Vol. 1: 133–142, 2015
doi: 10.3354/sedao00013

SEXUALITY AND EARLY DEVELOPMENT IN AQUATIC ORGANISMS
Sex Early Dev Aquat Org

Published online June 11

Extreme male-skewed sex ratios on spawning grounds for Atlantic cod *Gadus morhua* with typical coastal cod signatures of the *Pan* I (pantophysin) locus

Svein-Erik Fevolden[1,*], Jon-Ivar Westgaard[2], Torstein Pedersen[1]

[1]Department of Arctic and Marine Biology, Faculty of Biosciences, Fisheries and Economics, UiT – The Arctic University of Norway, 9037 Tromsø, Norway

[2]Institute of Marine Research, PO Box 6404, 9204 Tromsø, Norway

ABSTRACT: Large offshore and small inshore populations of Atlantic cod *Gadus morhua* L. display differences in migratory patterns and settling regimes, but little is known about possible differences in spawning behaviour. Cod presumably of the Norwegian coastal cod type were therefore sampled during 7 spawning seasons (2002–2013) in Malangen, northern Norway. A spawning site in neighbouring Balsfjord was sampled during 5 consecutive spawning seasons (2002–2006). Length, weight, sex and maturity stage were recorded for each individual fish (n = 995). To verify their population assignment, frequencies of the 2 different *Pan* I (pantophysin) alleles were measured in 6 sampling years in Malangen and 3 in Balsfjord (n = 728). During all survey years there was an extreme skewed sex ratio at the spawning sites. In 6 of 7 seasons in Malangen, 8–9 of 10 fish were males, and in Balsfjord 9 of 10 fish were males on average, indicating some form of lekking behaviour among the spawning fish. Average size of the fish varied among years and also between sexes in individual years, although not consistently. The smallest proportion of mature fish was seen in Malangen in the year with the earliest sampling date (23 February). This was also the sample with the least skewed sex ratio (6.5 of 10 fish were males). The frequency of the *Pan* I^A allele of the Malangen cod was highly stable among years at close to 90%, showing that this spawning ground is exclusively used by coastal cod. In Balsfjord, frequencies of the *Pan* I^A allele were lower (67–78%). This fjord is suggested to be more penetrable for the migratory NE Arctic cod with their high frequencies of the *Pan* I^B allele, making it a less exclusively coastal cod residence. In both fjords, length of mature males but not females varied among the *Pan* I genotypes.

KEY WORDS: Atlantic cod · Norwegian coastal cod · Spawning behavior · Sex ratio · Pantophysin · *Pan* I locus

INTRODUCTION

Spawning behaviour in marine demersal fishes is for many species insufficiently known; to a certain extent this also applies to the ecological and commercially important Atlantic cod *Gadus morhua* L. This broadcast spawner (females release thousands to millions of eggs) is distributed all over the North Atlantic. Major fisheries take place in part on the species' spawning grounds, with the possible consequence of sex-biased exploitation if lekking and thereby skewed sex ratios are common features in spawning aggregations. In the western Atlantic, skewed sex ratios have been reported in shoals of spawning Atlantic cod on the northern Grand Bank and the southern Scotian Shelf/Bay of Fundy (Morgan & Trippel 1996), and evidence of lekking and skewed sex ratios have been shown on spawning grounds off Newfoundland (Robichaud & Rose 2003, Windle & Rose 2007). Fine-scaled temporal and spa-

*Corresponding author: svein-erik.fevolden@uit.no

Publisher: Inter-Research · www.int-res.com

tial distributions have recently been reported from spawning grounds in the western Gulf of Maine (Siceloff & Howell 2013), also encompassing diel and gender-based differences in behaviour (Dean et al. 2014). Experimental studies of Atlantic cod collected near Sambro, Nova Scotia, and kept in a 60 m^3 tank, after having ensured they were in spawning condition, also showed clear evidence of lekking and vertical separation of the sexes, with males closest to the bottom (Hutchings et al. 1999). At the other side of the North Atlantic, e.g. along the cod-abundant Norwegian coast, there is only sporadic documentation in the scientific literature of sex ratios and lekking behaviour in natural populations. Nordeide & Folstad (2000) suggested lekking to occur among spawning Northeast Arctic cod in the Lofoten area, and recently, ultrasonic telemetry was used to study how the environment influenced the depth-related behaviour of cod spawning at Austevoll, western Norway, a known spawning ground for Norwegian coastal cod (Meager et al. 2012).

The migratory Northeast Arctic cod (NEAC) and the more resident Norwegian coastal cod (NCC) represent 2 major and coexisting populations of Atlantic cod that spawn in northern Norway (e.g. Rollefsen 1934, Bergstad et al. 1987). They differ not only in migratory mode but also in settling regimes for 0-group juveniles: NEAC juveniles settle in deep water, and NCC juveniles settle in shallow water (Fevolden et al. 2012). The NEAC represents today the largest commercial cod stock in the North Atlantic. Its main spawning grounds are off the coast of northern Norway from Vestfjorden (Lofoten) to the relatively shallow banks off Troms and Finnmark counties, with a tendency towards a more northeasterly spawning in recent years due to climate warming (Sundby & Nakken 2008). The NCC stock, much smaller in size, has spawning grounds in part overlapping with those of NEAC, but also spawns at long-known local spawning sites within fjords (Jakobsen 1987). Whereas the NEAC larvae and 0-group juveniles drift northeastward with coastal currents and eventually up into their nursery grounds in the Barents Sea (Bergstad et al. 1987), NCC tend to remain within the fjords where they were spawned or at least show a high degree of homing to their own fjord (Skjæraasen et al. 2011).

