strain, environment and incidences of the condition. As a result of the high negative correlations between growth rate and late maturation, selection for increased growth rate will therefore be expected to increase the proportion of fish maturing early. If selection for growth rate is to be applied in a breeding program, maturity and its correlation to growth rate must also be considered.

Trait	Estimated Heritability ± standard errors in parenthesis. Number of estimates in superscript		Reference
	Estimates from dam component of variance	Estimates from sire component of variance	
Maturity			
Age at Maturity	0.48 (0.20)		(Glebe and Saunders 1986)
Age at Maturity	0.39^{3} 0.49^{3}		(Gjerde and Gjedrem 1984)
Age at Maturity	0.21^{3} (0.05)	0.16^{3} (0.06)	(Wild et al. 1994)
Early maturity	0.34		(Gjerde and Gjedrem 1984)
Normal maturity	0.24		(Gjerde and Gjedrem 1984)
% non-grilse	0.19. 0.21, 0.22		
Body weight			
Weight, 190 days	0.46^{3} (0.39, 0.39, 0.59)	0.17^{3} (0.19, 0.15, 0.18)	(Refstie and Steine 1978)
Weight, 12 weeks 1978	0.44 (0.14)	0.35 (0.29)	(Bailey and Loudenslager
1979	0.93 (0.10)	0.89 (0.32)	1986)
Weight, 6months 1978	0.23 (0.08)	0.10 (0.17)	(Bailey and Loudenslager
1979	0.76 (0.13)	0.40 (0.26)	1986)
Weight, presmolts.	0.32 (0.05)		(Gjerde and Refstie 1984)
Weight, 15 months post hatch	0.75 0.16)	0.67 (0.32)	(Bailey and Loudenslager 1986)
Weight, 4 months at sea	0.31 (0.05)		(Gjerde and Gjedrem 1984)
Weight, 12 months at sea	0.30 (0.06)		(Gjerde and Gjedrem 1984)
Weight, 16 months at sea	0.15 (0.05)		(Gjerde and Gjedrem 1984)
Weight , 2 years at sea	0.31	0.31^{2}	(Gunnes and Gjedrem 1981)
Weight at slaughter		0.38 (0.15) 0.14 (0.07) 0.12 (0.06)	(Standal and Gjerde 1987)
Ungutted body weight	0.35 (0.10)	0.32 (0.09)	(Rye and Refstie 1995)
Gutted body weight	0.36 (0.10)	0.32 (0.09)	(Rye and Refstie 1995)
Ungutted body weight	0.38 (0.10)	0.55 (0.07)	(Gjerde and Gjedrem 1984)
Gutted body weight	0.44 (0.11)	0.53 (0.07)	(Gjerde and Gjedrem 1984)
Carcass quality			
Dressing percent	0.03 (0.02)	0.02 (0.02)	(Gjerde and Gjedrem 1984)
Meatiness, score	0.16 (0.05)	0.14 (0.03)	(Gjerde and Gjedrem 1984)
Meat colour, (score 0-5)	0.01 (0.03)	0.17 (0.04)	(Gjerde and Gjedrem 1984)
Fat content %	0.30 (0.09)	0.38 (0.10)	(Rye and Gjerde 1996)
Flesh colour (score 0-5)	0.09 (0.05)	0.16 (0.08)	(Rye and Gjerde 1996)
Average belly thickness	0.27 (0.09)	0.18 (0.08)	(Rye and Gjerde 1996)
Belly flap constitution	0.07 (0.05)	0.10 (0.07)	(Rye and Gjerde 1996)

Table 4-1 Estimates of heritability for economically important traits in Atlantic salmon

Flesh quality

The initial objectives of most breeding programs for salmonids, in the face of continuing price competition, has been has been to select for improved growth. However more recently, attention has begun to shift onto aspects of flesh quality that determine appearance, taste, texture and therefore value of the final product. Consumers will not buy low-quality products but at the same time are unwilling to pay too high a price for superior quality. Effective selection programs are needed to improve flesh quality and will also allow for differentiation of the final product to suit niche markets.

As well as offering a better product to the market , improving flesh quality will also result in savings to the producer. Better retention of carotenoid, for example, could mean lower carotenoid levels required in the feed while lower fat content means a higher yield and less downgrading. Another reason to introduce flesh quality traits into a selection index is to allow for the control of any undesirable correlations which may occur between increased growth and quality traits. Such correlations have been demonstrated between quality traits and growth (Rye and Gjerde 1996).

Flesh quality traits, like most traits, are under the influence of both environmental and genetic factors. Important environmental effects include; content of feed and feed regime (Wathne 1995; Johansen and Jobling 1998), treatment and handling of fish prior to slaughter and slaughter method (Einen and Thomassen M. S. 1998; Sigurgisladottir et al. 2001; Wathne 1995). Genetic parameters for flesh quality traits in Atlantic salmon have not been widely estimated (or at least published). A description of the most important flesh quality traits along with relevant studies of genetic parameters are given below.

Salmon at the point of sale is presented in the form of fillets or steaks. The appearance of the raw flesh is therefore of primary importance for the customer in making the buying decision. The fat, visible as bands in the flesh and deposited around the belly flaps, is of considerable importance. In general fat levels higher than 16% are considered too high, but this level will differ in different markets. A certain amount of fat in the flesh is important to maintain the distinctive taste of the fish and because of the beneficial effect of omega-3 and omega-6 lipids on health (Carroll 1991; Esterbauer 1992; Grundy 1993; Pietinen et al. 1997; Reddy 1993; Rose 1997). However, high fat levels are undesirable, particularly with uneven distribution, resulting in lower yields at processing, and have also been shown to increase 'gaping' in the flesh (Sheehan et al. 1996; Einen et al. 1999). Selection for faster growth in chickens and pigs has resulted in increased fat (Proudman et al. 1970; Hovenier et al. 1993). For this reason quality traits should always be included in the breeding objective to control undesirable side effects of selection for growth.

Heritability estimates for fat content are medium to high, demonstrating potential for genetic improvement in salmon (Rye and Gjerde 1996; Gjerde and Schaeffer 1989). Fat content in the flesh has been demonstrated to be highly correlated at the genetic and phenotypic level with growth rate with correlations of 0.42 and 0.63 respectively (Rye and Gjerde 1996). Therefore any increases in growth will likely result in increases in fat content in the flesh.

For consumers, pigmentation is regarded as the most important criterion after freshness for farmed salmonids (Koteng 1992). Although there is no standard colour in Atlantic salmon, it is traditionally held that 'pinkness' in the flesh is related to superior quality (Clydesdale 1993;

Sylvia et al. 1996). The characteristic pink colour of salmonid muscle is a result of the deposition of naturally occurring carotenoid pigments. Fish cannot synthesise these carotenoids *de novo* and to achieve pigmentation in the flesh the feed of farmed salmon is fortified with astaxanthin or canthaxanthin (Skrede et al. 1989). The retention of carotenoids in the flesh is relatively poor with only 10-18% being retained in the flesh (Torrissen et al. 1989; Nickell and Bromage 1998). This poor retention of carotenoid and its high price mean that by the time a salmon reaches market size (based on the price of extruded salmon feed supplemented by between 40 and 80mg astaxanthin per kg diet) the supplementation of carotenoids will have increased feed costs by 10-15% or 6-8% of total production costs. For this reason, flesh colour is an economically important trait in Atlantic salmon both because a high colour is desired by consumers and because of the high cost to the producer of carotenoids in the feed. Also, as salmon are exported throughout the world to markets with varying preferences for flesh pigmentation, it is therefore important that salmon can be sorted on the basis of flesh colour allowing for differentiation in the final product.

Heritabilities for colour in Atlantic salmon are low when estimates are based on subjective colour card measurements and medium when measurements are based on instrumental assessments (Chroma meter) (Torrissen and Naevdal 1988, Gjerde, 1984 ; Rye and Gjerde 1996). The low heritability recorded for colour from the subjective scores may be influenced by measuring conditions (Choubert 1982) and therefore have lower precision compared to instrumental recordings. When subjective score assessments and instrumental assessments are compared to chemical analysis of carotenoid concentration, the colour cards were found to provide a better prediction of carotenoid content than the Chroma meter when carotenoid contents are high (Christiansen et al. 1995). Genetic correlations between flesh colour and growth rate have been found to be positive (Rye et al. 1994; Rye and Gjerde 1996). Levels of

genetic variation have not been reported for carotenoid content in salmonids, however, significant differences in carotenoid levels between full- and half-sib groups of Atlantic salmon have been reported (Torrissen et al. 1984).

Fillet gaping occurs when connective tissue holding the muscle blocks together has been compromised resulting in the appearance of slits on the fillet surface. Salmon with gaping renders the product difficult to process and likely to be downgraded or not sold at all. This phenomenon is the cause of approximately 40% of all downgrading in the processing industry and, as such, represents a significant financial loss to the industry. A number of factors have been shown to be of importance including nutritional status (Einen et al. 1999), slaughter method (Skjervold et al. 2001a), feeding regime pre-slaughter (Einen et al. 1999), season (Lavety et al. 1988), stage of rigor prior to filleting (Skjervold et al. 2001b) and stress before slaughter (Sigholt et al. 1997). Fillet gaping is usually evaluated on a scale of 1-5 defined by (Andersen et al. 1994) which is commonly used in industry and in research institutes.
There has also been shown to be a strong relationship between gaping and soft flesh measured by breaking strength (Andersen et al. 1994). Salmon fillets show a wide phenotypic and genetic variation in texture of flesh (AKVAFORSK 1996) which may suggest a genetic component to gaping.

Measuring flesh quality

The measurement of flesh quality depends partly on the definition of the traits, i.e. colour of the fillet can be defined as the perceived colour or the carotenoid content in the fillet. For this reason, measurement of flesh quality is not as straightforward as with other traits and is more

subjective and prone to error. Ideally, a measurement should be able to be taken accurately and cheaply at time of slaughter. Because flesh quality traits can be defined in a number of ways we want to choose a measurement that best correlates with the economic value of the trait. For example, if fillets are sold at a higher price on the basis of the perceived colour then it is perceived colour that should be measured as opposed to actual carotenoid content.

There are two main possibilities of characterizing the degree of pigmentation of salmon flesh: by chemical analysis (Near infrared reflectance, NIR or HPLC analysis) or by definition of the colour appearance. Chemical analysis is expensive and time consuming but does give an accurate measure of the chemical content of carotenoid in the muscle. Definition of the colour appearance can be done by subjective colour perception or by characterization of the colour by measuring its composition instrumentally with a camera.

Within the industry the most commonly used system for visual determination of colour is colour cards such as the Roche Colour Card for Salmonids (scale 11-18) and the Roche Salmofan^{TM} (Hoffmann-La Roche Ltd, Basel, Switzerland) (scale 20-34) as described by (Skrede et al. 1990). Many processors and retailers specify Salmofan^{TM} scores of between 26-28 as the minimum acceptable colour. Other markets, for example, Japan, specify a higher minimum visual colour value. Since many different markets specify particular minimum colour levels that must be satisfied, the best colour is one that exceeds any minimum acceptable colour level thereby ensuring a consistently well pigmented product and minimal pale rejections. Changing light conditions throughout the day will affect the visual assessment of flesh colour. Colour measurements should therefore take place in standard light conditions preferably using a light box under fluorescent lighting under the specified colour temperature. The perception of colour varies between individuals and it is therefore advisable to use trained

assessors whenever possible. If more than one assessor is being used for the analysis it is necessary to make a record of this when the measurements are being done and in this way the effect of assessor can be taken account of in the final analysis.

For instrumental measurements, trimultus colorimeters (Chroma meter) measure the reflectance of light from the flesh compared to a calibration plate. The colour is characterised by the parameters: lightness (L*), the red/green chromaticity (a*) and the yellow/blue chromaticity (b*), according to the International Commission on Illumination, (CIE 1976). These three colour attributes together define the position of a specified colour within a 3D-colour space (Figure 4-1). In the measurement of salmon flesh, the a* value represents the redness and the b* value represents the yellowness of the sample (Warriss 1996). The intensity of red colouration of salmon flesh, as determined by the a* values produced from this colour meter, have been shown to be linearly correlated with the carotenoid pigment concentration of the flesh and most highly correlated (of the 3 values) with subjective colour scores (Skrede and Storebakken 1986; Skrede et al. 1990; Choubert et al. 1992; Christiansen et al. 1995).

It should be noted that chemical analysis and scoring colour are measuring different parameters, the former giving a measure of actual carotenoid in the flesh and the latter giving a value for perceived colour whether by human eye or by camera. The perceived colour may change depending on amount of water and fat in the flesh as these factors will affect the reflection of colour from the steak/fillet. The carotenoid content is a stable quantity but is not the quality on which the flesh will be valued and/or differentiated. No previous study has investigated the underlying genetic parameters and relationships of these three colour traits in Atlantic salmon.

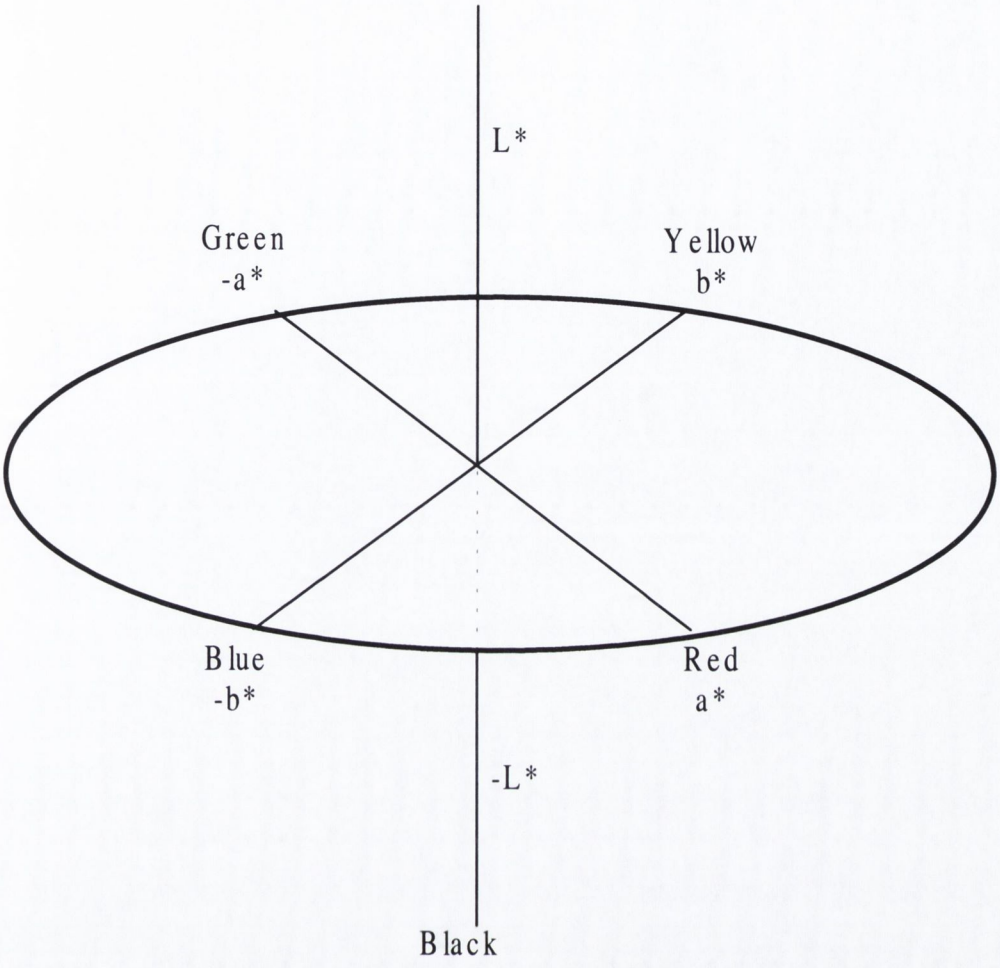


Figure 4-1 The CIE L* a* b* colour sphere

Fat content in the flesh is most commonly measured by chemical methods. Soxhlet (standard AOAC recommended method) Association of official Analytical Chemists (Chemists 1990) and the Folch method (Folch et al. 1957) provide reliable results but are time consuming and require skilled operators for their completion. In situations where large numbers of fish need to be sampled for fat content, such as is the case in a selective breeding program, an alternative method is desirable.

There is substantial variation in fat content between different segments in Atlantic salmon. Fillet fat decreases in the cranial-caudal direction, and the belly part of the fillet contains

about three times more fat than the rest of the fillet (Einen and Skrede 1998). Because of this substantial variation in fat distribution, analyses of fat content should be performed on a standard cut. The Norwegian quality cut (NQC) (see Figure 4-2) is a clearly defined fillet cut that meets the requirements for standardisation of sampling site and trimming of the fillet. NQC is the cutlet between the posterior end of the dorsal fin and the gut. Given the uneven distribution of fat within the fillet (Katikou et al. 2001), a clearly defined sampling point is necessary in order to compare fish and to compare data from other studies. Fat distribution can also be given a subjective score or measured by computerised tomography (Rye and Gjerde 1996), although this method is very expensive.

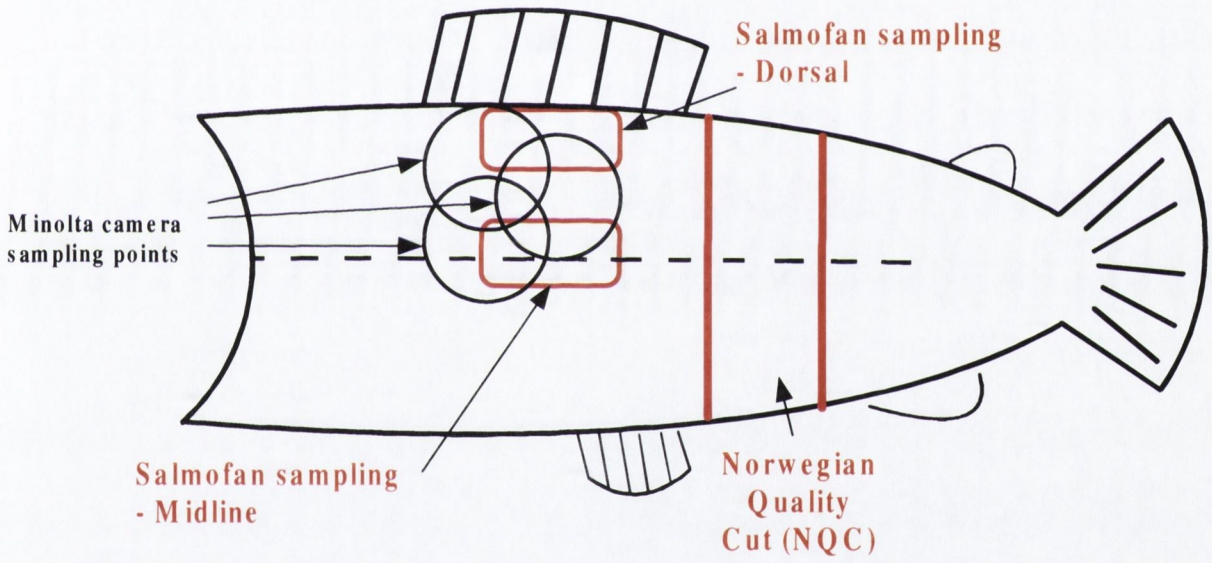


Figure 4-2 Sampling points on fillet for colour and fat analysis

The measurement of fat on a live fish would represent the most cost-efficient way of sampling. If fat content could be measured accurately in this way it would mean no waste of fillets/steaks at slaughter and also the trait could be measured on the breeding individual itself

as well as on full- and half-sibs. At the present time the only realistic alternative to destructive sampling is the Tory fat meter (Kent 1990). This instrument makes use of microwaves to estimate the lipid content of fish flesh. The main disadvantage with the instrument is that it has not been demonstrated to show very high accuracy (Douirin et al. 1998).

Another alternative to chemical analysis is Near infrared reflectance (NIR). The principle behind NIR is that chemical bonds are able to absorb light of specific energy, i.e. light with specific wavelengths (Mathias et al. 1987). The sample is illuminated by monochromatic light from a lamp that scans through wavelengths in the range from 1100nm to 2500nm. The light is partly absorbed by the sample by an amount which depends on the chemical bonds present in the sample. Using tailored statistical software enables calibration of the reflection of light to the lipid content determined by any fat extraction reference method. During the same time the instrument can also analyse a wide range of other fish quality parameters such as colour (carotenoid content) and water.

4-1-b Variance component estimation

The amount of variation in any trait is expressed as the variance of the trait. The total variance is described as the phenotypic variance and is the sum of the genetic variance and environmental variance. The genetic variance can be further divided into the variance due to the additive effects of genes and the variance due to the non-additive effects of genes. The relative importance of heredity in determining the phenotype of the individual is called the heritability of the character and is expressed as the ratio of the additive genetic variance to the total phenotypic variance (Falconer 1989).

