Original Article

"Real-time" genetic monitoring of a commercial fishery on the doorstep of an MPA reveals unique insights into the interaction between coastal and migratory forms of the Atlantic cod

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With the decline of many of the world's fisheries, increased regulation, including marine protected areas (MPA), forms an increasingly important role in promoting sustainable resource use. Here, we present a novel "real-time" genetic monitoring programme used to protect the depleted Norwegian coastal cod stock (NCC) in an MPA during the spawning season, while a fishery targeted at the sustainable Northeast Arctic cod stock (NEAC) operates immediately outside. In the period 2009–2016, >6800 cod from the fishery were genotyped with the *PanI* locus that is discriminatory between these two stocks. The estimated fraction of NEAC increased during the study period until 2014; however, it did not exceed 70% for any sustained period. Therefore, the MPA remained closed for commercial harvest. Genetic analysis of eggs revealed a distinctly lower fraction of NEAC than in the catch from the adult stock, both immediately outside and within the MPA itself. We suggest that this discrepancy is likely to reflect differences in spawning areas used by NCC and NEAC. Estimated fractions of NEAC/NCC using *PanI*, otolith classification, and 39 single nucleotide polymorphisms were similar, thus validating the use of *PanI* to estimate NEAC/NCC composition.

Keywords: conservation, DNA from egg, management units, mixed-stock fishery, population genetics, stock components, Taqman assay.

Introduction

A universal challenge in the effective management of mixed-stock fisheries is to assure sufficient protection to the most vulnerable components of the fishery (Allendorf *et al.*, 2008). One approach is to close the whole fishery as soon as one of the single species annual quotas is reached, although this is rarely implemented as it typically leads to underutilization of the primary target-species. A more demanding approach is to closely monitor the fishery and dynamically redirect the harvest to areas and seasons in a manner that optimizes the match between catch and quotas. However, this becomes a major challenge if the fishery includes two or more stocks of the same species that are morphologically similar or identical.

Diagnostic genetic markers, enabling identification of stocks or management units, provide us with an alternative management strategy to closing the fishery when the quota of the weakest resource is filled. However, while the use of DNA methods has revolutionized our understanding of population structure and

connectivity within the marine realm (Schwartz *et al.*, 2007; Hauser and Carvalho, 2008), examples of using DNA methods to actively manage fisheries "real-time" are few (but see Schwartz *et al.*, 2007). Genetic mixture analysis was first initiated for the marine salmon fisheries in the North Pacific. By sampling and estimating the fractions of the stock components, the fisheries have been regulated in a way that allowed effective commercial harvest of salmonids while ensuring enough mature fish enter their native rivers to spawn (Shaklee *et al.*, 1999; Dann *et al.*, 2013; Larson *et al.*, 2014). Grant *et al.* (1980) were the first to initiate such studies using allozymes in the 1970s, but today, DNA markers as single-nucleotide polymorphisms (SNPs) are primarily used for this task (Larson *et al.*, 2014).

A mixed-stock fishery for Atlantic cod (*Gadus morhua*) exists in the north of Norway. Here, cod are divided into two stock components: Northeast Arctic Cod (NEAC; Figure 1a) and Norwegian Coastal Cod (NCC; Figure 1b). Of these, NCC is the most vulnerable component. NCC spawn along the entire coast of Norway, but display limited migratory patterns between spawning and feeding areas (Jakobsen, 1987; Michalsen *et al.*, 2014). In contrast, NEAC performs long migrations from the Barents Sea to the Norwegian coast to spawn (Bergstad *et al.*, 1987; Michalsen *et al.*, 2014). The main spawning areas for NEAC are close to the Lofoten Islands and the Møre region (Bergstad *et al.*, 1987; Sundby and Nakken, 2008; Olsen *et al.*, 2010) which overlap with some of the spawning grounds for NCC (Figure 1). Furthermore, NCC are assumed to spawn at the same time as the NEAC (Berg and Albert, 2003). Consequently, it has been estimated that ~60–70% of the annual catch of NCC is during this mixed spawning fishery primarily targeted at the NEAC harvest (ICES, 2015a).

One of the pre-requisites for conducting genetic stock identification is that there is sufficient genetic differentiation among the different components of the fishery. In the early 1960s, both DNA and morphological markers such as otolith structure (Rollefsen, 1933; Berg *et al.*, 2005) have been used to distinguish between NCC and NEAC (for review of earlier work see Nordeide *et al.*, 2011). Most genetic markers show low but statistically significant structuring between NCC and NEAC (Westgaard and Fevolden, 2007; Wennevik *et al.*, 2008). Nevertheless, genetic markers presumed to be under some form of selection, and thus show increased differentiation among populations, can provide the tools required for the identification of stock components on a contemporary time scale. Specifically, for the differentiation of NCC and NEAC, good examples of informative genetic markers are available, including haemoglobin and allozymes (Mork *et al.*, 1985), Pantophysin (Fevolden and Pogson, 1997) and two microsatellites GMO34 and GMO132 (Westgaard and Fevolden, 2007; Michalsen *et al.*, 2014). Furthermore, recent genome-wide scans using SNP markers (Hemmer-Hansen *et al.*, 2013; Kirubakaran *et al.*, 2016; Berg *et al.*, 2016) have revealed that genetic differentiation between the two stocks is mainly located on three chromosomes. The SNP Pantophysin or *PanI* (previously *SypI*) is part of a tightly linked gene cluster located on chromosome one (Kirubakaran *et al.*, 2016), exhibits large allele frequency differences between samples of NEAC and NCC and is almost diagnostic for the two stocks (Fevolden and Pogson, 1997; Wennevik *et al.*, 2008). While NEAC are almost fixed for the *PanI*[B] allele ($p \approx 0.90$), NCC show high frequencies of the *PanI*[A] allele ($p \approx 0.80$), and this pattern is temporally stable (Fevolden and Pogson, 1997; Sarvas and Fevolden, 2005; Fevolden *et al.*, 2012).

Although the selective agent(s) shaping this *PanI* allele frequency difference between NEAC and NCC is not fully understood, the marker's contribution to elucidate stock structuring has been recognized (Pogson and Fevolden, 2002; Case *et al.*, 2006; Wennevik *et al.*, 2008; Michalsen *et al.*, 2014; Kirubakaran *et al.*, 2016).

Although NCC have been recognized as different from NEAC for more than 80 years (Rollefsen, 1933), it is only in the past decades that specific regulations protecting the lesser abundant NCC have been in operation. Traditionally, all cod sampled north of 62° (both from Norwegian fisheries and from Norwegian surveys) have routinely been assigned to NEAC or NCC on the basis of differences in otolith structure (Rollefsen, 1933; Berg and Albert, 2003). By using the otolith assignments of NCC, a time series of catch data back to 1984 and a survey time series back to 1995 have been established. Since 2001, ICES have provided advice for management of NCC, and due to a steep decline in abundance of NCC as revealed through annual surveys, they advised a zero harvest in the period 2004–2011 (ICES, 2010, 2013, 2015b). However, a complete stop in the harvest of NCC involves closing all coastal fisheries in Norway where NCC were captured through bycatch. This was not considered realistic in a mixed-stock fishery with NEAC by the Norwegian Directorate of Fisheries (NDF), the governmental body responsible for regulating fisheries in Norway. Therefore, the total annual quota of NCC was reduced from 40 000 to 20 000 tonnes, and regulations aimed at reducing NCC (by)catches were introduced. These regulations included, among others, redirecting fishing efforts from areas with NCC to areas and seasons with NEAC.

