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Natural Selection Against Offshore Atlantic Cod (*Gadus morhua*) in Skagerrak Fjords?

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Sammendrag

Det marine miljøet var tidligere sett på som et åpent system, og marine organismer var antatt å ha mye genflyt mellom populasjonene. Dette var i hovedsak på grunn av havets potensiale til å spre marine organismers egg og larvestadier over store distanser med de store havstrømmene, og på grunn av mangelen på tydelige barrierer som er mer vanlig i terrestriske miljøer. Til tross for dette har nyere forskning vist at er en generell oppdeling i bestander for flere marine organismer. To av de viktigste faktorene som bidrar til denne strukturingen er retensjon av egg og larver inne i fjordene, samt begrenset vandring av voksne dyr eller tilbakevandring til deres fødested for å reprodusere. Dette studiet ser på fordelingen av egg og juvenile torsk (*Gadus morhua*) i to ulike norske fjorder på sørlandet, Topdalsfjorden og Tvedestrandsfjorden, og om frekvensen av de to genetiske gruppene her kalt kysttorsk og Nordsjøtorsk forandres igjennom sesongen. I Tvedestrand viser tidligere modellstudier at det er en del miksing av vann gjennom sesongen, og også i dette studiet ser fjorden ut til å være et mer åpent system. I Topdalsfjorden var frekvensen av Norsjøtorsk generelt lavere, og fjorden ser også ut til å ha høyere retensjon av egg i de innerste delene av fjorden. På høsten ender begge fjordene opp med samme mønster, med de indre delene av fjordene dominert av en genetisk gruppe som trolig er fjordtorsk, mens de ytre stasjonene har noe frekvens av den genetiske gruppen som ligner Nordsjøtorsk genetisk. Resultatene kan ikke konkludere med at mønsteret som dannes i løpet av sesongen er forårsaket av seleksjon mot fisk som blir transportert inn til nye miljøer, men resultatene diskuteres i lys av nyere forsknings funn.

Abstract

The marine environment was previously presumed to be demographically “open”, and marine organisms were thought to have pronounced gene flow over vast areas due to their potential of dispersal during early life stages. However, recent studies have suggested a degree of self-recruitment within segments of coastal and offshore areas for several marine species. Two of the forces acting on this structuring are retention of early life stages and homing of adult individuals. This study looks at the distribution of early life stages of the Atlantic cod (*Gadus morhua*) in two Norwegian fjords divided into inshore-offshore transects, and study if the frequency of coastal cod and North Sea cod is changing over the season. In the early autumn, both fjords end up with the same pattern, with the highest frequency of genotypes resembling the North Sea in cod eggs in the outer stations, and genotypes probably being coastal cod dominating the inner stations. There was, however, a difference in how this pattern emerged. Tvedestrand seemed to have a more open system, with more mixing of the cod eggs than what was found in Topdalsfjord. In Topdalsfjord there seemed to be a higher retention in the inner basins, and generally there were less North Sea cod eggs and juvenile than what was found in Tvedestrand. The results cannot conclude if the pattern that emerges is caused by selection against fish transported into non-native areas, but the results are discussed in light of new research findings.

Preface

First, I would like to thank my supervisor, Halvor Knutsen, for excellent supervision over the last year! You have guided me so well, and you have patiently listened and answered my questions in times needed. For that I am so grateful, you definitely deserve a special thank you! I would also like to give an extra thank you to Sigurd Heiberg Espeland, for teaching me about egg sampling techniques, and for some awesome boat trips in beautiful weather along the Norwegian coast. Also, Sebastian Bosgraaf deserves an extra thank you, for helping me with the egg sampling, and for his good spirits. Hanne Sannæs deserve a special thanks for helping me in the laboratory, and giving great advice during the DNA extractions. I have learned so much from all of you. Thank you!

This last year have been a continuous learning process, and I have enjoyed the experiences so much. I would like to thank all my fellow students for great advice in times needed. Also, Linn Anette Haug and Beate Marlene Funk, thank you so much for all support and motivation! And thanks to Tor Fritjof for helping me sample the spawning fish in the icy waters of February.

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Kristiansand, 17.05.2016
Ann-Elin W. Synnes

Contents

Introduction

Genetic structure and potential for local adaptation

Understanding marine connectivity has been a long standing challenge, as the complex fluent environment of marine organisms offers a lot of possible ways for dispersal within and among populations (Cowen, 2009). In terrestrial and freshwater environments barriers often separate populations physically, making them demographically and genetically distinct from each other. In the marine environment, however, physical barriers are often absent, and continuous water masses disperse eggs and larvae by passive drift (Bradbury *et al*., 2008; Shanks, 2009). The marine environment was previously assumed to be demographically more ”open” panmictic systems due to marine organisms vast dispersal potential during the early pelagic life stages. Information on the true scale of larval dispersal was for many years limited, as the dispersal distances are very difficult to measure directly. However, increasing evidence suggesting a degree of self-recruitment within segments of coastal and offshore areas for several marine species (coral fish: Jones *et al*., 1999, Atlantic cod: Ciannelli *et al.,* 2010, Shrimp: Knutsen *et al*., 2015). The key mechanisms used to explain such population structure found in the marine environment include retention of eggs and larvae in complex current systems (Ciannelli *et al.,* 2010), high mortality of dispersing individuals (Freitas *et al.,* 2015), and homing of larval and mature fish to natal homing grounds (Jones *et al.,* 1999; Espeland *et al*., 2008).

Due to the high levels of gene flow in marine organisms, traditionally it was assumed that natural selection would be either absent or limited to form local populations (Pogson and Fevolden, 2003). However, the potential for selective forces acting on molecular gene

frequency should not be dismissed (Berg *et al.,* 2015). What most genetic studies of marine organisms have in common is the low level of genetic differentiation, F_{ST}, among presumed populations (Ward *et al*., 1994; Waples, 1998,). Low levels of genetic differentiation is most likely due to the extensive gene flow, which homogenizes genetic variation across habitat, making genetic structure hard to detect (Knutsen *et al.,* 2003). Also, the high number of individuals in many marine organisms constituting the effective population size (N_e) suggests genetic drift to be negligible or lower in the sea, as the intensity of genetic drift is linked with the number of breeding individuals in a population (Hellberg *et al*., 2002). However, there are examples of marine organisms where genetic drift may be an active force. Knutsen *et al.,* (2011) found that the effective size of a coastal cod population in a fjord was less than 200 or so individuals, and Hauser *et al*., (2002) found a marked difference between census population size and effective size in New Zealand snapper, making it reasonable to believe that genetic drift could have an effect in some cases.

Mechanisms maintaining structure and local adaptation

Studies of local adaptations gives us a greater understanding of the power of natural selection relative to gene flow and other evolutionary forces. Local adaptation arises from spatially and temporarily varying selection, as various populations may experience environmental variations in e.g. salinity, temperature, or river runoffs (Conover *et al*., 2006). For local adaptation to occur, selection must exceed the homogenizing effect of gene flow from other populations (Hendry *et al.,* 2001). Populations are locally adapted when individuals with local genotypes have higher fitness in their local habitats when compared to individuals with genotypes from alternative habitats (Kawecki & Ebert, 2004; Sotka, 2005). Environmental challenges reduce fitness and must be counteracted by either range shifts, by a phenotypic

response (phenotypic plasticity), or by evolutionary change (adaptation). Locally adapted populations can differ in the level of genetic variability they possess, as adaptation requires genetic variability in phenotypic traits (e.g., physiology, behavior, life history, morphology). It can, however, be a challenge to unravel the environmental effects on phenotypes from the genetics (Nielsen *et al*., 2009).

To understand the spatio-temporal dynamics of species and groups of species, it is essential to describe and identify the geographical extent of local populations (Knutsen *et al.,* 2003). Knowing the geographic extent of a population also opens the possibility to ask scientific questions regarding spatial scale for local adaptation. Two of the most important mechanisms influencing population connectivity in marine systems are retention of pelagic early life stages, and homing of adult individuals (Thorrold *et al.,* 2001; Olsen *et al.,* 2008). It is the combination of the processes acting on early life stages as well as the behavior of adult individuals that ultimately determine the spatial scale of population structuring and the degree of connectivity between regions (Rogers *et al*., 2014).

Retention of eggs and larvae

Eggs and larvae have the potential for long distance dispersal; however, a high potential for dispersal does not necessarily mean a high amount of gene flow (Avise, 1998). Genetic structure means that there must be restrictions in connectivity in all life history phases of a species, like dispersal ability for eggs and larvae (Knutsen *et al*., 2007) and adults (Rogers *et al.,* 2014). A fjord is a special type of estuary carved out by a glacier, meaning it is a semi-enclosed body of brackish water, where freshwater from river runoffs meets saline water from the ocean. When river runoffs dominate over tidal input, estuarine circulation develops,

characterized by a strong outflowing current at the surface and weak inflow in the deeper layers (Myksvoll *et al*., 2011). Thus, when the Atlantic cod spawn near the fjord environments, the horizontal transfer of the eggs is dependent on the vertical position of the eggs in the water column (Myksvoll *et al*., 2011). Evidence from a variety of studies indicate that retention may be much more common than previously thought, even in species with long larval duration (Warner and Cohen, 2002; Knutsen *et al.,* 2007; Ciannelli *et al.,* 2010). If pelagic larvae are retained near their natal populations by behavioral or physical mechanisms, persisting over many generations, the populations will have greater opportunities to develop genetic differentiation and local adaptation, and even new species (Taylor and Hellberg, 2003).

Adult migration

While dispersal is a demographic process that must be considered to understand the distribution and abundance of an organism, additionally, adult behavior can also be a crucial factor to consider (Rogers *et al.,* 2014). Long distance migration has evolved independently in many animals, such as birds and fish, mammals, reptiles, amphibians, insects and marine invertebrates. In many instances, migration is an adaptation for exploiting seasonal peaks of resource abundance, and to avoid resource declines. It has evolved independently a numerous of times, and requires genetic instructions about the timing and duration of movement, physiological and behavioral adaptations, as well as orientation and navigation (Alerstam *et al.,* 2003).

