Final Report

Project Title: Ecological diversity of Atlantic cod in the Gulf of Maine and its role in resiliency of a fishery

Grantee: Gulf of Maine Research Institute

Principal Investigator:
Dr. Lisa Kerr
Gulf of Maine Research Institute
350 Commercial St., Portland, ME 04101
lkerr@gmri.org

Co-Investigators:
Dr. Graham Sherwood
Gulf of Maine Research Institute
350 Commercial St., Portland, ME 04101
gsherwood@gmri.org

Captain David Goethel
23 Ridgeview Terrace
Hampton, NH 03842
egoethel@comcast.net

Dr. Adrienne Kovach
University of New Hampshire
Dept. of Natural Resources & the Environment
46 College Road, Durham, NH 03824
adrienne.kovach@unh.edu

Collaborators:
Dr. Douglas Zemeckis
Rutgers, The State University of New Jersey
Dept. of Agriculture and Natural Resources
1623 Whitesville Road, Toms River, NJ 08755, USA
zemeckis@njaes.rutgers.edu

Dr. Steven X. Cadrin
University of Massachusetts
School for Marine Science & Technology
200 Mill Road, Suite 325
Fairhaven, MA 02719
scadrin@umassd.edu

Zachary Whitener
Gulf of Maine Research Institute
350 Commercial St., Portland, ME 04101
zwhitener@gmri.org

A. Executive Summary

Biocomplexity of Atlantic cod (*Gadus morhua*) is known to be a key factor in the resilience and persistence of this fishery resource. Currently, this feature of population dynamics is compromised in US waters due to the extirpation of unique spawning components, which may limit the capacity of cod metapopulations to rebuild. We characterized the ecological diversity of the two major spawning complexes (winter and spring) of Atlantic cod in the Gulf of Maine. A combination of genetics, genomics, otolith chemistry, otolith structure, morphometric, and color analysis was applied to winter and spring spawning fish from the two main spawning locations in the Gulf of Maine (Ipswich and Massachusetts Bays) to characterize their genetic, spatial, and life history diversity. We also determined the relative contribution of these two spawning complexes to samples from the current commercial fishery and compared it with the composition of the fishery in two time periods in the past (1979-1982 and 1989-1992). Genetic analysis indicates significant neutral and adaptive genetic differentiation between winter and spring spawners sampled over multiple years, suggesting limited connectivity as well as ecological differentiation in these two spawning populations. Otolith chemistry analysis indicates significant differences in elemental ratios of winter and spring spawners within each Bay, both early in life and across their lifetime, suggesting differences in environmental conditions experienced early in life and habitat use by these groups over their lifetime. Morphometric analysis indicates that winter and spring spawning cod exhibit significant differences in body shape with winter spawners having features often associated with a more resident life history (deeper bodied and shorter head) than spring spawners. The three methods provided largely congruent results, and taken together, our findings point toward biocomplexity of Atlantic cod on a fine scale, consistent with local adaptation and ecological divergence. Genetic and otolith analyses indicated that the composition of the fishery has changed over time: a greater proportion of winter spawners comprise the fishery today compared with the past. Furthermore, genetic data suggest that the historical fishery may have been characterized by a greater diversity than it is today. Improved understanding of the ecological diversity of Gulf of Maine cod and how it has changed over time can inform potential rebuilding mechanisms and improvement of stock assessment and management practices.

B. Background

Atlantic cod is an iconic species in New England and historically was one of the most economically and socially important commercial fish in the North Atlantic. Recent extremely low estimates of cod biomass in the Gulf of Maine suggest that cod populations are at a small fraction (6-8 %) of their target biomass and the harvest of Gulf of Maine cod has been dramatically reduced to essentially a bycatch only fishery. One of the major gaps in our knowledge of cod in the Gulf of Maine is an understanding of the importance of individual populations and the unique role they play in the resilience of this fishery resource. Currently, there is evidence for the existence of two distinct spawning groups in the Gulf of Maine (winter and spring spawners in Massachusetts and Ipswich Bay) with historical evidence suggesting more unique groups were once prevalent. An understanding of ecological diversity of cod in the Gulf of Maine is necessary for reassessing current management practices and understanding the potential for recovery of depleted populations of cod in the Gulf of Maine. The complex spatial structure of Atlantic cod may be key to the resilience and persistence of the resource, as well as the fishery, in the face of changing environmental conditions. Currently, these features may be compromised due to contemporary and historical patterns in exploitation, as well as environmental change in the region.

The goal of our research was to characterize the ecological diversity (e.g., genetic variation, habitat use, and spatial behavior) of two major spawning populations of Atlantic cod in the Gulf of Maine and evaluate how the Gulf of Maine cod fishery interacts with these groups today and in recent history. We also aimed to characterize the importance of spawning populations/life history types of cod in current landings and in two periods in recent history when the estimated landings were at their peak (1979–1982 and 1988–1992). We explicitly tested three hypotheses in this study and have structured our reporting of findings from this study (Section D) around the hypotheses tested and the specific stock identification methods applied to test each (noted below each hypothesis). In addition, more complete descriptions of sampling and the results of each stock identification method can be found in the Appendices.

Hypothesis 1: The genetic differences previously identified between winter and spring spawners in the Gulf of Maine are temporally stable. We expect that genetic differences identified between winter and spring spawning cod collected during this project will be the same as those found in previous sampling (2003-2008).

1. Genetics

Hypothesis 2: Winter and spring spawning fish represent distinct ecological units with spring spawning fish adopting a resident life history strategy and residing in inshore regions and winter spawning fish adopting a migratory life history strategy and exhibiting broader dispersal/habitat use. Adaptive (functional) genetic differences will be consistent with these life history differences.

1. Genetics and Genomics
2. Otolith chemistry
3. Body color and morphometrics

Hypothesis 3: The relative contribution of genetic/ecological units of cod to the fishery has changed over time. We expect that current landings in Gulf of Maine are primarily composed of resident fish (spring spawners) and historical landings are composed of a broader mixture of sources.

1. Genetics and Genomics
2. Otolith chemistry

C. Summary of Atlantic Cod Sample Collection

Three types of Atlantic cod samples were collected to address our research questions: 1) spawning fish collections (2012-2016), 2) modern fishery collections (2015-2016), and 3) historical fishery collections over two time periods (1979-1982 and 1988-1992). A total of 1,489 samples were collected (**Appendix A, Table A**) in support of this research.

