View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by UNH Scholars' Repository

University of New Hampshire
University of New Hampshire Scholars' Repository

Master's Theses and Capstones | Student Scholarship

Winter 2008

Spawning stock identification of Atlantic cod (Gadus morhua) in US waters using Pan I and microsatellite genetic markers

Timothy S. Breton
University of New Hampshire, Durham

Follow this and additional works at: https://scholars.unh.edu/thesis

Recommended Citation
Breton, Timothy S., "Spawning stock identification of Atlantic cod (Gadus morhua) in US waters using Pan I and microsatellite genetic markers" (2008). *Master's Theses and Capstones*. 411.
https://scholars.unh.edu/thesis/411

This Thesis is brought to you for free and open access by the Student Scholarship at University of New Hampshire Scholars' Repository. It has been accepted for inclusion in Master's Theses and Capstones by an authorized administrator of University of New Hampshire Scholars' Repository. For more information, please contact nicole.hentz@unh.edu.

SPAWNING STOCK IDENTIFICATION OF ATLANTIC COD (*GADUS MORHUA*) IN U.S. WATERS USING *PAN* I AND MICROSATELLITE GENETIC MARKERS

BY

TIMOTHY S. BRETON

B.S., Suffolk University, 2006

THESIS

Submitted to the University of New Hampshire
in Partial Fulfillment of
the Requirements for the Degree of

Master of Science

in

Zoology

December, 2008

UMI Number: 1463213

INFORMATION TO USERS

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleed-through, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

UMI®

UMI Microform 1463213

Copyright 2009 by ProQuest LLC.

All rights reserved. This microform edition is protected against unauthorized copying under Title 17, United States Code.

ProQuest LLC
789 E. Eisenhower Parkway
PO Box 1346
Ann Arbor, MI 48106-1346

This thesis has been examined and approved.

Thesis Co-Director, Dr. Adrienne I. Kovach,
Research Assistant Professor of Natural Resources and the Environment

Thesis Co-Director, Dr. David L. Berlinsky,
Associate Professor of Biological Sciences

Dr. W. Huntting Howell,
Professor of Biological Sciences

12/1/08
Date

ACKNOWLEDGEMENTS

I would like to thank my committee members Drs. Adrienne I. Kovach, David L. Berlinsky, and W. Huntting Howell for allowing me to work on this project and for giving me valuable insight during the writing of this thesis. I would also like to thank Dr. Isaac Wirgin and Lauraine Maceda from the New York University School of Medicine for their collaboration and contribution in genotyping Atlantic cod samples. Funding for this project was provided by the Northeast Consortium and the UNH Graduate School.

This project was made possible by sampling contributions from many commercial fishermen, including Carl Bouchard, Jeff Carver, David Goethal, Frank Mirarchi, Chris Odlin, and Proctor Wells. Lou Van Eeckhaute from the Canadian Department of Fisheries and Oceans provided fin clips from Georges Bank. Jeremy King, David Salerno, and Mark Szymanski from the Massachusetts Division of Marine Fisheries and Dr. Steve Cadrin and Jon Loehrke from the University of Massachusetts Dartmouth provided valuable contributions in both juvenile and adult sampling.

I would like to thank Jobriah Anderson from the University of New Hampshire (UNH) Hubbard Center for Genome Studies for his assistance in genotyping. I also thank Cori Brauer, Justine Deming, Matthew Lubicky, Samantha Petren, and Chelsea Van Thof for their work in performing DNA extractions on cod samples. Many fellow graduate students at UNH also provided assistance during this project, including Heidi Colburn, Stephanie Coster, Lindsey Fendersen, Laughlin Siceloff, Abigail Walker, Jennifer Walsh, and Lauren Wyatt. Lastly, I thank my family, Cynthia Breton, Daniel Breton, and Dr. Beatrice Snow, for their support and guidance.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

SPAWNING STOCK IDENTIFICATION OF ATLANTIC COD (*GADUS MORHUA*) IN U.S. WATERS USING *PAN* I AND MICROSATELLITE GENETIC MARKERS

By

Timothy S. Breton

University of New Hampshire, December 2008

Most Atlantic cod (*Gadus morhua)* stocks within U.S. waters are currently in decline as a result of overexploitation and fishing pressure from commercial fisheries. A better understanding of the genetic structure of cod populations is essential to identify stocks for successful fisheries management. In this study, the genetic structure of cod from major temporally and geographically distinct spawning grounds in U.S. waters was investigated. Adult and juvenile cod were sampled from aggregations within the Gulf of Maine, Georges Bank, and southern New England waters in cooperation with commercial fishermen and state fishery biologists. Caudal fin clips were collected and analyzed using 11 microsatellite markers and the *Pan* I locus. Two spawning complexes of cod were identified. The northern spring complex was comprised of cod from coastal Gulf of Maine regions during the spring and summer seasons. The southern complex was comprised of cod collected during the winter months in the Gulf of Maine, on Stellwagen Bank in early spring, and at all southern locations. Georges Bank spawners were identified as a possible intermediate population between the complexes; they exhibited significant divergence from southern New England spawning aggregations, but not from

cod within the Gulf of Maine. Differentiation among these populations was stable over a two year study period and was consistent with previous results for Atlantic cod reported by Lage et al. 2004 and Wirgin et al. 2007. Juveniles collected from within the Gulf of Maine could be assigned as mixtures to parental spawning complexes. Divergence among adult and juvenile cod was primarily dependent on differentiation at the *Gmo*132 and *Pan* I markers, which are suspected to be under natural selection pressures. Local adaptation to environmental factors such as water temperature and salinity may therefore be the driving force of population differentiation. Natal homing and water currents that limit larval dispersal may also influence the stock structure, but cod migrations and dispersions among spawning aggregations likely limit reproductive isolation and neutral genetic drift of populations within U.S. waters.

CHAPTER 1

INTRODUCTION

Atlantic Cod Fishery and Management

The Atlantic cod (*Gadus morhua* L.) is one of the most commercially important marine fishes in the world and comprises a principal component of the northeastern United States (U.S.) groundfish assemblage. Cod are distributed throughout the temperate continental shelf waters of both the northwest and northeast Atlantic Ocean (Collette and Klein-MacPhee 2002). This species was once one of the most plentiful food fishes in the Gulf of Maine and acted as a mainstay of commercial fisheries since the 17th century (Mayo and Col 2006). The high desirability of cod led to dramatic over harvesting of the species, and recent fishing pressures have resulted in significant decline of most stocks across its range (Collette and Klein-MacPhee 2002).

Most cod stocks of the northwestern Atlantic are overexploited and are currently at low biomass levels (Mayo and Col 2006, O'Brien et al. 2006). For example, total stock biomass of the Gulf of Maine has decreased from 41,966 metric tons (mt) in 1990 to approximately 29,000 mt in 2004 (Mayo and Col 2006). Ames (2004) used anecdotal evidence to suggest that nearly half of the coastal spawning grounds for cod in the Gulf of Maine are now abandoned. Attempts to rebuild cod populations resulted in little to no increase in reproductive biomass despite 15 years of reduced fishing (Hutchings 2000). Successful restoration efforts may need to focus on identifying cod distributions and the characteristics of various stocks (Ames 2004). By gaining insight into cod

population structure in U.S. waters, it may be possible to more effectively manage this fishery.

Management plans for cod in U.S. waters are currently based on a two stock model: (1) a Gulf of Maine stock and (2) a Georges Bank and southward stock. Each management zone covers a broad distributional area with many habitats that may sustain multiple smaller and unique cod populations. Studies on cod movements found evidence of different migratory behaviors within the northwest Atlantic, including the existence of sedentary groups (Howell et al. 2008, Lindholm et al. 2007) that suggest a more complex population structure than previously believed. By tailoring fisheries guidelines to a flawed two stock model, management practices may be further depleting these smaller and more vulnerable cod stocks instead of restoring them (Ruzzante et al. 1999).

Differentiation among cod populations has been suggested to result from a variety of factors. Geographic features such as channels or trenches may serve as physical barriers to gene flow that limit dispersal and larval movements (Bentzen et al. 1996, Ruzzante et al. 1996a). Ocean currents and gyres may also be responsible for shaping population structure and retaining individuals within a geographic area (Ruzzante et al. 1998, Pampoulie et al. 2006b). The retention of larvae and juveniles can act as an imprinting episode for adult natal homing back to natal spawning grounds (Ruzzante et al. 1998). Some evidence of spawning site fidelity has been found within the Gulf of Maine (Howell et al. 2008). Even though groups may intermingle during summer and fall feeding migrations, stocks can remain genetically distinct through spawning site fidelity to unique spawning sites (Ruzzante et al. 1996b). Populations may also be separated on a temporal scale, where differences in peak spawning times can result in the

presence of two or more unique populations at a single geographic location (Wirgin et al. 2007). Genetic differentiation in marine fishes can also be the result of local adaptation at fine spatial scales (Conover et al. 2006). Environmental selection pressures such as differences in water temperature or salinity may act to overcome high levels of gene flow in the marine environment (Ward et al. 1994).

Molecular Markers for Genetic Stock Identification

Genetic stock identification provides a sensitive approach to evaluate and understand population structure. Many classes of molecular genetic markers have been developed, including allozymes, mitochondrial DNA markers (mtDNA), microsatellites, and single nucleotide polymorphisms (SNPs). These markers have been used extensively in many historical and recent studies (Avise 2004) to assay individuals and screen populations. Population variation is often greater in microsatellites and SNP loci than other genetic markers such as allozymes, mainly due to higher mutation rates (Avise 2004).

Microsatellites have become the most popular and most sensitive of the current methods in identifying population structure. Microsatellites are codominant molecular markers and most commonly consist of di-, tri-, and tetranucleotide tandem repeats within nuclear DNA. Polymorphisms in microsatellites are due to variations in these repeats, resulting in different allele lengths. Most microsatellites are assumed to be neutral to environmental selection pressures and significant differences among populations are the result of neutral genetic drift as opposed to local adaptation (Conover et al. 2006). Recent evidence suggests some Atlantic cod microsatellites may be under selection pressures (Westgaard and Fevolden 2007). Microsatellites in marine teleost

fishes often display longer allelic lengths and more variation than mammalian species (Brooker et al. 1994). This may reflect larger evolutionary effective population sizes and fewer barriers to gene flow in the more continuous marine environment (DeWoody and Avise 2000).

Many microsatellites have been developed for Atlantic cod over the past decade (Brooker et al. 1994, Miller et al. 2000, Jakobsdöttir et al. 2006). Although primer development is laborious and time consuming, microsatellites are easy to use in PCR-based assays and represent an efficient method of genotyping large numbers of individuals. A variety of studies used these markers to great effect in cod, including studies on larval aggregation (Ruzzante et al. 1996a), fishery stocks (Ruzzante et al. 1997), and aquaculture (Pampoulie et al. 2006a). Recent studies using microsatellites also utilized SNP markers (Wirgin et al. 2007, Pampoulie et al. 2008) as a complementary approach to identify stock structure.

Single nucleotide polymorphisms (SNPs) are a recently developed class of molecular markers that have also been useful in evaluating stock structure. SNPs are bi-allelic loci that represent specific base-pair variants, and they are abundant in most genomes. The Pantophysin I (*Pan* I) locus (Pogson 2001) has been used in many cod stock identification studies, often revealing several times greater levels of differentiation than microsatellites (Pampoulie et al. 2006a, 2006b, Wirgin et al. 2007, Pampoulie et al. 2008). The *Pan* I locus encodes a four domain integral membrane protein (Pantophysin) found in microvesicles and likely fills a role of basic structural functions (Haass et al. 1996). This marker has been suspected to be under a complex set of selection pressures (Fevolden and Pogson 1997, Pogson 2001, Karlsson and Mork 2003). Water temperature

and salinity have been correlated with *Pan* I allelic frequencies in cod (Case et al. 2005) and a latitudinal cline has been detected within the northwest Atlantic (Sarvas and Fevolden 2005). Although selection at *Pan* I is a likely cause of its high levels of differentiation compared to microsatellites, this locus may still provide insight into population differences on both local (Wirgin et al. 2007) and broad geographic scales (Pampoulie et al. 2008).

Recent cod genomics programs in Canada (Genome Canada, Ottawa, Ontario, Canada) and Norway (National Programme for Research in Functional Genomics – FUGE, Oslo, Norway) have been implemented for quantitative trait loci (QTL) development for cod breeding programs. This genomics research has increased the number of microsatellites (Stenvik et al. 2006b, Wesmajervi et al. 2007, Delghandi et al. 2008) and SNP markers (Moen et al. 2008) available for population genetic studies. These newly developed markers for Atlantic cod will provide more resolution of the stock structure and more precise estimates of effective population sizes and rates of gene flow (Moen et al. 2008).

Methods of Population Genetic Analysis

Population genetic analysis involves a variety of mathematical approaches, including traditional F-statistics (Wright 1951) and the latest Bayesian clustering probabilities. One of the most commonly applied method of estimating genetic differentiation between subpopulations is the measure of F_{ST}. F_{ST} is a population level approach that offers a convenient method of summarizing population structure (Weir and Cockerham 1984). F_{ST} estimates for populations of marine species are often lower than those observed in freshwater and terrestrial species because of larger effective population

sizes that increase the genetic variation at microsatellite markers (DeWoody and Avise 2000). Many cod genetic studies using microsatellite markers report low F_{ST} values (<0.01), which is characteristic of high gene flow and low differentiation among marine fish populations (Brooker et al. 1994). Although these population comparisons represent weak differentiation, they are often highly significant and may reveal important information about migration and larval dispersal over fine scales (Knutsen et al. 2003). Other measures such as Fisher's exact tests of allelic differentiation have been used as complementary statistics to F_{ST} (Hutchinson et al. 2001, Jorde et al. 2007, Wirgin et al. 2007) and offer a similar population level approach to examine stock differences. Fisher's exact tests were found to provide high resolving power when using microsatellite markers (Ryman et al. 2006).

One disadvantage to population level approaches and their applications is the *a priori* definition of populations. Population based estimates such as F_{ST} require the user to sort groups of individuals into predefined populations set by sampling regimes. These assumptions will introduce bias into the analysis if the real population structure is different from that of the predefined groups. Recently developed individual based analyses avoid *a priori* bias by comparing each individual to one another without the need for assumed population data (Manel et al. 2003). Individual based approaches offer an advantage over traditional genetic measures that may not detect hidden population structure.

Bayesian clustering programs are individual based analyses designed to identify the number of potential populations within a dataset without *a priori* bias. The most widely used program for clustering analysis is STRUCTURE (Pritchard et al. 2000).

STRUCTURE uses individual multilocus genotype data to minimize linkage disequilibrium and produce a probability that each individual originates from a potential population. STRUCTURE calculates the probability of different numbers of populations (K) in the dataset and determines the most likely K value. STRUCTURE has performed well in simulated tests (Waples and Gaggiotti 2006), complex population structure, and in populations with relatively high gene flow ($F_{ST} \geq 0.03$) among populations (Latch et al. 2006).

Assignment tests are another individual based approach and are most commonly used to assign individuals of unknown origin to previously identified populations. These statistical techniques do not avoid the *a priori* bias of population assumptions and depend on these definitions to calculate a probability value that each individual originated from a putative population. The program GENECLASS (Piry et al. 2004) is capable of using multiple Bayesian criteria and simulation algorithms to assign individuals to populations, but it may offer less power for resolving differences than other assignment methods when differentiation is low (Koljonen et al. 2005). Pure assignment tests such as GENECLASS assign genotypes based on highest likelihood ratios to certain populations, regardless of the assignment of other individuals in the collection. Newly developed conditional maximum likelihood programs such as ONCOR (Andersen et al. 2008) and the Bayesian program BAYES (Pella and Masuda 2001) add power to these assignments by incorporating mixture modeling prior to direct assignment. Mixture analysis provides a proportional assignment of the unknown collection to the populations, which influences the assignment and gives higher percent correct values by comparison (Koljonen et al. 2005). Low confidence in these assignment tests, however, may still be

present in cases where differentiation is extremely low ($F_{ST} \leq 0.01$), and individual assignments are only as valid as their respective mixture analyses.

