# The historical genetic variation in Atlantic cod (*Gadus morhua* L.) in Icelandic waters

Dissertation submitted in partial fulfillment of a
*Philosophiae Doctor* degree in Biology

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# Abstract

The population genetics, such as population structure, of Atlantic cod have been unravelled in the last two decades, at an ever finer scale. Yet, much is still unknown about the evolutionary genetics of cod and the possible impact of fisheries on the genetic structure, both temporal and spatial. The objectives of this thesis were to investigate the extent of temporal genetic differentiation in Icelandic cod over the latter half of last century and to correlate historical changes of genetic variation with known factors such as the potential impact of fisheries.

A prerequisite for such a study is access to historical genetic material and development of the appropriate genetic markers. DNA was obtained from dried tissue on stored cod otoliths, previously used for age determination. These were retrieved from the archives of the Icelandic Marine Research Institute. In the first part of the thesis, specific set of microsatellites markers were developed, suitable for the degraded cod DNA, usually obtained from dried tissue remains.

Secondly the connection between important behavioral phenotypes and genotypes at non-neutral marker *Pan* I was determined, using current-day genetic material. This analysis showed that the *Pan* $I^{AA}$ homozygotes could be assigned to a "coastal" type that appeared to follow seasonal trend in temperature, foraging in relatively shallow waters. On the other hand, most Pan $I^{BB}$ homozygotes were assigned to "frontal" type that is characterized by migrating to colder and deeper waters during feeding migration.

For the main part of the thesis historical data set of archived otoliths collected between 1948 and 2002 was used to jointly examine phenotypic and genetic data with the goal to examine historical trends in the genetic composition of the Icelandic cod stock. This analysis showed that there have been major changes in the genetic variation at the *Pan* I locus of Icelandic cod during the latter part of last century. These have occurred alongside changes in fishing patterns and pressure, and age composition of the stock, whereas no temporal changes were detected in neutral markers. The frequency of the *Pan* $I^{BB}$ genotype decreased over a period of six decades,

concomitant with considerable spatial and technical changes in fishing effort that resulted in the disappearance of older individuals from the fishable stock.

Analysis of the neutral historical genetic variation revealed considerable genetic variability. The genetic effective population size ($N_e$) of cod was estimated to be within the range of few hundreds to several thousand individuals i.e. several magnitudes smaller than the census size. The temporal variation in effective population size of Atlantic cod in Icelandic waters was considerable, likely determined by highly dynamic forces shaping the genetic variation of the population. Indications of subtle changes in genetic differentiation and in $N_e$ highlight the importance of repetitive temporal sampling for detection of any underlying trends which are difficult to detect by other means.

# Ágrip

Þekking á erfðafræðilegri stofngerð þorsks hefur aukist til muna á undanförnum áratugum. Hins vegar er enn lítið vitað um sögulegar breytingar í erfðasamsetningu þessa fisks eða möguleg áhrif fiskveiða á hana, hvort sem er í tíma eða rúmi. Markmið þessa doktorsverkefnis var að kanna erfðabreytileika þorsks á seinni hluta síðustu aldar og meta áhrif fiskveiða.

Unnt er að nýta gamlar kvarnir sem hefur verið safnað af Hafrannsóknastofnun til aldursgreininga, til að nálgast erfðaefni. Í fyrsta hluta verkefnisins er fjallað notkun nýrra erfðamarka en léleg gæði erfðaefnis sem einangrað var úr uppþornuðum vefjaleifum af yfirborði kvarnanna kallaði á þróun nýrra aðferða til að auðvelda notkun þess.

Í öðrum hluta ritgerðarinnar var hið valbundna *Pan* I erfðamark notað til að aðgreina erfðafræðilega tvo hópa þorsks sem sýnt hafa ólíkt atferli m.t.t. til fæðunáms. Til þessa var samtíma erfða efni notað bæði úr ferskum vef sem og af kvörnum. Þannig var sýnt fram á að *Pan* $I^{AA}$ arfgerð einkennir grunnfarsþorsk, sem heldur sig á grunninu allt árið um kring en *Pan* $I^{BB}$ arfgerð virðist einkenna djúpfarsþorsk sem leitar í dýpri sjó og í hitaskil til fæðuöflunar.

Í megin hluta ritgerðarinnar var notað erfðaefni af gömlum kvörnum frá 60 ára tímabili (1948-2002) auk líffræðilegra upplýsinga til að kanna langtíma breytingar á arfgerðatíðni *Pan* I erfðamarksins sem og hlutlausra erfðamarka (örtungl) í hrygnandi þorski við Ísland. Helstu niðurstöður voru þær að jafnframt því sem að meðalaldur fiska lækkaði á tímabilinu urðu breytingar á arfgerðatíðni *Pan* I erfðamarksins. Þessar niðurstöður bentu eindregið til þess að hlutfall *Pan I* $^{BB}$ arfgerðar sem einkennir djúpfarshópinn hafi minnkað í kjölfar aukins fiskveiðiálags á rannsóknartímabilinu.

Ítarleg rannsókn á langtíma breytileika í hlutlausum erfðamörkum (örtungl) leiddi í ljós töluverðan breytileika og erfðafræðilegan mun á milli ára, sér í lagi yfir seinni hluta tímabilsins. Erfðafræðileg stofnstærð ($N_e$) var metin vera á bilinu nokkur hundruð til þúsunda fiska sem er mjög lág miðað við eiginlegan hrygningarstofn. Niðurstöður bentu til þess að sá mikli breytileiki sem einkennir ýmis lífsöguleg atriði í íslenska þorskinum endurspeglist í breytilegri erfðasamsetningu en um leið tiltölulega lágri

erfðafræðilegri stofnstærð. Jafnframt sýna niðurstöður fram á mikilvægi endurtekinnar söfnunar yfir lengri tímabil svo greina megi erfðafræðilegar langtímabreytingar frá skammtímasveiflum.

*Tileinkað sonum mínum, Jakobi Alexander og Einari Tryggva*

# List of papers

The thesis is based on three published papers and a manuscript. The papers will be referred in the text by their respective numbers as following:

**Paper I***:*

Jakobsdóttir KB, Jörundsdóttir, ÞD, Skírnisdóttir S, Hjörleifsdóttir S, Hreggviðsson GÓ, Daníelsdóttir AK & Pampoulie C. 2006. Nine new polymorphic microsatellite loci for the amplification of archived otolith DNA of Atlantic cod, Gadus morhua L. *Molecular Ecology Notes*, **6**: 336-339.

**Paper II***:*

Pampoulie, C, Jakobsdóttir KB, Marteinsdóttir G, Thorsteinsson V. 2008. Are Vertical Behaviour Patterns Related to the Pantophysin Locus in the Atlantic Cod (Gadus morhua L.)? *Behavior Genetics*, **38**:76-81.

**Paper III*:***

Jakobsdóttir KB, Pardoe H, Magnusson A, Bjornsson H, Pampoulie C, Ruzzante DE, Marteinsdottir G. 2011. Historical changes in genotypic frequencies at the Pantophysin locus in Atlantic cod (Gadus morhua) in Icelandic waters: evidence of fisheries-induced selection? *Evolutionary Applications*, **4**: 562-573.

**Paper IV*:***

Jakobsdóttir KB, Pampoulie C, Ruzzante DE, Daníelsdóttir AK, Marteinsdóttir G. Temporal aspects of neutral genetic variation and effective sizes (Ne) of Atlantic cod (Gadus morhua) in Icelandic waters. Manuscript.

# Table of Contents

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# 1 Introduction

The importance of biodiversity, as well as the maintenance of diversity in life history traits is pivotal to ensure species or population resilience to habitat perturbation, climate change and harvest (Greene et al. 2010, Schindler et al. 2010). Studies on various fish species have contributed greatly to our understanding of genetic variation and evolutionary changes in wild populations, due to their remarkable display of adaptive radiation and persistence across a wide range of environment. Many of the larger marine fish populations have been shown to exhibit extraordinary high levels of intraspecific diversity and population differentiation and are subject to complex dynamic patterns of their life history

While the importance of biodiversity and genetic variation is clear, the relatively high biodiversity is being challenged by numerous ecological and genetic threats. Fishing activity is the predominant cause for depletion or collapse of fish populations and is also a major source of disruption in the marine environment. Worldwide, commercial fishing has led to dramatic decline in large, long-lived predatory fish, changes in stock structures, simplification of food webs and a tighter coupling between the abundance of fish stocks and environmental fluctuations (Hutchings 2000, Myers and Worm 2003, Ottersen et al. 2006, Pauly et al. 2002). Although the situation has improved slightly for some populations since the early nineties, a meta analysis indicated an overall 38 % decline of marine fish populations between 1970 and 2007 (Hutchings et al. 2010). Additionally, fishing has contributed to the life history changes within exploited stocks. Therefore, the exploration of potential evolutionary consequence of anthropogenic activities such as overexploitation is of great scientific and practical interest and of utmost importance to fishery management.

Indications of evolutionary response to fishing activities have emerged from studies of life-history evolution but the studies have not

always been conclusive with regard to the nature of the changes and the call for direct genetic evidence has been undisputed. The magnitude and rate of evolutionary response to fishing is also tightly entangled to the species population diversity and structure in space and time (Greene et al. 2010, Hilborn et al. 2003). Therefore, integration of genetic and life history information is pivotal for improved understanding of the dynamics shaping the populations diversity and response to various perturbations (Hutchinson 2008, Schindler et al. 2010). Genetic analysis of historical samples can provide invaluable insights into human-induced changes in the genetic composition of species or populations, including fish populations (Hansen et al. 2009, Nielsen and Hansen 2008). However, direct genetic evidence has been limited because of scarcity of historical material on which the genetic analysis need to be based for inference (Waples and Naish 2009). Another challenge has been the availability of suitable molecular markers because historical degraded material calls for specially designed markers of short fragments.

Atlantic cod (*Gadus morhua*) is one of the most valuable commercial species in the North Atlantic and has been heavily exploited across its geographic range. Like most cod stocks in the North Atlantic, Icelandic cod did experience a drastic reduction in abundance and spawning stock biomass (SSB) during the latter half of the last century. Alongside the decline in stock abundance, there have been notable alterations in the life history characteristics of Icelandic cod (Marteinsdóttir et al. 1998, Pardoe et al. 2009, Schopka 1994) that raise a concern for the stock's reproductive potential, and consequently its future ability to withstand harvesting and environmental changes (Árnason et al. 2009, Law 2007).

In this thesis genetic material obtained from the archived otolith collection of the Icelandic Marine Research Institute (MRI) was used to study temporal changes in the genetic composition of Atlantic cod at the population level over a decadal time scales. The objectives were to investigate the extent of temporal genetic differentiation over 60 years, to correlate historical changes of genetic variation with known changes in population size and to

evaluate the potential impact of fisheries on genetic composition (**paper III**, **IV**). To meet this challenge, suitable methodology for working with historical DNA had to be optimized (**paper I**) as well as the correlation between behavioural phenotypes and genotypes (**paper II**) to be explored.

## 1.1 Atlantic cod-life history

Atlantic cod is distributed throughout the North Atlantic occupying near shore waters, continental shelf and banks most often to depth of around 600m with the main limits to its distribution being depth and temperature. It is a long lived (up to 30 years) iteroparous species and characterized by wide variation in life history traits (Salvanes et al. 2004) and extreme fecundity such that hundreds of thousands or millions of eggs in multiple batches are released over prolonged period of time (Kjesbu et al. 1996, Kjesbu et al. 1998). The size age and physiological condition of the female plays an important role for survival of pelagic stages of eggs and larvae: Larger females produce more batches, spawn over longer periods of time and have increased egg size and realized fecundity as well as increased quality and survival of larvae (Kjesbu 1989, Kjesbu et al. 1996, Kjesbu et al. 1998, Marteinsdóttir and Begg 2002, Marteinsdóttir and Steinarsson 1998, Rowe and Hutchings 2003). All of these contribute to significant differences found in reproductive success between first-time and repeat–spawning cod (Trippel 1998). Following spawning, eggs and larvae drift with currents towards nursery grounds and in the absence of parental care face high rates of mortality during the first year of life.

Adults show both sedentary/resident behaviour and migrating behaviour where individuals migrate seasonally to spawn, sometimes over large distances, to and from feeding and overwintering grounds (Grabowski et al. 2011, Green and Wroblewski 2000, Neat et al. 2006, Robichaud and Rose 2004, Tamdrari et al. 2012a, Tamdrari et al. 2012b, Thorsteinsson et al. 2012, Westgaard and Fevolden 2007).

All of these above mentioned characteristics are of importance when investigating population genetics of cod. The extreme fecundity, external

fertilization, pelagic life stages and highly mobile and often migratory adults are all characteristics that create opportunity for considerable gene flow and high variance in offspring number with great impact on the species/populations genetic composition.

### 1.1.1 Atlantic cod in Icelandic waters –Icelandic cod

The main spawning grounds of Icelandic cod extend from the south coast (near Vestmannaeyjar) to the Snæfellsnes peninsula off the central west coast (Jónsson 1954, 1982, Sæmundsson 1924). More recently, numerous smaller regional spawning aggregations along the West, North and East coast are considered to make significant contributions to the productivity of the stock in a given year, depending on environmental and stock factors that determine dispersal and survival (Begg and Marteinsdóttir 2000, 2002a, b, Brickman et al. 2007, Marteinsdóttir et al. 2000, Thorsteinsson et al. 2012). This diversity in spawning components has been proposed as an explanation for the relatively stable recruitment around Iceland, compared to other stocks in the North Atlantic (Marteinsdóttir et al. 2000). The spawning on the main grounds in the southwest usually lasts from the middle of March until the middle of May with the older individuals spawning earlier and over a longer period (Marteinsdóttir and Björnsson 1999). Studies based on otolith chemistry and genetic markers have shown that two major spawning components likely exists, one located northeast (NE) and the other southwest (SW) of Iceland with limited intermixing between them (Jónsdóttir et al. 2006a, b, Pampoulie et al. 2006). These findings could explain life history differences between the regions with cod in the north growing more slowly, maturing larger and at an older age (Begg and Marteinsdóttir 2002a, Jónsdóttir et al. 2006b, Jónsdóttir et al. 2008, Marteinsdóttir and Begg 2002). Eggs and larvae spawned in the SW, drift with prevailing coastal and offshore currents clockwise to the nursery grounds off the north coast. Number of surviving juveniles from the main spawning grounds has been found to vary with the strength and timing of the coastal current and the inflow of the Irminger current onto the shelf (Brickman et al. 2007). A variable portion also episodically drifts to Greenland waters with the Irminger current, thus extending the nursery areas of Icelandic cod. This has

been found to have a positive effect on recruitment (Astthorsson et al. 2007, Begg and Marteinsdóttir 2000, Brickman et al. 2007, Schopka 1994). Prior to 1985, migration of cod from Greenland took place at fairly regular intervals while during the last two decades this has not been observed (Astthorsson et al. 2007).

Tagging studies have revealed that postspawning cod from the southwest (SW) region undertake long distance migrations to the main feeding regions located northwest (NW) and NE of Iceland, taking a route either west or east of the country while postspawning cod from the NE regions tend to stay more locally (Jónsson 1996, Pálsson and Thorsteinsson 2003).

Apart from the differences found between the NE and SW spawning components, evidence is accumulating that depth segregation is common in the SW region, i.e. in the main spawning area. Studies based on life history differences (e.g Marteinsdóttir et al. 2000), otolith shape, morphology, chemistry and increment growth (Jónsdóttir et al. 2006a, b, Pétursdóttir et al. 2006), *Pan* I variation (Jónsdóttir et al. 2002) show in general, that the larger, older, faster growing and most fecund spawning cod was present in more shallow water closest to coast. Further, depth differences between different genotypes of the *Pan* I locus was also observed (Árnason et al. 2009, Pampoulie et al. 2006). In recent years, evidence has been emerging from tagging studies using data storage tags that two groups of Icelandic cod associated with different foraging strategy and habitat selection during the feeding season occur within spawning location (Grabowski et al. 2011, **paper II**, Pálsson and Thorsteinsson 2003, Thorsteinsson et al. 2012). While both groups spawn at same location, the ‘coastal’ cod inhabit shallow shelf waters characterized by seasonal temperature trends all year round. In contrast, ‘frontal’ cod undertake migrations to cold, deep waters (>250m) where they forage at thermal fronts, making frequent vertical migrations between temperature extremes (<0 and >7 °C).

## 1.2 Post-war history of the fishery and fishery management

The Icelandic cod stock like most other cod stocks in the North Atlantic has experienced a drastic reduction in population sizes due to intensive fishing during the latter half of last century (Schopka 1994). The stock has been commercially exploited for centuries but during the World War II (WWII) the international fleet (mainly the British fleet) was absent from Icelandic waters creating a period of temporary rest with considerable lower fishing pressure. After the WWII the fishing increased again, concurrent with a considerable improvement of fishing technology and an increase in number and size of fishing vessels (Jónsson 1997). Prior to WWII, both international trawlers and the Icelandic fleet focused their fishing effort on the relatively shallow inshore areas (Þór 2005), with landings of cod reaching a maximum of *circa* 52,000 tonnes in 1933 (Anonymous 2009). Immediately after the war, the inshore cod fishery increased again due to the continued exploitation by the national fleet and return of international vessels, resulting in a peak catch of 548,000 tonnes in 1954 (Anonymous 2009). After 1955 there was an overall decline in the cod stock and its fishery accompanied by rising fishing mortality although fluctuations have occurred in both. Starting in the 1950s, the following decades were characterized by a gradual extension of the fisheries jurisdiction, based on the request of the Icelandic government for the right to protect its fish stocks. In 1952, Icelandic authorities banned the use of bottom trawls and Danish seine within four nautical miles of the shoreline. This was followed by the extension of fisheries jurisdiction to 12 nautical miles (nm) in 1958, 50 nm in 1972 and finally to 200 nm in 1976 (Anonymous 2004, Þór 2005). This, combined with the stricter inshore fishing regulations, improvements in fishing technology, and the introduction of larger vessels, resulted in the redirection of fishing effort into deeper offshore waters. Concurrently, trawlers were able to follow the seasonal migration of cod (Jónsson 1996), rather than being restricted to inshore breeding grounds during the spawning season. Since the 1950s and until the 1990s trawling has been responsible for about 60% of the catches of Icelandic cod and the proportion went down to ~ 40% after 1994. During the

latter half of last century gill nets catches were stable or about one fourth of the total landings.

The fishery management has been using technical measures such as closed areas since the beginning of the century and mesh size since the 1950s. In 1973 effort limitation in form of total allowable catch (TAC) system was introduced. However, the actual landings remained approximately 30% above that advised by MRI. In the seventies and in the early eighties the fishable stock (age 4+) and SSB reached lowest levels thus far (Jakobsson and Stefánsson 1998). Since the 1980s until the beginning of this century, SSB remained below the long-term average, rarely exceeding 200 000 tonnes (Schopka 1994, Anonymous 2009). A new management scheme consisting of individual transferable quotas (ITQs) and effort system was started in 1984 and prevailed until 1990 when the comprehensive Fisheries Management Act (No.38/1990) was introduced as legislation, leading to the adoption of ITQ system for all Icelandic fisheries. Following the historical lows in fishable stock and SSB, a harvest control rule (HCR) was implemented in 1995 setting TAC at 25% of the available biomass of fishable stock (fishable stock averaged over two adjacent calendar years). The HCR is under periodic revision and in agreement with the *precautionary approach* implemented in the ICES advice on fisheries management in 1998 (ICES 1997, 1998).

## 1.3 Evolutionary considerations of Fisheries

### 1.3.1 Life history approach to studies of fisheries induced evolutionary change

Life history theory is a concept that addresses how, in the face of trade-offs, organisms should allocate time and energy to tasks and traits in a way that maximizes their fitness. Change in life history traits is the subject of life history evolution and creates the theoretical background of fisheries-induced evolution.

Fishery management typically focuses on the overall numbers of fish captured or the harvest rate. However, harvest rate is normally not uniform across all types of individuals in a population. Fishing mortality is often selective, meaning that a wide range of traits can be fished with different intensity such as size and age, migration, timing and behaviour.

This can induce evolutionary response (genetic changes) with complex effects on the fishery yield and population viability. Hence, for conservation and management purposes it is an important issue to detect evolutionary responses in harvested populations and separate them from plastic changes in life histories (Kuparinen and Merila 2007).

#### 1.3.1.1 Known effects of fisheries on life history traits

Fisheries-induced evolution has been primarily discussed as selective mortality of fishing directly targeting certain phenotypes such as length-or weight-at-age by size –selective fishing or indirectly targeting phenotypes such as age-at-maturity due to age-at-entry into the fisheries or by location of fishery.

Harvesting of large individuals is proposed to select for maturation at a younger age and/or smaller size by reducing the proportion of old and/or large individuals in the spawning stock (Kuparinen and Merila 2007). Regardless of whether such life history trends are phenotypic or genetic, decreasing age and size at maturation can have serious effects on population dynamics. Earlier maturation can have negative implications for population growth rates (Hutchings and Fraser 2008). In addition, due to the general well-known positive correlation between body size and fecundity (Birkeland and Dayton 2005, Hutchings 2005, Trippel 1995), a lower age at maturity will reduce age specific sizes of adult individuals and consequently the reproductive potential of the population can be affected (Jørgensen and Fiksen 2006, Ratner and Lande 2001). If reduced average body size is not compensated for by increased population abundance, biomass and yield of the fish stock will decrease (Conover and Munch 2002, Stokes and Law 2000).

Knowing whether life history changes induced by fisheries are of plastic or genetic origin (evolutionary change) and moreover, at what pace such change is occurring, is important for the purpose of management and conservation of fish stocks. Evolutionary responses in life histories will alter genotype frequencies in a population and can lead to the loss of genetic variability. Reversing such changes can be very slow or even impossible (Dulvy et al. 2003, Petitgas et al. 2010). For evolution to take place selection must affect traits that are heritable. It has been shown in fish species that approximately 20-30% of the variation in life-history traits such as age-size at maturation is heritable (Dickerson et al. 2005, Funk et al. 2005, Stokes and Law 2000). Heritability can be measured (additive genetic variance divided by the total phenotypic variance) in common garden experiments but it is difficult to measure in a natural populations. Evidence for rapid evolutionary responses in fish has been demonstrated experimentally by several studies (reviewed by Conover and Baumann 2009). In natural populations however, studies of evolutionary response to selective harvesting are difficult as changes in phenotypic plasticity such as growth and timing of maturation might also be affected by environmental trends (Swain 2011).

A number of statistical methods have been developed to separate a potential genetic component in life history trends from phenotypic plasticity using retrospective studies (Edeline et al. 2007, Swain et al. 2007). The development of the probabilistic reaction norm (PMRNs) (Barot et al. 2004, Heino et al. 2002), have given rise to numerous studies investigating long-term trends in empirical field data (Jørgensen et al. 2007, Olsen et al. 2004, Pardoe et al. 2009). The PMNR approach is a statistical tool to help disentangle genetic variation in maturation from phenotypic plasticity resulting from variation in growth and survival (Heino and Dieckmann 2008). Life history variation arises from tight entanglement of genetic and environmental processes it can be difficult to separate evolutionary responses from plastic changes and major difficulty faced by investigations of fisheries-induced evolution in natural populations is that genetic changes and phenotypic plasticity are confounded in the phenotypic trait upon which most previous studies have had to rely (Law 2007). Only direct assessment of

genetic changes over time can help in improving our understanding of fisheries induced evolution (Allendorf et al. 2008, Kuparinen and Merila 2007).

#### 1.3.1.2 Known effects of fisheries on migration/behaviour traits

Together with life-history and underlying physiology, the behavioural variability among fish is one of the three main trait axes that determine the vulnerability to fishing (Alos et al. 2012). Most fisheries related studies have focused on evolution of life-history traits in response to size-selective harvesting. However, harvest will also be selective if the probability that an individual is caught (its catchability) depends on its migration pattern (Quinn et al. 2007) or behavior traits (Uusi-Heikkila et al. 2008). This would include vulnerability to capture by passive fishing gear such as gill-nets (Alos et al. 2012, Biro and Post 2008, Brauhn and Kincaid 1982), aggression level in attacking baited hooks (Askey et al. 2006, Cooke et al. 2007) or habitat choice such as preference for deep *vs*. shallow habitat as in diel vertical migration of cod (Olsen et al. 2012). These behaviour traits might constitute underappreciated mechanism for selection on growth rate or other life history traits which might be crucial for the maintenance of the population.

### 1.3.2 Genetic approach to studies of evolutionary change

Genetic variation (genetic diversity) describes naturally occurring genetic differences among individuals. It is generally believed that high genetic variation permits increased flexibility and higher likelihood of the survival of a population in changing environmental circumstances (Frankham 2002). Much of the development of genetics as a science has been the struggle to observe and understand the significance of genetic variation and to understand what evolutionary forces maintain genetic diversity in natural populations (Allendorf and Luikart 2007, Leffler et al. 2012). Advances in biochemical and molecular techniques in the latter half of the last century provided the development of molecular markers which are important tools for studies of genetic variation. To date, a large majority of genetic diversity

estimates have been based on neutral markers (Leffler 2012). However, there are two sides to the evolutionary coin: neutral variation and the adaptive divergence. The role of the adaptive variation in natural marine populations has, in general been less explored until recently (Nielsen et al. 2009) with an exception of cod where identification of *Pan* I (Fevolden and Pogson 1997, Pogson 2001) has initiated numerous studies of adaptive divergence in that species (see special chapter on Pan I below)

#### 1.3.2.1 Neutral variation and microsatellites

Neutral genetic variation describes diversity that arises from neutral processes i.e. generated by mutations, drift and gene flow. The neutral genetic variation which is estimated by employing molecular marker of neutral loci, can resolve geographic structure of populations without the confounding effects of selection or environmental influences (Avise 2004). The term "neutral" refers to a gene or locus that is not subject to natural selection and therefore has no effect on fitness e.g. in terms of offspring produced (Holderegger et al. 2006). In recent decades neutral genetic variation has been extensively studied in marine populations providing new insights into the structuring of these populations. With the arrival of highly polymorphic microsatellite loci in the 90s (Jarne and Lagoda 1996) the potential for detecting weak differentiation in high gene flow species increased substantially.

As for many other organisms, the implementation of microsatellite loci has revolutionized the perception of population structure in marine fishes and for past 15 years microsatellites have been commonly applied in the studies of population genetics. For a number of large marine fish populations that are characterized by high gene flow and large effective population sizes, significant genetic differentiation has been detected in species previously assumed to be genetically homogenous over large parts of the distributional ranges (Conover et al. 2006, Hauser and Carvalho 2008, Nielsen et al. 2006). Evidence of genetic structure at micro-geographical scales has been accumulating too (Bentzen et al. 1996, Knutsen et al. 2003, Knutsen et al.