In both eastern and western Atlantic there has been described two distinct ecotypes of cod, characterized as “migratory” and “stationary” ecotypes (Hemmer-Hansen *et al.,* 2013). The

Atlantic cod have a huge variation in migratory behavior, and the existence of these large differences within a species likely reflects local adaptations (Jørgensen *et al.,* 2008). While the Northeast Arctic cod is characterized by long distance migrations, the Norwegian coastal cod that inhabits the coast and fjord areas of Norway perform relatively short coastal migrations (Knutsen *et al.,* 2011; Rogers *et al*., 2014). In general, migratory ecotypes exploit deeper and offshore habitats, while stationary individuals can stay in the coastal waters their entire life (Hemmer-Hansen *et al*., 2013). In addition to differences in migration pattern, there is also a difference in feeding strategy, growth rate and age of maturity (Hemmer-Hansen *et al.,* 2013), and genomic architecture (Karlsen *et al*., 2013, Bradbury *et al.,* 2014, Berg *et al.,* 2016).

Objectives

Previous genetic studies of Atlantic cod from Skagerrak have revealed an overall low but significant level of genetic divergence, revealing some gene-flow, with a superimposed structure of slightly divergent components or populations. Ocean currents transfer pelagic eggs and larvae into coastal Skagerrak (Knutsen *et al*., 2004; Stenseth *et al*., 2006) from large oceanic spawning aggregates in the North Sea (Poulsen *et al*., 2007; Heath *et al*., 2014; Hemmer Hansen *et al*., 2013). Geographically fine-scaled genetic components have also been found along the coast (Knutsen *et al*., 2003; Jorde *et al*., 2007). Despite gene flow between the populations, there is evidence that this genetic structure is maintained by retention of pelagic early life stages (eggs and larvae) in fjords (Knutsen *et al*., 2007; Ciannelli *et al*., 2010), and restricted movement in older fish (Espeland *et al.,* 2008; Knutsen *et al*., 2011). Previously, there is also found indications for adaptive differences among coastal cod fjord populations in fitness-related phenotypic characters (Olsen *et al*., 2008). Also, a new study by

Sodeland *et al*. (2016) suggest that chromosomal inversions between coastal and offshore populations may be a key factor for local adaptation in this system.

In this study I wanted to test if there is a temporal stability of proportions of North Sea and coastal cod in the fjords during the different life stages, or if there is a gradual change in the distribution of over the season. Based on previous genetic studies on Atlantic cod, I define the two populations under investigation in this study as North Sea cod (NC) and coastal cod (CC) (Knutsen *et al.,* 2003, Sodeland *et al*., 2016). Here, temporal sampling was performed with several transects along an inshore-offshore gradient in two fjords, spanning over an area dominated by coastal cod (inside fjords), to the more exposed area dominated by offshore cod. Sampling was done at stations distributed along an inshore-offshore gradient, and spanned from spawning in February until October (eggs, and juveniles), as previous results indicate segregation in coastal and offshore components both temporally (Knutsen *et al*., 2011) and spatially (Sodeland *et al*., 2016).

Material and method

The study species

The Atlantic cod (*Gadus morhua*) is a demersal gadoid species, and is one of the most commercially important marine fish in the world. It is an ecological keystone species and is a top predator, interacting trophically with numerous other species (Frank *et al.,* 2005). It has a wide distribution area, ranging from the waters of the continental shelf in the North Atlantic, continuing northwards to Disco Bay, Spitsbergen, and the Labrador Sea, and southwards to Cape Hatteras and the Bay of Biscay. In the eastern Atlantic the cod also enters the very brackish waters of the Baltic Sea. Cod can be found in almost every salinity, from nearly fresh to oceanic water, and is also found in a wide range of temperatures, ranging from nearly freezing to 20°C (FAO, 1990).

Atlantic cod pass through a series of four life history stages as they develop: eggs, larvae, juvenile and adult. It is a seasonal batch spawner, with spawning usually taking place from January to April, depending on seawater temperature (Knutsen *et al*., 2003). In the North Sea and Skagerrak the spawning is usually from December to May, generally at depths of less than 50 m. (Knutsen *et al*., 2007; Bradbury *et al.,* 2000), and never beyond 200 m. Cod is one of the world's most fecund fishes, and a female cod can produce and release several million eggs depending on body size, distributed over several spawning events (Thorsen *et al.,* 2010). Larvae and postlarvae feed on plankton, while juveniles mainly feed on invertebrates, where crustaceans are considered to be very important (FAO, 1990). Older fish usually feed on invertebrates and other fish, including young cod, and fish is considered more important than crustaceans in the diet of older individuals (FAO, 1990). The larvae stay in the water column where they graze, until they metamorphose into juveniles in the early summer (Knutsen *et al.,*

2003). The pelagic juveniles feed in the water column until reaching a size of around 30 to 40 mm, before they begin to settle closer to the ocean bottom (Campana, 1996). The growth rate is rather high, and is highly variable from one area to another. Generally cod in open coastal areas are larger than cod inside the fjords (Rogers *et al.,* 2011).

The Atlantic cod displays a range of phenotypic and genotypic variations, and appears both as migratory and as stationary coastal forms with regards to spawning (Karlsen *et al*., 2013). Typically, the coastal cod is stationary, and complete their entire life cycle within a restricted area. In contrast, the oceanic cod may perform astonishingly long migrations up to several hundred kilometers (Hemmer-Hansen *et al.,* 2013). The population status for the coastal and oceanic cod has long been under discussion, and evidence concerning their genetic structure and connectivity is still debated (Berg *et al.,* 2016)

The study areas

The southern coast area of Norway consists of skerries and numerous small islands with medium sized fjords, shown to harbor multiple cod populations (Knutsen *et al.,* 2007). The fjords were formed during the last glacial period, and typically extend a few kilometers inland. Coastal cod in this area has been studied since the early 1900's with respect to e.g. population ecology and dynamics (Dahl, 1906), larval biology (Stenseth *et al.,* 2006), migratory behavior (Espeland *et al.,* 2008), oceanographic patterns (Ciannelli *et al.,* 2010), and more recently genetic structure (Berg *et al.,* 2016; Sodeland *et al.,* 2016)

Topdalsfjord (Figure 1a) is located outside of Kristiansand, and is approximately 11,5 km long. Largest recorded depth is just under 100 m. The fjord is known to hold several eelgrass beds, which is considered to be one of the most important nursery areas for Atlantic cod. The

fjord was chosen as it holds a viable population of cod and is also included in the annual beach seine survey performed by IMR along the Norwegian coast, and thereby provides valuable data for this thesis.

Tvedestrandfjord (Figure 1b) is approximately 8 km long, and has a maximum depth of 85 m. This fjord was chosen as it is an enclosed fjord, and harbors a known cod spawning area and nursery habitat. Studies have shown that cod-eggs are transported up fjord and retained within the inner basins (Knutsen *et al*., 2007; Ciannelli *et al.,* 2010). The length and topography of the fjord is also representative of fjord systems along the Skagerrak coast. The fjord has recently been protected as a MPA (marine protected area), including a no-take zone, containing the main spawning area. Disturbance of behavior due to fishing is thus expected to be negligible.

