Doctoral Thesis May 2014

Breeding Design for Atlantic Cod

(Gadus morhua L.)

Theódór Kristjánsson

Main supervisor: Þorvaldur Árnasson
Co-supervisors: Ágúst Sigurðsson and Sigurður Guðjónsson

Agricultural University of Iceland
Faculty of Land and Animal Resources

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Clarification of contribution

I hereby declare that the writing of the following thesis and the four accompanying papers is my work, done under the supervision and with assistance of my supervisors Dr. Þorvaldur Árnason, Dr. Ágúst Sigurðsson and Dr. Sigurður Guðjónsson.

The contribution of Theódór Kristjánsson to the papers included in this thesis was as follows:

Paper I: Kristjánsson collected all data which are presented in the paper. The data were collected when forming the base population for a cod-breeding program in Iceland 2004. Eggs from wild brood fish were collected from five locations off the Icelandic shore. Eggs were hatched at the Marine Research Institute (MRI) and later reared in cages off the Eastern coast of Iceland. Kristjánsson collected all data and wrote the manuscript and was responsible for correspondence with the scientific journal.

Paper II: Kristjánsson collected all data which are presented in the paper. The data were collected from three year classes of Atlantic cod (*Gadus morhua)* throughout their rearing off the Eastern coast of Iceland from 2004 – 2011. The growth and status of maturity were recorded during the rearing. Kristjánsson was responsible for carrying out the genetic analyses. Kristjánsson interpreted the results together with Árnason. Kristjánsson wrote the manuscript and corresponded with the scientific journal.

Paper III: Kristjánsson collected all data which are presented in the paper. Genetic parameters were estimated of economically important traits in Atlantic cod reared off the Eastern and Western coasts of Iceland. Moreover, genotype by environment interaction (G×E) among the two rearing locations was estimated. The cod was reared in cages from 2004 – 2005. Kristjánsson was responsible for carrying out the genetic analyses. Kristjánsson interpreted the results together with Árnason. Kristjánsson wrote the manuscript and corresponded with the scientific journal.

Paper IV: The simulation study was the prime responsibility of Kristjánsson. All programming, design and analysis of the simulation results were done by Kristjánsson. Kristjánsson interpreted the results together with Árnason. Kristjánsson wrote the manuscript and corresponded with the scientific journal.

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Theódór Kristjánsson

Abstract

Kristjánsson, T. 2014. *Breeding design for Atlantic cod (*Gadus morhua *L.)*. Doctoral thesis

This thesis summarizes the development of the Icelandic family breeding program for Atlantic cod (*Gadus morhua* L.*)*. The development of the base population began in the year 2003 when eggs were collected from wild brood fish from different spawning sites around Iceland. Approximately 350 viable families have been established and were later used to form the base population for the cod family selection program. Comparison of growth and early maturity of cod originating off the Northern and Southern coast of Iceland did not show significant differences.

The eggs were hatched at the Marine Research Institute at Staður in Grindavík and reared in land based facilities. At the age of 12 months the juveniles were transferred to sea cages in Berufjörður at the Eastern coast and Ísafjarðardjúp at the Western coast of Iceland. Throughout the rearing an estimation of genetic (co)variation of economically important traits and genotype by environment interactions (G×E) among the two rearing locations were explored.

Atlantic cod in Iceland shows high additive genetic variance for body weight (h^2 = 0.34), suggesting that selection for increased body weight is likely to be successful similar to what has been shown in Atlantic salmon (*Salmon salar* L.) farming. Genetic correlation between body weight in Ísafjarðardjúp and Berufjörður was high *(r_G = 0.95)*, which indicates low G×E for the trait. However, low heritability for process yields such at fillet yields (h^2 = 0.04) and the hepatosomatic index (h^2 = 0.061) is less promising for obtaining genetic improvement.

The strong phenotypic and genetic correlation (r_G = *0.*9) in body weight and maturation suggests that an increased growth rate will consequently lead to a higher proportion of mature individuals in the population. As a consequence, genetic manipulations to simultaneously increase growth and delay maturation may present a challenge.

DNA profiling was used at the start of the cod breeding program for constructing pedigree. The result revealed highly imbalanced representation of individual families. The survival rate among full-sib families in the early stages was highly skewed. In this study, the effect of an imbalanced family structure on breeding was explored. A stochastic model was used to simulate genetic gain in an imbalanced versus a balanced family structure. Balanced design had a significantly higher gain for normally distributed traits and binary trait for all

comparisons. The accuracy of estimated breeding values was not significantly different between balanced and imbalanced design. However the estimated breeding values were significantly higher for selected parentage fish in balanced design.

Keywords: Atlantic cod, *Gadus morhua*, base population, heritability estimates, G×E, simulation

Ágrip

Theódór Kristjánsson 2014. *Kynbótaskipulag fyrir eldisþorsk* (Gadus morhua *L.*)

Ritgerð þessi er samantekt um upphaf þorskakynbóta á Íslandi frá söfnun á erfðaefni í grunnstofn og síðar til mats á erfðastuðlum á mikilvægum eiginleikum í þorskeldi. Söfnun í grunnstofn stóð yfir á árunum 2003 – 2005 þar sem hrognum var safnað úr villtum þorski frá ellefu hrygningarstöðvum í kringum landið. Alls voru búnar til 350 fjölskyldur sem voru notaðar í áframhaldandi kynbætur. Samanburður á vexti og tíðni kynþroska í eldi á þorski, sem átti uppruna sinn frá hrygningarstöðvum fyrir Norður- og Suðurlandi, sýndi ekki marktækan mun í þessum eiginleikum.

Hrognin voru klakin og fyrstu mánuðir eldisins fóru fram hjá Tilraunastöð Hafrannsóknastofnunarinnar að Stað við Grindavík. Eftir 12 mánaða eldi frá klaki voru seiðin flutt í kvíar í Ísafjarðardjúpi og í Berufirði. Gerðar voru reglulegar mælingar á þorskinum allt frá útsetningu til slátrunar. Mat á arfgengi mikilvægra eiginleika sýndi að arfgengi vaxtar er hátt (h^2 = 0.34) og líklegt má telja að kynbætur fyrir auknum vaxtarhraða muni skila verulegum árangri líkt og raunin hefur verið í laxeldi. Samspil erfða og umhverfis fyrir vöxt í Berufirði og Ísafjarðardjúpi var lágt (r_G = *0.95*) og ekki líklegt til að hafa áhrif á val á einstaklingum til eldis í framtíðinni. Hins vegar var arfgengi slátureiginleika eins og flakanýtingar (h^2 = 0.04) og lifrahlutfalls (h^2 = 0.061) mjög lágt og ekki líklegt að kynbætur muni skila árangri fyrir þessa eiginleika.

Mat á arfgengi á tíðni kynþroska á fyrsta ári í sjókvíum sýndi að tíðni kynþroska hefur mjög háa erfðafylgni (r_G = *0.9*) við vöxt og sýna niðurstöður að erfitt verður að velja samtímis fyrir lækkun á tíðni kynþroska og auknum vexti. Þróa þarf aðrar aðferðir til að koma í veg fyrir kynþroska á fyrsta ári í sjókvíum.

Í upphafi þorskakynbóta var notast við DNA ætternisgreiningu. Niðurstöður úr ætternisgreiningum og síðar mat á lifun á seiðastigi þorsklirfa leiddu í ljós að lifun er mjög mismunandi á seiðastigi. Áhrif af mismunandi lifun á milli fjölskyldna á kynbótaframför voru prófuð með hermilíkani. Niðurstöður sýndu að verulega dró úr kynbótaframför þegar valið var úr fjölskyldukerfi með mjög mismunandi lifun.

Lykilorð: Þorskur, *Gadus morhua*, grunnstofn, erfðastuðlar, G×E, hermun

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Lastly I would to like to thank the Agricultural Productivity Fund and Westfjords Growth Agreement for their financial support, which helped to cover travel costs.

Contents

List of Original Papers

The present thesis is based on the following publications, which will be referred to by their Roman numerals.

Kristjánsson, T. 2013. Comparison of growth in Atlantic cod (*Gadus morhua*) originating from the northern and southern coast of Iceland reared under common conditions. *Fishery Research,* 139, 105-109.

Kristjánsson, T., Arnason, T. 2014. Strong phenotypic and genetic correlation between size and first maturity in Atlantic cod *Gadus morhua* L. reared in commercial conditions. *Aquaculture Research.*1-9. DOI: 10.1111/are.12377.

Kristjánsson, T., Arnason, T. 2014. Heritability of economically important traits in Atlantic Cod *Gadus morhua* L. *Aquaculture Research* (accepted with minor changes April 2014).

Kristjánsson, T., Arnason, T. 2014. Effects of imbalanced family structure and DNA profiling on genetic gain in Atlantic cod *Gadus morhua* L. breeding program. *Aquaculture Research* (submitted)**.**

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List of abbreviations

BLUP	Best Linear Unbiased Prediction
CF	Condition Factor
CnGV	Counter-Gradient Variation (growth)
dPH	Days at post hatching
EBV	Estimated breeding value
F_{ST}	Population differentiation
G×E	Genotype by environment interaction
HSI	Hepato Somatic Index
MCMC	Makrov Chain Monte Carlo
MAS	Marker Assisted Selection
PCR	Polymerase Chain Reaction
QTL	Quantitative Trait Loci
REML	Restricted Maximum Likelihood
SE	Standard Error
SNP	Single Nucleotide Polymorphism
YC	Year Class

1 Introduction

The Atlantic cod (*Gadus morhua* L.*)* is one of the most important cold-water marine teleosts for commercial fisheries (Norberg *et al*., 2004). Atlantic cod is an important fish stock caught all around Iceland and throughout the year. Long-term catching of cod has varied from 180,000 tons to 470,000 tons per year in the last 30 years, with an average of close to 290,000 tones. In the past few years the catch has been decreasing and the lowest quota was issued for the fishing year 2008/09 or only 130,000 tons. Since then the quota has increased to approximately 200,000 tons. Cod is by far the most economically important fish stock in Iceland. In recent years processed cod has accounted for 35-40% of the total seafood export (Statistics Iceland (http://www.statice.is)).

Cod fisheries in the Northern Atlantic Ocean go back for centuries. Decline of wild stocks has resulted in lower catches in the Atlantic Ocean in Northern Europe. Aquaculture is one way to compensate for lower catches. Presently, the farm production cost is even higher than the market price for the wild cod. The overall production cost of farmed cod is influenced by many factors, such as low survival of the juveniles, negative effects of maturity and poor growth, which, taken together, increase the production cost. In Northern Europe lower growth rate caused by cold seawater during the winter further adds to the overall production cost. It takes, for example, approximately 32 months for cod to grow from 150 grams to 4 kg off the North coast of Iceland but only approximately 26 months off the South coast. Moreover, a high percentage of the cod presumably matures within that time period (Kolbeinshavn *et al*., 2012). In comparison, farmed Atlantic salmon (*Salmon salar* L.) would have gained the same weight in 14 months off the South Icelandic coast. Higher temperature and increased feeding during the summer also increases the percentage of maturity. Cod in culture generally matures between one and two years of age. Mature cod ceases feeding and also loses body weight. The females can lose up to 30% of total body weight and males up to 14%. In both, the cessation of growth and the loss of weight means considerable increase in feed conversion rate, thus resulting in higher production cost (Fordham & Trippel, 1999).

The quality of farmed cod for different types of processing (fresh, for freezing and salting) can be determined by various measurements. Texture and color can be measured by sensory evaluation and by instrumental methods. Market surveys show that consumers consider farmed cod as good as wild cod. There is relatively little difference in the chemical composition of farmed cod and wild cod. On the other hand, there are more differences in physical properties of the flesh, especially with regard to lower pH-value of the flesh of

farmed cod compared to wild cod. Lower pH can cause more risk of gaping of fillets and less water binding abilities and tougher and drier texture after cooking (Love, 1980). Farmed cod has higher condition factor (ratio between length and weight) and larger liver compared to wild cod but by feeding wild caught cod with feed of lower energy content the liver proportion can be reduced (Karlsen *et al*., 2006). Farmed cod shows higher fillet yield and value in processing compared to wild cod.

Potential impact of cod farming may be especially important in the rural communities in Iceland. In the last decade the fishing of cod has reduced dramatically. This has left many rural areas vulnerable, as their income is heavily dependent on the fishing industry whether it be capturing, processing, marketing or services. Cod is potentially a good candidate for aquaculture and work provider for the rural community. Cod may in many ways be more relevant to the rural community than other marine species because of long fishing tradition and its economic importance in the last centuries. This in turn has led to very good fishery infrastructure and highly skilled workers in cod processing. In these areas farming of cod could help to replace the reduction of the wild catch. In addition, cod farming provides work in a wide range of expert fields, such as biology, engineering and various services needed by aquaculture companies. It is also important that the vision in cod farming development is sustainable, diverse, competitive and economically viable. Central to this development is the environmental sustainability of the industry. Cod farming would fit directly into such an assessment.

One way to increase productivity in cod farming is through selective breeding, whereas life history traits are most commonly improved in a breeding program. Growth is universally accepted as one of the first, and most important, traits chosen for improvement (Gjedrem, 1983; Gjerde, 1986; Kinghorn, 1983). Moreover, traits like age at maturity, disease resistance and flesh quality are quite often equally important.

In recent years the importance of selective breeding programs in aquaculture has been demonstrated. The gap between demand for fish and supply is widening due to a growing human population and decline in production from capture fisheries. Selective breeding has contributed greatly to the steadily increasing productivity in aquaculture.

The role of breeding in conventional agriculture has demonstrated tremendous increases in productivity over many decades (Eknath *et al*., 1991). This has also been tested in aquaculture. Through genetic improvement, and many other developments, the Atlantic salmon industry has grown to be the most significant farmed fish industry in Europe. Growth

rate has almost doubled in a 28-year period (seven generations) and had 25% better feed conversion efficiency (Gjøen & Gjerde, 1997).

First estimates of genetic variation in cod show that there appears to be substantial amount of additive genetic variation for body weight (Gjerde *et al*., 2004). This suggests that selection for increased body weight is likely to be successful.

1.1 Plans for a breeding program

Before we can plan a breeding program the knowledge about the biology and genetics of the animal in question needs to be established. The objective of the breeding program needs to be clear and what can be expected from genetic improvement. This can be summarized as follows:

1. Creation of a base population where animals are collected from wild populations
2. Reproduction of the animals must be known in order to close the life cycle
3. The breeding goal must be defined
4. Genetic variation associated with the selected trait and its heritability must be determined
5. Breeding method must be defined
6. Avoid inbreeding to maintain sustainability
7. Predict genetic improvement
8. Follow the improvement *in situ*

1.2 Base population

The first step to start a breeding program is to collect the genetic material from a wild population, which forms the base population. A base population is a group of animals with unknown parents in genetic evaluations. In Norway, genetic material for Atlantic salmon was sampled from 40 river strains (Gjedrem *et al*., 1991) and no restriction was placed on strain contribution during the first generations. This resulted in an initial selection between strains. An alternative is to primarily mate animals from different strains before starting selection and to apply low selection intensity during the first generations of selection as has been done for Nile tilapia (*Oreochromis niloticus* L.) in the Philippines (Bentsen *et al*., 1997). This may secure the maintenance of a broad genetic variability that would allow long-term selection response and stepwise inclusion of new traits in the breeding goal. In fish breeding, limited emphasis so far has been on the effect of the design of the base population on the magnitude

and variability of the long-term selection response and inbreeding. Response to short-term selection of a few generations depends on the intensity and accuracy of selection in these generations. High accuracy gives high response in the short-term but because of co-selection of family members it also gives a high rate of inbreeding, and consequently reduces the selection response in the long-term. Thus, methods achieving maximum short-term response do not necessarily optimize long-term response. Response to long-term selection also depends on how much genetic variability is maintained and how much inbreeding depression occurs over generations and therefore on the effective population size (Robertson, 1960). These are conflicting aims. Increasing selection intensity, thereby reducing the number of parents selected for given numbers recorded, reduces the effective population size. The use of family information to increase the accuracy of selection, thereby increasing co-selection of relatives within and across generations and variation in family size, also reduces effective population size. There has been extensive work on methods to select and mate animals to maximize the response condition on effective population size or rate of inbreeding, and algorithms have been developed (Brisbane & Gibson, 1995; Caballero *et al*., 1996; Grundy *et al*., 1998). These calculations are generally based on the infinitesimal model and do not include mutational effects. It is relatively easy to give figures for the cost of inbreeding depression while the cost of reduced variation is much harder to predict. Hence, in all analyses considering short and long-term responses some, perhaps rather arbitrary, relationship between short and long-term benefits has to be specified. Long-term response cannot be discussed without a consideration of time horizon. We do not know how demands for animal production will change, and we do not know what new technologies will come along. For example, a genetic conservation program in cart-horses would have had no impact in tractor age (Hill, 2000).

1.3 Genetic resources from wild populations

The Atlantic cod is now believed to be genetically diverse. Considerable effort has been made in the past to study the possible subdivision of cod populations in the North Atlantic and around Iceland. This has included the application of a variety of stock identification techniques, both genetic and morphological, which have produced contradictory results (Arnason *et al*., 2009; Jónsdóttir *et al*., 2001; Jónsdóttir *et al*., 1999; Pampoulie *et al*., 2006).

Age and size at sexual maturity are highly variable among cod stocks in the North Atlantic (Begg *et al*., 1999; Cardinale & Modin, 1999; Godø & Moksness, 1987; Holdway & Beamish, 1985; Marteinsdóttir & Begg, 2002; Trippel *et al*., 1997). In Iceland, a significant

sexual, spatial and temporal variation in age and size at maturity has been demonstrated among cod around the country. The greatest differences were displayed between cod at the North coast and South coast of Iceland. This variation appeared to be strongly affected by growth and condition (Marteinsdóttir & Begg, 2002). Faster growing cod in waters off the South coast of Iceland attained sexual maturity at a smaller size and younger age than slower growing cod off the North coast. Moreover, the influence of condition on maturity was more apparent for cod in waters off the North coast, particularly for size at maturity where distinct differences were detected between cod of variable condition. The influence of condition may be more significant for cod in the north where waters are cooler and more variable, and hence less conducive to growth and survival than waters in the south that tend to be on average at least 4°C warmer (Malmberg & Kristmannsson, 1992).

Although variations in age and size at maturation often reflect phenotypic plasticity in response to variations or changes in environmental conditions, the influence of the genetic composition of the population is also likely to have significant effects (Rijnsdorp, 1993). In fact, genetic variation in age at maturity has been found to be quite high among *Salmonidae*. For example, heritability for age at maturity was estimated around 0.3 in Atlantic salmon (Gjedrem, 1983).

1.4 Estimation of genetic variation

In animal BLUP (Best Linear Unbiased Prediction) models, if all genetic relationships are included back to the base population, and if all data are used in the analysis that were included in the selection decisions, Restricted Maximum Likelihood (REML) will provide estimates of the variance for the base population (Sorensen & Kennedy, 1984). It should be noted that including relationships does not only account for gametic disequilibrium due to selection Bulmer effect (Bulmer, 1971), but also for reduction of variance due to inbreeding and the buildup of covariance of related animals. It is debated whether it is possible to include relationships and data since the start of selection. The conclusion is that estimated genetic variances can be expected to be somehow biased by selection, generally more if more of the selection history is omitted from the analysis (Van der Werf & de Boer, 1990).

In a cod breeding program estimation of genetic parameters requires a full pedigree and rearing of a large number of fish from known families. Usually physical tagging has been used as an identification of individuals in a rearing unit, where each family has been held separately until the fish has reached the size of tagging. This requires large rearing facilities

with many rearing units. The increased availability of DNA markers has, however, revolutionized the possibilities of individual identification. For parental assignment microsatellites are the most commonly used genetic markers. The biggest advantage of this new method is the improved use of space and labor since the families of cod do not have to be kept in separate units after the hatching of ova.

DNA-profiling for pedigree assessment facilitates pooling families at fertilisation or shortly thereafter and thus eliminates the need for investment in costly multi-tank facilities for separate rearing of the families and minimizes possible common environmental effects (Doyle & Herbinger, 1994). However, the use of DNA profiling in fish breeding programmes carries some risks related to the representation of individual families. If the survival rate among full-sib families in the early stages is low and variable the resulting population structure may be imbalanced, making effective genetic improvement difficult.

The use of DNA-typing allows a full identification of any offspring early or late in the life cycle without the physical tagging. Allelic variation in offspring and parents is analysed and compared and subsequently individuals can be traced back to its original pair mating (family). During creation (full and half sibs) of the families a tissue sample has to be collected from every parent fish and stored for later analysis. A record of the mating scheme needs to be kept. DNA from the parents is then isolated and analysed for a set of DNA markers. The offspring from different pair mating (family) can then be reared collectively and traced back to its original pair mating (family) at any time by taking tissue sample and applying DNA-typing. This will enable the geneticist to make a full pedigree for the whole population necessary to evaluate the magnitude of genetic variation in valuable life history traits.

In order to follow individual cod in the whole population from early life (30 grams) to harvest, each individual cod in the project needs to be physically tagged with an electronic tag. This ensures that each individual only needs to be DNA-pedigree typed once. Thus, reading the electronic tag at each measurement one can monitor the whole life history.

The accuracy of parental assignment relies heavily on the reliability of marker information used in relationship inference. The exclusion of a given relationship because of its incompatibility with the observed genotypes is legitimate only when the genetic data are perfect. Unfortunately, however, genotype errors can be quite common in practice and are difficult to avoid. Even in the most favourable situation, where a large amount of high-quality DNA is available for repeated genotyping under optimised PCR (Polymerase Chain Reaction) conditions, relationship inference can still suffer from mutations that may occur at a rate as high as 1.4×10^{-2} for microsatellites (Talbot *et al*., 1995). In practice, typing errors may occur

frequently, especially when repeated typing is limited or even impossible due to the constraint of DNA amount or typing cost, when the quality of DNA is poor and/or PCR is not optimised. Such typing errors and mutations could have a devastating effect on relationship inference if they are not accounted for. A single scoring error (mutation) at just one locus of an individual may lead to its exclusion from being assigned the correct relationship with others no matter how many other loci of the individual are correctly scored. Some pair-wise approaches have been developed to account for typing errors and mutations in inferring parentage (Neff *et al*., 2002; San Cristobal & Chevalet, 1997) and other relationships (Douglas *et al*., 2000; Epstein *et al*., 2000). Several empirical studies verified the importance of typing errors in affecting parentage determinations (Blouin *et al*., 1996).

1.5 Genetic improvement

Present breeding programs in aquaculture are designed mainly on the basis of general knowledge from quantitative genetics theory and experiences from selection programs in traditional farmed animals. Due to the much higher fecundity in aquatic species, optimal designs for these species would be expected to differ from optimal designs for terrestrial animals. Therefore, the need for more knowledge on optimal designs of a selective breeding program for Atlantic cod is urgent. Furthermore, a methodology to determine the pedigree of individuals by using DNA-fingerprints is now available at a reasonable cost. Therefore, consideration of this technology when investigating the optimisation of breeding designs is necessary.

The distinctive reproductive characteristics in fish allow for accurate estimation of genetic effects and for high short-term selection responses, particularly through applying high selection intensities. However, these strategies can result in rapid accumulation of inbreeding, reduced performance and highly variable responses (Gjerde *et al*., 1996; Meuwissen & Woolliams, 1994). Also, the implementation of new technologies such as parental assignment using DNA markers may increase the many options in fish breeding programmes but also increase the risks. Studies on optimum designs of cod breeding programs, where current and new technologies are integrated and gain and risk is balanced, are therefore required.

1.6 Genetic improvement strategies

Selection within breeds or strains is one of the most important strategies available to alter the performance of animals. Particular advantages of this method are that improvements are made

permanent, cumulative and, in most cases, sustainable and highly cost effective. In general, investment in selection programs gives very high returns expressed in terms of interest on invested capital. In sheep, pig and beef cattle production full investment appraisals have shown net benefit/cost ratios ranging from 5/1 to 50/1 (Barlow, 1983; Mitchell *et al*., 1982).