Mixture analysis has also been used independently of individual assignments to aid biologists in stock assessment, conservation, and migration. Mixture analysis has given managers insights into the success of hatchery released brown trout (*Salmo trutta*) in Denmark (Ruzzante et al. 2004) and the juvenile migrations of hawksbill turtles (*Eretmochelys imbricata*) in the Caribbean Sea (Bowen et al. 2007). This method is also used in salmon (*Salmo* spp.) management in northwestern North America. Proportions of salmon stocks in a fishery can be estimated on a monthly or weekly basis (Beacham et al. 2005) to guide management on fishery closures. A maximum likelihood approach was used by Ruzzante et al. (2000a) to identify large contributions of summer Atlantic cod in the Gulf of St. Lawrence to overwintering mixed stock populations on both sides of the Laurentian Channel. The variety of applications offered by mixture analysis provides fishery biologists with a valuable tool to identify both juvenile and adult movements.

Previous Studies in Cod Stock Identification

Many studies have been conducted on cod genetic population structure within both the northwest and northeast Atlantic Ocean. Although gene flow is expected to be high in the marine environment (Ward et al. 1994), genetically distinct populations have been found within current management models (Ruzzante et al. 1998, Hutchinson et al. 2001) and within fine spatial and temporal scales (Knutsen et al. 2003, Wirgin et al. 2007). Fine scale population structure has been suggested to originate from oceanographic features (Ruzzante et al. 1998), current fronts of mixing warm and cold water (Pampoulie et al. 2006b), and isolation of relic populations (Hardie et al. 2006).

Differences in larval aggregations (Ruzzante et al. 1996a) and distinctions of farmed strains from wild populations (Pampoulie et al. 2006a) also provide evidence that Atlantic cod are not genetically homogeneous across their ranges.

Within Canadian waters, spatial variation in cod populations has been extensively studied. Weak but significant genetic differentiation has been found in several locations, including differences between cod on Hamilton Bank and Grand Bank (Bentzen et al. 1996), and between an inshore population in Trinity Bay, Newfoundland and offshore overwintering populations (Ruzzante et al. 1996b). The significant divergence among winter populations was temporally stable (Ruzzante et al. 1997) and persisted despite the mixing of these populations during summer and fall feeding migrations (Ruzzante et al. 1996b). Genetic differences have also been found over continental shelf scales and bay scales (Ruzzante et al. 1998, Ruzzante et al. 2000b). Cod from southern sites such as Georges Bank and the Scotian Shelf were genetically divergent from the cod in the Gulf of St. Lawrence (Ruzzante et al. 2000a), Grand Bank, and other northern Canadian waters (Ruzzante et al. 1998). Bay scale population structure has been revealed between aggregations in Gilbert Bay off Labrador and Trinity Bay on the coast of Newfoundland. Ruzzante et al. (2000b) suggested a link between these genetic differences and physiological features, citing that Gilbert Bay cod were smaller and less fecund for their age in comparison to cod from Trinity Bay. Beacham et al. (2002) further studied inshore and offshore locations around Newfoundland, and also found cod in Gilbert Bay to be unique from adjacent bays. Distinct populations have been found in extreme northern ranges, where relic populations of cod in the Canadian Arctic Lakes were strongly differentiated from Gilbert Bay cod and other southern waters (Hardie et al.

2006). Arctic populations exhibited the lowest genetic diversity of all studied sites, and the level of differentiation was two orders of magnitude higher in arctic populations than in southern Canada (Hardie et al. 2006).

Similar cases of population divergence have been observed in European waters. Broad scale differences were identified among Baltic Sea cod and other European samples using both microsatellite markers and the *Pan* I SNP locus (Pampoulie et al. 2008). Four genetically distinct populations of spawning cod were identified within the North Sea alone (Hutchinson et al. 2001), and fine scale structuring of cod was also found to be present within a 300 km area of the Skagerrak ocean basin off the coast of Norway (Knutsen et al. 2003). Observed levels of differentiation were weak in most cases ($F_{ST} < 0.01$) but statistically significant. Knutsen et al. (2003) found no spatial pattern to the genetic differentiation over such fine scales, and they suggested that the low levels of differentiation were due to differences in passive transport of eggs or larvae on ocean currents. Currents have also been suggested as a cause of divergence between populations of cod off Iceland, where clear separation was observed between northeastern and southwestern regions (Pampoulie et al. 2006b). This regional structuring was found to follow current fronts along the island, where mixing cold and warm water masses may act as a genetic barrier to dispersal. A tagging experiment of spawning cod in the region corroborated this observation and found that southwestern cod rarely mixed with those from the northeast (Pampoulie et al. 2006b).

Stock structure of Atlantic cod within U.S. waters has not yet been completely investigated, but two studies demonstrated differentiation involving the Nantucket Shoals, Long Island, and Ipswich Bay populations. Lage et al. (2004) found significant

differentiation between Georges Bank cod and a Nantucket Shoals population. Differences on a temporal scale within Ipswich Bay were detected by Wirgin et al. (2007), where an Ipswich Bay spring aggregation exhibited significant differentiation from both Ipswich Bay winter spawning cod and all other studied sites. This finding raised the question of whether or not the Ipswich Bay spring aggregation should be treated as a separate management unit from its winter counterpart. It also suggested that the Gulf of Maine may represent more than one stock and could be comprised of multiple unique populations separated on both spatial and temporal scales. These studies provided some evidence that stock structure within U.S. waters was highly complex, but more research was needed to fully investigate the fine scale population structuring. Unlike the plethora of genetic studies conducted in Canada and Europe, no study conducted in U.S. waters has examined the temporal stability of these differences. A multi-year sampling regime of spawning aggregations in this region was necessary to properly identify Atlantic cod stocks for successful fishery management. To this end, the present study was initiated to characterize the spawning stock structure of Atlantic cod in U.S. waters and to test for temporal stability among the aggregations over a two year study period.

Objectives

The purpose of this study was to examine the fine scale spatial and temporal genetic structuring of Atlantic cod populations within U.S. waters. All known major spawning aggregations in this region were sampled to more fully characterize the stock differences and to test for temporal stability over a two year period. Stability of the stock differences were further investigated by directly comparing the adult aggregations

sampled by Wirgin et al. (2007) to cod from the present study. Four main hypotheses were tested:

1) There is a consistent genetic difference between winter and spring aggregations in Ipswich Bay. Temporal stability of the seasonal variation should be evident in Ipswich Bay cod, and the genetic differences will be similar to previous results by Wirgin et al.(2007).

2) There is a consistent genetic difference between aggregations on Nantucket Shoals and Georges Bank. Lage et al. (2004) detected genetic differences between these populations that should be similar to data in the present study.

3) Further genetic structuring of cod in U.S. waters is present that has not been investigated by Lage et al. 2004 and Wirgin et al. 2007. These previous studies did not sample cod from all known major spawning aggregations in this region.

4) A subset of juvenile cod can be assigned to different adult spawning stocks. These assignments will be similar to expectations of larval drift based on the major water current patterns in the region.

CHAPTER 2

MATERIALS AND METHODS

Sample Collection

Cod were sampled over a two year study period from all known spawning locations in U.S. waters. Sampling was conducted in association with commercial fishermen and state fishery representatives (Massachusetts Division of Marine Fisheries) using bottom trawls and gill nets. Live adult cod were caught, and females in identifiable spawning condition were sampled whenever possible. Spawning condition was assessed by either visual inspection of the gonads or by observations of running milt or eggs. Female spawning condition was assessed using the National Marine Fisheries Service (Gloucester, Massachusetts, U.S.) ovarian staging criteria. Caudal fin clip samples (1 cm^2) were taken from each fish and preserved in 95-100% ethanol until later genetic analysis in the laboratory.

In Year 1 of the study, 701 individuals were sampled from December 2005 to December 2006. Adult cod were sampled from known spawning aggregations within Georges Bank, Ipswich Bay, Massachusetts Bay, Nantucket Shoals and Stellwagen Bank. Adult cod not in spawning condition (identified as resting individuals) were sampled from aggregations in Ipswich Bay and Platts Bank (Table 1, Fig. 1). In Year 2 of the study, 787 individuals were sampled from January 2007 to January 2008. Adult cod were sampled from known spawning aggregations within Bigelow Bight, Cox Ledge, Georges Bank, Ipswich Bay, Jeffrey's Ledge, Massachusetts Bay, Ipswich Bay, and Stellwagen

Table 1. Sample sites, dates collected, sample sizes (n), and condition of Atlantic cod adult and juvenile aggregations sampled during years 1 and 2 of the study.

Site	Name	Date	n	Condition
		Year 1 Adults		
Massachusetts Bay	MBW1	12/05-1/06	140	Spawning females
Georges Bank	GBW1	2/06	152	Spawning adults
Massachusetts Bay	MBS1	4/06	36	Spawning females
Ipswich Bay	IPS1	4/06-5/06	122	Spawning adults
Stellwagen Bank	SWS1	5/06	41	Spawning adults
Platts Bank	PBS	8/06	70	Resting adults
Nantucket Shoals	NTW	11/06	109	Spawning adults
Ipswich Bay	IPW	12/06	31	Resting adults
		Year 2 Adults		
Massachusetts Bay	MBW2	1/07	77	Spawning adults
Cox Ledge	CLW	1/07	158	Spawning adults
Georges Bank	GBW2	2/07-3/07	45	Spawning adults
New York Bight	NYS	3/07-4/07	47	Resting adults
Cox Ledge	CLS	4/07	118	Spawning adults
Stellwagen Bank	SWS2	4/07	74	Spawning adults
Ipswich Bay	IPS2	6/07	78	Spent females
Bigelow Bight	BBS1	7/07	70	Spent females
Jeffrey's Ledge	JLW	12/07	73	Spawning adults
New York Bight	NYW	11/07-1/08	47	Resting adults
Massachusetts Bay	MBS2	6/08	50	Ripe females
Bigelow Bight	BBS2	7/08	47	Spawning adults
		Juveniles		
Massachusetts Bay	MBWJ1	10/06	34	Age-0 juveniles
Cape Cod	CCWJ	10/06	69	Age-0 juveniles
Casco Bay	CBSJ	4/07	45	Age-1 immature
Massachusetts Bay	MBSJ	4/07	46	Age-0 juveniles
Massachusetts Bay	MBWJ2	10/07	48	Age-0 juveniles

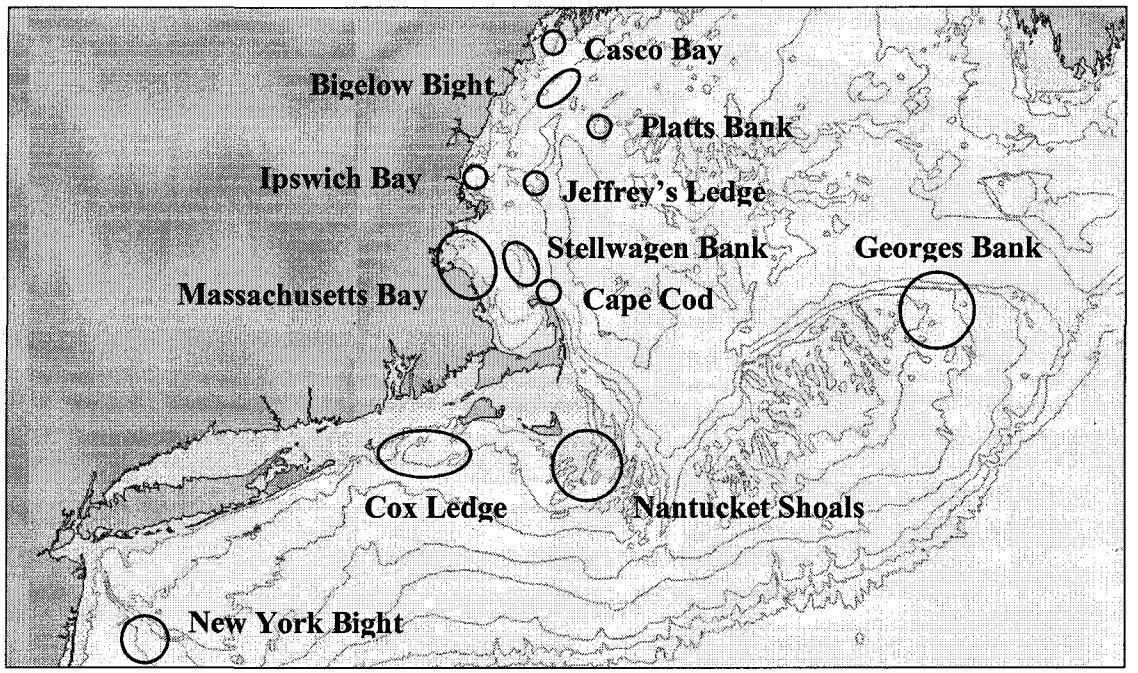

Fig. 1. Sample site locations for all Atlantic cod collected in the present study.

Bank. Resting adult cod were also sampled from seasonal aggregations within the New York Bight (Table 1, Fig. 1). To increase the sample size of the Massachusetts Bay spring population, an additional spawning aggregation was sampled in June 2008. The Bigelow Bight spawning aggregation was sampled again in July 2008 to provide an additional year of samples to test for temporal stability. These two summer 2008 aggregations were added to Year 2 (Table 1).

Young of year (age-0) juvenile cod were sampled by bottom trawl from Massachusetts Bay and the waters off Cape Cod by the Massachusetts Division of Marine Fisheries. Individuals from Massachusetts Bay were sampled in October 2006, April 2007, and October 2007. Individuals from Cape Cod were sampled in October 2006 (Table 1). Whole juveniles were frozen at -20°C and later thawed at University of New Hampshire (UNH) laboratories and fin clips were removed for analysis. A collection of immature (age-1) cod were sampled from Casco Bay in April 2007 by commercial fishermen using bottom trawls, and fin clips were taken from individuals prior to release. All juvenile fin clips (242 total samples) were preserved in 95-100% ethanol.

Genetic Analyses

Genetic analyses were conducted in collaboration with Dr. Isaac I. Wirgin at the New York University (NYU) School of Medicine (Tuxedo, NY, U.S.). Adult fin clip samples for DNA extraction and microsatellite analyses were divided between NYU and UNH. Juvenile fin clip analyses were conducted solely at UNH laboratories. DNA extractions at UNH and NYU were preformed using Qiagen DNeasy tissue kits (Qiagen, Valencia, CA, U.S.), and standard phenol/chloroform procedures (see Wirgin et al. 2007), respectively.

A total of 11 diagnostic microsatellite markers and the Pantophysin I (*Pan* I) SNP locus (Pogson et al. 2001) were used in the genetic analysis. Genotyping at 11 microsatellite markers was split between UNH and NYU laboratories. Analysis of *Gmo*02 (Brooker et al. 1994), *Gmo*19, *Gmo*36 (Miller et al. 2000), P*Gmo*34, P*Gmo*56, and P*Gmo*58 (Jakobsdóttir et al. 2006) was conducted at UNH, while analysis of *Gmo*132 (Brooker et al. 1994), *Gmo*35, *Gmo*37 (Miller et al. 2000), P*Gmo*32, and P*Gmo*38 (Jakobsdóttir et al. 2006) was conducted at NYU. The *Gmo*37 locus was genotyped using newly developed primers (see Wirgin et al. 2007) and all microsatellites at NYU laboratories were amplified using a polymerase chain reaction (PCR) approach as described in Wirgin et al. 2007.