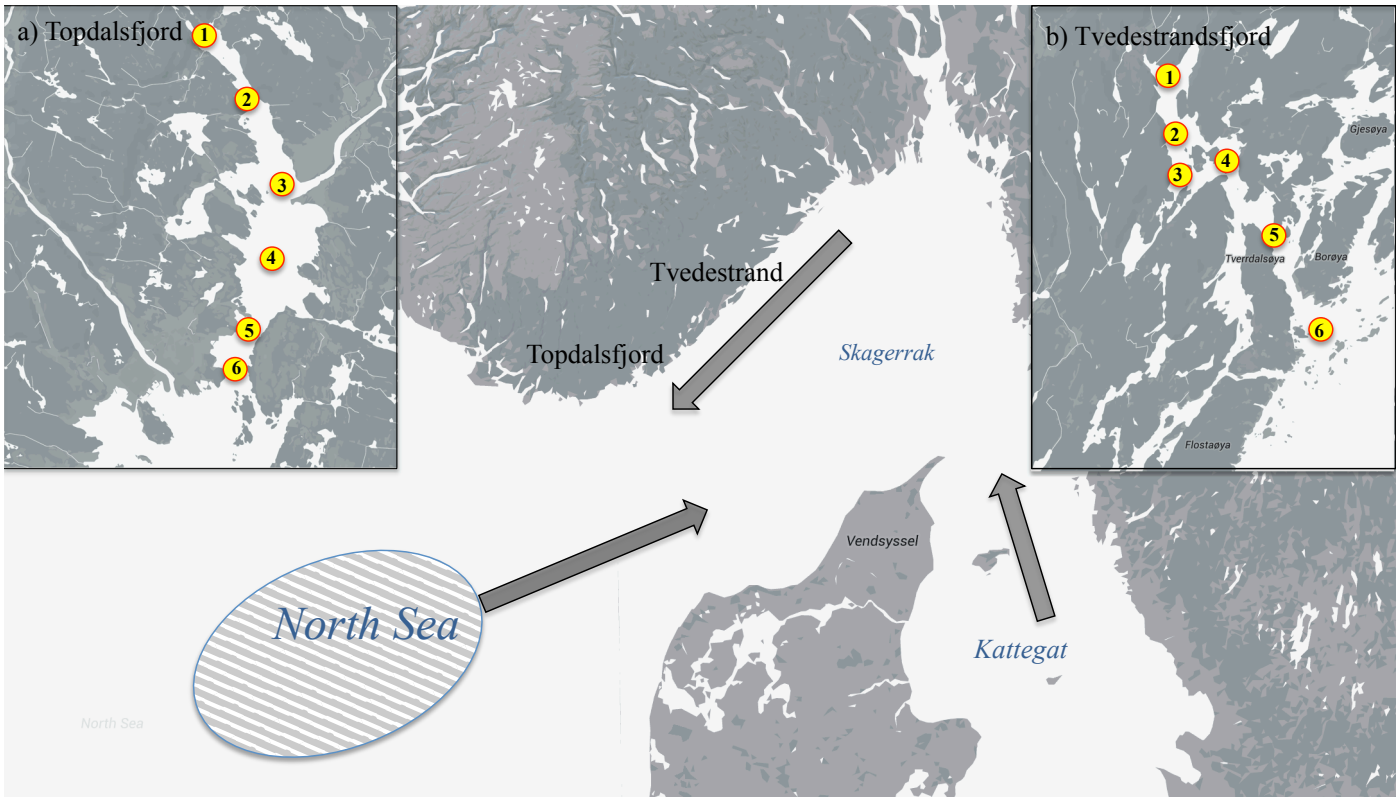

Figure 1. Map of study area with sample locations (yellow circles). The six stations are distributed along an inshore-offshore gradient, where station 1 is located in the inner parts of the fjords, and station 6 is located at the fjord inlets. Shaded area indicate one of the closest major spawning ground for North Sea cod, and grey arrows illustrate main direction of the ocean currents coming in from the North Sea before looping around and continuing down into Kattegat and around to the Norwegian coast.

Sampling

Cod eggs were sampled during the spawning season from February to late March, once in Topdalsfjord and mainly five times in Tvedestrand. At two occasions Tvedestrand was sampled at six stations. The sampling sites were distributed along an inshore-offshore gradient, and all egg samplings were done using a small open boat. Eggs were sampled with a WP2 planctonic net with a 500-µm-mesh size. The net was hauled vertically from 30 m depth to the surface at an optimal speed of 0,5 m/s. Cod eggs were identified among the plankton using a stereo loupe with measuring units, and eggs measuring 1,2-1,5 mm was pipetted out and sub sampled as cod eggs. Eggs was stored in 96% ethanol at -22 °C until DNA extraction was done.

Sampling of juveniles (0 group) was done first in early summer (June), then once again later in autumn (September and October) in both fjords, using a standardized protocol used for the annual beach haul survey by IMR along the Norwegian Skagerrak coast. Topdalsfjord was sampled for juveniles at six different stations, once in June and once in September. However on the sampling from September, the stations were listed as “inner” and “outer” stations. Tvedestrand was sampled for juveniles at five stations in June and three stations in October. Juveniles was stored in freezer at -22 °C until further analysis was done.

Spawning cod was sampled from Topdalsfjord during February 2015 with the help from a local fisherman. Sampling was done at 5 different locations within the inner parts of the fjord, and was collected over three days of fishing. Sampled cod was measured and sexed by visual examination. A small piece of the dorsal fin was subsampled for genetic analysis, and was stored in 96% ethanol at -22 °C until DNA extraction was done.

Genetic analysis

All cod eggs were extracted for DNA using the E.Z.N.A MicroElute Genomic DNA Kit, following the manufacturer's instructions for tissue samples. Extraction was done following published protocols with only one minor modification, i.e. the last elution buffer step was done twice through the same filter (25 µl was eluted). Genomic DNA from juvenile and spawning cod was extracted from a small piece of the dorsal fin, using E.Z.N.A Tissue DNA kit (Omega biotek) following the protocol. DNA from all individual cod samples was quality-verified and quantified with a NanoDrop instrument (NanoVue Plus, GE healthcare) before shipping.

A custom single nucleotide polymorphism small panel of 40 SNPs was selected from a SNP'CHIP developed as part of the Norwegian Cod SNP Consortium (CSC) to capture the population structure. These SNPs was screened for in all samples, however only 25 of these were scored as reliable. The SNP genotyping was performed at CIGENE (Centre for Integrative Genetics) at the Norwegian University of Life Sciences, using the Sequenom MassARRAY platform following the manufacturers protocol.

Statistical analysis

Within each population, estimates of observed (H_o) and expected (H_e) heterozygosity were calculated using GDA software (Table 2). Deviations from Hardy Weinberg was calculated for all SNPs estimated as F_{IS} (Weir and Cockerham, 1984) and were tested for by a probability test, both tasks using GENEPOP 4.2 on the web (Raymond and Rousset, 1995)

(Table 2). Number of alleles is not relevant for SNP's as they only have two allelic variants. Estimates of genetic differentiation (F_{ST}: Weir & Cockerham) and a heterogeneity test (exact G-test) for general structure in the data (for each locus) were performed by using the GENEPOP 4.2 software (Raymond and Rousset, 1995) (Appendix I). All P-values were corrected by FDR (Benjamini and Hochberg, 1995), correcting for "false discoveries" (reducing chance of type I errors).

Genetic assignment and outlier detection

Assignment of all individuals was estimated using the Bayesian assignment method in Geneclass II software (Pirya *et al*., 2004) using a mix of two samples from the North Sea and one sample from a mix of individuals inside three Skagerrak fjords as the two reference samples. All eggs, larvae and adults where individually assigned to either the North Sea (NC) or the coastal (CC) reference sample. Only scores higher than 80% and with over 15 loci were used to reduce the chance of misclassifying in the analysis, resulting in 57 individuals (12%) of the samples being left unassigned and removed from further investigation. Loci were tested for neutrality using the LOSITAN software (Beaumont and Nichlos, 1996; Anato *et al.,* 2008) which is a selection detection software based on the fdist F_{ST} outlier methods. Simulations were done using the Infinite Allele Method (IAM) with 1.000.000 simulations, a confidence interval of 0.95, and with a FDR of 0.1.

Results

330 cod eggs were sorted out from the 34 egg hauls done at 6 different locations in both fjords during the period from February to March. The 4 beach seine samplings done in June and October resulted in 76 fish in Tvedestrand and 20 fish in Topdalsfjord (Table 1). The local fisher in Topdalsfjord provided 52 cod from the inner parts of the fjord, while none were available in the no-take zone (MPA) in Tvedestrand. Some of the samples contained more eggs and juveniles than was noted on the sample, resulting in a total of 333 cod eggs, 100 juveniles and 52 spawning fish being extracted for DNA. 25 SNP loci were analyzed in all eggs, juveniles and adults. Of the total 485 individuals, we were able to genotype 475 individuals successfully from the two localities (97%)(Table 1). 418 individuals was successfully assigned to either coastal or oceanic reference sample, and a total of 68 cod (16%) was scored to the NC reference and 350 cod (84%) was scored to the CC reference.

The observed and expected heterozygosity was generally similar in all samplings performed at the different locations (Table 1). Genotype proportions varied somewhat among sites, however only one of the sites came out with negative F_{IS} estimate (Table 1). None of the localities deviated significantly from Hardy-Weinberg proportions after FDR correction (Table 1). Among loci there were greater differences, and all samples combined deviated somewhat from those expected under Hardy-Weinberg equilibrium, with a general deficiency of heterozygotes (Table 2). All loci except Gdist_220446_161, Gdist_545739_884, Gdist_205638_419 and Gdist_355999_102 had positive F_{IS} estimates (average over loci F_{IS}= 0.096: ranged from -0.088 at Gdist_545739_884 to 0.305 at Gdist_192507_8811; Table 2). However, only 3 loci showed significance when tested for deviation of genotype frequencies with a Hardy-Weinberg probability test, and none of the values came out significant after FDR correction (Table 2).

Population genetic structuring and loci under selection

Loci were chosen to segregate between cod from the North Sea and fjords of Skagerrak, and our results clearly illustrate that we did get both components, as overall level of genetic differentiation, F_{ST}, was high (F_{ST}= 0.022; P<<0.001) and significant even after FDR correction. Genic differentiation varied somewhat among loci, and 17 out of the 25 loci showed significant structure when tested with an exact G-test, resulting in a clear overall structure in the data (Appendix I). However, only 3 out of the 25 SNPs showed significant change in gene frequencies over the season with a slope being significantly different from zero (Appendix I). Out of the 25 loci, 8 were identified as outliers by the LOSITAN analysis (Beaumont and Nichlos, 1996; Anato *et al*., 2008), 3 of which were recognized as under positive selection (Figure 5, Appendix II) and 5 that were identified as under balancing selection (Figure 5, Appendix II). A total of 17 out of the 25 loci were identified as neutral (Figure 5). Also, variability in genotype frequencies was tested with a X^2-test, where 15 out of the 25 loci came out significant (Appendix I).

Frequency of cod eggs and juveniles

Tvedestrandfjord

In Tvedestrand, a total of 150 eggs were scored in the assignment test, 17 of the eggs were scored to the NC reference sample (11%), and 133 eggs were scored to the CC reference (89%). Of the juveniles, a total of 76 individuals were successfully assigned. 34 of the

juveniles were scored to the NC cod reference (45%), and 42 to the CC reference (55%)(Figure 2). In this fjord there were some NC eggs appearing in the system during the season, even in the inner basins. The highest frequency of NC eggs was early in March, with the inner station having the highest amount of NC eggs (Figure 2). Sampling done in early February and late March had lower number of NC eggs and also a lower number of eggs in general (Figure 2). The lowest frequency of CC was in June, and the inner stations consisted of both CC and NC, however there was a higher frequency of cod coming from the North Sea. The samples from the outer station consisted only of NC cod (Figure 3). In the last sampling done in early autumn, the frequency of North Sea cod is considerably lower, and coastal cod is again the dominating component (Figure 3).