The genetic gain per generation depends on three main factors: (i) selection intensity; (ii) the accuracy of predicted genetic merit of trait; (iii) the amount of genetic variation of the trait of interest. Thus, a basic requirement for this strategy is the existence of additive genetic variation. A continuous selection response relies on the maintenance of additive genetic variation. Only properly designed programs can produce responses over a large number of generations without serious loss of additive genetic variation due to inbreeding. This has been verified in long-term selection experiments (Enfield, 1974) and in several breeding programs in farm animals.

1.7 Selection methods

Different methods of selection are available each characterized by the type of information that the selection decision is based on. Three selection methods are important to consider in fish species: (i) individual selection (mass selection); (ii) family selection; (iii) combination of these two (combined selection).

1.7.1 Individual selection

Selection is based on the own performance of the individual or phenotype. Since records on relatives are not used, no tagging is required and individuals from different families can be reared together. However, a prerequisite for using individual selection is that the traits selected for can be measured on the breeding individual itself while being alive. This applies to traits carcass quality and binary traits, such as survival and early sexual maturity at high and low frequencies.

1.7.2 Family selection

When the trait of interest cannot be measured on the breeding candidates themselves while alive, selection decisions have to be based on the phenotypic records obtained from relatives. Whole families are selected or rejected as units according to the mean value of the family. Mean values of phenotypic records of sibs or of BLUP breeding values of the sibs could be used. The families may consist of full or half sibs, whereas families of more remote relationships are of little practical significance. To obtain an acceptable rate of genetic gain

and low rate of inbreeding, the number of family groups tested when applying family selection needs to be high.

1.7.3 Combined selection

This method optimally combines all available sources of information about the breeding value of an animal. In fish breeding this means information recorded on the breeding candidate itself and its full and half sibs. Combined selection maximizes the rate of genetic gain and is therefore generally considered to be the best selection method. Consequently, the need to restrict the number of selected individuals from each family is even more important in a combined selection program. In mass selection programs without tagging, the number of selected individuals from each family must be restricted at or shortly after fertilization. However, in populations, where family identity can be attained through physical tagging of DNA profiling, the restriction may be implemented after the performance test.

1.8 Restricting inbreeding

Compared to farm animals, the high fecundity in fish and shellfish allows greater genetic gains to be obtained by applying high selection intensities. This means that a very small number of individuals can make a large contribution to the genetic makeup of successive generations and, hence, the rate of inbreeding can be high. The detrimental effect of inbreeding is reduced fitness. In common carp (*Cyprinus carpio* L.) farming these negative effects are frequently experienced and farmers therefore often use wild fish to refresh their farmed stock (Eknath & Doyle, 1990).

The rate of inbreeding can be high even with selection procedures, which do not make use of family information (Gjerde, 1986). Also, the use of small number of parents can lead to high variability of response that is a measure of risk of the breeding program (Gjerde *et al*., 1996; Meuwissen & Woolliams, 1994; Villanueva & Woolliams, 1997). Consequently, it is very important to restrict the rate of inbreeding to limit its negative effect. However, within a population under selection the level of inbreeding will always increase over generations. The rate of inbreeding should be kept at a level that does not reduce the overall fitness (Meuwissen & Woolliams, 1994). This is an important part of the background for the overall objective of this proposal.

Recent developments have provided a general understanding of the optimum design for breeding programs to produce maximum genetic gain over a given time horizon while restricting the rate of inbreeding to a specific value (Gjerde *et al*., 1996; Grundy *et al*., 1998;

Meuwissen, 1997; Villanueva & Woolliams, 1997). These procedures incorporate, in a novel way, genetic gain and inbreeding within the same framework and give the optimal number of parents, the optimal contribution of parents to subsequent generations and the optimal weight assigned to information from relatives in the genetic evaluation. All this work has assumed normally distributed traits with records on all the selection candidates. Thus, the application of these methods for finding optimum design for selection programs to improve economically important traits in aquaculture species needs to be investigated.

1.9 Genotype by environment interaction (G×E)

For several aquaculture species farming is widespread and takes place under different climatic conditions and under a wide range of production systems. Selection within a nucleus may lead to lower-than-expected genetic gains in other production environments when G×E interaction exists but it is not introduced to the selection criteria. The most severe form of G×E interaction is re-ranking which means that ranking of genotypes changes across different environments (Lynch & Walsh, 1998). Re-ranking across environments can be estimated using a genetic correlation (r_G) between traits measured in two environments (Gjedrem & Baranski, 2009). G×E interaction is commonly considered to be biologically significant when genetic correlation is lower than 0.8 (Robertson, 1960).

If the level of re-ranking is important (when $r_G < 0.8$) independent selection may have to be carried out in two or more distinct sub-populations, each targeting specific types of actual production environments. Alternatively, a single breeding population may be serving a range of production environment types, if the selection is based on average performance across target environments. This strategy, however, requires that random sample of individuals from each of the genetic groups under evaluation are tested in several production environments, and thus substantially increasing the cost involved. However, due to the high fecundity, this is more feasible in aquatic species.

Even for situations, where no re-ranking of genotypes between production environments is seen, G×E may affect the absolute and relative magnitude of genetic, environmental and phenotypic variance and thus lead to heterogeneity of variance between environments. Dickerson (1962), Eisen & Saxton (1983) and others address this type of G×E interaction and its implication for design of breeding programs.

Both types of G×E are important for selection decisions. If a particular genotype is superior in one production environment but less superior in another, selection based on

performance in the first environment may not lead to less genetic improvement in the second. If the G×E is due to heterogeneous variances between the involved environments, a possible solution might be to base the selection within each class of environments on breeding value estimated on the basis of different sets of genetic parameters.

Although the magnitude of G×E in fish generally is considered to be low, several studies report on significant interactions for an important trait like growth (Ayles & Baker, 1983; Jónasson *et al*., 1997; Sylven *et al*., 1991). It is therefore important to address the possible consequences of G×E, especially with regard to the choice of number of test environments and type of environments and the need for the development of one versus several, specialized genetic lines targeting specific sets of production environments.

1.10 Selection on phenotype or genotype

In a cod farming breeding program the molecular genetic approach should also be considered. Since 1990 a considerable focus has been on Marker Assisted Selection (MAS). As a result the effort changed in animal breeding from quantitative to molecular genetics with the main emphasis on Quantitative Trait Loci (QTL) and MAS. These ideas generated optimism and enthusiasm that selection could be determined entirely based on genotype and without phenotype. The claim was that the molecular methods would replace BLUP, which is a traditional method for selection based on phenotype. However, results have been modest and some healthy scepticism is not out of place. This could be explained by several factors such as difficulty in gene identification and simplified assumptions in pattern of inheritance (Misztal, 2006).

In the last four years the emphasis has moved from using low density genetic mapping with the use of relatively low number of genetic markers, mainly microsatellites, to high density mapping with the use of hundreds of thousands or even millions of markers. This has been defined as Genomic Selection (GS). Microsatellites have been replaced by Single Nucleotide Polymorphism (SNP) as the microsatellites have turned out to be less stable (Ben Hayes, personal comm.). Moreover, an increased computational ability to work with thousands of observations has made it feasible to use methods of Bayesian analysis such as Markov Chain Monte Carlo (MCMC) and Gibbs sampling to generate draws from almost any distribution of interest, no matter how complex or multidimensional. With increased genetic marker availability it may seem a little ironic that the phenotype rather than genotype is the bottleneck.

The use of genomic selection can potentially decrease generation interval, by choosing individuals long before the trait appears. Perhaps the most important is to improve the accuracy, especially for lower heritability traits. In traditional BLUP selection the accuracy of selecting breeding candidates relies heavily on information from relatives through genetic relationship matrix. This means that related individuals are more likely to be selected, which leads to increased inbreeding. However, by using genomic selection the information from pedigree can be relaxed as the selection of candidates is based on genetic information rather than relationship.

Dense marker maps and high-throughput genotyping have become increasingly available in aquaculture species, especially for Atlantic salmon. Genomic selection has not been tested in aquaculture, but a simulation study has shown increased genetic improvement (Sonesson & Meuwissen, 2009; Villanueva *et al*., 2011). As today, the cost of genotyping thousands of markers is expensive. It is still a question whether the cost of this technique is economically feasible in aquaculture in the future. The technology may, however, become feasible and fish breeders should be aware of the possibility that it may offer.

1.11 Brood fish and hatchery

To obtain good egg quality in cod farming, it is necessary to provide the brood stock with good rearing conditions and nutritious diet. Good water quality must be maintained, preferably using a flow-through system with clean and cool seawater and oxygen saturation close to 100%, temperature 6-8°C and salinity 30-34‰.

Additional factors that have been suggested as possible determinants of egg quality include the following**:** the nutrition, size, stress, chemical composition, microbial colonization and the over-ripening of the egg (Kjørsvik *et al*., 1990). Nutritional status of the brood fish and over ripening, which is the aging process that occurs in an egg in the period following ovulation up to fertilization, have been clearly shown to influence egg quality. Shortage of omega-3 fatty acids, such as EPA and DHA, in the brood stock diet has resulted in low egg quality (Kjørsvik *et al*., 1990) The vitamin requirements of cod are not known but can be assumed to be similar to those of *Salmonidae* (Halver, 1989).

Egg quality is very important in aquaculture because of extremely high fecundity in fish species. This facilitates the concentration of available resources in a limited number of breeding centres. Genetic gain in the nucleus can be disseminated extensively throughout the entire industry with minimum delay through one or two levels of multipliers (see Figure 1).

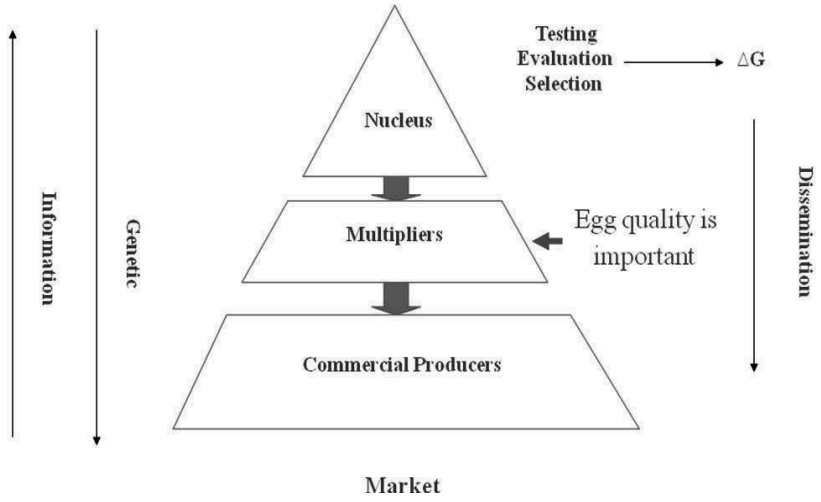

Figure 1. Schematic presentation of a breeding structure demonstrating the importance of egg quality for the multiplying stage.

1.12 Predicting the future

It is known from experiments with animals that additive genetic variance can be very large as was demonstrated by Weber in his experiments on wing-speed in *Drosophila* (Weber, 1990). Through the ingenious use of artificial wind tunnels, Weber was able to use much larger effective population sizes than most previous artificial selection experiments, and found that selection response continued for at least several hundred generations. This has also been shown in mice experiments where body weight continuously increased over 90 generations. At the end of the experiment the selected mice differed from unselected animals by about 12 phenotypic standard deviations (Hill, 2000).

The simplest way of predicting a selection response is to use "*the classic breeder's equation"*, first introduced by Jay Lush, which predicts that R gives the response to selection, or $R = h^2 S$ where S is the *selection differential*. Strictly speaking, the breeder's equation is valid only for a single generation of response from an unselected base population. In subsequent generations, selection and genetic drift change the genetic variances, and hence h^2 or the response to selection. The breeder's equation also requires a linear parent-offspring regression, which is guaranteed if the joint distribution of parental and offspring breeding values is multivariate normal (Bulmer, 1971). Selection can drive this distribution away from normality by generating kurtosis, potentially further altering the response relative to the

breeder‘s equation (Bürger, 1991; Turelli & Barton, 1990; Turelli & Barton, 1994; Zeng, 1987). However, in aquaculture, where individuals within families are much greater than the number of families, the Mendelian sampling deviation will counteract the kurtosis (Smith & Hammond, 1987).

It is, thus, not surprising that, while the breeder‘s equation typically provides a reasonable description of the first few generations of selection, it (at best) provides a very poor predictor of long-term response, even under BLUP which assumes that the additive variance changes over time. While REML provides an estimate of the additive variance for the base population (which is unbiased provided that the model assumptions hold), it does not immediately provide estimates of the actual additive variance in any particular generation of selection.

In most general biological settings, one cannot predict long-term response simply from knowledge of the base population variance components. However, in many instances, we can still gain significant insight into the course of response from some basic theory. We start by considering the infinitesimal model wherein each locus has only a small effect on the character, first introducing the basic model and then adding various layers of more realistic assumptions. One central theme throughout our review is that genetic drift is of fundamental importance in understanding long-term response. Finally, any review of long-term response would not be complete without at least mentioning that there have recently been several rather technical (but important) papers on selection response under very general settings (Barton & Turelli, 1987; Barton & Turelli, 1991; Bürger, 1991; Turelli & Barton, 1990).

1.13 Finding optimum designs

When the breeding system is large and complicated and it may not be feasible to make an experiment *in situ* due to cost and time, it is possible to simulate the behavior of the system, given some prior information about parameters and distribution. One way is to use MCMC methods where a sample is taken stochastically from a given probability distribution. For example, we can draw a stochastic sample for simulation when we have prior information about the genetic and phenotypic variation, and where the genetic variation is controlled by infinite number of unlinked genes each with an infinitesimally small additive effect. This, of course, is a simplification of the real world, but can provide a reasonable approximation. With the tremendous increases in computing power, stochastic models have become more and more

attractive and are used for the evaluation and analysis of breeding programs in both research and practice.

Techniques for optimizing selection decisions include tools both for designing *a priori,* and for operating the breeding scheme. Design tools involve stochastic models and enable us to find the optimal design of breeding schemes for maximizing genetic progress while restricting rates of inbreeding (Villanueva & Woolliams, 1997; Villanueva *et al*., 1996). This optimization is *a priori*, to maximize gain given the basic design variables (available resources, trait selected, time scale of interest and restriction on the rate of inbreeding). The ability to solve the problem depends on the facility for predicting both rates of gain and inbreeding. Operational tools involve stochastic simulations and are used to aid maximization of progress with constraints on rates of inbreeding in the routine operation of breeding schemes (Grundy *et al*., 1998; Meuwissen, 1997; Meuwissen & Sonesson, 1998; Sonesson & Meuwissen, 2000). Given a set of candidates available for selection, together with their estimated merit, the operational tools determine which candidates should be used and how widely they should be used. These tools take into account all available performance and pedigree information and use BLUP estimates as a predictor of merit.

The process of modeling optimal designs relies heavily on the definition of realistic structures and constraints. In addition, information on ongoing breeding programs for other fish species will be directly obtained from the industry. This will provide information on: i) the number of families and individuals in breeding nuclei and commercial populations, breeding objectives and selection criteria, evaluation and selection techniques, reproductive characteristics, mating designs and methods of dissemination of improved stock; ii) available estimates of genetic parameters and inbreeding depression; iii) current levels of rates of genetic progress for economically important traits and current levels of inbreeding.

The operational tools developed to date have assumed that the trait under selection is normally distributed. These tools will be applied (and modified if necessary) to investigate the optimal selection decisions for non-normally distributed traits. For binary traits an underlying continuous distribution will be assumed with a critical threshold value, which determines whether or not the trait is expressed. Different base population frequencies of the traits will be studied. The rates of gain and inbreeding and the variability of response obtained with optimized selection will be compared to those obtained with standard BLUP truncation selection to evaluate the benefits from these techniques.

Once the optimal group of selected parents has been identified in a base population it is necessary to determine the best design to mate them. The range of possible mating designs

is very large in fish species compared to terrestrial animals. Several mating systems have been proposed to reduce the rate of inbreeding, including factorial designs, minimum co-ancestry mating and compensatory mating (Caballero *et al*., 1996; Sonesson & Meuwissen, 2000). With factorial mating designs, each dam is mated to more than one sire. Minimum co-ancestry mating involves minimizing the relatedness of individuals that are mated. In compensatory mating, selected individuals from the largest families are mated to those from the smallest families, with families ranked according to the total number of selected individuals within each. Combinations of the different mating designs are also possible.

2 Objectives of the thesis

In the beginning of this century the Atlantic cod was considered to be a promising candidate for marine aquaculture. Earlier rearing experiments of Atlantic cod in Iceland indicated that poor growth, low survival rate of juveniles and early onset of maturity are major obstacles for the farming of Atlantic cod. In 2003 a full scale breeding was launched for Atlantic cod breeding in Iceland where the main breeding goals are therefore to improve these traits.. From the start of the cod breeding program, DNA profiling was used for constructing pedigree for the genetic estimates.

The specific objectives of this thesis are in four main parts:

- To estimate variation in growth, condition, maturation and population structure (F_{ST}) of Atlantic cod from five locations around Iceland (northeast, northwest and south) and their performance in sea-cages.
- To explore the genetic and phenotypic relationship between size and maturity in cod reared in commercial conditions in Iceland and the effect of maturation on growth.
- To estimate of the genetic (co)variation of economically important traits in Atlantic cod reared off the eastern and western coasts of Iceland and to explore the possible genotype by environment interaction (G×E) among the two rearing locations.
- To estimate the survival and heritability of cod juveniles at tagging size and to study the effect of different survival among families on genetic gain. A stochastic model was used to simulate genetic gain in an imbalanced versus a balanced family structure.

3 Summary of investigations

The thesis summarizes four papers. The first paper includes analyses on base population parameters in Atlantic cod in the Icelandic cod breeding program which started in 2003. The second paper explores the genetic analysis of maturity of cod in rearing and the effect of maturity on growth rate. The third paper includes the genetic analysis of economically important traits in cod framing, such as body weight, gutted weight, fillet yields and the percentage of liver weight to body weight or hepatosomatic index (HSI). The fourth paper includes a simulation study on the effect of DNA profiling in cod breeding where cod larvae are pooled shortly after hatching and low survival of cod larvae create a different representation of individual families in the breeding system.

3.1 Material

3.1.1 Animals used in the calculation

Wild cod were captured in April to May in the years 2003 – 2004 using gillnets brought onboard commercial fishing vessels. Five spawning areas located north and southwest of Iceland were chosen: Kópasker (63°36'58.46"N, 16°53'47.65"W), Trékyllisvík (66°4'33.14"N, 21°22'8.13"W), Blönduós (65°41'54.52"N, 20°19'59.28"W), Eyrarbakki (63°49'47.92"N, 21°9'35.01"W), and Selvogsbanki (63°28'33.61"N, 21°31'55.55"W) (Figure 2). All wild brood fish were hand-striped onboard the fishing vessels, and the eggs were fertilized half an hour later. The mating ratio was 1:2, with one male used to fertilize eggs from two females. Approximately 200 ml of eggs from each female were placed in a 1 L box, and after fertilization 800 ml of seawater were added. The eggs were then transferred to the Marine Research Institute (MRI) Hatchery at Staður, Grindavík. The brood fish were slaughtered and weighed and fin clips taken for genetic analysis and later used for parental assignment. Subsequently offspring from year classes 2003 and 2004 were used in breeding from 2006 – 2014. From 2003 each new hatching group has been identified with year class (YC) followed by the year of hatching.

After hatching and early rearing at MRI, the juveniles were transferred to a land-based station in Hafnir operated by the company IceCod and reared in one 40 m^3 circular tank with a flow-through system of seawater. The rearing temperature was kept constant at 8.3°C with 100% oxygen saturation and a salinity of 30-32‰ with seasonal light. Approximately 13 months after hatching, the juveniles were transferred to a 90-m circle, 18-m deep sea cage in

Berufjörður (64°43'42.02"N, 14°23'56.06"W) and Ísafjarðardjúp (66°0'32.29"N, 22°54'54.30"W) on the east and west coast of Iceland (Figure 2).

For the duration of rearing in the sea cages, the cod were fed commercially formulated feed from Fóðurblandan Ltd. in Iceland containing 18% fat and 45% protein. Feeding was regulated through appetite.

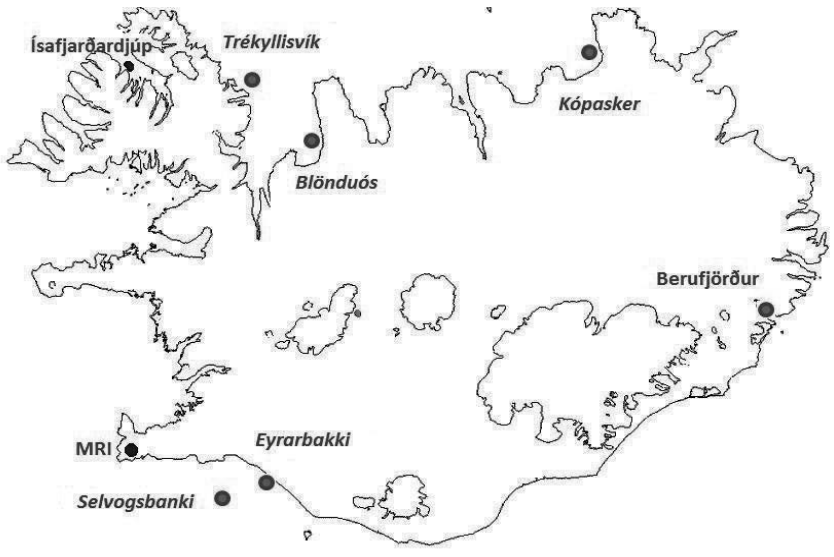

Figure 2. The base population was ordinated from five spawning grounds around Iceland, Trékyllisvík, Blönduós and Kópasker from the Northern coast and Selvogsbanki and Eyrarbakki from the Southern coast. The cod were hatched at the Marine Research Institute (MRI) and later reared to harvest size in Berufjörður and Ísafjarðardjúp.

3.1.2 Measurements of traits

During the rearing in sea cages we measured maturity, body weight, gutted weight, liver weight and fillet weight. Maturation was scored as 0 (not mature) if the gonads were undeveloped and as 1 (mature) when development had obviously started, which is evidenced by larger eggs and the ovaries filling more of the body cavity or by visible sperm or eggs. The body weight, gutted weight, length, liver weight, and fillet weight were measured on slaughered individuals, which were used for estimating the condition factor (CF) as follows:

100 × (body weight in grams) × (body length in cm^3)$^{-1}$. Hepato Somatic Index (HSI) as: 100 × (liver weight in grams) × (body weight in grams)$^{-1}$ and fillet yield as: 100 × (fillet weight in grams) × (gutted weight in grams)$^{-1}$. The fish were filleted using the Baader 189 filleting machine (www.baader.com).

For the analyses of cod larvae suvival in Paper IV field data from the Icelandic cod breeding program in 2010 – 2011 were used. Eggs from each female were kept separate in a 7 L silo until hatching approximately 14 days later. The cod larvae were moved to 70 L tanks three days after hatching and reared until they had reached the size tagging approximately 180 dPH at 15 g weight. The rearing temperature was kept constant at 8.5°C with 100% oxygen saturation and a salinity of 30-32‰. The total number of larvae, which were moved in to the tank, and the number of juveniles at tagging size was used to estimate the survival rate. In total the survival rate was estimated from 116 families.

3.2 Methods

3.2.1 Genetic model

In Papers II, III and IV REML was used to fit mixed linear models using VCE 5 software (Kovac & Groeneveld, 2003).The model (**Model 1**) used for the analysis was as follows: $\boldsymbol{y = Xb + Za + e}$, where $\boldsymbol{y}$ is the vector of the body weight, gutted weight, CF, length, fillet yield, HSI, proportion of maturity and survival of the fish and $\boldsymbol{b}$ is a vector of fixed effects, which included sex and the two rearing locations. As fillet yields were only estimated at Berufjörður the trait was treated as a missing value for Ísafjarðardjúp. The G×E analysis was performed for body weight only using Model 1. We assessed G×E by estimating genetic correlations between traits recorded in different environments. The weights were divided into two traits ($weight_1$ and $weight_2$), depending on whether individual fish from the same family were weighed at the location in Berufjörður or Ísafjarðardjúp. The vector $\boldsymbol{y}$ is the vector of the $weight_1$ and $weight_2$ and $\boldsymbol{b}$ is a vector of fixed effects, which included sex and the two rearing locations. The vector $\boldsymbol{a}$ is a vector of random additive genetic effects of individual animals.