Many means and methods have been used to separate these 2 stocks, from the earliest fishermen's categorization of the slim look of the long-distance swimmer NEAC versus the more stout appearance of the homebound NCC, via biometric markers such as otolith shape (Rollefsen 1934), to an array of molecular genetic markers (reviewed by Nordeide et al. 2011), and finally to the most recent studies using single nucleotide polymorphisms (SNPs) (Moen et al. 2008, Karlsen et al. 2014). One marker stands out as more successful in distinguishing the 2 stocks than others, namely the scnDNA locus coding for pantophysin (*Pan* I). Pantophysin was first described as a major synaptic vesicle protein (Haass et al.1996), but is also identified in non-neuronal tissues (e.g. Brooks et al. 2000), and thus does not necessarily act as a neurotransmitter. Its function has so far been poorly understood, but recent studies have revealed that the *Pan* I locus resides within the linkage group 1 in Atlantic cod (Borza et al. 2010, Hemmer-Hansen et al. 2013). This linkage group comprises several genes that show large divergence between NEAC and NCC, one of them being rhodopsin, whose variability is influenced by light conditions and seems to be strongly linked to *Pan* I (Andersen et al. 2015, Pampoulie et al. 2015).

NEAC and NCC were first included in a trans-Atlantic study using this locus (termed GM798 at the time) by Pogson et al. (1995), and shortly after in a comparison between cod caught in several fjords and those caught offshore in northern Norway (Fevolden & Pogson 1997; locus then termed *Syp* I). During the almost 2 decades that have since passed, a series of papers have included *Pan* I in comparative studies of these 2 stocks (see Fevolden et al. 2012 for the most recent overview). Two major features have emerged from these studies: (1) the 2-allelic class system of *Pan* I (Pogson & Fevolden 2003) is dominated in NCC by the *Pan* I^A class (≈80%) and in NEAC with correspondingly high or even higher frequencies of the *Pan* I^B allelic class (e.g. Sarvas & Fevolden 2005a, Fevolden et al. 2012), and (2) whereas the *Pan* I frequencies over the years are stable in NEAC, they tend to vary both over time and space in fjords, an attribute claimed to be due to variable intermingling of NEAC and NCC within the coastal/fjord system (Sarvas & Fevolden 2005a,b, Westgaard & Fevolden 2007).

During a research cruise to various spawning sites of coastal cod in 2002 we noticed an extreme skewed sex ratio of cod sampled with bottom trawls. One of these sites was Aursfjord in Malangen, just south of Tromsø. We decided to repeat sampling of cod at this site for the forthcoming years to see whether this sex skewness was consistent over years. In 2007 the catches had become very low due to a general decline in the size of the coastal stock over this period, and the yearly sampling was terminated. There were, however, signs of a gradual revitaliza-

tion of the coastal cod stock and the specific spawning site was revisited in 2013. For comparison, a second spawning site in the neighbouring Balsfjord was sampled in parallel with Malangen for the first 5 sampling years.

We believe observations of skewed sex ratios on Atlantic cod spawning grounds along the coast of Norway to be plentiful among fishermen, but since there is an apparent lack of documentation in the scientific literature, the first aim of this paper is to report extreme and stable male-skewed sex ratios at small inshore spawning sites stretching over an 11 yr (Malangen) or 5 yr (Balsfjord) sampling period. The second aim is to demonstrate an unusually stable frequency of the 2 *Pan* I allelic classes in cod spawning at one of these sites that is in close vicinity of spawning NEAC, providing clear evidence that this is a spawning site exclusively for NCC.

MATERIALS AND METHODS

Aursfjord in Malangen (69° 17.71′ N, 18° 38.90′ E) is located 30 nautical miles (nm) SW of Tromsø and 26 nm away from the open Atlantic Ocean (Fig. 1). The length of the trawling stretch was approximately 1.5 nm and the average depth is approximately 68 m. The Bergneset sampling site (69° 15.35′ N, 19° 22.35′ E) in the neighbouring Balsfjord is located 31 nm directly south of Tromsø, the trawl stretch was approximately 0.9 nm and the average depth is 90 m. Standard trawling time was set to 20 min at 2 knots using a bottom trawl with 50 m distance between the doors, an opening height of 8 m, and a cod-end mesh size of 38 mm. Length, weight, gutted weight, sex and maturity stage were recorded for all fish (n = 541 in Aursfjord, n = 454 at Bergneset). Only length was used as a measure of size herein. The maturity scale used was as follows: 1 = immature (no visible eggs or milk), 2 = mature (eggs and milk clearly visible), 3 = running (in spawning condition), and 4 = spent. The spawning season for cod in the survey area is known to stretch from February to May, with a peak in late March to early April (Falk-Petersen 1982).

To categorize the fish as either NCC or NEAC, in 6 of the 7 sampling years in Malangen and 3 of the 5 sampling years in Balsfjord, the fish (n = 428 and 300, respectively) were analyzed for variation at the locus coding for pantophysin (*Pan* I). DNA was extracted from ethanol-preserved gill tissue sampled from each fish onboard the ship immediately after each catch. DNA from samples up until 2007 was extracted by a modified salt lysis extraction method (Fevolden & Pogson 1997). DNA from the 2013 sample was isolated using the E-Z 96 Tissue DNA Kit (OMEGA Biotek) following the manufacturer's instructions. The 2-allelic-class (A and B) *Pan* I locus (Pogson & Fevolden 2003) is in itself an SNP, since a 1-nucleotide substitution decides the presence or absence of a *Dra-1* restriction site. The locus was analysed by a PCR-based method (Fevolden & Pogson 1997). The 2013 sample was analysed including *Pan* I on a multiplex of microsatellites and genotyped upon fragment analyses on an ABI 3130XL sequencer (Applied Biosystems) according to Stensvik et al. (2006).

Proportions of the 2 sexes were calculated as the number of individuals of each sex divided by the total number of all fish, and 95% confidence intervals of proportions were estimated according to Zar (1999). The Mann–Whitney *U*-test was used to compare 2-group samples. Relationships between fish length and different explanatory variables (sex, year, genotype) were estimated by general linear models (GLMs) in Systat (V. 13.1). Boxplots in Systat were used to describe length distributions in the various samples.