Topdalsfjord

In Topdalsfjord, a total of 119 eggs were scored in the assignment test, 9 eggs were assigned to the NC reference sample (8%), and 110 eggs were assigned to the CC sample (92%). A total of 21 juveniles were successfully assigned, 3 were scored to the NC reference (14%) and 18 were scored to the CC cod reference (86%)(Figure 4). In Topdalsfjord spawning fish had a very small quantity of NC (10%), and consisted mainly of CC (90%)(Figure 4). The egg sample from this fjord contained more eggs than from any of the egg samplings done in Tvedestrand. The fjord also show a clearer segregation, with a higher frequency of North Sea cod eggs in the outer inlet, and a tendency of lower frequency in the inner parts (Figure 4). This is also the case for the juvenile samples from both summer and early autumn, where the frequency of North Sea cod is generally higher in the outer parts of the fjord (Figure 4).

Table 1. The sample localities, number of individuals, life stage of individuals, and deviations from Hardy-Weinberg genotype proportions within samples (F_{IS}). An estimate of observed (H_O) and expected (H_E) heterozygosity was calculated using GDA software. P-values are displayed uncorrected.

				Deviations from Hardy-Weinberg proportions			
Sample site	Date	Sample size	Life stage	Average F_{IS}	P-value	H_O	H_E
Topdalsfjorden	19-25.01.15	52	Spawning	0.228	0.5753	0.262	0.335
Tvedestrand	20.02.15	2	Eggs	0.090	0.7645	0.352	0.387
Tvedestrand	27.02.15	49	Eggs	0.108	*0.0305*	0.355	0.397
Topdalsfjorden	05.03.15	119	Eggs	0.051	*0.0195*	0.361	0.380
Tvedestrand	06.03.15	46	Eggs	0.009	*0.0211*	0.403	0.406
Tvedestrand	13.03.15	27	Eggs	0.049	0.9750	0.385	0.405
Tvedestrand	24.03.15	25	Eggs	0.020	0.3600	0.385	0.393
Tvedestrand	08.06.15	50	Juvenile	0.120	0.4083	0.367	0.415
Topdalsfjorden	15.06.15	10	Juvenile	0.094	0.9987	0.377	0.416
Topdalsfjorden	15.09.15	11	Juvenile	-0.067	0.9972	0.428	0.402
Tvedestrand	12.10.15	26	Juvenile	0.039	0.9843	0.378	0.394

Table 2. Table showing expected (H_E) and observed (H_O) heterozygosity at individual SNPs, amount of variation among populations (F_{ST}) for each locus and deviations from Hardy-Weinberg (F_{IS}). The P-values refer to the Hardy-Weinberg probability test, estimated by Markov chain method. P-values are corrected by FDR, however average is displayed uncorrected. Loci that came out significant from the X^2-test are shown in italics.

Locus	H_E	H_O	F_{IS}	F_{ST}	P-value
Gdist_192507_8811	0.407	0.283	0.305	0.072	0.002
Gdist_187987_1900	0.373	0.300	0.193	-0.007	0.004
Gdist_08560_1753	0.369	0.265	0.283	0.101	0.006
Gdist_94561_5380	0.327	0.280	0.145	0.041	0.008
Gdist_565459_2052	0.325	0.284	0.126	0.019	0.01
Gdist_220446_161	0.415	0.437	-0.053	0.014	0.012
Gdist_340939_1382	0.455	0.428	0.059	0.046	0.014
Gdist_342952_3812	0.484	0.438	0.094	-0.002	0.016
Gdist_270696_5455	0.352	0.289	0.178	0.045	0.018
Gdist_24797_1444	0.326	0.296	0.091	0.001	0.02
Gdist_545739_884	0.499	0.544	-0.088	0.017	0.022
Gdist_205638_419	0.473	0.501	-0.058	0.007	0.024
NS_165637_4717	0.344	0.322	0.064	0.014	0.026
Gdist_355999_102	0.488	0.495	-0.013	0.019	0.028
Gdist_267492_1644	0.496	0.440	0.112	0.018	0.03
Gdist_68779_1970	0.408	0.365	0.105	0.003	0.032
Gdist_285988_206	0.478	0.426	0.108	-0.002	0.034
NS_108658_6546	0.496	0.448	0.096	0.023	0.036
NS_270695_1166	0.425	0.400	0.060	0.034	0.038
Gdist_565425_253	0.403	0.353	0.123	0.022	0.04
NS_207040_1618	0.500	0.494	0.012	0.019	0.042
GENE_06343_3566	0.495	0.457	0.075	-0.001	0.044
Gdist_580271_3190	0.379	0.366	0.034	0.029	0.046
Gdist_626723_12222	0.124	0.102	0.172	0.094	0.048
Gdist_141343_600	0.420	0.418	0.005	0.012	0.05
Average	0.410	0.377	0.096	0.021	0.733

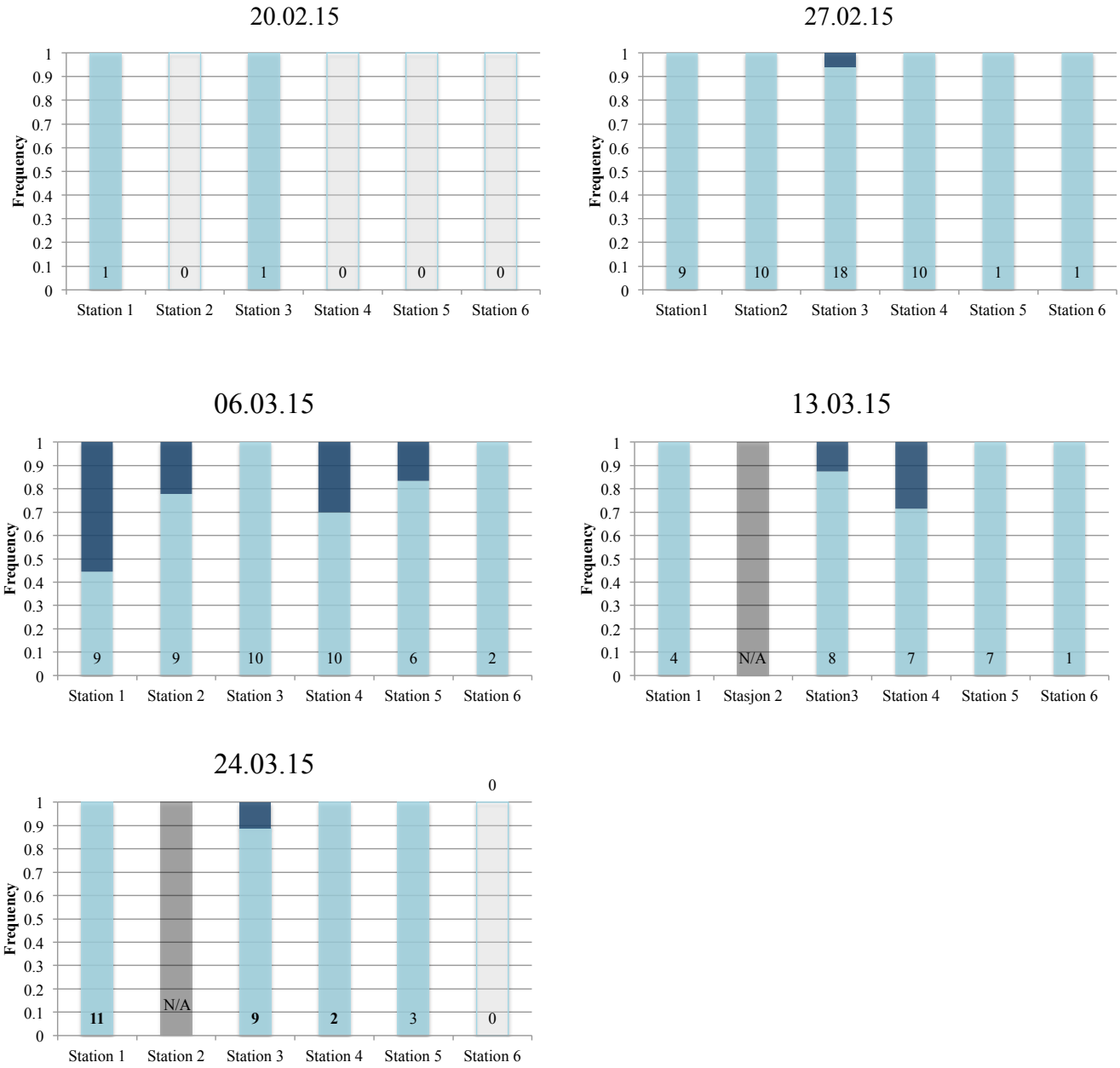

Figure 2. Frequency and distribution of cod egg at the different sample localities through spawning season in Tvedestrandfjord. Coastal cod frequency is shown in light blue, and North Sea cod is shown in dark blue. Dark grey bars indicate that no sampling was done at this station, and white bars indicate no eggs were sampled.

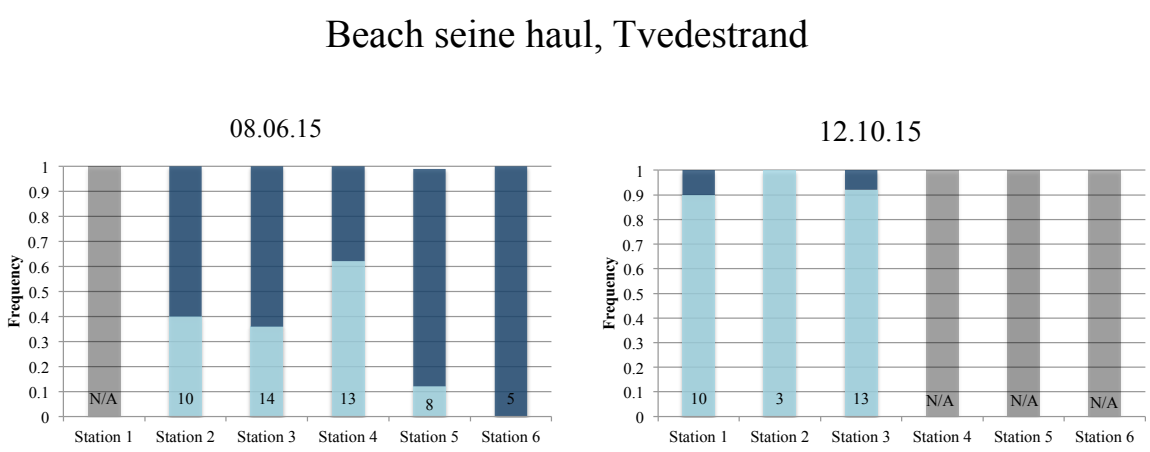

Figure 3. Frequency and distribution of juvenile cod (0-group) in Tvedestrand sampled from the different stations, early summer and early autumn. Lights blue bars show the frequency of coastal cod, and dark blue bars show frequency of North Sea cod. Dark grey bars indicate that no sampling was done at this station.

