Fine-scale spatial and temporal genetic structure of Atlantic cod off the Atlantic coast of the USA

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ABSTRACT: Atlantic cod *Gadus morhua* in US waters are currently managed as 2 stocks: (1) a Gulf of Maine stock and (2) a Georges Bank and south stock. This designation is decades old and warrants re-evaluation in light of concerns that fisheries management units may not reflect biologically meaningful population units. In this study, we used 10 microsatellite loci, the *Pan*I locus, and 5 single nucleotide polymorphism markers to characterize the population genetic structure of cod in US waters. We found significant differentiation among temporally and spatially divergent populations of cod (global F_{ST} = 0.0044), primarily stemming from 2 potentially non-neutral loci, and evidence for a population structure that strongly contradicts the current 2-stock management model. This genetic structure was stable over a 5 yr period. Our results indicate that cod in US waters are broadly structured into 3 groups: (1) a northern spring-spawning coastal complex in the Gulf of Maine (GOM), (2) a southern complex consisting of winter-spawning inshore GOM, offshore GOM and sites south of Cape Cod, Massachusetts, and (3) a Georges Bank population. The strongest differentiation occurs between populations in the northern and southern complex (mean F_{ST} = 0.0085), some of which spawn in the same bays in different seasons. By means of mixture analysis, young-of-the-year fish sampled on juvenile nurseries were assigned to the spawning complex of their origin. Our findings contribute to a growing body of knowledge that Atlantic cod and other marine fish populations are structured on a finer scale than previously thought and that this structure supports biocomplexity and locally adapted populations. As such, it may be warranted to re-evaluate current management units and tailor management plans toward this finer scale.

KEY WORDS: Atlantic cod · Microsatellite DNA · Single nucleotide polymorphism · Population genetic structure · Stock identification · *Gadus morhua*

INTRODUCTION

Marine fish populations have long been viewed as demographically open with high connectivity, owing to vagile, pelagic larval stages and high adult migratory potential. This classical view of marine fish species (Hauser & Carvalho 2008) was supported by tagging studies that demonstrated long distance migrations (Templeman 1974) and by early genetic studies that revealed high levels of gene flow (Ward et al. 1994). This perspective influenced management regimes, such that many marine fisheries are managed as broadly distributed, panmictic populations (Pampoulie et al. 2006). Overwhelming evidence now suggests that, rather than being demographically open, many marine species exhibit population subdivision on fine geographic and temporal scales (reviewed in Hauser & Carvalho 2008). A growing body of literature points to

the importance of processes that limit dispersal and promote self-replenishment of local populations, such as sedentary adult life history strategies (Robichaud & Rose 2004, Howell et al. 2008), spawning site fidelity (Taggart 1997), natal homing (Thorrold et al. 2001, Svedäng et al. 2007), egg and larval retention (Jones et al. 1999, 2005, Knutsen et al. 2007, Bradbury et al. 2008) and local adaptation (McIntyre & Hutchings 2003, Conover et al. 2006, Hutchings et al. 2007). The implications of such fine-scale population structure are important for effective management (Palumbi 2003, Bradbury et al. 2008, Reiss et al. 2009).

One marine fish species known to exhibit fine-scale population structure in parts of its distribution is Atlantic cod *Gadus morhua* L. (see reviews in Wirgin & Waldman 2005 and Hauser & Carvalho 2008). Atlantic cod is one of the most commercially important marine fishes in the world and comprises a principal component of the northeastern US groundfish assemblage. Cod are distributed throughout the temperate continental shelf waters of both the northwest and northeast Atlantic Ocean (Collette & Klein-MacPhee 2002). This species was once one of the most plentiful food fishes in the Gulf of Maine and has been a mainstay of fisheries since the 17th century (Mayo & Col 2006). The high desirability of cod led to dramatic overharvesting and significant decline of most stocks across its range (Collette & Klein-MacPhee 2002). There is growing concern that lack of recovery of cod stocks may be hampered by flawed management regimes that fail to recognize biologically meaningful population units (Smedbol & Stephenson 2001, Pampoulie et al. 2006). While cod population structure has been the subject of extensive study in the northeast Atlantic (e.g. Hutchinson et al. 2001, Knutsen et al. 2003, 2007, Nielsen et al. 2003, Jorde et al. 2007) and in Canadian waters (Ruzzante et al. 1996b, 1998, 2000, Beacham et al. 2002), it has received much less attention in US waters (Lage et al. 2004, Wirgin et al. 2007). Much of the genetic diversity in this species may reside in smaller sedentary populations that may be particularly vulnerable to overharvest (Robichaud & Rose 2004).

Atlantic cod in US waters are currently managed by a 2-stock model consisting of (1) a Gulf of Maine (GOM) stock and (2) a stock encompassing Georges Bank and areas southward, from southern New England to the mid-Atlantic coast. Recent evidence, including the results of tagging studies, suggests that cod movements do not conform to the 2-stock model (Tallack 2009). This is also supported by genetic data that suggest restricted gene flow among the Georges Bank and more southern populations as well as heterogeneity within the GOM (Lage et al. 2004, Wirgin et al. 2007). By tailoring fisheries guidelines to a flawed 2-stock model, management practices may negatively

affect the smaller and more vulnerable populations (Larkin 1977, Iles & Sinclair 1982, Ruzzante et al. 1999, Pampoulie et al. 2006, Bradbury et al. 2008).

In this study, we provided the most comprehensive analysis of Atlantic cod population structure in US waters to date. By means of microsatellite and single nucleotide polymorphism (SNP) markers and the *Pan*I locus, we expanded on our previous research (Wirgin et al. 2007) in which we found evidence for an alternate interpretation of management units. Previously, we found genetic differences on a temporal scale, such that a spring-spawning population of cod in Ipswich Bay, New Hampshire–Massachusetts, was differentiated from winter-spawning cod from all other sites within the GOM (including the same bay), Georges Bank and sites south of Cape Cod, Massachusetts. We also found evidence that cod spawning on the northeast peak of Georges Bank may be differentiated from populations south of Cape Cod. In the present study, we aimed to develop a more detailed model of stock structure of cod in US waters, by evaluating the genetic structure of all major identifiable spawning aggregations over a 2 yr period. Our objectives were to (1) characterize the fine-scale population structure of spatially and temporally separated spawning aggregates of Atlantic cod, (2) investigate the temporal stability of the genetic structure using replicate samples collected over a 2 to 5 yr period, and (3) determine whether young-of-the-year cod sampled at juvenile nurseries could be assigned effectively to their population of origin.

MATERIALS AND METHODS

Sample collection. Atlantic cod were sampled from known spawning locations from inshore and offshore GOM, Georges Bank and south of Cape Cod from December 2005 to July 2008. Adult cod (n = 1581; length, 54 to 127 cm) were captured with otter trawls, gill nets and hook and line, in collaboration with commercial and recreational fishers and fishery biologists. Spawning condition was assessed by visual inspection of the gonads or observations of 'running' milt or eggs by using the US National Marine Fisheries Service ovarian staging criteria (O'Brien et al. 1993). Caudal fin clip samples (1 cm^2) were taken from each fish and preserved in 95 to 100% ethanol.

Adult cod in spawning condition were sampled from spawning grounds at the northeast peak of Georges Bank, inshore GOM (in Bigelow Bight off the southern coast of Maine, Ipswich Bay and Massachusetts Bay), offshore GOM (on Jeffrey's Ledge and Stellwagen Bank) and south of Cape Cod (from the Nantucket Shoals and Cox Ledge) (Table 1, Fig. 1).

Table 1. *Gadus morhua.* Abbreviated sample names, locations, dates collected, sample sizes (n), fishing gear used in sample collection and sexes (for adults) or length ranges (for juveniles only) of Atlantic cod sampled in this study. Reproductive conditions of adults are mean (range, single value means all sampled fish were in the indicated reproductive condition) and refer to National Marine Fisheries Service (NMFS) ovarian staging criteria as follows: 1: immature; 2: resting; 3: developing; 4: ripe; 5: ripe and running; 6: spent. Sample name codes are as follows: BBS: Bigelow Bight spring; IPS: Ipswich Bay spring; IPW: Ipswich Bay winter; MBW: Massachusetts Bay winter; MBS: Massachusetts Bay spring; PBS: Platts Bank spring; JLW: Jeffrey's Ledge winter; SWS: Stellwagen Bank spring; GBW: Georges Bank winter; NTW: Nantucket Shoals winter; CLW: Cox Ledge winter; CLS: Cox Ledge spring; NYS: New York Bight spring; NYW: New York Bight winter; CBSJ: Casco Bay spring juveniles; MBWJ: Massachusetts Bay winter juveniles; MBSJ: Massachusetts Bay spring juveniles; CCWJ: Cape Cod winter juveniles

