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Genetic insights into the population composition of two regional inshore mixed stocks of Atlantic cod (Gadus morhua) in West Greenland

Master's thesis, July 2015

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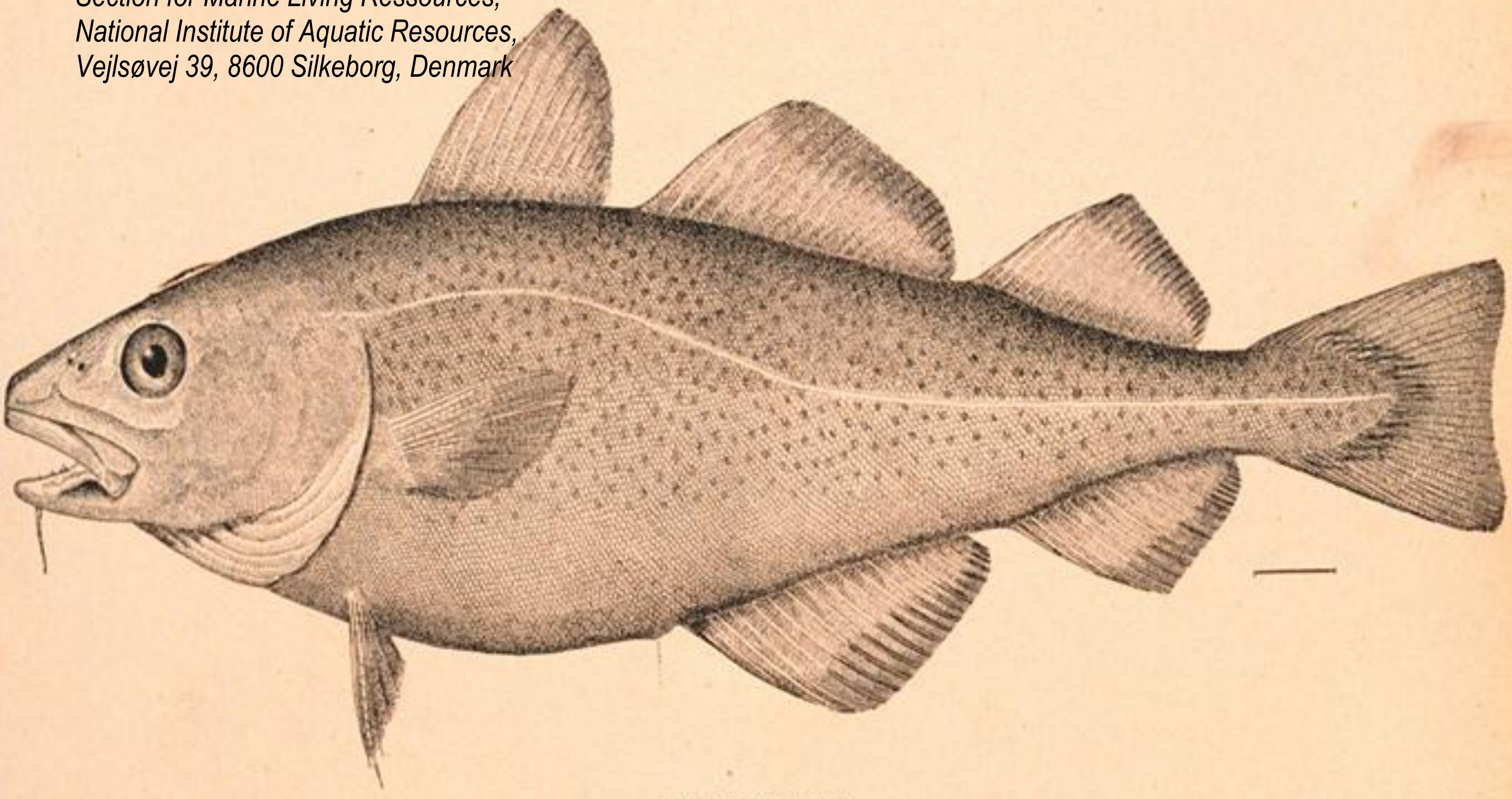

THE CODFISH.

Gadus morrhua, L. (p. 200.)

Drawing by H. L. Todd, from No. 10444, U. S. National Museum, collected at Eastport, Me., 1872, by U. S. Fish Commission.

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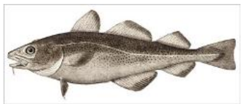
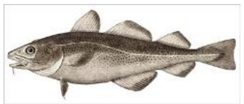




Preface

The thesis was submitted to the Technical University of Denmark (DTU) for partial fulfillment of the requirements for the candidature of a master's degree in Aquatic Science and Technology (cand.polyt.) from the National Institute of Aquatic Resources (DTU Aqua). The project can be divided into three parts affiliated with different geographical places; 1) collection of samples, 2) genetic work, and 3) analysis and writing.

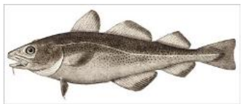
The sampling involved a field trip of approximately one month to Greenland in the summer of 2013 in order to collect fish from inshore waters and fjords around two locations, namely the settlements of Sisimiut and Nuuk. All the practical work associated with the genetic studies took place at the population genetics lab at DTU Aqua in Silkeborg, Denmark. Lastly, genetic analysis and the thesis write-up were conducted at DTU Aqua, Charlottenlund, Denmark. The goal of the thesis was to investigate the population composition of an assumed mixed stock fishery of Atlantic cod (*Gadus morhua*) in inshore coastal waters of West Greenland by applying advanced genetic tools. Briefly, findings included new insights into the spatial distribution of different genetic populations, both across and within regions in Greenland, which in turn might have important management implications for inshore fisheries.

Acknowledgments

This Thesis is the product of invaluable help and collaboration from numerous people and institutions, and to those I may forget here – Thank You.

First and foremost, I would like to thank my supervisor Einar Eg Nielsen for presenting this opportunity to conduct amazing and exiting research, for immense knowledge and inspirational presence, for continuous guidance and (both face-to-face and long-distance) support, for the enormous trust in my independence, for always taking the time necessary to answer questions, even up till the very last days and helping me to keep focus. I have yet much to learn. I am immensely grateful to Dorte Meldrup for the hard work she put into this project (unofficially dubbed the "Nightmare project"), for teaching me "how-to-be-a-geneticist", for believing in me and the great encouragement in the laboratory, and for spending hours extracting DNA from tissue and otoliths (also, thank you, Sara and Karen-Lise for the effort). A big shout-out for Sara Bonanomi for sharing her invaluable knowledge in population genetics, for assisting me with the majority of the analysis, for taking valuable time out (in the last hours of her PhD – btw, great work) to help me, for helping with otoliths and for all the good talks we have had. Also I want to show my gratitude to the rest of the population genetics group, Jakob the Co-SupV (thanks for insightful comments, assistance and help with R), Nina (thanks for providing and helping me with the SNP panel and for insightful emails), Karen-Lise (thank you for your efforts in lab), and Brian (thank for good and insightful talks).

I would also thank the Greenland Institute of Natural Resources for funding the project, for providing boat and accommodation in Nuuk and for collecting a vital portion of my samples. Here, a special thanks goes out to Rasmus Berg



Hedeholm for great assistance, for wonderful and helpful conversations, for always having time if I want to talk, for making me miss, and sometimes dream, of the wonderful wilderness of Greenland (I still owe you a Reindeer). Greenland would not have been the same without you.

Another thanks goes to the Arctic Technology Centre at DTU and in Sisimiut for arranging a wonderful course, for making it affordable for students to go to Greenland and for providing a boat. Thank you very much, Lis Bach for invaluable assistance in now two projects, for believing in me and for amazing guidance and valuable comments. At sea, Morten Holtegaard Nielsen and crew provided great working platform.

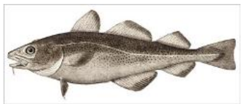
To the people in "the Castle", DTU Aqua Charlottenlund I would like to thank; Trine Marie Nielsen for our thesis-get-togethers, for your positive helpful and funny comments, for deep conversations, for always encouraging me, for being a wonderful friend and for reminding me what Justin Bieber once said – "Never say never"; Karin Hüssy for assistance with otoliths (even though, they were not used); Marie, Stine and Filipa for talks about babies and father/motherhood.

Thank you to my best friends (..and "real family"), Søren (aka Big B), Johan and Tina for always being there when I need you, and supporting me through everything. Also to my beautiful sister, Ulla, thank you for everything.

To my little daughter, Pil. Daddy loves you unconditionally and please, keep reminding him about the true values of life, simply through your mere existence. I look forward to some real quality-time with you, - "daddy's little marine biologist". Wuuuhuuu, paternity leave.

And finally, but never to forget, I am deeply grateful to you - my wonderful, beautiful and amazing partner, Trine. Words can never express how deeply grateful I am - you protect and uphold our stronghold in Nøddebogade with grace and always keep fighting when times are tough. Thanks, for the unconditional support, patience and time/space when science steal our time. Thank you for being a wonderful mother, best friend and amazing lover - I love you.

Thank you all



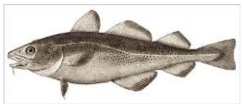
Thesis summary

The recognition of biocomplexity in a species might be essential in order to achieve a more sustainable exploitation of fishery resources, especially in the light of anthropogenic pressures such as fishery exploitations and climate change. Nevertheless, the notion of a species comprising many genetically distinct locally populations are frequently neglected in fisheries management. Therefore, management strategies that are more focused on biological unit rather than area are essential for effective fisheries management and conservation. A better understanding off the genetic diversity of fished stocks is of paramount importance for creating the settings for management improvements and reliable predictions that help preserve future biodiversity and fisheries yields.

The present thesis uses a catalogue of spatial population genetic approaches to explore the availability and methodology used for disentangling the genetic composition of mixed-stock samples. Primarily, the focus is to examine the spatial dynamics of Atlantic cod populations (*Gadus morhua*) in West Greenland. Provided contemporary samples collected by both "experts" (i.e. marine biologists) and "non-experts" (i.e. fishermen), the spatial composition in inshore regions around Nuuk and Sisimiut was investigated. The application of a panel of 96 gene-associated Single Nucleotide Polymorphisms (SNPs) was used to assign individual fish and estimate proportional contributions to previously identified populations.

Presented with different approaches for population genetics, tests of individual assignment and mixed-stock analysis showed that the suitability and application is case-specific. In particular, the evaluation of different approaches seems to be essential in the decision and selection of the most appropriate and accurate genetic tool. Here, the evaluation-process is presented and subsequently the most suitable approaches are chosen for estimating the origins at the level of individuals and populations.

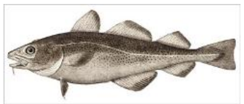
The individual assignment tests and mixed-stock analysis showed differences in the composition between the two regional inshore mixed stocks in West Greenland. Briefly, individuals originating from the "Iceland Offshore" population seemed to contribute significantly to inshore areas around Nuuk, and to a lesser degree around Sisimiut. The "West Greenland inshore" population was dominating both regions and constituted an important part of all sampled assemblages. Specifically, in the Nuuk region a small-scale genetic pattern emerged, where coastal and fjord samples showed substantially different genetic compositions. Most importantly, offshore individuals originating from both the "West Greenland Offshore"



and "Iceland Offshore" populations were confirmed within the fjord system. These findings are associated with important management implications which are thoroughly discussed and recommendations are put forward. Furthermore, these studies illustrate relevant aspects that are associated with recent tagging and genetic data, but also highlight the lack of information on the poor-studied population component residing inshore. In conclusion, the results provide important insights into the genetic composition of different fished assemblages of cod in inshore waters of West Greenland and have important implications for fisheries management. These findings along with other related studies of marine fish stress the need to continuously track the genetic origin of exploited marine fish in mixed assemblages.

Introduction

Over the last decades the primary objective of fisheries management has been 'sustainability' of marine fisheries resources (Sheperd et al. 1982; Brown et al., 1987). But fisheries have rarely been sustainable, and frequently resulted in depleted fish stocks over time (Pitcher & Pauly, 1998; Pauly et al., 2002). Nevertheless, the importance of marine resources seem indisputable in the light of global food production and security for a continuous growing human population (Rice & Garcia, 2011). Therefore, preserving biodiversity might be crucial for the persistence and stability of natural populations and future fisheries yields (Worm et al., 2006), especially in the light of climate change (Brander, 2007; Hollowed et al., 2013). The recognition of biocomplexity and associated complementary dynamics in a species, which can include many genetically unique populations adapted to different environments (Schindler et al., 2010), might promote resilience to future impacts of associated anthropogenic pressures (Hauser & Carvalho, 2008), mainly from fishery exploitation and global changes (Hilborn et al., 2003). Yet, almost all exploited marine species are managed and described by discrete traditional fisheries units, also termed stocks, and fail to acknowledge the biological complexity that might be apparent (Reiss et al., 2009). As a consequence, genetically distinct populations that might be susceptible to overexploitation may potentially be lost through fisheries practices. Briefly, many commercial marine fisheries exploit aggregations of mixed-population origin and in cases where exploitation rates are high and population productivity is unequal, population crash or extinction risks of the smallest populations would increase (Hindar, 2007). Hence, stock assessment models used for fisheries management require best-available, precise and accurate data of harvested populations in order to maintain viability of all contributing populations within a fishery (Kalinowski, 2004). The implication of such genetic structuring and potential loss of adaptive divergence could translate into detrimental consequences for the resilience in a species. Thus, information on spatial and temporal variations in the genetic composition for exploited stocks are essential for effective fisheries management and conservation (Begg & Waldman, 1999; Shaklee et al., 1999) - a key consideration on the path towards 'sustainable' fishery.



Focus of the thesis

The present thesis supports the candidature of a master's degree in Aquatic Science and Technology (cand.polyt.) from DTU Aqua at the Technical University of Denmark (DTU). Here, the current studies aims at investigating the population composition of an assumed mixed stock fishery of Atlantic cod (*Gadus morhua*) in inshore coastal waters of West Greenland by applying advanced genetic tools. This study adds to Bonanomi et al.'s (in prep.a)[1] findings for offshore mixed stocks in order to obtain a better overall resolution of the population composition and spatial dynamics for cod in Greenland and to improve fisheries management for this valuable exploited marine resource.

The next section (General background) presents a basic background in order to understand the application of genetics in fisheries science and management. Thereafter, the study species will be introduced (Study organism: The Atlantic cod (*Gadus morhua*), considering first general perspectives of the biology, fisheries exploitation and genetics, followed by specific sections on Atlantic cod in Greenland. The third section defines practicalities in relation to the research conducted, where also methods and approaches involving advanced genetic tools are described. Results are presented in the fourth section (Results) followed by the Appendix associated with the thesis. Next, the findings will be summarized and discussed (Discussion) in the view of previously published literature. Finally, overall management implications and conclusions will be presented (Management implications and conclusions) as well as perspectives for future research (Future perspectives).

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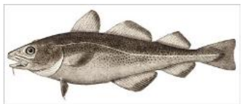
[1] Following two citations is yet to be published; Bonanomi et al. (in prep. a) are pending revisions for *Scientific Reports*; and Bonanomi et al. in (prep. b).
In the reference section, they are included in following PhD-dissertation; (Bonanomi, 2015)



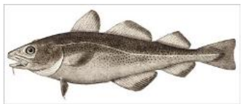
General background

Fisheries genetics

Fisheries genetics – it is the application that bridges the gap between molecular biology and fisheries research, by applying molecular tools in order to answer a range of fisheries-related questions. Topics that include evolutionary taxonomy and phylogenetics (de Pinna, 1999), population structure and connectivity (Cowen et al., 2006), spatial and temporal scale of population differentiation (Bonanomi et al. in prep.a), adaptive variation (Nielsen et al., 2009a), effective population size (Koljonen, 2001), fisheries-induced evolution (Kuparinen & Merilä, 2007) as well as other more specialized applications (Kochzius, 2009), are of great importance in order to enhance the understanding of mechanisms that shape marine fisheries resources. Likewise, fisheries genetics continuously adds to the conceptual framework of ecological and evolutionary theory. While classical fisheries approaches only capture short-term quantitative changes in populations, then genetic approaches captures a much larger spectrum of both short-term phenotypic variations and long-term evolutionary responses (qualitative changes) (Frankham, 2007). The rapid development and technological advancements within genetic research during recent decades have provided significant new insights, culminating in paradigm shifts that have changed the perception of the marine realm and the marine organisms therein (Hauser & Seeb, 2008; Hauser & Carvalho, 2008).

Paradigm shift in fisheries genetics

Until recently the classical paradigm that the marine environment is a demographically open realm with species comprising high mobility, great potential for substantial exchange via dispersal of egg and larvae and reproduction limited by no obvious barriers, has been a sole belief, and frequently still is, within marine sciences (Hauser & Carvalho, 2008). Marine fish has often been assumed to be more genetically homogenous than freshwater and diadromous fish, an assumption that was supported by earlier genetic studies to support (Gyllensten, 1985; Ward, 1994). Therefore, the expectations of oceans as inexhaustible resources with most marine fish populations having vast population sizes have been a common notion accepted for many decades. Fisheries biology and management has been influenced greatly hereby and consequently, the focus has been on the species-level, neglecting that species are made up of smaller units, "populations". Marine fish populations have been shown to often harbor unique adaptations to local



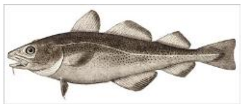
environments and exhibiting limited exchange rates on many levels, both temporally and spatially (Waples & Gaggiotti, 2006; Hauser & Carvalho, 2008). Realizing this, do not only enhance present knowledge of the mechanisms shaping fish abundances and distribution, but also contributes conceptually to evolutionary and ecological theory and aids in management and conservation of valuable marine resources.

The unit concept in fisheries: stock versus population

The concept and definition of fishery units as a basic measure for harvested species is central in fisheries management. Despite long-term established notions of sub-specific fishery units, there seem to be no universally applicable definition of associated terms describing these units, neither for so called "stocks" (Carvalho & Hauser, 1994) nor "populations" (Waples & Gaggiotti, 2006).

The concept of a stock has been the source to much debate in the scientific literature for at least two decades (Carvalho & Hauser, 1994; Booke, 1999; Ovenden et al., 2013). The debate rises due to the potential for multiple interpretation of the stock term presented through previous definitions given by a handful of authors (e.g. (Jamieson, 1973; Ihssen et al., 1981; Gauldie, 1988). The difference in interpretation is driven by conflicting factors, which can be defined as biological, socio-economical and even political considerations in the development of management strategies for fisheries (Carvalho & Hauser, 1994). The most frequent and largely dominating interpretation found in fisheries management seems to define stocks as a demographically distinct groups of marine organisms that might be accessible and available for exploitation within a given geographical area (Smith et al., 1990). Almost all exploited marine species are managed and described by such traditional discrete fisheries units (e.g. stocks), and fail to acknowledge the biological complexity that might be apparent (Reiss et al., 2009).

The use of these discrete fishery units, based mainly on geographical assumptions, has frequently on several occasions been dubbed as an insufficient management tool in the literature (Booke, 1999; Reiss et al., 2009). Consequently, the adoption of a more biologically relevant unit-definition has been recommended and Waples and Gaggiotti's (2006) review of population definitions has aided within this context. Although, the definition and interpretation of the population term varies along a continuum of ecological and evolutionary biology, the latter undoubtedly represents the solution to overcome the shortages of the traditional fisheries unit (Waples & Gaggiotti, 2006). Accordingly, a population viewed through the evolutionary paradigm can be defined as intraspecific genetically distinct groups produced by



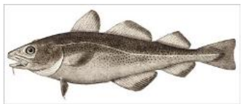
randomly mating individuals with temporal and spatial integrity (Ihssen et al., 1981; Carvalho & Hauser, 1994). Thus, a population concept based on evolutionary criteria needs to be adopted in order to account for complex spatial and temporal genetic structuring of adaptive significance, which can aid in reaching the primary objectives in fisheries; effective management, sustainable harvest and preservation of future resources. Here, stock and population will be used according to the above descriptions.

Genetic variation for stock identification

Stock identification is a central theme in fisheries science and represents a multidisciplinary field involving life history studies, biometrics and genetics (Begg & Cadrin, 2009). Taking a more technical note on these disciplines, they can largely be categorized into stock identification through life history traits (Begg et al., 1999), morphological variation (Cadrin & Friedland, 1999), environmental signals (Thorrold, 2003) and genetic variation (Shaklee, 1998). Although, all these stock identification approaches has experienced rapid advancements in the last decades, the use of genetic markers has provided some of the most promising results improving the resolution and coverage of stock composition over a wide range of commercially exploited mixed-stock fisheries (Ruzzante, 2000; Beacham et al., 2006; VanDeHey et al., 2010; Bekkevold et al., 2011). The practical advantages of genetic stock identification compared to other alternative techniques includes a nonlethal sampling procedure, simple and easy collection of samples by non-experts, statistical robust and accurate estimation of stock composition, fast processing speed potentially allowing genetic results the same day as samples arrive, and lastly the ability to use a range of fresh, preserved or highly processed material (Koljonen & Nielsen, 2007; Lago et al., 2012). Genetic methods and results are also now easily transferrable across laboratories making it possible to extend studies over large spatial and temporal scales, as well as supplementing with additional population samples and genetic information (Brumfield et al., 2003).

Genetic variation that reflects population structure

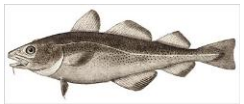
Genetic variation is the variation in allelic forms of genes representing the basis for the variation in heredity. Such variation is the product of past mutational events, which produced polymorphic regions in the genome inviting evolutionary forces (other than mutation) to act through heritable processes. The effect of



evolutionary forces in wild populations can be observed 1) through migration where gene-flow can lead to increased homogeneity among populations and 2), increased heterogeneity among populations can be the product of random genetic drift over several generations. Thirdly, selection will either generate increased or reduced differentiation among populations. The former two forces (i.e. drift and migration) are considered neutral and expected to exert uniform genome-wide effects, whereas selective forces are only expected to be site-specific affecting restricted regions linked to particular genes (i.e. adaptive variation). Accordingly, several authors have argued that neutral variation might be a poor estimate for local genetic differentiation and population viability (Merilä & Crnokrak, 2001; Leinonen et al., 2008). The lack of detectable neutral variation does not preclude population divergence as selection is permanently contextual, meaning that all neutral variation could potentially become future adaptive variation under a given environmental setting (Marty et al., 2015). Hence, evolutionary processes which have shaped the standing genetic variation will continue to optimize fitness in local populations through environmental selective stressors. Here, population structure might be observed in genes under selection by identifying adaptive variation distinguishing it from variation that is neutral (Hemmer-Hansen et al., 2007; Nielsen et al., 2009a) Therefore, neutral markers remain very useful when accompanied by markers associated with genes under selection (and vice versa), and furthermore serve as a good genome-wide estimators of levels of variation (Allendorf et al., 2010).