Spawning fish collections

Atlantic cod were collected in two areas (Ipswich Bay and Massachusetts Bay) and at two spawning times (spring and winter) to represent distinct cod spawning complexes (Figure 1). A total of 862 fish were sampled, with 761 of those fish being classified as spring or winter spawners based on the developmental stage of their gonads at time of capture (i.e., ripe, ripe and running, or spent, Table 1). All of the Ipswich Bay samples were collected during 2014 and 2015 at known spawning locations on five sampling trips conducted in collaboration with our fishing industry partner, Captain David Goethel, from his vessel, the *F/V Ellen Diane* (n = 232). Collections were made using a commercial groundfish bottom otter trawl deployed for approximately 60-minute tows at three knots, although the specific tow durations were determined by the captain based on the bottom characteristics and concentrations of fish determined using a commercial fishfinder. Spawning cod samples from Massachusetts Bay were collected aboard Massachusetts Division of Marine Fisheries' research vessels (i.e., *R/V Alosa* or *R/V Michael Craven*) or contracted commercial fishing vessels from 2009-2016 using bottom otter trawl, rod and reel, or longline gear (n = 630). Length to the nearest centimeter, sex, and maturity stage (Morse 1977 unpublished, described in Burnett et al. 1989) were recorded for each individual fish. Weight to the nearest gram was also recorded for Ipswich Bay fish. Each fish had at least one of the following samples collected for stock identification analysis: photographs for morphometric analysis, fin clips for genetic and genomic analysis, and/or otoliths for otolith structure and chemical analysis. All three stock id sampling methods were applied to the same individual fish when feasible. A more complete description of collections can be found in **Appendix A** (Tables B and C).

Table 1 *Summary of Atlantic cod sampled in Ipswich Bay and Massachusetts Bay at known spawning locations during known spawning times. Although fish were targeted at known spawning aggregation sites, only fish with gonads in spawning condition (i.e., ripe, ripe and running, and spent) were assigned to spawning complexes.*

Spawning Season	Spring		Winter		Total
Year	**Ipswich Bay**	**MA Bay**	**Ipswich Bay**	**MA Bay**	
2009		1			**1**
2010		39			**39**
2011		51		55	**106**
2012		67		2	**69**
2013		130		66	**196**
2014		46	73	103	**222**
2015	89		39		**128**
Total	**89**	**334**	**112**	**226**	**761**

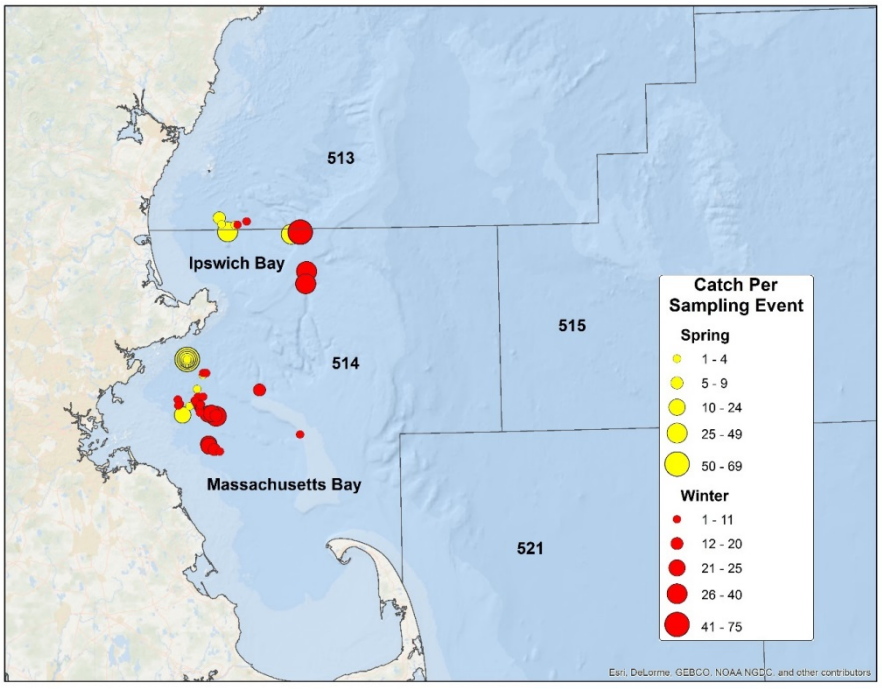


Figure 1. Map illustrating sampling locations and timing of spawning cod samples by season for this project. Each bubble represents numbers of fish sampled per discrete sampling event (date and location of capture). Statistical areas are denoted as well.

Modern fishery collections

A total of 324 fish were collected as representative samples of our modern fishery. We focused sampling in two statistical areas (513 and 514; Tables 2 and 3, Figure 2), from which the majority of commercial fishing landings have come from in the past decade, including >75% landings in 2011 (NEFSC 2013). The originally proposed sampling plan for collecting cod from the modern commercial fishery involved sampling fish caught as part of normal harvesting by the *F/V Ellen Diane*. However, the Gulf of Maine Cod and Haddock 2014 Interim and Emergency Actions enacted by the National Marine Fisheries Service halted the directed fishery for cod in the months before sampling was to be conducted (Department of Commerce 2014 and 2015). As such, we acquired a Scientific Research Letter of Acknowledgement from the NMFS to allow Capt. Goethel and the *F/V Ellen Diane* to conduct sampling activities that mimicked normal commercial fishing operations in 2015 and 2016. Some fish designated as representing the modern commercial fishery were collected during spawning aggregation and were included in this project due to the fact that commercial fishermen often targeted spawning aggregations in both Ipswich Bay and Massachusetts Bay when closures are not currently in effect, or were not in the past. Fish from an area known as The Cove in Ipswich Bay (n = 50) are one example, as this area was fished until the 2014 Interim and Emergency Actions. Some fish designated as representative of modern commercial fishery landings were collected by the Massachusetts Division of Marine Fisheries and UMass Dartmouth School for Marine Science and Technology (SMAST) to monitor spawning aggregations during the winter in Massachusetts Bay (n = 31). These fish were collected by the Massachusetts Division of Marine Fisheries' vessel *R/V Alosa.* More descriptions of commercial samples can be found in **Appendix A** (Tables D, E, F, and G).

Table 2. Number of fish samples collected to represent the modern and historical commercial fishery with at least one stock discrimination technique applied for each statistical area.

Type	Year	Statistical Area			Total
		513	514	515	
Modern Commercial	2015-2016	53	271		324
Historical Commercial	1979-1982	77	58		135
	1988-1992	56	56	56	168

Table 3. Summary of Atlantic cod sampled in Ipswich Bay and Massachusetts Bay to represent the modern fishery by season.

