Seascape ecology of Atlantic cod (Gadus morhua) in coastal Skagerrak: population structure, connectivity and role in fish assemblage

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Preface

Three years ago, I set sail and embarked on this incredible journey, full of excitement and exhilaration. Now the journey has come to an end, and I look back at these years as the most difficult, hardening, but also most valuable and rewarding years of my life. I am so grateful for everyone that took a part in this journey, and for everyone I met along the way.

First and foremost, I would like to thank my main supervisor, Even Moland, for being an incredible teacher and mentor. The times we spent during fieldwork are all precious memories to me, from learning how to distinguish between the fish species, and how to set out fyke nets for proper sampling, to wakeboarding and singing songs around the bonfire with “the crew” at Bolærne. Thank you for being patient with me and for giving me the tools that I needed to bring this thesis safe to shore.

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To all of you that I met along the way but are not mentioned here, thank you all for taking a part on this journey with me. I look forward to the next journey.

Ann-Elin Wåröy Synnes

Abstract

Atlantic cod populations along the Norwegian Skagerrak coast consist of two known ecotypes. During the last decade, there has been a dramatic decline of larger cod and other piscivorous fish species in eastern Skagerrak. In my PhD-project, I set out to study the outer Oslo fjord seascape with a main focus on the Atlantic cod (*Gadus morhua*) populations residing in the system, with the aim to generate fundamental knowledge on ecology, and evaluate potential for restoration of local populations. We conducted studies on genetic structuring of the two known ecotypes of cod present in this area, and combined genetic and oceanographic data to reveal potential spawning sites. Moreover, the absence of top predators gave the opportunity to investigate how the ecosystem compared to a contrasting seascape harboring a system of MPAs. First, we studied the potential for natural selection to act on a specifically designed single nucleotide polymorphism (SNP) panel used to differentiate between the two ecotypes of Atlantic cod, testing if these high-graded SNPs were unreliable for discriminating populations. We found no evidence for selection and concluded that high-graded marker panels under putative natural selection indeed represent a valid tool for identifying population structure in this species. Second, we investigated the fish assemblage in outer Oslo fjord to quantify and characterize the species present and compared the observed patterns to a protected seascape in southern Norway. We found evidence of a mesopredatory release in outer Oslo fjord, likely resulting from fishing down of the larger top predator species. Average length of cod sampled in the partially protected seascape was significantly larger than for cod caught in outer Oslo fjord. Mesopredatory fish species was in general more variable in the outer Oslo fjord seascape, and more stable in the Tvedestrand MPA. Third, we used genetic data from 0-group and 1-group cod coupled with a biophysical model of ocean drift to infer likely sources of cod recruits to the outer Oslo fjord seascape. Three potential spawning areas were revealed inside the sampling areas, and three potential extant sources were pointed out in the North Sea and Kattegat. We found that both ecotypes of Atlantic cod were present in outer Oslo fjord as both 0-group and as older fish. Fourth, we followed the 2016 cohort of Atlantic cod from 2017 to 2019 with the aim of distinguishing whether there was a higher survival of local 'fjord' cod compared to 'North Sea' assigned cod. We found that the 'fjord' ecotype had a higher proportion of individuals surviving their first year. However, both ecotypes disappeared from the system at around 3 years of age. This thesis demonstrates that: (1) The use of high-graded SNPs under putative selection can serve as a valuable tool in population identification. (2) Absence of larger top-predator species such as Atlantic cod act to increase the abundance of lower level carnivores, which can lead to a shift in ecosystem dynamics. (3) Both ecotypes of Atlantic cod are contributing to viable recruits in the area studied. (4) Both ecotypes experience high mortality, and few survive beyond age 3. Thus, present harvesting modes and -levels seem incompatible with rebuilding of age structure and biomass of local cod populations.

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1 Introduction

1.1 Historical decline of Atlantic cod and the present situation in eastern Skagerrak and outer Oslo fjord

For many centuries, Atlantic cod has been, and still is, one of the most commercially important fish species in the world. Traditional cod fishery dates back to the discovery of the New World and has been vital for settlement along the coast of Northern Europe as well as North America. After the earliest journeys of discovery to the northeastern coast of North America, exploitation of the cod in the sea around the Newfoundland area began immediately. The early explorers arriving at the coast of New England even named Cape Cod after the fish. Soon, the abundance of fish in the New World was common knowledge to the people of Europe. However, during the 16th to 18th centuries, fishing had limited impact on the cod stocks in Newfoundland, and annual harvest rates have been estimated to <5% (Rose, 2004). In the late 1800s, introduction of improved technology as cod traps and longlines, as well as the introduction of the bottom trawl technology in the early 1900s, gave fishermen the ability to harvest more efficiently with less effort. As the vessels grew in size and power, fishermen from Europe and East Asia were added to the fishery, resulting in catches of over 800,000 t in the late 1960s and early 1970s. Canada extended its jurisdiction to 200 miles from its previous 12-mile limit in 1977, eliminating most of the international vessels. In the years that followed, there was a small recovery of cod from the reduction of fishing, however, as the fishery management gave out excessive subsidizations, record-low levels of biomass lead to a fishing moratorium on the largest Canadian stocks in 1992. The result from the overfishing was devastating economic, social and ecological consequences, still visible to this day. Most of the cod stocks in the Northwest Atlantic are still recovering, and remain classified as ‘overfished’.

Today, most of the Atlantic cod populations in the North Atlantic are suffering from depletion (Svedäng & Bardon, 2003; Hutchings & Reynolds, 2004; Brander, 2007; Jakobsdóttir, 2011; Barceló et al., 2016). Although, while the declines in the Northwest Atlantic have been severe and resulted in a collapse of most cod stocks, it has not been as extreme in the Northeast. Atlantic cod stocks were heavily exploited in the North Sea after the famous ‘gadoid outburst’ in the 60s and 70s (Cushing, 1984), and great concerns was expressed about the decline in biomass (Cook et al., 1997). In recent years, there have been an increase in abundance of cod located in the northern part of the North Sea (Kjesbu et al., 2014), however, this increase is not observed for the stocks located in the southern parts (ICES, 2019).

I addition to fishing, synergetic effects of increasing temperature (Clark et al., 2003), ocean acidification (Frommel et al., 2012), habitat destruction (Lilley & Unsworth, 2014) and reduced oxygen (Holt & Jørgensen, 2015) are all affecting the cod populations. These environmental alterations have resulted in changes in recruitment (Beaugrand et al., 2003), growth (Fogarty et al., 2008) and natural mortality (Frommel et al., 2012). Since 1996, assessment has grouped the cod stocks in the Skagerrak, the North Sea and the eastern Channel (ICES, 2005). In recent years, recruitment from all areas in the North Sea has declined, and remains low (ICES, 2019). Also along the Norwegian Skagerrak coast recruitment has been weak, except for some occasionally stronger year classes. The variability in recruitment have been shown to have a negative correlation with temperature, where warm periods give weak year classes for the stocks located in the southern part of the North Sea, which is on the limit of the geographical range of cod in the northeast Atlantic (Olsen et al., 2011). These warmer periods and variability in recruitment have also been linked to the North Atlantic Oscillation index (Brander & Mohn, 2004).

Between the North Sea and the Baltic Sea lies the Skagerrak-Kattegat area (Figure 1). For the last decades, there has been a declining abundance of Atlantic cod as well as other demersal fish in this region. In particular, studies have shown a severe decline in the abundance of large, adult cod in eastern Skagerrak since the beginning of the 1980s (Hagström et al., 1990; Fromentin et al., 1998; Svedäng & Bardon, 2003; Svedäng, 2003). Previous research has revealed that populations which are mainly dominated by small individuals will have reduced reproductive potential and are more vulnerable to environmental fluctuations (Hutchings et al., 2004; Berkeley et al., 2004; Hsieh et al., 2006). Larger individuals provide stability to the population, and selective removal of larger fish will increase variability in terms of abundance (Hsieh et al., 2006). Studies have shown that the severe decline of >30 cm adult cod in Skagerrak and

Kattegat is mainly a result of unsustainable fishing pressure (Cardinale & Svedäng, 2004). In contrast, in Öresund, a small sound neighboring Kattegat, a restriction on trawl fishery has been implemented since 1932. Although Öresund is only a tenth the size of Kattegat, the total production of cod is considerably higher in this area, and have a much greater age diversity (Svedäng, 2010).

1.2 Connectivity in the Skagerrak-North Sea – and the coastal cod population-complex

Historically, local adaptation was thought to be a rare phenomenon in the marine environment, and marine species was traditionally viewed as demographically open populations. However, within the past decade, evidence that contradicts this concept has mounted, pointing to the existence of fine-scale population structure for several marine species in various marine environments all over the world (Pampoulie et al., 2004; Kovach et al., 2010; Hoffman et al., 2012; Carreras et al., 2017; Le Moan et al., 2019). Population genetic mechanisms generating local adaptation can arise due to differences between populations and the environments they inhabit. Species possess the means for local adaptation in their gene pool of genetic variation. As environments change, populations can evolve traits through natural selection, which can increase fitness and thus increase adaptation to local conditions (Palumbi et al., 2019). Populations are locally adapted when individuals with local genomes have higher fitness in their local habitats when compared to individuals with genomes from alternative habitats (Kawecki & Ebert, 2004). Genes that might be adaptive in one habitat may be selected against in another, thus, sympatric populations inhabiting separate niches within a single geographic area could also be subjected to divergent selection (Rundle & Nosil, 2005).

The life cycle for most marine organisms include a planktonic egg and larval stage. The small size of eggs and larvae coupled with the vast, fluid, ocean makes them susceptible to be carried far away from original spawning locations. High levels of gene flow are thought to dampen the effects of local natural selection through the introduction of maladaptive alleles from differentially adapted populations. Connectivity, defined as the exchange of individuals among marine populations, is a central topic related to marine ecology and conservation. Understanding of the mechanisms behind larval dispersal is crucial for management, as it is important to assess if local populations are solely dependent on self-recruitment or how much of the population is sustained from drift of eggs and larvae originating from a different population source. Connectivity between marine populations are influenced by multiple biological and hydrodynamic processes, as bathymetry, temperature and salinity, as well as egg buoyancy and larval characteristics of the species (Selkoe et al., 2010). Populations can persist when births and immigration into the population is equal or exceed deaths and emigration out from the population.

Along the Norwegian Skagerrak coast, two genetically distinguishable ecotypes of cod exist, an offshore, migratory type and a stationary ‘fjord’ type (Knutsen et al., 2018; Barth et al., 2019). Some years show a strong recruitment from the North Sea along the Norwegian Skagerrak coast, in particular when there is a high inflow of North Sea waters entering into the Skagerrak (Knutsen et al., 2004). Presence of juvenile fish and absence of older fish has brought the hypothesis that the North Sea cod is using the coastal area as a nursery habitats, where eggs and larvae drift in from spawning sites in the North Sea, and when mature venture back to parental spawning grounds (Robichaud & Rose, 2001; André et al., 2016). It is, however, still unknown to what extent the local ‘fjord’ populations located along the Norweigan Skagerrak coast is dependent on self-recruiting, or also from recruitment from external populations in the wider North Sea, or Kattegat.

1.3 The coastal fish assemblage in Skagerrak

Coastal ecosystems are heterogenous systems in the transition zone where land and ocean meet and characterized by a great variability of oceanographic conditions, consisting of an intricate patchwork of many specific habitat types. These areas are often incredibly diverse and are known for sustaining high numbers and production levels of fishes, many of which are economically important (Jackson et al., 2001). It serves as an essential ecosystem for numerous of important fish species, seeing that many fish use these areas for feeding and nursery habitats for juveniles (Munk et al., 2014; Staveley et al., 2017). In parallel, coastal marine habitats are subject to strong pressure from human activities. Although the coastal zone covers only about 20% of the earth's total land area, it is estimated that half of the world’s human population now lives within 60 km from the coast (Post and Lundin, 1996). In temperate coastal areas, over-fishing and nutrient enrichment are two of the biggest human-induced impacts (Jackson et al., 2001; Diaz & Rosenberg, 2008). This has resulted in a dramatic decrease of larger predatory fish species. When top predator carnivores are removed from the system, mesopredatory fish species increase in abundance, which can lead to a change in ecosystem dynamics known as trophic level dysfunction. Studies have shown that removal of large top predatory species can have a major effect on habitat and fish community in both marine (Parsons, 1992), and freshwater ecosystems (Brönmark & Weisner, 1996). In eastern Skagerrak, abundance of fish >30 cm is presently greatly reduced compared to historical records from the 1920s to 1970s (Svedäng, 2003). On the Swedish west coast, filamentous algae have increased in both distribution and abundance during the past decades (Jephson et al., 2008), which is seen to correlate with the absence of larger predatory fish and release of top-down control. In addition, when mesopredatory fish species increase in abundance due to the open niche that becomes available as the top predators are removed, there has been observed a higher mortality of eggs and juvenile of the top predators (Floeter et al., 2005). In the first years of life, the Atlantic cod is subject to become prey of these mid-level carnivores, as well as being exposed to cannibalism or predation from other higher trophic level species. This change in ecosystem dynamics and increased juvenile mortality could make it harder for the top predator to redeem its place in the ecosystem.

1.4 Background for the project

In outer Oslo fjord, there has been an extreme decline of local cod populations, as well as other important fish stocks. This has raised concern from both local and regional government. In 2017, a unique collaborative project was established, with the aim of collecting new knowledge on the fish community in the fjord and lay the foundations for effective management measures that can contribute to restore the ecosystem. Estuaries and coastal seas have been given increasing attention for development of ecosystem-based management and large-scale restoration projects (Halpern et al., 2007). Marine protected areas (MPAs) are emerging as effective tools for reducing fishing pressure and regulate human activities (Browman & Stergiou, 2004), and could also be a good strategy for management in outer Oslo fjord. As MPAs are very versatile, they can be custom-made to local circumstances. They have been proven to increase both numbers and size of species living inside the reserve (e.g., Lester et al., 2009; Fenberg et al., 2012; Moland et al., 2013; Fernández-Chacón et al., 2015), spillover of post-settled juveniles and adult individuals beyond the MPA borders (Abesamis & Russ, 2005; Kerwath et al., 2013) and enhanced production of eggs and larvae inside the reserves (Polacheck, 1990; Almany et al., 2007). However, it is crucial to include a large enough area for protection, as it has been proposed that protection-induced selection can lead to selection against fish that move out of the marine reserve, and thus reduce the potential spillover effects (Villegas-Ríos et al., 2017). Spatial and temporal variation in community structure (e.g., species composition and richness, relative species abundance) are affected by both physical (e.g., light, nutrients, habitat) and biological (e.g., recruitment, predation, competition) forces. To be able to successfully restore ecosystems, it is fundamental to gain a wider understanding of these two forces and how they act together to affect the community structure, as it is crucial for implementing the right design for the MPA (Claudet et al., 2006).

2. Aims and objectives

The overall objective of this thesis was to investigate the cod populations residing in outer Oslo fjord, mainly to gather more knowledge on which populations are inhabiting the outer Oslo fjord seascape, and if present, evaluate whether there would be potential for restoration of the local coastal cod population complex given appropriate management measures. In addition, it opened up a possibility for investigating the fish assemblage in the absence of a top predator species and use this information for additional knowledge on how to best protect and restore ecological function in the area. In **paper 1**, we show that the use of genetic markers under putative selection can be a valid and useful tool for investigating population differentiation in a highly fecund species as Atlantic cod. As marine populations are subject to high dispersal potential and gene flow, population differentiation is usually low, and the use of highly differentiated loci are often employed to increase statistical power. The paper aimed to investigate if assignment of individuals with the use of high-graded markers were driven by selective mortality. The objective of **paper 2** was to get an overview of the current fish assemblage of the outer Oslo fjord system and compare it that of a partially protected seascape harboring MPAs. In that paper, I aimed to investigate patterns, differences, and similarities in species composition, -richness and -abundance, as well as size distribution of top predators present in the two contrasting study systems. In **paper 3**, the main focus was to obtain more information regarding likely sources of recruits to the outer Oslo fjord seascape. Along the Norwegian Skagerrak coast, some years show plentiful recruitment, however, these strong year classes do not seem to replenish the adult cod population (Svedäng, 2003). It is hypothesized that the offshore cod originating from the North Sea use the coastal areas as nursery grounds, but eventually migrate back to parental spawning grounds (André et al., 2016). Finally, in **paper 4** I investigated whether recruits assigned to the ‘fjord’ reference dominated as older fish, under the assumption that these individuals might display higher fitness in the coastal environment than individuals originating from elsewhere.

3. Study system

3.1 Skagerrak

The Skagerrak is a sea forming the strait running between the southeast coast of Norway, the west coast of Sweden and the north coast of Denmark, connecting the North Sea, the Kattegat and the Baltic sea. It is a productive area, and considered a valuable area for fishing, both commercially and for recreational purposes. It is a heterogenous environment, and holds a great variety of habitats, from shallow sandy and stony reefs, to the deep in the Norwegian trench. The Norwegian trench has a maximum depth of 700 m and a sill depth of 270 m, and stretches from Stadt in the West to Ytre Hvaler in the outer Oslo fjord in the East. The Skagerrak receives water from three main water sources which holds different salinities. The surface waters are dominated by water coming from Kattegat, with salinities between 20 and 30 psu. Atlantic water with salinities exceeding 35 psu enters the Skagerrak with the Norwegian trench. From the North Sea, a mixture of waters enters the Skagerrak from the west and southwest, mainly as surface waters (Rydberg et al., 1996). Upwelling of nutrient-rich waters in the middle of Skagerrak spread horizontally in the surface layer, resulting in high primary productivity, and makes it a biologically productive zone (Fonselius, 1996).

3.2 The outer Oslo fjord seascape

Research for this thesis was conducted in shallow-water environments in two national parks situated at the opening of outer Oslo fjord, located in eastern Norway at the Skagerrak coast (Figure 1). On the west side of the fjord mouth lies Færder national park, covering an area of 340 km^2 with mainland, islands, skerries but mostly ocean and ocean bottom (Figure 1). Hvaler national park is located on the eastern side of the fjord mouth, almost directly opposite of Færder, covering an area of 354 km^2 of coastal landscape and ocean (Figure 1). The national parks are known to have a diverse seascape with several marine nature types, as kelp forests, soft-bottom areas, shell sand, and eelgrass beds, which are all important for the biodiversity found in this area. Worth mentioning is also the 1200 m long Tisler-reef which is one of the world's largest known cold-water reefs (*Lophelia pertusa*), and can be found in Ytre Hvaler national park. In both national parks there has been various marine protection initiatives implemented, as lobster reserves and recently a ban on fishing Atlantic cod.

Surface salinity in Outer Oslo fjord is dominated by Skagerrak waters, and varies between 25 and 32 ppt. Below 100 m. the water is dominated by Atlantic water, which normally have a salinity above 35 ppt. Both Færder- and Ytre Hvaler national park are fairly open areas, giving it a good water exchange between water masses. Average ocean temperature in winter is approximatley 3°C and can rise to 18°C in summer. Tidal amplitude in Skagerrak is low (~10 cm) and thus negligible.

Riverine input is strong in the outer Oslo fjord with Norway’s longest river, Glomma, having its outlet within the Hvaler archipelago. The river carries with it fresh water but also high amount of nutrients from agricultural runoff, and large amounts of sand and clay each year. Glomma’s water flow has increased significantly since 1990, and had one of the highest annual mean concentrations of phosphorous measured in Norway’s rivers in 2017 (13 ug/l) (Kaste et al., 2018). In general, outer Oslo fjord is an oceanographically complex region and provides a great study system for understanding species ecology and connectivity of marine organisms.

3.3 Tvedestrand fjord and seascape

The Tvedestrand fjord is located on the southern coast of Norway, extending approximately 8 km^2 inland. It includes several sills and basins, and hold a great variation of habitats, such as eel grass beds, mud flats and sparse kelp forests (Freitas et al., 2016). In 2012, Tvedestrand fjord and outer coastal areas was subject to a zoning process, in which ≈15% of the municipality waters were included in no-take- or partially protected areas. The no-take reserve covers approximately 1.5 km^2 and was mainly implemented for protection of lobster and fish against commercial fishing. On each side of this no-take zone, there is a partially protected zone, where only hook and line type gear are allowed. In addition, a 4.9 km^2 partially protected area extend from the outer islands to around 50 m depth.

The fjord has a two-layered structure, where in the inner zone there is a variable freshwater surface layer, where the temperature and salinity increase with depths down to 30 m (Ciannelli et al., 2010). The fjord also holds important nursery areas for cod, and an inshore spawning aggregation (Knutsen et al., 2007; Ciannelli et al., 2010).

3.4 Topdalsfjord

Topdalsfjord is a fjord located in southern Norway, outside of Kristiansand, and stretches approximately 10 km^2 inland. It is a part of a fjord system, where a 25 m deep sill (threshold) is located at the mouth of the fjord, separating Topdalsfjord from the Kristiansand fjord. It holds several sills and basins, and have a maximum depth of 78 m. It receives a substantial amount of freshwater from a nearby river, which forms a 1-3 m brackish water surface layer, depending on rainfall and snowmelt. It holds several eelgrass beds, which are known to be important nursery areas for cod. A viable population of cod is known to reside in the fjord, and there are known spawning sites in the northern parts of the system.

Figure 1 Location of study sites and associated papers (I – IV) in outer Oslo fjord (blue) in the Eastern parts of Skagerrak, and Topdalsfjord and Tvedestrand fjord (red) on the southern Norwegian coast. National parks in outer Oslo fjord is marked with circles, showing Færder national park (FNP) on the west side of the fjord, and Ytre Hvaler national park (YHNP) on the east side.

4. Study species

4.1 Atlantic cod

Figure 2 Juvenile Atlantic cod in eelgrass (*Zostrea marina*) bed. Foto: Lillian Tveit

Atlantic cod (*Gadus morhua*) is a demersal, benthopelagic cold-water species of the family Gadidae. It has a wide distribution range and inhabits most of the continental shelves and banks in the North Atlantic, spanning from Labrador Sea and Disco Bay to Cape Hatteras in the West, North to Spitsbergen and the North Sea down south to the Bay of Biscay. In the far East it also enters the more brackish waters of the Baltic Sea. Thus, it is distributed in various environments with respect to temperature and salinity. It is considered one of the most important fish species in the world, as it has historically supported one of the largest fisheries in human history. As with many other commercially important fish species, it is now in urgent need of improved management. Atlantic cod has undergone a significant reduction across its range since the early 1990s, as anthropogenic effects such as overfishing, pollution, habitat destruction, temperature increase and ocean acidification all are affecting the oceans, with unknown ecological and evolutionary consequences (Jackson, 2008).

The Atlantic cod also displays a wide range of phenotypic and genotypic variations and appears both as migratory and stationary coastal forms in regard to spawning (Rogers et al., 2014; André et al., 2016). In the North Sea and Skagerrak spawning usually starts in winter and last until late spring (December to May) with peak densities around 20 m in winter/early spring, and at 25-30 m in late spring (ICES, 2005). The Atlantic cod is a batch spawner and is one of the most highly fecund species in the world, with large individuals producing and releasing millions of eggs over several spawning events. Reproduction involves several complex behaviors from both sexes, where male Atlantic cod seem to establish a dominance hierarchy, determined by body size (Hutchings et al., 1999). Both females and males have been shown to achieve higher success when breeding with mates having a larger body size than themselves (Rowe et al., 2007). Larger, older females are known for producing larger eggs, which have a higher survival rate, than first-time spawners (Kjesbu et al., 1996). The eggs and larvae drift as pelagic plankton and is carried away with ocean currents until they metamorphose into juveniles in early summer. Pelagic juveniles feed in the water column until reaching a size of 30-40 mm, before settling closer to the ocean bottom (Campana, 1996). Larvae and post-larvae feed on zooplankton, while the juvenile diet consists of invertebrates, mainly crustaceans. Sub-adult fish generally feed on invertebrates, however other fish is considered to be the most important diet for adult cod, also including young of its own species. The length at which cod switch from mainly invertebrate to mainly vertebrate diet is around 40 cm (based on cod diet information from the Eastern Scotian shelf from 1999 to 2002, see Bundy & Fanning, 2005). Atlantic cod is considered to be a key-species in the ecosystem, and occupies the higher trophic role as a top predator. Top predators are known to influence their prey populations through top-down control, and have important impacts on the structure and functioning of the ecosystem they inhabit (Frank et al., 2005).

Atlantic cod display population structure on both large and small spatial scales. Research have revealed genetic differences between cod sampled across the Atlantic (O'Leary et al., 2007), between populations residing in the northern and southern Norway (Frydenberg et al., 1965), as well as between different ecosystems as the Baltic and the North Sea (Larsen et al., 2012). Several population genetic studies have shown that the Atlantic cod in Skagerrak is structured into two genetically distinguishable ecotypes, coastal and oceanic type (Knutsen *et al*., 2003; Jorde et al., 2007; Knutsen et al., 2007) which appear to coexist during a large part of their life cycle (Knutsen et al., 2018). These two ecotypes are known to differ in size and maturity at age (Olsen et al., 2008) as well as showing differences in behavior, and are usually characterized as migratory and stationary types (Espeland et al., 2007; André et al., 2016). Several mechanisms have been proposed to explain this observed population structure, as retention of eggs and larvae (Ciannelli et al., 2010), and homing of adult individuals (André et al., 2016). More recently, four chromosomal rearrangements, in the form of inversions, have been revealed in the Atlantic cod genome (Sodeland et al., 2016; Berg et al., 2017). Chromosomal rearrangements have previously been shown to promote and sustain population differentiation despite the presence of gene flow in several species (Noor et al., 2001; Lowry et al., 2010; Arostegui et al., 2019), as the inversions suppress meiotic recombination in heterozygous individuals. The inversions in the Atlantic cod genome have been shown to correlate with a latitudinal gradient on both sides of the Atlantic (Bradbury et al., 2010), behavior (Kirubakaran et al., 2016), as well as temperature and oxygen regulation (Berg et al., 2015).

5. Results and discussion

5.1 Absence of top-predators: a changing fish assemblage?

Earlier studies have shown that fishing and size-selective harvesting of larger predators in the ecosystem indirectly promote rapid increases in densities of their prey, even with only moderate fishing effort (DeMartini et al., 2008). Larger predators can strongly affect the habitat, biomass and structure of marine communities, and with the removal of larger top predatory species, the result can be a complete restructuring of the food web (Savenkoff et al., 2007). Highly diverse ecosystems were previously thought to be functionally redundant, and thus thought to be more resilient, resistant and stable. However, studies have shown that highly diverse fish assemblages can also have a low functional redundancy, making them vulnerable to the removal of keystone species (Bellwood et al., 2003; Guillemot et al., 2011). Surveying fish assemblages in the outer Oslo fjord seascape has revealed that there was a general dominance of shorthorn sculpin, viviparous eelpout and fivebeard rockling in the system (paper II). There also was a general absence of larger top predator species (paper II, IV). Coastal Atlantic cod populations are consisting of both a local ‘fjord’ ecotype, as well as an offshore ‘North Sea’ ecotype (papers I,III,IV). They both seem to be recruiting to the coastal zone (paper III), however none are found as larger individuals, and both seem absent from the study system after three years of age (paper IV). Paper II explored similarities and differences between the fished area of outer Oslo fjord compared to a partially protected seascape (Tvedestrandfjord and adjacent coastal areas). Results from this study indicated that the high abundance of mesopredatory species found in outer Oslo fjord might be due to the absence of functional top predators in the system. Tvedestrand also had significantly larger top predator species present (Figure 3), as well as a lower abundance of mid-level carnivores. Similar results have been found in previous studies from around the globe, showing an increased abundance of larger predatory species within MPAs or lightly fished areas, compared to exploited areas (Friedlander & DeMartini, 2002; Claudet et al., 2006; Watson et al., 2007). There was an exceptionally high abundance of shorthorn sculpin observed in outer Oslo fjord compared to the Tvedestrand seascape (Figure 3). This species is known to be a successful hunter, and could be a factor negatively affecting the cod populations’ recruitment success by predating on juvenile cod (0-group) in nursery areas. Equally high abundance of shorthorn sculpin and other mid-level carnivore species was not observed in the Tvedestrand seascape, which indicates that the protected area seems to have a more complete food web and thus increased stability, as the top predators are larger in size and still able to perform their functional role in the ecosystem by limiting smaller prey. The abundance of mid- and low-level carnivores also seemed to be more stable in the partially protected seascape, whereas it showed a higher variability between years in outer Oslo fjord. Implementing MPAs in areas showing sign of mesopredator release and trophic level dysfunction have previously shown promising results (Soler et al., 2015), particularly the increase of top predator abundance (Colléter et al., 2012; García-Rubies et al., 2013). Full protection of coastal marine seascapes could thus improve ecosystem functions and limit the potential for ecological phase-shifts and less desirable alternative states.

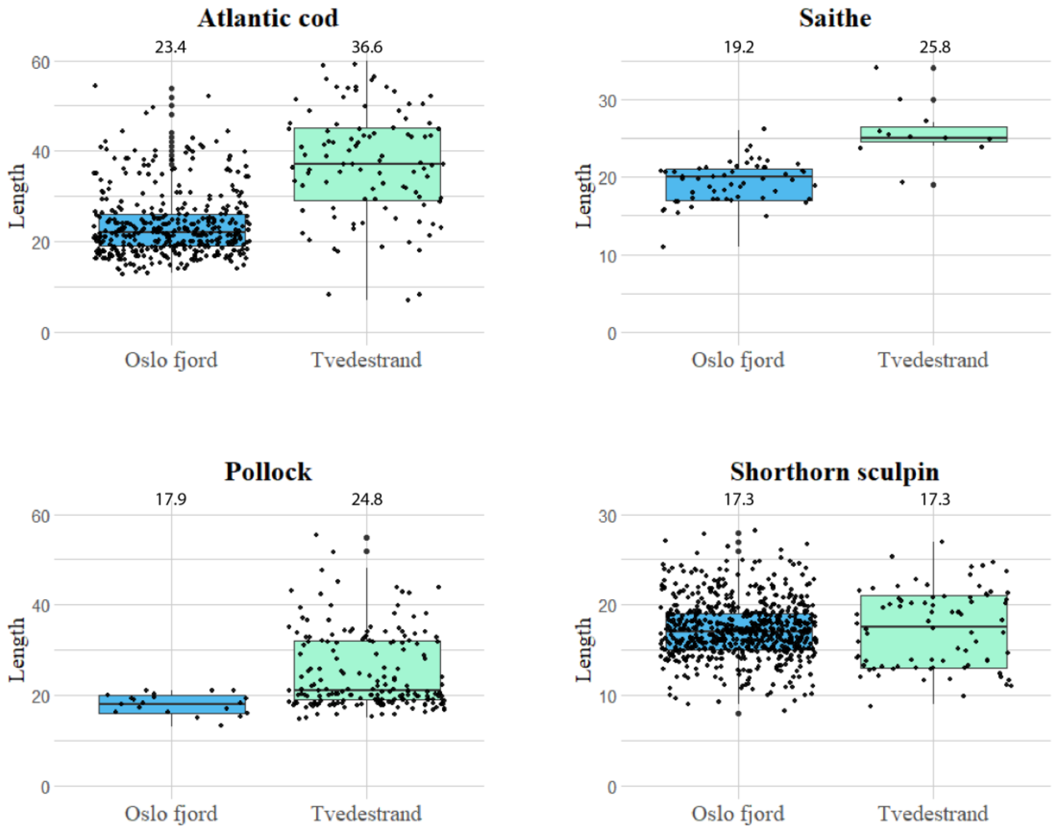

Figure 3 Length distribution for top-predator species Atlantic cod (*Gadus morhua*), saithe (*Pollachius virens*), pollack (*Pollachius pollachius*), and shorthorn sculpin (*Myoxocephalus Scorpius*) from samplings performed in Tvedestrand and outer Oslo fjord (samplings from both national parks pooled together) for all catches collected in 2017, 2018 and 2019. Box displays a confidence interval around the median, while average length is noted above the boxplots. (For further details, see paper II)

5.2 Putative sources of cod recruitment to outer Oslo fjord

Along the Norwegian Skagerrak coast, two genetically distinguishable ecotypes of Atlantic cod are known. The ‘fjord’ ecotype is known for being stationary and can complete their entire life cycle within a restricted geographic area (Rogers et al., 2014). The cod with an offshore, or North Sea origin, may perform longer migration routes to spawning areas, where eggs and larvae are transported with ocean currents to suitable nursery habitats. Previous studies have shown that in years with high influx of water coming from the North Sea, a larger fraction of the 0-group cod caught along the Skagerrak coast may have been of North Sea origin (Knutsen et al., 2004; Stenseth et al., 2006). Natal homing and spawning site fidelity have been proposed as mechanisms maintaining the observed genetic differentiation between these populations, and extreme spawning site fidelity have been observed in the ‘fjord’ populations of Atlantic cod (Espeland et al., 2007; Skjæraasen et al., 2011). Previous studies have also found inshore distribution of eggs, suggesting inshore spawning activity of ‘fjord’ cod, where eggs and larvae are retained by local currents (Espeland et al., 2007; Ciannelli et al., 2010). However, it is still unknown to what degree the local ‘fjord’ populations are dependent on external recruits from the North Sea, or solely on self-recruiting.