One part of the NCC protection and rebuilding management strategy was to establish Borgundfjord as a marine protected area (MPA) from 2009 onwards (Figure 2). The MPA is composed of three spawning areas: Aspevågen, Borgundfjorden central, and Åsefjorden, and is closed to commercial harvest during the spawning period from March to May (recreational angling within the MPA is however permitted). However, in some years, NEAC enter Borgundfjord in large numbers (Godø, 1977). Therefore, in parallel to establishing Borgundfjord as an MPA, a genetic-monitoring programme was initiated with the aim of opening the fjord to commercial harvest if and when catches of NEAC in Hessafjorden, which is immediately outside the MPA, exceed ~70% over time.

While genetic monitoring programmes typically make use of a panel of genetic markers (e.g. Larson *et al.*, 2014), the monitoring programme established in association with the Borgundfjord MPA only included the *PanI* locus that is diagnostic between NCC and NEAC. Based upon experiences from Lofoten where NEAC and NCC are also found overlapping during the spawning season (Wennevik *et al.*, 2008), we chose to estimate the fraction of NEAC in Hessafjorden using the frequency of the *PanI*[B] allele in the catch.

The present study had several objectives. First, to present the estimated fraction of NEAC in the cod catches in Hessafjorden (immediately outside the MPA) through the spawning season over the years 2009–2016. Second, to evaluate the accuracy of stock identification by *PanI* compared to assignments performed using otolith category, and genetic assignment based on a panel SNP markers recently identified as diagnostic between NEAC and NCC. Third, to estimate the fraction of NEAC in egg samples collected both in and immediately outside the MPA in order to investigate the relationship between adult presence in the region and recruitment.
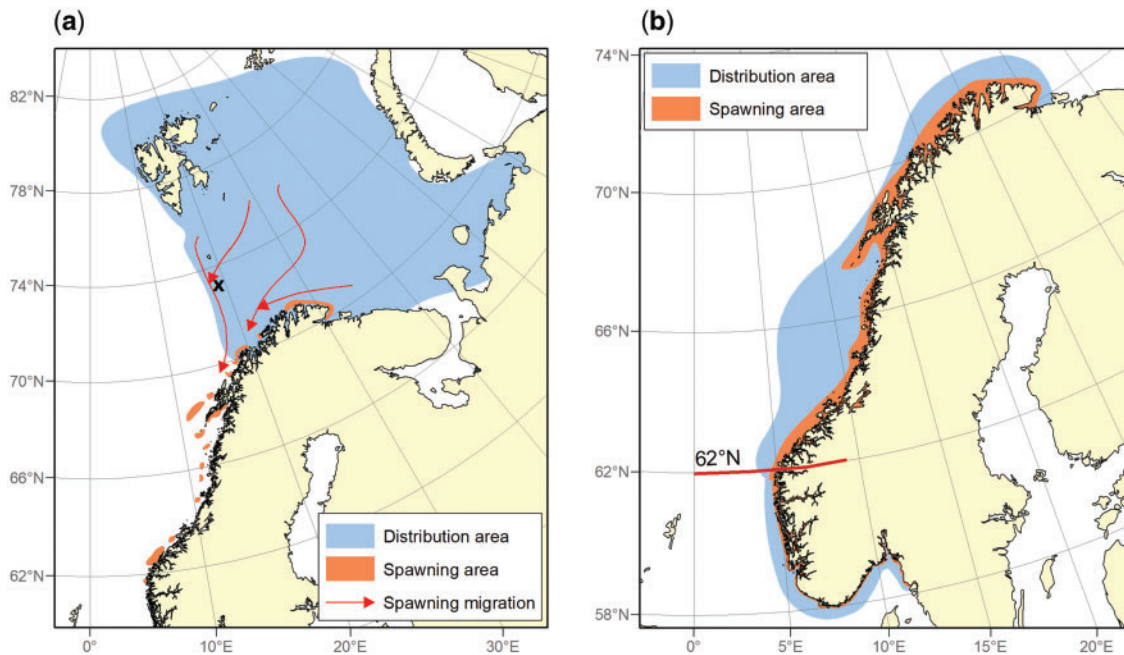
Figure 1. Distribution and life history of (a) Northeast arctic cod (NEAC) and (b) coastal cod. The Norwegian coastal cod (NCC) stock is distributed north of 62°N to the Russian border. X: reference sample of NEAC.
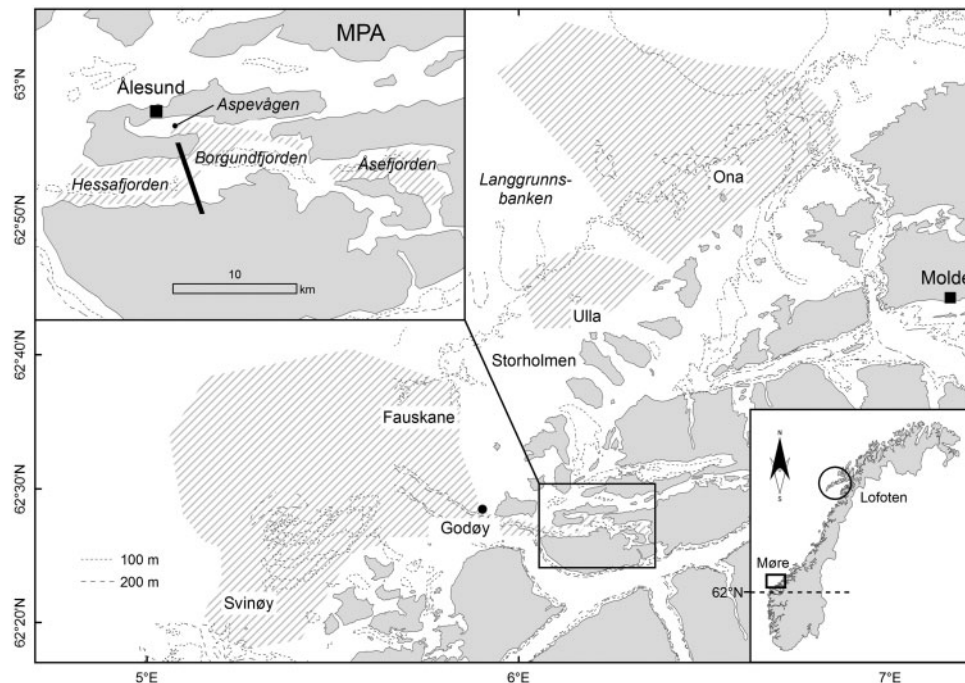

Figure 2. The Marine protected area (MPA: inside the solid line) is closed during spawning season (1 March to 30 May) and includes the spawning grounds: Aspevågen, Borgundfjord central, and Åsefjorden. Hessafjorden also belongs to location 0734, but is not incorporated in the seasonal closure. Genetic samples of adult fish were obtained from gill net vessels (<15 m) in Hessafjorden in the period 2009–2016. Egg samples were collected from the spawning grounds in the MPA + Hessafjord. ●: sampling station Godøy.

