

## **Northeast Consortium Cooperative Research Final Report**

**Project Title:** Genetic Identification of Atlantic Cod Spawning Stocks in U.S. Waters using Microsatellite and SNP DNA Markers

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Report prepared by Adrienne Kovach, September 30, 2010.



**Abstract:** In this collaborative research project, we formed a partnership of commercial fisherman and scientists from UNH and NYU to investigate stock definitions for Atlantic cod using DNA markers. Cod in U.S. waters are currently managed as two 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. To address this, we used 10 microsatellite and 6 SNP markers to characterize the population genetic structure of cod in U.S. waters. We found significant differentiation among temporally and spatially divergent populations of cod (global  $F_{ST} = 0.0044$ ,  $G'_{ST} = 0.0144$ ), primarily stemming from two non-neutral loci, and strong evidence for a population structure that contradicts the current two-stock management model. Our results indicate that cod in U.S. waters are broadly structured into three 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, MA, and 3) a Georges Bank population. The strongest differentiation occurs between populations in the northern and southern complex ( $F_{ST} = 0.0054 - 0.0156$ ), some of which spawn in the same bays in different seasons. This population genetic structure is stable over a 5-year period. We suggest a model of population structure that is maintained by geographic and seasonal differences in larval recruitment and differential adult life history strategies; local ecological adaptations may also be important. Our findings contribute to a growing body of knowledge that 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. We identified and began to fill additional research gaps: i.) characterizing remnant spawning aggregates in the midcoast and downeast Maine; ii.) increasing the resolution of our genetic markers for differentiating spawning complexes; and iii.) determining environmental factors that correlate with genetic differentiation among spawning complexes.

**Introduction:** Atlantic cod (*Gadus morhua*) is a principal component of the Northeast groundfish assemblage, and as such has supported tremendously productive commercial and recreational fisheries in the region for years. The social and economic importance of this fish is highlighted by Kurlansky (1997), who titled his history of the North Atlantic cod fisheries: “*Cod: A Biography of the Fish That Changed the World.*” Cod’s very desirability has led to rampant over harvesting across its range. North American cod stocks are in particularly poor shape. The recent collapse of the Canadian cod fisheries (Hutchings and Myers 1994) led to massive unemployment and social upheaval in Newfoundland. Within U.S. waters, cod stock biomass, commercial landings, and recruitment have fluctuated dramatically over the past four decades, with some of the lowest estimates to date recorded by the late 1990s. Today, many New England fishermen who rely heavily on cod are struggling to remain financially solvent as fishery management plans aimed at rebuilding stock biomass restrict harvesting.

A significant complication in the management of the Atlantic cod, and many other fishes, is uncertainty of their stock structure (i.e., number of stocks within a region, boundaries of stocks, and extent of stock mixing). In general, informed fisheries management should be built on detailed knowledge of the stock structure of harvested species, and should be based on discrete unit stocks (Cushing 1968). Management regimes should be tailored specifically for each stock to reflect differences in recruitment, growth, and mortality. This strategy has been called for

Atlantic cod (Ames 2002), but has not yet been implemented because of a void of information on its stock structure in U.S waters. To this end, the goal of this project is to address the uncertainty that exists about the stock structure and number of discrete spawning populations of Atlantic cod in U.S. waters.

Management of the cod fishery in U.S. waters usually assumes a two stock model: 1) the Gulf of Maine (GOM), and 2) Georges Bank and south. The empirical verification of this model is limited, however, and the studies upon which it is founded are decades old. This division is primarily based on limited and dated tagging studies reported in 1902, the 1930s and 1962 (Serchuk and Cohen 1990), frequencies of parasitic infestation (Sherman and Wise 1961), maximum spawning time data (Colton et al. 1979), growth rate variation (Penttila and Gifford 1976), and differences in age and length at sexual maturity (Fahay 1998). Additionally, patterns of recruitment of the Georges Bank stock are generally different from those observed in the GOM stock (Serchuk and Cohen 1990). These diagnostic biological characteristics are eco-phenotypic in nature, however, and the mark-recapture results were generated when these stocks were much larger and were not based on actively spawning fish tagged on the spawning grounds. During the past 50 years, cod stocks on Georges Bank and within the GOM have undergone tremendous fluctuations in abundances, which could compromise the applicability of earlier studies to current management. In recent years, both stocks have demonstrated decreases in landings, spawning stock biomass, and year class strength, although specific trends in these indices often differ between the two putative stocks.