Sample name	Collection site	Date	n	Gear used	Sex	Reproductive condition
Gulf of Maine inshore						
BBS	Bigelow Bight	Jul 2007	70	Trawl	Females	6 (6)
	Bigelow Bight	Jul 2008	47	Gill net	Mixed	5 (5)
IPS	Ipswich Bay	Apr–May 2006	122	Trawl	Mixed	5 (3–5)
	Ipswich Bay	Jun 2007	78	Trawl	Females	6 (6)
IPW	Ipswich Bay	Dec 2006	31	Trawl	Mixed	2 (2–5)
MBW	Massachusetts Bay	Dec 2005–Jan 2006	140	Gill net	Females	4 (3–6)
	Massachusetts Bay	Jan 2007	77	Trawl	Mixed	5 (3–5)
MBS	Massachusetts Bay	Apr 2006	36	Trawl	Females	4 (4–5)
	Massachusetts Bay	Jun 2008	49	Gill net	Females	5 (5)
Gulf of Maine offshore						
PBS	Platts Bank	Aug 2006	70	Gill net	Mixed	2 (2, 6)
JLW	Jeffrey's Ledge	Dec 2007	73	Trawl	Mixed	5 (3–5)
SWS	Stellwagen Bank	May 2006	41	Trawl, gill net	Mixed	4 (3–6)
	Stellwagen Bank	Apr 2007	74	Trawl	Mixed	5 (3–6)
Georges Bank						
GBW	NE Georges Bank	Feb 2006	152	Trawl	Mixed	5 (3–6)
	NE Georges Bank	Feb–Mar 2007	45	Trawl	Mixed	5 (3–6)
Southern New England						
NTW	Nantucket Shoals	Nov 2006	109	Trawl	Mixed	4 (3–6)
CLW	Cox Ledge	Jan 2007	158	Trawl	Mixed	4 (3–6)
CLS	Cox Ledge	Apr 2007	118	Trawl	Mixed	4 (3–6)
NYS	New York Bight	Mar–Apr 2007	47	Hook and line	Mixed	2 (2)
NYW	New York Bight	Nov 2007–Jan 2008	44	Hook and line	Mixed	2 (2)
Gulf of Maine inshore juveniles					Length range (cm)	
CBSJ	Casco Bay	Apr 2007	45	Trawl	36–47	1
MBWJ1	Massachusetts Bay	Oct 2006	34	Trawl	3–15	1
MBWJ2	Massachusetts Bay	Oct 2007	48	Trawl	3–5	1
MBSJ	Massachusetts Bay	Apr 2007	46	Trawl	3–5	1
CCWJ	Cape Cod	Oct 2006	69	Trawl	3–15	1

Cod were not sampled north of Bigelow Bight due to a lack of fishing effort on the depleted populations of mid-coast and southeast Maine. When spawning cod could not be located at a presumed spawning site, adult cod not in spawning condition (identified as resting individuals) were sampled from aggregations in Ipswich Bay and Platts Bank, offshore coastal Maine and 48 to 113 km (30 to 70 miles) offshore from coastal New Jersey in the New York Bight. At Ipswich Bay, Massachusetts Bay and Cox Ledge, spawning cod were sampled in both the spring and winter; these seasonal collections were separated for analysis. To facilitate testing for annual fluctuations in genetic variation, 6 of the 10 spawning sites were sampled in 2 subsequent years (Bigelow Bight, Ipswich Bay, Massachusetts Bay winter and spring samples, Stellwagen Bank and Georges Bank).

Juvenile cod (3 to 15 cm, age 0 to 1 based on age-at-length key of Kerr et al. 2009a) were sampled by bottom trawl from Massachusetts Bay in October 2006 and 2007 and April 2007, and off the coast of Provincetown, northeastern Cape Cod, in October 2006 (Table 1, Fig. 1). Whole fish were frozen at –20°C, thawed at the University of New Hampshire (UNH, Durham) and fin clips were removed for analysis. A collection of immature cod (36 to 47 cm, age 2 to 4) was sampled from Casco Bay in April 2007 by bottom trawl and fin clips were taken from individuals before being released.

Genetic analyses. DNA was extracted with Qiagen DNeasy tissue kits (Qiagen) or standard phenol/chloroform procedures. A panel of 11 microsatellite markers (*Gmo02* and *Gmo132*, Brooker et al. 1994; *Gmo19*, *Gmo35*, *Gmo36* and *Gmo37*, Miller et al. 2000; *PGmo-32*, *PGmo34*, *PGmo38*, *PGmo56* and *PGmo58*, Jakobs-
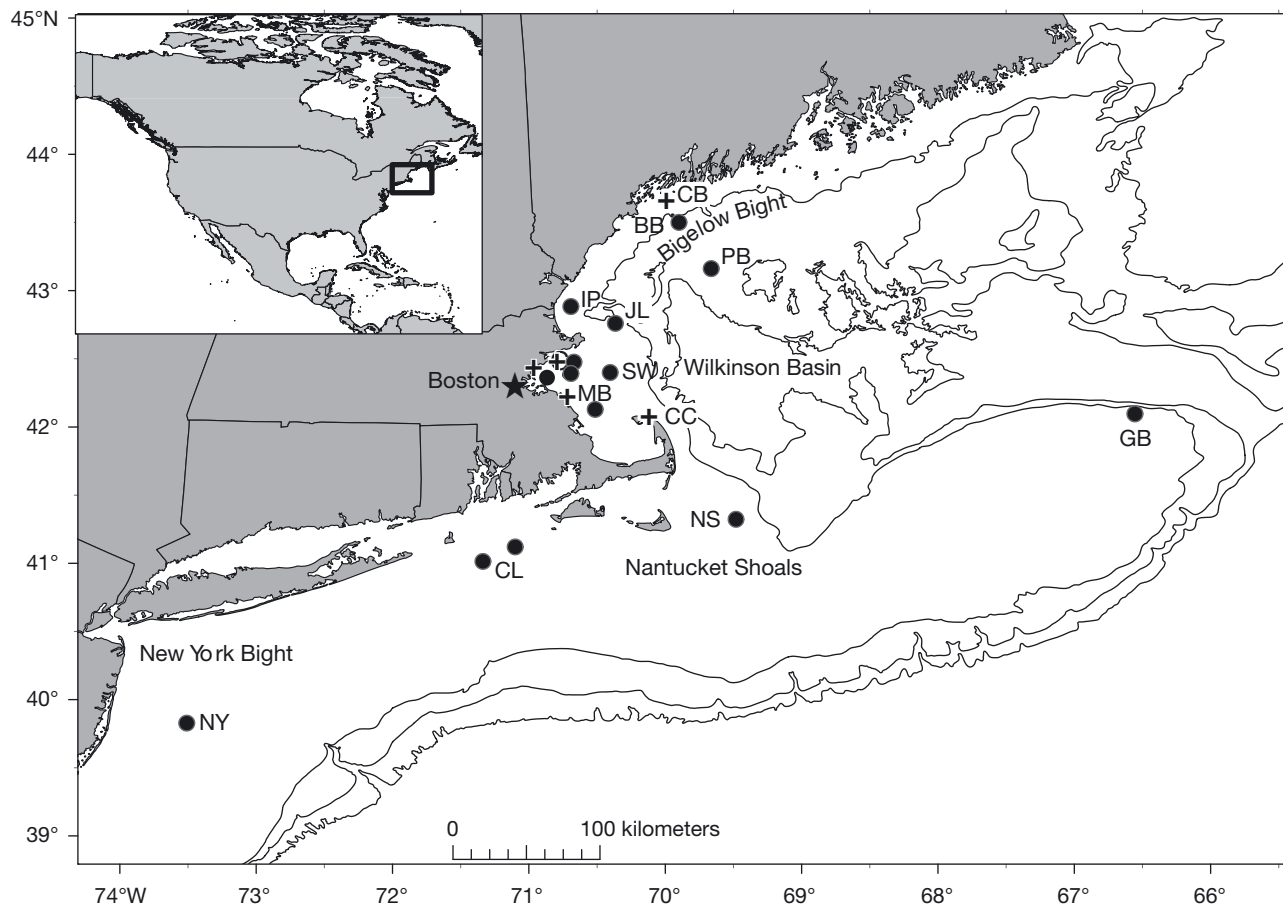
Fig. 1. *Gadus morhua*. Sample locations for (●) adult and (+) juvenile Atlantic cod collected in the present study. Abbreviated sample names are defined in Table 1

dóttir et al. 2006), the pantophysin I (*Pan*I) locus (Pogson 2001), the *AHR6* and *ARNT8* SNP markers (Wirgin et al. 2007) and 3 novel SNP markers, *ARNT1*, *CYP5* and *Kras*, were used. With the exception of the July 2008 Bigelow Bight samples and the juvenile samples, all samples were genotyped with the same suite of markers as follows: genotyping of microsatellites *Gmo02*, *Gmo19*, *Gmo36*, *PGmo34*, *PGmo56*, *PGmo58* and the *Pan*I locus were performed at UNH, and genotyping of microsatellites *Gmo132*, *Gmo35*, *Gmo37*, *PGmo32*, *PGmo38* and development and analysis of the SNPs were performed at New York University School of Medicine (NYU). The 2008 Bigelow Bight and juvenile samples were genotyped entirely at UNH by means of 10 microsatellite loci and the *Pan*I locus. To ensure consistency in the microsatellite allele scores for these samples relative to the remainder of the data set, a subset of samples (n = 123) were genotyped at *Gmo132*, *Gmo35*, *Gmo37*, *PGmo32* and *PGmo38* in laboratories at both institutions and the microsatellite allele scores at UNH were calibrated to those of NYU. Similarity of genotypes in the 2 temporal Bigelow Bight samples (see

'Results') suggest that calibration issues were not a concern.

Microsatellite analysis was conducted using procedures in Wirgin et al. (2007) or in 12 µl total volumes containing 2 µl DNA template (50 to 200 ng µl^{-1}), 0.5 µM of each primer, 1× GoTaq Flexi PCR Buffer (Promega), 0.2 mg ml^{-1} bovine serum albumin, 1 mM MgCl$_2$, 100 µM deoxynucleotide triphosphates (dNTPs) and 0.2 U GoTaq Flexi DNA Polymerase (Promega). At UNH, the forward or reverse primer of each microsatellite was fluorescently labeled with FAM, NED or HEX, and amplification parameters followed those described in the literature for each primer set. *Pan*I genotyping was conducted using the method of Stenvik et al. (2006a). Multiplex PCRs were conducted for loci with primers sharing common annealing temperatures (within 2°C). PCR products were diluted (15 to 20×) and electrophoresed in an ABI3130 automated capillary sequencer (Applied Biosystems). Alleles were scored manually using PeakScanner v. 1.0 software (Applied Biosystems). At NYU, characterization of microsatellite genotypes was performed in a CEQ^{TM}8000 capillary-based DNA sequencer (Beck-

man Coulter). Multiplexed PCR reactions were diluted up to 1:3 with Sample Loading Solution (SLS; Beckman Coulter). Diluted PCR reactions (0.5 to 2 μl) were loaded onto 96-well plates along with 0.5 μl of CEQ DNA Size Standard-400 (Beckman Coulter) and 40 μl of SLS and run with the FRAG 1 program (Beckman Coulter). MICRO-CHECKER (van Oosterhout et al. 2004) was used to test for the presence of null alleles, errors due to microsatellite stuttering and large-allele dropout.