Material and methods

Background for the MPA at Møre

In traditional, otolith-based monitoring of NEAC and NCC at Møre, the area is divided into eight statistical strata or locations (Supplementary Figure S1). Based upon background information described in detail in Supplementary Table S1, statistical location 0734 was identified as the area with the highest estimated fraction of NEAC, which also represents an important local spawning

ground for NCC in the region (Godø, 1977). To protect the spawning component of NCC in area 0734 (Borgundfjord), an MPA was established which included the following three spawning grounds: Aspevågen, Borgundfjorden central, and Åsefjorden (Figure 2). The MPA is not open to commercial exploitation, but Hessafjorden, immediately outside the MPA, remains open for commercial harvest.

Samples from the monitoring programme in Hessafjorden 2009–2016

Cod were collected from the commercial gill-net fishery operating in Hessafjorden, just outside the MPA by the NDF. Gill/fin clips, were collected at least three times a week (Monday, Tuesday, and Wednesday), preserved in 100% ethanol and sent to the Institute of Marine Research (IMR) on Thursday for genetic analysis. By Thursday afternoon, results from that week's samples were sent back to the NDF. On the basis of this information, the NDF had the ability to open the MPA if and when the fraction of NEAC exceeded 70% for more than two consecutive weeks. In the period 2009–2016, this sampling regime resulted in the analysis of >6800 cod captured in the fishery in Hessafjorden (Figure 2 and Table 1). At the start and end of the commercial fishing season in Hessafjorden, some fish were also collected from the permitted recreational fishery within the MPA. In addition, three samples of cod were also collected from the outer coastal areas in 2013 (Godøy in Table 1; Figure 2) to estimate the fraction of NEAC on the coast.

Egg samples from the MPA in 2012–2016

To test the fraction of NEAC spawning within the MPA, eggs were collected at three known spawning grounds within the MPA: Aspevågen, Borgundfjorden central (named Borgundfjord), and Åsefjorden. In addition, eggs were collected from Hessafjorden itself (Figure 2 and Table 1).

Eggs were sampled by a standard WP2 hand net with diameter 60 μl and mesh size of 500 μm. More details of sampling found in supplement material. Eggs mainly of developmental stage 1 (<2 days old) were used for the genetic analysis as they were assumed to be sampled close to the spawning location (as opposed to have drifted from outside). In total, 3478 eggs were sampled from the region in the period 2012–2016 (except for 2014).

Genetic analysis of *Pan*1

DNA from cod captured in Hessafjorden was extracted in 96-well plate format using the HotSHOT protocol (Truett *et al.*, 2000). During the study period, the *PanI* locus was genotyped using two methods. In the period 2009–2012, it was genotyped using allele-specific primers developed for the ABI sequencer (Stenvik *et al.*, 2006). From 2012 onwards, it was genotyped using an allele-specific TaqMan assay adapted to a Roche Lightcycler 480 II real-time PCR instrument (Roche diagnostics, Switzerland). This change in genotyping technology was implemented to speed up the analysis process. DNA from the eggs sampled in and around the MPA was extracted in 96-well plate format using a Chelex isolation method (modified from Bio-Rad Laboratories). Each egg was placed in 75 μl 5% Chelex solution [Chelex® 100 Molecular Biology Grade Resin (BioRad) in ultrapure water] and 15 μl of Proteinase K Solution (Qiagen, 20 mg ml⁻¹). The isolation mixture was placed at 56 °C for 1 h, followed by a 10-min incubation

at 96 °C. Prior to extraction, each egg was punctured with tweezers to free the embryo. These samples were genotyped using the ABI sequencer (Stenvik *et al.*, 2006).

Estimation of NEAC fraction in fish and egg samples

The fraction of NCC and NEAC is based upon the presence of genotypes at the *PanI* locus (AA, AB, and BB). The classification is made on a sample level, as this marker is not diagnostic on an individual level as BB and AB are present in NEAC, and all three genotypes are assumed present in NCC (Wennevik *et al.*, 2008; Michalsen *et al.*, 2014). The fraction of NEAC in each sample (*FractionNEAC*) was estimated by the equation:

$$FractionNEAC = \frac{FractionB - \alpha}{1 - 2\alpha}. \qquad (1)$$

FractionB is the observed fraction of *PanI*ᴮ allele in the sample and α is the assumed *FractionB* in a "pure" coastal cod sample. Previous studies in Lofoten (Wennevik *et al.*, 2008) indicated α close to 0.10, while other samples from fjords along the Norwegian coast indicated a smaller α, close to 0.05 (Sarvas and Fevolden, 2005). To quantify the associated uncertainty range, the *FractionNEAC* for each sample was calculated for both values of α, while the average of the two estimates was reported as the "best estimate."

Comparison of *PanI* and SNP-based assignments

Validating the accuracy of the NEAC/NCC classification by the *PanI* locus is important because the results directly influence management decisions. Therefore, in parallel with the primary monitoring programme where *PanI* was the sole marker used for classification, we compared the estimated fraction of NCC/NEAC between the *PanI* locus, otolith category, and newly developed SNP markers. Specifically, a subset of 364 adults and 455 eggs from Hessafjorden and Borgundfjord were analysed for a panel of 39 SNP markers (Table 1). In addition, an NEAC reference sample from the Barents Sea, comprising 100 adults, was included in this analysis. The SNPs were selected using the information from recent studies showing high differentiation between NEAC and NCC (Hemmer-Hansen *et al.*, 2013; Berg *et al.*, 2016; Kirubakaran *et al.*, 2016), and comprised 11 SNPs from chromosome one (LG1) and 28 SNPs from other chromosomes (LG2–16) (Kirubakaran *et al.*, 2016).

The 39 SNPs were genotyped on the above-mentioned samples using matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF MS) assays (Agena Bioscience, Inc., Hamburg, Germany). Primers used for genotyping are available from the authors. Genotyping was performed using the IPLEX protocol following the manufacturer's instructions (Agena Bioscience). MassARRAY Typer software was used for automated genotype calling (Agena Bioscience). Each allele was subsequently inspected manually. SNPs with more than 20% missing data per sample and a minor allele frequency below 0.05 were discarded. As *PanI* is included in the tightly linked inversion in LG1 (Kirubakaran *et al.*, 2016) a check for Linkage disequilibrium (LD) among the 11 selected SNP's from LG1 and *PanI* was performed in Genepop 4.3 (Rousset, 2008). This included calculation of the Pearson correlation coefficient between the allele frequency of the *PanI*ᴮ and the LG1 SNP's.