There is also much uncertainty regarding fine scale structuring of stocks within the GOM. Spawning within this stock occurs throughout the year with the exception of the summer months. Many areas have two or more spawning events within a single year (Berrien and Sibunka 1999, Howell et al. 2008) affording the possibility of more than one stock at a single geographic locale. Ames (2002) and others have reported that historically there were 91 spawning grounds in the GOM extending from Cape Ann in the west to Lurcher Shoals to the east. Today, spawning is only observed at approximately 40 of these locales, with the greatest reduction in activity observed at those sites in Downeast Maine (Berrien and Sibunka 1999). Ames (2002) proposed the historical existence of four major subpopulations of cod within the GOM, including a Western subpopulation from northern Massachusetts Bay to Sheepscot Bay, a Midcoast subpopulation in the area of Penobscot Bay, a Downeast subpopulation in the Mt. Desert Island area, and a Bay of Fundy subpopulation. However, evaluation of the genetic discreteness of these subpopulations has not been attempted prior to this study.

Genetic stock identification provides a sensitive approach to evaluate the stock structure of species in which spawning populations have been reproductively isolated for considerable times and between which contemporary gene flow is very low or non-existent. Genetic stock identification at selectively neutral loci (e.g. microsatellite DNA and SNP markers) offers a major advantage over other approaches in that genetic stock signatures are not subject to environmental influences and therefore remain stable over many generations. Once diagnostic genetic markers have been identified and their temporal stability over several generations confirmed, spawning stocks need not be revisited on a regular basis to reconfirm genotype frequencies. After the stock structure of spawning aggregations has been defined, mixed stock analysis can be performed to describe mixing and migration of fishes from spawning areas to

other areas. Furthermore, the genetic data can complement other biological data collected from the same populations, such as acoustic monitoring and tagging data, to better understand population structuring processes, such as migrations and spawning site fidelity.

With the goal of characterizing the genetic stock structure of cod in US waters, our project responded directly to recent stock assessments that reported overfishing and decline of both the GOM and Georges Bank stocks (May and Col 2002, O'Brien et al. 2002, NEFMC 2005). The depressed state of cod populations necessitates stock-specific management plans to maintain fishery sustainability; better information about cod stocks is crucial to effective rebuilding plans. The need for applying genetic and other biological data to re-evaluate stock boundaries and management units of commercially important fish species worldwide has been highlighted recently (Cadrin and Secor 2008, Hauser and Carvahlo 2008, ICES 2009, Reiss et al. 2009).

This project has involved commercial fisherman, researchers from UNH and NYU, and other partners (UMass-Dartmouth, Mass Division of Marine Fisheries) in a collaborative effort to plan and implement a robust sampling regime for Atlantic cod. Commercial fishermen's knowledge of the location and method of capture of spawning cod was critical to the success of this project. Both the goals and approaches of this project were directly relevant to programmatic goals of the Northeast Consortium. Our project addresses the topic area "commercial harvest and species sampling." Most specifically, our project addresses one of the highest priority research needs of the New England Fishery Management Council, which calls for "investigations into stock definition, stock movements, mixing, and migration, such as through tagging studies, DNA markers, morphological characteristics, etc" for Northeast Multispecies (Groundfish) assemblages. Our project also contributes to a number of the priorities outlined in the Northeast Fisheries Science Center section of the NMFS FY 2004-2009 strategic research plan (NMFS, 2004):

*"Determine the biological, environmental, and habitat processes controlling the reproductive success of important fishery resources."*

*"An outgrowth of the stock assessment peer review is continuing investigations of the appropriate assessment methodologies for Georges Bank/GOM cod stocks."*

**Project Objectives:** The overarching goal of our project is to identify the spatial and temporal stock structure of Atlantic Cod (*Gadus morhua*) in US waters, using genetic analyses.

Specifically, our objectives are to

- 1) Identify and sample major spawning aggregations throughout the Gulf of Maine, Georges Bank, and areas south of Georges Bank.
- 2) Determine if cod stocks on Georges Banks, GOM, and south of Georges Bank are genetically unique.
- 3) Characterize spatial or temporal genetic heterogeneity among spawning aggregations within these broad geographic areas (GOM, Georges Bank, and south).

*Overall Hypothesis:*

Cod spawning aggregations in northeast U.S. waters are genetically differentiated.

## **Participants:**

### *Scientists*

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### *Steering Committee*

- 5) Steven Cadrin, NOAA Northeast Fisheries Science Center, Cooperative Marine Education & Research, School for Marine Science & Technology, 200 Mill Road, Suite 32, Fairhaven, MA 02719 U.S.A. (508) 910-6358; Steven.Cadrin@noaa.gov
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### *Commercial Fishermen (advised and/or assisted with sample collection):*

- 8) David Goethel, F/V *Ellen Diane*, Hampton, NH & New England Fishery Management Council member
- 9) Proctor Wells, F/V *Tenacious*, Phippsburg, ME
- 10) Frank Mirarchi, F/V *Barbara L. Peters*, Scituate, MA
- 11) Chris Odlin F/V *Lydia and Maya*, Portland ME
- 12) Carl Bouchard F/V *Stormy Weather*, Hampton, NH
- 13) Jeff Carver, F/V *Saint Joseph*, Scituate, MA

*Student Participation:* This project has involved participation from 5 undergraduate students, 1 graduate student and 1 high school student.