The 5' nuclease reaction with TaqMan probes was used to genotype SNPs with Real-Time PCR. Primer and probe combinations for the 3 new SNP loci are as follows. *Ras* primers: GAA CCA TTT TGT GGA CGA GTA TGA and GGG ACC AAG AGG TTA ATA TGA AGG T; *Ras* probes: VIC-CAA CCA TCG AGG TAA A and FAM-AAC CAT TGA GGT AAA GT. *ARNT1* primers: CGC GTC AAA TAC AAC GAC TTG T and GGC CTA CAT AGC TTG ATA GTG ATA GCT; ARNT1 probes: FAM-TCC AGT TAA ACA ACC AAA A and VIC-CAA GTT AAA CAA CCA CAA. *CYP5* primers: TTT GAG TGG CAC CCA TAG CA and CCA TTT CCT GGA CAG CTG AAG; *CYP5* probes: FAM-TTG GTG ACA CGG CTT and VIC-TCT GTT GGT GAC AAG G. Amplifications occurred in an ABI 7300 Real-Time PCR instrument or in PTC-100^TM cyclers (MJ Research). Plates with amplicons from the PTC-100^TM cyclers were transferred after amplification to the ABI 7300 PCR instrument for allelic discrimination. Allele scoring was achieved with ABI Sequence Detection v. 2.1 software.

Statistical analyses. *Descriptive statistics:* Measures of diversity, including mean number of alleles, allelic richness, F_{IS} and observed and expected heterozygosities (H_o and H_e) were calculated with GDA (Lewis & Zaykin 2001) for each locus and population sampled. Locus-specific F_{ST} values and Fisher's exact tests of allelic differentiation were calculated in GENEPOP v. 3.4 (Raymond & Rousset 1995). We calculated the standardized measure of genetic differentiation G'_{ST} (Hedrick 2005) for each of the 3 types of markers (microsatellites, *Pan*I and SNPs), by dividing the observed F_{ST} value by its theoretical maximum, F_{max}, using the program RECODE DATA v. 0.1 (Meirmans 2006). Because at least 2 of the markers used in this study, *Gmo132* and *Pan*I, have previously been shown to be under selection in other Atlantic cod populations, we performed selection tests for all loci using an F_{ST} outlier approach (Beaumont & Nichols 1996) in LOSITAN (Antao et al. 2008). Tests of deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) per locus and population were conducted in GENEPOP by means of the Markov chain method with 10 000 iterations and 10 000 batches. Significance levels for multiple tests were adjusted to an α = 0.05 level with the standard Bonferroni method (Rice 1989).

Annual variation in genetic structure: Samples collected from the same spawning location and season in 2 separate years were examined for annual fluctuations in genetic variation using the F_{ST} estimator θ, of Weir & Cockerham (1984), produced in FSTAT 2.9.3 (Goudet 1995). Temporal stability was also assessed with a hierarchical analysis of molecular variance (AMOVA) conducted in ARLEQUIN 2.0 (Schneider et al. 2000) with 10 000 permutations. This method partitions the genetic variation among sites and between years. For population genetic structure to be meaningful, the differences among sites must be significantly greater than the differences between years within the same sites (Waples 1998). In the absence of significant differentiation, yearly samples from the same sites were pooled for further analyses.

Population genetic structure: To test for genetic differences among adult and juvenile samples, pairwise allelic differentiation tests in GENEPOP and F_{ST} calculations in FSTAT were used. Significance was adjusted with the standard Bonferroni correction. F_{ST} values for the pooled data set were also used in a principal component analysis (PCA) using GENALEX 6.1 (Peakall & Smouse 2006) to visualize the clustering of populations. For adult cod samples, analyses were conducted initially with only the spawning populations and secondarily with the addition of the resting populations. *A posteriori* analysis of population clusters identified by F_{ST} and PCA was conducted with a hierarchical approach in AMOVA to compare genetic variation within and among clusters.

To evaluate an isolation by distance (IBD) model, we used Mantel's tests implemented in GENALEX to test for a correlation of genetic distance (F_{ST}) and shortest ocean distance between sample locations. IBD was evaluated across all spawning populations overall and within the identified clusters. For samples with identical geographic coordinates (spawning sites sampled in 2 different seasons), IBD analyses were conducted with the exclusion of one seasonal sample.

Genetic barriers were identified with the program BARRIER 2.2 (Manni et al. 2004). This approach uses the Monmonier algorithm to identify geographic areas associated with genetic discontinuities among spatially connected populations. We created BARRIER maps from the geographic coordinates of the adult sample locations. To facilitate comparison of seasonal samples within a spatial framework, sites with identical geographic coordinates (sites from which samples were collected from the same location in different seasons) were placed adjacent to one another in the map. We conducted the analyses using 2 genetic distances: (1) one with bootstrapping over 100 matrices of Nei's

standard genetic distance D (Nei 1987) calculated in the program MSA (Dieringer & Schlötterer 2003); (2) the other with 16 F_{ST} matrices for each of the loci separately. A combination of the 2 approaches enabled us to evaluate the strength of the barriers and contributions of the different markers.

Five-year temporal stability: To address the question of temporal stability of allelic frequencies over a longer period (2003 to 2008), we compared our results with those from our previous work (Wirgin et al. 2007) in which we genotyped spawning cod from Ipswich Bay (n = 48 in both January and May 2003), the Great South Channel offshore of Chatham, Massachusetts (n = 40, November 2003), Stellwagen Bank (n = 27, January 2005), northeast Georges Bank (n = 100, February 2005) and resting (nonspawning) adults from western Long Island, New York (n = 80, January and February 2005). For this analysis, we used 8 of the 9 loci that were common to the 2 studies (*Gmo02*, *Gmo132*, *Gmo35*, *Gmo36*, *Gmo37*, *Pan*I, *AHR6* and *ARNT1*). Microsatellite data from 3 loci (*Gmo02*, *Gmo132*, *Gmo36*) were calibrated by reanalyzing a subset (n = 35) of the samples from Wirgin et al. (2007) to correct for differences in electrophoresis platforms among years. Data from the remaining loci were easily compared between studies due to consistency in the analysis platforms and because scoring of the biallelic loci (SNPs and *Pan*I) was not platform-dependent. A PCA was conducted with pairwise population F_{ST} values to visualize the genetic data. We used AMOVA to test for variation among temporal samples from the same locations.

Origin of juveniles: To determine the spawning population of origin of juvenile cod samples, pairwise population F_{ST} values for the juvenile and pooled spawning adult samples were used in a PCA in GENALEX. To determine whether juveniles could be assigned to their population of origin, mixture analysis was performed by means of the conditional maximum likelihood approach of ONCOR (Anderson et al. 2008). Confidence intervals (CIs) of mixture proportions were determined through 10 000 bootstraps. The accuracy of the genetic stock identification was assessed through self-assignment of adult individuals to their respective populations by using the leave-one-out cross-validation test and through 100% fishery simulations of 50 individuals and 1000 simulations. These methods provide less biased predictions of accuracy than conventional parametric bootstrapping procedures (Anderson et al. 2008) and probably give more realistic estimates of the power of assignments.

RESULTS

Descriptive statistics

Multilocus genotypes were compiled for 1534 Atlantic cod adults by using 10 of the 11 microsatellites, the *Pan*I locus and 5 SNP markers, and for 47 adults and 242 juveniles by using the 10 microsatellites and the *Pan*I locus. Reliable genotypes could not be obtained for the *PGmo56* microsatellite locus due to irregular scoring patterns and it was removed from further analyses. Descriptive statistics of the markers are shown in Table 2 and genetic diversity of the population samples is in Table 3. With the exception of *PGmo-*

Table 2. *Gadus morhua*. Number of alleles, allelic richness (AR), observed (H_o) and expected (H_e) heterozygosity, F_{IS}, F_{ST} and allelic differentiation p-values among adult and juvenile Atlantic cod for 16 genetic markers. **Bold** text indicates significance at the $\alpha = 0.05$ level. –: not analysed

Locus	Adults							Juveniles						
	Alleles	AR	H_o	H_e	F_{IS}	F_{ST}	p	Alleles	AR	H_o	H_e	F_{IS}	F_{ST}	p
Gmo02	25	10.3	0.784	0.792	0.010	0.0001	0.3894	18	12.1	0.838	0.797	–0.052	–0.0021	0.8792
Gmo19	74	27.1	0.958	0.963	0.005	0.0003	**0.0158**	46	29.9	0.936	0.961	0.026	–0.0005	0.1406
Gmo132	25	11.6	0.735	0.750	0.019	0.0327	**<0.0001**	19	13.0	0.777	0.776	–0.002	0.0408	**<0.0001**
Gmo35	13	7.0	0.770	0.773	0.004	0.0011	0.2552	9	7.7	0.746	0.771	0.033	0.0004	0.0687
Gmo36	14	4.0	0.577	0.617	0.064	0.0021	**0.0492**	6	4.0	0.546	0.570	0.042	0.0186	**0.0017**
Gmo37	18	10.3	0.857	0.861	0.005	0.0005	0.1595	17	11.8	0.834	0.867	0.037	0.0033	0.1786
PGmo32	5	3.3	0.389	0.373	–0.043	–0.0023	0.9279	4	3.5	0.417	0.366	–0.140	–0.0006	0.4775
PGmo34	13	5.5	0.585	0.588	0.005	0.0037	0.0523	10	6.3	0.563	0.582	0.033	–0.0080	0.9523
PGmo38	25	12.3	0.863	0.869	0.007	0.0016	0.1167	21	13.9	0.849	0.864	0.018	0.0009	0.1160
PGmo58	11	5.4	0.270	0.282	0.040	0.0022	**0.0027**	8	5.8	0.270	0.262	–0.030	0.0023	0.1127
*Pan*I	2	1.9	0.082	0.083	0.016	0.0527	**<0.0001**	2	2.0	0.083	0.080	–0.041	0.1042	**<0.0001**
AHR6	2	2.0	0.440	0.430	–0.024	0.0009	0.2947	–	–	–	–	–	–	–
ARNT1	2	2.0	0.178	0.204	0.131	0.0023	0.0990	–	–	–	–	–	–	–
ARNT8	2	2.0	0.237	0.307	0.226	–0.0007	0.3553	–	–	–	–	–	–	–
CYP5	2	2.0	0.332	0.330	–0.007	0.0028	0.0842	–	–	–	–	–	–	–
Kras	2	2.0	0.301	0.313	0.039	–0.0003	0.4367	–	–	–	–	–	–	–
Overall	14.7	6.8	0.523	0.533	0.020	0.0044	**<0.0001**	14.5	10.0	0.624	0.627	0.005	0.0071	**<0.0001**