Years	Season	Ipswich Bay	Mass Bay	Total
2015-2016	Spring	8	60	68
	Summer	75	16	91
	Fall	47	0	47
	Winter	6	112	118
Total		136	188	324

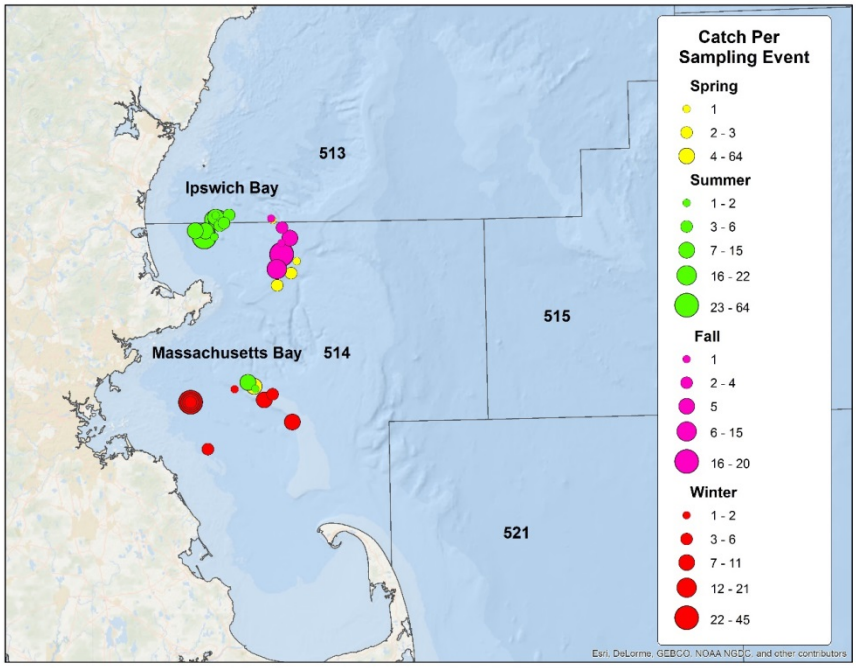


Figure 2. Map illustrating the locations of capture of modern fishery samples by season.

Historical fishery otolith collections

A total of 303 otoliths were collected from the NMFS sample archives at Woods Hole. These otoliths were port-sampled from commercial vessels during two time periods, 1979-1982 and 1988-1992 (Table 2). Samples were selected to represent and characterize commercial landings in the past, with a focus on statistical areas that comprised at least 75% of the commercial landings during the 1979-1981 (statistical areas 513 and 514) and the 1989-1991 (statistical areas

513, 514, and 515) time periods (NEFSC 2013). Length data were available for all samples and otoliths were aged by an experienced reader.

D. Findings

Hypothesis 1*:* The genetic differences previously identified between winter and spring spawners in the Gulf of Maine are temporally stable. We expect that genetic differences identified between winter and spring spawning cod collected during this project will be the same as those found in previous sampling (2003-2008).

Genetics

To test our hypothesis of temporally stable genetic differentiation between winter and spring spawning cod in Ipswich and Massachusetts Bays, we used microsatellite DNA markers to analyze recently collected cod samples for comparison with samples collected in a prior study of Kovach et al. (2010). First, a 12-locus microsatellite dataset was generated from samples of 65 spring-spawning fish (n = 32 from Ipswich Bay and n = 33 from Massachusetts Bay) and 95 winter-spawning fish (n = 48 from Ipswich Bay and n = 47 from Massachusetts Bay) collected during this study. Measures of genetic differentiation (F_{ST}) were small, but statistically significant for the winter and spring comparisons and similar to findings from our prior work in this system (Kovach et al. 2010). F_{ST} ranged 0.0077 – 0.011 for the four pairwise comparisons among winter-spring groups. Overall, the mean F_{ST} for winter vs. spring spawners, combined across the two bays, respectively, was 0.0091. Genetic differentiation between the two bays within the same spawning season were much smaller, with no difference between spring spawners in Ipswich and Massachusetts Bay, and a small, but significant difference between the winter spawners from Ipswich and Massachusetts Bay (F_{ST} = 0.0015). These findings are depicted by the results of a Principle Coordinates Analysis (PCA) of pair-wise population F_{ST}s in Figure 3 and Discriminant Analysis of Principle Components (DAPC) of the individual microsatellite genotypes for the 4 populations in Figure 4 and the winter and spring populations combined in Figure 5. Assignment indices from the DAPC method suggested high assignment probabilities for individual fish to the winter population (82%) and lower assignment scores to the spring population (52%). As expected, assignments to individual bays were lower, ranging 48-65% (see **Appendix B**).

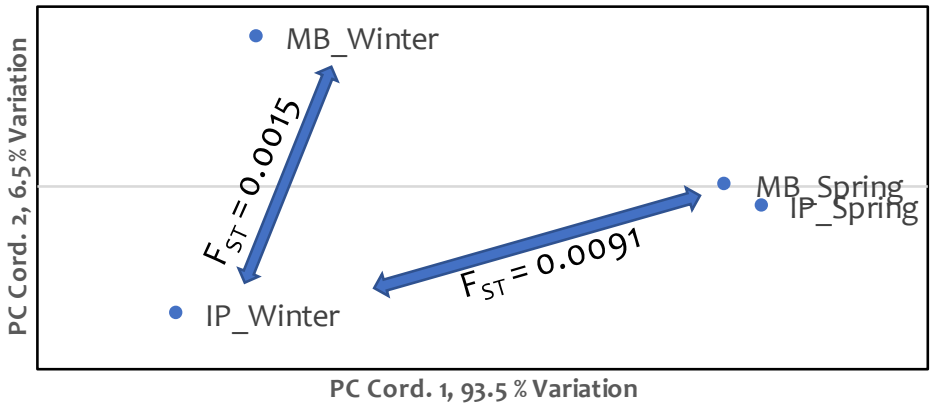


Figure 3. Principle Coordinates Analysis of genetic differentiation as measured by pair-wise population Fst values for spring and winter spawning populations of cod in Ipswich and Massachusetts Bays in 2014-2015.

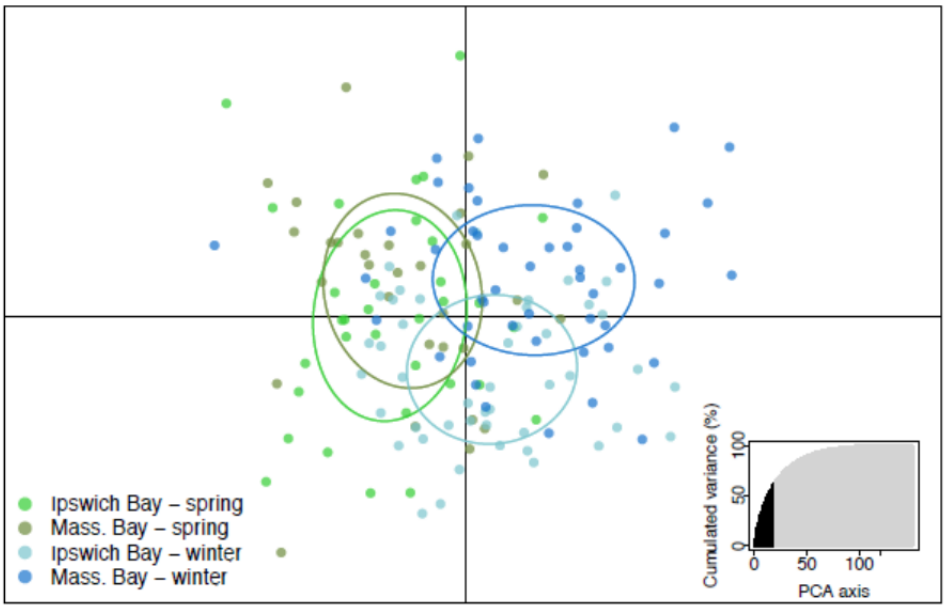


Figure 4. Discriminant Analysis of Principle Components of microsatellite genotypes of 170 winter and spring spawning cod populations in Ipswich and Massachusetts Bays in 2014-2015.