PCR reactions for microsatellites at UNH laboratories were conducted in 12 µl total volumes containing 2 µl DNA template (50-200 ng/ml), 0.5 µM of each primer, 1X GoTaq Flexi PCR Buffer (Promega Corp., Madison, WI, U.S.), 0.2mg/ml bovine serum albumin, 1 mM $MgCl_2$, 100 µM dNTPs, and 0.2 U GoTaq Flexi DNA Polymerase (Promega). The forward or reverse primer of each microsatellite was fluorescently labeled with FAM, NED, or HEX, and amplification parameters were followed as described in the literature for each primer set. *Pan* I genotyping was conducted at UNH using the method of Stenvik et al. (2006a). Three multiplex PCRs were conducted that shared annealing temperatures within 2°C. Multiplex PCR consisted of: 1) *Gmo*02, *Gmo*19, and *Gmo*36, 2) P*Gmo*34 and P*Gmo*56, and 3) *Pan* I and P*Gmo*58. PCR products were diluted (15 to 20X) and electrophoresed in an automated capillary sequencer (ABI3130, Applied Biosystems, Foster City, CA, U.S.) at the UNH Hubbard Center for Genome Studies. Sample genotyping was conducted manually using PeakScanner v.1.0

(Applied Biosystems) and checked for the presence of null alleles, errors due to microsatellite stuttering, and large-allele dropout using MICRO-CHECKER software (Oosterhout et al. 2004).

Statistical Analyses of Population Structure

To characterize population structure, microsatellite and *Pan* I genotypes from all individuals were compiled together into their respective Year 1 and Year 2 groups and subjected to a variety of population and individual-based analytical methods. Mean number of alleles per locus and observed heterozygosities were calculated using FSTAT 2.9.3 (Goudet et al. 1995). Locus specific F_{ST} values and Fisher's exact tests of allelic differentiation were calculated in GENEPOP version 3.4 (Raymond and Rousset 1995). Tests of deviations from Hardy-Weinberg equilibrium and linkage disequilibrium were conducted in GENEPOP. Markov chain Monte Carlo (MCMC) parameters for all calculations in GENEPOP were increased to 10,000 iterations and 10,000 batches prior to analysis.

To test for genetic differences among the populations, Year 1 and Year 2 collections were subject independently to pairwise allelic differentiation tests in GENEPOP and F_{ST} calculations using the estimator θ of Weir and Cockerham (1984) in FSTAT. Spawning aggregations sampled in both years of the study were compared using tests of allelic differentiation in GENEPOP and F_{ST} estimations in FSTAT to test for temporal stability from year to year. When no significant differentiation was observed from the same sample site, Year 1 and Year 2 samples were combined into a single population for analysis. Aggregations from Bigelow Bight in July 2007 and 2008 were also tested for temporal stability and combined into a single population. All spawning

populations from Year 1 and Year 2 were then combined into one matrix and subject to calculations of pairwise population F_{ST} and allelic differentiation. F_{ST} values were also used in principal component analysis (PCA) using GENALEX 6.1 (Peakall and Smouse 2006) to visualize the clustering of populations.

To test for differences among populations without *a priori* assumptions of population structure, individual genotypes of the combined spawning Year 1 and Year 2 groups were input into the Bayesian clustering program STRUCTURE v.2.2. The parameter set used in the analysis consisted of default admixture and independent allele frequency models with a Markov chain Monte Carlo (MCMC) burn-in length of 500,000 and a run length of 1,000,000. Clustering analysis was performed five times for each K value ranging 1-5. Individual $\ln(\Pr X \mid K)$ values for each K were averaged over the five runs. Probabilities of K were calculated from the average $\ln(\Pr X \mid K)$ values using the method of Pritchard et al. 2007.

The dataset from Wirgin et al. (2007) was also compared to samples in the present study to address the question of temporal stability of the stock structure over a longer time period (2003 to 2007). Spawning aggregations from Wirgin et al. 2007 were sampled from Ipswich Bay in January 2003 (48 samples) and May 2003 (48 samples), off of Chatham, Massachusetts in November 2003 (40 samples), on Stellwagen Bank in January 2005 (27 samples), and on northeast Georges Bank in February 2005 (100 samples). The resting adult aggregation sampled from Long Island in January-February 2005 (80 samples) was also included in the analysis. To make comparisons between the two studies, the microsatellite data were calibrated to correct for differences in electrophoresis platforms between years. The *Gmo*02, *Gmo*132, *Gmo*35, *Gmo*36, and

*Gmo*37 microsatellites were calibrated to the data from the present study by re-analyzing a subset of samples from Wirgin et al. 2007 using the above methods. *Gmo*19 could not be calibrated due to high levels of variability between studies and was removed prior to analysis. The *Gmo*19 locus was the least informative marker in either study (see Wirgin et al. 2007 and Table 2) and its removal was unlikely to affect the comparative analysis. The *Pan* I SNP locus was not calibrated between studies because the identification of the two alleles was not dependent on the electrophoresis platform. Microsatellite markers used in the present study but not in Wirgin et al. 2007 (P*Gmo*32, P*Gmo*34, P*Gmo*38, P*Gmo*56, P*Gmo*58) were not included in the analysis. A PCA was conducted using pairwise population F_{ST} values to visualize the genetic data.

To test for genetic differences among the juvenile collections, population F_{ST} estimates in FSTAT and allelic differentiation tests in GENEPOP were used. The genotype data were also tested for conformity to Hardy-Weinberg equilibrium in GENEPOP. F_{ST} values were calculated with a combined dataset of juvenile and spawning adult collections in GENALEX and used in a PCA. To assign the juvenile collections to adult spawning stocks, mixture analysis was performed using the conditional maximum likelihood approach of ONCOR. Confidence intervals 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 using the 'leave-one-out' procedure (Andersen et al. 2008) and through 100% fishery simulations of 50 individuals and 1,000 simulations. The ONCOR mixture analysis program was the preferred method for assigning juvenile proportions because it is a maximum likelihood approach that provides more power than pure

assignment tests such as GENECLASS (Koljonen 2005). The Bayesian method of mixture analysis in BAYES was not used because of the inability of the MCMC chains to converge as estimated by the Gelman and Rubin (1992) shrink factor. The failure of the shrink factor to converge indicated that this Bayesian method was not suitable for the weak differentiation present in the dataset. ONCOR does not have this limitation associated with MCMC chains, but assumes baseline allele frequencies without error.

CHAPTER 3

RESULTS

Descriptive Statistics

Observed per locus heterozygosities averaged over all Atlantic cod samples varied from 0.250-0.952 among the microsatellites and 0.093 at the *Pan* I locus (Table 2). The P*Gmo*32, PGmo56, and P*Gmo*58 markers exhibited the lowest heterozygosity values of all assayed microsatellites. The per locus heterozygosities of *Gmo*02, *Gmo*19, *Gmo*132, *Gmo*35, *Gmo*36, and *Gmo*37 were similar to the observed values from Wirgin et al. (2007). Mean number of alleles among the microsatellites ranged from 4.3-60.

The highest levels of differentiation in the present study were observed at the *Gmo*132 and *Pan* I markers (Table 2). Per locus tests of allelic differentiation detected highly significant ($p < 0.0001$) and consistent divergence at these markers within the Year 1, Year 2, and juvenile groupings of cod. Per locus F_{ST} values were also high at these markers relative to the others, ranging from 0.0279-0.0423 in *Gmo*132 and 0.0341-0.1091 in *Pan* I. Divergence at the *Pan* I locus was consistently higher than *Gmo*132 in all respective groupings. Only two other cases of significant per locus differentiation were observed in the present study, involving P*Gmo*56 in Year 2 and *Gmo*36 in the juvenile analyses. These differences were not consistent among groupings and per locus F_{ST} values were not as high in comparison to *Gmo*132 and *Pan* I. All other F_{ST} values for microsatellites were extremely low (≤ 0.0039) and did not indicate significant differentiation.

Table 2. Per locus mean number of alleles, observed heterozygosities (H_O), F_{ST} estimates, and allelic differentiation p-values for Atlantic cod. Mean alleles and mean H_O values are averaged over all populations in Years 1, 2, and juvenile groupings of the study. Bold indicates significance following standard Bonferroni correction ($p \leq 0.0042$).

			Year 1		Year 2		Juvenile	
Locus	Mean alleles	Mean H_O	F_{ST}	Differentiation	F_{ST}	Differentiation	F_{ST}	Differentiation
*Gmo*02	21.0	0.802	-0.0010	0.7350	0.0004	0.2072	-0.0032	0.7310
*Gmo*19	60.0	0.952	-0.0008	0.7712	0.0000	0.2437	0.0002	0.2313
*Gmo*132	22.0	0.758	0.0279	**<0.0001**	0.0423	**<0.0001**	0.0380	**<0.0001**
*Gmo*35	11.0	0.757	-0.0020	0.7298	0.0035	0.0285	0.0046	0.0569
*Gmo*36	8.3	0.571	0.0024	0.0286	0.0010	0.2765	0.0181	**0.0030**
*Gmo*37	17.0	0.851	0.0011	0.0712	0.0005	0.1278	0.0026	0.1243
P*Gmo*32	4.3	0.402	0.0006	0.5155	-0.0040	0.8746	-0.0015	0.5259
P*Gmo*34	11.3	0.577	-0.0003	0.6474	0.0039	0.0380	-0.0076	0.9462
P*Gmo*38	22.0	0.862	0.0010	0.4980	0.0013	0.2369	0.0010	0.3112
P*Gmo*56	6.0	0.250	0.0012	0.4801	0.0047	**0.0006**	-0.0028	0.1430
P*Gmo*58	9.7	0.268	0.0001	0.2862	-0.0012	0.3059	-0.0003	0.3633
Pan I	2.0	0.093	0.0341	**<0.0001**	0.0726	**<0.0001**	0.1091	**<0.0001**

Population Differentiation in Year 1

All populations in Year 1 were in Hardy-Weinberg equilibrium (Appendix B) and no linkage disequilibrium was detected (data not shown). Using MICRO-CHECKER software, no evidence of the presence of null alleles, stuttering, or large allele dropout was detected. Several cases of significant differentiation (Table 3) were detected using pairwise population F_{ST} estimates and tests of allelic differentiation. Significant differentiation was evident between Georges Bank cod and spawning adults on Nantucket Shoals. Spawning cod in Ipswich Bay exhibited significant differentiation from winter spawning females in Massachusetts Bay and spawning cod on Nantucket Shoals. Resting adults on Platts Bank also exhibited significant differentiation from the Nantucket Shoals population. Significant differences by allelic differentiation only were found between resting adults on Platts Bank and spawning females in Massachusetts Bay winter, and between spawning cod on Georges Bank and the Massachusetts Bay winter population. No significant differentiation was detected in these latter cases because pairwise F_{ST} estimates are likely more conservative in comparison to allelic differentiation. Fisher's exact tests of allelic differentiation have previously exhibited the highest power in resolving population differences (Ryman et al. 2006).

No significant differentiation was found among the spawning aggregations sampled from Stellwagen Bank, Massachusetts Bay spring, and the resting collection in Ipswich Bay winter. A high but non-significant pairwise F_{ST} value was found between the Ipswich Bay spring and winter collections (Table 3); the lack of statistical significance in these cases may be the result of low sample sizes of these three collections (n = 41, 36, and 31, respectively). Ruzzante et al. (1996a) suggested that

Table 3. Population differentiation of Atlantic cod in Year 1. Above diagonal are allelic differentiation p-values. Below diagonal are pairwise population F_{ST} values with p-values in parentheses. Abbreviated sample site names refer to Table 1. Bold indicates significance following standard Bonferroni correction ($p \leq 0.0018$).

	MBW1	GBW1	MBS1	IPS1	SWS1	PBS	NTW	IPW
MBW1	---	**<0.0001**	0.1501	**<0.0001**	0.5362	**0.0012**	0.2193	0.4503
GBW1	0.0023 (0.0071)	---	0.2535	0.0066	0.6856	0.0183	**0.0001**	0.0533
MBS1	0.0006 (0.2089)	-0.0001 (0.5679)	---	0.6376	0.3169	0.8281	0.0030	0.0434
IPS1	**0.0071 (0.0018)**	0.0024 (0.1607)	0.0004 (0.7446)	---	0.0168	0.7080	**<0.0001**	0.0424
SW1	-0.0012 (0.3268)	0.0010 (0.7429)	-0.0003 (0.4571)	0.0076 (0.1589)	---	0.0224	0.0643	0.0044
PBS	0.0041 (0.0625)	0.0007 (0.1429)	-0.0016 (0.7607)	-0.0002 (0.6339)	0.0050 (0.0750)	---	**<0.0001**	0.0258
NTW	0.0003 (0.0750)	**0.0039 (0.0018)**	0.0032 (0.0214)	**0.0098 (0.0018)**	0.0012 (0.0518)	**0.0086 (0.0018)**	---	0.2701
IPW	0.0042 (0.5946)	0.0053 (0.0179)	0.0057 (0.1232)	0.0090 (0.0429)	0.0071 (0.0352)	0.0064 (0.1196)	0.0017 (0.2571)	---

sample sizes of 50 or more individuals are required for identifying Atlantic cod population structure. Small collections of individuals may introduce sample size bias due to the low levels of differentiation expected for high gene flow marine species.

Population Differentiation in Year 2

No significant deviations from Hardy-Weinberg equilibrium (Appendix B) and no cases of linkage disequilibrium were detected in Year 2 of the study (data not shown). Using MICRO-CHECKER software, the possible presence of null alleles was detected within the winter spawning cod on Cox Ledge at P*Gmo*56 and within the first Bigelow Bight summer aggregation at *Gmo*02. However, because these populations conformed to Hardy-Weinberg expectations, this possibility was not considered in further analyses. Several cases of significant differentiation were detected within Year 2 using pairwise population F_{ST} estimates and tests of allelic differentiation (Table 4). All significant F_{ST} comparisons included spring spawning cod within coastal Gulf of Maine, which consisted of cod in Massachusetts Bay, Ipswich Bay, and Bigelow Bight. In most cases differentiation was observed between these spring spawning aggregations and the southern cod on Cox Ledge and within the New York Bight. Several cases of significant differentiation were also apparent between the Massachusetts Bay winter population and the spring populations in Massachusetts Bay and Ipswich Bay. The Stellwagen Bank spring population was more differentiated from the spring coastal Gulf of Maine populations than winter spawning cod in Massachusetts Bay or cod in southern New England waters. No significant differentiation was observed between spawning cod on Cox Ledge and resting adults in the New York Bight. The lack of differentiation in the

Table 4. Population differentiation of Atlantic cod in Year 2. Above diagonal are allelic differentiation p-values. Below diagonal are pairwise population F_{ST} values with p-values in parentheses. Abbreviated sample site names refer to Table 1. Bold indicates significance following standard Bonferroni correction ($p \leq 0.0008$).

	MBW2	CLW	GBW2	NYS	CLS	SWS2	IPS2	BBS1	JLW	NYW	MBS2	BBS2
MBW2	---	0.1777	0.0039	0.0525	0.1363	0.0540	**<0.0001**	0.0011	0.2299	0.0139	**<0.0001**	**<0.0001**
CLW	0.0001 (0.4682)	---	0.0050	0.0286	0.7491	0.0098	**<0.0001**	**<0.0001**	0.6509	0.0659	**<0.0001**	**<0.0001**
GBW2	0.0055 (0.0068)	0.0029 (0.0189)	---	0.7587	**0.0005**	0.0152	0.0549	0.6613	0.0895	0.6834	0.0081	0.1479
NYS	0.0029 (0.1038)	0.0011 (0.1189)	-0.0022 (0.5546)	---	0.0027	0.0042	**<0.0001**	0.0284	0.1307	0.7065	**<0.0001**	0.0032
CLS	0.0007 (0.3447)	0.0007 (0.8720)	0.0066 (0.0038)	0.0045 (0.0174)	---	0.0142	**<0.0001**	**<0.0001**	0.0214	0.0299	**<0.0001**	**<0.0001**
SWS2	0.0016 (0.0477)	0.0031 (0.0265)	0.0046 (0.0242)	0.0038 (0.0015)	0.0058 (0.0220)	---	**<0.0001**	**0.0002**	0.2281	0.1430	**<0.0001**	**<0.0001**
IPS2	**0.0118 (0.0008)**	**0.0122 (0.0008)**	0.0030 (0.0515)	**0.0075 (0.0008)**	**0.0155 (0.0008)**	**0.0111 (0.0008)**	---	0.3869	**<0.0001**	**0.0002**	0.2131	0.0376
BBS1	0.0054 (0.0136)	**0.0065 (0.0008)**	-0.0017 (0.5576)	0.0002 (0.0538)	**0.0100 (0.0008)**	0.0066 (0.0030)	0.0009 (0.1811)	---	0.0011	0.0135	0.1238	0.5483
JLW	0.0032 (0.3076)	0.0010 (0.5735)	0.0024 (0.1280)	0.0008 (0.1568)	0.0071 (0.0492)	0.0035 (0.5561)	0.0091 (0.0023)	0.0070 (0.0121)	---	0.0456	**<0.0001**	**0.0001**
NYW	0.0027 (0.0121)	0.0011 (0.0841)	0.0002 (0.8227)	0.0014 (0.3652)	0.0029 (0.1197)	0.0007 (0.2462)	0.0093 (0.0106)	0.0042 (0.0242)	0.0038 (0.0621)	---	**<0.0001**	0.0071
MBS2	**0.0243 (0.0008)**	**0.0248 (0.0008)**	0.0082 (0.0742)	**0.0146 (0.0008)**	**0.0313 (0.0008)**	**0.0208 (0.0008)**	0.0004 (0.1758)	0.0036 (0.2174)	**0.0191 (0.0008)**	**0.0175 (0.0008)**	---	0.8101
BBS2	0.0133 (0.0015)	**0.0118 (0.0008)**	0.0030 (0.2689)	0.0040 (0.0515)	**0.0169 (0.0008)**	0.0099 (0.0023)	0.0005 (0.0174)	-0.0015 (0.4750)	0.0097 (0.0114)	0.0079 (0.0939)	-0.0008 (0.5492)	---

Year 2 Georges Bank spawning population as compared to other aggregations may have been due to low sample size (n = 45).