38, the microsatellite markers of Jakobsdóttir et al. (2006) had substantially lower heterozygosities than did the markers used in our previous study of Atlantic cod (Wirgin et al. 2007). Heterozygosities for *Gmo02*, *Gmo19*, *Gmo132*, *Gmo35*, *Gmo36* and *Gmo37* were similar to the values reported by Wirgin et al. (2007). Numbers of alleles across all populations ranged from 4 to 74 for the microsatellite loci. Heterozygosity and allelic diversity were similar across spawning popula-

tions, and slightly higher among juvenile samples. *Pan*I^A allele frequencies ranged from 0 to 0.132 (Table 3).

For the adult population samples, F_{ST} values ranged from −0.0023 to 0.0527 per locus, and were highly significant overall with a global F_{ST} of 0.0044 (p < 0.0001) (Table 2). The highest levels of differentiation were observed at *Gmo132* and *Pan*I, with F_{ST} values of 0.0327 and 0.0527, respectively, and highly significant (p < 0.0001) allelic differentiation across all samples by the exact test. Consistent with previous studies (Pogson 2001, Nielsen et al. 2006), the results of the F_{ST} outlier test indicated that *Gmo132* and *Pan*I were candidates for positive selection (Fig. 2). All other markers were within neutral expectations. Among the neutral loci, *Gmo19*, *Gmo36* and *PGmo58* showed significant differentiation (0.0003 < F_{ST} < 0.0022). Differentiation for the neutral loci alone was also significant (global F_{ST} = 0.0011; allelic differentiation, p < 0.0001). G'_{ST} values were 0.015 for the microsatellites, 0.061 for *Pan*I and 0.001 for the SNPs.

Differentiation among juvenile samples was highly significant (p < 0.0001) across all loci (F_{ST} = 0.0071) and for the neutral loci alone (F_{ST} = 0.0014). As with the adult samples, *Gmo132* and *Pan*I displayed the highest per locus F_{ST} values (0.0408 and 0.1042, respectively) and exhibited highly significant allelic differentiation (Table 2). Differentiation among juvenile samples was

Table 3. *Gadus morhua*. Genetic diversity of adult and juvenile Atlantic cod samples. Column values are means across 16 markers for number of alleles, allelic richness (AR), observed (H_o) and expected (H_e) heterozygosity, F_{IS} values, and *Pan*I^A allele frequencies. Abbreviated sample names are defined in Table 1 and sample size is indicated by n

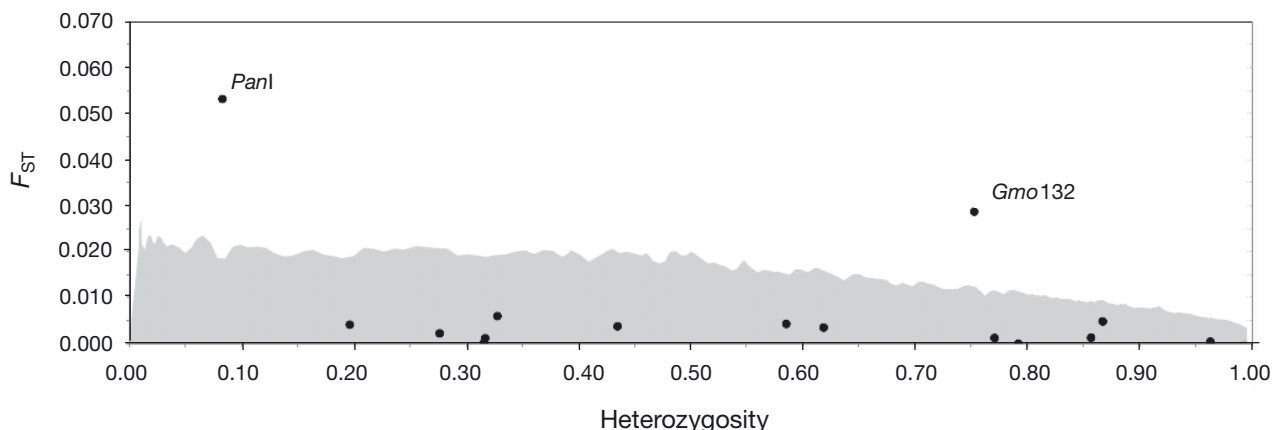| Population	n	Alleles	AR	H_o	H_e	F_{IS}	*Pan*I^A
Adults							
BBS	117	9.6	6.7	0.527	0.555	0.051	0.132
IPS	200	10.6	6.9	0.548	0.545	−0.007	0.103
IPW	31	7.0	6.7	0.486	0.514	0.055	0.016
MBW	217	10.7	6.8	0.516	0.528	0.022	0.021
MBS	85	8.6	6.6	0.539	0.547	0.013	0.129
PBS	70	8.8	6.7	0.552	0.543	−0.015	0.100
JLW	73	8.7	6.7	0.492	0.525	0.062	0.014
SWS	115	9.1	6.7	0.511	0.521	0.019	0.009
GBW	197	10.8	6.8	0.525	0.537	0.021	0.020
NTW	109	9.2	6.6	0.530	0.514	−0.030	0.000
CLW	158	10.3	6.6	0.507	0.514	0.014	0.006
CLS	118	9.4	6.7	0.508	0.526	0.033	0.000
NYS	47	8.4	7.2	0.535	0.541	0.012	0.032
NYW	44	7.9	6.8	0.511	0.526	0.029	0.023
Overall	113	9.2	6.8	0.520	0.531	0.020	0.043
Juveniles							
CBSJ	45	10.5	10.4	0.605	0.635	0.047	0.000
MBWJ	82	10.7	9.4	0.642	0.632	−0.017	0.122
MBSJ	46	10.8	10.7	0.623	0.622	−0.003	0.000
CCWJ	69	10.5	9.4	0.613	0.608	−0.007	0.000
Overall	61	10.6	10.0	0.621	0.624	0.005	0.031

Fig. 2. *Gadus morhua*. Selection test for 16 genetic markers; F_{ST} is plotted as a function of heterozygosity. Markers located within the light gray area are within the 95% CI for neutral markers; markers above this area are candidates for positive selection

greater than among adult samples, driven by the *Pan*I locus for which the F_{ST} value for the juvenile samples was almost double that of the adult samples. Among the neutral loci, only *Gmo36* showed significant differentiation (F_{ST} = 0.0186, p = 0.0017). G'_{ST} values for the juvenile samples were 0.019 for the microsatellites and 0.112 for *Pan*I (the SNPs were not used in the analysis of juvenile samples).

All adult samples conformed to HWE except those for Cox Ledge winter at the *ARNT8* locus (1 of 224 comparisons), which exhibited a significant heterozygote deficiency (p = 0.0001). The juvenile samples were all in HWE except those from the Massachusetts Bay collection from October 2007 at *Gmo37* (1 of 55 comparisons), which had a significant heterozygote deficiency (p < 0.0001). MICRO-CHECKER software detected no evidence of stuttering or large-allele drop out, but did detect the possible presence of null alleles within the adult samples from Massachusetts Bay winter (*Gmo36*), Georges Bank (*Gmo36* and *PGmo38*), Cox Ledge winter (*PGmo38*) and the Bigelow Bight (*Gmo02*). Because these populations conformed to HWE at these loci, this possibility was not considered in further analyses. LD was detected between the *ARNT1* and *ARNT8* markers for the adult samples (1 of 120 comparisons, p < 0.0001) (data not shown); however, no significant LD was detected for any sample when each of the adult populations was considered separately. LD was not detected among the juvenile samples.

Annual variation in genetic structure

Adult samples collected from the same site and season in 2 separate years were found to be genetically homogenous by both F_{ST} and AMOVA. Of the 6 comparisons, 4 had negative F_{ST} values (data not shown) and the 2 comparisons from Ipswich Bay and Massachusetts Bay spring had nonsignificant but positive F_{ST} values of 0.0006 and 0.0075, respectively. The Massachusetts Bay spring comparisons may have suffered from lack of precision due to low sample size of 1 of the 2 yearly samples (n = 36 for the April 2006 samples; a minimum of 50 individuals per sample is recommended, Ruzzante et al. 1996a). Pairwise F_{ST} comparisons of these samples using only neutral markers produced all negative values (data not shown). AMOVA results also demonstrated no significant variation between years for all loci as well as the neutral markers alone and indicated that annual fluctuations in genetic variation were minimal compared with the differences among sites (Table 4). While the differences among sites were significant for the neutral markers alone, the F_{CT} value was very small (0.001) and the pattern was much stronger when all loci were used (F_{CT} = 0.0044). Locus-by-locus AMOVA confirmed that this pattern of variance was largely driven by the 2 non-neutral loci (see Table S1 in the Supplement at www.int-res.com/articles/suppl/m410p177_supp.pdf). These results indicated stability in the genetic structure from year to year, and the replicate samples from the 2 yr of the study were pooled for further analyses.