2011, Pampoulie et al. 2006, Ruzzante et al. 2006, Ruzzante et al. 1999, Ruzzante et al. 2000)

In recent years, there has been a growing awareness that neutral molecular data, such as derived from microsatellites (i.e. interpretation of genetic drift and gene flow), normally do not refer to the adaptive potential of population or species and can therefore only provide partial insight into parameters such as genetic diversity, local adaptation or evolutionary potential. Presence of non-neutral microsatellite loci (subject to hitchhiking selection) has been empirically shown to exists (e.g. Nielsen et al. 2006). The findings of such non-neutral microsatellite loci initiated the consideration if such non-neutrality could indeed help to resolve population structure issue in high gene flow marine fish species where the level of neutral genetic differentiation was very low (Andre et al. 2010).

However, knowledge of the neutral variation is needed to resolve issues such as gene flow and use of neutral molecular markers are essential for studies of effective population size (**paper IV**) which is the most prevalent indicator of evolutionary potential in populations.

## Effective populations size

Genetic diversity within populations is influenced by a range of factors, the most important of which is effective population size (Kirk and Freeland 2011). It can only be measured with neutral markers as effective size ($N_e$) reflects the rate at which genetic drift alters allele frequencies from one generation to the next. The concept was introduced in the 1930s by Sewall Wright (Wright 1931) and reflects the size of an idealized population with some of the same properties as the actual one e.g., the same rate of genetic drift. This comparison with theoretically ideal population standardizes measurement of genetic drift and makes $N_e$ comparable across populations with very different life histories. Effective population size is therefore an indicator of long-term performance of a population regarding both diversity and inbreeding (Cervantes et al. 2011, Nikolic et al. 2009) and could potentially serve as an indicator of genetic condition for practical application in marine species management (Hare et al. 2011). In theory, the consequence

of a loss of genetic variation in a small population is straightforward: It leads to increasing effect of genetic drift and in extreme cases to fixation of deleterious or suboptimal alleles (inbreeding depression), even leading to extirpation of a population. In the wild, however, it is less clear how link can be established between population size and extinction risk due to genetic factors (Fraser et al. 2007, Palstra and Ruzzante 2008). Large marine populations pose a special problem as they often display relatively high $N_e$ in the range of hundreds or thousands (Cuveliers et al. 2011, Poulsen et al. 2006, Therkildsen et al. 2010, **paper IV** ) which are generally believed to be outside the range of immediate conservation concern (Palstra and Ruzzante 2008). Yet, these estimates are often in order of magnitudes lower ($10^{-3}$-$10^{-6}$) than the estimated census sizes $N$ (**paper IV**) and pose therefore a sharp contrast to theoretical considerations that suggest that the $N_e/N$ ratio should only rarely be less than 0.5 (Nunney 1993). $N_e/N$ ratio is believed to be an indicator of the extent of genetic variation expected in a population (Hedrick 2005) but neither the relationship between those numbers (Luikart et al. 2010) nor the mechanisms responsible for driving such low ratio are yet fully understood (Turner et al. 2006). Large variance due to sweepstake reproduction processes has empirically and theoretically been shown to exist (Hedgecock et al. 2007, Hedrick 2005) and has been proposed as an explanation for very low $N_e/N$ in numerous species (Waples and Naish 2009) linked with the life history of type III survivorship (i.e. high fecundity and high juvenile mortality). Shallow gene genealogy and large variance in reproduction success in form of sweepstake reproduction (Hedgecock 1994) have been suggested to be two of the main factors driving *N*e down in Atlantic cod (Árnason 2004). Advection has also been shown to play a major role in limiting $N_e$ in species with drifting developmental stages complementing the sweepstake recruitment hypothesis (Wares and Pringle 2008). Another important factor that can lead to considerable reduction of $N_e$ is an underlying subpopulation structure. Asymmetrical gene flow and unequal subpopulation sizes are assumed to be a likely scenario in natural systems (Palstra et al. 2009, Palstra and Ruzzante 2008). Under such conditions reproduction variance of individuals within subpopulation will be increased because of differential contributions of each subpopulation to the

reproduction. Lastly, several extrinsic factors can lead to increased variance in reproductive success such as perturbation of habitat or selective harvest (Saillant and Gold 2006, Turner et al. 2006). Taken together, the impact of those extrinsic mechanisms in large harvested fish population, are yet not fully understood, but the importance of highly dynamic processes shaping the genetic diversity and thereby the effective size of population such as the Atlantic cod in Icelandic waters is evident (**paper IV**).

Given that low $N_e/N$ ratio reflects the dynamics of biological systems concern has been raised over whether the evolutionary potential of the species in question is affected caution that marine fish could be at greater risk of losing genetic diversity as consequence of fishing practices than previously assumed (Hauser et al. 2002, Hutchinson et al. 2003). The temporal variation in effective population size of Atlantic cod in Icelandic waters is considerable (**paper IV**) and likely determined by highly dynamic forces shaping the genetic variation of the population and the pairwise $F_{ST}$ values indicated an increased level of differentiation in the latter period of the study suggesting that gene flow among years tend has become more restricted (**paper IV**). Although a loss of neutral genetic diversity is not apparent (**paper III**, **paper IV**) the adaptive potential is likely to have been altered by fishery practices (**paper III**) and therefore genetic monitoring with repeated temporal sampling is of utmost importance in such variable system.

#### 1.3.2.2 Adaptive variation and populations structure

Traditionally, population genetic research has focused on neutral molecular markers to estimate genetic divergence between populations based on levels of migration and gene flow (Hauser and Carvalho 2008). However new opportunities to study relationships between genotype and the environment have led to an increased interest in molecular markers that are under selection (Guinand et al. 2004, Schlötterer 2002, Schulte 2001).

Adaptive genetic variation describes how the genetic diversity of species/population is affected by selective processes. Genetic differences in fitness-related traits and their plasticity may reflect adaptations to the population's local environment. Improved understanding of local adaption

processes is not only important for the basic improvement of our understanding of evolution, but also critical in order to define management units and setting priorities for conservation and thus is highly relevant for issues regarding fishery management (Allendorf et al. 2008, Hauser and Carvalho 2008, Nielsen et al. 2009). Local adaptation occurs when genotypes in their native habitat have higher relative fitness than genotypes originating from other habitats. For adaptive divergence of populations to take place, the evolutionary force of directional selection should be stronger than random genetic drift and the homogenizing effect of gene flow among populations (Kawecki and Ebert 2004). Until recently, the belief was that because most marine species have highly dispersive and/or mobile life stages, gene flow would prevent local adaptation. This was supported by the low levels of genetic variation, (as measured by $F_{st}$ that is defined as a fraction of the total genetic variation attributable to differences among populations) commonly found among marine populations (Conover et al. 2006). However, it has been shown that apparent genetic homogeneity revealed by neutral markers exhibit heterogeneity in non-neutral markers (Andre et al. 2010, Larsen et al. 2007). Large marine populations such as Atlantic cod tend to have high effective sizes and local populations are hence little affected by genetic drift (in small populations the effect of random genetic drift is larger and can override effect of selection). This makes them more likely to respond even to weak selection as locally beneficial alleles have good chance of sweeping through the population. Recent studies illustrate that indeed adaptive population divergence may be prevalent, despite seemingly high levels of gene flow as found in most marine species (Luttikhuizen et al. 2003). In recent decades the understanding of effects of neutral processes on evolution of populations has improved considerably but this has also called for more insights on how selective processes are acting in shaping population diversity in space and time. Thus, studies of adaptive variation in natural fish populations using molecular markers under selection can help separating effects caused by neutral processes (drift-migration) from those caused by selection and are therefore able to give insights into local adaptation, behaviour patterns (**paper II**), adaptive response to global changes and evolutionary consequences of selective harvesting (Árnason et al. 2009, **paper III**)

## The Pan I locus – an example of adaptive variation

The *Pan* I locus has played an important role for studies of population genetics in Atlantic cod. The locus was first identified by Pogson et al. (Pogson et al. 1995) under the name GM798 and was shown to exhibit elevated levels of genetic differentiation. It was sequenced and identified as part of the synaptophysin gene (Fevolden and Pogson 1997) and later revised to pantophysin (Pogson 2001). Pantophysin is a membrane protein found in small synaptic transmitter vesicles (Haass et al. 1996) and the gene coding for the protein has been shown to be under positive Darwinian selection (Pogson 2001, Pogson and Mesa 2004). However, the exact function is not known or the mechanism underlying the positive selection on the gene. The two main allele classes within the *Pan* I locus, *Pan* $I^A$ and *Pan* $I^B$, differ by six fixed non synonymous substitutions and can be differentiated by a *Dra*I restriction site polymorphism.

Allele frequency or genotypic frequency has been the focus of numerous population genetics studies in cod e.g. (Fevolden and Pogson 1997, Fevolden et al. 2012, Jónsdóttir et al. 2001, Nielsen et al. 2007, Pampoulie et al. 2006, Pogson and Fevolden 2003, Sarvas and Fevolden 2005, Skarstein et al. 2007, Wennevik et al. 2008, Westgaard and Fevolden 2007). Studies comparing distribution of variation of *Pan* I with that of microsatellites have shown elevated levels of genetic structuring for the *Pan* I locus (Pampoulie et al. 2011, Pampoulie et al. 2006, Pampoulie et al. 2008, Skarstein et al. 2007). This has contributed to the general interpretation that this locus is a useful molecular marker for stock identification in weakly structured marine fishes, since it can reveal adaptation to local environmental changes earlier than strictly neutral loci (Fevolden and Pogson 1997, Hauser and Carvalho 2008).

Recently a number of different selective agents have been suggested to be responsible for the remarkably high levels of differentiation in cod *Pan* I, including temperature and salinity (Case et al. 2005), depth (Árnason et al. 2009, Pampoulie et al. 2006, Sarvas and Fevolden 2005) and fisheries (Árnason et al. 2009, **paper III**). Also, different *Pan* I genotypes have been suggested to influence various traits affecting local fitness such as growth (Case et al. 2006, Jónsdóttir et al. 2002, Pogson and Fevolden 1998, **paper III**), condition (Jónsdóttir et al. 2008) and behaviour (**paper II**).

### 1.3.2.3 Archived samples

Otoliths and scales play an essential role in fisheries biology, as they allow determination of age and growth patterns (Campana and Thorrold 2001) similar to growth rings in a tree. In past decade attention has been drawn to archived collections of otoliths and scales as a source for DNA for long-term temporal population genetic analyses of historical samples as they can provide invaluable insights into human-induced changes in the genetic composition of fish populations (Nielsen and Hansen 2008; Hansen et al. 2009).

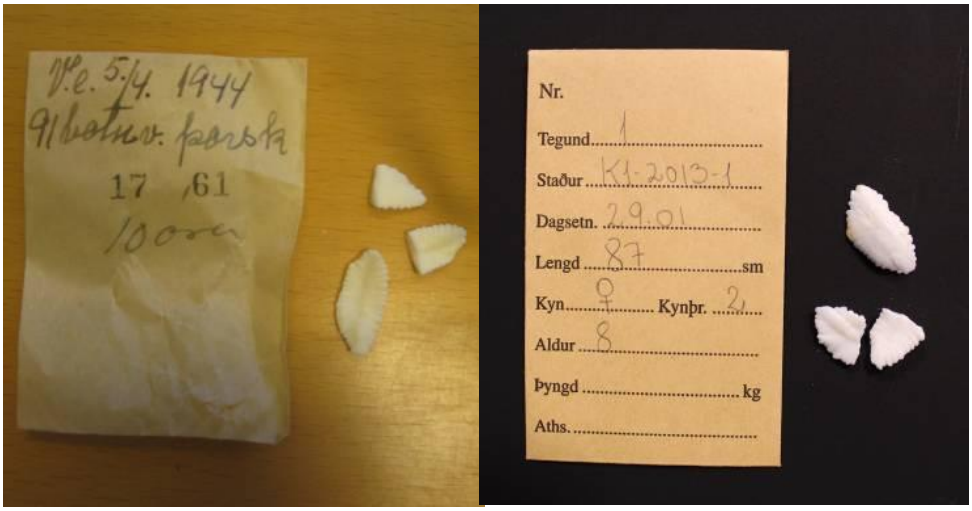


***Figure 1.*** *Otoliths and envelopes for sampling and storage from 1944 and from 2013. Relevant information (species, date, survey, length, gender and maturity) is added during sampling. One otolith has to be broken for age reading and afterwards otoliths are stored and archived. MRI has maintained archives of all otoliths sampled, some dating back 100 years. The intact otolith is used for the isolation of genetic material. Photo to the right with kind permission of Gróa Pétursdóttir.*

The importance for direct genetic approach in resolving issues of evolutionary change of fisheries is undisputed, but these types of studies are faced with several challenges. Resources are somewhat limited i.e. only few fishery research institutes have kept extensive archives of otoliths or scales needed to restore genetic historical data set suitable for genetic analysis on

population level. Another challenge for these types of studies is the poor quality of the DNA obtained from the dried rest of mucus/blood found on the surface which requires a specific set of molecular markers of short sequences suited to amplify DNA from short fragments (**paper I**). Despite these challenges by numerous studies have been conducted in order to resolve the issue of human-induced changes in genetic composition of harvested fish populations (Cuveliers et al. 2011, Hauser et al. 2002, Hoarau et al. 2005, Hutchinson et al. 2003, Nielsen et al. 2007, Palstra et al. 2009, Palstra and Ruzzante 2010, Poulsen et al. 2006, Ruzzante et al. 2001, Therkildsen et al. 2010, **paper III** and **paper IV**) .

#### 1.3.2.4 Temporal genetic approach

Earlier studies of genetic variation in historical data sets showed contradictory results. The first study of this sort on a large marine fish population was the study of Ruzzante et al. (2001). This study showed that despite suffering a total collapse the Newfoundland cod population still displayed genetic temporal stability. On the other hand, Hauser et al. (2002) showed significant decline in genetic diversity in a large population of New Zealand snapper (*Pagrus auratus*). They argued that very low $N_e/N$ sizes contributed to the loss of genetic variation. The study of Hutchinsson et al. (2003) on Atlantic cod in North Sea came to similar conclusions: They detected significant reduction in genetic diversity in the earlier period of their study (1954-1970) followed by recovery in the latter half (1970-1998). Another study on plaice (*Pleuronectes platessa L.*) conducted by Hoarau et al. 2005 indicated that the population had suffered genetic change as consequence from of the fishery i.e. displaying a low $N_e/N$ and inbreeding signal. However, the following studies of large marine fish populations that used historical data and neutral variation mostly indicated no change of genetic diversity or of effective population sizes. Study by Poulsen et al. (2006) on North Sea and Baltic cod found relative high levels of effective size and no signs of reduction in genetic diversity. The study of Therkildsen et al. (2011) on Atlantic cod in Gulf of St. Lawrence and the study of Cuveliers et al. (2011) on North Sea sole both estimated relative high effective population sizes and no genetic change despite heavy historical

exploitation. **Paper IV** describes relative high levels of effective population size in Icelandic cod. However the repeated temporal sampling shows that temporal change in pair wise $F_{st}$ is apparent and temporal variation in estimates of effective population size exists depending on what samples are used as a reference. Those studies of genetic effects of fisheries highlight the complexity of the issue such as species-specific properties, underlying population structure and demographic differences at population level that are needed to be accounted when analyzing genetic data in relation to fisheries activities. Another issue emerged with these studies. Neutral molecular markers are not likely to show change over relatively short time scales due to the initial large effective sizes and sometimes short evolutionary history of the populations under investigation (Case et al. 2006, Nielsen et al. 2009). Thus the need for genetic markers of loci under selection for unambiguous genetic evidence of contemporary evolution was called for (Conover 2006). However, until recently, the availability of adaptive estimates for different loci have been limited (Conover 2006, Nielsen 2009) and therefore only limited number of studies have been undertaken using historical data sets in order to infer about historical variation in adaptive estimates (Nielsen 2007, **paper III**). **Paper III** in this thesis, is the only study so far that has linked observed changes in genotypic frequencies of adaptive estimate to historical exploitation pattern in Atlantic cod. The findings revealed long-term significant trend in the genotypic frequencies of *Pan* I locus where proportion of *Pan* $I^{BB}$ genotypes decreased during the 60 year time period of study, likely as a result of fishery practices (**Paper III**). If the change did come about merely as a result of removal of older individuals from the stock or due to uneven fishing mortality of different behavioural groups (**paper II** , **paper III**) is not entirely conclusive from the available data, but the overall effects would be similar i.e. result in long-term changes in genotype frequencies (**paper III**).

#### 1.3.2.5 The behavioural aspects of genetic evidence

A study by Biro and Post (2008) is the first to show how genotypes linked to certain behaviour traits in rainbow trout (*Oncorhynchus mykiss*) suffered depletion because of selective–harvest of fishing. However, information on

behaviour and movement of individuals in natural marine fish populations as well as link between the genetic set-up and behavioural traits has been limited despite the importance for fishery management and for studies of FIE. In the North-Atlantic the concept of migratory and stationary ecotypes has emerged from findings of conventional tagging studies (Robichaud and Rose 2004). Development of data storage tags (DST) initiated a series of studies using DST to investigate the natural movement of individuals (Gröger et al. 2007, Hunter et al. 2004, Neat et al. 2006, Palsson and Thorsteinsson 2003, Thorsteinsson et al. 2012, Walsh and Morgan 2004). DST tags record depth and ambient temperature over extensive period of time (up to several years depending on battery life) and the information is then retrieved from the tag by recaptures in the fishery of tagged individuals. In the case of Atlantic cod, a general pattern of horizontal as well as vertical movement profiles have been extracted. These provide information on the behavioural processes responsible for the residential (coastal) or migratory (frontal) behaviour types (Pálsson and Thorsteinsson 2003, Grabowski et al. 2011, Thorsteinsson et al. 2012). The genetic analysis performed in this PhD study demonstrated differences at the selective *Pan* I locus between the two distinct behaviour types linking a certain ecotype (sedentary coastal foraging vs. foraging frontal type) to certain genotypes (*Pan* $I^{AA}$ and *Pan* $I^{BB}$ respectively) (**paper II**). Árnason et al. (2009) is the first study that provides an example of how the *Pan* I locus genotypes linked to certain behaviour trait may have suffered differently from intense fishing practices. In **paper III**, it is also argued that one plausible explanation for observed historical decrease of one genotype has come about because different behaviour traits have suffered different fishing intensity. However, these two studies interpret differentially the direction of the fishery selection. One study predicts decline of *Pan* $I^{AA}$ as a consequence of exploitation (Árnason 2009), while the other predicts the loss of *Pan* $I^{BB}$ (**paper III**). This difference is discussed in **paper III.** In summary, this discrepancy highlights the importance of investigating long-term trends in biology, genetics and fishery combined when examining changes in the genetic composition of populations that could have been potentially brought about by anthropogenic effects (**paper III**).

#### 1.3.2.6 Effect of population structure

It may not be possible to fully explain the causes of temporal changes in genotypic frequency of *Pan* I nor the variation in the genetic effective sizes until our understanding of the population structure of Atlantic cod improves further. The distinct variation in life history traits, behaviour and *Pan* I genotype frequencies of Icelandic cod is persuasive evidence that the current stock designation does not capture the actual geographic scale of population differentiation (Pampoulie et al. 2006, 2008; Marteinsdóttir and Pardoe 2008).

Traditionally, studies of genetic population structuring have utilized neutral molecular markers. Accordingly, the microsatellite analysis presented in this thesis did not indicate any spatial structure and the long-term genetic stability of the population under the assumption of neutrality could not be rejected. However, the absence of heterogeneity using neutral genetic markers does not contradict the hypothesized existence of different life history components in our data. Neutral markers are poorly suited to the detection of changes in genetic diversity because they segregate independently of the selected loci, and in populations with relatively large effective population sizes in particular, the level of genetic drift will be low (Allendorf and Hard 2009, Conover et al. 2006). Indeed, the population components might experience differential post-settlement selection, likely influenced by environmental factors, but still exhibit a relatively high level of gene flow as a consequence of larval dispersal or mixing on breeding sites, or both. However, if there is some degree of gene flow restriction between the stock components, as a recent study suggests (Grabowski et al. 2011), high effective population sizes and recent separation history are two non-exclusive explanations that could mask underlying stock structure (Case et al. 2006). If the Icelandic cod stock indeed comprises populations with limited gene flow and/or different selection schedules, then the changes in exploitation patterns described in chapter 1.2. will have affected the stock complex by removing the *Pan* $I^{BB}$ genotype at a faster rate than the *Pan* $I^{AA}$ genotype with the result in long-term changes in genotype frequencies.

## 1.4 Evolutionary considerations in fishery management

The importance of fisheries genetics to the science-based practices of fishery management has been recognized for a long time (Kapuscinski and Philipp 1988). The gradual development of the stock concept led to a greater appreciation of the importance of genetic considerations in conservation and management (Ryman and Utter 1987) and stock identification remains one of the major issue of fishery science (Cadrin et al. 2005, Shaklee and Currens 2003). More over, understanding the genetic changes and evolutionary responses of exploited populations is also crucial for the design of management aimed at sustainable exploitation of natural biological resources (Reiss et al. 2009, Walsh et al. 2006). Despite mounting evidence of evolutionary changes due to fishing practices and a recognition that evolutionary dimension to fisheries management needs to be established (Allendorf et al. 2008, Hutchings and Fraser 2008, Jørgensen et al. 2007, Kuparinen and Merila 2007, **paper III**, **paper IV**) evolutionary considerations of anthropogenic changes have received surprisingly little attention in the management. Firstly, the recognition is growing that evolution under exploitation can reduce population growth and viability and ultimately might reduce yield (Allendorf et al. 2008) and secondly such changes are likely to be irreversible on historical timescales (Petitgas et al. 2010).

Despite the progress in the field of fisheries genetics, genetics and genomics in the last decades, extension of evolutionary considerations into scientific advice can be challenging (Waples et al. 2008). A plausible reason for this limited amount of integration may be caused by a need of conceptional overlap between fishery genetics and management (evolutionary dimension involving both short-term long-term aspects of genetic variation *vs*. short-term demographic changes) (Hauser and Carvalho 2008). This lack of overlap comes about because the demographic unit (stock) does not necessarily coincide with the evolutionary unit in terms of migration and there is a mismatch between the management and biology (Reiss et al. 2009). The levels of migration (or gene flow) that can be most effectively studied

using genetic techniques are low compared to the levels that are typically of interest in stock identification (Carvalho and Hauser 1994, Waples 1998, Waples and Gagiotti 2006). In general, stock assessments assume demographic independence of the units under consideration, which means that population dynamics are driven more by recruitment and mortality than by immigration. It has been shown that the transition from demographic independence to dependence likely occurs with migration rate, $m$ =0.1 (10%) (Hastings 1993). These rates are very high in evolutionary term. As a consequence genetic methods typically have low statistical power to distinguish between migration rates that could have very different management implications (say 3% vs. 10%).

Sound knowledge of population subdivision is of importance when considering evolutionary change such as inflicted by fishery. In large mobile fish populations, estimates of effective size, census sizes and ratio thereof can be helpful for fishery management as they can be used to infer about the genetic variability over historical time scales (**paper IV**) but they are likely largely influenced by underlying subdivision and gene flow (Palstra and Ruzzante 2008, **paper IV**) and therefore the application as a indicator in fishery management needs to be evaluated by case to case basis.

Signals from selective markers is now thought to have more relevance to the ecological time scale of fisheries perspectives and they can infer about local adaptations which is crucial in order to define management units and setting priorities for conservation (Kirk and Freeland 2011, Nielsen et al. 2009). Adaptive population divergence may be prevalent despite seemingly high levels of gene flow, as found in most marine fishes (Nielsen et al. 2009) and highlights the importance of using both non-neutral and neutral markers when inferring about historical evolutionary response of a large marine fish population to anthropogenic impact of fishery practices (**Paper III**). The importance of behavioural perspective of fisheries (Árnason et al. 2009, Olsen et al. 2012, Uusi-Heikkila et al. 2008, **paper III**), has also been recently recognized and emphasizes the importance to genetically identify behavioural types (**paper II**) for implications of harvest-selection on behavioural traits (Olson et al. 2012, **paper II**). As in the case of Icelandic

cod such findings can point to the need of genetic monitoring both of spawning and harvested aggregations (Pampoulie et al. 2012).

## 1.5 Perspectives for conservation

The importance of biodiversity has recently been described in the terms of "portfolio" effect initially described by (Figge 2004) which is analogous to the effects of asset diversity on the stability of financial portfolio. It describes how species rich communities are considered to produce more temporally stable ecosystems because of the complementary or independent dynamics among species that perform similar ecosystem function. Recently it has been pointed out how such port-folio effect is also a fundamental concept of intraspecific diversity (biocomplexity) thus highlighting the importance maintaining population diversity for stabilizing ecosystem services and securing the economies and livelihood that depend on them (Greene et al. 2010, Rose et al. 2011, Schindler et al. 2010). Here, an important question arises about at what level the resilience of the Atlantic cod in Icelandic waters is dependent on its diversity both throughout its distributional range in the NA and on the diversity that seems to exist within the stock unit. An example of this diversity could be the proposed buffering effect of smaller regional spawning aggregations in the North/East/West of Iceland to the stock productivity possibly explaining the relatively stable recruitment in the Icelandic cod (Marteinsdóttir et al. 2000).

Biodiversity is dependant on underlying genetic diversity and loss of thereof is considered to be one of the most relevant evolutionary consequences of declining population size (Frankham 2002). Loss of genetic diversity can lead to inbreeding and thereby increased risk of extinction because inbreeding reduces reproductive fitness. However, controversy over the role of genetic factors in conservation management does exist and the significance of genetic factors in conservation have been questioned mainly because of the notion that demographic, ecological and behavioural considerations will drive wild populations to extinctions before genetic factors take effect (Lande 1988). One plausible reason for this view might be that available estimates of neutral variation (i.e. microsatellites) are at the

most weakly correlated with phenotypic or life-history variation (Coltman and Slate 2003). However, there is a clear theoretical background (Frankham 2002) and a growing body of experimental and empirical evidence that shows that evolutionary change can happen and is happening on decadal time scales. The evidence in large marine populations has been limited but as this thesis shows, such insights can be achieved. By using extensive historical data sets of biological and genetic information and by applying suitable set of markers, improved knowledge of the population's biology and genetic diversity (**paper II**, **III** and **IV**) can be gained. It is important to note the mutual reinforcement of genetic and biological considerations. Perspectives of population genetics are needed to be put in perspective to behavioural, ecological and environmental factors for sinful evaluations of the species/population diversity and meaningful and effective management or conservation actions.