Identification of Spawning Complexes

Spawning aggregations from both Year 1 and Year 2 were tested for temporal stability using allelic differentiation and F_{ST} estimates. No significant differentiation was detected between spawning aggregations sampled in both the Year 1 and Year 2 groupings (Appendix C). The comparison between Massachusetts Bay spring cod from both years exhibited a high but non-significant F_{ST} estimate that was likely due to sample size bias of the Year 1 collection (n = 36). The Ipswich Bay spring, Georges Bank winter, Massachusetts Bay winter, Massachusetts Bay spring, and Stellwagen Bank spring spawning aggregations from both years were then combined for analysis to identify potential spawning complexes in U.S. waters. No significant deviations from Hardy-Weinberg equilibrium conditions were detected at any of the combined populations (Appendix B).

Using cluster analysis of the combined spawning aggregations in STRUCTURE, no significant population structuring was detected, and K = 1 was identified as the most probable number of source populations (Table 5). However, significant differentiation among populations was detected using F_{ST} analysis and allelic differentiation. The combined spawning aggregations mirrored the Year 1 and Year 2 results and again separated the spawning aggregations of Bigelow Bight summer, Ipswich Bay spring, and Massachusetts Bay spring from cod in offshore Gulf of Maine locations and southern New England waters (Table 6). Significant differentiation was also evident between spawning female cod in Massachusetts Bay spring and Massachusetts Bay winter. The

Table 5. Number of sources populations (K) and mean probability of the dataset given K (ln(Pr X | K)) as determined by STRUCTURE. P-values for each K were calculated using the method of Prichard et al. 2007.

K	ln(Pr X | K)	p-value
1	-49578.7	1.0
2	-49877.3	0.0
3	-50224.1	0.0
4	-51413.7	0.0
5	-53549.5	0.0

Table 6. Population differentiation of spawning aggregations of Atlantic cod in combined years 1 and 2. Above diagonal are allelic differentiation p-values. Below diagonal are pairwise population F_{ST} values with p-values in parentheses. Abbreviated sample site names refer to Table 1. Bold indicates significance following standard Bonferroni correction ($p \leq 0.0011$).

	MBW	GBW	MBS	IPS	SWS	NTW	CLW	CLS	BBS	JLW
MBW	---	**<0.0001**	**<0.0001**	**<0.0001**	0.0852	0.0192	0.3885	0.1836	**<0.0001**	0.4233
GBW	0.0029 (0.0067)	---	**<0.0001**	**<0.0001**	0.0449	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.1279
MBS	**0.0105 (0.0011)**	0.0044 (0.0033)	---	0.5735	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.7457	**<0.0001**
IPS	**0.0089 (0.0011)**	0.0031 (0.0022)	-0.0015 (0.3022)	---	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.0619	**<0.0001**
SWS	0.0006 (0.0756)	0.0038 (0.0589)	**0.0097 (0.0011)**	**0.0088 (0.0011)**	---	0.0050	0.0034	0.0032	**<0.0001**	0.1147
NTW	0.0008 (0.0178)	**0.0038 (0.0011)**	**0.0148 (0.0011)**	**0.0107 (0.0011)**	0.0017 (0.0044)	---	0.0391	0.0161	**<0.0001**	0.0077
CLW	-0.0005 (0.3600)	**0.0034 (0.0011)**	**0.0129 (0.0011)**	**0.0105 (0.0011)**	0.0028 (0.0056)	0.0005 (0.0356)	---	0.7500	**<0.0001**	0.6518
CLS	0.0010 (0.4389)	**0.0071 (0.0011)**	**0.0178 (0.0011)**	**0.0144 (0.0011)**	0.0053 (0.0222)	0.0028 (0.0122)	0.0007 (0.8933)	---	**<0.0001**	0.0201
BBS	**0.0073 (0.0011)**	0.0028 (0.0022)	-0.0003 (0.4767)	0.0014 (0.0233)	**0.0081 (0.0011)**	**0.0116 (0.0011)**	**0.0088 (0.0011)**	**0.0129 (0.0011)**	---	**<0.0001**
JLW	0.0019 (0.2344)	0.0026 (0.0278)	0.0088 (0.0067)	**0.0079 (0.0011)**	0.0032 (0.2956)	0.0037 (0.0111)	0.0009 (0.6267)	0.0071 (0.0422)	**0.0082 (0.0011)**	---

combined Georges Bank spawning collection exhibited significant differentiation from the Nantucket Shoals and Cox Ledge spawning collections, but was not differentiated by F_{ST} analysis from any Gulf of Maine population. Significant divergence of the Georges Bank cod from the majority of populations in the Gulf of Maine, however, was detected using tests of allelic differentiation. Only the Stellwagen Bank spring and Jeffrey's Ledge winter spawning aggregations were similar to Georges Bank cod by both differentiation methods.

Using principal coordinate analysis of the spawning aggregations, two main clusters of populations (Fig. 2) were identified: a spring 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 the Nantucket Shoals and Cox Ledge. This apparent clustering of populations and consistent differentiation suggests the existence of two different spawning complexes of Atlantic cod within this region. The northern spring complex (NSC) is evident within coastal Gulf of Maine waters only in the spring and summer, while a southern complex (SC) is present year round in southern waters and at different locations and seasons within the Gulf of Maine (Fig. 3). The Georges Bank spawning collection was observed as an intermediate population between the two complexes (Fig. 2) and could not be placed in either grouping based on pairwise differentiation values (Table 6). This offshore population was highly distinct from spawning cod on Cox Ledge and the Nantucket Shoals, but showed some level of similarity with both the northern spring complex and southern complex of cod within the Gulf of Maine.

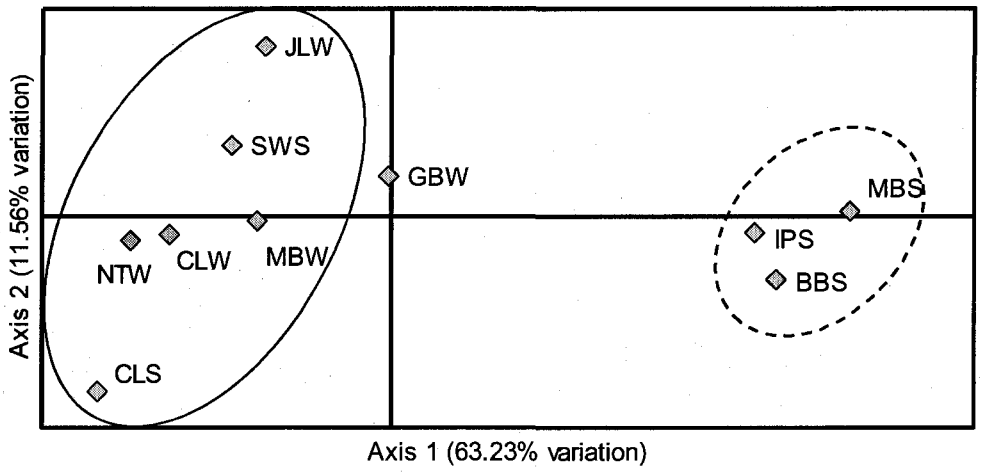

Fig. 2. Principal coordinate analysis of pairwise population F_{ST} values for combined years 1 and 2 spawning aggregations. Circles encompass similar populations with no significant pairwise F_{ST} values (see Table 5). Sample site names refer to Table 1. Percent values refer to the total percentage of variation explained by each axis.

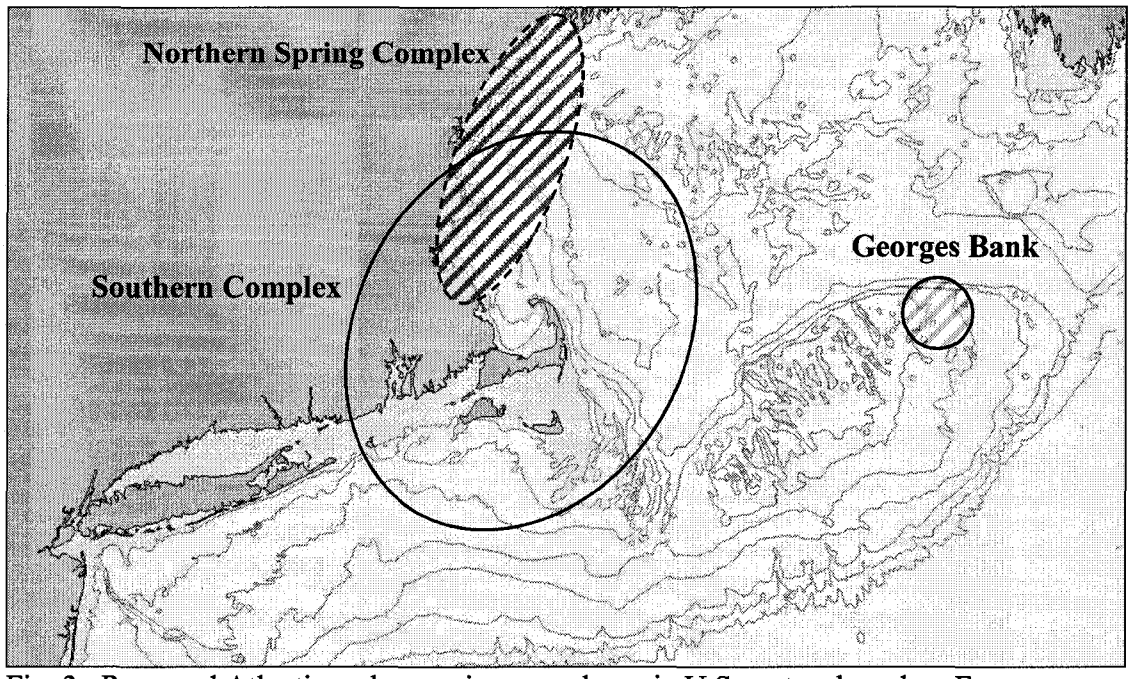

Fig. 3. Proposed Atlantic cod spawning complexes in U.S. waters based on F_{ST} estimates. Georges Bank represented a possible intermediate population and exhibited significant differentiation from southern New England but not within the Gulf of Maine.

Differentiation of Resting Adult Aggregations

The resting adult aggregations from Platts Bank, Ipswich Bay winter, and the New York Bight were found to cluster within the two spawning complexes (Fig. 4). The Platts Bank summer adults were observed to cluster within the northern spring complex. New York Bight spring and winter adults were observed to cluster closely with each other within the southern complex and Georges Bank. The Ipswich Bay winter adults clustered far from the southern complex, which was likely due to a bias of low sample size (n = 31). The large displacement of Ipswich Bay winter cod was largely due to variation along the second axis, which explained far less of the total variation (Fig. 4). Variation along this second axis was roughly one third that of the first and is therefore unlikely to represent a significant population difference. Variation along the first axis alone places the Ipswich Bay winter collection into the southern complex.

Temporal Stability of Spawning Complexes

A comparison with the spawning aggregation dataset of Wirgin et al. (2007) suggests that the population structure was temporally stable from 2003 to 2007 (Fig. 5). The 2003 Ipswich Bay spring spawning aggregation was observed to cluster with the other coastal Gulf of Maine samples into the northern spring complex. The Ipswich Bay winter, Chatham, Stellwagen Bank, Long Island, and Georges Bank cod clustered within the southern complex. Although the Chatham and Long Island cod do not have a temporal counterpart in this study, these additional aggregations are likely subpopulations within the southern complex.

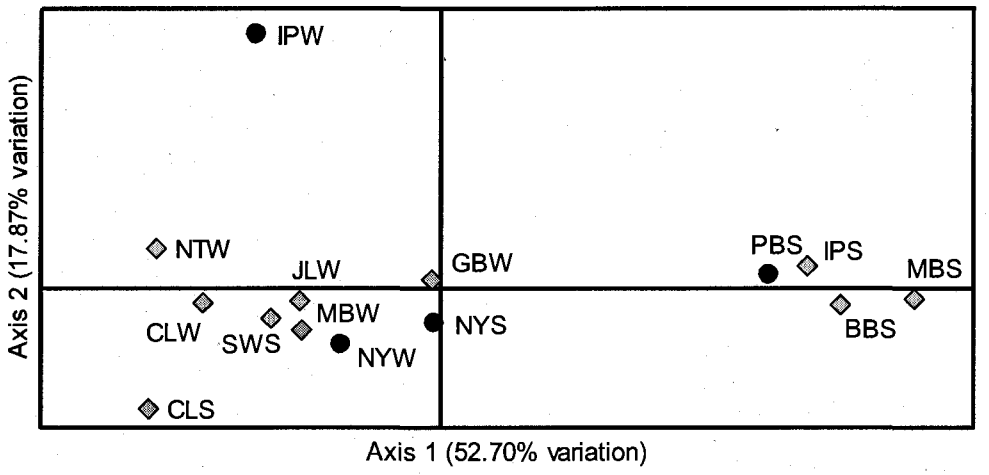

Fig. 4. Principal coordinate analysis of pairwise population F_{ST} values for combined years 1 and 2 spawning (◈) and resting adult aggregations (●). Sample site names refer to Table 1. Percent values refer to the total percentage of variation explained by each axis.

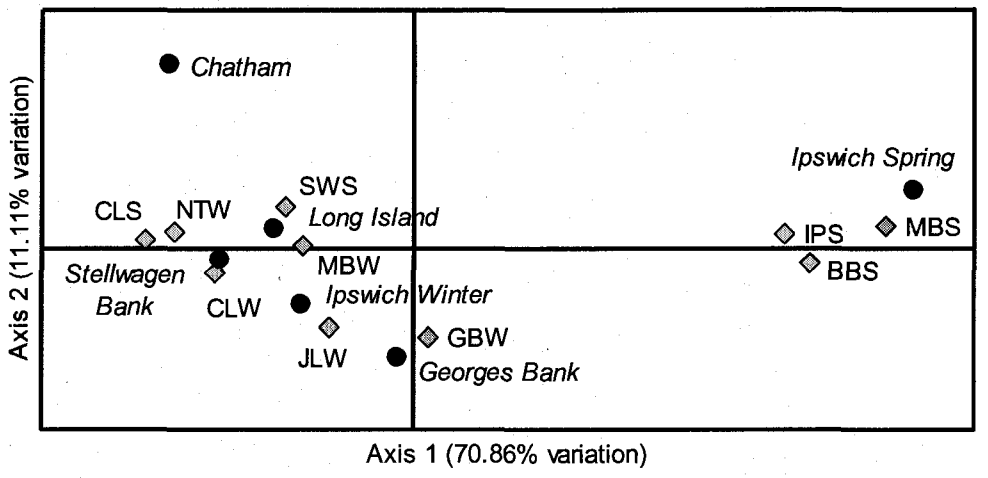

Fig. 5. Principal coordinate analysis of pairwise population F_{ST} values for combined years 1 and 2 spawning aggregations (◈) and spawning aggregations from Wirgin et al. 2007 (●). Spawning aggregation names from Wirgin et al. 2007 are in italics. Sample site names from the present study refer to Table 1. Percent values refer to the total percentage of variation explained by each axis.