Allele frequencies, conformity to Hardy–Weinberg equilibrium, as well as observed and expected heterozygosities were calculated using Genepop 4.1.4 (Rousset 2008). Wherever multiple tests were performed, the significance level was adjusted according to the false discovery rate (FDR) approach (Benjamini &

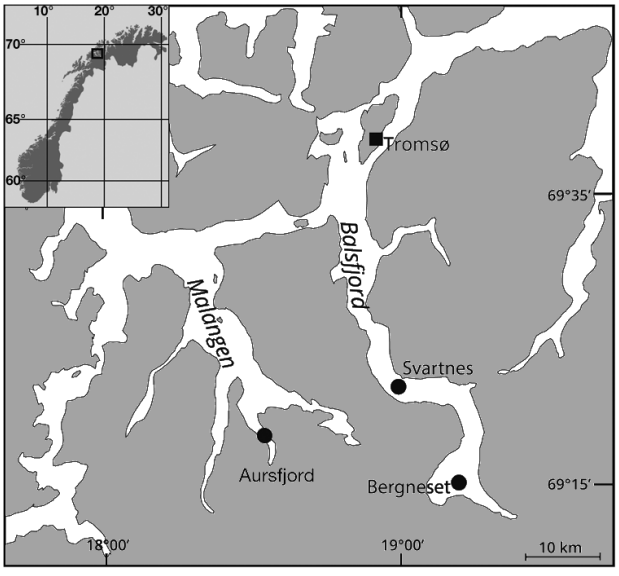

Fig. 1. Locations of the 2 surveyed *Gadus morhua* spawning grounds in northern Norway: Aursfjord in Malangen, and Bergneset in Balsfjord. 'Svartnes in Balsfjord is a trawling location outside the spawning ground where sex ratios were also measured

Yekutieli 2001.). Estimates of F_{ST} as a measure of genetic divergence between pairs of samples along with tests for allele frequency differences (*G*-tests) were carried out using Genepop 4.1.4 (Rousset 2008). Possible variation among *Pan* I genotypes in maturity was tested by Chi-square tests.

RESULTS

Malangen Aursfjord

Pronounced male-biased sex ratios were observed in all 7 sampling years (Table 1). In 6 of those years, between 8 and 9 out of 10 fish were males. The least skewed sex ratio, 6.5 males out of 10, was observed in the year with the earliest sampling date (2004, 23 February). The majority of the fish were sexually mature in all 7 survey years. Those still immature (n = 24 in total) and unlikely to spawn that season did not display male-skewed sex ratios (average proportion of males = 0.46), making the proportion of males among mature fish in some years even slightly higher than for the total sample set (Fig. 2). The highest proportion of immature fish was also in the year with the earliest sampling date (2004; Table 1). The highest proportion of fish in spawning mode or spent (maturity stages 3 and 4) was in the year with the latest sampling date (2013, 22 April).

The size of the fish caught at this spawning site varied among sampling years for both sexes (Fig. 3), but did not correlate with different sampling dates each year. In 5 of 7 years females were larger than males (Table 1), for mature fish significantly so only in 2002 and 2003 (Mann-Whitney *U*-test, $p < 0.01$). In 2013, the males were significantly longer than the 5 females encountered that year (Mann-Whitney *U*-test, $p = 0.04$). A GLM indicated that length varied significantly with sex, year, and the interaction of sex and year (Table 2). There was no consistent sex difference in maturity, only that females found in 2004 ($\chi^2 = 7.00$, df = 1, $p = 0.008$) and the mere 5 caught in 2013 ($\chi^2 = 11.8$, df = 2, $p = 0.003$) were less mature than their male counterparts.

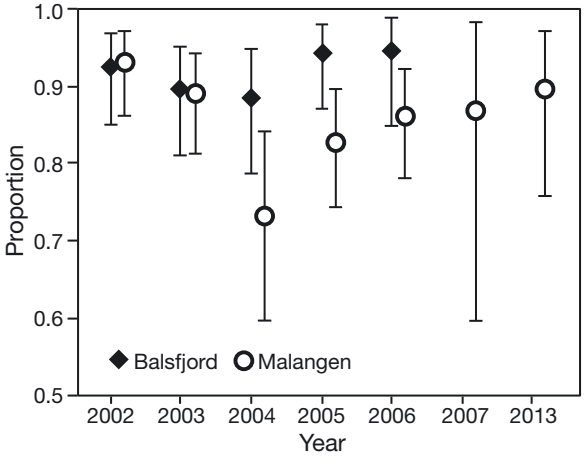

Fig. 2. *Gadus morhua*. Proportion of males among sexually mature fish at the spawning grounds in Malangen and Balsfjord in the different years of sampling. Error bars show 95% confidence intervals

Pan I allele frequencies were extremely stable throughout the sampling period, with the *Pan* I^A

Table 1. *Gadus morhua*. Sample details. Total number of specimens analysed (N) and percentage of males (M%) in each haul. Also shown are mean (±SD) length, and percentage representing the different maturity stages (1, 2, 3, and 4) for each sex

Date (d/mo/yr)	N	M%	Length (cm)		Maturity stage (%)							
					Females				Males			
			Females	Males	1	2	3	4	1	2	3	4
Malangen												
04/04/2002	100	93.0	76.3 (22.8)	57.8 (11.8)	0.0	100.0	0.0	0.0	0.0	98.9	1.1	0.0
13/03/2003	100	89.0	74.4 (15.4)	61.6 (10.0)	0.0	63.6	36.4	0.0	0.0	66.3	33.7	0.0
23/02/2004	72	65.3	61.6 (17.1)	55.7 (9.3)	40.0	40.0	20.0	0.0	12.8	85.1	2.1	0.0
16/03/2005	110	82.7	59.1 (10.4)	56.3 (10.3)	5.3	57.9	36.8	0.0	4.4	72.5	23.1	0.0
24/03/2006	104	87.5	61.4 (15.6)	58.0 (8.2)	0.0	71.4	28.6	0.0	1.1	49.4	48.3	1.1
16/04/2007	16	81.3	52.3 (19.6)	57.2 (9.4)	33.3	33.3	0.0	33.3	0.0	38.5	61.5	0.0
22/04/2013	40	87.5	53.6 (16.7)	68.1 (10.0)	20.0	40.0	40.0	0.0	0.0	8.6	82.9	8.6
Balsfjord												
02/04/2002	100	93.0	67.6 (19.4)	57.7 (10.2)	0.0	100.0	0.0	0.0	8.6	91.4	0.0	0.0
14/03/2003	100	88.0	57.6 (27.7)	52.6 (13.2)	25.0	33.3	41.7	0.0	12.5	37.5	50.0	0.0
05/03/2004	100	80.0	48.9 (17.0)	50.6 (15.2)	60.0	40.0	0.0	0.0	22.5	57.5	20.0	0.0
17/03/2005	96	90.6	48.0 (8.7)	52.2 (8.4)	44.4	33.3	22.2	0.0	5.7	80.5	13.8	0.0
20/03/2006	58	94.8	56.7 (5.7)	57.7 (11.9)	0.0	33.3	66.7	0.0	5.5	60.0	34.5	0.0