## 1.6 Summary and future perspectives

The overall purpose of this thesis was to investigate long-term genetic stability of spawning cod in Icelandic waters. By analyzing a large historical data set and suite of both neutral and non-neutral molecular markers it became possible to obtain an insight into the historical genetic variation of cod in Icelandic waters. A prerequisite for such a study are specific set of molecular markers of short sequences suited to amplify DNA from short fragments. This challenge is addressed in **paper I** by presenting new sets of short fragment molecular markers suitable for amplification of degraded DNA from cod. **Paper II** addresses important issue of genetic identification of different behaviour types and provides thereby an important background material for interpretation of results in **paper III** and **IV**. In **paper II** profiles from data storage tags (DST) were used firstly to confirm previous findings of two distinct behaviour types, shallow water (coastal) type and the frontal type. Secondly it was assessed whether distribution of *Pan* I genotypes was related to these different foraging types. The analysis revealed that most homozygous genotypes could be assigned to certain behaviour type where *Pan* $I^{AA}$ appeared to follow the seasonal trend in temperature for shelf waters, thus remaining in relatively shallow waters. Most *Pan* $I^{BB}$ genotypes moved

to colder and deeper waters during feeding migrations and thus remained in deeper waters most of the time. Heterozygous *Pan* $I^{AB}$ individuals showed a link to both types of behaviour. These findings are important for the future studies of Icelandic cod as they provide new insights into intraspecific diversity of the species. In **paper III**, historical data set of archived otoliths collected between 1948 and 2002 was used to jointly examine phenotypic and genetic data with the goal to examine historical trends in the genetic composition of the Icelandic cod stock. Clear evidence was found that major changes in genetic variation at the *Pan* I locus of Icelandic cod have occurred alongside those in fishing patterns and age composition of the stock, whereas no temporal changes were detected with neutral genetic markers. Another finding was the life history variation between genotypes. Fish of different genotypes mature at different ages such that the fish of *Pan* $I^{AA}$ genotype appeared to mature earlier in life and also grew faster than fish of *Pan* $I^{BB}$. The main implication from this study is a genetic evidence of evolutionary change, supporting the call for evolutionary consideration of fishery management. Further, life history variation found between genotypes indicated a new level of intraspecific diversity that also needs to be considered in management. In **paper IV** the neutral historical genetic variation was investigated, providing insights for temporal variation of genetic effective sizes in a large marine population such as cod. The genetic effective population size of cod is likely within the range of few hundreds to several thousand individuals i.e. likely several magnitudes smaller than the census size. However, temporal variation in effective population size of Atlantic cod in Icelandic waters is considerable and is likely determined by highly dynamic forces shaping the genetic variation of the population. Indications of subtle changes in genetic differentiation and in $N_e$ highlight the importance of repetitive temporal sampling for detection of any underlying trend which is difficult to detect by other means in such variable system.

The outcome of this thesis highlights the biological wealth that is kept in historical datasets of archived otoliths. These collections have proven their worth and illustrate the enormous and versatile potential of using historical DNA in population and conservation genetics.

The future perspectives of this thesis are tightly linked with technical advantages that have been made in recent years. Microsatellites that have been the main molecular markers for past decades are being rapidly replaced with genomic scans using the recent developed technology of second generation sequencing and SNP data. The genome of cod has been sequenced (Star et al. 2011) and extensive genomic research is already at pace. Currently there is much focus on identifying genomic signatures of selection in space and time. Insights into local adaptation, adaptive responses to global change and evolutionary consequences of harvest can now be generated through population genomic studies, allowing the separation of the effects invoked by neutral processes (drift-migration) from those due to selection (Nielsen et al. 2009). Such knowledge will improve our basic understanding of natural as well as human-induced evolutionary processes and can also be applied to predict the future and setting conservation priorities. The new technologies and markers have potential for large improvements of genotyping of poor quality samples such as historical samples (Helyar et al. 2011, Smith et al. 2011) so it is likely that analysis of historical data sets will become more prominent in the research and study of adaptive variation in space and time.

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# Paper I

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PRIMER NOTE

# Nine new polymorphic microsatellite loci for the amplification of archived otolith DNA of Atlantic cod, *Gadus morhua* L.

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**Abstract**

**Nine out of 22 microsatellite primers tested were successfully amplified on three samples of cod *Gadus morhua* L. (two contemporary and one archived otolith samples). All loci were polymorphic (5–23 alleles/locus). The average observed heterozygosity across loci and samples was 0.625, ranging from 0.294 to 0.895 at each locus. All loci were under Hardy–Weinberg equilibrium, except PGmo56 that showed significant excess of heterozygotes in all studied samples. The isolated loci were suitable for degraded DNA and therefore useful for conducting a long-term temporal study with DNA obtained from archived otoliths of cod.**

*Keywords*: archived DNA, Atlantic cod, *Gadus morhua*, microsatellite

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The Atlantic cod *Gadus morhua* is a benthopelagic marine species widespread in the North Atlantic. Because of high population sizes and availability on the continental shelves, the species has been heavily exploited for centuries throughout its distribution area (Christensen *et al*. 2003). The depletion of several cod stocks in the North Atlantic and the failure of recoveries (Hutchings 2000) have raised concern about how genetic diversity is affected by fishing pressure (Ruzzante *et al*. 2001; Hutchinson *et al*. 2003). The assessment of such an event requires, most of the time, a temporal genetic approach including several decades as changes in the effective population size and consequently loss of genetic diversity, might not be detectable on a short timescale (Ruzzante *et al*. 2001; Hutchinson *et al*. 2003).

Utilization of archived otoliths as a DNA source for a long temporal population study of marine fishes has recently gained interest (Rivers & Ardren 1998; Ruzzante *et al*. 2001; Hutchinson *et al*. 2003). However, one challenge is the correct amplification of microsatellite fragments from these DNA sources that were not preserved properly and have undergone serious degradation. Here we report the isolation of nine short microsatellite fragments (≈150 bp) that might be useful for the amplification of DNA from archived otoliths/scales.

Genomic DNA was isolated from fresh gill tissue preserved in 90% ethanol using Puregene DNA Isolation Kit (Gentra). To isolate microsatellite fragments both polymerase chain reaction (PCR) and whole genomic DNA libraries were constructed. The whole genomic DNA library was prepared by a blunt end library construction method of the whole genomic DNA by using the pBluescript II KS vector (Sambrook & Russell 2001). The PCR library was obtained by the PCR isolation of microsatellite arrays (PIMA) method that is based on random amplified polymorphic DNA (RAPD) PCR, cloning into a TOPO-TA cloning vector (Invitrogen) and then screening of the library for microsatellites by PCR with both vector primers plus a microsatellite specific primer (GT and GA repeats) (Lunt *et al*. 1999). Sequencing of positive insert PCRs was carried out on an ABI PRISM 3730 sequencer using the BigDye Terminator kit (Applied Biosystems) and by using vector primers. Sequences were analysed by the SEQUENCHER software (Gene Codes). Primers were designed from flanking sequences for 22 loci using the CLONE MANAGER 8 software (Scientific & Educational Software).

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**Table 1** Primer sequences of microsatellite loci developed for the amplification of historical samples of *Gadus morhua*

| Locus | Accession no. | Primer sequences 5′–3′ | Repeat motif | $T_a$ (°C) | Size (bp) | Alleles | IC $H_O/H_E$ | BS $H_O/H_E$ | ICH 1957 $H_O/H_E$ |
|---|---|---|---|---|---|---|---|---|---|
| PGmo32 | DQ191392 | F: 5′-CAATCGCCGTCCAACCAAC-3′<br>R: 5′-GGCGGCAGCAACGATTCTC-3′ | $(TTG)_6$ | 56 | 099–123 | 8 | 0.357/0.353 | 0.294/0.294 | 0.507/0.476 |
| PGmo34 | DQ191393 | F: 5′-GGCCAGGTTGTTCAAGG-3′<br>R: 5′-GACCTAAAGACTAAACATAGTGC-3′ | $(GA)_4(GG)_2(GA)_8$ | 56 | 114–132 | 8 | 0.643/0.630 | 0.587/0.594 | 0.680/0.612 |
| PGmo38 | DQ191394 | F: 5′-GCTGGAACGGGAATGACAGG-3′<br>R: 5′-AACAGACGGGTGTGTGTTGATG-3′ | $(GT)_{10}$ | 64 | 083–143 | 23 | 0.857/0.895 | 0.859/0.860 | 0.880/0.880 |
| PGmo47 | DQ191395 | F: 5′-CTTATAGGCTGCAATACACCACC-3′<br>R: 5′-GCTGCCTACAGTAGTGGTGAG-3′ | $(GT)_8$ | 56 | 121–129 | 5 | 0.515/0.514 | 0.340/0.326 | 0.490/0.539 |
| PGmo49 | DQ191396 | F: 5′-CCAGGAGACTATCAAGCATAGG-3′<br>R: 5′-ACATGATTGCAGTCTTACGTC-3′ | $(GT)_8(GC)_1$<br>$(TT)_1(GT)_5$ | 60 | 103–169 | 17 | 0.702/0.643 | 0.725/0.811 | 0.759/0.663 |
| PGmo53 | DQ191397 | F: 5′-ACATGTCCTGCCAGTACAAC-3′<br>R: 5′-GAAACTGAATTTCGTTCAGAGCC-3′ | $(GA)_5(GC)_1(GA)_9$ | 59 | 132–154 | 11 | 0.678/0.667 | 0.607/0.520 | 0.683/0.685 |
| PGmo55 | DQ191398 | F: 5′-ACTTTACAGAACACGTTTGCAC-3′<br>R: 5′-ACCAACAGTGGGACGAATG-3′ | $(CA)_5$ | 58 | 168–196 | 6 | 0.677/0.691 | 0.636/0.647 | 0.641/0.636 |
| PGmo56 | DQ191399 | F: 5′-CCAGCCACCTGTTATTTCG-3′<br>R: 5′-GTAAGCATGCCTCGTGTTTC-3′ | (CA) & (CT) mixed | 58 | 138–150 | 7 | 0.651/0.677* | 0.722/0.773* | 0.735/0.750* |
| PGmo58 | DQ191400 | F: 5′-CAGCAGATTGATGGGTTTAGC-3′<br>R: 5′-AGAGGAAACCCTAAGAACGAG-3′ | $(GT)_7(GC)_2(GT)_3$ | 60 | 131–157 | 10 | 0.596/0.688 | 0.569/0.666 | 0.482/0.478 |

*Significant $F_{IS}$ values after multiple test correction. $H_O$, observed heterozygosity; $H_E$, expected heterozygosity; IC, Iceland contemporary sample; BS, Baltic Sea contemporary sample; ICH, Iceland historical sample from the year 1957.

For the amplification process, DNA was extracted from fresh gills from contemporary samples using a Chelex extraction protocol (Walsh *et al*. 1991) and from otoliths using a Chelex/proteinase K protocol (Estoup *et al*. 1996). PCR was performed in 10 μL volumes containing 2 μL of DNA, 1 μL of 10 × buffer, 1 μL of 2.5 mM DNTP, 0.2–0.4 U of DyNAzyme DNA polymerase (Finnzymes) and 1–2 μM of each primer. PCR was performed on a GeneAmp 2700 thermal block as follows: initial denaturation step of 4 min at 94 °C followed by 35 cycles of 40 s at 94 °C, 40 s at annealing temperature (see Table 1), and 40 s at 72 °C, final elongation step of 4 min at 72 °C. PCR products were detected on an ABI automatic sequencer (ABI 377, Applied Biosystems) using GENESCAN version 3.1.2 (Applied Biosystems), and scored using GENEMAPPER version 3.0 (Applied Biosystems). Calculations for observed and expected heterozygosities and probability test for deviations from Hardy–Weinberg expectations were performed using GENEPOP version 1.2 (Raymond & Rousset 1995).

Out of 22 microsatellites tested, nine gave clear amplifications at optimum annealing temperature and were used for an analysis of polymorphism (Table 1) in contemporary samples from Iceland (IC, $n = 70$) and Baltic Sea (BS, $n = 94$), and one archived otolith sample from Iceland (ICH 1957, $n = 76$). All loci were polymorphic with allelic number ranging from five to 23. The observed heterozygosity was 0.625 across all loci. The lowest heterozygosity was observed at the locus PGmo32 (BS, $H_O = 0.294$) and the highest at PGmo38 (BS, $H_O = 0.859$). The observed average heterozygosity was $H_O = 0.631$ for the IC sample, $H_O = 0.593$ for the BS sample, $H_O = 0.651$ for ICH-1957. All loci were under Hardy–Weinberg equilibrium, except PGmo56 which showed significant excess of heterozygotes in all samples. This locus has an imperfect repeat pattern of mixed dinucleotide repeats that may lead to unreliable results. Permutation tests for linkage disequilibrium carried out in GENETIX version 4.03 (Belkhir *et al*. 1999) yielded few significant values ($0.01 < P < 0.05$) involving different pairs of loci in different samples, thus allowing allelic variation at all loci to be treated as independent.

These short fragment markers might prevent errors such as large allele dropouts when using degraded DNA. Therefore, their contribution to long-term temporal genetic studies of Atlantic cod will be of great value by providing markers that are suitable for DNA amplification from archived sources.

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# Paper II

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ORIGINAL PAPER

# Are Vertical Behaviour Patterns Related to the Pantophysin Locus in the Atlantic Cod (*Gadus morhua* L.)?

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**Abstract** Throughout their geographic distribution, marine fish species often form subpopulations with limited connectivity, among which individuals display a variety of migratory behaviours. Fish behaviour experiments using Data Storage Tags (DSTs) have been useful to define the natural movement of individuals. In Icelandic waters, such experiments have indicated the presence of two distinct behaviour types of the Atlantic cod *Gadus morhua*, related to vertical migrations and habitat choice in feeding migrations. Some individuals have been shown to stay most of the time in shallow waters characterised by the seasonal trend in temperature for the shelf waters, while other migrate to deeper and colder waters where most of them forage in temperature fronts characterized by highly variable temperatures. The analysis of the pantophysin locus (*Pan* I) of the same individuals revealed that individuals carrying the *Pan* $I^{AA}$ genotype are likely to display a shallow water feeding migrations while individuals carrying the *Pan* $I^{BB}$ genotype preferred deeper waters and forage near thermal fronts. The heterozygote exhibited both type of behaviours. This study therefore suggests that further research need to be done on the pantophysin locus and its potential effects on cod phenotypes to assess the potential relationship between this locus and the behavioural types described.

**Keywords** Cod · *Gadus morhua* · Tagging experiments · DST · *Pan* I locus · Behaviour · Vertical migration

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## Introduction

Despite the importance for fishery management, information on movement and behaviour of individuals of different stock components is often limited. Even more so, the interconnection between the genetic set-up of stocks and resulting behavioural adaptations is completely lacking. The Atlantic cod *Gadus morhua* L. is widely distributed throughout the continental shelf on both sides of the North Atlantic Ocean. Within its range, cod stocks have been characterised through a combination of techniques such as genetics, morphometric analyses and conventional tag-recapture programs (see Pampoulie et al. 2006; Marcil et al. 2006; Robichaud and Rose 2004 for a review). The application of neutral and non-neutral genetic markers and conventional tagging studies has shown that cod populations are not evenly distributed and do often form a web of subpopulations with different levels of connectivity. The application of data storage tags (DSTs) to fisheries science has been shown to be a powerful way to study the natural movement of individuals (Arnold and Dewar 2000; Thorsteinsson 2002; Neat et al. 2006). DSTs can be directly attached to the fish or inserted in the abdominal cavity where they record environmental parameters such as depth and ambient temperature over extensive periods of time. Such recording of both horizontal and vertical movements can provide information on the behavioural processes responsible for residential or migratory behavioural types of Atlantic cod.

Candidate genes, e.g., genes which are suspected to be involved in the expression of a particular trait, are also useful for behavioural ecology studies and can be used to assess the natural variation in individual behaviour within species (Fitzpatrick et al. 2005). Most studies in this field have been limited to model organisms where genomic information are available and some genes have been shown to display allelic variation that affects behaviour. For instance, in the nematode *Caenorhabditis elegans*, the expression of the *npr-1* gene, which encodes a predicted G-protein-coupled receptor similar to neuropeptide Y receptors, can induce a solitary feeding or a social feeding behaviour (de Bono and Bargmann 1998). Also, the aggression and courtship behaviour in the bluehead wrasse *Thalassoma bifasciatum* has been shown to be affected by the arginine vasotocin system expression (Semsar et al. 2001). In marine fishes, although its function is still poorly understood, one potential candidate gene implicated to be under selection (Pogson and Mesa 2004) is the pantophysin (*Pan* I) locus in the Atlantic cod *Gadus morhua*. It codes for an integral membrane protein expressed in cytoplasmic transport vesicles (Windoffer et al. 1999; Brooks et al. 2000). Depth as well as salinity and temperature have been suggested to be the driving forces behind the selection at this locus (Sarvas and Fevolden 2005; Case et al. 2005).

In Icelandic waters, DST experiments conducted on a spawning ground at the Southwest coast of Iceland described two distinct behaviour types of Atlantic cod (Pálsson and Thorsteinsson 2003). The two types were discriminated based on average time spent at depth below or above 200 m. Among individuals tagged within the same spawning area, some appear to stay most of the year in shallow coastal waters (<200 m) characterised by seasonal trend in temperature, while other make feeding migration to deeper waters where they forage in thermal fronts (west-northwest and east-southeast of the country where the cold and warm water masses meet, see Pampoulie et al. 2006).

In addition, the Icelandic cod stock has recently been suggested to be composed of at least two subpopulations with limited gene flow (Pampoulie et al. 2006). Although this study focused on potential migration at a relatively large geographical scale, a depth pattern was also described on a small geographical scale (see also Jónsdóttir et al. 2006a, b). Both microsatellite loci and the pantophysin locus (*Pan* I; previously shown to be under selection, see Pogson and Mesa 2004), clearly discriminated spawning cod from inshore and offshore spawning areas in the Southwest coast of Iceland (Pampoulie et al. 2006). During the spawning season, the majority of the *Pan* $I^{AA}$ homozygotes were caught in shallow waters (<125 m) while *Pan* $I^{BB}$ homozygotes were captured in deeper waters (>125 m), hence confirming recent genetic and biological observations (Jónsdóttir et al. 1999, 2006a, b). In the Northeast Atlantic region, one of the most striking results concerning the *Pan* I locus is the allelic distribution, e.g., the almost exclusive presence of *Pan* $I^{A}$ and *Pan* $I^{B}$ alleles in coastal and offshore spawning populations respectively (Jónsdóttir et al. 1999; Sarvas and Fevolden 2005). Although several studies have recently been published on the *Pan* I locus (Jónsdóttir et al. 2001, 2002; Karlsson and Mork 2003; Pogson and Fevolden 2003; Case et al. 2005, Sarvas and Fevolden 2005; Case et al. 2006), little is known about the exact function of this locus and the potential selective pressures leading to the observed genetic pattern. In addition, it is also of interest to note that coastal cod are often described as being more sedentary than their offshore counterpart based on tagging experiments (see Robichaud and Rose 2004 for a review). Therefore, another attractive approach would be to assess if the occurrence of the *Pan* I alleles is related to the existence of the different migration patterns described for these populations.

In an attempt to answer to this question, the present study focused on the behaviour of cod around Icelandic waters using DST data. Our objectives were (1) to provide additional DST data and to confirm the existence of two distinct behaviours, (2) to assess whether the two behaviour types related to foraging in different habitats were associated with the distribution of *Pan* I genotypes.

## Materials and Methods

### Data Collection

As a part of several large European projects (METACOD; CODYSSEY, STEREO), data (tagging and genetic sampling) were collected during excursions focusing on spawning cod, *Gadus morhua*, at several spawning locations around Iceland in 2003 and 2004. DST information and *Pan* I genotypes were recorded for a total of 69 cod tagged spawning on different spawning locations around Iceland (Fig. 1). Tagging with DST was conducted under the license No.0304-1901, issued by the Icelandic Committee for Welfare of Experimental Animals, Chief Veterinary Office at the Ministry of Agriculture, Reykjavík Iceland.

### Data Storage Tags Characteristics

The DST were manufactured by Star-Oddi, Reykjavík, Iceland, and were either the DST-CENTI or DST-MILLI equipped with 12-bit microprocessors. Tagging procedure has been fully described on the following web site: http://www.hafro.is/skrar/flokkar/merkingar_thorskur.pdf. The tags released in 2003 were mostly of the DST-CENTI

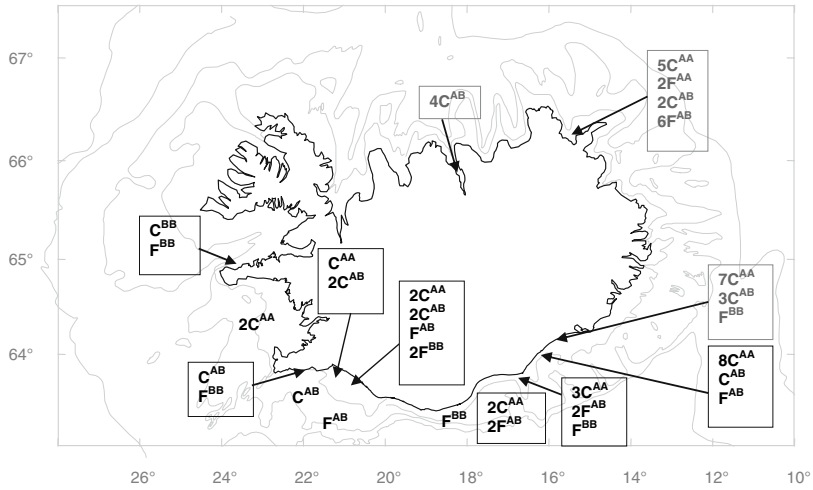


**Fig. 1** Release location of the shallow (C) and deep (F) waters individuals with their associated *Pan* I locus genotypes. Grey labels indicate individuals belonging to the Northeast spawning components; Dark labels, individuals belonging to the Southwest spawning components (according to Pampoulie et al. 2006)

(ext.memory) type, with a depth range of 0–700 m, a resolution of 0.21 m, and accuracy of ±0.40%. The range in temperature was −2 to 39°C, resolution 0.01°C, and accuracy ±0.20°C. The memory capacity of DST-CENTI is 130,000 records of temperature and depth.

## Definition of Coastal and Frontal Behaviours

In their previous study, Pálsson and Thorsteinsson (2003) defined two types of cod behaviours related to feeding migration, namely the shallow and deep water feeding cod, based upon average depth and ambient temperature profiles. In a further study (Thorsteinsson and Sæmundsson 2006), the depth range of these two behaviours sometimes overlapped creating a problem of definition. As a resolution coastal and frontal behaviours were defined according to the annual temperature history of the tagged individuals. Coastal types (C) spend at least 70% of their time in shallow waters showing annual rise in temperature to maximum in September–October and decline in temperature to reach minimum in February–March. The so called frontal type (F) shared the depth range of the coastal type during spawning migrations but during feeding migrations they inhabited in deep waters (250–600 m). The temperature history showed visits to thermal fronts and frequent vertical migrations moving between extremes in temperatures found at such locations (<0°C and >7°C).

To assess the potential relationship between these behaviours and the environmental parameters (temperature and depth), we carried out ANOVA analyses on data collected over the whole year as well as those representing only the feeding season, using Statistica 6.0 (Statsoft Incorporation 2001).

## Genetic and Statistical Analyses

DNA from individuals tagged with DST was extracted using a Chelex (Biorad 10%) extraction protocol (Walsh et al. 1991) from either gill filaments ($n = 33$) preserved in 1 ml of 96% ethanol or dried otolith preserved in envelopes ($n = 36$). A total of 69 individuals was genotyped at the *Pan* I locus. Polymerase chain reactions and digested PCR products analysis were performed as described in Pampoulie et al. (2006).

Individual genotypes were grouped into the two spawning components previously described (Northeast vs. Southwest, Pampoulie et al. 2006) and tested for Hardy-Weinberg expectation using exact tests in the program GENEPOP (version 3.1; Raymond and Rousset 1995).

To assess the potential relationship between the observed behaviour and the *Pan* I genotypes, we primarily carried out a Wilcoxon matched paired test (Siegel 1956) using Statistica 6.0 (Statsoft Incorporation 2001). We coded the Coastal and Frontal behaviours as 0 and 1, and the *Pan* I genotypes as 1 (*Pan* $I^{AA}$), 2 (*Pan* $I^{AB}$), 3 (*Pan* $I^{BB}$). We then performed one-way ANOVA to assess the potential relationship between depth, temperature and the *Pan* I locus genotypes using Statistica 6.0 (Statsoft Incorporation 2001) on the data collected during the year and during the feeding season only.

## Results

Of the 69 DST profiles retrieved during this study, 47 individuals stayed in shallow waters all year whilst 22 migrated to deeper waters during the feeding season (see electronic supplementary materials Appendix 1 for DST

information). The inspection of mean yearly temperature and depth (DST data representing the whole year) revealed that the shallow water feeding cod (C) usually occurred in warmer temperature than the deep water feeding cod (F) (ANOVA, $F_{[1,\ 66]} = 45.14$, $P < 0.001$; average temperature, C: $6.83 \pm 0.22$, F: $4.14 \pm 0.33$) but not necessarily in shallower water (ANOVA, $F_{[1,\ 67]} = 0.26$, $P = 0.613$). When analysing retrieved DST data representing only the feeding season, it could be seen that the shallow water feeding cod spent on average most of the time in warmer (ANOVA, $F_{[1,\ 60]} = 107.01$, $P < 0.001$; average temperature, C: $7.45 \pm 0.23$, F: $3.25 \pm 0.33$) and shallower (ANOVA, $F_{[1,\ 60]} = 144.82$, $P < 0.001$; average depth, C: $101.14 \pm 9.25$, F: $297.20 \pm 13.41$, $P < 0.001$) waters than their counterpart. DST profile from a typical shallow water feeding cod displaying a coastal temperature trends and a typical deep water feeding cod exhibiting feeding behaviour at thermal fronts are presented in Fig. 2. Further details of individual DST profiles of fish behaviour and genotypes are provided for 18 individuals (6 for each *Pan* I genotype) in electronic supplementary material, Appendix 2. Both behaviours were recorded among fish from the northeast and southwest spawning components (Pampoulie et al. 2006).