Differentiation of Juvenile Collections

The juvenile collections from Cape Cod, Casco Bay, and Massachusetts Bay were all in Hardy-Weinberg equilibrium conditions except for one significant deviation in the Massachusetts Bay collection from October 2007. This collection deviated from equilibrium conditions at the *Gmo*37 locus ($p = 0.0006$), and a significant heterozygote deficiency was detected (Appendix B). No linkage disequilibrium was detected in the juvenile collections (data not shown). Using MICRO-CHECKER software, no evidence were found of the presence of null alleles, stuttering, or large allele dropout.

Significant differentiation among the juvenile collections was detected using tests of F_{ST} and allelic differentiation (Table 7). The Massachusetts Bay cod from October 2006 were genetically distinct from the Cape Cod samples collected in the same month. The Massachusetts Bay cod from October 2007 exhibited significant differentiation from all other juvenile collections except for samples from the same location in the previous year. These two similar Massachusetts Bay collections from October were combined into a single population for further analyses. Significant differentiation between the Casco Bay immature fish and the Cape Cod collection was detect using tests of allelic differentiation, but this differentiation was not significant by F_{ST} analysis. The combined Massachusetts Bay collection clustered with the spawning adults of the northern spring complex when analyzed using principal coordinate analysis (Fig. 6). The Massachusetts Bay spring juvenile collection, Cape Cod juveniles, and the Casco Bay immature fish clustered within the southern complex.

Table 7. Population differentiation of juvenile Atlantic cod. Above diagonal are allelic differentiation p-values. Below diagonal are pairwise population F_{ST} values with p-values in parentheses. Abbreviated sample site names refer to Table 1. Bold indicates significance following standard Bonferroni correction ($p \leq 0.0050$).

	MBWJ1	CCWJ	CBSJ	MBSJ	MBWJ2
MBWJ1	---	**0.0013**	0.0085	0.0217	0.1133
CCWJ	**0.0068 (0.0050)**	---	**0.0023**	0.5781	**<0.0001**
CBSJ	0.0057 (0.0100)	0.0040 (0.0150)	---	0.1444	**<0.0001**
MBSJ	0.0088 (0.1250)	-0.0008 (0.6600)	0.0004 (0.4850)	---	**<0.0001**
MBWJ2	0.0036 (0.1150)	**0.0133 (0.0050)**	**0.0119 (0.0050)**	**0.0174 (0.0050)**	---

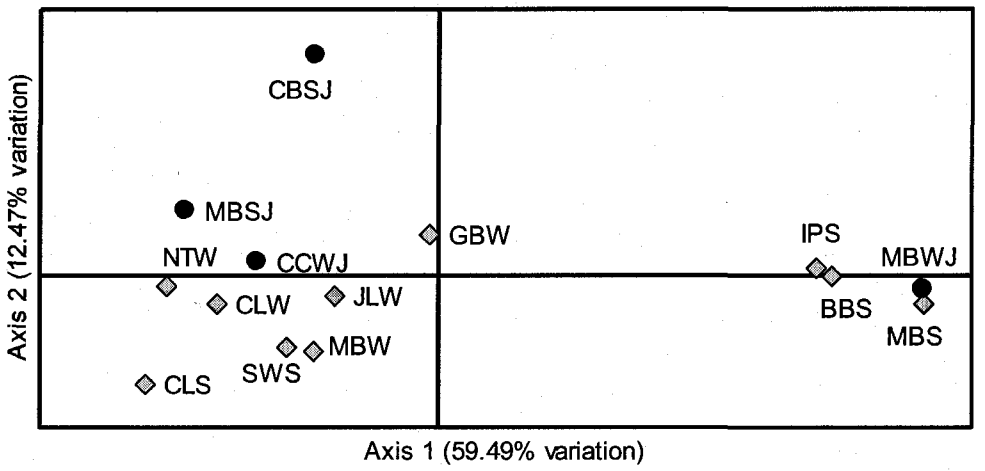

Fig. 6. Principal coordinate analysis of pairwise population F_{ST} values for combined years 1 and 2 spawning aggregations (◈) and juvenile collections (●). Sample site names refer to Table 1. Juvenile sites MBWJ1 and MBWJ2 were combined prior to analysis. Percent values refer to the total percentage of variation explained by each axis.

Mixture Analysis of Juvenile Collections

Mixture analysis was used to assign proportions of juvenile collections to spawning complexes. Individual adult populations were not used because of the lack of differentiation among the aggregations within a complex (see Table 6). The Georges Bank spawning population was removed prior to mixture analysis because it was unlikely that young of year juveniles from within the Gulf of Maine could originate from this adult population given ocean currents in the region.

Prior to mixture analysis, a leave-one-out cross validation test (Table 8) and a 100% fishery simulation test (Table 9) were conducted on the genetic data of the spawning complexes. These tests detected moderate power for the assignment of juvenile proportions. The southern complex percent correct scores from the cross validation test ranged from 74.0 to 83.9%, while the northern spring complex scores were lower and ranged from 54.1 to 65.5% (Table 8). These values indicated that a higher percentage of individuals could be assigned correctly to the southern complex than to 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 percent correct scores in this simulation were greater than the validation test in all cases, but the northern spring complex still produced lower values by comparison (Table 9). The low percent correct scores in these tests indicated that low levels of differentiation were present between the complexes.

Approximately 97% of the combined Massachusetts Bay fall collection was assigned to the northern spring complex through mixture analysis (Fig. 7). The high proportional assignment to this complex indicated that the majority of juveniles from

Table 8. Test of the accuracy of genetic stock identification using a leave-one-out cross validation test in ONCOR. Individuals from spawning Atlantic cod populations (see Table 5 and Fig. 2) were reassigned back to pre-defined reporting groups of northern spring complex (NSC) and southern complex (SC) (see Fig. 3). Percent correct values refer to the percentage of individuals correctly assigned back to each reporting group. The Georges Bank spawning population was removed prior to analysis. Abbreviated sample site names refer to Table 1.

Population	Assigned Reporting Group	Percent Correct
MBW	SC	78.3
MBS	NSC	65.5
IPS	NSC	56.9
SWS	SC	80.6
NTW	SC	80.4
CLW	SC	83.9
CLS	SC	82.6
BBS	NSC	54.1
JLW	SC	74.0

Table 9. Test of the accuracy of genetic stock identification using a 100% fishery simulation in ONCOR. Spawning Atlantic cod populations (see Table 5 and Fig. 2) were assigned to either the northern spring complex (NSC) or southern complex (SC) reporting groups (see Fig. 3). Mixture samples consisting entirely of single populations were simulated and then assigned to each adult complex. Percent correct refers to the percentage of these 100% mixtures assigned back to their correct reporting group. The Georges Bank spawning population was removed prior to analysis. Abbreviated sample site names refer to Table 1.

Population	Assigned Reporting Group	Percent Correct	Standard Deviation	95% Confidence Interval
MBW	SC	90.07	7.53	(72.52, 100.00)
MBS	NSC	86.37	7.97	(68.73, 99.85)
IPS	NSC	76.75	9.87	(56.18, 94.96)
SWS	SC	85.89	8.96	(65.95, 99.97)
NTW	SC	91.21	7.25	(74.18, 100.00)
CLW	SC	93.36	5.82	(79.41, 100.00)
CLS	SC	95.35	5.23	(81.98, 100.00)
BBS	NSC	76.49	9.89	(56.07, 94.26)
JLW	SC	83.48	9.52	(63.11, 99.68)

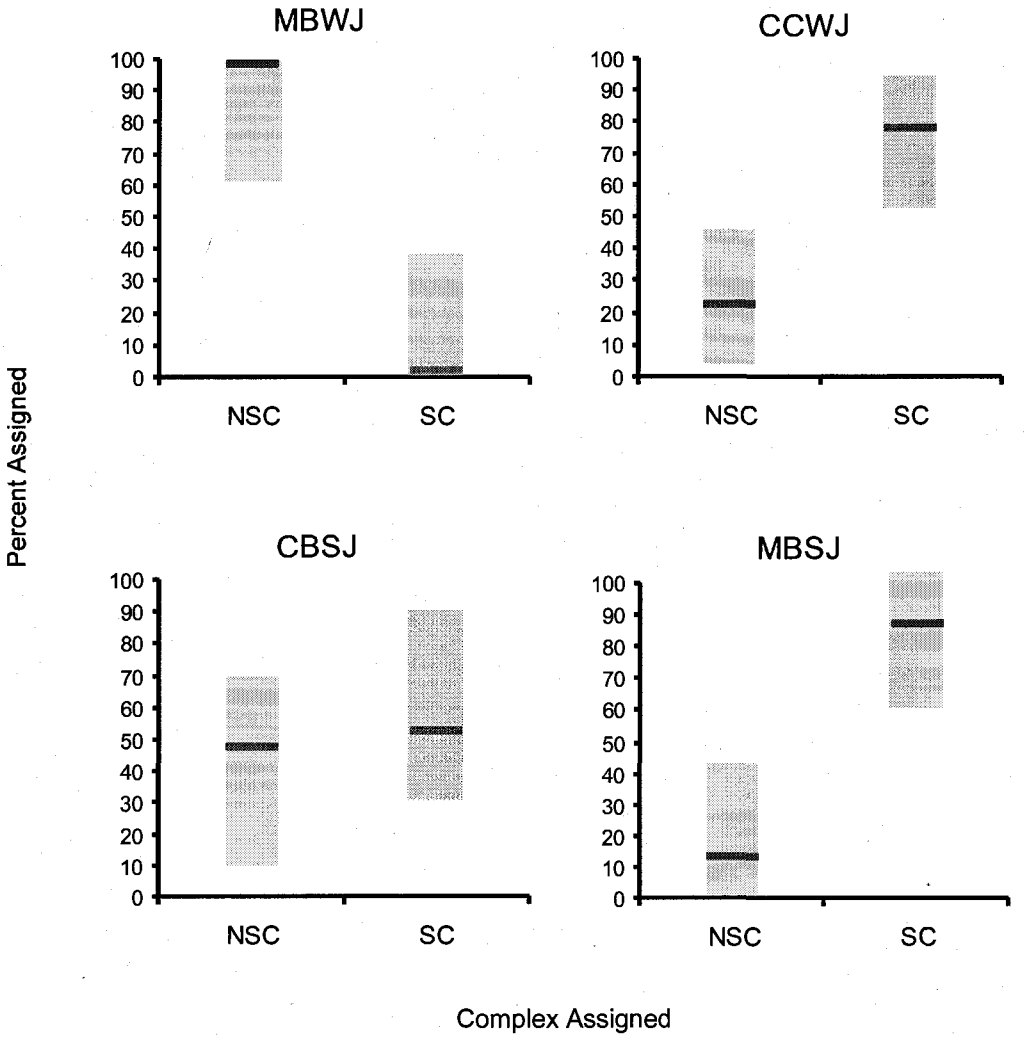

Fig. 7. Mixture analysis of juvenile collections to pre-defined spawning Atlantic cod reporting groups of northern spring complex (NSC) and southern complex (SC) (see Fig. 3). Black bars refer to percentages of juveniles assigned to each complex and shaded regions refer to 95% confidence intervals. Abbreviated site names refer to Table 1. Juvenile sites MBWJ1 and MBWJ2 were combined prior to analysis.

October in Massachusetts Bay originated from the northern spring complex. The seasonal shift of spawning complexes within the Gulf of Maine was evident in the Massachusetts Bay juveniles, and the majority (~ 87%) from the spring season were assigned to the southern complex. Although juveniles off Cape Cod were collected in the same month as the Massachusetts Bay fall collection, the majority in this case (~78%) were assigned instead to the southern complex (Fig. 7). Mixture analysis of the Casco Bay immature collection was equivocal, with roughly equal proportions of each complex present.

CHAPTER 4

DISCUSSION

Stock Structure of Atlantic Cod

Atlantic cod spawning aggregations in U.S. waters exhibited weak but statistically significant differentiation within the Gulf of Maine and Georges Bank management stocks. A pattern of genetic divergence was apparent that separated two main spawning complexes; a northern spring complex within coastal Gulf of Maine, and a southern complex present in both the Gulf of Maine and southern New England waters. The offshore Georges Bank spawning aggregation was genetically heterogeneous and could not be grouped into either complex. Differentiation among the complexes and Georges Bank spawning cod was similar to previous results found by Lage et al. 2004 and Wirgin et al. 2007. Comparisons of adult aggregation genetic data from Wirgin et al. 2007 to the present study indicate temporal stability of the stock structure over a five year period (2003-2007).

The spawning complexes were characterized by low levels of divergence ($F_{ST} < 0.01$) and population structuring was not evident when *a priori* assumptions of sampling regime were removed. The STRUCTURE clustering program is unable to identify population differentiation at $F_{ST} < 0.03$ (Latch et al. 2006), and the majority of pairwise population F_{ST} values in the present study were less than half of this value (Tables 3, 4, 5, and 7). Very little stabilization of $\ln(\Pr X \mid K)$ during the cluster analyses was evident at high K values (3-5), indicating that no structuring could be detected by the statistical

method. The assumptions of population division made by F_{ST} analysis and allelic differentiation give added power to these methods, but in return preclude the detection of hidden structuring outside the pre-defined sampling locations (see Pritchard et al. 2007).

Heterogeneity of Spawning Complexes in the Gulf of Maine

The northern spring complex of Atlantic cod occupied spring and summer coastal regions within the Gulf of Maine and consisted of spawning aggregations from Massachusetts Bay, Ipswich Bay, and the Bigelow Bight off southwestern Maine. These unique spawning aggregations were not present during the winter season and were replaced in Massachusetts Bay and Ipswich Bay by cod from the southern complex. Wirgin et al. (2007) previously studied the genetic variation of cod in Ipswich Bay and documented genetic divergence between spring and winter spawning aggregations in the area. Although few fish were sampled in Ipswich Bay during the winter months in the present study, the distinction between spring and winter remained evident (Fig. 4). This divergence was also temporally stable from 2003 to 2007 when the present study was compared to spawning aggregation data from Wirgin et al. 2007. These data are supported by a tag and recapture study conducted by Howell et al. (2008) that observed two different spawning groups present in Ipswich Bay during the spring and winter seasons. Ipswich Bay experienced a seasonal change in abundance, with strong movements of cod into the region during the spring and winter months for spawning. Spawning site fidelity was also evident in the spring spawning group, where tagged fish dispersed and returned to the released location (within 20 km range) one year later (Howell et al. 2008). The yearly migration events into the Ipswich Bay region and the

separate movements of winter and spring aggregations support the genetic distinctness of the two spawning complexes.

Robichaud and Rose (2004) reviewed many tagging studies and classified Atlantic cod aggregations according to four migratory behavior categories: sedentary residents, accurate homers, inaccurate homers, and dispersers. Cod exhibiting year round site fidelity were described as sedentary residents. Cod performing seasonal migrations to either small or large geographic areas were identified as accurate or inaccurate homers, respectively. Groups of cod without a clear pattern of migration and spawning were described as dispersers. Cod in Ipswich Bay have previously been assigned to the category of sedentary resident based on tag and recapture data (Howell et al. 2008). Recent data on cod migrations also provide evidence that cod in Ipswich Bay do not undertake extensive offshore movements, but instead stay within coastal Gulf of Maine regions (Tallack and Whitford 2008). Results from the present study were consistent with these findings and identified spring spawning cod in this region as part of a unique northern spring complex. Massachusetts Bay winter and spring aggregations followed similar seasonal variation to cod in Ipswich Bay (Table 5), and the Bigelow Bight summer spawners also cluster within this potential stock. This complex requires separate classification and is possibly composed of accurate homers. The spring spawning cod appear to exhibit spawning site fidelity to the coastal Gulf of Maine region during the spring and summer seasons, and these aggregations are not present during the winter spawning season when the southern complex predominates. The genetic similarity of spring spawning cod along the Gulf of Maine coast supports the idea that fidelity is to the general region and not just specifically to Ipswich Bay. Cod migrations between these

adjacent bays are known to occur (Tallack and Whitford 2008), and it is possible that spawning cod move along the Gulf of Maine coastline during the spring season.