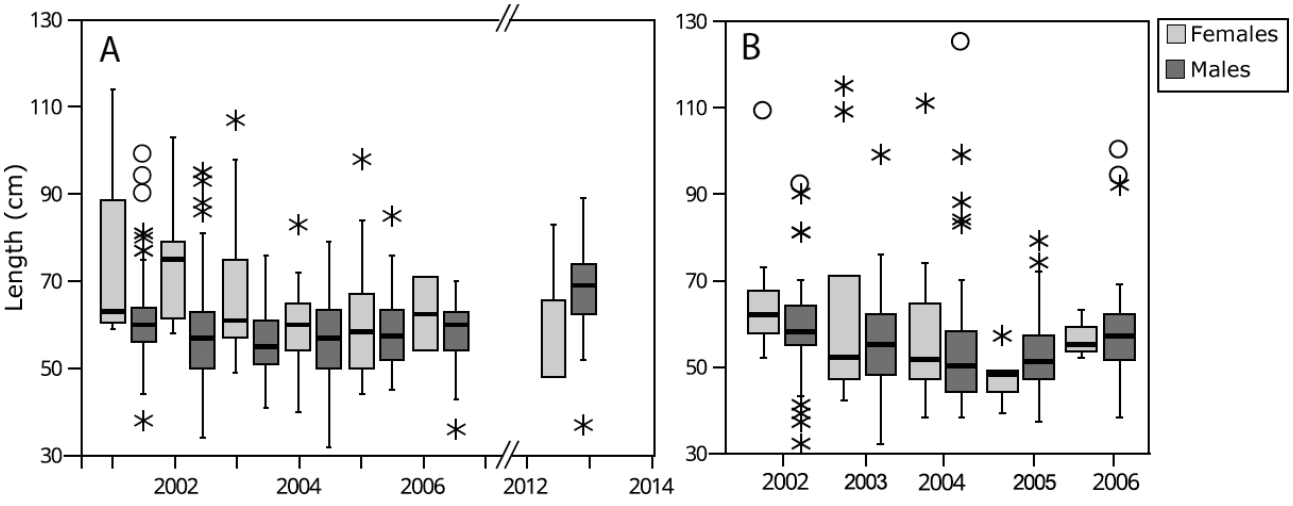

Fig. 3. *Gadus morhua*. Box plots of length of sexually mature cod at the 2 spawning grounds: (A) females and males in Malangen, (B) females and males in Balsfjord. Horizontal bars mark the median and the box shows the range of the central 50% of the values. The vertical lines (whiskers) show values up to 1.5× the height of the box. Asterisks show values that fall outside the whiskers and within 3× the height of the box, and circles show values further away from the median

allelic class varying slightly around 90% and that of the *Pan* I^B allelic class slightly around 10% (Table 3). Observed heterozygosities varied around 20% and were in no cases significantly different from those expected under Hardy-Weinberg equilibrium (Table 3). Pairwise F_{ST} estimates for the Malangen samples (Table 4) were insignificant, with the one exception of the comparison between the 2003 and 2006 samples. The 2 *Pan* I^{BB} homozygotes encountered in the 2006 sample contributed to this; when correcting for multiple tests according to the FDR approach, this pairwise comparison was no longer significant.

With the near absence of *Pan* I^{BB} homozygotes (only 3 were observed in total; Table 3), estimates of possible correlations between genotypes and size were carried out only for the *Pan* I^{AA} and *Pan* I^{AB} genotypes. For mature males (maturity stage > 1), *Pan* I^{AB} heterozygotes were significantly longer than *Pan* I^{AA} homozygotes (GLM: length = constant + year + *Pan* I genotype; $F = 8.6$, df = 1, 260, p = 0.004), but for mature females, length differences between the 2 genotypes were not significant. For the mature males and females together, *Pan* I^{AB} heterozygotes were still significantly longer than *Pan* I^{AA}

Table 2. General linear model (Type III) for length of mature cod *Gadus morhua* (maturity stage > 1) in Malangen: length = constant + sex + year + sex × year. Sum of squares (SS), degrees of freedom (df), mean squares (MS), Fisher *F*-statistic, and p-values are given

Source	SS	df	MS	*F*	p
Sex	1400.38	1	1400.38	11.91	0.00060
Year	2984.29	6	497.38	4.23	0.00036
Sex × Year	2998.89	6	499.81	4.25	0.00034
Error	59131.53	503	117.55		

Table 3. *Gadus morhua.* Basic genetic data for the samples analysed for the *Pan* I locus: genotype counts, frequencies of the 2 different allelic classes, observed (H_o) and expected (H_e) heterozygosities, inbreeding index (F_{IS}), and p-values for conformity to Hardy–Weinberg equilibrium

Date	*Pan* I^{AA}	*Pan* I^{AB}	*Pan* I^{BB}	*Pan* I^{A}	*Pan* I^{B}	H_o	H_e	F_{IS}	p
Malangen									
04/04/2002	77	22	0	0.89	0.11	0.21	0.19	−0.11	0.59
13/03/2003	83	14	0	0.93	0.07	0.13	0.12	−0.06	1.00
23/02/2004	56	16	0	0.89	0.11	0.22	0.20	−0.12	0.59
24/03/2006	80	22	2	0.88	0.12	0.21	0.22	0.04	0.65
16/04/2007	12	4	0	0.88	0.12	0.25	0.23	−0.11	1.00
22/04/2013	32	7	1	0.93	0.07	0.15	0.14	−0.07	1.00
Balsfjord									
02/04/2002	35	43	7	0.67	0.33	0.51	0.45	−0.13	0.33
14/03/2003	54	37	3	0.78	0.22	0.39	0.35	−0.11	0.39
05/03/2004	50	42	7	0.72	0.28	0.42	0.41	−0.04	0.81