All of the traits described in the section above are of economic importance and, with the exception of gaping in the flesh, have been shown to exhibit some level of genetic variation.

As well as estimates of genetic variance of each trait, it is important to estimate the genetic relationships between traits in order to know how changes in one trait will cause simultaneous changes in others. The extent of these covariances of traits will determine the breeding goal to a certain extent.

Estimates of genetic parameters for growth, maturity and flesh quality traits vary considerably between different strains of salmon, different rearing conditions and even between different year classes of the same strain (Table 4-1). The prediction of breeding values requires knowledge of the magnitude of the variances and covariances of the random effects in the statistical model. The purpose of this investigation was to estimate genetic parameters for body size, body composition, maturation and flesh quality traits in a pedigreed population of Atlantic salmon and to examine the effectiveness of different models and methods of doing so.

4-2 *Materials and methods*

4-2-a Analysis of variance (ANOVA) based estimation methods

Fisher (1922) described a method to estimate variance components for balanced data that has come to be known as an analysis of variance (ANOVA). The ANOVA corresponds to a model which describes the different sources of variation in the data. In an analysis of variance, variance components are obtained by partitioning the total sum of squares (sum of the squared deviations of individual measures from the mean) into components attributable to these sources of variation. A very simple model might have families as the main source of variation. The ANOVA would then give within and between family sum of squares. In a nested design with dams nested within sires, estimates of variance components can be obtained by equating the observed mean squares (scaled sum of squares) to their expectations, writing the expected mean squares as linear functions of the variance components and solving for the variance components. Variance and covariance component and heritability estimators are then given by the following equations

$$\sigma_s^2 = \frac{MS_s - MS_e - (k_2 / k_1)(MS_d - MS_e)}{k_3}$$ Equation 1 Sire variance

$$\sigma_d^2 = \frac{MS_d - MS_e}{k_1}$$ Equation 2 Dam variance

$$\sigma_e^2 = MS_e$$ Equation 3 Residual variance

$$\sigma_z^2 = \sigma_s^2 + \sigma_d^2 + \sigma_e^2$$ Equation 4 Total phenotypic variance

$$h^2 = \frac{4\sigma_s^2}{\sigma_z^2}$$ Equation 5 Heritability from the sire component of variance

M$=mean squares

where k_1, k_2 and k_3 are functions of the experimental design (equal to n, n and Mn under a completely balanced design, where n is the number of offspring per full-sib family, and M is the number of dams per sire). Formulae for estimating where k_1, k_2 and k_3 are given below.

$$k_1 = \frac{1}{N(\overline{M}-1)}\left(T - \sum_{i=1}^{N}\frac{\sum_j^{M_i} n_{ij}^2}{n_i}\right)$$

$$k_2 = \frac{1}{N-1}\left(\sum_{i=1}^{N}\frac{\sum_j^{M_i} n_{ij}^2}{n_i} - \frac{\sum_i^N \sum_j^M n_{ij}^2}{T}\right)$$

$$k_3 = \frac{1}{N-1}\left(T - \frac{\sum_i^N n_i^2}{N}\right)$$ Equation 6 Formulae for estimating k1, k2 and k3 in an unbalanced nested design

T=total number of individuals in the population

$\overline{M}$ =mean number of dams/sire

n_i = total number of offspring of the *i*th sire

ANOVA has the feature that the estimators for the variance components are unbiased regardless of whether the residuals are normally distributed, but it also has a number of serious limitations. Firstly, covariances among effects of genetically related animals, other than full- and half-sibs, cannot be accounted for. This results in biased estimates of variance components if data are from a population under selection (Sorensen and Kennedy 1984). Furthermore covariances between two traits can only be estimated from animals that have both traits measured. This yields biased estimates in cases where only animals that were selected on the first trait have a chance of expressing the second trait. ANOVA procedures are also based on the assumption that animals are a random sample from the population,

which will not be appropriate in selected populations such as in genetic improvement programmes. Also, because ANOVA yields unbiased estimates of variance components, sampling error associated with the mean squares sometimes causes the estimated among-family variance to be negative when σ_s^2 is small. This will result in negative estimates of heritability. Finally, ANOVA estimates of variance components require that sample sizes be well balanced, with the number of observations within each sub-group being essentially equal.

4-2-b Maximum Likelihood and Restricted Maximum Likelihood

Unlike ANOVA estimates, maximum likelihood estimators do not place any special demands on the design or balance of data (Hartley and Rao 1967) but maximum likelihood methods do require a prior distribution of errors, normally assumed to be normally distributed. Maximum likelihood (ML) methods can account for selection conditional on all the information, which has contributed to selection decisions, being included in the analysis. Even when this condition is not fully satisfied, parameters estimated with maximum likelihood are generally less biased then ANOVA estimates (Meyer and Thompson 1984). All of these analyses are based on the general mixed model a description of which is given in the section below. A general formulation of the mixed linear model in matrix terms is given in Equation 9. ML is conceptually very simple. For a specified model such as Equation 9 and a specified form for the joint distribution of the elements of y, ML estimates the parameters of the distribution that maximise the likelihood of the observed data. This distribution is almost always assumed to be multivariate normal. An advantage of ML estimators is their efficiency - they simultaneously utilise all of the available data and account for any nonindependance. One drawback with the usual variance component estimation using the ML approach is that all fixed effects are assumed to be known without error. As a consequence ML estimators yield

biased estimates of variance components (estimates of the residual variance tend to be biased downward). Unlike ML estimators, restricted estimated maximum likelihood (REML) (Patterson and Thompson 1971) estimates maximise only the portion of the likelihood that does not depend on the fixed effects. In this sense REML is a restricted version of ML. The elimination of bias with REML is analogous to the removal of bias that arises in the estimate of a variance component when the mean squared deviation is divided by the degrees of freedom instead of by the sample size. In the ideal case of a completely balanced design, REML yields estimates of variance components that are identical to those obtained by a classical ANOVA.

Selection changes the additive genetic variance of a character by generating changes in allele frequency. Likelihood methods such as REML at least partially account for biases introduced by selection, in part because the additive genetic relationship matrix A, corrects for the pattern of gene flow (of genetic information) from generation to generation (Schenkel and Schaeffer 1998). In this way REML estimates of genetic variance are unbiased by selection when all observed records and complete pedigree information are included in the analysis. Other variance component estimators, such as those derived from ANOVA, make no such correction.

The success of the mixed model in separating genetic and environmental effects is dependent on the genetic connectedness of individuals across environments, knowledge of phenotypic and genetic parameters, and the completeness of the data. Many fixed effects are removed directly, greatly reducing the level of environmental effects often ignored with standard least squares regression techniques (Kennedy and Sorensen 1988). When complete information is available for a fully pedigreed population, a major strength of the animal model approach is

that performance and genetic relatedness for all individuals in all generations is utilised simultaneously through the use of a numerator relationship matrix, A, resulting in the elimination of bias due to selection. REML overcomes the problem of not taking account of the degrees of freedom used to estimate the fixed effects when estimating the variance components. The basic idea is that the likelihood used to estimate the variance components is based on the residuals calculated after fitting, by ordinary least squares, only the fixed effects part of the model.

4-2-c General linear model versus general mixed model

ANOVA estimates variances based on the general linear model, whereas REML methods have been developed for variance component estimation under the general mixed model.

Under the general linear model:

$y = X\beta + e^*$ Equation 7 General linear model

where y is a vector of observations, β is a vector of fixed effects, X is an incidence matrix linking the observations to the fixed effects in the model and e* is the vector of residual effects assumed $\approx N(0,V)$.

Under mixed model the vector of residual effects is partitioned into two components, with $e^* = Zu + e$ giving:

$y = X\beta + Zu + e$ Equation 8 Mixed model equation in linear notation

where Z is an incidence matrix linking the observations to the random effects in the model, μ is a vector of random effects assumed $\approx N(0,G)$, e.g. additive genetic values and e is the vector of residual effects assumed $\approx N(0,R)$

implying:

$y \approx (X\beta, V) = (X\beta, ZGZ^T + R)$ for the mixed model we observe y, X and Z, while β, u, R and G are generally unknown. Thus, mixed model analysis involves two complementary estimation issues: (1) estimation of the vectors of fixed and random effects (unknown) and (2) estimation of the covariance matrices G and R. When y contains many thousands of observations, the inverse of V, V^{-1} can be difficult to obtain. Henderson solved this problem with a method for jointly obtaining $\hat{\beta}$ and $\hat{u}$. Minimizing the mean squared error gives the set of mixed model equations which need to be solved:

$$\begin{pmatrix} X^T R^{-1} X & X^T R^{-1} Z \\ Z^T R^{-1} X & Z^T R^{-1} Z + G^{-1} \end{pmatrix} \begin{pmatrix} \hat{\beta} \\ \hat{u} \end{pmatrix} = \begin{pmatrix} X^T R^{-1} y \\ Z^T R^{-1} y \end{pmatrix}$$ Equation 9 Mixed model equations in matrix notation

The known incidence matrices X and Z relate the observations to the corresponding fixed and random effects respectively.

Henderson (1975) assumes in the mixed model equations that covariance matrices G and R are known and that the densities of the vectors u and e are each multivariate normal with no correlations between them. These equations then yield maximum likelihood estimates of the fixed and random effects. The general mixed model forms the framework for BLUP analysis. There are a number of ways in which the model can be formulated: animal model, sire model and dam model. The animal model, which defines additive genetic effects for all animals individually and accounts for all (co)variances between them, is the one most commonly used now.

For the animal model since, $G^{-1} = \sigma_A^2 A^{-1}$

MME from above reduces to:

$$\begin{pmatrix} X^T X & X^T Z \\ Z^T X & Z^T Z + \lambda A^{-1} \end{pmatrix} \begin{pmatrix} \hat{\beta} \\ \hat{u} \end{pmatrix} = \begin{pmatrix} X^T y \\ Z^T y \end{pmatrix}$$ Equation 10 reduced mixed model

equations in matrix notation for the animal model

where $\lambda = \sigma_E^2 / \sigma_A^2 = (1\text{-}h^2)/h^2$

The mixed model equations can be easily extended to situations where two vectors of random effects are of interest e.g. in the estimation of both additive and dominance values or in the estimation of breeding values and maternal effects (Equation 11). With two vectors of random effects uncorrelated to each other, the mixed model becomes

$$y = X\beta + Z_1 u_1 + Z_2 u_2 + e$$

Equation 11 Mixed model equation for more than one random effect in linear notation

4-2-d Fish population

149 full-sib families were set up from crosses between 78 males and 149 females in November 1998. These fish were the first year class of the first generation of an Atlantic salmon breeding program initiated by Marine Harvest Ireland in 1998. Most of the crosses (138) were one male crossed to 2 females but a number of crosses were a result of one male crossed to one female (9 crosses) and one male crossed to three females (2 crosses). Immediately after fertilisation families were mixed within day degrees (number of days X mean temperature of water during the incubation period in ^{o}C) into 15 large batches. In April of 1999 all 15 batches were mixed evenly. Fish were transfered to sea in April 2000 into 8 sea cages. At all times during the rearing process, tanks, groups and cages received identical treatment in terms of feed, vaccination, temperature, light regimes and other environmental conditions. Fish were fed *ad libitum* (i.e. continuous access to food) with a feed conversion efficiency of approximately 1.2 (1.2 kg weight gain for every 1kg feed). From this period onwards one cage was reserved for genetic analysis of weight, maturation and flesh quality traits while the other 7 cages were graded further and reserved for broodstock selection for the spawning season on 2002/2003.

The potential broodstock were grilse graded and scanned for sex in January 2002. The cage of fish reserved for slaughter and measurement of traits was not graded from time of transfer to sea in order to ensure an even representation of families at measurement of traits. This cage of fish were kept on a normal grow-out diet with the exception that the carotenoid content (Canthaxanthin) content of the diet was reduced form the normal 75mg/kg to 35mg/kg from the time the fish reached an average weight of approximately 2kg (12 months before harvesting the fish). There is evidence that when salmon are fed high carotenoid diets, it becomes more difficult to distinguish differences in colour, particular when colour is being

measured using instrumental colour analysis (Christiansen et al. 1995). As a result of this loss of variation, at carotenoid diets > 50mg/kg additive genetic variation and hence heritability is close to zero (R. Neira, pers. comm). All fish were slaughtered between 7th January 2002 and 16th January 2002. The fish were starved for approximately 2 weeks prior to slaughter at which stage they were anaesthetised with carbon dioxide and the gill arches were cut.

4-2-e Number of fish sampled

Approximately 3100 fish were measured over a 5 day period, this number was estimated to give an average representation of 15 full sibs per family.(see Appendix A)

4-2-f Measurement of harvest traits.

All fish were transported to the secondary processing area of the processing plant. This area was set up over an 5 day period to measure harvest traits of 3100 fish (600/ fish a day). For practical and hygiene reasons, all gutting and removing gonads was done in the primary processing area of the processing plant. After fish had been gutted and washed they were transported to the secondary processing area. The layout of the primary and secondary processing areas for the purposes of these measurements can be seen in Figure 4-3 and some photographs of measurements being taken are shown in **Figure 4-4**.

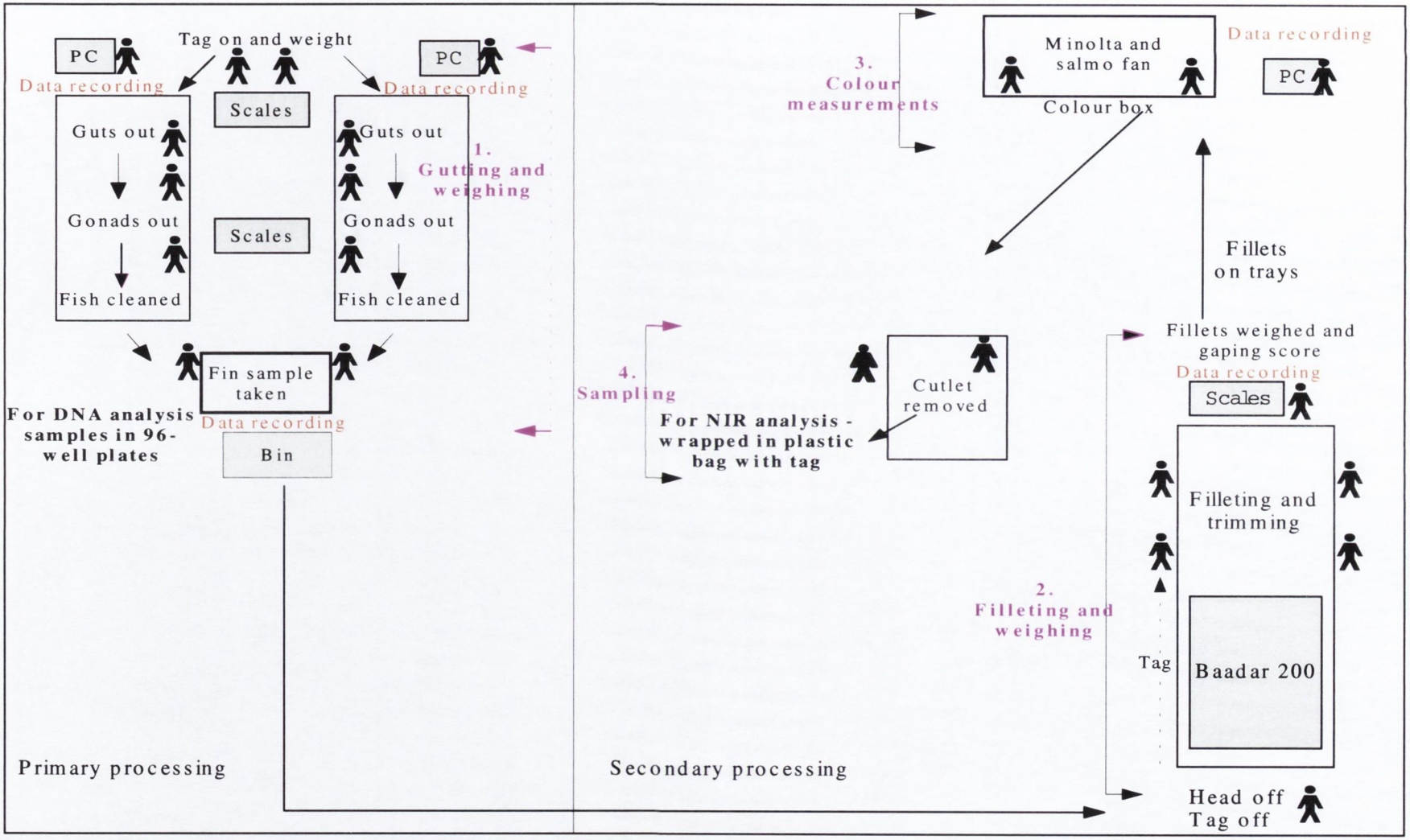


Figure 4-3 Outline of measurements in the processing plant

Body traits, sex and sexual maturation status.

12 hours after being slaughtered, fish were moved to the processing plant for primary and secondary processing. A consecutively numbered tag (PVC general ID tag, 7.5cm x 5cm) is put on the tail of the fish tied with a plastic band and each fish is weighed (round weight) and the weight recorded on a PC. Next, fish were gutted (viscera, liver, gonads and kidney removed) and gutted weight recorded and finally gonads were removed from the viscera mass and weighed. Two gutting lines are used (See Figure 4-3). Gutted fish are then cleaned and before being transported to the secondary processing room a fin sample (adipose fin) is taken (using special hand punchers with a 5 millimetre diameter die with a spring plunger attachment for extracting the sample product) from each fish and put into a labelled microwell plate, and tag number recorded was for each well and plate number. These samples would later be sent for analysis of genotype to determine pedigree. Approximately 8% of the fish were classified as grilse and not sampled except for one day when approximately 100 grilse were weighed and fin samples taken for analysis. A description of all body traits recorded on fish is given in Table 4-3.

All fish were filleted by either one of two teams of filleters using a combination of machine and hand filleting. Fish (heads off) were split in a 'splitting' machine (*Baader 200 Pelagic fish filleting machine*) and were later trimmed according to Norwegian Trim C. The right fillet from each fish was weighed. This same fillet was scored for gaping using the scale described by (Andersen et al. 1994) and demonstrated in Table 4-2 .

Score	Description
0	No gaping
1	Few small[1] slits (less than 5)
2	Some small slits (less than 10)
3	Many slits (more than 10 or a few large[2])
4	Severe gaping (many large slits)
5	The fillet falls apart

[1] small slits <2cm
[2] large slits >2cm
Table 4-2 Scale used to classify salmon according to gaping severity (Andersen et al. 1994)

Quality traits – colour, fat content and gaping.

Visual and instrumental colour measurements were done inside colour box as recommended by Roche (Ra >90, colour temperature > 5000K, colour of inside of colour box: Munsell N5). Visual colour assessment was done using Roche Salmo Fan ™ (Hoffmann-La Roche Ltd, Basel, Switzerland) at two locations: dorsal and midline as shown in Figure 4-2 The colour was evaluated using the 20-34 Scale (20 represents a low degree of pigmentation and 34 represents a highly pigmented fillet). Two scorers independently measured the colour of each fillet at two positions, dorsal and midline, shown in Figure 4-2 Assessors were carefully selected on the basis of testing each person's consistency in scoring a set number of fillets repeatedly. Photographs of the colour measurements being taken are shown in **Figure 4-4**

The colour of the flesh was also measured instrumentally using a tristimulus colorimeter (Minolta Chroma Meter, CR-2) which measures the reflectance of light from the flesh compared to a calibration plate. The average score of the three positions was taken.

After the fillets were assessed for colour parameters, a cutlet corresponding to the Norwegian Quality Cut (Figure 4-2) was taken from the fillet, placed, with the tag, into a plastic bag and frozen for NIR analysis of fat content and canthaxanthin concentration in the flesh (see **Figure 4-4**). A description of all body traits recorded on fish is given in Table 4-3.

There were four points of data recording in the room (Figure 4-3). At two of these points, data was recorded into a PC using the ELVIS database (designed by Nutreco Breeding Research Centre – IT). At the other two points measurements were recorded on paper and later recorded into the ELVIS database.