High-grade markers are characterized by selected loci that display a higher than average genetic differentiation. In paper I, we show that high-grade marker SNPs can be a useful and valuable tool for investigating population structure in Atlantic cod. Using these same SNPs, 1023 individual Atlantic cod, representing

0-group and 1-group juveniles were assigned to either North Sea or ‘fjord’ origin by comparing samples from outer Oslo fjord to previously sampled references collected in the inner parts of Norwegian fjords and in the North Sea (paper I, III). By combining these genetic data with a biophysical model of ocean drift, we found three potential spawning sites located inside the study area (Figure 4), and three potential sources of origin in the North Sea and Kattegat (Figure 5). Our model estimated that as much as 50% of the pelagic juveniles spawned in the Northeastern part of Skagerrak may have originated from Kattegat and settled in the outer Oslo fjord area. The potential spawning sites inside the study area are of particular interest considering potential for restoration of the local populations, and the possibility of future implementation of MPAs in this region. We find recruits of both ecotypes in the outer Oslo fjord seascape (paper III), suggesting that both ecotypes might be important for sustaining the coastal population in this area. Sympatric populations have previously been described in various species (Ford et al., 1998, Ravinet et al., 2016) including the Atlantic cod (Barth et al., 2017; Knutsen et al., 2018), and might be more common than previously thought (Jorde et al., 2018b). The offshore Atlantic cod ecotype cannot be distinguished genetically from the North Sea cod, thus, in addition to eggs and larval drift from the North Sea and Kattegat, we cannot exclude the potential for local spawning of both ecotypes (Jorde et al., 2018a).

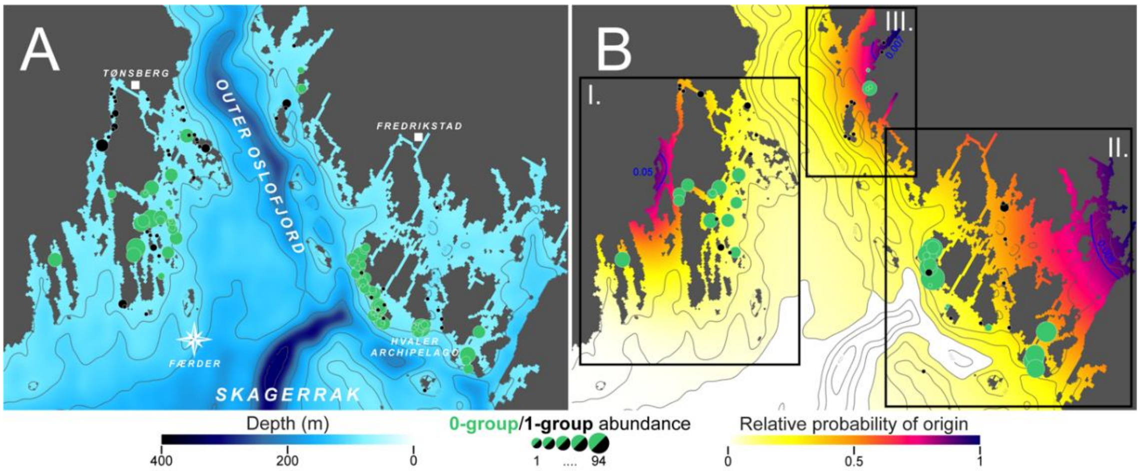

Figure 4 The 2017 Atlantic cod cohort sampled as 0-group recruits during summer and autumn 2017 (green circles), and as 1-group juveniles (≤30 cm) in spring 2018 (black circles). A: Bathymetry of outer Oslo fjord and recruits assigned to 'North Sea' origin. B: Recruits assigned to 'fjord' origin and heat map indicating the relative probability of local source locations (spawning sites) inside or adjacent to the national parks, predicted from the 2D GAM fitted to the geographical position of 0-group (presence/absence). Note that colored gradient within sub-areas denoted by roman numerals in panel B have to be scaled by: (I.) 0.053, (II.) 0.005, and (III.) 0.007; meaning that peak probability of recruitment to the sampled sites within the three sub-areas are: (I.) 5%, (II.) 0.5%, and (III.) 0.7% (see blue lines and numbers in panel B). (For further details see paper III).

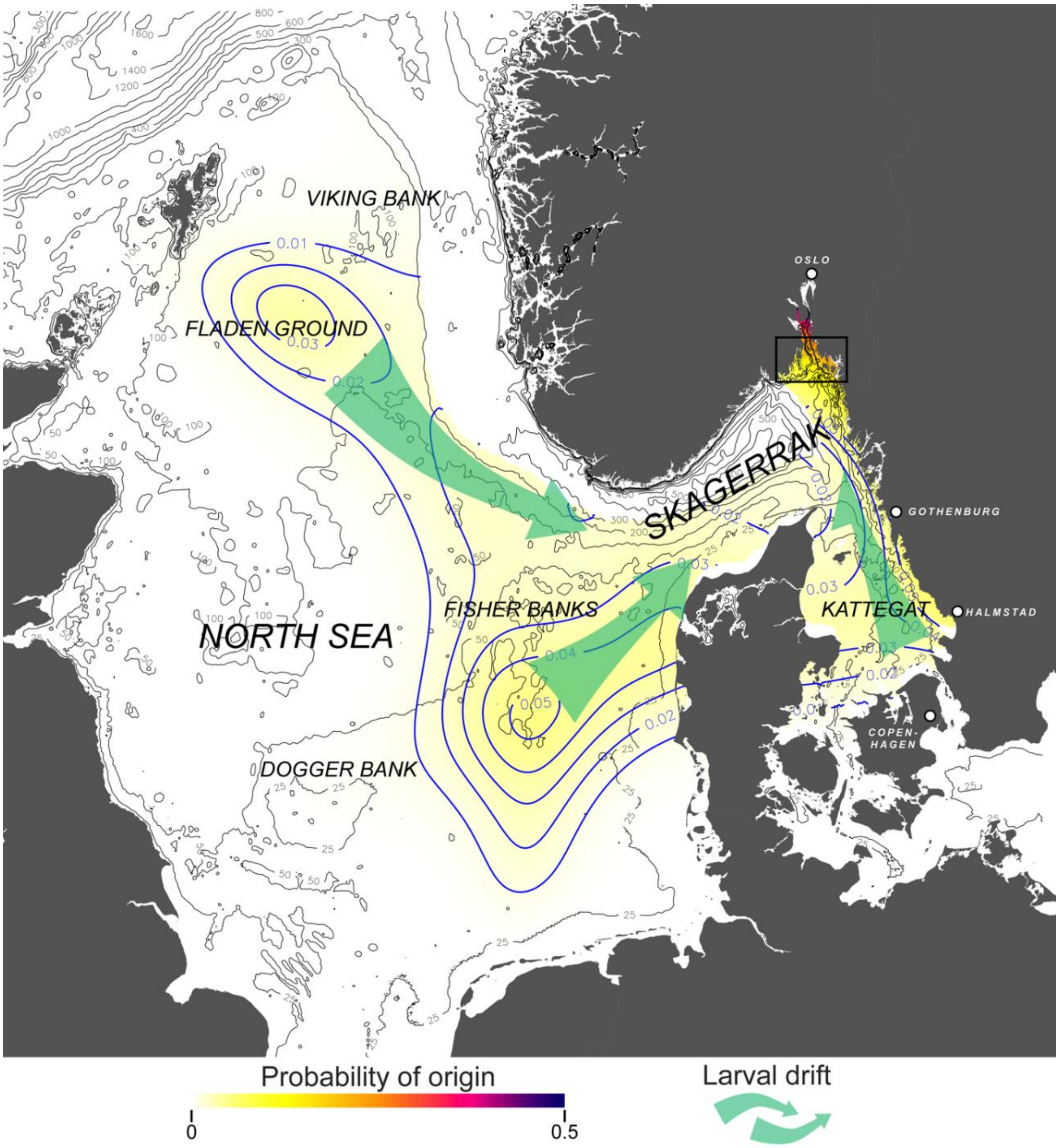

Figure 5 Predicted Atlantic cod spawning areas and idealized drift routes of cod originating from the North Sea or Kattegat. Heat map represents the probability of source sites for 2017 recruits, predicted from the 2D GAM fitted to the geographical position of 0-group presence/absence. Green arrows represent idealized drift routes for eggs and larvae originating from the North Sea or Kattegat. (For further details, see paper III).

5.3 Potential for restoration of local cod in outer Oslo fjord

In Skagerrak, several marine fish populations have suffered from depletion over the last decades, mostly as a consequence of overfishing (Cardinale & Svedäng, 2004; Cardinale et al., 2012). In Eastern Skagerrak, abundance of Atlantic cod is historically low after the 1900s, and individuals >30 cm are rare (Svedäng, 2003; Olsen et al., 2008). Results from our sampling revealed that most individuals are small in size and assumed to be 1-3 years of age (paper IV), and individuals >40 cm were rare in the samples (Figure 4). We do find a higher proportion of individuals with 'fjord' origin surviving their first year (paper IV), indicating that this ecotype might perform better in the coastal environment. Previous research has shown that cod with 'North Sea' genetic assignment might have lower fitness in the fjord environment (Barth et al., 2019). However, both ecotypes seem absent from the study area at 3 years of age (Figure 6), and the system is clearly depleted of larger individuals of several gadoid species (papers II, IV). Comparison of the outer Oslo fjord seascape with a partially protected seascape showed that top predator species sampled there were significantly larger than in outer Oslo fjord (Figure 3) (paper II). Recently, a restriction on cod fishing was implemented along the Norwegian Skagerrak coast, including our study area in outer Oslo fjord. Indeed, a reduction in fishing is crucial for population recovery, however, synergetic effects such as habitat destruction, increasing temperature and ocean acidification, as well as reduction in oxygen and a changing fish assemblage are all affecting the cod populations. Our genetic results show that both ecotypes are present in the outer Oslo fjord seascape both as 0-group individuals and older fish (papers III, IV). Implementing restriction on fishing efforts by implementing MPAs could be a good strategy for management, in particular, in the areas pointed out as potential spawning sites by our model (paper III), as this could increase the abundance of both ecotypes and also protect important nursery habitats. Increasing the abundance of top predatory fish species are expected to induce shifts in species assemblage, and they could eventually restore balance in the ecosystem (top-down control).

In summary; in this thesis I have demonstrated that both ecotypes of Atlantic cod are present in the outer Oslo fjord system, both as 0-group and adult individuals. By combining genetic data from a highly-graded SNP panel with a biophysical model of ocean drift, I showed that there are potential spawning grounds located inside the national park areas in outer Oslo fjord. I also showed that spawning grounds in the North Sea might be contributing viable recruits to the system. In particular, Kattegat seemed to be an important source of recruits. Moreover, I found a general absence of individuals >40 cm present in the system, which one could argue to be a direct effect of longevity overfishing, considering that most of the individuals of both ecotypes seem to disappear from the system as soon as they reach the minimum size limit for cod (40 cm). In addition, my results suggest that the ecosystem in outer Oslo fjord are experiencing a mesopredator release caused by the absence of larger top predator species. I argue that this observed increase in abundance of mid-level carnivore fish species could affect cod recruitment by causing a higher predation pressure on young-of-the-year and juvenile cod. For protection and restoration of the coastal cod population, I recommend implementation of MPAs with measures such as no-take zones and partially protected areas – also including spawning sites proposed by our model, could help limit the potential for ecological phase shifts, and ultimately be a first-step in restoring the coastal cod population in outer Oslo fjord back to a healthy state.

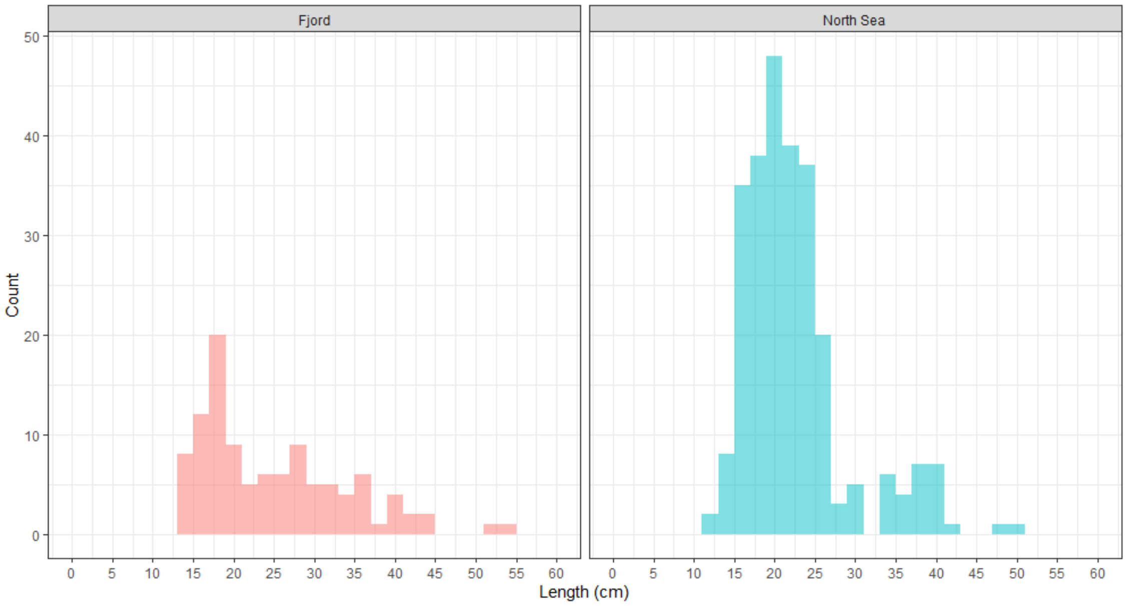

Figure 6: Frequency histogram of length distributions of all Atlantic cod samples collected in outer Oslo fjord during our three years of sampling in 2017, 2018 and 2019. Histogram display individuals assigned to ‘fjord’ (left, red) and ‘North Sea’ ecotype (right, cyan).

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7 List of papers

Paper I	Jorde, P.E., Synnes, A.E., Espeland, S.H., Sodeland, M., Knutsen, H. (2018). Can we rely on selected genetic markers for population identification? Evidence from coastal Atlantic cod. Ecology and evolution, 8(24), 12547 – 12558
Paper II	Synnes, A.E., Jorde, P.E., Olsen, E.M., Knutsen, H., Espeland, S.H., Moland, E. When size matters: Contrasting management regimes indicative of a mesopredatory release in temperate coastal fish assemblages. (*Manuscript*).
Paper III	Synnes, A.E., Huserbråten, M., Jorde, P.E., Knutsen, H., Espeland, S.H., Moland, E. Local recruitment of Atlantic cod and putative source spawning areas in a coastal seascape. (*Manuscript*).
Paper IV	Synnes, A.E., Olsen, E.M., Jorde, P.E., Knutsen, H., Moland, E. Fate of Atlantic cod (*Gadus morhua*) recruits assigned to two ecotypes in a Skagerrak coastal seascape (*Manuscript*).

Paper I

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ORIGINAL RESEARCH

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Can we rely on selected genetic markers for population identification? Evidence from coastal Atlantic cod

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Abstract

The use of genetic markers under putative selection in population studies carries the potential for erroneous identification of populations and misassignment of individuals to population of origin. Selected markers are nevertheless attractive, especially in marine organisms that are characterized by weak population structure at neutral loci. Highly fecund species may tolerate the cost of strong selective mortality during early life stages, potentially leading to a shift in offspring genotypes away from the parental proportions. In Atlantic cod, recent genetic studies have uncovered different genotype clusters apparently representing phenotypically cryptic populations that coexist in coastal waters. Here, we tested if a high-graded SNP panel specifically designed to classify individual cod to population of origin may be unreliable because of natural selection acting on the SNPs or their linked background. Temporal samples of cod were collected from two fjords, starting at the earliest life stage (pelagic eggs) and carried on until late autumn (bottom-settled juveniles), covering the period during summer of high natural mortality. Despite the potential for selective mortality during the study period, we found no evidence for selection, as both cod types occurred throughout the season, already in the earliest egg samples, and there was no evidence for a shift during the season in the proportions of one or the other type. We conclude that high-graded marker panels under putative natural selection represent a valid and useful tool for identifying biological population structure in this highly fecund species and presumably in others.

KEYWORDS
marine fishes, natural selection, population genetics, population of origin, statistical assignment

1 | INTRODUCTION

In order to increase statistical power to resolve weak population genetic structure, a select panel of loci with higher than average level of genetic differentiation is often employed (André et al., 2011; Banks, Eichert, & Olsen, 2003; Henriques et al., 2018; Johansen et al., 2018; Jorde, Kleiven, et al., 2018; Larson, Seeb, Pascal, Templin, & Seeb, 2014; Nielsen et al., 2012; Russello, Kirk, Frazer, & Askey, 2012). Such a high-graded panel is likely to include loci under divergent selection, raising concerns over their reliability as a tool for inferring demographic population structure (Luikart, England, Tallmon, Jordan, & Taberlet, 2003; Nielsen, Hansen, & Meldrop, 2006). Selected loci

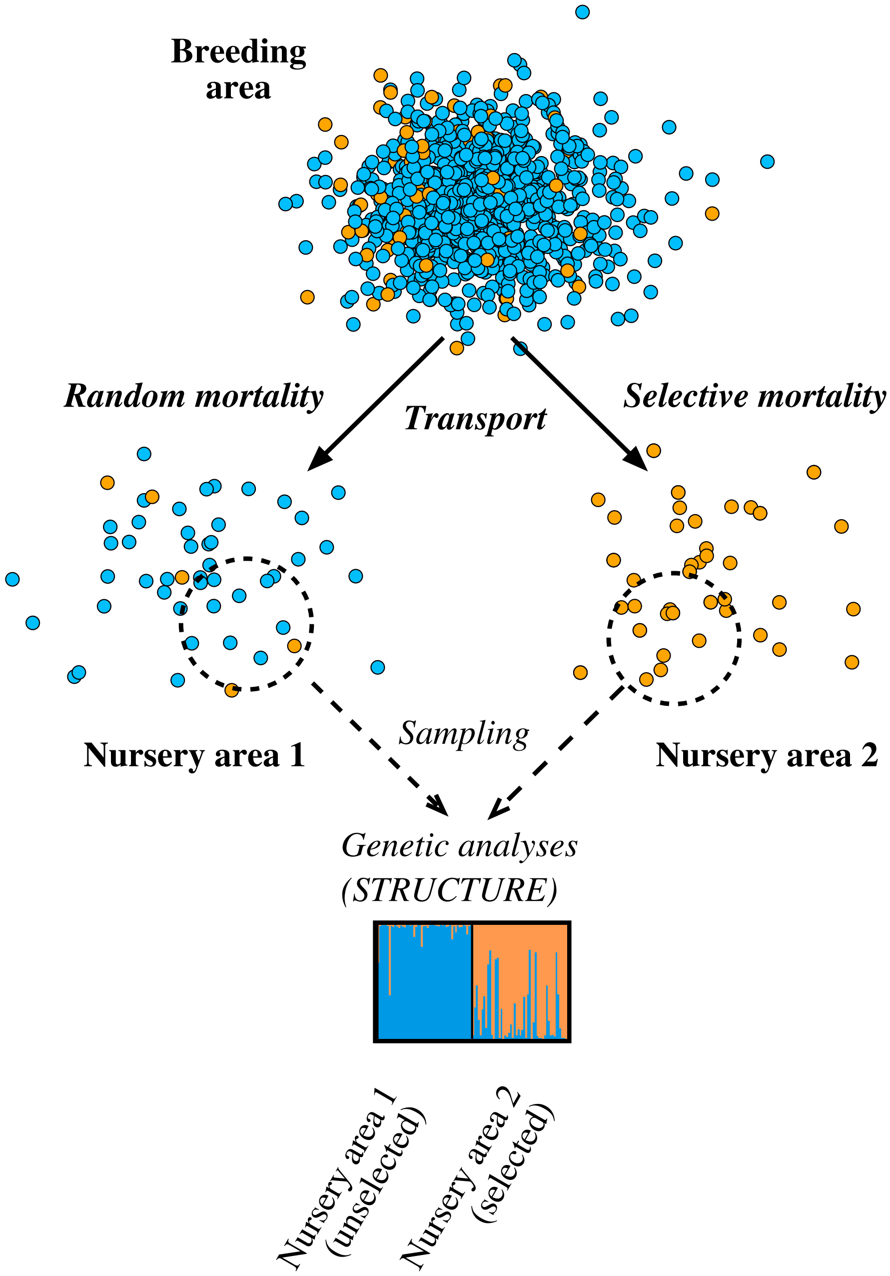

FIGURE 1 Hypothetical scenario of a breeding population distributing juveniles (e.g., seeds or larvae) to two nursery areas that differ in environmental conditions and thus in selective mortality. Selection is assumed to favor individuals that are homozygote in three particular loci (identified as orange dots) and in one nursery area (area 2) but not in the other (area 1). Below is a *Structure* plot of samples from the two hypothetical nursery areas. See Supporting Information for details

may nevertheless be excellent tools for the more restricted purpose of discriminating populations (Bekkevold et al., 2015; Lamichhaneya et al., 2012; Milano et al., 2014; Teacher, André, Jonsson, & Merilä, 2013) and for assigning individuals to population of origin (Banks et al., 2003; Freamo, O'Reilly, Berg, Lien, & Boulding, 2011; Helyar et al., 2011; Kavakiotis, Samaras, Triantafyllidis, & Vlahavas, 2017; Nielsen et al., 2012; Wilkinson et al., 2011). Challenges arise when selection on the markers is strong enough for environmental differences to override population demography on allele frequency dynamics. Individuals and genotypes sampled after an episode of selective mortality may poorly represent the parental generation and could lead to false impressions of population structuring. Such a scenario is illustrated in Figure 1, depicting the outcome of hypothetical selective mortality on genotype composition following transport of juveniles to different nursery areas. Upon sampling and genetic screenings of samples from the nursery areas, the results indicate genetically distinct groups that may be mistaken for separate biological populations, which they are not. While strong selection acting on a single or small number of marker loci is unlikely to have a great overall effect on a large panel of markers, the situation is different when using a small set specifically chosen for their high levels of divergence. This could be a problem especially when the true population structure is weak, absent or even moderate, as selection may generate patterns of genetic structure that trace environmental drivers rather than population processes (Lamichhaneya et al., 2012; Nielsen et al., 2006).

Strong selection in the form of non-random survival of genotypes is not unreasonable in organisms that combine extremely high fecundity with widespread dispersal of offspring into a diverse range of environments. High fecundity implies a high reproductive excess, for some organisms in the millions (Winemiller & Rose, 1992). To maintain population size, this excess must be balanced by high mortality, usually at early life stages. Thus, there is a potential for selective mortality in the offspring and the tiny fraction of individuals that survive and end up being sampled for genetic analyses may then poorly represent the parent population. While most mortality is likely to be unrelated to the individual's genotype and thus non-selective, even when, say, 99.9% of deaths are unrelated to genotype, there remains a reproductive excess on the order of 1,000 to cover the cost of natural selection if the excess was a million to begin with. Many highly fecund species also have a highly dispersive early life stage (e.g., seed plants [Nathan & Muller-Landau, 2000], marine invertebrates [Grantham, Eckert, & Shanks, 2003], and fishes [Cowen & Sponaugle, 2009]), and offspring may end up in environments their parents were not adapted to. Temporal fluctuations in environmental conditions could also contribute to create a mismatch between parental adaptation and optimal offspring genotypes, creating an option for selective mortality in offspring.

The use of high-graded markers is particularly attractive for marine organisms because population structure is typically weak within oceans (Hauser & Carvalho, 2008; Waples, 1998; Ward, Woodwark, & Skibinski, 1994). However, many marine species represent precisely the pattern of high fecundity and widespread dispersal followed by massive juvenile mortality that could cause problems for some genetic markers to provide reliable information on biological population structure and for correctly assigning individuals to population of origin. Here, we explore these issues empirically, using a panel of 27 SNP markers that were specifically developed for assigning Atlantic cod (*Gadus morhua*) along the south coast of Norway to population of origin, that is, to putative "North Sea" or "fjord" populations (Jorde, Kleiven, et al., 2018; Knutsen et al., 2018). We tested the hypothesis that such

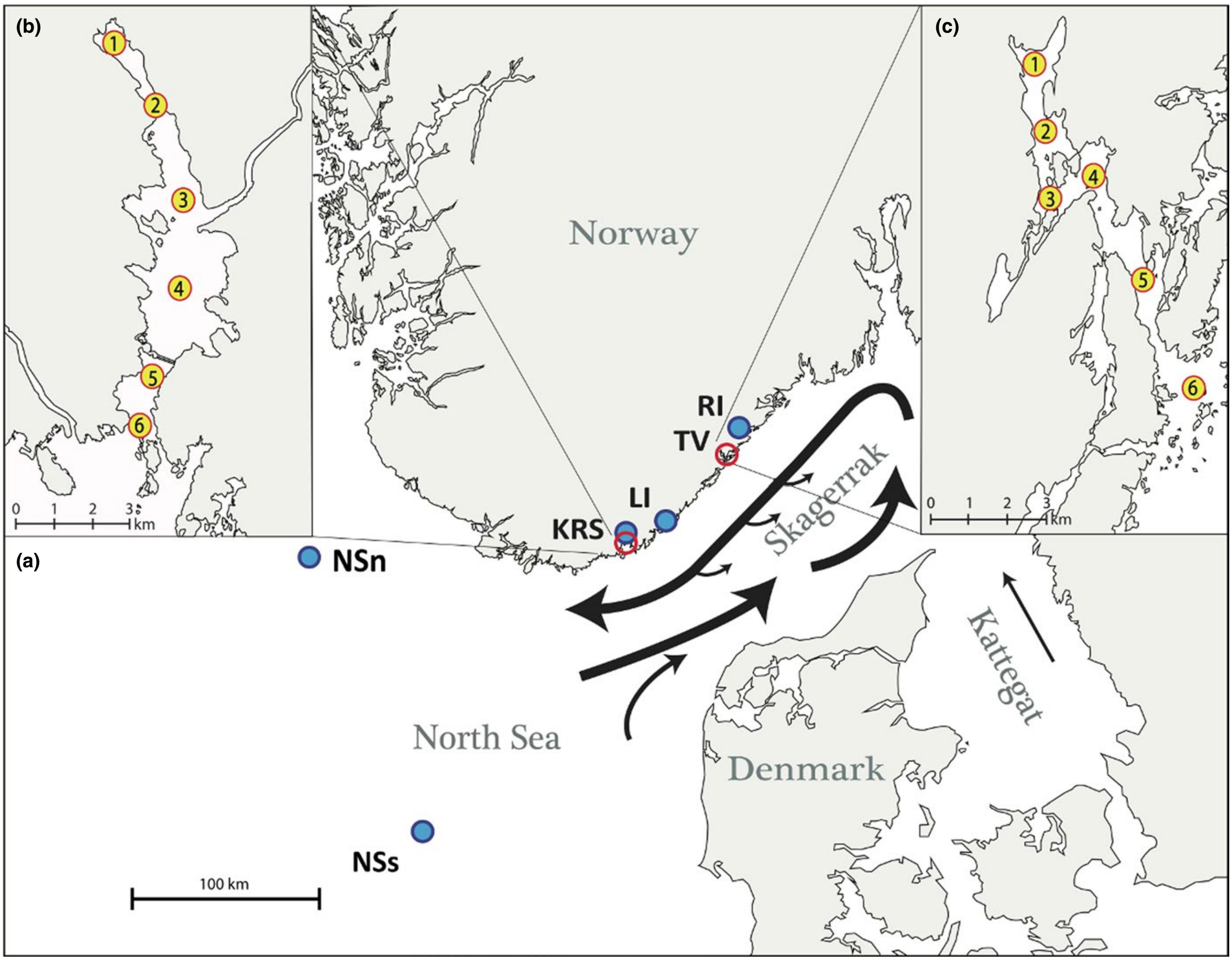

FIGURE 2 (a) Map of study area with sample locations for eggs and juveniles. Blue dots indicate position of reference samples in the North Sea (NSn and NSs) and within three fjords (KRS: Kristiansand; LI: Lillesand; RI: Risør). Black arrows indicate the dominant ocean currents (simplified from Danielssen et al., 1997). Insets: details of sampled fjords with sample locations (numbered yellow dots): (b) Topdalsfjord; (c) Tvedestrandsfjord

assignments were driven by selective mortality during the early life stages by monitoring genotype composition in eggs and juveniles over the time period (early spring to autumn) with highest natural mortality. The potential for selection on polymorphic loci in this highly fecund species lies in the extensive drift of pelagic eggs and larvae with ocean current and in the potentially contrasting environments where they settle and grow up. The alternative hypothesis is that genetic clustering and assignments of coastal cod is not unduly affected by ongoing selection on the SNP markers.

2 | MATERIAL AND METHODS

2.1 | The study species and experimental setting

The Skagerrak is an extension of the North Sea, situated between Denmark, Sweden, and southern Norway, bordering Kattegat (Figure 2). Spawning of Atlantic cod occurs in the North Sea, in the Kattegat, and in Skagerrak coastal waters during early spring (February to early April). The Atlantic cod is a highly fecund species, the female producing approximately half a million eggs per kg body weight (Kjesbu, 1989; May, 1967; Oosthuizen & Daan, 1974). Spawning products (eggs and larvae) are pelagic and subject to transport with ocean currents (Munk, Larsson, Danielsen, & Moksness, 1995), which in the Skagerrak form a counter-clockwise path from the North Sea along the Skagerrak coast (Figure 2). Thus, spawning products from the North Sea can and do reach the Skagerrak coast (Knutsen et al., 2004; Spies et al., 2018; Stenseth et al., 2006), and cod from the outer coastal areas in the Skagerrak appears to be genetically similar to or identical with North Sea cod (André et al., 2016; Barth et al., 2017; Jorde, Kleiven, et al., 2018; Knutsen et al., 2011; 2018; Sodeland et al, 2016). Eggs hatch after three to four weeks (von Westernhagen, 1970) and the larvae remain pelagic until early summer when they descend to the bottom and are referred to as 0-group. Mortality rates during early life stages of cod have been estimated to approximately 10.9% per day at the early larval stage, declining to 2.2% per day for larger larvae, and considerably lower than this for post-settled 0-group cod (Sundby, Bjørke, Soldal, & Olsen, 1989).