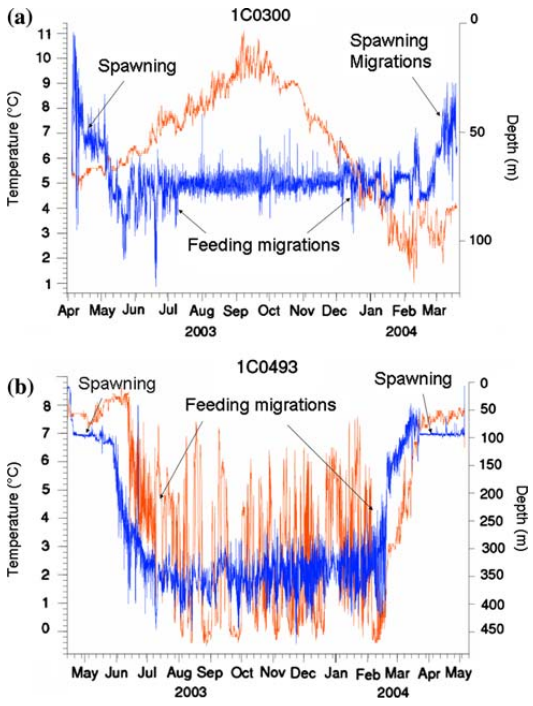


**Fig. 2** Typical DTS profile of (**a**) shallow water fish migration and (**b**) of deep water fish migration. Depth is represented by the blue line and temperature by the red one. Spawning and feeding migration are indicated by arrows. The fish DST tag number is indicated at the top of the figures. Notice different scales

Out of the 69 individuals analysed, 32, 29 and 8 exhibited a *Pan* $I^{AA}$, a *Pan* $I^{AB}$ and a *Pan* $I^{BB}$ genotype, respectively. All three genotypes were observed in both spawning components (Fig. 1; Northeast and Southwest defined in Pampoulie et al. 2006). When genotypic proportion were tested within the Northeast and Southwest spawning components, there was no evidence for departure from HWE (Northeast: $H_O = 0.500$, $H_E = 0.406$, $F_{IS} = -0.215$, $P = 0.376$; Southwest: $H_O = 0.359$, $H_E = 0.460$, $F_{IS} = 0.232$, $P = 0.175$).

The Wilcoxon match paired test revealed a highly significant difference in the behaviour displayed by the different *Pan* I genotypes ($n = 69$, $Z = 7.1149$, $P < 0.001$). A total of 30 out of 32 *Pan* $I^{AA}$ genotypes displayed typical shallow water feeding behaviour whilst the *Pan* $I^{AB}$ genotypes displayed both types of behaviour (see electronic supplementary materials Appendix 1 and 2). Out of 8 *Pan* $I^{BB}$ genotypes recorded, 7 were characterised as deep water feeding fish and 1 as shallow water feeding fish. When data retrieved from the DST during only the feeding season were considered, the ANOVA analyses revealed clear differences in the temperature and depth at which the different *Pan* I genotypes spent most of the time (ANOVA on temperature: $F_{[2,\ 54]} = 6.46$, $P = 0.003$, average temperature, *Pan* $I^{AA}$: $7.13 \pm 0.41$, *Pan* $I^{AB}$: $5.31 \pm 0.45$, *Pan* $I^{BB}$: $4.33 \pm 0.93$; ANOVA on depth: $F_{[2,\ 59]} = 12.39$, $P < 0.001$, average depth, *Pan* $I^{AA}$: $109.8 \pm 17.12$, *Pan* $I^{AB}$: $197.61 \pm 18.39$, *Pan* $I^{BB}$: $293.33 \pm 38.28$).

## Discussion

Although molecular techniques are routinely employed to answer behavioural questions (Owens 2006), linking behaviour types, and especially migratory pattern, to multilocus or single locus genotypes has been a challenging task for evolutionary biologists. Until recently behavioural genetic studies have mainly focused on mating systems of "model organism" reared under laboratory conditions for which genomic information were available. Very few studies have been conducted on wild populations. This is especially true for wild populations of fish (Semsar et al. 2001; Terai et al. 2006; Wright et al. 2006).

The combined use of DST and genetic markers has created new research opportunities that are likely to provide novel insights and means to assess the relationship between behaviour and genetics in wild populations. This is especially true when using candidate genes (Fitzpatrick et al. 2005). In contrast to conventional tagging experiment which can be used to evaluate locations of feeding grounds

and spawning site fidelity, the DST provide valuable information on the migratory behaviour of fish, involving migration from spawning to feeding areas and *vice-versa*. In Icelandic waters, the DST have revealed the existence of different behaviours related to feeding habit among cod from the same spawning area. The presence of these behaviours defined as shallow and deep waters fishes in a previous study (Pálsson and Thorsteinsson 2003) are clearly confirmed by observations in this study. In addition, the information obtained in the present study show that cod of different *Pan* I genotypes exhibited different pattern of seasonal migration. Regardless of the spawning region, most of the individuals carrying the *Pan* $I^{AA}$ genotypes appeared to follow the seasonal trend in temperature for the shelf waters, and consequently remain in relatively shallow waters. Similarly, most of the individuals with the *Pan* $I^{BB}$ genotypes moved to deeper and colder waters during feeding migration and therefore remained in deeper waters most of the time. Finally, individuals carrying the heterozygote genotypes exhibited both kind of behaviour. An exception to the general pattern described above was observed among three individuals. Two of these demonstrated deep water feeding migration but were classified as *Pan* $I^{AA}$ genotypes (individual 1C0599 and 1C0593) while one individual demonstrated a shallow water feeding migration and was classified as *Pan* $I^{BB}$ (individual 1C0265). This shows that the relation between the *Pan* I locus and the observed migration pattern is not perfect, and may merely reflect the association of this locus to other genes directly affecting the migratory behaviour of cod. The existence of shallow and deep-water cod has also been demonstrated in Norwegian waters and there they are considered as two distinct stocks, namely the north-east Arctic cod (NEAC) and the Norwegian coastal cod (NCC). The spawning areas of NCC are located in several coastal regions but also within the same spawning areas where NEAC spawns (Jakobsen 1987). However, NEAC appears to spawn in deeper water than NCC (Nordeide 1998). Recent genetic studies indicated that NCC and NEAC were distinguishable based on variation at the *Pan* I locus as well as variation at several microsatellite loci (Skarstein et al. 2007). Today, there are no evidence of a genetic divergence among coastal and frontal behaviour types of Icelandic cod at microsatellite loci, and further investigation are needed to potentially assess if the observed relationship between the *Pan* I locus and the migratory feeding behaviour is indirect (whether the *Pan* I locus is a marker for other genes affecting the feeding migration).

The observed relationship between the feeding migration pattern and the *Pan* I locus genotypes does not appear to be related to the degree of structure revealed within the stock (e.g., the northeast-southwest division) since the shallow and coastal feeding water behaviours were associated with *Pan* I genotypes in all spawning components previously described using "conventional" and "landscape" genetic approaches (Pampoulie et al. 2006). The exact function of the *Pan* I locus, which has been shown to code for an integral membrane protein expressed in cytoplasmic transport vesicles (Windoffer et al. 1999; Brooks et al. 2000), is still poorly understood. Depth was proposed to be linked to selection at this locus (Sarvas and Fevolden 2005; Case et al. 2005) as well as salinity and temperature (Case et al. 2005). These assumptions were mainly based on the observed distribution of the *Pan* $I^{B}$ and *Pan* $I^{A}$ alleles groups of cod spawning in deep and shallow waters respectively (Sarvas and Fevolden 2005). The existence of a relationship between the *Pan* I genotypes and the behavioural types of cod in Iceland suggests that the *Pan* I expression might be linked to traits that express migrational ability during the feeding season, therefore leading to the existence of differential behavioural units. Although we do not bring evidence that the pantophysin locus is directly involved in depth or temperature preferences of individual cod during the feeding migration, these results could reflect a classical codominance pattern related to the thermal optima for expression by different *Pan* I genotypes. Behavioral segregations have already been suggested as a possible explanation for the maintenance of the allelic cline in the pantophysin locus (Case et al. 2005), and cod populations were also described as behavioural units in the eastern North Sea region (Svedang and Svenson 2006). Although there was no clear evidence for the connection between these behaviour types and the role played by the *Pan* I locus, it is clear that future studies should attempt to take advantage of the insights provided by this study.

To our knowledge, this is the first time that the *Pan* I genotypes have been studied in relation to the complete migration pattern of individual cod. Our results clearly showed that Atlantic cod carrying different *Pan* I genotypes exhibited different feeding migrations, but in order to understand the underlying mechanism, further research need to be done on the pantophysin locus and its potential effect on cod phenotypes.

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*The following appendices accompany the article*

# Are vertical behaviour patterns related to the Pantophysin locus in the Atlantic cod (*Gadus morhua* L.)?

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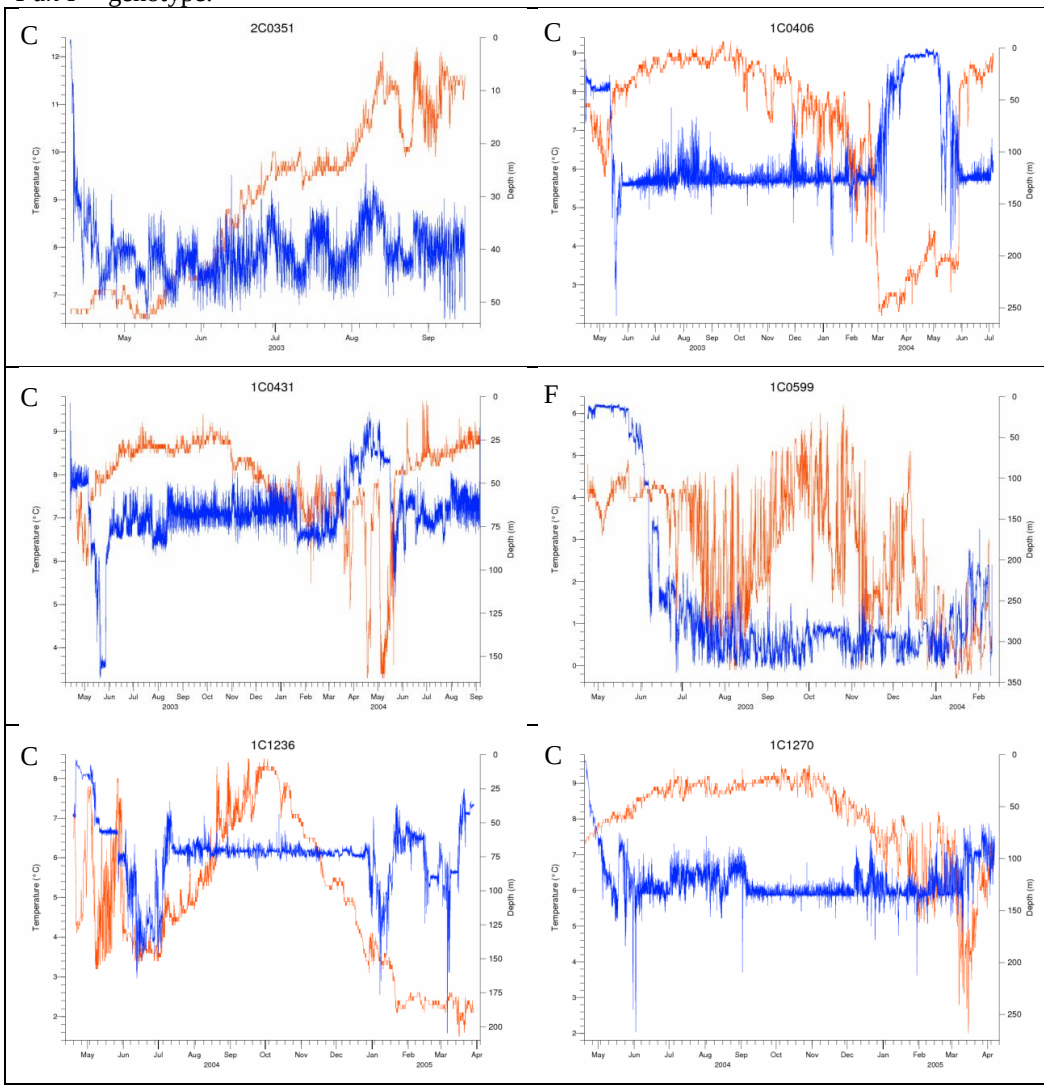
**Appendix 1.** Relevant information on the 69 data storage tags (DST): Number of DST (No), days at liberty ($d$), length (cm) at release ($L$) and recapture ($L_R$), mean and range of depth (m) ($D_{mean}$, $D_{range}$) and ambient temperature (°C) ($T_{mean}$, $T_{range}$) during the year, mean depth (m) ($DF_{mean}$) and ambient temperature (°C) ($TF_{mean}$) during the feeding season, *Pan* I locus genotypes (*Pan* I) and type of behaviour (Type).

| No | $d$ | $L$ | $L_R$ | $D_{mean}$ | $D_{range}$ | $T_{mean}$ | $T_{range}$ | $DF_{mean}$ | $TF_{mean}$ | *Pan* I | Type |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 265 | 385 | 89 | 96 | 168 | 1–572 | 6 | 3–8 | 229 | 7 | BB | C |
| 290 | 31 | 80 | 84 | 47 | 4–77 | 6 | 5–6 | NA | NA | BB | F |
| 326 | 172 | 75 | 75 | 128 | 4–502 | 7 | 0–10 | 277 | 6 | BB | F |
| 341 | 492 | 101 | 110 | 72 | 3–184 | 8 | 6–12 | 71 | 8 | AB | C |
| 351 | 160 | 84 | 99 | 40 | 0–54 | 9 | 7–12 | 40 | 10 | AA | C |
| 354 | 323 | 84 | 95 | 37 | 3–59 | 8 | 4–12 | 38 | 10 | AA | C |
| 364 | 74 | 102 | 102 | 181 | 1–432 | 7 | 2–8 | 303 | 5 | AB | F |
| 369 | 123 | 81 | 86 | 61 | 13–119 | 9 | 7–11 | 69 | 9 | AA | C |
| 374 | 307 | 100 | 103 | 134 | 10–304 | 7 | 1–8 | 179 | 7 | BB | F |
| 375 | 399 | 91 | 97 | 138 | 6–348 | 7 | 3–8 | 152 | 7 | AB | C |
| 377 | 346 | 90 | 97 | 100 | 1–159 | 8 | 6–10 | 118 | 8 | AA | C |
| 378 | 345 | 91 | 104 | 52 | 3–104 | 8 | 5–13 | 54 | 8 | AB | C |
| 389 | 124 | 100 | 100 | NA | NA | NA | NA | NA | NA | BB | F |
| 406 | 450 | 81 | 89 | 104 | 0–258 | 7 | 2–9 | 126 | 8 | AA | C |
| 410 | 152 | 90 | 93 | 71 | 21–138 | 8 | 6–10 | 48 | 9 | AA | C |
| 412 | 67 | 101 | 110 | 66 | 1–154 | 8 | 6–9 | NA | NA | AA | C |
| 415 | 159 | 103 | 103 | 44 | 1–114 | 7 | 4–13 | 22 | 10 | AA | C |
| 418 | 345 | 90 | 92 | 259 | 2–609 | 3 | -1–8 | 383 | 1 | BB | F |
| 420 | 23 | 86 | NA | 46 | 4–51 | 7 | 5–8 | NA | NA | AA | C |
| 422 | 324 | 106 | 108 | 86 | 2–218 | 7 | 3–9 | 70 | 7 | AB | C |
| 426 | 342 | 92 | 93 | 116 | 0–392 | 5 | 0–8 | 109 | 6 | AB | C |
| 431 | 512 | 96 | 98 | 66 | 4–163 | 8 | 3–10 | 67 | 8 | AA | C |
| 444 | 699 | 93 | 106 | 103 | 2–189 | 8 | 5–9 | 98 | 8 | AB | C |
| 476 | 426 | 80 | 93 | 91 | 1–123 | 8 | 7–9 | 96 | 8 | AA | C |
| 480 | 750 | 89 | 99 | 308 | 3–620 | 3 | -1–8 | 402 | 2 | AB | F |
| 493 | 387 | 94 | 94 | 263 | 7–472 | 4 | -1–9 | 344 | 3 | AB | F |
| 505 | 177 | 89 | 96 | 76 | 3–154 | 8 | 5–11 | 76 | 8 | AA | C |
| 514 | 506 | 101 | 106 | 74 | 0–197 | 8 | 3–10 | 79 | 8 | AB | C |
| 523 | 230 | 130 | NA | 100 | 8–200 | 6 | 4–8 | 113 | 6 | AA | C |
| 526 | 358 | 98 | 104 | 57 | 1–134 | 6 | 2–9 | 53 | 6 | AA | C |
| 533 | 316 | 110 | 112 | 106 | 7–289 | 7 | 3–9 | 133 | 8 | AB | C |
| 558 | 139 | 96 | 96 | 243 | 14–577 | 4 | -1–8 | 287 | 2 | AB | F |
| 584 | 284 | 74 | 74 | 156 | 0–507 | 3 | -1–5 | 283 | 1 | AB | F |
| 592 | 22 | 84 | 89 | 16 | 4–42 | 4 | 4–5 | NA | NA | AB | C |
| 593 | 683 | 78 | 87 | 206 | 1–470 | 3 | 0–6 | 258 | 2 | AA | F |
| 595 | 719 | 73 | 90 | 159 | 5–506 | 3 | 0–7 | 175 | 3 | AB | F |
| 599 | 293 | 70 | 75 | 249 | 8–342 | 3 | 0–6 | 294 | 2 | AA | F |
| 605 | 681 | 78 | 90 | 181 | 0–478 | 4 | -1–7 | 236 | 4 | AB | F |
| 616 | 326 | 69 | 75 | 190 | 3–412 | 3 | 0–6 | 235 | 3 | AB | F |
| 621 | 503 | 73 | 80 | 212 | 0–613 | 2 | -1–6 | 277 | 2 | AB | F |
| 1052 | 552 | 78 | 81 | 90 | 1–480 | 3 | -1–3 | 227 | 5 | AB | F |
| 1060 | 708 | 83 | 96 | 47 | 5–127 | 5 | 1–7 | 96 | 7 | AA | C |
| 1061 | 685 | 83 | 92 | 96 | 0–326 | 3 | 0–6 | 244 | 6 | AA | C |
| 1074 | 636 | 73 | 81 | 80 | 1–277 | 4 | 1–6 | 212 | 6 | AA | C |
| 1085 | 682 | 71 | 86 | 63 | 8–138 | 4 | 1–7 | 126 | 6 | AB | C |
| 1087 | 563 | 65 | 76 | 42 | 1–416 | 6 | 0–10 | 50 | 7 | AA | C |
| 1206 | 303 | 84 | 92 | 109 | 4–445 | 6 | 2–9 | 89 | 6 | AB | C |
| 1214 | 303 | 78 | 94 | 50 | 0–119 | 8 | 4–12 | 49 | 7 | AA | C |
| 1236 | 308 | 81 | 90 | 75 | 4–205 | 5 | 2–9 | 76 | 5 | AA | C |
| 1258 | 345 | 72 | 74 | 47 | 3–80 | 7 | 1–12 | 46 | 7 | AA | C |
| 1268 | 393 | 87 | 93 | 125 | 4–240 | 8 | 3–10 | 132 | 7 | AA | C |
| 1270 | 308 | 91 | 98 | 124 | 6–267 | 8 | 2–10 | 131 | 8 | AA | C |
| 1272 | 352 | 95 | 99 | 114 | 7–160 | 8 | 3–10 | 126 | 8 | AA | C |
| 1279 | 330 | 92 | 103 | 110 | 8–241 | 8 | 2–11 | 122 | 8 | AA | C |
| 1284 | 349 | 94 | 100 | 106 | 2–148 | 8 | 6–9 | 110 | 8 | AA | C |
| 1292 | 311 | 87 | 87 | 162 | 2–296 | 5 | 1–9 | 186 | 4 | AA | C |
| 1315 | 311 | 86 | 88 | 59 | 5–105 | 7 | 2–13 | 76 | 7 | AA | C |
| 1352 | 351 | 90 | 98 | 149 | 3–393 | 7 | 1–9 | 175 | 7 | AB | C |
| 1366 | 336 | 93 | 100 | 260 | 15–624 | 6 | 0–9 | 269 | 7 | AB | F |
| 1373 | 353 | 82 | 98 | 83 | 2–188 | 8 | 2–11 | 94 | 7 | AA | C |
| 1396 | 300 | 102 | 110 | 117 | 5–174 | 8 | 6–9 | 126 | 8 | AA | C |
| 1409 | 321 | 91 | 91 | 231 | 5–504 | 4 | 0–9 | 282 | 3 | BB | F |
| 1418 | 319 | 77 | 82 | 346 | 8–805 | 3 | 0–9 | 430 | 1 | AB | F |
| 1424 | 376 | 94 | 97 | 310 | 4–524 | 5 | 0–10 | 393 | 4 | AB | F |
| 1628 | 136 | 104 | 104 | 302 | 6–610 | 4 | 0–8 | 410 | 2 | BB | F |
| 4859 | 207 | 67 | 82 | 52 | 1–296 | 8 | 3–11 | 52 | 9 | AB | C |
| 4862 | 113 | 63 | 65 | 71 | 11–111 | 6 | 4–8 | 69 | 8 | AB | C |
| 4865 | 67 | 68 | 68 | 94 | 24–143 | 5 | 5–7 | NA | NA | AB | C |
| 4880 | 3 | 63 | NA | 25 | 15–46 | 4 | 4–6 | NA | NA | AB | C |

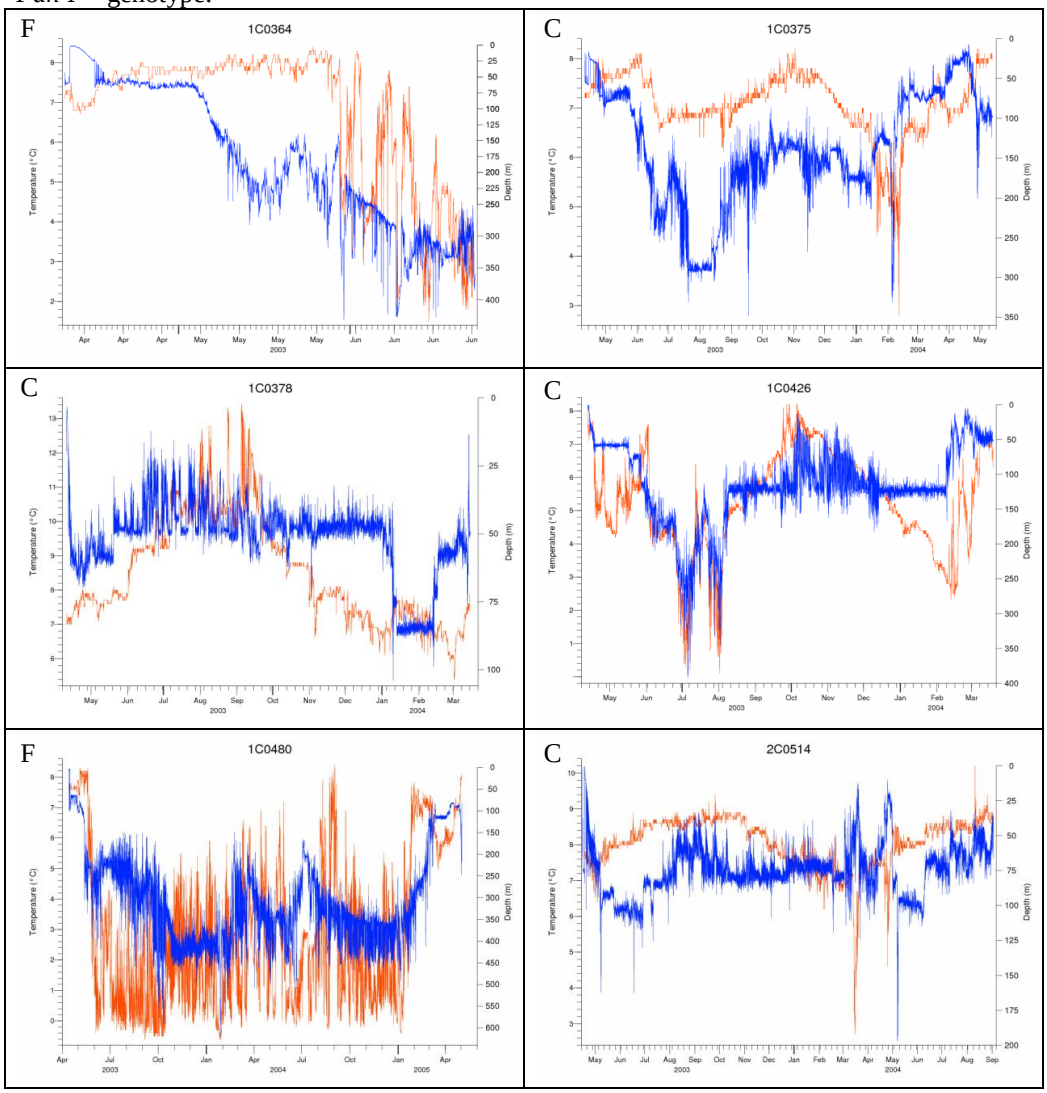
**Appendix 2.** DST profile of individual carrying different Pantophysin (*Pan* I) genotypes. C at the top right corner indicates shallow water feeding migration DTS profile while F indicates deep water feeding migration DST profile. The blue line represents the depth recorded, the red one the temperature. The fish DST tag number is indicated at the top of the figures. Notice different scales.