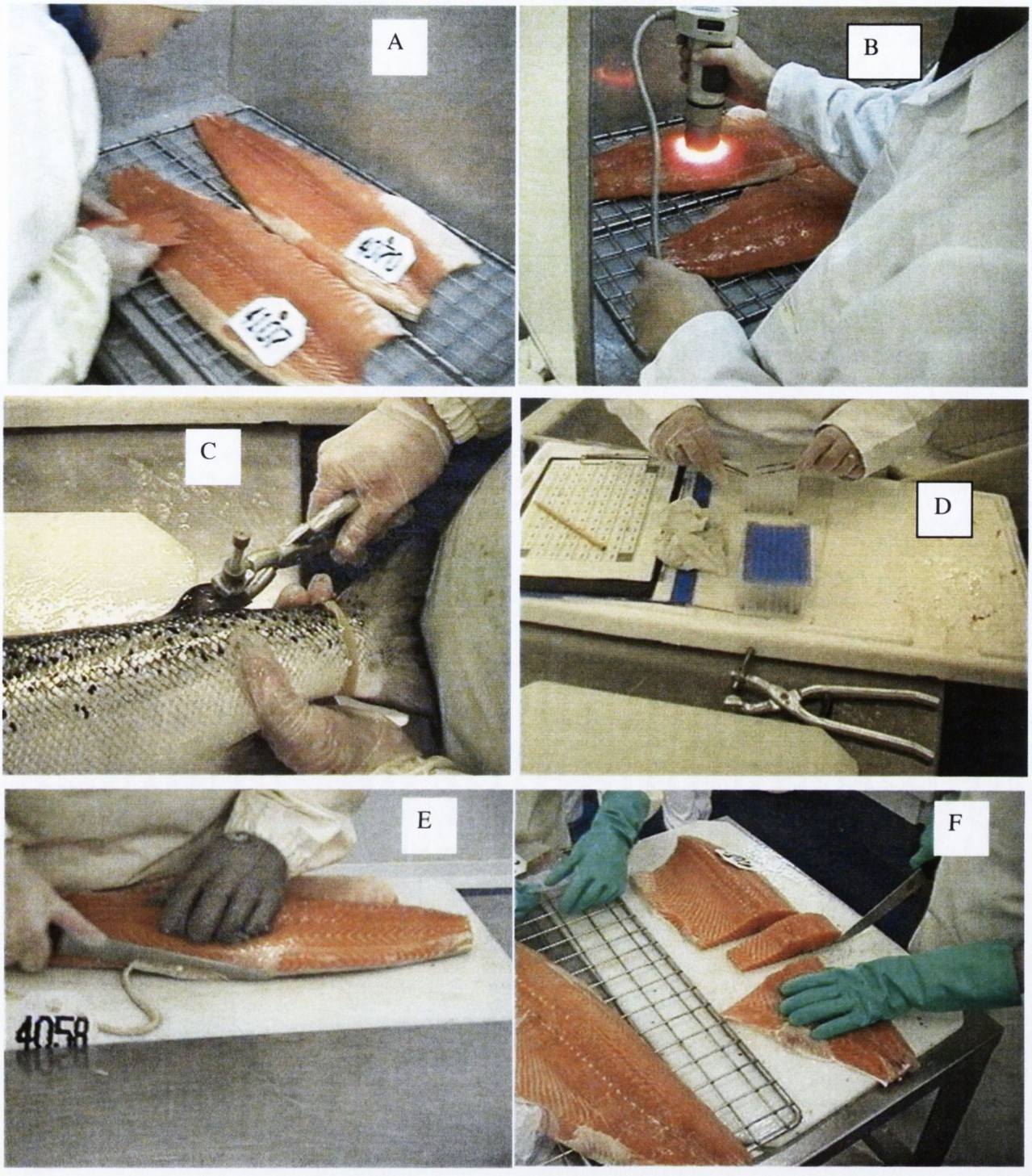


Figure 4-4 Photos of measurements being taken in the processing plant

A-SalmoFan^{TM} measurement, B-Minolta camera instrumental measurement, C-adipose fin sample removal, D-fin sample placed into microwell plates, E-trimming fillets, F-Norwegian Quality Cut taken for NIR analysis

NIR analysis of fat and canthaxanthin content

Before fat content and canthaxanthin content were analysed by NIR spectroscopy the spectroscoper was calibrated using 20 cutlets from 20 fish which were simultaneously analysed for fat content using the Soxhlet method and carotenoid content using high performance liquid chromatography (HPLC) analysis. Due to cost and time constraints, only 1000 flesh samples were analysed by NIR. Samples were chosen in order to ensure an even representation of 5-10 samples per family. After being defrosted, each sample was stripped from the skin, minced in a food blender and ~20g of the minced flesh placed in a NIR cell.

Traits recorded in the processing plant		
Abbreviation	Description of trait	units
Round wgt	Weight of bled fish	Kg
Gutted wgt	Gutted weight	Kg
Gonad wgt	weight of gonads	grams
Fillet Wgt	Fillet weight (weight of one fillet)	Kg
Gaping	Gaping score 1-5	units
A_Dor	Salmo Fan colour score-Dorsal (Scorer A)	Score 20-34
B_Dor	Salmo Fan colour score -Dorsal (Scorer B)	Score 20-34
A_Mid	Salmo Fan colour score -Midline (Scorer A)	Score 20-34
B_Mid	Salmo Fan colour score -Midline (Scorer B)	Score 20-34
L value	Lightness	
a* value	a* value - red/green chromaticity	
a*value	b* value - yellow/blue chromaticity	
Traits recorded on NIR spectroscoper		
Fat	Fat content	%
Cantha	Canthaxanthin content	mg/kg

Derived traits			
Abbreviation	Description of trait	units	Derivation formula
Viscera Wgt**	Weight of viscera, liver, kidney	grams	round wgt-(gutted wgt+gonad wgt)
Fillet %	% fillet wgt to gutted wgt	%	((fillet wgt)*2)/gutted wgt
GSI	gonadosomatic index	grams	gonad wgt*100/gutted wgt
LOG_GSI	Natural logarithm of GSI		Ln(GSI)
A_ave	Average Salmo Fan colour score for scorer A	Score 20-34	(A_Dor+A_Mid)/2
B_ave	Average Salmo Fan colour score for scorer B	Score 20-34	(B_Dor+B_Mid)/2

Table 4-3 Description of all traits recorded on slaughtered fish

* Average of three scores

**This trait will be referred to as Viscera weight although it also included weight of liver and kidneys

4-2-g Statistical analysis

Analysis of fixed and random effects

Data were initially analysed by an ANOVA based method [procedure GLM, (SAS 1990)] to determine significance of fixed, random and regressor effects and to estimate variance components. The fixed effects of sex, filleter, line, first feeding group, random effects sire and dam nested within sire were examined. Because body size and maturation status have been shown to be highly correlated with other traits (Shearer 1994; Rye and Refstie 1995; Rye and Gjerde 1996), and because of the part/whole nature of body size and composition traits, further analysis was performed on each trait to examine the effects of adjusting for body weight and/or maturation index (GSI) in the model.

Rather than adjusting for these traits in the model as covariates and in doing so remove a significant portion of the genetic variance, fish were classified into weight classes (9) and maturation index classes (4). All traits were examined for the effects of sex, age, weight class and maturation index. Significance of each effect was tested and different models were compared for best fit (R^2 – percentage of total variability in the data which is explained by the model). Using the same models, variance components were also estimated using restricted estimated maximum likelihood (REML) based on a sire and dam nested within sire model [Proc Mixed (SAS 1990)]. Heritabilities were derived from the sire component of variance. Multivariate analysis was carried out using REML on the basis of analytical gradients as described by (Neumaier and Groeneveld 1998) and fitting an animal model. A software package called REML VCE (Groeneveld 1996) was used to estimate variance components. Predicted breeding values were calculated by an animal model using the Prediction and Estimation program, PEST (Groeneveld 1990) and using the variance and covariance components estimated from the variance component analysis. The model used for both

variance component and breeding value estimation is shown (in matrix notation): in Equation 9.

Two multivariate models were applied to the data. Model I contained all body weight and composition traits and one colorimetric value (a* value) and the average subjective scores of the two scorers. In order to examine the relationships between both fillet weight and fillet percent and the other traits, and avoid confounding in the model, the two traits were included in separate runs of the analysis. Model II was used to compare all colour traits from the colorimetric analysis and all subjective scores on each fish as well as gutted weight, Log_GSI and fat and carotenoid content. Average colour scores over both fillet positions are best compared to colorimetric measurements which is also an average of three measurements taken in the same positions. The fixed and regression effects for both models are shown below *(see Table 4-3 for full names of traits)*

Model 1

Gutted wgt= $Y_{ij} = \mu + sex_i + bxage + a_j + \varepsilon_{ij}$

b_ave= $Y_{ijm} = \mu + sex_i + WS_j + a_m + \varepsilon_{ijm}$

a_ave= $Y_{jm} = \mu + WS_j + a_m + \varepsilon_{jm}$

Fillet wgt= $Y_{ijm} = \mu + sex_i + WS_j + a_m + \varepsilon_{ijm}$

Cantha= $Y_{km} = \mu + MS_K + a_m + \varepsilon_{km}$

Fat= $Y_{jm} = \mu + WS_j + a_m + \varepsilon_{jm}$

a* value= $Y_{jm} = \mu + WS_j + a_m + \varepsilon_{jm}$

Viscera wgt= $Y_{ijm} = \mu + sex_i + WS_j + a_m + \varepsilon_{ijm}$

Log GSI= $Y_{ikm} = \mu + sex_i + WS_j + a_m + \varepsilon_{ijm}$

Fillet percent= $Y_{im} = \mu + sex_i + a_m + \varepsilon_{im}$

Model 2

Gutted wgt: as above

Cantha: as above

Fat: as above

a* value: as above

L value= $Y_m = \mu + a_m + \varepsilon_m$

b* value= $Y_m = \mu + a_m + \varepsilon_m$

Log_gsi: as above

A_dor= $Y_{ijm} = \mu + sex_i + WS_j + a_m + \varepsilon_{ijm}$

A_mid= $Y_{ijm} = \mu + sex_i + WS_j + a_m + \varepsilon_{ijm}$

B_dor= $Y_{ijm} = \mu + sex_i + WS_j + a_m + \varepsilon_{ijm}$

B_mid= $Y_{ijm} = \mu + sex_i + WS_j + a_m + \varepsilon_{ijm}$

Where μ =overall mean
Sex_i=fixed effect of *i*th sex or ith sex within grilse class
Age=regression variable accounting for age
WS_j=fixed effect of *j*th weight score class
MS_k=fixed effect of *k*th weight score class
a_m=additive genetic effect of *m*th animal
e_{ijkm}=residual random effect

The distributions of random effects a and e were assumed multivariate normal with zero means and var(a)=Aσ_a^2, and var(e) Iσ_e^2 where A is the numerator of Wright's relationship matrix and I is an identity matrix. The expected values of these random effects and the covariances between them are assumed to be zero. Heritability was estimated as the proportion of the additive genetic variance to the total variance,

$h^2 = \sigma_a^2 / (\sigma_a^2 + \sigma_e^2)$

4-3 Results

4-3-a Structure of the data

Approximately 3100 fish were measured for body weight and gonad weight traits. Fin samples from all fish were sent for parentage analysis using a microsatellite multiplex genotyping system (Genomar AS). Out of ~3300 samples sent for parentage assignment, 18 fish were not assigned to any parent due to difficulty amplifying DNA or scoring errors/mutations. A further 40 fish were assigned to only the sire and greater than one dam. 73 offspring could not be assigned to parents with a probability of correct assignment of >0.99). Table 4-4 shows the structure of the data in terms of parentage of each fish.

	number	Average no. offspring/family	range	SD	Average no. dams/sire
Full-sib families	122	24.5	1-130	23	1
Paternal half-sib families	70	42.3	2-183	35	1.72

Table 4-4 Structure of the data with 122 families represented out of a possible 149

From these, approx. 3000 fish were classified as non-grilse and also measured for fillet weight and colour traits. From these 3000 fish approximately 1000 cutlets were analysed for fat content and cantha content. These 1000 cutlets were selected for NIR analysis on the basis of pedigree in order to ensure an even as possible distribution of NIR analysis across families. Maximum likelihood estimation methods require the specification of the distribution of the data (normality is usually assumed). Violation of these assumptions leads to bias in the genetic parameter estimation (Ibe and Hill 1988). All traits were tested for normality and gonadosomatic index (gsi) (gonad wgt/gutted wgt*100) was not normally distributed even

when tested separately for males and females. A natural log transformation was performed to homogenise variances and the new distribution, although bimodal for all fish, was closer to normality and normal for males and females separately (Figure 4-5). The probability plots for GSI and Log_gsi (Figure 4-6) confirm this analysis. As a result of the bimodality of this trait, all analysis was also performed separately for males and females.

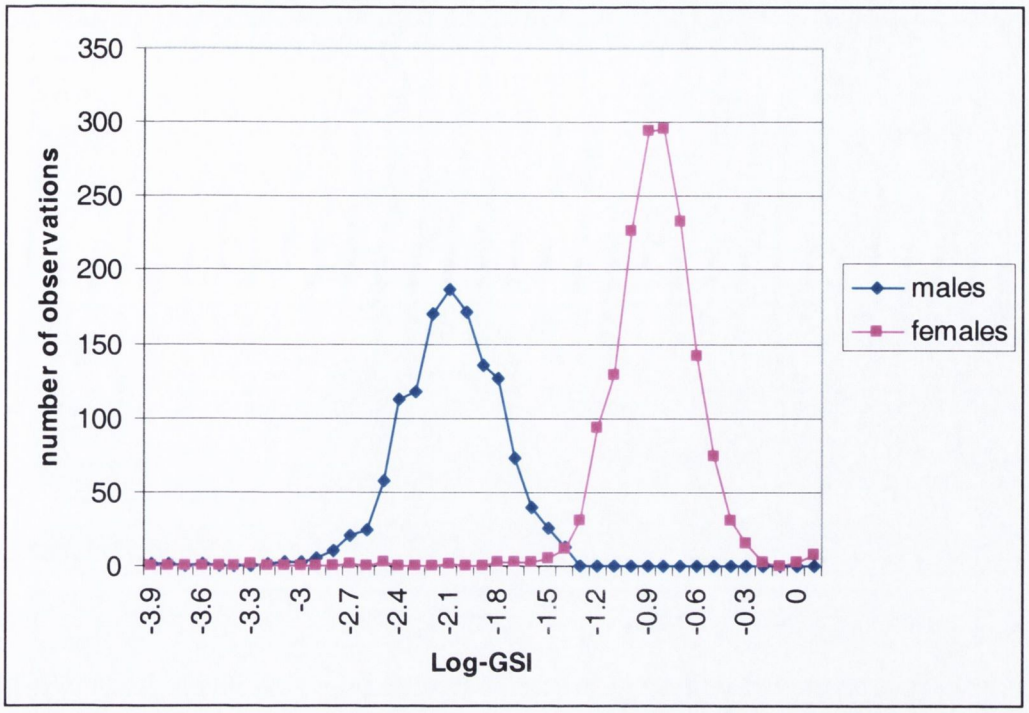


Figure 4-5 Histogram of GSI values for all females and males transformed to the logarithm scale

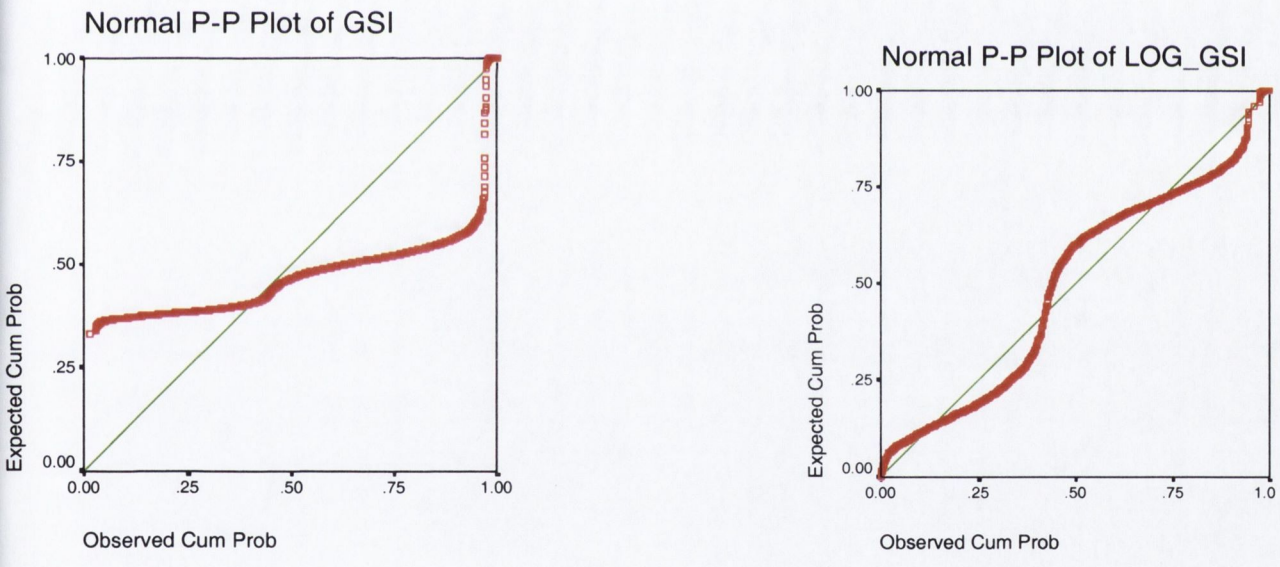


Figure 4-6 Probability plots for Gonadosomatic index and the log of gonadosomatic index for all fish

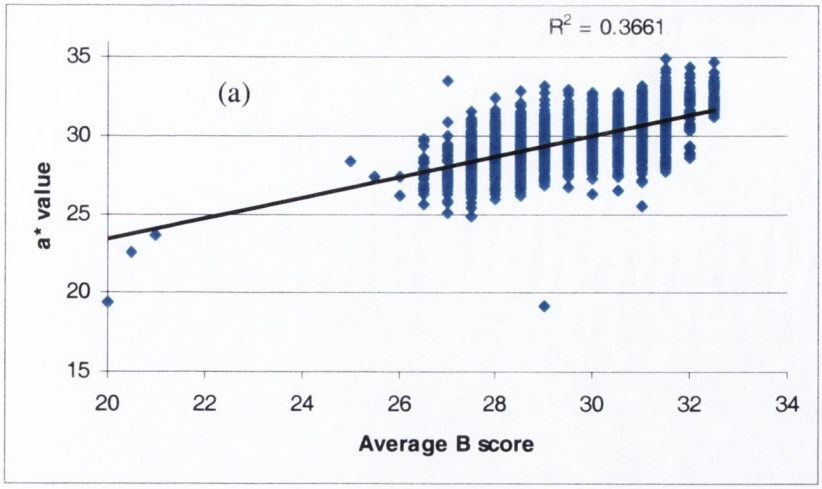
4-3-b Phenotypic means and variation

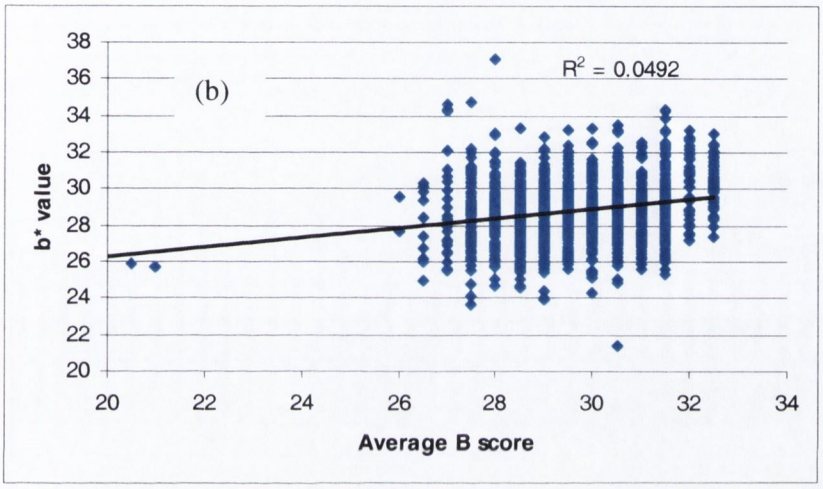
Large differences exist between non-grilse and the small number (90) of grilse fish sampled. The mean round weight of grilse was 4.24kg compared with 7.78 for non-grilse fish (Table 4-5) and for gonad weight the mean for grilse was 119g compared with 19 grams for non-grilse fish. This difference is explained by the maturation process where lipids are redistributed from the flesh and the intestines into the gonads.

Subjective colour scores with the SalmoFan ranged from 20 to 33. Scores on the midline position were significantly higher than scores on the dorsal position for both scorers ($p<0.001$) while scores given by scorer B are significantly higher than scores given by scorer A (Table 4-5). The colorimetric measurements (Minolta camera) are given in terms of L, a* and b* values. Figure 4-7 shows the relationship between the three colorimetric measurements and the salmofan scores taken by scorer B and averaged over the midline and dorsal position. The fit of the model, R^2 value, is also given for each plot of average scores. The strongest linear relationship was between the salmofan score and the average a* value, or redness of the flesh,. ($R^2=0.37$). The L value (lightness) and b* value (yellowness) did not exhibit any significant relationship with average scores ($R^2=0.07$ and 0.05 respectively).