Table 1. Samples of spawning cod ($n = 6802$) and eggs ($n = 3478$) collected during spawning season 2009–2016 in Hessafjorden and the MPA and the coastal area of Møre.

Location	Type	Marker type	Sampling year								Total
			2009	2010	2011	2012	2013	2014	2015	2016	
Hessafjorden	Adult	Samples	13	20	23	24	10	14	13	6	123
		Pan1 (n)	1 032	931	1 100	1 130	533	672	582	288	6 268
		SNP (n)	–	–	117	48	126	–	32	–	323
	Egg	Samples	–	–	–	5	5	–	7	8	25
		Pan1 (n)	–	–	–	182	46	–	175	609	1 012
		SNP	–	–	–	–	–	–	–	–	–
MPA locations			–	–	–	–	–	–	–	–	–
Aspevågen	Adult	Samples	–	–	2	2	–	–	1	–	5
		Pan1 (n)	–	–	56	66	–	–	48	–	170
		SNP (n)	–	–	–	–	–	–	48	–	48
	Egg	Samples	–	–	–	5	5	–	5	7	22
		Pan1 (n)	–	–	–	199	217	–	256	393	1 065
		SNP (n)	–	–	–	146	163	–	174	–	483
Åsefjorden	Adult	Samples	–	–	–	–	–	–	5	–	5
		Pan1 (n)	–	–	–	–	–	–	75	–	75
	Egg	Samples	–	–	–	3	3	–	–	7	6
		Pan1 (n)	–	–	–	128	104	–	–	299	531
		SNP	–	–	–	–	–	–	–	–	–
Borgundf.j Central	Adult	Samples	–	–	1	–	3	–	–	–	4
		Pan1 (n)	–	–	5	–	92	–	–	–	97
		SNP (n)	–	–	–	–		–	–	–	0
	Egg	Samples	–	–	–	3	3	–	7	7	20
		Pan1 (n)	–	–	–	108	37	–	224	501	870
		SNP (n)	–	–	–	–	–	–	–	–	–
Godøy (Møre)	Adult	Samples	–	–	–	–	4	–	–	–	4
		Pan1 (n)	–	–	–	–	192	–	–	–	192
		SNP (n)	–	–	–	–	48	–	–	–	48
NEAC (Barents Sea)	Adult	SNP (n)	–	–	–	–	100	–	–	–	100

The MPA includes the spawning grounds Aspevågen, Borgundfjorden central and Åsefjorden. All samples were analysed for *PanI*, and a sub-sample was also analysed for 39 SNPs (see Material and methods). *n*, number of individuals. NEAC is a reference sample from the Barents included in the SNP analysis.

We applied two assignment approaches using STRUCTURE 2.3.4 (Pritchard *et al.*, 2000) and GeneClass2 (Piry *et al.*, 2004), both relying on the Bayesian framework. STRUCTURE was used to estimate the number of population clusters (K) in our total dataset. We used default parameters with a burn-in of 200 000 followed by 500 000 MCMC steps. Ten independent runs for each K (1–5) were performed. The results from these runs were uploaded to STRUCTURE HARVESTER (Earl and Vonholdt, 2012), which returned the most likely value of K using the Evanno method (Evanno *et al.*, 2005). CLUMPP (Jakobsson and Rosenberg, 2007) found the optimal alignment of replicate cluster analysis of the same data and corrected for label switching in unsupervised cluster analysis. CLUMPP gives a mean individual membership probability (Q), based on ten independent runs, for belonging to each of the most likely clusters determined by STRUCTURE HARVESTER. We set the threshold for Q at 0.7, so that individuals with values above 0.7 were assigned to its most likely cluster. Assignments in GeneClass2 were made with the probability computation option, with simulations of 10 000 individuals following Paetkau *et al.* (2004). The individual Q value from STRUCTURE and the probability estimates from GeneClass2 were compared with Pearson's correlation coefficient.

For the samples of adults and eggs, each individual was assigned to one of the two clusters NEAC or NCC, as described above. Based on this assignment, the *PanI* genotype frequency for each cluster was calculated. Thereafter, the *PanI* genotype frequency for each of the two clusters was translated into a probability for each of the genotypes to belong to either NCC or NEAC. For the fishery samples, the fraction of NEAC was estimated with:

$$FractionNEAC = (countPanI^{AA}a) + (countPanI^{AB}b) + (countPanI^{BB}c). \qquad (2)$$

The variables $countPanI^{AA}$, $countPanI^{AB}$, and $countPanI^{BB}$ are the sums of the genotypes for a specific sample from the catches, and a, b, and c are the probabilities for each of the three *PanI* genotypes of belonging to the NEAC group. This enabled us to calculate the Pearson correlation coefficient between the estimates from Equations (1) and (2). To test the level of differentiation between locally assigned NEAC and NCC, we made an F_{ST} test based on Weir and Cockerham (1984) θ estimator for both LG1 and the total dataset.

Comparison of *PanI* and otolith-based assignments

NCC and NEAC have traditionally been identified and monitored in Norwegian fisheries using otolith structure (Rollefsen, 1933). This identification is based on morphological differences in shape and relative distance between the two innermost translucent zones in the otolith corresponding to cod older than two years.

The NCC are coded otolith type 1 and 2, while NEAC otolith type 4 and 5, where 2 and 4 are uncertain types (Berg and Albert, 2003). Otolith-classification corresponded well with the genetic assignment of NEAC/NCC from Lofoten (Wennevik *et al.*, 2008) and fjords farther north (Berg *et al.*, 2005; Stransky *et al.*, 2008). In the Hessafjorden gillnet fishery, most of the catch was headed and gutted prior to landing. Thus, otoliths in the primary genetic monitoring programme described above were not available for comparison to the *PanI* results. However, upon request, fish in a sub-sample of the genetic samples from the commercial catch were not headed before being landed and were sampled by the NDF. This included 847 cod that were used to compare the estimated fractions of NEAC/NCC with the results for *PanI*.

Tissue samples from the weekly catch in Hessafjorden were sent to IMR, samples were analysed, and the estimated fraction of NEAC reported back to the NDF within 8 h. This regime was implemented throughout the spawning season in the period 2009–2016, resulting in a unique dataset that included the allele frequencies of ~6500 cod sampled in Hessafjorden over multiple weeks and years.

Results

Estimation of NEAC in the commercial catch based upon *PanI*

The estimated fraction of NEAC in Hessafjorden typically varied between 40 and 65% in the period mid-March until the end of the spawning season (Figure 3). The estimated fraction of NEAC in Hessafjorden did not exceed the 70% threshold set by the NDF to open the MPA to commercial fishing for any sustained period, and consequently, the MPA remained closed in all years. In the years in which sampling was initiated in middle February, the estimated fraction of NEAC tended to be low, indicating that NCC initially dominated this region, followed by a pulse of NEAC. This is presumably a result of the NEAC arriving from its long-distance migration from the Barents Sea. The peak in the estimated fraction of NEAC in Hessafjorden was during March and April, before it once again tailed off towards the end of the sampling season in some years, presumably as NEAC left the region (Figure 3).