Genetic markers

Genetic markers are heritable, stable and discrete. The use of genetic markers dates back to the 1950's, and encompass a wide range of applications. Generally, genetic markers include protein and DNA analysis revealing variation in amino acids and DNA sequences, respectively. They can both provide high species resolution and are efficient in traceability schemes. However, the latter are progressively replacing protein-based analysis as technologies involving DNA is a fast developing field (Schlötterer, 2004). The application of DNA markers has become the standard approach in genetic research in fisheries, fish ecology, fish identification and even within the fish food industry (Hauser & Carvalho, 2008; Kochzius, 2009). The dominant genetic markers used nowadays for tractability purposes, such as species identification and population structure studies, are microsatellites and single nucleotide polymorphisms (SNPs). The uses of genetic markers for stock identification purposes are ideal because they possess several advantageous attributes (see Cadrin 2014), such as 1) independence of environmental variability

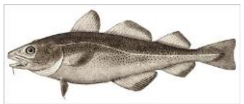


during a lifetime of an individual; 2) units are composed of discrete information making population differences readily quantifiable; 3) language of DNA is universal and is applicable to all forms of life; 4) achievable within reasonable cost and effort and 5) possibility for statistical analysis that provide estimates of error associated with the process (Shaklee et al., 1999). Although, at present the two genetic marker classes mentioned (see above and below) are the most frequently used in fisheries genetics, other markers such as reduced-representation genome sequencing, (e.g. RAD sequencing (Baxter, 2011) and genotyping-by-sequencing (Narum et al., 2013)) has started to appear and is certainly worth mentioning; however, the focus here will solely be on markers used in the thesis.

From microsatellites to SNPs

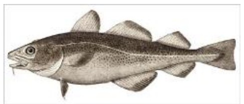
Microsatellites, also called short tandem repeats, are repetitive sequences of one to six base pairs in the genome. They represent non-coding DNA that is very abundant in the genome, they are inherited as codominant markers and attain high polymorphism with alleles distinguished by varying repeat numbers. These markers were entitled by their landmark paper *"Microsatellites: Genetic Markers for the Future"* (Wright & Bentzen, 1994) showing high potential for genetic origin assignment, because of their simplistic application through PCR followed by capillary electrophoresis, fast genotyping and high statistical power at affordable costs. They have since been widely used in fisheries, such as stock structure analysis and management (Hauser & Carvalho, 2008). Although, microsatellites still are the most commonly applied markers, disadvantages such as scoring error and difficulty in comparability among laboratories and studies are evident (DeWoody et al., 2006). Furthermore, other challenges include allele stuttering, allele dropout, null alleles (Van Oosterhout et al., 2004), hitchhiking selection (Nielsen et al., 2006) and more importantly homoplasy (Estoup et al., 2002).

An alternative that address these limitations is SNPs. SNPs are single base substitutions representing genome sites with more than one nucleotide present and are the most abundant polymorphism throughout a genome. They are codominant and can be located in both non-coding and coding regions and the genome (Morin et al., 2004). Therefore, a major advantage of SNPs is the possibility to detect both natural and adaptive variation (Bradbury et al., 2010; Limborg et al., 2012). The variability is smaller than for microsatellites, as normally only two alleles are present for each locus. However, the little information is compensated by their frequent occurrence and the fact that more SNPs can be used simultaneously.



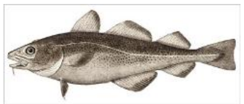
Advantages are also apparent for fragmented or degraded DNA, as SNPs only require a short flanking sequence of less than 100 base pairs per locus (Nielsen & Bekkevold, 2012). The information of SNPs is categorical, which means that data can be calibrated across laboratories and studies allowing temporal and spatial comparisons at all scales (Brumfield et al., 2003). Simultaneous analysis of over 1000 SNPs is now possible as a result of recently developed techniques, such as microarrays (Kochzius et al., 2010) or next-generation sequencing (Davey et al., 2010).

The application of gene-associated SNP makers has through several empirical studies demonstrated that a much more detailed resolution might be obtained about population subdivision compared to other neutral markers (Nielsen et al., 2009c; Poulsen et al., 2011; Limborg et al., 2012; Bradbury et al., 2010). Furthermore, Wirgin at al. (2007) also showed that SNP markers for distinguishing populations of cod provide higher resolution power for stock identification compared to microsatellites. Additionally, large increases in the statistical power for genetic stock identification have been proven on several other occasions (Bekkevold, 2012; Limborg et al., 2012; Nielsen et al., 2012). Potential problems seem to arise when attempting to estimate the genetic variation for population with markers developed for different populations from another geographical range, also referred to as ascertainment bias (Helyar et al., 2011). For panels with sufficient number of SNPs and restricted sized spatial range, ascertainment bias only tends to decrease the genetic information for specific SNPs in screened populations compared to the population that the developed panel was intended for (Rosenblum & Novembre, 2007); Bradbury et al., 2011). Another challenge involves the identification and selection of the most informative SNPs for empirical studies of population structure and genetic stock identification, which requires careful consideration in order to avoid the inclusion of loci that are either non-representative for all populations or have elevated assignment power that may lead to biased assignment (Waples, 2010; Helyar et al., 2011). Cross-validation approaches as suggested by Anderson (2010) could be employed in order to avoid such biases. Nevertheless, Nielsen et al. (2012) showed that with a carefully designed panel for specific target scenarios, even small SNP panels, including SNPs under selection provides unprecedented levels of assignment power for the identification of fish origins.



Individual assignment

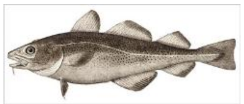
Individual assignment (IA) are methods that try to match an individual unknown fish to already known groups of fish that share similar characteristics driven by similar environmental factors, common genetic material or both (Manel et al., 2005). The groupings of fish are compared and subsequently assigned to representative populations. A genetic population is a interbreeding local group of fish of the same species leading to reproductive isolation and genetic differentiation from other groups of the same species (Waples & Gaggiotti, 2006). Genetically based individual assignment is using estimation of probabilities of matching characteristic genetic features that are apparent in individuals of unknown origin to a number of potential source populations (Paetkau, 1995). One assumption for assignment is evidently that potential source populations are known. The heritability and stability of genetic markers prove very useful in this context and baselines are commonly established (Limborg et al., 2012; Therkildsen et al., 2013; Milano et al., 2014). Statistical analysis and a wide selection of software have been developed, that via genetic data can determine population structure and assign individuals (Pritchard, 2000; Piry et al., 2004). The original assignment methods were frequency-based (Paetkau, 1995), but the more recent developed probabilistic Bayesian assignment methods have largely replaced the former (Rannala & Mountain, 1997; Piry et al., 2004). The baseline allele frequencies are estimated by assuming equal prior probability of occurrence for the allelic frequencies at each locus in each population and thereby accounts for potentially missing rare alleles. Another Bayesian-based assignment method involves clustering of groups of individuals in order to minimize Hardy-Weinberg and linkage disequilibria (Pritchard, 2000). The model then assumes a set of populations and assigns individuals to populations. The assignment may involve a joint allocation to two or more populations if their genotypes indicate that they are admixed. Lastly, a non-Bayesian clustering method has been developed, where multivariate statistics identifies the best supported number of groupings (Jombart et al., 2010). The assignment is based on discriminant functions by deriving posterior probabilities for each individual of membership in each of the inferred groupings, and therefore the method does not rely on any assumption of population genetics models or any particular type of substructure. Here, a standard probabilistic Bayesian assignment method and non-Bayesian clustering assignment method was used (i.e. implemented in GENECLASS2.0 and DAPC, respectively, see section for materials and method).



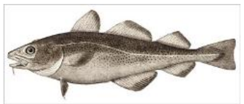
Genetic markers, such as microsatellites and SNPs, coupled with these newly developed statistical tools are effective for exploring population structure. Structuring of populations has been shown for many marine fish species, mostly commercially relevant for fisheries (Nielsen et al. 2009). Baselines for both cod and herring in the North Sea have already been established (Nielsen et al., 2001; Limborg et al., 2012). Origin assignment of marine fish remains challenging compared to their freshwater and diadromous relatives, but even at low genetic differentiation assignment power can be greatly improved by simply increasing number of markers screened (Manel et al., 2005). Nevertheless, regardless of the available genetic tool the power of assignment generally depends on following six parameters; 1) the level of genetic differentiation among populations, 2) type of genetic marker, 3) the number of loci studied, 4) the size of baseline samples, 5) the number of included populations and 6) the statistical method used for assignment (See Koljonen 2007).

Mixed-stock analysis

Mixed-stock analysis (MSA) provides genetic tools to estimate the proportional contribution of different baseline populations in a mixed sample comprising fish of unknown origin (Shaklee, 1990). Compared to IA, MSA takes into account the individual genotypes of each fish across multiple loci, the multilocus genotype distribution within both the baseline and mixture samples (Hansen, 2007). Briefly, the MSA methodology includes two probabilistic methods for estimation; maximum likelihood and Bayesian methods. Maximum likelihood estimation, which is based on multilocus genotype distributions in the known baseline populations and in the mixture samples, has traditionally been used for MSA. Hereby, the method estimate the relative proportional contributions of baseline populations associated with the highest likelihood of observed multilocus frequencies occurring in a mixture sample (Fournier, 1984; Millar, 1987) The reliability of the easy-assessable proportional estimates should subsequently be evaluated, which require information on sizes of baseline and mixture samples, as well as loci numbers (Koljonen et al., 2005). A more recent probabilistic method is based on Bayesian statistics, where prior to MSA information on multilocus genotype distribution is obtained from baseline samples, while no prior information is assumed for the population proportion in the mixture sample (Pella, 2001). The 'missing' knowledge is delivered from the multilocus genotype distribution in the mixture sample, and hereby estimates are returned of both the population compositions of the mixture sample and the genetic composition of the baseline samples.



MSA has been widely used in relation to fisheries management in Pacific salmon (*Oncorhynchus* spp.) (Beacham et al., 2012), Atlantic salmon (*Salmo sarr*) (Beacham et al., 2012), Atlantic cod (*Gadus morhua*) (Ruzzante, 2000), Atlantic hering (*Clupea harengus*) (Bekkevold et al., 2011), and many other species. Although widely utilized, it remains a great challenge in high gene-flow marine species to acquire enough genetic markers with sufficient high genetic separation for MSA. The recent development in population genetics has identified genetic variation of adaptive divergence and especially the accessibility to genomic markers, in particular SNPs, which are influenced by diversifying selection has provided unprecedented resolution in weakly structured fish (Bekkevold et al., 2015; Nielsen et al., 2012). SNPs has great potential for large-scale MSA, because their high-level divergence and bi-allelic nature provides for increased accuracy and precision as well as rapid and highly automated genotyping with low error rates (Schlötterer, 2004; Seeb, 2009).



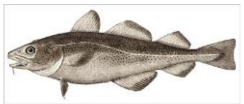
Study organism: The Atlantic cod (*Gadus morhua*)

Basic aspects of the biology

Many commercially harvested species are categorized as “classical” marine fish that are characterised by high fecundity, high dispersal potential as adults, large population sizes and pelagic egg and larval phases (Nielsen & Kenchington, 2001). The Atlantic cod (*Gadus morhua*, Linnaeus 1758) embrace this description with a wide distribution and few obvious barriers to prevent any intraspecific genetic exchange. It is a ray-finned teleost found in temperate clines ranging from shallow coastal areas to deep waters around the continental shelves throughout the North Atlantic. Longevity can be over 20 years and they can reach length up to approximately 130 cm and attain a weight of 25-35 kg. A few known cases have recorded fish up to 200 cm and 100 kg (Lough, 2004). They are omnivorous, feeding at dawn or dusk on a variety of invertebrates and fish (Glover et al., 2013), and even cannibalistic behavior has been described for some populations (Blom & Folkvord, 1997). Fecundity is correlated with size, where larger females produce more eggs. Accordingly, depending on their size, females may produce between 3 and 9 million eggs per spawning season. Large aggregations of cod are formed during spawning time, where females release eggs in multiple batches and males compete to fertilize the eggs near the bottom in water temperatures between 5 and 7 °C during winter and early spring (Bekkevold, 2002, 2006). There is substantial variation in life history traits between populations. Sexual maturity is attained around age 2 to 3 in most populations, but mean age at maturity may be up to 8 years for some populations (O'Brien, 1999). Variability is also evident in the migratory life-history strategies exhibited by populations ranging from sedentary to highly migratory and studies have even suggested homing behavior for some populations (Robichaud & Rose, 2004; Svedäng et al., 2007).

Historical overexploitation of a marine species: Cod fisheries

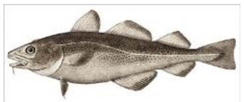
The exploitation and fishery of Atlantic cod dates back more than 1000 years to medieval Europe (Barrett et al., 2008) and it has undoubtedly been one of the most important commercial fish linked to human history (Kurlansky, 1997). The extensive distributional range and enormous population sizes of Atlantic cod throughout the North Atlantic has supported some of the world's largest and economically most important fisheries through history (Lear, 1998). Across the Atlantic Ocean, cod populations have declined



drastically over the last 50 years (Lilly et al., 2008; Daan, 1994). The classical example of overfishing, having become the 'poster child' of fisheries mismanagement, is from the Newfoundland cod fishery which collapsed in 1992 and has since almost reached legendary status having numerous published accounts to its name (Rose 2007; Harris 1998; Bavington 2011, along with numerous scientific publications, see below). These stocks have suffered declines of 99.9% compared to historic levels and the collapse of the Northern cod fishery ended the region's 500 year run having one of the largest and most productive fisheries ever seen in historical times (Hutchings & Reynolds, 2004; Davies & Rangeley, 2010). Precariously almost all cod fishery stocks are close to collapse throughout the species range (Cook et al., 1997; Christensen et al., 2003) and while several factors might have contributed to these overall declines, and some places failures of subsequent recovery, pressures from previous and contemporary fishing has been emphasized as one of the main causes (Hilborn et al., 2003). As a consequence, the extinction vulnerability has been assessed and several stocks has been listed threatened or endangered (Hutchings & Reynolds, 2004; Dulvy et al., 2005).

Population structure: What do we know?

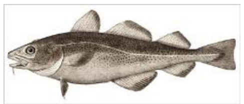
As a quintessential example of a "classical" marine fish, the Atlantic cod exhibit a wide distribution and few obvious barriers that should potentially prevent any interspecific genetic exchange. Nevertheless, classical notions of absence and rarity of local adaptation in marine environments due to swamping by the homogenizing effects of gene flow (Ward, 1994) has recently been proven false and recent decades of genetic research on Atlantic cod has challenged such traditional views (reviewed by Hauser and Carvalho 2008). Even weak levels of population structure offer differentiation that can be highly significant (Pogson, 1995). Historical attempts to elucidate population structure in Atlantic cod have included analysis on hemoglobin (Sick, 1965), allozymes (Mork et al., 1982; Mork & Sundnes, 1985), mtDNA (Smith et al., 1989), which all have provided variable results with lacking evidence of genetic differentiation. With the arrival of PCR-based markers and microsatellites (Schlötterer, 2004), weak, but significant population structure has been revealed numerous of times. Furthermore, the recent application of SNPs as genetic markers has provided additional evidence for population differentiation and adaptive divergence. The latter has given birth to the initial development of a catalogue of genes under directional selection. Several genes has already been identified and their allelic variation has been significantly associated with environmental



factors such as salinity (Larsen et al., 2012) and temperature (Bradbury et al., 2010). As well as findings of genomic signatures of selection on specific loci in wild populations (Nielsen et al., 2009b), common garden experiments has also provided evidence for the support of divergence in cod (Harrald et al., 2010b, 2010a). Strong local selections, homing behavior (Svedäng et al., 2007) and larval retention (Knutsen, 2007) have been outlined as mechanisms for producing these patterns. The wide-ranging population structure in Atlantic cod has been shown to be apparent at many spatial scales with an ancient cross-Atlantic division between European and North American cod populations (Bigg et al., 2008), followed by subdivisions into numerous smaller units on each side of the Atlantic and further down to the level of individual fjords (Jorde et al., 2007). In addition, Hemmer-Hansen (2013) identified a genomic region of strong population differentiation splitting ecologically distinct ecotypes of cod characterized by behavioral life-history traits. These ecotypes has been known for almost a decade and have been described as 'migratory' and 'stationary' cod (Pálsson & Thorsteinsson, 2003; Robichaud & Rose, 2004). Briefly, stationary ecotypes seem to spend their entire life in inshore coastal water habitats, whereas migratory make advantage of offshore and deeper habitats undertaking both long-distance travels and pronounced vertical movements in the water column, mainly associated with feeding or spawning behavior (See review, Nordeide et al. (2011).

The study population: Atlantic cod in Greenland

The waters surrounding Greenland represents the northern boundary for Atlantic cod and have been described as a marginal habitat for the species due to the highly fluctuating occurrence of cod over time (Buch, 1994; Rätz & Lloret, 2005). Furthermore, Greenland might also be the most recent colonized area of the current distribution of the species (Bigg et al., 2008). Historical records, mainly obtained from fisheries, have shown that the abundance of Atlantic cod in Greenland has experienced great fluctuations with episodic extreme peaks and lows during the past century (Hansen, 1949; Hovgard & Wieland, 2008). The commercial inshore fishery (i.e. defined as catches taken by the artisan Greenland fisheries operating within coastal areas and fjords) began near the coast in 1911 and early development was apparent during the 1920's (Horsted, 2000). In contrast to the development and rapid expansion of the offshore fishery the initial inshore fisheries has remained relatively trivial and catches has been maintained at levels between 10.000 and 35.000 tons. Since the 1970's inshore catches has generally followed the offshore astray towards the route of continuous stock decline. The inshore fishery reached historic low levels in 1998 with



yields less than 500 tons and an inevitable collapse of the offshore fishery became reality already in the beginning of the 1990's (Horsted, 2000; Hovgard & Wieland, 2008) (See overview Fig. 1).

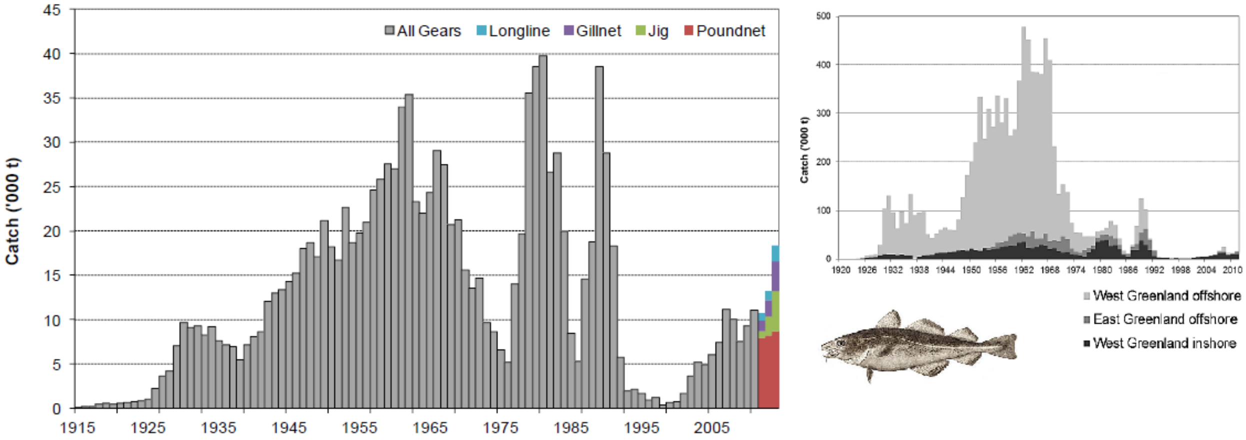
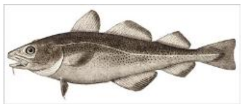


Figure 1 showing the historical commercial catches (in tones) of Atlantic cod in Greenlandic waters. The inshore landings are shown to the right, and the total fishery is shown to the left. In recent years fishing method has been separated according to type in order to get an overview of fishing gear used. Re-adapted from ICES (2013, 2015).

Traditionally, increases in cod biomass in West Greenland has been suggested to be a consequence of eggs and larval transport and/or adult migration from Iceland (Schopka, 1994; Astthorsson, 1994) and the multiple historical fluctuations has correlated with ocean temperatures (Buch, 1994; Stein, 2007). Recently, changes in cod biomass have been shown to be a combination of growth in resident populations and recurrent colonization by nearby populations (Therkildsen et al., 2013), and surely, the most recent outburst in biomass coincides with increases in ocean temperatures (Drinkwater, 2005). Indeed, a potential temperature-mediated effect providing favorable conditions for distinct populations (Riget, 1998) has recently been supported by both historical and predicted continuous climate change-induced migration by populations of Icelandic origin (Bonanomi et al. in prep.a). Accordingly, egg surveys (Bigg et al., 2008), tagging experiments (Storr-Paulsen et al., 2004), and genetic investigations (Bonanomi et al. in prep.b) have suggested irregular but substantial contribution of cod with Icelandic origin to offshore regions in West Greenland. However, the presence and abundance of individuals originating from the "Iceland Offshore" population in West Greenland coastal waters has yet to be documented. So far, the



actual origin of individuals and population structure in inshore areas remains uncertain and poorly investigated.