Canthaxanthin content in the flesh ranged from 6.5ppm to 27.9ppm, while average carotenoid content in the flesh was 12.6 mg/kg. This agrees well with the minimum acceptable carotenoid content for market size fish which is approximately 8-10mg/kg gutted weight (unpublished Roche Chile, 1997). No relationship was found between carotenoid content and the other colour measurements. Figure 4-8 plots the relationship between canthaxanthin content in the flesh and average SalmoFan score from scorer B (a) and colorimetric a* value. Linear and non-linear regression analysis were used to fit mathematical models to describe the relationship, and no significant relationship was found with any score or colorimetric

measurement. When the average canthaxanthin content was plotted against the average score the fit was considerably better (R^2=0.92). However, the high standard deviations demonstrate that prediction of cantha content from average B score would not be accurate.





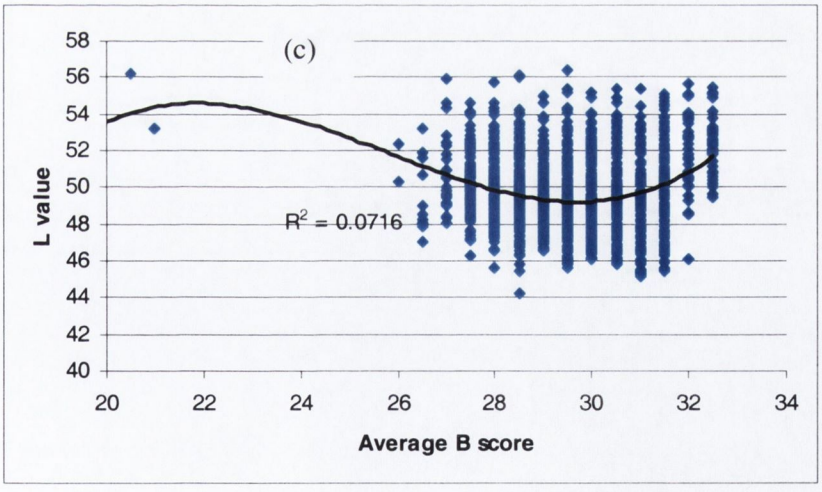
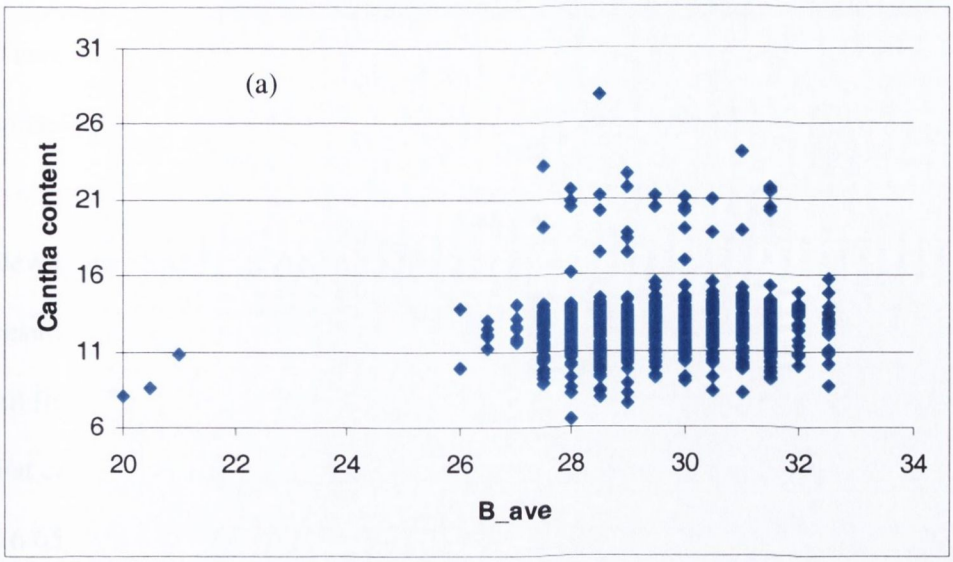


Figure 4-7 Relationship between average salmofan scores, and average a* value (a), b* value (b) and L value (c) in the flesh of Atlantic salmon (n=2820).
Vertical bars represent standard errors. Average scores are from scorer B averaged over dorsal and midline position.



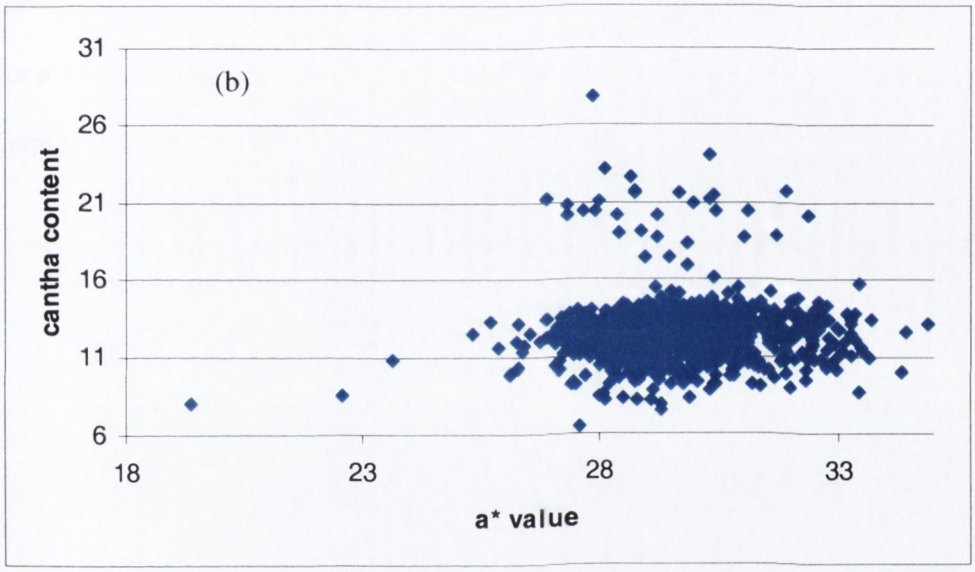


Figure 4-8 Relationship between canthaxanthin content in the flesh and a) average SalmoFan score, b) colorimetric a* value

Average scores are from scorer B averaged over dorsal and midline position.

4-3-c Effect of sex and sexual maturation status

There were considerable differences between sexes for a number of traits (Table 4-5). Round, gutted, fillet and viscera weights were all significantly ($p<0.001$) higher in males while gonad weight, GSI and fillet percent are significantly higher in females. Gonad structure and development differs in females in order to accommodate the development of the oocytes resulting in the difference in size between sexes. There was a large variation in GSI values for all fish particularly females (CV=205). Most colour scores were significantly higher in males. Fat content was also significantly higher in males. The average fat content of the flesh at 16.65 was higher than normal for harvest fish. However these fish, larger than average for this age, and therefore the fat content would be expected to be higher. Average gaping score for all fish was 0.23 with ~20% of fillets classified with a gaping score of greater than zero. Gaping score was not significantly different between sexes.

Trait	Non-grilse fish		Grilse		Males		Females		Sig.
Body weight and body composition traits									
	Ave n= 2972		Ave n= 90		Ave n= 1334		Ave n= 1607		
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	
Round wgt	7.78	1.74	4.24	1.33	8.51	1.83	7.14	1.44	***
Gutted wgt	7.12	1.60	3.88	1.28	7.80	1.68	6.52	1.32	***
Gonad wgt	19.36	11.97	119.97	85.09	9.90	5.19	28.11	22.68	***
Viscera wgt	617.98	187	222.69	196	684.99	206	586.80	155	***
GSI	0.29	0.20	3.33	3.35	0.13	0.09	0.42	0.17	***
Log GSI	-1.45	0.68	0.41	0.35	-2.08	0.44	-0.90	0.26	***
Fillet wgt (1 fillet)	2.47	0.56	----------		2.67	0.59	2.31	0.47	***
Fillet %	0.69	0.04	----------		0.68	0.04	0.70	0.04	***
Gaping	0.23	0.48	----------		0.23	0.49	0.24	0.46	
Flesh colour traits									
	Ave n= 2439				Ave n= 1086		Ave n= 1345		
a* value	29.63	1.43	----------		29.70	2.01	29.57	2.11	*
L value	49.70	2.07	----------		49.70	1.48	49.70	1.38	
b* value	28.79	1.66	----------		28.69	1.63	28.87	1.67	
A_dor	28.32	0.89	----------		28.41	0.93	28.25	0.85	*
A_mid	30.04	0.86	----------		30.11	0.91	29.97	0.82	**
B_dor	28.77	1.33	----------		28.80	1.33	28.74	1.34	*
B_mid	30.29	1.39	----------		30.34	1.41	30.24	1.37	
B_ave	29.53	1.32	----------		29.60	1.30	29.49	1.31	*
A_ave	29.18	0.83	----------		29.37	0.84	29.11	0.78	*
NIR traits									
	Ave n= 991				Ave n= 464		Ave n= 525		
Cantha	12.61	1.96	----------		12.62	1.95	12.60	1.96	
Fat	16.59	2.40	----------		17.04	2.51	16.19	2.22	**

*** $p<0.001$
** $p<0.01$
* $p<0.05$
Sig= significance of the effect of sex as part of a general linear model

Table 4-5 Means, standard deviations and numbers sampled for all traits. Significance of the difference between males and females is also shown.

The effect of sex was significant ($p<0.01$) for all traits with the exception of canthaxanthin content, L value and b* value. Where sex is significant, the difference between the sexes may be a result of real sex differences, body size differences or the maturation process. When the effect of sex was examined as part of a linear model with sire and dam as random variables and weight score or maturity score as fixed effects, the analysis was improved in most cases

(lower residual variance). Table 4-6 shows R^2 values and the error mean square term where traits were adjusted for either weight score or maturity score, for traits where the effect of these fixed effects was significant. This effect was most significant for fillet weight and viscera weight which are composition traits of gutted weight and are therefore likely to be highly correlated with gutted weight. For a number of traits; a* value, a_ave and canthaxanthin content, the effect of sex was no longer significant when weight score was included as a fixed effect, demonstrating that the difference between sexes is not independent of body weight or sexual maturity.

The effect of filleting method (hand or Baadar machine) did not have a significant effect on fillet percent. There was a significant effect of filleting method on fillet weight. However this result is confounded by the fact that only fish of < 6kg were passed through the Baadar filleting machine. Gaping score was influenced by filleting method, with a significantly higher gaping scores for hand filleting compared to machine filleting (hand filleting=0.27, Baadar filleting=0.19, $p<0.001$). However when the data was adjusted for gutted weight, the effect of filleter was no longer significant while the effect of gutted weight is ($p<0.001$).
All traits were also examined for the significance of age (days since fertilisation-range 1125 to 1162 days) and line in the processing plant. The effect of age was significant for whole body traits; round weight and gutted weight. Although the effect of age was significant for body composition traits until weight score was included in the model, in the final models, neither age nor line were significant for any other trait.

	Y=sire+dam(sire)+sex		New model		
	R^2	σ_e	R^2	σ_e	new model
Gutted wgt	0.35	1.73	0.42	1.71	Y=sire +dam(sire)+sex+age
Round wgt	0.34	2.13	0.39	2.08	Y=sire +dam(sire)+sex+age
Viscera wgt	0.11	77803	0.24	66948	Y=sire +dam(sire)+sex+ws
Fillet wgt	0.30	0.23	0.81	0.07	Y=sire +dam(sire)+sex+ws
Gonad wgt	0.56	65.90	0.60	60.58	Y=sire +dam(sire)+sex+ws+sex*ws
Log_GSI	0.82	0.09	0.83	0.09	Y=sire +dam(sire)+sex+ws
a* value	0.16	1.82	0.21	1.71	Y=sire +dam(sire)+ws
A_ave	0.13	0.63	0.24	0.55	Y=sire +dam(sire)+ws
B_ave	0.13	1.59	0.22	1.45	Y=sire +dam(sire)+sex+ws
Cantha	0.16	3.75	0.17	3.17	Y=sire +dam(sire)+ms
fat	0.21	5.26	0.39	4.05	Y=sire +dam(sire)+ws

Table 4-6 Proportion of variation explained by sex , and R^2 values and error mean squares for two models plus an interaction term

4-3-d Heritabilities

Heritabilities and their standard errors are shown for all traits analysed in a single trait analysis from both an ANOVA based method under the general linear model (Proc. GLM) and restricted maximum likelihood method (REML) (Table 4-7). Standard errors are not shown for heritabilities estimated from GLM model as they are not necessarily correct given the unbalanced data and the presence of nested random effects in the model. There is very little agreement between estimates of h^2 using the two methods although the models for both analyses are the same. Estimates of heritability are generally higher with least squared method compared to REML methods. For two traits, gaping and canthaxanthin content, estimates of h^2 are negative in the least squared method. Heritabilities from the general linear model were all based on the sire component of variance which in general was lower or similar to the dam component.

Estimates of heritability from the univariate analysis for body weight and body composition traits ranged from low to high (0.07-0.48). Heritabilities for scored colour traits were all of medium magnitude and all significantly higher in the ANOVA based analysis. From the three Minolta camera readings, a* value shows the highest heritability (0.22). The canthaxanthin content in the flesh, showed low heritability (0.06) while heritabilities for gaping and L value were not significantly different from zero.

A number of traits were adjusted for weight score or maturity score (see material and methods), Table 4-7 shows the difference in h^2 estimates when the fixed effects are included in the model. Fillet weight and viscera weight were significantly higher without the adjustment in the model demonstrating that a proportion of the additive genetic variance observed without the adjustment is a result of the high genetic covariance between fillet and viscera weight and gutted weight and gonadosomatic index respectively. The heritability of fillet weight adjusted for the effect of gutted weight was close to the heritability of fillet percent after the adjustment. In general the subjective colour scores were significantly lower once adjusted for weight score.

Table 4-7 shows the results of the multitrait analysis for two models. The traits round weight, gonad weight and gaping score were not included in the multivariate model. Round weight because it is effectively the same trait as gutted weight (correlation 0.99), gonad weight because the percentage trait, GSI, was used instead and gaping score because the heritability is not significantly different from zero. Model I includes the average score of both scorers rather than the individual scores and only one Minolta camera measurement (a* value). In model II each individual score for each fillet was analysed as well as all three Minolta measurements (L value, a* value and b* value) in order to compare all colour measurements and their

relationship to each other. Body composition traits; viscera weight and fillet weight were significantly higher in the multivariate model.

Heritability estimates for males and females analysed separately from a multivariate model (model 1) of 9 traits are shown in Table 4-7. A number of traits were significantly higher for females in particular; viscera weight, log_gsi and both NIR traits. Canthaxanthin content h^2 was low for the multivariate analysis with all fish and for males. For females, canthaxanthin content h^2 was of medium magnitude (0.22) and was similar to the heritability estimates of the other colour traits.

	Univariate analysis				Multivariate analysis-REML					
	ANOVA	REML-Proc Mixed			All		Females		Males	
Trait	h^2	h^2-ad	*SE*	h^2-unad	h^2	*SE*	h^2	*SE*	h^2	*SE*
Body weight and body composition traits										
Gutted wgt.[ab]	0.47	0.36	0.04	0.48	0.34	0.01	0.36	*0.03*	0.38	*0.02*
Round wgt	0.48	0.35	*0.04*	---------	0.41	*0.02*				
Viscera wgt[a]	0.13	0.17	0.02	0.15	0.24	*0.00*	0.21	*0.02*	0.19	*0.02*
Fillet %[a]	0.09	0.07	0.01	---------	0.06	0.01	0.07	*0.02*	0.09	*0.03*
Fillet wgt[a]	0.12	0.06	0.01	0.30	0.30	0.03	0.32	0.04	0.33	0.18
Gonad wgt	0.04	0.10	0.02	0.12	0.02	*0.07*				
Log GSI[ab]	0.22	0.18	0.03	0.15	0.17	0.09	0.25	*0.10*	0.14	*0.14*
Subjective and colorimetric colour traits										
a* value[ab]	0.20	0.20	0.03	0.22	0.20	*0.02*	0.21	*0.10*	0.28	*0.09*
b* value[b]	0.15	0.12	0.02	---------	0.18	*0.02*				
L value[b]	0.32	0.04	0.02	---------	0.05	*0.03*				
A_ave[a]	0.09	0.12	0.02	0.15	0.12	0.08	0.18	*0.02*	0.21	*0.02*
B_ave[a]	0.04	0.17	0.02	0.17	0.14	0.02	0.17	*0.02*	0.23	*0.02*
A_dor[b]	0.17	0.11	0.02	0.14	0.12	*0.01*				
A_mid[b]	0.41	0.11	0.02	0.20	0.12	*0.03*				
B_dor[b]	0.31	0.11	0.02	0.15	0.12	*0.02*				
B_mid[b]	0.18	0.12	*0.02*	0.17	0.14	*0.02*				
gaping	-0.01	0.01	*0.01*							
NIR traits										
Cantha[ab]	-0.06	0.06	0.03	0.06	0.07	*0.01*	0.21	*0.02*	0.09	*0.01*
Fat[ab]	0.24	0.15	0.03	0.15	0.12	*0.02*	0.24	*0.02*	0.14	*0.02*

ad=model adjusted for effect of body weight or maturity status
unad=model not adjusted for any effect other than sex

Table 4-7 Heritabilities (h^2) and standard errors for all traits from single trait and multi-trait analysis

4-3-e Correlations

Phenotypic and genetic correlations with standard errors are shown for both models in Table 4-8. The sign and magnitude of genetic and phenotypic correlations generally agreed, however, there were some exceptions. The genetic correlation between fat content and canthaxanthin content was very high and significant (0.81 $\pm$ 0.08) although the phenotypic correlation was zero. In general, the genetic correlations between body weight and body composition traits were all high (>0.82) as would be expected. Genetic and phenotypic correlations between body composition traits (gutted weight and fillet weight) and colour traits were all high with the exception of canthaxanthin content where these genetic correlation were not significantly different from zero.

Phenotypic correlations for subjective colour scores and colorimetric a* value with body composition traits were all significantly lower than the corresponding genetic correlations. The two average subjective colour scores had a very high genetic correlation (0.98) and a lower but significant phenotypic correlation. The average colour scores also had positive and significant correlation to the other measures of colour: canthaxanthin content and a* value. Genetic and phenotypic correlations of the natural log of gonadosomatic index were all negative and while not very high were mostly significantly different from zero (Table 4-8)

In model 2, the colour scores are broken down into the individual scores given to positions dorsal and midline by the two scorers. Also, the three values of the Minolta camera, L, a*, b* are included in the model. The a* value of Minolta showed the highest genetic correlation to the subjective colour scores and to canthaxanthin content. As in model 1, the genetic correlation of a* value to canthaxanthin content was positive and significant, whereas the

phenotypic correlation was not different from zero. L value (lightness) was negatively correlated to colour scores and canthaxanthin content although the magnitude of the correlation is significantly less than the correlation of colour scores to a* value. The genetic correlation of scorer B to a* value was higher at both positions than scorer A whereas the opposite was true for the colorimetric L value. The genetic correlations of the 4 scores taken on each fish are all highly significant (0.86-0.97), the same phenotypic correlations are similar where the same scorer is involved (e.g. B_dor to B_mid) but significantly lower (0.48-0.52) between scorers.

