Connectivity mechanisms in a population complex of coastal cod

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Preface

“Nothing is so boundless as the sea, nothing so patient. On its broad back it bears, like a good-natured elephant, the tiny mannikins which tread the earth; and in its vast cool depths it has place for all mortal woes. It is not true that the sea is faithless, for it has never promised anything; without claim, without obligation, free, pure, and genuine beats the mighty heart, the last sound one in an ailing world. And while the mannikins strain their eyes over it, the sea sings its old song. Many understand it scarce at all, but never two understand it in the same manner, for the sea has a distinct word for each one that sets himself face to face with it.”

A. Kielland 1880

In this thesis I have tried to understand the words from the old song of the sea. These are the words of the cod of the Skagerrak. It has not been an easy task, but it has most certainly been an enjoyable task. The work on my PhD thesis has been a journey that has led me trough highlights of both science and personal. The fieldwork has given me the opportunity to awe at the most beautiful sunrise glittering in the sea early mornings by the outermost barren rocks. Other times ice and snow nearly froze my fingers off. My work with the historical tagging data has given me the opportunity to seek trough old scribbled notes of fishery, bringing me closer to the daily life on the Skagerrak coast a hundred years ago. I have enjoyed carefully mixing chemicals on the genetics lab, setting up PCR cycles, and awaiting the secret genetic patterns of the cod population complex to appear. I have pounded over the theoretical challenges of fitting varying models to data and discovered new dimensions in the world of R. What I have enjoyed the most is the diversity of work, and the fact that I have been able to “stay in touch with the data” throughout the entire path from fieldwork to published results.

I am also lucky to have a couple of good translators, when trying to decipher, the words from the sea songs, Esben Moland Olsen and Halvor Knutsen. I am very grateful that Halvor asked me nearly ten years ago weather I would like to do a master on cod in Arendal. I am also very grateful to be taken into the group of Nils Chr. Stenseth, my main supervisor, who is a unique scientific resource. Finally I would like to thank Marianne and Oscar for giving me time to finally finish my thesis, and given me all kinds of highlights during the day.

Abstract

In this thesis I have studied connectivity processes affecting the population structure of coastal cod on the Norwegian Skagerrak coast. I have focused on three aspects of the processes. Firstly the dynamics and the integrity of the spawning grounds. This included potential retention mechanisms. There is a clear tendency for spawning site selection where adult cod chose to spawn in inshore areas where the current is likely to retain eggs and larvae close to the spawning site. The spawning sites are local and closely situated and the cod stay on the spawning ground throughout the spawning.

Secondly I have studied the scale of the spatial extent of the sub-population structure. Samples of mature cod from spawning areas along the coast demonstrated genetic differences between samples taken as close a 30 km apart. Dispersal of tagged cod suggested that the cross section of the 95 % recapture probability distribution to be 18 km. This suggests that Skagerrak coastal cod populations are structured at a fjord scale.

Finally I have studied movement and area utilization of adult cod. These studies suggested that the cod in some years may utilize a somewhat larger area for feeding and that there are some indications for a seasonal migration. However most cod in the fjord populations are stationary and only perform diel vertical migration and small scale horizontal movement as primary means for relocation.

Overall these findings suggest that the coastal cod populations along the Skagerrak coast has, for a mobile marine species, low connectivity and that population structure may be on a finer scale than previous reported

List of papers

PAPER I: *Home range and elevated egg densities within an inshore spawning ground of coastal cod* (2007). **S. H. Espeland**, A. F. Gundersen, E. M. Olsen, H. Knutsen, J. Gjøsæter and N. C. Stenseth. ICES journal of marine science 64 (5); 213-225

PAPER II: *Egg distribution, bottom topography and small-scale cod population structure in a coastal marine system* (2007). H. Knutsen, E. M. Olsen, L. Ciannelli, **S. H. Espeland**, J. A. Knutsen, J. H. Simonsen, S. Kreslet and N. C. Stenseth. Marine ecology progress series 333; 249-255.

PAPER III: *Spatial scale of genetic structuring in coastal cod Gadus morhua and geographic extent of local populations* (2007). P. E. Jorde, H. Knutsen, **S. H. Espeland** and N. C. Stenseth. Marine ecology progress series 343; 229-237.

PAPER IV: *New perspectives on fish movement: kernel and GAM smoothers applied to a century of tagging data on coastal Atlantic cod* (2008). **S. H. Espeland**, E. M. Olsen, H. Knutsen, J. Gjøsæter, D. S. Danielssen and N. C. Stenseth. Marine ecology progress series 372; 231-241.

PAPER V: *Maintenance of small-scale population structure in a marine population in relation to water circulation and egg characteristics* (2010). L. Cianelli, H. Knutsen, E. M. Olsen, **S. H. Espeland**, L. Asplin, A. Jelmert, J. A. Knutsen and N. C. Stenseth. Ecology, (in review).

PAPER VI: Vertical movement and temperature preference among young Skagerrak cod (2010). **S. H. Espeland**, A. Thoresen, E. M. Olsen, L. C. Stige, H. Knutsen, J. Gjøsæter and N. C. Stenseth. Marine ecology progress series (accepted).

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Introduction

What is a population? Patterns in time and space.

Trough the biological literature the importance of structuring different groups of individuals into populations has been recurrent. Some problems appear since a population is not an easy definable entity as for instance an individual (Berryman 2002, Jax 2006, Harwood 2009). Different population definitions have been put forward to suit different hypothesis. A biological population is usually considered to share some common natural trait or value, and thus deviates from a statistical population (the individuals about which we want to draw inference by sampling, [Snedecor & Cochrane 19679]). The natural traits are often cohesive forces that unite the individuals in a predefined group. The coherence may be mostly un-biological as in "A group of organisms of the same species occupying a particular space at a particular time" (Krebs 1994) or include more biological premises; "A group of individuals sufficiently isolated that immigration does not substantially affect the population dynamics or extinction risk over a 100-year time frame" (McElhany et al. 2000). Variations upon the population concept exists like; interaction group (Den boer 1979), demographic units (Brown & Ehrlich 1980) and the marine stock concept. Booke (1981) summarized the stock as: "a species group, or population, of fish that maintains and sustains itself over time in a definable area". The stock concept has existed since the 14th century (Booke 1999) and is closed linked to the management of marine resources. The resemblance to the population concept makes it possible to use them almost interchangeably. In this thesis I will study populations of cod rather than stocks, since a population is more closely linked to the biological processes. Management often relies on the behavior of a geographically defined stock although it may consist of several populations and thus have varying population dynamics. Marine resources, being the last wild populations harvested by large, are in need of a population definition suitable for sound management and conservation purposes.

The introduction of methods for assessing genetical variation trough screening of highly polymorphic DNA markers has made a revolutionary impact on the population concept. As generations go by the neutral genetic material in a finite population will change according to random processes (Wright 1931). This is genetic drift. In the absence of environamental changes a population arrives at allele frequency equilibrium between drift and gene flow. It is then possible to detect a population genetic difference between populations within a metapopulation. This focus on genetic variation has put emphasis on reproduction and gene flow between groups as a cohesive force in the population concept. Population definitions are for instance "A group of interbreeding individuals that exist together in time and space" (Hedrick 2000).

Movement and dispersal, what creates populations?

Besides recruitment and mortality; the movement of individuals is vital to population structure. The magnitude of migration between populations directly determines the level of genetic differentiation.

Movement or more specific relocation is a biological trait shared by all species, both plants and animals (Gaines 2007). In one or more life stages an individual must inevitably relocate itself away from its parental location.

Movement may be active, directional and self driven, or passive and driven by for instance the wind or water current. Active movement may be driven by the need to find food, the need to find a mate, to search for favorable conditions or to avoid being eaten. Active movement is often directional and has a purpose. Passive movement is common in the sea and applies to the entire life cycles (holoplankton) or only early life stages (meroplankton). One reproductive strategy among fish is to release large quantities of pelagic eggs and larvae. Movement may be small- or large scaled, and correspond to local and regional scale. Local scale, however, is a fuzzy concept usually dependent on the apriori choice of study and is so dependent on the perspectives of the researcher (Camus & Lima 2002). Movement may be large in relation to body size or in relation to population structure. If we define large scaled movement to be between populations it can be termed migration. Then small scaled movement will be within populations and will be best described by traditional behavioral studies not focusing on population dynamics.

Finally movement may be temporary or permanent. This only applies when considering movement done by an animal trough a period of time, and does not make sense when considering one single act of movement. Fish having separate feeding and spawning areas may undertake temporary migrations between these. A permanent relocation does ultimately involve that the animal never returns to the initial location

Population structure is sensitive to movement between populations regardless of considering demography, or a genetics. In all cases of movement the relocation may or may not have an actual affect on the population structure. If an animal migrates and reproduces in a new population it will effectively have transferred its genes to the new population. This represents gene flow between populations and will break down genetic differentiation. If the movement does not involve reproduction the animal will not be considered an immigrant to the population, in strict genetic sense. However animals may interact, trough competition or cannibalism, with conspecies of another population. This may for instance happen on a common feeding ground and affect mortality without involving transfer of genes. In this case movement will affect demography without being possible to detect genetically.

There has been a chiasm between ecology and evolution regarding the scale of important migration between populations (Waples & Gaggiotti 2006). In ecology, demographic processes usually scale with population size and 10% immigrating recruits each generation has been considered enough to affect demographic composition. In population genetics, evolutionary processes scales with the absolute number of migrants. In most cases demographic important migration is much larger than evolutionary important. This means that fish in two defined areas may act demographically as different populations although the gene flow between them is too large to support genetic differences (see further discussion Waples & Gaggiotti [2006]). Finally genetic differences may not be a sole good reason for managing fish from two samples as distinct populations (Waples 1998).

Theory and the real world

The real world never confines perfectly to theoretic models. There is an ongoing debate whether the theoretic population concept is applicable to the real world at all (Berryman 2002, Camus & Lima 2002). Even though the population concept may be flawed in some cases it still is a building block for ecology and a very useful concept. Sound management is in need of a managing unit that reflects biological significant processes.

Population genetic differences are usually measured by the F_{st} metric (or different variations [Wright 1951, Weir & Cockerham 1984]). In short this measure the lack of heterozygote individuals when two sub populations are considered as one, compared to two populations (within population heterozygosis versus between population heterozygosis). In an ideal island population the F_{st} is directly linked to migration by the formula: $F_{st} = \frac{1}{(4Nm + 1)}$ Where N is population size and m is migration rate (Neigel 2002). This assumes that populations are in Hardy Weinberg equilibrium. The Hardy Weinberg equilibrium requires among other things random mating between all individuals in a population. If these prerequisite are met is seldom the question. The questiont is rather how the flaws influence the results (Whitlock & McCauley 1999, Neigel 2002).

A practical question is how to sample a population. In biology inference is based on a sample and not the entire population. The critical point is then weather the sample is representative also for the unsampled part of the population. This depends on how the sample is taken, the size of the sample and the individuals in the sample. (Harwood 2009) claims that it is not possible to sample a population, only a specific geographic area. Further a geographical area may contain individuals from different populations, and not all individuals have the same chance of being sampled.

The marine environment has few absolute borders to population structure. Marine animals are also both difficult to observe and to sample. This makes inference about population structure difficult, and a mobile specie as the cod may not follow a strict population scheme. The understanding of populations in the marine environment requires a broad understanding of movement, genetic divergence and connectivity.

The Skagerrak coastal cod population complex, concepts applied.

STUDY AREA

The cod is an opportunistic predator that adopts different feeding regimes and habits according to different habitats. Movement and thus potential population structure varies widely with habitat (Comeau et al. 2002, Robichaud & Rose 2004). The north east arctic cod living in the Barents Sea (mean depth 230 m) undertake spawning migrations of several hundred kilometers. Cod in the North Sea (mean depth 90 meters) has been found to migrate intermediate distances and may move from coastal Sweden to the banks west of Denmark (Righton et al. 2007, Svedäng et al. 2007).

The North Sea is a relatively shallow sea, representing a homogenous feature in the cod perspective. Offshore the southern coast of Norway the Norwegian trench cuts along the Skagerrak coast with 500-700 meter depths (Figure 1). This coastal strip is composed of many small island and skerries, thus creating a complex area but without clear cut barriers to dispersal.

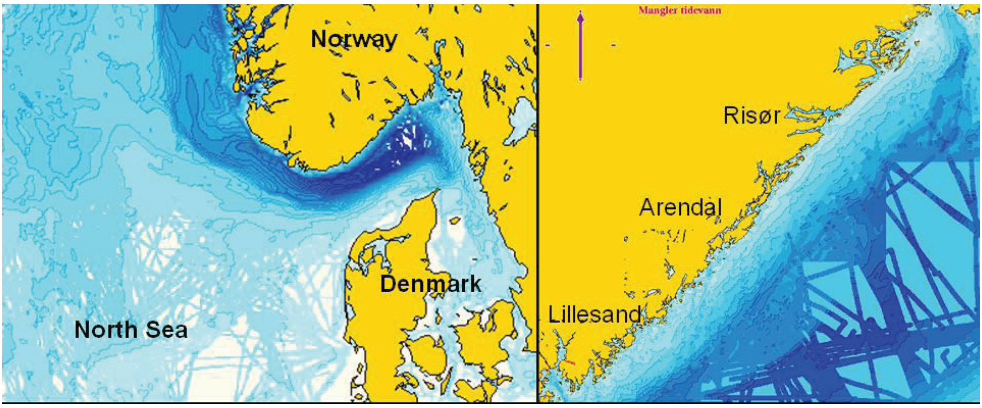

Figure 1: Left panel are depths from in the North Sea, showing the Norwegian coast and the Norwegian trench. Right panel is the Norwegian Skagerrak coast showing the numerous small islands and skerries.

POPULATION STRUCTURE

There has been a long debate whether the cod on the Skagerrak coast consists of one large panmitic population or several small fjord populations. The debate emerged when Captain G. M. Dannevig released hatched larvae on a large scale along the coast in the late 19th century. Capture mark recapture studies indicated that the cod may be confounded to different fjords, thus substituting different sub populations. An important step was the discovery of genetic differences of cod sampled from different locations along the coast (Knutsen et al. 2003). Samples from six different locations along the coast demonstrated small but significant genetic differences between locations. This suggests that there is a population structure on the genetical scale in the Skagerrak region, with limited gene flow. These studies did however not show isolation by distance. Further studies have strengthened this population perspective by demonstrating life history variation along the coast (Olsen et al. 2004). Still the cod has a high potential for dispersal and exists on a continuum between the sampled populations with no obvious boundaries between them.

MOVEMENT AND RELOCATION

The cod lifecycle may be divided in four distinct stages; eggs, larvae, juvenile and adults. The Skagerrak cod spawn on spawning grounds from February to April, with a peak in March and April (Kjesbu 1989). The eggs are pelagic trough a two to three week period before hatching. The larvae are primarily pelagic but develop swimming capabilities before settling during early summer. Trough this passive drift period some spawning fields may be interlinked with exchange of recruits by water movement and currents. Others may be nearly isolated from each other even though they may be geographically closely situated. Finally adult cod, being a batch spawner, may move between different spawning areas, thus linking these together genetically in addition to passive drift of eggs and larvae.

From juvenile stage and on the cod is a good swimmer and may move freely along the coast. Several capture mark recapture studies has been performed on the Skagerrak coastal cod during the last century. Already in 1905 Knut Dahl tagged adult cod in Risør to shed light on the question whether the cod on the Skagerrak coast was composed of one panmitic population or several small fjord populations. He concluded that a large portion of the cod did not undertake long migrations, but that some displayed migration out of the fjords to the open sea (Dahl & Dannevig 1906). Further CMR studies have been undertaken and the overall conclusion regarding the migration of the cod is that "…the cod on Norwegian Skagerrak coast consist of non-migratory populations." (Danielssen & Gjøsæter 1994). These studies have yet not investigated the dynamics of recaptures trough time, and have thus not been suited for investigating different area utilization, including possible feeding migration, spawning site fidelity or natal homing. To further complicate the population structure, there has been documented an effect of inflow of eggs and larvae from the North Sea on the juveniles on the coast (Knutsen et al. 2004, Stenseth et al. 2005). If these return to spawn in the North Sea or are assimilated in the populations is not yet established.

Objectives of this study

The main objective of this thesis was to study connectivity processes affecting the population structure of coastal cod on the Norwegian Skagerrak coast. We focused on three aspects of the processes. First we studied the dynamics and the integrity of the spawning grounds. This includes potential retention processes. Secondly we investigated the scale of the spatial extent of the sub-population structure. Finally we studied movement, migration and area utilization of adult cod.

Paper I: In this study we did a combined investigation of egg dynamics and adult cod spawning ground behavior. The objectives of the paper were to describe the spatial extent and dynamic of one spawning ground. Another objective was to investigate adult cod spawning site fidelity.

Paper II: This study was a general investigation of the egg patterns in transects out fjords throughout Norway. The main objective of the study was to describe patterns in egg distribution by bottom topography and general geographical features.

Paper III: In this study we utilized samples of tissue from adult cod to determine genetic differences. The objective of the study was to find the lower geographic threshold were genetic differences appear and study the genetic extent of populations.

Paper IV: This study use capture mark recapture data from several studies the last century to calculate capture probability distributions. The objectives of the study were to determine the geographic extent and area utilizations of populations of cod along the coast.