Topdalsfjord

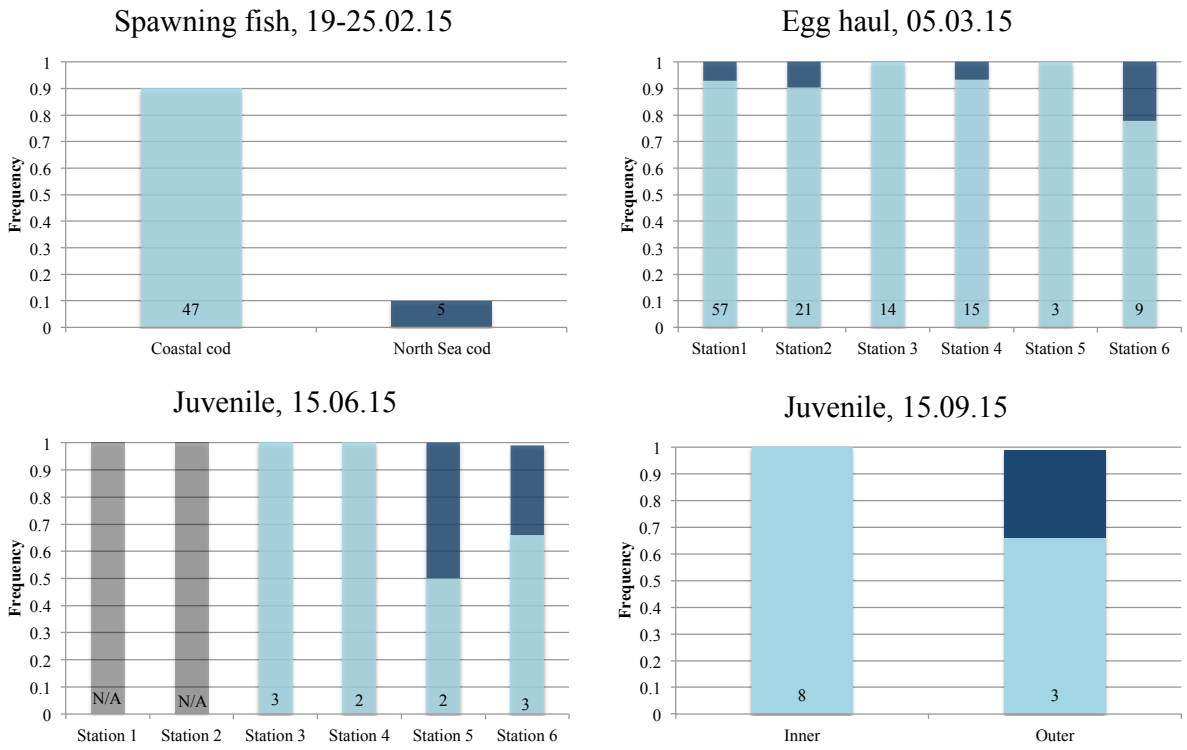

Figure 4. Frequency and distribution of spawning fish, eggs and juvenile sampled from Topdalsfjorden during the season, from February until September. Juvenile sample from September is sorted as “inner” and “outer, and all spawning fish was caught near the stations assigned to the inner stations of the fjord. Inner stations are located in the inner part of the fjord at around station 2-4, while outer stations are located around station 5-6.

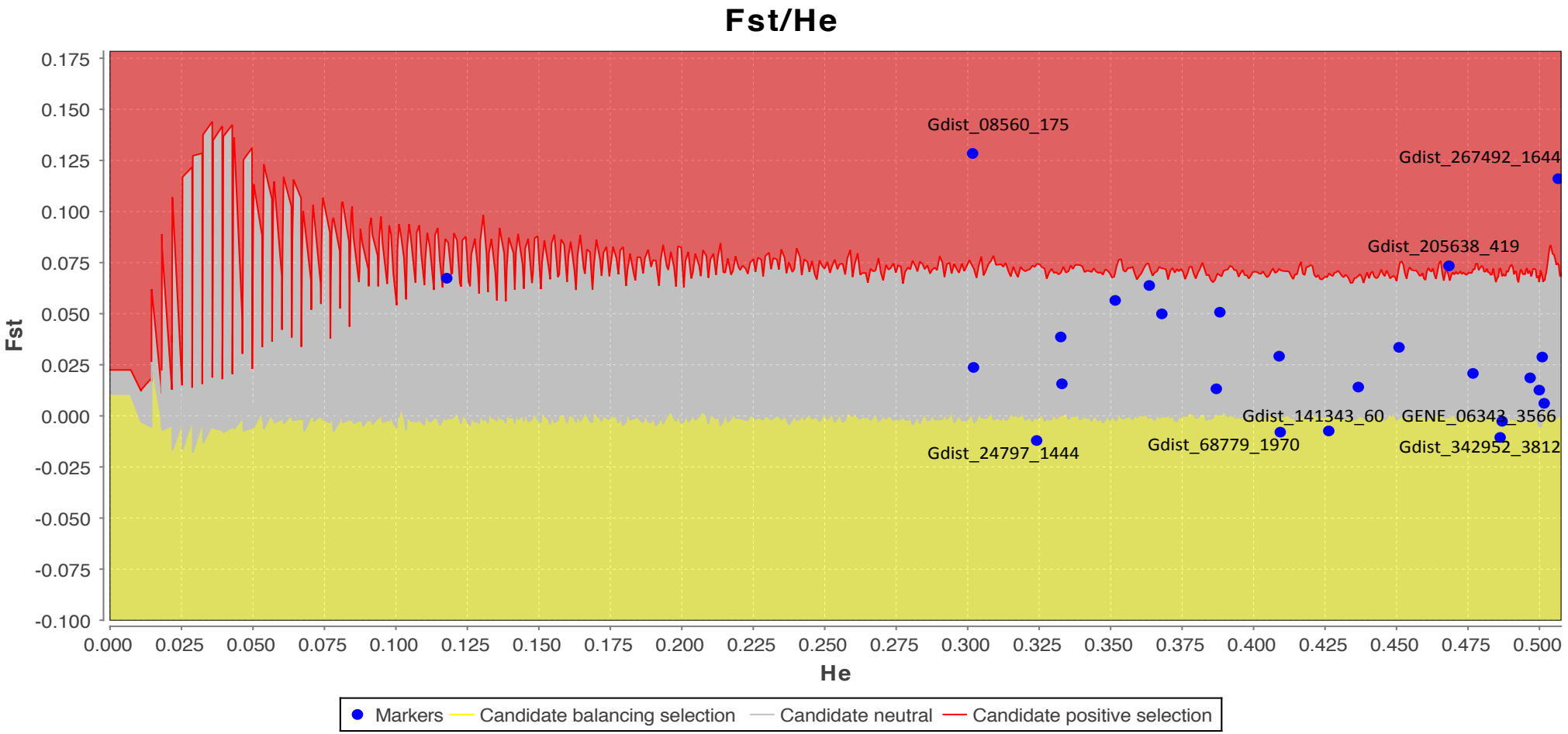

Figure 5. LOSITAN analysis, displaying the 25 SNPs and their level of neutrality. Red area indicates loci under positive directional selection, yellow area indicates loci under balancing selection and grey area indicates neutral loci. In total, 3 loci were displayed as under positive directional selection, and 5 loci came out as under balancing selection. 17 out of the 25 loci were displayed as neutral (See Appendix II

Discussion

Coastal marine systems are complex, as physical parameters as topography and currents, along with biological ones interplay in how populations are structured. Understanding such complex systems require combination of approaches targeting different elements of the mystery. This thesis combine extensive field work with novel genomic analysis, and give new information about how cod originating from offshore and coastal areas interplay the first months after spawning in coastal areas. I find that the frequency of the genetically distinct groups does change over the season, and there is a lot of variability in relation to the distribution of coastal and offshore cod components on our coast. In both fjords, cod ended up being dominated by local fjord populations inside the fjords and offshore cod dominating the outer parts, however the two fjords varied in how this pattern emerged. Below I discuss the findings in relation to the biology of cod and to other findings.