*Graduate Student:* Timothy S. Breton, University of New Hampshire

*Undergraduates:* Corinne Brauer, Drew University, Madison, NJ

Justine Deming, University of New Hampshire

Matthew Lubicky, University of New Hampshire

Samantha Petren, University of New Hampshire

Katrina Papanastassiou, University of New Hampshire

*High School Student:* Chelsea Van Thof, Seacoast School of Technology, Exeter, NH

**Methods:**

*Sampling:* During December 2005 – July 2008, 1581 adult cod were captured via otter trawl, gill net or hook and line, in collaboration with commercial and recreational fishermen and fishery biologists. Spawning condition was assessed by visual inspection of the gonads or observations of running milt or eggs using the National Marine Fisheries Service ovarian staging criteria. Caudal fin clip samples (1 cm<sup>2</sup>) were taken from each fish and preserved in 95-100% ethanol.

Spawning cod were sampled from the following sites: northeast peak of Georges Bank, the inshore Gulf of Maine in Ipswich Bay, Massachusetts Bay, and Bigelow Bight, ME, the offshore Gulf of Maine at Jeffrey's Ledge and Stellwagen Bank, and south of Cape Cod from Nantucket Shoals, and Cox Ledge. At Ipswich Bay, Massachusetts Bay and Cox Ledge, distinct spawning aggregates were identified and sampled in both the spring and winter. Additionally, adult fish not in spawning condition (resting individuals) were sampled from Ipswich Bay, Platts Bank and New York Bight. Six of the spawning aggregates were sampled in 2 subsequent years, enabling us to test for stability in the structure (see Table 1 in Appendix and Fig. 1, below).

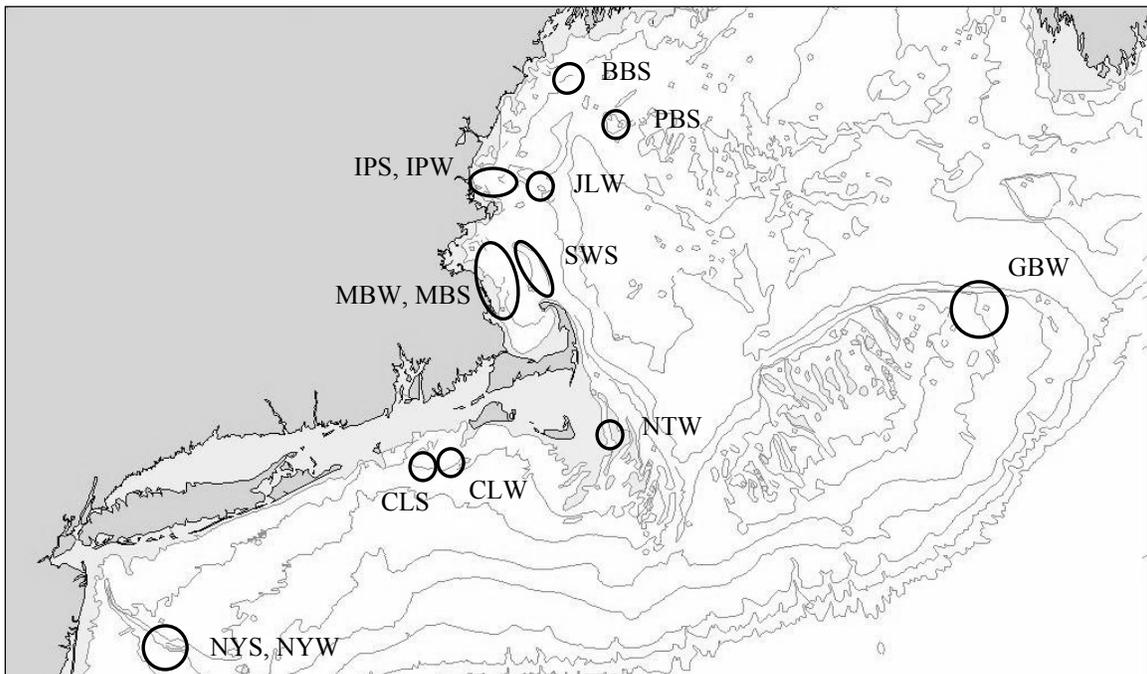


Figure 1. Sample site locations for Atlantic cod genetic stock identification in this study. Abbreviated sample site names refer to Table 1 of Appendix.