Paper V: This study was an in depth study of the egg distribution in a spawning ground on the Skagerrak coast. The objective of the study was to describe egg patterns by using measures of water circulation.

Paper VI: This study used acoustic transmitters to investigate vertical behavior of young adult cod. The objectives of the study were to describe some causal explanations for vertical migration.

Methodological considerations

Field work, sampling problems

The field work of this thesis have included sampling of eggs from spawning grounds, sampling of adult cod for tagging both with traditional and acoustic transmitters and sampling of adult cod for genetic screening. The main point when drawing inference about a population from a sample is weather the sample is representative for the scope of the study. This depends both on the size of the sample compared to the sampled population and the methods used to achieve the sample.

Egg sampling was done with vertical net hauls. One haul presumably contain a very small proportion of the entire population of eggs in a fjord. Few numbers of eggs in each haul may suggest that the egg samples are mostly stochastic and with little information on the true density of eggs. In these studies a total of more than 1200 hauls were conducted. Two areas have been sampled a throughout the spawning period and several fjords (Tvedestrand, Risør and Lillesand) have been sampled more than one year. The absolute magnitude of egg density varied, but neighboring stations very usually similar. Patterns of increasing densities towards spawning areas were clear and suggest that patterns found are real features in egg densities.

Sampling of adult cod is usually done by fishing either by eel pots or gill nets. These are set at 5-10 meter depths in sheltered locations. This methodology makes samples somewhat biased towards fish from good fishing locations, and induces a higher probability for a tagged fish to come from a fjord population than from a population in the outer parts of the coast.

The genetic sampling (Knutsen et al. 2003) include one sample from the outer parts (Bjelland, also included in analysis in paper III). Although this sample may not be a spawning population, the assignment test suggests that it represent a true coherent group compared to the inshore samples. The tests further suggest that there are true population differences between fjords on the coast, and that the fjord populations are not just the stationary part of a more mobile offshore population. There may be population structure in the outer parts of the coast not fully described by studies in this thesis and others (Knutsen et al. 2003, Olsen et al. 2004). Further in this thesis most data concern fjord populations and we will focus on the fjord populations. We are aware that there might be a cross coast population structure in addition to the studied along-shore structure.

Semi-parametric models

Most types of biological data rarely fit a parameterized strict linear relationship. In several of the papers (Paper I, Paper II, Paper IV, Paper V and Paper VI) in this thesis we have used semi-parametric models to describe the relation between signal and noise in the field data (Worton 1989, Hastie & Tibshirani 1990). They are not parametric in the sense that they don't assume a linear mathematical relationship between predictor and response variables throughout the dimensions of the data. Instead they evaluate the predictor data in several "neighborhoods" to make inference about the response (Wood 2004, Gitzen et al. 2006). They are not completely non-parametric as they depend largely on one smoothing parameter, determining the width of the neighborhood. Having a too small smoothing parameter makes the neighborhood small, overfitting the data and thus explaining noise (a further discussed of the smoothing methods can be found in paper IV).

These models may seem very dependent on the nature of the predictor variables and useless for finding a general relationship between variables compared to linear models (for instance when the predictors of egg models are latitude and longitude, they apparently cannot be used as a general prediction model given any fjord). Firstly predicting outside the range of the data should always be done with caution. Having a linear mathematical prediction model does not guarantee the equivalent relationship outside the data. Secondly most models in this thesis are not used as prediction models, but description models. They are mainly used to investigate general biological relationships between predictor and response variables. Latitude and longitude are not interesting in themselves but represent biological variation that can be qualitatively evaluated from the model.

Results and discussion

SPAWNING GROUNDS, EXTENTS AND DYNAMICS.

Cod spawn pelagic eggs with a relatively long pelagic period. During this period the potential for dispersal and mixing of eggs and larvae is large. For the genetic structure along the coast to persist the cod must either spawn in areas where the eggs are retained on the spawning ground by currents or spawn downstream of the nursery habitat with a later homing to the natal spawning ground.

Spawning grounds for cod have previously been documented by using interview information from local fishermen and preliminary egg surveys (Knutsen et al. 2000). Figure 2 show some of the spawning areas for cod mapped in the Tvestrand and Arendal municipality (Data from "National program for mapping marine habitats in Norway").

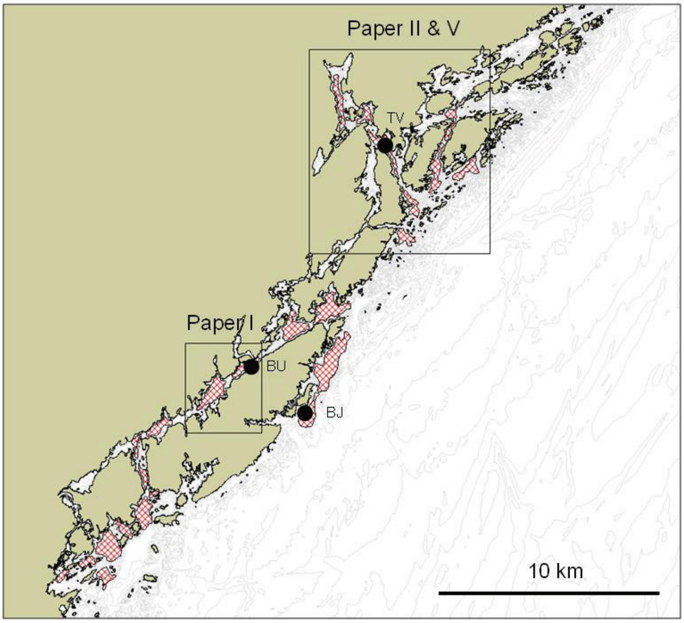

Figure 2: Registered spawning areas for cod in Arendal and Tvedestrand municipality (Red cross shaded areas). Black boxes show different spawning areas studied in the different papers. The black dots demonstrate some of the sampling locations for paper III.

These do not necessarily represent equal potential for cod recruitment, nor does necessarily the recruitment potential scale with the size of the spawning area. The areas may also represent good fishing areas for mature cod rather than areas with high recruitment potential. Most of these spawning areas are small and closely separated. Given the close proximity of these spawning areas to each other, strong current would easily mix eggs from different areas. The unity, for recruitment to the local population, would then be a group of spawning areas rather than the individual areas drawn in figure 2.

Given the high mobility for cod, a spawning individual could easily move between these spawning areas within one spawning season spawning batches in different areas. Even in the presence of retention the spawning areas would appear to be interlinked genetically. Ultimately the fish would also need to show lifelong spawning site fidelity.

In paper I we approached these two problems by studying egg distribution on one spawning ground (Figure 2) and the site fidelity of mature cod on the same spawning ground. This study demonstrated that the egg densities in the area showed clear geographical patterns even on a small scale. There were clearly more eggs in the northern basin of the studied area (Figure 3), while the mature cod were highly stationary slightly south of the high egg distributions. There was a slightly northward shift in egg distribution either due to weak current moving eggs northward or a very small scaled shift in geographic spawning pattern. Altogether the spawning area was ~1 km^2, while the mature cod utilized areas even smaller; on average 0.27 km^2.

This first study was not a causal explanation of the egg densities given geographical variables; however this study did prove that the recruitment to local populations could be by smaller spawning areas than previously documented. This study did also strengthen the hypothesis that population structure on the Skagerrak coast was promoted by egg retention and spawning site fidelity of adult cod. This study also suggests that there is a potential for even smaller population structure than previously documented on the coast (Knutsen et al. 2003).

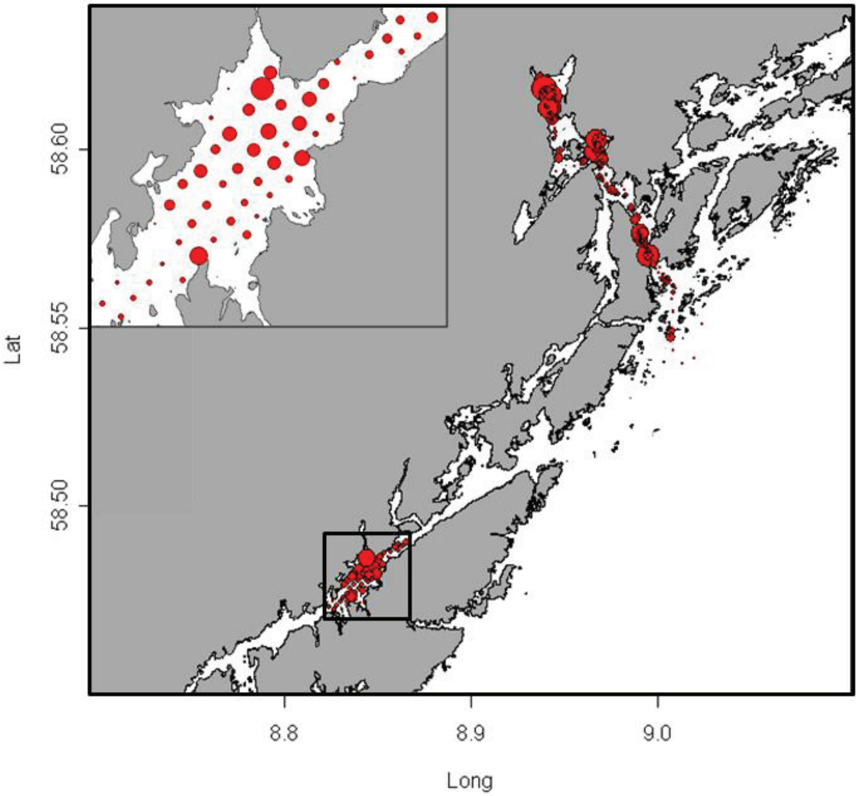

Figure 3: A joint map of egg distributions from Tvedestrand 2007 (Paper V) and from Tromøysund (paper I). Circles corresponds to number of eggs found summarized at the station trough the entire study period.

In paper II we extended the study of egg distributions to include presumed spawning areas in 20 fjords along the Norwegian Coast. The purpose of the study was to use general geographical features to describe the egg distribution across several fjords. Although small scaled genetic structures in cod populations have only been extensively studied on the Norwegian Skagerrak coast, the egg distribution patterns were similar in different regions of Norway. The general pattern was considerably more eggs inshore than offshore. Some additional features were also found in this study. The egg distributions gradually decreased when moving offshore in all but the northernmost fjords. In these fjords the densities leveled out suggesting that eggs from North East Arctic cod were sampled. This demonstrates that we could detect spawning areas offshore, however in western and southern Norway no such were found. The distance from a sill in the fjord had a significant effect on the density of cod eggs. Considerably more eggs were found just inside sills and this effect was more pronounced when the sill was shallow (threshold value of 37.05 m). This Sill effect probably reflects the water circulation pattern in fjords with sills. Water and eggs are retained in the basins on the inside of the sills.

In study II we indirectly demonstrated an effect of water current on the retention of eggs. In study V we investigated egg distribution in relation to observations on water circulation and egg characteristics in a fjord on the Skagerrak coast (Figure 2 & 3). The objective was to further explain the retention process of eggs in a typical fjord. The general pattern was in accordance with paper II. The majority of eggs (~80 %) were found in the upper 15 m of the water column. In this part of the water column the patterns were more eggs inshore than offshore. Below 15 m depth there were no apparent patterns in egg distribution. On general the water was flowing inwards in the upper 15m while outwards in the lower 15 m (that is the opposite of the classic estuarine circulation). The current strength was also decreasing when moving inwards in the fjord. These results suggest that the cod display a spawning site selection. Areas and depths were eggs are transported inwards are preferred over areas where eggs are transported out of the fjord.

Overall these studies do describe some important features of the spawning grounds on the Norwegian Skagerrak coast. Spawning Adults do both display a spawning site selection and spawning site fidelity. Spawning site selection make sure eggs are retained in the area and the patterns of egg distribution suggest that the spawning areas may be smaller than previously documented. These mechanisms all promote population structure on a small scale.

SPATIAL EXTENTS OF POPULATIONS

Many community processes and trophic interactions are spatially correlated processes. Given the depleted state of the populations of cod on the coast, this will also be important to management, for instance when constructing marine protected areas (Palumbi 2004).

As only areas, not populations directly, may be managed; the delineation of population will become increasingly important.

To study the geographical extent of cod populations we used two approaches. In paper III we used the geographical patterns in genetical differences along the coast to find the lowest distance where genetical distances appear. In paper IV we used probability distributions of tagged cod do determine the spatial extent of populations. These two methods were also compared in the last paper.

The genetical material utilized were samples of mature cod from five to seven spawning areas along the coast (Figure 4). When the between sample F_{st} was compared to distance in samples situated less than 30 km apart, no genetic structure appeared in 3 of 4 comparisons. Positive F_{st} values were found between samples situated more than 30 km apart. This suggests that fish sampled more than 30 km apart usually will belong to different populations, and the extent of the populations may be on a smaller scale than previously reported. Assignment of individuals in this study showed consistently higher assignment to capture site than to any other site even when comparing populations situated less than 30 km apart. This suggests that even population closely situated may represent (slightly) differentiated populations. The sample from Bjelland (Figure 4) displayed a positive F_{st} value when compared to the sample from

Buøya even though these are very closely separated (8 km). The sample from Bjelland may however not be from a typical spawning area described in the previous chapter. This sample may thus not represent a true spawning population.

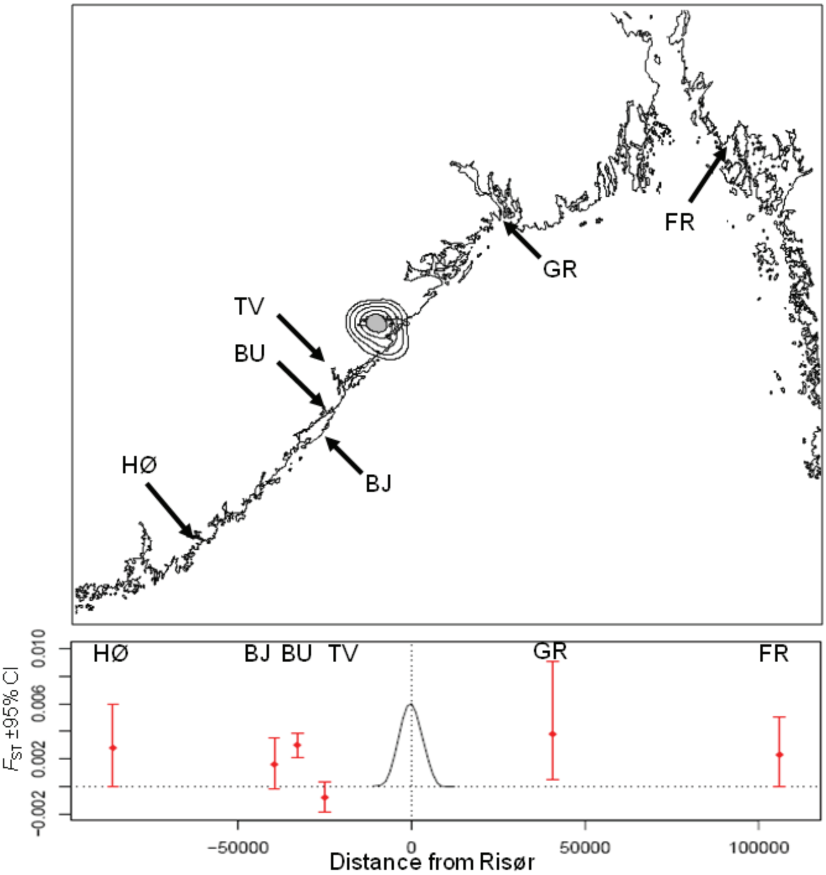

Figure 4: An illustration of the spatial extent of a population of coastal Skagerrak cod (figure adopted from paper IV). The upper figure shows the isolines connecting equal probability of capturing a tagged cod around Risør. The innermost isoline representing the 0.25 probability area is represented by a grey filled polygon. Arrows indicate locations of samples used for genetic comparison. The lower panel demonstrates the distance along coast from Risør and the Fst values between Risør and the given sample (see also figure caption paper IV).

In paper IV (and partly in paper III) we used capture mark recapture data from the last century to estimate the extent of populations along the coast. Assuming that tagged cod belonged to the population at the capture site the distribution of recaptures was used to estimate a probability distribution of recaptures. The 95 % probability area of recapturing a tagged cod (that would exclude 5 % of the recaptures, reflecting tagged fish not belonging to the target population and fish displaying abnormal behavior) where considered the spatial extent of the population. In paper III a slightly different approach was used were an asymptotic value of a minimum convex polygon (MCP) based on the recaptures after one year was used. These two methods both suggest that a population of tagged cod in Risør would extend ~18 km and ~13 km respectively along the coast.

These two studies reflect two aspects of the spatial extent of a population; interaction trough movement and gene transfer to new generations by spawning. Recaptures from tagging do describe the area the population will use and the area where individuals will interact, but not necessarily where they will spawn. The genetic information on the other hand describes the distance between populations exchanging genes but not how these interact between spawning. Together they demonstrate that populations of cod on the Norwegian Skagerrak coast are fjord bound and extend only a few kilometers along the coast. These two methods also have very similar results where F_{st} values appear some distance outside the extent of the recoveries.