Genetic structure of spawning populations

Since our primary objective was to characterize the genetic structure of spawning populations of Atlantic cod, we first considered only the samples from sites at which spawning individuals were collected (n = 10 spawning sites, including spawning, spent and ripe condition categories described in Table 1). Pairwise F_{ST} comparisons of the spawning populations yielded 16 of 45 significant values, ranging from 0.0029 to 0.0156, and 26 of 45 significant tests of allelic differentiation (p ≤ 0.0011, following Bonferroni adjustment) (Table 5). Significant differentiation was primarily due to divergence between the spring spawning populations from the inshore Gulf of Maine (Bigelow Bight, Ipswich Bay and Massachusetts Bay) and the majority of the remaining spawning sites, and divergence between the Georges Bank sample and the southern New England samples. Employing the less conservative criterion of p ≤ 0.05 yielded another 18 significant comparisons for F_{ST} values ranging from 0.0005 to 0.0076. Overall, 35

Table 4. *Gadus morhua*. Analysis of molecular variance (AMOVA) of genetic variation among sites, years and samples for 6 sites sampled in 2 yr using all loci and neutral markers only (*Pan*I and *Gmo132* are excluded). **Bold** text indicates significance (p < 0.05)

Source of variation	df	Variance components	% variation	Fixation indices		p
All loci						
Among sites	5	0.0153	0.44	CT	0.0044	**<0.0001**
Among years	6	0.0015	0.04	SC	0.0004	0.2638
Within samples	1850	3.4503	99.52	ST	0.0048	**<0.0001**
Total	1861	3.4670				
Neutral markers only						
Among sites	5	0.0032	0.10	CT	0.0010	**0.0341**
Among years	6	−0.0028	−0.09	SC	−0.0009	0.9259
Within samples	1850	3.0320	99.99	ST	0.0001	0.5202
Total	1861	3.0323				

Table 5. *Gadus morhua*. Population differentiation of spawning populations of Atlantic cod. Allelic differentiation p-values are shown above the diagonal break; pairwise population F_{ST} values with p-values in parentheses are shown below the diagonal. Abbreviated sample names are defined in Table 1. **Bold** text indicates significance following standard Bonferroni correction (p ≤ 0.0011). Significance at the 0.05 and 0.01 levels are indicated by * and **, respectively

	BBS	IPS	JLW	MBW	MBS	SWS	GBW	NTW	CLW	CLS
BBS		0.1810	**0.0002**	**<0.0001**	0.7559	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
IPS	0.0007 (0.0367)*		**0.0006**	**<0.0001**	0.7593	**<0.0001**	0.0001	**<0.0001**	**<0.0001**	**<0.0001**
JLW	0.0063 (0.0078)**	0.0067 (0.0044)**		0.6299	**0.0001**	0.1640	0.2598	0.0074**	0.7171	0.0193*
MBW	**0.0054** **(0.0011)**	**0.0071** **(0.0011)**	0.0016 (0.2989)		**<0.0001**	0.1731	**<0.0001**	0.0349*	0.4910	0.3408
MBS	−0.0005 (0.4856)	−0.0017 (0.4256)	0.0076 (0.0156)*	0.0093 **(0.0011)**		**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
SWS	0.0065 (0.0022)**	0.0071 (0.0022)**	0.0026 (0.3300)	0.0004 (0.0778)	0.0080 **(0.0011)**		0.2046	0.0121*	0.0369*	0.0054**
GBW	0.0017 (0.0044)**	0.0025 (0.0056)**	0.0020 (0.0456)*	0.0018 (0.0167)*	0.0036 (0.0056)**	0.0026 (0.1044)		**<0.0001**	**<0.0001**	**<0.0001**
NTW	**0.0107** **(0.0011)**	**0.0097** **(0.0011)**	0.0039 (0.0067)**	0.0005 (0.0244)*	**0.0138** **(0.0011)**	0.0012 (0.0067)**	**0.0036** **(0.0011)**		0.0562	**0.0010**
CLW	**0.0078** **(0.0011)**	**0.0090** **(0.0011)**	0.0009 (0.6544)	−0.0005 (0.4133)	**0.0109** **(0.0011)**	0.0016 (0.0178)*	**0.0029** **(0.0011)**	0.0005 (0.0267)*		0.4156
CLS	**0.0097** **(0.0011)**	0.0124 **(0.0011)**	0.0061 (0.0533)	0.0007 (0.4200)	**0.0156** **(0.0011)**	0.0047 (0.0156)*	**0.0056** **(0.0011)**	0.0036 (0.0033)**	0.0014 (0.7378)	

of 45 comparisons were significant by one or both differentiation methods at the p ≤ 0.05 level (not corrected). When the selected loci *PanI* and *Gmo132* were excluded from the analyses, overall differentiation among sites was evident using the neutral markers alone, but was much weaker (data not shown).

Principal component analysis of the F_{ST} values from the spawning samples identified 2 main clusters (Fig. 3A): one comprising the spring spawning coastal Gulf of Maine populations of Bigelow Bight, Ipswich Bay and Massachusetts Bay and another cluster comprising spring spawners on Stellwagen Bank, winter spawners on Jeffrey's Ledge and Massachusetts Bay, and southern aggregations on Nantucket Shoals and Cox Ledge. Cod spawning on Georges Bank fell intermediately between these 2 clusters as they were distinct from the southern sites of Cox Ledge and Nantucket Shoals, but were only somewhat differentiated from the inshore Gulf of Maine populations in both winter and spring and similar to the offshore Gulf of Maine populations on Stellwagen Bank and Jeffreys Ledge.

A posterior analysis of hierarchical structure of the population clusters identified by F_{ST} and PCA gave support to these genetic groupings when all loci were included in the analysis (Table 6). Although the majority of the variation was attributable to differences within samples, the remaining variation was due primarily to differences among clusters. The percent variation (0.95%) and fixation index ($F_{CT} = 0.0095$) were much larger among than within clusters (0.03%, $F_{SC} =$

0.0003). This pattern was not evident when *PanI* and *Gmo132* were excluded from the analysis (see Table S1 in the Supplement for locus-by-locus AMOVA). Mantel tests for correlation of geographic and genetic distances were all nonsignificant (p > 0.05), even when samples with identical geographic locations were excluded. Similarly, no IBD was detected within the clusters identified by F_{ST} and PCA.

BARRIER analysis identified genetic discontinuities that were largely consistent using both distance methods, Nei's *D* and F_{ST}. The first-order barrier corresponded with the separation of the spring spawning coastal GOM population of Bigelow Bight, Ipswich Bay and Massachusetts Bay from the offshore and winter spawning GOM samples, with 72 to 82% bootstrap support from Nei's *D* and support from 5 to 7 of 16 single locus F_{ST} matrices (the support for the boundaries between each pair of populations along this barrier varied slightly). The continuation of this first-order barrier separated the spring spawning coastal GOM cod samples from the Georges Bank samples and was supported by 49% bootstrapping and 5 loci. Second- and third-order barriers were identified between the Cox Ledge spring samples and the Nantucket and Cox Ledge winter samples and between Georges Bank and Nantucket and the offshore GOM. These lesser barriers were supported by 18 to 43% bootstrapping and only 1 to 5 loci.

The results of our above analyses are synthesized in Fig. 4, in which we demonstrate the 3 primary genetic
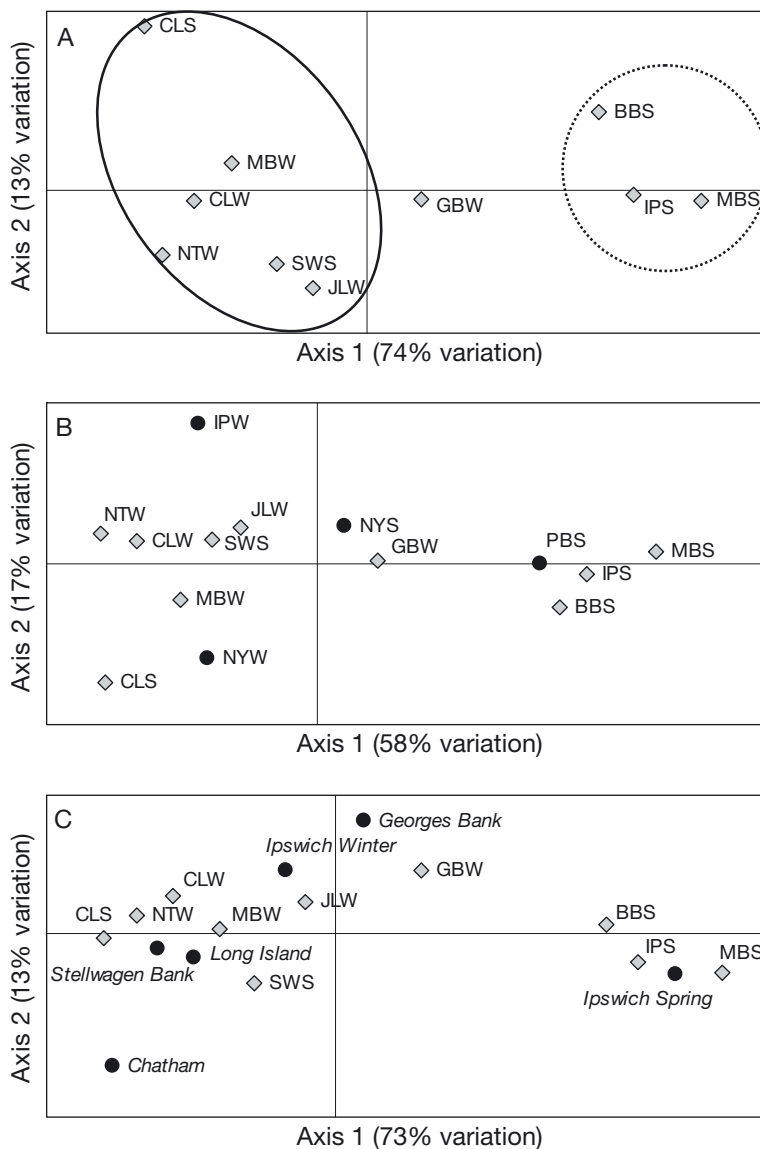
Fig. 3. *Gadus morhua*. Principal component analyses of pairwise population F_{ST} values of Atlantic cod samples. (A) Comparison of (◆) spawning samples; circles surround genetically similar populations. (B) Comparison of (◆) spawning and (●) resting samples. (C) Comparison of (◆) spawning populations of the present study and (●) those sampled in Wirgin et al. (2007), labels in italics. Abbreviated sample names are defined in Table 1. Percent values refer to the percentage of variation explained by each axis

values between the complexes are 0.0085 between the northern spring and southern complexes, 0.0028 between the northern spring complex and Georges Bank and 0.0025 between the southern complex and Georges Bank.