Table 4-9 shows the genetic and phenotypic correlation for males and females, all non-grilse, for model 1. For males, there were significant negative genetic correlations between gonadosomatic index (Log_gsi) and fat content (-0.48 ± 0.09) For females this correlations was positive although not significantly different from zero(0.08 ± 0.09). Genetic parameters for canthaxanthin content in the flesh differed significantly between males and females. Heritabilities for males and females were 0.21 and 0.09 respectively (Table 4-7), genetic correlations for cantha content and body composition traits for males were high while for females, the same genetic correlations were not different from zero.

a)

	Gutted wgt	Viscera wgt	± SE	Fillet %	± SE	Fillet wgt*	± SE	B_Ave	± SE	A_Ave	± SE	Cantha	± SE	Fat	± SE	a* value	± SE	Log_GSI	± SE
Gutted wgt		0.84	0.02	-0.08	0.10	0.99	0.00	0.34	0.04	0.33	0.04	0.03	0.06	0.43	0.13	0.30	0.04	-0.14	0.06
Viscera wgt	0.78			-0.14	0.10	0.82	0.02	0.39	0.03	0.43	0.03	0.00	0.06	0.33	0.11	0.20	0.04	-0.19	0.07
Fillet %*	0.11	0.01				------		0.18	0.13	0.21	0.02	0.11	0.13	0.00	0.11	0.20	0.05	-0.21	0.09
Fillet wgt	0.97	0.78		------				0.35	0.04	0.36	0.04	0.04	0.06	0.40	0.13	0.34	0.04	-0.17	0.06
B_Ave	0.14	0.14		0.06		0.15				0.98	0.02	0.53	0.06	0.29	0.05	0.73	0.03	-0.05	0.06
A_Ave	0.18	0.18		0.05		0.18		0.56				0.46	0.06	0.22	0.07	0.62	0.05	-0.11	0.07
Cantha	0.05	0.05		0.00		0.05		0.06		0.11				0.79	0.08	0.41	0.08	-0.21	0.09
Fat	0.39	0.30		0.10		0.39		0.06		0.09		0.00				0.41	0.06	-0.21	0.07
a* value	0.13	0.08		0.03		0.14		0.57		0.29		0.02		0.14				-0.08	0.07
Log_GSI	-0.18	-0.14		-0.02		-0.17		-0.04		-0.09		-0.08		-0.11		-0.05			

b)

	Gutted wgt	B_Dor	± SE	B_Mid	± SE	A_Dor	± SE	A_Mid	± SE	Cantha	± SE	Fat	± SE	a* value	± SE	b* value	± SE	L value	± SE	Log_GSI	± SE
Gutted wgt		0.22	0.10	0.31	0.05	0.29	0.05	0.38	0.13	0.05	0.11	0.39	0.06	0.28	0.05	0.16	0.04	0.12	0.04	-0.12	0.04
B_Dor	0.06			0.97	0.10	0.94	0.10	0.86	0.14	0.48	0.09	0.23	0.13	0.70	0.09	0.03	0.09	-0.14	0.09	-0.09	0.08
B_Mid	0.15	0.86				0.96	0.06	0.93	0.10	0.54	0.08	0.26	0.09	0.71	0.07	0.04	0.08	-0.16	0.08	-0.11	0.08
A_Dor	0.18	0.50		0.52				0.92	0.08	0.53	0.11	0.20	0.08	0.55	0.05	-0.02	0.05	-0.21	0.04	-0.16	0.04
A_Mid	0.18	0.48		0.51		0.76				0.40	0.09	0.26	0.09	0.61	0.08	-0.06	0.09	-0.29	0.05	-0.20	0.09
Cantha	0.05	0.04		0.08		0.12		0.09				0.67	0.06	0.39	0.04	0.11	0.07	-0.01	0.05	-0.20	0.07
Fat	0.38	0.01		0.07		0.05		0.13		-0.01				0.41	0.09	0.12	0.09	0.04	0.09	-0.18	0.09
a* value	0.13	0.55		0.55		0.27		0.27		0.03		0.13				0.25	0.02	0.05	0.02	-0.09	0.01
b* value	0.09	0.12		0.10		-0.01		-0.05		0.01		0.05		0.42				0.96	0.03	0.08	0.02
L value	0.02	0.05		0.01		-0.20		-0.26		-0.05		0.04		0.28		0.77				0.18	0.01
Log_GSI	-0.21	-0.04		-0.05		-0.09		-0.12		-0.09		-0.12		-0.05		0.03		0.06			

Table 4-8 Estimates of phenotypic (below diagonal) and genetic correlations (above diagonal) with standard errors for model I (a) and model II (b).

* fillet percent included in a separate analysis.

Males	Gutted wgt	Viscera wgt		Fillet wgt		B_Ave		A_Ave		Cantha		Fat		a* value		Log_GSI	
			± SE		± SE		± SE		± SE		± SE		± SE		± SE		± SE
Gutted wgt		0.66	0.04	0.99	0.10	0.51	0.05	0.54	0.04	0.37	0.12	0.56	0.06	0.51	0.04	-0.07	0.09
Viscera wgt	0.62			0.65	0.10	0.41	0.07	0.47	0.03	0.39	0.12	0.27	0.09	0.20	0.08	-0.28	0.10
Fillet wgt	0.98	0.64				0.53	0.10	0.56	0.04	0.38	0.13	0.59	0.15	0.54	0.09	-0.14	0.06
B_Ave	0.31	0.23		0.31				0.98	0.02	0.61	0.17	0.67	0.07	0.83	0.03	-0.22	0.08
A_Ave	0.35	0.27		0.35		0.60				0.59	0.11	0.69	0.14	0.76	0.13	-0.21	0.12
Cantha	0.06	0.04		0.06		0.09		0.11				0.69	0.12	0.65	0.07	-0.22	0.13
Fat	0.43	0.29		0.42		0.16		0.19		-0.03				0.81	0.06	-0.48	0.09
a* value	0.33	0.17		0.33		0.65		0.39		0.10		0.27				-0.27	0.10
Log_GSI	-0.15	-0.11		-0.15		-0.04		-0.09		-0.07		-0.10		-0.07			

Females	Gutted wgt	Viscera wgt		Fillet wgt		B_Ave		A_Ave		Cantha		Fat		a* value		Log_GSI	
			± SE		± SE		± SE		± SE		± SE		± SE		± SE		± SE
Gutted wgt		0.85	0.02	0.99	0.00	0.74	0.05	0.81	0.03	-0.07	0.05	0.76	0.05	0.53	0.05	-0.14	0.04
Viscera wgt	0.78			0.85	0.02	0.72	0.05	0.84	0.03	-0.02	0.05	0.60	0.05	0.35	0.06	-0.19	0.05
Fillet wgt	0.97	0.78				0.73	0.05	0.81	0.04	-0.05	0.05	0.75	0.05	0.53	0.05	-0.16	0.05
B_Ave	0.32	0.28		0.32				0.97	0.02	0.36	0.10	0.70	0.05	0.73	0.05	-0.14	0.07
A_Ave	0.45	0.41		0.44		0.59				0.32	0.08	0.70	0.05	0.71	0.06	-0.14	0.07
Cantha	0.03	0.08		0.03		0.05		0.11				0.37	0.08	0.32	0.10	-0.10	0.11
Fat	0.59	0.45		0.58		0.17		0.27		0.03				0.55	0.08	0.08	0.09
a* value	0.25	0.17		0.25		0.56		0.30		-0.03		0.18				-0.09	0.10
Log_GSI	-0.27	-0.31		-0.27		-0.11		-0.16		-0.06		-0.22		-0.07			

Table 4-9 Estimates of phenotypic (below diagonal) and genetic correlations for males and females

4-4 Discussion

Phenotypic means and variation body weight, composition and colour traits

The significance of the difference between the sexes for all body composition traits shows the importance of taking the effect of sex into account when calculating genetic parameters and has also been demonstrated in rainbow trout (Crandell and Gall 1993). Not surprisingly, body weight and viscera weight (included internal organs) are higher in males. However, the percent of fillet is higher for females (Table 4-5) indicating that the relationship between sex and body size is not necessarily the same for sex and body composition. When the final product of processing is fillets rather than gutted salmon, there is less wastage per fish from females compared to males. While most of the colour scores and the a* value were higher for males than for females, this difference was mostly due to the effect of size rather than sex (Table 4-6). The colour scores on the midline were significantly higher that the scores on the dorsal position. This unequal distribution of carotenoid is typical in Atlantic salmon with the colour of muscle lightening from the head towards the tail and from the midline of the fish towards the external of the fish (Schmidt and Cuthbert 1969; Choubert et al. 1997). For both subjective and colorimetric colour scores, therefore, it is important to measure more than one position in order to get an accurate reflection of overall colour. The colorimetric measurements are an average score over 3 positions which span the dorsal and midline subjective score positions. The most realistic comparison between colorimetric and subjective scores will therefore be obtained using the subjective score averaged over dorsal and midline positions.

Despite the low dietary level of carotenoid, 35ppm, for the final year before harvesting these fish, the carotenoid content in the flesh, Minolta readings and the subjective colour scores were comparable with other studies where carotenoid concentration in the diet is 60-80ppm

(Christiansen et al. 1995; Einen and Thomassen M. S. 1998; Buttle et al. 2001; Veninga, Pers. Comm). Canthaxanthin content in the flesh is also normal at 12ppm. From the 3 colorimetric measurements, a* value shows the strongest and most linear relationship with other colour traits (Figure 4-7). This agrees with other studies where the three colorimetric measures were compared (Christiansen et al. 1995; Buttle et al. 2001). Unlike Christiansen et al. (1995) who found a significant non-linear relationship between carotenoid content (astaxanthin) and colorimetric measurements, L, a* and b* and between carotenoid content and subjective scores, no significant relationship was found here for canthaxanthin content and any other colour trait at the phenotypic level. This result agrees with Storebakken and Fjalestad (1992) who found no relationship between carotenoid content and visually scored colour when carotenoid concentration exceeded 10mg/kg and Wathne et al. (1998) who found the same result when carotenoid concentration exceeded 6-8mg/kg. It may be that the human eye has a limited capacity to distinguish differences in flesh colour when carotenoid content exceeds a certain level as suggested by Torrissen et al. (1989). However, this would not explain the correlation between subjective scores and colorimetric measurements demonstrated here and by Christiansen et al. (1995) even at higher colour scores. One explanation is that higher perceived and colorimetric colour are not only a result of higher carotenoid content in the flesh but also a result of higher fat levels, body size and interactions between all of these factors.

Effects of sex, sexual maturation and size

The present study indicates the effect of sex on many body weight and composition traits under investigation. The largest differences were for traits related to body size where males were larger than females with the exception of mature males. Stage of sexual maturation (measured by gonadosomatic index) or size of the fish are also causing differences between

sexes and these effects may be confounded with the actual effects of sex. When the data were adjusted for these effects, the effect of sex (for a* value) and filleter (for gaping) was no longer significant and the fixed effect explained the proportion of variation previously explained by the sex and filleter. For most traits the difference between sexes was reduced or eliminated by adjusting for the effects of body weight or sexual maturation (Table 4-6). Differences between sexes for scored colour traits and colorimetric measurements was, in some cases explained by weight score (Table 4-6) showing that this sex difference is dependent on body weight.

Scored flesh colour traits have been found to be significantly different between sexes by Gjerde (1989) and by Rye and Gjerde (1996) but not by Gjerde and Gjedrem (1984). Comparisons are often difficult to make between studies on scored flesh colour due to the different scoring techniques. All the above studies employed a scoring system from 1-5 whereas the present study used the Roche Salmofan™ (Hoffmann-La Roche Ltd, Basel, Switzerland) which scored colour on a scale of 20-34. No evidence of a difference between sexes in colorimetric measurements has been reported in Atlantic salmon. However, in rainbow trout a difference was reported (Choubert et al. 1997) for Hue angle ($H^{o}_{ab} = \tan^{-1} b^{*}/a^{*}$), which is a function of both a* and b* values, where males exhibited higher values than females.

Previous studies have demonstrated the increase in body weight prior to the onset of maturation and the subsequent decrease in body weight and increase in gonad weight as sexual maturity is reached (Naevdal 1983; Gjerde 1984; Aksnes et al. 1986; Rye and Refstie 1995). This process occurs at different rates in males and females and its onset is dependant on environmental effects such as photoperiod, temperature, size etc.. (Aksnes et al. 1986;

Bjornsson et al. 1994; Taranger et al. 1998; Taranger et al. 1999). The time in which the measurements are taken and environmental conditions will determine to a certain degree the level of differences between males and females and the effect of maturation status on other traits. These results demonstrate the importance of adjusting the statistical model for both sex and sexual maturation when estimating (co)variance components.

Heritabilities

ANOVA vs. REML

Estimates for heritability under the general linear model differed significantly from estimates under the animal model using REML method for most traits (Table 4-7). In general there was no trend for heritability estimates under the animal model (REML) to be higher than under the general linear model (sire), although this was the case for a number of traits. Where relationships trace back to the base population (as is the case here), animal model estimates of heritability are expected to be estimates of variance prior to selection and therefore to be higher than other methods. No evidence was found here to conclude that animal model estimates are higher that sire model estimates, perhaps indicating lack of selection and hence no reduction in genetic variance.

In the case of a completely balanced design (equal number of offspring per full and half-sib family and equal number of dams mated to sires) and in an population not under selection ANOVA methods should yield estimates of variance components identical to those obtained by REML. However, with unbalanced data, such as the data presented here, there are a number of ways to partition the sums of squares and as the mean squares do not have straightforward definitions, the derivations of expressions for the expected mean squares are quite complicated (Henderson 1953). In an ANOVA design, these different methods of computing sums of squares, based on the factoring out of model effects, can yield rather

different results. In none of these approaches are the observed mean squares necessarily independent and their expectations do not yield to definitions of simple ratios (as is expected under ANOVA where numerators and denominators are equal under a null hypothesis). As a result, in a breeding design where certain conditions of balance are not met, estimators of fixed and random effects will be biased (Henderson 1975).

Selection changes the additive genetic variance of a character by generating gametic phase disequilibrium can changes in allele frequency. Likelihood methods at least partially account for biases introduced by selection, in part because the additive genetic relationship matrix, A corrects for the pattern of gene flow of genetic information from generation to generation. Other variance component estimators, such as those derived from ANOVA, make no such correction. This difference between the two estimation methods could also contribute to the difference in estimates. Heritability estimates for a number of traits were negative under the general linear model. This can occur with ANOVA methods when σ_s^2 is small (sampling error associated with the mean squares causes the estimated among-family variance to be negative).The possibility of negative estimates is true of all applications of ANOVA methodology.

Univariate vs. multivariate

In the univariate analysis, where traits were not adjusted for the effect of weight or maturation score, genetic variances were generally higher compared to the adjusted trait (Table 4-7) and in each case R^2 values increased with the inclusion of the fixed effect. The decrease in additive genetic variance was also associated with a decrease in the error variance term as the fixed effect was now explaining a considerable portion of the overall variation. This increase was greatest in body composition traits where the part-whole nature of these traits to body

weight was resulting in a portion of variation being assignment to either the additive or residual component of variation. This was also reported by Kause et al. (2002) in rainbow trout where body composition traits were adjusted for body weight. The choice of fixed effect or covariate to use may affect the heritability estimates obtained and will partly depend on an understanding of what is affecting the data and by testing the model for a number of effects. Adjusting the data for covariates is particularly important when dealing with body composition traits in order to calculate the genetic variation independent of the genetic variation in whole body traits. Adjusting for the effects of body size and maturation traits may reduce the additive genetic variation, inflated by genetic covariance between traits, resulting in lower heritabilities.

Heritability estimates for the multivariate analysis under both models were in general not significantly different from the univariate analysis adjusted for the effects of body size or maturity (Table 4-7). Two exceptions to this were viscera weight and fillet weight where higher additive genetic variances and lower residual variances resulted in significantly higher heritabilities in the multivariate model. The information provided by the genetic covariances between traits (these two traits have high genetic covariances with body size) may have improved the accuracy of variance component estimation because correlated characters provide information about each other. Alternatively, estimates of heritability may have been inflated by the inclusion of body size traits of which viscera weight and fillet weight are part of. If such correlations between traits are not appropriately handled then some correction for non-independent tests must be applied to control type I errors (Ducrorq and Besbes 1993). Non-independence occurs because the variance for fillet weight, which is highly correlated to body weight, is now maintained in the multitrait model. In the unitrait model this variance is explained by the weight class fixed effect.

For fillet weight, this result suggests that there is very little genetic variation which is independent of the genetic variation in body weight and therefore available for selection for increasing fillet weight. The low heritability for fillet percent agrees with Kause et al. (2002) in rainbow trout but not with Rye and Gjerde (1996) who found significant additive genetic variation for fillet percent. Heritability of a ratio trait (such as fillet percent) is a function of the genetic variances and covariances of the two traits (Simm 1983). When heritabilities are very similar, as is the case here (see Table 4-7), and the genetic correlation is close to unity (0.99 ± 0.09) then the expected heritability of the ratio trait will be close to zero. If fillet weight is to be included in a selection goal for Atlantic salmon it should be included as a percentage of body weight, otherwise selection on the basis of breeding values for fillet weight is actually selection for increased body size.

Body size and body composition traits

Heritabilities presented here for body size and composition traits were all medium to high and in general agree with other reported estimates in Atlantic salmon (Gjerde and Gjedrem 1984; Gjerde et al. 1994; Rye and Refstie 1995). Heritability for round weight (not shown) was identical to that for gutted weight with a genetic and phenotypic correlation of 0.99, this agrees with previous studies in Atlantic salmon and rainbow trout (Gjerde and Gjedrem 1984; Gjerde 1989).

Heritability for fat content was lower than that previously reported for Atlantic salmon (Rye and Gjerde 1996) and for rainbow trout (Gjerde and Schaeffer 1989) although the methods of measurement in these studies were different (computerised tomography and score respectively). Similar estimates were reported for percent fillet fat (weight) and abdominal fat

(score) in rainbow trout (Kause et al. 2002). Difference in estimates may be due to the differences between strains/species and/or timing and methods of measurement.

Farm reared Atlantic salmon typically have a slaughter yield (gutted to round weight ratio) of 90% (Rora et al. 1998). In this study the loss though gutting over all fish was 8.5% (result not shown). The gutting loss through slaughter, a composite trait made up of liver and kidney weight and viscera fat, has been demonstrated to be correlated to body shape and weight (Rora et al. 1998). Variations in slaughter yield or gutting loss also have a genetic origin with 11-23% of the total variance in slaughter yield due to family differences (Gjerde 1996; Rye and Gjerde 1996). The results presented here show high additive genetic component for gutting loss with h^2 of 0.24 $\pm$ 0.00 for males and females together in the multivariate model. While there is significant potential for improvement in this trait as well as significant incentive as all gutting loss is a waste, reducing gutting loss may have deleterious physiological and/or reproductive impacts (Aksnes et al. 1986) and therefore selection for a reduction in gutting loss should be considered carefully.

Colour traits

Heritabilities for scored colour traits were all low to medium (0.12-0.15) and agreed well with most other studies in Atlantic salmon and rainbow trout (Gjerde 1989; Rye and Gjerde 1996; Kause et al. 2002) with the exception of Gjerde and Gjedrem (1984) which found no significant genetic variation, based on the sire component, for visually assessed score. All of the above studies employed a different system of scoring to the one used here. Of the three instrumental colorimetric measurements heritability was highest for a* value which is not surprising given that this variable is most strongly correlated with perceived colour (Choubert 1982; Christiansen et al. 1995). The heritability of a* value was significantly higher than the subjective scores. Instrumental measurements of colour are not prone to the subjectivity of

visual scores and therefore would be expected to show higher heritability (i.e. are less confounded by environmental variation of the scorer). However, the instrumental measurement is likely to be affected by inter-myotome fat which is removed to a lesser or greater degree by filleter and, given that it is white in colour, would result in a higher lightness and lower redness reading (scorer can ignore this layer of fat when present and therefore will not give a lower score). This may explain why the heritability for colorimetric redness is not higher. Both scorer and instrument are under environment influences which are difficult or impossible to account for and, therefore, to eliminate from the error variance term resulting in lower heritabilities.

Sexual maturity

Gonadosomatic index is used commonly as an indication of likelihood of sexual maturity developing over a given period. Early maturity, a binary trait; is a measure of the actual onset of sexual maturity at a given period. Heritability estimates reported in Atlantic salmon for early and late maturity on a binary scale and on the underlying liability scale vary from 0.09 to 0.35 (Gjerde and Gjedrem 1984; Wild et al. 1994). Estimates of heritability for gonad weight and gonadosomatic index presented here were low to medium and agree with previous heritability estimates for gonad weight (Gjerde 1989) although no estimates for gonadosomatic index have been presented in the literature. The variation observed for both early maturity and gonadosomatic index will depend greatly on the time of year, the conditions preceding the measurement as well as the strain of salmon, as large differences exist for this trait between commercial strains (Glebe and Saunders 1986).

The results presented here indicate the potential for improvement in this trait through selection, although intense selection for lower gonadosomatic index may delay the maturation

process beyond the time of normal maturation for breeding. Further research is needed on the genetic relationship between gonadosomatic index and percent early maturation in different environments and at different times of the growth cycle in order to fully understand, and best apply selection for late maturation. To optimise selection for the trait, data from the processing plant on GSI should be combined with actual maturation figures through the ongrowing phase at sea and from broodstock.

Difference between sexes.

When the analysis of variance components was split into separate datasets for each sex, heritabilities for a number of traits were significantly higher for females. In particular, log_GSI and both NIR traits; fat and canthaxanthin content (Table 4-7). For log_gsi and fat content the phenotypic variance was significantly higher in males (Table 4-5) resulting in lower contribution of the additive genetic variances and hence lower heritabilities. The difference in heritability estimates for canthaxanthin content for males and females cannot be explained by difference in overall variances or non-normal distributions. One possible explanation is based on the relationship between dietary carotenoid content and body size. The absorption and retention of pigment is different for fish of different sizes (Torrissen et al. 1989; Torrissen 1995). In general, females would have been smaller than males when the dietary pigment content was reduced (one year before harvest) and although the average canthaxanthin content was the same at harvest for both sexes, this size difference may be confounded with the additive genetic component of canthaxanthin in the flesh for females. Estimates of heritability were also significantly different between the sexes for the natural logarithm of gonadosomatic index (Log_GSI) with lower genetic variation for males compared to females. All grilse, with the exception of 94 fish, were eliminated from the

study. Most grilse are male (in 94 grilse only 14 were female) and the effect of removing them from the study, was to reduce the variation in the trait, a significant portion of which will likely be genetic. In order to include all genetic variation for this trait, grilse fish should be measured for gonad weight.