Table 4. Estimated F_{ST} values (below the diagonal) for the *Pan* I locus in a pairwise comparison of cod *Gadus morhua* samples from Malangen (Mal) and Balsfjord (Bal) in the various years (6 from Malangen, 3 from Balsfjord). The corresponding p-values from the exact *G*-test (Rousset 2008) are above the diagonal. Bold values represent uncorrected significant values, while bold values with asterisks represent significant values after false discovery rate correction. *p < 0.01, **p < 0.05, ***p < 0.001

	Mal '02	Mal '03	Mal '04	Mal '06	Mal '07	Mal '13	Bal '02	Bal '03	Bal '04
Mal '02	–	0.145	1.000	0.642	0.758	0.511	**0.000*** **	**0.003** **	**0.000*** **
Mal '03	0.0076	–	0.160	**0.039**	0.256	0.791	**0.000*** **	**0.000*** **	**0.000*** **
Mal '04	−0.0052	0.0099	–	0.742	1.000	0.485	**0.000*** **	**0.008***	**0.000*** **
Mal '06	−0.0030	0.0175	−0.0049	–	1.000	0.294	**0.000*** **	**0.011***	**0.000*** **
Mal '07	−0.0145	0.0124	−0.0162	−0.0187	–	0.467	**0.020***	0.246	0.058
Mal '13	−0.0024	−0.0071	−0.0014	0.0038	−0.0048	–	**0.000*** **	**0.003** **	**0.000*** **
Bal '02	0.1417	0.2113	0.1266	0.1167	0.0832	0.1565	–	**0.019***	0.310
Bal '03	0.0450	0.0966	0.0378	0.0291	0.0130	0.0646	0.0258	–	0.204
Bal '04	0.0907	0.1513	0.0797	0.0699	0.0456	0.1088	0.0014	0.0044	–

homozygotes (GLM: F = 5.9, df = 1, 299, p = 0.016; Fig. 4). Immature cod displayed no significant effect of *Pan* I genotype on fish length. Chi-square tests performed for each sex showed no significant association between maturity rate and *Pan* I genotype for either males or females (p > 0.05).

Balsfjord Bergneset

On average, 9 of 10 fish caught at Bergneset were males, both for the total sample set (Table 1) and for the mature fish only (Fig. 2). The number of immature fish here was higher than in Malangen (n = 62), and samples exhibited higher male-skewed sex proportions than the Malangen immatures (0.69 versus 0.46). All samples but one from early April (2002) were taken in March, and as in Malangen, the proportion of immature fish was highest at the earliest sampling date (5 March 2004). Also as in Malangen, this earliest sample was the one with the highest proportion of females (20%), which displayed a significantly lower maturity rate than the males that year (χ^2 = 12.4, df = 2, p = 0.002), as did females in 2005 (χ^2 = 14.4, df = 2, p = 0.0007). In the 3 other survey years there was no gender-specific difference in maturity rate.

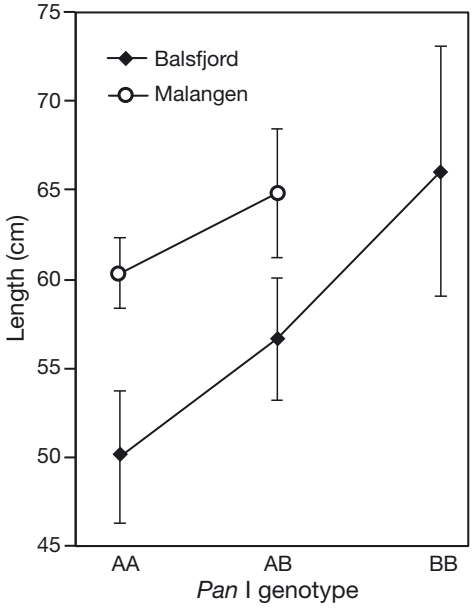

Fig. 4. *Gadus morhua*. Least square mean lengths for the different *Pan* I genotypes for sexually mature cod (both sexes) from Malangen and Balsfjord based on a general linear model for length. Error bars show 95% confidence intervals

As in Malangen, differences in size among survey years were evident for both sexes (Fig. 3), but no significant difference in size between sexes was observed for single years (Mann–Whitney *U*-test, p > 0.05,). When all Balsfjord samples were considered together, however, a significant sex influence on length was revealed, with females being longer than males (GLM: length = constant + year + sex; F = 4.8, df = 1.384, p = 0.029).

The Balsfjord samples differed from those of Malangen in *Pan* I allele frequencies. The proportion of the *Pan* I^A allele was lower and varied between 67 and 78% for the 3 years for which *Pan* I data are available (Table 3). Observed heterozygosities were higher in Balsfjord than in Malangen, but none of the samples were out of Hardy–Weinberg equilibrium. Pairwise F_{ST} estimates were significant when the 2002 and 2003 samples were compared, and 13 of 15 comparisons between Balsfjord and Malangen samples were significant (Table 4).

As to size–genotype interactions, length of mature males varied significantly among *Pan* I genotypes (GLM: length = constant + year + *Pan* I-genotype; F =

19.9, df = 2, 204, p = 1×10^{-7}). *Pan* I^{AA} homozygotes were smallest, *Pan* I^{AB} heterozygotes were intermediate in length and the *Pan* I^{BB} homozygotes were the longest. Length of mature female cod did not vary with *Pan* I genotype, but when males and females were pooled there was again a significant effect of *Pan* I genotype on length (GLM: $F = 13.55$, df = 2, 225, p = 3×10^{-6}; Fig. 4). Also for the immature cod, *Pan* I^{BB} homozygotes were longer than the other genotypes (GLM: $F = 3.94$, df = 2, 44, p = 0.026). There was no indication of genotype–maturity interactions in Balsfjord.