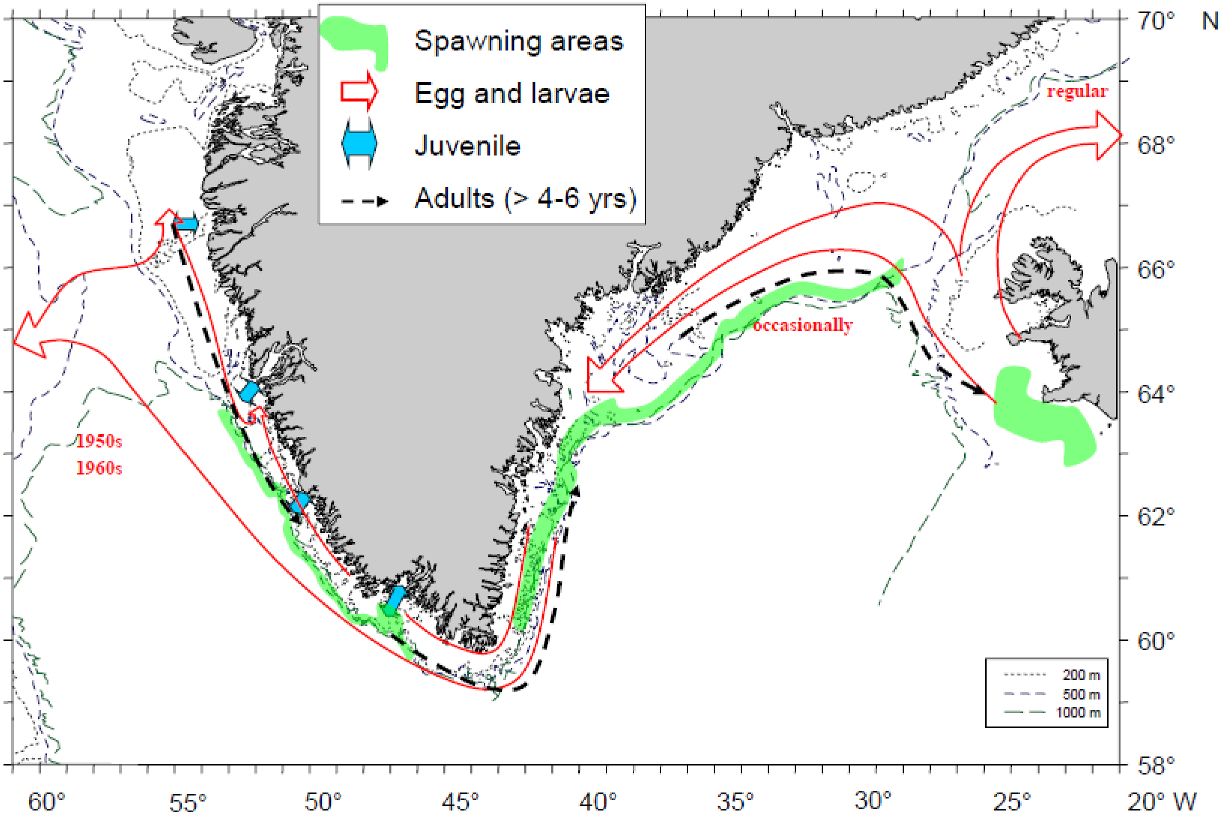
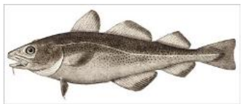
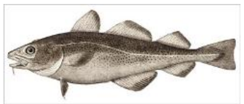


Figure 2 Spawning areas, egg and larval transport, and migration of Iceland adults of Atlantic cod (*Gadus morhus*) in Greenlandic and Icelandic waters. ICES (2015), originally re-adapted from Wieland & Hovgard (2002)

Therkildsen et al. (2013) applied SNP's for establishing the initial baseline for populations of cod in Greenland and hereby identifying four genetically distinct groups known as West Greenland Inshore, West Greenland Offshore, Iceland Offshore (also known as East Greenland/Iceland offshore population (ICES, 2015)) and Iceland Inshore population (re-adapted from Therkildsen et al. (2013)), which exhibits different spatial distributions with considerable mixing and overlap. Known significant spawning grounds include (See Fig. 2): the banks north of 62°N in East Greenland and on the southwest coast of Iceland for the Iceland Offshore population (Begg, 2000; Marteinsdóttir, 2000); many local spawning grounds of Iceland, such as



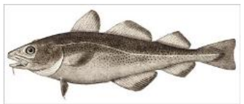
northwest and north of Iceland are assumed to be of significance of Iceland Inshore populations (Jónsdóttir et al., 2007); most fjords between Sisimiut (66°N) and Paamiut (62°N), and on a particular note a major spawning site has been described near the settlement of Kapisillit within the fjord system (i.e. Godthåbsfjord) in Nuuk, for West Greenland Inshore (Smidt, 1979; Storr-Paulsen et al., 2004); banks along the Western shoreline and the shelf of Southeast and South Greenland The main spawning grounds for West Greenland Offshore populations (Wieland & Hovgard, 2002). These populations have most recently been confirmed contributing substantially to mixed stock fisheries historically, as well as contemporarily (Bonanomi et al. in prep.a). Furthermore, historical tagging experiments have suggested that mixing of different populations occurs along West Greenlandic inshore waters where cod populations share feeding grounds (Hansen, 1949; Hovgård & Christensen, 1990; Storr-Paulsen et al., 2004). Moreover, genetic studies on coastal feeding aggregations in West Greenland have confirmed coastal assemblages to be of mixed origin of at least an offshore and inshore component (Pampoulie et al., 2011). Accordingly, a mixing zone in inshore waters comprising different population components might be possible, but genetic studies are needed to support this hypothesis.



Thesis objectives

The primary aim of this thesis was to investigate the spatial contribution of different cod populations to regional inshore mixed stocks in West Greenland. To accomplish this, a catalogue of different genetic approaches was explored and evaluated, thus becoming a vital part of the thesis. Furthermore the local sampling in Nuuk provided opportunities for investigating the spatial genetic pattern in the transition from coastal inshore waters into fjord systems. Accordingly, addressing the following questions became the main questions:

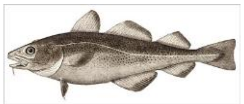
1. *What is the population mixture composition of inshore coastal waters?*
2. *How does the population mixture composition differ spatially*
   - *2.1. - at regional scales in Sisimiut and Nuuk?*
   - *2.2. - at local scales in the transition from coastal inshore waters into fjord systems?*
3. *How do different genetic approaches differ and what are the most appropriate tool for estimating the origins at the level*
   - *3.1. - individuals?*
   - *3.2. - populations?*



Materials and methods

Sampling

Tissue samples of Atlantic cod were collected at different locations within the coastal regions of Western Greenland. A total of 565 individuals were collected from inshore waters and fjords around two locations, namely the settlements of Sisimiut and Nuuk (Fig. 3). Hereof, 220 samples were collected by boat provided by the Arctic Technology Centre (ARTEK) in Sisimiut (N=100) and Greenland Institute of Natural Resources (GINR) in Nuuk (N=120). Gill tissue was taken from each individual and placed in 1.5ml vials with 95% ethanol. Supplementary 326 genetic samples were provided by commercial fishing vessels collaborating with GINR and collected at sites around Nuuk inshore waters and fjords. Fish were subsequently transported to the GINR in Nuuk, where fin clips were taken from the anal fins and placed in 1.5ml vials with 95% ethanol. Otoliths were also collected from all fish to allow ageing of the fish and as an alternative source for genetic material. All samples were collected in 2013, where gill tissue samples was collected in August and samples provided from commercial fisheries was collected between September and December.



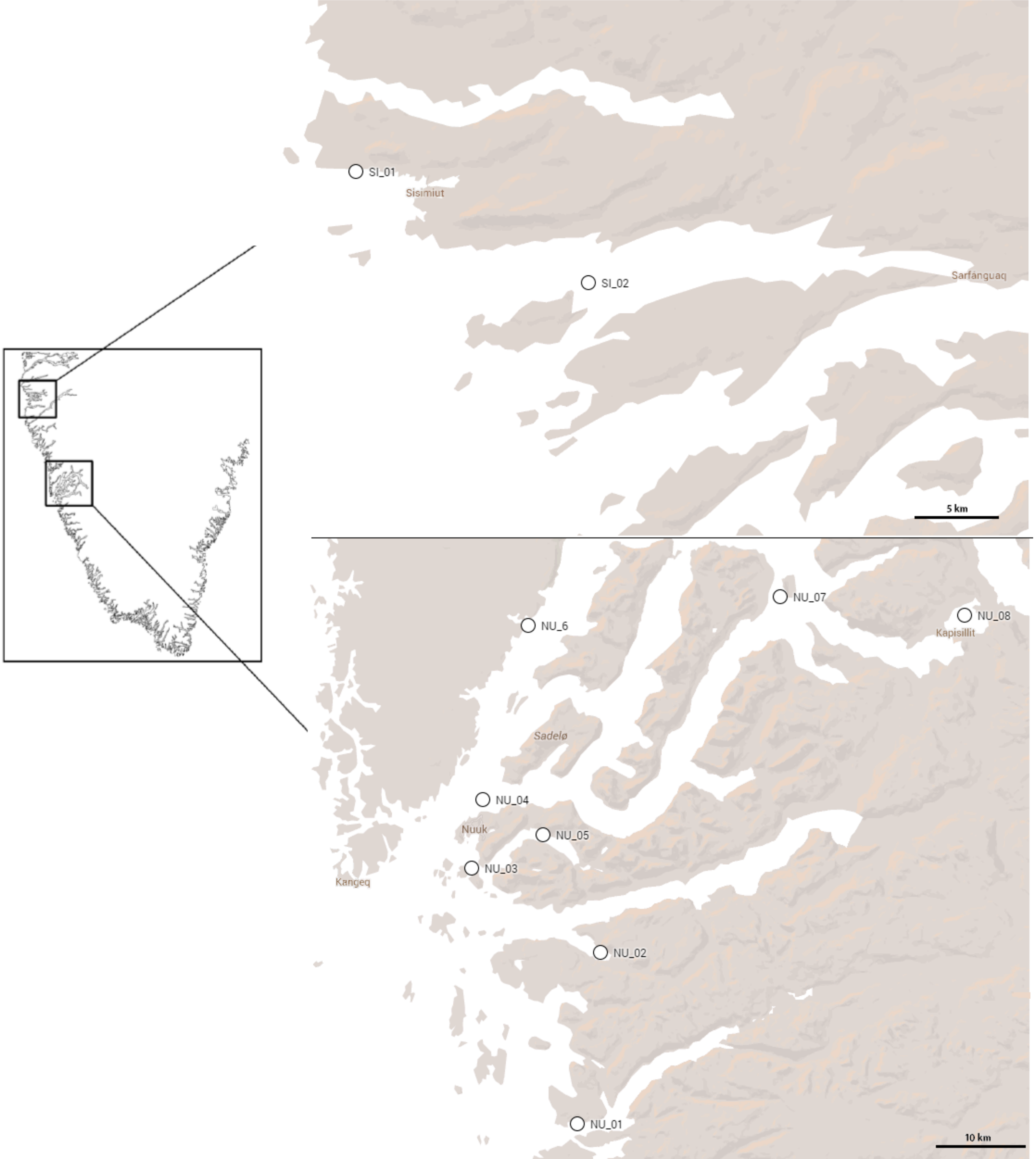


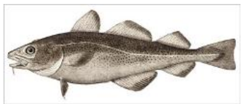
Figure 3 Map of sampling sites in Sisimiut (SU-sites) in the northwest region and Nuuk (NU-sites) in the middle southwest region of Greenland.

DNA Extraction

All practical work associated with the genetic studies took place at the population genetics lab at DTU Aqua in Silkeborg, Denmark. DNA was extracted from sample tissue using E.Z.N.A.® Tissue DNA Kit by following the instructions and protocol provided by the manufacturer, Omega Bio-Tek, Inc., USA (see E.Z.N.A.® Tissue DNA Kit Product Manual for details). In summary, tissue was dissolved through lysis processes using a digestion buffer and proteinase K incubated at 55 °C. Depending on DNA source and tissue material lysis time varied from a few hours for gill and fin tissue to overnight incubation for otoliths. Subsequently, a three-step procedure was performed involving binding buffer and ethanol addition, respectively, to the supernatant. This was followed by a short incubation period between additions, and finally a transfer of liquid and any precipitates into HiBind DNA Mini Columns. Thereafter, two washing steps were employed in order to firstly remove water-soluble impurities and secondly to concentrate and purify DNA. Multiple centrifugation steps were used between all liquid additions and after the final centrifugation, assuring a dry pellet. Heated elution buffer was used to collect and preserve DNA ensuring long-term storage and chemical stability. DNA extractions from gill and fin tissue were performed in a standard laboratory for genetic studies, whereas work with otoliths took place in a specialized container housing a sterile laboratory environment where no other extraction was processed simultaneously. DNA concentration and the ratio of absorbance in extracts from tissue and otoliths were measured with a Nanodrop 2000 Spectrophotometer (Thermo Scientific, USA). Potential cross-contamination among samples was assessed through PCR amplification success and fragment analysis of four microsatellite loci (Tch11, Tch14: (O'Reilly et al., 2005); Gmo-C18: (Stenvik et al., 2006); and PGmo38:(Jakobsdóttir et al., 2006)).

SNP panel and genotyping

A panel of 96 SNPs (Single Nucleotide Polymorphisms) was selected as the most informative for population assignment. The panel is the most recent and updated based on screenings by Therkildsen et al. (2013) of 935 SNPs from spawning population samples collected throughout Greenlandic and Icelandic waters. Minimum assays with maximum power can be achieved with the reduced panel which includes loci that attained the highest pairwise differentiation (F_{st}) between four distinct spawning groups previously identified (Therkildsen et al., 2013). In order to validate possible improvements and associated power in the newly developed and updated panel, comparisons was done with the previous panel that included 81 loci,

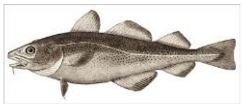


which previously has been used in genetic assignment studies involving Atlantic cod in Greenland (Bonanomi et al., in prep.a). The associated power for each panel for each genetic method (see section; Individual assignment) was tested following Anderson et al.'s (2010) 'training-set/holdout-set' protocol. The panels was assessed by dividing the data panel into a 'training' and 'holdout' set and subsequently assigning 'holdout' individuals back to populations defined by reference individuals from the 'training-set'. Each individual population in the baseline panel was divided into 'training' and 'holdout' datasets. The allocation into sets happened in a randomized selection process, where 10 'training-set/holdout-set' for each panel was produced. Subsequently, individual assignment was performed applying conservative criteria (i.e. probability cutoff of origin >0.9) for each genetic software package (see section; Individual assignment).

All cod were genotyped using Fluidigm® 96.96 Dynamic Array™ IFC (i.e. integrated fluidic circuit) system applied to the Biomark™ HD System by following the instructions and protocol provided by the manufacturer, Fluidigm® Corporation and according to methods described in Seeb at al. (2009). The genotyped data was normalized, so that the intensities of the assays roughly were plotted in a square, and checked against internal sample quality controls using the Fluidigm® SNP Genotyping Analysis Software (see Fluidigm® SNP Genotyping Analysis User Guide for details). The clustering of genotypes could be manually edited and quality crosschecking was assured through multiple independent reviews of the data by different persons and comparisons between individual data reviews.

Individual assignment

Four different genetic approaches were applied for individual assignment in order to explore the performance of different packages (see below) that has previously been used for genetic investigations (Bonanomi in prep.a, b). Furthermore, different criteria for each approach for assignment were explored and lastly, the best suitable approach was to estimate the contribution of different populations to fished mixed stocks in West Greenland. These approaches involved two genetic software packages using different methodology for assignment tests. Firstly, the program *GENECLASS2* (GC; (Piry et al., 2004), which provides a range of assignment calculations, was used for assignment purposes. These tests were based on a Bayesian probability approach (Rannala & Mountain, 1997). The approach evaluates the likelihood of an individual sample belonging to candidate populations based on observed genotype frequencies, and a



Monte-Carlo resampling algorithm for probability computation (Paetkau et al., 2004), which simulates a number of multilocus genotypes for each population from baseline allele frequencies. Thereafter, the method generates expected distributions of likelihoods within populations in order to compare with estimated individual likelihoods of genotypes to be assigned. 10.000 individuals were simulated (type I error, α = 0.01) to evaluate whether a multilocus genotype originated from one of four previously identified baseline populations (Therkildsen et al. 2013).

Secondly, Discriminant Analysis of Principal Components (DAPC; (Jombart et al., 2010)) was employed. It is a multivariate method to identify and describe the best supported groups of genetically similar individuals through K-means and discriminant functions, constructing synthetic variables that maximizes differences between and minimizes differences within groups. Posterior probabilities derived hereof can describe each individual according to membership of each of the inferred groupings. DAPC is included in the *adegenet R* package, which implements a number of different methods for analyzing population structure using multivariate statistics, graphics and spatial statistics.

The different genetic approaches involved either one of GC or DAPC or a combination of the two assignment methods inspired by assignment procedures previously used by Bonanomi et al. (in prep.a, b) (See Table 1 for summary of abbreviations and approaches). A range of set criteria cutoffs was explored; no cutoff (NC), >0.5., >0.6, >0.7 >0.8 and >0.9, assuming that probability likelihoods and posterior probabilities obtained from GC and DAPC, respectively, represented equivalent measures for comparisons,

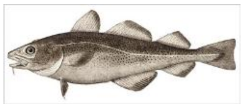
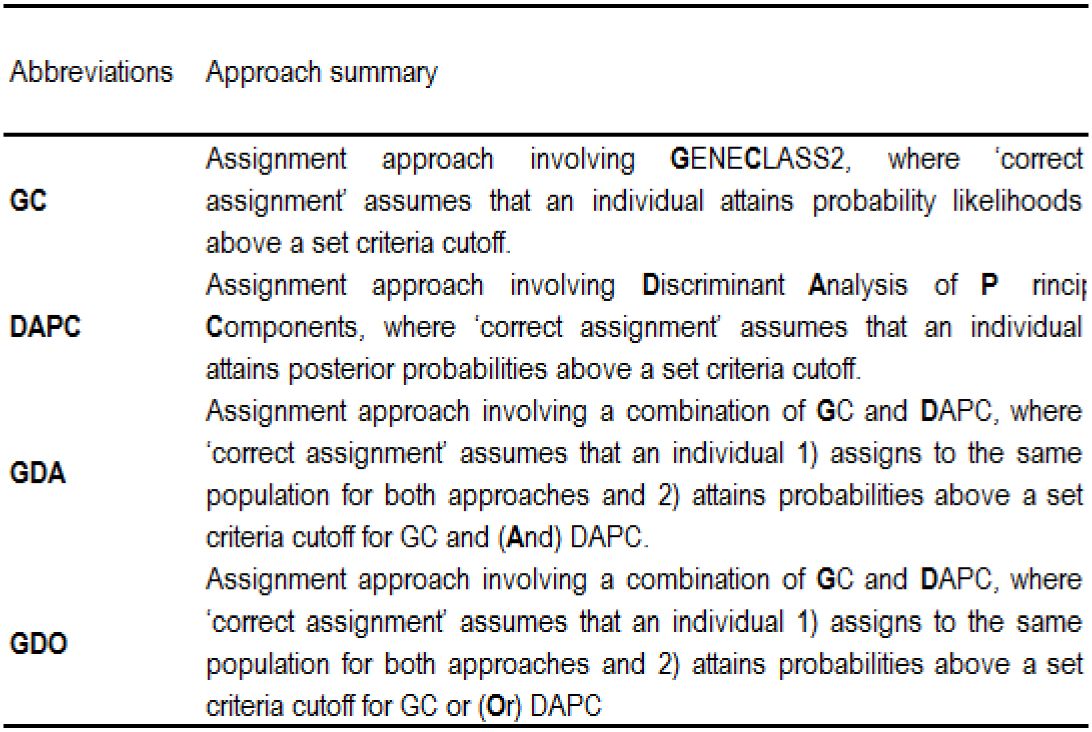


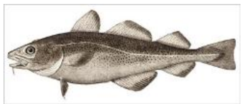
Table 1 Details for each genetic assignment approach and their abbreviations



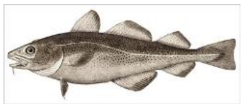
Abbreviations	Approach summary
GC	Assignment approach involving **G**ENE**C**LASS2, where 'correct assignment' assumes that an individual attains probability likelihoods above a set criteria cutoff.
DAPC	Assignment approach involving **D**iscriminant **A**nalysis of **P** rinci **C**omponents, where 'correct assignment' assumes that an individual attains posterior probabilities above a set criteria cutoff.
GDA	Assignment approach involving a combination of **G**C and **D**APC, where 'correct assignment' assumes that an individual 1) assigns to the same population for both approaches and 2) attains probabilities above a set criteria cutoff for GC and (**A**nd) DAPC.
GDO	Assignment approach involving a combination of **G**C and **D**APC, where 'correct assignment' assumes that an individual 1) assigns to the same population for both approaches and 2) attains probabilities above a set criteria cutoff for GC or (**O**r) DAPC

Mixed-stock analysis

In order to compare relative contributions associated with the different IA approaches, mixed-stock analysis (MSA) was applied. MSA represents a statistical approach especially suited for estimating relative contributions of different population (Koljonen et al., 2005). An approach based maximum-likelihood (Millar, 1987) was used, implemented in the program *ONCOR* (S. Kalinowski, www.montana.edu/kalinowski/Software/ *ONCOR*. htm). In short, *ONCOR* require baseline information of allele frequencies for all populations and hereby estimates mixed-stock proportions. It estimates the stock composition using a conditional maximum likelihood approach, which applies Rannala & Mountain's (1997) methods for defining the accuracy level for which genetic markers are most suitable for estimating a stock based on baseline information. After obtaining empirical contribution estimates, the performance and accuracy of MSA was tested through simulation analysis in two ways. Firstly, a so-called '100% simulation' was performed. The method simulates a 'fishery sample' in which all the individuals are from the baseline, where 100 individuals is drawn with replacement and subsequently pooled to form a single sample. This



was then repeated 1000 times for each population and MSA was performed for each simulated sample sequentially. Secondly, a more realistic scenario was simulated using different management scenarios (See Results section; Comparisons between MSA and IA). Here, simulations that match empirical sample sizes of each of four management scenarios was performed by sampling alleles, with replacements, from proportions obtained from IA. Finally, "Three-way error decomposition" was performed, which assesses error rates associated with MSA from three error sources: baseline genotypes (size), where larger error is associated with lower number of genotypes; similarity of allele frequencies in the baseline, where error rates is associated with lower differentiation between populations; and mixed-stock sample size, where errors ra associated with lower numbers. All analysis for accuracy of MSA was conducted in *ONCOR*.

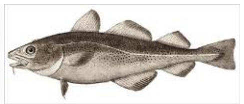


Results

Assessment of the assignment variability between panels

The training-set/holdout-set protocol showed significantly different assignment between SNP panels for the two genetic assignment packages. In general improved assignment was observed for the updated 96 loci panel compared to the previous 81 loci panel, assigning ten randomly selected hold-out sets back to populations of origin. For simplicity, these panels are in this section referred to as the 'New panel' and the 'Old panel'. The improvement in assignment was observed by applying conservative criteria (i.e. probability cutoff >0.9) for the two genetic software packages. The mean number of successfully assigned individuals from the ten assignment trials was significantly higher (Student's t-test; $p < 0.000$) for the New panel using GC. Accordingly, on average 90.0% (±0.8SEM; SEM is the standard error of the mean) of all individuals were correctly assigned with the New panel compared to the Old panel which averaged 84.9% (±0.8SEM) correct assignment (Fig. 4). Although, no significant difference between panels was detected for DAPC, correct assignment was still higher for the New panel, which successfully assigned more individuals 7 out of 10 times.

The misassignment of individuals (i.e. assigned to another population than the population of origin) was also significantly improved (Student's t-test; $p = 0.006$) for GC. Accordingly, the New panel assigned fewer individuals (6.7%±0.5SEM) under the set criteria compared to the Old panel (5.3%±0.5SEM). Again, for DAPC the misassignment was not significantly improved with the New panel. No significant difference was observed for non-assigned individuals (below set thresholds) for both DAPC and GC. The absence of significant improvements was similar for all DAPC-related comparisons between panels. Nevertheless, improvements was still observed, where the New panel displayed fewer individuals with low assignment probabilities 9 out of 10 times for GC and 7 out of 10 times for DAPC. In view of these tests, the most recent panel that includes 96 SNPs provided enhanced performance in overall assignment, successfully allocating more individuals back to their correct population of origin, misassigning fewer individuals and reducing the proportion of individuals not assigning for the set threshold criteria.