*Pan* $I^{AA}$ genotype:



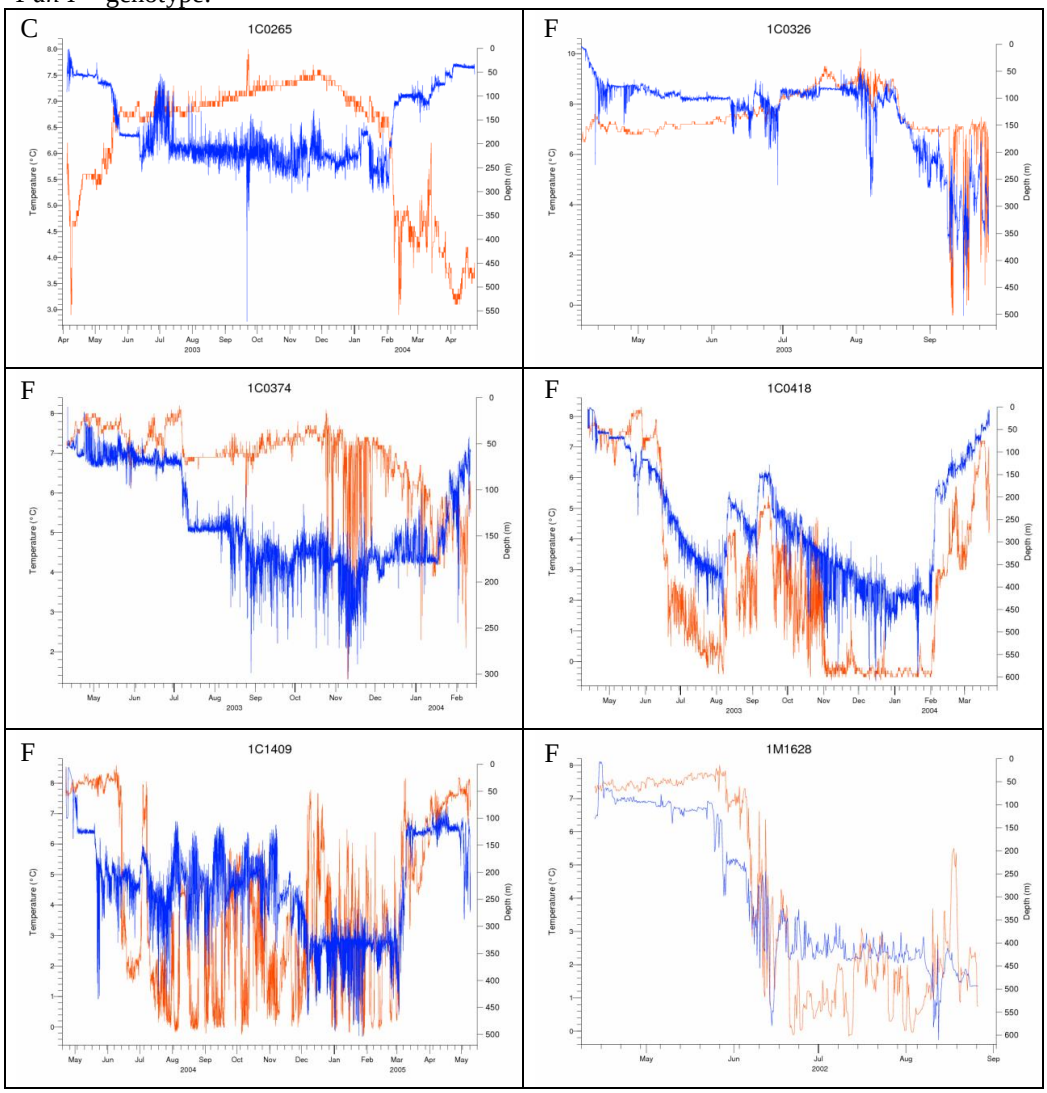
**Appendix 2.** continued

*Pan* $I^{AB}$ genotype:



**Appendix 2.** continued

*Pan* $I^{BB}$ genotype:



# Paper III

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ORIGINAL ARTICLE

# Historical changes in genotypic frequencies at the *Pantophysin* locus in Atlantic cod (*Gadus morhua*) in Icelandic waters: evidence of fisheries-induced selection?

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**Keywords**
cod, fisheries selection, *Gadus morhua*, genetic composition, Iceland, *Pan* I locus, temporal trend.

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## Abstract

The intense fishing mortality imposed on Atlantic cod in Icelandic waters during recent decades has resulted in marked changes in stock abundance, as well as in age and size composition. Using a molecular marker known to be under selection (*Pan* I) along with a suite of six neutral microsatellite loci, we analysed an archived data set and revealed evidence of distinct temporal changes in the frequencies of genotypes at the *Pan* I locus among spawning Icelandic cod, collected between 1948 and 2002, a period characterized by high fishing pressure. Concurrently, temporal stability in the composition of the microsatellite loci was established within the same data set. The frequency of the *Pan* $I^{BB}$ genotype decreased over a period of six decades, concomitant with considerable spatial and technical changes in fishing effort that resulted in the disappearance of older individuals from the fishable stock. Consequently, these changes have likely led to a change in the genotype frequencies at this locus in the spawning stock of Icelandic cod. The study highlights the value of molecular genetic approaches that combine functional and neutral markers examined in the same set of individuals for investigations of the selective effects of harvesting and reiterates the need for an evolutionary dimension to fisheries management.

## Introduction

Worldwide, there have been significant changes in life history traits of fish stocks that are consistent with a response to fishing pressure (Jørgensen et al. 2007). However, in most cases it remains inconclusive whether these changes reflect an evolutionary response to fishing mortality or merely phenotypic plasticity (Marshall and Browman 2007; Kuparinen and Merilä 2008). Evidence in support of contemporary fisheries-induced evolution has accumulated from both experimental (reviewed by Conover and Baumann 2009) and retrospective studies (Walsh et al. 2006; Conover and Munch 2007). The latter include investigations of long-term trends in empirical field data (Edeline et al. 2007; Kendall et al. 2009), which have most frequently utilized observations on the age and size at which individuals mature (Jørgensen et al. 2007). The analysis of such data has been facilitated by the development of the probabilistic maturation reaction norm (PMRN) approach (Heino et al. 2002; Barot et al. 2004), a statistical tool developed to help disentangle genetic variation in maturation from phenotypic plasticity, resulting from variation in growth and survival (Heino and Dieckmann 2008). Indeed, the major difficulty faced by investigations of fisheries-induced evolution in natural populations is that genetic changes and phenotypic plasticity are confounded in the phenotypic trait upon which most previous studies have had to rely. The need for a direct genetic approach has been noted (Kuparinen and Merilä 2007; Allendorf et al. 2008), but unambiguous genetic evidence of contemporary evolution in marine fish populations has proven elusive because of the limited availability of adaptive variation estimates, as well as historical molecular data sets (Conover et al.


 

2006). Population genetic analyses of historical samples can provide invaluable insights into human-induced changes in the genetic composition of fish populations (Nielsen and Hansen 2008; Hansen et al. 2009). In recent years, attention has thus been drawn to archived collections of otoliths and scales as a source of DNA for long-term temporal genetic studies of natural fish populations (Nielsen and Hansen 2008; Palstra et al. 2009; Palstra and Ruzzante 2010). Although genetic studies have commonly focused on neutral variation, recent approaches have shown that non-neutral or functional markers (loci subject to selection) are more likely than neutral markers to reveal processes leading to changes in allele frequencies over short temporal scales (Conover et al. 2006). Indeed, because of the large effective population sizes of exploited marine fishes, neutral markers are unlikely to exhibit changes in a signal over a relatively short time period, while loci under selection might readily respond to changes in selection regime.

Like most cod (*Gadus morhua*) stocks in the North Atlantic, Icelandic cod has experienced a drastic reduction in abundance and spawning stock biomass (SSB) during the last few decades. SSB has declined from about 1 million tonnes in the early 1950s to <200 000 tonnes at the beginning of this century (Fig. 1). Since the 1980s, estimates of SSB have remained below the long-term average, rarely exceeding 200 000 tonnes. Concurrently, there has been a long-term increase in fishing mortality ($F$), with exploitation rates exceeding 0.8 during three different time periods (Fig. 1). These levels of $F$ are considerably higher than the target of 0.3–0.4, which would enable a sustainable fishery that is within biologically safe limits (Marine Research Institute 2007).

Prior to World War II (WWII), both international trawlers and the Icelandic fleet focused their fishing effort on the relatively shallow inshore areas around Iceland

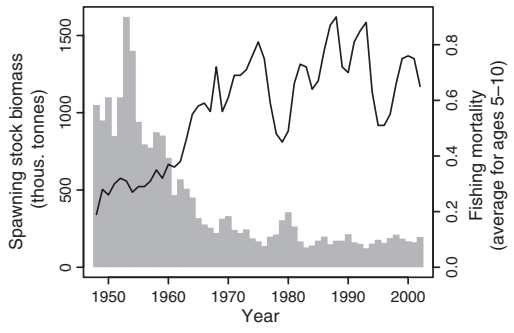


**Figure 1** Estimated total spawning stock biomass (histogram bars) and average fishing mortality of age groups 5–10 (line) of cod in Icelandic waters during the period 1948–2002 (Marine Research Institute 2009).

(Þór 2005), with landings of cod reaching a maximum of just short of 52 000 tonnes in 1933 (Marine Research Institute 2009). During the war, the international fleet was absent from Icelandic waters and therefore fishing pressure was considerably lower. Since WWII there have been substantial spatial and seasonal changes in the exploitation pattern of Icelandic cod. Immediately after the war, the inshore cod fishery increased again because of the continued exploitation by the national fleet and return of international vessels, resulting in a peak catch of 548 000 tonnes in 1954 (Marine Research Institute 2009). In 1952, Icelandic authorities banned the use of bottom trawls and Danish seine within four nautical miles of the shoreline. This regulation was followed by a gradual extension of the national fishery jurisdiction, culminating at 200 nautical miles in 1976 (Ministry of Fisheries 2004; Þór 2005). This, combined with the stricter inshore fishing regulations, improvements in fishing technology, and the introduction of larger vessels, resulted in the redirection of fishing effort into deeper offshore waters. Concurrently, trawlers were able to follow the seasonal migration of cod (Jónsson 1996), rather than being restricted to inshore breeding grounds during the spawning season.

Alongside the declines in stock abundance, there have been notable alterations in the life history of Icelandic cod, whereby far fewer fish now survive beyond 7 years of age, resulting in severely truncated age and size distributions (Schopka 1994; Marteinsdóttir and Thorarinsson 1998). Furthermore, Icelandic cod now reach maturity at younger ages and smaller sizes (Marteinsdóttir and Begg 2002). A recent study, which estimated PMRNs for 36 cohorts of Icelandic cod, found evidence that a shift towards maturation at smaller sizes and younger ages has occurred independently of changes in growth, condition and temperature (Pardoe et al. 2009). This change in the maturation schedule of Icelandic cod, along with the loss of older and larger repeat spawners, raises concerns for the stock's reproductive potential, and consequently its ability to withstand harvesting and future environmental change (Law 2007; Árnason et al. 2009). An investigation into whether the fishery has also had a long-term effect on genetic variation in the Icelandic cod stock is thus imperative.

In this study, we used archived otoliths, collected between 1948 and 2002, to jointly examine phenotypic and genetic data. Our goal was to examine historical trends in the genetic composition of the Icelandic cod stock using both selected and neutral markers. Temporal stability in the allele frequency distribution of neutral markers would reflect stability in population structure and minimal drift, as is expected when effective population sizes are large. When such stability at neutral markers is accompanied by temporal changes in allele frequency distributions at functional loci then one can infer the population has been sub-

jected to changes in selection regimes. We examined polymorphism at the Pantophysin (*Pan* I) locus (Pogson et al. 1995; Pogson 2001), a genetic marker known to be under selection or closely linked to a gene under selection, as well as a suite of six microsatellite loci assumed to be neutral. The two main allele classes within the *Pan* I locus, *Pan* $I^A$ and *Pan* $I^B$, are differentiated by a *Dra* I restriction site (Pogson 2001). The distribution of this diallelic system has been the focus of numerous population genetics studies in cod (Pampoulie et al. 2006, 2008; Nielsen et al. 2007; Skarstein et al. 2007; Árnason et al. 2009 and references within these studies).

We found clear evidence that major changes in genetic variation at the *Pan* I locus of Icelandic cod have occurred alongside those in fishing patterns and age composition of the stock, whereas no temporal changes were detected with neutral genetic markers. These results strongly demonstrate the importance of long-term genetic data, and more specifically the use of both functional and neutral genes, when studying anthropogenic disturbances in a highly dynamic system such as that characterizing the Icelandic cod stock.

## Material and methods

### Sampling

A total of 16 samples, comprising 1471 spawning or near-spawning individuals, were collected from three relatively shallow locations known to be major spawning grounds for Icelandic cod (South West = SW, Faxaflói Bay = FAX and the West Coast Bay, Breidafjördur = BRE; see Table 1 for exact details on location). DNA from 1286 of those individuals was successfully amplified and used for genetic analysis. Otoliths collected during the period 1948–1996 were recovered from the archived collections at the Icelandic Marine Research Institute (MRI). Otoliths collected prior to 2000 were stored in dry paper envelopes, while the more recent historical samples (collected in 2000) were stored in specially designed plastic storage wells. A contemporary sample (2002) was obtained from fresh gill tissue. The choice of sample years was based on the availability of individuals (sample size) stratified by years of known high and low stock size. Biological information, i.e. total length, age, sex and maturity status, for all individuals used in the genetic analyses was retrieved directly from the MRI database. Individuals were pooled into 10-year cohort classes based on their year of birth (1931–1940 through to 1991–2000).

### DNA extraction and analysis

DNA was extracted from the otolith surface using a Chelex/Proteinase K protocol (Estoup et al. 1996). The DNA was preserved at 4°C during this study and subsequently

**Table 1.** Overview of Icelandic cod samples used in this study: year, date and subarea of sampling (see Note for corresponding coordinates), sampling depth (meters; entries with asterisks show depth distributions [10–90% quantiles] within the subarea for those samples where actual depth of the catch was not available), number of individuals ($n$), amplification success ($A_{succ}$), proportion of females ($P_{fem}$), mean total length (cm; $TL_{mean}$), length range (cm; $TL_{range}$), mean age (years; $A_{mean}$), age range (years; $A_{range}$). For all samples, DNA was derived from archived otoliths with the exception of 2002 for which DNA was obtained from fresh gills.

| Year | Date | Subarea | Depth | $n$ | $A_{succ}$ | $P_{fem}$ | $TL_{mean}$ | $TL_{range}$ | $A_{mean}$ | $A_{range}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 1948 | 18/4–22/4 | BRE | 100 | 132 | 68.9 | 83.9 | 87.48 | 67–150 | 9.84 | 5–16 |
| 1957 | 20/3–2/4 | SW | 37 | 111 | 95.6 | 40.7 | 93.95 | 73–136 | 9.38 | 5–19 |
| 1959 | 14/4 | SW | 37–74* | 90 | 83.3 | 21.3 | 96.22 | 78–115 | 9.64 | 6–14 |
| 1966 | 15/4 | SW | 37–74* | 95 | 94.7 | 51.1 | 91.81 | 74–112 | 8.42 | 5–13 |
| 1972 | 6/4–8/4 | FAX | 38–83* | 94 | 88.3 | 44.6 | 87.12 | 75–100 | 8.54 | 4–11 |
| 1973 | 3/5 | FAX | 68 | 95 | 64.2 | 59.0 | 90.37 | 76–124 | 8.84 | 6–12 |
| 1973 | 26/4 | SW | 70 | 86 | 97.7 | 46.4 | 88.26 | 76–103 | 8.84 | 6–11 |
| 1976 | 19/4 | FAX | 55 | 88 | 77.5 | 55.1 | 88.27 | 61–121 | 7.26 | 4–13 |
| 1976 | 10/4 | SW | 45 | 90 | 100.0 | 30.0 | 88.77 | 47–130 | 6.93 | 3–11 |
| 1979 | 26/4 | FAX | 50 | 85 | 97.6 | 34.9 | 85.39 | 69–115 | 6.98 | 6–10 |
| 1985 | 18/4 | SW | 37–74* | 93 | 100.0 | 54.8 | 96.53 | 70–129 | 8.84 | 5–18 |
| 1996 | 16/4–19/4 | BRE | 63 | 87 | 82.2 | 50.0 | 86.28 | 57–128 | 7.26 | 4–11 |
| 1996 | 25/3–9/4 | FAX | 55 | 98 | 68.4 | 56.7 | 97.24 | 79–130 | 7.36 | 5–14 |
| 1996 | 27/3 | SW | 56 | 85 | 100.0 | 43.0 | 101.20 | 82–128 | 7.55 | 5–13 |
| 2000 | 6/4–8/4 | BRE | 150 | 68 | 97.1 | 45.5 | 77.50 | 50–104 | 6.75 | 5–11 |
| 2002 | 5/4 | SW | 54 | 74 | 98.5 | 40.5 | 85.54 | 53–117 | 6.18 | 3–12 |
| | | | | 1471 | 87.4 | 47.4 | | | | |

Note: Exact coordinates and mean depth of subareas are as follows: subarea South West (SW): 63.5–64°N, 20–21°W; subarea Faxaflói bay (FAX): 64–64.5°N, 22–23°W; subarea West Coast Bay, Breiðafjörður (BRE): 65–65.5°N, 23–24°W.


 

moved to a −80°C freezer for long-term storage. Primers specially designed to amplify a short fragment (142 bp) of the *Pan* I locus from DNA that has experienced considerable degradation (Nielsen et al. 2007; forward: 5′-GGCAA ATGAAACCCAGAAAA, rev: 5′-ATGACACTTGTGGCAA GCAG) were used for the polymerase chain reaction (PCR). PCR was performed in a 17 μL volume containing 3 μL of DNA product, 1.7 μL 10× buffer, 0.51 μL of 1.5 mM MgCl2, 1.7 μL of 2.5 mM DNTP, 0.25 μL of each 10 μM primer solution, 0.25 μL of 20 mg/mL BSA (Fermentas) and 0.5 units of DyNAzyme polymerase (Finnzymes Oy, Espoo, Finland). Cycles were performed on GeneAmp2700 thermal block using 'Touchdown' procedures as follows: initial denaturation step of 2 min at 95°C followed by 10 cycles of 30 s at 94°C, 45 s of annealing temperature that decreases in each cycle by 0.5°C until 55°C was reached and 30 s of 70°C. This was followed by 25 cycles of 30 s at 94°C, 50 s at 55°C and 30 s at 70°C. A final elongation step of 5 min at 72°C was performed. Immediately after the PCR, a restriction analysis was carried out on the PCR product (17 μL) using 18 units of the enzyme *Dra*I (Fermentas) and accompanying buffer (2 μL). *Dra*I cuts at the diagnostic restriction sites in the PCR product, discriminating between the *Pan* $I^A$ and the *Pan* $I^B$ alleles. Fragments were visualized on a 3.5% agarose gel stained with ethidium bromide. As expected, the digestion pattern was similar to that described by Nielsen et al. (2007): a single band at 142 bp for the *Pan* $I^{AA}$ homozygote, two bands of 40 and 102 for the *Pan* $I^{BB}$ homozygote and all three bands for the heterozygotes.

We also analysed genetic variation at six microsatellite loci assumed to be neutral using a subset of those individuals from the *Pan* analysis: Gmo2 (Brooker et al. 1994), Gmo8, Gmo19 (Miller et al. 2000), Tch5, Tch14 and Tch 22 (O'Reilly et al. 2000). PCR were performed in 10 μL volumes containing 2 μL of DNA, 1 μL of 10× Buffer, 1 μL of 2.5 mM DNTP, 0.2–0.4 units of DyNAzyme™ DNA polymerase (Finnzymes) and 1–2 μM of each primer. PCR were performed on a GeneAmp®2700 thermal block using the 'Touchdown' procedure described earlier. PCR products were multiplexed and detected on an ABI-automatic sequencer (ABI 377; Applied Biosystem) using GeneScan 3.1.2 (Biosystems 2000) and scored using GeneMapper 3.7 (Biosystems 2004). The occurrence of genotypic errors resulting from technical artefacts (null alleles) or DNA quality (large allele dropouts) was assessed using the program MICRO-CHECKER (Van Oosterhout et al. 2004).

Extra care was taken to minimize chances of potential contamination during laboratory procedures. Samples were processed in a designated area that was decontaminated between procedures using a chlorine solution and UV light. Negative controls were run, and comparisons to microsatellite readings were conducted to test for possible contaminations between neighbouring DNA wells.

### Statistical analyses

Changes in the age distribution between sampling years were tested using chi-square tests. In situations of low expected values (<5), samples from cod aged 6 years or younger, and eleven years or older, were pooled together. The changes in *Pan* I genotype frequencies between age groups and cohort classes were also tested using chi-square tests. For these tests, individuals aged 5 years or younger, and aged twelve years or older, were pooled, while all intermediate age classes were kept separate.

To investigate the long-term consistency of genotypic change by age, we pooled the samples in three time periods. The earliest period consisted of samples collected in 1948, 1957, 1959 and 1966 when average fishing mortality is estimated to have been <0.3. The middle period, characterized by an average fishing mortality of 0.6, contained samples from 1972, 1973, 1976 and 1979. The latest period comprised samples collected in 1985, 1996, 2000 and 2002 and featured several peaks of very high fishing mortality (>0.8).

We used a multinomial log-linear model (Venables and Ripley 2002) to explore changes in genotype frequencies with time. The purpose of the model was to describe the long-term changes in genotype frequencies of cohorts 1932–1999, after taking into account that some cohorts were only sampled at a younger age and other cohorts only at an older age. In a single model, we fitted the frequencies of *Pan* I genotypes *Pan* $I^{AA}$, *Pan* $I^{AB}$ and *Pan* $I^{BB}$ as a smooth function of both cohort and age. The smooth functions for cohort and age were natural cubic splines, each with 3 degrees of freedom. The multinomial model ensures that the predicted relative frequencies of the three *Pan* I genotypes sum to one within each cohort-age stratum.

Potential differences in growth between the *Pan* I genotypes were investigated by analysing length-at-age data, combined for all years. The analyses included cod aged 7–9 years only since those age classes contained sufficient individuals from all three *Pan* I genotypes in all time periods. To test whether differences in growth of Icelandic cod were related to their *Pan* I genotype, mean length was modelled using analysis of covariance (ANCOVA) with *Pan* I genotype as the categorical independent variable and age as continuous covariate.

For the suite of six microsatellite loci, observed ($H_O$) and expected ($H_E$) heterozygosity were calculated in GENETIX 4.03 (Belkhir et al. 1999). Tests for deviations from Hardy–Weinberg equilibrium (HWE) were conducted for both the *Pan* I locus and the suite of six microsatellite loci using the exact test in GENEPOP 3.1 (Raymond and Rousset 1995).

Genetic differentiation between sampling sites and all pairs of populations was estimated with pairwise $F_{ST}$ estimates following Weir and Cockerham (1984), and 95% confidence intervals were determined by bootstrapping over loci. The program FSTAT 2.9.2 (Goudet 1995) was used for this analysis. An analysis of molecular variance (AMOVA) was carried out for both the *Pan* I locus and the suite of six microsatellite loci in ARLEQUIN 3.0 (Excoffier et al. 2005) to assess hierarchical partitioning of genetic variance. The genetic relationships among samples were further analysed using principal component analysis and visualized with a multidimensional scaling (MDS) plot based on a matrix of pairwise $F_{ST}$'s.

## Results

A hierarchical AMOVA revealed that overall variation at the *Pan* I locus was because of temporal ($F_{SC} = 0.122$, $P < 0.001$) rather than spatial variation ($F_{CT} = -0.025$, $P = 0.92$) (Table 2). Furthermore, no trend was detected in sampling depth (Table 1). Therefore, in the following analyses, samples were grouped into sample years (or cohort classes) independently of sampling location (and depth). Results from the analysis of genetic variation at the *Pan* I locus are presented in Table 3. Genetic diversity among sample years was highly variable, with observed heterozygosities ranging from 0.153 (2002) to 0.988 (1979), with an average value of 0.617. Genetic diversity within cohort classes was relatively moderate, with observed heterozygosities ranging from 0.395 (1991–2000 cohort class) to 0.761 (1951–1960 cohort class). Significant deviations from HWE were found in 50% of the sample years, whereas five of seven cohort classes contained samples that were not in HWE. Heterozygote excess was the cause of all of the deviations from HWE (Table 3).

As has been previously observed with an independent data set (Marteinsdóttir and Thorarinsson 1998), the age composition of Icelandic cod changed markedly through the study period. The observed shift towards a narrower age distribution resulted from the loss of older age classes and an overall reduction in mean age ($\chi^2_{[55]} = 851.0$, $P < 0.001$) (Table 1, Fig. 2). Moreover, our analyses revealed that the *Pan* I locus has been subject to major changes in allele frequencies during the study period, in relation to both age and cohort class. Within cohorts, the frequencies of the homozygous genotypes were age dependent (Figs 3 and 4). The *Pan* $I^{BB}$ genotype was more common among the older spawning cod, while the *Pan* $I^{AA}$ genotype was most frequently observed among the younger age classes ($\chi^2_{[14]} = 187$, $P < 0.001$; Fig. 3). In fact, no spawning cod younger than 6 years of age carried the *Pan* $I^{BB}$ genotype. These age-dependent trends in homozygote frequency were still present when the data were split into three different time periods (Fig. 4). A high proportion of heterozygote individuals were found in all age classes (Figs 3 and 4). Indeed, as stated earlier, a lack of HWE both within sample years and cohort classes was mainly because of heterozygote excess (Table 3).

At the inter-cohort level, there was a gradual but strong decline in the frequency of *Pan* $I^{BB}$ over the study period ($\chi^2_{[12]} = 199.3$, $P < 0.001$) (Fig. 5). Concomitantly, the *Pan* $I^{AA}$ genotypes increased in frequency, exceeding 50% in the last 20 years of the study at the expense of the *Pan* $I^{BB}$ and *Pan* $I^{AB}$ individuals ($\chi^2_{[4]} = 85.9$, $P < 0.0010$) (Fig. 5). For those ages represented in our data throughout the study period, predicted genotype frequencies from a multinomial model showed similar temporal trends (Fig. 6). According to the model (likelihood ratio test versus null model $\chi^2_{[12]} = 276.4$, $P < 0.001$), the gradual increase in *Pan* $I^{AA}$ genotype frequency started in the 1960s and was accompanied by decreases in the relative proportions of the two other genotypes.

Distinct differences were observed in length-at-age among the *Pan* I genotypes, whereby Icelandic cod carrying the *Pan* $I^{AA}$ genotype had significantly higher length-at-age on average than cod of the *Pan* $I^{AB}$ and *Pan* $I^{BB}$ genotypes ($P = 0.048$) (Fig. 7).