DISCUSSION

Two major findings emerged from this study: (1) large male-biased sex ratios, consistent over years, occur at small-scale inshore spawning grounds for Atlantic cod in northern Norway, and (2) the cod spawning at one of the 2 sites studied herein, Aursfjord in Malangen, are exclusively of the Norwegian coastal cod type. The topography of this spawning ground comprises a relatively flat bottom with an average depth of approximately 68 m surrounded by upward slopes of various slope angle on all sides. In a study of spawning cod in Vestfjorden (Lofoten area), it was suggested that female cod dived into aggregations of males when they were ready to spawn and then disappeared again after having spawned (Nordeide & Folstad 2000). This is similar to what we believe happens in Aursfjord, where the females reside somewhere up in the slopes, and dive into the bottom shoaling males on an individual basis, not as promiscuous group spawners.

A somewhat different scenario was described on spawning grounds at the Grand Bank and Scotian Shelf/Bay of Fundy. There, both male- and female-dominated shoals of Atlantic cod were observed, the females in deeper water than the males (Morgan & Trippel 1996). More females were in spawning condition when caught in the male-dominated shoals. In the female-dominated shoals, more of them were spent, making the authors suggest that after having visited the male aggregations and spawned, females return to deeper and warmer water.

Where the females go after having spawned in Aursfjord is unclear. Since the Atlantic cod is a batch spawner, the females may temporarily remain in the slopes above the flat bottom, which would enable them to mate repeatedly with additional males, supposedly displaying a female controlled mate choice, as described by Hutchings et al. (1999). After the last dive into the male shoal, the females may eventually cross the shallower areas and escape into deeper water in the main Malangen fjord. However, during the 15 years studying Atlantic cod in this particular area, we have never encountered female-dominated shoals in the vicinity of Aursfjord, either in the spawning season or at other times of the year. In Balsfjord, in contrast, where sex ratios at the Bergneset spawning site were just as profoundly skewed as in Aursfjord, a nearby trawling ground (Svartneset; Fig. 1) displayed female proportions varying between 51 and 76% in 5 sampling years (2003–2006 and 2013; authors' unpubl. data). This site is not considered a spawning area and is much deeper (185 m) than Bergneset. Thus, such localities could represent a post-spawning escape for spent females.

The fact that females in 5 of 7 survey years in Aursfjord were larger on average than the males could of course indicate that the older females get ready to spawn earlier than the younger ones, which is consistent with reports from coastal Newfoundland waters, where older females spawn earlier but also later than younger females (Lawson & Rose 2000). The measures of maturity stage during the present survey showed no conclusive trend other than that the least mature fish were encountered in the years with the earliest catch, and the highest proportion of fish in spawning condition, or already spent, were encountered in the years with the latest catch. Spawning of Atlantic cod in these waters is known to continue at least into May (Falk-Petersen 1982). The least skewed sex ratio for both fjords was also at the earliest sampling dates, suggesting the presence of individuals in pre-spawning condition, when the sexes have yet not fully segregated.

In a large-scale survey of a heavily exploited stock of cod in the Irish Sea, male-skewed sex ratios were observed on the spawning grounds, whereas commercial midwater trawl catches of cod in the same area had equal or even female-biased sex ratios, suggesting that males and females have different vertical migration behaviour (Armstrong et al. 2004). From Placenthia Bay, Newfoundland, male-skewed sex ratios were observed on a small-scale spawning ground (25 km^2) in 5 of 6 survey years early in the season (Windle & Rose 2007). The proportion of spawning females and spent males were significantly higher in the male-dominated fishing sets, whereas sets with equal sex ratios had significantly higher proportions of immature males and spent females. Those surveys provide solid evidence consistent with the hypothesis that there is lekking behaviour in spawning Atlantic cod on both large-scale and small-

scale spawning grounds. The present study adds to this by demonstrating that this is indeed true also for what might be called local micro-scale spawning grounds.

The ability of the *Pan* I locus to distinguish between the 2 major stocks of Atlantic cod that occur in coastal and offshore waters in northern Norway was first demonstrated 2 decades ago (Pogson et al. 1995, Fevolden & Pogson 1997). In the early days of using this locus as a genetic marker some debate arose due to its selective mode, but this was before the advantage of using markers with selective adaptability in revealing population divergences was fully acknowledged.

The more stationary and smaller NCC stock has high frequencies (≈80%) of the *Pan* I^{A} allelic class, whereas the migratory and far larger NEAC stock possesses very high frequencies of the *Pan* I^{B} allele class (≥90%) (e.g. Sarvas & Fevolden 2005a, Fevolden et al. 2012). A complicating factor in nearshore waters in northern Norway, even within fjords, is that the migratory NEAC may intermingle with NCC, giving the impression that populations with intermediate *Pan* I allele frequencies exist. This possibility cannot be dismissed, but the major reason for variable *Pan* I allele frequencies in catches of cod from these waters, both spatially and temporally, is probably attributable to varying proportions of NEAC and NCC in the catches. This holds true for both adult fish (Sarvas & Fevolden 2005a,b, Westgaard & Fevolden 2007) and 0-group juveniles (Fevolden et al. 2012).

The Balsfjord population has previously been shown to exhibit temporal variation in *Pan* I allele frequencies (e.g. Sarvas & Fevolden 2005a). The innermost known spawning site of that fjord, Bergneset, displayed sex ratios that were at least as skewed as in Malangen (9 of 10 fish on average over all study years were males) but the *Pan* I^{A} frequencies were lower, down to 57% one year, as compared to the steady 90% in Malangen. It is difficult to say whether the *Pan* I^{BB} genotypes, typical of NEAC, caught in Balsfjord were actually potential spawners or were accidental roamers that just happened to be there at the time of trawling. Based on allele frequencies and Hardy–Weinberg expectations of genotype distribution (see Table 3), there is no sign of a Wahlund effect (mixing of populations with different allele frequencies) in the present Balsfjord samples. No difference among genotypes in maturity stage was observed. The fact that the *Pan* I^{BB} homozygotes were larger than the other genotypes could indicate that they belong to a different population than the local one. More intriguing is the observation that the heterozygotes both in Malangen and Balsfjord also were larger than the *Pan* I^{AA} homozygotes, albeit in line with previous observations both for adult (Fevolden & Pogson 1995) and juvenile (Fevolden et al. 2012) cod, providing conditional support for a heterosis effect.