*Additional Sampling:* In addition to the above samples, which were used in our characterization of the genetic stock structure of Atlantic cod, we have collected additional samples for further study. These samples consist of sites in midcoast and downeast Maine, collected during the summer of 2010, as well as collections from Chatham/western Georges Bank during winter 2009-2010 and additional samples from another spring-spawning population in Massachusetts Bay in 2010. The genetic characterization of these samples is part of ongoing and future research efforts.

*Project Design:* The collection sites targeted in this project followed our initial proposed sampling scheme, which built upon our prior NEC-funded research (Wirgin et al. 2007): actual collection sites were influenced by discussions with steering committee members (led by Dr. Steve Cadrin, NMFS/SMASST) and availability of spawning cod. This led to some additional collection opportunities beyond our original proposal, including a site on Nantucket Shoals and separate spring and winter spawning aggregations on Cox Ledge (resulting in 3 collection sites in the south, rather than the 1 originally proposed). We also identified and sampled an unanticipated spring spawning aggregate in Massachusetts Bay (in addition to the winter spawning fish in that bay). Offshore sites of Stellwagen Bank and Jeffreys Ledge were also included in our GOM collections (although not in our original proposal), due to the potential for differentiation among inshore and offshore populations.

*Limitations:* In our initial efforts (2006 – 2009), we were unable to locate spawning (or any adult) cod in eastern Maine, due to a lack of fishing effort on these depleted populations. Our northern/eastern most collection site, therefore, was Bigelow Bight. We were also initially unable to obtain a sufficient sample of spawning cod from the western Georges Bank area, despite targeted collection trips. Therefore, these sites were not included in our evaluation of stock structure (Wirgin et al. 2007, Kovach et al. 2010). In 2009 and 2010 we were successful in obtaining samples from western Georges Bank and a limited number of samples from midcoast Maine. Analyses of these samples are ongoing and will be a component of future publications. We also found that the winter spawning site in Ipswich Bay, which we characterized in our previous Northeast Consortium-sponsored research (Wirgin et al. 2007), has not been an active spawning site in the last several years; instead, these fish are thought to be aggregating and spawning in Massachusetts Bay during this time (December-January; D. Goethel, personal communication). We therefore sampled winter-spawning fish in Massachusetts Bay and resting fish in Ipswich Bay in the winter.

*Genetic Analyses:* DNA was extracted using Qiagen DNeasy tissue kits (Qiagen, Valencia, CA, U.S.) or standard phenol/chloroform procedures. Genetic analysis was performed using a panel of 10 microsatellite markers (*Gmo02*, *Gmo132*, Brooker et al. 1994; *Gmo19*, *Gmo35*, *Gmo36*, *Gmo37*, Miller et al. 2000; *PGmo32*, *PGmo34*, *PGmo38*, and *PGmo58*, Jakobsdóttir et al. 2006), and 6 SNPs (Pantophysin I (*Pan I*), Pogson et al. 2001, *AHR6*, *ARNT8*, Wirgin et al. 2007, and *ARNT1*, *CYP5*, and *K ras*, characterized in this study). To characterize population genetic structure, the genotypic data were analyzed by several statistical population genetic methods, including F-statistics ( $F_{ST}$ , a measure of genetic variation among populations), allelic differentiation exact tests (statistical tests to compare the allele frequency distributions among populations), and molecular analysis of variance (AMOVA), to test for hierarchical structure and temporal variability (see below). To test for assumptions of neutrality, we performed selection tests for all loci using an  $F_{ST}$  outlier approach (Beaumont and Nichols 1996) in LOSITAN (Antao et al. 2008) with a calculation of the neutral mean  $F_{ST}$  using 95,000 simulations.

To test for genetic differences among adult populations, pairwise allelic differentiation tests in GENEPOP version 3.4 (Raymond and Rousset 1995) and  $F_{ST}$  calculations using the estimator  $\theta$  of Weir and Cockerham (1984) in FSTAT 2.9.3 (Goudet et al. 1995) were used. Spawning aggregations sampled from the same site and season in two separate years were examined for annual fluctuations in genetic variation using  $F_{ST}$  estimations in FSTAT. Temporal stability was

also assessed using an 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). After verification of temporal stability, yearly samples from the same sites were pooled for further analyses of pairwise population  $F_{ST}$  and allelic differentiation.  $F_{ST}$  values for the pooled dataset were also used in a principal coordinate analysis (PCA) using GENALEX 6.1 (Peakall and Smouse 2006) to visualize the clustering of populations. A *posteriori* analysis of population groupings identified by significant  $F_{ST}$  and PCA clustering were conducted using a hierarchical approach in AMOVA to identify significant genetic variation within and among groups.

*Screening of additional loci:* We have also screened the following additional loci in order to identify additional markers to add to our current suite, to improve our resolution for differentiation among the stock complexes: GmoC94, GmoC102, GmoC115, GmoC121, GmoC122, GmoC123, GmoC18, GmoC20, GmoC50, GmoC71, GmoC76, GmoC78, GmoC80, GmoC82, GmoC83, and GmoC88 (Delghandi et al. 2008, Stenvik et al. 2006).