The majority of variation among the populations could be explained by the *Pan*I locus and 2 alleles at *Gmo132*. The northern spring complex was characterized by higher allelic frequencies of *Pan*I[A] (>0.100) in comparison with the southern complex that was nearly fixed for the *Pan*I[B] allele. The southern complex also exhibited higher frequencies of the *Gmo132* 135 bp allele, while higher frequencies of the 117 bp allele were found in northern spring complex cod. Georges Bank cod were heterogeneous between the complexes and exhibited similar *Gmo132* allelic frequencies to the northern spring complex but near fixation for the *Pan*I[B] allele (Fig. 5).

Comparison of spawning and resting adult samples

When the resting adult cod samples from Platts Bank, Ipswich Bay winter and the New York Bight were added to the analyses, they clustered primarily within 1 of the 2 spawning complexes (Fig. 3B). The Platts Bank summer adults clustered within the northern spring complex. New York Bight spring and winter samples clustered within the southern complex, although the spring sample was also similar to the Georges Bank samples. The Ipswich Bay winter adults clustered within the southern complex based on variation along the first axis, but exhibited a large displacement along the second axis. Variation along this second axis was approximately one-third that of the first axis and may not represent a significant population difference. Furthermore, this result may have been affected by a low sample size (n = 31).

Five-year temporal stability of genetic structure

Comparative analysis with the data set of Wirgin et al. (2007) showed stability in the population structure from 2003 to 2008. AMOVA detected no significant

groupings of Atlantic cod: a northern spring complex spawns within coastal Gulf of Maine waters in the spring, a southern complex spawns in different inshore and offshore locations and seasons within the Gulf of Maine and in southern waters, and a Georges Bank population, which maintains some connectivity with populations spawning in the Gulf of Maine, especially its offshore spawning sites, but less connectivity with spawning aggregations south of Cape Cod. The F_{ST}

variation between samples collected from the same sites in different years (see Tables 7 & S1). By PCA, the 2003 Ipswich Bay spring spawning sample clustered with the spring coastal GOM samples of this study (northern spring complex, Fig. 3C; pairwise population

F_{ST} values are provided in Table S2). The Ipswich Bay winter, Chatham, Stellwagen Bank and Long Island samples clustered with the southern complex. The Georges Bank samples were very similar between the 2 studies and clustered together with a placement intermediate to the 2 complexes.

Table 6. *Gadus morhua*. Analysis of molecular variance (AMOVA) of genetic variation between the spawning complexes (see 'Results — Genetic structure of spawning populations' and Fig. 4), within the complexes and within samples of adult Atlantic cod using all loci and neutral markers only (*Pan*I and *Gmo132* are excluded)

Source of variation	df	Variance components	% variation	Fixation indices	
All loci					
Among complexes	1	0.0341	0.95	CT	0.0095
Among samples within complexes	7	0.0011	0.03	SC	0.0003
Within samples	2375	3.5495	99.02	ST	0.0098
Total	2383	3.5847			
Neutral markers only					
Among complexes	1	−0.0025	−0.08	CT	−0.0008
Among samples within complexes	7	0.0017	0.05	SC	0.0005
Within samples	2375	3.1628	100.02	ST	−0.0002
Total	2383	3.1621			

Population origin of juveniles

Pairwise F_{ST} (0.0070 to 0.0184) and allelic differentiation tests detected significant variation among the 5 juvenile cod samples from the 4 sites and 2 seasons (Table 8). The Massachusetts Bay juveniles from October 2007 exhibited highly significant differentiation from the other juvenile samples, except a sample from the same season and location made in the previous year; the 2 October Massachusetts Bay collections were pooled in further analyses. Using the less conservative criteria ($p \leq 0.05$), differentiation was also

Fig. 4. *Gadus morhua*. Population structure of Atlantic cod spawning in US waters in relation to stock boundaries, 4X: Scotian Shelf; 5Y: Gulf of Maine; 5Z: Georges Bank. Three genetically similar population complexes and the first-order genetic barrier (see 'Results — Genetic structure of spawning populations' for explanation) are shown. Abbreviated sample names are defined in Table 1
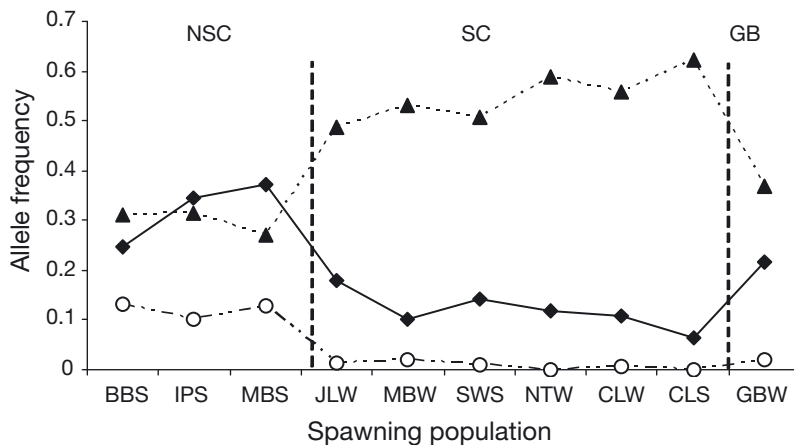
Fig. 5. *Gadus morhua*. Frequencies of the (◆) *Gmo132* (117 bp) allele, (▲) *Gmo132* (135 bp) allele and (○) *PanI*[A] allele of spawning Atlantic cod samples in the northern spring complex (NSC), southern complex (SC) and Georges Bank (GB). Abbreviated sample names are defined in Table 1

Table 7. *Gadus morhua*. Analysis of molecular variance (AMOVA) of genetic variation among sites, years and samples for 3 populations (IPS, IPW, GBW; see Table 1 for abbreviation definitions) compared between the present study and Wirgin et al. (2007). **Bold** text indicates significance (p < 0.05)

Source of variation	df	Variance components	% variation	Fixation indices	p
Among sites	2	0.0150	0.65	CT 0.0065	**<0.0001**
Among years	3	0.0016	0.07	SC 0.0007	0.2230
Within samples	1202	2.2909	99.28	ST 0.0072	**<0.0001**
Total	1207	2.3075			

apparent between the Casco Bay and Cape Cod juveniles (F_{ST} = 0.0043). When visualized in a PCA with the data from the adult spawning complexes, the pooled Massachusetts Bay October cod samples clustered within the northern spring complex, and the juveniles from Massachusetts Bay spring, northeastern Cape Cod and Casco Bay clustered within the southern complex (Fig. 6).

Mixture analysis was used to assign proportions of the juvenile samples to the 2 spawning complexes. Assignments to specific spawning aggregations within the complexes were not attempted due to the low levels of differentiation among some of the aggregations within a complex (Table 5). The Georges Bank spawning population was not used in the mixture analysis because it was only weakly differentiated from the Gulf of Maine populations and, given the anticyclonic circulation (Lough et al. 2006), was unlikely to be the source population for young-of-the-year juvenile cod on nursery grounds in the Gulf of Maine.

Before mixture analysis, a 'leave-one-out' cross-validation test and a 100% fishery simulation test were

conducted to assess the power of the genetic data for assignment of juvenile proportions to the spawning complexes (Table 9). The percent scores from the cross-validation test ranged from 75.3 to 83.9% for the southern complex and 54.1 to 63.1% for the northern spring complex. The 100% fishery simulation consisted of simulating a mixture sample composed entirely of one population and then assigning those individuals back to the same population. The percentage of correct scores in this simulation was greater than in the validation test and ranged from 82.6 to 95.4% in the southern complex and from 76.2 to 86.0% in the northern spring complex.