ADULT MOVEMENT AND AREA UTILIZATION

Adult cod has a high potential for dispersal. Fish tagged on the Norwegian Skagerrak coast have been recaptured in Smøgen in Sweeden, Thyborøn, Læsø and Rønner in Denmark and Sassnitz in Germany near the Polish border. Active movement by adults may thus have important implications for connectivity processes between populations along the coast. Adults cod must show homing to their natal spawning area, site fidelity within spawning seasons, between spawning seasons and spawning site selection favoring sites where eggs are retained. These behaviors relating to spawning have been discussed in the previous chapters. Behaviors between spawning seasons do not affect transfer of genes between generations, but may indirectly affect population structure. Populations may utilize overlapping areas between spawning, and compete for common resources. Descriptions of movement are also important when managing areas and imposing restrictions on fishery.

Paper IV studied movement and behavior in populations of fish using recaptures of tagged cod trough the last century. A total of 1679 recaptures from seven different studies were investigated using probability distributions of recaptures and models describing distances moved. Dahl (Dahl and Dannevig 1906) suggested that recapture locations varied by season reflecting seasonal migration. This pattern partly appears in density distributions of tagged cod from different parts of the year (Figure 3) and will reflect different utilization of the areas in spawning season (Jan-May) and during the rest of the year. These patterns rely however on few numbers of recaptures, and may as well reflect geographic stochastic variation or variation in fishery intensity.

Fish tagged in nearly the same locations in different years utilized different areas of the coast. Cod tagged in Risør in 1939 were recaptured in an area stretching 10 km along the coast, while fish tagged in Risør in 1970 and 71 were recaptured in an area covering 55 km of the coast. The fish in 1971 was generally tagged further offshore than in 1939, however the models applied to the distances moved revealed no clear pattern of offshore fish moving larger distances. Overall the models explained only 4 – 23 % of the variation of movement and large residuals appeared early after the fish was tagged and released. This suggest that the cod had a potential for rapid relocation within the population extent and that individual behavior could be more important than for instance number of days at liberty.

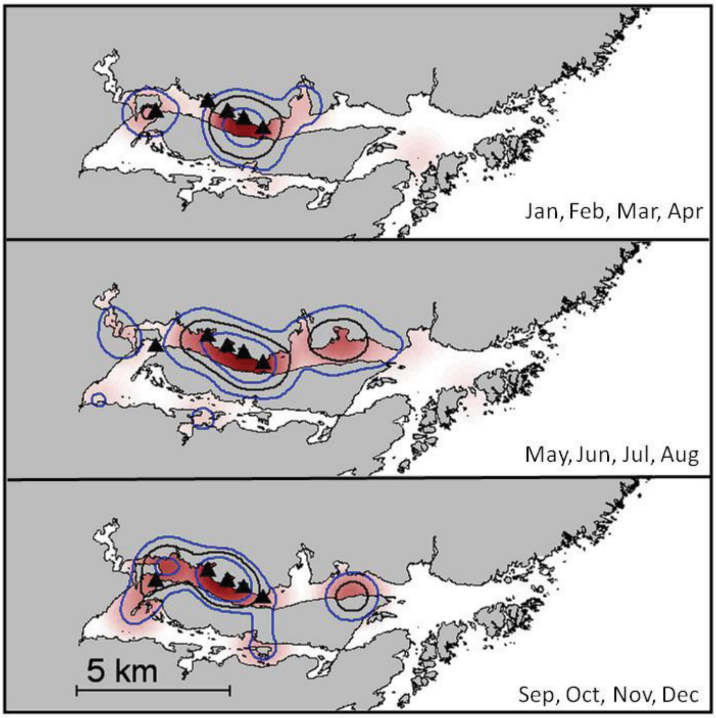

Figure 3: Map of Risør showing kernel density distributions of recaptures of tagged cod released at the black triangles in 1905. The upper panel show recaptures done in the first third of the year (winter and spawning season). The middle show recaptures done the second third (summer months). The lower panel shows the recaptures done during autumn early winter.

Most of the fish tagged in this study was sedentary, moving only short distances. Still adult cod in the area may move rapidly within their population extent utilizing different areas for feeding and spawning. There is however no evidence for a collectively movement, as for instance feeding migration, and there are probably large individual differences.

In all these studies tagged fish were caught no deeper than 10 meters. This could have introduced a bias if fish did not move considerably vertically. Paper VI investigates the vertical component of movement on an individual basis. A total of 30 fish were equipped with acoustic tags transmitting the residence depth of the fish. In this paper we demonstrated a clear year round diel vertical migration (DVM) pattern. Fish constantly selected shallow areas, probably for feeding, during night. During day the fish descended to the deeper areas. There was also large seasonal variation in the vertical behavior. During winter when the water was cold they consistently selected the deepest available habitat, where the water was warmer. During summer in warm water they utilized shallower depths, but avoided the warmest waters. The vertical behavior was also probably affected by food availability.

This study demonstrated how cod utilize variable depths according to environmental factors. This suggests that populations along the coast are not confined to specific water layers, but interact also in the vertical dimension. This study also demonstrates some of the flexibility in cod behavior. The fish may adopt different behavior in response to different environmental stimuli (for instance temperature and food availability). Together these two studies suggest that many factors affect the movement and behavior of Skagerrak cod. There may be individual differences in behavior. The cod is an opportunistic feeder with plastic behavior. Although mostly sedentary they may perform local migrations both vertically and horizontally.

The Skagerrak cod population complex

Populations in a meta-population complex are linked by connectivity processes. These processes may have both genetic and demographic consequences trough transfer of genes and interaction of individuals, and will ultimately shape population structure. Mechanisms promoting population structure include; Adult spawning site selection, retention of pelagic life stages by water circulation, homing to natal spawning area, within-spawning site fidelity and between spawning site fidelity. These mechanisms may function at different scales thus creating global, regional or local meta-population structure.

In this thesis we have studied mechanisms affecting population structure and dynamics in cod on the Norwegian Skagerrak coast. The cod has a high dispersal capability and a long pelagic duration. Due to this population structure has been expected to be large scaled with a high degree of connectivity between areas. In this thesis we have demonstrated that cod populations on the Norwegian Skagerrak coast are structured in small populations with mean population home ranges of 195 km^2. These populations have unique spawning areas where eggs and larvae are retained by current. The adult have lifelong site fidelity and select spawning sites to minimize advection of offspring. All these behavior promote population structure on a small scale.

The mechanisms discussed here apply to many species and are not specifically linked to cod. Connectivity processes are not unique for cod only in this area. Here we describe how a high potential dispersal specie have populations structure on the scale of tens of kilometers and are mostly fjord bound. Similar patterns and population structure may thus be found in cod in other parts of its range as well as in other species with a potential for high dispersal.

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Egg distribution, bottom topography and small-scale cod population structure in a coastal marine system.
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Egg distribution, bottom topography and small-scale cod population structure in a coastal marine system

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ABSTRACT: Coastal marine species with pelagic egg and larval stages, such as the Atlantic cod *Gadus morhua*, can be structured into genetically distinct local populations on a surprisingly small geographic scale considering their dispersal potential. Mechanisms responsible for such small-scale genetic structure may involve homing of adults to their natal spawning grounds, but also local retention of pelagic eggs and larvae. For example, spawning within sheltered fjord habitats is expected to favour local retention of early life stages. Here, we studied the distribution of cod eggs along inshore–offshore transects in 20 Norwegian fjords. The general pattern exhibited across all fjords was a higher concentration of cod eggs inside the fjords than further offshore. In particular, fjords with shallow sills (model threshold 37 m) show an abrupt reduction in egg density over the sill. This study provides empirical support for an offspring retention hypothesis, which may help to explain the maintenance of local population structure in pelagic marine systems.

KEY WORDS: Atlantic cod · *Gadus morhua* · Egg distribution · Population structure

INTRODUCTION

According to Thorrold et al. (2001), the local population structure of coastal species may result from physical barriers limiting dispersal among habitats behavioural features such as homing of adults to their natal area. It has been proposed that the existence of a highly heterogeneous population structure is inversely proportional to the species dispersal potential (e.g. Waples 1998). However, it is also acknowledged that a prolonged dispersal potential (for example measured by the duration of the mobile early life stages) does not always result in populations that are genetically similar over large distances (e.g. Kettle & Haines 2006).

Marine organisms face a special challenge in that ocean currents may transport pelagic eggs and larval stages passively over vast distances, potentially moving them toward unfavourable habitats. Indirect evidence for the ecological and genetic impact of larval drift is the observation that many marine species are genetically similar over large geographic distances as compared to terrestrial and freshwater organisms (e.g. Waples 1998, Knutsen et al. 2004). However, recent studies have also revealed that marine species with pelagic early life history stages can be structured into local populations on surprisingly small geographical scales (Campana et al. 1999, Knutsen et al. 2003). Potential mechanisms explaining such local population structure include (1) high site-fidelity of both juveniles and adults, (2) homing of mature individuals to their natal area, and (3) spawning site selection minimising the advection of pelagic offspring. These mechanisms need not be mutually exclusive.

In coastal marine systems, there is evidence supporting the site-fidelity hypothesis (Green & Wroblewski 2000) and the homing hypothesis (Thorrold et al. 2001). The offspring retention hypothesis has not been fully investigated, particularly in temperate marine systems. Recent modelling work shows that coastal hydrodynamics is potentially important for generating non-

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random dispersal of pelagic early life stages, affecting the balance between gene flow and local adaptation in coastal marine species (Cowen et al. 2006). Innovative field analyses showed evidence for a high degree of self or local recruitment in a variety of coral reef species (Jones et al. 2005), emphasizing the importance of the offspring retention hypothesis. However, coral reef fish species have a relatively short pelagic life cycle when compared to more temperate or subpolar species, and many of them have demersal egg stages. These features alone would not favour a long-distance drift of early life stages. Therefore, it is not yet clear to what extent similar patterns of early life retention and self-recruitment also apply to more temperate species with long-lasting pelagic egg and larval phases.

With regard to more temperate systems, the seminal study of Iles & Sinclair (1982) on the north Atlantic herring *Clupea harengus*, also a species with demersal eggs, suggests the existence of local circulation patterns that favour larval retention and the maintenance of population structure across the entire north Atlantic. Bailey et al. (1997) propose similar mechanisms to explain the genetic structure of walleye pollock *Theragra chalcogramma*, a species with pelagic eggs, across the north Pacific. However, it is in small-scale settings that the existence of local population structure is most intriguing. Ruzzante et al. (1996) has shown that genetic structure is recognizable also within a patch of cod larvae, presumably originating from different local spawning aggregations, but there is no causal explanation of how such structure is generated in first place. Thus, the role of the early life retention hypothesis in relation to small-scale population structure still remains untested, particularly in temperate or subarctic seas.

Here, we study the significance of the egg retention hypothesis for maintaining a local population structure in Norwegian coastal cod by sampling cod eggs in transects of fjords. Sampled habitat types range from sheltered fjord basins inside sills to more open coastal habitats, throughout the western and southern coast of Norway. The Norwegian coastal cod spawn in excess of a million eggs, distributed over multiple spawning events during February–April. The eggs hatch after 2 or 3 wk and the larvae stay in the water column where they feed on zooplankton. The larvae metamorphose and settle in demersal nearshore habitats in early summer (April–June) when they are about 3 to 5 cm long. The Norwegian Coastal Current (Skjoldal 2004) has the capacity to rapidly mix pelagic eggs and larvae over vast distances. Still, Norwegian coastal cod is composed of local populations, some of which are separated by only a few tens of kilometers (Knutsen et al. 2003), which also differ in adaptive traits such as age and size at maturation (Olsen et al. 2004). Further, tagging studies indicate that the Norwegian coastal cod has a site fidelity comparable to the extent of the local populations as inferred from genetic studies (Danielsen & Gjøsæter 1994).

MATERIALS AND METHODS

We sampled cod eggs in inshore–offshore transects from 20 fjords along the Norwegian coast during the cod spawning period in March–April 2005 (Fig. 1, Appendix 1 available at: www.int-res.com/articles/suppl/m333p249_app.pdf). Two of the fjords, Lillesand (18) and Risør (19), were also sampled in March 2004. The eggs where collected with a WP2 plankton net (diameter: 60 cm; mesh width: 500 µm). The net was hauled vertically from 50 m depth (in shallower waters just above the bottom) to the surface at a speed of 0.5 ms^{-1}, filtering about 14 m^3 of water in each tow. This depth range was chosen based on preliminary hauls in all geographic areas (southern, western, and northern Norway) split into vertical depth segments of 75 to 50, 50 to 25 and 25 to 0 m; none or very few eggs below 50 m depth either inside or outside fjords for all areas were detected (H. Knutsen pers. obs.). Cod eggs were identified among the plankton following Russel (1976; see also www.larvalbase.org). Early stage cod eggs cannot readily be distinguished from those of haddock *Melanogrammus aeglefinus* based on visual inspections of egg size and appearance. Older egg stages of cod and haddock, however, can be distinguished. Among the older stage eggs in our samples only 1% were identified as haddock (23 out of 2287 cod + haddock eggs). This indicates that haddock eggs were not an important source of bias in the estimates of total cod egg abundance. At each sampling site, we recorded latitude, longitude, bottom depth and the distance to the sill of the fjord.

We tested the effect of local geographic features on egg distribution using different formulations of Generalized Additive Models (GAM; Wood 2006). The response variable (y) was the natural logarithm of egg density (the sum of all stages), standardized by volume filtered. The inspected predictor variables (hereafter covariates) were the natural logarithm of bottom depth (z, the logarithm was taken to achieve a uniform distribution of sampling depths), distance in meters from the sampling station to the sill location (d), depth (s) and latitude (l) of the sill. The distance from the sill was negative if the sampling station was inside of the sill and positive otherwise. Only tows located at less than 10 km from the sill (either side) and with positive egg count were included in the analysis. Removal of tows outside of the 10 km range (7 out of 233) was necessary to reach a uniform spread of samples on either side of the sill. Removal of zero-egg tows (9 out of 233) was necessary to stabilize the variance and normalize the distribution. All but one of the discarded zero-egg

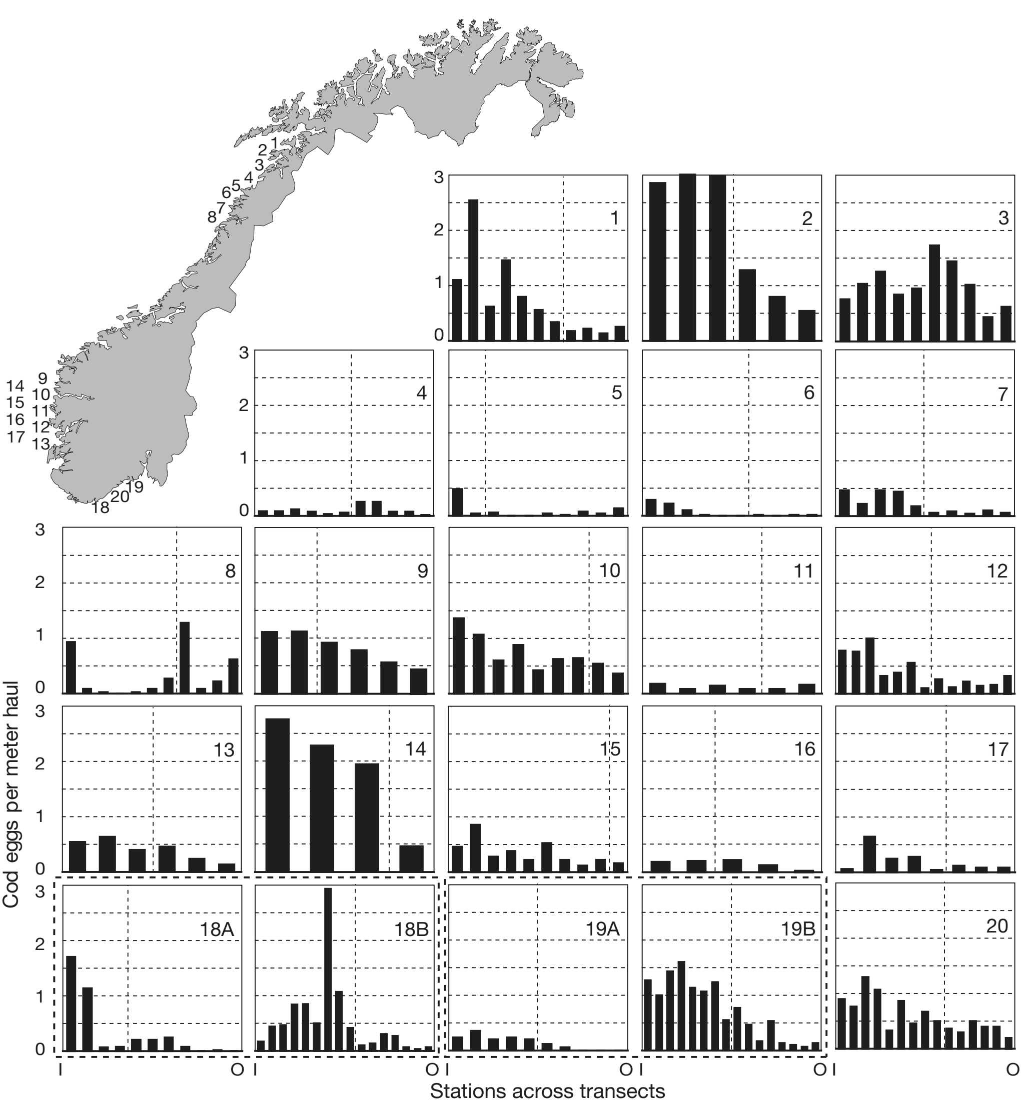

Fig. 1. *Gadus morhua.* Raw data showing number of eggs m^{-1} haul in transects from 20 fjords in Norway. Stations arranged on *x*-axis from innermost (I) to outermost (O) station in transect. Vertical dotted lines: location of sill for each fjord. Transects—North Norway: (1) Hopen, (2) Reipå, (3) Bjerangsfjorden, (4) Storfjorden, (5) Okfjorden, (6) Sørfjorden, (7) Brekosen, (8) Beiarnfjorden; west Norway: (9) Landviksosen, (10) Hellosen, (11) Fanafjorden, (12) Kolltvettosen, (13) Davængvågen, (14) Lurosen, (15) Austerfjorden, (16) Skogsvågen, (17) Lysefjorden; south Norway: (18A,B) Lillesand in 2004 and 2005, respectively; (19A,B) Risør in 2004 and 2005, respectively, (20) Tvedestrand. NB the vessel was prevented from entering the area on the fjord side of the sill in Bjerangsfjorden (Transect 3). See Appendix 1 for detailed maps

samples were located offshore of the sill, while one sample, in Beiarnfjorden (8), was located about 10 km outside of the sill. This fjord was rather unique, in that it was very long and with a very shallow sill (6 m). The egg distribution pattern along this fjord will be dealt in greater detail in the 'Results' and 'Discussion'. The final number of stations used in the data set was 217.