The maternal effect was estimated for body weight. The model (**Model 2**) used for the analysis was as follows: $\boldsymbol{y = Xb + Za + Wm + e,}$ where y is the body weight, $\boldsymbol{b}$ is a vector of fixed effects, which includes rearing location and sex. Vector $\boldsymbol{a}$ is random additive genetic effects of the animals and $\boldsymbol{m}$ is a vector of random maternal genetic effects (mother). The matrices **X, Z** and **W** are known design matrices assigning observations to levels of **b, a**, and **m**, respectively. The vector **e** represents the random residual effects in both models.

3.2.2 Statistical model

All statistics were calculated by using R 2.14.0 software (R Development Core Team, 2011). In Paper I the growth of the 2004 year-class was compared with that predicted by Björnsson and Steinarsson (2002). The model is $G = (0.5735T) \times W^{(-0.1934 - 0.02001T)}$ where G = specific growth rate, T = rearing temperature and W = weight of the fish. The predicted weight Wt_2 after t days, then, is $Wt_2 = Wt_1 \times e^{(G \times t / 100)}$, where G = specific growth rate and Wt_1 = present weight. The predicted values from the model versus the measurements were tested with two-sample *t*-tests. In Papers II and III the effects of gender and rearing location on body weight, gutted weight, CF and HSI were tested with two-way ANOVA. An α level of 0.05 was used to test for significant differences.

In Papers I and II the difference in proportion of maturation among year classes and the difference due to gender were tested using the multiple proportions test (Newcombe, 1998). An α level of 0.05 was used to determine significance. In Paper II Gibbs sample was used for regression analysis between maturity status and weight, where the model then becomes $P(yi = 1) = \Phi(\beta 0 + \beta 1_{weight} + \beta 2_{sex})$. In Paper IV the survival among families was fitted using the software Easyfit 5.5 © (www.mathwave.com).

3.2.3 Parental assignment and genetic distance

Parentage assignment described in Papers I, II and III was performed using 6-16 microsatellites: Gmo8, Gmo19, Gmo37 (Miller *et al*., 2000), Tch11, Tch14 (O'Reilly *et al*., 2000), Gmo38 (Jakobsdottir *et al*., 2006), PGmo38, PGmo49, PGmo61-FRb, PGmo71, PGmo74, PGmo87, PGmo94, PGmo100, PGmo124 and PGmo134 (Skirnisdottir *et al*., 2008). The microsatellite markers were scored by the company Prokaria Reykjavík. The software COLONY 2.0 (Jones & Wang, 2010) was used to estimate the probability of paternity and maternity among the sacrificed fish, based on microsatellite markers. Individuals with an inferred probability of maternity or paternity below 95% were excluded from the analysis.

The population differentiation (F_{ST}) was determined from the sixteen microsatellites listed above using the R package HIERFSTAT (Goudet, 2005).

3.2.4 Simulation study

In Paper IV the statistical analysis and heritability of survival were used to simulate genetic gain in a cod breeding program. The first scheme simulated genetic improvement in two traits in a family breeding system with 50, 100 and 200 families, each with 100 and 150 individuals within family. This represents traditional breeding in aquaculture where families are reared in separate tanks and tagged at approximately 15 g weight. This will be referred to as **balanced**

design. The second scheme simulated genetic gain for the same number of families where all families were pooled shortly after hatching and the number of individuals within families was reflected by different survival among families. This represents a family structure which is assigned by DNA profiling and will be referred to as **imbalanced design** (Figure 3).

The general assumption is that offspring of each family are equally divided between the breeding station and the test cage. The number per family kept at the breeding station is always 50, but the number of offspring from each family moved to test cage is either 50 or 100. Fish moved to test cages serve as sib-test and the fish are not used as breeding candidates. We define three traits for each individual: 1) body weight in the breeding station; 2) body weight in test cages (sib test); 3) disease resistance (binary trait) in test cages (sib test). The breeding candidate gets an overall weighted index of these three breeding values. Selected breeding candidates are selected only from fish in the breeding station.

The heritability for both the normally distributed traits was set $h^2 = 0.35$ and genetic correlation r_G between them was set as 0.7. For the binary distributed trait three heritability values were tested, $h^2 = \{0.10, 0.20 \text{ and } 0.30\}$, and the disease resistance trait was assumed to have no genetic correlation with the other two traits. The aggregate breeding value of individuals was estimated in an overall index, where 30% relative weight was set on EBV for 'weight at the breeding station', 30% weight on EBV for 'weight in test cage' and 40% weight on EBV for the binary trait. To restrict inbreeding the number of individuals, that were chosen from each family, were limited to 4, 5 and 6 for breeding systems of 50, 100 and 200 families, respectively. The mating ratio is 1:2 where one male was mated to two females. As a consequence, the population was constructed of half sibs and full sibs. Figure 3 summarizes the setup of the simulation scheme.

The phenotype y_{ij} of fish $i = 1,2,...k.$ and three traits $j = 1,2,3$ can be expressed as $y_{ij} = a_{ij} + e_{ij}$, whereas $a_{ij} \sim \boldsymbol{N(0, G)}$ and $e_{ij} \sim \boldsymbol{N(0, R)}$. The matrix $\boldsymbol{G}$ is the genetic covariance matrix amongst the three traits. Furthermore $\boldsymbol{G = K^T K}$, where $\boldsymbol{K}$ is a Cholesky decomposition in an upper triangular matrix. Similarly, the environmental effect can be expressed as $\boldsymbol{r \sim N(0,R)}$, where $\boldsymbol{R = M^T M}$ and again $\boldsymbol{M}$ is a Cholesky decomposition in an upper triangular matrix. The vector r is a random standard normal density such as $\boldsymbol{r \sim N(0,I)}$, where $\boldsymbol{I}$ is an identity matrix. Then we can sample from distribution a_{ij} such $a^s_{ij} = \boldsymbol{K^T} r$. Later generations can be expressed such $a_{ij} = ½\ a_{father} + ½\ a_{mother} + m$, where m represents "Mendelian segregation" and $a_{ij} \sim \boldsymbol{N(0,d_i G)}$, where $d_i = ½(1-(½F_{father} + ½F_{mother}))$ and F is the inbreeding coefficients of the father and mother $(f_i = 2\Theta_{ii} - 1)$ (Lynch & Walsh 1998).

The phenotype of the threshold character was created assuming underlying scale from standard normal density as $y_i \sim N(0,1)$, at given the threshold value λ within standard normal density. Simulated number $y_i < \lambda$ gives 0 but otherwise 1.

All simulations were performed using the statistical software R 2.14.2 (R Development Core Team, 2011).

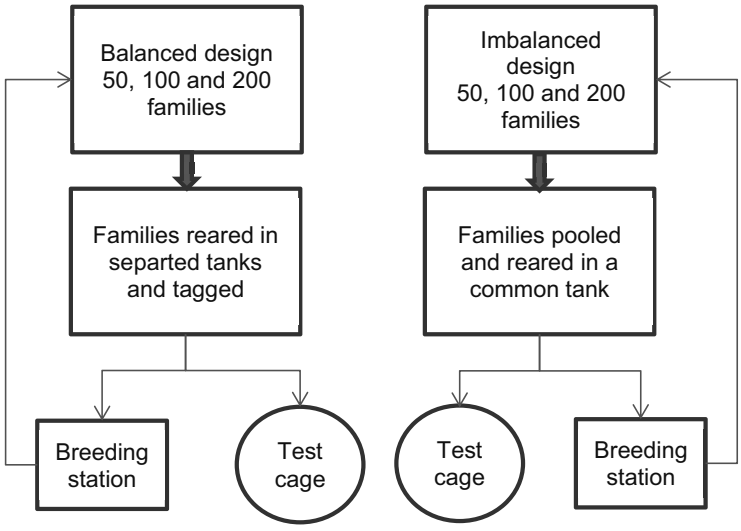

Figure 3. Schematic overview of the simulation scheme.

4 Main findings

4.1 Base population and measurements of live history traits

The main findings of Paper I are that measurements taken at approximately 952 days post hatch (dPH) did neither show significant differences in weight among Atlantic cod originating from the North and South coast of Iceland nor quantitative differences in growth, proportion of maturation or length (Table 1). The pair-wise genetic differentiation F_{ST} among all five groups showed a low value, indicating extensive gene-flow among the five locations and a large effective population size (Table 2). The proportion of hatching success variety ranged from 13% to 40% among the brood fish (females) from these five locations and was not significant ($P = 0.07$). Neither did the weight of the brood fish differ significantly among the same locations ($P = 0.40$) (Table 3). Paper II presents the effects of early maturation on growth. High proportion of maturity during first winter in sea cages depressed growth significantly (Figures 4 and 5). Tables 4 and 5 summarize the main phenotypic mean and variance for the most important traits in cod farming that are described in Paper III. The rearing locations had significant effects on growth due to higher rearing sea water temperature in Ísafjarðardjúp (Figure 6).

Table 1. Measurements taken where age of the five groups was from 950 – 963 days post hatch. Weight is given in grams and length in centimeters and both with ± one standard error (SE). Predicted (g) indicates the predicted value for growth from the Björnsson & Steinarsson (2002) growth model. CF is the condition factor; dPH is the days post-hatch. N stands for number of individuals measured.

Traits	Trékyllisvík	Blönduós	Kópasker	Eyrarbakki	Selvogsbanki
Weight (g)	1414 ± 56	1504 ± 30	1426 ± 49	1402 ± 44	1477 ± 25
Predicted (g)	1471	1446	1400	1475	1443
Length (cm)	48.8 ± 0.51	48.6 ± 0.30	47.1 ± 0.39	48.1 ± 0.35	47.9 ± 0.25
CF	1.30 ± 0.03	1.37 ± 0.04	1.49 ± 0.06	1.42 ± 0.05	1.43 ± 0.03
Maturation	100	100	100	100	100
dPH	960	953	933	963	950
N	68	194	87	141	265

Table 2. Estimates of pair-wise genetic differentiation (FST) among cod samples and P-values. Significance levels were adjusted according to the G-statistics test (Goudet, 2005). The F_{ST} values are in the lower diagonals of the matrix, and the P-values are in the upper diagonals.

F_{ST}	Trékyllisvík	Blönduós	Kópasker	Eyrarbakki	Selvogsbanki
Trékyllisvík	0	***	***	***	***
Blönduós	0.0055	0	***	***	***
Kópasker	0.0058	0.0046	0	***	***
Eyrarbakki	0.0043	0.0051	0.0045	0	***
Selvogsbanki	0.0043	0.0042	0.0031	0.0023	0

*** $P < 0.0001$

Table 3. Summary of the wild brood cod used to form a base population for cod breeding in 2004. Weight and hatching success are given ± standard error (SE).

Location	Weight g	Hatching success %	Number of females
Trékyllisvík	5726 ± 892	38 ± 30	16
Blönduós	8107 ± 796	23 ± 15	28
Kópasker	5342 ± 286	30 ± 21	37
Eyrarbakki	7268 ± 452	40 ± 29	43
Selvogsbanki	7991 ± 400	13 ± 14	52

Table 4. Descriptive statistics for body weight, gutted weight and condition factor (CF), and number of individuals, measured in Berufjörður and in Ísafjarðardjúp. All figures are shown with one standard deviation (SD).

Location	Gender	Body weight (kg)	Gutted weight (kg)	CF	Number
Berufjörður	Male	1.670 (0.295)^{a}	1.252 (0.241)^{c}	1.40 (0.23)^{e}	295
Berufjörður	Female	1.684 (0.387)^{a}	1.296 (0.341)^{c}	1.39 (0.26)^{e}	304
Ísafjarðardjúp	Male	1.914 (0.422)^{b}	1.435 (0.364)^{d}	1.36 (0.22)^{f}	390
Ísafjarðardjúp	Female	1.952 (0.488)^{b}	1.501 (0.412)^{d}	1.37 (0.20)^{f}	413

Different letters in superscript within a column and trait indicate significant differences, two way ANOVA *($P < 0.001$).* Adjusted R-squared for body weight, gutted weigth and CF were *0.077, 0.059* and *0.0061,* respectively.

Table 5. Descriptive statistics for hepatosomatic index (HSI) and fillet yields, and number of individuals, measured in Berufjörður and in Ísafjarðardjúp. All figures are shown with one standard deviation (SD).

Location	Gender	HSI	Fillet yields	Number
		%	%	
Berufjörður	Male	13.0 (2.4)[a]	56.0 (6.6)[c]	295
Berufjörður	Female	12.6 (2.4)[b]	57.1 (4.9)[c]	304
Ísafjarðardjúp	Male	12.4 (2.3)[b]		390
Ísafjarðardjúp	Female	12.6 (2.5)[b]		413

Different letters in superscript within a column and trait indicate significant differences, HSI was tested with two way ANOVA *(P < 0.01),* fillet yields tested with one way ANOVA *(P = 0.19).* Adjusted R-squared for HSI and Fillet yields were *0.004* and *0.0011,* respectively.

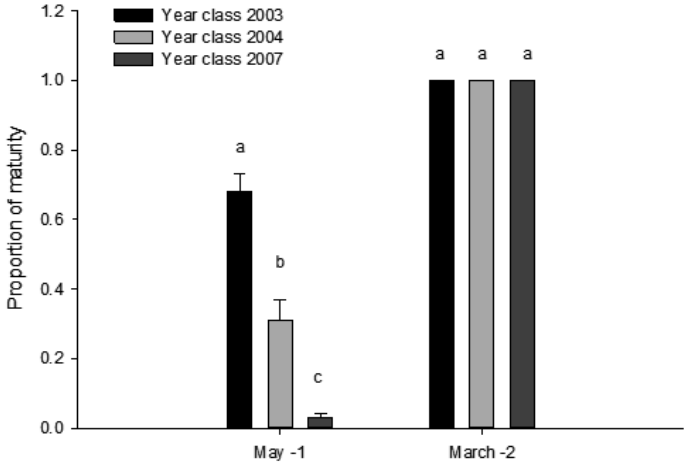

Figure 4. The estimates of maturity during first sea-winter (May-1) and second sea-winter (March-2). Vertical whiskers indicate SE. Different letters denote significant differences (*P < 0.001*).

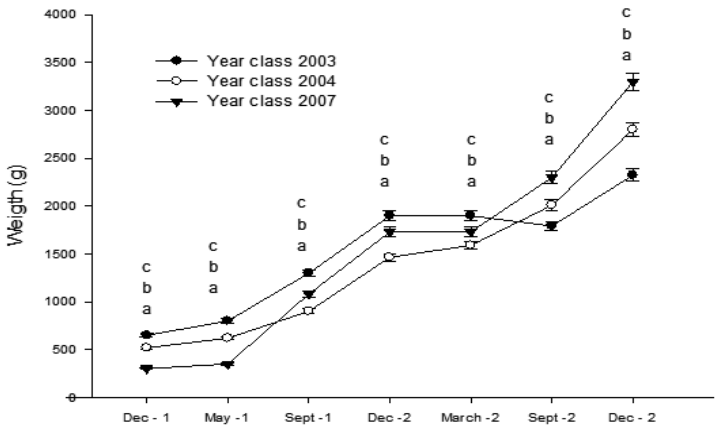

Figure 5. The mean body weight of each year class measured seven times over 24 months in rearing. Vertical whiskers indicate SE. Different letters denote significant differences (one way ANOVA, $P < 0.05$).

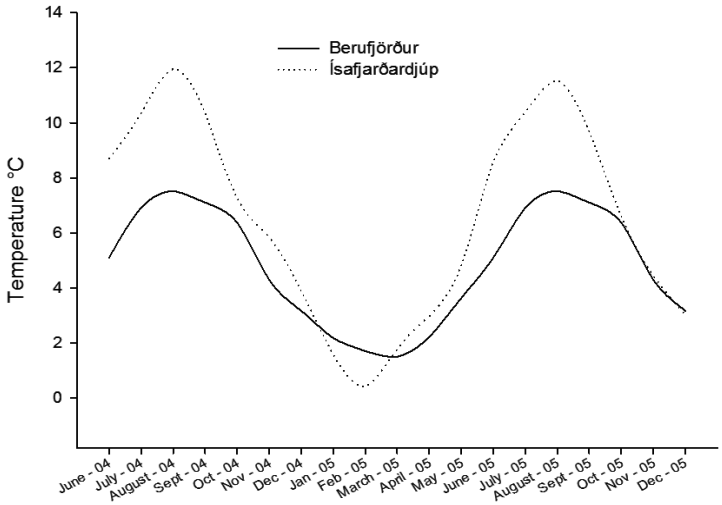

Figure 6. Rearing temperatures in Berufjörður and Ísafjarðardjúp from June 2004 to December 2005.

4.2 Genetic analyses

In Paper II the heritability on an observed scale for maturation at the first winter was estimated to be h^2_o = 0.17 ± 0.04 and proportion of maturation among all families was estimated at 0.30 ± 0.06. Transforming heritability to underlying scale results in h^2_u = 0.28 ± 0.06. The heritability for body weight was estimated as h^2 = 0.34 ± 0.04. The genetic correlation between body weight and maturation was relatively high at r_G = 0.90 (Figure 6). Gender had no notable effect on weight or maturation. In Paper III the heritability (h^2) of body weight, estimated by Model 2, was moderate and significantly different from zero at the 5% level of significance, whereas the estimated maternal effect (m^2) was not significant (Table 6). The heritabilities for fillet yields and HSI, estimated with Model 1, were not significantly different from zero, whereas the estimated heritabilities for gutted weight and CF were both significantly different from zero (Table 6). The genetic correlations between body weight, gutted weight, CF, HSI and fillet yields, as estimated with Model 1, were all significant from zero (Table 7).

The genetic correlation between body weights at the two rearing locations is described in Paper III and was estimated with Model 1 as r_G = 0.95 ± 0.06. The heritability for body weight in Berufjörður ($weight_1$) was 0.44 ± 0.13 and in Ísafjarðardjúp ($weight_2$) 0.40 ± 0.13. All estimates were significantly different from zero.

In Paper IV it was shown that survival was best fitted by gamma distribution which gave the best fit for α = 0.66 and β= 5.34. The fit did not depart from gamma distribution given Anderson-Darling test *($P < 0.01$)*. Approximately 50% of all individuals were within 14% among the largest families (Figure 2). The heritability for survival at 180 dPH was estimated to be h^2 = 0.02 with a standard error (SE) = 0.10. The mean survival among 116 families was 3.5% with a maximum survival of 22% and a minimum of 0.1% (Figure 8). Genetic gain for body weight was significantly higher for the balanced design when compared with the imbalanced design for all family sizes. The genetic gain for the disease resistance trait was significantly higher in balanced design for all family sizes and significantly higher when the heritability of 0.2 and 0.3 was compared with the gain at heritability of 0.1 (Figures 9 and 10). The accuracy of the selected parent for body weight and disease resistance was not significantly different between balanced and imbalanced design. For the normally distributed trait the accuracy was at maximum 0.72 and minimum 0.70. For the disease resistance trait the accuracy was maximum 0.55 and minimum 0.50.

Table 6. The estimates (± SE) of genetic parameters. The variance components for body weight (kg) were estimated with Model 2, while variance components for gutted weight (kg), condition factor (CF) and hepatosomatic index (HSI) were estimated with Model 1.

Component	Symbol	Body weight	Gutted weight	CF	HSI	Fillet yields
Animal	h^2	0.31± 0.06	0.34 ± 0.04	0.24 ± 0.06	0.06 ± 0.04	0.04 ± 0.04
Mother	m^2	0.03 ± 0.02				
Residuals	e^2	0.66 ± 0.07	0.66 ± 0.05	0.76 ± 0.06	0.94 ± 0.06	0.96 ± 0.05
Total	σ^2_p	0.16 ± 0.02	0.11 ± 0.02	0.06 ± 0.02	66.4 ± 0.28	10.9 ± 0.55

h^2, m^2 *and* e^2 = proportion of phenotypic variance associated with additive genetic, maternal and residuals effects, σ^2_p = phenotypic variance.

Table 7. The estimate (± SE) of the genetic correlation between body weight, gutted weight, condition factor (CF) and hepatosomatic index (HSI) using Model 1.

Traits	Body weight	Gutted weight	CF	HSI
Gutted weight	0.99 ± 0.01			
CF	0.31 ± 0.15	0.40 ± 0.10		
HSI	0.67 ± 0.25	0.42 ± 0.20	0.42 ± 0.12	
Fillet yields	0.82 ± 0.11	0.80 ± 0.15	0.80 ± 0.21	0.69 ± 0.24

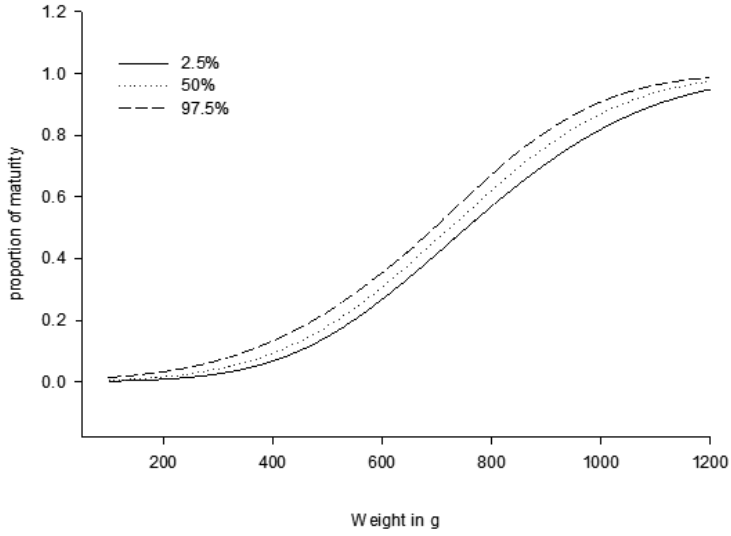

Figure 7. Posterior distribution of mature proportion with increasing body weight. The 2.5th, 50th and 97.5th percentiles of the posterior distribution are plotted for each weight. The interval between the dashed lines corresponds to a 95% interval estimate for proportion of maturity.

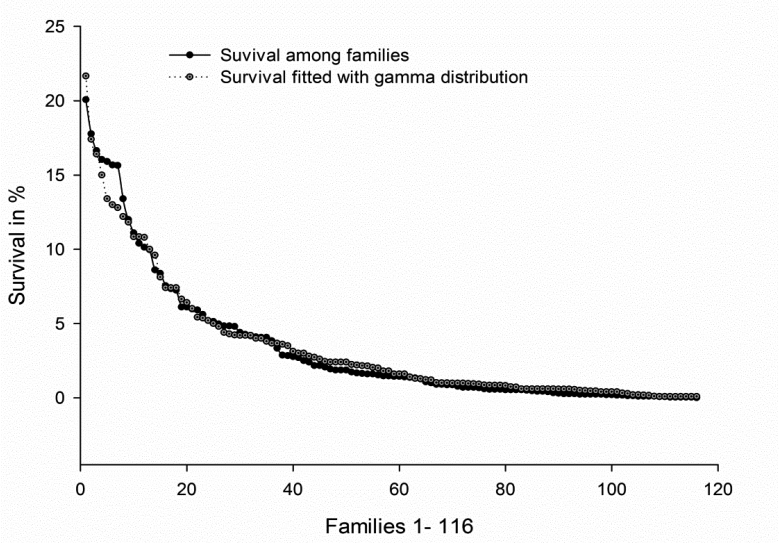

Figure 8. Field data of 116 families fitted with gamma distribution. The data are from the Icelandic cod breeding program and represent the results for survival estimated at 180 days post hatching.