**Data:** Per locus  $F_{ST}$  values ranged -0.0023-0.0327. The highest levels of differentiation were observed at *Gmo132* and *Pan I*, with  $F_{ST}$ s 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 under positive selection. All other markers were within neutral expectations. The mean neutral  $F_{ST}$ , calculated by LOSITAN after removal of *Gmo132* and *Pan I*, was 0.0020. Across all loci, differentiation was highly significant ( $p < 0.0001$ ), with a global  $F_{ST}$  of 0.0044 and a  $G'_{ST}$  (Hedrick 2005) of 0.0144. There was no significant variation between the yearly collections from the same sample locations and variation among sites was significantly greater than annual variation within sites. These results indicated stability in the genetic structure from year to year, and the replicate samples were pooled for further analyses.

When the pooled data from all spawning aggregates were compared by pair-wise  $F_{ST}$  analysis, 16 of 45 population comparisons were significant. The primary source of differentiation occurred between the spring spawning coastal aggregates of the inshore Gulf of Maine (Ipswich Bay, Massachusetts Bay and Bigelow Bight) and sites in the offshore Gulf of Maine, winter spawning inshore Gulf of Maine and southern New England sites (Nantucket and Cox Ledge). Significant  $F_{ST}$  values ( $P < 0.001$ ) for these comparisons ranged from 0.0054-0.0156. Additionally, Georges Bank was significantly differentiated from the southern sites ( $F_{ST} = 0.0029-0.0056$ ) and was differentiated by allelic differentiation but not  $F_{ST}$  analysis from the majority of the Gulf of Maine populations. The two offshore Gulf of Maine populations of Stellwagen Bank and Jeffrey's Ledge were similar to the Georges Bank collection by both differentiation methods. Employing less conservative criteria (e.g.  $p < 0.01$  or  $p < 0.05$ ) yielded additional population differences primarily following the pattern above (see Table 4 for details) as well as a fine-scale difference between the southern populations of Cox Ledge (spring-spawning) and Nantucket Shoals ( $F_{ST} = 0.0036$ ;  $p = 0.0033$ ). Overall, 35 of 45 comparisons were significant by one or both differentiation methods at the  $p \leq 0.05$  level.

Principal coordinate analysis of the  $F_{ST}$  data from the spawning aggregates identified two main clusters of populations (Fig. 2A): a spring spawning coastal Gulf of Maine population of Bigelow Bight, Ipswich Bay, and Massachusetts Bay and another cluster comprised of 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 two clusters, as they were highly distinct from the southern sites of Cox Ledge and Nantucket Shoals, but 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. When the resting adult aggregations from Platts Bank, Ipswich Bay winter, and the New York Bight were added to the analyses, they were found to cluster primarily within one of these 3 groupings (Fig. 2B). The Platts Bank summer adults clustered with the spring spawning GOM populations. New York Bight spring and winter collections clustered with the other GOM populations, although the spring collection was also similar to Georges Bank. The Ipswich Bay winter adults clustered with the other winter-spawning GOM populations, 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 and may not represent a significant population difference.