We compared results from 3 different model formulations, each addressing a specific hypothesis (Table 1). In Model 1 (the 'null' model) it is assumed

Table 1. Generalized Additive Model (GAM) equations used to analyze the *Gadus morhua* egg distribution data. Model 1 is a fully additive formulation, while Models 2 and 3 are non-additive formulations in which the relationship between egg density (y) and distance from the sill (d) is allowed to change depending on whether the latitude (l, Model 2) and depth (s, Model 3) of the sill are above or below a threshold value (r) estimated from the data. k_t and k_f are year- and fjord-specific constant coefficients, ε is the error term, assumed to be normally distributed with finite variance and equal among the 2 regimes of Models 2 and 3. The subindices i and t identify the sample and the year, respectively. z is the natural logarithm of bottom depth in meters, and the gs are smooth functions (i.e. natural cubic splines) linking the covariates with the response variable

Model type	Equation	Threshold
Model 1	$y_{i,t} = k_t + k_f + g_1(z_i) + g_2(d_i) + \varepsilon_i$	No threshold
Model 2	$y_{i,t} = k_t + k_f + g_1(z_i) + \begin{cases} g_2(d_i) + \varepsilon_i & \text{if } l \le r \\ g_3(d_i) + \varepsilon_i & \text{otherwise} \end{cases}$	Latitude
Model 3	$y_{i,t} = k_t + k_f + g_1(z_i) + \begin{cases} g_2(d_i) + \varepsilon_i & \text{if } s \le r \\ g_3(d_i) + \varepsilon_i & \text{otherwise} \end{cases}$	Sill depth

that the distribution of eggs along a fjord (i.e. relationship between egg density and distance from the sill) is similar across all sampled fjords. This hypothesis is in contrast to those of Models 2 (the 'geographic' effect model) and 3 (the 'sill' effect model), in which the distribution of eggs along a fjord can change according to a threshold value of either the latitude or the depth of the sill, respectively. The selection of the threshold was based on the minimization of the generalized cross validation (GCV) among a variety of GAM models spanning the entire range of possible threshold values. For more details on threshold estimation see Ciannelli et al. (2004). The GCV, although suitable for comparing models having a similar formulation (e.g. models with thresholds), does not properly account for the presence of additional parameters (i.e. the threshold) when comparing models with different formulations. Thus, to properly compare models with and without a threshold, we numerically computed the cross validation score (hereafter referred to as genuine cross validation, CV) as follows. A random selection of 20 data points (about 10% of the entire data set) was excluded from the model calibration. The resulting model was then used to predict these 20 out-of-sample observations, and the mean-squared predictive error was calculated. The operation was repeated 500 times, with the final CV being the mean among the 500 evaluations of the mean-squared predictive error. Low CV values indicate models with better fit to the data and lower complexity.

RESULTS

The data showed a clear signal where most fjords have a high egg density in inshore stations (inside the sill or at the sill) and with decreasing levels further offshore (cf. Fig. 1, Appendix 1). Beiarnfjorden (8) deviated from the expected pattern. This, could be due to a very shallow entrance sill (6 m depth), and that the 'outside' of this sill is the inner part of another larger fjord (see Appendix 1 for map details). Other deviating results were the low number of eggs in Storfjorden, Okfjorden and Sørfjorden (Fjords 4, 5 and 6 in Fig. 1). This is probably due to the fact that these 3 fjords were sampled last in the study, when cod spawning was already decreasing. We were not able to sample inside the sill of Bjerangsfjorden (3) as a power line was too low to pass under with the research vessel. Fanafjorden (11) at Hordaland had multiple deep sills and the shallowest one was noted.

In agreement with the raw data, all examined model formulations indicate a clear effect of distance from the sill on egg distribution. In Model 1 (i.e. no difference of egg distribution among fjords), the density of eggs reaches a peak slightly inshore of the sill location, and quickly decreases offshore of it (Fig. 2). Results of Model 2 indicate that the effect of distance from the sill vary according the geographic location of the examined fjord. In particular, fjords located south of 60.8° N exhibit a constant and linear decline of egg density, going from inshore to offshore locations, while fjords north of 60.8° N exhibit a peak of eggs slightly inshore of the sill and a slight decline tapering off to a plateau afterwards (Fig. 3, Appendix 2 available at: www.int-res.com/articles/suppl/m333p249_app.pdf). Similarly, results from Model 3 indicate that the egg density in fjords with a sill depth shallower than 37.05 m reaches a pronounced peak inshore of the sill and a decline offshore of it, while the egg density in fjords with a sill deeper than 37.05 m constantly decline with the increase in distance from the sill (Fig. 4, Appendix 2). In spite of their similar pattern of egg distribution, southern fjords or fjords with a deep sill constituted 2 different groups (cf. legend of Figs. 2 & Fig. 3). Based on the CV score, the 'sill effect' (CV = 0.547, R^2 = 72.2%) model is marginally superior to the 'geographic effect' (GCV = 0.576, R^2 = 71.7%) model, and both superior to the 'null' (GCV = 0.618,R^2 = 68.7%) model. The effect of bottom depth was similar among all

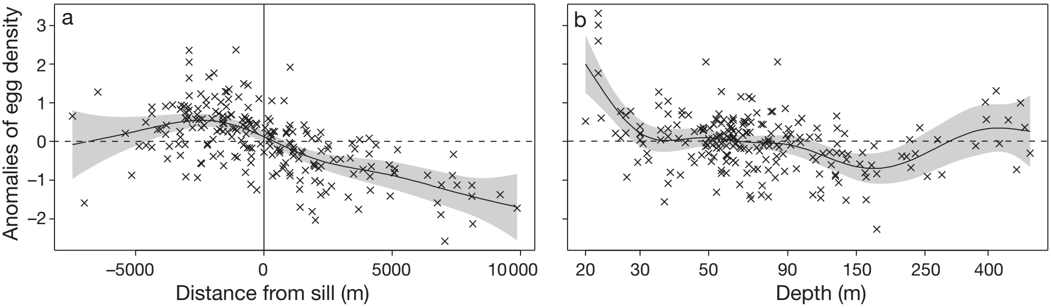

Fig. 2. *Gadus morhua.* Results from the first model formulation from Table 1 (Model 1, null model). (a) Average effect (solid line) of distance from the sill (negative for stations located inshore of the sill and positive otherwise) on anomalies of egg density. (b) Average effect (solid line) of bottom depth (natural log transformed). Shaded regions indicate the point-wise 95% CIs around the predicted effect; scatterplots are the partial residuals (i.e. those obtained from a model which includes all the covariates, except that being examined) around the average effect. Horizontal dashed lines indicate the 'zero-effect' response and the vertical line in (a) indicates the location of the sill. The model predicted values are given by the sum of the 2 additive effects plus the fjords- and year-specific coefficients (k_f and k_t from Table 1)

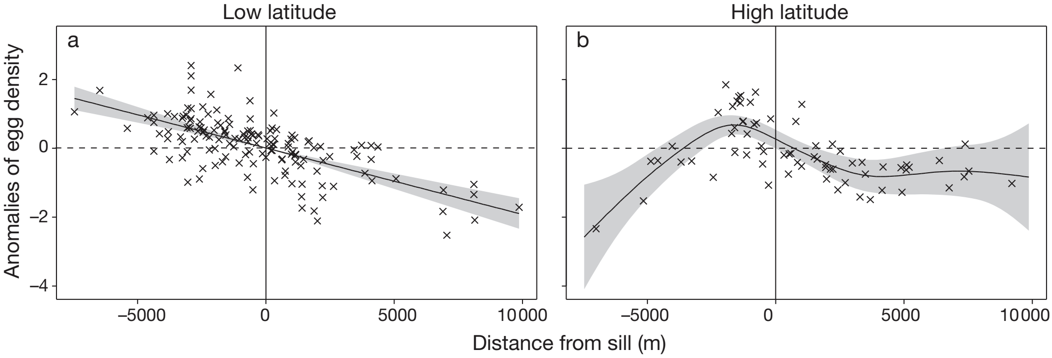

Fig. 3. *Gadus morhua.* Results from the second model formulation from Table 1 (Model 2, geographic effect). Average effect along with the partial residuals of distance from the sill on anomalies of egg density, for fjords located (a) south and (b) north of 60.8° N, respectively. See Fig. 2 legend for further details. Fjords north of the latitude threshold were: 1–8. Fjords south of the latitude threshold were: 9–20

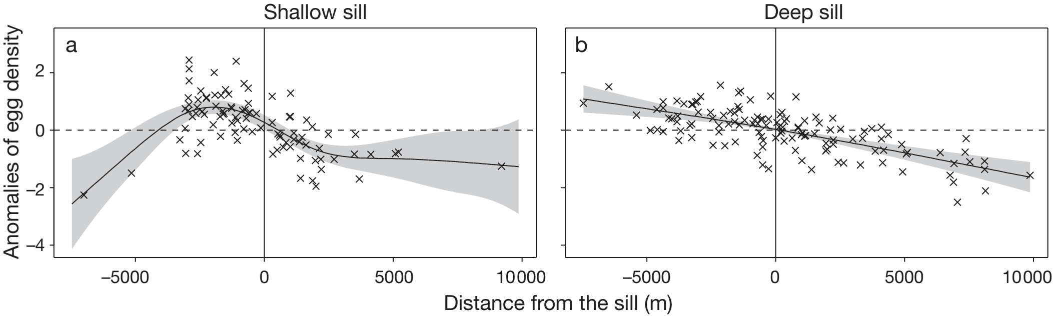

Fig. 4. *Gadus morhua.* Results from the third model formulation from Table 1 (Model 3, geographic effect). Average effect along with the partial residuals of distance from the sill on anomalies of egg density, for fjords having a sill depth (a) shallower or (b) deeper than 37.05 m, respectively. See Fig. 2 legend for further details. Fjords with a sill depth shallower than the threshold were: 1, 2, 7, 8, 10, 18 and 20. Fjords with sill depth deeper than the threshold were: 3, 4, 5, 6, 9, 11, 12, 13, 14, 15, 16, 17 and 19

model formulations and here it is shown only for the null model (Fig. 2). From visual inspection of the residuals, none of the models showed any heteroscedasticity or strong departure from normality.

DISCUSSION

This study demonstrates a pattern with a higher density of pelagic eggs in sheltered fjord habitats along the Norwegian coast, and decreasing levels offshore. The fjord sill, i.e. the bottom topography, plays a significant role in determining the spatial pattern of egg distribution. Higher concentrations of eggs are found inside the sill, where the depth of the sill is also of importance. Fjords with shallow sills (model threshold of 37 m) show an abrupt reduction in egg density over the sill.

A pattern of higher egg densities within fjords could be the results of (1) adult cod selecting sheltered sections of the fjord for spawning and eggs being retained and/or transported further inwards; (2) adult cod spawning outside the fjord and eggs and larvae being transported inside. The typical estuarine circulation found in most Norwegian fjords transports the surface water out the fjords and the water layer beneath the wind-driven water inwards (Farmer et al. 1983). The buoyancy of the eggs of Norwegian coastal cod (Vikebø 2005) indicate that these will mainly stay below the less saline surface waters, although egg buoyancy may vary considerably among local populations and will also depend on egg volume and whether the egg is spawned early or late in the season (Kjesbu et al. 1992). Also, cod eggs tend to occur at greater depths as egg development progresses (Ouellet 1997), which could increase the retention probability of eggs as they may be located deeper than the sill depth. For comparison, spawning upstream of nursery grounds is well documented for oceanic migratory cod (Bergstad et al. 1987), potentially reflecting an adaptive use of ocean hydrodynamics.

We have direct observations of spawners and non-spawners in one fjord, Tvedestrand, where from a total of 314 coastal cod, 77 to 90% were mature inside the fjord, whereas 33% were mature in the outer more exposed part of the fjord (H. Knutsen pers. obs.). Inshore spawning of cod has also been observed elsewhere (Green & Wroblewski 2000). An alternative explanation is that advection from the offshore would facilitate a high egg/larval drift among neighbouring fjords, which is not supported by genetic data which indicates limited contact among fjords (Knutsen et al. 2003).

Although the highest egg densities were consistently found in the inshore sections of the sampled fjords, some eggs where also found offshore. This raises the interesting question of where these offshore eggs originate. No firm conclusion can be reached from the data available in our study; however, it is still worthwhile to discuss some potential mechanisms. The origin of offshore eggs most likely differs for the different regions sampled. In southern Norway, the eggs found offshore may have been transported from the inshore locations due to occasional events of reverse estuarine fjord circulation in the typical fjord circulation. Alternatively, offshore eggs in southern fjords may have originated in the North Sea, having been entrained in the fast coastal flow (Knutsen et al. 2004, Stenseth et al. 2006), or they may have been the result of some limited offshore spawning of costal cod. In northern Norway, the model depicts a slightly different picture in that the egg density stabilises at a plateau outwards, and does not further decrease. This is expected, as oceanic migratory Arcto-Norwegian cod are present in this northern area and spawn in offshore locations. These cod have a life-history strategy of performing long spawning migrations from the Barents Sea and south along the Norwegian coast, where they spawn. Because the eggs float (Sars 1879), they are exposed to the strong ocean currents in this region which transport them towards the nursery areas in the Barents Sea.

We found a consistent picture of an increase in egg density in both very shallow and very deep stations. The depth pattern should be interpreted as a residual effect, after accounting for distance from the sill. Although there is a correlation between distance from the sill and bottom depth (i.e. deeper offshore), not all fjords have the same length–depth profile. Hence, the depth variable still conveys important information in the analysis. We interpret the higher egg density in shallow and deep stations as a result of higher than average spawning activity of either the coastal (in shallow stations) or the offshore cod populations (in deep stations), respectively. For stations <50 m deep there could also be a direct sampling effect. This is because egg density was pooled for the upper 50 m of the water column while the distribution of eggs is unlikely to be homogeneous within this column. If the eggs are concentrated in the upper parts of the water column, then the density of eggs (given as eggs m^{-1} haul) will tend to be higher for shallow stations where <50 m of water could be sampled.

In most fjords, egg sampling was limited to one spawning season; however, both abiotic and biotic conditions vary among years. Nevertheless, the general pattern of egg distribution was visible throughout a large geographic area, covering major parts of the Norwegian coast, suggesting that the biological signal we describe is an important one. The egg samples from a second spawning season from 2 of the fjords also supported the general picture of decreasing egg density along an inshore offshore transect.

In conclusion, we find a pattern with higher density of cod eggs within sheltered fjord basins in keeping with the previously detected genetic structure of adult individuals (Knutsen et al. 2003). The genetic structure in Norwegian coastal cod among fjords is small, but significant, meaning that demographically more or less independent populations exist. In order to get such a genetic effect, egg/larval drift among fjords is most likely also impeded, as only a small level of gene flow would erase such a genetic pattern (cf. Waples 1998). This is not to say that egg/larval drift among fjord does not occur, only that there are strong natural forces acting to retain the early life stages. The present study thus provides evidence for one mechanism which can generate a small-scale population structure, even in temperate species with a long early-life drift phase. However, more data is still needed to determine the origin of the genetic structure in Norwegian coastal cod. We need to learn more about egg distribution in relation to local currents throughout the spawning season. Furthermore, genetic or otolith microchemistry analyses on egg and larval samples from different locations along the fjord will help untangle questions about the spawning origin of these specimens. Finally, the fate (i.e. survival) of the eggs and larvae found inshore compared to those found further offshore is still unknown.