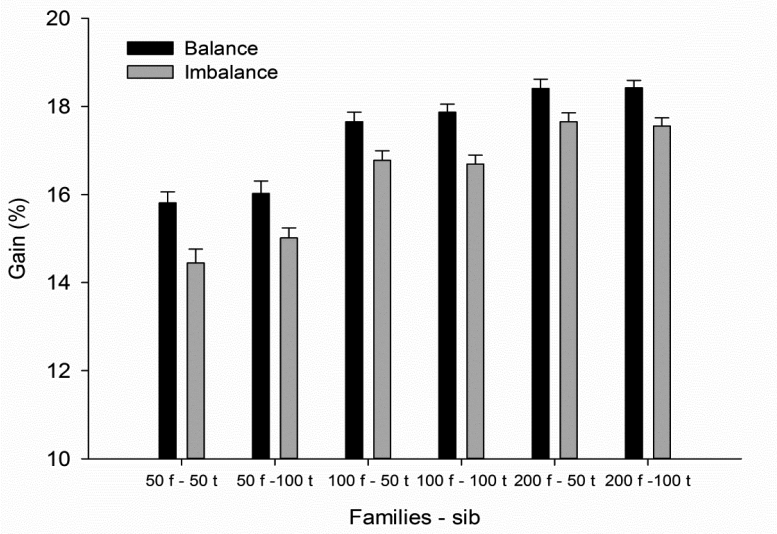

Figure 9. Mean of genetic gain (ΔG) tested for six different family sizes for balanced and imbalanced design. The x axis is marked with the letters f and t, where letter f stands for number of families and letter t stands for number of individuals in a sib test. Vertical whiskers show SE.

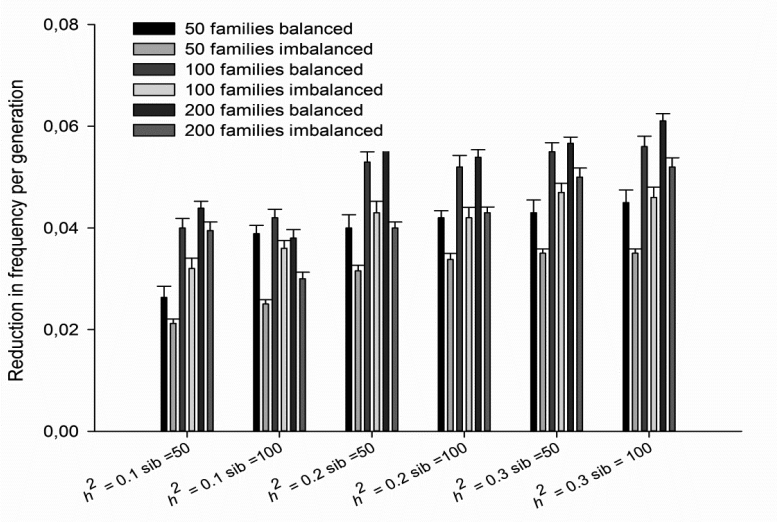

Figure 10. Different genetic gain for three heritabilities h2 = {0.1, 0.2 and 0.3}. The comparisons were done for 50, 100 and 200 families. Each family is tested for both 50 and 100 sib. Vertical whiskers show SE.

5 General discussion

The main conclusion is that Atlantic cod originating from the North and South coast of Iceland show no quantitative differences in growth, proportion of maturation or length. Moreover, it should be noted that in the current study the body weight increase in all five groups is in accordance with the growth model presented by Björnsson & Steinarsson, (2002). The current result does not correspond well with the Counter-gradient variation in growth (CnGV hypothesis), which proposes that cod populations from higher-latitudinal waters have higher capacity for growth as adults (Brander, 1995), juveniles (Suthers & Sundby, 1996), and larvae (Hunt von Herbing & Boutilier, 1996).

The pair-wise genetic differentiation F_{ST} among all five groups showed a low value, indicating extensive gene flow among the five locations and a large effective population size. This finding is similar to recent publications on population structure in Atlantic cod (Nielsen *et al*., 2003; Pampoulie *et al*., 2006). However, it should be noted that genetic diversity based on neutral markers such as microsatellites yield little useful information for animal breeders because of the lack of information regarding the quantitative genetic variation of a population (Reed & Frankham, 2001).

In Paper II a strong phenotypic and genetic relationship was demonstrated between body weight and maturity for cod in rearing at the first sea winter. The relationship between these two traits was stronger than those that have been published for Atlantic cod and *Salmonidae* (Crandell & Gall, 1993; Gjerde, 1986; Su *et al*., 1999). Still, the estimates of heritability for both body weight and maturity are in agreement with earlier findings. The heritability for maturation was 0.17 ± 0.07, which was estimated based on an observed scale and is not significantly different from previous studies in Norway (Kolstad *et al*., 2006). Heritability for body weight $h^2 = 0.34$ is similar to that in an earlier publication (Gjerde *et al*., 2004).

Size seems to be the most important factor for determining maturity in cod. A doubling in body weight at first winter in sea cages increases the proportion of maturity from 3% to above 60%. The importance of size while determining fish maturity has been studied for a variety of fish species, and the effect of age and size has often been debated (Taranger *et al*., 2010). There seems to be no indication of fixed age or size for fish species like *Salmonidae*, which have been studied most extensively (Morita & Fukuwaka, 2006). However, the significance of genetics has been demonstrated in breeding and has already

been implemented in Atlantic salmon and rainbow trout (*Oncorhynchus mykiss* W.) breeding programs (Gjedrem & Baranski, 2009).

Paper III demonstrates moderate additive genetic variance for body weight of Atlantic cod in Iceland, suggesting that selection for increased body weight is likely to be successful. The heritability estimate for body weight at harvest was 0.31 ± 0.06 and maternal effect was 0.03 ± 0.02. This agrees with other published data on heritability for cod (Tosh *et al*., 2010). In fact, the heritability for body weight in both *Salmonidae* and marine fish universally seems to be 0.3-0.4 (Elvingson & Johansson, 1993; Gjedrem, 2000; Jónasson *et al*., 1997; Kolstad *et al*., 2006; Tosh *et al*., 2010; Winkelman & Peterson, 1994). The results indicate a high genetic correlation (r_G *=0.95*) for body weight between two rearing locations, reflecting a low G×E interaction with no re-ranking of families between the two locations, indeed genetic correlation above 0.7 is considered to have negligible or marginal effects on the G×E interaction.

The heritability of fillet yields in this study was estimated as $h^2 = 0.04 \pm 0.04$ and was not significant from zero. Direct selection for increased fillet yield would thus not be a feasible option in a breeding program. The low heritability for fillet yields in cod is in line with what has been estimated for *Salmonidae*. Kause *et al*. (2002) estimated the heritability of fillet yields in rainbow trout as $h^2 = 0.03$ and Neira *et al*. (2004) estimated the heritability of fillet yields as $h^2 = 0.11$ in coho salmon (*Oncorhynchus kisutch* W.). Both these studies reveal a high genetic correlation between body weight and fillet yields.

One limitation of this study is that the estimates are taken in December and it is not known whether the time of harvest would have given different results. It is known from cod fisheries that the fillet yield is considerably influenced by the time of catching (Margeirsson *et al*., 2007). This may also be relevant in cod farming and therefore the effects caused by the time of harvest in farmed cod need to be studied in more detail. However, farmed cod shows higher yield in processing compared to wild cod (Rätz & Lloret, 2003). This gives the opportunity of controlling the time of harvest and feeding for maximizing fillet yields through management.

The heritability estimate for HSI was also low and the trait can therefore not be expected to be improved with breeding. Farmed cod, which are fed high energy feed, have a tendency for larger energy stores in the liver and have a higher HSI compared to wild cod (Karlsen *et al*., 2006). Lipid and protein intake by farmed cod is the main factor influencing HSI. Different feed could have altered the variance and heritability observed implying needs

for further study. The estimated HSI in this study is consistent with earlier research on HSI in farmed cod in Iceland (Árnason *et al*., 2010).

Both fillet yields and HSI revealed a high genetic correlation between body weight and those two traits. It should be noticed that genetic correlation between body weight, CF, HSI and fillet yield have high standard errors. This indicates that more data may be needed for more accurate estimates. These factors may be difficult to separate with the genetic model used in this study so alternative statistical methods may be needed to avoid this problem. Estimating the heritability at a standard weight instead of a standard age could be an option to reduce the effect of body weight (Powell *et al*., 2008). Whether such an alteration will yield better estimates of the heritability and variance components is yet to be tested in cod farming.

In Paper IV we estimated the survival among cod families at early stages in rearing and the results demonstrated that a high mortality rate led to highly imbalanced family structure. The estimated heritability for survival of $h^2 = 0.02 \pm 0.10$ is low and not significant from zero. This is in line with earlier findings for Atlantic cod. Gjerde *et al*. (2004) reported heritability for survival in cod larvae at 200 dPH to be zero. Heritability for survival at early stages in Atlantic salmon has also been reported low or from 0.04-0.09 (Rye *et al*., 1990).

Genetic gain was significantly reduced both for body weight and disease resistance in the imbalanced design when compared with the balanced design as shown in Figures 2 and 6. It is clear that the genetic gain is reduced regardless of number of families. Figures 4 and 8 clearly indicate that the imbalanced design is lacking in selection intensity in BLUP-EBV when compared with the balanced design. For the imbalanced design, the number of individuals of each family is governed by a gamma distribution, and it is therefore stochastic whether a family with a high breeding value has many or few individuals. A family with a high BLUP-EBV can therefore have few individuals from which to select. This has implications for the selection intensity and applies regardless whether the breeding value is estimated on live candidates or based on sib test. The overall accuracy of selected parents is not affected by the imbalanced structure. Alteration in sire dam ratio may be a way to increase the intensity in an imbalanced design where males with high BLUP -EBV can be used to contribute to families with low number of individuals. This needs to be looked into more carefully should DNA profiling become the chosen method in fish breeding. Optimal contribution theory could also be an approach to deal with an imbalanced family structure.

6 Conclusions

- The results of this study show that there is no significant difference in mass at harvest due to geographical origin in the Atlantic cod around Iceland. Wild brood fish show low genetic distance calculated from microsatellite markers, indicating a high gene flow among the five populations.

- Body weight and maturity in cod are highly linked. This makes the simultaneous selection for increased body weight and a lowered proportion of maturity in cod farming impractical. The likelihood of maturation in cod farmed in cages exceeds 50% as cod reaches 600 g at the first sea-winter. The effect of the first maturity in rearing is mild but the second maturity has an immense effect on growth rate.

- There is substantial genetic variation for body weight in the Atlantic cod. Therefore, a breeding program could be successful, as has been well-documented in Atlantic salmon. However, the low heritability of processing traits, such as fillet yields and HSI, provides little promise of genetic improvement.

- The unequal contribution of families in a breeding program in aquaculture can be difficult to account for, especially when the survival is low. Highly imbalanced family structure due to low survival can result in less genetic gain.

7 Future research

At the moment there is a reduced interest in cod farming in Iceland and Norway. Poor growth, negative effect of maturity on growth and high losses has made cod farming less attractive for fish farmers. Growth rate has increased during the last decade, and the negative effect of maturity has been minimized with management and rearing technology such as light. It is doubtful that genetic improvement will contribute much to that solution, because of the strong correlation between growth and maturity. However, the biggest problem in cod farming today is the high losses in sea cages especially from furunculosis. Atlantic cod has a very different immune system from salmon and it seems that vaccination will not become an option in cod farming. Breeding may be the solution to improve the overall survival and increase the disease resistance. The conventional breeding methods will take time but implementing a genomic selection can increase the genetic improvement considerably. Genomic selection may become an optional tool in future cod breeding.

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Comparison of growth in Atlantic cod (*Gadus morhua*) originating from the northern and southern coast of Iceland reared under common conditions

Theódór Kristjánsson*

Stofnfiskur Staðarberg 2-4, 221 Hafnarfjörður, Iceland

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ABSTRACT

Counter-gradient variation in growth has been documented for several commercial fish species. This phenomenon was tested for Atlantic cod (*Gadus morhua*) when forming the base population for a cod-breeding program in Iceland. In 2004, wild brood fish from five locations off the Icelandic coast were captured using gillnets from commercial fishing vessels. The eggs were hatched at the Marine Research Institute (MRI) and transferred to sea cages at 322 days post hatch dPH. Growth rate, maturation and conditional factor were measured at a commercial scale among the fishes originating from these five locations. The measurements taken at 322 days post hatch (dPH) showed a significant different in weight, but measurements taken at 729 and 952 dPH showed no difference in growth rate, length or maturation.

Analysis of gene diversity among the brood fish showed a significant genetic structure, but all F_{ST} were below 0.006 and were significant. Moreover, the hatching success among the females from these locations was not significant.

The main conclusion is that Atlantic cod originating from the North and South coast of Iceland show no quantitative differences in growth, proportion of maturation or length. These results need to be considered when forming the base population of Atlantic cod.

1. Introduction

The hypothesis that fish vary in growth performance and other life history traits on a latitudinal scale was put forward by Conover (1990) and Conover and Present (1990). According to the hypothesis, northern populations should display higher growth performance at higher temperatures. This variation is explained by adaptation to the different growth conditions, such as day length and average annual temperature, at different latitudes. Counter-gradient variation in growth (CnGV) has been documented for several commercial species, including turbot (*Scophthalmus maximus*), largemouth bass (*Micropterus salmoides*) (Williamson and Carmichael, 1990), Atlantic salmon (*Salmon salar*) (Nicieza et al., 1994) and striped bass (*Morone saxatilis*) (Conover et al., 1997). Temperature and light varies considerably through the distribution range of Atlantic cod (*Gadus morhua*), giving the potential for local variation in growth patterns (Brander, 1995; Suthers et al., 1999).

Recent studies have found considerable geographical variation in the life history and genetic structuring of Atlantic cod populations, even at small geographical scales (Arnason et al., 2009; Knutsen et al., 2007; Pampoulie et al., 2006; Salvanes et al., 2004). Population variation in growth has also received recent interest. Svåsand et al. (1996) found different growth performances in Norwegian coastal cod and Arcto-Norwegian cod. Another study on Norwegian cod found evidence for counter-gradient variation in life history traits, including growth and feeding performance (Salvanes et al., 2004). Similarly, growth experiments of cod populations from the North American coast found that cod from a cold environment had better growth performance at colder temperatures and an overall broader range of optimal growth temperature (Dutil et al., 2008). However, a recent study on juvenile cod from the same area did not find evidence for CnGV, although growth patterns appeared genetically determined (Wijekoon et al., 2009).

In general, the effect of temperature on the growth patterns of cod has been well documented during different life stages (Björnsson and Steinarsson, 2002; Imsland et al., 2005). According to the model of Björnsson and Steinarsson (2002) for the growth of wild cod, the optimal temperature varies with weight from 17 °C for a juvenile of 2 g to approximately 7 °C for a two kg cod. The sea temperature around Iceland varies in both longitude and latitude; for example, there are 1100 more degree days in one year (days multiplied by average sea temperature) in the southwest than in the northwest (Astthorsson et al., 2007). Based on the growth model, 150 g juveniles transferred to sea-cages in spring will take approximately 32 months to reach a 4 kg slaughter size (Björnsson and Steinarsson, 2002). As for cod farming, optimizing growth in sea-cages is important and moreover, recent reports of geographical variation in Atlantic cod highlight the need to record geographical

* Corresponding author. Tel.: +354 5646300; fax: +354 5646301.
E-mail address: theodor@stofnfiskur.is

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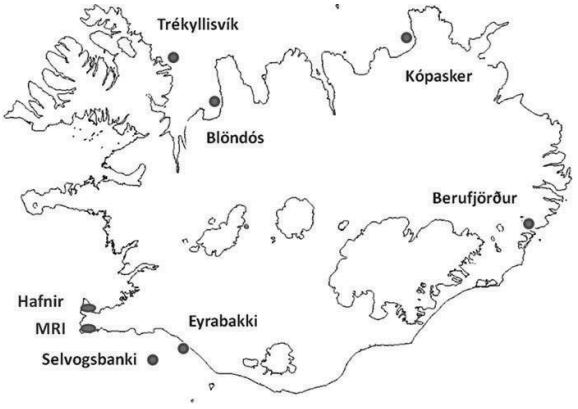

Fig. 1. Overview over the origin of the base population sampled in 2004 and the rearing location at Marine Research Institute (MRI), land-based station in Hafnir and in cages at Berufjörður.

variation in growth and other economical important traits under industrial conditions.

The main objective of this study was to estimate any variation in growth, condition and maturation of Atlantic cod from five locations around Iceland (northeast, northwest and south) and their performance in sea-cages to select individuals for creating the base population of a cod breeding program.

2. Materials and methods

2.1. Sampling and rearing conditions of juveniles

Wild cod were captured in April–May 2004 (year class 2004) using gillnets brought onboard commercial fishing vessels. Five spawning areas located north and southwest of Iceland were chosen: Kópasker, (NE Iceland – N63°36′58.46″, W16°53′47.65″). Trékyllisvík (66°4′33.14″N, 21°22′8.13″W), Blöndós (65°41′54.52″N, 20°19′59.28″W) Eyrabakki (63°49′47.92″N, 21°9′35.01″W) and Selvogsbanki (63°28′33.61″N, 21°31′55.55″W) (Fig. 1.). All wild brood fish were hand-striped onboard the fishing vessels, and the eggs were fertilized 1/2 h later. The mating ratio was 1:2, with one male used to fertilize eggs from two females. Approximately 200 ml of eggs from each female were placed in a 1 l box, and after fertilization 800 ml of seawater was added. The eggs were then transferred to the Marine Research Institute (MRI) Hatchery at Staður, Grindavík. The brood fish were slaughtered and weighed and fin clips taken for genetic analysis.

The rearing temperature at the MRI was kept constant at 8 °C during the first week post-hatch, to optimize the yolk conversion and facilitate proper filling of the swim bladder, after which the temperature was gradually raised to 10–14 °C. The growth rate peaked at 5 weeks post-hatch (15–20%/day), while the length increment was largest at a size of 6–7 cm (up to 1.7 mm/day). The juveniles at the MRI were fully weaned at 8 weeks post-hatch (33 mm). During the rearing time at the MRI, tests were made to measure to survival of eggs from each female.

The 2004 year-class was transferred from the MRI to the land-based station at Hafnir in November 2004 at a size 30–40 g. The juveniles were reared in one 40-cubic-meter circular tank with a flow-through system of seawater. The rearing temperature was kept constant at 8.3 °C with a 100% oxygen saturation and a salinity of 30–32‰ with continuous light.

The 2004 year-class was transferred in May 2005 from the land-based farm to a 90-m round, 18-m-deep sea cage in Berufjörður at the east coast of Iceland (64°43′42.02″N, 14°23′56.06″W) (Fig. 1). During growth in the sea-cage, the cod were fed once each day from June to November and once every three days over the coldest period from late November to May using a commercially formulated feed from Fóðurblandan Ltd., Iceland, containing 18% fat and 45% protein. Feeding was regulated depending on appetite. The seawater temperature was measured twice weekly throughout rearing (Fig. 1).

2.2. Data handling and statistical analysis

The fish were measured three times during rearing. The first measurement was made on the 25th of March, 2005, at the land-based rearing stage in Hafnir, where random samples of 2794 individuals were measured. The second measurement was made on the 6th of May, 2006, where a random sample of 756 individuals taken from the sea-cage in Berufjörður were killed and measured. The last measurements were made on the 15th of December, 2006. A random sample of 755 individuals was taken from the sea-cage in Berufjörður and killed and measured. The weight, maturation length and condition factor were compared between individuals based on the original locations of the brood fish. The condition factor (CF) was calculated as ungutted body weight (g) × 100/(body length cm)^3. The maturation stage was determined visually on the 6th of May, 2006, and the 15th of December, 2006. The slaughtered fish were gutted and classified as mature if eggs or sperm were clearly observed.

The analysis data for body weight, length and CF were tested with one way ANOVA, and the proportion of maturation multiple proportions test (Newcombe, 1998). Interaction between groups and days of measurements were tested with two ways ANOVA with interaction. All statistics were calculated by using R 2.14.0 software (R Development Core Team, 2011). The growth of the 2004 year-class was compared with that predicted by Björnsson and Steinarsson (2002). The model is $G = (0.5735\mathrm{T}) \times W^{(-0.1934 \text{ to } 0.02001T)}$, where G = specific growth rate, T = rearing temperature and W = weight of the fish. The predicted weight Wt_2 after t days, then, is $Wt_2 = Wt_1 \times e^{(G \times t/100)}$, where G = specific growth rate and Wt_1 = present weight. The predicted values from the model versus the measurements were tested with two-sample t-tests in R 2.14.0. An α level of 0.05 was used to test for significant differences.

2.3. DNA profiling

In the beginning of the rearing, all eggs were mixed at 7 day, post fertilization, and at each measurement a tissue samples were collected from the measured fish. Tissue samples from brood-fish were collected when the fish was stripped. Pedigree was constructed using the microsatellite markers listed in chapter 2.4. The offspring were matched to their parentage, and their origin, based on their scoring and the record of mating (marker scoring was performed at Prokaria Ltd., Reykjavik). Parental assignment was performed using MasterBayes R software (Hadfield et al., 2006).

2.4. Genetic distance (F_{ST})

The genetic distance for the 2004 year-class was determined from sixteen microsatellites (Gmo8, Gmo19, Gmo37 (Miller et al., 2000), Tch11, Tch14 (O'Reilly et al., 2000), and Gmo38 (Jakobsdottir et al., 2006), PGmo38, PGmo49, PGmo61-FRb, PGmo71, PGmo74, PGmo87, PGmo94, PGmo100, PGmo124, and PGmo134 (Skirnisdottir et al., 2008)). Analysis of gene diversity in the subdivided populations was performed as described by Nei (1973) and using the algorithm in R (Goudet, 2005). Significant genetic structures among the populations were tested using the

Table 1
Summary of the first measurement, taken on the 25th of March, 2005, at the land-based station in Hafnir. Values are given with ± one standard error (s.e.). Predicted indicates the predicted value for growth from the Björnsson and Steinarsson (2002) growth model. C.F. is the condition factor; dPH is the days post-hatch.

Traits	Trékyllisvík	Blöndós	Kópasker	Eyrabakki	Selvogsbanki
Weight (g)	109 ± 1.54	106 ± 1.70	101 ± 1.35	107 ± 1.22	100 ± 1.12
Predicted	108	102	94	111	101
Length (cm)	22.4 ± 0.39	22.1 ± 0.11	23.4 ± 0.45	21.8 ± 0.10	23.0 ± 0.08
C.F	1.08 ± 0.02	1.04 ± 0.02	0.97 ± 0.02	1.10 ± 0.02	0.88 ± 0.01
dPH	330	323	303	333	320
Number	444	398	523	682	747

Table 2
Summary of the second measurement, taken at Berufjörður on the 6th of May, 2006. Values are given with ±one standard error (s.e.). Predicted indicates the predicted value for growth from the Björnsson and Steinarsson (2002) growth model. C.F. is the condition factor; dPH is the days post-hatch.

Traits	Trékyllisvík	Blöndós	Kópasker	Eyrabakki	Selvogsbanki
Weight (g)	647 ± 25	630 ± 12	631 ± 13	660 ± 15	625 ± 15
Predicted	643	633	602	647	631
Length (cm)	40.4 ± 0.47	39.3 ± 0.22	37.6 ± 0.21	40.0 ± 0.25	38.2 ± 0.26
C.F.	0.97 ± 0.02	1.01 ± 0.02	1.16 ± 0.01	1.01 ± 0.01	1.09 ± 0.01
Maturation	30% ± 20	42% ± 6	30% ± 6	44% ± 6	32% ± 7
dPH	736	731	709	739	728
Number	48	216	218	111	163

G-statistic test (Goudet et al., 1996). The analysis data for brood-fish body weights and hatching success were tested with one-way ANOVA.

3. Results

3.1. Growth

The increase in weight over the 322 day period at post-hatch (dPH), did show a significant difference in weight ($F_{.05,4,2789} = 7.9$, $P = 2.2e{-}6$). At 322 (dPH), the cod from Kópasker and Selvogsbanki were significantly lower in weight than those from Blöndós, Trékyllisvík and Eyrabakki. The second measurement, taken at 729 (dPH), showed no significant difference in weight among the five groups ($F_{.05,4,751} = 0.76$, $P = 0.55$), and the third measurement, taken at 952-day (dPH) did not show a significant difference in weight among the five groups ($F_{.05,4,750} = 0.02$, $P = 0.88$). Interaction between days and groups did not show significant interaction effect on weight ($F_{.05,8,4290} = 0.48$, $P = 0.86$).