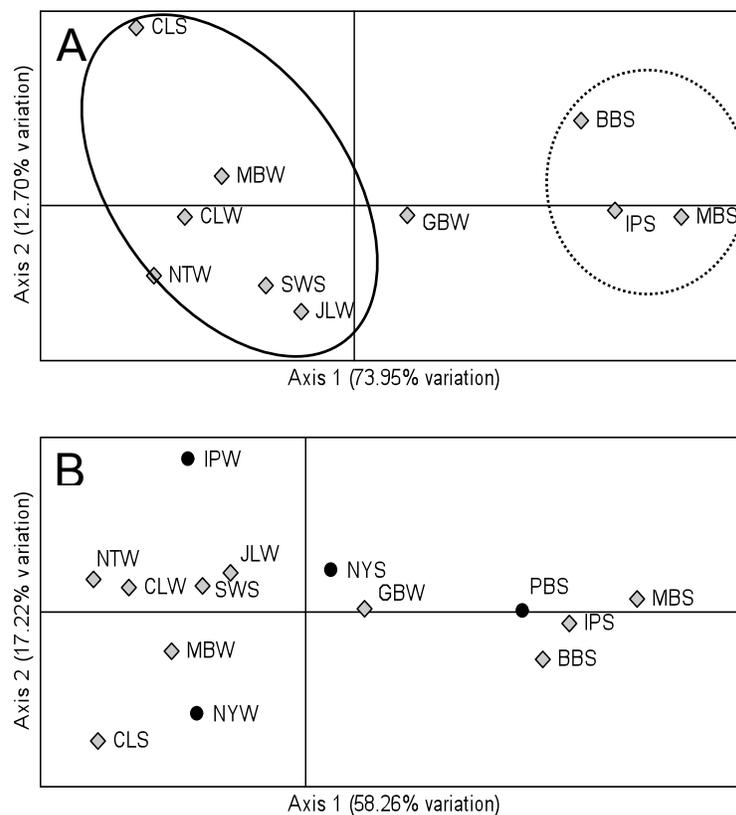


Figure 2. Principal coordinate analyses of pair-wise population  $F_{ST}$  values. (A) Comparison of spawning Atlantic cod populations (♦); circles surround genetically similar populations. (B) Comparison of spawning Atlantic cod populations and resting collections (●).

We tested for hierarchical structure of the population clusters identified by  $F_{ST}$  and PCA. This *a posteriori* analysis with AMOVA demonstrated significant genetic variation between the two population clusters when all loci were included in the analysis (Table 5; Georges Bank was not included in this analysis as it was the only population in its grouping and therefore not relevant to hierarchical structure). Significant variation was also evident within the clusters, indicating the presence of finer scale structure. For additional data see Kovach et al. (2010).

**Results and Conclusions:** Our results are synthesized in Fig. 3, which shows that the majority of the genetic variation among cod spawning in U.S. waters can be explained by three major groupings: 1) a northern spring coastal complex (NSC), which spawns in coastal GOM waters from Massachusetts Bay to Bigelow Bight in the spring and summer; 2) a southern complex (SC), which spawns within the inshore GOM 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 (NGB), which spawns in the late winter and is strongly differentiated from the populations south of Cape Cod, only weakly differentiated from the inshore GOM, and similar to the offshore GOM. Finer scale population structuring also occurs within the complexes, including weak differentiation between 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).

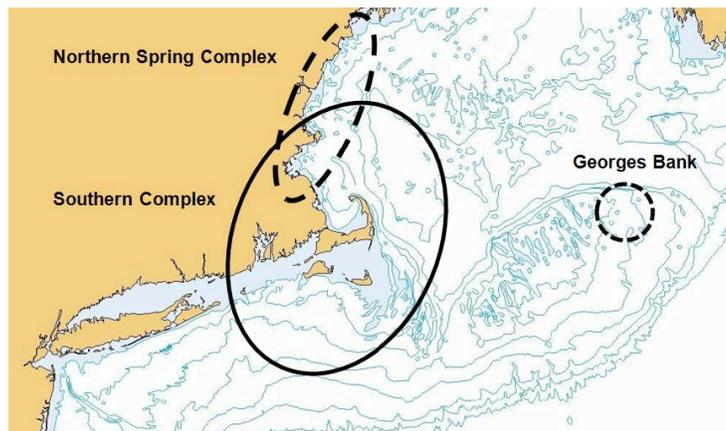


Figure 3. Proposed Atlantic cod spawning complexes in U.S. waters based on genetic differentiation (see text for explanation). Abbreviated sample site names refer to Table 1 of Appendix.

One of our most significant findings is that of genetic differentiation among temporally divergent spawning groups that overlap spatially in the GOM. In earlier NEC-supported 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 we found this population structure to be stable over a 5-year period. This 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 fish (the 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 points toward greater potential gene flow among populations in the SC, which may be a consequence of early life stage dispersal patterns or adult migrations.

The genetic population structure observed in this study is consistent with recent findings about adult movements and early life history ecology of cod in the GOM. Tagging and acoustical tracking studies have shown that two distinct groups of fish spawn in the inshore GOM in the spring and winter (Howell et al. 2008). Some of these fish (likely the NSC of this study) do not undertake extensive migrations, but rather have limited movements along the GOM coast (Tallack 2009). The tagging data also support migrations between some GOM fish (likely the SC of this study) and the Nantucket Shoals (Gröger 2007, Tallack 2009), and are consistent with the genetic data in indicating a role for the Great South Channel in separating Georges Bank from populations in southern New England waters (Lage et al. 2004, Wirgin et al. 2007, Tallack 2009). Larval dispersal models show seasonal differences in advection/retention that may provide another mechanistic explanation for the genetic patterns we observed (Churchill 2009). Genetic distinctiveness of NSC cod may be promoted by self-recruitment within Massachusetts and Ipswich Bays as a result of downwelling winds that favor larval retention in May and June (Churchill 2009). In contrast, upwelling favorable winds in January and February lead to advection of eggs and larvae to downstream and offshore sites (Churchill 2009), thereby promoting genetic connectivity within the SC.

The genetic differences in this study were due primarily to two non-neutral loci (*Gmo132* and *Pan I*). Population differentiation at selected loci requires different interpretation than that at neutral loci (Nielsen et al. 2006), as it is indicative of local ecological adaptation 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 vs. neutral loci (Beaumont and Balding 2004). Selected loci thus 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 and Carvalho 2008, ICES 2009). Future studies should focus on determining the selective forces shaping the adaptive population divergence among cod populations.