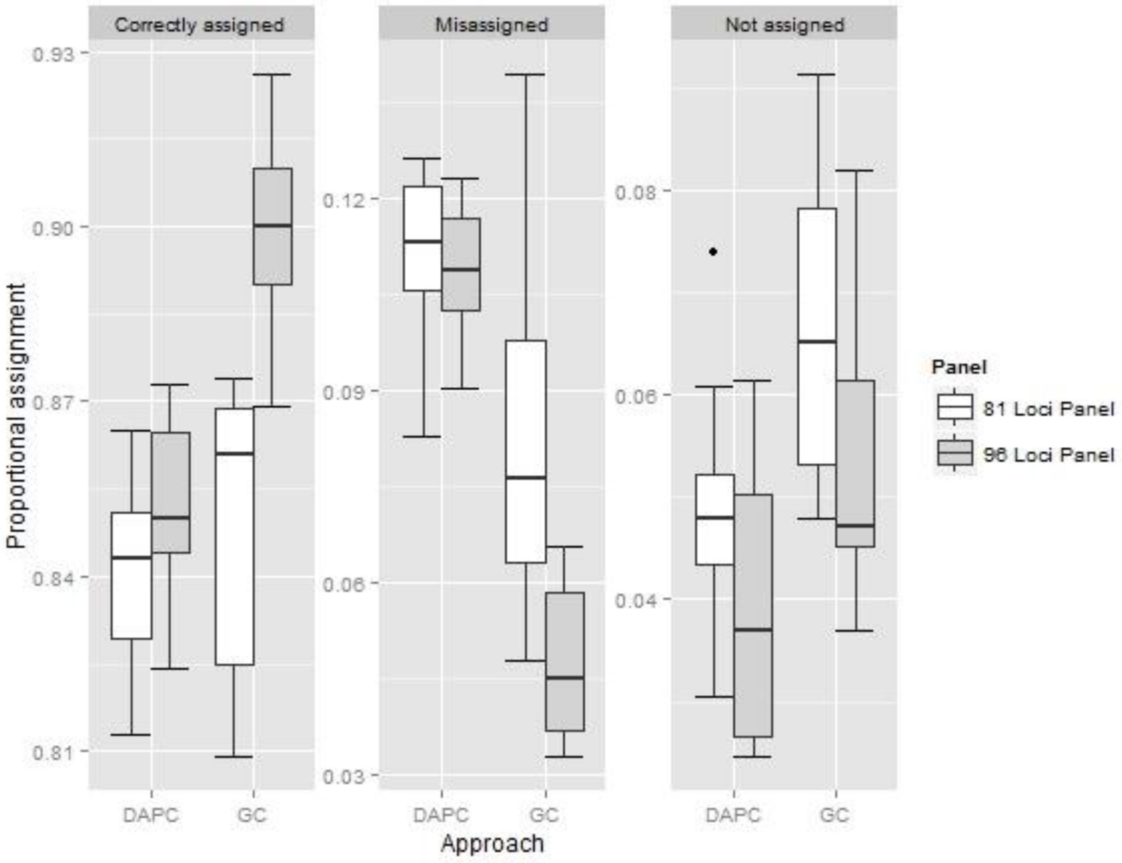
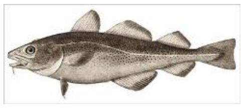


Figure 4 Box plots of the mean proportional assignment of Atlantic cod (*Gadus morhua*) for ten runs following Andersen et al.'s (2010) training/holdout protocol. Mean proportional assignment are shown for individuals that are correctly assigned (>0.9), not assigned (<0.9) and misassigned (i.e. assigned to another population than the population of origin) for two different SNP-panels (i.e. 96 loci panel and 81 loci panel) using two different genetic packages (i.e. GENECLASS2 and DAPC). The horizontal band in each box represents the median, the bottom and top of the boxes represent the 25th and 75th percentiles, and the error bars define the 5th and the 95th percentiles. Outlier data points are marked by dots.

In addition, the New informative panel also provided improved assignment at the level of individual populations. Assignment was significantly improved for Iceland Offshore (Student's t-test; p = 0.002), Iceland Inshore (Student's t-test; p = 0.007) and West Greenland Inshore (Student's t-test; p = 0.024) using GC. For DAPC, only Iceland Offshore (Student's t-test; p = 0.007) showed significantly better assignment



when using the New panel (Fig. 5). The New panel revealed improved assignment always attaining higher averages all comparisons between panels with the exception of assignment to West Greenland Inshore using DAPC.

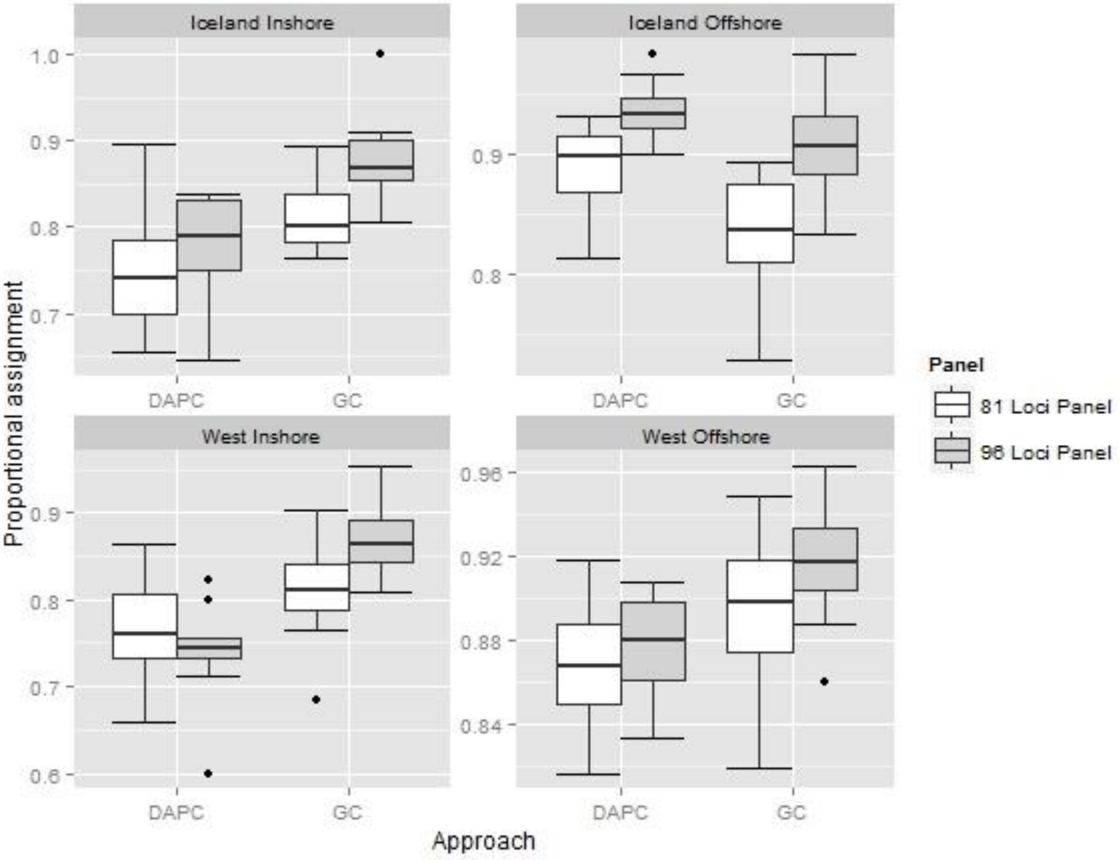
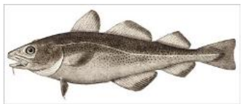


Figure 5 Box plots of the mean proportional assignment of Atlantic cod (*Gadus morhua*) for ten runs following Andersen et al.'s (2010) training/holdout protocol. Mean proportional assignment are shown for individuals that are correctly assigned (>0.9) for two different SNP-panels (i.e. 96 loci panel and 81 loci panel) using two different genetic packages (i.e. GENECLASS2 and DAPC) to one of four populations. The horizontal band in each box represents the median, the bottom and top of the boxes represent the 25th and 75th percentiles, and the error bars define the 5th and the 95th percentiles. Outlier data points are marked by dots.

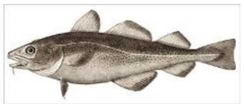


Assignment of empirical samples; variability between software packages

Overall individual assignment produced different outcomes depending on which genetic software package and associated assignment method applied for IA. In general, more conservative methods involving stringent cutoffs reduced the proportion of individuals assigned. However, the proportional contributions among assigned individuals of each population only changed marginally within each software package.

GC positively assigned more individuals compared to DAPC for all the criteria with a set cutoff.The proportion of individuals successfully assigned to populations under each criterion ranged from 81.6% to 99.4% for GC (404 to 492 of 495) and from 75.5% to 98.4% for DAPC (378 to 483 of 495) (Fig. 6). Consequently, the individual proportions that were not assigned over the array of different cutoffs reached maximums of 18.4% (91 of 495) for GC and 23.4% (117 of 495) for DAPC.

The most pronounced difference between packages was revealed in the proportions assigned back to the previously defined baseline populations. Briefly, for equivalent thresholds assignment was always higher for GC t to West Greenland Inshore, and DAPC assigned more individuals to Iceland Offshore.. In detail, GC assigned on average 43.0% to the West Greenland Inshore component, whereas DAPC assigned substantially less averaging 32.5%. In contrast, DAPC allocated on average 30.4% of to the Iceland Offshore population, whereas GC only assigned 23.9% on average. Furthermore, DAPC on average assigned marginally more individuals o (8.9%) to Iceland Inshore compared to GC (8.3%). Assignment to West Greenland Offshore had similar assignment rates for both GC and DAPC for all criteria averaging 18.3% and 18.0%, respectively.



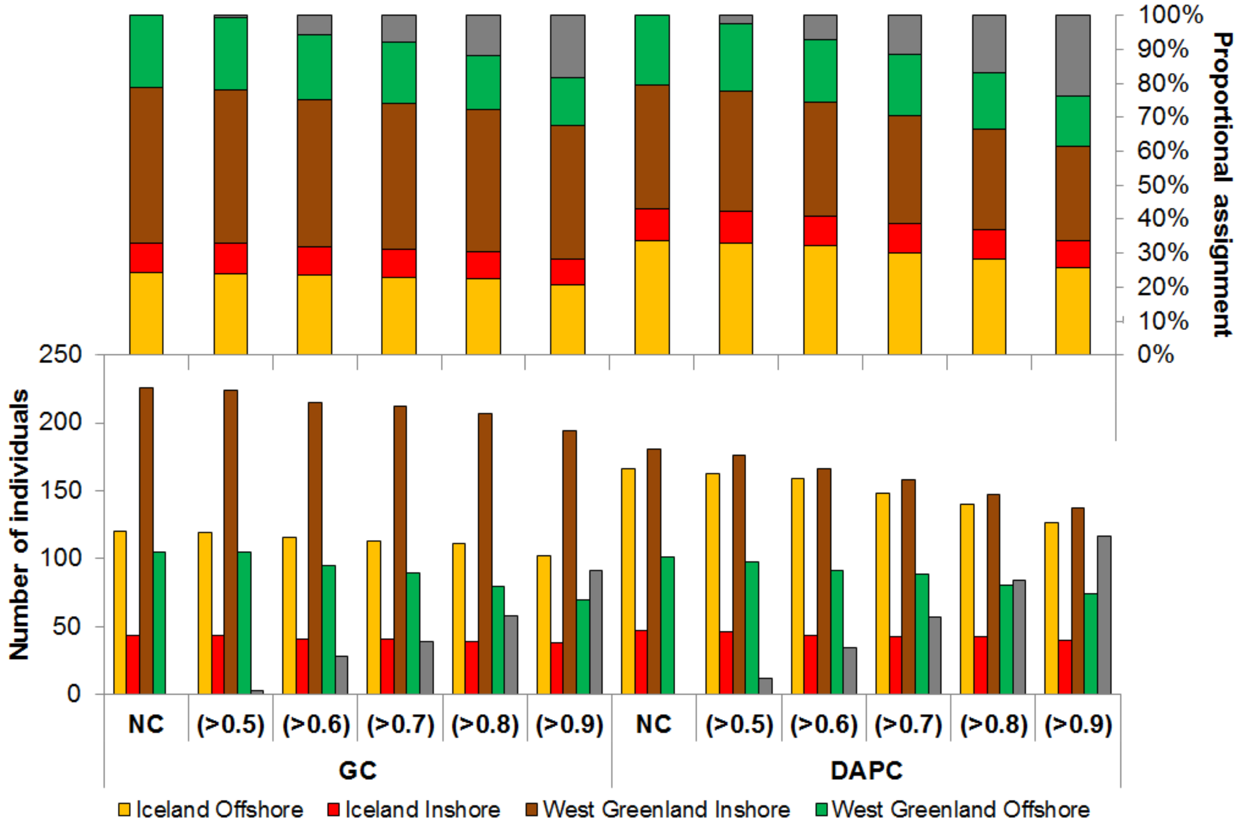
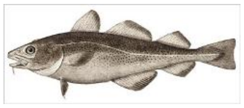


Figure 6 Individual assignment of Atlantic cod (*Gadus morhua*) from West Greenland involving two genetic software packages (i.e. GENECLASS2 and DAPC). Clustered columns show the number of individuals that are not assigned for different set criteria cutoffs (grey) and individuals correctly assigned to one of four populations (Iceland Offshore, Iceland Inshore, West Greenland Inshore and West Greenland Offshore). Stacked columns show the proportional assignment.



A similar pattern as seen for the total assignment was detected for the proportional assignment considering only assigned individuals. A consistent pattern revealing pronounced differences between software packages for assignment to West Greenland Inshore and Iceland Offshore was apparent. The exclusion of individuals falling below the different cutoff criteria ranged from 0.6% to 18.4% for GC and from 2.4% to 23.6% for DAPC. The results for only the positively assigned individuals returned an overall less variable output for the proportional contributions to populations within each package (Fig. 7). Still higher average assignment (46.5%) was attained for West Greenland Inshore for GC compared DAPC (36.2%). Mean assignment to Iceland Offshore produced the opposite patterb with DAPC assigning 33.8% compared to 24.8% for GC. Once again, DAPC also assigned slightly more individuals to Iceland Inshore compared to GC (9,9% versus 9.0%). The mean proportional contributions for assigned individuals to West Greenland Offshore were very similar for DAPC (20.0%) and for GC (19.7%). There was no effect of sampling site on the variability between packages (See Appendix, Fig. S1).

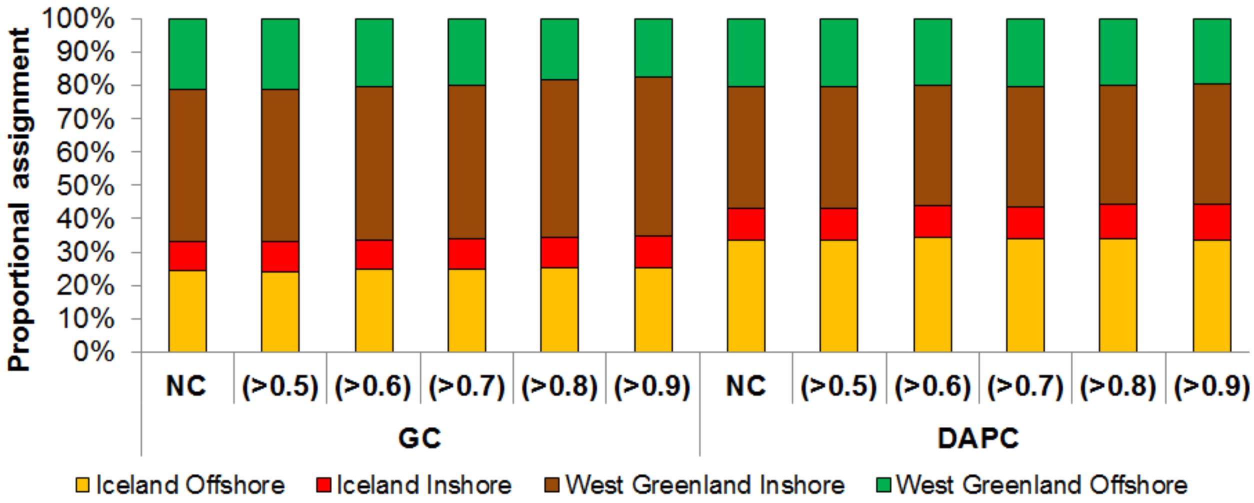
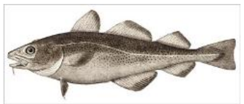


Figure 7 Individual assignment of Atlantic cod (*Gadus morhua*) from West Greenland based on different approaches involving two genetic software packages (i.e. GENECLASS2 and DAPC). Stacked columns show the proportional assignment of individuals that are correctly assigned to one of four populations (Iceland Offshore, Iceland Inshore, West Greenland Inshore and West Greenland Offshore) for different set criteria cutoffs.

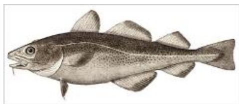


Assignment of empirical samples; approaches involving combined genetic assignment methods

Assignment approaches involving a combination of the genetic assignment methods (i.e. GDA and GDO) revealed differences between approaches in the proportion of individuals assigned to populations. Whereas GDO was relatively insensitive to increased stringency in the criteria, the number of individuals assigned with GDA was significantly reduced as the stringency was increased.

The mean assignment of GDO across criteria was 69.4%, while GDA assigned 63.3%. The exclusion of individuals that achieved probabilities too low for successful assignment ranged from 30.0 to 46.1% for GDA (148 to 228 of 495) and 29.9 to 32.7% for GDO (148-162 of 495) (Fig. 8).

The proportions of correctly assigned individuals followed the expectations that the lowest assignment power of 53.9% (267 of 495) was obtained for the most conservative approach (GDA; >0.9). Thus, the more stringent criteria for GDA (both method above the threshold) generally provided low proportions of assigned individuals , but proportions progressively increased with less rigorous probability cutoffs. Likewise, the contribution of each population increased marginally with less strict assignment thresholds for GDO. The average assignment to all populations was always lower for GDO in all comparisons with corresponding criteria.



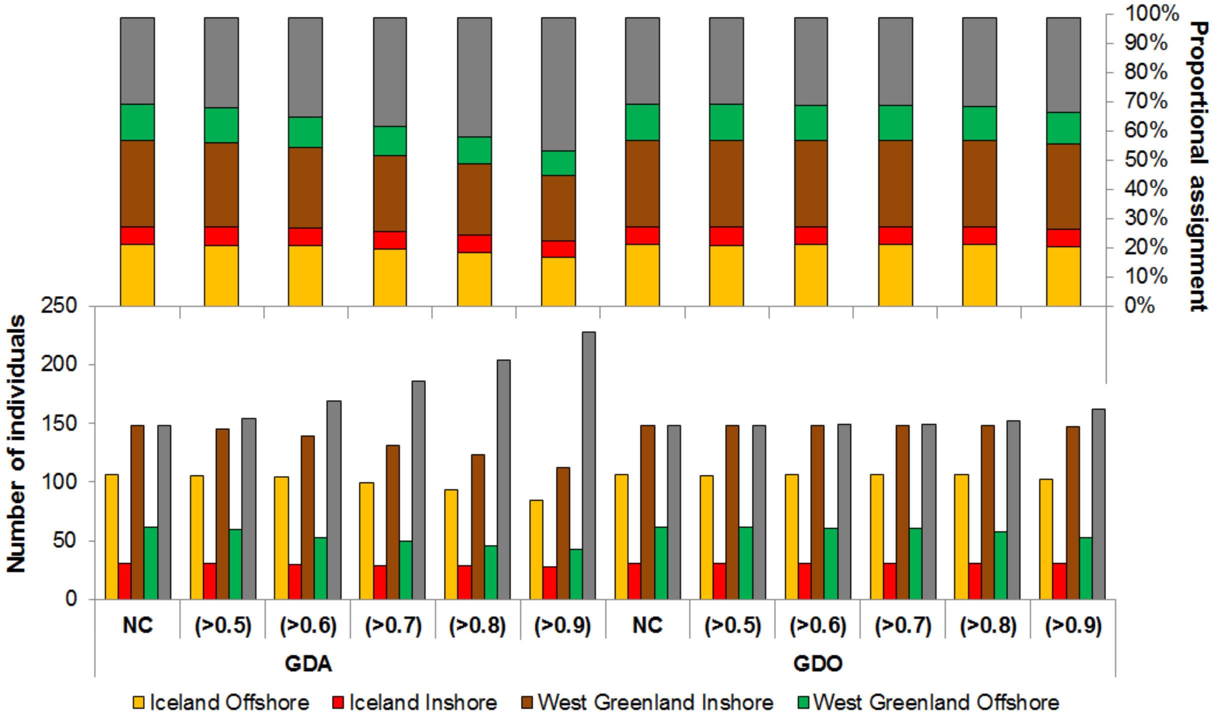
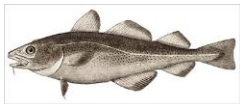


Figure 8 Individual assignment of Atlantic cod (*Gadus morhua*) from West Greenland based on two different approaches involving a combination of two genetic software packages (i.e. GENECLASS2 and DAPC). Clustered columns show the number of individuals that are not assigned for different set criteria cutoffs (grey) and correctly assigned to one of four populations (Iceland Offshore, Iceland Inshore West Greenland Offshore West Greenland Inshore). Stacked columns show the proportional value for assignment.

The proportional assignment, considering only positively assigned individuals, differed from the overall assignment proportions (i.e. clear differences between GDA and GDO, Fig. 8). Here, the proportional relationships only varied marginally across criteria and between methods (Fig. 9). GDA attained marginally higher average assignment for both Icelandic populations, whereas GDO showed marginally higher average assignment for Greenlandic populations. In detail, Icelandic mean proportions for offshore and inshore individuals, respectively, comprised 31.4% and 9.5% for GDA and 30.6% and 9.0% for GDO. Greenlandic mean proportions offshore and inshore individuals, respectively, comprised 16.6% and 42.2% for GDA and 43.4% and 17.3% for GDO. It is important to be aware that the proportional estimates are associated with pronounced variability in individual numbers that are positively assigned under the various



criteria, ranging from 267 to 347 individuals for GDA and 333 to 347 individuals for GDO. As for the comparison of the genetic packages, there was no effect of sampling site on the variability between approaches (See Appendix, Fig. S2).