The growth of cod from Kópasker at 322 (dPH) was significantly higher than expected from the growth model (Björnsson and Steinarsson, 2002) ($t = 5.06$, $df = 522$, $P = 5.7e{-}7$), but all other measurements were in accordance with the model ($P < 0.05$) (for further details, see Tables 1–3).

3.2. Length, condition factor and maturation

The measurement, taken at 322 (dPH) showed a significant difference in length among the five groups ($F_{.05,4,2789} = 7.82$, $P = 2.8e{-}6$), and condition factor showed a highly significant difference ($F_{.05,4,2785} = 30.7$, $P = 2.2e{-}16$). The second measurement, taken at 729 (dPH), showed no significance in length among the five groups ($F_{.05,4,751} = 2.45$, $P = 0.11$), but the condition factor was highly significant ($F_{.05,4,751} = 49$, $P = 4.9e{-}12$.) Third measurement, taken at 952-day (dPH), showed no significant difference in length among the five groups at day 952 post-hatch (dPH) ($F_{.05,4,750} = 2.32$, $P = 0.055$), and the condition factor was not significantly different ($F_{.05,4,750} = 2.75$, $P = 0.097$) (for further details, see Tables 1–3).

The proportion of mature cod among the five groups in the second measurement showed no significant difference (*X*-squared = 7.5177, *df* = 4, *P*-value = 0.1109). All the fishes were clearly mature in the last measurement (Table 3).

3.3. Brood fish hatching success and genetic distance F_{ST}

The genetic distances for the brood-fish showed a genetic structure. The F_{ST} among the groups showed a maximum of 0.0055 and a minimum of 0.0023. The greatest genetic distance was found between Trékyllisvík and Kópasker (Table 5). All F_{ST} were tested as significant according to the *G*-statistical test (Goudet et al., 1996).

The proportion of hatching success variety ranged from 13% to 40% among the females from these five locations and was not significant ($F_{.05,4,76} = 2.25$, $P = 0.07$), nor did the weight of the brood fish differ significantly among the same locations ($F_{.05,4,174} = 0.67$, $P = 0.40$) (Table 4).

4. Discussion

The main conclusion of the current study is that Atlantic cod from NW, NE and SW Iceland reared at a commercial scale showed no significant differences in weight, length and proportion of maturation at harvest size. Although the current results may indicate geographical variation in growth capacity or growth patterns, this does not correspond well with the CnGV hypothesis. It has been

Table 3
Summary of the third measurement, taken at Berufjörður on the 15th of December, 2006. Values are given with ± one standard error (s.e.). Predicted indicates the predicted value for growth from the Björnsson and Steinarsson (2002) growth model. C.F. is the condition factor; dPH is the days post-hatch.

Traits	Trékyllisvík	Blöndós	Kópasker	Eyrabakki	Selvogsbanki
Weight (g)	1414 ± 56	1504 ± 30	1426 ± 49	1402 ± 44	1477 ± 25
Predicted	1471	1446	1400	1475	1443
Length (cm)	48.8 ± 0.51	48.6 ± 0.30	47.1 ± 0.39	48.1 ± 0.35	47.9 ± 0.25
C.F.	1.30 ± 0.03	1.37 ± 0.04	1.49 ± 0.06	1.42 ± 0.05	1.43 ± 0.03
Maturation	100	100	100	100	100
dPH	960	953	933	963	950
Number	68	194	87	141	265

Table 4
Summary of the wild brood-cod used to form a base population for cod breeding in 2004. Weight and hatching success are given ± standard error.

Location	Weight (g)	Hatching success (%)	Number of females
Trekyllisvík	5726 ± 892	38 ± 30	16
Blöndós	8107 ± 796	23 ± 15	28
Kópasker	5342 ± 286	30 ± 21	37
Eyrabakki	7268 ± 452	40 ± 29	43
Selvogsbanki	7991 ± 400	13 ± 14	52

Table 5
Estimates of pair-wise genetic differentiation (F_{ST}) among cod samples and *P*-values. Significance levels were adjusted according to the *G*-statistics test (Goudet et al., 1996). The (F_{ST}) values are in the lower diagonals of the matrix, and the *P*-values are in the upper diagonals.

F_{ST}	Trekyllisvík	Blöndós	Kópasker	Eyrabakki	Selvogsbanki
Trekyllisvík	0	***	***	***	***
Blöndós	0.0055	0	***	***	***
Kópasker	0.0058	0.0046	0	***	***
Eyrabakki	0.0043	0.0051	0.0045	0	***
Selvogsbanki	0.0043	0.0042	0.0031	0.0023	0

*** $P < 0.0001$.

found that cod populations from higher-latitude waters have a higher capacity for growth as adults (Brander, 1995), juveniles (Suthers and Sundby, 1996), and larvae (Hunt von Herbing and Boutilier, 1996). Although not in line with the CnGV hypothesis, it is possible that the differences observed in the present study indicate an inherent difference in growth patterns among the cod stock from NW, NE and SW Iceland. Intrinsic to the CnGV hypothesis is that populations at higher latitudes exhibit faster growth during the relatively shorter growing season (Conover and Present, 1990; Conover et al., 1997; Hunt von Herbing and Boutilier, 1996). Had the present study continued, the populations in this study may have made up for the apparent lapse in growth. Moreover, as the observed growth was noted only in one measure, i.e., after 32 months, further long-term studies are necessary to confirm a consistent pattern.

The variation in growth of cod populations from different localities might require local stock selection or on-growth of local stocks only. However, it should be noted that in the current study the general mass increase in all five groups was in accordance with Björnsson and Steinarsson's (2002) growth model, and from a commercial perspective the difference in weight was not substantial in relation to the rearing time. Therefore, even in the case of there being inherent differences in the growth capacity or growth patterns of cod from different geographical localities across Iceland, these differences may not be substantial enough at the time of harvest to make local stock selection for aquaculture needed. However, any localized variation in the growth patterns of Atlantic cod has important implications for stock management, which in itself warrants further study. This result provides a significant addition to the data on the geographical partitioning and potential local adaptation of the Icelandic cod stock.

For stock comparison, especially in farming, the rearing condition may influence the performance of individuals and families. Therefore, it is possible that maternal effects can contribute to the growth of some families, which has been shown in earlier studies (Tosh et al., 2010). Although the brood-fish in this study were collected from different locations and, therefore, could have been in a different condition prior to stripping, which could have had a different maternal effect, this factor does not satisfy a strict common garden setup, where the parental fish would have needed to be reared in a common environment to minimize possible differences the maternal affect. However, such maternal effects have been shown to decline in cod aquaculture, as the fish grow closer the harvest (Tosh et al., 2010). The differences among the brood fish from these five locations were not significant in weight or in hatching success. Research on brood-fish nutrition has, in some cases, shown controversially results. Lambert and Dutil (2000) showed that nutrition and fecundity is strongly correlated in cod, but Ouellet et al. (2001) noted only limited effects of maternal nutritional condition on hatching success.

Moreover, the pair-wise genetic differentiation F_{ST} among all five groups showed a low value, indicating extensive gene-flow among the five locations and a large effective population size. This finding is similar to recent publications on population structure in Atlantic cod (Nielsen et al., 2003; Pampoulie et al., 2006). However, it should be noted that genetic diversity based on neutral markers such as microsatellites yield little useful information for animal breeders because of the lack of information regarding the quantitative genetic variation of a population (Reed and Frankham, 2001).

In summary, the results of this study show there is no significant difference in mass at harvest due to geographical origin in the Atlantic cod around Iceland. Wild brood-fish show low genetic distance calculated from microsatellite markers, indicating a high gene flow among the five populations.

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II

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Strong phenotypic and genetic correlation between size and first maturity in Atlantic cod *Gadus morhua* L. reared in commercial conditions

Theódór Kristjánsson[1] & Thorvaldur Arnason[2]

[1]Stofnfiskur, Hafnarfjörður, Iceland

[2]Agricultural University of Iceland, Borgarnes, Iceland

Correspondence: T Kristjánsson, Stofnfiskur, Staðarberg 2-4, Hafnarfjörður 221, Iceland. E-mail: theodor@stofnfiskur.is

Abstract

Three-year classes of Atlantic cod *Gadus morhua* were studied throughout their rearing in the eastern coast of Iceland from 2004 to 2011. The growth and status of maturity were recorded during the rearing. For one of the year classes, genetic parameters for body weight and maturity status were estimated from 757 individuals, which were the offspring of 40 dams and 20 sires. The estimate for heritability of body weight was $h^2 = 0.34$ at the average weight of 630 g, and heritability for proportion of maturity was $h^2 = 0.17$ given the same weight. The relationship between body weight and the proportion of mature individuals at first winter revealed a strong genetic correlation of $r_G = 0.90$. The phenotypic relationship between body weight and proportion of maturity was estimated with a Bayesian logistic regression as $P(yi = 1(\text{mature})) = \Phi(\beta 0 + \beta 1_{\text{weight}} + \beta 2_{\text{sex}})$. The best fit yielded $\beta 0 = -2.9320$ with a 95% interval between -3.2807 and -2.5394, $\beta 1_{\text{weight}} = 0.0041$ with a 95% interval ranging from 0.0035 to 0.0046 and $\beta 1_{\text{sex}} = -0.0201$ with a 95% interval from -0.2003 to 0.1445. The gender had no notable effect. This strong phenotypic and genetic correlation in body weight and maturation suggests that an increased growth rate will consequently lead to a higher proportion of mature individuals in the population. As a consequence, genetic manipulations to simultaneously increase growth and delay maturation may present a challenge.

Keywords: *Gadus morhua*, Atlantic cod, heritability, maturity, genetic correlation

Introduction

The Atlantic cod, *Gadus morhua*, in the north Atlantic displays a high variation in age at the onset of maturity that depends on size and environment (Holdway & Beamish 1985; Godø & Moksness 1987; Crandell & Gall 1993; Trippel, Morgan, Frechet, Rollet, Sinclair, Annand, Beanlands & Brown 1997; Begg, Hare & Sheehan 1999; Cardinale & Modin 1999) This relationship has been well-documented for cod offshore of the Icelandic coast. Significant sexual, spatial and temporal variations in age and size at maturity have been demonstrated among cod around the country. Extensive differences have been observed between cod at the north and south coasts of Iceland. This variation appeared to be correlated with growth (Marteinsdóttir & Begg 2002). Due to a decreasing temperature gradient from south to north, the cod grow faster in the warmer sea at the south coast of Iceland and attain sexual maturity at a smaller size and younger age than the slower-growing cod off of the north coast. The water temperature is critical given that a 2°C decrease increases the age at maturity by ~1 year (Drinkwater 2002).

Cod farmed in sea cages show a similar pattern. Well-fed cod can become sexually mature at 1 year of age, and high proportions of cod in sea cages achieve sexual maturity at 2 years old or at the first sea winter in cages (Karlsen, Holm & Kjesbu 1995). During the autumn before the farmed cod enter the first sea winter, changes in photoperiod, temperature and size seem to be key factors that determine maturation (Taranger,

Carrillo, Schulz, Fontaine, Zanuy, Felip, Weltzien, Dufour, Karlsen, Norberg, Andersson & Hansen 2010). When a fish reaches sexual maturity, it will start producing gonads. The production of gonads and spawning is associated with a high reduction in feed intake (Fordham & Trippel 1999). In cod farming, this leads to a considerable loss of body weight and a decrease in flesh quality. The weight of the gonads in a sexually mature cod constitutes 20–25% of its body weight, and the loss of body weight may be reduced by more than 30% during spawning (Karlsen *et al.* 1995). The decreased age at maturity in farmed cod is likely due to the favourable food availability, which leads to faster growth and larger energy stores than in wild populations, most notably evidenced by a higher liver index in farmed cod compared to wild cod (Karlsen, Norberg, Kjesbu & Taranger 2006).

Studies on the Atlantic cod indicate that maturation is both genetically and phenotypically correlated to increased growth. In a Norwegian investigation, the heritability of age at sexual maturity was estimated to be 0.21(Kolstad, Thorland, Refstie & Gjerde 2006). The records were taken on 2 year-old fish reared in three localities. The per cent of fish that were sexually mature at 2 years of age varied between 5% and 88% in the different localities. At recording, the average weight of the fish was just above 2 kg, and the genetic correlations between body weight and sexual maturity were found to be small but positive.

Heritability for age at maturity has been widely studied in fish and especially in Salmonidae, and it has been reported in the range of $h^2 = 0.15–0.48$ in Atlantic salmon (Gjerde, Simianer & Refstie 1994; Wild, Simianer, Gjoen & Gjerde 1994; Gjedrem 2000) and from 0.12 to 0.35 in rainbow trout *Oncorhynchus mykiss* (Walbaum) (Gjerde & Schaeffer 1989; Kause, Ritola, Paananen, Mantysaari & Eskelinen 2003). Moreover, several studies have examined the genetic correlation between growth and sexual maturity in salmonids, and these h^2 values range from 0.11 to 0.5 (Gjerde 1986; Crandell & Gall 1993; Su, Liljedahl & Gall 1999).

The focus of this study is to explore the genetic and phenotypic relationship between size and maturity in cod reared in commercial conditions in Iceland and the effect of maturation on growth. These assessments were performed during the initial establishment of the base population for cod breeding in Iceland.

Materials and methods

Animals used in the calculation

In this study, the following 3-year classes were examined: year class 2003, year class 2004 and year class 2007. The year class 2003 (YC 2003) was hatched at the Marine Research Institute (MRI) in May of 2003. To make YC 2003, a nested mating design was used in which one male was used to fertilize eggs from two females, creating groups of full-sibs and paternal half-sibs. In total, 336 females were mated with 168 males.

After hatching and early rearing at MRI, the juveniles were transferred to a land-based station in Hafnir operated by the company IceCod and reared in one 40-cubic-meter circular tank with a flow-through system of seawater. The rearing temperature was kept constant at 8.3°C with 100% oxygen saturation and a salinity of 30–32‰ with seasonal light. Approximately 13 months after hatching (22 June 2004), the juveniles were transferred to a 90-m circle, 18-m deep sea cage in Berufjörður (64°43′ 42.02″ N, 14°23′ 56.06″ W) on the east coast of Iceland (Fig. 1). The average weight was 270 g.

The year class 2004 (YC 2004) was hatched at MRI in May 2004. The mating and rearing procedures were the same as for the YC 2003. In total, 200 females were mated with 100 males. YC 2004 was transferred to sea cages at 9 months after hatching (2 February 2005) or earlier to a 90-m circle, 18-m-deep sea cage in Berufjörður. The average weight was 100 g.

Year class 2007 (YC 2007) was composed of offspring of YC 2004 and was hatched at MRI in May 2007. The mating and rearing procedures were the same as for the YC 2003 and YC 2004. YC 2007 was transferred ~11 months after hatching (27 April 2008) to a round 80-m circle by 18-m deep sea cage in Berufjörður. The average weight was 100 g.

For the duration of rearing in the sea cage, the cod were fed commercially formulated feed from Fóðurblandan Ltd. in Reykjavík, Iceland containing 18% fat and 45% protein. The regulation of feeding was dependent on appetite. The total rearing time in sea cages was ~32 months for each year class. During that time the fish was reared through two winters which are referred to as first sea winter and second sea-winter. Sea water temperature was measured twice every week throughout rearing. Table 1 gives an overview of the year classes.

Figure 1 Overview of the hatchery and the rearing location of the 3-year classes.

Table 1 Overview of animals in the study

Year classes	Hatched	In Cages	Harvest	Genetic estimates
YC 2003	May 2003	June 2004	Dec 2006	No
YC 2004	May 2004	Feb 2005	Dec 2007	Yes
YC 2007	May 2007	April 2008	Dec 2010	No

Measurements

Weight and maturation status were measured regularly from the transfer to sea cages to harvest. To examine a fish for maturation, the fish was sacrificed, its abdomen was opened, and the sex organs were visually examined. Maturation was scored as 0 (not mature) if the gonads were undeveloped and as 1 (mature) when development had obviously started, which is evidenced by larger eggs and the ovaries filling more of the body cavity or by visible sperm or eggs. An overview of the dates of assessment is given in Table 2.

Heritability and genetic correlation

Heritability and genetic correlation between body weight and maturation were estimated for YC 2004. In May 2006, a random sample of 1000 individuals from the sea cages were sacrificed and assigned to families by DNA genotyping. From this sample, 757 individuals were assigned to 40 families that are 40 females and 20 males. Genotyping was determined from the following 16 microsatellites: Gmo8, Gmo19, Gmo37 (Miller, Le & Beacham 2000), Tch11, Tch14 (O'Reilly, Canino, Bailey & Bentzen 2000), Gmo38 (Jakobsdottir, Jorundsdottir, Skirnisdottir, Hjorleifsdottir, Hreggvidsson, Danielsdottir & Pampoulie 2006), PGmo38, PGmo49, PGmo61-FRb, PGmo71, PGmo74, PGmo87, PGmo94, PGmo100, PGmo124 and PGmo134 (Skirnisdottir, Pampoulie, Hauksdottir, Schulte, Olafsson, Hreggvidsson & Hjorleifsdottir 2008).

The genetic parameters were analysed using REML to fit mixed linear models using VCE 5 software (Kovac & Groeneveld 2003). The model used for the analysis was as follows: $y = Xb + Za + e$, where y is the vector of individual body weights and status of maturation scored 0 or 1. The factor b in this model is a vector of fixed effects, which includes gender; a is the vector of random additive genetic effects of individual animals; and e is the uncorrelated residuals error. Transformation of heritability of maturation from observed to underlying scale was done according to (Dempster & Lerner 1950) where $h^2_o = h^2 \times \{p(x_p)^2 \ (\Phi_p(1 - \Phi_p)^{-1}\}$. Whereas h^2_o is heritability on observed scale, h^2 is heritability on underlying scale, Φ_p is frequency of incidence and $p(x_p) = (2\pi)^{-1/2} \exp(-x^2_p/2)$ is the height of the standardized normal distribution at truncation point.

Statistics

All statistics were calculated using R 2.14.0 software (R Development Core Team 2013). The difference in body weight among the three-year classes was tested with a one-way ANOVA. The difference in proportion of maturation among the

Table 2 Overview of the timing of measurements

Time of measure	Maturation estimated	Weight measured	YC 2003 number	YC 2004 number	YC 2007 number	Days from hatching
Dec-1		X	150	150	150	550
May-1	X	X	200	1000	200	730
Sept-1		X	100	100	100	880
Dec-2		X	100	760	100	950
March-2	X	X	200	200	200	1050
Sept-2		X	100	150	100	1200
Dec-2		X	16.300	21.800	9000	1300

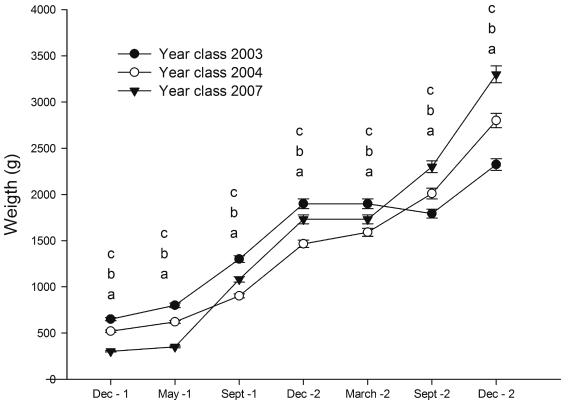

Figure 2 The mean body weight of each year class measured seven times over 24 months in rearing. Vertical whiskers s.e. Different letters denote significant differences (one-way ANOVA, $P < 0.05$).

3-year classes and the difference due to gender were tested using the multiple proportions test (Newcombe 1998). An α level of 0.05 was used to determine significance.

The YC 2004 was used for regression analysis between maturity status and weight, and it was fitted with a binary response regression with a profit link using Gibbs sampler where maturation (scored 0 or 1) is a binary observation $y_1, \ldots, y_{757}$ associated with the ith response of weight and gender of the fish. Model 1 then becomes $P(yi = 1) = \Phi(\beta 0 + \beta 1_{\text{weight}} + \beta 2_{\text{sex}})$. This regression was fitted using the package bayesm in R 2.14.0. The regression model was compared with the observed proportions of maturity in year classes YC 2003 and YC 2007. The estimates of the proportion of maturity were performed with Bayesian inference assuming that the observations were binomially distributed, where posterior distribution is $p(\theta|m) = \beta(\alpha + m, \beta + n - m)$; prior is uniformed ($\alpha = 1$, $\beta = 1$); m is the number of mature fish in the sample and n is the total number of fish in the measured sample. Extract posterior quintiles were obtained by direct posterior simulation and given a 95% interval.

Results

Growth and maturation

Significant differences in weight were observed among the three groups for all measurements between the transfer to sea cages to the harvest (see Fig. 2). The estimates of maturity at the first sea-winter were significantly different among all 3-year classes ($\chi^2 = 182.9172$, df = 2, $P < 2.2e^{-16}$), and all year classes were mature at the second sea-winter (Fig. 3).

Heritability of traits

The heritability on observed scale for maturation at the first winter was estimated to be $h^2_o = 0.17$ with a SE = 0.041 given the incidence of maturation among all families was estimated at 0.30 with SE = 0.06. Transforming of heritability to

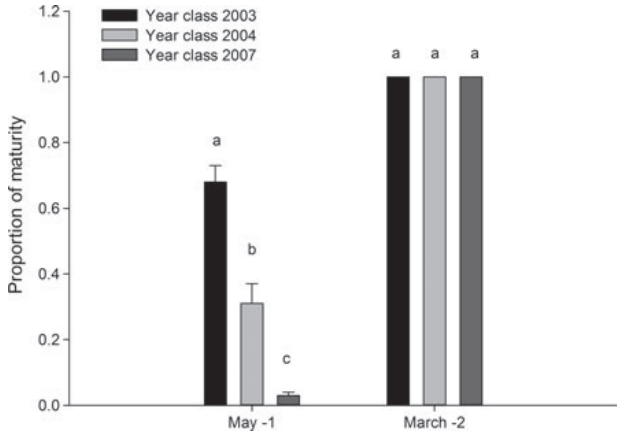

Figure 3 The estimates of maturity. May-1 denotes the estimate at first sea-winter, and March-2 is the estimate at second sea-winter. Vertical whiskers s.e. Different letters denote significant differences ($P < 0.001$).

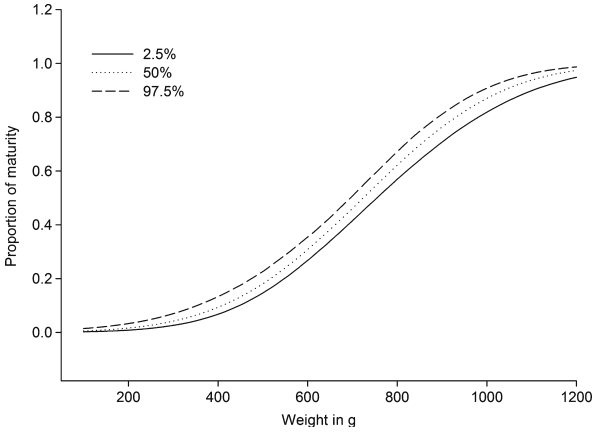

Figure 4 Posterior distribution of mature proportion with increasing body weight. The 2.5th, 50th and 97.5th percentiles of the posterior distribution are plotted for each weight. The interval between the dashed lines corresponds to a 95% interval estimate for proportion of maturity.

underlying scale is $h^2 = 0.28$ with SE = 0.06. The heritability for body weight was estimated as $h^2 = 0.34$ with SE = 0.04. The genetic correlation between body weight and maturation was relatively high at $r_G = 0.90$. Gender had no notable effect on weight or maturation.

Phenotypic correlation between weight and proportion of maturation

Logistic regression between maturity and weight yielded $\beta 0 = -2.9320 + \beta 1_{weight} = 0.0041 + \beta 2_{sex} = -0.0201$. Table 3 lists the quintiles for the β parameters. The effect of gender is not significant, while zero is within the 95% credibility interval. Figure 4 shows the graph of the likelihood of maturity as a function of weight.