Importantly, our study found strong evidence for a fine-scale spatial and temporal population genetic structure that contradicts the two-stock management model. The Gulf of Maine stock consists of two temporally divergent spawning groups, while the Georges Bank population is divergent from the southern New England and perhaps mid-Atlantic populations, with which it is currently grouped for management purposes. The southern New England populations maintain connectivity with winter spawning inshore and both spring and winter spawning offshore populations of the GOM. Using the definition of stocks as demographically independent units (Waples et al. 2008), our results do not support the current stock delineations for Atlantic cod in U.S. waters. Rather, our results suggest that cod are broadly structured into 3 groups: 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 population. This population structure is temporally stable and the magnitude of

differentiation, while not large, is comparable to that observed for cod populations in other geographic locations (e.g. Beacham et al. 2002, Knutsen et al. 2003, Westgaard & Fevolden 2007, Pampoulie et al. 2008).

Our findings are in accordance with a recent awareness of a mismatch between biological and management units for a number of commercially important marine fish (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 and 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 and Secor 2009, ICES 2009, Reiss et al. 2009). Our project has made a significant contribution to the Northeast Consortium's goal of investigating stock definitions using DNA markers and our findings are directly relevant to fisheries management practices in the GOM.

**Partnerships:** Our fishermen-scientist partnerships have proven critical in sample collection of Atlantic cod. This project has involved collaboration of over a dozen individuals, including university, NOAA and state agency scientists and commercial fisherman (see Participants section). Numerous fisherman were helpful not only in obtaining samples, but in advising on sample site selection and availability of spawning fish at given times and locations. In addition, we have and continue to communicate our findings and future work plans via meetings, seminars and workshops. Many of these venues are well attended by our commercial fisherman partners. Our partnership with Dr. Steven Cadrin (UMASS Dartmouth) has also been invaluable and has resulted in not only additional sample collections from Cox Ledge and Massachusetts Bay, which tie in to ongoing tagging studies, but also critical advice and logistical help with coordination of sampling trips. Additionally, Jeremy King, David Salerno, and Mark Szymanski from the Massachusetts Division of Marine Fisheries have assisted in sampling in Massachusetts Bay. Overall, our research has generated broad interest in New England from those involved in the fishing industry as well as those involved in fishery management (agency biologists).

**Impacts:** Our research involves collaboration between scientific researchers, managers and commercial fisherman. We have been working closely with our steering committee members in sample collection and have made efforts to communicate our preliminary findings through reports, scientific publications, seminars and meetings. Our project is generating much interest and we anticipate that our findings will be incorporated into the scientific information used to develop fisheries management plans. At a recent workshop on Exploring Fine-scale Ecology for Groundfish in the Gulf of Maine and Georges Bank held in York, ME on April 2, 2009, our findings were germane to discussions about the need to rethink and reassess stock boundaries for groundfish, especially Atlantic cod, and to move potentially towards fine-scale management. Marine scientists, fisheries managers, fisherman and conservation agencies, such as the Penobscot River Restoration Trust, would benefit from knowing our findings.

**Related projects:** Not Applicable.

**Presentations:** Results of this and ongoing NEC-funded research were presented at the Workshop on Exploring Fine-scale Ecology for Groundfish in the Gulf of Maine and Georges Bank held in York, ME on April 2, 2009. The presentation, entitled “Genetic Insights into the Stock Structure of Atlantic Cod” was made by Adrienne Kovach.

Runge, J.A., A.I. Kovach, J. Churchill, L. Kerr, and others. Understanding Climate Impacts on Recruitment and Spatial Dynamics of Gulf of Maine Atlantic cod stock complex. Cod and Climate Change Workshop, 3<sup>rd</sup> GLOBEC Ocean Science Meeting, Victoria, B.C. June, 2009

Breton, T.S., A.I. Kovach, I. Wirgin and D. Berlinsky. 2008. Spawning Stock identification of Atlantic cod (*Gadus morhua*) in U.S. Waters using Microsatellite Markers. Northeast Regional Fish & Wildlife Conference, Galloway, New Jersey.

Isaac Wirgin, Lorraine Maceda, Nirmal Roy, Adrienne Kovach, and David Berlinsky. 2006. Stock Structure of Atlantic Cod in U.S. Using Microsatellite and SNP DNA Analysis. Presented at 62<sup>nd</sup> Northeast Fish & Wildlife Conference, Burlington, VT, April 23-26.

Kovach, A.I., D.L. Berlinsky, and I. Wirgin. 2005. Genetic Identification of Spawning Stocks of Atlantic Cod in US Waters. Abstract, 5<sup>th</sup> Annual Northeast Consortium Participants Meeting, Portsmouth, NH.