On the basis of the above patterns up to 2013, it was decided that beginning in 2014, cod would be sampled only in Hessafjorden during the period in which NEAC was expected to arrive in significant numbers (i.e. March–April). In 2014, the fraction of NEAC in the first sample (14 March) was 71.8%. However, it decreased in subsequent samples, remaining below 70% for the rest of the sampling period. Although, the fraction of NEAC in 2014 was larger than in previous years (more than 40% until early-April), it decreased to below 20% by the end of the season, as in previous years, once again illustrating the exodus of NEAC from the region. In 2013, three additional samples of cod were collected in the coastal waters outside Hessafjorden (Godøy, Figure 2). These samples showed a larger fraction of NEAC early in the season than samples taken from Hessafjorden at the same time (Figure 3).

Over all sampling years, the fraction of NEAC in the catches in Hessafjorden increased steadily from 2009 with a peak fraction of NEAC in 2014 (Figure 4). Interestingly, this trend was clearer for the March samples than for the April samples. The curve in April may be more strongly influenced by the difference in timing of NEAC leaving the region between years, while the curve for March more closely reflected the NEAC peak.

Estimation of NEAC in the rod and line fishery permitted inside the MPA

Approximately 350 cod were captured by rod and line inside the MPA. These were also genotyped with *PanI* to estimate frequencies in NEAC (Figure 3). With few exceptions, samples from either Aspevågen, Borgundfjorden central, or Åsefjord, displayed a similar, or as in most cases, lower estimated fraction of NEAC than in Hessafjorden at the same time. This is not surprising given that NEAC migrate through Hessafjorden on their way into the MPA, but nevertheless reveals a large frequency of NCC within the MPA. Importantly, none of the samples from the MPA revealed NEAC fractions greater than 70%, confirming results from immediately outside the MPA that it should remain closed to commercial harvest.

Estimation of NEAC in the egg samples

Eggs were sampled four years at three spawning locations inside the MPA, as well as in Hessafjorden (Figures 1 and 5). In all four years, and at all four locations, the abundance of stage-I cod eggs peaked in the second half of March. Peak egg production coincided with the approximate timing of the peak NEAC fraction in the region. In 2013, the largest abundance of eggs was from the spawning ground at "Aspevågen." However, in the other years, the abundances of eggs were similar among the sampled regions.

The *PanI* estimates indicated that the majority of the eggs sampled within the three localities inside the MPA, as well as in Hessafjorden, originated from NCC spawning in all four years sampled and that the NEAC contribution was typically <30% (Figure 6). Furthermore, with a few exceptions, no clear difference in the fraction of NEAC in the egg samples was observed among the four locations (except for data from 2015). Thus, the relative contribution of NEAC to the egg recruitment was similar within and immediately outside the MPA. Collectively, these data demonstrate that NEAC may spawn at all three locations within the MPA, as well as in Hessafjorden outside the MPA, but nevertheless, egg production in this region predominantly originates from NCC spawning.

A comparison of the NEAC fraction in eggs from the four locations with the adult cod sampled in the same years indicated that the fraction of NEAC adults in Hessafjorden was larger than that in the eggs (compare Figures 3 and 6). For example, late March estimates in adults and eggs in Hessafjorden in 2013–2015 indicated that adults were typically 40–60% NEAC (Figure 3), but estimates of NEAC eggs were approximately half (Figure 6).

Comparison of *PanI*- and SNP-based assignments

Nine hundred and two eggs and adults were genotyped with 39 SNPs, in addition to *PanI*, to compare the two methods. We successfully genotyped 364 of 419 adults, and 455 of 483 eggs while permitting up to a maximum of 20% missing SNP data per individual (Figure 5; Table 1). LD was observed among the 11 SNP's in LG1and *PanI*, in the NEAC reference sample (22 of 66 pairwise comparisons), but the few LD observed (below 5%) in the MPA and Hessafjorden samples were randomly distributed. Positive correlation was found between the *PanI*[B] allele and the number of linked SNP's (Pearson $r = 0.83$, $p < 0.001$) in the total SNP dataset.
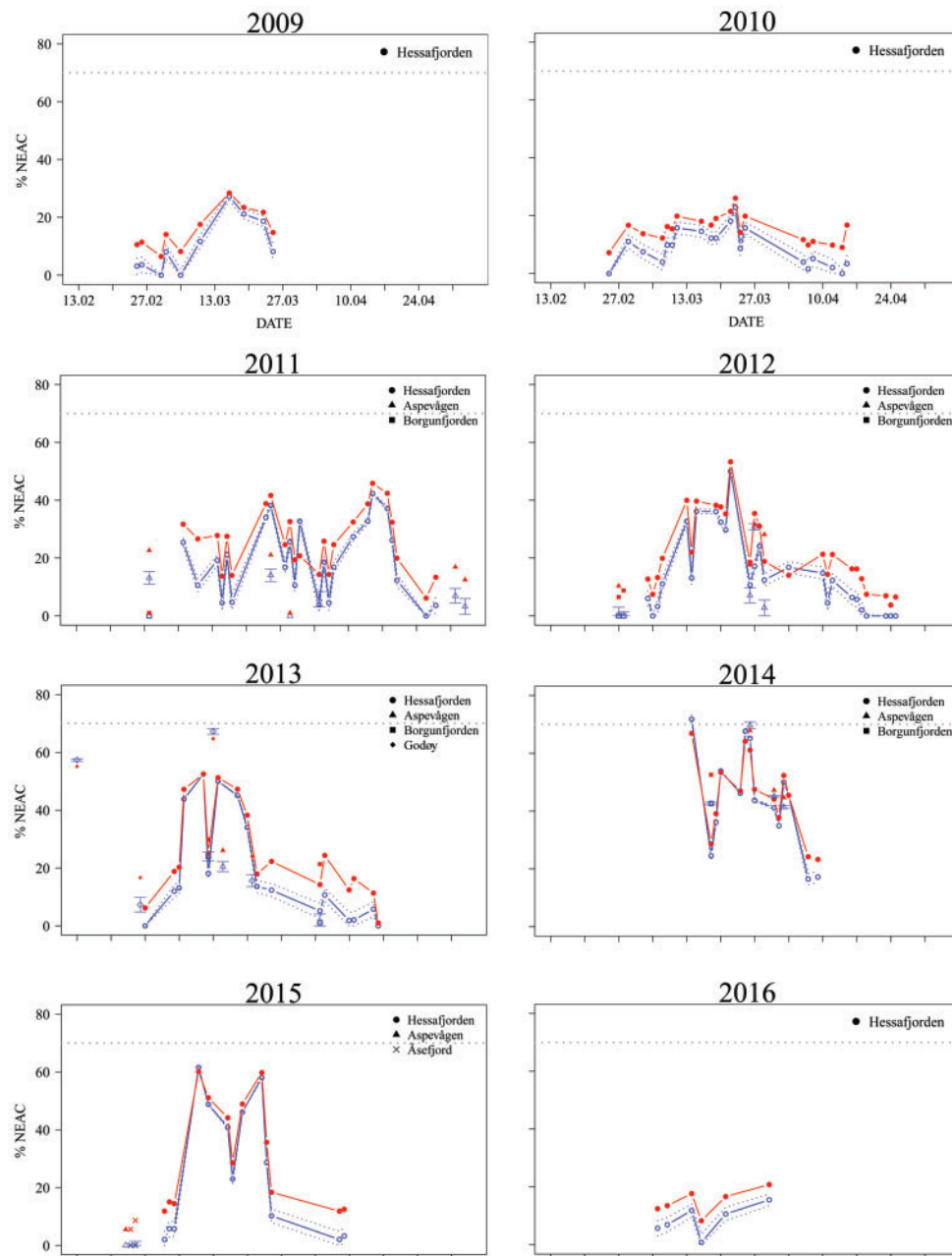
Figure 3. Estimated fraction NEAC in the catches from Hessafjorden, located just outside the MPA. Samples from within the MPA (Aspevågen, Borgundfjorden, and Åsefjorden) were collected in some year and in 2013 also outside Hessafjorden (Godøy see Figure 2). The estimated fraction is on the basis of Equation (1) (open symbols) and Equation (2) (filled symbols)—see text for details. The hatched line represents error rate: the $\alpha = 0.05$ (upper line) and $\alpha = 0.10$ (lower line). The horizontal dotted line shows 70% (for details see text).