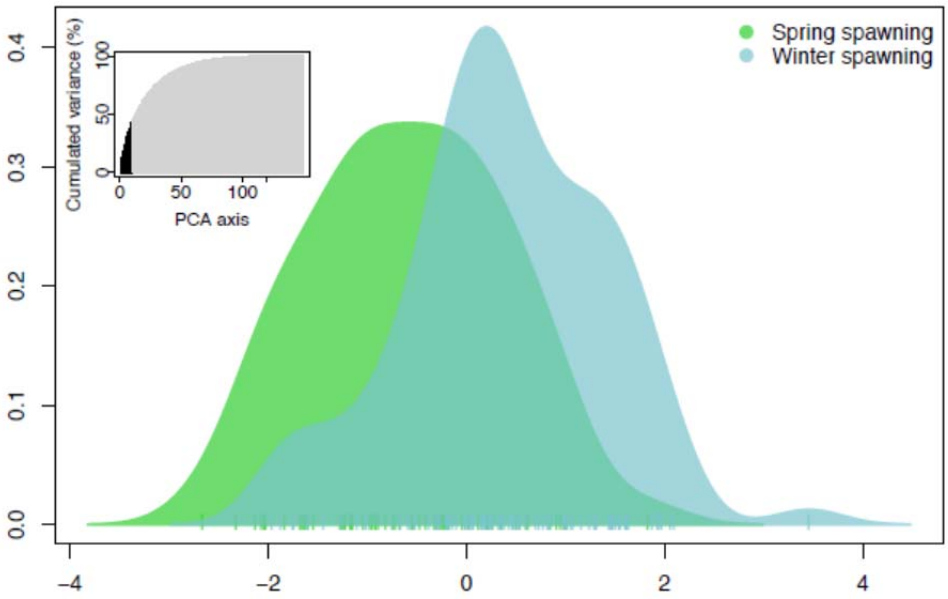


Figure 5. Discriminant Analysis of Principle Components of microsatellite genotypes of 170 cod grouped as winter and spring-spawning populations in 2014-2015.

*Table 4. Pairwise-population F_{ST} values of genetic differentiation for spring and winter spawning cod from Ipswich and Massachusetts Bays sampled from two time periods: 2006-2008 (labeled 2010; samples from Kovach et al. 2010) and 2014-2015 (labeled 2015; samples collected during this study). IP = Ipswich Bay and MB = Massachusetts Bay. Values highlighted in bold are significant after Bonferroni correction; P <0.001786. Values with * are significant at P <0.5 and ** are significant at P<0.01.*

IP_Spring _2010	IP_Spring _2015	MB_Spring _2010	MB_Spring _2015	IP_Winter _2010	IP_Winter _2015	MB_Winter _2010	MB_Winter _2015	
0								IP_Spring-2010
-0.0001	0							IP_Spring_2015
0.0009	0.0016	0						MB_Spring_2010
-0.0017	-0.0026	-0.0031	0					MB_Spring_2015
0.0123	**0.009	**0.0168**	**0.0081**	0				IP_Winter_2010
0.0192	*0.0094	**0.0217**	**0.0105**	0.0019	0			IP_Winter_2015
0.0125	**0.0065	**0.0157**	**0.0061**	-0.0009	0.0001	0		MB_Winter_2010
0.0147	*0.008	**0.0182**	**0.0077	0.0015	0.0015	*0.0028	0	MB_Winter_2015

To test for temporal stability in the population structure over time, we genotyped an additional 274 archived samples from 2006-2008 from the two bays and spawning seasons (n = 83 Ipswich Bay spring, n = 84 Massachusetts Bay spring, n = 31 Ipswich Bay winter, n = 76 Massachusetts Bay winter; samples from Kovach et al. 2010). Measures of genetic differentiation by F_{ST} were significant for all pair-wises comparisons of winter vs. spring spawning groups (Table 4; Figure 6). Genetic differentiation of the same populations between years was not significant for any

comparisons except the two winter Ipswich Bay collections (F_{ST} = 0.0028, P = 0.02; not significant after Bonferroni correction). Results of an Analysis of Molecular Variance revealed that a small but significant amount of variation was explained by differences among the 4 populations (1%; Frt = 0.009; P=0.001) and no measurable variation could be explained by sampling years (0%; Frs = 0; P=0.273).

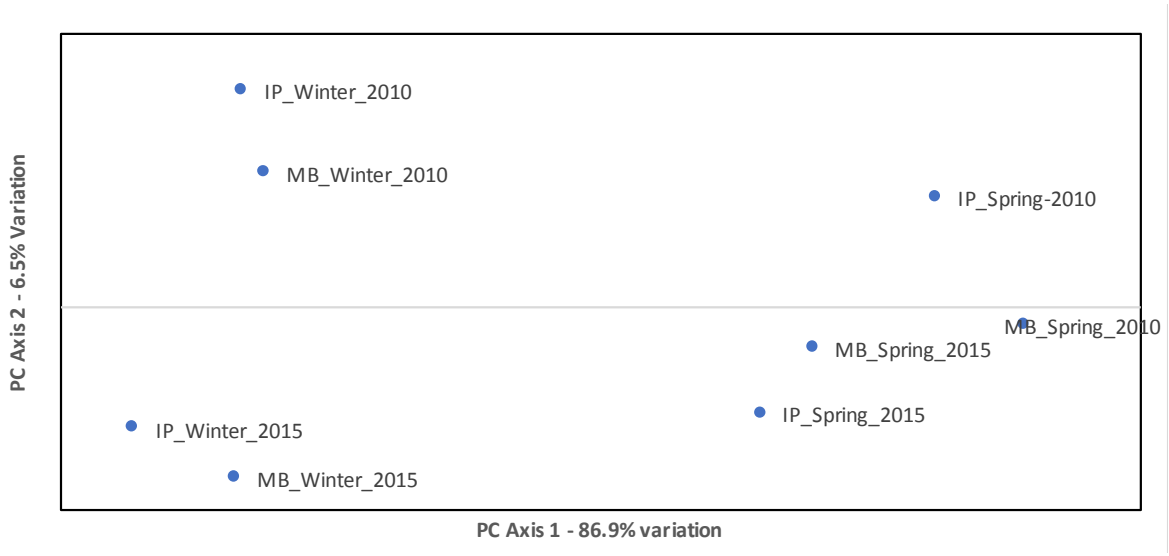


Figure 6. PCA of pair-wise population FST values from Table 4.