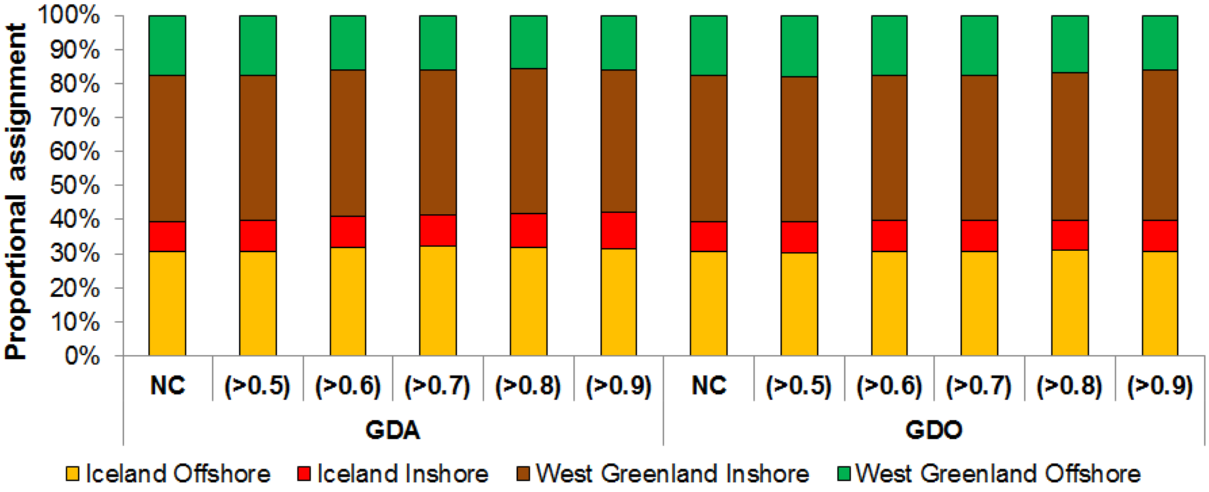
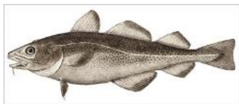


Figure 9 Individual assignment of Atlantic cod (*Gadus morhua*) from West Greenland based on different approaches involving a combination of two genetic software packages (i.e. GENECLASS2 and DAPC). Stacked columns show the proportional assignment of individuals that are positively assigned to one of four populations (Iceland Offshore, Iceland Inshore West Greenland Offshore West Greenland Inshore) for different set criteria cutoffs.

Variability between all IA approaches

The overall proportion of individuals positively assigned for the range of set criteria was significantly different for the four IA approaches (ANOVA; p > 0.000), and post hoc test revealed that approaches involving a combination of the two assignment methods differed from approaches with only one method (Fig. 10). The highest mean assignment rate was returned by GC (92.6%±2.8%SE) and DAPC (89.7%±3.7%SE), which also both displayed the largest variability across criteria. GDA representing the most rigorous and conservative approach attained the lowest mean assignment rate (63.3%±2.5%SE), and the variability in assignment rate was much higher compared to the related combined-method GDO (69.4%±0.4%SE) and significantly different.



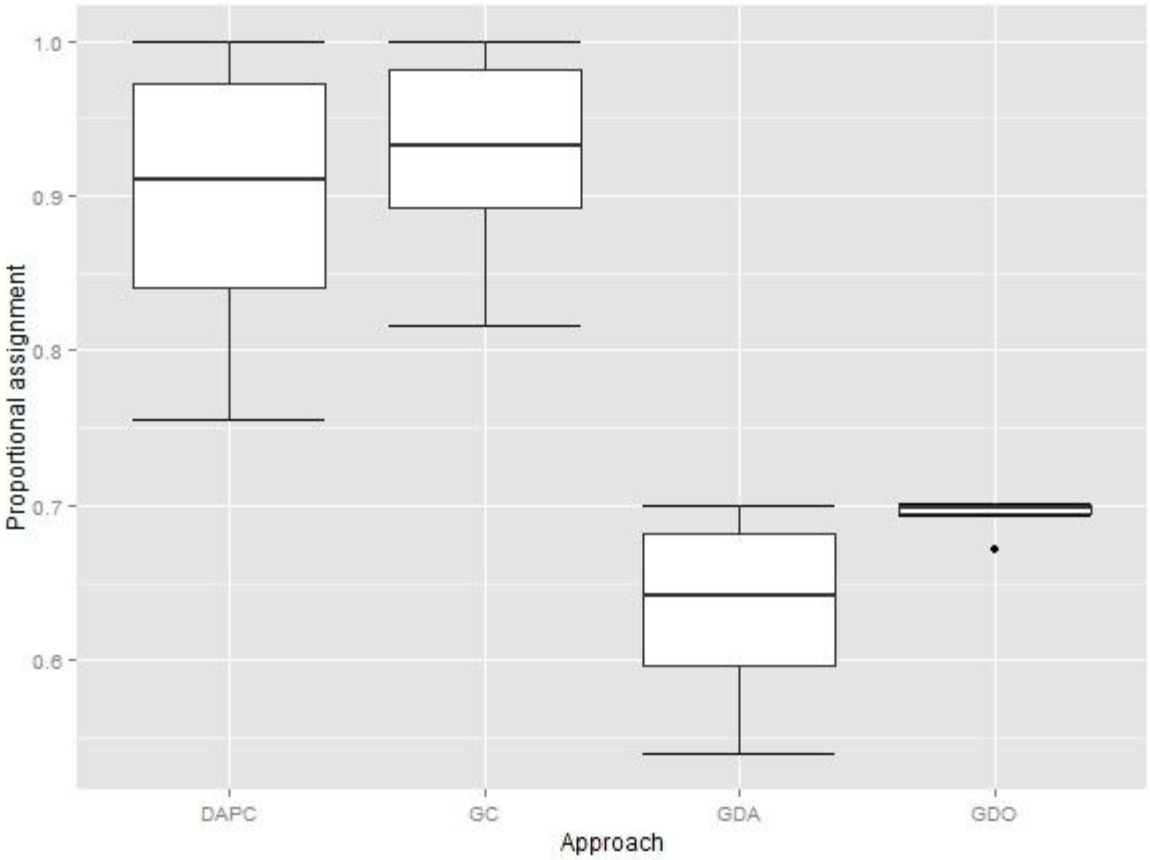
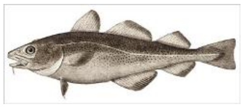
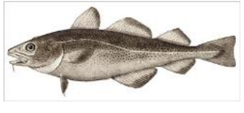


Figure 10 Box plots of the proportional assignment of Atlantic cod (*Gadus morhua*) for four different genetic assignment approaches (DAPC, GC, GDA and GDO) involving a single or a combination of two genetic software packages (GENECLASS2 and DAPC). Mean proportional assignments are shown for individuals that are positively assigned for range of set criteria cutoffs. The horizontal band in each box represents the median, the bottom and top of the boxes represent the 25th and 75th percentiles, and the error bars define the 5th and the 95th percentiles. Outlier data points are marked by dots



Likewise, the average proportional assignment for positively assigned individuals varied among approaches and significant difference were found for all populations (ANOVA; $p < 0.000$) (Fig. 11). The overall trends seen for the two genetic software packages were clearly also reflected in the average assignment back to individual populations. For the Iceland Offshore component, DAPC and GC significantly differed from the combined approaches (GDA and GDO) (Tukey's HSD; $p < 0.000$) with DAPC assigning significantly more and GC significantly less individuals to Iceland Offshore. . In contrast, GC assigned significantly more individuals to West Greenland Inshore, whereas DAPC assigned significantly fewer compared to all other approaches for this population (Tukey's HSD; $p < 0.000$). In addition, DAPC assigned significantly more individuals to Iceland Inshore compared to GC (Tukey's HSD; $p = 0.002$) and GDO (Tukey's HSD; $p = 0.002$; $p = 0.003$). Assignment to West Greenland Offshore were significantly lower for the two combined approaches compared to approaches utilizing a single-method (Tukey's HSD; GDO-GC, $p = 0.002$; all other significant comparisons, $p < 0.000$). Noticeably, no significant differences were found between GDA and GDO for any of the populations.



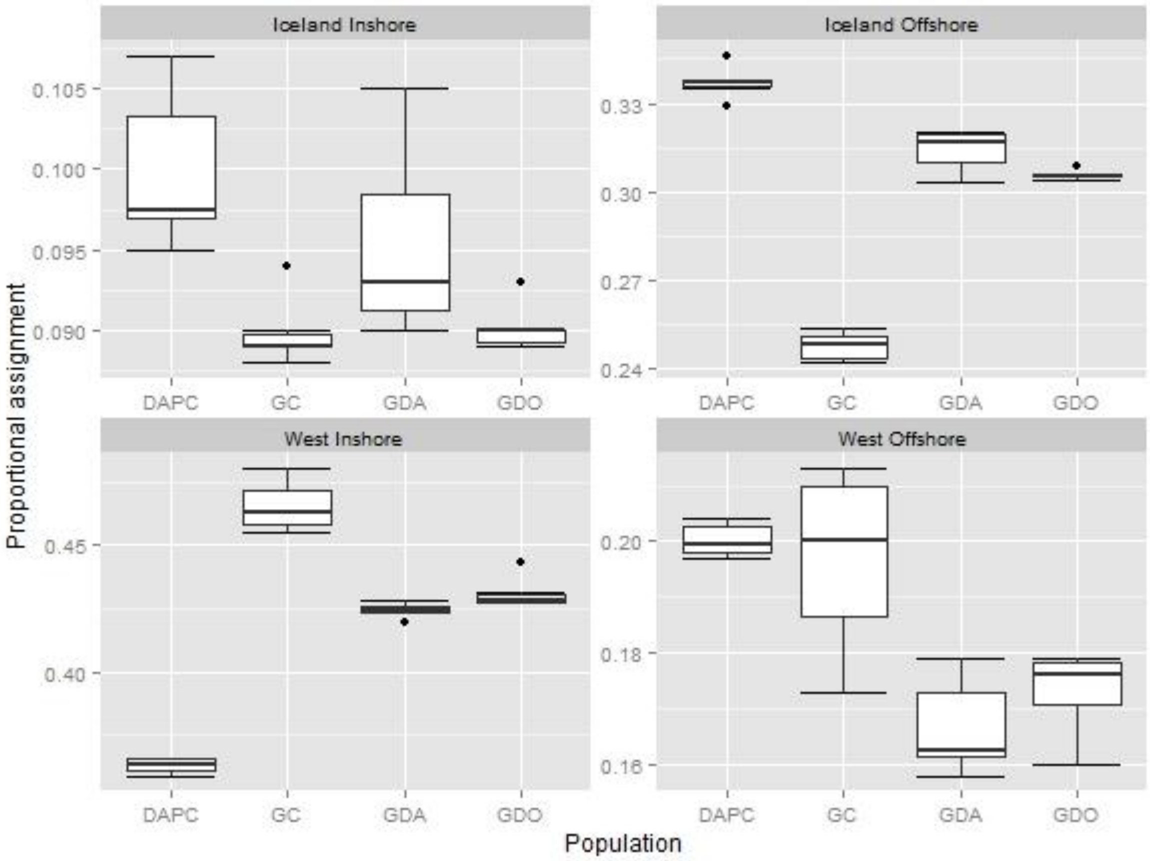
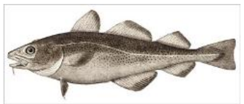


Figure 11 Box plots of the mean proportional assignment of Atlantic cod (*Gadus morhua*) for four different genetic assignment approaches (DAPC, GC, GDA and GDO) involving a single or a combination of two genetic software packages (GENECLASS2 and DAPC). Mean proportional assignment is shown for individuals that are correctly assigned for range of set criteria cutoffs to one of four populations (Iceland Offshore, Iceland Inshore West Greenland Offshore West Greenland Inshore). The horizontal band in each box represents the median, the bottom and top of the boxes represent the 25th and 75th percentiles, and the error bars define the 5th and the 95th percentiles. Outlier data points are marked by dots



Comparisons between MSA and IA

MSA results provided estimates of mixture contributions that generally were similar to the proportions attained with GC, but different from DAPC (Table 2 & 3). The combined IA approaches returned mixture contributions that were less similar to MSA than to GC, but most different from DAPC. The mean difference between MSA and summed IA approaches varied with each population. The most pronounced difference between MSA and IA was observed for DAPC, with estimates of proportions diverging 10.9% for Iceland Offshore and 13.9% for West Greenland Inshore on average. GDA and GDO both returned similar mean percentage differences in estimated proportions to MSA, averaging 6.9% and 6.5% for Iceland Offshore, and 8.4% and 7.2% for West Greenland Inshore. For West Greenland offshore the mean percentage difference was lower, averaging 6.3% for DAPC, 3.6% for GDA and 3.2% for GDO. The most similar estimates of proportions to MSA were observed for GC, where mixture contributions always attained the lowest average difference among all IA-approaches. Accordingly, the mean difference between MSA and GC was 1.7% for Iceland Offshore, 0.8% for Iceland Inshore, 2.3% for West Greenland Inshore and 1.7% for West Greenland Offshore. The difference between IA approaches and MSA was not pronounced for Iceland Inshore. Overall confidence intervals of MSA correlated with sample size, thus providing narrow intervals for large sample sizes and wide intervals for small sample sizes. The '100% simulations' estimation accuracy for MSA was always high for all populations averaging >0.99 over 1000 populations (Appendix, Table S3). For a more realistic mixed stock simulation of different management scenarios, the simulated proportions were almost identical to the empirical input MSA proportions (Table 4). As for the empirical MSA contribution, confidence intervals were dependent on sample sizes. The 'Three-way error decomposition' analysis showed that uncertainty was unrelated to the size of mixed stock samples (Appendix, Table S4). The estimation error for Iceland Inshore was more or less equally distributed between the baseline sample size and genetic differentiation. For all other populations, error sources were mainly associated with baseline sample size.

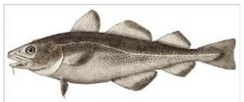


Table 2 Individual assignment of mixed-feeding aggregations showing numbers of Atlantic cod assigning to one of four populations. The proportional contributions (not including unassigned individuals) are given in parentheses. Assignment was performed with GENECLASS (GC) applying probability cutoff criteria >0.7. Numbers and percentages are also given for fish not assigned for criteria.

Approach	Region	Location	ID	Zone	Date	*n*	Iceland offshore	Iceland Inshore	WG Inshore	WG offshore	Not assigned
GC	Sisimiut	Tømmermandsøen	SI_01	Coastal	Aug-13	50	3 (6.8%)	10 (22.7%)	22 (50.0%)	9 (20.4%)	6 (12.0%)
		Amerloq	SI_02	Coastal	Aug-13	50	7 (16.3%)	6 (13.9%)	18 (41.9%)	12 (28.0%)	7 (14.0%)
	Total mixed stock			Coastal		100	10 (11.5%)	16 (18.4%)	40 (46.0%)	21 (24.4%)	13 (13.0%)
	Nuuk	Bukse Fjord	NU_01	Coastal	Aug/July-13	55	24 (45.3%)	3 (5.7%)	18 (34.0%)	8 (15.1%)	2 (3.6%)
		Præstefjord	NU_02	Coastal inlet	Aug-13	82	31 (40.2%)	10 (13.0%)	22 (28.6%)	14 (18.2%)	5 (6.1%)
		Hundeø	NU_03	Coastal	Aug-13	11	4 (40.0%)	1 (10.0%)	5 (50.0%)	0 (0.0%)	1 (9.1%)
		Sadelø	NU_04	Coastal	Aug-13	49	18 (38.3%)	8 (17.0%)	16 (34.0%)	5 (10.6%)	2 (4.8%)
		Coastal mixed stock		Coastal		197	77 (41.2%)	22 (11.8%)	61 (32.6%)	27 (14.4%)	10 (5.1%)
		Kobbefjord	NU_05	Fjord	Aug-13	8	3 (37.5%)	0 (0.0%)	3 (37.5%)	2 (25.0%)	0 (0.0%)
		Terte	NU_06	Fjord	Dec-13	78	4 (5.3%)	1 (1.3%)	62 (82.7%)	8 (10.7%)	3 (3.9%)
		Uummannaq	NU_07	Fjord	Nov-13	77	14 (21.2%)	2 (3.0%)	32 (48.5%)	18 (27.3%)	11 (14.3%)
		Kapisillit	NU_08	Fjord	Dec-13	35	5 (15.2%)	0 (0.0%)	14 (42.4%)	14 (42.4%)	2 (5.7 %)
		Fjord mixed stock		Fjord		198	26 (14.3%)	3 (1.6%)	111 (61.0%)	42 (23.1%)	16 (8.1%)
	Total mixed stock			Coastal/Fjord		395	103 (27.9%)	25 (6.8%)	172 (46.6%)	69 (18.7%)	26 (6.6%)

Table 3 Mixed stock analysis of mixed-feeding aggregations showing estimated percentage contributions of Atlantic cod to one of four populations. 95% bootstrap confidence intervals are given in parentheses.

Region	Location	ID	Zone	Date	*n*	Iceland offshore	Iceland Inshore	WG Inshore	WG offshore
Sisimiut	Tømmermandsøen	SI_1	Coastal	Aug-13	50	6.3% (0.0-15.1)	22.2% (12.9-39.5)	55.2% (38.5-70.3)	16.3% (3.9-25.4)
	Amerloq	SI_2	Coastal	Aug-13	50	19.0% (7.2-31.8)	13.0% (5.1-26.6)	40.4% (27.7-59.7)	27.6% (9.9-37.3)
Total mixed stock			Coastal		100	12.3% (5.5-20.1)	17.8% (12.4-29.3)	48.1% (37.6-60.5)	21.8% (9.9-27.5)
Nuuk	Bukse Fjord	NU_1	Coastal	Aug/July-13	55	42.3% (29.1-55.0)	5.4% (0.0-13.9)	36.1% (23.1-50.6)	16.2% (5.6-26.3)
	Præstefjord	NU_2	Coastal inlet	Aug-13	82	41.3% (30.4-52.2)	12.0% (5.9-20.8)	29.1% (19.6-42.4)	17.7% (4.8-24.3)
	Hundeø	NU_3	Coastal	Aug-13	11	36.4% (9.1-63.6)	9.1% (0.0-27.3)	54.5% (27.3-81.8)	0.0% (0.0-0.5)
	Sadelø	NU_4	Coastal	Aug-13	49	38.2% (23.7-52.4)	16.9% (7.7-3.3)	35.5% (20.7-50.9)	9.2% (0.0-17.1)
	Coastal mixed stock		Coastal		197	40.6% (33.1-46.7)	11.2% (7.5-17.2)	34.1% (28.2-42.9)	14.1% (6.7-17.4)
	Kobbefjord	NU_5	Fjord	Aug-13	8	37.01% (0.0-74.9)	0.0% (0.0-24.8)	37.6% (12.2-75.0)	25.4% (0.0-60.1)
	Terte	NU_6	Fjord	Dec-13	78	5.2% (0.1-11.2)	0.0% (0.0-2.8)	87.9% (80.8-97.2)	6.9% (0.0-12.4)
	Uummannaq	NU_7	Fjord	Nov-13	77	19.2% (10.8-31.1)	1.6% (0.0-9.5)	51.3% (38.9-68.0)	27.9% (10.3-36.5)
	Kapisillit	NU_8	Fjord	Dec-13	35	10.4% (0.0-25.3)	0.0% (0.0-5.5)	46.5% (29.3-67.3)	43.1% (20.8-57.0)
	Fjord mixed stock		Fjord		198	13.0% (8.1-18.9)	0.0% (0.0-4.7)	65.1% (57.9-75.7)	21.8% (11.5-25.9)
Total mixed stock			Coastal/Fjord		395	27.1% (22.7-32.1)	6.0% (4.3-10.5)	49.2% (44.1-57.3)	17.7% (10.2-20.5)

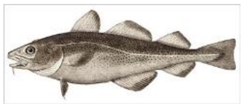
Table 4 Simulation of realistic management scenarios of mixed-feeding aggregations showing estimated percentage contributions of Atlantic cod to one of four populations. Numbers in bold show true numbers of estimated populations. 95% bootstrap confidence intervals are given in parentheses.

Region	**Scenario**	***n***	**Iceland offshore**	**Iceland Inshore**	**WG Inshore**	**WG offshore**
Sisimiut	Coastal mixed stock	100	**11.5**/11.5% (5.9-18.0)	**18.4**/18.4% (10.9-26.0)	**46.0**/46.0% (35.5-55.9)	**24.1**/24.2% (16.0-33.6)
Nuuk	Coastal mixed stock	163	**41.2**/41.0% (34.3-47.9)	**11.8**/11.8% (7.5-18.7)	**32.6**/32.6% (25.9-38.9)	**14.4**/14.5% (9.6-19.6)
	Fjord mixed stock	232	**14.1**/14.0% (9.6-19.2)	**1.6**/1.7 % (0.5-4.1)	**61.0**/61..0 % (53.0-66.5)	**23.1**/23.2 % (18.4-29.9)
	Coastal/fjord mixed stock	395	**27.9**/28.0.0% (23.5-32.5)	**6.8**/6.7 % (4.3-9.3)	**46.6**/46.6% (41.5-51.4)	**18.7**/18.7 % (14.6-22.5)

Proportional contribution of populations to mixed samples; Approach and results

Based on the previous tests on accuracy and reliability for estimation of proportional contributions provided by MSA , both MSA and the very similar proportions attained from the summed IA with GC could be selected as the most robust and accurate estimation approach for proportional estimation of inshore waters. Specifically, for GC using the probability >0.70 criteria the results for the two approaches are very similar. . Thus by using GC to estimate proportions, the maximum number of individuals would be positively assigned within a statistically (verified through MSA) robust assignment procedure for estimation of mixture contributions. MSA would be an equally robust tool for estimating proportions, but given that one of the goals also was to evaluate IA as a tool for mixed-stock studies, it is here preferred over MSA. GC successfully assigned 92.1% individuals (456 of 495) providing a high assignment rate for the estimation of proportional contributions by the different populations. For all individuals positively assigned, IA returned mixture compositions diverging only 1.7% on average from contributions provided from MSA.

Spatial differences were detected and a clear regional pattern was revealed, where the 'Sisimiut region' (Fig. 13) seemed to have a different composition and associated contributions of different populations compared to the 'Nuuk region' (Fig. 12; for comparisons with MSA contributions, see Appendix, Fig. S3). The samples from Sisimiut were dominated by a large contribution from the West Greenland Inshore population (46.0%) comprising the majority of individuals. A substantial contribution representing roughly a quarter for West Greenland offshore (24.4%) was also apparent together with a considerable contribution of Iceland Inshore (18.4%). Iceland Offshore only comprised a small fraction (11.5%). Similarly, the Nuuk mixed stock predominantly comprised a large contribution of West Greenland Inshore (46.6%). In

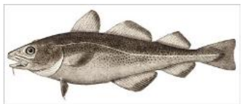


contrast to the Sisimiut region Iceland Offshore contributed with 33.9%, whereas smaller proportions of West Greenland offshore (18.7%) and Iceland (6.8%) were also present. In contrast to Sisimiut the Iceland Inshore population generally contributed very little in the Nuuk region.

While the regional differences were clear for the total mixed stocks, a pattern which became even more apparent when, only comparing the coastal samples in Sisimiut with equivalent coastal samples from Nuuk (Table 2). The Nuuk coastal mixed stock samples returned an even more pronounced spatial pattern shifting proportional assignments to comprise predominately to the Iceland Offshore population (41.2%) and the West Greenland Inshore population attaining much smaller assignment rates (32.6%).

Locally, in particular for Nuuk sites, small-scale spatiogenetic patterns was apparent with assemblages around coastal inshore waters and the mouths of fjords comprising genetic compositions that differed from population compositions observed within the fjords.