Correlations

In salmonids, as maturity approaches, viscera weight and fat content in the flesh decrease as fat is re-distributed from flesh and viscera into gonads (Aksnes et al. 1986). In the present study the maturation index (Log_GSI) is negatively correlated at the genetic and phenotypic level with all body weight, composition, fat and colour traits. These results indicate that fish with larger gonads were in the process of maturing resulting in lower body weight, fat and colour in the flesh. At an earlier stage in the growth cycle it is likely that this correlation would have been reversed with larger gonad size associated with faster growing fish, as was observed by Gjerde (1989). As the maturation process develops, energy is diverted from muscle growth to gonad development resulting in the negative correlation observed here. When maturation status (mature/immature) is recorded throughout the seawater growth phase, a negative correlation is found between body weight and sexual maturity, demonstrating that larger fish have a tendency to mature earlier than smaller fish (Naevdal 1983; Thorpe et al. 1983; Skilbrei 1989; Gjerde et al. 1994; Rye and Refstie 1995). A few months before maturity is reached maturing fish are on average 30% heavier than non-maturing fish (Rye and Refstie 1995). However, as with gonad development, this trend reverses as soon as a critical point is reached where further growth is sacrificed for gonadal development.

The relationships between gonadosomatic index, maturity and body weight will therefore depend greatly on the time of sampling and the environmental conditions prior to sampling. In the present study carotenoid content, colorimetric measurements and subjectively scored colour traits were all negatively correlated with gonadosomatic index (Table 4-8 and Table 4-9). This is a consequence of carotenoids migration from the muscle into the gonads (Aksnes et al. 1986) and has been demonstrated previously in salmonids (Crozier 1970; Sivtseva and Dubrovin 1981; Kitahara 1983; Torrissen et al. 1984; Torrissen and Naevdal 1988). However, if the maturation process has not reached a critical period at the time of sampling (where carotenoids migrate from the flesh), no influence will be found of maturation status or index on colour (Rye and Gjerde 1996). Selecting for late maturation, therefore, will not necessarily result in better pigmented fish but rather later maturing fish at the same pigmentation level. A better understanding of sexual maturation and its interaction with growth, fat, colour and other quality traits will allow for classification of individuals into maturation classes within sex which will reduce correlations such as the one demonstrated above. This understanding will be helped when the analysis of other year classes (within which there are genetic links) are included as these fish will have been slaughtered and sampled at different times in the growth/maturation curve.

The present results indicate a positive genetic relationship between percent fat content and body weight (r_g =0.40-model I, 0.39-model II). Similar relationships have been reported in rainbow trout by Ayles et al. (1979) and who found a strong genetic correlation between growth rates and percent fat. However, Gjerde (1989) and Kause et al. (2002) reported a negative correlation and zero correlation respectively between the traits at the genetic level in rainbow trout. In Atlantic salmon, a significant, positive correlation between muscle fat, measured by computerised tomography and body weight was also reported (Rye and Gjerde

1996). These results indicate that selection for increased growth rate may result in increased fat storage in the flesh and that fat content should be included in the breeding objective if an increase in fat content is undesirable. However, if selection for increased growth rate results in harvesting fish at an earlier age, as opposed to a larger size, then fat content may not be increased despite the unfavourable relationships (Rye and Gjerde 1996).

Less fat in the viscera is important from a sales point of view as fish are sold ungutted and all gutting loss is waste. The present results indicate a strong positive genetic relationship between gutting loss (adjusted for maturity index) and body weight and fillet weight (0.84 and 0.82 respectively). These findings suggest that selection for increased gutted weight will result in corresponding increases in gutting loss. The genetic correlation to fat content in the flesh was not very high (Table 4-8) indicating that although the viscera is largely composed of fat, genetic mechanisms of fat deposition in the intestines is different to that in the muscle. Viscera weight would not therefore be a good candidate trait to select for lower fat in the fillet (as an alternative to chemical analysis of fillet fat). The genetic correlations reported here agrees with Rye and Gjerde (1996) who found high genetic correlation for intestinal fat weight (+intestines) and size (0.90 ± 0.06) but not for intestinal fat index (ratio of intestinal fat to round weight) where the genetic correlation was negative (-0.64 ± 0.19) as was the genetic correlation to fat content in the fillet (-0.67 ± 0.18). This same result was reported by Gjerde (1989) in rainbow trout, suggesting that while increasing body size will increase intestinal weight, the relative proportion of intestinal weight to overall body weight will decrease. This finding suggests that fat is redistributed to the fillet from the intestines as body size increases and therefore selection for decreased fat in the intestines may result in higher fillet fat which is not desirable from a marketing point of view.

In salmonids, as an individual approaches maturity, fat is redistributed from the body tissue and viscera into the gonads (Aksnes et al. 1986). As a result, the weight of the viscera is likely to be highly dependant on the stage of maturation. In this study there was a low, but significant, negative genetic correlation between maturity index (Log_GSI) and gutting loss adjusted for Log_GSI (-0.19 ± 0.07). If reduction in gutting loss was included in a selection goal this may result in inadvertent increase in gonad weight.

Table 4-8 shows the genetic and phenotypic correlations for fillet weight (adjusted for gutted weight) and other traits in model I. Genetic correlations for fillet weight to body weight and composition were all high as would be expected given that fillet mass is the main component of gutted weight (69%). A separate analysis was performed (due to confounding of fillet weight and fillet percent, genetic correlation =0.99) for fillet percent. Unlike fillet weight, fillet percent shows a negative genetic correlation with gutted weight (-0.08 ± 0.1), implying that although the general observation is for fillet percent to increase with increasing body weight, the underlying genetic trend is the opposite. As fish reach maturation a proportion of fat from the flesh will be redistributed into the gonads, and this process has a strong genetic component resulting in the corresponding negative genetic correlation between fillet percent and body size (and fillet percent and maturation index (-0.21 ± 0.09)). In rainbow trout Kause et al. found medium positive correlations between percent fillet weight and percent gutted weight (r_p=0.23, r_g=0.47). The differences between these result could arise from strain differences or more likely the maturation stage of fish at the time of measurement.

Three different methods to measure colour in salmon fillets were employed in this study; subjective, visual scoring, colorimetric measurement and carotenoid content measured by NIR analysis. Although phenotypic relationships between colorimetric variables and visually

assessed colour (Little et al. 1979; Christiansen et al. 1995) and carotenoid content (Foss et al. 1984; Christiansen et al. 1995) have been reported, this is the first study to investigate the genetic relationships between the colour traits.

The challenge for the industry is to find a cost efficient and practical method of measuring redness in the flesh which best explains the underlying genetic variation and which will allow for selection for improved coloration as perceived by the customer, or same level of coloration at lower carotenoid content in the feed. Two different scorers were used in this study both of whom had been tested for repeatability of scoring colour. The use of two scorers allowed an illustration of the effect of scorer and the relationship between different subjective scores and other colour measurements on the same fillet. The effect of scorer for both positions on the fillet was significant ($p<0.001$). The genetic correlation between the average scores of scorer A and B was close to unity (0.98 ± 0.02) despite the medium phenotypic correlation (0.56) suggesting that although the two scorers are not always consistent in scoring, the underlying genetic variation is being explained by both scorers in a consistent way. Genetic correlations between average scores, adjusted for gutted weight, and all body weight and composition traits are medium (0.34-0.46) and are all higher than the corresponding phenotypic correlations and higher than other reported correlations in Atlantic salmon and rainbow trout (Gjerde 1989; Rye and Gjerde 1996; Kause et al. 2002). The fish used in this study were fed a high pigmented diet (75ppm) until ~2kg weight when the carotenoid content was reduced to 35ppm for the final year of growing.

One hypothesised model for carotenoid absorption in salmonids suggests that the rate of carotenoid deposition is curvilinear throughout the life of the fish with a rapid initial increase in flesh pigmentation from the start of carotenoid inclusion in the diet followed by a plateau in

absorption as the fish becomes larger (Torrissen et al. 1989; Torrissen 1995). Under this model, fish which had not reached a certain body size before the dietary carotenoid concentration was reduced would exhibit lower colour compared to larger fish. As growth rate is obviously under strong genetic control, the genetic correlation between growth rate and colour would be expected to be high. Genetic correlations between average colour scores and percent fat content are also significantly positive which contrasts with previous studies in rainbow trout (Gjerde 1989; Rye and Gjerde 1996) but is not surprising given the strong positive genetic relationship between growth and colour reported here.
Relationships between the colorimetric value for redness (a*) and average scores for both scorers are all positive and of medium to high magnitude (Table 4-8). Scores from scorer B show a significantly higher genetic correlation to a* value than scorer A.

The high genetic correlation between canthaxanthin content and fat content is not consistent with the phenotypic relationship, which is not significantly different from zero. This result is surprising given the lipid soluble nature of carotenoids and the relationship between dietary fat concentration and increased carotenoid digestibility (Choubert et al. 1991). Carotenoid content is based on the Norwegian quality cut of the fillet which is located further towards the tail where the amount and pattern of pigmentation is less consistent (Schmidt and Cuthbert 1969; Choubert et al. 1997) than the section where colorimetric measurements and subjective scores are taken (Figure 4-2). This could explain the low phenotypic correlation of canthaxanthin content to other colour traits while, as the same genes are likely to be involved in flesh pigmentation and carotenoid retention, the underlying genetic correlations are significantly positive.

In model II correlations between all colorimetric measurements and colour scores are given. The a* value, or redness, shows the strongest genetic relationship to body weight, composition traits and maturation index. Also, of the three colorimetric measurements, the a* value also has the highest correlation to the four subjective scores. Lightness score (L value) is negatively correlated with subjective scores but of a lower magnitude to a* value and b* value or yellowness has the lowest correlation to colour scores. The a* value also has the highest genetic correlation to carotenoid content in the flesh suggesting that the a* value, or redness, colorimetric measure is explaining more of the genetic variation for visually assessed colour and for the concentration of carotenoids in the flesh than the other colorimetric measures. The genetic correlations between scorer B assessed colour and a* value were higher over both positions than scorer A while the opposite was true for L value, or lightness of the flesh. This may be the result of differences in how two people perceive colour with one person associating scores with redness and another with lightness of the flesh. There is no 'correct' score for colour and although the phenotypic correlation between the four scores were variable (0.51-0.86), the scorers were both explaining the underlying genetic variation in a consistent way. The high genetic correlations between the four subjective colour scores (0.86-0.97) suggest that both scorers are essentially measuring the same trait and only one scorer should be necessary.

Males and females separately

The most remarkable differences in the relationships between traits for males and females is for the traits maturation index (log_GSI) and canthaxanthin content in the fillet. The negative genetic correlation observed between log_GSI and fat content is significantly higher in males than in the analysis for all fish (Table 4-9) whereas for females, this relationship is now

significantly positive. These result suggest that in males the genes governing the process of maturation are also involved in the redistribution of fat from the flesh into the developing gonads. For females, the timing and physiologically effects of the maturation process are different (Aksnes et al. 1986; Taranger et al. 1999) resulting in the positive genetic correlations seen here. The relationship between maturation and other traits, particularly body size and fat content, will depend on the sampling point in the growth/maturation curve and the environmental conditions preceding harvest. In general, for females, maturation is later and more gradual which is evidenced here by the fact that increased gonad index is not yet influencing the process of redistribution of fat and the fact that out of 94 grilse fish sampled, only 12 were females. Classifying fish by maturation status within sex and using this variable as a fixed effect would partly eliminate his effect.

The high genetic correlations between canthaxanthin content and body size and composition traits for males is surprising given the low additive genetic variance for canthaxanthin content. However, when males and females are analysed separately, the numbers of individuals per full-sib family are now <5, resulting in higher standard errors (Table 4-9). Although the difference is still significant, significance levels attached to a single correlation assume independence of the pair traits from all others, adjusting significance tests to account for multiple comparisons is straightforward when the different tests involve independent data (Rice 1989). This is not the case here and significance levels for genetic correlations should not be over interpreted. Separating the data set into males and females, as has been done here, will result in less robust and less stable parameters, particularly with a small dataset such as this which lacks sufficient pedigree structure (only full- and half-sibs). This can result in mis-

allocation of genetic variation to other variance sources or fixed effect particularly when the dataset is further reduced by splitting it between sexes.

In conclusion, until more data from other year classes and generations is available, all data from both males and females should be included together in the analysis to ensure larger sample sizes within effects and better connectiveness in the data. For unbalanced data such as this, maximum likelihood methods should always be chosen over least squares methods. Traits which have a part-whole relationship with other traits should be analysed carefully with correct adjustments and/or conversion to percentage traits in order to avoid the mis-assignment of the variance and inflated heritabilities. Once the second generation of fish have been reared and measured, non-additive genetic effects can also be investigated helping to explain more of the variation present.

This study provides estimates of heritabilities and genetic correlations between economically important harvest traits, many of which have not been investigated at the genetic level previously. Results presented here will influence the type, and manner of measurement, of flesh quality traits to be included in the breeding objectives of subsequent years. Following research will further elucidate the complex genetic and phenotypic relationship between body size, body composition, maturation and quality traits in Atlantic salmon. Knowledge of the relationship between some of these traits at other stages of the life cycle, other than at harvest, will further increase understanding of the biological processes involved and allow for a more efficient selection program.

4-5 Appendix A

Sample size

When designing an experiment to measure genetic parameters such as heritability and genetic correlations between traits, it is important to give some consideration to the design of the experiment. Different mating designs will exert a major influence on the genetic parameters estimated and their standard errors. Design in this case refers to the optimum initial family number and optimum number of full and half-sibs to be tested in order to estimate genetic parameters. In the breeding design described here, the number of families is limited to an initial maximum of 200 (due to the number of individual hatching cylinders available) which is later reduced to 150 families due to unequal survival to hatching of different families.

As a result of the high reproductive capacity of fish, there is almost an unlimited number of full sibs from each family available for testing and/or selection. However, each fish tested in the processing plant has an associated cost (NIR analysis, genotyping, labour, tagging etc..) and the facilities for tested are limited. Therefore, the problem is to determine the minimum sample size which will not compromise our estimates of genetic parameters. This is a balance between cost and accuracy.

In a half sib design such as this, Robertson (1959) first addressed this problem of optimal allocation of effort in half sib design. Assuming total number of individuals that can be measured (T) is equal to N*n, optimum family size n~1/t (intraclass correlation of half sibs) $=t = \sigma_s^2 / \sigma_z^2$, where σ_s^2 = variance due to sires (half sib design) σ_z^2 is the total phenotypic variance and σ_a^2 is the additive genetic variance Also, since $\sigma_s^2 = \sigma_a^2 / 4$, the paternal half sib estimator of heritability is h^2~4t. therefore n~4/ h^2. Similarly the standard error of estimates of heritability in paternal designs is 4 times the standard error of estimates of the intraclass correlation. Osborne and Patterson (1952) showed that the large-sample variance of the intraclass correlation from a balanced one-way ANOVA is:

$$Var(t) \cong \frac{2(1-5)^2[1+(n-1)t]^2}{Nn(n-1)}$$

Equation 12 sample variance of the intraclass correlation of half sibs

The expected standard error of the heritability is

$$SE(h^2) \cong \left(1-\frac{h^2}{4}\right)\sqrt{\frac{2h^2}{T}}$$

Equation 13 Standard error of the heritability from a half sib design

$SE(h^2)$ becomes more sensitive to the design as h^2 gets larger. Plotting $SE(h^2)$ for 4 realistic estimates of h^2 (0.1 to 0.4) we can see that for all estimates of h^2, the standard error levels of after ~30 paternal half sibs. Optimum number of full and half sibs to include in a testing station can also be investigated in terms of genetic gain achieved under different scenarios. Martinez et al. (2001) determined rates of genetic gain from different breeding programs where selection is based on truncation on an index of weighted breeding values. Genetic gain was determined using the prediction error variance-covariance matrix of the traits included in the best linear prediction. Full-sib family size for 200 full-sib families ranging in size from 7 to 30 (1400 to 4,500 tested individuals) were used for performance testing in the simulation. In this simulation, the authors found that once the total number of individuals tested at the performance station exceeded 15 full-sibs per family, the accuracy of breeding value estimation and hence genetic gain levels off.

A simulation was performed in order to observe the increase in accuracy of selection as the number of paternal half sibs increased. The simulation was performed in the multi-trait index selection software program MSSEL (developed by Marc J. M Rutten and Piter Bijma, Animal breeding and Genetics group, University of Wageningen, 2000). The response and accuracy of selection from selecting on an index for growth and colour with different numbers of

paternal half sibs was simulated for an index of two traits; harvest weight and flesh colour. Response to selection under with different number of full-sibs per family is shown in Figure 4-9. Response to selection, for both traits, increases rapidly as the number of sibs increases from 5 to 20 but after this point the increase in gain from including more sibs in the analysis starts to decrease.

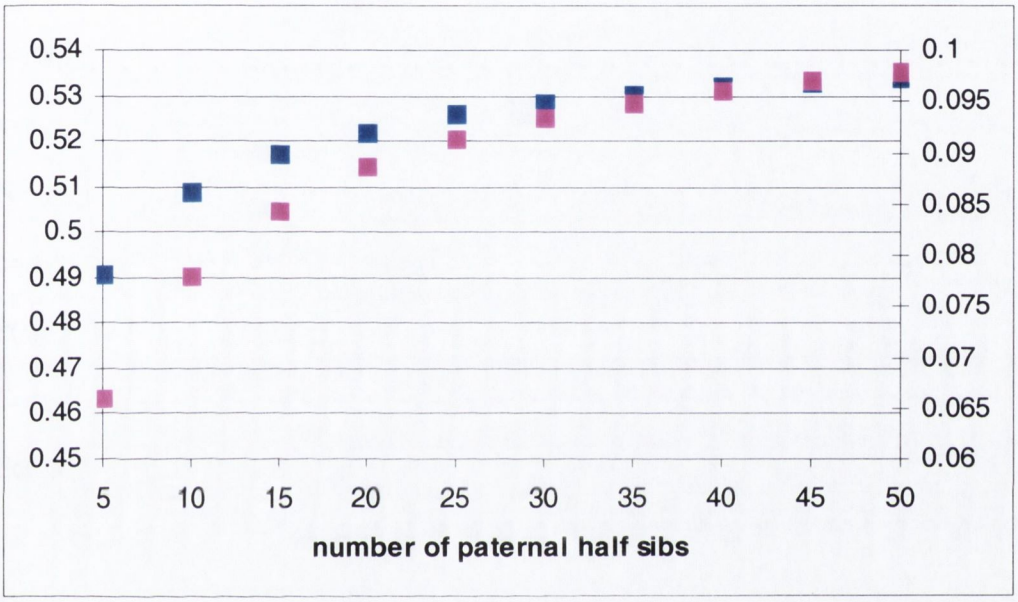


Figure 4-9 Response to selection for two traits estimated using different numbers of paternal half sibs

Based on these figures a sample size of 15 full-sibs (and automatically 15 half sibs) per family is sufficient in order to ensure minimum standard errors of genetic variance parameters within the confines of a commercial breeding program. In the breeding program described here, the families of fish are all mixed and there is no way of knowing which family a fish is from at the time of sampling. In order to estimate how many fish in total need to be sampled to ensure a family size of 15 full-sibs (paternal family size of 30) it is necessary first to assume equal

variance of family size. Where sampling is carried out without replacement (as is the case here), a hypergeometric distribution is assumed where x (in this case, number of fish from a particular family) is distributed as:

$$P(x) = \frac{C_x^k \, C_{n-x}^{N-k}}{C_n^N}$$

where

f=number of families

k=number of fish in family i

x=number of fish of family i in the sample

N =total number of fish in the population

n=total number of fish in sample

P(x) probability of obtaining x

C= Combinatorial symbol

Equation 14 Multivariate hypergeometric sampling distribution

This distribution approximates to a binomial distribution where N and n are very large. In this situation, it is not that important whether a sample is taken with or without replacement.

$$\frac{C_x^k \, C_{n-x}^{N-k}}{C_n^N} = \binom{n}{x} p^x (1-p)^{n-x}$$

$N \to \infty$

$k \to \infty$

$k / N \to p$

Equation 15 Approximation of the hypergeometric distribution to the binomial distribution

Estimates were made from both the hypergeometric and binomial sampling distributions as to the sample number required to achieve >95% probability of sampling at least 15 full-sibs per family. These probabilities are shown in Table 4-10 for two values of n (3000 and 3300) out of a cage of ~7000 fish from 150 families. From Table 4-10 under a hypergeometric distribution there is a greater than 95% probability of obtaining at least 15 full-sibs from a sample size of 3000 and a greater than 95% probability by sampling 2900 individuals. However, under the binomial sampling distribution it is necessary to sample 3100 individuals to achieve the same probability.