Taken together, these findings give strong support that the genetic divergence between winter and spring spawning cod are stable over time (2006-2015). Note, our prior work showed stability of this structure between 2003-2008 as well (Kovach et al. 2010), suggesting a consistent, long-term stability in populations structure. Our findings also suggest that finer scale differences occur between the two Bays within season, particularly for the winter spawning groups. The temporal comparisons suggest some variability in these fine-scale patterns, or perhaps that larger sample sizes and higher resolution genetic markers are needed to track these fine-scale differences consistently over time.

Given the finding of temporal stability, we combined the genotypes for the 2006-2008 samples with the samples from this study (2014-2015) to generate a full microsatellite dataset of 434 individuals. With the increased sample sizes, the dataset had higher resolution for discriminating among populations, with an F_{ST} of 0.0135 for winter and spring spawners overall. Pair-wise population F_{ST}s were slightly larger for all between-season comparisons and lower for all within-season comparisons, relative to the smaller 2015 dataset described above (Table 5). DAPC of the individual genotypes are shown in Figures 7 and 8. Assignment scores were 78% and 74% to the winter and spring populations, respectively, and 42-63% to the specific Bays and seasons (see **Appendix B**). Note, these assignment scores reflect the power to assign individual fish to a

population of origin and are not identical to (typically lower than) the power of a mixed stock analysis (see Hypothesis 3 for the latter).

Table 5. Pairwise population F_{ST} values for the four spawning cod populations in the Gulf of Maine, for samples collected in 2006-2008 combined with samples collected in 2014-2015.

	Ipswich Bay Spring	Massachusetts Bay Spring	Ipswich Bay Winter	Massachusetts Bay Winter
Ipswich Bay Spring	-			
Massachusetts Bay Spring	0.0009	-		
Ipswich Bay Winter	0.0140	0.0171	-	
Massachusetts Bay Winter	0.0108	0.0138	-0.0007	-

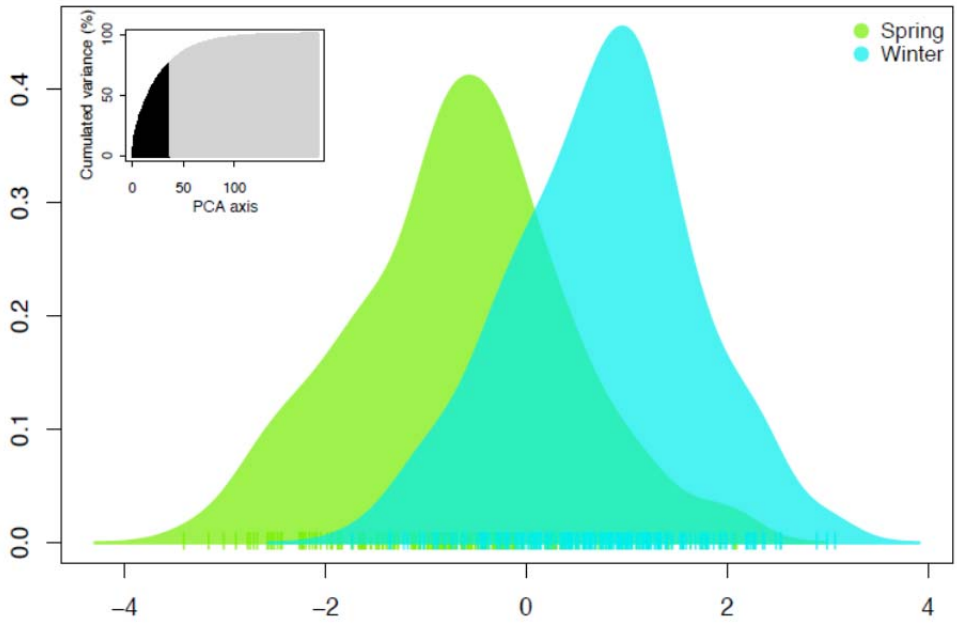


Figure 7. DAPC of microsatellite genotypes of 434 Gulf of Maine cod from a combined dataset of 2006-2008 and 2014-2015 individuals grouped as winter and spring spawning populations.

***Hypothesis 2**:* Winter and spring spawning fish represent distinct ecological units with spring spawning fish adopting a resident life history strategy and residing in inshore regions and winter spawning fish adopting a migratory life history strategy and exhibiting broader dispersal/habitat use. Adaptive (functional) genetic differences will be consistent with these life history differences.

Genetics and Genomics

To investigate adaptive genetic differentiation between winter and spring spawning Gulf of Maine cod, we used a RAD Sequencing approach to identify genome-wide SNPs. This approach revealed 1408 SNPs across the four spawning populations. We used DAPC to characterize the genetic structure identified by these SNPs. These analyses corroborated the genetic structure we identified with the microsatellite loci, with a higher resolution and higher discriminatory power (Figures 8 & 9). The higher resolution SNP data enabled discriminating not only between the seasonally distinct spawning groups, but also spatially between Ipswich and Massachusetts Bays in the winter and to a lesser degree in spring. Assignment scores for the two groups were 94.9% for the spring spawners and 95.8% for the winter spawners. Assignment probabilities for the four populations (separate bays and seasons) were lower, ranging 66.7% to 87.5% (higher for the winter spawners in both bays). F_{ST}s were similar and slightly larger than for the microsatellite dataset, with an overall F_{ST} of 0.016 for comparison of the winter and spring spawners (0.016-0.02), and 0.002 and 0.009 for comparisons of Ipswich and Massachusetts Bays in the spring and winter, respectively.