Similar assignments of two clusters ($K = 2$) were observed with Geneclass 2.0 and STRUCTURE (Pearson $r = 0.82$, $p < 0.001$). Based on this result, we used only STRUCTURE to estimate further assignments. The NEAC from the Barents Sea were all assigned to one cluster together with some fish from the Godøy and the MPA samples (green bars in Supplementary Figure S1). The threshold for assigning individuals to NEAC and NCC was set to a Q-value of 0.7 and 0.3, respectively. Only small differences were observed in the estimates between adults and eggs. The level of differentiation between the NEAC and NCC group on the basis of the SNP markers was highly significant based on the SNPs

from both LG1 ($F_{ST} = 0.31$, $p < 0.001$) and LG2–16 ($F_{ST} = 0.24$, $p < 0.001$). Individuals assigned to the second cluster were interpreted as NCC (red bars in Supplementary Figure S1). The frequencies of the *PanI* genotypes (AA, AB, and BB) for each of the fish groups assigned to NEAC and NCC are presented (Table 2).

For the *PanI* genotype frequencies on the basis of the SNP assignment, we grouped the 6900 fish into NEAC and NCC and obtained an independent estimate of NEAC in the catches of adult cod (filled symbols: Figure 3) and the egg samples (filled symbols or red lines: Figure 6). We found a high correlation

between the estimated fraction of NEAC based upon the SNP assignment and by using *PanI* directly (Pearson $r = 0.99$, $p < 0.001$).

Comparison of *PanI* and otolith-based assignment

The 847 cod for which both *PanI* and otoliths were sampled were primarily captured in Hessafjorden. A high correlation was observed between the *PanI*^AA genotype and the fraction of NCC estimated with otoliths, and between the *PanI*^BB genotype and the fraction of NEAC estimated otoliths (Table 3). The large number of individuals with both *PanI*^AA and otolith type 1 is evidence of a high fraction of NCC. In general, assignments based on genetic information alone (Table 2) were correlated with
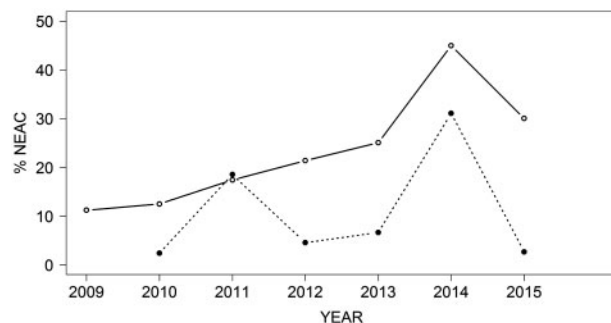
Figure 4. Estimated yearly average fraction of NEAC for March (solid line) and April (broken line) in the commercial catches from Hessafjord, located just outside the MPA, in the period 2009–2015. This is based upon the capture of more than 6500 adult cod analysed with the locus *PanI*.

assignments based on otolith morphology (Table 3). An ideal correspondance between *PanI* and otolith typing would give few fish in otolith assumed uncertain NCC (type 2) or assumed uncertain NEAC (type 4). Among fish with otolith type 4, the *PanI*^AA, allele occurred with a frequency of 50%, indicating the uncertainty of genetic classification. However, only 20 fish were classfied as type 4 so this conclusion may be weak.

Discussion

Increased regulation of harvest is a natural consequence of man's legacy of over-exploitation of many of the world's fisheries. As a component of this, MPAs can provide fish with a safe haven, which may result in recruitment and spillover effects into neighbouring seas (Villegas-Rios *et al.*, 2017). However, when MPAs contain both fragile resources in need of protection, as well as resources which could in-principle be sustainably harvested, commercial, and biological interests may conflict. The "real-time" genetic monitoring described here provides a tool to help manage this conflict in the MPA and neighbouring seas. Some of the salmon fisheries in the North Pacific have been actively managed using similar approaches for several decades (Schwartz *et al.*, 2007; Dann *et al.*, 2013; Larson *et al.*, 2014). However, together with the commercial NEAC/NCC fishery in operation in Lofoten, northern Norway, which we also are managing by "real-time" genetic methods (G. Dahle and T. Johansen, unpublished data), the programme described here represents the only marine fishery managed "real-time" in the Atlantic to our knowledge.

Has the MPA been successful?