Genetic studies of 0-group and older cod along the Norwegian Skagerrak coast have found genetic differences mainly between inner fjords and outer skerries (Knutsen et al., 2011; Øresland & André, 2008). This spatial pattern of genetic variability has been attributed to the existence in the Skagerrak of genetically distinct forms or putative ecotypes of cod (Barth et al., 2017), co-occurring in coastal waters (Knutsen et al., 2018; Sodeland et al., 2016). Based on a panel of >9,000 SNPs, Jorde, Kleiven, et al. (2018) developed a small panel of 27 SNPs for cost-efficient assignment of coastal cod from Skagerrak into two ecotypes, referred to as “fjord cod” and “North Sea cod”, respectively. The panel was developed by ranking loci according to levels of genetic divergence (Nei’s G_{ST}) in their study area, which broadly overlapped the present one, while avoiding closely linked (composite linkage disequilibrium, CLD > 0.5) loci. Thus, the 27 SNP panel represents a high-graded subset of genetic markers specifically developed to provide high levels of divergence among cod in the present study area.

2.2 | Study areas

The present study areas include two nearby fjords on the Norwegian Skagerrak coast, the Topdalsfjord and Tvedestrandsfjord (Figure 2). Topdalsfjord (Figure 2b) is located near the city of Kristiansand, and is approximately 11 km long until it opens significantly to the semi-open sea, and has a largest depth of about 100 m. The fjord is known to hold several eelgrass beds which are considered to be one of the most important nursery areas for Atlantic cod. Tvedestrandsfjord (Figure 2c) is located outside the city of Tvedestrand and is approximately 8 km long with a maximum depth of 85 m. Studies of current patterns in this fjord indicate that pelagic eggs and larvae on average tend to experience an inward transport by estuarine circulations and thus become retained within the inner fjord basins (Ciannelli et al., 2010; Knutsen et al., 2007). Tvedestrandsfjord has recently been protected as a marine protected area (MPA), including a no-take zone, and fishing mortality during the present study is expected to be negligible.

2.3 | Sampling

Cod eggs were sampled during the spawning season from February to late March 2015, once in Topdalsfjord and five times in Tvedestrandsfjord. Six sampling sites or “stations” were arranged in the form of transects from the innermost to the outer part of the fjords (Figure 2b,c). Eggs were sampled with a WP2 planktonic net (Fraser, 1968) with 60 cm diameter and 500 µm mesh size. The net was hauled vertically from 30 m depth to the surface at a speed of 0.5 m/s. Eggs were identified and determined to species according to size and pigmentation (Hiemstra, 1962). Cod eggs were considered to be 1.2 mm to 1.5 mm in diameter (Thompson & Riley, 1981). Eggs were stored in 96% ethanol at −22°C until DNA extraction.

Sampling of young-of-the-year 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 for the annual beach survey by the Institute of Marine Research (IMR) along the Norwegian Skagerrak coast (Barceló, Ciannelli, Olsen, Johannessen, & Knutsen, 2016). The Topdalsfjord was sampled for juveniles at six different stations, once in June and once in September but the latter employed somewhat different sampling stations (corresponding approximately to stations 3 and 6: Figure 2b) to comply with the annual IMR beach seine program. Tvedestrandsfjord was sampled for juveniles at five stations (no. 1 through 5) in June and three stations (1 through 3: Figure 2c) in October. Juveniles were stored frozen at −22°C until DNA extraction.

Mature, supposedly spawning, cod were sampled from Topdalsfjord during February 2015 with the help from a local fisher. Sampling was done at five different locations within the inner parts of the fjord (approximately stations 1 through 4: Figure 2b) over three days of fishing. Sampled cod were sacrificed, measured, and sexed by visual examination of gonads. A piece of the dorsal fin was saved for genetic analysis and was stored in 96% ethanol at −22°C until DNA extraction.

2.4 | Reference samples

As genetic references for cod in the study area we used two previously sampled and genotyped sets of individuals from the Norwegian Skagerrak coast and from the North Sea, respectively (Jorde, Kleiven, et al., 2018). The two reference samples consisted of a (n = 143) sample of juvenile cod from the inner part of three fjords (including Topdalsfjord and two other nearby fjords, sampled in 1997–2010) and a sample (n = 91) of adult cod from two locations (sampled in 2002 and 2012, respectively) in the North Sea (Figure 2).

2.5 | DNA extraction

Sampled cod eggs were extracted for DNA using the E.Z.N.A MicroElute Genomic DNA Kit (Omega Bio-tek, Norcross, GA), following the manufacturer's instructions for tissue samples with only one minor modification: the last elution buffer step being 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).

2.6 | Genotyping

A total of 333 cod eggs, 100 young-of-the-year juvenile cod, and 52 adult cod were genotyped for the present study (Table 1). Genotyping of the 27 SNPs was carried out on a Sequenom MassARRAY platform at the Centre for Integrative Genetics, Norway (https://cigene.no). We dismissed individuals with 10 or more missing genotypes as having poor DNA quality, resulting in 76 individuals (70 eggs, 6 juveniles, 0 spawners) being removed from further analyses, which were based on the remaining 409 individuals (Table 1). We consistently got genotypes only from 25 of the 27 SNPs, with two SNPs (ss1712301578 and ss1712299621: www.ncbi.nlm.nih.gov/SNP/) often failing, and all statistical analyses were therefore limited to 25 SNPs.

2.7 | Statistical analyses

Correlations of alleles within individuals relative to the sample (F_{IS}) and among samples relative to the total (F_{ST}) were calculated according to Weir and Cockerham (1984), separately for each SNP and as averages over loci, using the Genepop software (v. 4.2.1: Rousset, 2008). Genotype proportions within samples were tested for conformation to Hardy–Weinberg expectations with the chi-square goodness-of-fit

TABLE 1 The target samples from the Topdalsfjord and Tvedestrandsfjord

		Sample sizes			Assigned to	
Date (DD.MM.YYYY)	Life stage	n_1	n_2	F_{IS}	NS	fjord
Topdalsfjord						
19–25.02.2015	Adult	52	52	0.019	5	47
05.03.2015	Egg	126	120	0.046*	9	111
15.06.2015	Juvenile	10	9	0.080	2	7
15.09.2015	Juvenile	11	10	−0.094	1	9
			$\chi^2 = 2.308$, $df = 3$, $p = 0.511$			
Tvedestrandsfjord						
20.02.2015	Egg	7	2	NA	0	2
27.02.2015	Egg	77	46	0.012	0	46
06.03.2015	Egg	61	45	0.094*	11	34
13.03.2015	Egg	33	25	−0.024	3	22
24.03.2015	Egg	29	25	−0.012	1	24
08.06.2015	Juvenile	54	50	0.094	31	19
12.10.2015	Juvenile	25	25	0.038	2	23
			$\chi^2 = 69.31$, $df = 6$, $p = 0.000$			
Total		485	409		65	344

Note. For each sample are given date of sampling, life stage sampled, sample sizes (n_1 = total number of genotyped individuals; n_2 = number of those that were successfully genotyped, i.e., with <10 genotypes missing), average F_{IS} over 25 loci (NA = not calculated due to low sample size; asterisks indicate significance at the 5% level with *Genepop* probability test), and numbers assigned by *Geneclass2* to the "North Sea" (NS) and "fjord" types. χ^2 refers to the contingency chi-square test for homogeneity of proportions assigned to the two types at different sample times and life stages.

test. Individuals were clustered on the basis of their multilocus genotypes using *Structure* (v. 2.3.4: Pritchard, Stephens, & Donnelly, 2000) with the correlated allele frequencies model (Falush, Stephens, & Pritchard, 2003). For each predefined number ($K = 1$ to 5) of clusters, *Structure* was run with 1 million MCMC iterations following 1 million burnins. The distribution of ln prob(data|K) was evaluated for assessing the most likely number K. Individual *Q*-values (i.e., the estimated membership coefficients for each individual) were plotted graphically with *Distruct* (Rosenberg, 2004). *Geneclass2* (v.2.0.g: Piry et al., 2004) was used to assign individuals to the aforementioned two reference samples, employing the Bayesian method of Rannala and Mountain (1997).

We used individual cluster memberships, as assigned by *Geneclass2*, and tested for change over time and space in the proportion of eggs and 0-group juvenile cod that were assigned to the fjord and North Sea reference samples. Under the hypothesis of selective change in genotypic proportions, we expect a decline in proportions of individuals that were assigned to the North Sea population and a corresponding increase in the proportion assigning to the fjord population for samples taken inside the fjords. Such selective shifts, if they exist, must take place largely after the release of eggs to the environment, which occurred around our first sampling date, and before late autumn when the last samples were taken, as these dates span the period with high levels of natural mortality. For Topdalsfjord, one date of eggs (March 5; six sampling sites: Figure 2b) and two temporal replicates of juveniles (June and September) were available for testing (Table 1), resulting in three temporal samples from this fjord. In addition, a sample of adult spawners was available for comparison from the inner part of the Topdalsfjord. For Tvedestrandsfjord, heterogeneity in proportions of the two genotype clusters was tested in five temporal replicate samples for eggs (February 20 to March 24) and two temporal replicates for juveniles (June and October), for a total of seven temporal samples (Table 1).

To test for difference among temporal samples in proportions of individuals assigned to each genetic cluster, we used standard chi-square heterogeneity tests and regression analyses. We chose logistic regression with *Geneclass2* score as response variable and date of sampling and position of sampling site in the fjord as explanatory variables. The model is logistic because score is a binary variable (1 = individual belong to the North Sea cluster, 0 = individual belong to the fjord cluster) and we used regression because the two explanatory variables are ordinal, and regression is then statistically more powerful than alternative approaches that ignore this information (Agresti, 2013, p. 87). The first explanatory variable was day of sampling, counted as the number of days after the first sampling date, and was taken to represent the time of exposure to the fjord environment. Clearly, this is not exactly so, as eggs may have been spawned at different dates, but these differences should be relatively minor (a few weeks) considering the total time-span of the study (eight months). The second explanatory variable was sampling position in the fjord (Figure 2: 1 = inner part of fjord, 6 = outer part), which was assumed to represent any of a number of environmental gradients running from the inner to the outer part of the fjords. These gradients could reflect differences in temperature, salinity, oxygen level, prey availability and species composition, parasite prevalence, and so on (cf. Schulze, 2006) that might induce selective mortality on genotypes. The two fjords were analyzed separately, and spawning fish (Topdalsfjord) were not included in the regression analysis, which was based on the following logistic model:

$$s_i = \frac{\exp(a + bx_i + cy_i)}{1 + \exp(a + bx_i + cy_i)}, \tag{1}$$

where the response variable (*s*) is the *Geneclass2* score and explanatory variables (*x* and *y*) are sampling date and station number, respectively, and *i* index individuals. The model parameters (*b* and *c*) were estimated and tested for significance with the *glm* function in the R statistical environment (R Core Team, 2016).

3 | RESULTS

A total of 409 individuals, representing adults, eggs and juveniles, were genotyped successfully, in the sense that >15 SNPs produced a valid genotype (i.e., <10 SNPs failed). Eggs typically had more missing genotypes than did juveniles and adults, and the number of missing genotypes was greater for eggs with low DNA concentration (Supplementary Information Figure S1). The few eggs that were obtained at the first sampling event, on February 20 in Tvedestrandsfjord, all had very low DNA concentration, presumably reflecting recent spawning (Espeland & Sannæs, 2018). The distribution of egg DNA concentration, and hence age distribution, in Tvedestrandsfjord, was much wider already at the next sampling event a week later (February 27), and by early March tended to be wider than seen in Topdalsfjord at the same date (cf. Supplementary Information Figure S1).

Most SNPs displayed a deficiency of heterozygotes in the pooled sample ($n = 409$), with positive F_{IS} estimates at 21 out of 25 SNPs (Figure 3). For ten of the SNPs deviation from Hardy–Weinberg (HW) genotype proportions were significant at the 5% level in Tvedestrandsfjord, while three SNPs deviated significantly in Topdalsfjord, two of them in common between fjords. Deficiencies of heterozygotes were also evident from positive average F_{IS} estimates in seven out of ten temporal samples from within fjords, two of the ten samples reaching significance at the 5% level (Table 1). The deviations from HW within loci appeared to be linked to the locus' level of genetic diversity in this geographic region, as single-locus F_{IS} estimates correlated significantly with levels of divergence (F_{ST}) between the North Sea and fjord reference samples ($r = 0.578$, $p = 0.0017$: Figure 3). The average F_{ST} over the 25 SNPs was 0.174 between the fjord and North Sea reference samples and ranged among SNPs from 0.059 to 0.414.

3.1 | Number of clusters

Results from *Structure* software were consistent with the existence of two genetic clusters or populations of cod in the samples, with a

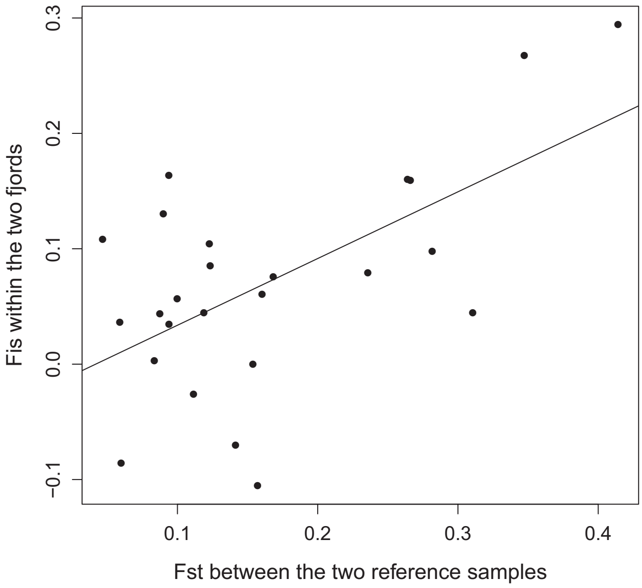

FIGURE 3 Single-locus deviations (black dots) from Hardy–Weinberg (F_{IS}) within fjords (vertical axis) as a function of their level of differentiation (F_{ST}, horizontal axis) between the two reference samples. Pearson's correlation coefficient $r = 0.578$, $t = 3.562$, $p = 0.0017$. Average F_{ST} over all 25 SNPs between the two reference samples was 0.174

TABLE 2 Estimation of number of populations in the combined samples from Topdalsfjord and Tvedestrandsfjord

K	Ln Prob(data|*K*)	Prob(*K*|data)
1	−11,409.0	0
2	−10,847.5	1
3	−10,960.8	0
4	−11,150.3	0
5	−11,649.2	0

Note. Numbers depict the log probability of data given various numbers (*K*) of hypothetical clusters or populations, Ln Prob(data|*K*), as reported by *Structure*, and the corresponding estimate of the posterior probabilities of *K*, Prob(*K*|data).

maximum Ln Prob(data|*K*) for $K = 2$ (Table 2). Estimated membership to either of the $K = 2$ clusters displayed a clear dichotomy with most individuals having either a high ($Q > 0.8$) or a low ($Q < 0.2$) probability of membership to each cluster (Figure 4). Comparison of the Topdalsfjord and Tvedestrandsfjord samples with the two reference samples revealed that the larger of the two clusters coincided with the fjord type (cf. Figure 4c,d) and the smaller cluster with the North Sea type.

3.2 | Change in cluster proportions

The test of constant proportions of the two genotype clusters in temporal samples from Topdalsfjord included adults, eggs, early (June) and late (October) juveniles and revealed no difference among life stages (contingency chi-square test, $p = 0.511$: Table 1). Cod of the putative North Sea type was present in all samples in low proportions, with the highest proportion (two out of seven sampled individuals, or 29%) in the early juvenile sample. The logistic model (Equation 1) revealed a non-significant ($p = 0.148$) trend with increasing proportion of the North Sea type toward the outer part of the fjord (higher station number) but little or no change with time ($p = 0.614$; Table 3; Figure 5 left). In Tvedestrandsfjord, which included five replicate egg samples but no adults, there was a highly significant heterogeneity among temporal samples in proportions of the two types ($p < 0.0001$: Table 1). In this locality, heterogeneity was observed both among egg samples ($\chi^2_{df=4} = 16.14$, $p = 0.0028$), between the two juvenile samples ($\chi^2_{df=1} = 17.593$, $p < 0.0001$), with a higher number of North Sea types in the early (June) than in the late (September) juvenile sample (cf. Table 1), and between egg and juvenile samples pooled ($\chi^2_{df=1} = 30.253$, $p < 0.0001$), with a higher proportion of the North Sea type among juveniles than among eggs (42 of 75 = 56% vs. 15 of 143 = 10%). These differences among temporal samples resulted in a statistically significant ($p = 0.014$) increase in North Sea proportions with sampling date in the logistic regression model for this fjord (Table 3; Figure 5 right) but without any clear trend in the spatial dimension ($p = 0.587$). Inspection of the distribution of individual *Structure* *Q*-values (Figure 4d) indicated that the observed temporal trend in Tvedestrandsfjord to a large extent reflected an elevated proportion of juveniles of the North Sea type in the June sample; a component that was not seen in the later, October sample.

4 | DISCUSSION

Strong selection acting on standing genetic variation could in principle lead to different clusters of genotypes, predominating in different environments, that could be mistaken for genetically differentiated biological populations (cf. Figure 1). If selective survival of members from a common gene pool was responsible for generating genetic clusters of Atlantic cod in Skagerrak coastal waters, the shift in genotypic composition would be expected to take place during a period of strong natural mortality. Given the very high mortality characterizing early life stages in this broadcast spawner, we expected genetic shifts to occur sometime during our first (egg stage) and last (bottom-settled juvenile fish) sampling times.

In Topdalsfjord, we found no evidence for the predicted genetic changes and members of both clusters were presented in apparently constant proportions during all life stages, including the adult spawners that presumably gave rise to the present offspring cohort. Moreover, the fjord type was the by far most numerous type at all sample times. We therefore reject the hypothesis of selective mortality as an explanation for the observed genetic clusters in this fjord. The situation was more complicated in Tvedestrandsfjord where proportions of the two clusters varied significantly over time, although not in a consistent direction. While temporal samples also in this fjord were dominated by the fjord genetic cluster, episodes of increased presence of individuals of the North Sea cluster occurred

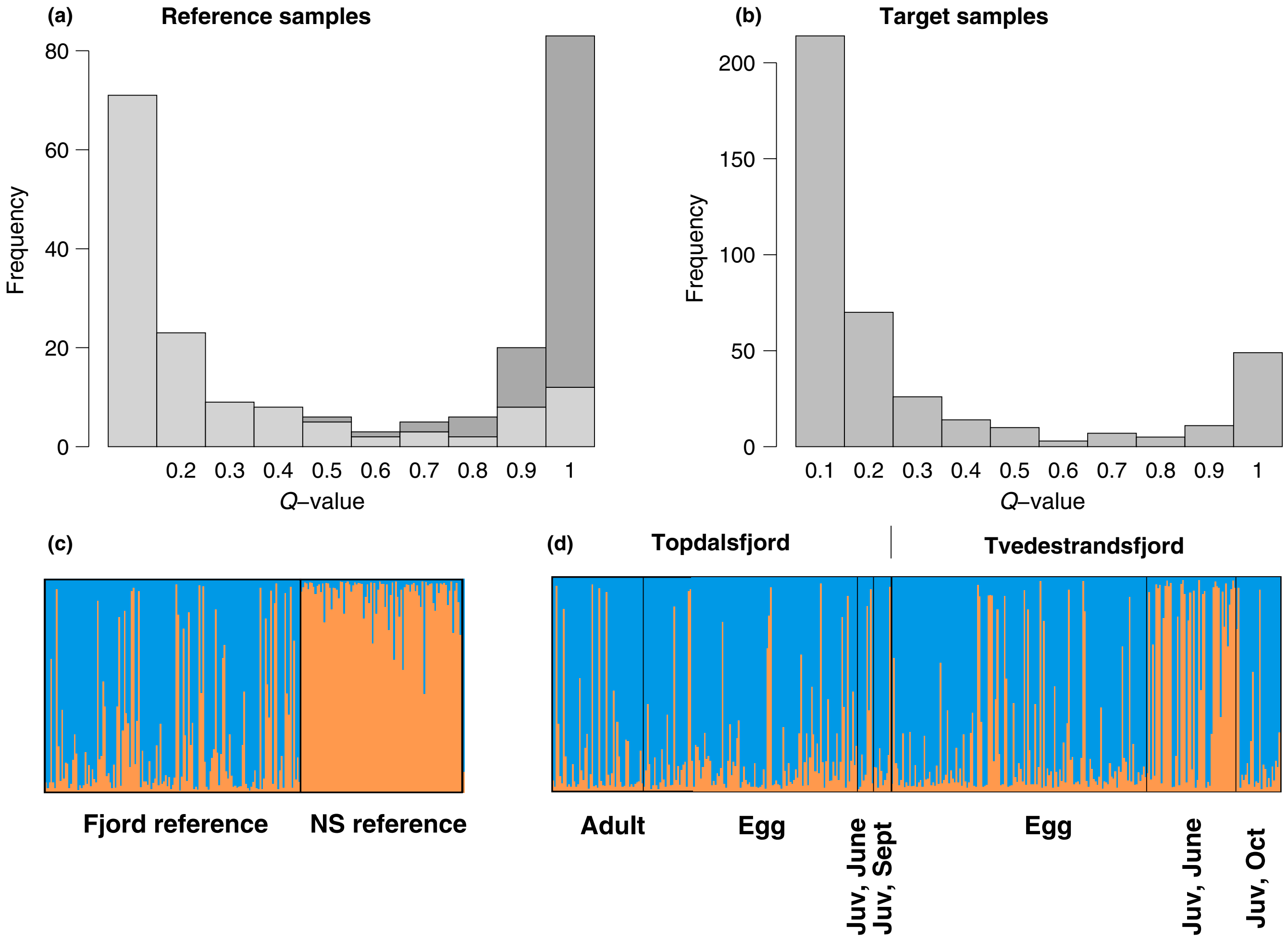

FIGURE 4 Classification of individual cod into two genetic clusters. Figure panels depict estimated probabilities (*Q*-values) of individual cod to belong to the North Sea cluster, calculated from 25 SNP genotypes with the *Structure* software. Top panels: (a) frequency histograms for reference samples (light and dark gray for fjord and North Sea reference samples, respectively), and (b) for target samples from the two fjords. (c,d) same data as in a and b, respectively, depicted as individual barplots (orange bars: North Sea cluster; blue bars: fjord cluster), with sample and life stages indicated

TABLE 3 The importance of location (station number) and time (date of sampling) on the proportion of individuals assigned to the North Sea reference sample (*Geneclass2* assignments)

	Topdalsfjord			Tvedestrandsfjord		
Explanatory variable	Estimate	*SE*	*p*	Estimate	*SE*	*p*
Station number	0.260	0.180	0.148	0.094	0.133	0.480
Sampling date	0.002	0.005	0.614	0.005	0.002	0.014*

Note. Numbers given are the estimated parameters of the logistic regression model (Equation 1) for each fjord, with standard errors (*SE*) and *t* tests for significance (*p*: asterisk indicates significance at the 5% level).

both at the egg (in early March) and early juvenile (June) stages. Presumably, these episodes reflected events of inflow of eggs or larvae of North Sea origin into the Tvedestrandsfjord or movement of early juvenile fish. The subsequent decline of North Sea members in later (October) juvenile samples may be suggestive of selective removal of North Sea genotypes in the fjord environment, but cannot explain the dominance of the fjord type already manifested in the earliest, recently spawned egg samples. This latter observation verifies that the two genetic clusters in Tvedestrandsfjord were, as in Topdalsfjord, established already prior to the onset of high natural mortality and potential for strong selection.

If the two genotype clusters are not the result of strong selective survival in different environments of members of the same gene pool they must instead be manifestations of two genetically differentiated lineages or populations, possibly representing different ecotypes with partially overlapping ranges in Skagerrak (Knutsen et

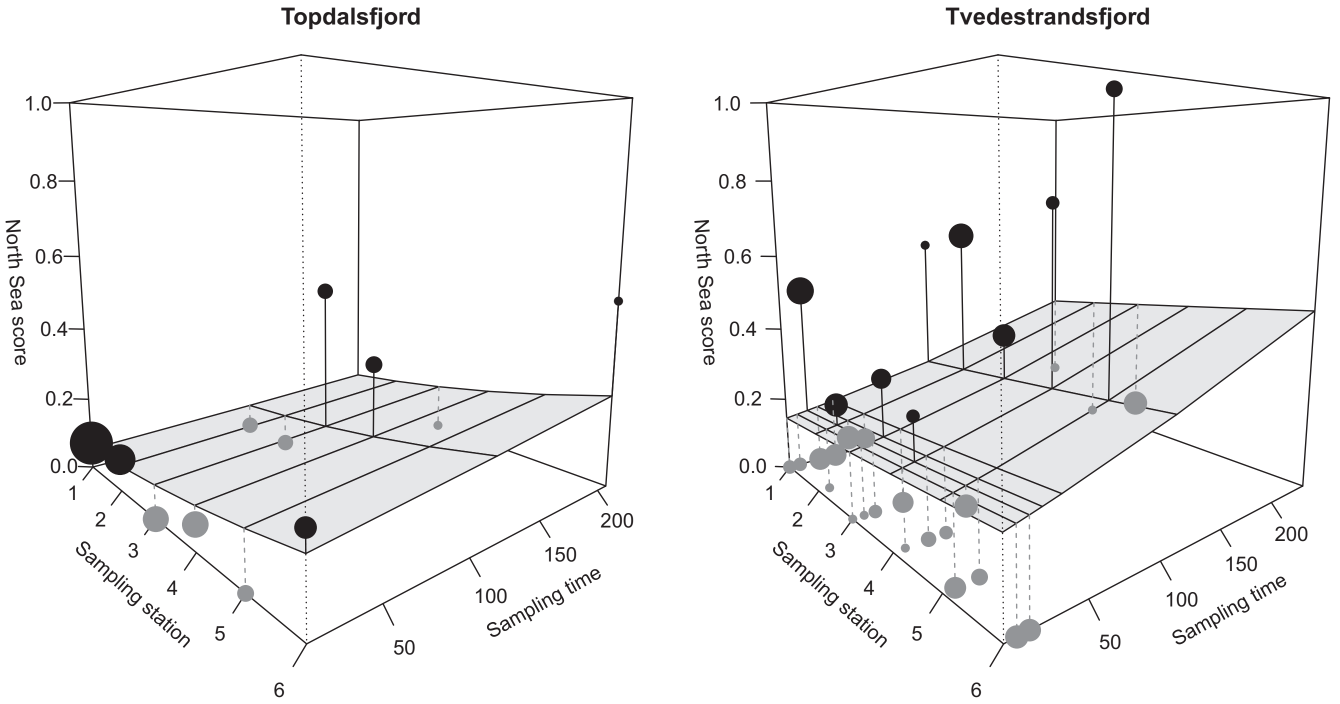

FIGURE 5 Effects of time (number of days after first sample date) and position in fjord (sample station number) on proportion of individual eggs and juveniles that were scored (*Geneclass2*) to the North Sea type (vertical axes). The shaded plane represents the effects predicted by the model (Equation 1, dots represent data for single samples scaled in proportion to sample size, and other graphical elements are visual aids. Parameter estimates and test statistics are given in Table 3

al., 2018). This interpretation is consistent with the finding (Figure 3) of a strong correlation among loci in deficiency of heterozygotes and level of genetic divergence, indicating a Wahlund effect (i.e., population mixture) within fjords.

Of the two putative ecotypes, the North Sea type is the only one thus far observed in the North Sea proper (cf. Figure 4c, NS reference sample) and its presence also within fjords may represent drift of pelagic eggs or larvae from the North Sea cod population to the Skagerrak coast (Knutsen et al., 2004; Stenseth et al., 2006). Local spawning of this type on the coast cannot be excluded, however, and nearly 10% (5 out of 52: Table 1) of the adult and presumably mature cod in Topdalsfjord were of this type. We do not know if these individuals actually spawned inside the fjord or represent strayers from other areas, but local spawning of this type could explain why we found apparently very young egg also of the "North Sea" type within fjords (cf. Supplementary Information Figure S1). The drift time from North Sea spawning grounds into the (inner) Skagerrak has been estimated to at least 10 days (Munk et al., 1995).

Since the fjord genetic cluster dominates the inner fjord samples it likely represents a unique lineage of cod. There is evidence that this lineage may be related to the western Baltic cod stock (Barth et al., 2017). Whatever its origin, this type must be largely reproductively isolated from North Sea cod in order to maintain its genetic characteristics where the two types coexist. Apart from the putative indications for selective removal of North Sea cod from within Tvedestrandsfjord, the circumstances allowing co-occurrence of two types of cod in coastal Skagerrak remain unknown. Similar phenomena of coexisting types have been described for coastal and migratory cod along northern Norway (Johansen et al., 2018; Kirubakaran et al., 2016; Sarvas & Fevolden, 2005; Westgaard & Fevolden, 2007), Iceland (Halldórsdóttir & Árnason, 2015), Greenland (Therkildsen et al., 2013), and Canada (Berg et al., 2017), and thus appear to be common for this species. Phenotypically cryptic, coexisting lineages or ecotypes may be common also in other species but may be under-reported because their detection requires either highly informative markers or extensive sampling to detect the often weak statistical signals of heterozygote deficiency and admixture linkage disequilibrium (Jorde, Andersson, Ryman, & Laikre, 2018).

A number of studies have explored population genetic differentiation patterns between panels of putative neutral and selected loci and found largely consistent, yet more pronounced differentiation and/or differentiation at finer geographic scales for selected loci (Bekkevold et al., 2015; Larson et al., 2014; Milano et al., 2014). This consistency may be interpreted in support of the notion that selected markers loci represent a valid, and highly informative, tool for population studies in species with low levels of neutral structure. On the other hand, there is little evidence that gene loci generally follow a clear dichotomy into purely neutral and selected classes, and different statistical tools used for discriminating among such locus classes often yield conflicting results (Lotterhos & Whitlock, 2014; Narum & Hess, 2011). The present study does not rely on comparisons of spatial differentiation patterns among putative distinct classes of loci as a means of assessing their reliability as population markers. Instead, our aim was to test the hypothesis that observed differentiation in a high-graded SNP marker panel might be attributed to recurrent, strong selection.

Despite the high potential for selective shifts of high-graded SNPs in a species as fecund as the Atlantic cod, we reject this hypothesis. This does not imply that selection on these SNPs or on their linked genomic background is not occurring, but the magnitude of selective mortality during a single season is clearly too small to be detected in the present experimental setting, and also too small to affect the statistical assignment of individuals to population of origin. Hence, this selected SNP panel may be considered valid and highly useful markers for certain population studies, including detection of population subdivisions and assignment of individuals to population of origin. By implication, high-graded panels should be useful for addressing similar questions also in other areas and for other species, the great majority of which have lower fecundity than the cod and less potential for rapid selective shifts. Of course, due considerations need to be made to the scientific question at hand when employing such a panel.

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CONFLICTS OF INTEREST

The authors declare no conflict of interests.