Retention and population structuring

Previous studies have shown that NC cod eggs and larvae most likely drift into coastal waters (Knutsen *et al*., 2004; Stenseth *et al*., 2006). However, there are genetic indications that these early life stages do not mix extensively with cod from sheltered fjords (Knutsen *et al*., 2011; Sodeland *et al*., 2016). In this study, I wanted to look at if the structure between cod from sheltered and exposed areas are present from spawning until autumn, or if there is a mixture of cod from different origin in the entire fjord at first, that later are segregated into structure. Results from the two fjords chosen in this study both showed that oceanic cod eggs do penetrate into sheltered areas of the both fjords, however, there seems to be differences in the

magnitude (Figure 2, Figure 4). In Tvedestrand there was a higher frequency of the NC eggs during the spawning season, indicating that this fjord system could be more open to oceanic water (Figure 2). This is also in line with the findings of Ciannelli *et al*. (2010), that fjord currents do retain eggs and larvae, but with several "leaking events", mixing water in large parts of the fjord. However, since there are no spawning fish sampled from Tvedestrand, it is not possible to say if the eggs have drifted in with currents from the North Sea, or if there actually was cod with genes resembling NC cod spawning inside the fjord, as we do find a few adult cod in fjords resembling NC cod genetically. In this study, cod were assigned to either the NC or to the CC reference sample. Knutsen *et al*. (2011) have shown that cod populations inhabiting the outer skerries show less structuring against the North Sea than the cod populations inside the fjord, and could represent a local population receiving a substantial amount of gene flow from the North Sea. In this assignment done by the GENECLASS II software (Pirya *et al*., 2004), it is not possible to distinguish between cod from the outer areas or the North Sea but with more research being done in this field, e.g. from full genome sequencing, this might be possible in the future.

In Topdalsfjord we generally found a clearer segregation of the two components than we did in Tvedestrand through the whole season, indicating that retention in this area is stronger (Figure 2, Figure 3, Figure 4). Atlantic cod is a seasonal batch spawner, meaning it distributes its eggs over multiple spawning events. The spawning season lasts from February until May, and thus it is expected that there will be some pulses of eggs coming into the fjords from the ocean during this time period. This life history strategy spreads the risk for e.g. fjord cod to not "loose" their eggs out of the sheltered areas due to bad timing when pulses of water go out the fjord. Because an egg sampling gives a sort of snapshot of the egg distribution at exactly that point in time, some of the influxes from offshore areas may be missed, due to the fact that it is unfortunately not possible to be out sampling at all times.

In June there is a higher number of juveniles in both fjords than found in the sampling done later in September and October (Figure 3, Figure 4). However, the two fjords contrast in the frequency of the two major genetic groups. We found juveniles originating from offshore and fjord systems in both fjord transects, however more mixing is evident in the Tvedestrand fjord in June than for the Topdalsfjord (Figure 3, Figure 4). As this pattern is also present for the egg stage, it is likely that Topdalsfjord have a stronger retention of early life stages than Tvedestrand. Mixing of water masses in Tvedestrand has also been shown in Ciannelli *et al*. (2010) where water exchange in the entire fjord takes place under circulation reversal events. These reversal events might be transporting the eggs coming from the North Sea further into the inner basins, as well as transporting coastal cod eggs out of the fjord, providing more mixing in Tvedestrand than what is found in Topdalsfjord. In both fjords mortality is high over the summer, in line with previous studies showing that the mortality rate of juveniles is extremely high during the warm months of July (Johannessen, 1989; Freitas *et al*., 2015), and only a few survive into the months of autumn. We thus expected that natural selection would be stronger in this period, selecting for cod that originate in either fjords or offshore areas.

Local adaptation and selection

The results from this thesis show that the genic differentiations for most of the SNP's is variable during the season, and do indeed change in frequency over the time-period we sampled (Appendix I). Note that for both our fjords, we do end up with genetic structure between inner and outer areas, possibly a result of natural selection. However, the advantage of the genes located on the recently identified inversions or other undetected loci under selection is probably not that extensive, as we would expect to see a much clearer pattern where one population eventually would disappear or go extinct. When testing for loci under

selection, only 3 out of the 25 loci was identified as positive outliers, however as these loci are not sampled by chance, the elevated level of genetic differentiation here could bias the analysis in showing less loci under directional selection than is actually the case (Figure 5, Appendix II). The three loci that was identified as positive outliers by the LOSITAN analysis had the highest F_{ST} values overall, however, the loci did not come out as significant when tested for directional change in gene frequencies (positive slope in regression analysis) over the season (Appendix I, Appendix II). The five loci that came out as balancing outliers all had negative F_{ST} values, and also much lower R^2 values, clearly showing less variability in the frequency distribution through the season (Appendix I, Appendix II). Most of the loci from the LOSITAN analysis came out as neutral, although some of them had a slightly higher F_{ST} value (Appendix II). Also, several of the loci behaving neutral in the LOSITAN analysis were still displaying significant heterogeneity in the data (Appendix 1), indicating that maybe not all loci that is under selection was detected by the LOSITAN analysis. New research is mounting evidence that recurrent adaptations in the Atlantic cod, from migratory to stationary forms (Berg *et al.,* 2016; Kirubakaran *et al.,* 2016) and from offshore to coastal ecotypes (Sodeland *et al.,* 2016) are also facilitated by large (several megabases), polymorphic chromosomal inversions. These inversions may be protecting adaptive loci from recombinating, and the affected genomic regions may capture multiple loci involved in adaptation to contrasting habitat types or life-history strategies (Sodeland *et al*., 2016). Thus, the survival of eggs and juveniles in this thesis might be affected by the chromosomal inversions, providing a structure in genetic origin during the summer when mortality is very high.

Conclusion

In summary, this study shows that there is a change in frequency in the distribution of eggs and larvae in different fjords during the season. Both fjords ended up with roughly the same pattern with coastal cod dominating the inner parts of the system, and North Sea cod in the outer areas. This pattern that emerges does indicate that it is likely that there is selection against cod being transported in to non-native areas. However, due to the small geographic scale of the study and limited sample size (fjords and eggs) no clear conclusion can be drawn, and further investigations are needed.

Litterature

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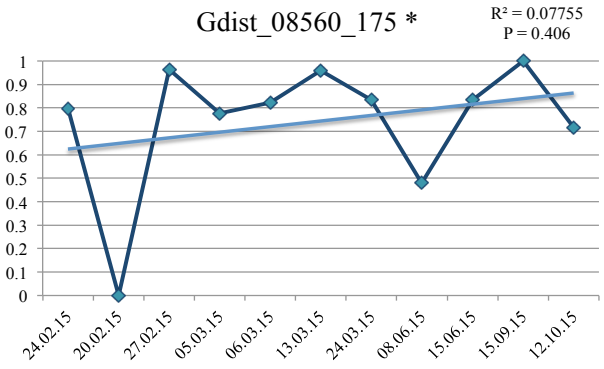
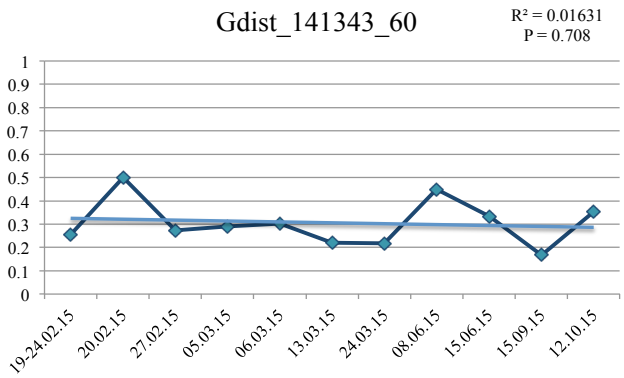
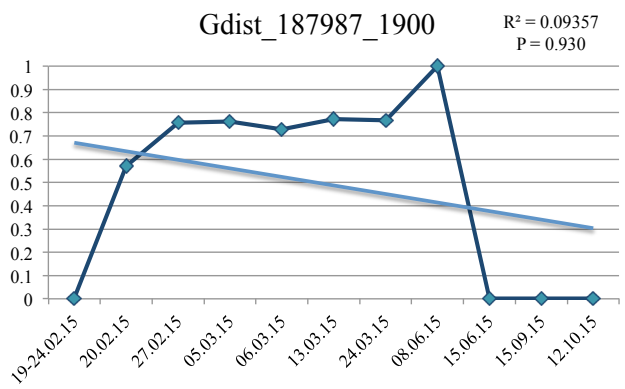
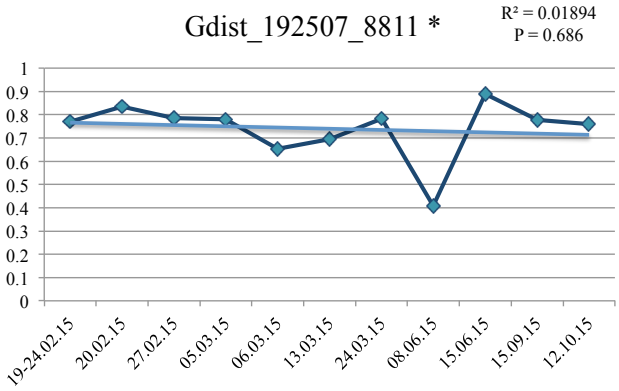
Appendix 1