The observed maturity proportions in YC 2003 and YC 2007 are not significantly different from the predicted values calculated from the regression model. The observed proportion of maturity in YC 2003 at the first sea winter is 0.66 with a 95% interval from 0.592 to 0.722. The proportion of maturity predicted by the model is 0.620 with a

Table 3 Quintiles for β parameters based on 9000 valid draws (burn-in = 1000)

β parameters	2.5%	5%	50%	95%	97.5%
β0	−3.2807	−3.2305	−2.9320	−2.6012	−2.5394
$\beta 1_{weight}$	0.0035	0.0036	0.0041	0.0045	0.0046
$\beta 2_{sex}$	−0.2003	−0.1705	−0.0201	0.1187	0.1445

95% interval between 0.569 and 0.672. The observed proportion of maturity of YC 2007 at the first sea winter is 0.03 with a 95% interval of 0.013–0.050, and the predicted value is 0.05 with a 95% interval of 0.035–0.078 (Fig. 5).

Discussion

The results of this study show a strong phenotypic and genetic relationship between body weight and maturity for cod in rearing at the first sea winter. The relationship between these two traits was stronger than those that had been published for Atlantic cod and Salmonidae (Gjerde 1986; Crandell & Gall 1993; Su *et al.* 1999). Still, the estimates of heritability for both body weight and maturity are in agreement with earlier findings. The estimated heritability for maturation was 0.17± (SE = 0.07), on an observed scale which is not significantly different from previous studies in Norway (Kolstad *et al.* 2006). The heritability estimate for body weight $h^2 = 0.34$ is similar to that in an earlier publication (Gjerde, Terjesen, Barr, Lein & Thorland 2004). A remaining question is whether an interaction exists between growth rate and maturity, e.g., maturity enhances or decreases growth. These factors may be difficult to tease apart with a genetic model. However, in this study, Fig. 2 indicates that none of the 3-year classes are affected by the different maturity proportions at the first sea-winter, but a great difference is seen in growth after the second sea-winter. A second consideration is the rearing environment. The average sea temperature from January

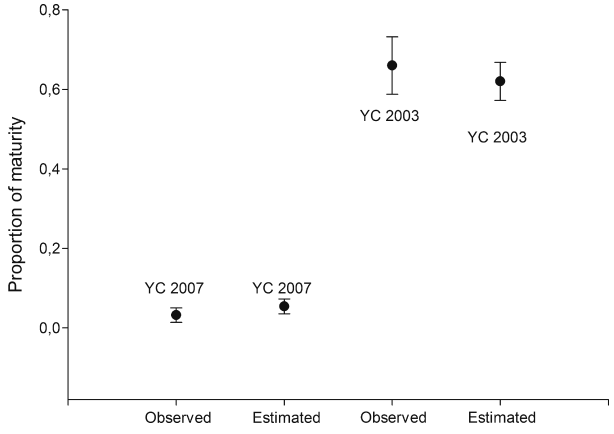

Figure 5 Comparison of the observed proportion of maturity in year classes YC 2003 and YC 2007 to the estimated proportion from the regression model. Vertical whiskers on estimated and observed values are the 95% interval.

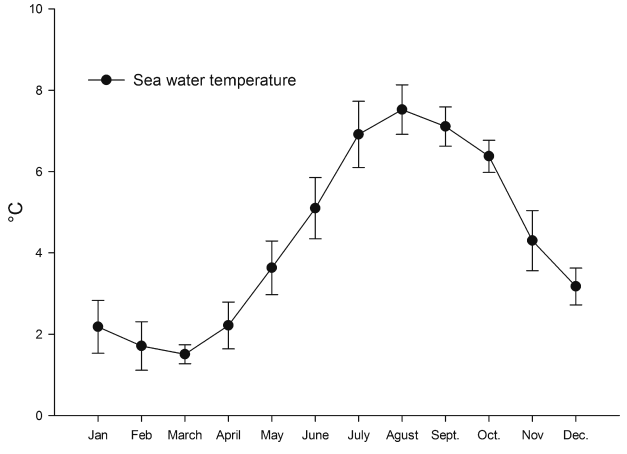

Figure 6 Sea water temperature measured at rearing side in Berufjörður. Vertical whiskers are the standard deviation of the temperature in the given month.

to May is 1.9°C (Fig. 6), which consequently leads to a low metabolic rate, slowing down somatic growth and the development of gonads. At higher rearing temperatures surrounding the time of maturity or the first sea winter, the interaction between somatic growth and maturity may become more significant, which may be a necessary consideration in a genetic valuation model.

Size seems to be the most important factor in the determination of maturity in the cod. Figure 5 demonstrates that, the difference between YC 2007 and YC 2003 is approximately double in body weight, and the proportion of maturity increases from 3% to above 60%. The importance of size in the fish maturity determination process has been studied for a variety of fish species, and the effect of age and size in that process has often been debated (Taranger *et al.* 2010). The phenotypic plasticity in the determination of maturity is considerable, and it seems to be no indication for fixed age or size for fish species like Salmonidae, which have been the most studied (Morita & Fukuwaka 2006). However, the significance of genetics has been demonstrated in breeding and, especially in salmon and rainbow trout, has been implemented in breeding for those species (Gjedrem & Baranski 2009).

Light also has a strong influence on maturity both to prevent and induce maturity. Continuous light treatment in an indoor tank can prevent maturity in cod (Hansen, Karlsen, Taranger, Hemre, Holm & Kjesbu 2001; Norberg, Brown, Halldorsson, Stensland & Björnsson 2004; Karlsen *et al.* 2006), but this has turn out to be less successful in sea cages, where the strong ambient light in the cages relative to the artificial light may cause interference (Taranger, Aardal, Hansen & Kjesbu 2006). However, recent findings have shown that the timing of the artificial light exposure in cages is critical, and can prevent maturation of cod (Imsland, Hanssen, Foss, Vikingstad, Roth, Bjørnevik, Powell, Solberg & Norgerg 2013). For cod in indoor facilities, as for brood-fish, it is relatively easy to alter the photoperiod to get off-season spawning, which is a common practice in brood-fish management and breeding (Taranger *et al.* 2010).

Researches, on the effects, of nutrition on maturation show controversial results. Some studies have showed that maturation depends on nutritional status of the fish (Kjesbu, Klungsøyr, Kryvi, Witthames & Walker 1991; Lambert & Dutil 2000), but Karlsen *et al.* (1995) found that 9 weeks of starvation did not significantly affect the occurrence of early maturation. Moreover, the variation of protein, fat and carbohydrate content in the diet did not affect early sexual maturation in cod (Karlsen *et al.* 2006).

The prediction model that was made from YC 2004 holds for predicting the proportion of maturity in YC 2003 and YC 2007 (see Fig. 5). It is easy to photo-manipulate cod brood-fish, and therefore, the weight of the cod juveniles before entering the first sea-winter can be controlled with precision. Assuming that the model holds, controlling the size of juveniles before entering the first sea-winter is one way to minimize the harmful effect maturity has in cod farming.

In conclusion, body weight and maturity in cod are highly linked. This makes the simultaneous selection for increased body weight and a lowered proportion of maturity in cod farming impractical. The likelihood of maturation in cod farmed in cages exceeds 50% as cod reaches 600 g at the first sea-winter. The effect of the first maturity in rearing is mild but the second maturity has an immense effect on growth rate.

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III

HERITABILITY OF ECONOMICALLY IMPORTANT TRAITS IN THE ATLANTIC COD *Gadus morhua* L.

Theódór Kristjánsson*

* Corresponding author

E-mail address:

theodor@stofnfiskur.is

Telephone: 354-5646300

Fax: 354-5646301

Stofnfiskur

Staðarberg 2-4

IS-221 Hafnarfjörður, Iceland

Thorvaldur Arnason

Second author

E-mail Address

info@ihbc.se

Telephone +46 -22460109

Agricultural University of Iceland

IS-311 Borgarnes, Iceland

Abstract

During the development of breeding program for Atlantic cod *Gadus morhua* L., in Iceland, genetic parameters were estimated for 1402 individuals, which were assigned with DNA profiling to 140 dams and 70 sires. The cod was reared in cages on the eastern and western coasts of Iceland from 2004 - 2005. At the average body weight of 1.8 kg, the estimated heritability (h^2 ± s.e.) for body weight, gutted weight and the condition factor (CF) were 0.34 ± 0.04, 0.34 ± 0.04 and 0.24 ± 0.06, respectively. Genetic correlation (r_G) in body weight between the two rearing locations was estimated as 0.95, which reflects a low GxE interaction.

The estimated heritability for hepatosomatic index (HSI) and fillet yields was 0.061 ± 0.04 and 0.04 ± 0.04, respectively. HSI and fillet yields were highly genetically correlated with body weight or 0.67 and 0.82, respectively. The genetic correlation between the CF and body weight was estimated as 0.31.

There appears to be substantial amount of additive genetic variation for body weight suggesting that selection is likely to be successful. Low heritability for fillet yields and the HSI indicates less promise of genetic improvement. Assigning of parentage to individuals with DNA profiling was 80% successful.

Keywords: *Gadus morhua*; Atlantic cod; Heritability; DNA profiling

Introduction

The Atlantic cod *Gadus morhua* has been identified as a suitable candidate for marine aquaculture in Northern Europe because of its high market value and declining natural stocks. This has led to an increased interest in the intensive cultivation of cod, both by aquaculture and sea ranching (Norberg, Brown, Halldorsson, Stensland & Björnsson 2004). The domestication of cod began in Iceland at the beginning of the last decade. Test rearing of cod in sea cages has shown that the growth rates need to be improved. It takes approximately 26-32 months for 150 g juveniles to grow to 3 kg (Björnsson, Steinarsson & Árnason 2007; Kristjánsson 2013). By using thermal growth coefficient (TGC) of 3.4 as for salmon, this is half the expected growth rate for salmon under the same conditions (Johnston, Bickerdike, Xuejun, Dingwall, Nickell, Alderson & Campbell 2007). However, with selective breeding the growth rate of Atlantic salmon *Salmon salar* L. has approximately doubled in seven generations, and the expected genetic improvement is approximately 14% for every generation (Gjøen & Gjerde 1997). Atlantic salmon is a good example of how selective breeding has increased productivity in aquaculture, and it is believed that selective breeding could play a similar role in cod farming (Gjedrem & Baranski 2009).

An increased growth rate is most often the first trait chosen for improvement in aquaculture (Kinghorn 1983; Hershberger, Myers, Iwamoto, McAuley & Saxton 1990; Gjedrem 1983; Gjerde 1986). Larger fish at harvest are considered a key factor in increasing productivity in aquaculture. However, even though they are less important than the growth rate, traits involving processing could also become valuable in cod farming. Fillet yield is probably the most important factor in cod processing. Yields are generally higher in farmed cod than in wild cod, but it should be noted that various factors influence the yields of wild cod, such as age, diet and the time the fish were caught (Margeirsson, Jonsson, Arason & Thorkelsson 2007; Love 1980).

Moreover, processing pre- or post-rigor influences yields in cod farming, and processing of cod pre-rigor has been chosen due to less gaping and better yields. The trend in wild catching seems to be that morphology is linked with fillet yields; therefore, a higher condition factor leads to thicker muscle and higher yields (Eyjolfsson, Arason, Þorkelsson & Stefánsson 2001). This phenomenon has also been observed in fresh water fish such as common carp *Cyprinus carpio* L. (Cibert, Fermon, Vallod & Meunier 1999).

The percentage of liver weight to body weight or hepatosomatic index (HSI) in farmed cod is usually higher than in wild cod (Karlsen, Norberg, Kjesbu & Taranger 2006). High energy feed tends to increase the HSI, but the liver is a less valuable product than flesh. Feed with varying protein and lipid content has not revealed an altered protein and lipid ratio in flesh (Jobling, Knudsen, Pedersen & Dos Santos 1991). An increased lipid intake by cod increased the lipid in the liver but not in the flesh. In this perspective marine fish differ from salmonids since salmon tend to store extra energy as lipid in the flesh. Although the liver could be a valuable by-product in cod farming, it is important that the feed used results in high somatic growth rather than increased lipid content of the liver.

Little is known about how genetics affects these traits in cod and how much these traits are inter-correlated or correlated with growth. Moreover, it is not known if increased body weight as a consequence of breeding has an effect on other production traits. Research in farmed salmonids, such as coho salmon *Oncorhynchus kisutch* L. and rainbow trout *Oncorhynchus mykiss* (Walbaum), has revealed low heritability of processing traits such as fillet yields (Neira, Lhorente, Arande, Diaz, Bustos & Alert 2004; Kause, Ritola, Paananen, Mantysaari & Eskelinen 2002). This has led to low expectations for improving fillet yields with the help of genetic selection programs.

The primary aims of this study were to estimate the genetic (co)variation of economically important traits in Atlantic cod reared on the eastern and western coasts of Iceland and to explore the possible gene-environment interaction (GxE) among the two rearing locations. This study was conducted during the development of the Icelandic cod breeding, where the base population was developed from wild stocks around Iceland.

Materials and methods

Animals.

The fish in this study were offspring of wild fish caught off the south and north coasts of Iceland between April and May 2003. The broodfish were captured using gillnets brought onboard commercial fishing vessels. They were hand stripped onboard the vessels and killed post-stripping; a fin clip sample was taken for DNA analysis. Approximately 200 ml of eggs were collected from each of 336 females and placed in a 1000 ml box. Within half an hour the eggs were fertilized and 800 ml of seawater added. After one hour the eggs were cleaned and new seawater added. Subsequently, the eggs were transferred to the Icelandic Marine Research Institute (MRI). A nested mating design was used where one male was used to fertilize eggs from two females, creating groups of full-sibs and paternal half-sibs. Eggs from all females were mixed on day 5 after fertilization and DNA profiling was later used to assign individual fish to families. The eggs hatched in May 2003. After hatching the larva and juveniles were reared in 40 cubic meters tanks on a land-based station. Approximately 13 months after hatching the juveniles were transferred to sea cages. In June 2004, 9,500 juveniles were transferred to sea cage in Ísafjarðardjúp (66° 0'32.29"N, 22°54'54.30"W) and 37,000 juveniles were transferred to sea cage in Berufjörður (64°43'42.02"N, 14°23'56.06"W) (Figure 1). At both locations the sea cages were 90-m in circumference, 18-m deep. After transfer to cages the fish were fed with commercial feed from Fóðurblandan Ltd. in Reykjavik formulated for cod, containing 53% protein and 18% lipid. The feeding regime depended on appetite and sea temperature with high consumption during the summer and lower during the winter (Figure 2).

Traits

After 18 months of rearing in cages (December 2005) a random sample of 1750 fish were sacrificed from cages at both rearing locations. In total 750 individuals were sacrificed at Berufjörður and 1000 at Ísafjarðardjúp. The body weight, gutted weight, condition factor (CF), HSI and fillet yields were measured on all individuals. CF was calculated as 100 x (body weight in grams) x (body length in $cm^3)^{-1}$, HSI was calculated as 100 x (liver weight in grams) x (body weight in grams)$^{-1}$ and fillet yield, was calculated as 100 x (weight of both fillet, in grams) x (gutted weight in grams)$^{-1}$. The fish were filleted using the Baader 189 filleting machine (www.baader.com).

Fillet yields were only estimated at Berufjörður, and the effects of gender were tested with one-way ANOVA. The effects of gender and rearing location on body weight, gutted weight, CF and HSI were tested with two-way ANOVA in R version 2.15.3 (R Development Core Team 2013).

Statistical model

The genetic parameters were analyzed for 1402 individuals, which were assigned to 140 dams and 70 sires with DNA profiling. Parentage assignment was performed using six microsatellite markers (Gmo8, Gmo19 and Gmo37 (Miller, Le & Beacham 2000), Tch11 and Tch14 (O'Reilly, Canino, Bailey & Bentzen 2000) and Gmo38 (Jakobsdottir, Jorundsdottir, Skirnisdottir, Hjorleifsdottir, Hreggvidsson, Danielsdottir & Pampoulie 2006). The microsatellite markers were scored by the company Prokaria Reykjavík. The software COLONY 2.0 (Jones & Wang 2010) was used to estimate the probability of paternity and maternity among the sacrificed fishes, based on microsatellite markers. Individuals with an inferred probability of maternity or paternity below 95% were excluded from the analysis.

REML was used to fit mixed linear models using VCE 5 software (Kovac & Groeneveld 2003).The model (**Model 1**) used for the analysis was as follows: $\boldsymbol{y = Xb + Za + e}$, where $\boldsymbol{y}$ is the vector of the body weight, gutted weight, CF, length, fillet yield and HSI of the fish and $\boldsymbol{b}$ is a vector of fixed effects, which included sex and the two rearing location. As fillet yields were only estimated at Berufjörður the trait was treated as a missing value for Ísafjarðardjúp. The Genotype-environment interaction (GxE) analysis was performed for body weight only, using Model 1. We assessed GxE by estimating genetic correlations between traits recoded in different environments. The weights were divided into two traits ($weight_1$ and $weight_2$), depending on whether individual fish from the same family were weighed at the location in Berufjörður or Ísafjarðardjúp. The vector $\boldsymbol{y}$ is the vector of the $weight_1$ and $weight_2$ and $\boldsymbol{b}$ is a vector of fixed effects, which included sex and the two rearing location. The vector $\boldsymbol{a}$ is a vector of random additive genetic effects of individual animals.

The maternal effect was estimated for body weight. The model (**Model 2**) used for the analysis was as follows: $\boldsymbol{y = Xb + Za + Wm + e,}$ where y is the body weight, $\boldsymbol{b}$ is a vector of fixed effects, which includes rearing location and sex. Vector $\boldsymbol{a}$ is random additive genetic effects of the animals and $\boldsymbol{m}$ is a vector of random maternal genetic effects (mother). The matrices **X, Z** and **W** are known design matrices assigning observations to levels of **b, a**, and **m**, respectively. The vector **e** represents the random residual effects in both models.

Results

In total 348 individuals out of 1750 sacrificed individuals, about 20% of all samples were excluded from analysis because they fell below the 95% of inferred probability of paternity or maternity. The remaining 1402 individuals included in the analysis were assigned to 140 dams and 70 sires.

Body weight, gutted weight and CF were influenced by rearing location ($P < 0.001$) but not gender (Tables 1 and 2). The fish were significantly heavier at Ísafjarðardjúp than in Berufjörður as a consequence of higher rearing temperatures (Figure 2), while the CF was higher in Berufjörður (Table 1). Sires at Berufjörður had significantly higher HSI ($P < 0.01$) but a fillet yield was not effected by gender (Tables 1 and 2).

The direct heritability (h^2) of body weight, estimated by Model 2, was moderate and significantly different from zero at the 5% level of significance, whereas the estimated maternal effect (m^2) was not significant (Table 3). The heritability for fillet yields and HSI, estimated with Model 1, were not significantly different from zero, whereas the estimated heritability for gutted weight and condition factor were both significantly different from zero.

The genetic correlation between body weight, gutted weight, CF, HSI and fillet yields, as estimated with Model 1, were all significant from zero (Table 4).

The genetic correlation between body weights at the two rearing locations, as estimated with Model 1, was $r_G = 0.95 \pm 0.06$. The heritability for body weight in Berufjörður ($weight_1$) was 0.44 ± 0.13 and for body weight in Ísafjarðardjúp ($weight_2$) was 0.40 ± 0.13. All estimates were significantly different from zero.

Discussion

Atlantic cod in Iceland shows moderate additive genetic variance for body weight, suggesting that selection for increased body weight is likely to be successful. The heritability estimate for body weight at harvest was 0.31 ± 0.06 and maternal effect was 0.03 ± 0.02. This agrees with other published data on heritability for cod (Tosh, Garber, Trippel & Robinson 2010). In fact, the heritability for body weight in both salmonids and marine fish universally seems to be 0.3-0.4 (Jónasson, Gjerde & Gjedrem 1997; Elvingson & Johansson 1993; Winkelman & Peterson 1994; Gjedrem 2000; Kolstad, Thorland, Refstie & Gjerde 2006; Tosh *et al.* 2010). The results indicate a high genetic correlation ($r_G = 0.95$) for body weight between two rearing locations, reflecting a low GxE interaction with no re-ranking of families between the two locations, whereas genetic correlation above 0.7 is consisted to have negligible or marginal effects of GxE interaction (Gjedrem & Baranski 2009). Although the magnitude of GxE in fish generally is considered to be low (Maluwa, Gjerde & Ponzoni 2006; Dupont-Nivet, Vandeputte, Vergnet, Merdy, Haffray, Chavanne & Chatain 2008; Reddy, Gjerde, Tripathi, Jana, Mahapatra, Gupta, Saha, Sahoo, Lenka, Govindassamy, Rye & Gjedrem 2002; Standal & Gjerde 1987), several studies report significant interactions for growth traits (Ayles & Baker 1983; Sylven, Rye & Simianer 1991; Jónasson *et al.* 1997). It is therefore important to address the possible consequences of GxE, especially with regard to choice of selection criteria, types and number of test environments and the development of one versus several, specialised genetic lines targeting specific sets of production environments. Our finding has an implication in controlling the costs of cod breeding systems in locations such as Iceland.

223 Although growth is better at Ísarfjarðardjúp, due to higher rearing temperature,
224 it should be noted that in the current study the body weight increased at both rearing
225 locations in accordance with the growth model of Björnsson *et al.* (2007), which has
226 proven accurate for growth of cod in the Faroe Islands, Northern Norway and Iceland
227 (Kolbeinshavn, Vestergaard, Patursson & Gislason 2012; Kristjánsson 2013).
228 The heritability of fillet yields in this study was estimated at $h^2 = 0.04 \pm 0.04$
229 and was not significant from zero. Direct selection for increased fillet yield would
230 thus not be a feasible option in a breeding program. One limitation of this study is that
231 the estimates are taken in December and it is not known whether the time of harvest
232 could have given different results. It is known from cod fisheries is that the fillet yield
233 is considerably influenced by the time of catching (Margeirsson *et al.* 2007). This may
234 also be relevant in cod farming and therefore the effects caused by the time of harvest
235 in farmed cod need to be studied in more detail. However, farmed cod shows higher
236 yield in processing compared to wild cod (Rätz & Lloret 2003). This gives the
237 opportunity of controlling the time of harvest and feeding for maximizing fillet yields
238 through management.
239 Studies on fillet yields that have been conducted in salmonids have shown low
240 heritability estimates in line with this study. Kause *et al.* (2002) estimated the
241 heritability of fillet yields in rainbow trout at $h^2 = 0.03$ and Neira *et al.* (2004)
242 estimated the heritability of fillet yields at $h^2 = 0.11$ in coho salmon. Both of these
243 studies reveal a high genetic correlation between body weight and fillet yields.
244 In an Icelandic study conducted in wild cod, a high positive phenotypic
245 correlation was shown between condition factors and fillet yields (Eyjolfsson *et al.*
246 2001). The current study illustrated a high genetic correlation between condition
247 factors and fillet yields. Additional studies need to be carried out to determine how the

condition factor correlates with the overall thickness of the head and tail regions of the fillet, whereas the head region is the more valuable part of the fillet. Furthermore, whether the condition factor can be used as an indirect indicator of thicker and more valuable fillets in a cod breeding program needs to be studied in greater detail. The estimated heritability for the condition factor was $h^2 = 0.24$; therefore, one can expect a considerable response from selection for that trait.

The heritability estimate for HSI was low and the trait can therefore not be expected to be improved with breeding. Farmed cod, which are fed high energy feed, have a tendency for larger energy stores in the liver and have a higher HSI compared to wild cod (Karlsen *et al.* 2006). Lipid and protein intake by farmed cod is the main factor influencing HSI. Different feed could have altered the variance and heritability observed implying needs for further study. The estimated HSI in this study is consistent with earlier research on HSI in farmed cod in Iceland (Árnason, Björnsdottir, Arnarsson, Árnadottir & Thorarensen 2010). Liver in farmed cod has a high proportion of omega – 3 fatty acids and therefore can be an important by-product in cod farming. Improved feeding management seems to be a more efficient way to lower the HSI than breeding.