AUTHOR CONTRIBUTIONS

This work represents part of AES's Master thesis. The study was designed by HK, AES, PEJ, SEH and MS. AES did the field work, DNA extraction, initial data analyses and wrote the first draft. MS designed the SNP panel. SEH assisted in egg sampling and beach seine hauls. PEJ did the statistical analyses and wrote the present version with assistance from all authors.

DATA ACCESSIBILITY

SNP genotypes and metadata are available at Dryad https://doi.org/10.5061/dryad.k718h66.

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SUPPORTING INFORMATION

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Paper II

When size matters: Contrasting management regimes indicative of mesopredator release in temperate coastal fish assemblages

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Abstract

Absence of functional top-predators has been proposed as a mechanism acting to shape fish assemblages in temperate marine ecosystems, with cascading effects on lower trophic levels. The trophic and functional status of fish assemblages in a region on the eastern Norwegian coast was assessed by comparing patterns with a nearby fjord that harbors a system of marine protected areas (MPAs) including a no-take zone. Demersal fish assemblies were sampled using fyke-nets over three consecutive seasons. Atlantic cod (*Gadus morhua*) is the most dominant top-predator and a key-species in this ecosystem. Historically, Atlantic cod and other gadids have been targeted by the full range of former and present fisheries, and comparisons of size distributions between the protected and unprotected fjords showed that average size of the Atlantic cod were significantly larger in the former (mean ± SD: 36.6 cm ± 14.38 vs. 23.4 ± 7.50; $p<0.001$). The unprotected seascape also showed a higher abundance of mesopredator fish species than what was found in the zoned fjord, indicating that protection of top predators within MPAs control the mesopredator populations. This study suggests that the present trophic and functional state of the coastal fish assemblage in eastern Norway is driven by top predator release.

Introduction

Trophic cascades and food web structure

Since the onset of the industrial fishing era, mean trophic levels of fisheries landing around the world has declined, both in marine and inland waters (Pauly et al., 1998). Apex consumers (i.e. top predators), defined as predators that occupy the higher trophic links in an ecosystem, often have a strong effect on the trophic dynamics and diversity of the system in which they occur (Moksnes et al., 2008; Baden et al., 2010). Reduction of large, piscivorous species can alter ecosystem productivity, and result in cascading effects down the food web and thereby affect community structure as well as ecosystem functioning and processes (e.g., Steneck, 2012; Donadi et al., 2017). Marine food webs have been intensely studied with respect to the interactions of consumers (“top-down control”) and the resources available to them (“bottom-up control”). The historical view of the marine ecosystems was that the oceans were structured by bottom-up control, meaning that the food web was mainly controlled by resource limitation (Cushing, 1975). Even though primary producers and bottom-up processes are mainly controlling all food webs, presence of predators is also crucial for a well-functioning ecosystem, as they strongly affect habitat, biomass and structure of marine communities.

Recent studies have drawn attention to the importance of top predators and their role in the food web, by suppressing populations of smaller predators (mesopredators), and thereby reducing predation on smaller prey species (top-down control) (Ritchie & Johnson, 2009; Baden et al., 2012; Östman et al., 2016). For example, sea otter (*Enhydra lutis*) overexploitation in Alaska led to a dramatic increase of their sea urchin prey. As a result, kelp forests were destroyed by overgrazing of the sea urchins, and the ecosystem showed signs of altered productivity and species assemblages. Once the sea otter population recovered, the ecosystem transformation was reversed. Trophic cascades caused by such top-down control have been demonstrated in various ecosystems, as kelp forests (Estes et al., 2004), lakes (Persson et al., 2003) and streams (Bechara et al., 1992), as well as in oceanic systems (Shears & Babcock, 2002; Frank et al., 2005; Myers & Worm, 2005; Baum & Worm, 2009).

The definition of top predators and mesopredators can be considered relative, as a top predator in one system might be a mesopredator in another. Trophic level interactions between various predators create complex relationships as intraguild predation (IGP). IGP is the killing and eating of species that use similar resources, and are thus potential competitors (Polis et al., 1989). IGP often occurs among species that compete for basic food resources but differ in body size, such that the smaller species, or stage, falls within the normal prey size range of the larger. IGP may arise both among, and within species (cannibalism). The juveniles of a species are thus vulnerable both to predation from conspecific adults and predation from other species with whom the adults may compete. IGP often occurs as life-history omnivory, when growth in body size during different life stages generally results in changes in diet (Woodward & Hildrew, 2002). These diet shifts are often associated with shifts in habitat use and a general change in interaction patterns of the individual with its environment, as it increases an individual's capacity to exploit new resources (Juanes et al., 2002).

In recent decades, human activity has driven the functional extinction of many top predators, and several studies have indicated subsequent ecosystem changes that are complex, unpredictable and largely unknown (Floeter et al., 2005; Frank et al., 2005; Ellingsen et al., 2015).

The Skagerrak coastal ecosystem and management of cod

The Norwegian Skagerrak coastal system includes only a few higher trophic fish species, where Atlantic cod (*Gadus morhua*) is one of the most dominant top predators. During the last decade, there has been a substantial decline in the abundance of larger cod and piscivorous fish in the North Sea and Skagerrak waters, as well as in Kattegat (Svedäng, 2003; Svedäng & Bardon, 2003, Rogers et al., 2017). On the Swedish Skagerrak coast, abundance of demersal fish >30 cm, including cod, in the inshore fish community has been extremely low compared to historical records from 1920's and 1970's (Svedäng, 2003). The same decline of large cod and piscivorous fish has been observed along the Norwegian Skagerrak coast, especially in the eastern part of Skagerrak and the areas around outer Oslo fjord. This has raised concern from both local and regional government, as well as among recreational and commercial fisheries located in this region. The government is now raising the question if the stocks of local cod populations could be restored and brought back to the state they were in before the collapse observed in the early 2000s.

Using data collected over three survey years, our aim was to investigate patterns, differences and similarities in species composition, -richness and -abundance, and size distribution of top predators among two contrasting study areas along the Norwegian Skagerrak coast: within a recently established MPA and a nearby exploited area. As the Atlantic cod is considered the dominant predator in this region, our main focus was aimed at the presence and size of this species, although other top predator species were also investigated. Generalized linear models (GLMs) were employed to assess if there was a higher probability of catching cod in the MPA compared to the exploited outer Oslo fjord area, and classical linear models were used to compare diversity indexes between sites and years. A high abundance of mesopredatory fish in the exploited area motivated a further investigation into the relationship between the most abundant mesopredatory fish species, shorthorn sculpin, and Atlantic cod. Last, we assessed whether absence of top predator species in the study system is the likely cause for an apparent mesopredator release. We discuss our findings in light of potential for restoration of top predator populations and recovery of the former species assemblage.

Material and methods

Study Area

Outer Oslo fjord seascape

This study was conducted in outer Oslo fjord for the years 2017, 2018 and 2019. Oslo is the capital of Norway, and the land surrounding the Oslo fjord region is the most densely populated area in Norway. In this area, two national parks were established to protect habitats and arrest development in the coastal zone: Ytre Hvaler National Park (hereafter YHNP) in 2009, and Færder National Park – (hereafter FNP) in 2013, situated on the eastern and western side of the fjord mouth, respectively (Fig. 1). The area covered by our fyke net survey was approximately 200 km^2 on the west side (FNP), and 190 km^2 on the east side of the fjord (YHNP). The outer Oslo fjord seascape consists of archipelagos, and several smaller fjords bays and estuaries. It is an exposed area, which is influenced by different water masses, where the upper layers of the water column consist mainly of brackish water due to river discharge and inflow of brackish water from Kattegat and the Baltic sea (<25.0 psu). Underneath this brackish water layer there is a mixing of water masses from the North Sea and surface layer (25-35 psu), while high saline, nutrient rich, Atlantic water (>35 psu) flows up from the Norwegian trench, and is usually found on depths greater than 70-80 m. Tidal amplitude in Skagerrak is generally low (less than 0.5 m) and is thus negligible.

Outer Oslo fjord is defined as an eutrophicated area partly due to the increased supply of nutrients from Norway's two largest rivers, Drammenselva and Glomma, and also smaller river systems in the inner parts of Oslo fjord. Due to increased rainfall during the last decades, these rivers carry high amounts of soil particles, nitrogen and phosphorous which are released into the sea (Walday et al., 2017). The outer Oslo fjord area is also affected by long-term fishing pressure, from both commercial and recreational fisheries, having depleted larger bodied piscivorous fish, including gadoids (Cardinale & Svedäng, 2004; Casini et al., 2005).

The zoned seascape – Tvedestrand

Tvedestrand municipality is situated 120 km southwest of the outer Oslo fjord. In 2012, the Tvedestrand fjord and outer coastal areas was subject to a zoning process in which ≈15% of municipality waters were included in no-take- or partially protected areas. The Tvedestrand fjord proper is a small fjord including several sills and basins, extending approximately 8 km inland. It includes a great variation of habitats, such as eel grass beds, soft corals, mud flats and also sparse kelp forests (Freitas et al. 2016). It also harbors inshore spawning aggregations and nursery areas for coastal cod (Knutsen et al., 2007; Ciannelli et al., 2010). In 2012, a 1.5 km^2 no-take reserve was implemented in this fjord for protection of fish and lobsters against commercial and recreational fishing. This reserve effectively protects 40-80 % of the home ranges of at least two resident aquatic top predators: the anadromous brown trout and the Atlantic cod (Thorbjørnsen et al., 2019; Villegas-Ríos et al., 2017). On each side of the no-take zone is a partially protected zone, where only hook and line type gear are allowed. In the northeastern part of municipality waters, a 4.9 km^2 partially protected area extend from the outer islands to approximately 50 m depth. The Tvedestrand seascape covered by the fyke net survey measure a total area of approximately 17 km^2, with depths reaching 87 m, and a topography that is representative of fjord-to-coast systems along the Norwegian Skagerrak coast (Fig. 1). The fjord has a two-layered structure, where in the inner zone there is a variable freshwater surface layer, below which the temperature and salinity increases with depth down to ~30 m (Ciannelli et al., 2010), whereas in the outer exposed areas, the freshwater layer is absent.

Study periods and sampling method

To assess the fish assemblage in Outer Oslo fjord, sampling was performed on the eastern (YHNP) and western (FNP) side of the fjord mouth, located approximately 20 km apart. Sampling was done using fyke-nets with green mesh, 55 cm openings and 25 mm mesh size. Fyke nets were deployed in gentle slopes or level habitat, with the cod-end towards the deep, usually in depths <6 m. Experimental fishing was conducted in early May in the years 2017, 2018 and 2019. A total of 930 fyke nets were hauled during the three surveys, as well as 111 large collapsible baited fish traps (130 × 80 × 120 cm) for 'control' sampling of deeper habitat (>10 m). Soak time was approximately 24 hours for both fyke nets and traps. Catches were recorded directly on board, and all fish were counted and identified to species level and measured to nearest cm (fork length), before being released back into the sea. A tissue sample was collected from all *G. morhua* individuals for population genetics analyses (to be reported elsewhere). After sampling, the fishing gear was relocated to a new, randomly chosen position, before being hauled again the next day. In outer Oslo fjord, each site was sampled for four days except for YHNP in 2018 (3 days) (Table S1). In order to be consistent from a taxonomic point of view, non-fish organisms have been excluded from the data analysis.

The zoned seascape (Tvedestrand) was sampled using fyke nets in May for the years 2017, 2018 and 2019. A total of 606 fyke nets were deployed during the three years of sampling, using the same sampling procedure as used in outer Oslo fjord. Sampling was carried out for 6 days in 2017 and 2018, and 7 days in 2019. The fjord was sampled both inside the no-take- and partially protected zones, as well as outward to the exposed areas beyond the fjord mouth (Fig.1).

Data analyses

To compare the fish communities sampled in the outer Oslo fjord and Tvedestrand seascapes, fish species' relative abundance (catch-per-unit-effort; CPUE), representing densities of fish species (N/fyke nets/days) was calculated for both juvenile and adult life stages for the most abundant families (Fig. 2, Fig. 3). For each sampling year, the Shannon diversity index, Simpson index, and species evenness were calculated for all sampling sites to assess differences between sites and years (Table 3). In addition, sampling sites were clustered together into 11 and 12 different clusters following a north-south gradient, where cluster No. 1 was situated in the northern part of the national parks and No. 12 in the southernmost part. This was done to look for patterns in occurrence of species on the eastern and western side of outer Oslo fjord, and potential ecosystem “hot spots”. Clustering was done under the assumption that islands and land areas close to each other would have somewhat similar fish assemblages

(Table S3). Shannon- and Simpson diversity indexes and evenness were also calculated for the same 11 and 12 clusters (Table S3). The degree of similarity in frequencies of all species found in the two national parks and adjacent areas was calculated using a heatmap analysis with dendrograms showing similarity between species abundance, sites and years (Fig. S1).

We used classical linear models (McCullagh, 2018) to analyze differences in species diversity indexes and evenness between the three sampling regions Tvedestrand, FNP and YHNP. Plotting the raw data indicated similar variance among regions in each sampling year, with seemingly shared year-to-year differences. We thus chose to run models with an interaction effect between region and sampling year, with year modelled as a factor. Residual plots indicated that models fitted the data adequately. We tested for an effect of region and sampling year on species diversity (Shannon and Simson indexes) using the following model structure:

$$\text{Diversity} = \text{Region} \times \text{Year} \qquad \text{eq. 1}$$

The same model structure was used to test for an effect of region and sampling year on evenness.

Generalized linear models (McCullagh, 2018) were used to investigate effects of contrasting management regimes on cod abundance and average body size. Preliminary analyses showed that a large proportion of the fyke net hauls did not contain any cod. Therefore, cod catch (CC) was analyzed as a binary process (i.e. the probability of catching at least one cod per fyke-net). Sampling year was added as a factor to control for temporal variation in catches:

$$CC = Region + Year + (Region \times Year) \qquad \text{eq. 2}$$

Next, we used the same model structure to test for effects of contrasting management regimes on presence of above legal-size cod (>40 cm).

A model without explanatory variables (null model) was also fitted in order to test the hypothesis that none of the variables influenced the abundance or size of the top predators. Both variables “region” and “year” was also tested separately (Table 9). A model selection based on Akaike’s Information Criterion (AIC) was used to determine the most parsimonious model, and the model with the lower AIC was selected as the best one (Table 9).

Differences in size distribution for top predator species between outer Oslo fjord and the MPA was tested with a Welsh two-sided t-test. In addition, a chi-square test (without correction) was conducted for Atlantic cod and shorthorn sculpin to test for a positive association between these species (Table S2). All data analyses were conducted using the open-source language R 3.6.1 (R Core Team, 2019), using the package vegan for calculation of diversity indexes (Oksanen et al., 2019).

Trophic levels and life stage

Each fish species was assigned a trophic level using information from FishBase (www.fishbase.org), and grouped into categories as low-, mid- or high-level carnivore. Low-level carnivores were identified as species with a trophic level ranging from 3 - 3.5, and mid-level carnivores were identified as species with a trophic level ranging from 3.5 - 3.9. Predators grouped into high-level carnivores were identified as species with a trophic level ≥ 4.0 (as done in Essington et al., 2006), hence including the gadoids cod, saithe, whiting and pollack, as well as the species from the Scopthalmidae family; garfish and great weever (Table 2). To test if there was a difference in proportions of the trophic level species between the exploited area in outer Oslo fjord and the protected area of Tvedestrand, we used a two proportion Z-test with continuity correction.

Life stage categories of adult and juvenile were based on the species-specific length at maturity according to FishBase (Froese and Pauly, 2016; Staveley et al., 2017). For species where maturity data was unobtainable, a method commonly used to determine life stage was used, and individuals that were ≤ 1/3 of their maximum length (according to FishBase) were recorded as juveniles (Nagelkerken & Van der Velde, 2002; Dorenbosch et al., 2006; Staveley et al., 2017).

Results

Fish assemblage in the outer Oslo fjord seascape

A total of 7959 individual fish comprising 34 species from 19 families were recorded from 930 fyke net hauls at the east and west side of outer Oslo fjord. Of these, seven species (19%) were classified as high-level predators and 25 species (81%) were classified as mesopredators (low and mid-level carnivores) (Table 2, Fig. 4). Of the total fish assemblage, 6% were classified as high-level carnivores and 94% of all fish caught were classified as mesopredators (mid and low-lever carnivores) (Table 1, Fig. 4). The Labridae and Cottidae families had the highest abundances throughout all years of sampling (Fig. 2). Goldsinny wrasse was the most abundant species in both national parks for all years, while corkwing wrasse, shorthorn sculpin and fivebeard rockling also had a generally high abundances in hauls (Fig. 2). The Labridae family was especially abundant in the 2018 sampling (Fig. 2). Atlantic cod accounted for 11% of the fish community (by numbers) in 2017, 3.2% in 2018 and only 0.8% in 2019 (Fig. 3). Notably, most of the gadid species were small in size and considered juvenile (Fig. 3). In contrast, the dominating mesopredators were mostly classified as adult individuals (Fig. 2). Species only present in outer Oslo fjord were whiting (Gadidae), megrim (Scopthalmidae), sprat (Clupeidae), montagu's sea snail (Liparidae), lumpsucker (Cyclopteridae), rock gunnel (Pholidae), hooknose (Agonidae) and common dragonet (Callionymidae).

For the control sampling of deeper areas in outer Oslo fjord using baited fish traps, a total of 304 individual fish comprising 10 species from 6 different families were caught using traps set at 10 - 30 m deep from 111 sampling locations. Of these, 6 species were classified as low or mid-level carnivores (60%), while 4 species were classified as high-level predators (40%). The Pleuronectidae had the highest abundance throughout all years, with common dab (Pleuronectidae) as the most abundant species.

Fish assemblage in the zoned seascape - Tvedestrand

In Tvedestrand fjord and adjacent areas, a total of 6035 individual fish comprising 34 species from 16 families were registered from 606 fyke net hauls. Of these, nine species (26%) were classified as high-level carnivores and 25 species (74%) were classified as mesopredators (low and mid-level carnivores) (Table 2, Fig. 4). Of the total fish assemblage, 5% were classified as high level carnivores and 95% of all fish caught were classified as mesopredators (mid- and low-level carnivores) (Table 1, Fig. 4). Similar to outer Oslo fjord, Labridae was the most abundant family in Tvedestrand in all years, with corkwing and goldsinny wrasse as the most dominant species (Fig. 2). Results from Tvedestrand also show that abundance of the Labridae species was especially high for the 2018 survey. Species that were present in Tvedestrand, but not in Outer Oslo fjord included poor cod (Gadidae), great weever (Trachinidae), Nilsson's pipefish (Sygnathidae), trout (Salmonidae), lemon sole and American plaice (Pleuronectidae) as well as topknot and Norwegian topknot (Scopthalmidae).

Comparison of fish assemblages in Oslo fjord and Tvedestrand seascapes

Fish communities showed similar patterns between east and west side of outer Oslo fjord for the years 2017 and 2018, however, the eastern side had generally lower abundances of fish in the 2019 hauls (Table 1, Fig. 2). Average species richness was highest on the western side of the Oslo fjord, and lowest on the eastern side (Table 3). Tvedestrand had equal richness for all years of sampling (Table 3). Results from samples clustered into 11 or 12 sites based on a north-south gradient showed little difference in Shannon or Simpson indexes or Evenness, and appeared similar (Table S3). The linear model (eq. 1) test results showed that neither the Shannon nor Simpson diversity indexes were different between the three sampling regions Tvedestrand, eastern- (FNP) or western outer Oslo fjord (YHNP) (Table 6, Table 7). However, all regions shared somewhat lowered Shannon and Simpson ($p < 0.05$) indexes in 2019. Species Evenness ranged from 0.56 to 0.81 among years and areas (Table 3), where the eastern side of outer Oslo fjord (FNP) had the overall highest Evenness and the western side of outer Oslo fjord (YHNP) had lower Evenness overall (Table 3). The linear model test result showed this measure of species diversity was only different between regions in 2019, with significantly lower Evenness for the western outer Oslo fjord (Table 8).

Density of fish species assigned to trophic level (low, mid and high-level carnivores) showed high variability between years for the sample sites in outer Oslo fjord, but seemed to be more stable in Tvedestrand (Fig. 4). Low-level carnivores dominated the catches most years, especially in the Tvedestrand seascape, and Mid-level carnivores showed a higher abundance in the fished area in Oslo fjord than in the protected area in Tvedestrand (Table 1, Fig. 4, Fig. 5). High level carnivore species had the highest abundance in 2017 for all sampling locations (Table 1, Fig. 4). A two proportion Z-test showed that there was a significantly greater proportion of mid-level carnivores ($p<0.001$), and less low-level carnivores in outer Oslo fjord compared to Tvedestrand ($p<0.001$), however, no difference was found for proportions of top predators ($\chi^2 = 3.52$, df = 1, $p = 0.06$).

For key predatory fish species present, body size was on average 62, 30 and 34% greater in the Tvedestrand seascape compared to the outer Oslo fjord national parks for Atlantic cod, pollack and saithe, respectively (Fig. 6). For Atlantic cod, the 90th percentile length (the length that 90% of the fish are less than) was 35.3 cm in outer Oslo fjord compared to 54 cm in Tvedestrand. A Welch two-sided t-test of top predator species body lengths that were comparable between the sites (had a large enough sample size) showed significant differences between Tvedestrand and outer Oslo fjord (Atlantic cod: t= -9.06, df = 118.94, p<0.001; Pollack: t= -8.58, df = 94.13, p<0.001; Saithe: t= -5.51, df=11.99, p<0.001).

Abundance of shorthorn sculpin was lower in the Tvedestrand seascape compared to outer Oslo fjord (Fig. 6). The chi-square test results showed that there was a significant association between the shorthorn sculpin and Atlantic cod in the western side of Oslo fjord (FNP) all years of sampling (Table S2). On the east side (YHNP), we found a significant association in 2018, however not for the other sampling years (Table S2). We found no association between the species for the samplings done in Tvedestrand MPA (Table S2).

Atlantic cod dominated the top predator catch and accounted for 80% of the top predators in outer Oslo fjord, and 33% in Tvedestrand (Table 1). We explored the possibility of explaining a significant interaction effect between the geographic location and years of sampling, with the main goal of answering the probability of catching at least one cod per fyke net in the outer Oslo fjord compared to the Tvedestrand seascape. The best model for predicting presence of cod (eq. 2) supported a regional effect that also varied among years (i.e., a region × year interaction term, Table 9). Overall, fyke net hauls in the Tvedestrand seascape had a lower probability of cod catch compared to the national parks (Table 4). There was a significant decrease in cod catch for 2018 and 2019 (p<0.001) (Table 4). Also, the western side of outer Oslo fjord (YHNP) had a higher abundance of cod in 2018, and lower abundances in 2019, compared to the 2017 sampling (p<0.01) (Table 4). The eastern side of outer Oslo fjord (FNP) had a higher abundance of cod in 2018 compared to 2017 ($p<0.05$), however no difference was found for 2019 (Table 4). For cod above the legal-size limit (>40 cm), both eastern (FNP) and western side (YHNP) of the outer Oslo fjord had a significantly lower abundance of cod in 2017 compared to Tvedestrand (Table 5). The western side of outer Oslo fjord had a significantly higher abundance of cod above legal-size limit compared to Tvedestrand in 2018 and in 2019. The eastern side of the fjord had significantly higher abundance of cod above legal-size limit in 2018 but no significance was found for 2019 (cf. Table 5).

Discussion

Fish assemblages in outer Oslo fjord- and Tvedestrand seascapes

Tvedestrand had a higher abundance of mature Gadoids than outer Oslo fjord in 2017, however no apparent difference in abundance patterns could be seen for the other years of sampling. Nevertheless, top predators in the Tvedestrand seascape had a larger body size than those sampled in outer Oslo fjord, and we also found a difference in proportions of low and mid-level carnivores between the sites. In concordance with our results, similar changes have been reported in previous studies from all around the world, showing an increase in abundance of larger predatory species within MPAs or lightly fished areas, compared to exploited areas (Friedlander & DeMartini, 2002; Claudet et al., 2006; Watson et al., 2007), as well as an increase of lower trophic-level species in ecosystems experiencing substantial declines of top predator species (i.e highly fished areas) (Friedlander & DeMartini, 2002; Eriksson et al., 2011). When larger predators disappear from a system, the ecosystem responds with an increase in densities of smaller predatory fish species, and with it follows marked changes in ecosystem structure and function (Jackson et al., 2001).

Our results indicated a reduction of Atlantic cod presence during our three years of sampling in both outer Oslo fjord and Tvedestrand seascapes. This is not unexpected, as it is known from previous studies that there is high natural variability in Atlantic cod recruitment and presence (Smith and Page, 1996; Stenseth et al., 2006; Johannessen et al., 2012). The variability and reduction of Atlantic cod seen in our data could be due to random chances as well as normal temporal variation, e.g. as a response to temperature and other natural fluctuations affecting where the cod favor to be present in space and time.

Signs of trophic dysfunction and mesopredator release in the outer Oslo fjord seascape

There were more species assigned as high-level predators in the Tvedestrand seascape samples compared to outer Oslo fjord. The most abundant were Pollack and Atlantic cod, however, several of the other top predator species only occurred once or twice in the fyke net hauls. These species could still be present in higher numbers than what was shown in the results from our sampling done for this study. Some species may be poorly sampled as the fish needs to swim into the net and are not actively targeted. In addition, although fyke net is a well-used method to capture distribution of fish residing in shallow coastal habitat, species that might have influenced the results could well be underestimated through our choice of method.

The lower abundance of mid-level carnivore fish in Tvedestrand seascape might indicate that the higher trophic level species in this area still have a functional role in the ecosystem by limiting their prey (see Table 1, Fig. 4). Earlier research has argued that removal of top predator species from complex marine food webs with many interacting species may weaken the top-down effects, and trophic cascades arise only in simple food webs lacking functional redundancy (Shurin et al., 2002; Donadi et al., 2017). Our results show that the fish assemblage in outer Oslo fjord contain a higher abundance of mid-level carnivores compared to the Tvedestrand seascape (see Fig. 4, Fig. 5, Table 1). Also, the results indicate that the abundance of mid- and low-level carnivores is more stable in the Tvedetrand seascape, whereas it showed more variability between years in outer Oslo fjord (Fig. 4). These results might indicate that outer Oslo fjord is suffering from a trophic level dysfunction, where the large top predator (with Atlantic cod being the dominant species) have been extirpated, and the mesopredatory fish species have taken over the trophic niche that was freed as the top predators were declining (Floeter et al., 2005; Bourque et al., 2008).

It should be noted that, although these areas are located approximately 120 km apart, outer Oslo fjord and Tvedestrand seascapes are assumed to have somewhat similar fish assemblage as for rest of the North Sea and Skagerrak. However, it should be recognized that these areas are two different seascapes. Outer Oslo fjord is in generally a more open area compared to Tvedestrand fjord, which is a rather closed seascape. Although samples were also collected outside of the no-take- and partially protected zones in the more exposed areas in the inlet of the fjord, it is a seascape less affected by high human population densities and other anthropogenic effects. In addition, the area sampled in Tvedestrand was considerably smaller (17 km^2) than the two national parks sampled in outer Oslo fjord (FNP: 300 km^2, YHNP:190 km^2).

Some species dominated over others in abundance, and most conspicuous was the high abundance of the Cottids shorthorn sculpin (*M. scorpius*) and longspined bullhead (*T. bubalis*) observed in outer Oslo fjord. Both fish species are known to be successful piscivore hunters, and especially the shorthorn sculpin is known for being capable of eating fish almost as big as its own body size. Result from the nearby Swedish west coast by Wennhage and Pihl (2002) indicate that, depending on habitat, there might be intraguild competition for the resources shared between the Atlantic cod, shorthorn sculpin and longspined bullhead. Although no diet analysis was done in the present study for the sculpins and Atlantic cod in outer Oslo fjord, we did find an association between the species in outer Oslo fjord that was not seen for the Tvedestrand seascape.

When a dominant predator population declines, it's prey should increase in abundance. In the 1980s and 1990s, Atlantic cod populations in the North Atlantic collapsed. In the same time period, Atlantic herring populations increased drastically (NEFSC, 1998). In the Baltic Sea, a collapse of the Atlantic cod populations was followed by an increase in abundance of the European sprat (Köster et al., 2003). None of these populations have recovered, although fishing exploitation has been reduced. It is hypothesized that predation on cod eggs and larvae from these lower level species is a significant factor preventing the recovery of the cod populations (Köster et al., 2003). The high abundance of shorthorn sculpin we observed in outer Oslo fjord could be a factor negatively affecting the cod populations recruitment success by predating on eggs and larvae, as well as newly settled young-of-the-year (0-group) cod. The high abundance of this species might thus represent yet another impediment for the Atlantic cod to redeem its place as a top predator in this ecosystem.

Recovery of Atlantic cod in Outer Oslo fjord

Fish stock collapses can result in large changes to marine ecosystems, as trophic cascades and eventually regime shifts (Pershing et al., 2015; Donadi et al., 2017). Regime shifts alter the energy flow in the system, as it spans over multiple trophic levels. Since the early 1990s, several cod stocks in the northwest Atlantic has experienced a collapse and has failed to respond to complete cessation of fishing (Frank et al., 2005). Previous research has shown great decline of Atlantic cod and other piscivorous fish >30 cm in Swedish Skagerrak west coast (Svedäng, 2003). Concurrent with this decline, the abundance of mesopredatory fish such as gobids and labrids have increased in coastal Skagerrak (Eriksson et al., 2011; Bergström et al., 2016; Barceló et al., 2016). When a stock is depleted to the level of collapse, it may take several decades to recover (Neubauer et al., 2013). The recent implementation of restriction on cod fishing in along the Norwegian Skagerrak coast (including outer Oslo fjord) could potentially have a positive effect on restoration of local cod populations. However, if the abundance of other mesopredatory fish increase, or the ecosystem is already experiencing a “mesopredator release” and trophic dysfunction, as indicated by our results, the mortality rate of juvenile cod might exceed the production in this area.

Implementing MPAs could be a possible solution to improve ecosystem functions, as an increase of larger top predator species could aid to suppress lower trophic groups once the predator populations are recovering. Implementing MPAs in areas that are showing signs of ecosystem dysfunctions have previously shown promising results (Soler et al., 2015), especially for increase of top predator abundance (Colléter et al., 2012; García-Rubies et al., 2013). As greater diversity in species result in more complete food webs (Worm & Duffy, 2003; Rooney et al., 2006), MPAs offer better prey choices and availability which leads to increased abundance and better diet composition of species (Dell et al., 2015). Greater phenotypic diversity (see Fernández-Chacón et al., 2020) of protected species may also confer ecosystem benefits, reinforcing the effect of functional roles changing throughout ontogeny and lifetime of long lived, large-bodied species often absent from heavily harvested seascapes. The findings reported herein point to an increased proportion of larger individuals of top predator species as an effect of reduced fishing pressure, and lower abundance of mesopredatory species as a result of higher predation inside and around the MPAs in Tvedestrand. Thus, full protection of temperate coastal marine seascapes could act to improve piscivore predation and functional redundancy of predatory species which could limit the potential for ecological phase shifts and less desirable, alternative states.