Approximately 98% of the pooled Massachusetts Bay fall juvenile cod collection was assigned to the northern spring complex through mixture analysis (Fig. 7). The majority (~85%) of the juveniles from the Massachusetts Bay spring collection were assigned to the southern complex. Similarly, the majority (~79%) of the Cape Cod juveniles were assigned to the southern complex. Mixture analysis of the Casco Bay immature collection was more equivocal, with approximately 63 and 37% assignment to the southern and northern spring complexes, respectively. The confidence intervals for these assignments, however, overlapped extensively and

Table 8. *Gadus morhua*. Population differentiation of juvenile Atlantic cod. Allelic differentiation p-values are shown above the diagonal break; pairwise population F_{ST} values with p-values in parentheses are shown below the diagonal. Abbreviated sample names are defined in Table 1. **Bold** text indicates significance following standard Bonferroni correction (p ≤ 0.005). Significance at 0.05 and 0.01 levels are indicated by * and **, respectively

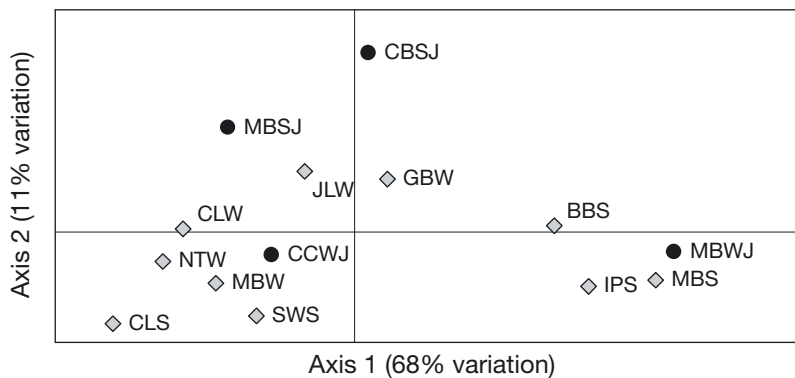|	CBSJ	MBWJ1	MBWJ2	MBSJ	CCWJ
CBSJ		0.0073**	**<0.0001**	0.2795	**0.0032**
MBWJ1	0.0058 (0.0100)*		0.0859	0.0135*	**0.0015**
MBWJ2	**0.0124 (0.0050)**	0.0043 (0.1200)		**<0.0001**	**<0.0001**
MBSJ	0.0000 (0.5500)	0.0099 (0.0800)	**0.0184 (0.0050)**		0.6810
CCWJ	0.0043 (0.0200)*	**0.0070 (0.0050)**	**0.0140 (0.0050)**	−0.0011 (0.7300)	

Fig. 6. *Gadus morhua*. Principal component analysis of pairwise population F_{ST} values for (◆) spawning and (●) juvenile Atlantic cod samples. Abbreviated sample names are defined in Table 1. Percent values refer to the percentage of variation explained by each axis

Table 9. *Gadus morhua*. Test of the accuracy of genetic stock identification using a leave-one-out cross-validation test and a 100% fishery simulation test in Oncor. Percent correct values refer to the percentage of individuals correctly assigned to each of the 2 reporting groups, northern spring complex (NSC) and southern complex (SC). The Georgia Bank population was not included in this analysis (see 'Results'). SD values and 95% CI refer to the 100% simulation. Abbreviated sample names are defined in Table 1

Population	Assigned reporting group	Percent correct Leave-one-out test	Percent correct 100% simulation	SD	95% CI
BBS	NSC	54.1	76.8	9.89	56.09, 94.46
IPS	NSC	55.9	76.2	10.01	55.11, 94.50
MBS	NSC	63.1	86.0	8.17	68.19, 99.76
JLW	SC	75.3	82.6	9.77	61.49, 99.88
MBW	SC	77.3	90.0	7.63	72.53, 100.00
SWS	SC	77.7	85.4	9.23	65.00, 99.99
NTW	SC	80.8	91.6	7.10	75.45, 100.00
CLW	SC	83.9	93.8	5.70	80.36, 100.00
CLS	SC	82.7	95.4	5.20	82.37, 100.00

suggested that the Casco Bay fish were of heterogeneous origin. Notably, the Casco Bay fish were of an older and more mobile life stage and, therefore, were not probably sampled from a juvenile nursery, as were the young-of-the-year collections.

DISCUSSION

Spawning stock structure

In this study, we found evidence for fine-scale genetic differentiation among spatially and seasonally divergent spawning populations of Atlantic cod in US waters. This differentiation was stable over a 5 yr period. Documentation of temporal stability of the genetic structure of fish populations is critical for identifying meaningful population units for management

(Waples 1998, Waples et al. 2008, ICES 2009).

We showed that the majority of the genetic variation among Atlantic cod spawning populations in US waters can be partitioned into 3 major complexes: (1) a northern spring coastal complex, which spawns in coastal Gulf of Maine waters from Massachusetts Bay to Bigelow Bight in the spring; (2) a southern complex, which spawns within the inshore Gulf of Maine in the winter and at different offshore locations and seasons within the GOM and south of Cape Cod; and (3) a population on the northeast peak of Georges Bank, which spawns in the late winter and is differentiated from the populations south of Cape Cod and the inshore GOM, but similar to the offshore GOM. Finer scale population structuring also occurs within the complexes, including weak differentiation between cod populations in the offshore GOM and southern New England waters (south of Cape Cod), as well as differentiation between populations south of Cape Cod (e.g. Cox Ledge and Nantucket Shoals).

One of our most significant findings is that of genetic differentiation among seasonally separated spawning groups of cod that overlap spatially in the GOM. In earlier work (Wirgin et al. 2007), we found a genetically divergent spring spawning population in Ipswich Bay. Herein we determined that this genetically distinct group of cod occurs in inshore GOM waters from Massachusetts Bay north to at least Bigelow Bight, and this population structure is stable over a 5 yr period. This northern spring complex (NSC) appears to have fidelity to the coastal GOM region during the spring and early summer months. During the winter months, a genetically distinct group of cod, the southern complex (SC), spawns in the same geographic locations (Massachusetts and Ipswich bays). The populations that spawn in the inshore GOM in the winter are genetically similar to spring and winter spawning populations on Stellwagen Bank, Jeffreys Ledge and the southern New England waters of Cox Ledge and Nantucket Shoals. This indicates greater potential gene flow among populations in the SC, which may be a consequence of early life stage dispersal patterns or adult migrations.

The weak but statistically significant differentiation observed in this study (overall $F_{ST} = 0.0044$ and $F_{ST} =$
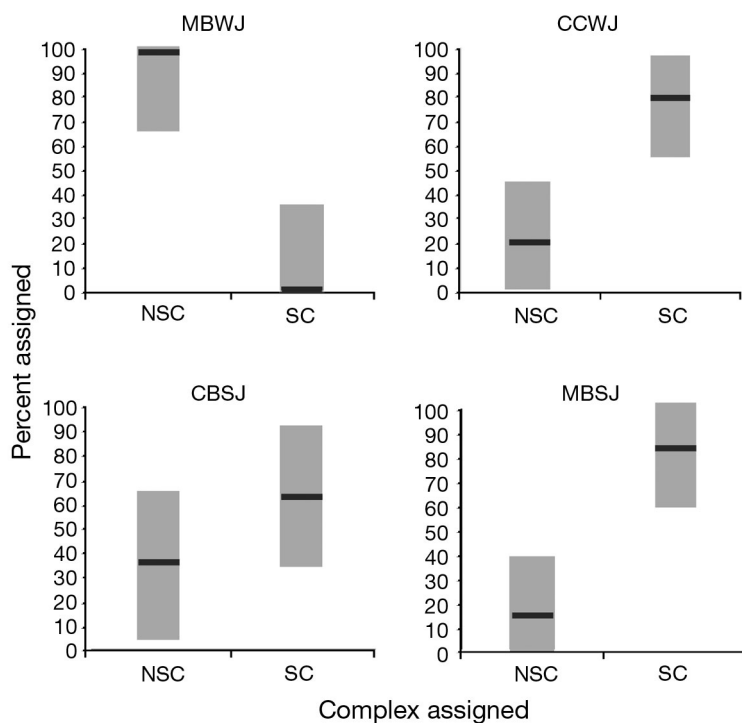
Fig. 7. *Gadus morhua*. Percentage (black bars) and 95% confidence intervals (gray rectangles) of juvenile samples assigned by mixture analysis to reporting groups of northern spring complex (NSC) and southern complex (SC). Abbreviated sample names are defined in Table 1

Mechanisms of population structuring

Processes that contribute to gene flow and thereby influence the structure of marine fish populations operate across the entire life cycle; they include adult migratory behaviors, site fidelity, dispersion of early life stages and settlement on juvenile nursery grounds (Metcalfe 2006). The genetic population structure we observed in the present study is consistent with recent findings about adult movements and early life history ecology of Atlantic cod in the GOM.

Using conventional tagging and acoustic tracking studies, Howell et al. (2008) documented the presence of 2 distinct groups of cod spawning in Ipswich Bay during the spring and winter. The spring spawning fish (NSC of our study) do not appear to undertake extensive migrations, but have limited movements along the GOM coast, exhibit spawning site fidelity and have been characterized as 'sedentary residents' (after Robichaud & Rose 2004, Howell et al. 2008, Tallack 2009). Cod comprising the SC appear to display more extensive adult movements than do those of the NSC. Cod from Cape Cod and the Great South Channel migrate both into the GOM and south towards the Nantucket Shoals and beyond (Tallack 2009). Cod from the southern GOM region may also use the Great South Channel as a migration corridor to the Nantucket Shoals (Gröger et al. 2007). The genetic similarity of the resting cod aggregations from New Jersey to the SC suggest that there may be demographic connections that extend as far south as the New York Bight. The tagging data also show limited migration between Georges Bank and southern New England waters (Tallack 2009), which is consistent with the genetic data and indicates a role for the Great South Channel in separating Georges Bank cod from these other populations (Lage et al. 2004, Wirgin et al. 2007).

Temporal differences in spawning time combined with differences in migratory behavior may serve to separate populations from the NSC and SC demographically despite some spatial overlap in their geographic locations at certain times of the year. Similarly, in the northeastern Atlantic Ocean, 2 genetically distinct groups of Atlantic cod, the northeastern Arctic cod and the northern coastal cod, differ in migratory behavior with the latter being characterized as a more sedentary inshore population (Westgaard & Fevolden 2007). These findings are consistent with an expanding body of research that recognizes biocomplexity and

0.0085 between the 2 most divergent spawning complexes) is consistent with findings from other studies of Atlantic cod populations over similar geographic scales, although direct comparisons between studies are confounded by the use of different markers. Beacham et al. (2002) employed many of the same markers as we did in this study, including *Gmo132* and *Pan*I, and found an average F_{ST} of 0.008 among populations in Newfoundland and Labrador, Canada. Studies employing the *Pan*I locus in northeastern Atlantic and Icelandic waters, however, found much greater differentiation than we found herein (e.g. Skarstein et al. 2007, Pampoulie et al. 2008a,b). In the present study, we also found differences among discrete populations within a spawning complex (F_{ST} = 0.0039 to 0.0061) that were consistent with fine-scale differences documented among nearby fjords (separated by 60 to 300 km) in Norway (F_{ST} = 0.0013 to 0.0054, *Pan*I not used, Knutsen et al. 2003) and among cod in Icelandic waters (F_{ST} = 0.0030, excluding *Pan*I, Pampoulie et al. 2006). Weak genetic differentiation may be a result of the recent origin of cod populations (Pampoulie et al. 2008a). Yet, even very small F_{ST} values among marine fish populations can coincide with migration rates that are sufficiently low to suggest demographic independence (Waples et al. 2008).

life-cycle diversity in fish (e.g. Ruzzante et al. 2006, Kerr et al. 2009b).