The monitoring programme presented here was designed to provide the Norwegian Directorate of Fisheries with the ability
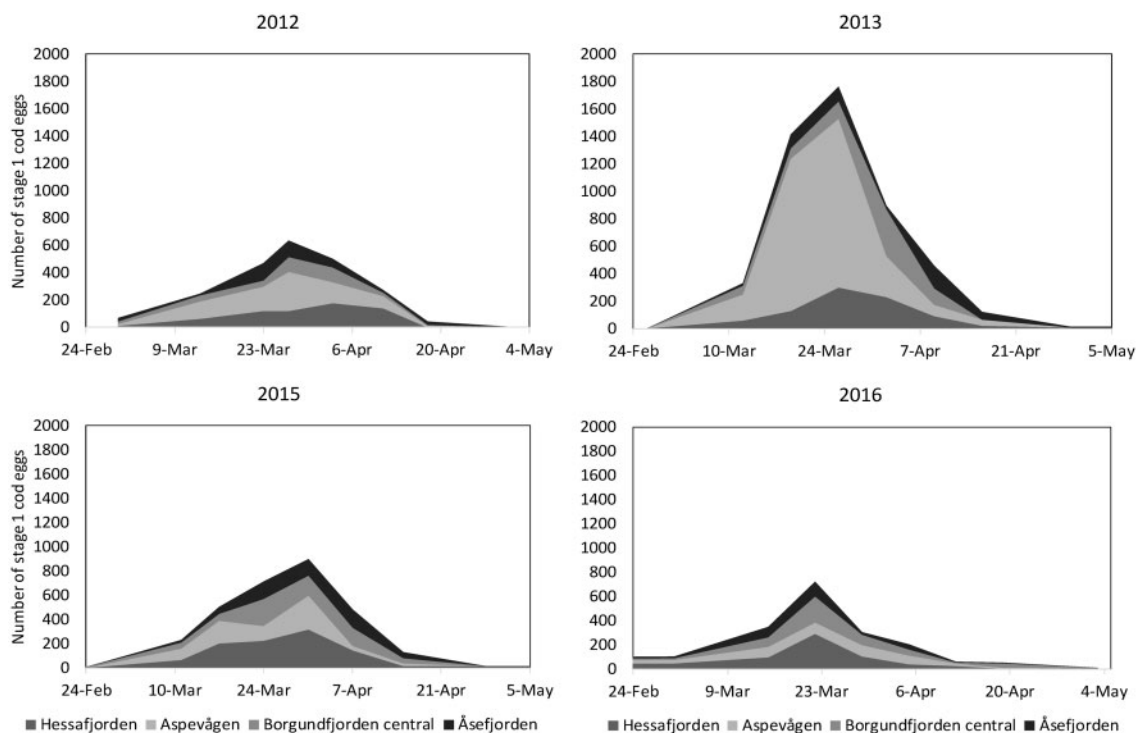
Figure 5. Estimated abundance of stage-I cod eggs sampled throughout the spawning season at three locations within the MPA (Aspevågen, Bogundfjorden central, Åsefjorden), in addition to Hessafjorden just outside the MPA. Peak spawning in all years was towards the end of March. No eggs were sampled in 2014.
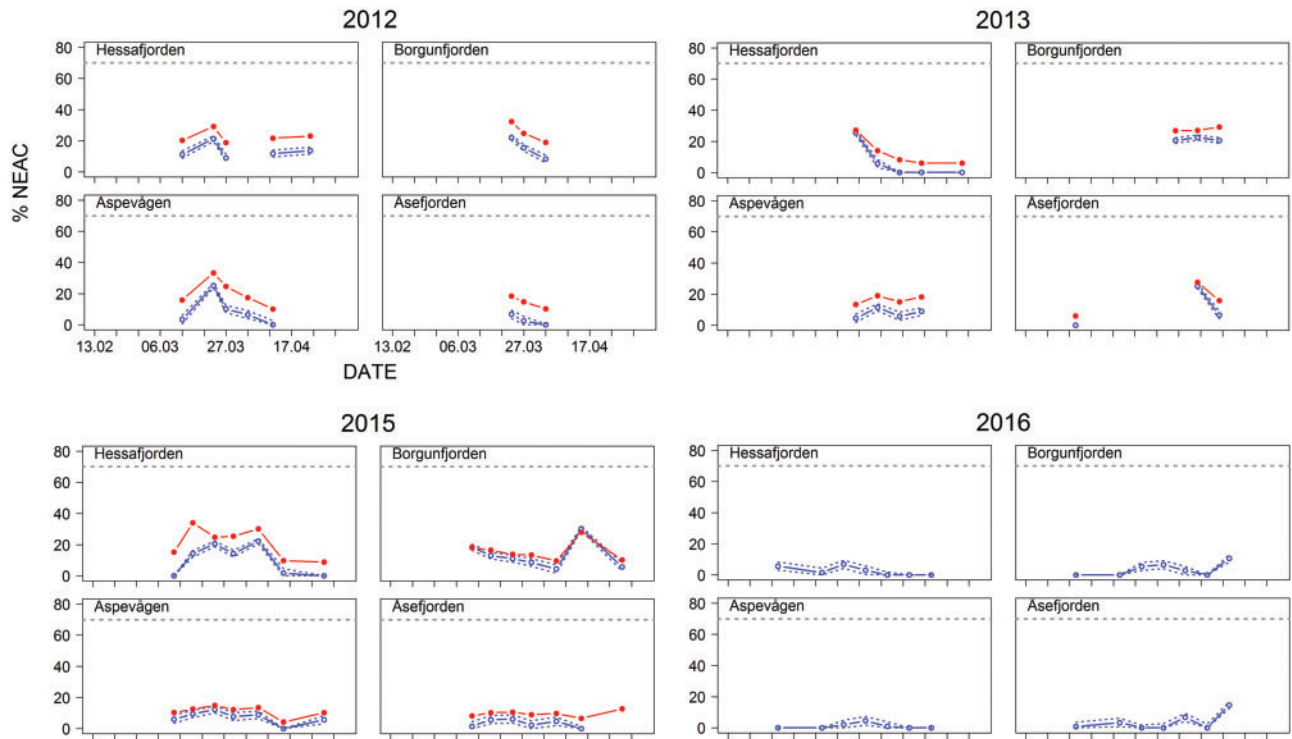
Figure 6. Estimated fraction of NEAC in samples of eggs collected in 4 years from spawning locations within the MPA (Aspevågen, Åsefjorden, and Borgundfjorden central), and in Hessafjorden, throughout the spawning season. The estimated fraction is on the basis of Equation (1) (open symbols) and Equation (2) (closed or filled symbols)—see text for details. The hatched line represents the $\alpha = 0.05$ (upper line) and $\alpha = 0.10$ (lower line) for the *PanI* method. See text for details. The horizontal dotted line: 70%.

Table 2. Pan genotypes (in numbers) shown for NEAC and NCC as classified by 39 SNP loci as assigned by STRUCTURE (Pritchard et al. 2000).

Genotype	NCC	NEAC	Total	% NEAC
Adult				
*PanI*AA	213	5	218	20
*PanI*AB	7	11	18	61
*PanI*BB	4	45	49	91
Total (*n*)	224	61	285	–
Egg				
*PanI*AA	258	3	261	1
*PanI*AB	6	10	16	61
*PanI*BB	1	17	18	94
Total (*n*)	265	30	295	–

The cod were classified into NEAC and NCC for both adult cod and eggs. % NEAC, percent among the *PanI* genotypes.

Table 3. For 857 cod collected for genetic analyses we also collected otoliths.

	Otolith type					
	NCC		NEAC			
Genotype	1	2	4	5	Total (*n*)	% NEAC
*PanI*AA	464	20	10	18	512	5
*PanI*AB	101	7	5	63	176	39
*PanI*BB	13	3	5	138	159	90
Total (*n*)	578	30	20	219	847	–

On the basis of Rollefsen (1933) the otoltith typing 1 and 2 is in assessment assigned NCC and 5 and 4 assigned to NEAC. The table compare the assignment of cod on the basis of otoliths with *PanI* genotypes. High correlation was observed between genotype *PanI*AA common in NCC and *PanI*BB most common in NEAC as in Wennevik et al. (2008). % NEAC, percent among the *PanI* genotypes.

to open the MPA for commercial harvest, if the proportion of the cod in the fishery just outside the MPA exceeded a 70% NEAC contribution. While there was an increase in NEAC fraction during 2009–2014 (Figure 4), it never exceeded 70% for any sustained period in any of the years (Figure 3). In 2016, the fraction of NEAC did not exceed 20% throughout the spawning period. Therefore, the MPA remained closed, thus fulfilling the primary objective of protecting the NCC stock component from potential overharvest within the MPA. For this primary objective, the programme has been a huge success and is still in operation.