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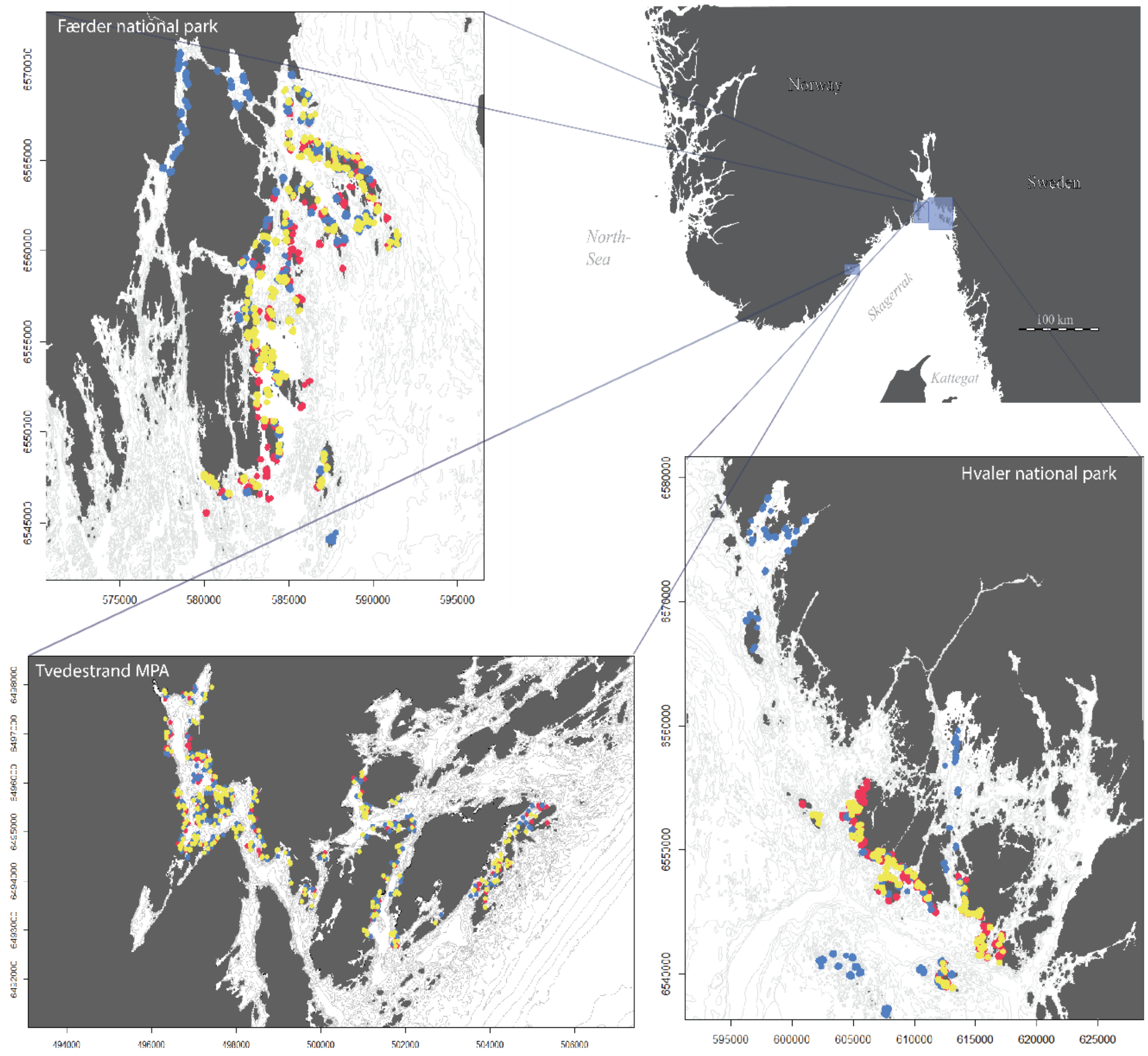

Figure 1 Map of Norwegian Skagerrak coast and sampling sites on east and west side of outer Oslo fjord, and Tvedestrand MPA located further south on the coast. Red dots represent sampling sites from 2017, blue dots represent sampling sites from 2018, and yellow dots represent sample sites from 2019.

Table 1 Sample overview from all fyke net hauls performed during three years of sampling, displaying total sample size (all fish caught), mesopredator (all individuals assigned to low and mid-level carnivores), top predator, and cod abundance from all years of sampling.

Region	Year	Total sample size	Mesopredator		Top-predator	Atlantic cod
			Low	Mid		
FNP	2017	1076	345	561	170	156
	2018	2184	946	1125	113	75
	2019	1216	673	538	5	3
YHNP	2017	1230	356	758	116	97
	2018	1598	652	893	53	42
	2019	649	322	314	14	12
Total		**7953**	**3294**	**4189**	**471**	**385**
Tvedestrand	2017	1067	560	378	129	66
	2018	3159	1653	1376	130	25
	2019	1808	1284	471	53	14
Total		**6034**	**3497**	**2225**	**312**	**105**

Table 2 List of species caught in fyke nets in outer Oslo fjord and Tvedestrand fjord assigned to trophic level and -category. Low = low-lever carnivore, Mid = mid-level carnivore, High = high-level carnivore. Information on species specific trophic levels and standard errors derived from fishbase.org.

Family	Species	Common name	Trophic level	SE	Trophic category
Gadidae	*Gadus morhua*	Atlantic cod	4.1	±0.2	High
	Merlangius merlangius	Whiting	4.4	±0.2	High
	Pollachius virens	Saithe	4.3	±0.3	High
	Pollachius pollachius	Pollack	4.3	±0.4	High
	Raniceps raninus	Tadpole fish	3.8	±0.56	Mid
	Trisopterus minutus	Poor cod	3.7	±0.2	Mid
Labridae	*Centrolabrus exoletus*	Rock cook	3.5	±0.50	Low
	Labrus bergylta	Ballan wrasse	3.2	±0.0	Low
	Symphodus melops	Corckwing wrasse	3.4	±0.1	Low
	Ctenolabrus rupestris	Godsinny-wrasse	3.6	±0.2	Mid
	Labrus mixtus	Cuckoo wrasse	3.9	±0.62	Mid
Pleuronectidae	*Limanda limanda*	Common dab	3.4	±0.64	Low
	Platichthys flesus	European flounder	3.3	±0.2	Low
	Hippoglossoides platessoides	American plaice	4.1	±0.0	High
	Pleuronectes platessa	European plaice	3.2	±0.50	Low
	Microstomus kitt	Lemon sole	3.2	±0.33	Low
Scopthalmidae	*Scophthalmus rhombus*	Brill	4.4	±0.1	High
	Lepidorhombus whiffiagonis	Megrim	4.3	±0.1	High
	Phrynorhombus norvegicus	Norwegian topknot	4	±0.60	High
	Zeugopterus punctatus	Topknot	4	±0.66	High
Gobidae	*Gobisculus flavescens*	Two-spotted goby	3.2	±0.34	Low
	Gobius niger	Black goby	3.3	±0.2	Low
Syngnathidae	*Entelurus aequoreus*	Snake pipefish	3.5	±0.44	Low
	Syngnathus rostellatus	Nilsson's pipefish	3.7	±0.40	Mid
	Syngnathus acus	Greater pipefish	3.3	±0.2	Low
Clupeidae	*Clupea harengus*	Atlantic herring	3.4	±0.1	Low
	Sprattus sprattus	European sprat	3	±0.07	Low
Cottidae	*Myoxocephalus scorpius*	Shorthorn sculpin	3.9	±0.0	Mid
	Taurulus bubalis	Longspined bullhead	3.6	±0.52	Mid
Liparidae	*Liparis montagui*	Montagu’s seasnail	3.5	±0.57	Low
Soleidae	*Solea solea*	Common sole	3.2	±0.1	Low
Gasterosteidae	*Spinachia spinachia*	Fifteen-spined stickleback	3.5	±0.37	Low

Table 2 (continued)

Anguillidae	*Anguilla anguilla*	European eel	3.6	±0.2	Mid
Lotidae	*Ciliata mustela*	Fivebeard rockling	3.5	±0.3	Low
Cyclopteridae	*Cyclopterus lumpus*	Lumpsucker	3.9	±0.0	Mid
Pholidae	*Pholis gunnellus*	Rock gunnel	3.5	±0.46	Low
Belonidae	*Belone belone*	Garfish	4.2	±0.4	High
Zoarcidae	*Zoarces viviparus*	Eelpout	3.5	±0.49	Low
Agonidae	*Agonus cataphractus*	Hoocknose	3.4	±0.3	Low
Callionymidae	*Callionymus lyra*	Common dragonet	3.3	±0.38	Low
Trachinidae	*Trachinus draco*	Greater weever	4.2	±0.71	High
Salmonidae	*Salmo trutta*	Sea trout	3.4	±0.1	Low

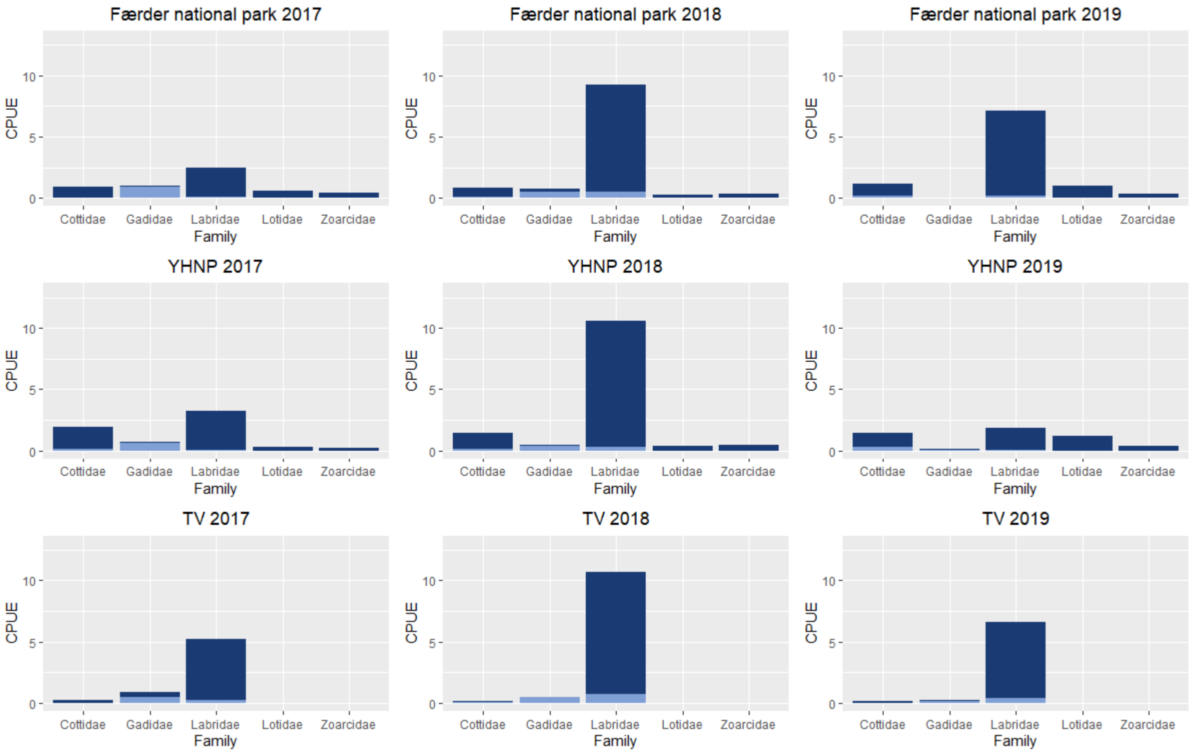

Figure 2 CPUE (N/total fyke nets/days) split into life stages for the most common taxonomic groups present at both national parks (FNP, YHNP) and Tvedestrand fjord (TV) for all years of sampling. Dark blue bars represent CPUE of adults, while light blue bars represent CPUE of juveniles.

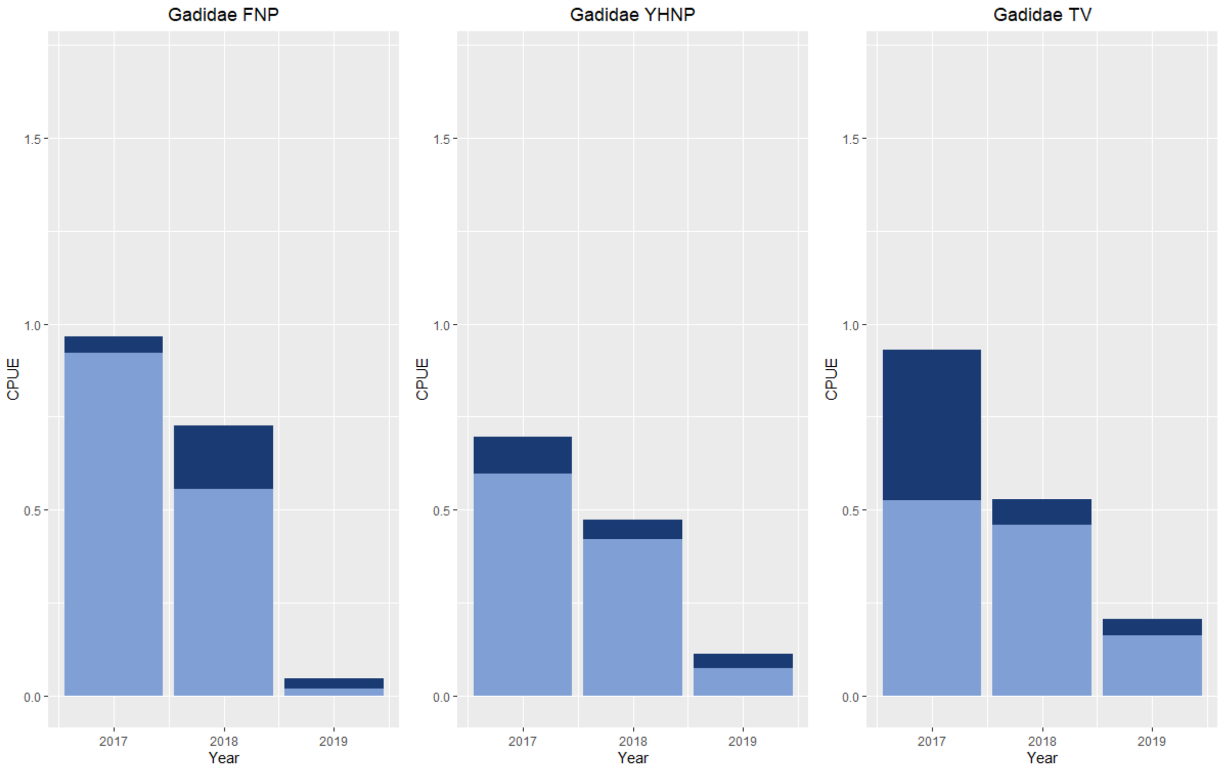

Figure 3 CPUE (N/total fyke nets/days) for the Gadidae family, representing the group holding the most important top-predator species for the study areas, present at both national parks (FNP, YHNP) and Tvedestrand fjord (TV) for all years. Dark blue bars represent CPUE of adult individuals while light blue bars represent CPUE of juveniles.

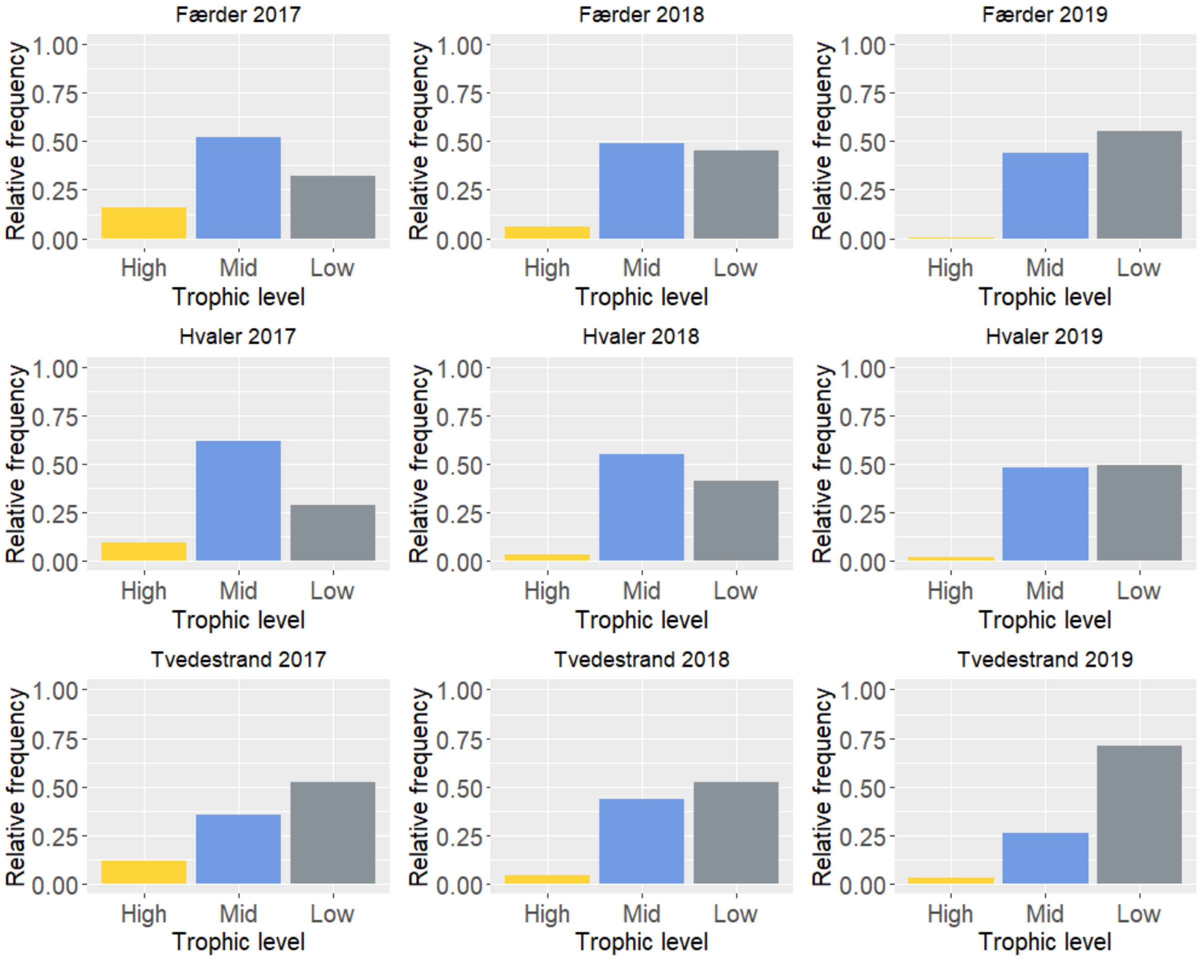

Figure 4 Relative frequency of trophic levels for all fish species caught in the outer Oslo fjord and in the Tvedestrand MPA during three years of sampling. Abundance of high-level carnivores is displayed as yellow bar, mid-level carnivores is displayed as blue bar, and low-level carnivores are displayed as grey bar.

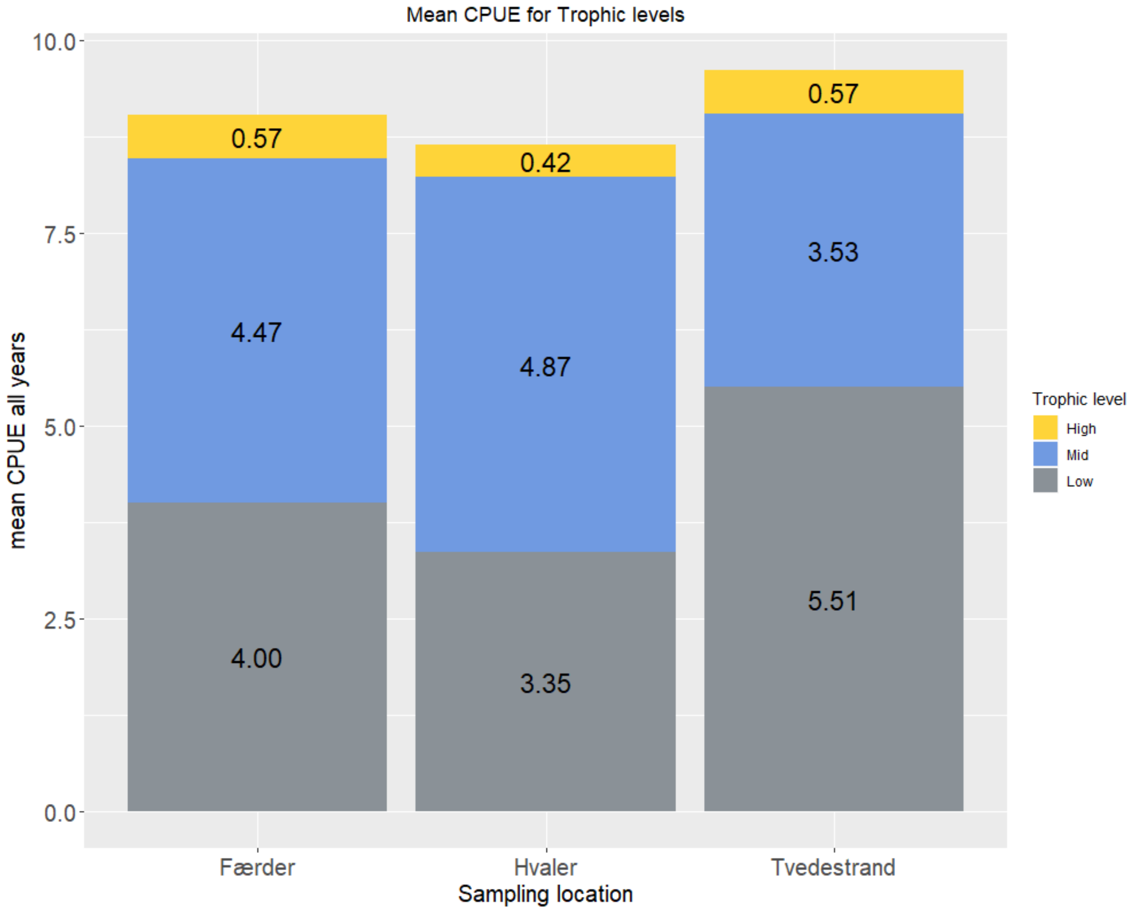

Figure 5 Mean CPUE (N/total fyke nets/days) for all species caught from the three sampling sites and all years grouped into low (grey), mid (blue) and high-level (yellow) carnivores based on diets (www.fishbase.org).

Table 3 Summary of average Diversity indices, evenness and richness for east and west side of outer Oslo fjord and protected area of Tvedestrand fjord for all sampling years.

Site	Year	Shannon index (H)	Simpson index (D)	Evenness	Richness
Oslo fjord west	2017	2.22	0.17	0.68	26
	2018	2.02	0.22	0.61	27
	2019	2.1	0.18	0.66	24
Oslo fjord east	2017	2.47	0.16	0.81	21
	2018	1.89	0.25	0.63	20
	2019	2.28	0.14	0.74	21
Tvedestrand MPA	2017	2.22	0.16	0.68	26
	2018	2.21	0.15	0.68	26
	2019	1.82	0.24	0.56	26

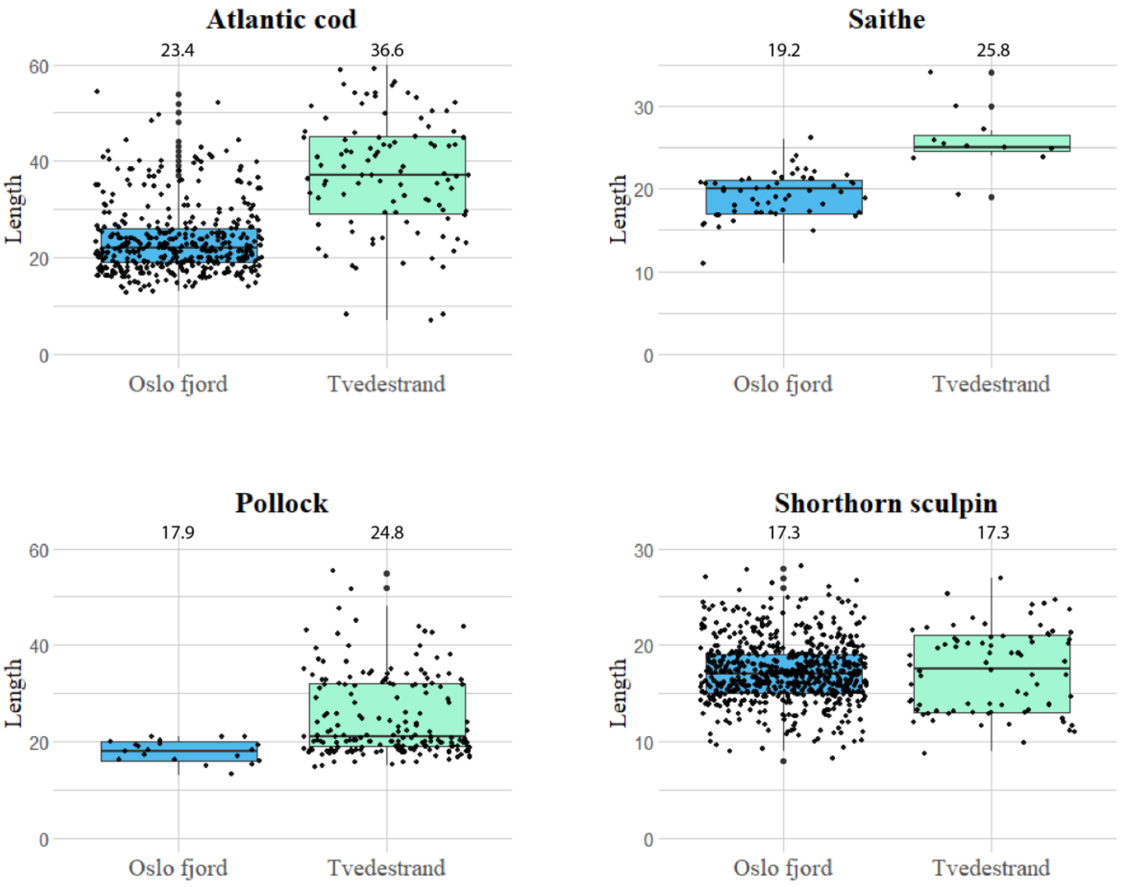

Figure 6 Length distribution for top-predator species Atlantic cod (*Gadus morhua*), saithe (*Pollachius virens*), pollack (*Pollachius pollachius*), and shorthorn sculpin (*Myoxocephalus Scorpius*) from samplings performed in Tvedestrand and outer Oslo fjord (samplings from both national parks pooled together) for all catches collected in 2017, 2018 and 2019. Box displays a confidence interval around the median, while average length is noted above the boxplots.

Table 4 Results from GLM (Cod presence predicted by region and fyke-nets used). Region Tvedestrand and year 2017 were set as reference levels (coded as zero) in the model. (Presence ~ Region * factor(Year)).

Coefficients:	Estimate	Std. Error	z value	Pr(>|z|)	
Intercept	-0.63	0.1769	-3.562	0.000368	***
RegionFNP	0.542	0.2309	2.348	0.01889	*
RegionYHNP	0.1892	0.2378	0.796	0.426223	
factor(Year)2018	-1.7309	0.2932	-5.902	3.58E-09	***
factor(Year)2019	-2.2878	0.3451	-6.629	3.38E-11	***
RegionFNP:factor(Year)2018	1.1163	0.3691	3.024	0.00249	**
RegionYHNP:factor(Year)2018	1.1201	0.3973	2.82	0.004809	**
RegionFNP:factor(Year)2019	-1.4744	0.6939	-2.125	0.033612	*
RegionYHNP:factor(Year)2019	0.3106	0.5152	0.603	0.546529	
R^2	0.16				

Table 5 Results from GLM (Cod >40 cm) presence predicted by region and fyke-nets used) for the three study areas. Region Tvedestrand and year 2017 were set as reference levels (Presence ~ Region * factor(Year)).

Coefficients:	Estimate	Std. Error	z value	Pr(>|z|)	
Intercept	-1.3083	0.1455	-8.992	< 2e-16	***
RegionFNP	-3.1915	0.5234	-6.098	1.08 e -09	***
RegionYHNP	-3.0984	0.5237	-5.917	3.28 e -09	***
factor(Year)2018	-2.1619	0.3079	-7.021	2.21 e -12	***
factor(Year)2019	-2.3293	0.3266	-7.131	9.98 e -13	***
RegionFNP:factor(Year)2018	3.4165	0.6589	5.185	2.16 e -07	***
RegionYHNP:factor(Year)2018	1.859	0.9232	2.014	0.0441	*
RegionFNP:factor(Year)2019	1.8946	0.929	2.039	0.0414	*
RegionYHNP:factor(Year)2019	-11.8301	450.1	-0.026	0.979	

Table 6 Results from linear model predicting Shannon index using region and year as predictor variables (Shannon ~ Region * factor(Year)). Region FNP and year 2017 were set as reference levels (coded as zero) in the model.

Coefficients	Estimate	Std.Error	t-value	Pr(>|t|)	
(Intercept)	1.14093	0.0281	40.597	<2e-16	***
Region TVE	0.06183	0.04325	1.429	0.1531	
Region YHNP	0.03966	0.04056	0.978	0.3284	
factor(Year)2018	-0.02408	0.04086	-0.589	0.5558	
factor(Year)2019	-0.09419	0.04102	-2.296	0.0218	*
RegionTVE:factor(Year)2018	-0.00568	0.0579	-0.098	0.9219	
RegionYHNP:factor(Year)2018	-0.05938	0.06253	-0.95	0.3425	
RegionTVE:factor(Year)2019	-0.09851	0.05896	-1.671	0.095	.
RegionYHNP:factor(Year)2019	-0.0772	0.05985	-1.29	0.1974	
R^2	0.03				

Table 7 Results from linear model predicting Simpson index using region and year as predictor variables (Simpson ~ Region * factor(Year)). Region FNP and year 2017 were set as reference levels (coded as zero) in the model.

Coefficients	Estimate	Std.error	t-value	Pr(>|t|)	
(Intercept)	0.621134	0.01108	56.057	<2e-16	***
RegionTVE	0.022441	0.017052	1.316	0.1884	
RegionYHNP	0.015649	0.015993	0.979	0.328	
factor(Year)2018	-0.030493	0.016111	-1.893	0.0586	.
factor(Year)2019	-0.038742	0.016173	-2.395	0.0167	*
RegionTVE:factor(Year)2018	0.012429	0.022826	0.544	0.5862	
RegionYHNP:factor(Year)2018	-0.01925	0.024653	-0.781	0.4351	
RegionTVE:factor(Year)2019	-0.039041	0.023244	-1.68	0.0933	.
RegionYHNP:factor(Year)2019	-0.009461	0.023598	-0.401	0.6885	
R^2	0.02				

Table 8 Results from linear model predicting Evenness using region and year as predictor variables (Evenness ~ Region * factor(Year)).

Coefficients	Estimate	Std.error	t-value	Pr(>|t|)	
(Intercept)	0.899087	0.010107	88.954	< 2e-16	***
RegionTVE	0.001038	0.015555	0.067	0.94683	
RegionYHNP	-0.008851	0.014589	-0.607	0.54416	
factor(Year)2018	-0.087323	0.014697	-5.942	3.64E-09	***
factor(Year)2019	-0.034964	0.014753	-2.37	0.01794	*
RegionTVE:factor(Year)2018	0.039109	0.020821	1.878	0.06057	.
RegionYHNP:factor(Year)2018	0.009685	0.022488	0.431	0.6668	
RegionTVE:factor(Year)2019	-0.02595	0.021203	-1.224	0.22122	
RegionYHNP:factor(Year)2019	0.061227	0.021526	2.844	0.00452	**

Table 9 Summary of Generalized Linear Models (GLMs) tested for Altantic cod presence in outer Oslo fjord and Tvedestrand MPA. Bold version shows GLM with lowest AIC score that was used for analysis.