In general, comparing potential fishery management scenarios in the Nuuk area the contribution of Iceland Offshore populations was much more pronounced around the mouth of the fjords towards coastal zones, whereas large contributions of West Greenland Inshore individuals was observed further into the fjord system. Presented above in comparison with the Sisimiut region, the coastal mixed stock in the Nuuk region had higher contributions of Iceland Offshore compared to West Greenland Inshore. West Greenland Offshore (14.4%) and Iceland Inshore (11.8%) contributed smaller proportions. In contrast, the fjord mixed stocks was dominated by West Greenland Inshore (61.0%), whereas West Offshore (23.1%) had considerable proportions and Iceland Offshore (14.3%) had smaller comparable contributions. Iceland Inshore was generally absent within the fjords in Nuuk which was in contrast to coastal samples.



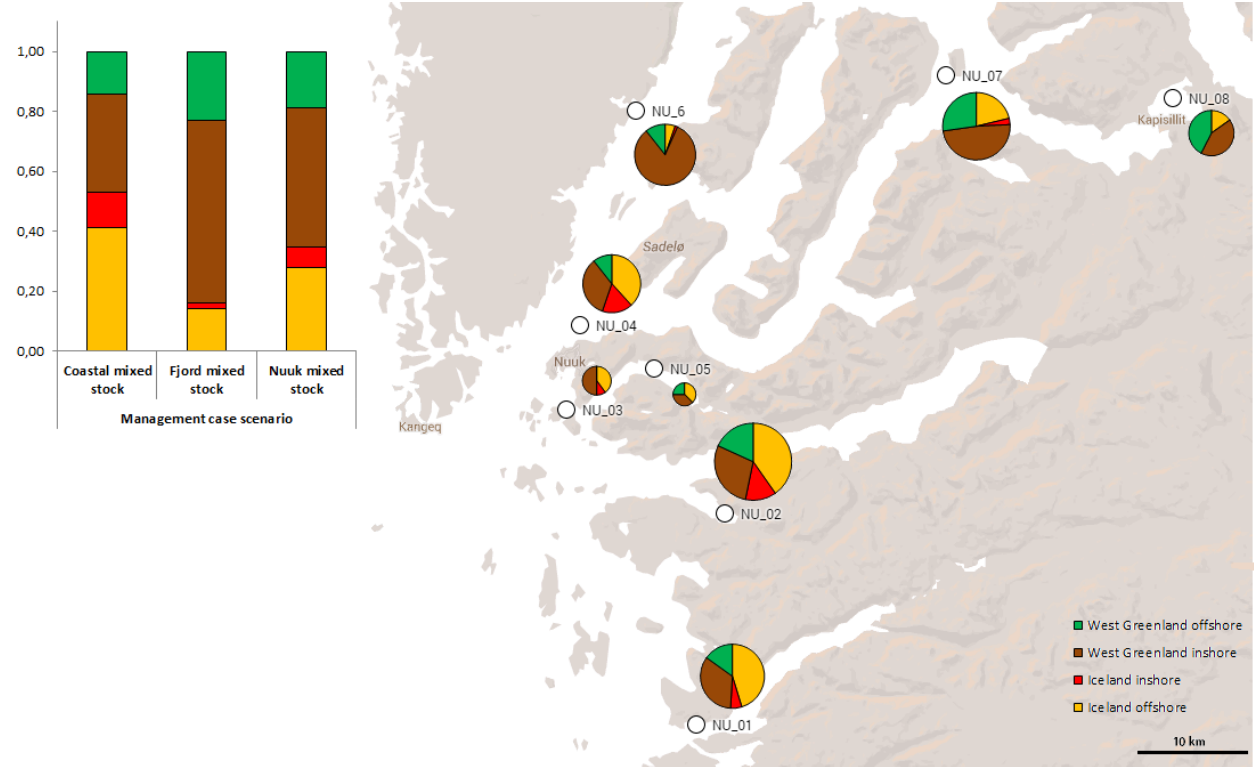


Figure 12 Individual assignment (GC; >0.7) of Atlantic cod (*Gadus morhua*) from the inshore waters around Nuuk. The stacked columns show overall individuals that are correctly assigned to one of four baseline populations (Iceland Offshore, Iceland Inshore, West Greenland inshore and West Greenland offshore) for different proposed management scenarios. The pie charts display the proportional contribution of each population at eight sites. The size of each pie signifies sample sizes.

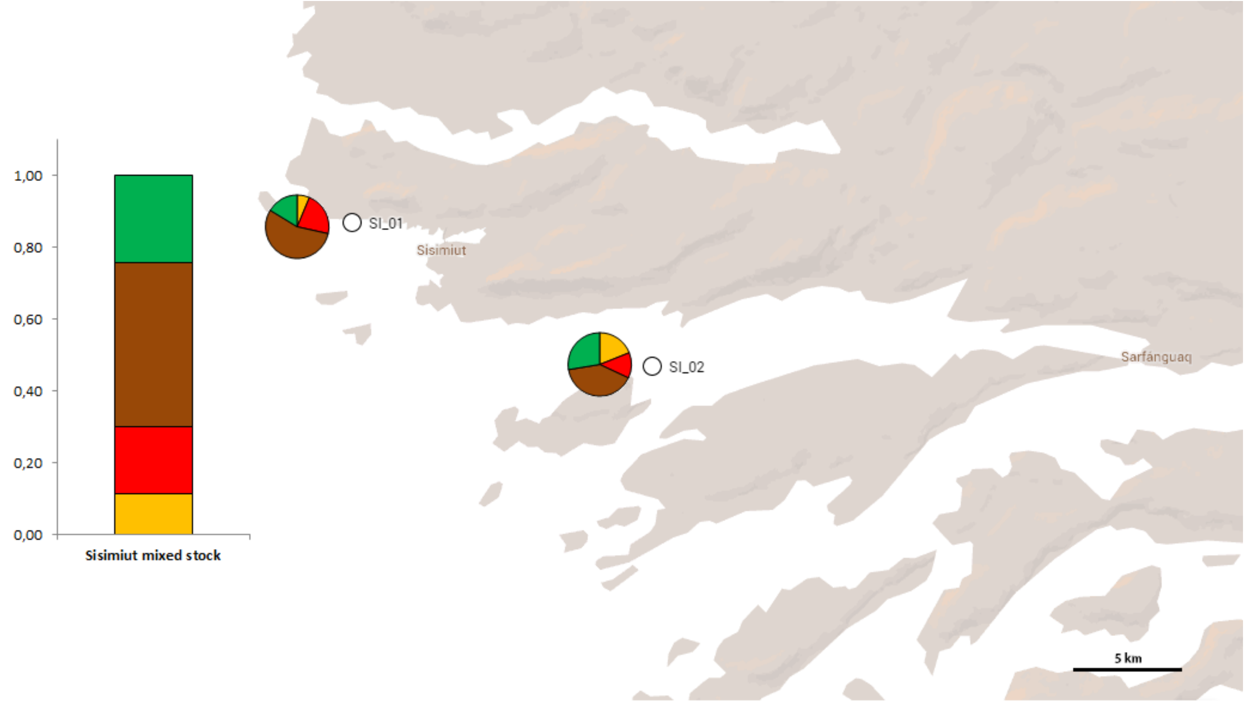


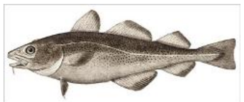
Figure 13 Individual assignment (GC; >0.7) of Atlantic cod (*Gadus morhua*) from the inshore waters around Sisimiut. The stacked column show overall individuals that are correctly assigned to one of four baseline populations (Iceland Offshore, Iceland Inshore West Greenland Offshore West Greenland Inshor). The pie charts display the proportional contribution of each population at two sites. The size of each pie signifies sample sizes.

Individual assignment of inshore mixed samples; Approach and results

Based on the evidence, that the different approaches returned similar proportional contributions to populations, and being aware of pronounced differences in assignment between packages, a conservative and precautionary GDO approach was selected for the assignment of individuals. Specifically, GDO (>0.7) was chosen for assignment, where positive assignment of an individual was attained when it was assigned to the same population with both GC and DAPC and a probability >0.70 for at least one of the methods. Thereby, the maximum number of individuals was assigned within a statistical robust assignment procedure at the level of the individual. GDO successfully assigned 69.9% individuals (346 of 495) providing a relatively high assignment rate. The majority of all individuals that was positively assigned included large contributions from the West Greenland Inshore (42.8% or 148 of 346) and Iceland Offshore (30.6% or 106 of 346) components. Individuals assigning to West Greenland Offshore (17.6% or 61 of 346) also contributed a significant proportion, whereas assignment to Iceland Inshore (9.0% or 31 of 346) provided the smallest proportion among assigned individual fish.

Fish not assigning because of low probabilities attained similar proportions to the total of 'unassigned' individuals (30.1%). Generally unassigned fish was distributed equally among sites averaging 34.0% and 27.3% for Sisimiut (Fig. 15) and Nuuk (Fig. 14) respectively, although, two sites, SI_01 (40.0%) and NU_06 (46.7%), was associated with noticeably high numbers of unassigned individuals. NU_05 (37.4%) also showed a low assignment rate, but here sample size were very small. 148 out of the 149 individuals was not assigned because they attained the highest posterior membership for different populations with each of the two methods.

Focusing solely on assigned individuals (Table 5), clear differences in origin assignment were detected across sites, both between the regions of Nuuk and Sisimiut, and among sampling sites in the Nuuk region. The overall regional and local pattern of proportional contributions returned by MSA and GC was similar to GDO. Briefly, summarizing the most important differences, both regions returned high IA proportions for the West Greenland Inshore population, comprising 39.4% (26 out of 66) for Sisimiut and 43.6% (122 out of 280) for Nuuk. Again, the main regional difference was observed in the assignment of the Iceland Offshore. The region around Nuuk assigned considerable amounts of individuals to Iceland Offshore (33.9% or 95 out of 280), whereas in Sisimiut they comprised a small fraction of population contributions (16.7% or 11 out of 66). Both regions have large proportions of West Greenland Offshore,

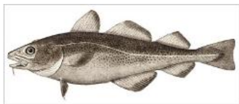


where Nuuk assigned more (43.6% or 122 out of 280) than in Sisimiut (39.4% or 26 out of 66). Furthermore on a regional scale, Iceland Inshore showed a surprisingly high assignment in Sisimiut (19.7% or 13 out of 66) compared to Nuuk (6.4%, or 18 out of 280). Splitting the Nuuk region into zones, when only considering coastal sites, assignment returned mixed stocks of individuals predominately assigned to Iceland Offshore (48.1% or 76 out of 158). This was in strong contrast to contributions observed regionally in Sisimiut, and locally in the fjords (15.6% or 19 out of 122). In the fjord mixed stock, proportions of West Greenland Inshore was very high (66.4% or 81 out of 122) compared to coastal mixed stocks (25.9% or 41 out of 158).

In summary, individual assignment was very similar proportionally to MSA and GC, but was associated with high numbers of unassigned individuals in each region, where 34 out of 100 individuals and 115 out of 395 individuals was not assigned. No obvious geographical pattern was detected for these individuals. Nevertheless, high statistical power is associated with the 346 individuals assigned individuals in total.

Table 5 Individual assignment of mixed-feeding aggregations showing numbers of Atlantic cod assigning to one of four populations. The proportional contributions (not including unassigned individuals) are given in parentheses. Assignment approach involved a combination of two genetic software packages (i.e. GENECLASS2 and DAPC) applying cutoff criteria >0.7.Numbers and percentages are also given for fish not assigned for criteria.

Approach	Region	Location	ID	Zone	Date	*n*	Iceland offshore	Iceland Inshore	WG Inshore	WG offshore	Not assigned
GDO	Sisimiut	Tømmermandsøen	SI_01	Coastal	Aug-13	50	3 (10.0%)	8 (26.7%)	13 (43.3%)	6 (20.0%)	20 (40.0%)
		Amerloq	SI_02	Coastal	Aug-13	50	8 (22.2%)	5 (13.9%)	13 (36.1%)	10 (27.8%)	14 (28.0%)
	Total mixed stock			Coastal		100	11 (16.7%)	13 (19.7%)	26 (39.4%)	16 (24.2%)	34 (34.0%)
	Nuuk	Bukse Fjord	NU_01	Coastal	Aug/July-13	55	29 (54.7%)	3 (0.6%)	14 (30.4%)	7 (13.2%)	7 (12.7%)
		Præstefjord	NU_02	Coastal inlet	Aug-13	82	30 (46.2%)	8 (12.3%)	14 (21.5%)	13 (20.0%)	17 (20.7%)
		Hundeø	NU_03	Coastal	Aug-13	11	4 (50.0%)	1 (12.5%)	3 (37.5%)	0 (0.0%)	3 (27.3%)
		Sadelø	NU_04	Coastal	Aug-13	49	18 (48.7%)	6 (16.2%)	10 (27.0%)	3 (8.1%)	12 (24.9%)
		Coastal mixed stock		Coastal		197	76 (48.1%)	18 (11.4%)	41 (25.9%)	23 (14.6%)	39 (19.8%)
		Kobbefjord	NU_05	Fjord	Aug-13	8	2 (40.0%)	0 (0.0%)	2 (40.0%)	1 (20.0%)	3 (37.5%)
		Terte	NU_06	Fjord	Dec-13	78	3 (5.4%)	0 (0.0%)	47 (83.9%)	6 (10.7%)	22 (28.2%)
		Uummannaq	NU_07	Fjord	Nov-13	77	13 (31.7%)	0 (0.0%)	20 (48.8%)	8 (19.5%)	36 (46.7%)
		Kapisillit	NU_08	Fjord	Dec-13	35	1 (5.0%)	0 (0.0%)	12 (60.0%)	7 (35.0%)	15 (24.5%)
		Fjord mixed stock		Fjord		198	19 (15.6%)	0 (0.0%)	81 (66.4%)	22 (18.0%)	76 (38.4%)
	Total mixed stock			Coastal/Fjord		395	95 (33.9%)	18 (6.4%)	122 (43.6%)	45 (16.1%)	115 (29.1%)



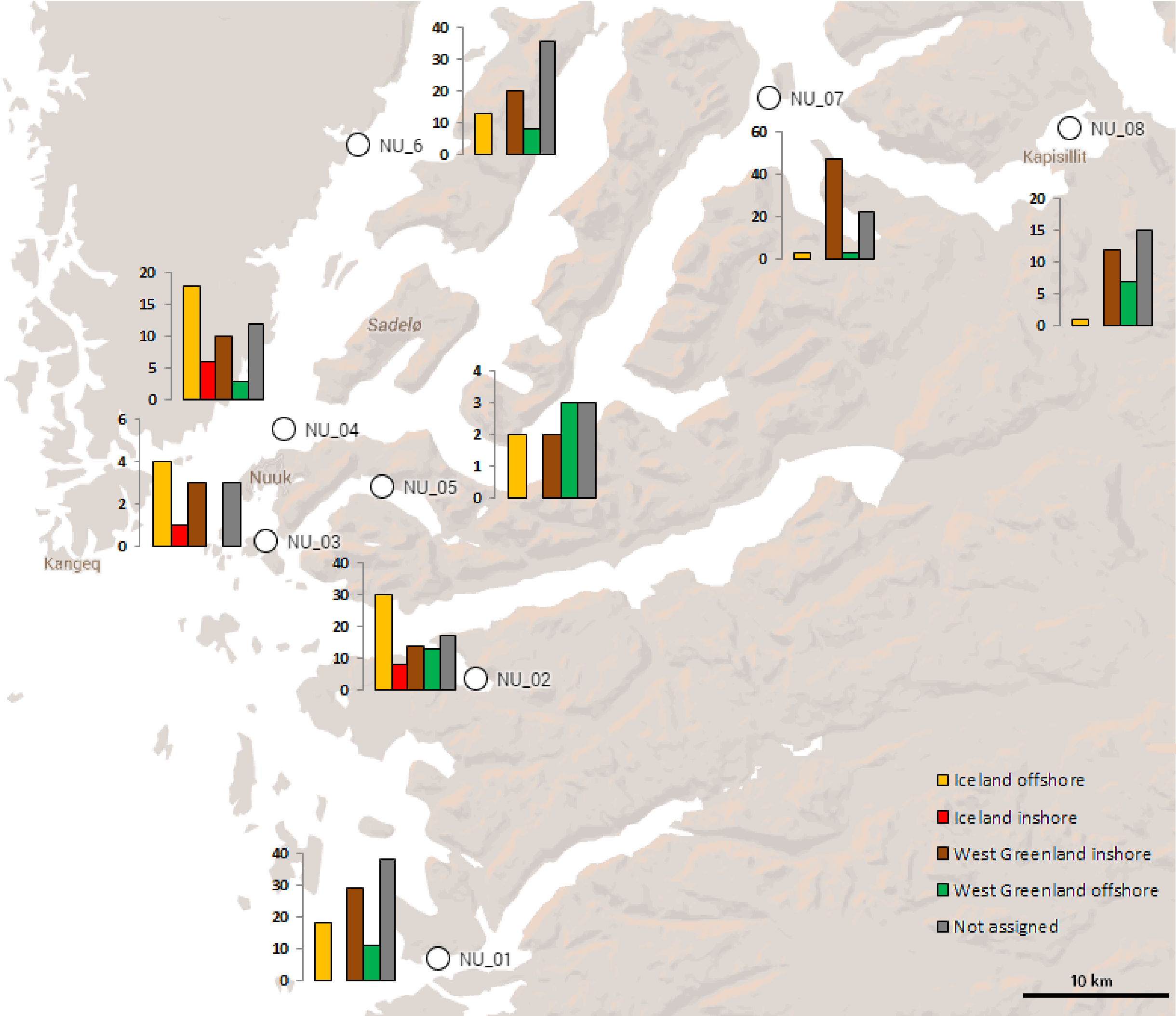
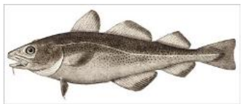


Figure 14 Individual assignment (GDO; >0.7) of Atlantic cod (*Gadus morhua*) from the inshore waters around Nuuk. The bar columns show the number of individuals that are correctly assigned to one of four baseline populations (Iceland Offshore, Iceland Inshore, West Greenland inshore and West Greenland Offshore).



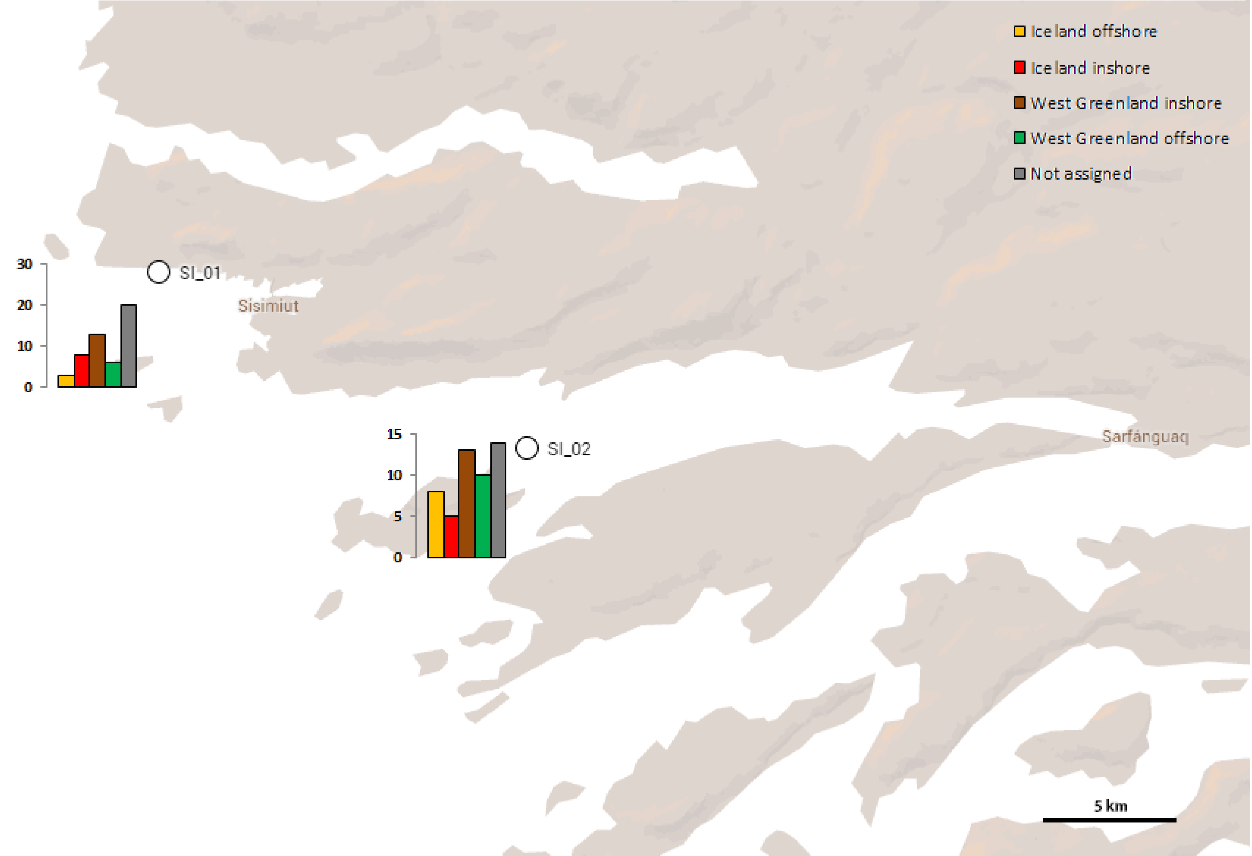
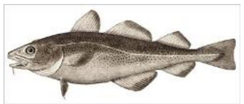


Figure 15 Individual assignment (GDO; >0.7) of Atlantic cod (*Gadus morhua*) from the inshore waters around Sisimiut. The bar columns show the number of individuals that are correctly assigned to one of four baseline populations (Iceland Offshore, Iceland Inshore, West Greenland inshore and West Greenland offshore).

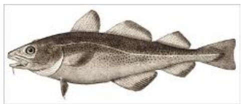


Discussion

IA: Methods and approaches

As expected, the assignment accuracy was dependent on the set criteria cutoff, where increasing thresholds decreased the rate of assigned individuals (i.e. proportion of successfully assigned individuals) and thus positively assigned fewer individuals for all four assignment approaches (GC, DAPC, GDA and GDO). Assignment rate was generally high for both assignment tools, GC and DAPC. , A substantial decrease assignment rate was observed when combining methods.. The substantial number of unassigned individuals for approaches combining GC and DAPC is in contrast with the recent IA approach performed in two studies by Bonanomi et al. (in prep a, b). Involving the same populations of Atlantic cod investigated here, they attained high assignment rates of of 90.5% and 92.3%. Using the same criteria here (GDA; >0.9) for assignment yielded a rate of 54% in this study. They used an 81 loci panel, which was also tested here, and accordingly, was apparently outperformed by a new updated panel consisting of 96 loci.. Although, the 96 loci panel includes 39 new loci with high pairwise differentiation (F_{st}) between the four distinct spawning groups previously identified (Therkildsen et al., 2013), it cannot be ignored that the exclusion of 24 low differentiated loci or/and inclusion of new loci might have had an effect on assignment. The majority of individuals that were allocated to different populations by GC and DAPC in this study, and therefore categorized as unassigned, may have represented a more admixed or uninformative genotype batch, which was not present in the studies by Bonanomi et al.. In addition, the assignment accuracy is highly sensitive to baseline design, and crucial genetic variation might have been lost in the exclusion/inclusion of loci or genotypes. It is likely that widening the genetic base, i.e. including more loci and individuals of the reference populations would improve assignment for atypical genotype profiles that assigns differently for GC and DAPC. In addition, it t is still uncertain to what extent all major population clusters is included within the panel, in particular with emphasis on the West Greenland inshore cluster (See below).