These estimates assume that there is no variation in family size at the time of sampling, i.e. mortality is the same for all families. This will not necessarily be the case as mortality from disease, grilsing etc.. may be higher in some families than others. Given that there will be variation in family survival, it will be necessary to sample more than 3100 individiuals in order to achieve 15 full-sibs per family.

	Binomial	Binomial	Hypergeometric	Hypergeometric
x	n=3000	n=3100	n=2900	n=3000
10	0.9979	0.9987	0.9996	0.9990
11	0.9952	0.9968	0.9989	0.9989
12	0.9899	0.9931	0.9969	0.9997
13	0.9803	0.9862	0.9924	0.9970
14	0.9646	0.9744	0.9830	0.9912
15	**0.9403**	**0.9556**	**0.9654**	**0.9795**
16	0.9056	0.9279	0.9353	0.9595
17	0.8591	0.8894	0.8886	0.9249
18	0.8003	0.8392	0.8224	0.8738
19	0.7302	0.7774	0.7364	0.8021
20	0.6511	0.7052	0.6339	0.7113

Table 4-10 Cumulative probabilities of obtaining x or more from a sample size of n

Chapter 5

Estimation of breeding values, accuracy of breeding values and response to selection

5-1 *Introduction*

5-1-a BLUP estimation of breeding values

Accurate prediction of breeding values is of great importance in an animal improvement enterprise since response to selection is linearly dependent on the accuracy of the breeding value of individuals being evaluated. Advances in computer technology and the concomitant improvements in statistical procedures have enabled animal breeders to improve both the estimation of (co)variance components and the genetic evaluation of animals BLUP allows for the separation of genetic and environmental effects across all animals in the population and environments while correcting for selection over generations (Henderson 1973) and the technique now dominates the analysis of data from animal improvement programs.

The general mixed model forms the framework for BLUP analysis. The mixed model and its statistical properties have been described in detail for applications involving data from both selected and unselected populations (Henderson 1975; Henderson 1976; Henderson 1984). The genetic properties of parameter estimates have been discussed by (Kennedy and Sorensen 1988) (for a more detailed description of the statistical properties of mixed model equations see Chapter four - Materials and methods). In summary, inferences about fixed effects are called estimators and those concerning random effects are known as predictions. The procedure originally described by (Henderson 1973) for obtaining such estimators and predictors are Best Linear Unbiased Estimation (BLUE) and Best Linear Unbiased Prediction (BLUP). They are *best* in the sense that they minimize the sampling variance, *linear* in that they are linear functions of the observed phenotypes y, and *unbiased* in the sense that E[BLUE(β)]=β and E[BLUP(u)]=u. Once variance components are known (covariance matrices G and R), BLUP is a general method which yields maximum likelihood estimates of

random effects (u) such as breeding values and maternal effects and fixed effects (β) from the mixed model equations (MME) in the form of Chapter three equation 7 (Henderson et al. 1959; Henderson 1973; Henderson 1984).

There are a number of ways in which the model can be formulated: animal model, sire model and dam model. The animal model, which defines additive genetic effects for all animals individually and accounts for all (co)variances between them (Meyer and Hill 1991), is the one most commonly used in animal breeding practices now. When complete information is available for a fully pedigreed population, a major strength of the animal model approach is that performance and genetic relatedness for all individuals in all generations is utilized simultaneously through the use of a numerator relationship matrix. The use of an animal model for the prediction of breeding values requires prior knowledge of the additive genetic and phenotypic variances of the trait concerned (Kennedy 1981). If variance components are known, realisations of the random additive genetic effect, or breeding values, can be predicted (BLUP) (Henderson 1984). REML estimates these variance components from the mixed model framework as described in Chapter four.

Information from several traits can be incorporated into the mixed model equations, for the prediction of breeding values. The success of the mixed model in separating genetic and environmental effects is dependent on the degree of confounding of individuals across environments, knowledge of phenotypic and genetic parameters, and the completeness of the data. In general, multitrait BLUP increases the accuracy of selection and the difference between single trait and multitrait evaluation is based on the magnitude of the genetic parameters involved (Thompson and Meyer 1986). Traits included in the selection objective may not be measurable on the breeding individuals themselves, such as carcass quality traits.

Information on the genetic covariances between measured traits and traits in the selection objective can be incorporated into the mixed model equations, for the prediction of breeding values for all traits in the selection objective. However, the accuracy of breeding value estimation, when measurements cannot be performed on the individual themselves, will always be less compared to when traits have been recorded.

The accuracy of BLUP breeding values is the correlation between the true and predicted breeding values, the calculation of which requires the inverse of the mixed model equations as shown by (Henderson 1975). The purpose of this investigation is to compare single trait and multitrait breeding value estimation for body size, body composition and quality traits in Atlantic salmon and to compare the accuracy of such evaluations under different designs.

5-2 *Materials and methods*

5-2-a Measurement of live end weight

One week after the harvest measurement were recorded on slaughtered fish, live end weight measurements were recorded on the siblings of these fish still in sea cages. Approximately 2,000 males and 3,500 females (previously sex-determined through ultrasound) were selected on the basis of size and appearance from approximately 20,000 potential broodstock. These fish were all anaesthetized, tagged by injecting with passive integrated transponders (PIT) tags, weighed and a fin sample taken. Fin samples from all fish were sent for parentage analysis using a microsatellite multiplex genotyping system (Genomar AS)

5-2-b Analysis of fixed and random effects

Multivariate and univariate analysis of variance components for live end weight was carried out using REML and fitting an animal model. A software package called REML VCE (Groeneveld 1996) was used to estimate variance components. Predicted breeding values were calculated by an animal model using the Prediction and Estimation program, PEST (Groeneveld 1990) and using the variance and covariance components estimated from the variance component analysis.

Estimation of variance components and breeding values was performed as a univariate analysis under the model shown below and as part of a multivariate model (model I) with 9 other traits as shown in chapter four (materials and methods).

Live end weight= $Y_{ij} = \mu + sex_i + b\ x\ age\ + a_j + \varepsilon_{ij}$

Where μ =overall mean

Sex_i=fixed effect of *i*th sex

Age=regression variable accounting for age

a_j=additive genetic effect of *j*th animal

e_{ij}=residual random effect

A second multivariate analysis was also performed with the 8 traits most likely to be included in a breeding goal and for these traits the predicted error variance and accuracy of breeding value estimates was also calculated. The predicted error variance (PEV) is a measure of the variation about the predicted breeding value or can be described as the fraction of the additive genetic variance not accounted for by the prediction or the variance of the difference between the estimated breeding value and the true breeding value. (Henderson 1975) showed that if the coefficient matrix of the MME equation in equation 7 (chapter four) is represented as:

$\begin{bmatrix} C_{11} & C_{12} \\ C_{21} & C_{22} \end{bmatrix}$ and a generalized inverse of the this matrix as $\begin{bmatrix} C^{11} & C^{12} \\ C^{21} & C^{22} \end{bmatrix}$

then the PEV is:

$$\text{PEV}=C^{22}\sigma_e^2=(1-r^2)\sigma_a^2$$

Where r^2 is the squared correlation between the true and estimated breeding values and σ_a^2 and σ_e^2 are the additive genetic and residual variance components respectively. The accuracy of the estimated breeding values, r, is the square root of this correlation:

$$r=(1-PEV/\sigma_g^2)^{1/2}$$

The predicted response to selection based on BLUP breeding values can be calculated from the accuracy of the estimated breeding values by:

$$R=ir_{IA}\sigma_A$$

where i is the intensity of selection, r_{IA} is the square root of the correlation between the breeding values and index values, or the accuracy and σ_A is the square root of the additive

genetic variance for the trait in question. Response to selection was calculated for a number of traits under different scenarios given an intensity of selection of 2.6 (selected proportion of 1%).

5-3 *Results*

5-3-a Structure of the data and variance components analysis

In total 4950 fish were measured for live end weight. Fin samples from all fish were sent for parentage analysis (Genomar AS). Out of these samples sent for parentage assignment, 29 fish were not assigned to any parent due to difficulty amplifying DNA or scoring errors/mutations. A further 24 fish were assigned to only the sire and to greater than one dam. 77 offspring could not be assigned to parents with a probability of correct assignment >0.99. Table 5-1 shows the pedigree structure of the data and statistics for live end weight.

Live end weight		
Number males	1661	
Number females	3289	
Mean-all fish	7.98	± 1.73
Mean-males	8.75	± 2.45
Mean-females	7.58	± 0.99
Number full-sib families	133	
Mean no. full-sibs/family	35	

Table 5-1 General statistics on live end weight and family structure for approximately 5000 fish. Standard deviation of averages also shown.

Considering the samples taken from harvested fish, the total number of samples sent for parentage assignment was ~8250. Eight microsatellites were used for parentage assignment, Table 5-2 shows the breakdown of assignment of these 8250 offspring to parents. A small percentage of offspring (2.4%) were assigned to more than one parental pair, and in most of these cases, the sire was the same in all assignments but the dam could have been > 1 possible dams. The overall assignment rate for all 8250 samples agrees well with the investigation of

part-real, part-hypothetical parental assignment in chapter three (Table 3-2). More females were measured and recorded than males in order to provide sufficient females for breeding. The average weight of both males and females was significantly ($p<0.001$) higher than average round weight of males and females in the processing plant.

	Result
% of offspring assigned to true parents and no others	**96%**
% of offspring assigned to >1 sire or dam	**3.4%**
% of offspring assigned to no parental pair	**0.6%**

Table 5-2 Assignment of 8250 offspring to 227 parents from 8 microsatellite loci

Heritability of Live end weight (LEW) was 0.29 ± 0.03 and 0.25 ± 0.04 under a univariate and multivariate model respectively. These estimates are similar in magnitude to those for whole body size traits at harvest shown in chapter four. Genetic correlations of LEW to other traits recorded at harvest are shown in Table 5-3. Correlation to gutted weight at harvest was high as would be expected (0.91 ± 0.02) and correlations were medium to high for all other body composition traits.

Trait	r_g	SE
gutted wgt	0.91	0.02
viscera wgt	0.77	0.04
fillet wgt	0.88	0.02
b_ave	0.28	0.06
m_ave	0.32	0.06
cantha	-0.04	0.09
fat	0.27	0.12
a_score	0.23	0.04
log_gsi	-0.10	0.06

Table 5-3 Genetic correlations of harvest traits to live end weight and their standard errors for LEW and other traits in model I

5-3-b Estimated breeding values for single trait and multi trait BLUP analysis.

Table 5-4 shows correlations between single trait EBVs and multitrait EBV for all traits in model I plus live end weight. The correlation is lowest for body composition traits: viscera weight and fillet weight (0.43 and 0.04 respectively). The difference in average EBVs between single and multitrait BLUP for these traits can be explained by the large difference in the additive genetic components of variation for these two traits in the univariate and multivariate analysis of variance (chapter four). Fat content and canthaxanthin content exhibit medium to high correlations between uni and multivariate EBVs (0.67 and 0.59 respectively), while all other traits show high correlations between the two types of analysis (>0.9).

Trait	Correlation
gutted wgt	0.95
viscera wgt	0.43
fillet wgt	0.04
fillet %	0.95
b_ave	0.94
m_ave	0.91
cantha	0.59
fat	0.67
a_score	0.97
log_gsi	0.99
LEW	0.96

Table 5-4 Correlation of EBVs from uni-and multivariate analysis for all traits in model 1. All correlations are statistically significant from zero ($p<0.001$) except fillet wgt.

5-3-c Accuracy of EBVs

Accuracy of estimated breeding values was compared under single and multitrait models. The multitrait model used here is composed of 7 traits which are the most economically important and the ones most likely to be included into a breeding goal for Atlantic salmon. The analysis was also performed to compare the accuracy of breeding values of harvest traits between live fish (for which no harvest measurements were taken) and harvested fish. This difference is the gain in accuracy obtained when a trait can be recorded on a breeding animal compared to taking the measurement only from full- and half-sibs. The results of average accuracy of estimated breeding values under these conditions are presented in Table 5-5 and Figure 5-2. For fat content and canthaxanthin content, the accuracy is significantly improved in the multi trait model. For all other traits, the increase in accuracy in multi trait BLUP is not significant.

When all fish included in the analysis are separated into fish from which harvest measurements were taken and live fish from which only live end weight was recorded, accuracies from harvested fish were consistently higher than from live fish. The difference was highest for fat content and canthaxanthin content. The distribution of accuracy of EBVs for a single trait, B_ave colour score, (as part of a multitrait model) for fish on which the measurement was taken and for live fish where only measurements from full- and half-sibs is shown in Figure 5-3. This figure demonstrates graphically that only approximately 12% of fish reach an accuracy of 0.7 or above when performance cannot be measured directly compared to 62% when this trait (B_ave colour score) can be measured directly on the individuals. The distribution is skewed for both datasets as a result of the smaller number of accuracies under ~ 0.4. This is because most families had at least 10 full sibs represented in the data set resulting in the unequal distribution of accuracies.

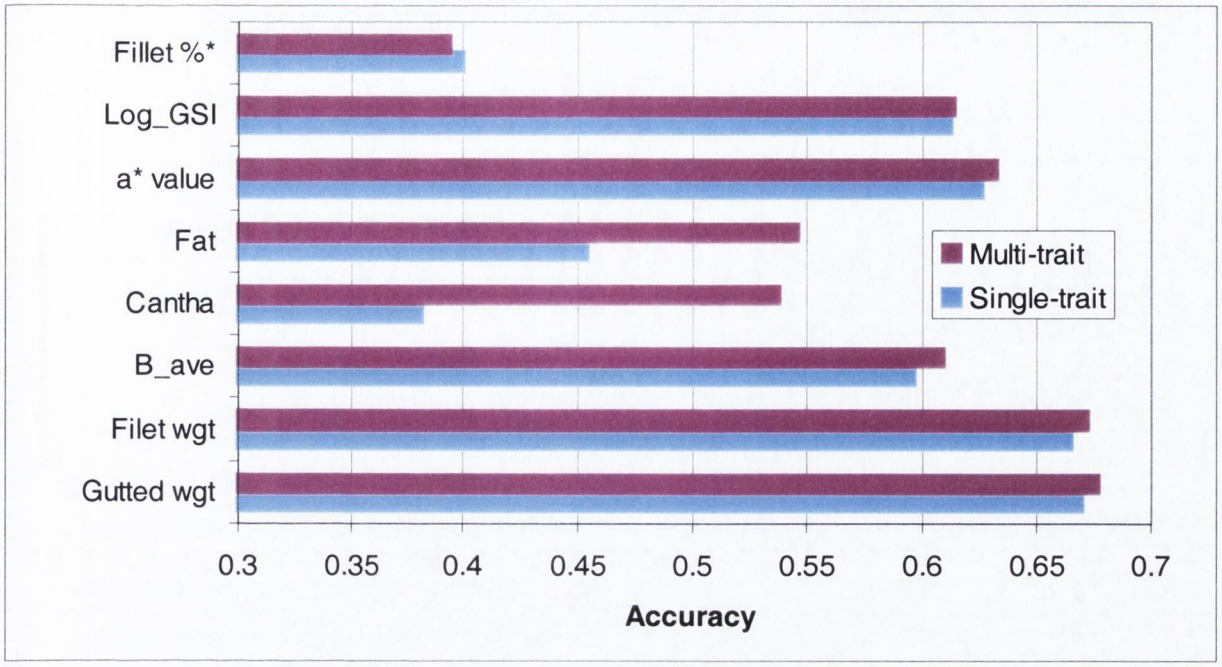


Figure 5-1 Accuracy of EBVs from single trait and multi trait BLUP

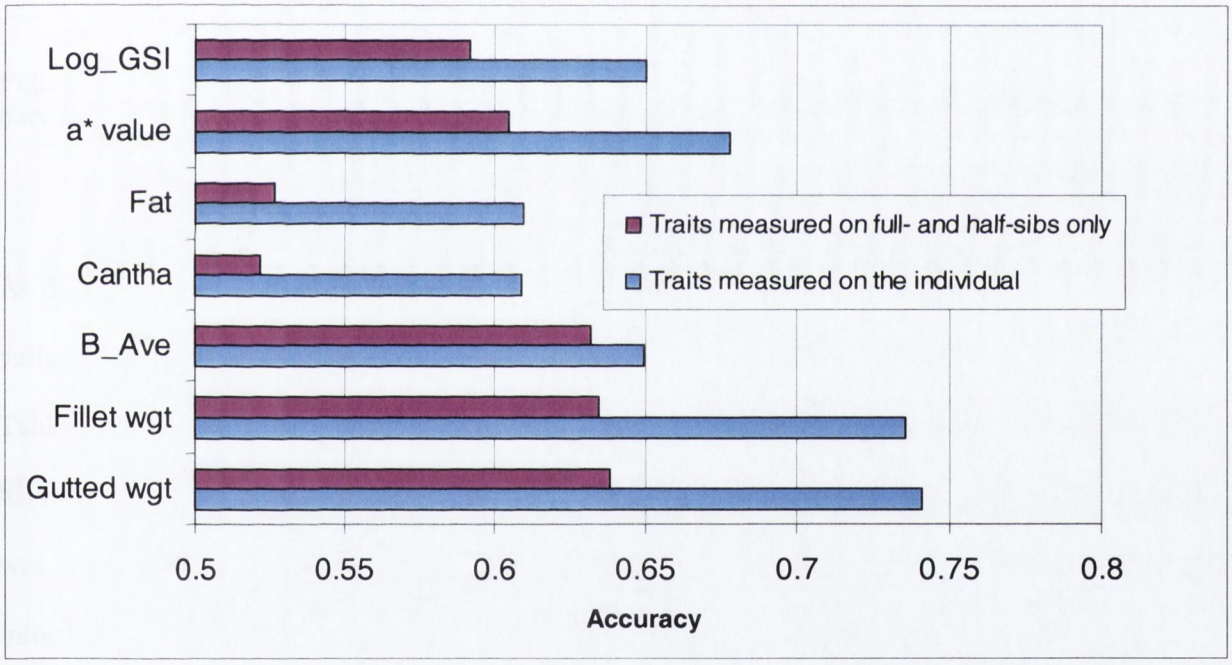


Figure 5-2 Accuracy of EBVs when traits are and are not measured directly on the animal

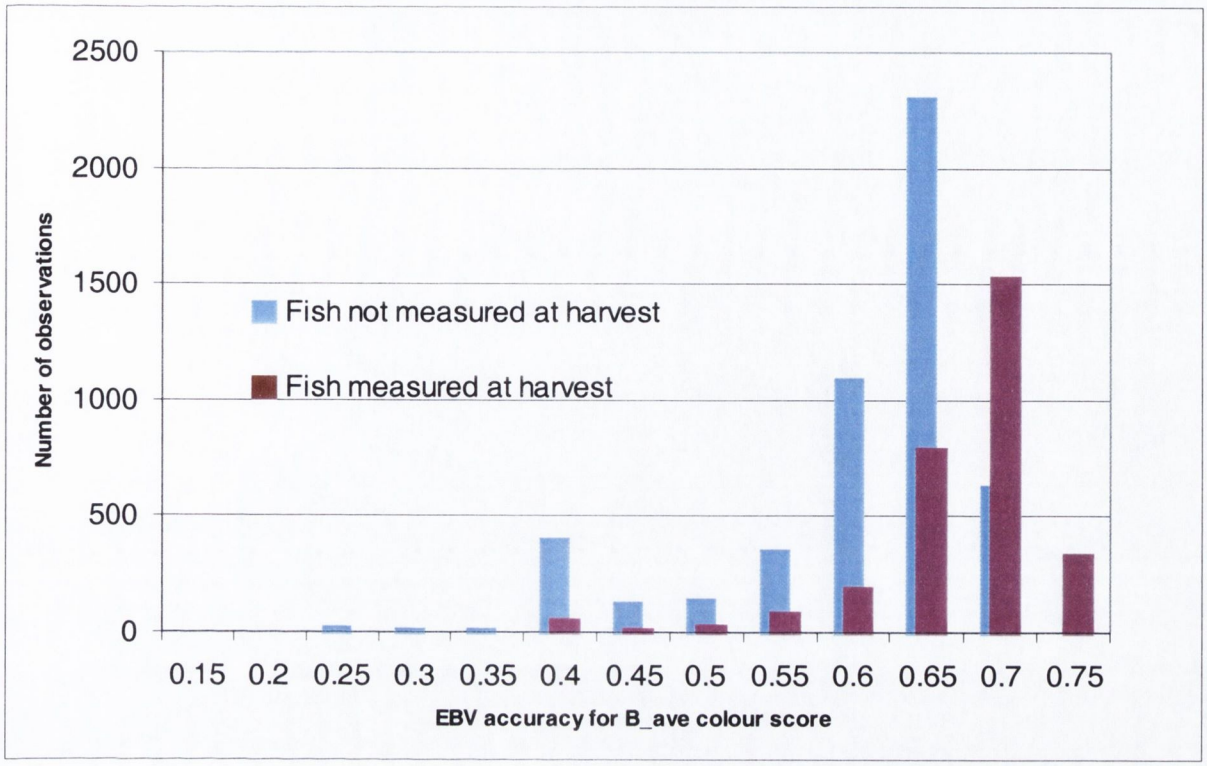


Figure 5-3 Distribution of EBV accuracies the trait B_ave colour score for fish measured at harvest and for fish where EBV was calculated from full and half sibs.