Top-predator species	Models	AIC
Cod	**GLM= Presence ~ Region * factor(Year)**	**1335.3**
	GLM= Presence ~ Region + factor(Year)	1350.6
	GLM= Presence ~ Region	1592.6
	GLM= Presence ~ factor(Year)	1373.5
0-model	GLM=Presence ~ 1	1730.1
Cod > 40 cm	**GLM= Presence ~ Region * factor(Year)**	**754.9**
	GLM= Presence ~ Region + factor(Year)	770.1
	GLM= Presence ~ Region	915.9
	GLM= Presence ~ factor(Year)	947
0-model	GLM=Presence ~+1	1034.4

Supplementary

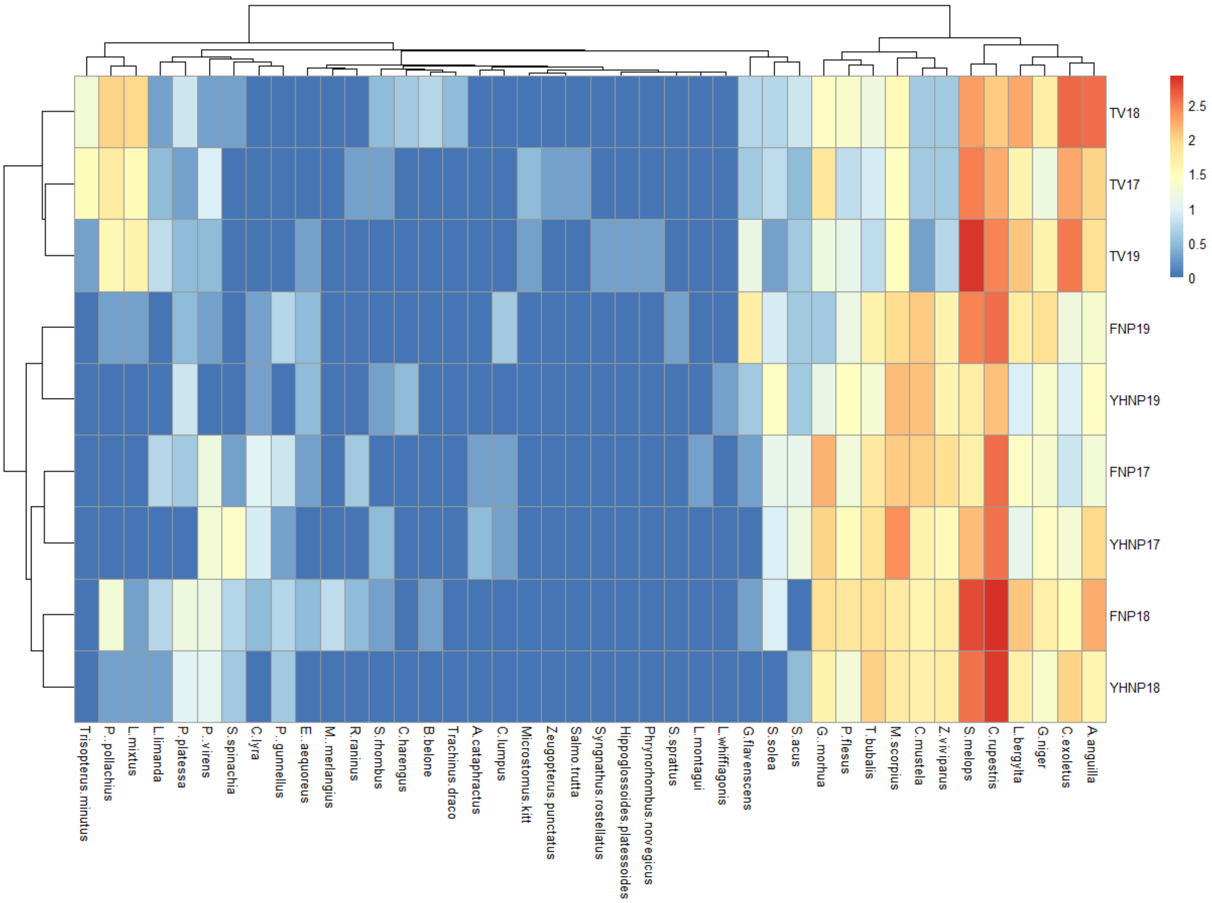

Figure S1 Heatmap of frequency of species caught in fyke nets (log10 transformed) located in outer Oslo fjord (FNP, YHNP) and Tvedestrand (TV) for all years. Colors suggest a positive or negative relation between the national parks and the abundance of the different species. Top dendrogram shows which species appeared most similar, side dendrogram shows which years appeared most similar.

Table S1. Summary of sampling years, total catch and number of fyke nets and traps used in the two national parks and in Tvedestrand

Sample site	Year	Fyke nets	Trap	Sampling days	Total catch (all species)
Færder national park (FNP)	2017	181	21	4	1076
	2018	159	20	4	2184
	2019	156	20	4	1216
Ytre Hvaler National park (YHNP)	2017	166	19	4	1230
	2018	112	14	3	1598
	2019	156	17	4	649
Tvedestrand MPA (TV)	2017	141	0	6	1067
	2018	231	0	6	3159
	2019	234	0	7	1808
Total		**1536**	**111**	**42**	**13987**

Table S2. Summary of numbers of shorthorn sculpin (*Myoxocephalus Scorpius*) and Atlantic cod (*Gadus morhua*) caught at the national parks and Tvedestrand, chi-square test for association between the species and fishers exact test.

Area	**Year**	**χ2**	**p**	**Fishers exact test**
Fæder	2017	5.6	**0.01**	**0.02**
Hvaler	2017	2.73	0.09	0.1
Tvedestrand MPA	2017	0.004	0.94	1
Færder	2018	4.89	**0.02**	**0.03**
Hvaler	2018	3.96	**0.04**	0.06
Tvedestrand MPA	2018	0.34	0.55	0.47
Færder	2019	3.66	**0.05**	0.09
Hvaler	2019	0.005	0.9	1
Tvedestrand MPA	2019	0.59	0.44	0.34

Table S3 Summary of Shannon-, Simpson- and evenness indices for sample sites in both national parks for all years, divided into 11 or 12 clusters based on north-south gradient, where 1 is located in the northernmost part, and 12 is located in the southernmost part.

National park	Cluster	Shannon index (H')			Simpson's index			Evenness (J)		
		2017	2018	2019	2017	2018	2019	2017	2018	2019
Færder national park	1	2.04	1.84	2.22	0.8	0.74	0.84	0.77	0.65	0.78
	2	1.83	2.12	2.13	0.77	0.81	0.86	0.71	0.74	0.89
	3	2.37	1.66	1.95	0.88	0.64	0.82	0.85	0.63	0.81
	4	2.22	1.43	1.97	0.86	0.65	0.81	0.84	0.53	0.82
	5	1.7	1.19	1.53	0.69	0.55	0.7	0.66	0.51	0.78
	6	1.83	1.46	1.82	0.79	0.62	0.76	0.83	0.64	0.79
	7	2.09	1.53	1.75	0.84	0.59	0.74	0.84	0.58	0.73
	8	1.72	2.11	1.77	0.7	0.82	0.75	0.64	0.8	0.74
	9	2.2	1.6	1.8	0.84	0.69	0.74	0.81	0.59	0.66
	10	1.65	1.65	1.55	0.73	0.72	0.73	0.75	0.69	0.75
	11	2.35	1.52	1.51	0.88	0.69	0.65	0.83	0.63	0.61
	12	2.09	1.44	1.33	0.85	0.7	0.6	0.91	0.69	0.64
Ytre Hvaler national park	1	2.05	1.92	2.04	0.82	0.82	0.85	0.8	0.87	0.89
	2	2.25	1.61	1.88	0.86	0.64	0.79	0.81	0.61	0.85
	3	1.63	1.71	2.13	0.77	0.8	0.83	0.91	0.88	0.86
	4	2.17	1.43	2.06	0.84	0.68	0.81	0.78	0.65	0.83
	5	1.78	1.41	1.72	0.75	0.67	0.78	0.71	0.59	0.88
	6	2.02	1.3	2.17	0.84	0.64	0.86	0.79	0.59	0.9
	7	1.93	1.32	2.02	0.82	0.62	0.83	0.87	0.55	0.79
	8	2.09	2.13	2.09	0.84	0.85	0.84	0.79	0.86	0.84
	9	2.09	1.35	1.69	0.84	0.62	0.75	0.84	0.65	0.73
	10	2	1.37	1.97	0.81	0.64	0.82	0.74	0.59	0.77
	11	2	1.62	2.07	0.8	0.72	0.81	0.76	0.65	0.8
	12	1.79	-	1.71	0.74	-	0.77	0.72	-	0.82

Paper III

Local recruitment of Atlantic cod and putative source spawning areas in a coastal seascape

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Abstract

Good understanding of realized dispersal is fundamental to measure connectivity between populations, and determining factors driving this exchange are crucial to our understanding of the population dynamics, genetic structure and biogeography of many coastal species. Recruitment in marine systems are known to be highly variable, with large year-to-year variations. In Skagerrak, beach seine time-series data available from 1919 to present show high variability in recruitment and settlement of Atlantic cod (*Gadus morhua*). Along the Norwegian Skagerrak coast, two ecotypes of cod co-exist, assigned to either "fjord cod" or "North Sea cod" based on genetic divergence. Recent research points to evidence of these ecotypes being sympatric populations inhabiting the coastal areas, separated by genomic and behavioral barriers to gene flow. In outer Oslo fjord, a decline of Atlantic cod has been observed since the 1990s. However, although larger cod (>40) cm are now considered rare, some years show a strong recruitment signal. This raises the question of which population is contributing recruitment in this area. By combining genetic assignment data for recruits sampled in 2017 and 2018 (as 0- and 1-group cod, respectively) with a biophysical model for the Skagerrak region over the time period from spawning to settlement in 2017, we located the most probable spawning locations of Atlantic cod recruits by “back-tracking” larval drift trajectories. We found putative source areas on both sides of the outer Oslo fjord, as well as potential upstream sources in the North Sea and Kattegat. Findings are discussed in light of suitable management strategies and potential for restoration of coastal cod populations.

Introduction

Using seascape genetics to infer dispersal and connectivity in marine populations

The collapse of many of the world’s fisheries has fueled an interest of a reevaluation of existing fisheries management strategies, as the effects of human impact on the environment is now leading to an Anthropocene mass extinction in the ocean (Jackson et al., 2001). Synergetic effects of overfishing, habitat destruction, anthropogenic global warming, new species introductions, ocean acidification, toxins and river runoffs containing high amounts of nutrients are all affecting the oceans, with unknown ecological and evolutionary consequences (Jackson, 2008). This has triggered a call for immediate and effective conservation action, where reducing exploitation, restoring habitats and establishing marine protected areas (MPA’s) and no-take zones is crucial for protection of marine species and habitats (CBD, 2010). For successful restoration of species populations and designs of MPA’s, it is fundamental to describe the spatial distribution and the demographic connectivity of marine organisms (Palumbi, 2003). The use of genetic tools gives the opportunity to study the variation of allele frequencies within and among groups to identify populations. New methods of combining genetic data with other fields of knowledge, also referred to as “multidisciplinary seascape genetics”, provide a contemporary approach that could reveal novel insights into principles behind marine population structure and connectivity, which could be crucial for implementing better strategies for fisheries management and marine conservation networks (Selkoe et al., 2008).

Many marine species are known for their large effective population sizes, in an environment where physical barriers are largely absent. Marine organisms are generally broadcast spawners with pelagic egg and larvae stages, where ocean currents may carry them far from their original spawning site. This high dispersal potential is often associated with only mild genetic differentiation over large spatial scales due to extensive gene flow; however, some level of genetic structure is still prevalent in many marine species (Mariani et al., 2005; Purcell et al., 2006; Knutsen et al., 2011). Understanding of dispersal is fundamental for determining the connectivity between populations. Population connectivity, defined as the exchange of individuals among geographically separated populations, plays a central role in local and metapopulation dynamics (Lipcius et al., 2008). Connectivity and gene flow between marine populations is influenced by multiple biological and hydrodynamic processes involved in the transport of eggs and larvae, such as bathymetry, temperature and salinity, as well as egg buoyancy and larval characteristics of the species (Selkoe et al., 2010). Pelagic larval duration (PLD), defined as the amount of time the larvae remain in the water column, and their interaction with ocean current speed and direction prior to settlement, has a direct effect on the potential distance larvae are dispersed (Fiksen et al., 2007; Cresci et al., 2019). One of the biggest challenges studying population connectivity lies in the questions regarding the physical oceanography, especially in coastal environments. Nearshore environments are affected by several coastal processes, as coastal topography, stratified water columns, tidal forces, wind, buoyancy, surface waves, and turbulence, which all can have direct or indirect effects on larval transport (Cowen & Sponaugle, 2009). In addition, it is important to consider that even though larvae from outside populations might get carried to new grounds, this does not necessarily translate to successful recruitment (in terms of realized dispersal) (Pineda et al., 2007). These questions are crucial considering proper management of marine species, as it is essential to assess if the local populations are solely dependent on their own recruitment, or how much of the population is sustained from drift of eggs and larvae from another population source.

Atlantic cod along the Skagerrak coast

The Atlantic cod (*Gadus morhua*) is a benthopelagic, cold-water gadoid species. It is distributed on both sides of the Atlantic Ocean, extending northward to Spitsbergen, Disco bay and Labrador, and southwards to Cape Hatteras and the Bay of Biscay. It also enters the brackish Baltic Sea in the east. Historically it has supported one of the largest marine fisheries in the world and, as with many other commercially exploited fish species, is now in need of improved management. Atlantic cod has undergone a significant reduction across its range since the “gadoid outburst” ended in the mid-1980s, reaching historically low biomass in the 1990s. Over-exploitation as well as a changing ocean climate to a warmer state, has left several cod stocks depleted (Myers et al., 1996; Cardinale & Svedäng, 2004; Drinkwater, 2005). Despite measures to reduce fishing mortality, several stocks have shown little or no recovery during the past decade (Hutchings et al., 2004), with some exceptions such as northern North Sea (ICES, 2017) and the Barents Sea/ NEAC cod (Kjesbu et al., 2014). In the Skagerrak neighborhood, Öresund – where bottom trawling has been banned since 1932 – stands out as a rare example of a local cod population characterized by low fishing mortality and old growth age structure (Lindegren et al., 2010, 2013; Svedäng et al., 2010).

The Atlantic cod exhibits population structuring on both small and large spatial scales. Studies have revealed genetic differences between cod sampled across the Atlantic (O'Leary et al., 2007), as well as between populations residing in different ecosystems as the Baltic and the North Sea (Nielsen et al., 2003). More recently, population genetic studies have revealed that in coastal areas, Atlantic cod is present in two genetically distinct ecotypes, which appear to coexist during a large part of their life cycle (Knutsen et al., 2018). While coastal cod generally display high site fidelity (Skjæraasen et al., 2011; Rogers et al., 2014), individuals assigned to the 'fjord' ecotype share characteristics with reference samples collected from individuals found inside fjords, while individuals assigned to the 'North Sea' ecotype are more similar to an oceanic North Sea reference sample (see *Materials and methods*). Similar division of ecotypes have been described in the western Atlantic (Siceloff & Howell, 2013), Iceland (Thorsteinsson et al., 2012), northern Norway (Berg et al., 2016) and along the Norwegian Skagerrak coast (Knutsen et al., 2003, 2011; Jorde et al., 2018). Not only are genetic differences observed between the fjord cod and oceanic cod, but fjord cod in Norway have been found to display a small, but significant genetic structure throughout its entire range (Dahle et al., 2018), including also on finer scales such as adjacent fjords (Knutsen et al., 2003; Jorde et al., 2007; Barth et al., 2017).

Recruitment in marine systems is known to be highly variable, with large year to year variations. Transport of eggs and larvae are affected by wind and internal waves, which makes it sporadic and patchy. The Atlantic cod is a highly fecund batch spawner, capable of producing as many as 9 million eggs within a spawning season, in up to 20 egg batches (Kjesbu, 1989). Cod larvae stay in the water column for some weeks after hatching and are exposed to the ocean currents for at least one month before settling to the bottom, making them susceptible to be carried far away from parental spawning location (Huserbråten et al., 2018). In Skagerrak, the now 100-year-old beach seine survey data performed yearly by the

IMR along the Norwegian Skagerrak coast since 1919, show high variability in recruitment and settlement of Atlantic cod. There is a clear indication of a major transport of eggs and larvae of cod from the North Sea, some years more than others (Knutsen et al., 2004; Stenseth et al., 2006). Population genetic studies suggest that spawning aggregations of cod in the eastern North Sea could supply recruits to Skagerrak, based on evidence of an extensive transport of cod larvae in this direction (Svedäng, 2003; Knutsen et al., 2004). However, along convoluted coastlines – such as the Norwegian Skagerrak coast, Atlantic cod showed high site fidelity and had limited movement ranges in studies using acoustic telemetry (Olsen and Moland, 2011; Aalvik et al., 2015; Freitas et al., 2016; Villegas-Ríos et al., 2017). Longer term mark-recapture-recovery work conducted along the same coastline has shown median movement well below 1 km between release and recapture for individuals at liberty for at least 1 yr (Rogers et al., 2014). Atlantic cod are pelagic broadcast batch spawners, known to migrate to specific spawning sites with extremely high fidelity (Skjæraasen et al., 2011). Resident local behavioral units or -populations with spawning aggregations in fjords and coastal lagoons are a likely cause for the structuring that has been documented, with significant along-coast population structure on scales of 10-30 km (Jorde et al., 2007; Knutsen et al., 2011). Positioning of pelagic eggs in water layers with a net inward/ fjord-ward flow are one mechanism by which cod propagules might be retained (Ciannelli et al., 2010) – increasing the chance for juvenile settlement in fjords or less exposed coastal areas. The degree to which settled juveniles stay and recruit to the local adult population, and whether such populations are mostly self-recruiting is not known. However, the relatively stable coexistence of at least two distinct ecotypes indicate that several populations use the convoluted Skagerrak coastline for spawning, as nursery, and for longer term (> 1 yr) residence (Rogers et al., 2014; Knutsen et al., 2018; Barth et al., 2017).

In eastern Skagerrak, abundance of cod and other demersal fish species > 30 cm has declined dramatically since the 1970s. However, this same decline is not found for juvenile cod; occasionally, good levels of recruitment are observed along the Skagerrak coast (Aglen et al., 2016). It is, however, still not known why these years of strong recruitment do not seem to replenish local populations in the long term. Inshore spawning aggregations of cod have been observed along the Norwegian coast (Knutsen et al., 2000) as well as off the Swedish coast (Hallbäck et al., 1974). However, it is still unknown if local populations of coastal Atlantic cod are sustained mostly by self-recruitment, or if the persistence of populations is dependent on supply from upstream sources. Abundance of juvenile fish and absence of older fish might suggest that local cod populations in the coastal areas are regulated by recruitment from offshore areas (Svedäng, 2003).

Herein, we utilized the abundant cohort of young-of-the-year (YOY) cod observed as recently settled 0-group juveniles in summer and autumn of 2017, and as highly stationary 1-group juveniles in spring 2018 – to make inference regarding likely sources of recruits to nursery areas in the outer Oslo fjord seascape. Genetic assignment of individuals sampled as 0- or 1-group juveniles, was combined with a high- and low-resolution hydrodynamic model of ocean drift for the North Sea and Skagerrak during the time period cod spawning to putative settlement of cod recruits in 2017. By 'back-tracking' larval drift trajectories from observed juvenile cod sampled in autumn and following summer, the aim of this study was to gain insight into which population of origin is the most probable source of recruits contributing to replenishment of coastal cod populations found inhabiting the outer Oslo fjord region. For the purpose of this study – taking assignment at face value – we tested whether "fjord cod" assignments were indeed likely to originate from local, proximal spawning populations, and from where "North Sea" assignments would likely originate if indeed imported to outer Oslo fjord from upstream spawning populations. We discuss our findings in light of population connectivity and its implications for restoration of depleted local spawning populations.

Materials and Methods

Study area

This study was conducted in the outer Oslo fjord (N58°9’ E 010°38’), a section of coastal Skagerrak in Southeast Norway. Here, recently designated national parks, Færder- and Ytre Hvaler national park, cover mainland coastlines and archipelagoes along the western and eastern shores of the Oslo fjord, respectively (Figure 1). Both national parks include areas high in biodiversity and nature types, which in the marine realm consist of several eelgrass beds, kelp forests, soft-bottom areas, and shell sand beaches. The outer Oslo fjord is an exposed coastal area influenced by different water masses. Due to river runoffs and inflow of waters from Kattegat and the Baltic Sea, the upper layer of the water masses consists of brackish water (>25.0 ‰), while underneath this layer, there is a mixing of water masses from the North Sea and the surface layer (25-30 ‰). High saline water (>35.0 ‰) of Atlantic origin is found at depths larger than 70-80 m transported into Skagerrak from the southern part of the Norwegian trench, which ends in the Hvaler deep within Ytre Hvaler national park. Tidal amplitude in Skagerrak is generally less than 0.5 m and is thus negligible. In general, the outer Oslo fjord is an oceanographically complex region and serves as a great study system for coastal species. The Oslo fjord region is highly populated, and as much as 40 % of the Norwegian population lives closer than a 45 min drive to the area. It is a widely used region for recreational boating and camping, particularly in the summer months. The outer Oslo fjord is affected by long-term fishing pressure, from both commercial and recreational fisheries, having depleted larger-bodied piscivorous fish, including gadoids. Bottom trawling for northern shrimp (*Pandalus borealis*) remains the last economically viable fishery in inner Skagerrak, and all suitable areas are subject to bottom trawling.

Sampling and sampling methods

To obtain maximum spatial and temporal coverage of the 2017 coastal Atlantic cod cohort we employed three modes of sampling. During July (Hvaler) and August (Færder), young-of-the-year cod (hereafter referred to as ‘0-group’) was sampled with the help of two local wrasse fishers. Sampling was performed using wrasse traps placed in groups of 5-6 traps on each sampling site located close to shore and not deeper than 7-8 meters, to keep any fish from getting damaged by pressure differences. In Ytre Hvaler national park, sampling was performed during nighttime until morning the next day (2:00-12:00) for 5 days. In Færder, sampling was performed during daytime (09:00-15:00) for 2 days. All 0-group cod caught as bycatch was measured to the nearest cm, and fin-clipped for genetic analysis before being released back into the sea. Tissue samples were stored in 99% ethanol for further DNA extraction.

In September, we utilized the beach seine survey conducted annually since 1919, with several fixed stations in the outer Oslo fjord and 15 additional stations added specifically for the purpose of this study (Figure 1). The beach seine covers an area of up to 700 m^2 per haul and is used in suitable nearshore habitats (<15 m depth). In each haul, all fish were counted and identified to species. All cod was measured to nearest mm. In addition, for this survey, all 0-group cod were collected and frozen whole on-board within 30 min of sampling. In September, individuals ranging from 5-15 cm are assumed to be 0-group cod, and >15 cm is classified as young and older adults.

Sampling of fully recruited subadult cod (hereafter referred to as ‘1-group’) from the 2017 cohort was performed during 01 to 08 May of 2018 using fyke-nets with 55 cm openings and 18 mm cod end mesh size. Sampling was performed during daytime (08:00-18:00) and fyke-nets were soaked for approximately 24 hours before being hauled and moved to a new randomly chosen site (Figure 1). All fish were counted and identified to species level and measured to nearest cm (fork length, FL) before being released back into the ocean. All Atlantic cod captured was fin-clipped for genetic analysis, and tissue samples were stored in 99% ethanol for further DNA extraction. In May 2018, all cod ≥ 30 cm were considered 1-group recruits from the 2017 cohort.

DNA extraction and genotyping

Fin-clips were stored at 4°C in pure ethanol prior to DNA extraction. All samples were extracted for DNA using the E.Z.N.A tissue kit (Omega Bio-tek), following manufacturers protocol for tissue DNA extraction. A sub sample of 15-20 samples pr. 96 well plate was quality-verified and quantified using Qubit assays (ThermoFisher) before genotyping. A total of 1022 cod tissue samples from 0-group and 1-group cod catches from 2017 and 2018 were genotyped for the present study. Genotyping of the 26 SNPs was done on a MassARRAY platform (Sequenom Inc.) at the IMR laboratory in Bergen, Norway. The SNPs used in this study were previously specially developed to distinguish among individuals from coastal and North Sea ecotypes (Jorde et al. 2018). Originally 27 SNP’s were used to discriminate between populations, however, one SNP (Gdist_68504_1675) were discarded due to repeated failing. In this paper, all analysis was therefore limited to 26 SNPs.

Population genetics - assignment of individuals

As genetic reference for the fish sampled in outer Oslo fjord, we used two previously sampled and genotyped sets of individuals from the Norwegian Skagerrak coast and from the North Sea (see Jorde et al., 2018), representing a ‘fjord’ reference and a ‘North Sea’ reference, respectively. Genetic assignment was computed using the Bayesian assignment method using the GeneClass2 software. Scores lower than 80% (n=46) and individuals that had a score with <20 loci (n=33) were omitted from further analysis, resulting in 1023 individuals being scored successfully.

Statistical analyses

Estimates of genetic differentiation (F_{st}: Weir & Cockerham) and a heterogeneity test (exact G-test) for general structure in the data were preformed using GENEPOP 4.7 on the web (Raymond and Rousset, 1995). Pairwise F_{st} estimates between samples collected from the east and west side of the Oslo fjord were calculated for all fjord individuals and North Sea individuals separately (Table S2, Table S3). Correlation between geographic and genetic distances was computed using pairwise comparisons of individuals ($\hat{a}$ statistic; Rousset, 2000) using GENEPOP 4.2 on the web. For each data set, a Mantel test with 10000 permutations was used to test for significance of geographic and genetic distance between all fjord individuals from the sampling locations, under the null hypothesis of independence between genotype and geographical location (Rousset, 2008). Geographic distances between individuals represent linear geographic distances in km. Our data was analyzed in the following way: all fjord individuals from east, all fjord individuals from west, and all individuals from both locations combined. (Note: 5 individuals of ‘fjord’ cod sampled in July on the west side of the fjord was grouped together with samples from September due to low sample size.)

Biophysical model

Ocean model and Lagrangian particle advection scheme

The hydrodynamic models used to represent the ocean currents in the study area was based on the Regional Ocean Modeling System (ROMS, http://myroms.org), a free-surface, hydrostatic, primitive equation ocean general circulation model (Shchepetkin & McWilliams 2005; Haidvogel et al., 2008). The large scale ROMS model was run with a horizontal resolution of 4 km x 4 km in an orthogonal, curvilinear grid covering parts of the North Atlantic and all the Nordic and Barents seas over the time period 1960-2017 (Lien et al. 2013, as applied in for example Lien et al. 2014). The small scale ROMS model used to simulate local cod spawning was run with a horizontal resolution of 160 m × 160 m, and was forced using hourly values of currents and hydrography along the open boundaries from a larger-scale model covering the whole Norwegian coast (Albretsen et al., 2011, as for example applied in Huserbråten et al. 2018), high-resolution wind fields (Skamarock et al., 2008) and realistic freshwater discharge from all rivers in the model domain (provided by the Norwegian Water Resources and Energy Directorate, see Beldring et al., 2003)

To model the advection of particles in the horizontal plane we applied the fourth order Runge-Kutta scheme LADIM (Ådlandsvik & Sundby, 1994, as for example applied in Myksvoll et al., 2018) coupled with the velocity fields from the ROMS models. Here particles were released randomly across the North Sea, Skagerrak and Oslo fjord in areas shallower than 200 m, every day from the 1st to 31st of March 2017 (see Brander, 1994 and Heath et al., 2008 for review of spawning times in the North Sea). The particles drifted at a fixed depth, uniformly distributed between 1 m and 20 m throughout the simulations. In total 1,240,000 particles were released in the two model domains (620,000 in each of the two domains) and were allowed to drift until 20th of July, as settlement period of cod in this area has been found to last from start of June to mid-July (see Johannessen, 2014, but also Huserbråten et al., 2018 for modelled drift period)–giving a drift period of 62-141 days.

To locate the most probable spawning locations of cod in the two model domains we "back-tracked" larval drift trajectories from observed 0-group/1-group cod in autumn to the most probable spawning locations in spring (see method applied in Huserbråten et al., 2019). More specifically, an objective search algorithm identified drift trajectories (evaluated from all random particle release locations) that intersected the 0-group/1-group observations (within a radius of ≈1600 m) of the beach seine hauls, within the settlement phase 1st of June to 20th July (see previous paragraph). Due to the coarser resolution of the large-scale model we used the entire outer Oslo fjord area as settlement area when locating/"back-tracking" the North Sea spawning areas. The ability of the drift trajectories to explain the observed 0-group/1-group abundance was thus interpreted as a confirmation of spawning at a given release point and a high larval survival integrated over the drift phase. Subsequently we fitted a 2D Generalized Additive Model (GAM) to the successful/unsuccessful drift intersection of a given release location with the identified settlement areas, with x and y coordinates of release point as covariates, as implemented in R-package "MGCV" (Wood, 2011).

Results

Assignment and size distribution of the two ecotypes

A total of 1023 individuals, representing 0-group and 1-group juvenile cod sampled in summer and autumn 2017 and spring 2018, respectively, were genotyped successfully and had an assignment score above 80% and >20 SNPs producing a valid genotype. Assignment tests showed that overall, there was a slightly higher proportion of cod assigned to the 'North Sea' reference (n=531) compared to the coastal 'fjord' reference (n=492) in our data.

Sampling of 0-group cod performed in July and August 2017 aboard wrasse fisher vessels caught a considerably higher proportion of individuals assigned to the 'North Sea' reference (98%). Mean size (cm ± SD) of sampled 0-goup cod was 9.37 ± 1.48 and 10.1 ± 1.43, in July and August, respectively. (Figure 2 upper panels, Table 1).

Sampling conducted in September showed a higher abundance of 0-group cod assigned to the coastal fjord reference in both national parks (Figure 2 middle panels, Table 1). In Færder national park, a majority (90 out of 158, or 57%) of the 0-group cod caught in the beach seine was assigned to the fjord reference, and 43% to the North Sea reference. Along the Ytre Hvaler national park, the proportion of 'fjord cod' was even higher (363 out of 431 or 84%) with only 16% of the beach seine samples assigned to the 'North Sea' ecotype. Mean size (cm ± SD) of sampled 0-goup cod in September was 8.2 ± 1.39.

In May 2018, a total of 96 cod considered older than 1 year (14 to ≤30 cm FL) were caught, ranging in size from 14 to 30 cm, mean 21.7 ± 4.2 SD. Overall, there was a higher proportion of individuals assigned to the 'North Sea' reference (65%). Body size, on average, was larger for 1-group cod assigned to 'North Sea' than those assigned to 'fjord' in Ytre Hvaler national park (Figure 2, lower left panel), whereas the average size of both ecotypes were similar in Færder national park (Figure 2, lower right panel). Of the total cod catch from 2018, 33 individuals were >30 cm and considered to be older than 1 year, ranging in size from 31 to 54 cm. Of these, 14 individuals were assigned to the 'fjord' reference (mean 39.6 ± 5.4 SD), and 19 individuals was assigned to the 'North Sea' reference (mean 37.8 ± 4.0 SD).