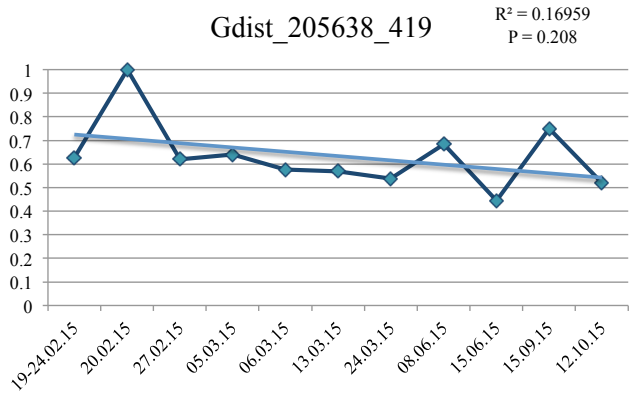
Genic differentiation

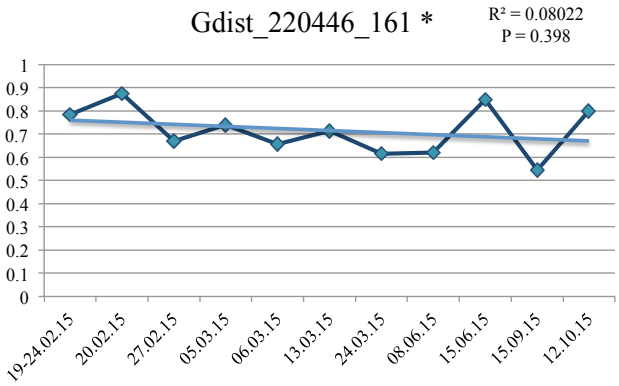


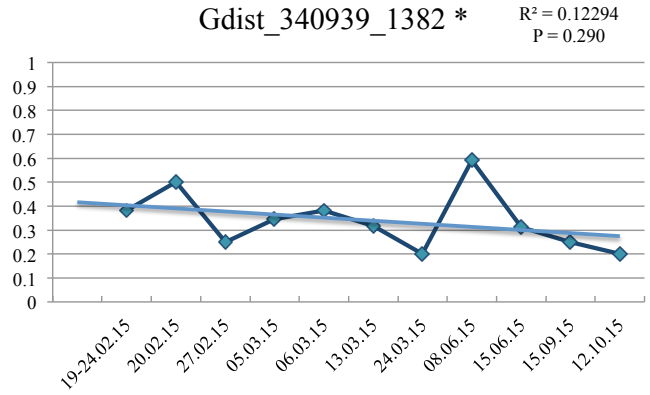











Gdist_340939_1382 *
R² = 0.12294
P = 0.290
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0.2
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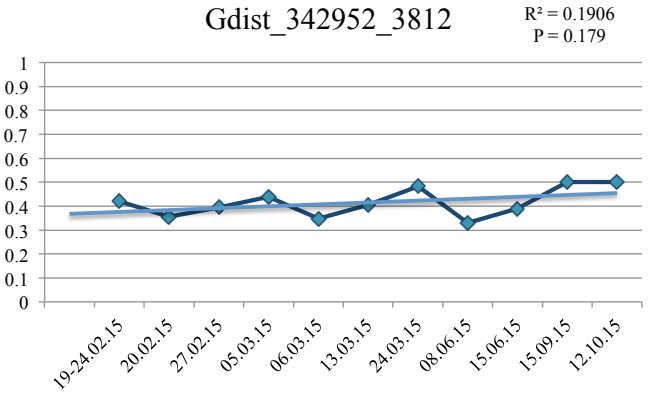
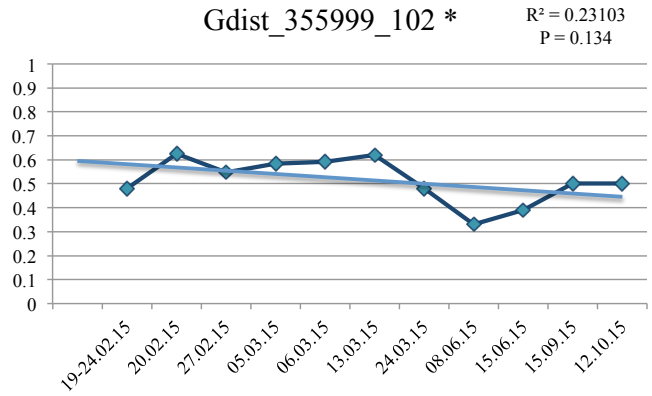
05.03.15
06.03.15
13.03.15
24.03.15
08.06.15
15.06.15
15.09.15
12.10.15


Gdist_342952_3812
R² = 0.1906
P = 0.179
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
19-24.02.15
20.02.15
27.02.15
05.03.15
06.03.15
13.03.15
24.03.15
08.06.15
15.06.15
15.09.15
12.10.15


Gdist_355999_102 *
R² = 0.23103
P = 0.134
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
19-24.02.15
20.02.15
27.02.15
05.03.15
06.03.15
13.03.15
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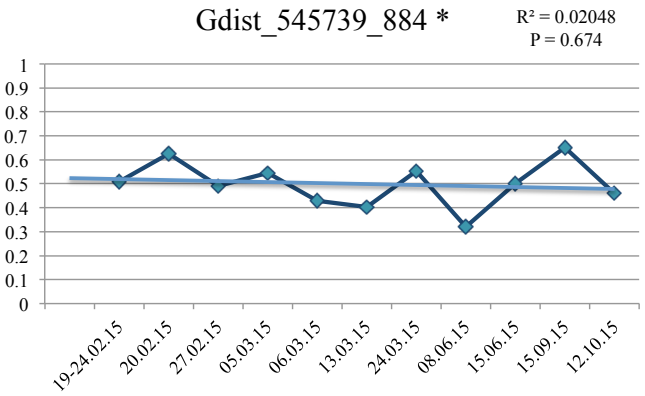
08.06.15
15.06.15
15.09.15
12.10.15


Gdist_545739_884 *
R² = 0.02048
P = 0.674
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
19-24.02.15
20.02.15
27.02.15
05.03.15
06.03.15
13.03.15
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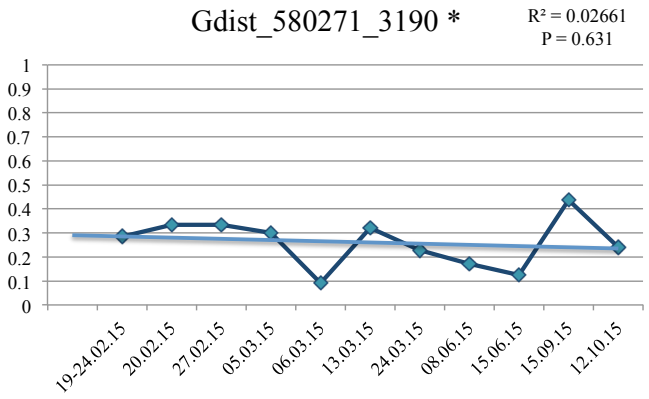
08.06.15
15.06.15
15.09.15
12.10.15


Gdist_580271_3190 *
R² = 0.02661
P = 0.631
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
19-24.02.15
20.02.15
27.02.15
05.03.15
06.03.15
13.03.15
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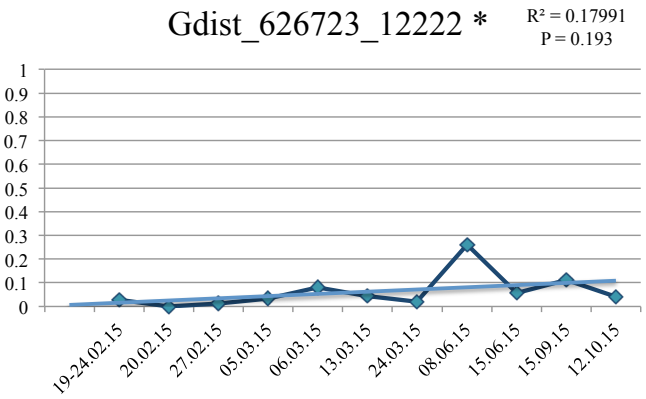
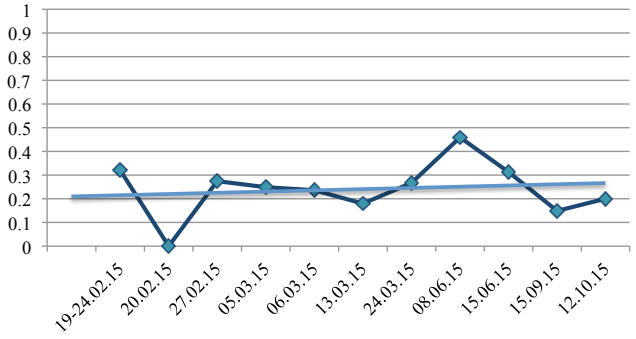
08.06.15
15.06.15
15.09.15
12.10.15


Gdist_626723_12222 *
R² = 0.17991
P = 0.193
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
19-24.02.15
20.02.15
27.02.15
05.03.15
06.03.15
13.03.15
24.03.15
08.06.15
15.06.15
15.09.15
12.10.15