The Platts Bank resting adult collection also clustered within the northern spring complex (Fig. 4). These resting fish were sampled in late August at approximately 58 km offshore, far from the expected coastal spawning aggregations. They may have been spring spawners from inshore areas in the Gulf of Maine that have dispersed away from coastal regions after spawning. If this occurs, then cod from the northern spring complex may be present at other offshore locations in late summer, when spawning events have ended and dispersal is ongoing. Howell et al. (2008) observed local postspawning movements from the Ipswich Bay area over a six to eight month timeframe in which the mean distance from the spawning site was 70-90 km. The summer resting adults on Platts Bank are located within this range and these cod may be one example of local dispersal that is characteristic of the northern spring complex.

Low differentiation at neutral microsatellite loci observed in the present study (Table 2) indicate that moderate to high levels of gene flow are likely ongoing in this region, and dispersers are likely present that homogenize the genetic variation. The non-significant F_{ST} values observed at neutral microsatellites cannot describe the levels of gene flow (Conover et al. 2006), but they do indicate that reproductive isolation is unlikely to be the driving force of differentiation. Despite the existence of spawning site fidelity and sedentary groups of cod in the western Gulf of Maine (Lindholm et al. 2007, Howell et al. 2008), transient groups or individuals may also be present at spawning times that introduce moderate levels of gene flow. Although Lindholm et al. (2007) were able to identify high site fidelity among cod on boulder reefs, a larger percentage of

tracked individuals were observed to depart the area rapidly upon release. This variability in cod migratory behavior may explain how genetic variation is homogenized between sedentary and more transient groups. The northern spring complex of spawning cod is likely composed of fish having multiple different migration and spawning strategies.

Heterogeneity of the Southern Complex and Georges Bank

The southern complex of spawning cod identified in this study (Fig. 3) did not fit the current model of cod stocks in U.S. waters (Mayo and Col 2006, O'Brien et al. 2006). Winter spawning aggregations in the Gulf of Maine, spring spawners on Stellwagen Bank, and aggregations in southern New England waters were all genetically similar. Unlike the northern spring complex, the southern complex consisted of both winter and spring spawning events and exhibited a wide spatial range. This similarity between Gulf of Maine populations and cod in southern New England waters is supported by recent data from tag and recapture studies. Cod from the Cape Cod and Great South Channel region were found to migrate both into the Gulf of Maine and in a southern direction towards the Nantucket Shoals (Tallack and Whitford 2008). Resting aggregations in Long Island waters and within the New York Bight were also similar to the southern complex and do not appear to represent a distinct population. These data suggest that the southern complex of cod extends further down the mid-Atlantic coast than what is presented in Fig. 3.

Significant differentiation between cod on Georges Bank and southern New England waters was consistent with previous studies in U.S. waters (Lage et al. 2004, Wirgin et al. 2007). The consistent and stable divergence between these areas likely

indicates that adult cod in southern New England waters and the mid-Atlantic region are not the product of larval spillover from Georges Bank (Lough 2004), but instead represent a separate stock. Recent data on cod movements support this hypothesis and show limited migration from Georges Bank to southern New England waters (Tallack and Whitford 2008). The stability of this divergence may also indicate that the clockwise gyre on Georges Bank is enough of a retention mechanism to limit larval movements in a southwestern direction towards the mid-Atlantic coast.

Georges Bank spawning cod were only weakly differentiated from adults within the Gulf of Maine, including both aggregations of the northern spring and southern complexes (Table 5). The offshore spawning cod on northeastern Georges Bank did not cluster clearly within either spawning complex (Fig. 2) and appeared to be genetically heterogeneous. This finding may be explained by the drift of eggs and larvae on the western Gulf of Maine current. The counterclockwise gyre of the Gulf of Maine may cause larvae to drift south and eastward towards Georges Bank (Collette and Klein-MacPhee 2002). The spawning aggregations on Stellwagen Bank and Jeffrey's Ledge exhibited the highest similarity with Georges Bank cod (Table 5), and these offshore Gulf of Maine subpopulations would be likely to have their larvae drift along the western Gulf of Maine current in a southeast direction. Inshore Gulf of Maine cod from both complexes were only weakly differentiated from cod on Georges Bank (Table 5). They may also be connected to Georges Bank via larval drift. Dispersal modeling data provided evidence that larvae from these inshore Gulf of Maine spawning grounds may drift in a southern direction towards eastern Georges Bank and the Great South Channel (Huret et al. 2007).

Heterogeneity of Juveniles within the Gulf of Maine

The stock composition of juveniles within the Gulf of Maine was heterogeneous and varied depending on season and location. A large proportion of the Massachusetts Bay fall juvenile collection was assigned to the northern spring complex, while spring juveniles from the same location were assigned to the southern complex (Fig. 7). This seasonal change in mixture proportions reflects the differences in adult spawning complexes present within the Gulf of Maine. Juveniles spawned from the northern spring complex appear to be retained within inshore Gulf of Maine regions through the fall season. The high retention of juveniles within Massachusetts Bay is likely a result of its inshore location that reduces exposure to the western Gulf of Maine current and limits larval dispersal (Huret et al. 2007). This retention may act as an imprinting episode for natal homing during spring spawning events in this region.

A large proportion of the Cape Cod collection was assigned to the southern complex, and it is likely that the majority of these juvenile cod were spawned from spring spawners on nearby Stellwagen Bank. The predominant western Gulf of Maine current in the area would likely be capable of moving larvae and juveniles off Stellwagen Bank and in a southern direction towards Cape Cod.

The mixture proportions of the Casco Bay immature cod cannot be explained in the same manner. These age-1 juvenile fish did not represent a young of year collection and therefore were not dependent on ocean currents for movements. Any number of individuals within this collection could have originated from either stock with equal chance due to their ability to freely migrate and disperse within the region. The equal mixture proportions of the Casco Bay collection (Fig. 7) reflects this potential dispersing

behavior of immature individuals. Age-1 juveniles from both stocks may perform seasonal migrations into inshore locations during the spring season (Hanson 1996). The Casco Bay collection may consist of juveniles moving from overwintering sites in deep waters to more shallow locations in the spring.

Differentiation at Non-Neutral Loci

The majority of the genetic differentiation observed in this study was due to two markers (*Gmo*132 and *Pan* I) suspected to be under selection pressures (Fevolden and Pogson 1997, Karlsson and Mork 2003, Pogson and Fevolden 2003, Sarvas and Fevolden 2005, Nielsen et al. 2006). The *Gmo*132 microsatellite marker has been used in many Atlantic cod population studies, exhibiting several times higher levels of differentiation than other assumed neutral markers (e.g. Lage et al. 2004, Wirgin et al. 2007). The high levels of differentiation present at this locus (see Table 2) is inconsistent with neutral expectations that assume neutral genetic drift causes relative similar levels of differentiation across all microsatellite loci. Nielsen et al. (2006) found evidence of hitch-hiking selection at *Gmo*132, which means that the microsatellite is linked to a gene under selection pressures. Assumed neutrality at this locus is invalid and the levels of differentiation are inflated with respect to neutral genetic drift and reproductive isolation. The high genetic divergence at *Gmo*132 may be caused by local adaptation that is capable of overcoming the effects of gene flow (Conover et al. 2006). The temporal stability of differentiation at *Gmo*132 in U.S. waters (Lage et al. 2004, Wirgin et al. 2007) suggests that selection pressures are driving local adaptation in northwest Atlantic cod, despite the presence of at least moderate levels of gene flow acting to homogenize variation at neutral microsatellite markers.

Natural selection is a likely cause of high differentiation at the *Pan* I locus in relation to other SNP markers and neutral microsatellites (see Wirgin et al. 2007). Nucleotide and protein level differences between the *Pan* I^A and *Pan* I^B alleles have previously been documented, including differences by 19 nucleotide mutations and four amino acids on the protein level (Pogson 2001). These differences may contribute to differential expression or functioning of the protein in certain tissues (Pogson 2001). Local adaptation favoring certain alleles may lead to high levels of genetic divergence. Selection at this locus has been reported to cause both spatial divergence among populations (Case et al. 2005, Sarvas and Fevolden 2005, Pampoulie et al. 2006b, 2008) and differences among cohorts and sexes (Karlsson and Mork 2003). Differences in water temperature, salinity, and depth among spatially and temporally segregated groups may drive selection pressures that favor physiological differences between the two alleles. Selection at this SNP locus has been described as a complex combination of balancing and directional selection (Pogson 2001), and the effects of these pressures on local scales likely produce the high levels of divergence observed among populations, cohorts, and sexes.

Variation at the *Pan* I locus has been used extensively to discriminate between two divergent groups of cod in the Barents Sea, identified as northeast Arctic cod and Norwegian coastal cod. Recent diversifying selection at *Pan* I within these populations (Pogson and Fevolden 2003) has resulted in the dominance of the *Pan* I^B allele within Arctic cod while the *Pan* I^A allele is predominant in Norwegian coastal cod (Fevolden and Pogson 1997, Sarvas and Fevolden 2005). These two groups of cod separated by broad geographic scales can also be distinguished by the suspected non-neutral

microsatellite loci *Gmo*34 and *Gmo*132 (Westgaard and Fevolden 2007). Divergence of the two groups is probably the product of different environmental conditions found inshore versus offshore in Norwegian waters. Sarvas and Fevolden (2005) observed a strong negative correlation between the frequency of the *Pan* I^A allele and depth at sampling among combined Arctic and coastal cod. Pampoulie et al. (2006b) found a similar correlation between *Pan* I^A allele frequency and sampling depth in Icelandic cod. In both cases, the dominance of the *Pan* I^B allele was evident at greater depths (up to 450 m) and reached average frequencies of approximately 0.90.

Differentiation at the *Pan* I locus was not as profound in U.S. waters, which may be due to the finer spatial scales analyzed in the present study. A similar trend in *Pan* I variation, however, was evident between the northern spring complex and the southern complex. The coastal Gulf of Maine spawners of the northern spring complex exhibited higher *Pan* I^A frequencies (0.06-0.16) than more offshore aggregations of the southern complex (0.00-0.02). Atlantic cod in U.S. and European waters may be under similar selection pressures. The lower levels of divergence in this study relative to northeastern cod stocks may be due to smaller depth and temperature differences in U.S. waters.

Water temperature and salinity have been observed to have a significant correlation with *Pan* I^A allele frequency (Case et al. 2005). These factors may explain the differences between the Norwegian cod stocks at individual fjord scales (Fevolden and Pogson 1997). Case et al. (2005) studied variation at *Pan* I in northeast Atlantic cod and observed a positive trend between *Pan* I^A allele frequency and temperature, while a negative trend was evident between *Pan* I^A allele frequency and salinity. These selection pressures may also be present within coastal U.S. waters, where higher spring

temperatures and lower salinities result in higher *Pan* I^A allele frequencies in cod. Spring river runoff into the Gulf of Maine likely plays an important role in depressing ocean salinity in coastal regions. Coupled with high inshore spring and summer temperatures, these two selection pressures may drive local adaptation of spring spawners. The exclusion of spring spawners on Stellwagen Bank from the northern spring complex may be explained by its more offshore location. Increased salinity and decreased temperature in relation to inshore Massachusetts Bay and Ipswich Bay may play a role in separating these populations. Steep transition zones between *Pan* I^A allele frequency and temperature and salinity have been observed (Case et al. 2005), indicating even a small difference in these environmental factors could potentially result in a dramatic increase or decrease in allele frequency. Slightly elevated salinity or lower temperature on Stellwagen Bank could therefore be one reason for the divergence of these cod from the northern spring complex.

Temperature and salinity differences that drive local adaptation at *Pan* I likely have an effect on the fitness of cod from different populations. The association between *Pan* I allele frequencies and growth has previously been studied, but the relationship remains unclear and likely depends on several environmental parameters (Jónsdóttir et al. 2008). Case et al. (2006) examined northeast Atlantic cod larvae and found that *Pan* I^{AB} heterozygotes exhibited significantly higher mean dry weights, standard lengths, and RNA/DNA ratios than *Pan* I^{BB} homozygotes. These data suggested that the *Pan* I^A allele was associated with faster growth in natural populations, although a direct comparison with the *Pan* I^{AA} genotype was not possible in the study (Case et al. 2006). A comparative study between the Norwegian coastal cod and northeast Arctic cod also

provided evidence that coastal cod larvae grow faster and exhibit greater weight at age than larvae originating from the northeast Arctic stock (Otterlei et al. 1999). This stock-specific growth difference was found across a wide range of temperatures (4-14°C) and suggested that higher growth rates of larvae from *Pan* I^A dominated populations is not limited to high environmental temperatures. These studies provide evidence that directional selection is present at this locus and that cod with the *Pan* I^A allele have increased fitness. Selection pressures acting on *Pan* I, however, are likely complex (Pogson 2001) and directional selection alone cannot explain the presence of *Pan* I^B dominated cod populations. The maintenance of both *Pan* I alleles indicates the presence of ongoing balancing selection that favors different alleles under different environmental conditions. Pampoulie et al. (2006b) suggested the presence of divergent selection at the *Pan* I locus in adult cod around Iceland, where cod with the *Pan* I^B allele predominate in warmer, highly saline southern waters and cod with the *Pan* I^A allele are more common in low salinity northern waters. Jónsdóttir et al. (2008) found variations in growth associated with *Pan* I allele frequencies in different environments and suggested that different physiological properties of the pantophysin proteins could be acting in different areas across Icelandic waters. The relationships between *Pan* I and fitness of Atlantic cod in U.S. waters has not yet been investigated. Higher spring and summer temperatures in coastal Gulf of Maine regions may contribute to increased fitness of the *Pan* I^A allele in these populations.

Differential growth rates are not the only fitness effects to result from differences in water temperature. Temperature may also be a selective factor for population differences in antifreeze production (Ruzzante et al. 1996b, Goddard et al. 1999) and the

distribution of polymorphic haemoglobin (HbI-1 and HbI-2 alleles) in Atlantic cod. Haemoglobin allele frequencies have been observed to differ substantially across the ranges of Atlantic cod (Sick 1965a, b), especially along the coast of Norway where a clear cline of HbI-1 frequencies was evident (Frydenberg et al. 1965). Patterns of haemoglobin variation in this region are similar to divergence observed at the *Pan* I locus (see Sarvas and Fevolden 2005), where *Pan* I^A and HbI-1 alleles dominate at warmer temperatures. Jónsdóttir et al. (1999) examined *Pan* I and haemoglobin variation in southern Icelandic waters and observed that the two markers varied independently but showed similar distinction among populations. The small but significant level of divergence at *Pan* I within U.S. waters may also indicate that selection pressures from water temperature may vary antifreeze production and haemoglobin genotypes in cod populations within the Gulf of Maine. If differentiation within cod haemoglobin is similar to *Pan* I variation, then HbI-1 allele frequencies would be expected to be higher in cod from the northern spring complex than the southern complex. Considering that cod with different haemoglobin genotypes are known to prefer different temperatures (Petersen and Steffensen 2003), the divergence between these two complexes may represent important stock differences if variation in haemoglobin exists. Local adaptation based on selection pressures from water temperature likely plays an important role in shaping Atlantic cod spawning complexes within U.S. waters. Future studies attempting to differentiate between these complexes will need to focus on non-neutral genetic markers and potential physiological differences.