Connectivity

Results from the low-resolution/large-scale biophysical model pointed to three likely source areas for long distance transport of eggs and larvae imported to nursery areas in outer Oslo fjord, from the North Sea (Fladen ground and Fisher banks) and Kattegat (Figure 3). Fladen ground, a shallow bank located in the northern North Sea, is the potential source located furthest away from Skagerrak, and also seems to be the least liable source of recruits according to our results with a maximum probability of 3% of settling in the outer Oslo fjord. Fisher bank, a sand bank located off the west coast of Denmark, seems to be the most probable source of origin for transport of cod eggs and larvae from the North Sea into the outer Oslo fjord region, with recruits spawned there having a maximum probability of 5% of settling in the outer Oslo fjord. In addition, Kattegat also stands out as a potential source of recruits, with particles released along the coastal stretch between Halmstad and Gothenburg having up to 5% chance of settling in the outer Oslo fjord. On the other hand, the model also suggested a high probability (up to 50%) of more local sources of recruits from the north-eastern Skagerrak and the outer Oslo fjord itself.

The spatial distribution of genetically assigned individuals showed that both ecotypes seem to recruit to overlapping locations in the national parks, with no apparent distinction in habitat preference (see Figure 4). Results from the high-resolution/small-scale biophysical model suggests three possible sources of origin of local ‘fjord’ cod pointed out by the model inside the study areas, one on the west side of the fjord and two on the east side. Here the tentative spawning area on the western side of the fjord (i.e. within the Færder NP) had a maximum probability of 5.3% of supplying larval recruits to the western sampling sites (Figure 4, B-I); the eastern spawning area (i.e. within the Ytre-Hvaler NP) a maximum probability of 0.5% of supplying larval recruits to the eastern sampling sites (Figure 4, B-II);

and the north-eastern spawning area a 0.7% maximum chance of recruiting to the north-eastern sampling area (Figure 4, B-III).

Population genetic structure

Loci used for this study was chosen to segregate between the two cod ecotypes and results clearly illustrates that both components were present in both east and west side of the fjord (Figure 5). Overall genetic differentiation for the fjord ecotype between the east and west side of the fjord was highly significant ($F_{st} = 0.0054$, $p < 0.0001$). Similarly, for the ‘North Sea’ ecotype, genetic differentiation between the east and west side of the fjord was also evident ($F_{st} = 0.0032$, $p = 0.0054$). Pairwise F_{st} values showed a general higher differentiation between ‘fjord’ populations compared to ‘North Sea’ population (Table S1, S2). A t-test for the mean F_{st} across loci between the ‘fjord’ and ‘North Sea’ types found no significance (t=1.275, df=45.9, p=0.2). The relationship between genetic differentiation and geographical distance was assessed between all fjord individuals both within sampling location and between sampling locations. No significance was found between genetic and geographic distances, whether measured among fjord individuals in the east (Mantel test: p=0.355), or in the west (p=0.303).

Discussion

Genetic assignment of 0- and 1-group cod sampled in this study provide evidence that both ecotypes deliver viable recruits to areas in the outer Oslo fjord seascape in eastern Skagerrak, which supports findings reported in earlier genetic studies (Stenseth et al., 2006; Knutsen et al., 2018).

High resolution back-tracking of larval dispersal trajectories based on 0- and 1-group cod assigned to ‘fjord’ origin revealed three likely spawning sites for fjord cod within- or adjacent to the areas sampled for this study (cf. Figure 4B). Moreover, genetic differentiation of assigned ‘fjord’ cod between sampling locations on the eastern and western side of the fjord was highly significant. We note that the loci used in this study was originally designed to segregate between the ‘fjord’ and ‘North Sea’ ecotypes, without regard to the ability of these loci to segregate further between potential sub-components or local populations. Nonetheless, the differentiation found is of interest considering the short distance (≤ 22 km) between the sampling sites and the possibility of egg and larvae drift between them. Our findings thus indicate a lower exchange rate between populations than might have been expected, although the mechanisms behind this remains unknown. Fjord cod is known to be highly stationary and can live its whole life inside a fjord, and is assumed to be mostly self-recruiting (Rogers et al., 2014). Cod from the North Sea cannot be distinguished genetically from cod in offshore coastal areas. Thus, in addition to larval drift from one or more spawning grounds in the North Sea proper, this type could potentially also spawn in more coastal or near-coastal areas (Barth et al., 2017; Jorde et al., 2018; Barth et al., 2019). Hence, we cannot know exactly from where our ‘North Sea’ ecotype originate. For future studies, we recommend that genetic samples from the sources suggested by the biophysical model should be included as “outgroups” for comparison. Spawning site fidelity as well as retention of eggs and larvae have been proposed as mechanisms contributing to the observed population structure (Espeland et al., 2007; Ciannelli et al., 2010; Skjæraasen et al., 2011). More recently, three large chromosomal inversions have been described between fjord and offshore samples of Atlantic cod on the Norwegian Skagerrak coast (Sodeland et al., 2016). These inversions have been identified on both sides of the Atlantic Ocean (Bradbury et al., 2010).

SNPs within these inversions have been linked to temperature (Bradbury et al., 2010), as well as salinity and oxygen regulation (Berg et al., 2015).

Results from our large-resolution model pointed out three potential spawning grounds in the North Sea and Kattegat possibly supplying off-shore recruits to the study area (Figure 3A). Previous studies have revealed significant genetic structure between populations of cod in the North Sea (Hutchinson et al., 2001; Heath et al., 2014). Fisher bank in the North Sea was suggested as the most probable source of origin by our model, as well as Kattegat. Fisher bank is the closest bank which also holds a known spawning ground for cod (Munk et al., 2009). Fladen ground, which was also suggested as a potential source of recruits, is located outside the southwest coast of Norway, and the distance outer Oslo fjord is most likely too far for recruits to drift and eventually settle. Although some eggs and larvae might make the distance, it’s unlikely that this spawning ground is impacting the coastal areas in eastern Skagerrak in a manner that could be as impactful as previously observed for years with high inflow of North Sea water, where cod of ‘North Sea’ ecotype are known to dominate the 0-group catches (Knutsen et al., 2004; Stenseth et al., 2006). In addition, Kattegat was also included out as a potential source of recruits in our model. Cod in the southern Kattegat is genetically quite similar to the coastal ‘fjord’ cod ecotype and may well contribute to recruitment on the Skagerrak coast (Barth et al. 2017). While the degree of genetic connectivity between cod populations in Kattegat and outer Oslo fjord is largely unknown, there is a great potential for eggs and larvae to be transported northward with the Baltic outflow, before settling into nursery habitat in the outer Oslo fjord (Jonsson et al., 2016). This was also supported by our model, which predicted that 5% of recruits that was spawned in this area could potentially settle in the outer Oslo fjord region. Although this all seems probable, due to the limitations of the SNPs used for this study we could not investigate this any further in this study.

Our sampling protocol was successful in obtaining a substantial number of tissue samples from the 2017 Atlantic cod cohort settling into nursery areas in the outer Oslo fjord, with a wide temporal and spatial coverage. Our oval sampling season in 2017 revealed some interesting temporal patterns. Sampling performed in late summer (July and August) were dominated by cod assigned to the North Sea reference (Table 1, Figure 2). From the sampling performed in September and October, the catches were dominated by cod assigned to 'fjord' reference (Table 1, Figure 2). It should be noted that different gear was used for the late summer (wrasse traps) and early fall (beach seine) sampling, which could have influenced the results. Nevertheless, our results show that both ecotypes of cod are present in the system as 0-group cod, and both ecotypes are thus contributing recruits into the coastal population in this area. When considering batch spawning during an oval spawning season in larger cod, the temporal differences in assignment ratios could be due to different batches of recruits having reached different sub-areas (habitats) in the seascape at different times. Also, the different gear target different habitats in the seascape and it is likely that there is unknown and unobserved heterogeneity in habitat specificity of settling recruits operating at finer scales. However, to disentangle such heterogeneity was beyond the scope of this study.

Although data on older cod are not shown herein, individuals >30 cm were rare in both ecotypes (to be reported elsewhere). Presence of juveniles despite absence of older fish indicate that fishing mortality in this area is high. Juvenile cod are known to aggregate in nursery habitats, as eelgrass beds, which serves as a refuge with high abundance of food and shelter to hide from other predators. However, these areas are often vulnerable to fragmentation due to anthropogenic disturbance. Outer Oslo fjord is a widely used area for recreational activities, by locals, summer residents and tourists, especially during the summer months. In addition, bottom trawling for shrimp (*Pandalus borealis*) is the last remaining economically viable commercial fishery in eastern Skagerrak, including outer Oslo fjord.

Adult cod constitute a high-value by-catch in this fishery. Bottom trawling is known to destroy or alter habitats and will affect demersal fish directly and indirectly. Availability of suitable habitats, providing protection from predators, is known to be an important factor for fish mortality. Reduction of mean age and size is also known to be a result of overfishing. Larger individuals provide stability to the population, and selective removal of larger fish will increase variability (Hsieh et al., 2006). Populations that are mainly dominated by small individuals will have reduced reproductive potential and are more vulnerable to environmental fluctuations (Hutchings et al., 2004; Berkeley et al., 2004). In addition to fishing, cod is facing several other challenges, as habitat destruction, eutrophication, increased temperature and ocean acidification. Thus, improved management is crucial for the protection and restoration of the species. Marine protected areas (MPAs) are valuable tools for management, as they can be custom made for targeted species. MPAs have the possibility to increase productivity and improve resilience of the ecosystem, which makes the ecosystem able to better withstand future environmental changes. Our results points to three areas of interest located inside the study area, which would be good candidates for marine reserves. Protection of these areas might help the coastal cod population by giving suitable protective habitats for juveniles, and with implementation of a no-fishing zone this could also increase abundance of larger individuals of both ecotypes.

In conclusion, results reported in this paper shows that both ecotypes of Atlantic cod are present in the outer Oslo fjord seascape, both as 0-group and 1-group individuals. Genetic results combined with the oceanographic simulations, does not rule out the occurrence of local spawning in coastal sites inside both sampling areas. Verification of suggested local spawning areas and implementation of MPAs in the seascape studied could promote restoration of both ecotypes by increasing abundance and size of protected individuals, as well as preserve nursery habitats.

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Table 1. Sample overview of 0-group cod collected in 2017, and 1-group individuals collected in 2018, assigned to “fjord” and North Sea ecotype from Færder and Ytre Hvaler national park.

Year	Month	Life stage	Sample size	Assigned to	
				NS	CC
Færder mational park					
2017	August	0-group	115	115	0
2017	September	0-group	158	68	90
2018	May	1-group	58	44	14
Ytre Hvaler national park					
2017	July	0-group	223	218	5
2017	September	0-group	431	68	363
2018	May	1-group	38	18	20
Total			1023	531	492

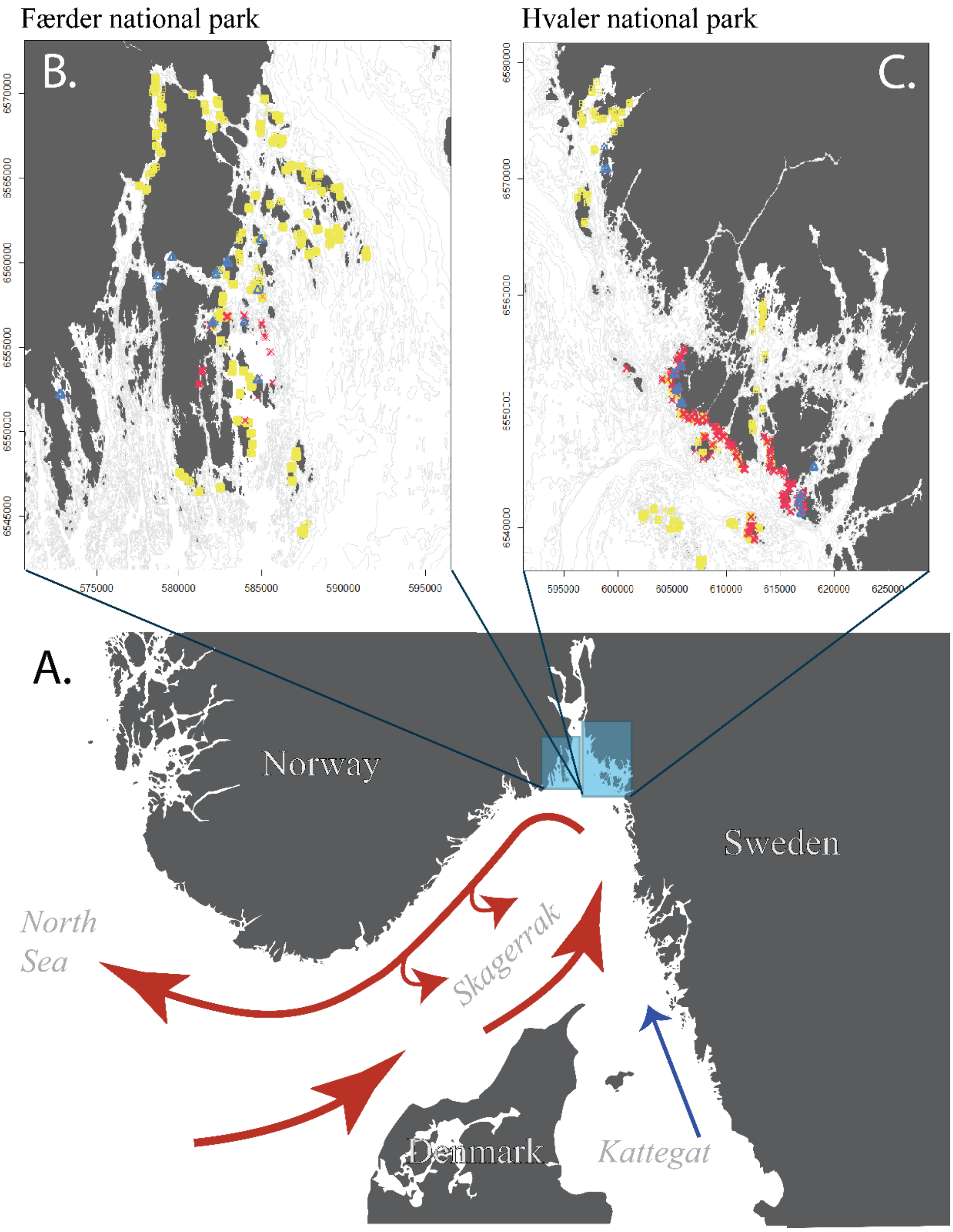

Figure 1 Map of study area (A.) and sampling locations for the two national parks located on the west (B.) and east (C.) side of outer Oslo fjord. Crosses represent sampling sites from July-August 2017 (red). Sampling performed in September and October 2017 are shown as triangles (blue). Squares represent sampling sites from May 2018 (yellow). Main currents in Skagerrak are displayed as red- (North Atlantic current) and blue arrow (Baltic current).

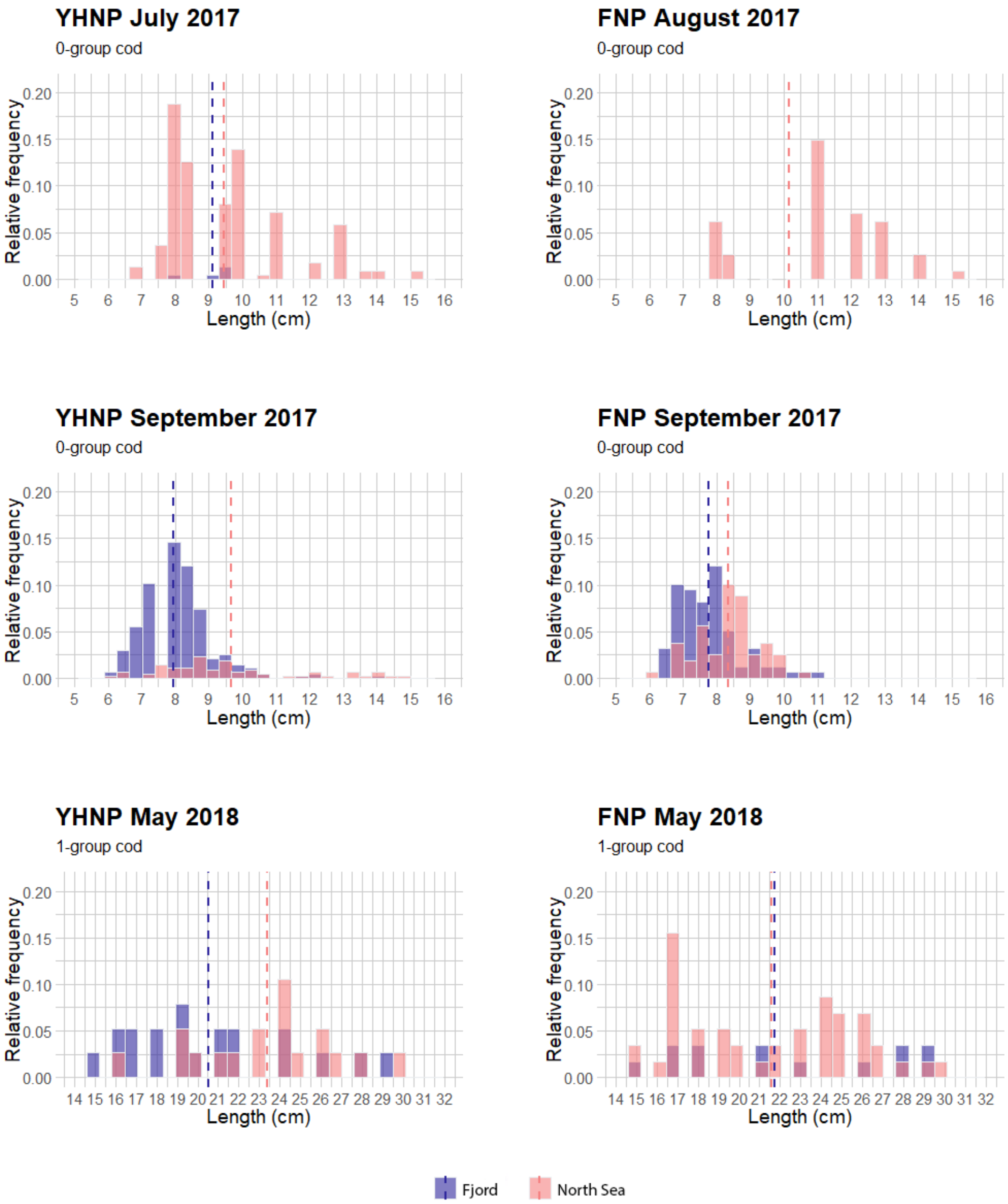

Figure 2 Relative frequency histogram showing length distribution of 2017 young-of-the year 'fjord' cod (blue) and 'North Sea' cod (red) for Færder- and Ytre Hvaler national park sampled in 2017 (0-group) and 2018 (1-group). Average length is shown as dotted bars for both ecotypes. Relative frequency is displayed on the y-axis and length is displayed on the x-axis. Notice the different scaling of the x-axis in relation to whether 0- or 1-group cod are displayed.

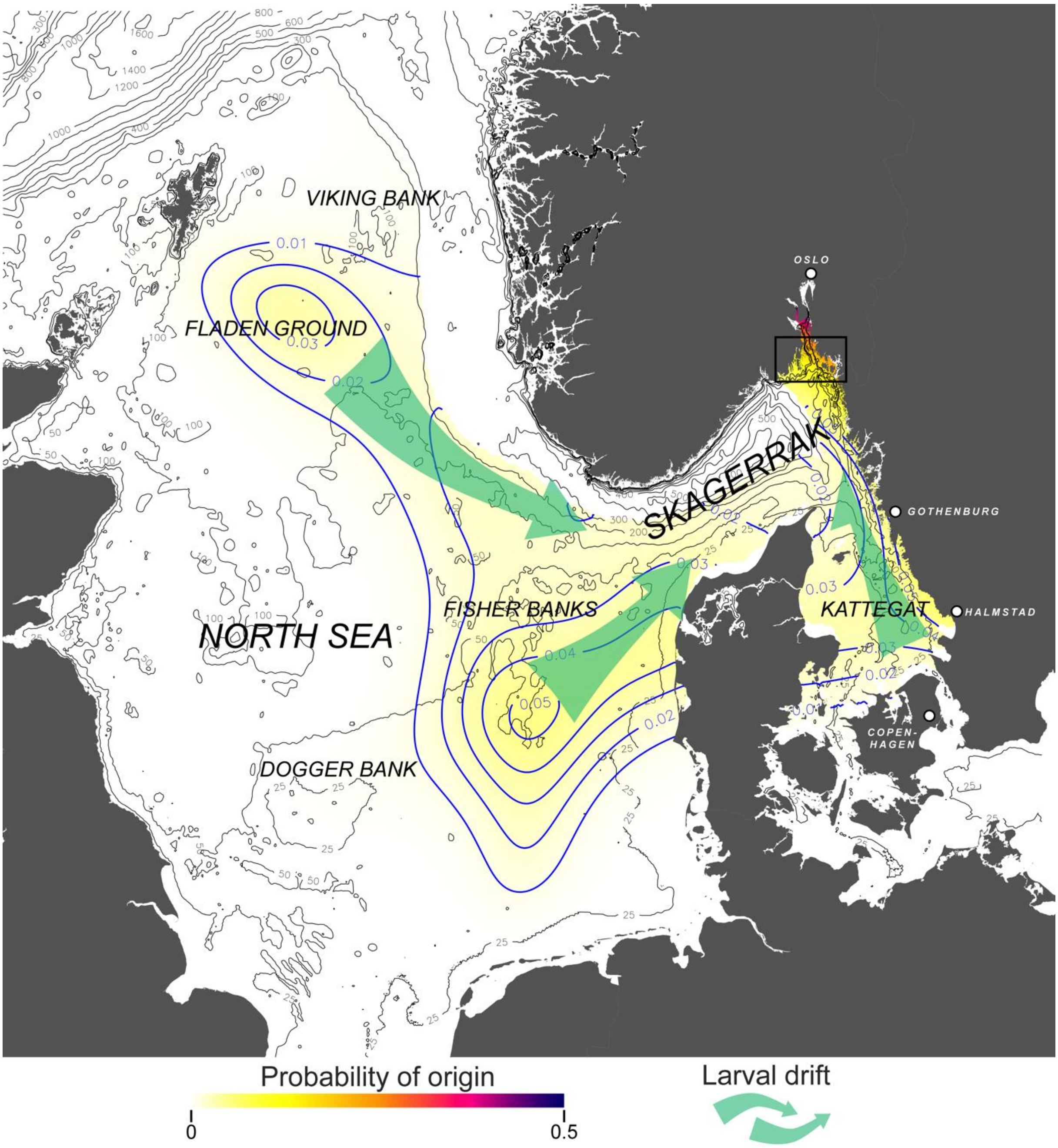

Figure 3 Predicted Atlantic cod spawning areas and idealized drift routes of cod originating from the North Sea or Kattegat. Heat map represents the probability of source sites for 2017 recruits, predicted from the 2D GAM fitted to the geographical position of 0-group presence/absence. Green arrows represent idealized drift routes for eggs and larvae originating from the North Sea or Kattegat.

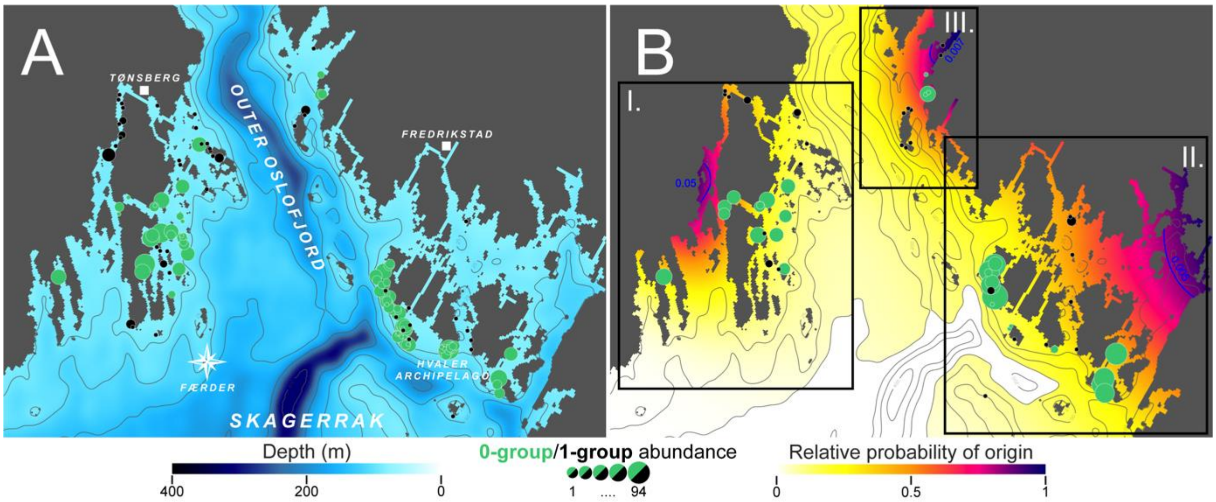

Figure 4 The 2017 cod cohort sampled as 0-group recruits during summer and autumn 2017 (green circles), and as 1-group juveniles (≤30 cm) in spring 2018 (black circles). A: Bathymetry of outer Oslo fjord and recruits assigned to 'North Sea' origin (see Figure. 3). B: Recruits assigned to 'fjord' origin and heat map indicating the relative probability of local source locations (spawning sites) inside or adjacent to the national parks, predicted from the 2D GAM fitted to the geographical position of 0-group (presence/absence). Note that colored gradient within sub-areas denoted by roman numerals in panel B have to be scaled by: (I.) 0.053, (II.) 0.005, and (III.) 0.007; meaning that peak probability of recruitment to the sampled sites within the three sub-areas are: (I.) 5%, (II.) 0.5%, and (III.) 0.7% (see blue lines and numbers in panel B).

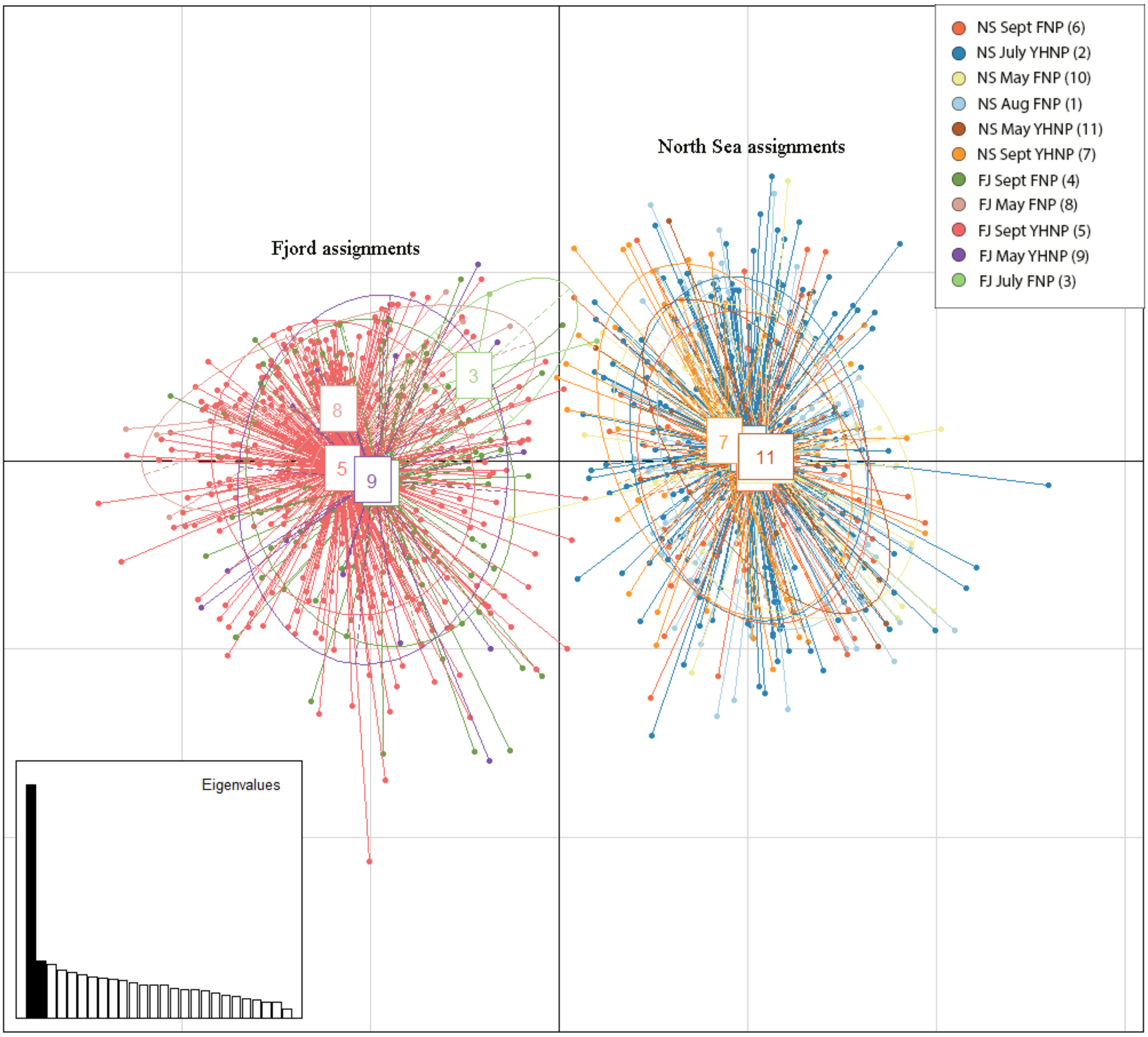

Figure 5 PCA plot of all individuals assigned to "fjord" or North Sea reference from east and west side of outer Oslo fjord. Each dot is an individual, and colors are explained in legend, top right, where "FJ" are individuals assigned to "fjord" reference, and "NS" are individuals assigned to North Sea reference. Clustering of points show a clear segregation between individuals assigned to the two ecotypes. X and Y axis of the scatterplot describe first and second component (explaining 22.4% and 5,4% of genetic variance, respectively). Inset shows relative eigenvalues of the first 25 principal components, with represented components in black. (Note: FJ July FNP (3) consisted of only 5 samples, and was later added to the FJ Sept FNP samples before doing pariwise F_{st} analysis)

Appendix

Table S1 Pairwise F_{st} for individuals assigned to "fjord" reference. F_{st} values are shown in bottom and corresponding p-values from G-test shown in top. All p-values are corrected by FDR (Benjamini and Hochmeg, 1995), and significant values are highlighted as bold.

Pairwise F_{st} for all fjord individuals				
	east 0-group	west 0-group	west 1-group	east 1-group
east 0-group		0.07	0.06	0.06
west 0-group	0.002		0.07	**0.03**
west 1-group	0.0125	0.0081		0.06
east 1-group	0.0119	0.0151	0.0202	

Table S2 Pairwise F_{st} for individuals assigned to the North Sea reference. F_{st} values are shown in bottom and corresponding p-values from G-test shown in top. All p-values are corrected by FDR (Benjamini and Hochmeg, 1995).