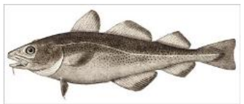
Comparing the different IA approaches, GC assigned obtained highest proportional assignment achieving significant improved assignment with the 96 loci panel (i.e. training-set/holdout-set protocol), and assigning most individuals for the empirical samples. Likewise, DAPC as a single performed well, but the combination of the two methods for IA approaches showed low assignment rate. In this case, it does not suggest that GDA and GDO represent inefficient approaches, but rather that they are the most conservative



approaches applying the precautionary principle and providing the highest statistically confident result for IA. I.e. when the statistical certainty of individuals rather than the mixture proportions are important, these methods should be applied. On the other hand GC and DAPC individually are excellent tools for well-differentiated populations, but caution should be taken in situations where one would expect considerable contributions from potentially admixed, uninformative or containing slightly differentiated "ghost populations" (e.g. unidentified coastal cod populations not included in the baseline). A single-method approach can be very efficient for routine applications and investigations of overall proportions, but for populations with low levels of differentiation and displaying biocomplexity, comparisons with other known genetic assignment methods should be explored and the variability among approaches assessed.

Comparisons between IA and MSA

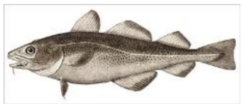
The genetic resolution was overall similar between MSA and summed IA's, which provides a high level of confidence in the overall results. All analysis tests for accuracy were high, and MSA returned generally similar mixture contributions to GC. Furthermore, estimates derived from the conservative GDA and GDO approaches did not deviate much from MSA estimates, whereas the summed IA from DAPC provided noticeable different results. Nevertheless, the overall spatial pattern did not demonstrate qualitative differences between MSA and any of the IA approaches (For comparisons tables with DAPC and GDA; See Appendix, Table S1 and S2). It is perhaps not surprising that MSA provided similar results to summed IA returned by GC. MSA performed in ONCOR and IA in GC represents conceptually different statistical approaches, but they have their foundation in methods that rely on assumption of population genetics models (Millar, 1987; Rannala & Mountain, 1997). Accordingly, the DAPC approach which is based on discriminant functions represented the most differentiated in methodology (i.e. do not rely on any assumptions of population genetics models or any particular type of substructure) returned overall mixture proportions that was least similar to MSA. Issues can occur when retaining too many principal components in DAPC, which can lead to overfitting the discriminant analyses and thus bias assignment (Jombart et al., 2010; Blair et al., 2012). This is, however, not considered the case, as both a-score optimization (Jombart et al., 2010) and several assignment tests with different numbers of principal components yielded similar results in the present study. MSA is expected to provide maximal accuracy for the relative proportional composition of interest, whereas IA is presumably most useful for the direct origin on the level of individuals.



The summing of individuals over fractional allocation methods, where uncertainty on the classification of individuals may lead to estimation bias, seem to be the major disadvantage associated with IA. Hence, MSA would be the most appropriate for most cases in fishery assessments, where the interest often lies in the best estimated proportional contribution (Koljonen et al., 2005). However, these results also show that summed IA estimates provide very similar estimates (for GC) which can be used for genetic stock identification. When combined in more stringent approaches they can provide a statistical conservative and powerful alternative or supplement to MSA, which often can be associated with relatively broad confidence intervals, in particular for small samples. Consequently, deciding which approach that is most appropriate should be case-, and question-specific, thoroughly assessed and considered critically. Here, uncertainty in firstly individual assignment methods and secondly in the population structure (i.e. whether the West Greenland Inshore population constitutes a single population) was evident and therefore, the application of GDO was utilized representing a conservative, but statistical powerful approach of IA, when the focus is on the identification of the origin of individuals.

Regional distribution patterns

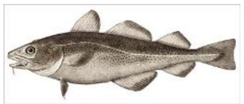
This is the first study to genetically identify the origin of mixed feeding aggregations of cod in West Greenlandic inshore waters around Nuuk and Sisimiut. The genetic assignment indicate that the majority of individuals originate from the local West Greenlandic inshore population. Interestingly, this study revealed that West Greenland inshore coastal areas might display regional distribution patterns similar to the most recent spatiotemporal genetic analysis conducted in West Greenland offshore waters. In the offshore waters the 'local' West Greenland offshore population has rebounded in central and northwestern areas, while the Iceland Offshore population has remained historically high in southwestern areas (Bonanomi, in prep.a). Indeed, the results showed that Northwest Greenland inshore waters (represented here by the Sisimiut region) might be dominated by a 'local' West Greenland Inshore population, whereas Iceland Offshore individuals might gradually increase in southwestern regions (represented here by the Nuuk region) during the feeding season. The inferred genetic composition is consistent with previous studies, where contemporary and historical coastal cod samples have revealed a clear genetic separation between populations that are present in inshore and offshore regions (Pampoulie et al., 2011; Therkildsen et al., 2013). Furthermore, these results highlights new aspects related to Bonanomi et al.'s (in prep.a) findings of



the significant contributions of Iceland Offshore individuals in mixed stock fisheries of Greenland. Hence, the contributions of the Iceland Offshore population seem to be apparent not just in offshore areas, but also in inshore coastal areas and even into fjords of West Greenland. Whether the proposed (see Bonanomi et al. a) distributional trend for the different cod populations, translating into a gradual north-south trend in contribution of populations with West Greenland Inshore and Iceland Offshore origin, respectively, is also fully transferrable to coastal assemblages along the westcoast of Greenland, is uncertain based on above results, but such hypothesis is certainly supported with the data presented here.

Local distribution patterns

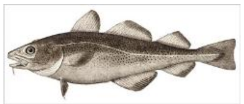
The regional distribution patterns for northwest and southwest assemblages in Greenland revealed here and by previous studies (Therkildesen et al. 2013, Bonanomi in prep.a) was also detected locally in samples from the inshore coastal regions. In addition, these findings also provide a more detailed resolution of population distributions for the inshore areas around Sisimiut and Nuuk. Within the region around Nuuk the study explored the proportion of different populations in assemblages distributed along a transition from coastal inshore waters into the fjord system. In fjords, an expected very large proportion of West Greenland Inshore individuals were found, but surprisingly also individuals with an offshore origin. Of particular interest to management is that individuals from the Iceland and West Greenland offshore populations were contributing within fjords as well as in the inshore coastal zone. These findings provide valuable knowledge in relation to previous non-genetic studies on these populations suggesting that the system is very dynamic. Accordingly, historical tagging data dating back to the early 20th century, has reviewed and analyzed in several publications (Hansen, 1949; Hovgård & Christensen, 1990; Storr-Paulsen et al., 2004), suggests that different migratory behaviors exist among the cod populations in Greenland. The most recent available tagging data, , mainly focusing on the inshore region (ICES, 2012), and recent genetic investigation of the tagging records (Bonanomi et al., in prep.b) have illustrated similar patterns as previous publications (See review by Storr-Paulsen et al. (2004). These comprehensive tagging studies have shown that cod tagged at West Greenlandic banks display migrating behavior, with most recaptures in western offshore areas in Greenland, southwest Iceland and East Greenland, and to a lesser degree in the inshore areas of West Greenland. In contrast, cod tagged in the fjords have been predominately recaptured in either the same fjord as tagged or in the adjacent coastal inshore area, which would indicate that these inshore cod



assemblages might display a more sedentary behavior. Thus, a high degree of sedentary behavior for fjord populations, as proposed by Storr-Paulsen et al. (2004), could restrict populations to individual fjords and therefore the contribution of migrating individuals of inshore origin to offshore regions in West Greenland could be very limited. Here, assignment indicates that migration (in the opposite direction) of offshore individuals to inshore regions and fjords might be of a reasonable magnitude, contributing significantly to the total biomass found in inshore coastal waters and fjord systems. Together with a comparable study from the Norwegian coast (Godø, 1995), they have suggested that the integrity between population components of cod can be maintained even if they share feeding grounds. The insights on population proportions and the apparent integrity of the populations investigated here support these views.

Icelandic cod in Greenlandic inshore waters; why are they here?

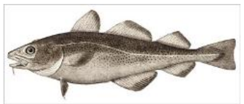
Recent studies have suggested strong population divergence between pairs of ecotypes found for Atlantic cod, characterized by a migratory and stationary behavior, respectively (Hemmer-Hansen et al., 2013; Karlsen et al., 2013, 2014). These ecotypes have been well-described both in Norway and Iceland (Pálsson & Thorsteinsson, 2003; Nordeide et al., 2011). In Iceland these types are also known as 'frontal cod' for the migratory type and 'coastal cod' for the stationary type, which translate into individuals from Iceland Offshore and Iceland Inshore, respectively, for the current studies. The migratory ecotypes undertake migrations associated with feeding and spawning.. These migrations have been documented for Icelandic individuals both vertically in the water column during feeding season (Pálsson & Thorsteinsson, 2003; Pampoulie et al., 2008) and long-distance migrations during spawning season (Svedäng et al., 2007; Bonanomi et al. b). For cod with a presumed Icelandic origin in Greenland, it has previously been suggested that individuals could be limited to juvenile cod arriving to Greenland as a consequence of eggs and larval transport from Iceland (Schopka, 1994; Astthorsson, 1994). In relation to the arrival of juveniles, the coastal zone has previously been suggested to serve as an adolescent feeding habitat where many distinct genetic populations would form mixed feeding aggregations. Tagging studies support these notions (See review; Storr-Paulsen et al., 2004), where returned individuals for coastal tagged cod represent all areas (i.e. fjord, coast, bank, Iceland and East Greenland) and age-specific analysis that has shown a spawning migration upon maturity to offshore areas or Iceland. Furthermore, natal homing behavior from West to East Greenland waters has previously been suggested (Rätz, 1994; Schopka, 1994). Likewise, several studies



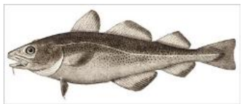
have indicated that individuals that are presumed to originate from spawning grounds in East Greenland and Southwest Iceland undertake long-distance eastward migrations from West Greenland (Shepard & Pope, 1993; Schopka, 1994; Storr-Paulsen et al., 2004). Recently, Bonanomi et al (in prep.b) found strong evidence of natal homing in Iceland Offshore cod tagged in Greenlandic waters. The findings of Icelandic Offshore cod in inshore waters further supports the hypothesis by Bonanomi et al., of egg and larval drift from Iceland to West Greenland (Buch, 1994; Storr-Paulsen et al., 2004), and a return migration to offshore waters in East Greenland and Iceland. The apparent occurrence of Iceland Inshore individuals as far north as in the Sisimiut region is a somewhat puzzling finding. It is difficult to explain how this inshore population has spread in significant numbers to the more northerly regions of West Greenland. Nevertheless, one might speculate that these individuals are a product of one or a few infrequent massive transports of eggs and larvae from Iceland to Greenland, which previously has been associated with strong year-classes in the fishery (Horsted, 2000; Storr-Paulsen et al., 2004). Alternatively, if there is population structure within the Greenland Inshore component, the inshore population around to Sisimiut may show a higher genetic similarity to the Iceland Inshore population than the population around Nuuk. Thus a proportion of the Sisimiut inshore population is (erroneously) assigned to Iceland inshore. However, only a genetic survey of spawning individuals in the Sisimiut region can reveal the most probable cause of the apparent high occurrence of Icelandic Inshore individuals (see also discussion below).

West Greenland inshore populations; shared evolutionary history or convergent adaptation

It is uncertain to what extent the samples included in the current baseline for population assignment is representative for all inshore populations in West Greenland. Since the identification of several distinct populations in Greenlandic waters (Therkildsen et al., 2013), the evolutionary history of the 'presumed local', inshore population West Greenland has not been investigated in detail. The high degree of sedentary behavior observed for cod populations in fjord systems could suggest the presence of multiple inshore cod populations in Greenland that may exhibit unique genetic signatures. Therkildsen et al.'s (2013) investigations of cod populations in Greenland showed that the individuals forming a West Greenland Inshore component (name re-adapted from 'Nuuk inshore') were noticeably differentiated from other components, supporting previous historical abundance records, tagging data and egg distribution records (Buch, 1994; Storr-Paulsen et al., 2004). Furthermore, Therkildsen et al. (2013) described inshore regions



around Sisimiut to hold cod from the West Greenlandic inshore component, corroborating this study. However, the West Greenlandic inshore component has so far been defined genetically solely by individuals with genotypes originating from, presumably, the most important spawning ground for the West Greenland inshore population at Kapisillit within the bottom of the fjord system (i.e. Godthåbsfjord) around Nuuk (Smidt, 1979; Storr-Paulsen et al., 2004). Therefore, at present, no inshore spawning individuals from other inshore spawning grounds have been included in the genetic baseline. Hence, it is still uncertain if the genetic assignment of individuals from Sisimiut inshore areas actually represents a shared evolutionary history or convergent adaptation to an inshore environment. Previous, studies have inferred microgeographical differentiation of cod revealing population structuring along coastal segments (Knutsen et al., 2003) and even at fine-scale levels of individual fjords (Jorde et al., 2007). Moreover, tagging experiments showed that cod with sedentary behavior only involve travelling distances of less than 50km in Canada (Howell et al., 2008) and Norway (Jorde et al., 2007), which likely would make frequent movement between fjords unlikely. Thus, an inshore population component in West Greenland only represented by a single population seems rather unlikely. However, still the fact that a large proportion of cod in the Sisimiut area are assigning to the population defined by the inshore 'Nuuk' component suggests a common ancestry of any potential coastal populations. In regard to inshore individuals in West Greenland, the Sisimiut region had a considerable portion of individuals assigning to Iceland Inshore. These individuals are unlikely to be a statistical artifact as even the most rigorous IA approach assigned them to Iceland Inshore. One might speculate that the common origin of "inshoreness" between stationary ecotypes of cod (Andersen et al., 2015), might bias assignments by "mis-assigning" individuals from a "ghost population", (i.e. a distinct 'local inshore' population with allelic variation not yet included in the baseline) which bears some resemblance similar to Iceland Inshore cod. Hence, a more detailed investigation is called for to elucidate the inshore coastal component. Such investigations should be the next focal point to any future research considerations for the inshore component in order to determine how 'local' the inshore cod populations in West Greenland really are. Regardless of this uncertainty, this study provides important insights into the distribution and spatial dynamics of cod populations in West Greenland inshore waters.

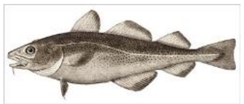


Management implications and conclusions

Currently, three biological units of Atlantic cod have been managed as fisheries entities in Greenland waters; 1) offshore unit composed of West Greenland offshore stocks (NAFO, 1A-1E), 2) offshore unit composed of one Southwest Greenland offshore stock (NAFO, F1) and several stocks in East Greenland (ICES subarea XIV), and 3) inshore unit comprising inshore stocks from West Greenland (majority of catches is from NAFO 1B-1D) (ICES, 2015). Since the offshore cod fishery collapse in West Greenland, closures of all offshore areas has been implemented with the main objective of rebuilding the stocks and thus, any fishery effort has mainly been conducted as experimental surveys. Nevertheless, dispensations have recently been given and the most recent catches amounted to a total of 116 tons for West Greenland offshore (ICES 2015). The division of the offshore component for cod in Greenland into two separate units in 2014 reopened fisheries in offshore waters, officially conducted as experimental fishery, which yielded total catches of approximately 7.900 tons (TAC of 10.000 tons) for South and East Greenland. At present, the most important fishery for cod is covered by inshore operations (TAC of 18.500 tons). Recently, inshore coastal catches have gradually increased, reaching peak catches of 18.400 tons in 2014 for inshore fisheries representing an increase of 39% compared to 2013.

However, even with recent increases in catches and a more optimistic management plan, the use of the current management units of the cod fisheries in Greenland could be discussed. Recent investigations, that have identified the population structure of cod in Greenland (Therkildsen et al., 2013; Pampoulie et al., 2011) and new insights into the spatiotemporal dynamics and mechanistic drivers (i.e. fishing and global warming) for offshore populations (Bonanomi, in prep.a) have raised issues in relation the future management of Greenlandic cod populations. Of particular note, the use of traditional discrete fishery units mainly based on geographical assumptions, such as NAFO subdivisions and ICES subareas, has been subjected to debate and frequently dubbed as an insufficient management tool in the literature on several occasions (Booke, 1981; Stephenson, 1999; Reiss et al., 2009). It may be advisable to attempt conducting the stock assessment based on biological populations and subsequently to regulate fisheries quotas based on the knowledge of spatiotemporal distribution of biological populations.

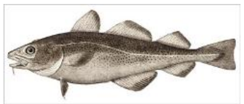
The current findings could aid in the management of West Greenland inshore fisheries. Present findings show that the inshore fishery have exploited feeding aggregations of mixed origin and indeed, the identified composition here might be useful for implementing a better assessment and fisheries regulation,



such as the preceding offshore fishery in West Greenland (Horsted, 2000). 'Classic' overfishing have frequently been visited in the literature (Worm et al., 2006), most likely due to a mismatch between biological and traditional management units (see Reiss et al. (2009)). Hereby, valuable knowledge about the composition of an exploited stock, which usually includes many genetically distinct populations locally adapted to different environments (Nielsen et al., 2009a), could aid in preserving biodiversity that might be essential for the persistence and stability of marine fisheries resources (Worm et al., 2006; Conover & Munch, 2002).

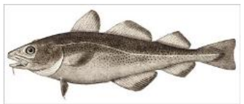
Here, the snapshot of population composition of fished mixed assemblages might indicate that the increase and the apparent recovery of the recent observed biomass of West Greenlandic inshore populations might not exclusively be attributed, to a sudden growth of the resident inshore population, but also to a considerable input of Icelandic offshore individuals. Based on the present results and the migratory behavior and spawning patterns of inshore and offshore populations (Storr-Paulsen et al. 2004; Bonanomi et al., in prep.b), then using inshore abundance indices of pre-recruit cod obtained from gillnet surveys (ICES 2015) as a proxy for local recruitment processes only may be overestimating the fishery potential due to the 'false immigration'. Therefore, accordingly, a substantial proportion of juveniles with a possible Icelandic origin might be included in inshore abundance indices, despite the fact that these individuals would potentially move offshore as they mature and eventually migrate back to their natal spawning grounds (Bonanomi et al. in prep.a). The substantial recruitment of cod with an Icelandic offshore origin into the fishery in some areas, such as regions around Nuuk (Area 1D, ICES, 2015), would most likely bias the fishery impact in other inshore areas and thus caution should be taken. For instance, the fishery effort in areas where vulnerable inshore population represent the majority of a mixed stock, such as regions Sisimiut (Area 1B, ICES, 2013) or fjord waters in Nuuk, should be adjusted accordingly. Indeed, the current investigations of inshore mixed stocks provide a preliminary baseline for management of inshore fisheries, where separate management strategies for regional coastal assemblages along the West Greenland shoreline should be implemented. Furthermore, harvest strategies that separates regions into coastal and fjord zones should be recommended, ensuring that fishery efforts are not concentrated in limited areas.

Recent investigations (Therkildsen et al. 2013; Bonanomi et al., in prep.a) support the notion of include separate exploitation strategies for each population component and have argued that the acknowledgement of the full biocomplexity and associated complementary dynamics, also termed the



portfolio effects (Schindler et al., 2010, 2015), might involve such implementations in order to minimize impact on vulnerable distinct populations, This, in turn would preserve genetic variation and secure future fishery yields, which might prove vital especially in synergy with climate change (Perry et al., 2005).

Nevertheless, together with the latest genetic studies (Therkildsen 2013; Bonanomi, in prep a, b), the present findings have provided new important insights into the genetic composition of the fishery and spatial dynamics of Atlantic cod in West Greenland inshore waters. These insights was achieved through the application of advanced genetic tools on sampled tissue collected by mostly non-experts with relatively little cost and effort. The SNP-based genetic stock identification method provided statistically robust and clear results, which other phenotypic character-based stock identification methods rarely archives. Other practical advantages include that the method and results are transferrable across laboratories and SNP genotyping platforms, providing opportunities for addition of samples and genetic markers (Araujo et al., 2014). Furthermore, other than fresh tissues of otoliths and fin clips, a range of processed tissue might be used (Lago et al., 2012). Even historical archived (Therkildsen et al., 2010) and non-tissue material, such as tags can be used (Bonanomi et al., 2014). For all these reasons, continuous tracking of the genetic origin of exploited cod populations in Greenland is recommended. Thereby, continuously improving the spatial management, and avoiding variable biased impacts on populations of cod in Greenland – some key considerations on the path towards a long term sustainable and high yield fishery.



Future perspectives

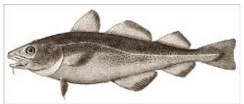
The investigations of inshore populations of Atlantic cod in West Greenland represents the aftermath of many previous studies revealing declining fisheries (Horsted, 2000), dynamic movement patterns (Storr-Paulsen et al., 2004), genetic variation (Pampoulie et al., 2011), population structure (Therkildsen et al., 2013), and recently spatial and temporal population composition (Bonanomi et al., in prep.a). As for previous findings, the presented results here should encourage new further investigations further expanding existing knowledge. This thesis provides valuable new insights into the resolution of the population composition of marine fishes and also adds to the genetic methodology developed for genetic studies. Here, a brief outline of two key focal points and follow-up investigations associated with the existing results is presented.

Better spatial and temporal resolution

The spatial genetic resolution in West Greenlandic inshore regions needs to be more thoroughly investigated in order to elucidate complex dynamics of inshore cod assemblages. Specifically, focus should be on patterns along the West Greenlandic coastline and the transition from coastal waters into fjord systems in order to understand the spatial distribution dynamics. Investigations of the proposed regional pattern of variable population contributions along the coastline of West Greenland for inshore waters would logically be the next step. Furthermore, other more typical fjord systems, such as the Sisimiut area, might be investigated to confirm current patterns observed in the Nuuk area. The promising results might also encourage studies on a temporal scale, where especially an annual replication of the current study would provide deeper insights into the compositional dynamics within and between years, which would be of high value for fishery management, providing implementation tools to help in decision making in relation to setting restrictions of certain areas or periods.