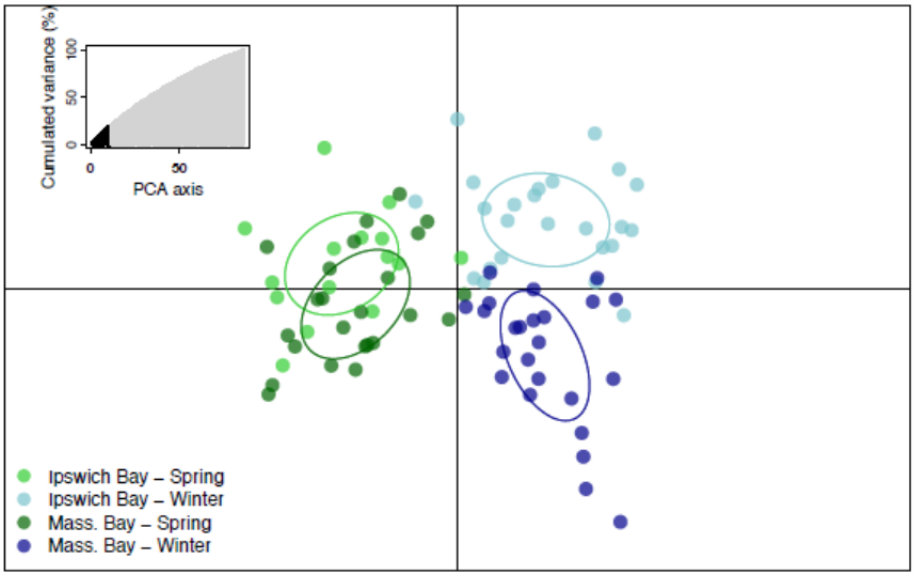


Figure 8. Discriminant Analysis of Principle Components for 1408 SNP loci of winter and spring spawning cod populations in Ipswich and Massachusetts Bays in 2014-2015.

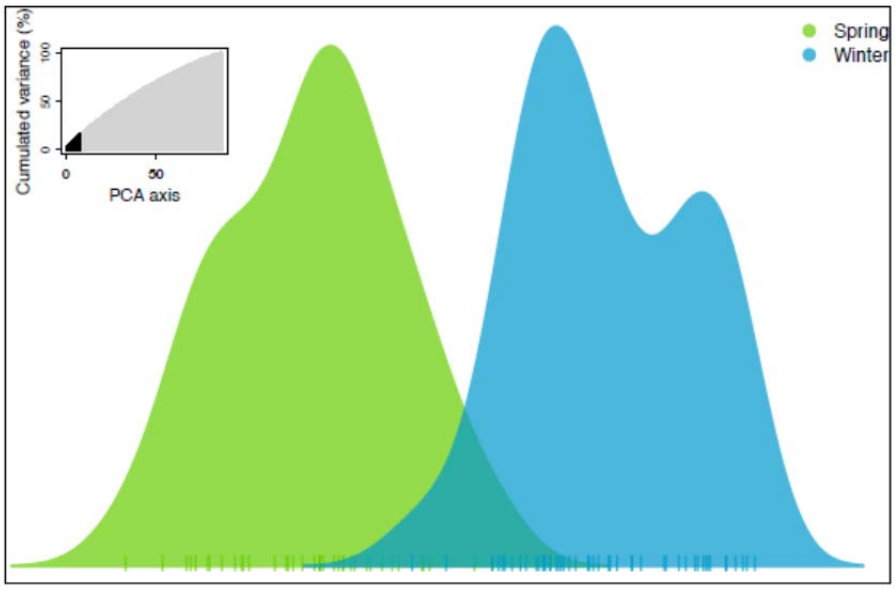


Figure 9. DAPC of 1408 SNP loci of winter and spring spawning Gulf of Maine cod populations.

To explore the influence of adaptive genetic variation in driving this genetic structure, we determined the distribution of F_{ST} s for all SNPs (see **Appendix B**) and defined outlier loci as those SNPs with a higher F_{ST} than the upper 99 percentile of this distribution (F_{ST} cutoff = 0.195). This resulted in the identification of 15 outlier loci, which we considered candidates for association with adaptive gene loci. We then ran DAPC on the 1393 putatively neutral loci that we retained with the exclusion of these outliers. The population structure remained evident with the putatively neutral dataset and, notably, differentiation of winter spawners in Massachusetts and Ipswich Bays was supported by the neutral loci alone (Figure 10). F_{ST}s between the winter and spring spawners overall was 0.008 for this dataset. Interestingly, with the neutral SNPs, the divergence between winter spawners in Ipswich and Massachusetts Bays was the same magnitude (F_{ST} = 0.009) as the divergence between some of the comparisons between winter and spring spawners. These findings suggest that gene flow is restricted on a fine-scale between these populations and adaptive differences driven by the divergence of the winter and spring spawning populations. Bayesian clustering analyses in STRUCTURE corroborated these findings, by showing strongest support for two distinct populations by season, and additional support for separation of the Ipswich and Massachusetts winter populations (see **Appendix B**).

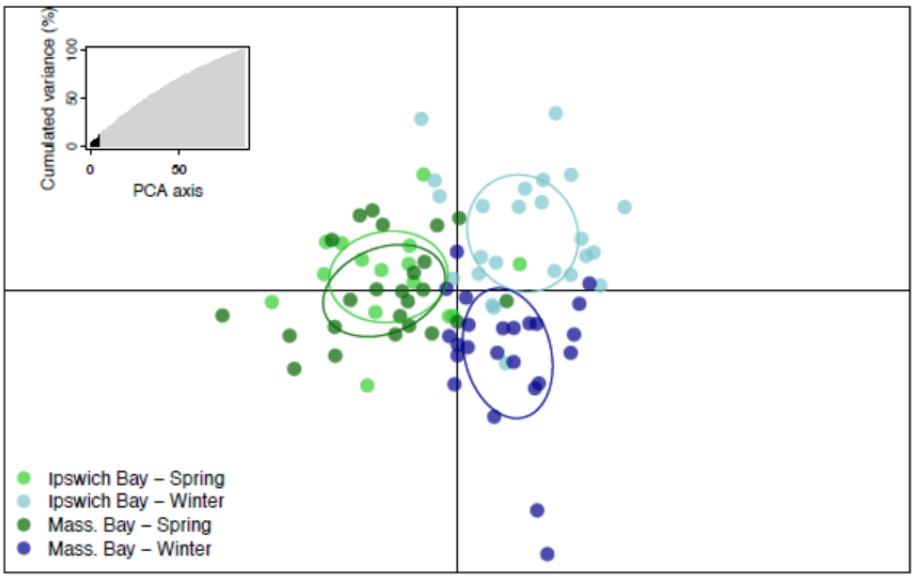


Figure 10. Discriminant Analysis of Principle Components for 1393 putatively neutral SNPs of winter and spring spawning cod populations in Ipswich and Massachusetts Bays in 2014-2015.

Lastly, to evaluate our hypothesis of ecotype differences between winter and spring spawning fish, we focused on the gene annotations for the genome sequences associated with the 15 outlier SNPs that we identified. Ten of the 15 loci were found to occur on linkage group (LG) 7 of the cod reference genome. An additional outlier was located on LG 12. These 2 linkage groups have been associated with ecotype differences of cod in the Northeast Atlantic and specifically associated with temperature, salinity and depth differences. The remaining 4 outliers were located on LG 2 and 18. The annotations for the identified genes include immune response, DNA repair, metabolism, cartilage and bone development, among other cellular and developmental processes (See **Appendix B2** for Table of all gene annotations).