### **Published Reports and Papers:**

Kovach, A.I., T.S. Breton, D.L. Berlinsky, L. Maceda, and I. Wirgin. 2010. Fine-scale and temporal genetic structure of cod off the Atlantic coast of the USA. *Marine Ecology Progress Series*. 410: 177-195.

Runge, J.A., A.I. Kovach, J. Churchill, L. Kerr, and 17 others. *In Press*. Understanding Climate Impacts on Recruitment and Spatial Dynamics of Atlantic Cod in the Gulf of Maine: Integration of Observations and Modeling. *Progress in Oceanography*.

A recording of the presentation noted above, made at the Workshop on Exploring Fine-scale Ecology for Groundfish in the Gulf of Maine and Georges Bank held in York, ME on April 2, 2009 along with a technical abstract, can be accessed at the following website:  
<http://www.gmri.org/community/display.asp?a=5&b=14&c=160>

A portion of this research comprised a Masters thesis by Timothy Breton:  
Breton, T.S. 2008. Spawning Stock Identification of Atlantic Cod (*Gadus morhua*) in U.S. Waters Using Pan I and Microsatellite Genetic Markers. Masters Thesis, University of New Hampshire, Durham, NH, 75 pp.

Wirgin, I., A. I. Kovach, L. Maceda, R. Nirmal, J. Waldman, and D.L. Berlinsky 2007. Stock identification of Atlantic cod in U.S. waters using microsatellite and single nucleotide polymorphism (SNP) DNA analyses. *Transactions of the American Fisheries Society* 136: 375-391.

**Future Research:** We have identified 3 additional directions for future research and have begun addressing efforts to fill these knowledge gaps. First, efforts should continue to identify and monitor the locations of spawning aggregates, throughout the Gulf of Maine, and especially within the depleted populations of midcoast and downeast Maine. Determining the genetic structure of these populations will test Aimes' (2004) hypothesis of multiple, historic, self-sustaining spawning populations in this area. Second, there is an additional need for future work to identify more informative markers to enhance our ability for differentiating among subpopulations within the stock complexes. 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. We have recently been working on characterizing a subset of these in our lab. Lastly, future research should focus on understanding the ecological implications of the genetic population differentiation. Given our finding that the majority of differentiation was explained by genetic markers influenced by natural selection, the role of adaptive differentiation should be explored. To this end, investigating correlations of genetic structure with environmental features, such as salinity, depth and temperature will provide predictive power for population persistence in the face of global change.

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## Appendix

Table 1. Abbreviated sample site names, collection sites, dates collected, sample sizes (n), and sexes of Atlantic cod used for genetic stock identification. Length ranges (cm) of juvenile cod from each location are given in place of sexes. Reproductive conditions of cod collections are given in parentheses as mean; range and refer to National Marine Fisheries Service (NMFS) ovarian staging criteria: 1 = immature, 2 = resting, 3 = developing, 4 = ripe, 5 = ripe and running, 6 = spent.

Name	Collection Site	Date	n	Sex (condition)
<i>Gulf of Maine inshore</i>				
BBS	Bigelow Bight	7/07	70	females (6; 6)
	Bigelow Bight	7/08	47	mixed (5; 5)
IPS	Ipswich Bay	4/06-5/06	122	mixed (5; 3-5)
	Ipswich Bay	6/07	78	females (6; 6)
IPW	Ipswich Bay	12/06	31	mixed (2; 2, 5)
MBW	Massachusetts Bay	12/05-1/06	140	females (4; 3-6)
	Massachusetts Bay	1/07	77	mixed (5; 3-5)
MBS	Massachusetts Bay	4/06	36	females (4; 4-5)
	Massachusetts Bay	6/08	49	females (5; 5)
<i>Gulf of Maine offshore</i>				
PBS	Platts Bank	8/06	70	mixed (2; 2, 6)
JLW	Jeffrey's Ledge	12/07	73	mixed (5; 3-5)
SWS	Stellwagen Bank	5/06	41	mixed (4; 3-6)
	Stellwagen Bank	4/07	74	mixed (5; 3-6)
<i>Georges Bank</i>				
GBW	NE Georges Bank	2/06	152	mixed (5; 3-6)
	NE Georges Bank	2/07-3/07	45	mixed (5; 3-6)
<i>Southern New England</i>				
NTW	Nantucket Shoals	11/06	109	mixed (4; 3-6)
CLW	Cox Ledge	1/07	158	mixed (4; 3-6)
CLS	Cox Ledge	4/07	118	mixed (4; 3-6)
NYS	New York Bight	3/07-4/07	47	mixed (2; 2)
NYW	New York Bight	11/07-1/08	44	mixed (2; 2)