Pairwise F_{st} for all North Sea individuals						
	west aug 0	east jul 0	west sept 0	east sept 0	west may 1	east may 1
west aug 0		0.18	0.18	0.18	0.63	0.99
east jul 0	0.0027		0.18	0.65	0.18	0.83
west sept 0	0.0014	0.0001		0.63	0.49	0.83
east sept 0	0.0083	0.0013	0.0029		0.49	0.58
west may 1	0.0019	0.0053	0.0053	0.0043		0.83
east may 1	-0.0059	0.0054	0.0081	0.0165	0.008	

Paper IV

Fate of Atlantic cod (*Gadus morhua*) recruits assigned to two ecotypes in a Skagerrak coastal seascape

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Abstract

Although marine populations often experience fairly open environments without geographical barriers to gene-flow, sympatric ecotypes for some species still occur, with mechanisms separating them being largely unresolved. For Atlantic cod, the present state of knowledge suggests both behavioral- and structural genomic barriers to interbreeding between ecotypes inhabiting the southern Norway coastal zone. Evidence suggest differing adaptations to fjord- and offshore oceanic environments, with implications for the expected success and survival for co-existing Atlantic cod ecotypes in Skagerrak. Here, we explore the processes influencing connectivity and population structure by 1) examining the spatial and temporal variation in population genetic structure of sub-adult (1-group) and adult cod (*Gadus morhua*) in the outer Oslo fjord during three years of sampling and 2) apply this information to test for differences in proportions of the two ecotypes recruited from the 2016 cohort. Both ecotypes where encountered as fully recruited 1-group, and as older (>1 yr) sub-adults in all years of sampling, with high variability among years. Adults above the minimum legal size (40 cm TL) were rare in samples (N = 20, 5.4 % of total). For the 2016 cohort, the ‘fjord’ ecotype was underrepresented as 1-group recruits (21.2 % of total), but overrepresented as 2-group sub-adults (52.9 % of total). Relative reductions in sample size suggested 11% and 28 % apparent survival from the 1- to 2-group stage, for ‘North Sea’ and ‘fjord’ cod ecotypes, respectively. Our findings suggest that both ecotypes recruit to- and mature in the coastal seascape of outer Oslo fjord. Apparent survival is low, suggesting that: 1) fisheries and predators are removing both ecotypes at a high rate, or 2) both ecotypes undertake ontogenetic movements away from the sampled areas. Implications for sub-stock management are discussed.

Introduction

Atlantic cod (*Gadus morhua*) is one of the most intensively studied fish species worldwide. It is a demersal bentho-pelagic species and is distributed on both sides of the Atlantic Ocean, and, is known to be one of the most important commercial fish species in the world. Abundance of Atlantic cod is known to fluctuate extensively on large spatial and temporal scales and is affected by both interannual fluctuations as well as long-term trends (Mello & Rose, 2005; Kristiansen et al., 2011). Several factors contribute to the long-term trends in abundance, as e.g. human exploitation (Cardinale & Svedäng, 2004) and alterations of habitats (Juanes, 2007), or abiotic environmental variation as changes in ocean currents and temperature and salinity (Fromentin et al., 1996; Drinkwater, 2005). The year-to-year variation of the observed abundance is mostly a result of juvenile survivorship. Many teleost fishes, including Atlantic cod, is characterized by high fecundity and egg production, releasing more than a million eggs in several batches during spawning season. Spawning season is also known to last for several months, enhancing survival of recruits by using a “bet hedging” strategy (Lambert et al., 1984). During the early life stages, cod experience a high mortality rate, where as much as 99.99% of a cohort dies. However, although mortality for most marine species is highest during the first months of life, mortality can also be high during the first years of the sub-adult life. Typically, from 1 million ripe cod eggs, about 800 individuals will survive to age 1, and about 6 to age 3 (Bogstad et al., 2016). During the first years of life, various processes are taking place that can considerably influence mortality, which can be highly variable. For a better understanding of the productivity of a fish stock, knowledge about the survival of individuals during their sub-adult life is very important, especially if the stock is subject to high exploitation. Thus, mortality in later stages of the life history is also important to consider for maintenance of healthy fish stocks.

Atlantic cod displays a range of phenotypic and genotypic variations, which includes offshore migratory types as well as stationary coastal types. Such variation has been described across its distribution range, on both sides of the Atlantic (Ruzzante et al., 2000), Icelandic waters (Petursdottir et al., 2006), Greenland (Storr-Paulsen et al., 2004) as well as in northern Norway (Pogson & Fevolden, 2003). Along the Norwegian Skagerrak coast, two ecotypes are found (Sodeland et al. 2016; Barth et al. 2017). Coastal cod along the Norwegian Skagerrak coast are assigned to either ‘fjord’ or ‘North Sea’ reference populations based on genetic markers (see Materials and Methods section) and the two types are found co-existing in coastal habitats (Knutsen et al., 2018; Barth et al., 2019). Coastal cod – regardless of ecotype, are characterized by limited movement ranges as demonstrated in mark-recapture studies (Knutsen et al. 2011; Rogers et al. 2014), although some level of movement have been described (Rogers et al., 2014). Coastal cod populations display complex population structure along the Norwegian coast, where populations seem to be geographically restricted at spatial scales down to the order of local fjords (Jorde et al., 2007; Dahle et al., 2018). Local cod populations display high spawning site fidelity (Skjæraasen et al., 2011) and are assumed to be mostly self-recruiting, although their habitats do receive recruits from offshore cod stocks from the North Sea (Huserbråten et al., 2018). This inflow of North Sea cod larvae seems to be highly variable between years, as it is affected by the strength of ocean currents transporting the larvae into Skagerrak coastal waters (Knutsen et al., 2004; Stenseth et al., 2006; Huserbråten et al., 2018). The North Sea cod are found offshore in the Skagerrak and North Sea. It is not considered to be a homogenous stock, as some sub-structure has been detected within the North Sea, and these populations are assumed to be spatially distinct during spawning season (Hutchinson et al., 2001; Nielsen et al., 2009). The North Sea cod is known to move over longer distances, although not in the same degree as for the North East Arctic cod, which carry out long distance migrations between feeding areas in the Bartents Sea and spawning areas along the coast of North Norway (Bergstad et al., 1987). The North Sea cod is also known for having a higher growth rate than the ‘fjord’ cod during the early life stages and is found to be generally larger than the ‘fjord’ cod (Gjøsæter & Danielssen, 2011). Recent studies have reported four large chromosomal rearrangements in the Atlantic cod genome (Hemmer-Hansen et al., 2013; Sodeland et al., 2016) from both sides of the Atlantic (Bradbury et al., 2010), three of which are segregating in the present study area. These chromosomal regions appear to correlate with latitudal and climatic gradients (Bradbury et al., 2010), behavior (Hemmer-Hansen et al., 2013), as well as temperature and oxygen regulation (Berg et al., 2015).

In outer Oslo fjord and eastern Skagerrak, concern has grown that the local spawning populations of cod are severely declining along the coast. Results from several studies show that abundance of Atlantic cod in eastern Skagerrak is historically low after the 1900s, and individuals > 40 cm are presently rare (Svedäng, 2003; Olsen et al., 2008). Although some years still yield strong recruitment signals, these recruits do not seem to replenish the adult population over time. It is hypothesized that individuals originating from offshore or up-stream population components are exported into coastal nursery areas during the pelagic egg and larval phase but migrate back to natal spawning grounds before or upon reaching maturity (Svedäng et al., 2007; André et al., 2016). Thus, for preserving and restoring local populations of cod found in Southern Norway fjords, protecting the local, stationary, coastal cod has been a highlighted management advice in recent years.

The aim of the present study was to investigate the proportional success of cod recruits assigned to ‘fjord’ and ‘North Sea’ ecotypes within the study system, originating from the 2016 cohort. We treated our samples as cohorts based on assumptions of body size distribution and examined the proportional survival of the two ecotypes throughout three years of sampling. Are recruits assigned to the ‘fjord’ type surviving better than the ‘North Sea’ type in coastal nursery habitats and dominating as older fish? We discuss the potential mechanisms that could be responsible for the observed variability, and give our thoughts considering appropriate management strategies for restoration of the cod population complex in coastal Skagerrak.

Materials and methods

Study area

Sampling was conducted in areas within or adjacent to two national parks located on the east (Ytre Hvaler national park) and west (Færder national park) side of the outer Oslo fjord mouth, during the years 2017, 2018 and 2019 (Figure 1). The Oslo fjord is located in the south-east part of Norway and is a part of the Skagerrak which connects the North- and Kattegat Seas. The outer Oslo fjord area is a heterogenic environment, consisting of several islands and skerries, and is known for harboring high biodiversity both on land and in the sea. It is also one of the most densely populated areas in Norway, and subject to heavy boat traffic ranging from larger ships and ferries to fishing boats and smaller boats used for recreational purposes, especially during the summer months. The area is also affected by high long-term fishing pressure, both commercial and recreational. This has dramatically reduced the abundance of larger fish in the fjord system, and today just a few people are making their living of fishing in this area, mainly from bottom-trawling for northern shrimp with fish landed as bycatch. In 2009, the Ytre Hvaler national park on the east side of the fjord was established, covering 354 km^2 of the coastal landscape, most of which is in the sea. Later, in 2013, Færder national park was established on the west side of the fjord, covering 340 km^2, where 325km^2 is ocean. Both national park areas are exposed and subject to strong weather, with smooth rock formations and skerries shaped by glacial scouring. The convoluted coastline and archipelago harbor a diverse marine environment with several important nature types, as eelgrass beds, soft-bottom areas and kelp forests.

Sampling method

Sampling was performed for the years 2017, 2018 and 2019 in the beginning of May, using fyke nets with green mesh and a total length of 2.75 m (3.5 m leading net), 25 mm mesh size and 55 cm openings, and baited collapsible fish traps (130 × 80 × 120 cm). A total of 930 fyke nets fishing at 1 – 6 m, and 111 traps for sampling of deeper areas (10 – 30 m) were hauled over three years of sampling. Fyke nets and baited traps were deployed at random locations and soaked for approximately 24 hours before being hauled the next day. For each haul, catches were recorded directly on board, and all fish caught were identified to species level, counted and length-measured to the nearest cm (FL), before being released back into the sea. In addition, all cod were fin clipped for DNA analysis, by clipping a small piece of tissue along the distal margin of the caudal fin, which was then stored in 99% ethanol for DNA extraction. The effort was four days on each side of the fjord mouth in every year (exception: Ytre Hvaler national park and adjacent areas were sampled only 3 days in May 2018), yielding a total of 8 sampling days per year.

DNA extraction

Fin-clips was stored at 4°C in pure ethanol prior to DNA extraction. All samples were extracted for DNA using the E.Z.N.A tissue kit (Omega Bio-tek), following manufacturers protocol for tissue DNA extraction. A sub sample of 15-20 samples pr. 96 well plate was quality-verified and quantified using Qubit assays (ThermoFisher) before genotyping.

Genotyping and assignment of individuals

A total of 398 cod tissue samples from cod sampled over three years of sampling were genotyped for the present study. 27 SNPs were previously developed to segregate between fjord and North Sea individuals and there were genotyped on a MassARRAY platform (Sequenom Inc.) at the IMR laboratory in Bergen, Norway. Only 26 SNPs gave consistent results, with the one of the SNPs (Gdist_68504_1675) often failing, and all analyses were therefore based on 26 SNPs. Genetic assignment of individual cod to ecotype was computed using the GeneClass2 software, using previously sampled reference populations of ‘fjord’ and ‘North Sea’ cod (see Jorde et al., 2018 for additional information). Scores lower than 80% (n=24) and individuals that were genotyped at <20 SNPs (n=3) were omitted from further analysis, resulting in 371 individuals being assigned successfully.

Cohort analysis

A density plot of length frequencies was made for determining putative size intervals for different stages of growth, using all data collected from the three years of sampling of both ecotypes pooled (Figure 2). Cut-offs were made visually at 33 cm and 47 cm (FL), which was then used to sort different growth-stages of sampled individuals into 1-group (13-33 cm), 2-group (34-47 cm) and 3-group (> 47 cm).

A two-proportion Z-test with continuity correction was used to compare the observed proportions of the two ecotypes sampled as 1-group in 2017 and as 2-group in 2018, using the R software. The null hypothesis was expecting the same proportions (H_0: P1=P2) from the two years of sampling.

In addition, using data from all years of sampling, a Mann-Whitney U test with continuity correction was used to test for significant differences in mean length between the two ecotypes.

Results

Size distribution

Results from our sampling of Atlantic cod in outer Oslo fjord show a strong left-skewed size frequency distribution (Figure 3). The majority of sampled individuals ranged between 14-25 cm. Mean length of cod assigned to the ‘fjord’ evotype was 25.4 cm ± 9.0 SD, while for cod assigned to the ‘North Sea’ ecotype the mean was 22.9 cm ± 6.5 SD. In total, 63 individuals were >= 30 cm, where 31 (49%) was assigned to the ‘fjord’ reference, and 32 (51%) was assigned to the North-Sea reference. Only 20 individuals were equal or above the legal-size limit for cod (40 cm), of which 10 (50%) were assigned to the ‘fjord’ reference and 10 (50%) to the ‘North Sea’ reference.

In 2017, 80% of individuals sampled were assigned to the ‘North Sea’ reference, and 20% to the ‘fjord’ reference. In 2018 more individuals were assigned to the North-Sea reference (63%), compared to individuals being assigned to the fjord reference (37%). 2019 had the lowest abundance of all years of sampling (n=15), and only 2 individuals were assigned to the North-Sea reference, while the remaining 13 individuals were assigned to the fjord reference. Mean length of cod assigned to the fjord reference was larger than for the cod assigned to the North-Sea reference for all years of sampling (Figure 3), but the difference was not statistically significant (Mann-Whitney U test: $p > 0.1$).

Cohort analysis

Based on visual inspection of the pooled density plot, the lowest troughs occurred at approx. 33 and 47 cm (see Figure 2). We assumed that individuals between 13-33 cm were 1-group cod spawned the previous year. Following the 2016 cohort sampled as 1-group cod in May 2017, results from assignment test show that 36 1-group individuals (21.8 %) were assigned to the ‘fjord’ reference, and 170 individuals (78.2 %) to the ‘North Sea’ reference (Table 1, Figure 2). Catches of putative 2-group cod sampled in 2018 yielded 9 (52.9 %) individuals assigned to the ‘fjord’ reference and 17 (47.1%) to the ‘North Sea’ reference (Table 1, Figure 2). Results from the two-proportion Z-test found significant difference in proportions of the two ecotypes as 1- and 2-group cod ($Z = 4.77$, $df = 1$, $p < 0.05$). Reductions in sample size from 1-group ($n = 170$) to 2-group ($n = 17$) was 89 % for ‘North Sea’ cod, while for ‘fjord’ cod, reductions in sample size from 1-group ($n = 37$) to 2-group ($n = 9$) was 72 %. Yield from the 2019 sampling was very poor (n = 15), and no individuals caught were above 47 cm (i.e., no 3-group cod from the 2016 cohort were observed in the samples: see Figure 3).

Discussion

Overall, there was a higher abundance of cod genetically resembling a North Sea origin in our data (72%) compared to cod with a supposed local, ‘fjord’ origin (28%). These cod could either truly be originating from the North Sea, or spawn offshore the Skagerrak coast, but with sufficient geneflow with the North Sea cod that make them indistinguishable genetically. The North Sea ecotype dominated in the 2017 and 2018 samples, while this had shifted to the ‘fjord’ cod being in majority in the small sample obtained in 2019. The large proportion of cod assigned to the ‘North Sea’ reference in 2017, classified as 1-group individuals, indicating that 2016 might have been a year with high influx of larvae from the North Sea, which have been reported in previous studies (Knutsen et al., 2004; Stenseth et al., 2006).

As we found a higher proportion of cod assigned to ‘fjord’ reference in the 2-group, it could indicate that this ecotype is better adapted to the coastal environment, and have higher fitness in this habitat than the ‘North Sea’ ecotype. Results from previous studies have found a higher proportion of eggs with ‘fjord’ genetics in the inner, more sheltered locations of the fjord system (Jorde et al., 2018). As reported previously, the two types differ in relative frequencies of three large (several megabases) chromosomal inversions (Berg et al., 2015; Sodeland et al., 2016) and these could contribute to local adaptation. Some of these rearrangements have been inferred to play an important role for the adaptive abilities of Atlantic cod, for example in salinity and oxygen regulation (Berg et al., 2015) and temperature (Bradbury et al., 2010; Berg et al., 2015). Studies have shown that North Sea cod and individuals homozygous for the LG12 inversion may be selected against in the fjord environment, as results have indicated that they have a lower fitness for the habitat (Barth et al., 2019). Interestingly, we found that the mean length of cod assigned to the ‘fjord’ reference was consistently larger through all years of sampling, in contrast to studies that have shown a higher mean growth of cod recruits (0-group) with ‘North Sea’ assignment (Knutsen et al., 2018). The observed difference in this study, however, was not statistically significant.

Our results showed that both ecotypes disappeared from areas sampled during their first three years of life. This could indicate either high mortality or that both ecotypes migrate out of the settled habitats at some point. While such ontogenetic habitat shifts, or -expansions, are likely, several studies have shown that coastal cod are stationary, some living their entire life within a restricted geographical area (Espeland et al., 2007; Rogers et al., 2014). The more common explanation is therefore that both ecotypes are subject to high mortality. In eastern Skagerrak there has been a dramatic decline of fish >30 cm (Svedäng, 2003), and cod abundance in this area is historically low (Cardinale & Svädeng, 2004). The left-skewed distribution in length frequency in our results indicate that the most abundant size group present in the outer Oslo fjord seascape are cod between 14-30 cm, and probably 1-group individuals. Most cod disappear from the areas before reaching 3 years of age. A previous study done by Olsen and Moland (2011) along the southern Norwegian coast, estimated that as much as 75% of the cod catch died within the study area after 1 year, and only 5% of individuals were estimated to be older than 3 years of age. Our findings are in concordance with these results, as only 1% of the fish caught over our years of sampling was above 47 cm, and thus considered to be at least 3 years old. Most fisheries only target individuals above a certain size, and restrictions are usually in the form of minimum legal size-limits and minimum mesh sizes. Prolonged periods of exploitation can affect life history traits, as size-selective fishing removes the large, older individuals from the population.

Potential for recovery

Most fisheries target large individuals, and fishing-induced mortality is often highly size-selective (Kuparinen & Merilä, 2007). Our data from this study points to a depletion of large individuals of both ecotypes, indicating that fishing mortality in this area may be high. A reduction of fishing is therefore necessary for fish recovery. Studies of Atlantic cod have shown that older and larger females produce higher quality eggs, leading to enhanced survival of larvae (Kjesbu, 1989; Marteinsdottir & Steinarsson, 1998). Minimum mesh-size and minimum legal-size limits regulate fisheries in such a way that smaller fish have a higher probability than larger ones to breed and spawn before being caught. However, first time spawners have been shown to breed for shorter periods, have a lower fecundity and egg production, and produce smaller eggs that have a lower fertilization- and hatching rate (Trippel, 1998). Increasing abundance of older and larger individuals that would be able to spawn several times during their lifetime would enhance local spawning and thus contribute to an increased number of recruits supplied to the area.

In addition, there is an increasing recognition that removal of large individuals can have dramatic impacts in community composition and ecosystem functioning. Atlantic cod is a top-predator and considered to be a key-species in the ecosystem it inhabits. Depletion of functionally important consumer species can result in cascading effects down the food web, with an increase of mesopredator species. When top predators are removed from the system, mesopredators are able to benefit from the freed ecological niche that becomes available. This in turn leads to a higher predation on lower trophic species, as grazers, which can result in increased production of filamentous algae, reducing the quality of important habitats as eelgrass beds (Baden et al., 2012; Östman et al., 2016).

The ocean environment is changing rapidly, with temperatures increasing, bringing with it associated challenges as ocean acidification, changes in salinity, pH and introduction of new species and pathogens. These changes in the physical and chemical composition of seawater is impacting several marine species, also the Atlantic cod. More genetic variation leads to a better resistance against these future challenges. Implementing restrictions on fishing efforts through MPAs could be a good strategy for management considering preserving and restoring the coastal cod population in outer Oslo fjord – while at the same time aim at maximizing the resilience of the seascape (Roberts *et al*. 2017). This could increase abundance of larger- and older individuals of cod, which in turn contribute to more offspring as well as providing the ecosystem function of larger top-predatory fish.

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Table 1. Summary of Atlantic cod sampled in outer Oslofjord during 2017 to 2019. Total sample size and assignment of individuals to ‘fjord’ or ‘North Sea’ reference and age group. Numbers in bold are samples assigned to the 2016 cohort.

			Assigned to	
Year	**Month**	**Stage**	**Fjord**	**North Sea**
2017	May	**1-group**	**36**	**170**
		2-group	9	8
		3-group	0	1
total			45	179
2018	May	1-group	37	63
		2-group	**9**	**17**
		3-group	2	1
total			48	81
2019	May	1-group	10	2
		2-group	3	0
		3-group	**0**	**0**
total			13	2

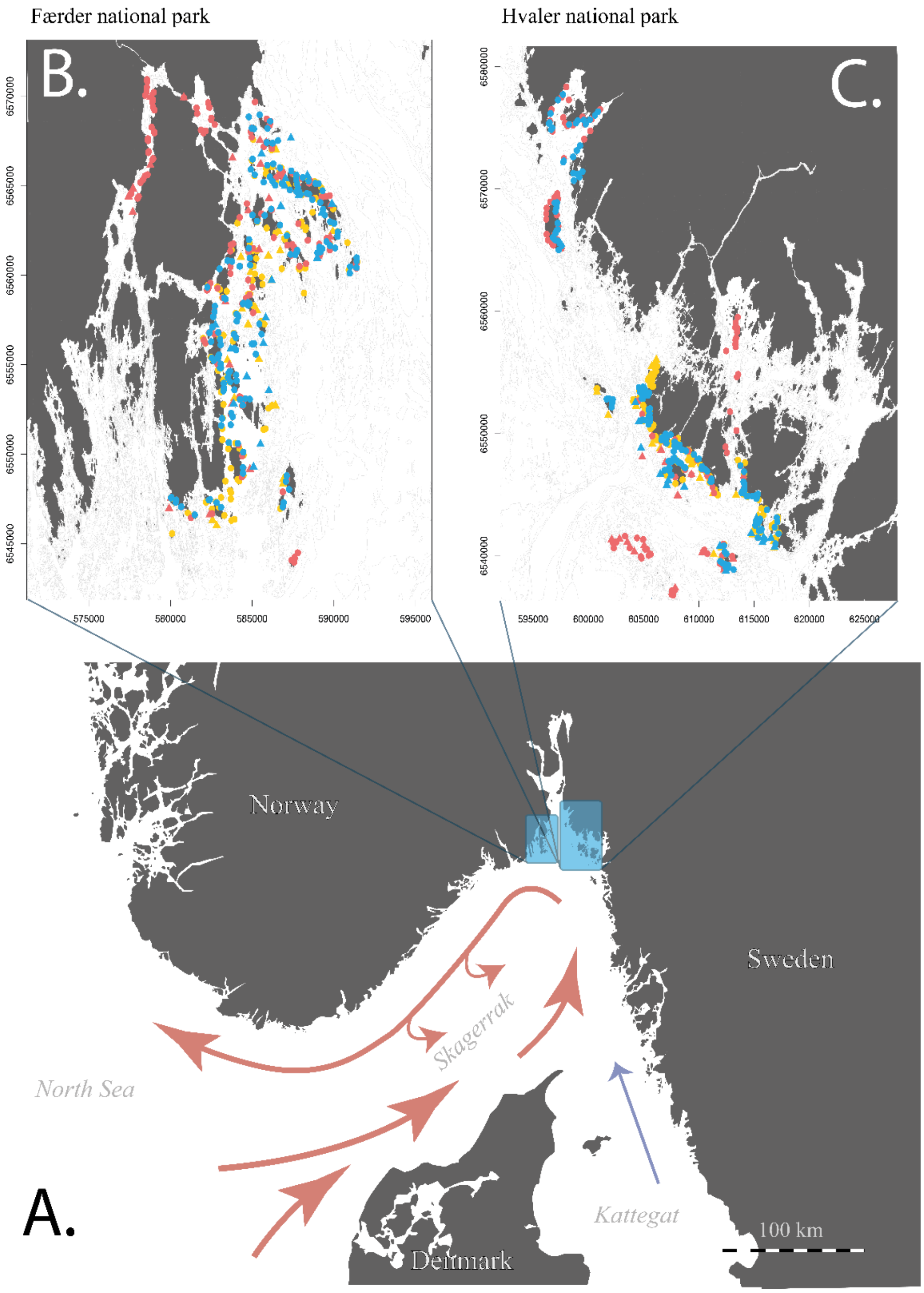

Figure 1 Map of study area and sampling sites. Southern Norwegian coast and sampling sites (A). Main currents in the Skagerrak Sea are displayed as arrows. Sampling sites in Færder (B) and Ytre Hvaler (C) national parks and adjacent areas. Dots represents deployment of fyke nets (1-6 m), and triangles represent traps used for sampling deeper areas (10 – 30 m). Yellow: 2017, red: 2018 and blue: 2019.

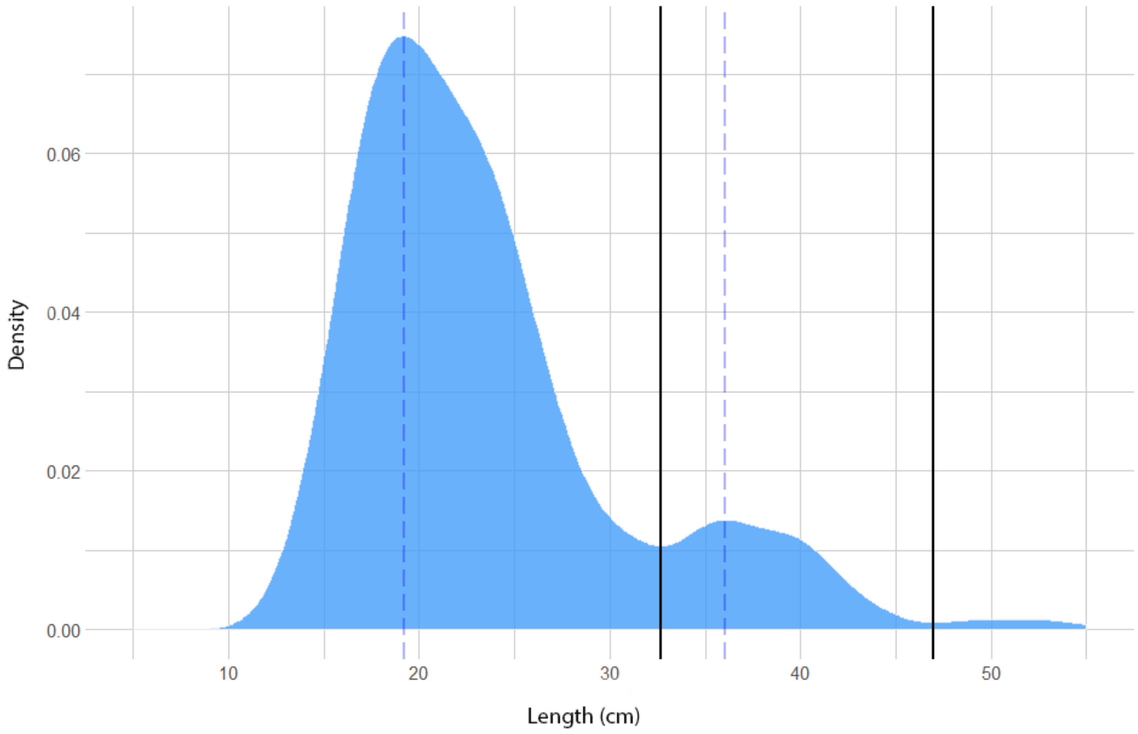

Figure 2 Density plot of body size for pooled samples (both ecotypes) in all years, showing assumed size intervals for the three different age groups (vertical black bars). Stippled vertical bars denote median body size in 1- and 2-group cod.

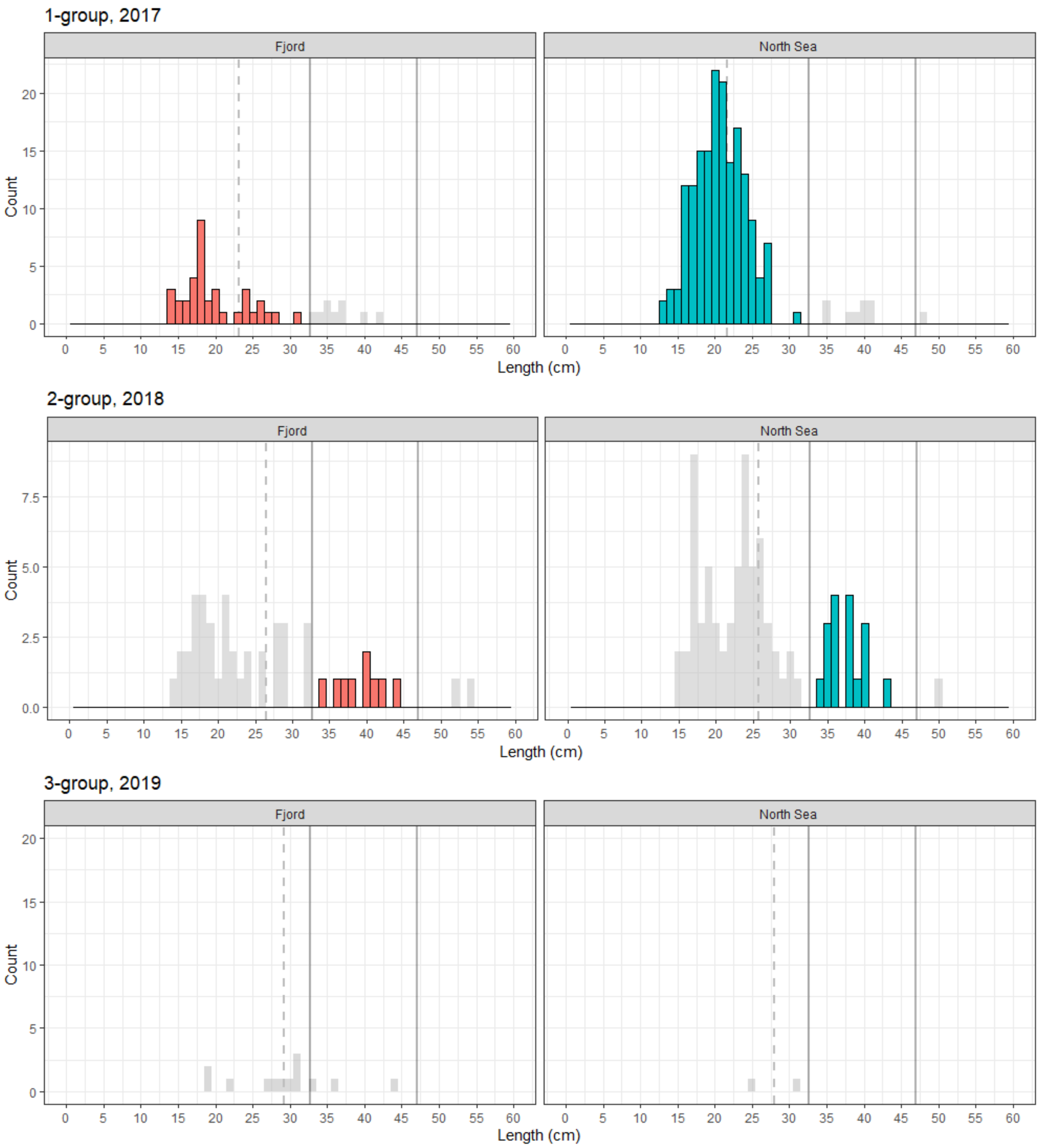

Figure 3 Frequency histogram of length distributions of Atlantic cod assigned to 'fjord' (left) and 'North Sea' ecotype (right) in outer Oslo fjord 2017 - 2019. Average length of ecotype in each year is displayed as vertical stippled lines. Filled vertical lines denote size range (33 and 47 cm) of putative year classes (see Figure 2), allowing grouping into 1-group, 2-group and 3-group individuals displayed as colored bars ('fjord' = orange, 'North Sea' = cyan). Shaded bars display samples that were outside of the selected size-range for the different groups.