The evolutionary history of, a 'presumed local', inshore population

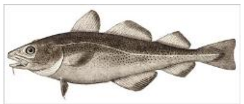
The uncertainty of the evolutionary history of inshore cod populations in West Greenland seems to be the next big question. Specifically, addressing a hypothesis of shared evolutionary history or convergent adaptation to an inshore environment is the center of attention. Informative SNP panels can be adjusted to



accommodate various scales of genetic differentiation and the addition of spawning samples from other inshore population and specific 'inshore-differentiated' loci might provide greater resolution to detect potential additional distinct inshore population components. The increasing throughput of sequencing and genotypic technologies in synergy with decreasing costs also enables a more dense and broad genome-wide coverage (Allendorf et al., 2010), uncovering signatures of selection that are needed for differentiating populations. It has already been demonstrated that the 'inshore-offshore divergence' are associated with the well-studied Pan-I polymorphism, which has split offshore from inshore populations in many regions (Norway, (Wennevik et al., 2008); Iceland, (Pampoulie et al., 2006); and Greenland, (Therkildsen et al., 2013)), and the search for genomic regions that separate "inshoreness" has already begun. Divergence between pairs of ecotypes found for Atlantic cod (Hemmer-Hansen et al., 2013; Karlsen et al., 2013, 2014) separating migratory, also called offshore, and stationary, also called inshore, and most recently the genes responsible for the stationary behavior has been investigated (Pampoulie et al., 2015; Andersen et al., 2015).

Exciting future ahead

At the current rate of technological advances in the field of molecular biology the relative value and importance of genetic information would indisputably increase in the future (Hauser & Seeb, 2008). Within a decade highly polymorphic markers has completely transformed the genetic scene by 1) shifting paradigms uncovering population structure in low-differentiated marine organisms, 2) detecting adaptive variation in non-model organisms, 3) having the ability to screen whole genomes for signatures of adaptive selection, and 4) providing continuous supply recently developed and improved genetic tools for possible investigations. The aftershocks of the most recent revolution of population genomics have already been observed, where research has been disentangling the temporal dimensions and discovering selection through time. Now major challenges include a more holistic understanding of the genetic signatures observed, linking; genotype with phenotype; phenotype with fitness; and fitness with selective drivers – a challenge rarely achieved for natural populations (Barrett & Hoekstra, 2011). Although confronted with many puzzles that have to be put in the right places, the tremendous opportunities that are associated with the rapid advances in biotechnologies will most certainly address these critical questions and open up exiting new avenues for upcoming future research.



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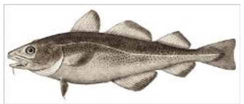

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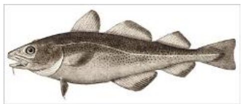

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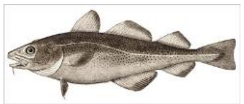

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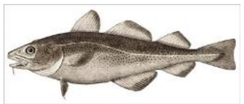

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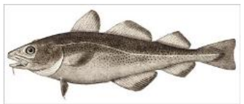

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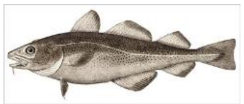

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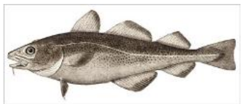

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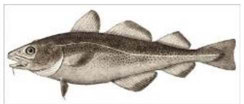


Appendix

SUPPORTING INFORMATION for following Thesis

"Genetic insights of the composition of two regional inshore mixed stocks of Atlantic cod (Gadus morhua) in West Greenland"

Ole Henriksen

This Appendix contains:

Supplementary Figures

Supplementary Tables

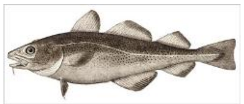
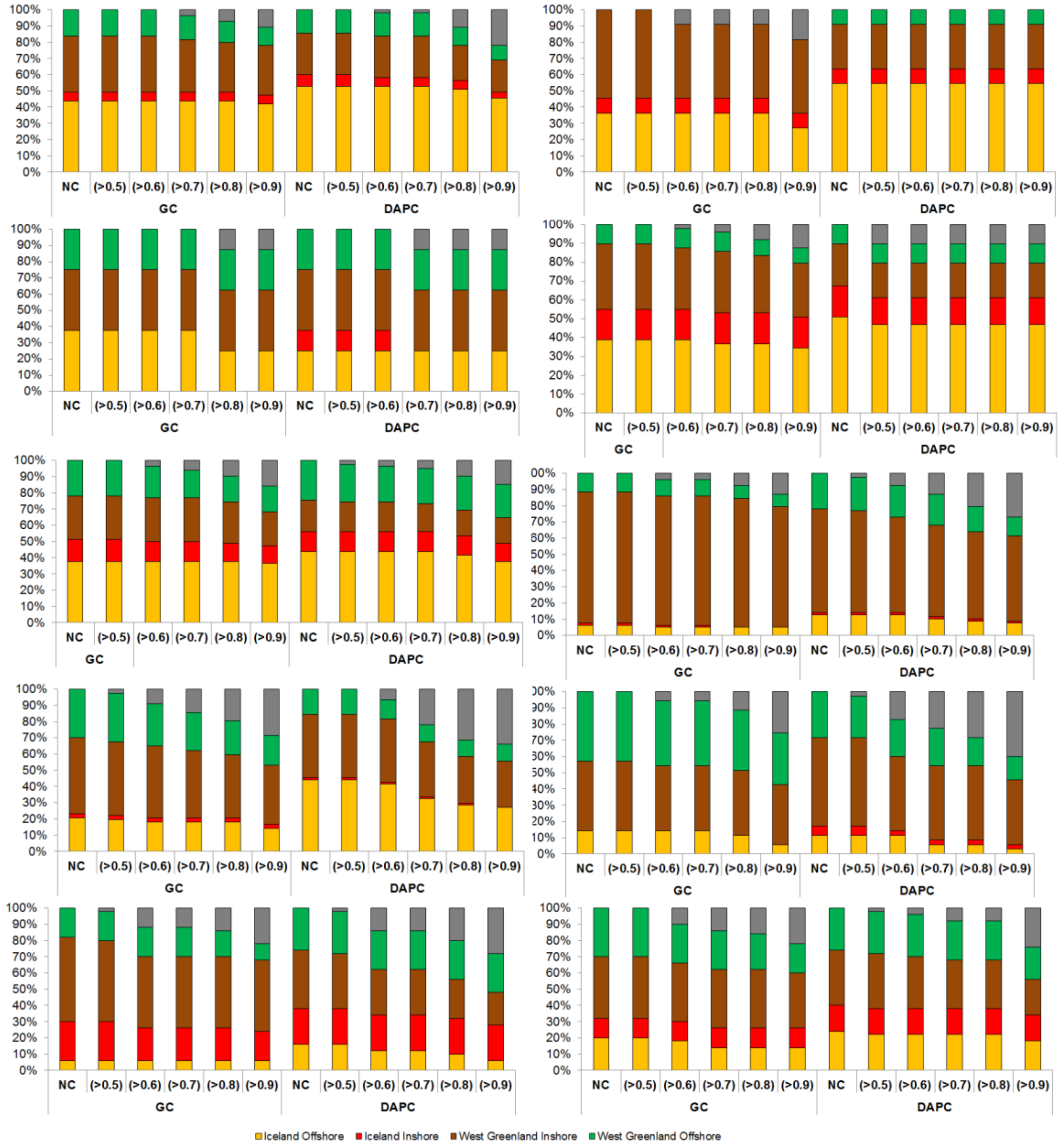


Figure S1 Proportions attained from individual assignment of Atlantic cod (*Gadus morhua*) from all sites in Nuuk (from right side counting in row-direction; NU-01, NU_02, NU_03, NU_04, NU_05, NU_06, NU_07 and NU_08) and Sisimiut (bottom row counting from right side; SI_01 and SI_02). Assignment was based on different approaches involving one of two genetic software packages (i.e. GENECLASS2 and DAPC). Assignment of individuals that are correctly assigned, were allocated to one of four populations (Iceland Offshore, Iceland Inshore, West Greenland Inshore and West Greebland Offshore) for different set criteria cutoffs.



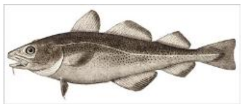
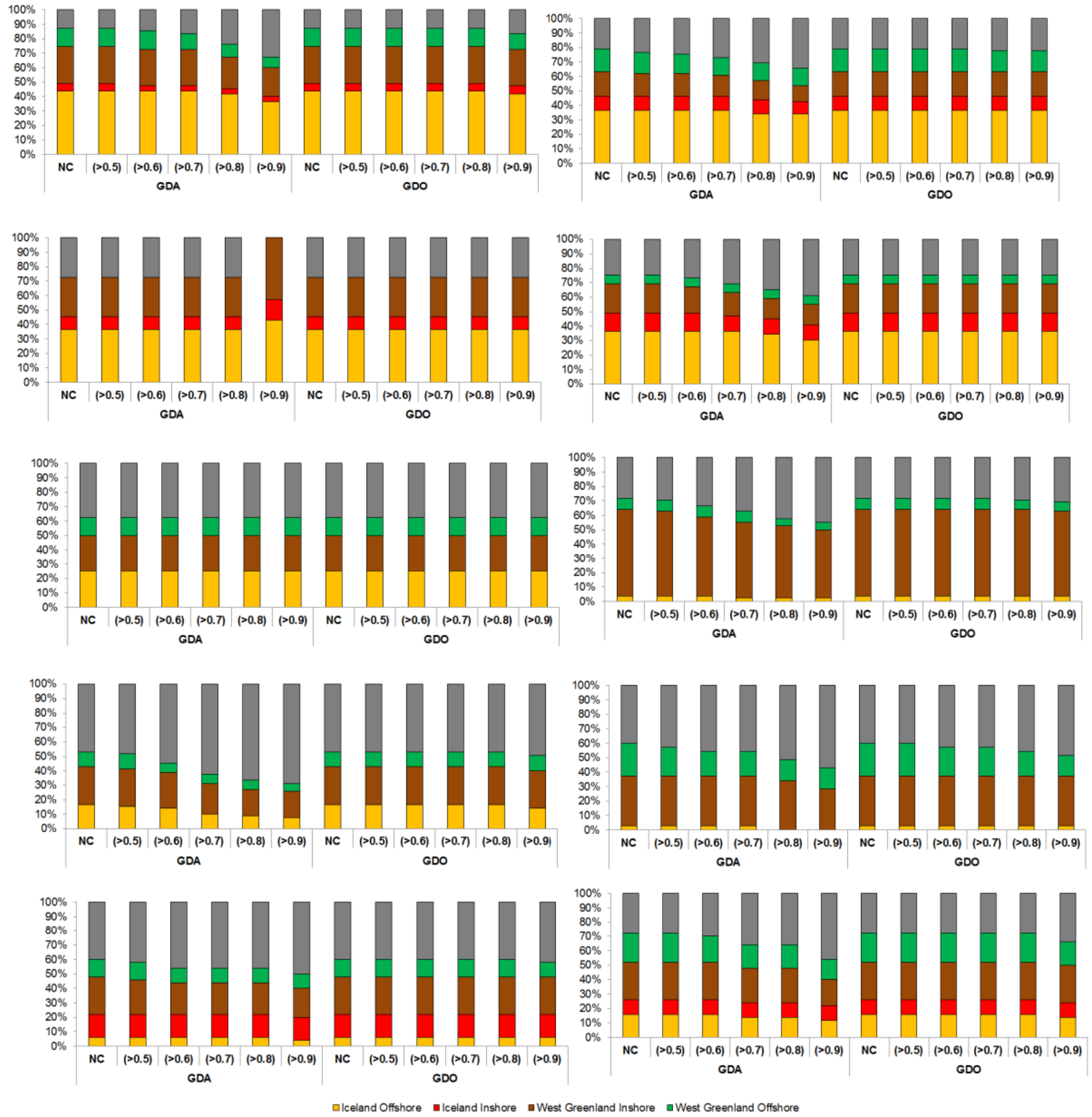
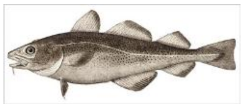


Figure S2 Proportions attained from individual assignment of Atlantic cod (*Gadus morhua*) from all sites in Nuuk (from right side counting in row-direction; NU_01, NU_02, NU_03, NU_04, NU_05, NU_06, NU_07 and NU_08) and Sisimiut (bottom row counting from right side; SI_01 and SI_02). Assignment was based on different approaches involving one of two genetic software packages (i.e. GENECLASS2 and DAPC). Assignment of individuals that are correctly assigned, were allocated to one of four populations (Iceland Offshore, Iceland Inshore, West Greenland Inshore and West Greebland Offshore) for different set criteria cutoffs.





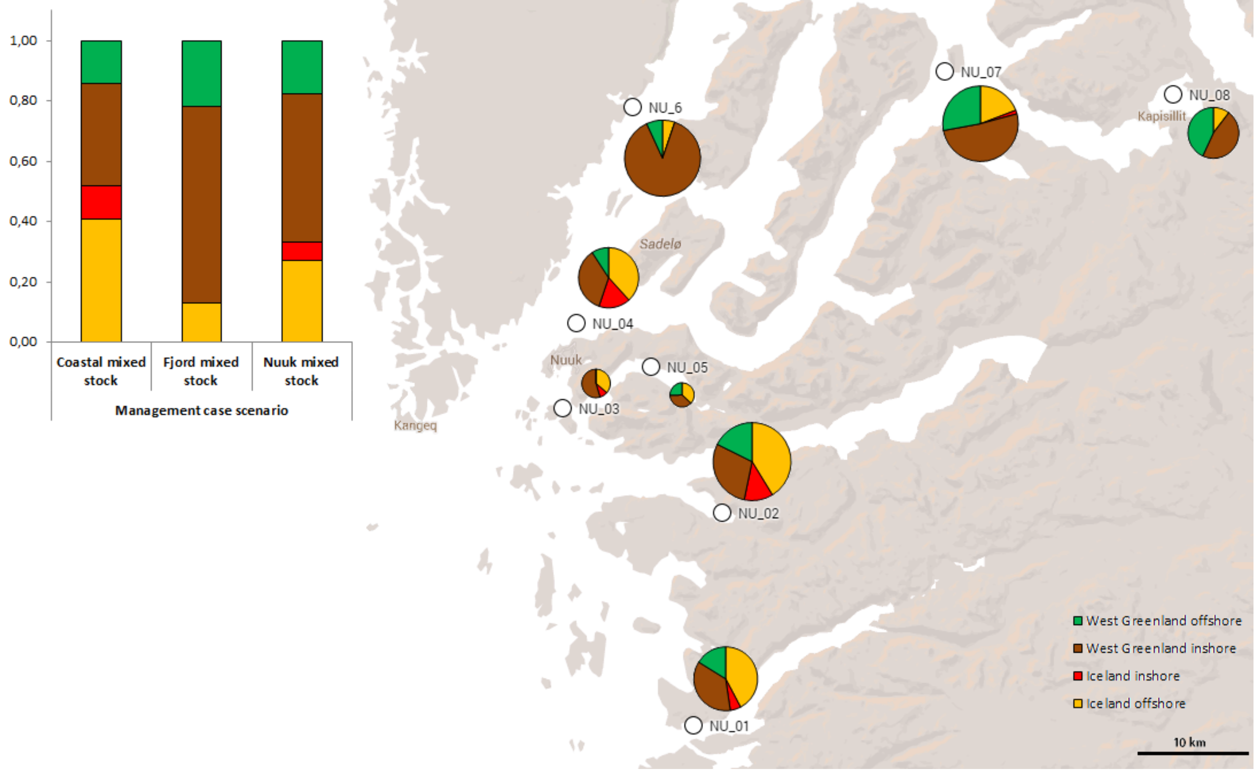


Figure S3 Mixed-stock analysis of Atlantic cod (*Gadus morhua*) from the inshore waters around Nuuk. The stacked columns show overall contributions to one of four baseline populations (Iceland Offshore, Iceland Inshore, West Greenland inshore and West Greenland offshore) for different proposed management scenarios. The pie charts display the proportional contribution of each population at eight sites. The size of each pie signifies sample sizes.

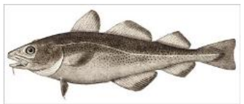


Table S1 Individual assignment of mixed-feeding aggregations showing numbers of Atlantic cod assigning to one of four populations. he proportional contributions (not including unassigned individuals) are given in parentheses. Assignment was performed with DAPC applying cutoff criteria >0.7._Numbers and percentages are also given for fish not assigned for criteria.

Approach	Region	Location	ID	Zone	Date	*n*	Iceland offshore	Iceland Inshore	WG Inshore	WG offshore	Not assigned
DAPC	Sisimiut	Tømmermandsøen	SI_01	Coastal	Aug-13	50	6 (14.0%)	11 (25.6%)	14 (32.6%)	12 (27.9%)	7 (14.0%)
		Amerloq	SI_02	Coastal	Aug-13	50	11 (24.0%)	8 (17.4%)	15 (32.6%)	12 (26.1%)	4 (8.0%)
	Total mixed stock			Coastal		100	17 (19.1%)	19 (21.3%)	29 (32.6%)	24 (27.0%)	11 (11.0%)
	Nuuk	Bukse Fjord	NU_01	Coastal	Aug/July-13	55	29 (53.7%)	3 (5.5%)	14 (25.9%)	8 (14.8%)	1 (1.8%)
		Præstefjord	NU_02	Coastal inlet	Aug-13	82	36 (46.2%)	10 (12.8%)	14 (17.9%)	18 (23.1%)	4 (4.9%)
		Hundeø	NU_03	Coastal	Aug-13	11	6 (54.5%)	1 (9.1%)	3 (27.3%)	1 (9.1%)	0 (0.0%)
		Sadelø	NU_04	Coastal	Aug-13	49	23 (52.3%)	7 (15.9%)	9 (%)20.4	5 (11.4%)	5 (10.2%)
		Coastal mixed stock		Coastal		197	94 (50.3%)	21 (11.2%)	40 (21.4%)	32 (17.1%)	10 (5.1%)
		Kobbefjord	NU_05	Fjord	Aug-13	8	2 (28.6%)	0 (0.0%)	3 (42.9%)	2 (28.6%)	1 (12.5%)
		Terte	NU_06	Fjord	Dec-13	78	8 (25.9%)	1 (1.5%)	44 (64.7%)	15 (22.1%)	10 (12.8%)
		Uummannaq	NU_07	Fjord	Nov-13	77	25 (41.7%)	1 (1.7%)	26 (43.3%)	8 (13.3%)	17 (22.1%)
		Kapisillit	NU_08	Fjord	Dec-13	35	2 (7.4%)	1 (3.7%)	16 (59.3%)	8 (29.6%)	8 (22.9%)
		Fjord mixed stock		Fjord		198	37 (22.8%)	3 (1.9%)	89 (54.9%)	33 (20.4%)	36 (18.2%)
	Total mixed stock			Coastal/Fjord		395	131 (37.5%)	24 (6.9%)	129 (37.0%)	65 (18.6%)	46 (11.6%)

Table S2 Individual assignment of mixed-feeding aggregations showing numbers of Atlantic cod assigning to one of four populations. The proportional contributions (not including unassigned individuals) are given in parentheses. Assignment approach involved a combination of two genetic software packages (i.e. GENECLASS2 and DAPC) applying cutoff criteria >0.7.Numbers and percentages are also given for fish not assigned for criteria.

Approach	Region	Location	ID	Zone	Date	*n*	Iceland offshore	Iceland Inshore	WG Inshore	WG offshore	Not assigned
GDA	Sisimiut	Tømmermandsøen	SI_01	Coastal	Aug-13	50	3 (11.1%)	8 (29.6%)	11 (40.7%)	5 (18.5%)	23 (46.0%)
		Amerloq	SI_02	Coastal	Aug-13	50	7 (21.9%)	5 (15.6%)	12 (37.5%)	8 (25.0%)	18 (36.0%)
	Total mixed stock			Coastal		100	10 (16.9%)	13 (22.0%)	23 (39.0%)	13 (22.0%)	41 (41.0%)
	Nuuk	Bukse Fjord	NU_01	Coastal	Aug/July-13	55	24 (52.2%)	2 (4.3%)	14 (30.4%)	6 (13.0%)	9 (16.4%)
		Præstefjord	NU_02	Coastal inlet	Aug-13	82	30 (50.0%)	8 (13.3%)	12 (20.0%)	10 (16.7%)	22 (26.8%)
		Hundeø	NU_03	Coastal	Aug-13	11	4 (50.0%)	1 (12.5%)	3 (37.5%)	0 (0.0%)	3 (27.3%)
		Sadelø	NU_04	Coastal	Aug-13	49	18 (52.9%)	5 (14.7%)	8 (23.5%)	3 (8.8%)	15 (30.6%)
		Coastal mixed stock		Coastal		197	76 (51.4%)	16 (10.8%)	37 (25.0%)	19 (12.8%)	49 (24.9%)
		Kobbefjord	NU_05	Fjord	Aug-13	8	2 (40.0%)	0 (0.0%)	2 (40.0%)	1 (20.0%)	3 (37.5%)
		Terte	NU_06	Fjord	Dec-13	78	2 (4.1%)	0 (0.0%)	41 (83.7%)	6 (12.2%)	29 (37.2%)
		Uummannaq	NU_07	Fjord	Nov-13	77	8 (27.5%)	0 (0.0%)	16 (55.2%)	5 (17.2%)	48 (62.3%)
		Kapisillit	NU_08	Fjord	Dec-13	35	1 (5,3%)	0 (0.0%)	12 (63.2%)	6 (31.6%)	16 (45.7%)
		Fjord mixed stock		Fjord		198	13 (12.7%)	0 (0.0%)	71 (69.6%)	18 (17.6%)	96 (48.5%)
	Total mixed stock			Coastal/Fjord		395	89 (35.6%)	16 (6.4%)	108 (43.2%)	37 (14.8%)	145 (36.7%)

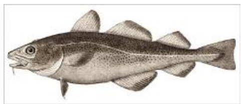


Table S3 Results for '100% simulations' analyses generated with ONCOR, where samples of 100 fish 'originating' from a single collection (i.e. true proportion is 1.00 in all cases) were simulated using baseline allele frequencies. Estimates are based on 1000 simulations for each of the four populations.

Population	**Average**	**STD**	**95% percentage intervals**
Iceland offshore	0,9999	0,0009	(1,0000, 1,0000)
Iceland Inshore	0,9999	0,001	(0,9999, 1,0000)
WG Inshore	0,9998	0,0012	(0,9995, 1,0000)
WG offshore	0,9997	0,0014	(0,9948, 1,0000)

Table S4 Three-way decomposition of error on MSA using 3 x 1000 simulated mixtures where each of the populations, 12, 24 or 48 fish, giving total mixture sample sizes of respectively 48, 96 and 192. Numbers show the respective percentage of error on mixed stock estimates, contributed by mixture sample size ('Mixture size'), numbers of genotypes in baseline ('Baseline size'), and baseline resolution ('Baseline genotypes').

Error source	**Iceland offshore**	**Iceland Inshore**	**WG Inshore**	**WG offshore**
Mixture size	0/0/0	0/0/0	0/0/0	0/0/0
Baseline size	75.2% / 73.6% / 74.9% / 70.5%	56.8% / 67.6% / 58.6% / 63.5%	77.1% / 72.4% / 70.2% / 67.0%	81.4% / 69.7% / 77.0% / 70.5%
Baseline genotypes	24.8% / 26.4% / 25.1% / 29.5%	43.2% / 32.4% / 41.4% / 36.5%	22.9% / 27.6% / 29.8% / 33.0%	18.6% / 30.3% / 23.0% / 29.5%

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