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III

Spatial scale of genetic structuring in coastal cod Gadus morhua and geographic extent of local populations.
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Spatial scale of genetic structuring in coastal cod *Gadus morhua* and geographic extent of local populations

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ABSTRACT: We estimated the spatial scale of genetically distinguishable populations of coastal Atlantic cod *Gadus morhua* using microsatellite DNA markers. Significant overall heterogeneity in allele frequencies was found among 5 cod samples (n = 493) along a 79 km segment of the Norwegian Skagerrak coast ($F_{ST} = 0.0013$; p = 0.021). Most (3 out of 4) samples separated by less than 30 km were genetically highly similar ($F_{ST} < 0$), whereas more distantly separated samples were typically genetically different. This genetic differentiation pattern indicates a patchy population structure with local coastal cod populations being limited in geographic extent to approx. 30 km or less. The spatial structure is thus on the scale of local fjords, suggesting a role for local topography in shaping population structure. The population structuring of coastal cod is more fine-scaled than hitherto reported, but is consistent with mark–recapture studies and data on egg distributions, and emphasises the need to focus on local populations in the management of marine fishes.

KEY WORDS: Spatial scale · Coastal Atlantic cod · *Gadus morhua* · Local populations · Genetic differentiation · Microsatellites

INTRODUCTION

Structuring of species into local populations and the spatial scale of such structures have important implications for demography and population dynamics, ecological interactions, and evolution of the species. Spatial substructuring implies that the local breeding unit, or population, is numerically smaller than the total number of specimens in the general area may suggest. Stochastic processes in demography and genetics (random genetic drift: Wright 1978 and references therein) then assume more important roles than if the species consisted of a larger, panmictic unit. Reduction in the geographic extent of the habitat patch, or home range, also influences population dynamics because of the increased perimeter to interior area ratio, and there may exist a critical smallest patch area below which populations cannot be maintained (Holmes et al. 1994). Spatial substructuring further alters the stability of species interactions and opportunities for coexistence in both predator–prey and competitive systems (Kareiva et al. 1990). Spatial processes may significantly modify the dynamics of local populations by individual dispersal, by community processes and trophic interactions, and by spatially correlated factors (Bjørnstad et al. 1999). Such spatial processes are scale-dependent, and the issue of spatial scale is central to many contemporary topics in ecology and evolution.

The prevalence and importance of spatial structuring have also become increasingly apparent for marine organisms, as more powerful methods have been developed and applied to detect such structure. In the Atlantic cod *Gadus morhua*, early genetic investigations uncovered a weak, but large-scale, substructuring spanning thousands of kilometres and depicting an apparently linear increase in genetic differentiation with increasing distance (Mork et al. 1985). Later large-scale studies confirmed and expanded on those find-

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ings, employing different genetic markers such as cDNA RFLP patterns (Pogson et al. 1995), microsatellite DNA polymorphisms (Pogson et al. 2001), and mitochondrial DNA sequence variation (Árnason 2004). The quantitatively weak structure and the apparent linear pattern by which genetic differentiation increases with increasing distance suggest (cf. Rousset 1997) that gene flow is prevalent in this species and that conspecifics are isolated primarily by geographic distance (but see Pogson et al. 2001 and Árnason 2004 for alternative explanations). The aforementioned genetic studies were all based on samples that were collected from a large geographic area, typically covering several thousand kilometers, and the observed genetic differentiation pattern at that spatial scale yields little information on the issue of local populations and their structuring.

In more or less continuous habitats characterizing parts of a species' range, the extent of local populations may be defined as the area over which local recruitment dominates over external sources. In Atlantic cod, as for many other marine species with a pelagic larval phase, the traditional view has been that external sources dominate throughout, also in coastal areas, due to larval drift with ocean currents (Hjort 1914, Caley et al. 1996). This view is challenged by recent findings of genetic substructuring (implying distinct local populations) in coastal cod, occurring on a spatial scale of a few hundreds of kilometers (Ruzzante et al. 2000, 2001) or less (Knutsen et al. 2003). Contrary to the pattern of isolation by distance that characterizes large-scale structures, no evidence has been found for the occurrence of increased genetic differentiation with increasing distance at this finer spatial scale (Fig. 1; data from Knutsen et al. 2003). The absence of an isolation-by-distance pattern in this area, consisting of the Norwegian Skagerrak coast (see Fig. 2), is probably caused by larval drift from off-shore spawning grounds in the North Sea. Such larval drift has recently been detected directly, by observation of juvenile (0-group) cod genetically assigned to North Sea spawning samples (Knutsen et al. 2004), and indirectly, by its significant effect on juvenile coastal cod abundance in time series data from the Norwegian Skagerrak coast (Stenseth et al. 2006). Larval drift, if it produces successful recruits to the recipient coastal populations, represents gene flow that is expected to counteract the build-up of genetic differentiation among populations. The fact that we nevertheless do find detectable, albeit low, levels of genetic differentiation, shows that the coastal populations are not entirely swamped by gene flow. This observation suggests the existence of a spatial domain wherein local recruitment dominates, allowing some differentiation among local gene pools. The present study sought, by utilizing and expanding on the material of Knutsen et al. (2003), to test this hypothesis and to estimate the geographic extent of local coastal cod populations.

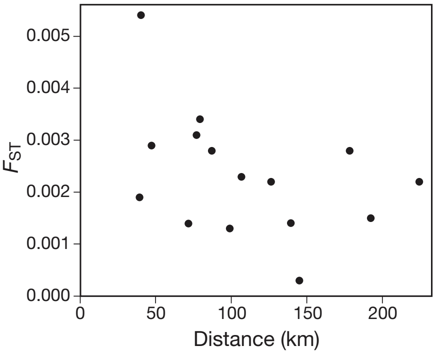

Fig. 1. *Gadus morhua*. Spatial pattern of genetic differentiation (F_{ST}) among Skagerrak coastal cod reported by Knutsen et al. (2003; data based on 10 microsatellite loci from their Table 4). Note that pairwise estimates are low, but consistently >0 throughout this spatial range

MATERIALS AND METHODS

Our study area was the Norwegian Skagerrak coastal range (Fig. 2) previously studied by Knutsen et al. (2003) and shown to harbour multiple cod popula-

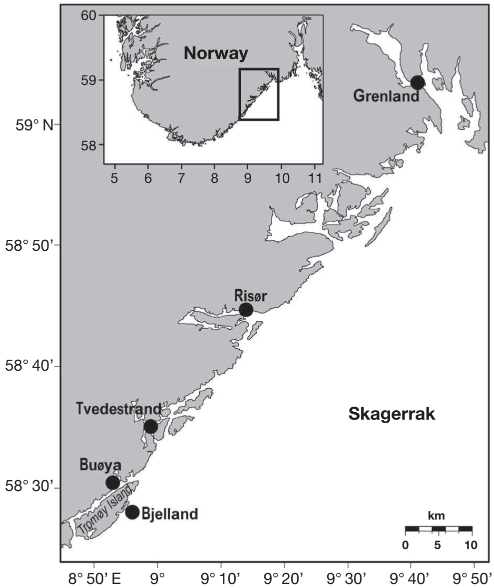

Fig. 2. Sampling area. (●) Sampling sites; inset: southern Norway, sampling area boxed

tions. The coastline consists of numerous small islands and skerries with small fjords or fjord-like basins extending up to a few km inland. Because Knutsen et al.'s (2003) study indicated that the level of local genetic differentiation was the same along the coast, we picked a sub-area for the present study that was conveniently situated and readily available for sampling. The sub-area comprised a 79 km segment of the coast that included 3 previous sample localities (Bjelland, Risør and Grenland) reported by Knutsen et al. in 2003. We expanded the sampling effort by including 2 additional localities (Buøya and Tvedestrand), situated among the 3 earlier ones (Fig. 2). The 5 sampling localities were separated by 8 to 79 km (measured as the shortest straight line between them) and represent a finer geographic scale than hitherto available for genetic analyses in coastal Atlantic cod. From each of the 5 localities about 100 adult Atlantic cod were sampled with gill nets, near presumed spawning sites in or near fjords. Sampling took place during the spawning season, in January to March, and was carried out using 3 to 4 gill nets (150 m each) over approximately 1 wk until the required sample size was obtained (see Table 1).

All sampled cod were sexed by visual examination of the gonads, and assigned to a 5-point index of sexual maturity: cod were defined as mature if the gonads where either growing (Maturity Stage 2), ripe (Stage 3), or if the fish was spawning (Stage 4) or spent (Stage 5). The age was estimated for each individual from the number of hyaline zones deposited in the otoliths in late winter or spring, as observed under the microscope. White skeletal muscle was collected from each individual and stored in 96% ethanol until DNA extraction and genetic analyses.

DNA was extracted from muscle tissue using the DNEASY kit (QIAGEN). The previous analyses of Knutsen et al. (2003) were based on 10 microsatellite loci. We expanded the number of loci to improve the statistical power to discriminate among populations, and included an additional 3 loci (Gmo8, Tch5, and Tch22) that have proved useful in other studies of Atlantic cod (e.g. Beacham et al. 2002, Hardie et al. 2006). Hence, a total of 13 microsatellite loci were amplified from extracted genomic DNA and screened for genetic variability, using PCR conditions that were slightly modified from those published (by excluding bovine serum albumin and by using a different brand of *Taq* polymerase and buffer [QIAGEN]): Gmo2 and Gmo132 (Brooker et al. 1994); Gmo3, Gmo8, Gmo19, Gmo34, Gmo35, Gmo36, and Gmo37 (Miller et al. 2000); Tch5, Tch12, Tch13, and Tch22 (O'Reilly et al. 2000). Microsatellite DNA fragments were separated and sized on an ALFexpress II automatic sequencer (Amersham Pharmacia Biotech).

Allele and genotype frequencies were estimated from genotype counts in the samples. Deviations from Hardy-Weinberg genotype proportions were quantified by F_{IS} (estimator f of Weir & Cockerham 1984), and tested for using exact probability tests summed over loci using Fisher's summation procedure (GENEPOP software Version 3.3d, Raymond & Rousset 1995). Amounts of genetic variation were characterised by average gene diversities within samples (H_S) and in the combined material (H_T) (Nei & Chesser 1983). Genetic differentiation among samples was quantified by F_{ST}, using estimator θ of Weir & Cockerham (1984), and tested with an exact test for allele frequency heterogeneity among samples in GENEPOP. The joint null-hypothesis of no differentiation at any locus was tested by combining the single-locus p-values by Fisher's summation procedure, i.e. summing twice the negative logarithms of the single-locus p-values, as recommended by Ryman & Jorde (2001). As an additional test for spatial differentiation we applied a permutation test for F_{ST}, using the GENETIX software (Belkhir et al. 2002) with 10 000 permutations. The permutation test was carried out both for each locus separately and over all 13 loci jointly. Finally, each fish was assigned to the most likely geographic origin (sample location) on the basis of its genotype and the spatial distribution of the other genotypes, using an assignment test in the WHICHRUN software (Banks & Eichert 2000). In this assignment analysis the fish being assigned was not included in the base line data, i.e. we used the 'leave-one-out' procedure.

Possible relationships between genetic differentiation and geographic distance were tested by estimating F_{ST} (Weir & Cockerham 1984) between pairs of samples and regressing these pair-wise $F_{ST}/(1–F_{ST})$ values against shortest linear geographic distance between samples (Rousset 1997). Confidence limits for F_{ST} were calculated by bootstrapping loci (10 000 replicates), using the GDA software (Lewis & Zaykin 2001). An AMOVA test (Excoffier et al. 1992) in the Arlequin software (Schneider et al. 1997) was used to decompose genetic variability into 2 components: within and among cohorts, respectively. This test, which was carried out in order to check temporal stability in the spatial genetic structure, utilised the fact that the cod samples included several different year classes or cohorts. However, some cohorts were represented by very few individuals in each sample (range 1 to 67 ind.) and we restricted the analyses to cohorts with at least 15 fish. Applying stricter statistical criteria and increasing this lower limit would yield too few cohorts for temporal comparisons in most samples. In addition to the AMOVA test we also estimated F_{ST} pair-wise among cohorts within the sample localities, and among localities for the same cohort, in order to separate and compare temporal and spatial genetic variability components. In this latter analysis we included cohorts with at least 10 ind. (rather than 15) to permit comparison of at least 1 cohort from each geographic sample (because the samples had somewhat different cohort compositions). All pair-wise comparisons were tested with an exact test for allele frequency differences, using the GENEPOP software.

RESULTS

The total sample comprised 493 fish from 5 different localities and scored at 13 microsatellite loci. The amount of genetic variability was very similar among sites (H_S: Table 1); however, individual loci differed greatly in variability (Table 2), with H_T ranging from 0.181 (Gmo3) to 0.927 (Tch5) and the total number of observed alleles ranged from 4 (Gmo36) to 49 (Gmo8).

The genotypic proportions within samples generally conformed to Hardy-Weinberg expectations (cf. Table 1). Without correcting for multiple tests there were apparently significant heterozygote deficiencies in the Risør sample at loci Gmo8, Gmo19, and Tch22 (each significant at the 5% level), in Buøya at Gmo36 ($p < 0.01$), and in Tvedestrand at Gmo37 ($p < 0.05$). A significant heterozygote excess was observed at Locus Gmo2 in the Risør sample ($p < 0.05$). Confounding factors such as segregation of null-alleles are often invoked as explanations for apparent heterozygote deficiencies in microsatellites (e.g. Pemberton et al. 1995), but were unlikely in the present case because all samples had quite similar allele frequencies, as reflected in the low (but significant: see below, this section) F_{ST}-values. Hence, if present, null-alleles should segregate at the same locus and affect all samples to a similar extent, which was not the case. Likewise, we tentatively ruled out potential external forces such as natural selection—directly or acting through hitchhiking—because such effects should again be limited to particular loci (Gmo132 has been suspected; Nielsen et al. 2006). Summing over all 13 loci, no sample deviated significantly from Hardy-Weinberg expectations.

There were statistically significant differences in allele frequencies among the 5 samples (Table 2), with an average F_{ST} = 0.0013 over loci. The permutation test and the exact test both rejected the null hypothesis of no differentiation at 2 loci each, although there was some difference between the tests with regard to which loci were significantly different (Gmo36 and Gmo132 in the permutation test versus Gmo34 and Gmo36 in the exact test). Nearly significant genetic

Table 2. *Gadus morhua*. Genetic variability at individual microsatellite loci, with observed number of alleles (*a*), mean gene diversity (H_T), and amount of variation among populations (F_{ST}) for each locus and averaged over all 13 loci. The null hypothesis (F_{ST} = 0) was tested with a permutation test (p-perm) and with an exact test for allele frequency heterogeneity (p-exact). **Bold**: significant

Locus	Within total		Between localities		
	a	H_T	F_{ST}	p-perm	p-exact
Gmo2	20	0.852	0.0000	0.456	0.184
Gmo3	8	0.181	0.0014	0.231	0.185
Gmo8	49	0.878	0.0011	0.082	0.161
Gmo19	24	0.913	0.0013	0.131	0.263
Gmo34	9	0.619	0.0028	0.100	**0.039**
Gmo35	9	0.830	0.0020	0.111	0.088
Gmo36	4	0.502	0.0078	**0.032**	**0.048**
Gmo37	20	0.843	0.0022	0.078	0.159
Gmo132	33	0.910	0.0028	**0.018**	0.205
Tch5	32	0.927	0.0005	0.266	0.136
Tch12	15	0.290	−0.0003	0.452	0.541
Tch13	37	0.920	−0.0009	0.794	0.914
Tch22	8	0.597	−0.0023	0.791	0.694
Mean	20.6	0.712	0.0013	**0.003**	**0.021**
SE	3.8	0.070	0.0007		

Table 1. *Gadus morhua*. Sampled localities, no. of ind., percentage of mature ind., estimates of genetic variability (H_S), and deviations from Hardy-Weinberg genotype proportions within samples (F_{IS}). p: results of exact probability tests (GENEPOP: using 10 000 dememorisation steps, 1000 batches, and 10 000 iterations per batch, and averaged over 5 replicate runs), carried out for each locus and summed over loci using Fisher's summation procedure. Loci with significant (not corrected for multiple tests) deficiencies or excesses of heterozygotes are indicated (*$p < 0.05$; **$p < 0.01$)

Locality	n	% Mature	Avg H_S	Deviations from Hardy-Weinberg proportions			
				Avg F_{IS}	p	Deficiencies (F_{IS} pos.)	Excesses (F_{IS} neg.)
Bjelland	92	46.7	0.716	0.027	0.16		
Buøya	100	78.0	0.709	−0.025	0.26	Gmo36**	
Tvedestrand	100	84.0	0.716	0.008	0.24	Gmo37*	
Risør	101	57.5	0.720	0.014	0.07	Gmo19* Tch22* Gmo8*	Gmo2*
Grenland	100	64.0	0.698	0.013	0.26		
Avg	98	66.0	0.712	0.007			
SE			0.004	0.009			

differences ($p < 0.10$) were observed at 3 other loci (Gmo 8, Gmo35, and Gmo37; Table 2). When evaluating the joint null hypothesis of no differentiation at any locus, both the permutation and the exact tests yielded significant results ($p = 0.003$ and 0.021, respectively; Table 2). The results of the assignment test (Fig. 3) were consistent with the results of the heterogeneity tests and assigned a much higher fraction of individuals to the locality at which they were sampled (range 51 to 70%) than to any other site (range 4 to 14%). Hence, genetic differentiation prevails even at a spatial scale <79 km in coastal Atlantic cod.