It has been reported that male cod that are more than 25% longer than spawning females have a lower probability of fertilizing eggs compared to males more similar in size to the females (Rakitin et al. 2001, Bekkevold et al. 2002). The mean differences in length between females and males found in the present study were smaller than this, and females were often larger than males, suggesting that sex-related size differences were unlikely to act as spawning barriers within the present coastal cod populations. However, the NEAC becomes sexually mature at a much larger size (ca. 70–80 cm) than the NCC (45–50 cm) (Berg & Pedersen, 2001), and these size differences may limit interbreeding between the two. It is well known that the NCC stock will also spawn at some of the larger offshore spawning grounds for the NEAC stock in northern Norway, e.g. Lofoten, but there is limited evidence that the 2 groups interbreed. Spawning of NEAC inside fjords remains to be documented. The mere presence of cod with NEAC signatures at the *Pan* I locus in a fjord sample was suggested above to be the most likely reason for variable *Pan* I allele frequencies. The divergent and variable *Pan* I allele frequencies in the present Balsfjord samples cannot, however, be taken as unambiguous evidence for mechanical mixing of populations. In contrast, the extremely stable and high *Pan* I^{A} frequencies in all 7 survey years in Aursfjord Malangen indisputably demonstrate absence of population mixing at that spawning ground. So, in conclusion, this survey has shown that Atlantic cod spawning at a very small-scale spawning ground, and being of the NCC gene pool, employ lekking behaviour at spawning that may be very comparable to that of large commercial stocks of cod spawning at offshore banks and in seas. Thus, spawning behaviour of cod may represent an adaptive feature that has evolved across populations, in contrast to settling regime and migratory behaviour, which differ between populations of high *Pan* I^{A} or *Pan* I^{B} frequencies both in Norwegian (Sarvas & Fevolden 2005a, Fevolden et al. 2012) and Iceland/Greenland waters (Pampoulie et al. 2008, 2011). Although spawning behaviour seems to be similar across populations dominated by each of the 2 *Pan* I allelic classes, there is no evidence to suggest when this feature evolved

in the 2 million year old (Pogson & Mesa 2004) history of the 2 *Pan* I alleles. The adaptive success of the evolvement has, however, been upheld into modern times.

The different spatial behaviour patterns of males and females at spawning grounds combined with the recognized different vulnerability of sexes in the various fishing gears used at spawning grounds (Rollefsen 1953) may have unforeseen management consequences. Disruption of Atlantic cod spawning aggregations has been reported from gill-net fisheries (Dean et al. 2012), and it seems quite obvious that extensive use of any type of bottom-based fishing gear targeting small-scale spawning aggregations such as those studied herein would lead to a male-biased exploitation and possible depletion of the local population.

Acknowledgements. This study was supported by funding from the Norwegian Research Council, plus internal funding from UiT, the Arctic University of Norway. We thank the crew onboard the RV 'Johan Ruud' for professional assistance in sampling cod, and T. Hanebrekke, F. Tonning, and L. Nunez Aguillero for excellent lab assistance.

LITERATURE CITED

Andersen Ø, Johnsen H, De Rosa MC, Præbel K and others (in press) (2015) Evolutionary history and adaptive significance of the polymorphic Pan I in migratory and stationary populations of Atlantic cod (*Gadus morhua*). Mar Genomics, doi:10.1016/j.margen.2015.03.009

Armstrong MJ, Gerritsen HD, Allen M, McCurdy WJ, Peel JDA (2004) Variability in maturity and growth in a heavily exploited stock: cod (*Gadus morhua* L.) in the Irish Sea. ICES J Mar Sci 61:98–112

Bekkevold D, Hansen MM, Loeschcke V (2002) Male reproductive competition in spawning aggregations of cod (*Gadus morhua*, L.). Mol Ecol 11:91–102

Benjamini Y, Yekutieli D (2001) The control of the false discovery rate in multiple testing under dependency. Ann Stat 29:1165–1188

Berg E, Pedersen T (2001) Variability in recruitment, growth and sexual maturity of coastal cod (*Gadus morhua* L.) in a fjord system in northern Norway. Fish Res 52:179–189

Bergstad OA, Jørgensen T, Dragesund O (1987) Life history and ecology of the gadoid resources of the Barents Sea. Fish Res 5:119–161

Borza T, Higgins B, Simpson G, Bowman S (2010) Integrating the markers Pan I and haemoglobin with the genetic linkage map of Atlantic cod (*Gadus morhua*). BMC Res Notes 3:261

Brooks CC, Scherer PE, Cleveland K, Whittermore JL, Lodish HF, Cheatham BT (2000) Pantophysin is a phosphoprotein component of adipocyte transport vesicles and associates with GLUT4-containing vesicles. J Biol Chem 275:2029–2036

Dean MJ, Hoffmann WS, Armstrong MP (2012) Disruption of an Atlantic cod spawning aggregation resulting from the opening of a direct gill-net fishery. N Am J Fish Manage 32:124–134

Dean MJ, Hoffman WS, Zemeckis DR, Armstrong MP (2014) Fine-scale diel and gender-based patterns in behaviour of Atlantic cod (*Gadus morhua*) on a spawning ground in the Western Gulf of Maine. ICES J Mar Sci 71:1474–1489

Falk-Petersen IB (1982) Ecological investigations on the zooplankton community of Balsfjord, northern Norway. Planktonic eggs and larvae from March 1978 to February 1980. Sarsia 67:69–78

Fevolden SE, Pogson GH (1995) Differences in nuclear DNA RFLPs between the Norwegian coastal and the North-East Arctic population of Atlantic cod. In: Skjoldal HR, Hopkins C, Erikstad KE, Leinaas HP (eds) Ecology of fjords and coastal waters. Elsevier Science B.V., Amsterdam, p 403–415

Fevolden SE, Pogson GH (1997) Genetic divergence at the synaptophysin (*Syp* I) locus among Norwegian coastal and north-east Arctic populations of Atlantic cod. J Fish Biol 51:895–908

Fevolden SE, Westgaard JI, Pedersen T, Præbel K (2012) Settling-depth vs. genotype and size vs. genotype correlations at the *Pan* I locus in 0-group Atlantic cod *Gadus morhua*. Mar Ecol Prog Ser 468:267–278

Haass NK, Kartenbeck J, Leube RE (1996) Pantophysin is a ubiquitously expressed synaptophysin homologue and defines constitutive transport vesicles. J Cell Biol 134: 731–746