Gene flow is not only a function of adult behaviors, but also of egg and larval dispersal. The classical view holds that demographic connections are maintained across wide geographic locations for marine organisms with pelagic eggs and larvae, which may disperse widely with ocean currents. Yet, recent research shows that larval recruitment for some marine organisms, including cod, may occur on a local scale. In Atlantic cod, local recruitment has been linked to fine-scale habitat associations (Bradbury et al. 2008) and egg and larval retention has been hypothesized as a mechanism promoting fine-scale genetic structure (Knutsen et al. 2003, 2007, Jorde et al. 2007). Norwegian cod may select spawning sites within sheltered fjords to minimize advection of pelagic larvae (Knutsen et al. 2007). In the GOM, acoustic tracking of spring-spawning cod in Ipswich Bay revealed that spawning activity is focused at vertically elevated bathymetric features where adults aggregate during the spawning period (Siceloff 2009). Spawning in proximity to these features may promote local retention of larvae within the bay.

The outcome of egg and larval transport (dispersal) is a function of spawning location in relation to the coastal currents in the western GOM (Huret et al. 2007). The general cyclonic circulation of the Gulf of Maine Coastal Current does not favor local retention, but rather a southwestward flow to downstream or offshore from juvenile nursery sites. This circulation pattern supports connectivity among spawning grounds within the GOM and downstream nurseries within Massachusetts and Cape Cod bays and the Nantucket Shoals (Huret et al. 2007). For offshore spawning sites, advection of larvae is likely to occur to sites farther offshore (Huret et al. 2007). Larval transport varies both annually and seasonally, however. In May and June, winds favorable for downwelling may cause the coastal currents to bypass spawning grounds within Ipswich and Massachusetts bays, thereby promoting larval retention within these bays, while upwelling winds in January and February sweep larvae offshore (Churchill 2009). These seasonal differences in advection and retention are another possible mechanistic explanation for the genetic patterns we observed. Larval retention of NSC cod promotes the genetic distinctiveness of these populations via self-recruitment, while greater connectivity within the SC is facilitated by advection of eggs and larvae. These differential larval transport patterns are supported by the results of our juvenile mixture analysis. Juvenile cod, collected in Massachusetts Bay in the fall and spring, originated from spawning in the preceding season within the inshore GOM of the NSC or SC, respectively, and were retained within the bay. Juveniles collected in the fall from Cape Cod Bay, however, originated from the SC; they were probably spawned on Stellwagen Bank during the previous spring and drifted into Cape Cod Bay via the western GOM coastal current.

Larval recruitment in relation to ocean currents on Georges Bank supports the genetic distinctiveness of these fish from the populations south of Cape Cod, but not from the GOM. The anticyclonic, clockwise gyre on Georges Bank (Lough et al. 2006) may serve to retain larvae locally and limit their dispersal to either the inshore GOM or southern New England waters. Larvae spawned within the GOM, however, especially on offshore or nearshore sites, may drift with the coastal currents toward Georges Bank (Huret et al. 2007), resulting in some weak connectivity among these spawning populations despite limited adult movements. Consistent with this explanation, the spawning aggregations on Stellwagen Bank and Jeffrey's Ledge were the most genetically similar of all stocks to the Georges Bank cod.

Natural selection and local adaptation

The genetic differences in this study were due primarily to 2 potentially non-neutral loci (*Gmo*132 and *Pan*I). *Gmo132* is assumed to be under hitch-hiking selection with a gene of unknown function (Nielsen et al. 2006). The *Pan*I locus codes for an integral membrane-trafficking protein (pantophysin) expressed in cytoplasmic microvesicles (Haass et al. 1996); however, its exact function and the basis for selection at this locus are unknown (Jónsdóttir et al. 2008). The population structure we detected with these 2 selected loci could not be detected with the neutral loci alone, although the BARRIER analysis showed that the major genetic discontinuities driven by *Gmo132* and *Pan*I were also supported by a subset of the neutral loci. Similarly, selected loci, including *Pan*I, *Gmo132*, and other non-neutral microsatellites, have been found to drive the genetic differences found among other cod populations (e.g. Beacham et al. 2002, Pampoulie et al. 2006, Westgaard & Fevolden 2007; but see Pampoulie et al. 2008a,b for differentiation detected with neutral markers alone).

Population differentiation at selected loci is interpreted differently than at neutral loci (Nielsen et al. 2006), as it is indicative of local ecological adaption to environmental selection pressures. Adaptive divergence can occur over much shorter time scales than can neutral genetic differentiation (Endler 1986, Cano et al. 2008), resulting in genetic differentiation of a greater magnitude for selected versus neutral loci (Beaumont & Balding 2004). Although selected loci are

therefore inappropriate for making inferences about population processes, such as migration rates and effective population size, they can serve an important function in genetic stock identification (Nielsen et al. 2006, Westgaard & Fevolden 2007, ICES 2009). The adaptive differences underlying variation at selected loci may have important management implications (Crandall et al. 2000, Conover et al. 2006, Hauser & Carvalho 2008, ICES 2009).

Variation in *Pan*I has been correlated with environmental factors, including water temperature, salinity, depth, latitudinal gradients and inshore/offshore migrations (Beacham et al. 2002, Case et al. 2005, Sarvas & Fevolden 2005, Pampoulie et al. 2006, Westgaard & Fevolden 2007), with potential fitness effects reported (Otterlei et al. 1999, Case et al. 2006, Jónsdóttir et al. 2008). The patterns are not consistent across studies and geographic regions, however, indicating that the selective pressures may be complex (Jónsdóttir et al. 2008).

On a macrogeographic scale, Pampoulie et al. (2008a) found that *Pan*IBB genotypes occur only in the northwestern Atlantic Ocean (Iceland and Canada), while the northeastern Atlantic is dominated by AA and AB genotypes. In Canadian waters all 3 genotypes are present, with a trend for higher frequencies of the B allele in offshore and southern populations, while A is more prevalent in northern and inshore populations (Beacham et al. 2002). Our results show an extension of the latitudinal and inshore/offshore gradient found in Canadian waters. Genotype AA is extremely rare in the populations in the present study (occurs in 3 of 1581 adults, all in the NSC), and a higher frequency of the A allele occurs in the NSC, which is thought to be a resident coastal population, than in the SC and Georges Bank, which consist predominantly of BB genotypes and include offshore and presumably more migratory populations. Our findings are consistent with studies hypothesizing a selective advantage of the B allele in Atlantic cod with offshore migrations into deeper and higher salinity waters (Pampoulie et al. 2006, 2008c, Westgaard & Fevolden 2007, Jónsdóttir et al. 2008, Árnason et al. 2009). It is important to note, however, that the differences in *Pan*I allele frequencies in this study were small relative to the differences found in other studies, which might be at least in part a function of the small spatial scale.

Notably, the differences in *Pan*I allele frequencies in this study were greater in the juveniles than the adults (*Pan*I F_{ST} value for the juvenile samples was nearly double that of the adults, Table 2). This result may be suggestive of post-settlement selection on the early life stages, which occurs in the face of ongoing gene flow. Further research into the environmental correlates and selective forces acting upon *Pan*I is warranted.

Conclusion and management implications

The definition of stocks as demographically independent units is at the core of marine conservation and management (Waples et al. 2008). By this definition, our results do not support the current stock delineations for Atlantic cod in US waters. Consistent with previous studies (Lage et al. 2004, Wirgin et al. 2007), we found strong evidence for a fine-scale spatial and seasonal population genetic structure that contradicts the 2-stock management model. The Gulf of Maine stock consists of 2 seasonally divergent spawning groups, while the Georges Bank population is divergent from the southern New England and perhaps New York Bight populations, with which it is currently grouped for management purposes. The southern New England populations maintain connectivity with the winter-spawning inshore GOM population and both spring- and winter-spawning offshore populations of the GOM. Our results suggest, therefore, that Atlantic cod are broadly structured into 3 complexes: (1) a northern spring-spawning coastal complex in the GOM, (2) a southern complex consisting of winter-spawning inshore GOM, offshore GOM and sites south of Cape Cod, and (3) a Georges Bank complex. This population structure is temporally stable and the magnitude of genetic differentiation, while not large, is sufficient to assign juveniles to their complex of origin via mixture modeling.

Our findings are in accordance with a recent awareness of a mismatch between biological and management units for a number of commercially important marine fishes (Reiss et al. 2009). Our results also add to the growing body of knowledge that marine fish populations do not conform to the classical panmictic population view, but rather are characterized by population structure on a much finer scale than expected from their dispersal and migratory abilities (Hauser & Carvalho 2008). It may be warranted to re-evaluate current management units and tailor management plans toward this finer scale in light of the importance of managing for biologically meaningful units, based on concepts of ecological exchangeability and maintaining biodiversity and biocomplexity (Crandall et al. 2000, Ruzzante et al. 2006, Bradbury et al. 2008, Cadrin & Secor 2009, ICES 2009, Reiss et al. 2009).

Future studies should focus on determining the selective forces shaping the adaptive population divergence and identifying additional informative markers to enhance our ability for differentiating among subpopulations within the US stock complexes of Atlantic cod. To this end, some of the recently identified gene-associated loci (e.g. Stenvik et al. 2006b, Wesmajervi et al. 2007, Delghandi et al. 2008, Moen et al. 2008) may prove useful.

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