Multilocus genotype information from six microsatellites was obtained from 892 of the individuals utilized in the *Pan* I analyses, representing the same sampling years and sites (SW, FAX, BRE; Table 1). Genetic diversity in the samples was moderate to high, with observed heterozygosities ranging from 0.594 (Tch22) to 0.951 (Tch14). The numbers of alleles ranged from 5 (Tch22) to 35 (Tch14). Deviations from HWE were detected in 3 of 105 tests after Bonferroni correction for multiple tests and

**Table 2.** Hierarchical partitioning of genetic variance at the *Pan* I locus, based on an analysis of molecular variance (AMOVA) of samples of Icelandic cod (see Table 1).

| Source of variation | d.f. | Variance components | Percentage variation | Fixation indices | *P*-values |
|---|---|---|---|---|---|
| Among locations | 2 | −0.006 | −2.49 | CT = −0.025 | 0.917 |
| Among years, within locations | 13 | 0.031 | 12.47 | SC = 0.122 | <0.001 |
| Within years | 2556 | 0.220 | 90.02 | ST = 0.100 | <0.001 |
| Total | 2571 | 0.244 | 100 | | |


 

**Table 3.** Observed ($H_O$) and expected ($H_E$) heterozygosity and observed frequencies of *Pan* $I^B$ allele in Icelandic cod samples (*n*: sample size) by cohort class and sample year. Bold values indicate significant deviations of $F_{IS}$ values from Hardy–Weinberg expectations, after correction for multiple tests.

| Cohort class | *n* | $H_O$ | $H_E$ | $F_{IS}$ | Freq. of *Pan* $I^B$ allele |
|---|---|---|---|---|---|
| 1931–1940 | 62 | 0.629 | 0.490 | −0.278 | 0.573 |
| 1941–1950 | 182 | **0.621** | 0.499 | −0.242 | 0.525 |
| 1951–1960 | 113 | **0.761** | 0.495 | −0.534 | 0.549 |
| 1961–1970 | 383 | **0.640** | 0.496 | −0.290 | 0.453 |
| 1971–1980 | 184 | **0.712** | 0.500 | −0.422 | 0.497 |
| 1981–1990 | 215 | **0.502** | 0.385 | −0.302 | 0.261 |
| 1991–2000 | 147 | 0.395 | 0.377 | −0.044 | 0.252 |
| | 1286 | | | | |
| Sample year | *n* | $H_O$ | $H_E$ | | Freq. of *Pan* $I^B$ allele |
| 1948 | 91 | 0.637 | 0.497 | −0.277 | 0.539 |
| 1957 | 106 | **0.670** | 0.499 | −0.338 | 0.476 |
| 1959 | 75 | 0.480 | 0.453 | −0.053 | 0.653 |
| 1966 | 90 | **0.867** | 0.499 | −0.734 | 0.522 |
| 1972 | 83 | **0.723** | 0.484 | −0.490 | 0.590 |
| 1973 | 145 | **0.731** | 0.493 | −0.480 | 0.559 |
| 1976 | 158 | 0.449 | 0.375 | −0.195 | 0.250 |
| 1979 | 83 | **0.988** | 0.500 | −0.976 | 0.506 |
| 1985 | 93 | 0.559 | 0.495 | −0.124 | 0.548 |
| 1996 | 224 | **0.500** | 0.384 | −0.301 | 0.259 |
| 2000 | 66 | 0.652 | 0.486 | −0.333 | 0.417 |
| 2002 | 72 | 0.153 | 0.187 | −0.188 | 0.104 |
| | 1286 | | | | |

were not because of any specific loci or sample. The microsatellite analysis revealed that there was no genetic differentiation ($F_{ST}$) among sampling sites ($F_{ST}$ = 0.0004, $P$ = 0.100). Of 105 pairwise ($F_{ST}$) comparisons among all samples, none were found to be significantly different from zero and there was an overall $F_{ST}$ = 0.0018 ($P$ = 0.538). This pattern was also evident in a hierarchical AMOVA, which showed that only 0.03% of total genetic variance ($F_{CT}$ = 0.0003) could be attributed to variance among sampling locations. The variance between temporal samples was slightly higher (0.13%, $F_{SC}$ = 0.0013). However, both values were nonsignificant ($P$ > 0.05). This lack of differentiation confirms long-term temporal stability under the assumption of neutrality and a MDS analysis (not shown) further confirmed the lack of spatial or temporal pattern in the data.

## Discussion

Our study is the first, to our knowledge, to analyse functional and neutral genetic markers in combination with fisheries data, over a multidecadal time period, in an attempt to explain distinct changes in genetic and phenotypic variation in a fish stock that has been heavily exploited since the 1940s and 50s. By isolating DNA from archived cod otoliths, gathered over a 60-year period at spawning sites on the SW coast of Iceland, we were able to detect long-term temporal changes in genotype frequencies at the Pantophysin (*Pan* I) locus, whereas no changes were observed at six microsatellite loci. The significant decline in the *Pan* $I^{BB}$ genotype, which represented 26% of the sampled population in the 1930s but

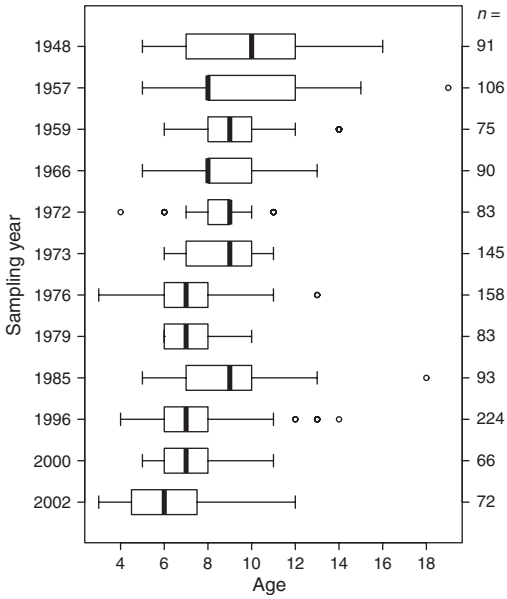


**Figure 2** Box and whiskers plot of the age distribution of Icelandic cod within each sampling year. The corresponding sample size is shown on the right axis.

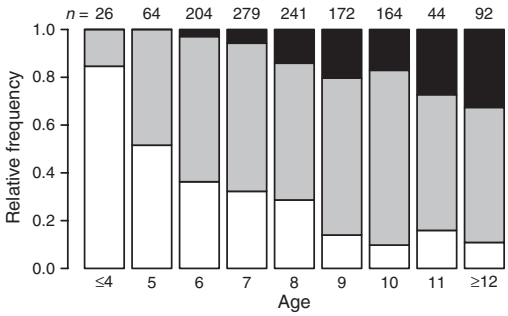


**Figure 3** Age-specific distributions of *Pan* I genotype frequencies (white: *Pan* $I^{AA}$, grey: *Pan* $I^{AB}$, black: *Pan* $I^{BB}$) in samples of Icelandic cod collected between 1948 and 2002. The corresponding sample size is listed above each column.

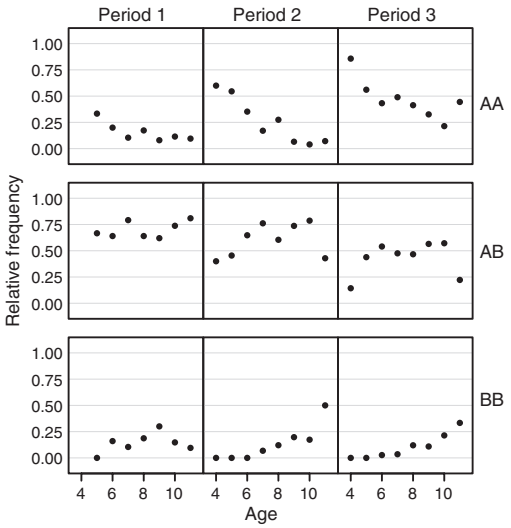


**Figure 4** Observed age-specific frequencies of *Pan* I genotypes (*Pan* $I^{AA}$, *Pan* $I^{AB}$ and *Pan* $I^{BB}$) for Icelandic cod sampled in three time periods: Period 1, 1948–1966; Period 2, 1972–1979; Period 3, 1984–2002 (see Table 1 for actual years sampled within each period).

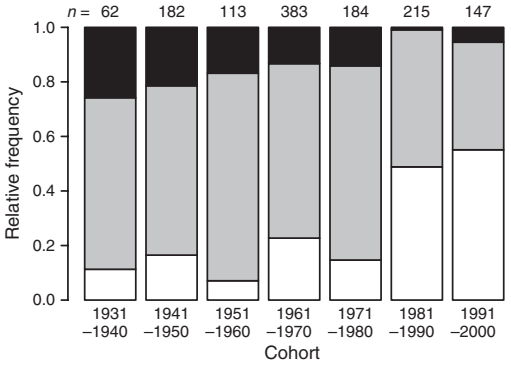


**Figure 5** Observed *Pan* I genotype frequencies (white: *Pan* $I^{AA}$, grey: *Pan* $I^{AB}$, black: *Pan* $I^{BB}$) for Icelandic cod from different (10 year) cohort classes. The corresponding sample size is listed above each column.

only 5% in the 1990s, followed changes in the exploitation pattern, which were characterized by increased effort in deeper waters facilitated by larger ships and technological advancements in fishing gear.

In the current study, the frequencies of *Pan* I genotypes varied both within and between cohorts. Within cohorts, the frequency of the *Pan* $I^{BB}$ genotype increased with age (Figs 3 and 4). Currently, the age-specific distribution of the *Pan* I genotypes is unknown for immature Icelandic cod. However, this result strongly indicates that fish of different genotypes mature at different ages. The absence of the *Pan* $I^{BB}$ genotype among spawning fish aged 3–5 years is therefore most likely due to cod carrying that genotype not having matured yet and thus not being present on the spawning sites, which provided the data for this study. The gradual increase in the frequency of the *Pan* $I^{BB}$ genotype from 6 to 9 years of age is thus likely to reflect differences in the rate of maturation. The potential existence of life history variation between cod of different *Pan* I genotypes was also supported by the observation that cod exhibiting the *Pan* $I^{AA}$ genotype grew at a significantly higher rate than those carrying the *Pan* $I^{AB}$ and *Pan* $I^{BB}$ genotypes (Fig. 7). Furthermore, our findings complement emerging evidence from tagging studies using data storage tags of two groups of Icelandic cod associated with foraging strategy and habitat selection during the feeding season (Pálsson and Thorsteinsson 2003; Pampoulie et al. 2008). The so-called coastal cod inhabit shallow shelf waters characterized by seasonal temperature trends. In contrast, 'frontal' cod undertake migrations to cold, deep waters (>250 m) where they forage at thermal fronts, making frequent vertical migrations between temperature extremes (<0 and >7°C). Pampoulie et al. (2008) revealed that individuals carrying the *Pan* $I^{AA}$ genotype are most likely to be classified as coastal cod based on their behaviour during feeding migrations, while individuals carrying the *Pan* $I^{BB}$ genotype most frequently exhibit the migratory behaviour of frontal cod. The heterozygotes were found to exhibit mixed feeding migration strategies.

In the present study, we have shown a gradual decline in the frequency of the *Pan* $I^{BB}$ genotype among spawning cod during the last 60 years. Concurrently, the *Pan* $I^{AA}$ genotype increased in frequency, exceeding 50% in the last 20 years of the study at the expense of the *Pan* $I^{BB}$ and *Pan* $I^{AB}$ individuals (Fig. 5). We cannot be certain of the primary driving force behind these changes in the proportions of the different genotypes. However, four different hypotheses, three of which can be linked to changes in exploitation patterns, warrant our scrutiny.

First, changes in genotypic frequencies may have resulted from changes in stock composition because of the consistent removal of older fish during a period of increased fishing mortality (Marteinsdóttir and Thorarinsson 1998; Fig. 2 in this study). By the end of the last century, relatively few cod survived to 10 years of age in Icelandic waters (Marine Research Institute 2009). Accordingly, the mean age of spawning cod declined from 10 to 6 years during the study period (Table 1, Fig. 2). As previously discussed, the oldest cod in our samples were predominantly of the *Pan* $I^{BB}$ or *Pan* $I^{AB}$ genotype, whereas relatively few cod carrying the *Pan* $I^{AA}$ genotype were found to be aged 9 years or older. Therefore, the truncation of the


 

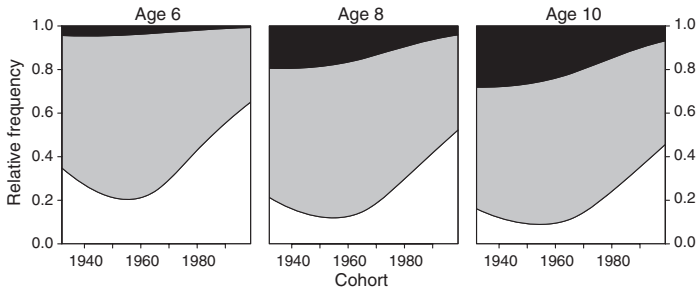


**Figure 6** Predicted distributions of cohort-specific *Pan* I genotype frequencies for Icelandic cod aged 6, 8 and 10 years, from a multinomial model (smoothing allowing for three degrees of freedom).

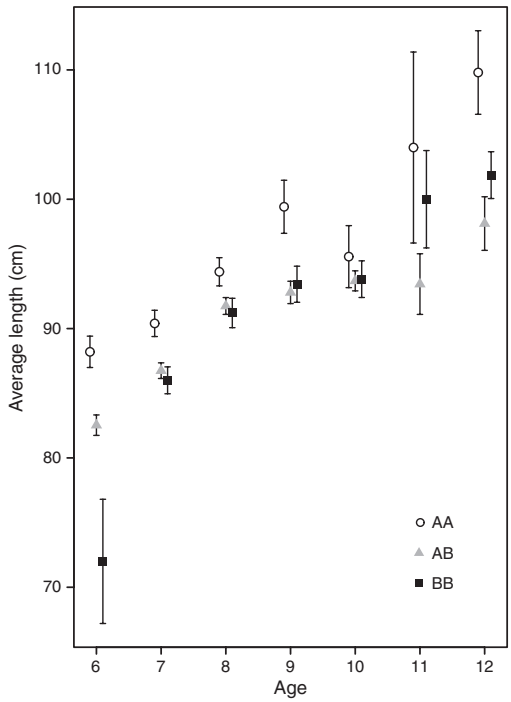


**Figure 7** Average length (±1 SE) of Icelandic cod aged 6–12 years, sampled in the period 1948–2002, and carrying either *Pan* $I^{AA}$, *Pan* $I^{AB}$ or *Pan* $I^{BB}$ genotype.

age distribution of Icelandic cod, a common feature of commercially exploited fish stocks, is likely to have resulted in the removal of the *Pan* $I^{BB}$ genotype from the spawning stock at a proportionally faster rate than that of the *Pan* $I^{AA}$ genotype.

Secondly, the gradual decline in the frequency of the *Pan* $I^{BB}$ genotype may also have resulted from the substantial spatial and seasonal changes in fishing effort on Icelandic cod that have occurred since WWII. Following the termination of fishing by Danish seine and bottom trawl within four nautical miles of the coast, the fishery jurisdiction was gradually extended to 200 nautical miles (see also the Introduction). At the same time, the introduction of larger vessels resulted in the redirection of fishing effort into deeper offshore waters. This also enabled trawlers to follow the seasonal migration of adult cod, rather than being restricted to inshore breeding grounds during the spawning season (Jónsson 1996). The bathymetric distribution of the *Pan* I genotypes has been recorded for both spawning and migrating cod around Iceland (Pampoulie et al. 2006, 2008; Árnason et al. 2009), while this information for juvenile cod is unfortunately scarce. Spawning cod sampled in 2002–2003 in the SW of Iceland, the area covered by this study, ranged from being predominantly of the *Pan* $I^{AA}$ genotype at the shallowest stations to predominantly of the *Pan* $I^{BB}$ and *Pan* $I^{AB}$ genotypes at the deeper stations (Pampoulie et al. 2006). Similar changes in genotypic frequency with depth, at stations all around Iceland, were observed by Árnason et al. (2009). Therefore, changes in the exploitation pattern of Icelandic cod during the study period are likely to have resulted in relatively greater fishing mortality in deeper water and on the feeding grounds and thus may have led to the disproportionate removal of the *Pan* $I^{BB}$ genotype from the stock by the commercial fishery.

Thirdly, we may not be able to fully explain the causes of these long-term temporal changes in genotypic frequency in Icelandic cod until our understanding of the population structure further improves. The distinct variation in life history traits, behaviour and *Pan* I genotype frequencies of Icelandic cod is persuasive evidence that the current stock designation does not capture the actual geographic scale of population differentiation (Pampoulie et al. 2006, 2008; Marteinsdóttir and Pardoe 2008). Traditionally, studies of genetic population structuring have

utilized neutral molecular markers. Accordingly, our microsatellite analysis did not indicate any spatial structure and the long-term stability of the population under the assumption of neutrality could not be rejected. However, the absence of heterogeneity using neutral genetic markers does not contradict the hypothesized existence of different life history components in our data. Neutral markers are poorly suited to the detection of changes in genetic diversity because they segregate independently of the selected loci, and in populations with relatively large effective population sizes in particular the level of genetic drift will be low (Conover et al. 2006; Allendorf and Hard 2009). Indeed, the population components might experience differential postsettlement selection, likely influenced by environmental factors, but still exhibit a relatively high level of gene flow as a consequence of larval dispersal or mixing on breeding sites, or both. However, if there is some degree of gene flow restriction between the stock components, as a recent study suggests (T. B. Grabowski, B. McAdam, V. Thorsteinsson and G. Marteinsdóttir, unpublished manuscript), high effective population sizes and recent separation history are two nonexclusive explanations that could mask underlying stock structure (Case et al. 2006). If the Icelandic cod stock indeed comprises populations with limited gene flow and/or different selection schedules, then the changes in exploitation patterns described above will have affected the stock complex by removing the *Pan* $I^{BB}$ genotype at a faster rate than the *Pan* $I^{AA}$ genotype. Furthermore, the different populations may also have been targeted by the fishery in an uneven manner. The overall effects are similar, i.e. they result in long-term changes in genotype frequencies.

Finally in our fourth hypothesis, we recognize that the genotype distribution at the *Pan* I locus may have changed because of selection by other agents such as those of climatic origin. Temperature is the only available long-term proxy for potential environmental changes during this time period. Examination of temporal changes in sea surface temperature (SST) off the northern coast of Iceland (Hanna et al. 2006) did not reveal any significant correlations with changes in frequency of the *Pan* I genotypes ($P > 0.05$). This is perhaps not surprising as ocean conditions in this area have fluctuated extensively during the study period. Between 1925 and 1964, there was a high inflow of warm Atlantic water onto the Icelandic shelf resulting in relatively mild temperatures (Malmberg 1986; Astthorsson et al. 2007). In the late 1960s, the climate shifted dramatically because of increased inflow of icy freshwater from the Arctic (Jakobsson 1980; Malmberg 1986; Dickson et al. 1988). During this period, identified locally as the 'Ice Years', temperatures dropped far below the long-term average, reaching the lowest recordings in 1968–1969 (Astthorsson et al. 2007). Conditions subsequently remained more stable, until the current situation of gradual warming, which started in 1996 (Astthorsson et al. 2007). These striking fluctuations in temperature and salinity of Icelandic waters thus do not appear responsible for the gradual and consistent changes in the genotype frequencies observed in this study. Other studies have also shown that there does not appear to be a direct link between *Pan* I genotype frequencies in cod and water temperature (Nielsen et al. 2007). Unfortunately, the quantitative investigation of this potential relationship for Icelandic cod is limited by the quality and quantity of available environmental data. Although the SST time series from northern Icelandic waters (Hanna et al. 2006) is thought to be relatively representative of the average environmental conditions experienced by the stock in general, Icelandic cod are confronted with highly fluctuating environmental conditions throughout their lifespan (Jakobsson and Stefánsson 1998); variability that can probably only be reliably captured through data storage tags. Furthermore, the life history stage(s) at which temperature affects the genotypic distribution of the *Pan* I locus of cod is unknown. In conclusion, although the observed changes in genotype frequencies at the *Pan* I locus could be the result of simultaneous selection by both trends in temperature and fishing, the information that does exist indicates that although there have been persistent changes in environmental conditions in Icelandic waters, those changes alone are insufficient to explain the strong selection against the *Pan* $I^{BB}$ observed during the last 20 years of the study.

A recent study has also attempted to explore the effects of exploitation on genetic diversity at the *Pan* I locus in Icelandic cod using samples from spawning areas around Iceland over a 3-year period (2005–2007) (Árnason et al. 2009). The authors estimated the fitness across age groups at the inter-cohort level and concluded that, in contrast to our study, the changes in the frequencies of the *Pan* $I^{AA}$ and *Pan* $I^{BB}$ genotypes were because of selection against the *Pan* $I^{AA}$ genotype (the coastal component) by high near-shore fishing pressure. Two potential explanations for the contrasting conclusions from these studies are evident. First, the changes in *Pan* $I^{BB}$ homozygote frequencies observed in our historical time series appear concomitant with the known changes that took place in the fishery during that period. It is, in principle, possible for an abrupt shift in the selection regime to have taken place in recent years, and this is the conclusion reached by Árnason et al. (2009) in their study using contemporary, but not historical, samples. It is worth noting though that the frequencies of the *Pan* $I^{AA}$ genotype did not appear to have declined in our contemporary sample (collected in 2002) in comparison with the earlier years. Secondly, our study indicates that the genotypic variation observed within cohorts is because of differences in matu-

  

ration rates, with cod carrying the *Pan* $I^{BB}$ genotype more likely to mature at a later age. Árnason et al. (2009) also detected distinct changes in *Pan* I genotype frequencies at the intra-cohort level, i.e. the *Pan* $I^{BB}$ genotype increased in frequency with age of the cod. Therefore, the reported change in the frequency of the *Pan* $I^{AA}$ genotype over the 3-year period in their study may actually have been because of a gradual increase in the number of *Pan* $I^{BB}$ individuals as they matured and entered the spawning grounds, rather than the intense removal of *Pan* $I^{AA}$ cod by the fishery. A thorough investigation of maturation rates of cod in relation to Pantophysin genotypes would therefore help resolve the conflicting conclusions from these two studies. What is apparent is that historical or temporally replicated data, such as that used in our study, are of the utmost importance when examining changes in the genetic composition of populations that could have been potentially brought about by anthropogenic effects.

Significant excess of *Pan* I heterozygotes was observed in five of seven cohort classes and 50% of sampling years. Heterozygote excess at the Pantophysin locus has been reported in other studies of Atlantic cod (Jónsdóttir et al. 1999, 2001; Beacham et al. 2002; Karlsson and Mork 2003; Árnason et al. 2009); however, the magnitudes in the present study are higher than typically reported. The possibility of genotyping errors because of incomplete enzyme digestion, leading to inflated heterozygote scores (this would not affect *Pan* $I^{AA}$ homozygotes) was thus examined. However, repetitions of samples (ca. 30%) showed consistent results thus rendering the hypothesis of mis-scoring because of partial digestion unlikely. Another explanation for the heterozygote excess observed in this study is that heterozygotes have a selective advantage i.e. in a highly fluctuating environment, such as that inhabited by Icelandic cod, preservation of both alleles could be beneficial both in the short and in the longer term. The fact that polymorphisms have been maintained at the *Pan* I locus for at least two million years (Pogson and Mesa 2004) indicates there are ongoing natural selection processes influencing the genotypic distribution (Canino et al. 2005). However, heterozygotes do not appear to have an advantage in growth or longevity. Indeed, even if a fluctuating environment can provide a favourable basis for protected polymorphisms, the conditions required for this are often quite restrictive (Hedrick 2006). The strength of selection needed to explain such departures from HWE would probably thus be unrealistically elevated. Significant heterozygote excess at the *Pan* I locus has also been attributed to the confounding effect of sexes (Karlsson and Mork 2003); however, this was not supported by our data. We therefore propose another explanation: if heterozygotes have maturation rates that are similar to those of *Pan* $I^{AA}$ homozygotes, i.e. allele A exhibits some dominance (Fig. 3), the recruitment of *Pan* $I^{AA}$ and *Pan* $I^{AB}$ cod to the spawning grounds would occur before the *Pan* $I^{BB}$ homozygotes and would thus generate excesses of heterozygotes. We therefore find it likely that the observed heterozygote excess represents a dynamical system influenced by behavioural differences in the stock (Árnason et al. 2009). Once again, this hypothesis could be examined through future investigations of maturation rates associated with the *Pan* I genotypes.

In conclusion, decades of high fishing pressure have resulted in evident changes in the age composition of Icelandic cod (Marteinsdóttir and Thorarinsson 1998; this paper) and are also likely to have altered the maturation schedule of this important fish stock (Pardoe et al. 2009). In the present study, we also show that commercial fishing has likely led to loss of adaptive variation at the Pantophysin locus over a period of six decades. The analysis of historical genetic material from archived otolith collections, and more specifically non-neutral markers such as the *Pan* I locus, has thus proved their worth in the assessment of the long-term effects of exploitation on commercial fish stocks such as Atlantic cod. Our results suggest that fisheries can shape the genetic composition of a fish population over a relatively short time period, thus supporting the calls for an evolutionary dimension to fisheries management (e.g. Conover 2000; Law 2000; Jørgensen et al. 2007; Kuparinen and Merilä 2007), ideally through the establishment of genetic monitoring programs based on an examination of functional as well as neutral markers (Bradbury et al. 2010) and incorporation of genetic perspectives (André et al. 2010) into management objectives that are concerned with the long-term sustainability of harvested populations.

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# Paper IV

# Temporal aspects of neutral genetic variation and effective sizes ($N_e$) of Atlantic cod (*Gadus morhua*) in Icelandic waters

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Abstract

Fisheries genetics have added a new dimension to our understanding of biology of large marine fish stocks but implementation of this knowledge in scientific advice has been challenging. An estimate of effective size, $N_e$, is an indicator of genetic condition that can be crucial to management decisions because it integrates genetic effects with the life history of the species. Based on analysis of DNA extracted from archived otolith samples we used a suite of seven neutral microsatellite loci to infer about temporal genetic variation and effective size among spawning Atlantic cod in Icelandic waters collected between 1948 and 2000. This time period is characterized by rapid post-war increase in fishing-intensity that resulted in marked changes in abundances and demography of the stock. By using several estimators of $N_e$ we suggest that the effective size of Icelandic cod is likely to be in range from few hundreds to thousands which is several magnitudes smaller than the census size. Temporal

approach indicated a subtle decrease in $N_e$ over the period although no such decrease was detected with other indicators of genetic diversity. Variations in temporal estimates reflect the high variability that characterizes the life history of the Icelandic cod stock and underscores the necessity of repetitive temporal sampling for insights.

## INTRODUCTION

Effective fishery management depends on a sound understanding of population ecology and genetics (Hare et al. 2011). However, despite the progress in the field of fisheries genetics, implementation of genetics and genomics tools into scientific advice have been challenging (Waples et al. 2008). A plausible reason for this limited amount of integration may be caused by absence of conceptional overlap between fishery genetics and management (Hauser and Carvalho 2008) as well as the lack of reliable indicators for genetic condition (Hare et al. 2011).

The Icelandic cod stock (*Gadus morhua*) like most other cod stocks in the N-Atlantic has experienced a drastic reduction in population sizes due to intensive fishing during the latter half of last century (Schopka 1994). The stock has been commercially exploited for centuries but the effort increased substantially with improved fishing technology and increase in number (and size) of vessels following the World War II (Jónsson 1997) (Fig. 1). Estimated census size (i.e. stock size of four years and older) declined from 700 millions in the early 1950s to 200 millions at the beginning of this century (Anonymous 2012). Concurrently high levels of fishing pressure are believed to have led to evident changes in the age and genetic composition of Icelandic cod (Jakobsdóttir et al. 2011, Marteinsdóttir et al. 1998) and caused alteration in the maturation schedule (Pardoe et al. 2009) or even loss of adaptive variation (Jakobsdóttir et al. 2011) during the time period under study. The dramatic decline of the Icelandic cod stock during last century has raised concern in terms of a general

loss of genetic diversity where such a loss is considered to be one of the most relevant evolutionary consequences of declining population size (Frankham 2003).