This study revealed a high genetic correlation between body weight and both fillet yields and HSI. It should notice that genetic correlation among body weight, CF, HSI and fillet yield have high standard error (table 4). This indicates that more data may be needed for more accurate estimates. These factors may be difficult to separate with the genetic model used in this study so alternative statistical methods may be needed to avoid this problem. Estimating the heritability at a standard weight instead of a standard age could be an option to reduce the effect of body weight (Powell, White, Guy & Brotherstone 2008). Whether such an alteration will yield better

estimates of the heritability and variance components is yet to be tested in cod farming.

The current results highlight a potential concern regarding the exclusion power of parental assignment. In this study, more than 20% of the population fell below the given exclusion threshold. The accuracy of parental assignment relies heavily on the reliability of marker information used in the relationship inference. However, both genotype and human errors can be quite common in practice and are difficult to avoid (Wang 2004). Such typing errors and mutations can have a devastating effect on relationship inference if they are not accounted for. Several empirical studies verified the importance of typing errors in affecting parentage determinations (Blouin, Parsons, Lacaille & Lotz 1996; O'Reilly, Herbinger & Wright 1998). This should be explored carefully by implementing DNA profiling as a way of pedigree reconstruction in a breeding system, such as cod breeding.

In conclusion, there is substantial genetic variation for body weight in the Atlantic cod. Therefore, a breeding program could be successful, as has been well-documented in salmonids. However, the low heritability of processing traits, such as fillet yields and HSI, provides little promise of genetic improvement.

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Figure legends

Figure 1. Overview of the hatchery location at the Marine Research Institute (MRI) and the rearing locations at Berufjörður and Ísafjarðardjúp.

Figure 2. Rearing temperatures in Berufjörður and Ísafjarðardjúp from the time of transfer to cages (June 2004) to the time of harvest (December 2005).

Table 1. Descriptive statistics for body weight, gutted weight and condition factor (CF), and number of individuals, measured in Berufjörður and in Ísafjarðardjúp. All figures are shown with one standard deviation (SD).

Location	Gender	Body weight (kg)	Gutted weight (kg)	CF	Number
Berufjörður	Male	1.670 (0.295)[a]	1.252 (0.241)[c]	1.40 (0.23)[e]	295
Berufjörður	Female	1.684 (0.387)[a]	1.296 (0.341)[c]	1.39 (0.26)[e]	304
Ísafjarðardjúp	Male	1.914 (0.422)[b]	1.435 (0.364)[d]	1.36 (0.22)[f]	390
Ísafjarðardjúp	Female	1.952 (0.488)[b]	1.501 (0.412)[d]	1.37 (0.20)[f]	413

Different letters in superscript within a column and trait indicate significant differences, *two way ANOVA (P < 0.001), Adjusted R-squared* for body weight, gutted weigth and CF were *0.077, 0.059 and 0.0061,* respectively.

478 **Table 2**. Descriptive statistics for hepatosomatic index (HSI) and fillet yields, and number of
479 individuals, measured in Berufjörður and in Ísafjarðardjúp. All figures are shown with one
480 standard deviation (SD).

Location	Gender	HSI %	Fillet yields %	Number
Berufjörður	Male	13.0 (2.4)[a]	56.0 (6.6)[c]	295
Berufjörður	Female	12.6 (2.4)[b]	57.1 (4.9)[c]	304
Ísafjarðardjúp	Male	12.4 (2.3)[b]		390
Ísafjarðardjúp	Female	12.6 (2.5)[b]		413

481 Different letters in superscript within a column and trait indicate significant differences, HSI was
482 tested with *two way ANOVA (P < 0.01),* Fillet yields tested with *one way ANOVA (P = 0.19).*
483 *Adjusted R-squared* for HSI and Fillet yields were *0.004 and 0.0011,* respectively.

Table 3. The estimates (±s.e) of genetic parameters. The variance components, for body weight (kg) were estimated with model 2, while variance components for gutted weight (kg), CF = condition factor and HSI = hepatosomatic index were estimated with model 1.

Component	Symbol	Body weight	Gutted weight	CF	HSI	Fillet yields
Animal	h^2	0.31± 0.06	0.34 ± 0.04	0.24 ± 0.06	0.061 ± 0.04	0.04 ± 0.04
Mother	m^2	0.03 ± 0.02				
Residuals	e^2	0.66 ± 0.07	0.66 ± 0.05	0.76 ± 0.06	0.94 ± 0.06	0.96 ± 0.05
Total	σ^2_p	0.16 ± 0.02	0.11 ± 0.02	0.06 ± 0.02	66.4 ± 0.28	10.9 ± 0.55

h^2, m^2 *and* e^2 = proportion of phenotypic variance associated with additive genetic, maternal and residuals effects, σ^2_p = phenotypic variance.

490 **Table 4**. The estimate (±s.e) of the genetic correlation between body weight, gutted weight,
491 condition factor (CF) and hepatosomatic index (HSI) using Model 1.

Traits	Body weight	Gutted weight	CF	HSI
Gutted weight	0.99 ± 0.01			
CF	0.31 ± 0.15	0.40 ± 0.10		
HSI	0.67 ± 0.25	0.42 ± 0.20	0.42 ± 0.12	
Fillet yields	0.82 ± 0.11	0.80 ± 0.15	0.80 ± 0.21	0.69 ± 0.24

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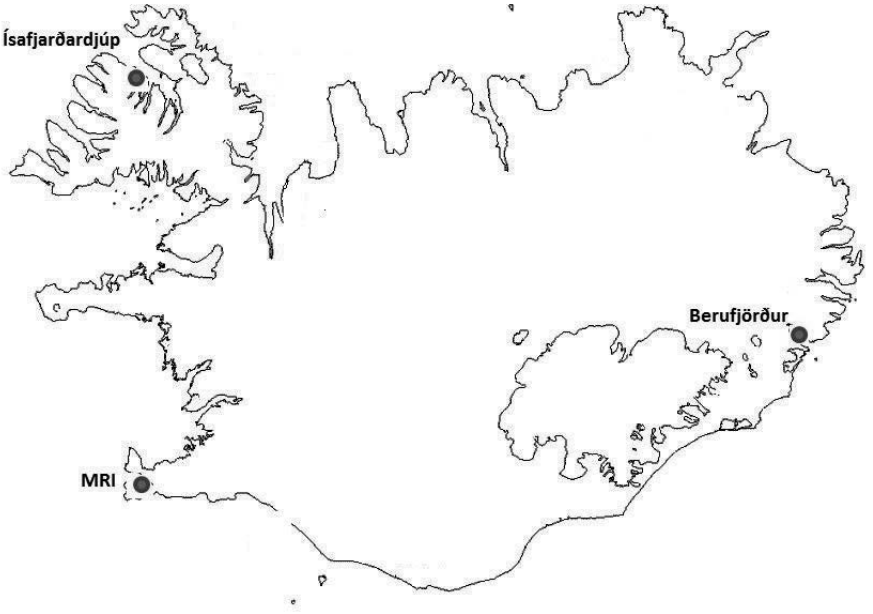

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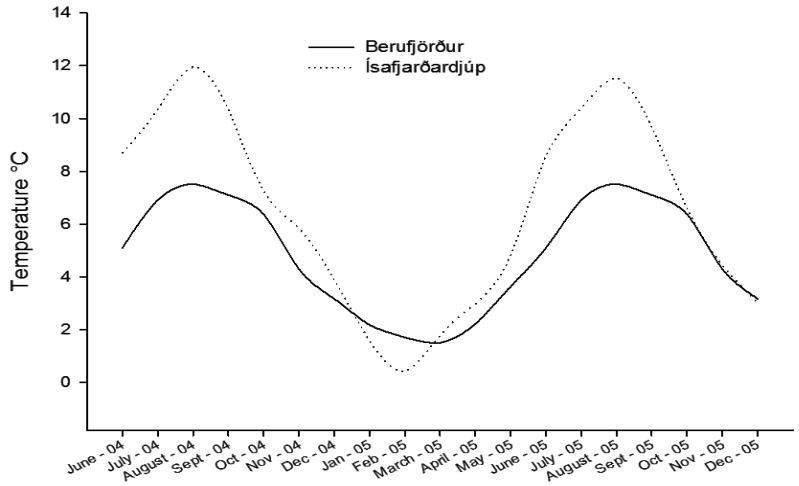
Berufjörður
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Temperature °C
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Feb - 05
March - 05
April - 05
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IV

Effects of imbalanced family structure and DNA profiling on genetic gain in Atlantic cod *Gadus morhua* L breeding program.

Theódór Kristjánsson*

* Corresponding author

Email address:

theodor@stofnfiskur.is

Telephone: 354-5646300

Fax: 354-5646301

Stofnfiskur Staðarberg 2-4

221 Hafnarfjörður, Iceland

Thorvaldur Arnason

Second auther

Email Adress

info@ihbc.se

Telephone +46 22460109

Agricultural University of Iceland,

IS-311 Borgarnes, Iceland.

Abstract

We estimate the survival in Atlantic cod at tagging size 180 days at post hatch (dph) reared in commercial cod breeding program from 2010 - 2011. The survival among families were approximated with gamma distribution which gave a best fit $\alpha = 0.0.64$ and $\beta = 5.34$. Approximately 50% of all individuals were within 14% largest families. The estimated heritability, (± s.e.), for survival at 180 dph was $h^2 = 0.02$ (± 0.10) thus not significantly different from zero.

The effect of different survival among families gave an imbalanced family structure. The genetic gain was explored in a simulation model and compered with model where the survival was balanced among families. The comparisons involved 50, 100 and 200 families, two normally distributed traits and one binary trait.

Balanced design had a significantly higher gain for normally distributed traits and binary trait for all comparisons. The accuracy of estimated breeding values was not significantly different between balanced and imbalanced design. However the estimated breeding values were significantly higher for selected parentage fish in balanced design. The rate of inbreeding (ΔF) among all comparisons did not differ significantly ranging from 0.86 to 1.10

Keywords: *Gadus morhua*; Atlantic cod; survival; genetic gain; accuracy; simulation

Introduction

Family selection has become standard methodology for large scale selective breeding programs in aquaculture, where BLUP (Best linear unbiased prediction) are used to predict the genetic merit for each individual within the pedigree. The usual implementation of family breeding program in aquaculture is to rear each family separately until the fish are large enough to be physically tagged. Such a multi-tank facility is costly in terms of space and labor, and has been argued to introduce environmental effects common to full sibs. In Canadian cod breeding approximately 10 % of the total variation for body weight at a mean tagging size of 20 g was due to such effects (Tosh, Garber, Trippel & Robinson 2010). Similar findings have been reported in two studies with rainbow trout where the effect common to full sibs accounted for 17 % (Henryon, Jokumsen, Berg, Lund, Pedersen, Olesen & Slierendrecht 2002) and 13 % (Kause, Ritola, Paananen, Wahlroos & Mantysaari 2005) of the total variation in body weight at an tagging size of 176 g and 50 g, respectively. These confounding effects of genetics and environmental effects common to full sibs can lead to reduced genetic gain and thus less efficient breeding programs. However in family breeding system in aquaculture, a nested design is often used, where every male is mated with two or more females creating full and half sib groups which may give evaluations of the male across several different tanks.

A way around this problem is to use of DNA profiling for family identification and pedigree assessment which facilitates pooling of families at fertilization or shortly thereafter. This eliminates the need for investment in costly multi-tank facilities for separate rearing of the families and minimizes the tank affect (Doyle & Herbinger 1994). DNA profiling in fish breeding programs involves some risks related to the representation of individual families. Low survival rates following the pooling of family groups may result in large variations between families in the number of surviving fish at later stages of life, creating an imbalanced population structure.

65 Seemingly the DNA profiling has been proven successful reconstructing pedigree, but in many
66 cases, has pooling shortly after fertilization revealed imbalanced families structure, (Garcia de
67 Leon, Canonne, Quillet, Bonhomme & Chatain 1998). Vandeputte & Launey (2004) reported
68 from a complete factorial cross of 24 sires and 10 dams in the common carp. They observed
69 unequal contributions of both sires and dams to the offspring generation despite a determined
70 effort to standardise egg volumes at the start of the experiment. Survival rate may differ greatly
71 between fish species, and reflect different evolution strategies for existence. Where for Salmonids
72 the expected to be 60 – 70 % from fertilization to harvest size compare to marine species as
73 Atlantic cod where survival is approximately 10% or lower (Rosenlund & Halldorsson 2007) .
74 The genetic improvement in the short term is dependent on the amount of genetic
75 variation in the trait of interest, selection intensity and accuracy of the predicted genetic merit of
76 the trait (Wei, Caballero & Hill 1996). Studies on the effects of such imbalanced population
77 structure in fish breeding on genetic improvement have not been published.
78 The aim of this study was to estimate the survival and heritability of cod juveniles at
79 tagging size and to study the effect of different survival among families on genetic gain. A
80 stochastic model was used to simulate genetic gain in an imbalanced versus a balanced family
81 structure.
82

Material and methods

Data analysis and statistical methods

The survival of cod larva was obtained for field data from the Icelandic cod breeding program in 2010 – 2011. Eggs from each female were kept separated in a 7 liters silo until hatching at approximately 14 days. The Cod larva were moved to 70 liters tanks 3 days after hatching and reared until the they had reach the tagging size approximately 180 dph at the weight 15 gr. The rearing temperature was kept constant at 8.5° C with 100% oxygen saturation and a salinity of 30-32‰. The total number of larva which was moved in to the tank and the number of juveniles at tagging size was used to estimate the survival rate. In total the survival rate was estimated from 116 families. The distribution for the survival among all families was fitted using the software easy fit 5.5 © (www.mathwave.com)

The heritability estimates for survival from hatching to tagging size was analyzed using REML to fit mixed linear models with the VCE 5 software (Kovac & Groeneveld 2003). The model used for analysis was: $\boldsymbol{y} = \boldsymbol{Xb} + \boldsymbol{Za} + \boldsymbol{e}$, where $\boldsymbol{y}$ is the vector of survival in each family, The factor $\boldsymbol{b}$ in this model is a vector of fixed affects in this case year class. $\boldsymbol{a}$ is the vector of random additive genetic effect of individual animals and $\boldsymbol{e}$ is the vector of uncorrelated residuals .

Setup of simulation schemes

In this study two simulation schemes were applied. The first scheme simulated genetic improvement in two traits in family breeding systems with 50, 100 and 200 families each with 100 and 150 individuals within family. This represents a traditional breeding in aquaculture where families are reared in separated tanks and tagged at approximately 15 gr weight which will be referred to in the text as **balance design**. The second scheme simulated genetic gain for the same numbers of families where all families were pooled shortly after hatching and the number

of individuals within families was reflected by different survival among families. This represents a family structure which is assigned by DNA profiling and will be referred to as **imbalance design.** (Figure 1)

The general assumption is that offspring of each family are equally divided between the breeding station and the test cage. The number per family kept at the breeding station was always 50, but the number of offspring from each family moved to test cages was either 50 or 100. Fish moved to test cages serve as sib-test and these were not used as breeding candidates. We defined three traits for the each individual: 1) body weight in breeding station; 2) body weight in test cages (sib test); and 3) disease resistance (binary trait) in test cages (sib test). The breeding candidates got an overall weighted index of these three breeding values. Selected breeding candidate are selected only from fish in the breeding station.

The heritability for both the normally distributed traits was set $h^2 = 0.35$ and genetic correlation r_G between them was set as 0.7. For the binary distributed trait the three heritabilities were tested $h^2 = \{0.10, 0.20 \text{ and } 0.30\}$ and the disease resistance trait was assumed to have no genetic correlation with the other two traits. The aggregate breeding value of individuals was estimated in an overall index where 30% relative weigh was set on EBV for 'weight at the breeding station', 30% weight on EBV for 'weight in test cage' and 40 % weight on EBV for the binary trait. To restrict inbreeding the number of individuals that were chosen from each family were limited to 4, 5 and 6 for breeding systems of 50, 100 and 200 families respectively. The mating ration is 1:2 where one male was mated to 2 females. As a consequence, the population was constructed of half–sibs and full–sibs. The table 1 summarizes the setup of the simulation scheme.

Simulation procedure

The phenotype y_{ij} of fish $i = 1,2,...k.$ and three traits $j = 1,2,3$ can be expressed as $y_{ij} = a_{ij} + e_{ij}$ whereas $a_{ij} \sim \boldsymbol{N\ (0,\ G)}$ and $e_{ij} \sim \boldsymbol{N\ (0,\ R)}$. The matrix $\boldsymbol{G}$ is the genetic covariance matrix amongst the three traits. Furthermore $\boldsymbol{G = K^T K}$ where $\boldsymbol{K}$ is a Cholesky decomposition in an upper triangular matrix. In similar way the environmental effect can be expressed such $\boldsymbol{r \sim N(0,R)}$ where $\boldsymbol{R = M^T M}$ where again $\boldsymbol{M}$ is a Cholesky decomposition in an upper triangular matrix. The vector r is a random standard normal density such as $\boldsymbol{r \sim N(0,I)}$ where $\boldsymbol{I}$ is an identity matrix Then we can sample from distribution a_{ij} such $a^s_{ij} = \boldsymbol{K^T} r.$ Later generations can be expressed such $a_{ij} = ½\ a_{father} + ½\ a_{mother} + m$, where m represents "Mendelian segregation" and $a_{ij} \sim \boldsymbol{N(0,d_i G),}$ where $d_i = ½(1-(½F_{father} + ½F_{mother}))$ where F is the inbreeding coefficients of the father and mother $(f_i = 2\Theta_{ii} - 1)$, (Lynch & Walsh 1998).

The phenotype of threshold character was created assuming underlying scale from standard normal density such as $y_i \sim \boldsymbol{N(0,I)}$, such as, at given the threshold value $\boldsymbol{\lambda}$ within standard normal density. Simulated number $y_i < \boldsymbol{\lambda}$ gives 0 but otherwise 1.

All simulations were performed using the statistical software, R 2.14.2 (R Development Core Team 2011).

The genetic improvement was simulated for 10 (r) generations from the base population, and each test was repeated ten times (R). Breeding candidates were selected based on BLUP – EBV (estimated breeding value). The accuracy was obtains by correlating the true breeding value in the simulation and estimated breeding value in the BLUP – EBV prediction. The rate of inbreeding per generation ΔF was obtained from the diagonal of genetic relationship matrix, $\Delta F = (F_r - F_{r-1})/(1 - F_{r-1})$, where F_r is the mean inbreeding at generation r. The BLUP-EBV was standardized with the mean equal 100 and with one standard deviation as 10.

The genetic gain for body weight is given as a percentage increase in body weight (ΔG) which was calculated as $\Delta G_{mean,\ body\ weight} = \frac{1}{R(r-3)} \sum_{r=3}^{r} \sum_{R=1}^{R} \Delta G_{\boldsymbol{r.R}}$ Where $\Delta G_{\boldsymbol{r.R}}$ genetic gain in generation r and replicate R. The mean in BLUP-EBV and accuracy for body weight is given as an average from generation 3 to generation 10 over 10 replicates in total $R(r-3)$ estimates. The variance of ΔG is the variance over $\Delta G_{\boldsymbol{r.R}}$. The genetic gain for binary trait is given as mean reduction per generation in frequency from generation 3 to generation 7 and average over all replicates. The mean in BLUP-EBV and accuracy for the binary trait is given as an average from generation 3 to generation 7 over 10 replicates in total $R(r-6)$ estimates.

The differences between the balanced versus imbalanced scheme were tested with a *one way ANOVA*. An α level of 0.05 was used to test for significant differences.

Results

Data analyses

The survival was best fitted by gamma distribution which gave the best fit for $\alpha = 0.66$ and $\beta = 5.34$. The fit did not departures from gamma distribution given Anderson-Darling test *($P < 0.01$)*. Approximately 50% of all individuals were within 14% largest families (figure 2).The heritability for survival at 180 dph was estimated to be $h^2 = 0.02$ with a standard error (s.e.) = 0.10. The mean survival among 116 families was 3.5% with maximum survival 22% and minimum 0.1%.

Simulations

Genetic gain in body weight was significantly higher for the balanced design when compared with the imbalanced design for all families sizes (figure 3). The genetic gain for disease resistance trait was significantly higher in balanced design for all families sizes and significantly higher when the heritability 0.2 and 0.3 compared with the gain at heritability 0.1 (figure 6). The accuracy of selected parent for body weight and disease resistance was not significantly different between balanced and imbalanced design (figures 5 and 7). For the normally distributed trait the accuracy was at max 0.72 and min 0.70. For the disease resistance trait the accuracy was max 0.55 and min 0.50.

The mean BLUP-EBV of selected parents was significantly lower in imbalanced design for both the normally distributed trait and the disease resistance trait (figures 4 and 8)

The rate of inbreeding (ΔF) did not differ significantly among all family sizes or between balance and imbalance design. The maximum ΔF was 1.10 % per generation and min ΔF was 0.87% per generation (figure 2).

Discussion

Mortality among cod families at early stages in rearing is high leading to highly imbalanced family structure. The estimated heritability for survival estimated $h^2 = 0.02 \pm 0.10$ and is low and not significant from zero. This is in line earlier finding in Atlantic cod, where heritability for survival in cod larva at 200 dph reported to be zero (Gjerde, Terjesen, Barr, Lein & Thorland 2004). Heritability survival at early stages in salmonids has been reported low from 0.04 – 0.09 (Rye, Lillevik & Gjerde 1990).

Unequal contribution of families has been observed and reported for other species, including both marine fish and salmonids (Herbinger, Doyle, Pitman, Paquet, Mesa, Morris, Wright & Cook 1995; Garcia de Leon *et al.* 1998). A plausible explanation can be the difference in female and male fertility and maternal effect. For Atlantic cod the larval stage is most vulnerable causing high mortality during the first weeks after fertilization. The mortality in the first 100 days after fertilization in intensive culture has ranged from 80% to 90% (Rosenlund & Halldorsson 2007). Much has been speculated about the effect of egg quality in early survival of cod. Poor egg quality which can steam from parental condition, ages, diet stress, poor water quality or over-ripening can contribute to low survival during the early life history stages for many marine fishes (Kjørsvik, Mangorjensen & Holmefjord 1990; Brooks, Tyler & Sumpter 1997). Low heritability seem to indicate that environmental factors are dominant factors which determent overall survival for cod larva.

Genetic gain was significantly reduced both for body weight and disease resistance in the imbalanced design when compared with the balanced design as shown in figures 2 and 6. It is clear that the genetic gain is reduced regardless of number of families. Figures 4 and 8 clearly indicate that the imbalanced design is lacking in selection intensity in BLUP-EBV when compared with the balanced design. For the imbalanced design, the number of individuals of each

family is governed by a gamma distribution, and therefore, it is stochastic whether a family with a high breeding value has many or few individuals. A family with a high BLUP-EBV can therefore have few individuals to select from. This has implications on the selection intensity. This applies to both traits, where the estimated breeding value is estimated on live candidates or based on sib test. The overall accuracy of selected parent is not affected by the imbalanced structure. Alteration in sire dam ratio may be a way increase the intensity in an imbalanced design, where males with high BLUP -EBV can be used to mate with more than two females. However altering the mating ratio can also be beneficial in balance design. This needs to be looked into more carefully should DNA profiling be chosen in fish breeding. Optimal contribution theory could also be applied in an imbalanced family structure. In this study the difference in mortality among cod families are large. Weather such large imbalance applies for all marine fishes in culture is not known, and in what extents the family structure, in fish breeding, needs to be balanced to minimize the effect of genetic gain, needs to be study in more detail.

Much research has been conducted on the practical use DNA profiling in aquaculture. Successful results have been reported for a number of commercially important species including the rainbow trout (Herbinger *et al.* 1995), Atlantic salmon (O'Reilly, Herbinger & Wright 1998), turbot (Estoup, Gharbi, SanCristobal, Chevalet, Haffray & Guyomard 1998) and shrimp (Jerry, Preston, Crocos, Keys, Meadows & Li 2004). The emphasis has mainly been on how exclusively individuals can be assigned to their parents. However a little has been speculated about the effect of unequal contribution on the genetic gain. The main argument for DNA profiling in aquaculture is to reduce the effects which are common to full-sibs to a minimum. It has been demonstrated in fish breeding that the events that occur in early stages can follow the individuals for a considerable time. The family tank effect was detectable in cod but declined close to zero as the fish got closer to harvest (Tosh *et al.* 2010). Moreover, the genetic correlation between tagging

size and harvest size vary can between 0.5 – 0.8 (Gjedrem & Baranski 2009). This mean that estimates at tagging size gives limited information for selection of breeding candidates at harvest size.