The importance of adaptive variation in driving the genetic differentiation is corroborated by the microsatellite dataset, which included several markers that were known to be gene-associated (non-neutral markers). Four of the 12 markers used were found to be largely driving the genetic patterns and all four of these markers are known to be gene-associated (Appendix B). Two of them, microsatellite *Gmo132* and the *PanI* SNP have been previously associated with genetic differences correlated with salinity, temperature and water depth. The other two, *GmoC94* and *GmoC123* were derived from an EST database. *C123* is linked with a gene that functions in cell division and *C94* is correlated with a gene of unknown function (Delghandi et al. 2008).

In sum, the genome-wide SNPs corroborated the genetic structure obtained by the microsatellite dataset and provided higher resolution for discriminating among the populations, revealing more clearly the finer scale structure between the bays within the same season (especially for winter spawning populations). For both SNP and microsatellite datasets, genetic differentiation is driven

largely by markers putatively under selection, indicating that adaptive differences are important in separating the winter and spring-spawning cod. Identification of outlier loci associated with regions of the cod genome known to be associated with ecotype differences, including migratory/resident and temperature-driven adaptations, provide support for the hypothesis that winter and spring spawning cod have important ecological and life history differences.

Otolith Structure and Chemistry

To examine whether winter and spring spawning fish represent distinct ecological units with potentially different migratory strategies, we analyzed the otolith chemistry of spawning fish collected in spring and winter in Ipswich and Massachusetts Bays. There is a considerable body of work that has established the utility of otolith chemistry as a useful natural marker of fish stock structure (including alternative life history types) and tracer of fine-scale habitat use of fish (Kerr and Campana 2014).

Adult cod in spawning condition were collected in spring and winter months from Ipswich Bay (2014-2015) and Massachusetts Bay (2012-2016). Otoliths were photographed, aged and annual growth increments were measured using ImagePro image anlysis software. Otoliths were prepared and analyzed for trace element analysis using laser ablation ICPMS. Otoliths of spawning fish were ablated along the longest growth axis in a transect from the edge of otolith through the core to the opposite edge of age one otolith growth. Otoliths were analyzed for a suite of isotopes, including ^{25}Mg, ^{48}Ca, ^{55}Mn, ^{86}Sr, ^{87}Sr, ^{88}Sr, ^{137}Ba, ^{138}Ba, ^{114}Cd, ^{68}Zn, and ^{63}Cu. In total, 132 spawning cod otoliths were analyzed with the LA-ICPMS system. Data were standardized and associated with the respective year of growth of the fish.

Spawning Fish: Otolith Structure Analysis

The relative growth of otolith annuli are known to be related to growth of the fish and changes in fish and otolith growth are expected to occur ontogenetically, but will also vary spatially and temporally in response to the different ocean conditions. Large differences in the width of the first annuli of winter and spring spawners have been recognized in winter and spring spawners in Massachusetts Bay (M. Dean pers comm.). In this study, significant differences in otolith growth at age were identified between winter and spring spawning cod within each location ($p=0.03$, Figure 11) with pairwise comparisons revealing differences in growth across all ages, with the exception of age three. Age one and two growth was higher for winter spawners compared to spring spawners, but growth of winter spawners tended to be lower at older ages. The largest differences in growth were evident in the width of the age one annulus between winter and spring spawning cod (Figures 11 & 12). Applying a discriminant function analysis classification approach with jackknife prediction to age one otolith growth information we were able to assign winter and spring spawners to their known spawning group with good classification accuracy (78%). Classification accuracy of fish to location (54%) and spawning time at location (44%) based on age one otolith growth was considerably lower. The large growth difference at age one

likely has to do with the spawning phenology of cod and how we define the first annulus of an otolith. We count one opaque (fast growth period ~ spring and summer) and translucent zone (slow growth period ~ fall and winter) as a year. Because of their time of spawning, winter spawned fish experience a longer growing period (e.g., December to January) compared to spring spawners (e.g., May to January) during what we count as the first annulus (age one). We also expect that winter and spring spawners experience very different early growth conditions due to starting life at different time periods in seasonally variable Gulf of Maine waters which also likely influences differences in early growth.

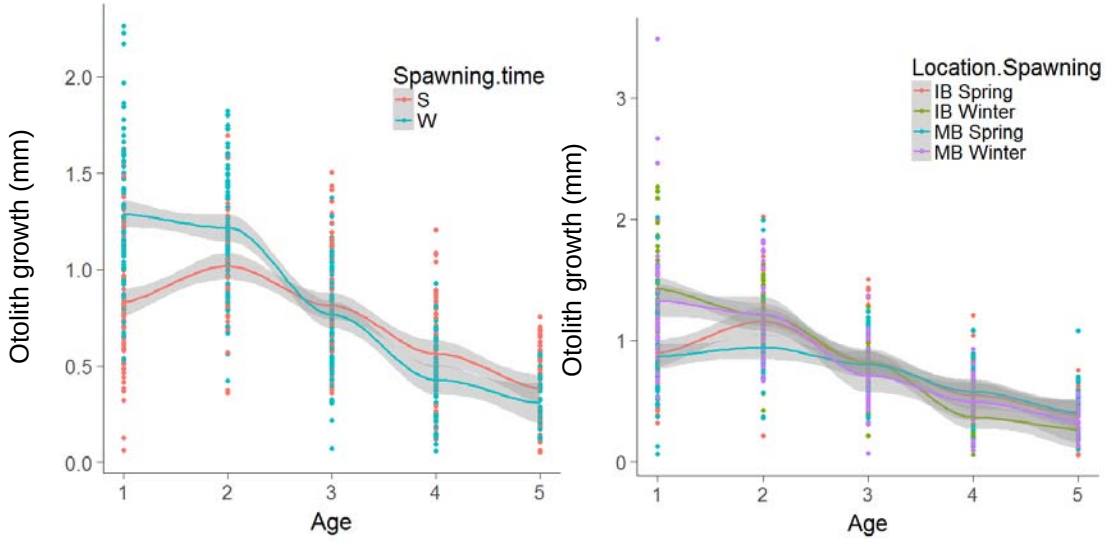


Figure 11. Annual growth increments widths of winter and spring spawning cod fit with Lowess smoothing function.