CHAPTER 5

CONCLUSIONS

Two divergent complexes of spawning Atlantic cod were identified in U.S. waters. A northern spring complex occurred in coastal regions of the Gulf of Maine and was present only during the spring and summer seasons. A southern complex of cod occurred in both the Gulf of Maine and southern New England waters during both winter and spring spawning seasons. These two complexes overlap geographically in Massachusetts and Ipswich Bay but are separated temporally by different spawning seasons. Georges Bank was identified as an intermediate population between the two complexes; it was strongly divergent from southern sites but similar to both spring and winter spawning populations in the Gulf of Maine. These differences were temporally stable over a two year period and were consistent with preliminary studies conducted in this region (Lage et al. 2004, Wirgin et al. 2007). The present study differed from previous analyses by sampling all known major spawning aggregations in U.S. waters and testing for temporal stability of the stock differences. Juvenile collections within the Gulf of Maine exhibited similar levels of genetic heterogeneity and mirrored the adult spawning complexes from which they were spawned. Juveniles could be assigned as groups to the spawning complexes from which they originated.

Differentiation among the complexes is likely driven by local adaptation acting to overcome the effects of gene flow. Although spawning site fidelity, larval retention in inshore areas, and limited migration patterns are evident among cod groups, at least

moderate levels of gene flow are present among populations that homogenize the genetic variation at neutral loci. A combination of high water temperatures and a decrease in salinity within spring coastal regions of the Gulf of Maine may drive adaptation in the northern spring complex. Physiological differences may be present in cod from the northern spring complex that increases their fitness under these environmental conditions.

Many unanswered questions concerning the stock structure of Atlantic cod remain. Although there is evidence of spawning site fidelity among cod groups in the Gulf of Maine, the presence of natal homing to spawning grounds remains unknown. Larval retention in inshore areas may allow for imprinting and natal homing as adults, but the exact levels of retention were not tested in the present study. Larvae from within the Gulf of Maine may also drift on ocean currents to more offshore sites such as Georges Bank. Additional cod populations from different spawning grounds on Georges Bank will need to be assayed using genetic markers to more clearly identify stock composition and larval recruitment to this area. Future studies will also need to focus on the physiological differences between the two spawning complexes and Georges Bank. The selection pressures acting on cod in U.S. waters need to be studied further to identify important population differences for effective management and conservation.

LITERATURE CITED

Ames, E.P. (2004) Atlantic cod structure in the Gulf of Maine. Fisheries 29: 10-28.

Andersen, E.C., R.S. Waples, and S.T. Kalinowski. (2008) An improved method for predicting the accuracy of genetic stock identification. Canadian Journal of Fisheries and Aquatic Sciences 65: 1475-1486.

Avise, J.C. (2004) Molecular markers, natural history, and evolution. Second Edition. Sinauer Associates, Inc. pp. 92-97.

Beacham, T.D., J. Brattey, K.M. Miller, K.D. Le, and R.E. Withler. (2002) Multiple stock structure of Atlantic cod (*Gadus morhua*) off Newfoundland and Labrador determined from genetic variation. ICES Journal of Marine Science 59: 650-665.

Beacham, T.D., J.R. Candy, B. McIntoch, C. MacConnachie, A. Tabata, K.M. Miller, and R.E. Withler. (2005) DNA-level variation of sockeye salmon in southeast Alaska and the Nass and Skeena Rivers, British Columbia, with applications to stock identification. North American Journal of Fisheries Management 25: 763-776.

Bentzen, P., C.T. Taggart, D.E. Ruzzante, and D. Cook. (1996) Microsatellite polymorphism and the population structure of Atlantic cod *(Gadus morhua*) in the northwest Atlantic. Canadian Journal of Fisheries and Aquatic Sciences 53: 2706-2721.

Bowen, B.W., W.S. Grant, Z. Hillis-Starr, D.J. Shaver, A. Bjorndal, A.B. Bolten, and A.L. Bass. (2007) Mixed-stock analysis reveals the migrations of juvenile hawksbill turtles (*Eretmochelys imbricata*) in the Caribbean Sea. Molecular Ecology 16: 49-60.

Brooker, A.L., D. Cook, P. Bentzen, J.M. Wright, and R.W. Doyle. (1994) Organization of microsatellites differs between mammals and cold-water teleost fishes. Canadian Journal of Fisheries and Aquatic Sciences 51: 1959-1966.

Case, R.A., W.F. Hutchinson, L. Hauser, V. Buehler, C. Clemmesen, G. Dahle, O.S. Kjesbu, E. Moksness, H. Otterå, H. Paulsen, T. Svåsand, A. Thorsen, and G.R. Carvalho. (2006) Association between growth and *Pan* I* genotype within Atlantic cod full-sibling families. Transactions of the American Fisheries Society 135: 241-250.

Case, R.A., W.F. Hutchinson, L. Hauser, C.V. Oosterhout, and G.R. Carvalho. (2005) Macro- and micro-geographic variation in pantophysin (*Pan*I) allele frequencies in NE Atlantic cod *Gadus morhua*. Molecular Ecology Progress Series 301: 267-278.

Collette, B.B. and G. Klein-MacPhee. (2002) Bigelow and Schroeder's Fishes of the Gulf of Maine. Smithsonian Institute Press. pp. 228-235.

Conover, D.O., L.M. Clarke, S.B. Munch, and G.N. Wagner. (2006) Spatial and temporal scales of adaptive divergence in marine fishes and the implications for conservation. Journal of Fish Biology 69: 21-47.

Delghandi, M., J. Stenvik, F. Nilsen, M.S. Wesmajervi, K.T. Fjalestad, and B. Damsgård. (2008) Identification and characterisation of nine new gene-associated microsatellite markers for Atlantic cod (*Gadus morhua* L.) Conservation Genetics 9: 747-749.

DeWoody, J.A. and J.C. Avise. (2000) Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. Journal of Fish Biology 56: 461-473.

Fevolden, S.E. and G.H. Pogson. (1997) Genetic divergence at the synaptophysin (*Syp* I) locus among Norwegian coastal and north-east Arctic populations of Atlantic cod. Journal of Fish Biology 51: 895-908.

Frydenberg, O., D. Møller, G. Nævdal, and K. Sick. (1965) Haemoglobin polymorphism in Norwegian cod populations. Hereditas 53: 257-271.

Gelman, A. and D.B. Rubin. (1992) Inference from iterative simulation using multiple sequences. Statistical Science 7: 457-511.

Goddard, S.V., M.H. Kao, and G.L. Fletcher. (1999) Population differences in antifreeze production cycles of juvenile Atlantic cod (*Gadus morhua*) reflect adaptations to overwintering environment. Canadian Journal of Fisheries and Aquatic Sciences 56: 1991-1999.

Goudet, J. (1995) FSTAT (version 1.2): a computer program to calculate F-statistics. Journal of Heredity 86: 485-486.

Haass, N.K., J. Kartenbeck, and R.E. Leube. (1996) Pantophysin is a ubiquitously expressed synaptophysin homologue and defines constitutive transport vesicles. Journal of Cell Biology 134: 731-746.

Hanson, J.M. (1996) Seasonal distribution of juvenile Atlantic cod in the southern Gulf of St. Lawrence. Journal of Fish Biology 49: 1138-1152.

Hardie D.C., R.M. Gillett, and J.A. Hutchings. (2006) The effects of isolation and colonization history on the genetic structure of marine-relict populations of Atlantic cod (*Gadus morhua*) in the Canadian arctic. Canadian Journal of Fisheries and Aquatic Sciences 63: 1830-1839.

Howell, W.H., M. Morin, N. Rennels, and D. Goethal. (2008) Residency of adult Atlantic cod (*Gadus morhua*) in the western Gulf of Maine. Fisheries Research 91: 123-132.

Huret, M., J.A. Runge, C. Chen, G. Cowles, Q. Xu, and J.M. Pringle. (2007) Dispersal modeling of fish early life stages: sensitivity with application to Atlantic cod in the western Gulf of Maine. Marine Ecology Progress Series 347: 261-274.

Hutchings J.A. (2000) Collapse and recovery of marine fishes. Nature 406: 882-885.

Hutchinson, W.F., G.R. Carvalho, and S.I. Rogers. (2001) Marked genetic structuring in localised spawning populations of *Gadus morhua* in the North Sea and adjoining waters, as revealed by microsatellites. Marine Ecology Progress Series 223: 251-260.

Jakobsdóttir K.B., P.D. Jörundsdóttir, S. Skínidóttir, S. Hjörleifsdóttir, G.O. Hreggvidsson, A.K. Daníelsdóttir, and C. Pampoulie. (2006) Nine new polymorphic microsatellite loci for the amplification of archived otolith DNA of Atlantic cod, *Gadus morhua* L. Molecular Ecology Notes 6: 337-339.

Jónsdóttir, I.G., A.K. Imsland, A.K. Daníelsdóttir, V. Thorsteinsson, and G. Nævdal. (1999) Genetic differentiation among Atlantic cod in south and south-east Icelandic waters: synaptophysin (*Syp* I) and haemoglobin (*HbI*) variation. Journal of Fish Biology 54: 1259-1274.

Jónsdóttir, I.G., G. Marteinsdóttir, and C. Pampoulie. (2008) Relation of growth and condition with the *Pan* I locus in Atlantic cod (*Gadus morhua* L.) around Iceland. Marine Biology 154: 867-874.

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

Karlsson, S. and J. Mork. (2003) Selection-induced variation at the pantophysin locus (*Pan*I) in a Norwegian fjord population of cod (*Gadus morhua* L.) Molecular Ecology 12: 3265-3274.

Knutsen, H., P.E. Jorde, C. Andre, and N.C. Stenseth. (2003) Fine-scaled geographic population structuring in a highly mobile marine species: the Atlantic cod. Molecular Ecology 12: 385-394.

Koljonen, M.L., J.J. Pella, and M. Masuda. (2005) Classical individual assignments versus mixture modeling to estimate stock proportions in Atlantic salmon (*Salmo salar*) catches from DNA microsatellite data. Canadian Journal of Fisheries and Aquatic Sciences 62: 2143-2158.

Kurlansky, M. (1997) Cod: a biography of the fish that changed the world. Pengiun Group. pp. 1-294.

Lage, C., K. Kuhn, and I. Kornfield. (2004) Genetic differentiation among Atlantic cod (*Gadus morhua*) from Browns Bank, Georges Bank, and Nantucket Shoals. Fishery Bulletin 102: 289-297.

Latch, E.K., G. Dharmarajan, J.C. Glaubitz, and O.E. Rhodes Jr. (2006) Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. Conservation Genetics 7: 295-302.

Lindholm, J., P.A. Auster, and A. Knight. (2007) Site fidelity and movement of adult Atlantic cod *Gadus morhua* at deep boulder reefs in the western Gulf of Maine, USA. Marine Ecology Progress Series 342: 239-247.

Lough, R.G. (2004) Atlantic cod, *Gadus morhua*, life history and habitat characteristics. Second Edition. NOAA Technical Memorandum NMFS-NE-190.

Manel, S., M.K. Schwartz, G. Luikart, and P. Taberlet. (2003) Landscape genetics: combining landscape ecology and population genetics. Trends in Ecology and Evolution 18: 189-197.

Mayo, R.K. and L.A. Col. (2006) The 2005 assessment of the Gulf of Maine Atlantic cod stock. U.S. Department of Commerce, Northeast Fisheries Science Center Reference Document 02-6.

Moen, T., B. Hayes, F. Nilsen, M. Delghandi, K.T. Fjalestad, S.E. Fevolden, P.R. Berg, and S. Lien. (2008) Identification and characterisation of novel SNP markers in Atlantic cod: evidence for directional selection. BMC Genetics 9:18.

Miller, K.M., K.D. Le, and T.D. Beacham. (2000) Development of tri- and tetranucleotide repeat microsatellite loci in Atlantic cod (*Gadus morhua*). Molecular Ecology 9: 237-244.

Nielsen, E.E., M.M. Hansen, and D. Meldrup. (2006) Evidence of microsatellite hitch-hiking selection in Atlantic cod (*Gadus morhua* L.): implications for inferring population structure in nonmodel organisms. Molecular Ecology 15: 3219-3229.

O'Brien, L., N. Shepherd, and L.A. Col. (2006) Assessment of the Georges Bank Atlantic cod stock. U.S. Department of Commerce, Northeast Fisheries Science Center Reference Document 06-10.

Oosterhout, C.V., W.F. Hutchinson, D.P.M. Wills, and P. Shipley. (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4: 535-538.

Otterlei, E., G. Nyhammer, A. Folkvord, and S.O. Steffansson. (1999) Temperature- and size-dependent growth of larval and early juvenile Atlantic cod (*Gadus morhua*): a comparative study of Norwegian coastal cod and northeast Arctic cod. Canadian Journal of Fisheries and Aquatic Sciences 56: 2099-2111.

Pampoulie, C., T.D. Jörundsdóttir, A. Steinarsson, G. Pétursdóttir, M.O. Stefánsson, and A.K. Daníelsdóttir. (2006a) Genetic comparison of experimental farmed strains and wild Icelandic populations of Atlantic cod (*Gadus morhua* L.). Aquaculture 261: 556-564.

Pampoulie, C., D.E. Ruzzante, V. Chosson, T.D. Jörundsdóttir, L. Taylor, V. Thorsteinsson, A.K. Daníelsdóttir, and G. Marteinsdóttir. (2006b) The genetic structure of Atlantic cod (*Gadus morhua*) around Iceland: insight from microsatellites, the *Pan* I locus, and tagging experiments. Canadian Journal of Fisheries and Aquatic Sciences 63: 2660-2674.

Pampoulie, C., M.O. Stefánsson, T.D. Jörundsdóttir, B.S. Danilowicz, and A.K. Daníelsdóttir. (2008) Recolonization history and large-scale dispersal in the open sea: the case study of the North Atlantic cod, *Gadus morhua* L. Biological Journal of the Linnean Society 94: 315-329.

Peakall, R. and P.E. Smouse. (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6: 288-295.

Pella, J. and M. Masuda. (2001) Bayesian methods for analysis of stock mixtures from genetic characters. Fisheries Bulletin 99: 151-167.

Petersen, M.F. and J.F. Steffensen. (2003) Preferred temperature of juvenile Atlantic cod *Gadus morhua* with different haemoglobin genotypes at normoxia and moderate hypoxia. Journal of Experimental Biology 206: 359-364.

Piry, S., A. Alapetite, J.-M. Cornuet, D. Paetkau, L. Baudouin, and A. Estoup. (2004) GENECLASS2: A software for genetic assignment and first-generation migrant detection. Journal of Heredity 95: 536-539.

Pogson, G.H. (2001) Nucleotide polymorphism and natural selection at the Pantophysin I (*Pan* I) locus in Atlantic cod, *Gadus morhua* (L.) Genetics 157: 317-330.

Pogson, G.H. and S.E. Fevolden. (2003) Natural selection and the genetic differentiation of coastal and Arctic populations of Atlantic cod in northern Norway: a test involving nucleotide sequence variation at the pantophysin (*Pan*I) locus. Molecular Ecology 12: 63-74.

Pritchard, J.K., M. Stephens, and P. Donnelly. (2000) Inference of population structure using multilocus genotype data. Genetics 155: 945-959.

Pritchard, J.K., X. Wen, and D. Falush. (2007) Documentation for STRUCTURE software: Version 2.2 pp. 12-14.

Raymond, M. and Rousset, F. (1995) GENEPOP (version 1.2) population genetic software for exact tests and ecumenicism. Journal of Heredity 86: 248-249.

Robichaud, D. and G.A. Rose (2004) Migratory behaviour and range in Atlantic cod: inference from a century of tagging. Fish and Fisheries 5: 185-214.

Ruzzante, D.E., M.M. Hansen, D. Meldrup, and K.E. Ebert. (2004) Stocking impact and migration pattern in an anadromous brown trout (*Salmo trutta*) complex: where have all the stocked spawning sea trout gone? Molecular Ecology 13: 1433-1445.

Ruzzante, D.E., C.T. Taggart, and D. Cook. (1996a) Spatial and temporal variation in the genetic composition of a larval cod (*Gadus morhua*) aggregation: cohort contribution and genetic stability. Canadian Journal of Fisheries and Aquatic Sciences 53: 2695-2705.