As fish were selected at random for measurement of harvest traits, there was considerable variation in the number of full- and half-sibs per family measured at harvest (see chapter four Table 4-4). The average accuracy was estimated for each size of half-sib group measured at harvest for three traits and this plot is shown in Figure 5-4. Canthaxanthin content in the flesh was measured for only 1000 fish (maximum 10 fish per full-sib family) after pedigree information was available. For the three traits, the EBV accuracy increases as the number of half-sibs per family measured increases until the number of half-sibs reaches about 20, after this point there is little further gain in accuracy in measuring more individuals. Average accuracy for gutted weight is higher than for the other traits due to the higher heritability. Canthaxanthin content was measured on between 5 to 10 full-sibs per family and within that range the average accuracy increased from 0.42 to 0.55.

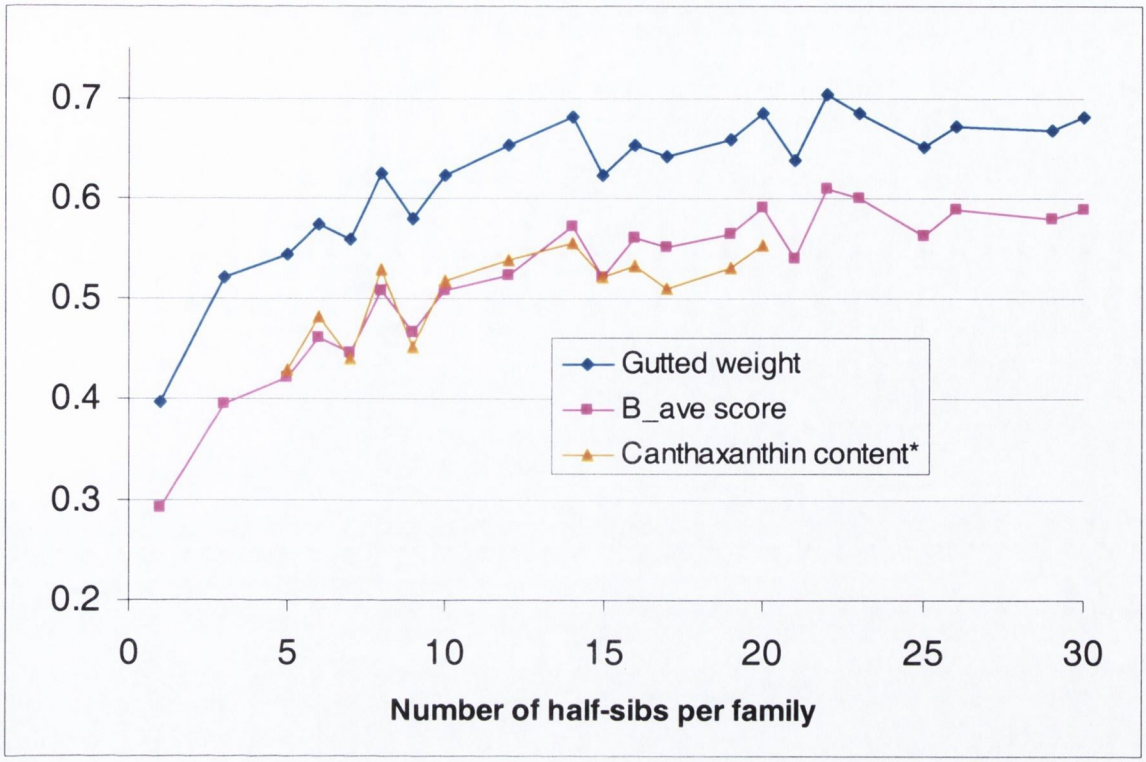


Figure 5-4 Plot of average accuracy against number of half-sibs per family
Standard error bars also shown
* for canthaxanthin content and fat content, only ~1000 fish were measured

5-3-d Predicted response to selection

The predicted response to selection, in the units of the three traits, is presented in Table 5-5 where the measurements are made on individuals themselves compared to when measurements can only be made on relatives. This table also shows the effect on response when increasing numbers of half-sibs are included in the analysis. As with accuracy of selection, the predicted response increases as the number of half-sibs measured increases. However, the relative gain becomes less after approximately 20 half-sibs have been measured. Without measurements on the individuals themselves the response to selection is always lower than when the individuals themselves are included in the analysis. The difference is greatest when lower numbers of half-sibs are measured. There is an expected difference in body size

at harvest of 324g when the trait of body size at harvest (or a correlated trait such as live end weight) is recorded directly on breeding individuals compared to when the measurement is taken from 5 half-sibs. This difference reduces to 188g when 40 half-sibs are measured (Table 5-5).

Trait	Gutted weight (kg)	B_ave score (units)	Cantha. content (%)
Traits measured on the individual:			
5 half-sibs measured	1.09	0.51	0.38
10 half-sibs measured	1.14 (4.6)	0.50 (-4)	0.42 (9.3)
15 half-sibs measured	1.15 (6.3)	0.49 (-6)	0.42 (11.3)
20 half-sibs measured	1.19 (9.3)	0.54 (4.2)	0.42 (11)
30 half-sibs measured	1.18 (8.7)	0.53 (3.4)	----------
40 half-sibs measured	1.21 (11.1)	0.56 (8.3)	----------
Traits only measured on full and half sibs:			
5 half-sibs measured	0.76	0.34	0.27
10 half-sibs measured	0.91 (20)	0.36 (5.8)	0.35 (27.2)
15 half-sibs measured	0.93 (22)	0.37 (10.3)	0.36 (30)
20 half-sibs measured	0.99 (29.6)	0.40 (17.2)	0.37 (35.2)
30 half-sibs measured	0.99 (30.3)	0.39 (16.4)	----------
40 half-sibs measured	1.02 (33.7)	0.42 (23.7)	----------

Table 5-5 Predicted response to selection for selection based on EBV and intensity of selection of 2.6 for 3 traits under different scenarios; traits measured on the individual and traits measured only on full and half sibs.
The percentage difference in response to selection compared to measuring 5 half sibs is shown in parenthesis.

5-4 Discussion

Although live end weight was significantly higher than round weight at harvest (3% and 5%, males and females respectively), this difference can be explained by a combination of the weight difference between full fed (live fish) and briefly starved (harvested fish) and the weight loss through bleeding (Einen and Thomassen M. S. 1998). The heritability of live end weight is of medium magnitude and similar to heritability estimates of whole body weight traits shown in chapter four. The high correlation between live end weight and gutted weight (0.91) would indicate that selection for gutted weight will result in a corresponding increase in live end weight. This does not eliminate the necessity of measuring live end weight, however, because as it is the only trait recorded on live fish , its measurement will increase connectedness in the data and hence the accuracy of breeding values for all other traits. The genetic correlation of LEW to other traits (Table 5-3) were in general not different from the genetic correlations of gutted weight to the same traits, indicating that the same genetic relationships are involved.

The correlation between univariate and multivariate BLUP was high for all traits where the additive and residual components of variance were of similar magnitude between the univariate and multivariate analysis. Where correlations are low, for fillet weight and viscera weight in particular, differences in ranking of broodstock for these traits will be expected. In analysing traits like these which are part of, or closely related to body weight, using a univariate model, the inclusion of body weight in the model absorbs much of the genetic variation for the trait analysed resulting in lower estimates of the additive genetic variance and therefore lower heritabilities. If, on the other hand, a parallel multivariate model is used, the maximum likelihood algorithm reallocates this variance to the other trait (which formally was

a covariate) resulting in higher estimates of the additive genetic variance and heritability. As a result of the differences in genetic parameters between the univariate and multivariate model, significant differences will exist in estimated breeding values between the two models as evidenced by the low correlations observed here for these traits. Estimated breeding values for fillet percent, however, do not change significantly between single and multivariate analysis (correlation = 0.95). In a multitrait model, therefore, body composition traits should be converted to percentages of body weight rather than using the actual weights.

The low correlations for a number of traits between univariate and multivariate BLUP is partly explained by the change in accuracy of EBVs between the two methods seen in Table 5-4. In general, multitrait BLUP increases the accuracy of selection but this is also dependent on the model and traits used as evidenced by the discussion above. The gain in accuracy is dependent on the absolute differences between the genetic and residual correlations between the traits. The larger the differences in these parameters, the greater the gain in accuracy from multitrait evaluation (Schaeffer et al. 1978; Thompson and Meyer 1986). For example, if all traits in the multivariate evaluation have approximately equal heritabilities, genetic and environmental correlations, then multivariate predictions are equivalent to those from a univariate analysis for each trait. Furthermore, traits with lower heritabilities benefit more when analysed with traits with higher heritabilities in a multitrait model as can be observed here with canthaxanthin and fat content in the fillet. The increase in accuracy in these cases results in better connections in the data, due to residual covariance between traits (Thompson and Meyer 1986). In addition, traits with less performance information, such as canthaxanthin and fat content, will benefit from information from correlated traits in a multivariate analysis more than traits where a lot of performance information is available (e.g. gutted weight).

For traits such as fillet percent, which exhibit low, insignificant genetic correlations to other traits in the model, accuracy will not be improved in the multivariate analysis as no genetic connectedness exits with traits of higher heritability. The increased accuracy for some traits in the multitrait model is a reflection of the difference in EBV between the single trait and multitrait models. To achieve maximum genetic gain, by optimising the ranking of animals in the overall index, multivariate models with all relevant traits should be used although considerably more costly in computer time.

The increase in accuracy of EBV estimation with the increase in the number of half-sibs measured is most pronounced for traits of lower heritability. As the number of measurements per animal increases, the prediction error variance decreases, while the variance of the predicted breeding values increases. After the number of half sibs measured reaches approximately 20 (approx. 10 full-sibs per family) further gains in accuracy are very small. Accuracies are a reflection of both the number of observations available and the genetic parameters involved (Thompson and Meyer 1986). As the selection program advances past the first generation, provided there are links between year classes, the amount of information from relatives available for each fish will increase and accuracy will quickly reach an optimum level.

Information from performance of the individual itself also results in higher accuracies and higher response to selection compared to when information from relatives is used to compute breeding values (Figure 5-3 and Table 5-5). This is because the relationship between the individual, for which we are computing the EBV, and the individuals for which we have measurements will always be less than 1, thereby reducing the correlation between true and estimated breeding values. The difference in accuracy under the different experimental

designs is responsible for the expected differences in response to selection for each trait (under the presumption that each trait is given the same selection pressure). The gain in response to selection from measuring quality traits directly on the individual, combined with the cost necessitated by the harvest of fish to measure traits, could warrant the development of new technology (e.g. colour probes, fat meters) to facilitate the measurement of fat and colour on live animals.

Chapter 6

Conclusions

6-1 *Molecular genetic variation in Atlantic salmon and application of microsatellites to aquaculture*

The investigation into levels of genetic variation between and within farmed and wild strains of Atlantic salmon demonstrates firstly the high levels of polymorphism detectable with microsatellite loci even within farmed strains of Atlantic salmon. In an aquaculture situation this technology has the potential to be of great use in monitoring levels of genetic variation between different stocks and also as a selective breeding program progresses. These results also show that average number of alleles is a more sensitive measure of differences in genetic variation between wild and farmed populations than overall heterozygosity, as previously observed in hatchery salmon and farmed rainbow trout (Butler and Cross 1996; Tessier et al. 1998). This is likely to be a result of the loss of low frequency alleles when new populations are founded from larger primary ones. This founder effect combined with subsequent artificial breeding practices results in the fact that the clearest differentiation between all strains is between farmed and wild rather than on geographical origin (Irish or Norwegian). Differences in genetic variation observed between farmed stocks may be a result of different breeding regimes and population sizes.

This study also demonstrates the feasibility of identifying parentage and estimating relatedness in a mixed aquaculture situation using microsatellite markers. This approach offers new alternatives for the design and implementation of fish breeding programs where identification of pedigree has traditionally been a hindrance. For mass selection programs, significant genetic progress can be achieved for traits which can be measured directly on the individual (Gjerde et al. 1996; Villanueva et al. 1996). Some restriction on inbreeding, however, must be applied in the absence of pedigree information (Villanueva et al. 2000). Relatedness estimates from microsatellites genotypes could be used on top breeders to avoid

crossing related individuals. As was demonstrated here, the probability of detecting full and half sibs with 8 microsatellite loci is very high.

Although the direct application of DNA markers to fish breeding has been investigated and suggested by a number of authors (Doyle and Herbinger 1995; O'Reilly and Wright 1995; de Leon et al. 1998; Norris et al. 2000), this is the first example of the application of DNA markers, for the purposes of identifying pedigree, to a large scale, commercial breeding program. In the present study, out of over 8000 samples sent for parentage assignment, 96% were assigned to one parental pair with a probability of correct assignment greater than 99%. Less than 1% of samples were not assigned to any parent and the remainder of samples could only be assigned to one parent, usually the sire as a result of the hierarchical mating system of one sire and two dams. These figure agrees remarkable well with the estimate of assignment of offspring in the small study described in chapter three. Estimation of genetic parameters in the breeding program described here should, on the basis of previous research (Herbinger et al. 1999b), be less biased as a result of the elimination of the family effect of rearing sibs together, which is confounded with genetic effects. However, a direct comparison of genetic parameters on this basis is not reasonable due to the variability of genetic variation parameters which may result from genetic differences among strains/populations of Atlantic salmon, different environments, sampling error or the way in which the measurements were taken (Gjedrem 1983; Gjerde and Gjedrem 1984; Gjerde 1986).

6-2 Quantitative genetic variation for economically important traits

Despite the inherent variability of genetic parameters, in general, the results presented here agree with the few studies in harvest traits already carried out for Atlantic salmon (Gjerde et

al. 1994 Rye and Gjerde 1996; Rye and Refstie 1995). The importance of defining the correct model for many traits, particularly body composition traits, has been illustrated here. There is clearly a complex interaction between growth rate and maturation status on many harvest traits. The measurements described here were performed over one time period in the growth phase. To fully understand the interaction of growth and maturity, harvest traits need to be investigated over a longer time period of the growth cycle or, on many year classes of fish, with genetic links between them, reared in different environments and harvested at different times. Also measurements of maturity status at harvest time should be combined with measurements of maturity before harvest time and in broodstock after harvest time. A better understanding of the genetic and physiological relationships between these traits will help to optimise selection strategy and management within a selection programme.

Interpretation of genetic parameters for body composition traits (fillet weight, viscera weight) should be treated with some caution due to the dependence of these traits on body size. Despite the correction for body size at harvest, genetic components of variation are still inflated due to the confounding genetic and environmental effects. Furthermore, selection for decreased gutting waste may result in higher fillet fat (as the distribution of fat from the viscera to the flesh is partly genetically determined), and may result in impaired physiological processes as the liver, kidney and intestine weights are reduced.

The use of the multivariate model for variance component estimation did not differ significantly for most traits, with the exception of body composition traits, with the univariate estimates of the adjusted models. However, apart from allowing for the estimation of correlations between traits, multivariate analysis may improve the accuracy of estimation of variance components by the additional information correlated characters provide to each

other. Balancing the advantages of multivariate analysis are computational difficulties that increase with the numbers of characters under consideration. For the data set of one year of measurements (~10,000) and pedigrees, computation requirement is not an issue of practical concern. However, a multivariate model with 6 traits and 30,000 animals would result in ~1.5 million mixed model equations to be solved requiring 20 mega bytes of memory. After a number of generations, computer time and capability may become a factor in choosing univariate over multivariate analysis of variance components.

Although splitting a dataset into males and females may help elucidate the processes of maturation and their effects on other traits, for variance component and breeding value estimation, it is better to include variance components estimated from all data in order to obtain a more generalized and robust model. Also dividing the data set will also result in higher standard errors of the estimates as the number of individuals within each fixed effect class is now halved.

While there have been a number of investigations in Atlantic salmon and rainbow trout, into the phenotypic relationship between fillet colour measured by different methods (Christiansen et al. 1995; Warriss 1996; Choubert et al. 1997; Hatlen et al. 1998), this is the first reported study on the genetic parameters underlying these traits. The importance, to the industry, of obtaining a reliable, accurate and practical method of measuring fillet colour, which reflects the genetic variation for both pigment retention and perceived colour cannot be understated. The Roche Salmofan^{TM} (Hoffmann-La Roche Ltd, Basel, Switzerland) has become the industry standard and any measurements/improvements in a selection program should be in reference to this. Visually and colorimetrically assessed colour both demonstrate significant components of additive genetic variation implying that this trait could be improved by BLUP

selection. Surprisingly, given that perceived colour must be dependant on the pigment content in the flesh, carotenoid retention in the flesh shows significantly lower heritability. This result would seem to suggest that retention of pigment is under the control of different genes, and under less genetic influence, compared to perceived colour. It may be that fillet colour is more influenced by factors other than pigment retained in the flesh. (Johnston et al. 2000a) demonstrated a significant positive relationship between muscle fibre density (number mm^{-2} white muscle cross sectional area) and visualized colour in two strains of Atlantic salmon. In fact, fibre density was shown to explain between 33% and 44% of the total variation for visually assessed colour. Since patterns of muscle fibre recruitment and density have been shown to differ between strains of farmed salmon (Johnston et al. 2000b) this trait must be under some genetic control. Muscle fibre density was also shown to be related to other flesh quality traits such as gaping, texture and lipid content of the fillet (Johnston et al. 2000a). It may be that the trait of perceived flesh colour is composed of genes governing both pigment retention and muscle fibre density/recruitment. In this case, the measurement and inclusion of muscle fibre density in a selective breeding program will allow for more efficient selection of flesh quality traits, particularly colour, in Atlantic salmon.

Another possible explanation for the low heritability for carotenoid content could be the low levels of phenotypic variation for this trait, despite the low content of carotenoid in the diet. A further reduction in dietary carotenoid content may be required to observe the inherent variation in pigment retention. Where fillet colour is part of a breeding goal, visually assessed colour, either by human eye or by camera, is a better selection candidate for both the reason given above and because perceived colour is the only colour trait measured by processors and retailers. Both colorimetric and subjective colour scores are open to bias (subjective, by the scorer) or error (colorimetric, from the whiteness of intermuscular fat) which can be

eliminated to a certain extent but not completely. Scoring colour accurately (when compared to a camera measure) is a very particular skill, and not many people have the ability to score thousands of fillets consistently and in an unbiased manner. The two scorers used in this study show very high correlations at the genetic level to each other and to colorimetric measurements. People perceive colour in different ways, for example, based on the results presented here, one scorer was perceiving the redness of the fillet better (more highly correlated with the camera 'redness') while the other scorer was perceiving the lightness of the colour better. In general, visually assessed colour is the most important colour trait, as it is on this basis that fish are classified. Although instrumentally measured colour is a useful measure to include in a breeding objective, particularly as it could partially compensate for the bias of the subjective scorer, it should never be completely substituted for colour perceived by the human eye.

Other than live end weight (which has a genetic correlation to round weight of 0.90) no other economically important harvest trait can, at present, be accurately recorded on live fish. The high to medium genetic correlation of this and other body size traits to harvest quality traits such as fat and colour will allow for efficient selection of quality traits on live fish. The accuracy of this selection could be improved by the ability to measure these traits directly on the breeding individuals themselves. Not only would this improve the accuracy of selection, evidenced by the increased accuracy of the estimated breeding values, but it would also eliminate the need to slaughter large numbers of fish in order to take the measurements. The financial implications of direct measurements on live fish may encourage the development of such tools within the industry.

The use of a multivariate analysis of estimated breeding values has a significant effect on traits where the heritability is low as a result of genetic correlations to other traits in the evaluation. As a result, the breeding values for fat and carotenoid content in a univariate analysis may not rank animals the same as breeding values computed in a multivariate analysis. An optimal selection strategy will include all economically important traits in an index of total merit.

In conclusion, given the magnitude and levels of genetic variation for the traits under investigation, a selection strategy for improved; growth, late maturation and quality traits should be feasible at reasonable selection intensities and without compromising on inbreeding. The positive relationship between body size and fat content will demand that restrictions be applied to increases in growth rate if a correlated increase in fat content is to be avoided. Selection for better coloration in the fillet is compatible with selection for body size and should yield genetic progress for this trait. Selection for larger fish should be possible without sacrificing an increase in grilsing rates if selection for smaller gonad size is also included in the index. However, this relationship and the relationship between gonad development and grilsing require further investigation throughout the growth phase of the fish in the sea.

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