Hemmer-Hansen J, Nielsen EE, Therkildsen NO, Taylor MI and others (2013) FISHPOPTRACE Consortium A genomic island linked to ecotype divergence in Atlantic cod. Mol Ecol 22:2653–2667

Hutchings JA, Bishop TD, McGregor-Shaw CR (1999) Spawning behaviour of Atlantic cod, *Gadus morhua*: evidence of mate competition and mate choice in a broadcast spawner. Can J Fish Aquat Sci 56:97–104

Jakobsen T (1987) Coastal cod in Northern Norway. Fish Res 5:223–234

Karlsen BO, Emblem A, Jorgensen TE, Kilingan KA, Nordeide JT, Moum T, Johansen SD (2014) Mitogenome sequence variation in migratory and stationary ecotypes of North-east Atlantic cod. Mar Genomics 15:103–108

Lawson GL, Rose GA (2000) Small-scale spatial and temporal patterns in spawning of Atlantic cod (*Gadus morhua*) in coastal Newfoundland waters. Can J Fish Aquat Sci 57:1011–1024

Meager JJ, Skjæraasen JE, Karlsen Ø, Løkkeborg S and others (2012) Environmental regulation of individual depth on a cod spawning ground. Aquat Biol 17:211–221

Moen T, Hayes B, Nilsen F, Delghandi M and others (2008) Identification and characterisation of novel SNP markers in Atlantic cod: evidence for directional selection. BMC Genet 9:18

Morgan MJ, Trippel EA (1996) Skewed sex ratios in spawning shoals of Atlantic cod (*Gadus morhua*). ICES J Mar Sci 53:820–826

Nordeide JT, Folstad I (2000) Is cod lekking or a promiscuous group spawner? Fish Fish 1:90–93

Nordeide JT, Johansen SD, Jørgensen TE, Karlsen BO, Moum T (2011) Population connectivity among migratory and stationary cod *Gadus morhua* in the Northeast Atlantic — A review of 80 years of study. Mar Ecol Prog Ser 435:269–283

Pampoulie C, Jakobsdóttir KB, Marteinsdóttir G, Thorsteinsson V (2008) Are vertical behaviour patterns related to

the pantophysin locus in the Atlantic cod (*Gadus morhua* L.). Behav Genet 38:76–81

Pampoulie C, Daníelsdottír AK, Storr-Paulsen M, Hovgård H, Hjörleifsson E, Steinarsson BÆ (2011) Neutral and nonneutral genetic markers revealed the presence of inshore and offshore stocks components of Atlantic cod in Greenland waters. Trans Am Fish Soc 140:307–319

Pampoulie C, Skirnisdottir S, Star B, Jentoft S, and others (2015) Rhodopsin gene polymorphism associated with divergent light environments in Atlantic cod. Behav Genet 45:236–244

Pogson GH, Fevolden SE (2003) Natural selection and the genetic differentiation of coastal and Arctic populations of the Atlantic cod in northern Norway: a test involving nucleotide sequence variation at the pantophysin (*Pan* I) locus. Mol Ecol 12:63–74

Pogson GH, Mesa KA (2004) Positive Darwinian selection at the pantophysin (*Pan* I) locus in marine gadid fishes. Mol Biol Evol 21:65–75

Pogson GH, Mesa KH, Boutilier RG (1995) Genetic population structure and gene flow in the Atlantic cod *Gadus morhua*: a comparison of allozyme and nuclear RFLP loci. Genetics 139:375–385

Rakitin A, Ferguson MM, Trippel EA (2001) Male reproductive success and body size in Atlantic cod *Gadus morhua* L. Mar Biol 138:1077–1085

Robichaud D, Rose GA (2003) Sex differences in cod residency on a spawning ground. Fish Res 60:33–43

Rollefsen G (1934) The cod otolith as a guide to race, sexual development and mortality. Rapp P-V Reùn Cons Int Explor Mer 88:1–15

Rollefsen G (1953) The selectivity of different fishing gears used in Lofoten. J Cons Int Explor Mer 19:191–194

Rousset F (2008) Genepop`007: a complete re-implementation of the Genepop software for Windows and Linux. Mol Ecol Resour 8:103–106

Sarvas TH, Fevolden SE (2005a) Pantophysin (*Pan* I) locus divergence between inshore *vs* offshore and northern vs southern populations of Atlantic cod in the NE Atlantic. J Fish Biol 67:444–469

Sarvas TH, Fevolden SE (2005b) The scnDNA locus *Pan* I reveals concurrent presence of different populations of Atlantic cod (*Gadus morhua* L.) within a single fjord. Fish Res 76:307–316

Siceloff L, Howell WH (2013) Fine-scale temporal and spatial distributions of Atlantic cod (*Gadus morhua*) on a western Gulf of Maine spawning ground. Fish Res 141: 31–43

Skjæraasen JE, Meager JJ, Karlsen Ø, Hutchings JA, Fernø A (2011) Extreme spawning-site fidelity in Atlantic cod. ICES J Mar Sci 68:1472–1477

Stensvik J, Wesmajervi MS, Damsgard B, Delghandi M (2006) Genotyping of pantophysin (*Pan* I) of Atlantic cod (*Gadus morhua* L.) by allele-specific PCR. Mol Ecol Notes 6:272–275

Sundby S, Nakken O (2008) Spatial shifts in spawning habitats of Arcto-Norwegian cod related to multidecadal climate oscillations and climate change. ICES J Mar Sci 65:953–962

Westgaard JI, Fevolden SE (2007) Atlantic cod (*Gadus morhua* L.) in inner and outer coastal zones of northern Norway display divergent genetic signature at non-neutral loci. Fish Res 85:306–315

Windle MJS, Rose GA (2007) Do cod form spawning leks? Evidence from a Newfoundland spawning ground. Mar Biol 150:671–680

Zar JH (1999) Biostatistical analysis. Prentice Hall, Upper Saddle River, NJ

Editorial responsibility: Thavamani Pandian, Tamilnadu, India

Submitted: December 22, 2014; Accepted: April 15, 2015
Proofs received from author(s): May 12, 2015