Genetic diversity depends on a population's genetically effective size $N_e$, one of fundamental parameters in evolutionary biology. The concept of $N_e$ was first introduced by Sewall Wright (Wright 1931), who defined it as the size of an imaginary theoretically ideal population affected by genetic drift at the same rate per generation as the population being studied. This comparison with theoretically ideal population standardizes measurement of genetic drift and makes *N*e comparable across populations with very different life histories. Effective population size is therefore an indicator of long-term performance of a population regarding both diversity and inbreeding (Cervantes et al. 2011, Nikolic et al. 2009) and could potentially serve as an indicator of genetic condition for practical application in marine species management (Hare 2011). In a small population, the consequence of a loss of genetic variation is rather straightforward: It can lead to increasing effect of genetic drift and in extreme cases to fixation of deleterious or suboptimal alleles (inbreeding depression) eventually leading to extirpation of a population. In the wild, however, it is less clear how often a link can be established between population size and extinction risk due to genetic factors (Fraser et al. 2007, Palstra and Ruzzante 2008). Large marine populations pose a special problem as they often display relatively high $N_e$ in the range of hundreds or thousands which are generally believed to be outside the range of immediate conservation concern (Palstra and Ruzzante 2008 and references within). Yet, these estimates are often in order of magnitudes lower ($10^{-3}$-$10^{-6}$) than the estimated census sizes and pose therefore a sharp contrast to theoretical considerations (Waples 2009). The mechanisms responsible for driving such low ratio are yet not fully understood (Turner et al. 2006) but mainly they are believed to increase (or maintain) large variance in reproductive success and therefore lowering *N*e considerably. A Fisheries related aspect would be that such low $N_e/N$ ratio might explain in

part the poor relation between spawning stock size and recruitment often observed in large fish populations (Hauser et al. 2002). Given that such extreme $N_e/N$ reflect the dynamics of biological systems the concern has been raised if the evolutionary potential of the species in question is affected (Hauser et al. 2002, Hutchinson et al. 2003) caution that marine fish could be at greater risk of losing genetic diversity as consequence of fishing practices than previously assumed.

The main objective of this study was to estimate genetic diversity and effective population size of the Atlantic cod in Icelandic waters during time of steep increase and maintenance of high fishing intensity. In order to do this, DNA was isolated from archived otoliths compiling a large historical data set spanning six decades with repetitive temporal sampling. Results among numerous indicators were relatively consistent and indicated moderate effective population sizes but of extremely low $N_e/N$ ratio. The results are discussed in context with life history of cod and methodological aspects of estimators highlighting the need for longer term temporal repetitive sampling for genetic monitoring purposes.

## MATERIAL AND METHODS

### *Sampling*

This study is based on DNA extracted from historical cod otoliths collected over a period of six decades from the 1940s to the 2000s. Otoliths were retrieved from the archives of the Icelandic Marine Research Institute (MRI) covering a period of sixty years (1948-2000). A total of 16 samples were obtained comprising 1471 spawning or near spawning individuals from three locations known to be major spawning grounds for Icelandic cod. As the genetic variability among sampling locations was lower than the temporal variance (Jakobsdóttir et al. 2011), individuals from several sampling localities were clustered into 11 sample years (n = 1337) to maximize the genetic information content and to minimize the sampling bias. The

sampling overlaps a time period of drastic change in the Icelandic cod stock such that the oldest samples were obtained from time periods characterized by moderate fishing mortality following the World War II (Fig. 1) and in contrast, the most recent sample (2000) represents the stock after an extended period of intensive fishing (Fig. 1). The choice of sample years was based on the availability of individuals (sample size) stratified by years of known high and low stock size. Biological information such as total length, sex, age and maturity stage were retrieved from the MRI database. In several cases, potential temporal changes were tested by dividing samples into periods. The earlier period consisted of samples collected in 1948, 1957, 1959, 1966 , 1972, 1973 when average fishing mortality was almost constantly increasing from ~ 0.2 to 0.7 with an average ~0.4 throughout the period. The later period comprised samples collected in 1976,1979,1985, 1996 and 2000 and featured several peaks of high fishing mortality (>0.7).

*DNA extraction and analysis*

DNA was extracted from the dried tissue covering the otolith surface using Chelex/Proteinase K protocol (Estoup et al. 1996). Effort was made to preserve otoliths in original state and adjustments made to procedure to account for the large size of the otoliths without compromising volumes used for the extraction. The samples were genotyped at 7 microsatellite loci, namely Gmo2 (Brooker et al. 1994), Gmo8, Gmo19, Gmo34 (Miller et al. 2000), Tch5, Tch14 and Tch22 (O'Reilly et al. 2000). PCR were performed in 10 µl volumes containing 2µl of DNA, 1 µl of 10xBuffer, 1µl of 2.5 mM DNTP, 0.2–0.4 units of DyNAzyme$^{TM}$ DNA polymerase (Finnzymes) and 1–2 µM of each primer. PCR cycles were performed on GeneAmp®2700 thermal block using "Touchdown" procedures as follows: Initial denaturation step of 2 min at 95°C followed by 10 cycles of 30 s at 94°C, 45 s of

annealing temperature that decreases in each cycle by 0.5°C until 55°C was reached and 30 s of 70°C. This was followed by 25 cycles of 30 s at 94°C, 50 s at 55°C and 30 s at 70°C. A final elongation step of 5 min at 72°C was performed. PCR products were multiplexed and detected on an ABI-automatic sequencer (ABI 377, Applied Biosystem) using GeneScan 3.1.2 (Biosystems 2000), and scored using GeneMapper 3.7 (Biosystems 2004). The occurrence of genotypic errors due to technical artifacts (null alleles) or sample quality (large allele dropout) was assessed using the program MICRO-CHECKER (Van Oosterhout et al. 2004). Out of the 1471 cod processed, DNA was successfully amplified for 1337 individuals. Amplification success was on average 91 % (See Table 1).

Special precautions were taken to minimize chances of potential contamination during laboratory procedures. Samples were processed in designated area that was decontaminated between procedures using a chlorine solution and UV-light. Negative controls were run and a proportion of samples were rerun to confirm consistency of the sequencing.

*Statistical methods*

Genetic diversity was assessed by observed ($H_o$) and expected ($H_e$) heterozygosities per locus using GENETIX version 4.03 (Belkhir 1999) and -allelic richness (AR) using FSTAT (Goudet 1995). Deviation from Hardy-Weinberg equilibrium (HWE) at each locus and genotypic disequilibrium for each pair of loci were performed with Fisher's exact tests in GENEPOP (Rousset 2008). Differentiation between samples was estimated in FSTAT using pairwise $F_{ST}$ values following (Weir and Cockerham 1984) and confidence intervals were determined by bootstrapping 10000 times over loci Significance of the global $F_{ST}$ was tested using ARLEQUIN 3.0 (Excoffier et al. 2005). Significance levels for multiple comparisons were adjusted with sequential Bonferroni correction (Rice 1989). To assess the consistency of

Fst values over the study period sample pairs from earlier period was tested against the values of the latter using student's t test (see also Table 3).

Neutrality assumption of genetic markers is crucial for effective population size ($N_e$) estimates (LD signals or allele frequency drift arises only from drift) and therefore the software FDIST2 (Beaumont and Nichols 1996) was used to detect potential outliers loci as it has been shown to perform well (Narum and Hess 2011). Coalescent simulations were performed with samples of the same size as the observed samples assuming an island model of 100 islands, and generating 100000 independent loci with the infinite allele mutation model. Simulated distribution was thus compared to observed $F_{ST}$ values to identify potential outlier loci.

*Effective population size*

Numerous estimates of $N_e$ have been published over the last decade (reviewed in (Luikart et al. 2010). We chose several methods for contemporary estimates enabling comparisons among methods with the same data set and also to enable comparisons to other studies of $N_e$ in Atlantic cod (Árnason 2004, Hutchinson et al. 2003, Poulsen et al. 2006, Rowe and Hutchings 2003, Therkildsen et al. 2010). Estimates were obtained based on year samples drawn from spawning (or near spawning) individuals from the same cod population over fifty year period. The data set also allowed for grouping of six single-cohorts with sufficient sample sizes: 1949 (n = 57), 1963 (n = 98), 1964 (n = 63), 1970 (n = 69), 1988 (n = 54) and 1989 (n = 99). One single-sample method and three temporal methods were used in the present study. The single sample method LDNE (Waples and Do 2010) calculates $N_e$ based on linkage disequilibrium (LD) under the theoretical expectations that LD (i.e. nonrandom association of different loci) will arise from drift alone at neutral, unlinked loci in isolated randomly mating populations and hence reflect the $N_e$ of the population (Waples

2006, Waples and Do 2010). Estimates were obtained both on annual samples and on single-cohorts as they may contain different information for species with overlapping generations i.e. samples derived from single cohort represents effective number of parents or breeders that gave rise to the sample ($N_b$) (Waples 2005), while a sample of mixed ages are expected to display per generation effective population size (Robinson and Moyer 2012). Allele frequencies less than 0.02 were screened out since high numbers at very low frequencies will bias the estimation (Waples and Do 2010). Estimates were compared between period (see above) using Student´s *t* test.

Temporal methods use two samples separated in time (preferably separated by several generations) to calculate changes in allele frequency between the samples. Temporal estimates were compared over the whole period (1948-1996 or 1948-2000) and between numerous pairs of samples. We also divided the period of study in two time intervals. The earlier time interval (1940´s to 1970´s) corresponds to a period of increasing fishing mortality (F increasing from ~ 0.2 to ~ 0.7) while the second interval (1970´s to 1990's) corresponds to a period of maintained high fishing mortality (mean F ~ 0.7, Fig. 1).

First, we estimated $N_e$ with TempoFs (Jorde and Ryman, 2007); this method is a modified version of the moment estimator of Waples (1989) and estimates the variance of $N_e$ based on unbiased estimators Fs' and was developed explicitly for data with many rare alleles (Palstra and Ruzzante 2008). Calculations were made assuming a "sample plan 1", i.e., individuals were all mature and spawning and therefore assumed to have been sampled after reproduction. The TempoFs was also applied with a cohort model for iteroparous species using six single-cohorts (see above) following the temporal method (Jorde and Ryman 2007) correcting for age structure (by using life table information to calculate a correction factor and estimating generation lengths). This method takes into account the changes in allele frequencies that take place in age structured populations exhibiting overlapping generations.

In such systems the changes in allele frequencies depend not only on $N_e$ but also on the demographic characteristics of the population which influence the proportion of individuals that breed each year (Jorde and Ryman 1995). Generation length and the correction factor C were calculated according to method described in Jorde and Ryman (1995, 1996) using the software *FactorC2* (Jorde 2012). Life table information was extracted from age-based estimates of census size, mean weight and maturity for cod (Anonymous 2007, 2010).

Generation length can change in harvested fish populations (Cuveliers et al. 2011) and is thus likely to have varied over the time period of drastic demographic changes in the population. We found that generation length in the earlier period of the study (1950´s) was 7.51 years and had been reduced to 6.25 years in the nineties. An average value of 6.9 was therefore used when comparing among time intervals.

For calculations of $N_e/N$ ratio we used the Spawning stock estimates (Spawning Stock Biomass, SSB) of the Icelandic cod stock as a reference census size ($N$), as $N$ is proposed to reflect the number of reproductively mature adults in a population (Palstra and Fraser 2012). Thus we translated SSB estimations to number of individuals using estimates of mean weight of mature cod (Anonymous 2010).

In addition to contemporary estimates, estimates of long term $N_e$ which is influenced by demographic processes over historical time scales, were also made applying the software MIGRATE (Beerli 2008), which uses coalescent approach to estimate the relative effective population size $\Theta N_e$ over long periods of time (approximately $4N_e$ generations in the past; 1000's of years). Average mutation rate was set to $5*10^{-4}$ which is an average value of reported mutation rate for microsatellite loci (Ellegren 2000)

## RESULTS

### *Genetic diversity*

Comparisons of the simulated distribution and the observed $F_{ST}$ estimates did not detect any outlier loci suggesting the neutrality assumption was met for all genetic markers (Fig. 2).

There were no indications of temporal change in allelic richness or expected heterozygosity (AR or $H_e$) among sampling years (Table 1). Average AR based on 57 individuals was similar in all temporal samples with an overall mean value of 16.31. Multilocus genotype information for sampling years showed high $H_e$ values ranging from 0.755 to 0.801 with an overall mean value of 0.774 (Table 1). Mean $H_e$ was slightly higher in more recent samples but not significantly so ($p = 0.137$). Genetic diversity varied among loci, with $H_o$ ranging from 0.214 (Gmo34) to 0.988 (Tch14). The number of alleles ranged from 10 (Gmo34) to 53 (Gmo8) (Table 2). Deviation from HWE was detected in 2 of 77 exact tests after Bonferroni correction (10 were out of HWE before correction).

The global $F_{ST}$ among sample years was 0.003 (95% CI: 0.001-0.007) and not significantly different from zero ($p = 0.751$). Pairwise $F_{ST}$ estimates varied from 0 to 0.012 with confidence intervals ranging from -0.002 to 0.030 (Table 3). All of the high pairwise values of differentiation were found between samples in the more recent time period i.e. 1976 and onward ($t_{(10,3)} = -4.14$, $p < 0.01$). Thus, most of the 1979 samples, 1996 samples and the entire 2000 sample showed values of $F_{ST}$ exceeding the global one of 0.003.

### $N_e$ estimates

### *Single sample estimates*

Three out of eleven single sample estimates with the LDNE method using annual collections of individuals of mixed ages were infinite, estimates ranged from 313 (year 2000) to 1689 (1973) (Table 4). Confidence intervals were broad with lower confidence limits ranging from

136 (year sample 2000) to 1590 (year sample 1972) and upper confidence limits ranging from 1722 (year sample 2000) to infinite (8 samples) (Table 5). No differences in estimates was detected when comparing two time periods (1948-1973 vs. 1976-2000, $t_{(3,\ 794)} = 1.54$, $p = 0.2031$).

We also estimated $N_e$ using six cohort samples (Table 5). Two out of these six estimates (cohorts 1949 and 1964) could not be distinguished from infinity and both belonged to the earlier period of the study (i.e. 1948-1973). Regardless of whether we estimated $N_e$ with LDNE using samples of mixed ages or cohort samples, approximately 1/3 of the estimates were infinite.

*Temporal methods*

The estimates of $N_e$ on sample years varied considerably between samples and methods (Table 6). In general the estimates by TempoFs were in the hundreds with a harmonic mean of 316 but the estimates by MLNE and TM3 were mostly in the thousands with harmonic mean of 1042 and 1432 respectively (Table 6). An estimate for the whole study period was obtained evaluating the allele frequency change between the oldest (1948) and one of the two most recent samples (1996 and 2000). Thus, the overall estimates for the period using year sample 1948 and 1996 assuming 7 generations varied from 551 (TempoFs) to 1982 (MLNE) and 1467 (TM3) (Table 6). When using year sample 2000 (assuming 8 generations) all three estimates were lower or ranging from 331 (TempoFs), to 980 (MLNE) and 1061 (TM3) (Table 6). Confidence intervals were broad with lower confidence limits from 145 (TempoFs) to 1080 (TM3) and upper confidence limits mostly showing infinite values. Using more iterations for the Bayesian coalescent approach, TM3, helped to obtain convergence (curve symmetry) of the posterior distribution, however the curve was always highly skewed. (Supplementary Material Fig 1 a-c). Comparing earlier period (1948-1973) to the latter period

of the study (1973-1996 or 2000), estimates of earlier samples and sample pairs mostly had no upper confidence limits whereas the more recent samples appeared to give better precision of estimates. Estimates from latter period were mostly lower than from earlier time period and therefore a reduction in $N_e$ is detected in most temporal estimates. When using sample year 2000 the reduction is considerable (679 to 145 in TempoFs method, 1566 to 493 in MLNE method and 2720 to 1012 using TM3 method) (Fig. 3). A reduction over the time period was more subtle when the more recent period was defined between 1973 and 1996 instead of 2000 (679-407 for TempoFs method, 2720 to 1953 for TM3), while MLNE estimates were similar between the two periods (1566 to 1654). Therefore, all estimates showed high variation depending on method and samples used, yet, an overall of decrease in $N_e$ over the time period is observed.

The cohort analysis correcting for overlapping generations revealed extreme variation of $N_e$ estimates depending on the cohort considered (Table 7). Lowest value observed was $N_e$ = 8 between the consecutive cohorts 1988 and 1989 and the highest value observed was $N_e$ = 1852 between cohorts 1949 and 1963.

*Ne/N ratio*

Spawning stock biomass (SSB) has decreased considerably over the latter half of last century. Thus, in 1955, the stock was estimated to be about 940 thousand tonnes and had gone down to about 168 thousand tonnes in 2000 (Anon. 2010) (Fig.1). This translates into $1.5 * 10^8$ mature individuals in 1955 and $2.3* 10^7$ mature individuals in 2000. In this study estimates of $N_e$ of Icelandic cod stock appear to be between few hundreds to thousands and therefore, the contemporary $N_e$ of Icelandic cod stock are likely to be several orders of magnitude ($10^{-5}$ -$10^{-6}$) lower than the adult census sizes.

*Long term estimates*

The long term estimates of $N_e$ ranged from 14000 to 17000 across loci, depending on samples (Table 8) assuming average mutation rate of $5*10^{-4}$. This would indicate a manifold reduction in $N_e$ compared to contemporary temporal estimates of few hundreds to several thousands.

## DISCUSSION

The main objective of the present study was to estimate genetic diversity and $N_e$ of the Atlantic cod in Icelandic waters during time of steep increase and maintenance of high fishing intensity, in order to assess possible impact of fisheries as well as demographic parameters on different $N_e$ estimates. The results showed that $N_e$, which is likely to be in range of few hundred to few thousand for the Icelandic cod stock (indicating low $N_e/N$ ratio), is strongly affected by the demographic characteristics of the population. All three temporal estimates used indicated a trend toward a reduction in $N_e$ over the investigated time period (Fig. 3). At the same time no evidence of decrease in estimates of genetic diversity such as allelic richness or heterozygosity were detected and an overall temporal stability was confirmed at neutral genetic markers. The results are discussed below in details.

Despite reduction in population sizes over the time period no decrease in estimates of genetic diversity such as allelic richness or heterozygosity was detected and overall temporal stability was confirmed. However all three temporal estimates indicated a trend toward reduction in $N_e$ over the investigated time period.

*Genetic diversity and temporal stability at neutral markers*

Genetic diversity based on neutral assumptions appears to have been maintained in the Icelandic population as a whole despite heavy exploitation in the last 50-60 years (but see Jakobsdóttir 2011). The high level of heterozygosity was in congruence to the level found in

other studies carried out in Iceland, North and Baltic Seas cod using similar set of markers (Pampoulie et al. 2006, Poulsen et al. 2006). Deviation from HWE, detected in two samples could have resulted from technical artefacts such as genotyping errors which are of particular concern when working with historical material (Poulsen et al. 2006, Therkildsen et al. 2010). However, no evidence for deviations from expected frequency distributions of allele sizes and genotypes suggested that genotyping error was not responsible for any of the deviation in our results. Another explanation would be a lack of panmixia in years that significantly departed from HWE. Indeed overall tendency of negative $F_{IS}$ values (which indicates the level of inbreeding) in all years could indicate some level of subtle gene flow from neighbouring population although the source is not known. The global $F_{ST}$ of 0.003 was surprisingly high considering that the same population is being repeatedly sampled over the time period investigated. The pairwise $F_{ST}$ values however indicated an increased level of differentiation in the latter period of the study, especially in samples from 1979, 1996 and 2000. This could suggest that gene flow among years tend has become more restricted in the latter time period of the study. This observation is reflected in the $N_e$ estimates of temporal methods and will be discussed later.

*$N_e$ estimation*

*Single sample method (LDNE)*

The values obtained using single sample estimation varied from hundreds to infinite (Table 4) without any pattern or trend making this data inconclusive about possible changes in $N_e$ over time. However, the magnitude of the estimates was in congruence with those from the temporal methods. Fairly large $N_e$ are expected for Icelandic cod (Árnason 2004) and therefore caution should be held in interpreting the results from this method. Moreover, other studies have shown that this estimator does no perform well when $N_e$ are large (Antao et al.

2011, Robinson and Moyer 2012, Waples and Do 2010). However, for several samples the upper bound of confidence interval was reached, indicating a good precision in at least several estimates. Therefore, if general requirement are met in regard to samples sizes and loci (Waples and Do 2010) fairly large $N_e$ can be estimated with this method although very large $N_e$ (~ $10^4$) as reported in Therkildsen et al. (2010) are likely outside its limits. One potentially major source for bias is the assumption of closed population. A recent study has shown that violation of this assumption appears not to bias this estimates except if migration rate exceeds $m$ > ~5-10% (Waples and England 2011).

*Temporal estimates*

Temporal methods showed estimates within range of few hundred to several thousand but the results were inconclusive about the exact $N_e$ or the amount of change observed. Temporal estimates calculate shift in allele frequencies between two temporal separated samples normally under the assumption that drift is the only driver of that change. The limitations of these methods have been extensively reviewed elsewhere (Jorde and Ryman 2007, Luikart et al. 2010, Palstra and Ruzzante 2008); however, in the context of our study several issues need to be addressed.

First, genetic estimates of relatively large $N_e$ are known to have relatively poor precision because the drift signal will be lowered by high $N_e$ and thereby reducing the signal-to-noise ratio considerably (Waples 1989, 1998). In these cases, likelihood methods such as MLNE and TM3 are believed to be more powerful as they use more information concealed in the data, especially when data contains many rare alleles (Palstra and Ruzzante 2008). Larger sample sizes and increasing number of generations between samples have also been proposed to improve precision (Ovenden et al. 2007). In the present study, estimates obtained by the MLNE and TM3 methods were fairly accurate and statistically powerful as validated by finite

bounds of observed values although in several cases upper confidence limit was lacking (exceeded to infinitive value).

Second, skewed allele frequencies appear to be source of upward bias of $N_e$, in particular when $N$ exceeds $N_e$ (Jorde and Ryman 2007, Ovenden et al. 2007). Therefore, in the present study, $N_e$ estimated using MLNE or TM3 might be overestimated. The unbiased estimator TempoFs exhibits values that are lower than the other two methods but the precision of this method is constrained as all estimates lack upper finite bounds (likely because weighing of alleles increases standard deviation of F values, lowering precision considerably (Jorde and Ryman 2007)). Therefore, a trade-off between accuracy and precision appears to be evident when selecting estimators for $N_e$ with temporal methods. Hence, it appears crucial to use several methods to obtain fairly good trends in $N_e$ variation.

Third, temporal models normally assume discrete generation and violation of this assumption (i.e. when population has overlapping generations and age structure) can lead to considerable bias because the amount of temporal shift of allele frequencies depends not only on $N_e$ but also on the demographic characteristics of the population (Palm et al. 2003). Our dataset allowed for temporal sampling 4 to 8 generations apart which is generally believed to be long enough time lapse to avoid severe bias because of age structure (Ovenden et al. 2007, Palstra and Ruzzante 2008, Waples and Yokota 2007).

Correcting for age structure and overlapping generations with the cohort model of the temporal method revealed extremely variable values depending on cohort pairs. Large allelic frequency differences among consecutive cohorts could imply large variance in reproduction success. However these large differences were not only observed among consecutive cohorts. Another plausible explanation for the allelic differences among cohorts could be that intermittent migration events have caused an increase in allelic frequencies change. Consequently estimates of $N_e$ would be highly biased if migration is ignored (Wang and

Whitlock 2003). The cohort based method is also limited by the assumption of reasonably constant demographic parameters (Jorde 2012, Jorde and Ryman 1995), which is in our case violated as census sizes, mortality rates and age structure are all factors that have changed considerably over the period investigated (Jakobsdóttir et al. 2011, Marteinsdóttir et al. 1998). Despite this limitation we conclude that comparing the sample based estimates with cohort based estimates highlights the role of demographic influences affecting $N_e$ in Icelandic cod and identification of such relationships remains a crucial task for management (Osborne et al. 2012)

Fourth, the role of gene flow is normally ignored in temporal methods as most estimates assume reproductive isolated population (but see method from Wang and Whitlock 2003). However, the effect of gene flow is an important factor affecting the $N_e$ such that estimates can be biased if applied to subpopulation that has considerable genetic connection to neighbouring populations (Fraser et al. 2007, Palstra et al. 2009, Palstra and Ruzzante 2008). Ignoring gene flow can lead to bias in either direction depending on the direction and intensity of the migration taking place (Palstra et al. 2009, Wang and Whitlock 2003, Waples and England 2011).

Temporal estimates revealed variable outcome depending on which sample pairs were used and the pairwise $F_{ST}$ values indicated increased level of differentiation in the latter period of the study. These observations render a discussion about how unresolved population structure could be influencing gene flow within the cod population over the period of study. A certain amount of connectivity exists between the cod stocks from East Greenland and Iceland, and periodic spawning migrations are likely. Such immigration events took more often place prior to 1970 than during the following decades (Schopka 1994). However, to what extent genetic differentiation between the Icelandic and East Greenland component exists is not known and remains to be studied if mixing of these two components on spawning

grounds would lead to a genetic signal such as increased allele frequency changes or even homozygote deficiency such as to cause deviation from HWE as observed for 1976 and 1979 sample. Besides the connectivity to Greenland stock, there is the possibility of other underlying unresolved structuring. Behavioural groups that have been shown to differ in adaptive variation (Jakobsdóttir et al. 2011, Palsson and Thorsteinsson 2003, Pampoulie et al. 2008) appear to also show temporal variation in the onset of migration from and to the spawning ground (Thorsteinsson et al. 2012) or bathymetrical differentiation in spawning events (Grabowski et al. 2011). Here, a possibility of occasional increased allele frequency change can arise. Unresolved structuring could also lead to changes in genetic differentiation if fishing practices are affecting subpopulations in a different manner (Jakobsdóttir et al. 2011).