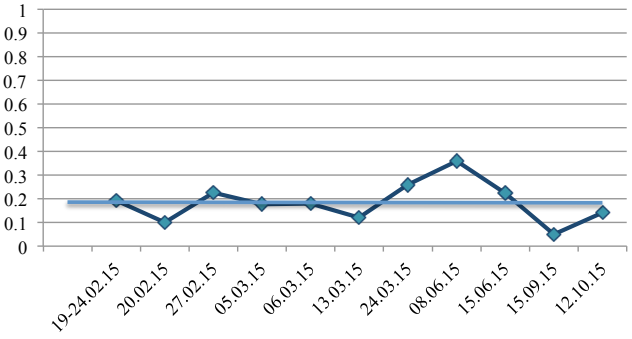
Gdist_565425_253 *

$R^2 = 0.02195$
$P = 0.663$



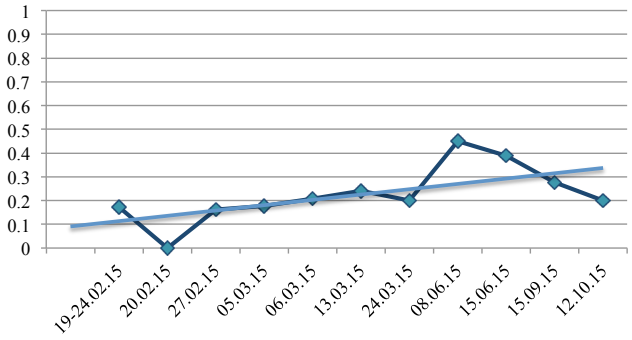
Gdist_565459_2052 *

$R^2 = 0.00011$
$P = 0.975$



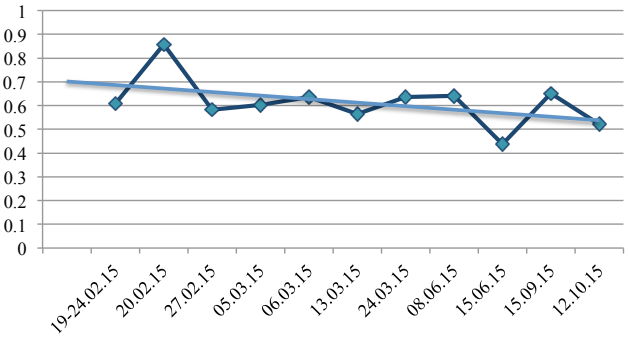
Gdist_270696_5455 *

$R^2 = 0.38952$
$P = 0.040$



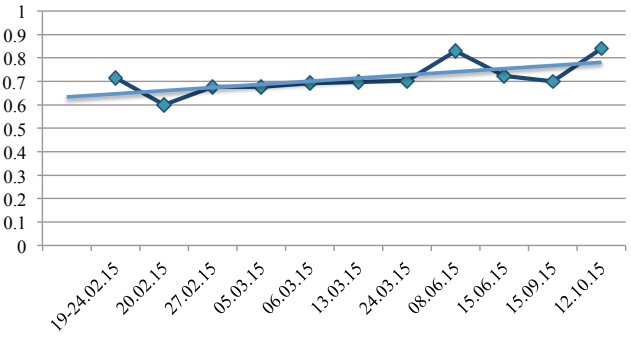
Gdist_285988_206

$R^2 = 0.23019$
$P = 0.153$



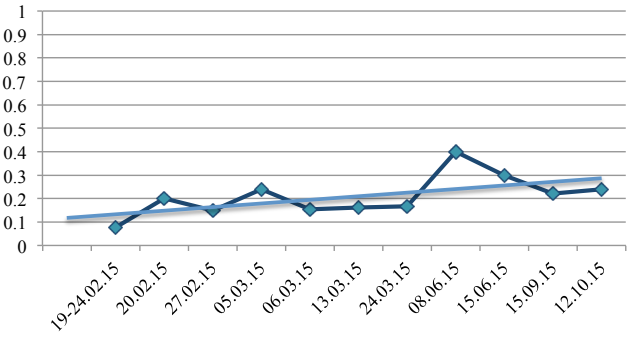
Gdist_68779_1970

$R^2 = 0.42855$
$P = 0.028$



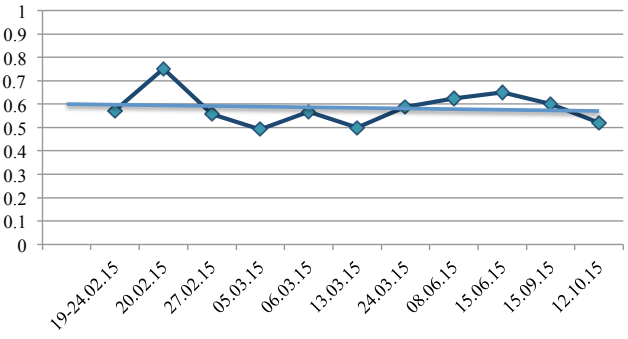
Gdist_94561_5380 *

$R^2 = 0.34671$
$P = 0.056$



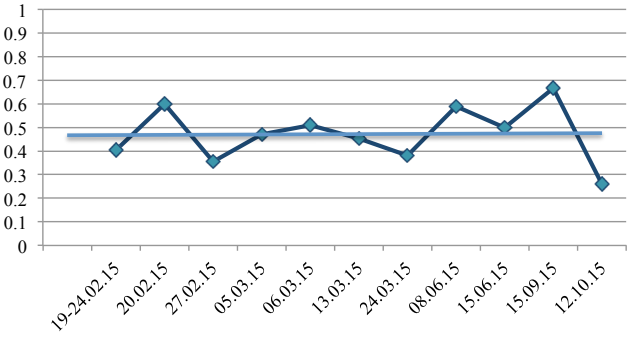
GENE_06343_3566

$R^2 = 0.01456$
$P = 0.723$



NS_108658_6546 *

$R^2 = 0.0005$
$P = 0.947$



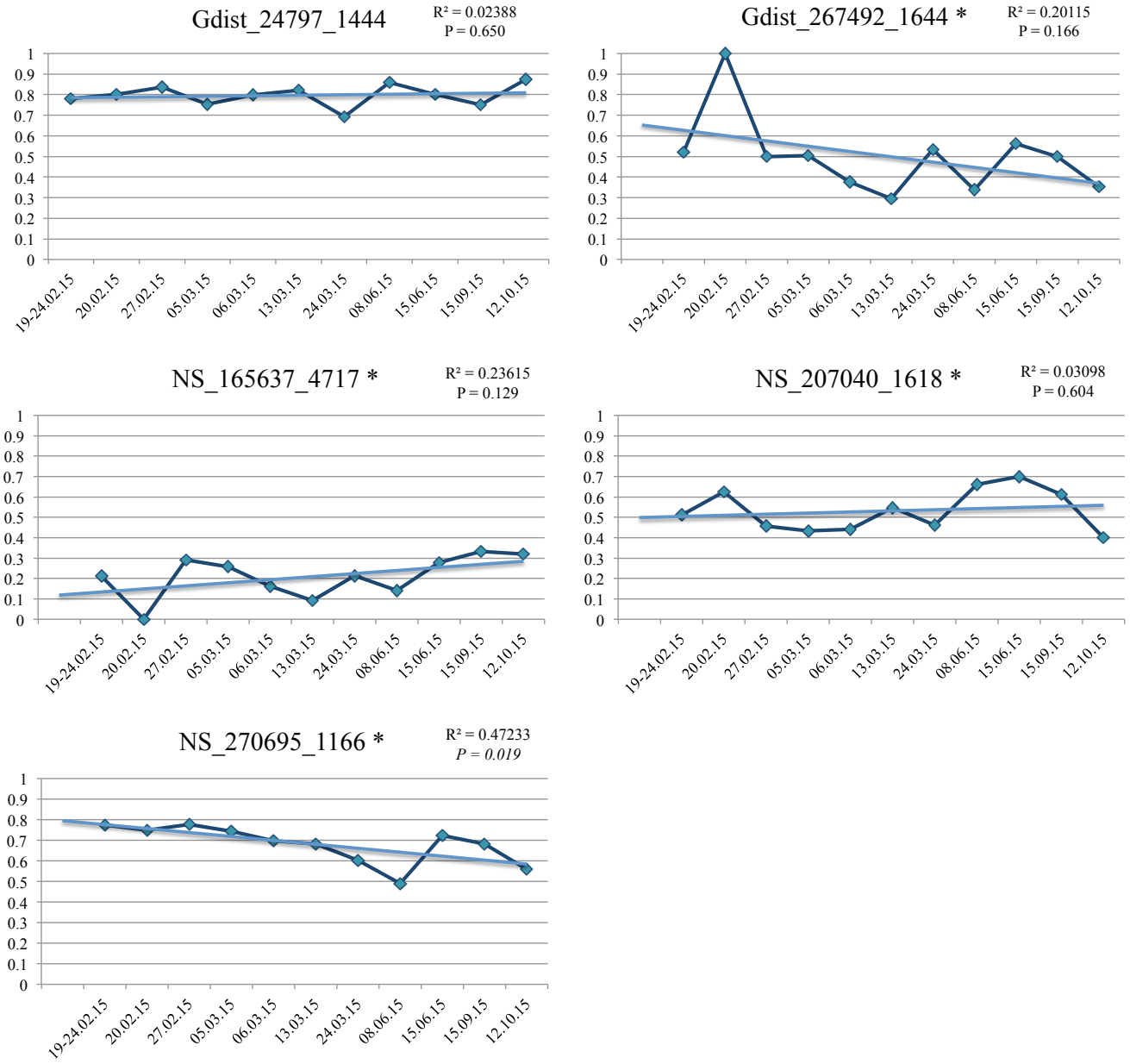


Figure 1 Genic differentiation for all loci over the season. Loci that came out significant from the exact G-test is marked with a *. The overall p-value for all loci came out highly significant (P<<0.001). The R^2 value and p-value from the simple regression analysis is displayed in top right corner (uncorrected), with loci that came out with a slope being significantly different from zero displayed in italics.

Appendix II

Table 1. Table displaying heterozygosity (H_{ET}), and amount of variation among populations (F_{ST}) for each locus, with P-value and FDR correction. Loci showing positive selection is displayed in red, and loci showing balancing selection is displayed in yellow.

Locus	H_{ET}	F_{ST}	P(Simul F_{ST}<sample F_{ST})
Gdist_08560_175	0.301	0.128	0.999
Gdist_141343_60	0.426	-0.007	0.003
Gdist_187987_1900	0.363	0.063	0.953
Gdist_192507_8811	0.388	0.050	0.886
Gdist_205638_419	0.468	0.073	0.908
Gdist_220446_161	0.408	0.029	0.559
Gdist_24797_1444	0.324	-0.012	0.001
Gdist_267492_1644	0.506	0.116	0.999
Gdist_270696_5455	0.351	0.056	0.913
Gdist_285988_206	0.476	0.020	0.406
Gdist_340939_1382	0.450	0.033	0.662
Gdist_342952_3812	0.486	-0.010	0.003
Gdist_355999_102	0.496	0.018	0.363
Gdist_545739_884	0.501	0.006	0.049
Gdist_565425_253	0.367	0.049	0.872
Gdist_565459_2052	0.302	0.023	0.422
Gdist_580271_3190	0.386	0.013	0.227
Gdist_626723_12222	0.117	0.067	0.889
Gdist_68779_1970	0.409	-0.007	0.001
Gdist_94561_5380	0.332	0.015	0.337
GENE_06343_3566	0.486	-0.002	0.019
NS_108658_6546	0.500	0.028	0.612
NS_165637_4717	0.332	0.038	0.730
NS_207040_1618	0.499	0.012	0.262
NS_270695_1166	0.436	0.014	0.257