In view of the small amount of differentiation among the samples ($F_{ST} = 0.0013$), there is the possibility that observed differentiation was confounded by temporal genetic change within sample localities, i.e. by differences among age classes or cohorts (Waples 1998). Temporal fluctuations in allele frequency occur in all finite populations because of random genetic drift, generating some allele frequency differences among cohorts within the population (Jorde & Ryman 1996). When the samples do not have exactly the same age composition the observed differences among samples will include a temporal component that may be mistaken for spatial differentiation. In the present case, however, it was possible to measure the temporal component directly, utilizing age information to classify each fish to a birth cohort. Applying AMOVA to the cohort data yielded a significant, positive estimate of $F_{ST} = 0.0017$ among geographic samples ($p = 0.023$, based on 10 000 permutations), whereas the estimate between cohorts within samples was negative (–0.0021) and not significantly larger than zero. The pair-wise F_{ST} (Table 3) added more details to this picture and uncovered predominantly positive estimates of differentiation among cohorts (Table 3: along diagonal), indicating some temporal change within samples. The estimated magnitude of this change (0.0023 averaged over pair-wise estimates) was, however, not significant within any locality (exact test, p-values ranging from 0.18 in Grenland to 0.97 in Buøya), nor for the average over samples ($p = 0.14$). Further, this temporal component was on average considerably smaller than that estimated within cohorts among *different* samples (Table 3: below diagonal; average $F_{ST} = 0.0041$). Several of these latter pair-wise tests for spatial differentiation were significant (Table 3: below diagonal; 7 out of 10 tests were significant at the 5% level or better). Hence, both the results of the AMOVA and the pair-wise F_{ST} analyses of cohorts demonstrate that the temporal component to the observed genetic differentiation in this study was smaller than the spatial component, and the observed spatial differentiation therefore cannot be explained by temporal change alone.

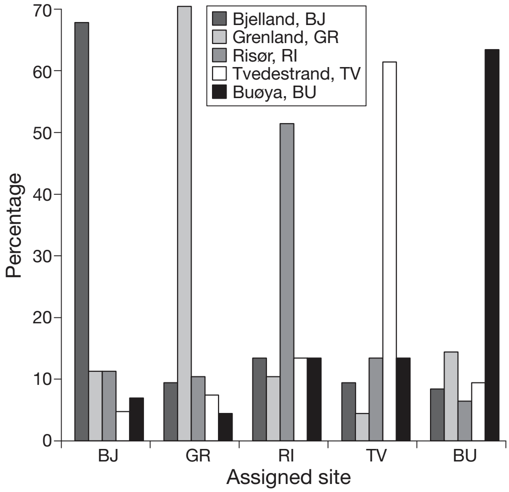

Fig. 3. *Gadus morhua*. Distribution of statistical assignment of sampled individuals based on genotypes at 13 microsatellite loci, showing percentage of individuals assigned to each sampling site. For all sampled localities a much higher fraction of individuals (51 to 70%) was assigned to site of capture than to any other site (4 to 14%)

In view of the above findings, demonstrating that the observed genetic differentiation cannot be explained by temporal fluctuations among age classes, the following analyses utilised whole samples. Combining all age classes within samples we found that several sample-pair comparisons were statistically significant

Table 3. *Gadus morhua*. Mean pair-wise F_{ST} estimated from 13 microsatellite loci, among and within year classes (cohorts). Above diagonal: comparison of different cohorts in different geographic samples (avg. $F_{ST} = 0.0042$); along diagonal (**bold**): comparison of different cohorts within samples (i.e. temporal component, average $F_{ST} = 0.0023$); below diagonal: comparison of different geographic samples within same cohorts (i.e. spatial component, average $F_{ST} = 0.0041$). Only year classes with ≥10 ind. are included in the table. *p < 0.05; **p < 0.01 based on exact tests of allele frequency heterogeneity

	Bjelland (3)	Buøya (3)	Tvedestrand (3)	Risør (4)	Grenland (3)
Bjelland	**0.0036**	0.0050*	0.0010	0.0013*	0.0062
Buøya	0.0010*	**–0.0003**	0.0030*	0.0069**	0.0002
Tvedestrand	0.0024	0.0041*	**0.0034**	0.0023	0.0046*
Risør	0.0068*	0.0111**	0.0020	**0.0013**	0.0094**
Grenland	0.0034*	0.0006	0.0039*	0.0106**	**0.0047**

Table 4. *Gadus morhua*. F_{ST} values among pairs of samples (below diagonal) and exact tests for differentiation (p, above diagonal). Parentheses: sample size (all age classes combined). **Bold**: significant

	Bjelland (92)	Buøya (100)	Tvedestrand (100)	Risør (101)	Grenland (100)
Bjelland	–	**0.050**	0.687	0.250	0.070
Buøya	0.0031	–	0.247	**0.007**	0.139
Tvedestrand	−0.0003	−0.0001	–	0.452	0.210
Risør	0.0016	0.0030	−0.0008	–	**<0.001**
Grenland	0.0020	0.0000	0.0008	0.0038	–

from each other (Table 4). The linear regression of pair-wise F_{ST} values against geographic distance was close to zero (b = 0.000014), and not significant. However, there was an apparent pattern, with most (3 out of 4) closely situated sample pairs (separated <30 km apart) being genetically very similar (estimated $F_{ST} < 0$ among them), whereas more distant pairs had positive F_{ST} and were thus somewhat differentiated (Fig. 4). There were 2 exceptions to this general pattern (Fig. 4; Table 4): the closely situated sample pair Bjelland and Buøya (8 km apart) were significantly different from one another, whereas the more distantly separated (at 72 km) pair Buøya and Grenland apparently were not.

DISCUSSION

Genetic methods are highly appropriate for delineating local populations because each generation's genes are transferred to the next generation, resulting in higher genetic similarity within the population boundary if members are predominantly recruited from local offspring. Herein, we have exploited this principle to tentatively delineate local populations of Atlantic cod along a short segment of coastline. Delineating local populations of marine species has typically been a difficult task because of the weak genetic differentiation characterizing many marine organisms (Ward et al. 1994, Waples 1998). Genetic differentiation is indeed weak among the present Atlantic cod populations also, and our success in detecting differences at this spatial scale can be ascribed to the high statistical power resulting from employing relatively large numbers of microsatellites and sampled fish (see Ryman et al. 2006, Waples & Gaggiotti 2006). As a consequence of the low level of differentiation, the point estimates (F_{ST}) are uncertain, as indicated by their wide CI (Fig. 4), and we would therefore expect that some pairwise comparisons would, by chance, be significant whereas others would not. The non-positive F_{ST} estimate between Buøya and Grenland, 72 km apart, most likely reflects such stochastic errors or 'noise', because other explanations seem to be biologically less plausible. Apart from this single pair, the tendency was for the more distant samples (>30 km apart) to be significantly differentiated genetically, reiterating our earlier findings (Knutsen et al. 2003 and present Fig. 1) and confirming the existence of multiple local cod populations along the Norwegian Skagerrak coast. This conclusion is strengthened by our demonstration herein that the observed differences among sample localities cannot be explained by genetic differences among age classes, and thus represent real spatial substructuring.

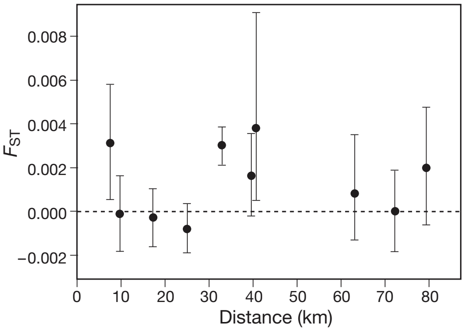

Fig. 4. *Gadus morhua*. Mean genetic differentiation (F_{ST}, averaged over 13 microsatellite loci: Table 4) among pairs of cod samples as a function of (straight-line) geographic separation. Vertical bars: 95% CI for estimated F_{ST}, calculated by bootstrapping individual loci

Among samples that were collected <30 km apart we found no consistent genetic differentiation. On the contrary, 3 of the 4 pairwise comparisons below that distance yielded zero or negative estimates of F_{ST} and only 1 was positive and significant (between Bjelland and Buøya, 8 km apart). Again, statistical noise is a possible explanation for a deviating point estimate, especially since the p-value in question was very close to the nominal alpha level of 5% (Table 4). On the other hand, it is also possible that the Bjelland sample (situated outside Tromøy Island; Fig. 2), is partly isolated from its neighbours and that Tromøy Island acts as a 'barrier' to free exchange between the 2 localities. Bathymetric and hydrodynamic structures have been shown to represent such barriers in Atlantic cod, e.g. between the Georges Bank and Browns Bank, about 80 km apart (Ruzzante et al. 1998) and over a saline gradient (Nielsen et al. 2003). It may also be of relevance that Bjelland was sampled further outside the fjords than were the other samples (perhaps outside the spawning area, as suggested by recent egg-density counts; Knutsen et al. 2007). This sample also contained a lower percentage of mature fish (<50%: Table 1), and it is unclear to what extent it represented a spawning population. The assignment analysis (Fig. 3), (which showed consistently higher assignment to capture site than to any other locality, including neighbouring sites), indicated that all 5 samples might have represented (slightly) differentiated populations. A similar interpretation seems reasonable from the results of the spatio-temporal analyses (Table 3 and AMOVA), which did not uncover any tendency for neighbouring samples to vary temporally in a concerted fashion, as might be expected were they drawn from the same biological population (e.g. Jorde & Ryman [1996] found highly correlated temporal allele frequency changes in brown trout from 2 interconnected lakes). Hence, there is a possibility for differentiation at an even finer geographic scale than the 30 km inferred from Fig. 4.

The observed genetic differentiation pattern indicates a patchy population structure in coastal Atlantic cod, with the geographic extent of the population unit being limited to ≤30 km. Assuming that individual fish for the most part restrict their movements within the population boundary (or 'home range'), we compared our estimate with the large body of traditional capture–mark–recapture (CMR) data that have accumulated on Norwegian Skagerrak coastal cod (Løversen 1946, Moksness & Øiestad 1984, Danielssen & Gjøsæter 1994). We applied the 'minimum convex polygon' method (MCP; Mohr 1947) to estimate area utilization of coastal cod for CMR data from Danielssen & Gjøsæter (1994). The data represent 1624 cod that were captured, marked, and released in the Risør fjord (in the middle of the present study area: Fig. 2) during 1988 and 1989, and yielded a total of 455 recaptures, 95% of which were found within a polygon area estimated to be 123 km^2. This estimate represents the approximate asymptotic value, achieved after the fish had been at sea for about 1 yr (375 d) before recapture, and corresponds to ~13 km of coastline. The estimate is clearly consistent with our present genetically determined size of local populations, and also with the earlier studies (Løversen 1946, Moksness & Øiestad 1984, Danielssen & Gjøsæter 1994) that concluded that coastal cod is 'highly stationary'. Other evidence for a restricted geographic extent of coastal populations of cod is provided by egg density studies. Knutsen et al. (2007) found strong evidence for retention of eggs within separate fjords along the Norwegian coast, in the Skagerrak, and elsewhere. Their findings imply restricted mixing of pelagic eggs and larvae, which should facilitate the build-up of genetic differentiation among coastal populations located in separate fjords. Both our genetic findings and those based on traditional methods are thus congruent in suggesting that coastal cod are structured on a spatial scale of the order of local fjords. This observation suggests that fjords and other coastal structures (bays, islands, etc.) may play an important role in shaping the structure and geographic extent of fish populations in coastal areas.

The broader picture of coastal cod populations and related off-shore breeding stock(s) that emerges from the present and recent studies is that of a complex of geographically restricted coastal populations. Coastal populations in the Skagerrak appear largely self-recruiting, as indicated by their partial (i.e. weak) genetic distinctness, but seem partly open to (and most likely receive) recruits from off-shore sources in the North Sea. An average number of 108 recruits successfully entering each coastal population per generation would be sufficient to explain the observed level of genetic differentiation among cod in the Skagerrak (Stenseth et al. 2006). However, the number of larvae (and recruits) probably fluctuates widely among years, and the inflow of North Sea larvae may be restricted to years when ocean currents during the spawning period are favourable for transportation of larvae into Skagerrak coastal waters (Knutsen et al. 2004). Current research focuses on the temporal aspect of larval drift and on recruitment of cod of North Sea origin to the coastal populations. Beyond its direct impact on the dynamics and ecology of the recipient coastal populations (Stenseth et al. 2006) there are also interesting evolutionary implications for such larval drift (e.g. Strathmann et al. 2002). We are currently approaching these and related questions with a combination of genetic analyses, CMR studies, oceanographic modelling, and time series analyses of juvenile cod abundance along the Skagerrak coast.

Knowledge of the spatial extent of local populations has numerous practical applications, both for research and for management. Such knowledge is instrumental in devising proper sampling strategies for estimating ecological parameters (including rates of reproduction, mortality and dispersal) as well as genetic parameters (including sampling baseline populations for statistical assignment and for mixed fishery analyses). The findings of the present study were directly applied in a recent project involving mathematical modelling of time-series data (Stenseth et al. 2006). Such modelling requires *a priori* partitioning of the sampling 'stations' into population units (Chan et al. 2003), and our present results show that data from quite small segments of a coastline (e.g. single fjords) may be joined in such statistical modelling.

The small geographic extent of local populations in coastal Atlantic cod provides a possible explanation for the strikingly dissimilar pattern of decline in cod abundance characterizing different areas of the Skagerrak coast (cf. Svedäng 2003). With distinct coastal populations and restricted movement among them, as indicated by our findings, there may not be sufficient export of spawners to rebuild overexploited or otherwise depleted cod populations (e.g. Waples 1998). The implication of these observations for the management of Atlantic cod and other marine species is clear and there is an obvious need to focus on local populations (Wroblewski et al. 2005).

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Diel vertical migration patterns in juvenile cod from the Skagerrak coast.
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Diel vertical migration patterns in young Skagerrak coastal cod.

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Abstract

We demonstrate a clear year-round diel vertical migration pattern (DVM) of small (26-45 cm) Skagerrak coastal cod on a small geographic scale. Generalized additive models (GAM) were used to make inferences about depth movements from a large dataset of repeated depth measurements of acoustically tagged cod.

The general pattern in vertical behaviour was closely linked to sunrise and sunset, and the timing varied with the length of the day. The absolute magnitude of vertical movement was seasonally variable and probably influenced by temperature and food availability. Fish constantly selected deeper residence depths during the day than at night. Fish selected deeper residence depths during winter than summer.

This study utilizes flexible models to make generalisation of the varying nature of the diel vertical migration patterns in juvenile coastal cod.

Keywords: Diel vertical migration, DVM, generalized additive models, GAM, coastal cod.

1

Introduction

Diel vertical migration (DVM) is a widespread phenomenon among aquatic animals (Sims et al 2009). Typical DVM behaviour involves a repeated shift between shallow-water occupancy during night and deep-water occupancy during day (Gliwicz 1986). DVM has ecological effects through the interaction between species and through direct physiological consequences for the individual animal. On the ecological scale DVM may affect the ecological impact of one species on others both through competition and/ or predation (Hays 2002). Hunting strategies are often affected by light intensities varying with depth and time of day. Different species may thus experience varying hunting efficiencies and varying predation risks inducing DVM patterns. Furthermore DVM patterns in one species may induce DVM in either their prey or their predators. On the physiological scale the DVM patterns are important for food intake and metabolic processes. This is due both to varying food availability and the associated environmental factors of the different depths (Hays 2002).

Potential non exclusive explanations for DVM as an evolved strategy include (1) a trade-off between food availability and predation risk (Gliwicz 1986), where both are generally more pronounced in shallow water, while predation risk from visually hunting animals is reduced during nighttime. (2) A thermoregulatory strategy allowing ectotherms to lower their metabolic rates in cold deep waters (Wurtsbaugh & Neverman 1988, Sims 1996).

Explanation (1), predicts that animals with high predation risk should ascend to shallow waters to feed at night, while descend to deeper waters during day with low light conditions to reduce predator risk. This predator evasive behavior is a strategy to avoid predators using visual hunting strategies (Gliwicz 1986). Larger predators on the other hand will move vertically to find food and optimize their foraging strategies. DVM in prey species may therefore induce a similar vertical migration in the predator species as a food driven DVM pattern.

Explanation (2) is based on the fact that the metabolic processes of digesting food and growth are affected by temperature (Imsland et al. 2005). Fish can regulate metabolic rate by actively choosing the depth with an optimal ambient temperature (Kristiansen et al. 2001); this “Hunt warm, rest cool” behaviour has been shown in many marine fish species (Sims et al. 2006, Mortensen et al. 2007).

Novel technology in data storage tags (DST) and acoustic transmitters with depth sensors have allowed detailed studies of the vertical positions of marine animals. Given long battery lifetime and good storage capabilities some of these tags may produce 100 000 – 200 000 depth observations from a single fish. This provides, coupled with low cost, the opportunity to acquire large datasets on DVM on aquatic animals. Making inference on “typical” movement, excluding variable individual effects and abnormal behaviour, may be challenging in large datasets. Generalized additive models (GAM) are ideal for describing patterns in biological datasets with complicated structure. Instead of predefining particular linear or nonlinear functional forms, such models “let the data tell” by fitting local piecewise polynomials to the data. This makes them more flexible than parametric models as they do not require any a priori definition of the shape of the relationship between predictors and response (Hastie & Tibshirani 1990).

In this paper we study the diel vertical migration of juvenile cod on the Norwegian Skagerrak coast and evaluate the factors influence vertical movement in coastal cod. We expect the DVM patterns to be affected by the following factors: light intensity, ambient temperature and food availability. These factors exhibit large seasonal variations. We use GAMs as a tool for describing the vertical position of a typical fish and the temperature experienced. Predators may also affect the vertical migration, although numbers of possible predators are few.