While the monitoring programme has been a success from a fishery-regulation point of view, it is pertinent to ask whether the MPA established in Borgundfjord since 2009 has led to an increase in NCC in the region. This was not evaluated in the present study. Nevertheless, in March and April each year, NCC was the most abundant of the cod stocks in the MPA (based upon samples from the MPA itself and immediately outside in Hessafjorden), and not least, egg production in the MPA was clearly dominated by NCC during peak spawning in this area (Figures 5 and 6). If the MPA had been opened to commercial harvest at this time, a large number of NCC, which provide most

of the recruitment in the MPA, would have been harvested and thus the overall recruitment of NCC would have been reduced in the region.

Catchability of cod is greater during spawning due to the aggregation of individuals. In general, reducing fishing pressure in a spawning area can benefit fish populations by reducing mortality directly (Gruss *et al.*, 2014), and by reducing disturbance (Morgan *et al.*, 1997). Clarke *et al.* (2015) protected a spawning ground for local cod west of Scotland, but found no effect in terms of abundance, biomass, and reduced mortality. They indicated that this may have been due to the bycatch of juvenile cod in the local *Nephrops* fishery, and further suggested that efforts to halt the decline in the local cod stock may have been too late. Continuation of our NEAC/NCC time series in Borgundfjord is imperative to be able to evaluate the status of the NCC stock in the MPA. However, this would have to be combined with other methods of assessing success, such as estimates of adult abundance in the region, and estimates of the local spawning stock biomass on the basis of egg abundance combined with genetic analyses as described here. This work is in progress and provides a non-invasive and cost-effective way to measure spawning stocks in coastal areas. In the meantime, redirection of the commercial fleet to areas and seasons that optimises the match between quota and actual catch by stock is encouraged.

The observed discrepancy between adult abundance and egg recruitment

The monitoring programme implemented in this study has not only permitted regulation of the fishery and its potential influence with the MPA, but has also revealed insights into the interaction between NEAC and NCC in this region in time and space. One of the most striking results is that the estimated fraction of NEAC in the eggs sampled was consistently lower than the estimated fraction of NEAC in the adults in the same period, both within the MPA and in Hessafjorden (compare Figures 3 and 6). To illustrate, the estimated fraction of NEAC in the adult catch in Hessafjorden in 2013–2015 was typically 40–60% during late March during peak spawning in the area (Figure 5). In contrast, the estimated fraction of NEAC in the predominantly stage-1 eggs recruited in Hessafjorden at the same time was typically less than 20–30% (Figure 6). This reflects about a ≥2-fold discrepancy between the estimated fraction of NEAC adults in the region and the estimated contribution of NEAC eggs.

There may be several potentially interlinking factors influencing the discrepancy between adults and eggs. We suggest that the most likely explanation is that while NEAC do indeed migrate into Hessafjorden and the MPA, and to some degree overlap on the spawning grounds with NCC in this region, many of the NEAC caught in this area may have migrated back out to the outer-coastal areas to spawn in areas such as Ona, Ulla, and the region between Svinøy and Fauskane, which are known spawning grounds for NEAC (Figure 2). Earlier, it was assumed that NEAC and NCC shared the same spawning grounds in this region (Godø, 1977). A way of testing this could be to tag individual cod captured in the MPA, and see if any are recaptured in the commercial fishery on these known spawning banks in the outer coastal areas.

Peak NCC spawning seems to take place 23–24 March each year. The spawning peak of NEAC, as estimated in Lofoten, is later and varies little between years (Olsen *et al.*, 2010). This may be the first indication that time of spawning is different between NEAC and NCC. An extensive egg sampling regime could be used to look for potential differences in timing and location of NEAC and NCC spawning-recruitment in the Møre area.

Previous time series have followed the development of a population on the basis of juvenile cod within a fjord system in the south of Norway by sampling the sites once a year (Knutsen *et al.*, 2003; Knutsen *et al.*, 2011), but not to the same extent by collecting adults and egg during the whole spawning period as in the present study. Clarke *et al.* (2015) evaluated spawning grounds for cod west of Scotland (without DNA sampling), but did not collect egg samples. In our study, we collected egg samples over 3 months in 4 years. Although we observed increased amount of cod eggs (Figure 4) we did not see increased fractions of NEAC (Figure 6). Even though we observe some increase of egg production within the MPA, more research is needed to evaluate whether the number of NCC spawners is really increasing.

Temporal increase in estimated NEAC fraction

The estimated fraction of NEAC captured in the commercial fishery in Hessafjorden displayed a distinct increase with time in the period 2009–2014 (Figure 4). This trend coincides with the increase in the NEAC spawning stock biomass measured in the Barents Sea by ICES (ICES, 2015c).

NEAC migrate from coastal areas of Norway where they are recruited, to the Barents Sea for feeding, and then back to the Norwegian coastline to spawn (Olsen *et al.*, 2010). Each year, we observed an increase in the estimated NEAC fraction in Hessafjorden from February onwards, with a peak in late March. The timing of the NEAC "arrival" in this region overlaps with the peak in the estimated NEAC fraction in Lofoten, northern Norway (G. Dahle and T. Johansen, unpublished data, see Figure 2). single-stock of NEAC could in principle explain why we appear to observe a temporal increase in NEAC fraction in multiple areas simultaneously both within and among years. To test this theory, more extensive genetic comparisons between NEAC captured on the spawning grounds in different areas of Norway is required.

Methodological validation

The high correlation between *PanI* genotypes and assignments with SNP markers strongly supports that the *PanI* locus is a good indicator of NEAC/NCC fraction in the catch, and can be used to rapidly estimate this in both adult and egg samples. The *PanI* SNP is found in LG1 and appears to be linked to a cluster of more than 500 genes (Kirubakaran *et al.*, 2016) of which we included 11 loci in our SNP panel. Similar differentiation between NEAC and NCC is also observed in other linkage groups as LG2, LG7, and LG12 (Hemmer-Hansen *et al.*, 2013; Berg *et al.*, 2016), but only the cluster of SNPs in LG1 is assumed to be linked to adaptation in NEAC. We therefore tested the eleven SNPs found in LG1 combined with the 28 assumed neutral SNPs from the other LGs when assigning individual cod to NEAC and NCC for identifying the local *PanI* frequency. We demonstrated that both panels show similar estimates of NEAC (Figures 3 and 6; Table 2). This is supported by a recent study which found similar differentiation between NEAC and NCC both by assumed selective and neutral markers on the basis of a panel of more than 9000 SNPs (Berg *et al.*, 2016).

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