It is not known whether the common environmental effect will disappear completely by pooling all of the families shortly after incubation, particularly with an active cannibalistic fish like the Atlantic cod. In fact, faster growing families may be cannibal on slower growing families. Such cannibalism is likely to be selective with respect to body size rather than random. The outcome is that the slower growing families will suffer higher mortality resulting in a skewed distribution of family mean survival. High stocking density can have an effect on the social behavior of fish, as has been found among salmonids and other species (Macintosh & De Silva 1984; Wallace, Kolbeinshavn & Reinsnes 1988). In addition, the cod spawn in batches over several weeks (Sundby 2000; Sundby, Bjørke, Soldal & Olsen 1989; Lambert, Yaragina, Kraus, Marteinsdottir & Wright 2003). Therefore, the individual egg quality can vary over that time. This leads to age and size differences, making the pooling of all families an unrealistic option.

Conclusions

The unequal contribution of families in a breeding program in aquaculture can be difficult to account for, especially when the survival is low. Highly imbalanced family structure due to low survivals can result in less genetic gain.

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Figure legends

Figure 1. Schematic overview of the simulation scheme

Figure 2. Field data of 116 families fitted with gamma distribution. The data are from the Icelandic cod breeding program and represent the resulting survival estimated at 180 days at post hatching.

Figure 3. Mean of genetic gain *(ΔG)* tested at six different families sizes for balance and imbalance design. The x axis is marked with letter f and t, where letter f stands for number of families and letter t stands number of individuals in a sib test. Balanced design have significantly higher genetic gain than imbalanced design (*one way ANOVA*, $P < 0.05$). Vertical whiskers SE.

Figure 4. Mean of estimated BLUP-EBV for body weight of fish selected as parents, tested at six different families sizes for balance and imbalance design. The x scale is marked with letter f and t, where letter f stands for numbers families and letter t stands number of individuals in a sib test. Balanced design have significantly higher BLUP - EBV than imbalanced design (*one way ANOVA*, $P < 0.05$). Vertical whiskers SE.

Figure 5. Mean accuracy in estimated breeding value of body weight of fish selected as parents, tested at six different families sizes for balance and imbalance design. The x scale is marked with letter f and t, where letter f stands for numbers families and letter t stands number of individuals in a sib test. No significant difference between balanced and imbalanced design (*one way ANOVA*, $P < 0.05$). Vertical whiskers SE.

Figure 6. Different genetic gain for three heritabilities $h^2 = \{0.1, 0.2 \text{ and } 0.3\}$. The comparisons involved 50, 100 and 200 families. Each family is tested for both 50 and 100 sib. Balanced design had significantly higher genetic gain (*one way ANOVA*, $P < 0.05$). Vertical whiskers SE.

Figure 7. Comparisons of accuracy for three heritabilities $h^2 = \{0.1, 0.2 \text{ and } 0.3\}$. The comparisons involved 50, 100 and 200 families. Each family is tested for both 50 and 100 sib. None significant difference was found between balanced and imbalanced design (*one way ANOVA*, $p = 0.12$). Vertical whiskers SE.

Figure 8. Different BLUP - EBV for three heritabilities $h^2 = \{0.1, 0.2 \text{ and } 0.3\}$. The comparisons involved 50, 100 and 200 families. Each family is tested for both 50 and 100 sib. Balanced design had significantly higher BLUP - EBV (*one way ANOVA*, $P < 0.05$). Vertical whiskers SE.

Figure 9. Mean rate of inbreeding, tested at six different families sizes for balance and imbalance design. The x scale is marked with letter f and t, where letter f stands for number of families and letter t stands for number of individuals in a sib test. No significant difference between balanced and imbalanced design (*one way ANOVA*, $P = 0.21$). Vertical whiskers SE.

Tables

Table 1. Number of individuals and families in the simulation scheme. The breeding candidates are those which are selected for breeding. The individuals in sib – test are those used for estimating BLUP – EBV for disease resistance and body weight in test cages.

Families	Within families	Breeding candidates	Sib-test	Total in sib - test	h^2 growth	h^2 disease res.
50	50	2500	50	2500	0,35	0.1, 0.2, 0.3
50	50	2500	100	5000	0,35	0.1, 0.2, 0.3
100	50	5000	50	5000	0,35	0.1, 0.2, 0.3
100	50	5000	100	10000	0,35	0.1, 0.2, 0.3
200	50	10000	50	10000	0,35	0.1, 0.2, 0.3
200	50	10000	100	20000	0,35	0.1, 0.2, 0.3

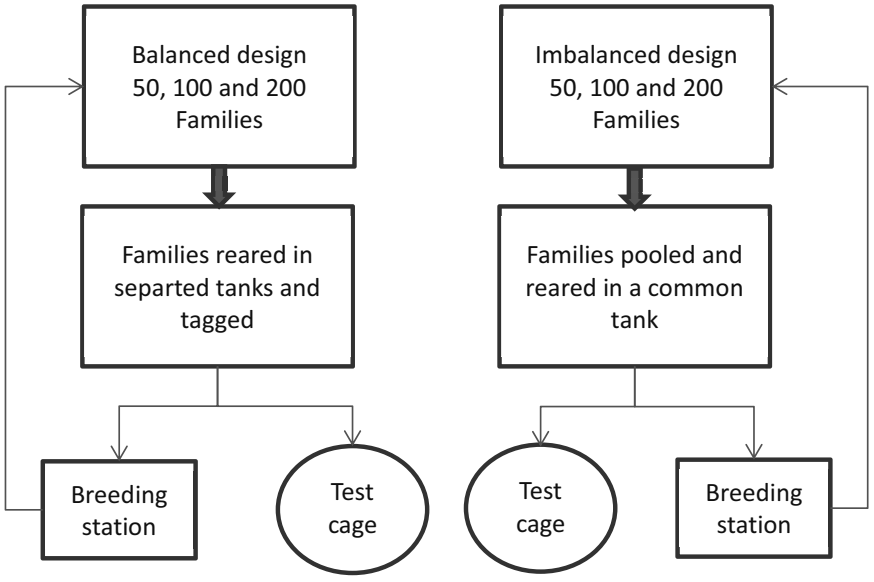
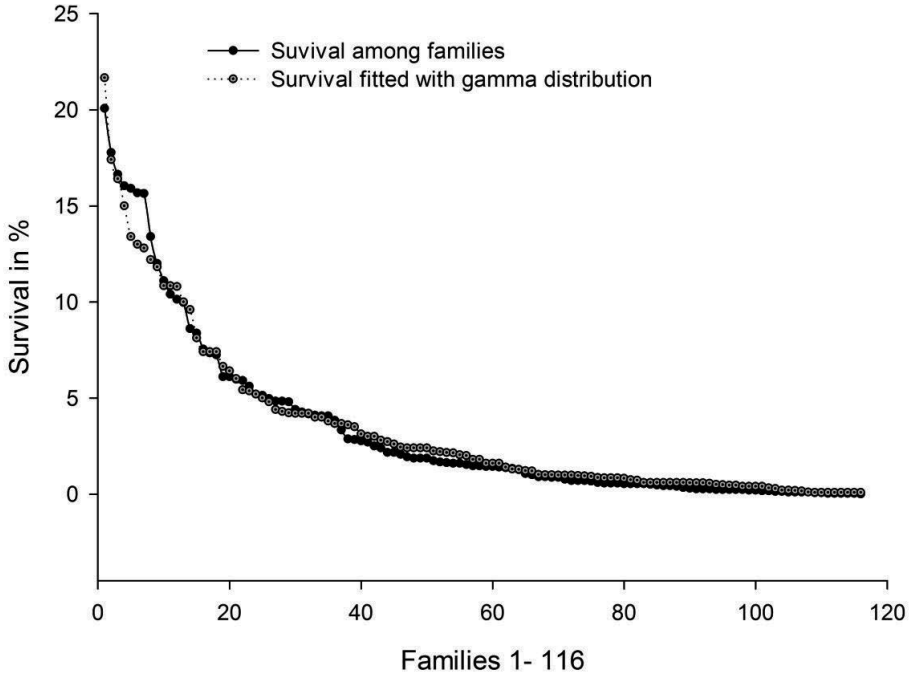

Balanced design
50, 100 and 200
Families
Families reared in
separted tanks and
tagged
Breeding
station
Test
cage
Imbalanced design
50, 100 and 200
Families
Families pooled and
reared in a common
tank
Test
cage
Breeding
station

Figure 1.

429



430
431
432 **Figure 2.**
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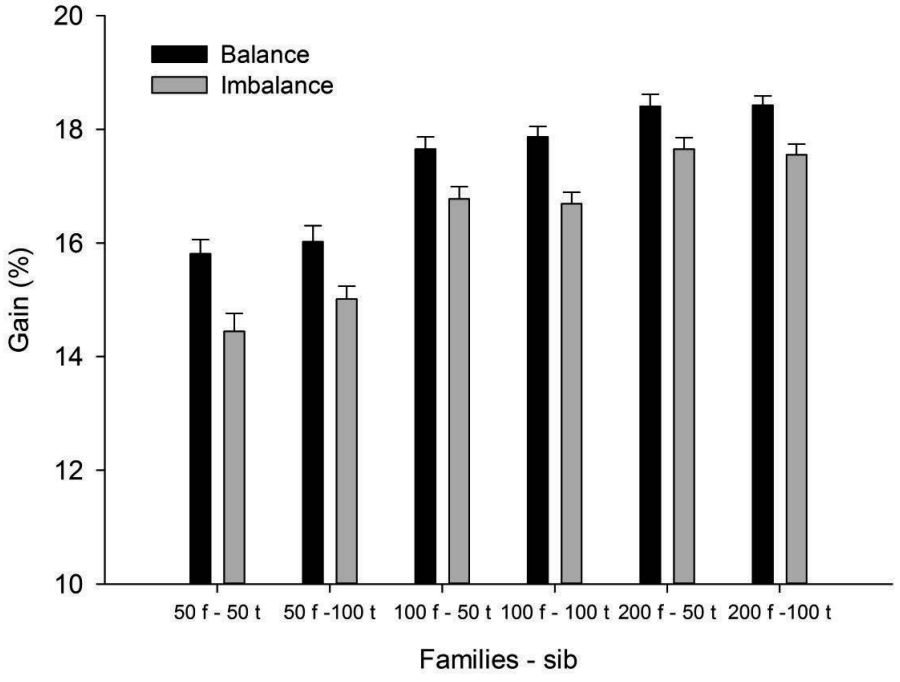

435
436 **Figure 3.**
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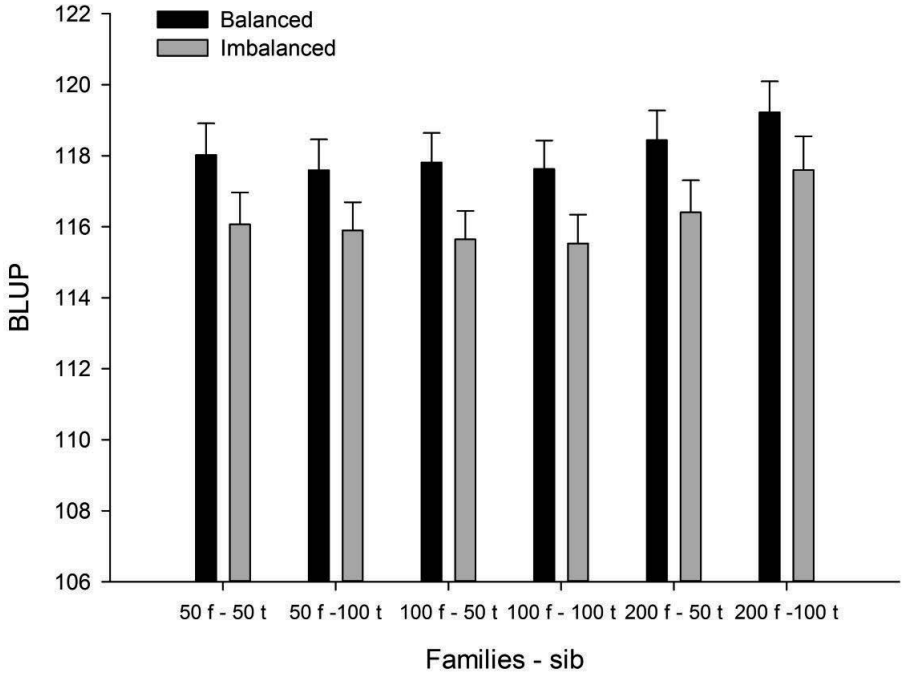

Balanced
Imbalanced
BLUP
122
120
118
116
114
112
110
108
106
50 f - 50 t
50 f -100 t
100 f - 50 t
100 f - 100 t
200 f - 50 t
200 f -100 t
Families - sib

Figure 4.

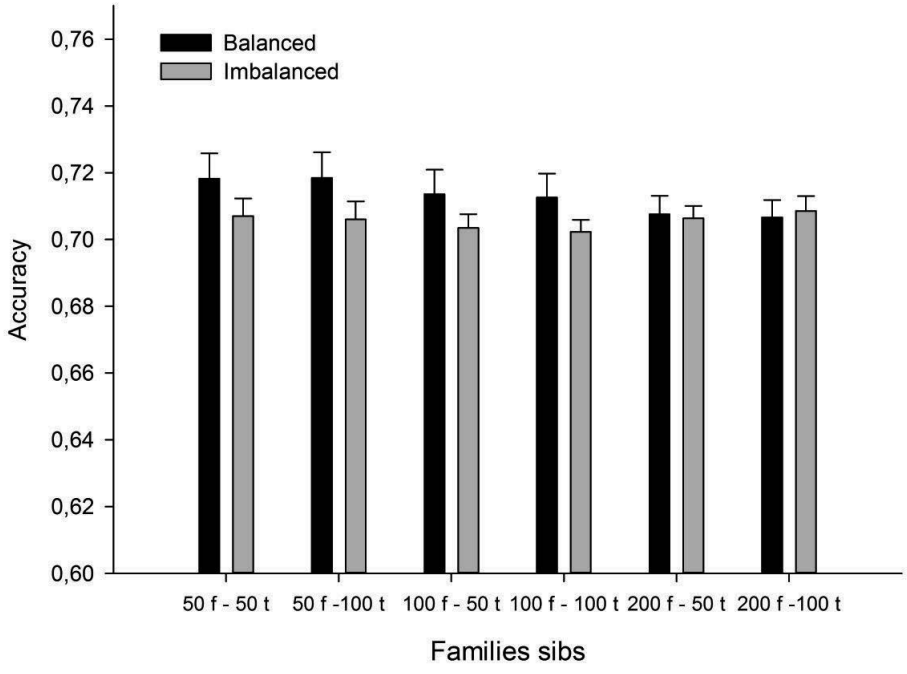
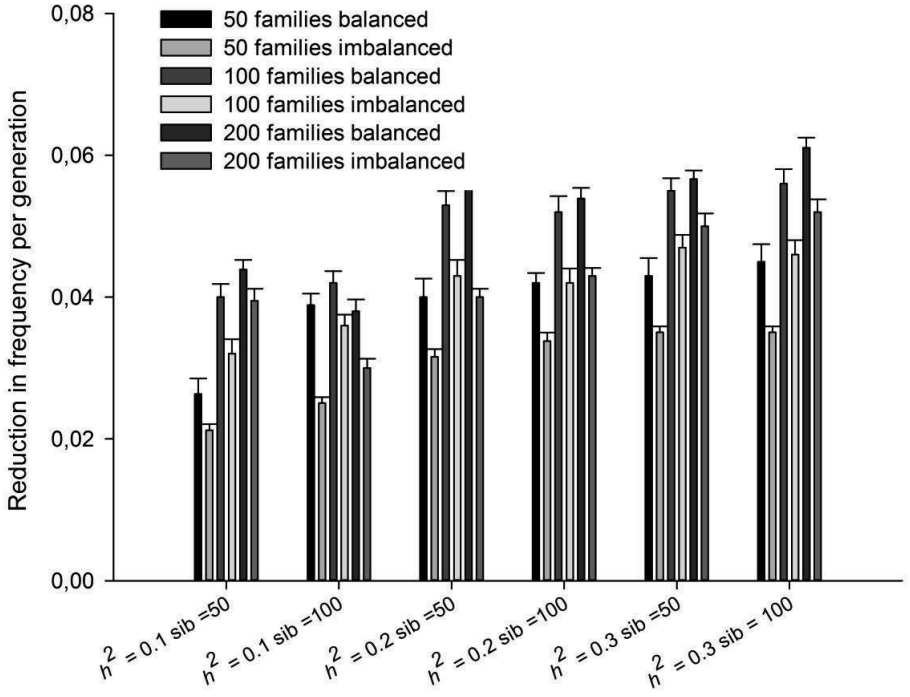

Balanced
Imbalanced
0,76
0,74
0,72
0,70
0,68
0,66
0,64
0,62
0,60
Accuracy
50 f - 50 t
50 f -100 t
100 f - 50 t
100 f - 100 t
200 f - 50 t
200 f -100 t
Families sibs

Figure 5.

446



447
448 **Figure 6.**
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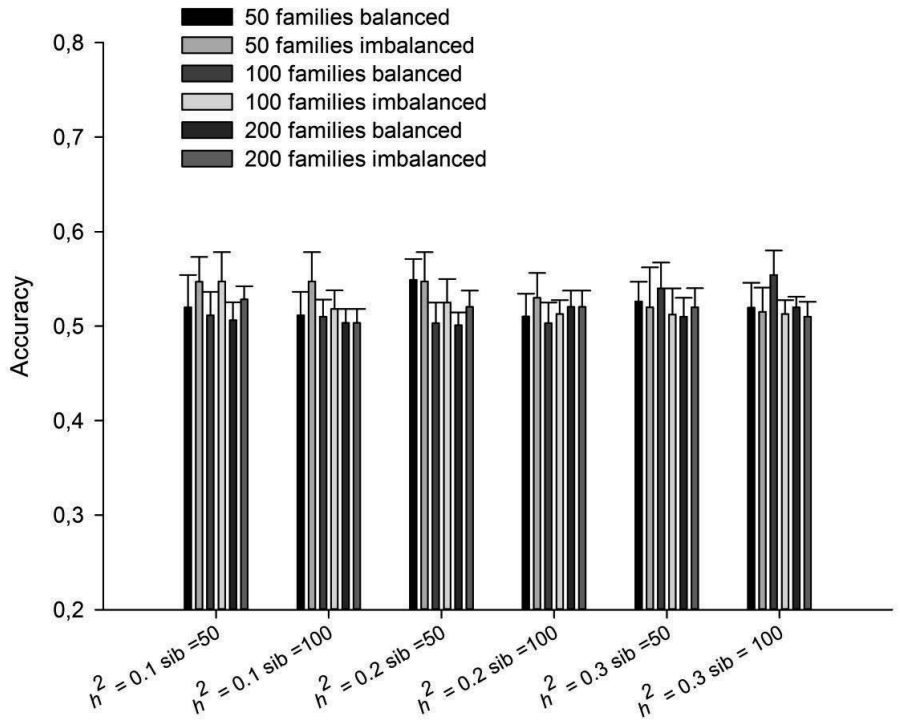

50 families balanced
50 families imbalanced
100 families balanced
100 families imbalanced
200 families balanced
200 families imbalanced
Accuracy
0,8
0,7
0,6
0,5
0,4
0,3
0,2
h2 = 0.1 sib =50
h2 = 0.1 sib =100
h2 = 0.2 sib =50
h2 = 0.2 sib =100
h2 = 0.3 sib =50
h2 = 0.3 sib = 100

Figure 7.

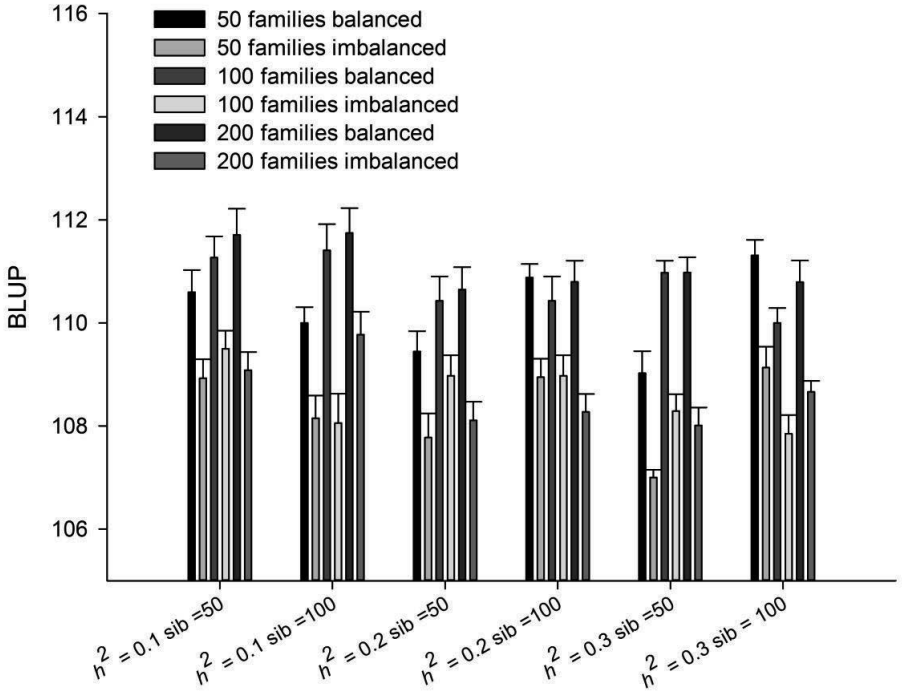
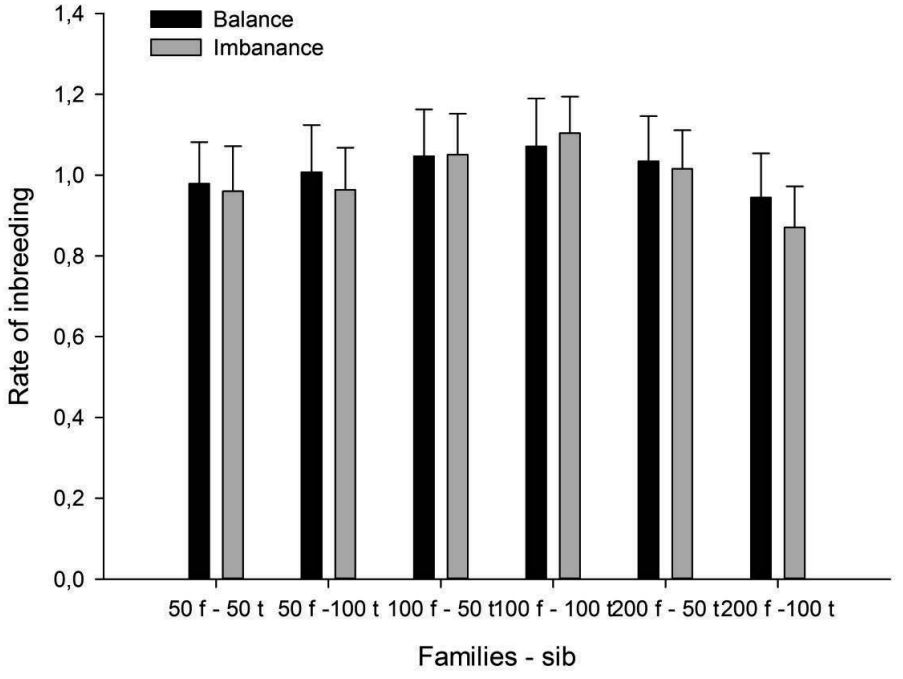

50 families balanced
50 families imbalanced
100 families balanced
100 families imbalanced
200 families balanced
200 families imbalanced
BLUP
116
114
112
110
108
106
h2 = 0.1 sib =50
h2 = 0.1 sib =100
h2 = 0.2 sib =50
h2 = 0.2 sib =100
h2 = 0.3 sib =50
h2 = 0.3 sib = 100

Figure 8.



460
461 **Figure 9.**
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