*$N_e$/N ratio*

We report that in Icelandic cod stock, $N_e$ is likely to be several orders of magnitude smaller than the actual adult census sizes. Such low ratio has been previously reported (Árnason 2004) for trans-Atlantic data set of several Atlantic cod stocks using mitochondrial DNA. A decrease in $N_e$ over the time period is likely to have happened although the amount of change remains inconclusive. $N_e/N$ ratio is an indicator of the extent of genetic variation expected in a population (Hedrick 2005) but the relationship between those numbers (i.e. $N_e$ and $N_c$) are not fully understood (Luikart et al. 2010). Shallow gene genealogy and large variance in reproduction success in form of sweepstake reproduction (Hedgecock 1994) have been suggested to be two of the main factors driving *N*e down in Atlantic cod (Árnason 2004). Large variance because of sweepstake reproduction process has empirically and theoretically been shown to exist (Hedgecock et al. 2007, Hedrick 2005) and has been proposed as an explanation for very low $N_e/N$ in numerous species (Waples 2009) linked with the life history

of type III survivorship (i.e. high fecundity and high juvenile mortality). Advection has also been shown to play a major role in limiting $N_e$ in species with drifting developmental stages complementing the theory of Sweepstake hypothesis (Wares and Pringles 2008). Another important factor that can lead to considerable reduction of $N_e$ is a potential underlying subpopulation structure. Asymmetrical gene flow and unequal subpopulation sizes are assumed to be a likely scenario in natural systems (Palstra et al. 2009, Palstra and Ruzzante 2008). Under such conditions reproduction variance of individuals within subpopulation will be increased because of differential contributions of each subpopulation to the reproduction. Lastly, several extrinsic factors can lead to increased variance in reproductive success such as human perturbation of habitat or selective harvest (Saillant and Gold 2006, Turner et al. 2006). However, the impact of those extrinsic mechanisms and the consequence of these in large harvested fish population are yet not fully understood.

*Long-term $N_e$*

Coalescent genealogy sampling estimates historical $N_e$ of cod to be tenfold higher than the contemporary estimates shown in the present study which could indicate a considerable reduction in $N_e$ since historical times. We did not detect bottleneck in our data, which would be expected if the decline was a recent one (Nei et al. 1975). Intermittent gene flow from adjacent populations could also cause an increase in genetic diversity that would inflate the long term estimates (Ovenden 2007). Large difference between contemporary and long-term $N_e$ can also arise if mutation rate is not correctly estimated; all of these are issues that cannot be resolved by this study.

Estimates of $N_e$ are usually of concern for conservation and management purposes as such measure usually give important information on the status of commercial species through

demographic and evolutionary processes. Similar study with comparable dataset on North Sea sole (Cuveliers et al. 2011) led to opposite results, namely despite heavy exploitation $N_e$ remained stable. The contrast of these two studies highlights the importance of species specific life history and biology when inferring $N_e$ from genetic data and the relationship of demography and genetic variation is thus of crucial importance to the management of such exploited natural resources (Osborne et al. 2012). Various studies have reported $N_e$ estimates of Atlantic cod in the range of hundreds (Hutchinson et al. 2003, Knutsen et al. 2011) or thousands (Árnason 2004, Poulsen et al. 2006, Therkildsen et al. 2010). These different magnitudes for the same species indicate that $N_e$ can be population specific and demographic characteristics of each population are likely to affect the $N_e$ number considerably.

*Conclusion*

Temporal variation in effective population size of Atlantic cod in Icelandic waters is considerable and is likely determined by highly dynamic forces shaping the genetic variation of the population. Indications of subtle changes in genetic differentiation and in $N_e$ highlight the importance of repetitive temporal sampling for detection of any underlying trend which is difficult to detect by other means in such variable system. Estimators of $N_e$ can be helpful for fishery management as they can be used to infer about the genetic variability. For these purposes the importance of long-term monitoring and application of several methods needs to be stressed. Future studies are recommended to infer about the role of gene flow and meta-population structure in influencing effective population sizes.

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TABLES

**Table 1**. Overview of Icelandic cod samples used in the study. *n* = sample size; Asucc = amplification success; $H_e$ = expected unbiased heterozygosity; $H_o$ = observed heterozygosity; AR = allelic richness; $F_{IS}$ = inbreeding coefficient. Bold values indicate significant deviations of $F_{IS}$ values from Hardy-Weinberg expectations, after correction for multiple tests.

| Sampling year | n | Asucc | He | Ho | AR | $F_{IS}$ | Mean Age | Range |
|---|---|---|---|---|---|---|---|---|
| 1948 | 78 | 91.0 | 0.783 | 0.793 | 16.84 | -0.006 | 10.2 | 5-11 |
| 1957 | 114 | 94.8 | 0.763 | 0.769 | 16.21 | -0.002 | 9.4 | 5-19 |
| 1959 | 89 | 89.6 | 0.749 | 0.763 | 16.35 | -0.013 | 9.6 | 3-14 |
| 1966 | 95 | 91.1 | 0.770 | 0.776 | 15.97 | -0.002 | 8.4 | 5-13 |
| 1972 | 94 | 93.5 | 0.758 | 0.774 | 16.15 | -0.016 | 8.5 | 4-11 |
| 1973 | 181 | 82.0 | 0.765 | 0.774 | 16.62 | -0.009 | 8.8 | 6-12 |
| 1976 | 178 | 92.1 | 0.801 | 0.816 | 16.27 | **-0.016** | 7.1 | 3-13 |
| 1979 | 87 | 87.9 | 0.755 | 0.806 | 14.80 | **-0.061** | 6.9 | 3-10 |
| 1985 | 93 | 94.3 | 0.764 | 0.770 | 16.82 | -0.002 | 8.8 | 5-18 |
| 1996 | 258 | 85.8 | 0.800 | 0.823 | 16.82 | -0.027 | 7.4 | 4-14 |
| 2000 | 70 | 94.6 | 0.801 | 0.813 | 16.59 | -0.008 | 6.6 | 5-11 |
| Mean | 121.5 | 90.6 | 0.774 | 0.789 | 16.31 | -0.015 | 8.3 | |

**Table 2**. Summary statistics per locus by sampling year. *n* = number of individuals; AR = allelic richness based on 57 individuals; Na = number of alleles, number in parenthesis indicate unique number of alleles; $H_e$ =expected unbiased heterozygosity; $H_o$ =observed heterozygosity; HWp = p-value corresponding to Hardy-Weinberg test.

| Sample<br>Locus | 1948 | 1957 | 1959 | 1966 | 1972 | 1973 | 1976 | 1979 | 1985 | 1996 | 2000 | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gmo2 | | | | | | | | | | | | |
| N | 78 | 111 | 88 | 95 | 94 | 168 | 172 | 84 | 87 | 250 | 68 | *118* |
| AR | 13.8 | 10.9 | 9.9 | 9.6 | 11.5 | 10.1 | 10.8 | 8.4 | 12.1 | 10.4 | 10.5 | *10.8* |
| Na *(18)* | 15 | 12 | 11 | 10 | 13 | 13 | 13 | 9 | 14 | 14 | 11 | *11* |
| He | 0.852 | 0.803 | 0.773 | 0.797 | 0.781 | 0.765 | 0.812 | 0.736 | 0.802 | 0.805 | 0.862 | *0.799* |
| Ho | 0.897 | 0.793 | 0.773 | 0.737 | 0.830 | 0.833 | 0.802 | 0.762 | 0.793 | 0.828 | 0.824 | *0.807* |
| HWp | 0.310 | 0.872 | 0.324 | 0.724 | 0.222 | 0.009 | 0.002 | 0.000 | 0.198 | 0.007 | 0.138 | |
| Gmo8 | | | | | | | | | | | | |
| N | 68 | 107 | 79 | 88 | 87 | 159 | 168 | 81 | 88 | 240 | 69 | *112* |
| AR | 24.8 | 23.9 | 26.2 | 20.4 | 21.5 | 22.3 | 22.7 | 20.1 | 23.2 | 26.1 | 26.6 | *23.4* |
| Na *(53)* | 27 | 29 | 30 | 24 | 24 | 33 | 31 | 22 | 26 | 42 | 29 | *29* |
| He | 0.926 | 0.918 | 0.907 | 0.908 | 0.921 | 0.906 | 0.918 | 0.900 | 0.914 | 0.922 | 0.917 | *0.914* |
| Ho | 0.956 | 0.907 | 0.949 | 0.898 | 0.920 | 0.881 | 0.941 | 0.938 | 0.966 | 0.925 | 0.942 | *0.929* |
| HWp | 0.715 | 0.497 | 0.952 | 0.883 | 0.522 | 0.004 | 0.014 | 0.069 | 0.610 | 0.001 | 0.298 | |
| Gmo19 | | | | | | | | | | | | |
| N | 71 | 105 | 80 | 85 | 94 | 138 | 171 | 76 | 92 | 235 | 64 | *110* |
| AR | 22.6 | 18.8 | 19.9 | 20.7 | 20.3 | 21.6 | 21.4 | 18.9 | 22.3 | 21.2 | 22.6 | *20.9* |
| Na *(31)* | 24 | 20 | 21 | 22 | 22 | 27 | 26 | 20 | 26 | 26 | 23 | *23* |
| He | 0.919 | 0.907 | 0.920 | 0.906 | 0.904 | 0.903 | 0.922 | 0.872 | 0.930 | 0.910 | 0.898 | *0.91* |
| Ho | 0.958 | 0.905 | 0.900 | 0.929 | 0.947 | 0.899 | 0.918 | 0.934 | 0.902 | 0.915 | 0.938 | *0.92* |
| HWp | 0.613 | 0.753 | 0.252 | 0.766 | 0.888 | 0.115 | 0.200 | 0.520 | 0.358 | 0.697 | 0.771 | |
| Gmo34 | | | | | | | | | | | | |
| N | 78 | 113 | 89 | 94 | 91 | 175 | 178 | 86 | 93 | 248 | 70 | *120* |
| AR | 7.2 | 6.1 | 5.5 | 6.4 | 5.5 | 6.2 | 6.7 | 6.3 | 6.6 | 7.2 | 6.0 | *6.34* |
| Na *(10)* | 8 | 7 | 7 | 7 | 6 | 8 | 9 | 7 | 8 | 10 | 6 | *8* |
| He | 0.320 | 0.245 | 0.199 | 0.298 | 0.243 | 0.329 | 0.467 | 0.373 | 0.299 | 0.525 | 0.542 | *0.349* |
| Ho | 0.321 | 0.239 | 0.214 | 0.330 | 0.264 | 0.331 | 0.500 | 0.430 | 0.301 | 0.569 | 0.529 | *0.366* |
| HWp | 0.660 | 0.131 | 1.000 | 1.000 | 1.000 | 0.428 | 0.915 | 0.969 | 0.299 | 0.450 | 0.654 | |
| Tch5 | | | | | | | | | | | | |
| N | 67 | 105 | 78 | 84 | 90 | 145 | 151 | 57 | 90 | 235 | 61 | *106* |
| AR | 18.6 | 17.5 | 19.3 | 21.0 | 21.6 | 19.3 | 18.9 | 18.0 | 18.8 | 19.2 | 16.0 | *18.9* |
| Na *(33)* | 19 | 20 | 21 | 23 | 24 | 23 | 22 | 18 | 20 | 24 | 16 | *21* |
| He | 0.925 | 0.930 | 0.925 | 0.931 | 0.937 | 0.928 | 0.927 | 0.923 | 0.933 | 0.930 | 0.914 | *0.927* |
| Ho | 0.866 | 0.914 | 0.923 | 0.917 | 0.889 | 0.903 | 0.868 | 0.895 | 0.922 | 0.932 | 0.951 | *0.907* |
| HWp | 0.597 | 0.572 | 0.044 | 0.531 | 0.146 | 0.928 | 0.030 | 0.455 | 0.136 | 0.231 | 0.413 | |
| Tch14 | | | | | | | | | | | | |
| N | 72 | 106 | 83 | 84 | 86 | 119 | 167 | 72 | 90 | 217 | 62 | *105* |
| AR | 25.8 | 30.4 | 28.5 | 27.8 | 26.6 | 30.1 | 27.5 | 27.0 | 28.9 | 28.0 | 28.6 | *28.1* |
| Na *(44)* | 26 | 35 | 31 | 30 | 29 | 34 | 33 | 28 | 32 | 35 | 29 | *31* |
| He | 0.955 | 0.954 | 0.954 | 0.954 | 0.949 | 0.960 | 0.952 | 0.956 | 0.951 | 0.947 | 0.960 | *0.954* |
| Ho | 0.944 | 0.972 | 0.952 | 0.988 | 0.942 | 0.916 | 0.946 | 0.958 | 0.944 | 0.949 | 0.952 | *0.951* |
| HWp | 0.265 | 0.908 | 0.169 | 0.425 | 0.730 | 0.063 | 0.058 | 0.089 | 0.094 | 0.662 | 0.020 | |
| Tch22 | | | | | | | | | | | | |
| N | 77 | 112 | 86 | 95 | 92 | 172 | 177 | 84 | 93 | 251 | 70 | *119* |
| AR | 5.0 | 5.8 | 5.0 | 5.8 | 6.1 | 6.7 | 5.8 | 5.0 | 5.8 | 5.6 | 5.8 | *5.7* |
| Na *(13)* | 5 | 6 | 6 | 6 | 7 | 10 | 8 | 6 | 7 | 7 | 6 | *7* |
| He | 0.619 | 0.611 | 0.593 | 0.623 | 0.603 | 0.580 | 0.623 | 0.559 | 0.549 | 0.571 | 0.554 | *0.590* |
| Ho | 0.610 | 0.652 | 0.628 | 0.632 | 0.630 | 0.657 | 0.735 | 0.726 | 0.559 | 0.641 | 0.557 | *0.639* |
| HW | 0.796 | 0.205 | 0.155 | 0.818 | 0.861 | 0.463 | 0.028 | 0.005 | 0.515 | 0.113 | 0.087 | |

**Table 3.** Level of genetic differentiation ($F_{ST}$) between all pairs of samples with 95% confidence limits in parentheses.

| | **1957** | **1959** | **1966** | **1972** | **1973** | **1976** | **1979** | **1985** | **1996** | **2000** |
|---|---|---|---|---|---|---|---|---|---|---|
| **1948** | 0.002 [-0.001-0.004] | 0.002 [0.0-0.004] | 0.002 [0.001-0.003] | 0.001 [-0.001-0.003] | 0.002 [0.00-0.005] | 0.002 [0.00-0.005] | 0.005 [0.002-0.009] | 0.002 [0.00-0.003] | 0.003 [0.000-0.009] | 0.006 [0.001-0.014] |
| **1957** | | 0.003 [-0.000-0.006] | 0.002 [-0.000-0.004] | -0.000 [-0.002-0.002] | 0.001 [-0.000-0.002] | 0.004 [0.001-0.011] | 0.005 [0.003-0.009] | 0.000 [-0.001-0.001] | 0.005 [-0.00-0.016] | 0.010 [0.003-0.023] |
| **1959** | | | 0.000 [-0.002-0.002] | 0.000 [-0.001-0.000] | 0.002 [-0.000-0.005] | 0.006 [0.001-0.015] | 0.005 [0.001-0.010] | 0.002 [-0.001-0.004] | 0.008 [0.001-0.024] | 0.012 [0.003-0.03] |
| **1966** | | | | 0.001 [-0.001-0.004] | 0.002 [-0.000-0.004] | 0.002 [-0.001-0.007] | 0.003 [-0.000-0.006] | 0.003 [0.000-0.007] | 0.005 [0.000-0.013] | 0.01 [0.004-0.019] |
| **1972** | | | | | 0.000 [-0.001-0.000] | 0.003 [-0.000-0.009] | 0.004 [0.002-0.006] | 0.001 [-0.001-0.002] | 0.004 [-0.00-0.021] | 0.008 [-0.0-0.023] |
| **1973** | | | | | | 0.002 [0.000-0.005] | 0.003 [0.001-0.004] | 0.001 [-0.001-0.003] | 0.002 [-0.00-0.008] | 0.009 [0.002-0.019] |
| **1976** | | | | | | | 0.005 [0.002-0.009] | 0.003 [0.000-0.008] | 0.001 [-0.000-0.002] | 0.005 [0.002-0.008] |
| **1979** | | | | | | | | 0.006 [0.002-0.011] | 0.006 [0.003-0.009] | 0.011 [0.001-0.025] |
| **1985** | | | | | | | | | 0.003 [-0.001-0.010] | 0.008 [0.001-0.017] |
| **1996** | | | | | | | | | | 0.004 [-0.0-0.008] |

**Table 4.** Single sample estimator (LDNE) of effective population size based on linkage disequilibrium (Waples and Do, 2010). Estimates were estimated for each sample year. S = mean sample size, confidence limits (CI-95%; CI+95%) were computed by jackknifing over loci. Alleles at frequencies < 0.02 were screened out.

| Sample year | *LDNE* Estimation | S | CI-95% | CI+95% |
|---|---|---|---|---|
| 1948 | inf | 66 | 399 | ∞ |
| 1957 | 444 | 101 | 233 | 2478 |
| 1959 | 1227 | 75 | 287 | ∞ |
| 1966 | 795 | 82 | 265 | ∞ |
| 1972 | inf | 86 | 1590 | ∞ |
| 1973 | 1689 | 122 | 257 | ∞ |
| 1976 | 619 | 156 | 288 | ∞ |
| 1979 | inf | 62 | 136 | ∞ |
| 1985 | 753 | 87 | 334 | ∞ |
| 1996 | 706 | 216 | 370 | 3668 |
| 2000 | 313 | 61 | 166 | 1722 |

**Table 5**. Single sample estimator LDNE of effective population size based on linkage disequilibrium (Waples and Do, 2010). Estimates were obtained for six single cohorts. S = mean sample size, (CI-95%; CI+95%) = confidence limits. Alleles at frequencies < 0.02 were screened out. LD estimation of single cohort indicates the effective number of breeders ($N_b$) that gave rise to that cohort. This value multiplied by generation time gives approximately $N_e$. Generation time G = 7.51 used for cohort 1963 and 1970 and G = 6.25 used for more recent samples.

| cohort | *LDNE* $N_b$ | S | CI-95% | CI+95% | Nb xG |
|---|---|---|---|---|---|
| 1949 | inf | 49 | 253 | ∞ | - |
| 1963 | 802 | 77 | 235 | ∞ | 6023.0 |
| 1964 | inf | 48 | 439 | ∞ | - |
| 1970 | 1262 | 61 | 181 | ∞ | 9477.6 |
| 1988 | 556 | 45 | 167 | ∞ | 3475 |
| 1989 | 556 | 81 | 218 | ∞ | 3475 |

**Table 6.** Estimates of $N_e$ in Icelandic cod for the time period 1948 -2000 using three different temporal approaches: TempFs (Jorde and Ryman, 2007), MLNE (Wang 2001) and TM3 (Berthier et al. 2002). Confidence limits are presented within brackets. Temporal points are chosen firstly to show both an overall estimate for the whole period of 50 years and secondly to show estimates for different time intervals i.e. earlier (1948-1973) and more recent period (1973-1996/2000). G = Generations, $T_g$ = generation time, Fs' = allele frequency shifts, SE = Standard error.

| | | | Jorde and Ryman | | MLNE | TM3 |
|---|---|---|---|---|---|---|
| Sample pairs | G | Tg | (2007) | Fs' (SE) | | |
| 1948 -1973 | 4 | 7.51 | 679 [227- inf] | 0.003 (0.003) | 1566 [605-inf] | 2720 [321-inf] |
| 1948 -1996 | 7 | 6.90 | 551 [219-inf] | 0.006 (0.005) | 1982 [1019-8437] | 1467 [938-4533] |
| 1948 -2000 | 8 | 6.90 | 331 [154-inf] | 0.012 (0.007) | 980 [510-3632] | 1061 [428-4072] |
| 1973 -1996 | 4 | 6.25 | 407 [145-inf] | 0.005 (0.005) | 1654 [970-inf] | 1953 [1080-7320] |
| 1973 -2000 | 5 | 6.25 | 145 [69-inf] | 0.021 (0.013) | 493 [291-1136] | 1012 [400-6641] |

**Table 7**. Observed allele frequency shifts among (Fs') among several cohorts of spawning Icelandic cod and estimates of effective size ($N_e$) averaged over 7 microsatellite loci (Jorde and Ryman 2007). Correction factor (C) and generation length ($T_g$) were extracted from life history data calculated according to Jorde and Ryman (1996, 2012). SE = standard error.

| Cohort pairs | | years | $T_g$ | C | Fs' (SE) | Ne |
|---|---|---|---|---|---|---|
| 1949 | -1963 | 14 | 7.5 | 50.1 | 0.002 (0.004) | 1852 |
| 1949 | -1964 | 15 | 7.5 | 46.2 | 0.112 (0.052) | 28 |
| 1963 | -1964 | 1 | 7.5 | 30.3 | 0.143 (0.069) | 14 |
| 1963 | -1970 | 7 | 7.5 | 31.0 | 0.102 (0.062) | 20 |
| 1963 | -1989 | 26 | 6.9 | 53.0 | 0.010 (0.011) | 391 |
| 1970 | -1988 | 18 | 6.9 | 65.7 | 0.220 (0.128) | 22 |
| 1988 | -1989 | 1 | 6.3 | 27.4 | 0.272 (0.129) | 8 |

**Table 8.** Long term estimates. Theta estimation based on averaging values over loci.

| Locus | Theta θ 1948 | Theta θ 2000 | Theta θ 1996 |
|---|---|---|---|
| Gmo2 | 19.0 | 21.8 | 4.5 |
| Gmo8 | 81.5 | 0.0 | 11.2 |
| Gmo19 | 54.9 | 53.9 | 7.7 |
| Gmo34 | 6.5 | 5.6 | 3.1 |
| Tch5 | 40.6 | 29.0 | 6.6 |
| Tch14 | 54.4 | 96.8 | 8.5 |
| Tch22 | 9.5 | 7.0 | 3.1 |
| Theta all | 28.2 | 33.7 | 5.7 |
| Long term Ne | 14075 | 16865 | 2825 |

FIGURES

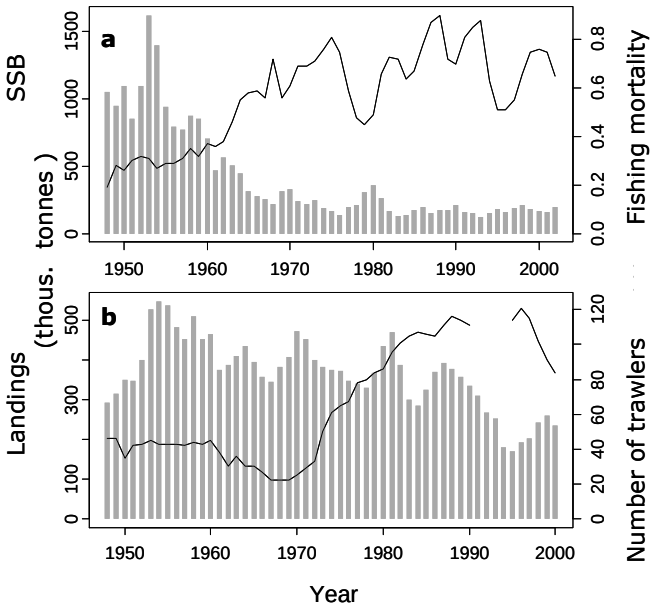


**Figure 1.a.** Estimated total spawning stock biomass (SSB, columns) and average fishing mortality of age groups 5–10 (line) of cod in Icelandic waters during the period 1948–2002. **Fig.1.b**. Estimated landings (columns) and number of active trawlers (line) during the study period. (Marine Research Institute 2009 and Jónsson 1997)

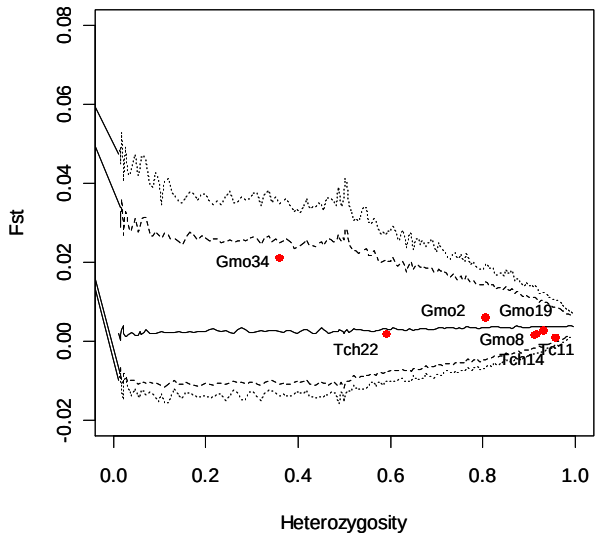


**Figure 2.** Simulated distribution of the level of genetic differentiation ($F_{ST}$) and heterozygosity in Atlantic cod under the infinite allele model using the weighted heterozygosity for assumed neutral loci. The median is enclosed by 95% (dashed lines) and 99% (punctuated lines) confidence limits of $F_{ST}$.

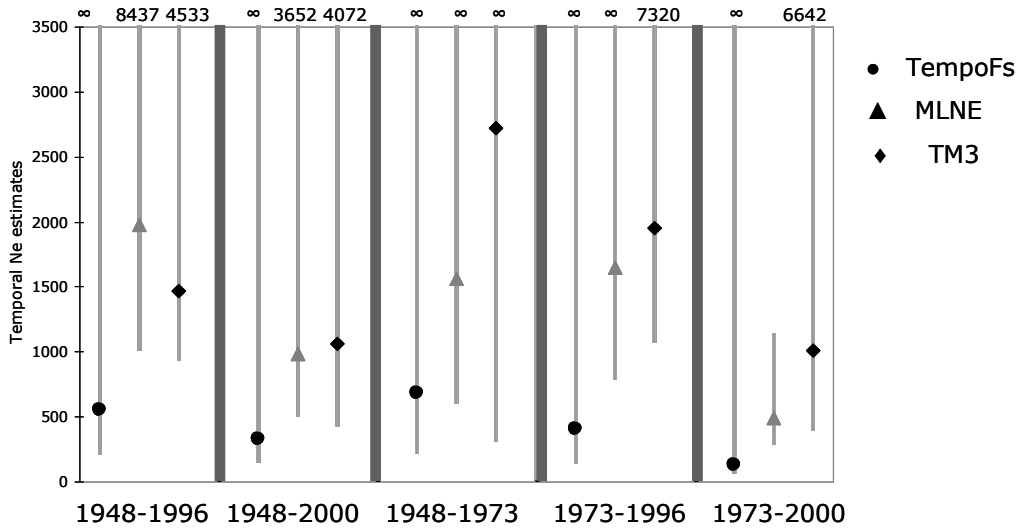


**Figure 3**. Point estimates (symbols) and confidence limits (dotted lines) of $N_e$ obtained with three different temporal estimators. Each symbol represents different method i.e. TempoFs (Jorde and Ryman 2007), MLNE (Wang 2007) and TM3 (Berthier et al. 2003). Each section of the Figure shows different intervals between temporal points. Exact numbers can be found in Table 6.