Material and methods

DATA

During fall 2006 (Oct. 11. – Nov. 4.), 20 cod (mean length 325) were captured in the Sømskilen area west of Arendal on the Norwegian Skagerrak coast (Figure 1) and brought back to Institute of Marine Research, Flødevigen. The fish were tagged with acoustic transmitters (V9P-2L, Vemco ltd. Shad bay, Nova Scotia, Canada, Lokkeborg 1998, Lindholm & Auster 2003, Espeland et al. 2007) and released back in the same area as caught. During April 2007, another 10 cod (mean length 405 mm) were tagged and released in the same area using the same procedure as for the first 20. (For a complete overview of all 30 tagged cod; see Table I.)

Transmitters were 9×30 mm and set to transmit a signal every 20–60 sec, with a random interval to reduce code collision (i.e. two or more fish simultaneously transmitting a signal to the same receiver). The projected battery life of these transmitters was 230 days. The depth of the fish was transmitted along with an identity code for each cod. Transmitters were surgically implanted in the abdominal cavity. Fish were anesthetized in clove oil (King et al. 2005), a small incision was made posterior to the pelvic fins and the transmitter inserted. The wound was closed using absorbable suture material. Fish were kept from 3 to 13 days in captivity prior to release to ensure they recovered from the surgical procedure. All fish were released close to the point they were captured (<100m).

Previous studies have shown that the Skagerrak coast hold several genetically distinct populations of cod (Knutsen et al. 2003, Jorde et al. 2007), where fish generally display a high degree of site fidelity (Espeland et al. 2009). The Skagerrak cod are subject to intensive fishing pressure (Julliard et al. 2001), inducing a skewed age- and size- structure, with few old, large fish and reduced phenotypic diversity (Olsen et al. 2004, 2009). The Skagerrak cod reach maturity at the age of 2-4 years (Olsen et al. 2004). Due to their relatively small body size, we expect the fish in our study to be immature (see Olsen et al. 2008), so they will not display any spawning behaviour.

The area of Sømskilen, where all fish were released, is a semi-sheltered basin with several small islands and rocks (Figure 1). The surface area is approximately 3 km^2, excluding permanently dry land. Sømskilen is influenced by the river Nidelva, which has one of its outlets in the northern sector of the basin (Figure 1).The freshwater discharge into Sømskilen is variable, but limited to the surface layer. The minimum salinity at 2m depth was 18.8. The water column from 2 to 30m depths had salinities from 18.8 to 37.8 (mean 31.5). The island of Jerkholmen is part of a moraine extending obliquely across the basin from the southwest, at Hasseltangen, towards the northeast (Figure 1). This creates a shallow, but passable, barrier sheltering the area from the open ocean. The south-western part of the basin extends down to 10m depth while the north-eastern parts are somewhat deeper (maximum depth of approx. 30m).

To acquire data regarding temperature and salinity of the area, weekly STD (salinity, temperature, depth) measurements were taken in five different locations (Figure 1) by lowering a mini STD probe. The STD measurements started 21. Dec. 2006 and continued to 17. Nov. 2007.

General habitat category mapping was performed in the area and divided into two sediment types (rocky or fine) and two macro-photosynthetic community types (sparse and lush). The habitat categorization was done based on ROV video film from 20 transects (total length 2.7 km) and 30 fixed stations in a grid net pattern. The upper 1-2 meters were usually rocky habitat inhabited by several macroalgae. The depths between 5 and 3 meters were either rocky or sandy, but usually lush with respect to macro-photosynthetic community. Several large eelgrass beds dominate the shallow (<5-7 m depth) flat areas. Photosynthetic communities became gradually sparser down to 10 meters and were more or less absent further down, so the deeper basins were barren muddy flats.

Ultrasonic receivers (Vemco ltd. VR2) were used to register signals emitted from the ultrasonic transmitters. Two receivers were anchored in the area from 27 Oct. 2006, four more were deployed on 29 Nov. 2006, while a further two were deployed in the area on 13 Feb. 2007. These eight buoys (Figure 1) were anchored with the receiver positioned 1-2 meters below the surface.

Since there were times when many fish were present in a small area, codes had a tendency to collide even though they had a random interval delay. This will sometimes lead to the erroneous recording of a transmitter. Furthermore, some transmitters started to send erroneous signals after an off time. These signals were usually easily detected and removed from the dataset (e.g. signals presenting larger depths than the max depth of the area). Observations were plotted for every fish and inspected for erroneous signals, such as singleton signals with no other signals present for days prior or subsequent.

ANALYSIS

Manual STD measurements resulted in approx. 15000 recordings of temperature salinity and depth. Since changes in temperature through the year was not assumed to be linear in the periods between days of observations, a Generalized Additive Model (GAM) (Hastie & Tibshirani 1990, Hastie et al. 2001) was fitted to the STD data. The model was created using the gam function in the mgcv library (version 1.3-31) in the statistical package R (R-2.7.0: R Development core team 2006). Using this function the optimal degrees of freedom for each smooth function is estimated by minimizing the generalized cross validation score (Wood 2006). The STD model was given by the equation:

$$T_{ilr} = \beta_l + s_1(D_i, P_r) + \varepsilon_{ilr} \quad (1)$$

Predicted temperature (T_{ilr}) at day i, location l (Figure 1) and depth r was determined by a nonlinear interaction effect of day and depth (D_i,P_r) and a location specific intercept β_l. The depth was modelled as a negative number to allow for more intuitive presentation of the data: shallow depths are represented by small numbers and therefore are positioned higher on the vertical scale in figures. The error term ε_{ilr} is assumed to be identically and independently distributed (*iid*). The s_1 is a 2-dimensional smoothing function modelled as a tensor product of two natural cubic spline basis functions (Wood 2006). The *D* and *P* basis functions had 9 and 19 maximal degrees of freedom (df), respectively (i.e., 10 and 20 knots). We allowed for relatively high df in order to be able to capture the steep temperature gradient in the upper part of the water column.

This model explained 96.9 % of the variation in the temperature data. There was generally little difference between sampling locations; only the station closest to the river outlet was significantly different with an effect of -0.06 degrees Celsius.

The dataset of depth observations of all fish, as recorded by the VR2 buoys, consisted of a total of approx. 2.2 million recordings. To reduce the amount of data a 10 min mean depth were calculated for all fish and all time periods the fish were observed resulting in 363 000 mean depths for all fish. The CTD model (1) was used to predict the ambient temperature associated with each mean depth observations for each fish (C_{ijk} in equation 3). Temperatures for the fish were predicted according to the CTD station effect of the station closest to the buoy recording the signal.

In order to obtain a more balanced data set 1000 data points for each fish were randomly selected from the mean depth data set, giving a total dataset of 30 000 recordings for all fish.

To describe the depth movement of fish throughout a year, we constructed a GAM based on the subsampled depth observations dataset. The model was given by the equation:

$$-P_{ijk} = s_1(D_i, M_j) + \beta_k + \varepsilon_{ijk} \qquad \varepsilon_{ijk} \sim N(0, \sigma^2) \qquad (2)$$

-P_{ijk} represents the negative value of the depth of fish k at day i at time j. The β_k is the fish specific intercept. The D_i and M_j are the day and time of day associated with the 10-min mean depth. Day was defined as day in the year (running from 1 [Jan. 1] to 365 [Dec. 31]). The time of day was defined in decimal hours, running from 0 to 23.99. S_1 is a 2-dimensional smoothing function mapping the nonlinear interaction effect of day and time. In this case the smoother was modelled as a tensor product of two cyclic cubic spline basis functions (Hastie & Tibshirani 1990). Cyclic basis functions allowed effects to be continuous at the upper and lower bounds of the covariates. The smoother was modelled with 13 and 12 maximal df for the D and M basis functions, respectively. ε_{ijk} is an *iid* error term. Note that the model did not account for positive temporal autocorrelation. This is not expected to have large influence on the estimation of the mean response, but nominal p-values and confidence intervals tend to be overly optimistic and are therefore not shown. To indicate the representativeness of model predictions compared to among-individual variation in behavior, we plotted model predictions along with observed 10-min mean depths of individual fish at three representative dates (Fig. 3).

To describe the predicted ambient temperature experienced by each fish a GAM of experienced temperature given time at day and day in year was constructed.

$$C_{ijk} = s_1(D_i, M_j) + \beta_k + \varepsilon_{ijk} \quad \varepsilon_{ijk} \sim N(0, \sigma^2) \qquad (3)$$

Here the ambient temperature C_{ijk} for fish k at day i and time j, is given by the fish-specific intercept β_k and the interaction between day (D_i) and time (M_j). The s_1 is the smoothing function modelled as a tensor product of cyclic cubic spline basis functions of D and M (maximal df 13 and 12, respectively). $\varepsilon_{\mathrm{ijk}}$ is an *iid* error term. The predicted ambient temperatures for the fish were compared to the predicted available temperature range from the STD model (1).

All models were computed using University of Oslo's TITAN computer cluster through the freely available bioportal (www.bioportal.uio.no).

Results

All 30 fish recovered from surgery, and were successfully released in the study area. Eight fish were lost during the first 100 days after release (Table I). This could be due to permanent emigration, transmitter failure or unreported harvest mortality. Two fish were declared dead as they ceased to show any vertical activity and were manually tracked to the exact same location on several occasions. Three of the fish were harvested by fishers (reported to us), while one transmitter clearly malfunctioned producing spurious measurements. A total of 12 fish stayed in the area either until the battery expired or the study ended.

Sea surface temperature in the area ranged from nearly 0°C during February, up to 18°C during July (Figure 2). Bottom temperatures (30m depth) varied somewhat less; coldest bottom temperatures occurred late in March (6 - 8°C), whereas warmest bottom temperatures occurred mid August (12 - 14° C). Some periods in spring and autumn exhibited narrow temperature ranges.

Overall, the tagged cod were observed throughout most of the depth range available within the study area. The deepest available habitat (25-30 m) was occupied only during the winter months of January through March. The cod generally stayed in deep layers during day and then ascended to more shallow areas at night (Figure 3 and 4). Furthermore, there were clear seasonal tendencies in the extent and timing of the vertical movements of the 30 fish (Figure 4).

Model (2) describing the 10 min mean depth of a fish as a function of the interaction between time at day (hour) and time at year (day number) explained 57% of the variation and had a GCV of 15.318. Modelling day and time as additive effects instead of an interaction increased the GCV to 19 and only explained 46% of the variation.

Figure 4 shows predicted depth of the fish in response to day and time. During winter (January – February), the day residence depth (given as the area in the middle of the figure above sunrise and below sunset) was much deeper than during early summer (May and June). Also the nightly residence depth varied; with somewhat deeper depths during winter than summer. The period with the deepest overall depths was in the middle of the day during February.

In winter and early spring the ambient temperatures at day were in the warm end of the available temperature range, and higher than the ambient temperatures at night (Figure 5). In late spring and during summer the ambient temperatures at night were higher than the ambient temperatures at day, however both were at the warm end of the available temperature range.

Discussion

In this study we have investigated both depth preferences and ambient temperatures for wild juvenile cod in a semi-sheltered natural basin. The cod displayed a consistent diel vertical migration pattern within a limited geographic area, moving from deeper areas during day to shallow water habitats, such as kelp and eelgrass beds, at night. We discuss potential proximate and ultimate factors influencing this behaviour. Specifically, the habitat use is viewed against temperatures experienced by the fish.

Studies of DVM in cod have been ambiguous and inconclusive. Large regional, seasonal, and individual differences have been found (Righton et al. 2001, Neat et al. 2006, Svedäng et al. 2007). Cod have been found to feed both during the night and the day (Gregory & Anderson 1997, Løkkeborg 1998), and select depths to regulate temperature (Clark & Green 1990, 1991, Righton et al. 2001). Coastal cod in Newfoundland have been shown to hunt in shallow warm water, and rest in cool deep water when the water column is stratified. When the column is isothermic, they consistently prefer shallow, prey-rich waters (Clark & Green 1990, 1991). In the North Sea vertical behaviour has also been found to be closely linked to horizontal movement (Hobson et al 2007, Hobson et al 2009).

In this study we found that the seasonal shift in the timing of DVM was highly correlated with the seasonal shift in sunrise and sunset, i.e., throughout the year, the fish ascended from deep waters close to sunset and descended from shallow waters close to sunrise. Fish may partly select ambient temperature by selecting a given depth. When considering ambient temperature, the temperature at night closely follows the surface temperature, as it is cold during winter and warm during summer (Figure 5).

Previous studies on the feeding ecology of cod in the area indicate regional and seasonal differences in stomach contents. Fish in this size range preferred small fish (mainly gobids) and crustaceans (decapods and isopods) as their primary food source during winter. During spring the proportion of polychaetaes increased to more than 50% with a corresponding reduction in decapods (Hop et al. 1992, Hop et al. 1993). Crustaceans and the small fish are expected to be more numerous in the denser habitat from 10 meters depth and upwards.

Behaviour with regard to vertical position was found to vary between day and night. Vertical position at day showed the largest seasonal fluctuation. The deepest occupied daily depths were found in the period January to March (from 20 to 24m depths). This is the period with the lowest overall temperatures and the fish occupy the warm end of the available temperature range (Figure 5). These depths are lacking macro-photosynthetic communities and only have low prey densities, mainly echinoderms (ophiura) and polychaetes. During the day in May and June the fish usually occupy intermediate depths (8-12 meters). During this period the water column is nearly isothermic. The depths are in the lower part of the depth range for the macro-photosynthetic communities and may contain variable numbers of food items. Later in the summer (August) the fish utilize slightly deeper depths at day. Vertical positions at night had less variation through the year, but were constantly shallower than depths during daylight. The deepest vertical positions during night were found in February and March (10 – 14 meters) while the rest of the year they varied from two to eight meters. These depths represent the middle and the lower zones of the lush coastal macro-photosynthetic communities, usually inhabited by small fish and invertebrates. The temperature experienced by fish during day closely follows the surface temperature except in February and June where the fish may avoid the extreme ends of the temperature range.

From these previous studies on cod feeding and this study it appears that night is the primary foraging period. There may be avoidance of very low temperatures during February as the night foraging depths are from 6 to 12m, instead of the shallower cooler water.

This study suggests that daytime is used for resting, but feeding may also occur especially during spring when cod feed on polychaetes and there is little variation between day and night vertical positions.

Some of these vertical behaviours could be driven by predator risk. Cannibalism is known to play a role in cod biology, however in this area there are few large conspecies. For these fish the most numerous predators would be cormorants and seagulls. However when the cod reach a size of 30-40 cm (as in this study), they are too large to be taken by birds. Also during the period around mid June when the night is short and light, they prefer shallower depths than during the rest of the year. If vertical migration was linked to predator avoidance behaviour, shallow depths would be most dangerous during this period.

Altogether this study suggests that the fine scale diel vertical migration is influenced by food availability and temperature. Night is used as the primary feeding period in unfavourable temperatures at shallow depths. Daytime is used as the resting period in deeper waters. Due to the lack of accurate observation of fish behaviour (resting or feeding) and only on depth we are prevented from drawing strong conclusions on causality of vertical movement. A further extent of this study could be to specifically study feeding in wild (Bestley et al. 2008, Fosette et al. 2008).

We have shown how large datasets on vertical behaviour may be analyzed using generalized additive models. Using these models we specifically demonstrate how the typical pattern in vertical behaviour is closely linked to environmental cues. The timing of the vertical behaviour was closely linked to sunrise and sunset. Still the overall behaviour is flexible and the total depth utilization may be affected by other factors in addition to temperature and food availability.

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Tables

Table I: Information on released data. ID is the transmitter serial number identifying all depth codes. Release date is the date the fish was released after surgery. Length is the total body length measured during surgery. End date was the day when the last valid signal was recorded. Days in sea, was the number of days between release and the last signal. #obs was the number of valid depth measurements made during the period the fish was in sea. #10min were the number of 10 min mean observations calculated from the raw data. The comments on end date give footnotes to the reason why the signal did not record any more signals.

ID	Release Date	Length	End date	Days in sea	# obs.	#10min
233	04.11.2006	315	20.03.2007	108	60541	10380
234	04.11.2006	340	04.04.2007[3]	241	84981	12967
235	04.11.2006	325	28.08.2007[5]	297	193422	28564
236	25.10.2006	350	21.04.2007[1]	179	117629	17614
237	25.10.2006	330	20.07.2007[5]	268	252870	31557
238	25.10.2006	305	25.12.2006[4]	61	19875	2283
239	25.10.2006	295	31.03.2007	157	85146	14704
240	25.10.2006	320	16.11.2007[2]	387	55345	15236
241	25.10.2006	320	05.03.2007	131	19051	6112
242	16.10.2006	375	23.01.2007	99	15549	3762
243	16.10.2006	320	01.01.2007	83	15210	3524
244	16.10.2006	353	21.01.2007	97	47043	8968
245	16.10.2006	319	03.04.2007	169	152479	21195
246	16.10.2006	335	22.01.2007	98	29808	5445
247	16.10.2006	358	11.05.2007[4]	208	96408	18289
248	11.10.2006	265	03.01.2007	79	11086	2497

249	16.10.2006	348	05.03.2007	140	19439	4885
250	11.10.2006	285	17.04.2007[5]	188	72596	15237
251	11.10.2006	298	04.09.2007[5]	328	96946	21255
252	16.10.2006	350	27.04.2007[1]	198	87783	12679
223	27.04.2007	400	16.11.2007[2]	203	18522	3623
224	27.04.2007	440	16.11.2007[2]	203	151492	14415
225	27.04.2007	445	27.10.2007	183	142683	18063
226	27.04.2007	435	22.06.2007[1]	56	107900	8155
227	27.04.2007	435	16.11.2007[2]	203	46583	9203
228	27.04.2007	400	19.08.2007	114	73873	11161
229	27.04.2007	375	30.06.2007	64	63737	5667
230	27.04.2007	355	16.11.2007[2]	203	49414	8009
231	27.04.2007	395	15.07.2007	69	29928	4125
232	27.04.2007	370	05.05.2007	8	13091	1246

Comments: 1: Removed from area by fishermen, 2: Transmitter present in the area at the end of study, 3: Transmitter malfunction after given day, 4: declared dead by given date, 5 battery assumed expired during study.