Ruzzante, D.E., C.T. Taggart, and D. Cook. (1998) A nuclear DNA basis for shelf- and bank-scale population structure in northwest Atlantic cod (*Gadus morhua*): Labrador to Georges Bank. Molecular Ecology 7: 1663-1680.

Ruzzante, D.E., C.T. Taggart, and D. Cook. (1999) A review of the evidence for genetic structure of cod (*Gadus morhua*) populations in the NW Atlantic and population affinities of larval cod off Newfoundland and the Gulf of St. Lawrence. Fisheries Research 43: 79-97.

Ruzzante, D.E., C.T. Taggart, D. Cook, and S. Goddard. (1996b) Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua*) off Newfoundland: microsatellite DNA variation and antifreeze level. Canadian Journal of Fisheries and Aquatic Sciences 53: 634-645.

Ruzzante, D.E., C.T. Taggart, D.Cook, and S. Goddard. (1997) Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua*) off Newfoundland: a test and evidence of temporal stability. Canadian Journal of Fisheries and Aquatic Sciences 54: 2700-2708.

Ruzzante, D.E., C.T. Taggart, S. Lang, and D.Cook. (2000a) Mixed-stock analysis of Atlantic cod near the Gulf of St. Lawrence based on microsatellite DNA. Ecological Applications 10: 1090-1109.

Ruzzante, D.E., J.S. Wroblewski, C.T. Taggart, R.K. Smedbol, D. Cook, and S.V. Goddard. (2000b) Bay-scale population structure in coastal Atlantic cod in Labrador and Newfoundland, Canada. Journal of Fish Biology 56: 431-447.

Ryman, N., S. Palm, C. Andre, G. R. Carvalho, T. G.Dahlgren, P. E. Jorde, L. Laikre, L. C. Larson, A. Palme, and D. E. Ruzzante. (2006) Power for detecting genetic divergence: differences between statistical methods and marker loci. Molecular Ecology 15: 2031-2045.

Sarvas, T.H. and S.E. Fevolden. (2005) Pantophysin (*Pan* I) locus divergence between inshore *v.* offshore and northern *v.* southern populations of Atlantic cod in the north-east Atlantic. Journal of Fish Biology 67: 444-469.

Sick, K. (1965a) Haemoglobin polymorphism of cod in the Baltic and the Danish Belt Sea. Hereditas 54: 19-48.

Sick, K. (1965b) Haemoglobin polymorphism of cod in the North Sea and the North Atlantic Ocean. Hereditas 54: 49-69.

Stenvik, J., M.S. Wesmajervi, B. Damsgård, and M. Delghandi. (2006a) Genotyping of pantophysin I (*Pan*I) of Atlantic cod (*Gadus morhua* L.) by allele-specific PCR. Molecular Ecology Notes 6: 272-275.

Stenvik, J., M.S. Wesmajervi, K.T. Fjalestad, B. Damsgård, and M. Delghandi. (2006b) Development of 25 gene-associated microsatellite markers of Atlantic cod (*Gadus morhua* L.). Molecular Ecology Notes 6: 1105-1107.

Tallack, S.M.L., and S.L. Whitford. (2008) An overview of findings from data collected through the northeast regional cod tagging program: a five year, international, collaborative study into cod migration in the Gulf of Maine region. Accessed 24 October 2008 from www.codresearch.org/News_&_Updates.htm.

Waples, R.S., and O. Gaggiotti. (2006) What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. Molecular Ecology 15: 1419-1439.

Ward, R.W., M. Woodwark, and D.O.F. Skibinski. (1994) A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. Journal of Fish Biology 44: 213-232.

Weir, B.S. and C.C. Cockerham. (1984) Estimating F-statistics for the analysis of population structure. Evolution 38: 1358-1370.

Wesmajervi M.S., T. Tafese, J. Stenvik, K.J. Fjalestad, B. Damsgård, and M. Delghandi. (2007) Eight new microsatellite markers in Atlantic cod (*Gadus morhua* L.) derived from an enriched genomic library. Molecular Ecology Notes 7: 138-140.

Westgaard, J.I. and S.E. Fevolden. (2007) Atlantic cod (*Gadus morhua* L.) in inner and outer coastal zones of northern Norway display divergent genetic signature at non-neutral loci. Fisheries Research 85: 306-315.

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

Wright, S. (1951) The genetical structure of populations. Annals of Eugenics 28: 114-138.

APPENDICES

APPENDIX A. ANIMAL CARE AND USE APPROVAL DOCUMENTATION

University *of* New Hampshire

Research Conduct and Compliance Services, Office of Sponsored Research
Service Building, 51 College Road, Durham, NH 03824-3585
Fax: 603-862-3564

28-Nov-2006

Berlinsky, David
Zoology, Spaulding Life Science Center
Durham, NH 03824

IACUC #: 061006
Project: Genetic Identification of Atlantic Cod Spawning Stocks in U.S. Waters using Microsatellite and SNP DNA Markers
Category: B
Approval Date: 17-Nov-2006

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category B on Page 5 of the Application for Review of Vertebrate Animal Use in Research or Instruction - *the study involves either no pain or potentially involves momentary, slight pain, discomfort or stress.*

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of the original approval.

Please Note:
1. All cage, pen, or other animal identification records must include your IACUC # listed above.
2. Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and their affiliated personnel, employees of the University and students alike. A Medical History Questionnaire accompanies this approval; please copy and distribute to all listed project staff who have not completed this form already. Completed questionnaires should be sent to Dr. Gladi Porsche, UNH Health Services.

If you have any questions, please contact either Roger Wells at 862-2726 or Julie Simpson at 862-2003.

For the IACUC,

Jessica A. Bolker, Ph.D.
Chair

cc: File

APPENDIX B. TESTS OF HARDY-WEINBERG EQUILIBRIUM

P-values of probability of tests of Hardy-Weinberg equilibrium of Year 1 Atlantic cod. Sample site names refer to Table 1. No significant deviations from equilibrium conditions were present ($p \leq 0.0005$).

Locus	Site							
	MBW1	GBW1	MBS1	IPS1	SWS1	PBS	NTW	IPW
*Gmo*02	0.3064	0.7179	0.1992	0.5411	0.6830	0.7605	0.1169	0.4457
*Gmo*19	0.3535	0.2089	0.3106	0.8286	0.0637	0.9847	0.0500	1.0000
*Gmo*132	0.1374	0.6766	0.6618	0.7854	0.3053	0.7517	0.2207	0.1808
*Gmo*35	0.7451	0.4867	0.4906	0.8573	0.0823	0.4062	0.4256	0.8172
*Gmo*36	0.1829	0.1106	0.3110	0.8186	0.3340	0.2077	0.2207	0.8711
*Gmo*37	0.7588	0.0517	0.8005	0.2418	0.5994	0.6782	0.4586	0.0941
P*Gmo*32	0.7186	0.3295	0.5524	0.6634	0.1285	0.4125	0.2423	0.7328
P*Gmo*34	0.5641	0.7432	0.6634	0.7543	0.1391	0.0444	0.7011	0.0851
P*Gmo*38	0.4965	0.2300	0.9330	0.7643	0.5170	0.5495	0.4943	0.8796
P*Gmo*56	0.7694	0.0052	0.1594	0.3440	0.3716	0.3745	0.2743	1.0000
P*Gmo*58	0.0237	0.5776	0.0537	0.8965	0.4305	0.2853	0.1879	0.1175
Pan I	1.0000	1.0000	1.0000	1.0000	---	0.5136	---	---

P-values of probability tests of Hardy-Weinberg equilibrium of Year 2 Atlantic cod. Sample site names refer to Table 1. No significant deviations from equilibrium conditions were present ($p \leq 0.0003$).

Locus	Site											
	MBW2	CLW	GBW2	NYS	CLS	SWS2	IPS2	BBS1	JLW	NYW	MBS2	BBS2
*Gmo*02	0.2116	0.8384	0.2424	0.1474	0.5728	0.2735	0.6952	0.2198	0.0395	0.0831	0.2098	0.7046
*Gmo*19	0.3153	0.7052	0.9896	0.2960	0.8249	0.5038	0.3907	0.3685	0.0804	0.9550	0.4395	0.6740
*Gmo*132	0.9400	0.1794	0.3555	0.3932	0.5161	0.2526	0.7960	0.3644	0.1723	0.3401	0.3453	0.8348
*Gmo*35	0.1347	0.8461	0.3362	0.8130	0.3061	0.5217	0.2577	0.7502	0.1483	0.7541	0.9247	0.5161
*Gmo*36	0.2833	0.5882	0.3746	0.3277	0.3086	0.1750	0.6490	0.6194	0.5420	0.1013	0.6820	0.2720
*Gmo*37	0.6341	0.7681	0.6022	0.3741	0.0136	0.3833	0.9846	0.7837	0.9431	0.9357	0.3102	0.0732
P*Gmo*32	0.0913	0.6236	0.7172	0.7048	0.0574	0.6066	0.8116	0.1407	0.5211	0.8392	0.0908	0.4976
P*Gmo*34	0.1382	0.1119	0.5601	0.7171	0.2116	0.9685	0.9438	0.3774	0.1421	0.0852	0.0501	0.4054
P*Gmo*38	0.9342	0.0164	0.1153	0.4213	0.7893	0.4725	0.4790	0.3968	0.6380	0.1256	0.6639	0.3508
P*Gmo*56	0.7652	0.3033	1.0000	0.1188	0.0305	0.0878	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
P*Gmo*58	1.0000	0.0807	1.0000	0.1424	0.1810	0.8926	0.4776	0.1316	0.1411	0.1958	0.8133	1.0000
Pan I	1.0000	1.0000	1.0000	1.0000	---	1.0000	0.6805	1.0000	1.0000	1.0000	0.3259	1.0000

P-values of probability tests of Hardy-Weinberg equilibrium of combined Year 1 and Year 2 spawning Atlantic cod. Sample site names refer to Table 1. No significant deviations from equilibrium conditions were present ($p \leq 0.0004$).

Locus	Site									
	MBW	GBW	MBS	IPS	SWS	NTW	CLW	CLS	BBS	JLW
*Gmo*02	0.2125	0.5396	0.2070	0.6613	0.5906	0.1165	0.8435	0.5705	0.3005	0.0399
*Gmo*19	0.2195	0.5265	0.1846	0.3848	0.2450	0.0485	0.7056	0.8218	0.4832	0.0730
*Gmo*132	0.6280	0.5513	0.2616	0.9451	0.2980	0.1319	0.1791	0.5134	0.5926	0.1786
*Gmo*35	0.5106	0.3924	0.8358	0.5661	0.3564	0.4247	0.8468	0.3061	0.7270	0.1492
*Gmo*36	0.1472	0.0812	0.6110	0.7293	0.1769	0.2208	0.5868	0.3094	0.5658	0.5420
*Gmo*37	0.8539	0.2624	0.8745	0.5767	0.0708	0.4564	0.7669	0.0135	0.9106	0.9424
P*Gmo*32	0.4275	0.8030	0.1332	0.6711	0.5187	0.2419	0.6233	0.0575	0.4358	0.5206
P*Gmo*34	0.2035	0.8684	0.2533	0.8643	0.8860	0.7023	0.1110	0.2120	0.2720	0.1424
P*Gmo*38	0.8389	0.1915	0.8077	0.3920	0.5910	0.4958	0.0157	0.7913	0.7189	0.6374
P*Gmo*56	1.0000	0.0151	0.6229	0.6370	0.0565	0.2736	0.3040	0.0303	1.0000	1.0000
P*Gmo*58	0.1874	0.6938	0.1656	0.8675	0.7644	0.1862	0.0797	0.1828	0.4017	0.1426
Pan I	1.0000	1.0000	0.3443	0.7001	1.0000	---	1.0000	---	1.0000	1.0000

P-values of probability tests of Hardy-Weinberg equilibrium of juvenile collections of Atlantic cod. Sample site names refer to Table 1. Bold indicates significance following standard Bonferroni correction ($p \leq 0.0008$).

Locus	Site				
	MBWJ1	CCWJ	CBSJ	MBSJ	MBWJ2
*Gmo*02	0.4380	0.5785	0.2968	0.7426	0.1371
*Gmo*19	0.2429	0.5728	0.4066	0.2872	0.8222
*Gmo*132	0.8669	0.5977	0.5900	0.7705	0.8191
*Gmo*35	0.8889	0.4116	0.6025	0.0074	0.0588
*Gmo*36	0.9155	0.8123	0.1348	0.7705	0.8008
*Gmo*37	0.2628	0.1403	0.8207	0.1301	**0.0006**
P*Gmo*32	1.0000	0.2694	0.7116	0.8110	0.3864
P*Gmo*34	0.2099	0.3029	0.0565	0.7650	0.3926
P*Gmo*38	0.4207	0.1801	0.7558	0.5793	0.1114
P*Gmo*56	0.0711	0.3646	1.0000	0.6312	0.3594
P*Gmo*58	1.0000	0.8448	0.3171	1.0000	1.0000
Pan I	1.0000	---	---	---	0.3228

P-values of heterozygote deficiency and heterozygote excess tests of Hardy-Weinberg equilibrium of juvenile collections of Atlantic cod. Sample site names refer to Table 1. Bold indicates significance following standard Bonferroni correction ($p \leq 0.0008$).

Heterozygote Deficiency

Locus	Site				
	MBWJ1	CCWJ	CBSJ	MBSJ	MBWJ2
*Gmo*02	0.9150	0.7468	0.4863	0.9477	0.1248
*Gmo*19	0.6295	0.3220	0.1300	0.0297	0.0737
*Gmo*132	0.9830	0.7887	0.0459	0.1087	0.7501
*Gmo*35	0.6453	0.3023	0.2082	0.3795	0.0022
*Gmo*36	0.6325	0.5230	0.0510	0.5672	0.3444
*Gmo*37	0.3985	0.6109	0.8960	0.0206	**<0.0001**
P*Gmo*32	1.0000	0.9770	0.4826	0.8112	1.0000
P*Gmo*34	0.9906	0.4197	0.0728	0.6328	0.1742
P*Gmo*38	0.7455	0.0890	0.7301	0.6894	0.3748
P*Gmo*56	0.0416	0.1812	1.0000	0.4687	0.2408
P*Gmo*58	1.0000	0.8420	0.2689	1.0000	1.0000
Pan I	1.0000	---	---	---	1.0000

Heterozygote Excess

Locus	Site				
	MBWJ1	CCWJ	CBSJ	MBSJ	MBWJ2
*Gmo*02	0.0928	0.2572	0.5132	0.0670	0.8758
*Gmo*19	0.4293	0.6964	0.8906	0.9714	0.9289
*Gmo*132	0.0283	0.2254	0.9559	0.9002	0.2548
*Gmo*35	0.3934	0.6981	0.7952	0.6246	0.9977
*Gmo*36	0.4015	0.5026	0.9584	0.4926	0.6855
*Gmo*37	0.6112	0.3895	0.1094	0.9794	1.0000
P*Gmo*32	0.3084	0.0703	0.7783	0.2418	0.0430
P*Gmo*34	0.0198	0.6184	0.9365	0.4236	0.8626
P*Gmo*38	0.2581	0.9115	0.2907	0.3295	0.6281
P*Gmo*56	0.9930	0.9398	0.4303	0.8438	0.9320
P*Gmo*58	0.6285	0.4437	0.7885	0.3123	0.6554
Pan I	0.9111	---	---	---	0.2230

APPENDIX C. TESTS OF TEMPORAL STABILITY

Tests of temporal stability of Atlantic cod spawning aggregations sampled in both Year 1 and Year 2 groupings. Sample site names refer to Table 1. No significant differentiation is present among population comparisons ($p \leq 0.0083$).

Population Comparison	F_{ST} (p-value)	allelic differentiation p-value
MBW1 & MBW2	0.0009 (0.0750)	0.1224
GBW1 & GBW2	-0.0006 (0.0636)	0.0318
MBS1 & MBS2	0.0081 (0.0242)	0.0085
IPS1 & IPS2	0.0003 (0.1515)	0.0650
SWS1 & SWS2	-0.0003 (0.0303)	0.0870
BBS1 & BBS2	0.0036 (0.4652)	0.5462