Figure captions

Figure 1: The study area of Sømskilen. Grey shaded areas represent dryfall (less than 0.5 m depth), while light grey depth contours are 5m, 10m, 15m, 20m, 30m and so on. The Island of Gjervoldsøy splits the river outlet of Nidelva in two. Black circles represent release points for the fish. The serial number of each fish is given. White filled triangles are VR2 receiver buoy positions. White filled squares are stations were salinity, temperature and depth (STD) measures were made weekly. The upper left map indicate where in the Skagerrak region the study area was located.

Figure 2: Modelled temperature throughout the year at different depths. The surface plotted is the interaction effect between depth and day as given by the GAM model (1). Isolines connect day and depth combinations with equal predicted temperature. Moving vertically in the figure describes how temperature changes with depth at a given day. Moving horizontally, describes how temperature at a given depth varies throughout the year. The dotted line indicates 1. January 2007.

Figure 3: Comparison of model predictions and data for three two day periods. One panel is shown for each two day period (early February, mid May, and late August). Points in panels represent 10 minute running mean depths for fish present during the period. The number of fish present varied (14 during 2-3. February, 13 during 9-10. May, 6 during 20-21. August). Red lines represent model predictions for the same fish. The thick black line represent predictions from the average fish (predictions with average fish specific offset). The dotted vertical line indicates time of sunrise, while the thick vertical line indicates sunset.

Figure 4: Depths occupied by a typical fish through the day and year. Contour lines show how predicted depth varies in response to day and time, and represents a 2-dimensional surface estimated by model (2). Moving vertically along the figure, one sees how the occupied depth at a given time of year varies through the day. Fig. 3 shows predictions for the dates represented by the three vertical lines. Moving horizontally, one sees how the occupied depth at a given time at day varies throughout the year. Dark areas in the figure correspond to periods when the fish occupy deep waters and light colours correspond to shallow waters. Red contour lines connect day and time combinations with equal predicted depth. The thick black line indicates time of sunset, while the dotted black line indicates time of sunrise. Depth is given in meters below surface as a negative number. Predictions are shown for a fish with near-average mean depth; predictions for other fish differ by fixed constants.

Figure 5: Predictions of temperature experienced by a typical fish trough the year. The thick black line is the predicted temperature experienced (from model (3)) during night (at 0 h), while the dotted line is the predicted temperature experienced (from model (3)) during day (at 12 h). The two grey lines are maximum and minimum temperatures in the area as predicted by model (1). Since STD measurements were not done a complete year and model (1) do not use a cyclic smoother no estimate is presented for the period from 17. Nov to 21. Dec.

Figure 1:

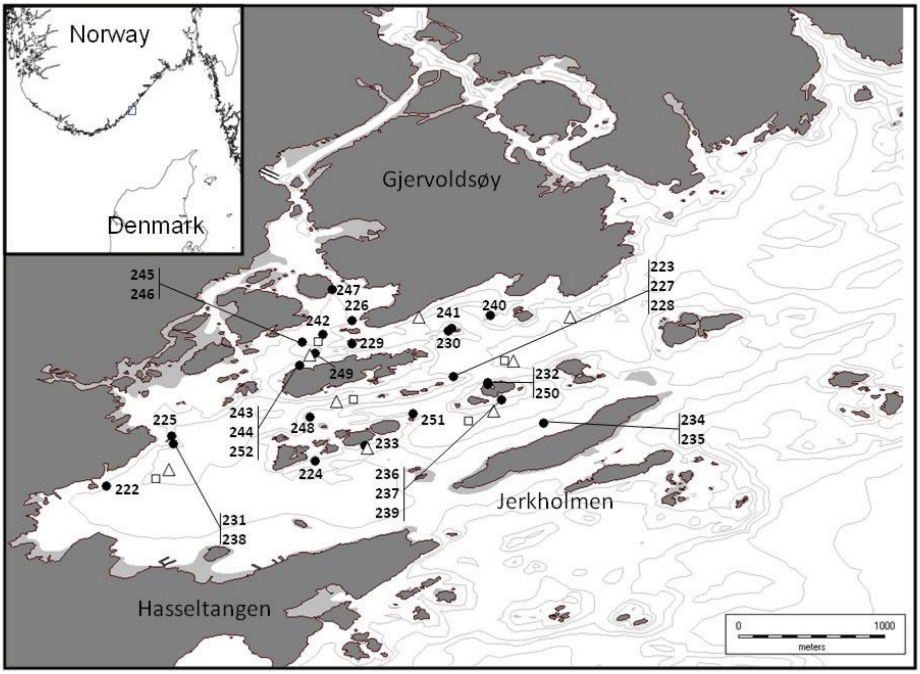

Norway
Denmark
Gjervoldsøy
Jerkholmen
Hasseltangen
245
246
247
226
242
229
249
241
240
230
223
227
228
232
250
243
244
252
248
251
233
224
236
237
239
234
235
225
222
231
238
0
1000
meters

Figure 2:

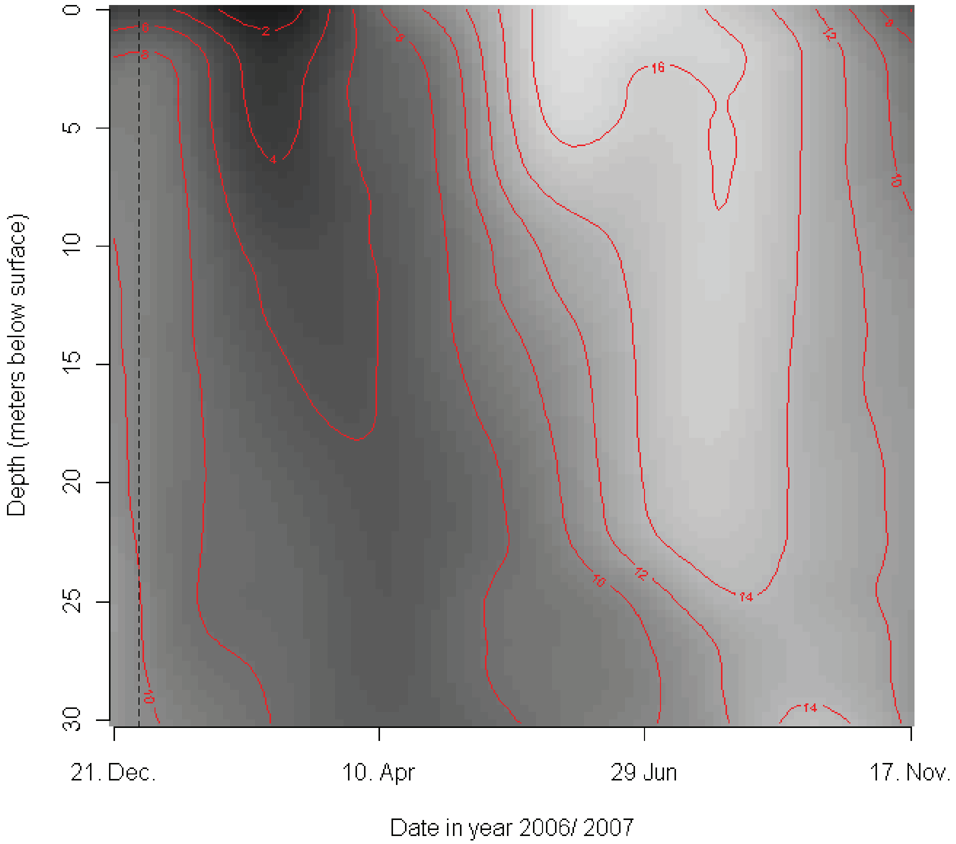

Depth (meters below surface)
0
5
10
15
20
25
30
21. Dec.
10. Apr
29 Jun
17. Nov.
Date in year 2006/ 2007
2
4
6
8
10
12
14
16

Figure 3:

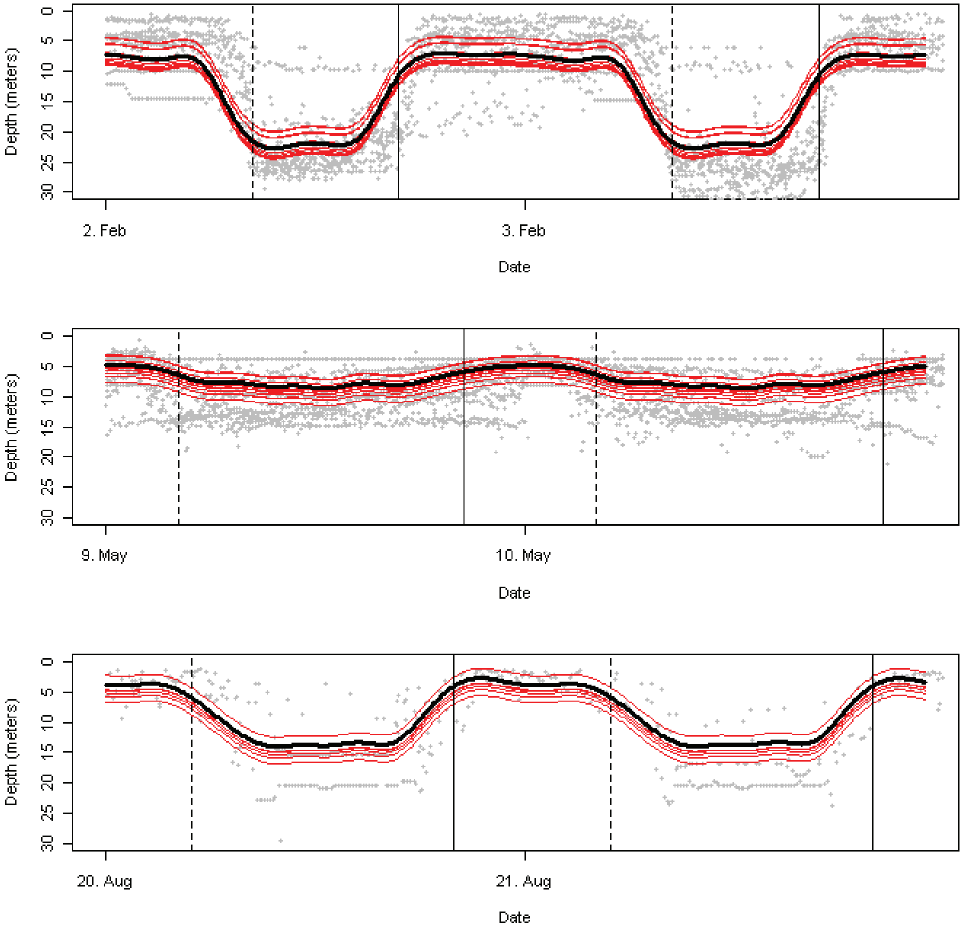

Depth (meters)
0
5
10
15
20
25
30
2. Feb
3. Feb
Date
Depth (meters)
0
5
10
15
20
25
30
9. May
10. May
Date
Depth (meters)
0
5
10
15
20
25
30
20. Aug
21. Aug
Date

Figure 4:

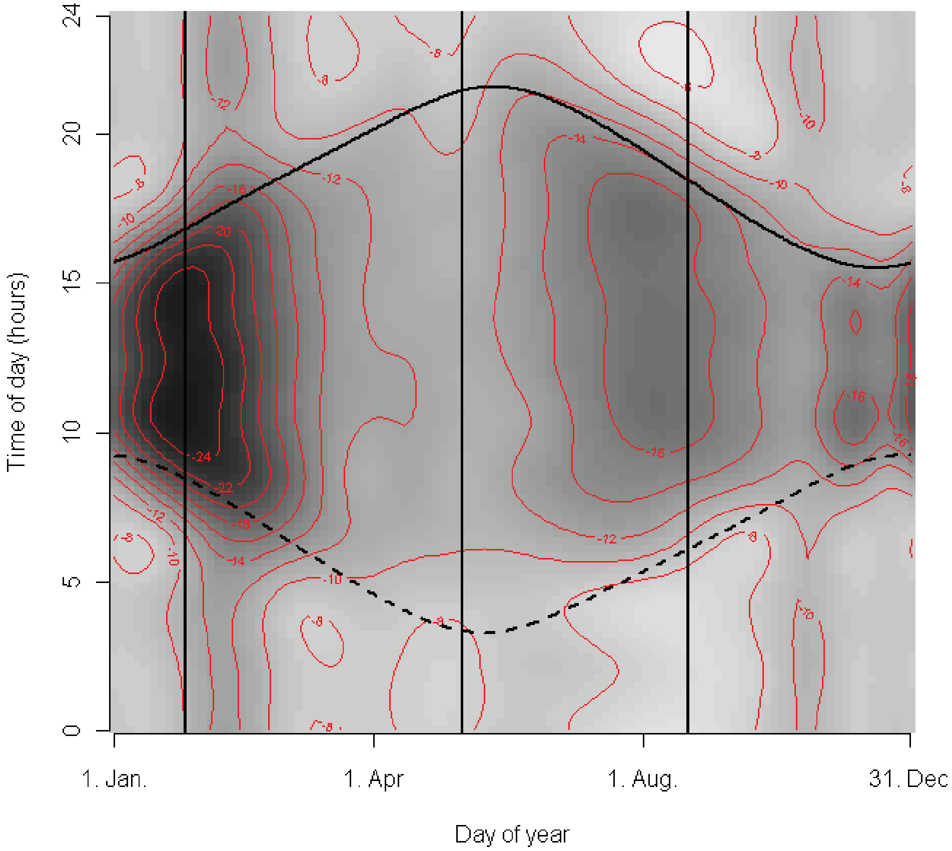
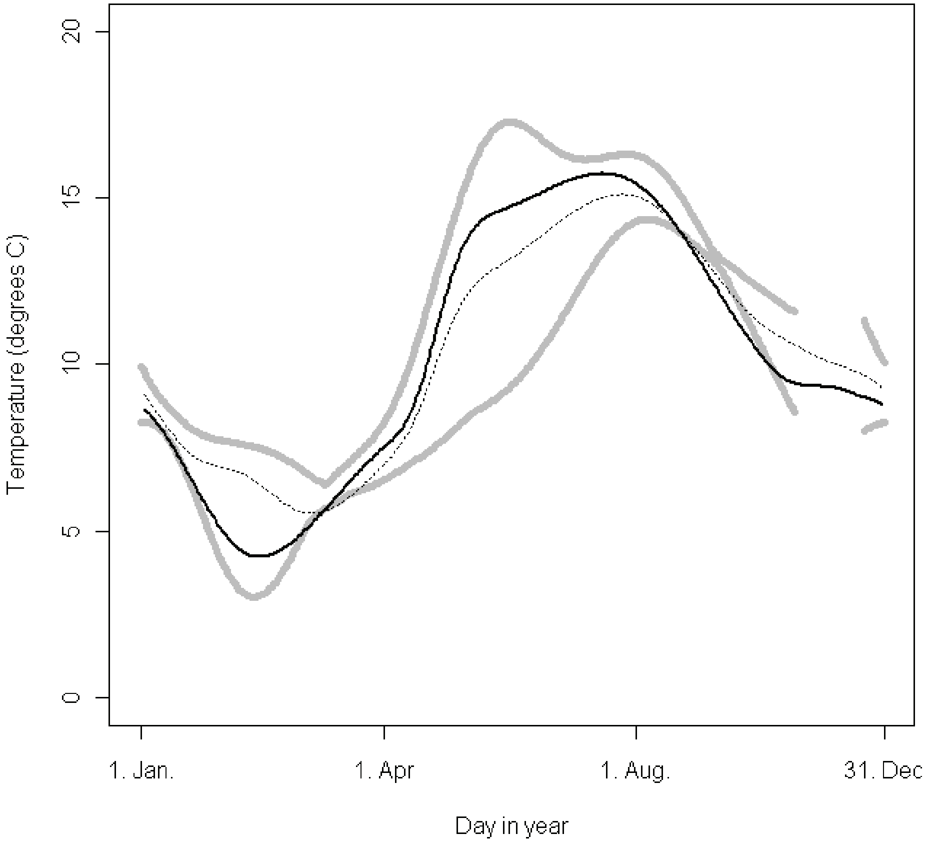

Time of day (hours)
0
5
10
15
20
24
1. Jan.
1. Apr
1. Aug.
31. Dec
Day of year
-8
-10
-12
-14
-16
-18
-20
-22
-24

Figure 5:


20
15
10
5
0
Temperature (degrees C)
1. Jan.
1. Apr
1. Aug.
31. Dec
Day in year