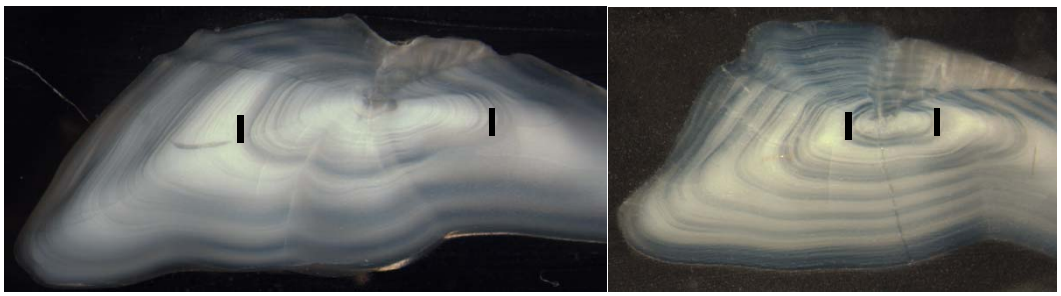


Figure 12. Left photo: Image of winter spawner depicting large age one annulus. Right photo: Image of spring spawner with comparatively small age one annulus. Black bars denote width of first annulus.

Our sample of spawning fish encompassed several recent year classes, with reasonable samples sizes in the years 2008 to 2012 for the purpose of comparing trends in growth. Significant differences in age one growth were found based on spawning group ($p<0.0001$) and the

interaction of spawning group and year (p=0.01). Winter spawners exhibited increasingly higher growth over this short time period compared to spring spawners (Figure 13).

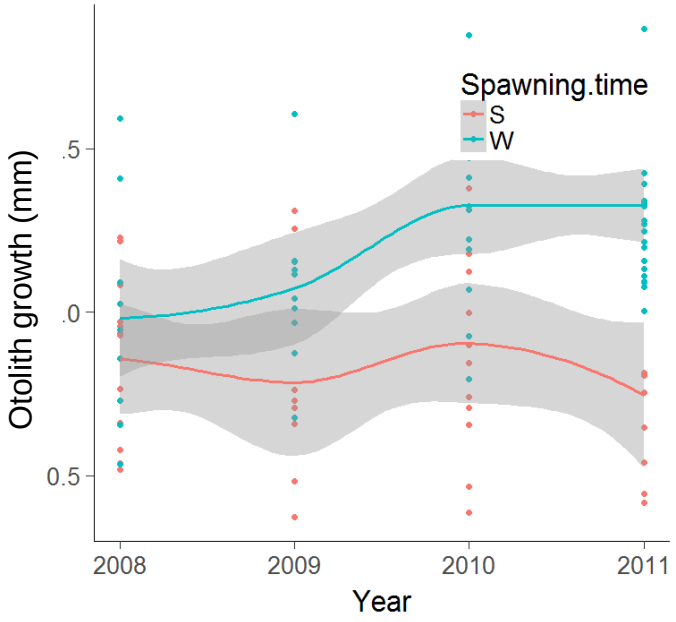


Figure 13. Trends in age one Atlantic cod otolith growth over time for winter and spring spawning fish.

Spawning Fish: Otolith Chemistry Analysis

Significant differences in the median values of the suite of elemental ratios measured in age one growth of cod otolith were evident for the main factors of spawning time and capture location, as well as the interaction of these factors. An examination of the individual response of isotopes indicated significant differences in Sr:Ca, Mg:Ca, Ba:Ca, and Mn:Ca based on spawning time (Figure 14, Appendix C: Tables 15 & 16). Spring spawners had lower Ba:Ca, Mg:Ca, and Mn:Ca values and higher Sr:Ca values compared to winter spawners (Figure 14,). In addition, significant differences were identified in Mn:Ca and Cu:Ca based on capture location and significant differences in Sr:Ca and Cu:Ca based on the interaction of these factors (Appendix C: Table 15 & 16). Linear discriminate function analysis with jackknife prediction was used to classify fish based on age one otolith chemistry to spawning time (winter and spring), location (Ipswich and Massachusetts Bay), and the interaction of these factors. Stepwise linear discriminate function analysis was used to select the parameters providing the most discrimination based on scale of classification. Classification success of Atlantic cod to spawning time was relatively high at 74%, compared to classification rates to capture location (48%), and to spawning times within each location (46%).

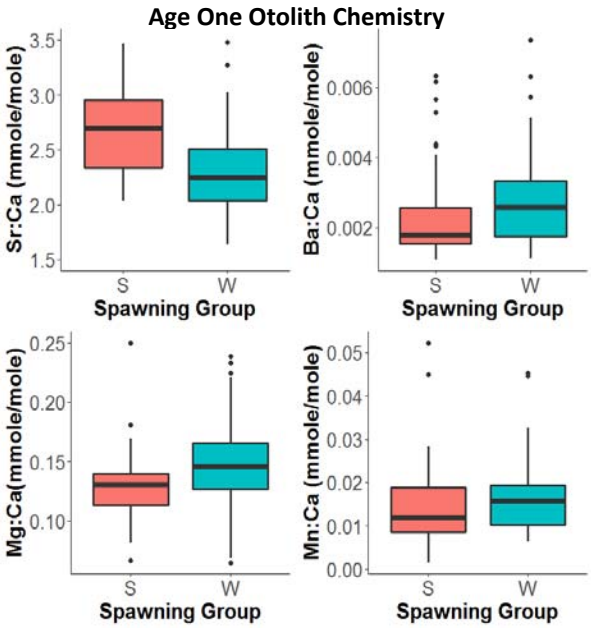


Figure 14. Elemental ratios of age one otolith growth in winter and spring spawning cod.

Significant differences in the combined chemistry of whole cod otolith growth were evident for the main factors of spawning time and location ($p<0.001$) and the interaction of these factors ($p < 0.001$) based on a two-way MANOVA. Examination of elemental ratios indicated significant differences Sr:Ca, Ba:Ca, Mg:Ca, Mn:Ca, and Cu:Ca based on spawning time, and significant differences in Sr:Ca, Ba:Ca, Mg:Ca, and Mn:Ca based on location, and significant differences in Cu:Ca based on the interaction of terms. Classification success of Atlantic cod to spawning time was higher (70%), than classification rate to capture location (65%), or classification rate to spawning times within each location (44%). Classification accuracy of spawners based on their whole otolith chemistry was slightly lower than the classification rate based on age one otolith chemistry alone.

All elemental ratios (Sr:Ca, Ba:Ca, Mg:Ca, Mn:Ca) demonstrated differences across age, indicative in ontogenetic changes in habitat use (Figure 15; see Appendix C). Examination of otolith elemental ratio differences at-age revealed differences in Sr:Ca values for fish age 3-5, in Mn:Ca values for age 3-4, and in Ba:Ca values for fish age 4 between spawning groups within each location (Figure 15; see Appendix C). The fact that differences extend beyond the first year of life supports the hypothesis that winter and spring spawning groups from each location experience different habitats over their lifetime aligns with ecotype differences.

Overall, we found the most robust differences in otolith growth and chemistry occurred between spawning groups, with more subtle differences based on location. Age one differences in growth and chemistry offered the best classification accuracy for the purpose of stock identification. Significant differences in otolith chemistry of winter and spring spawning groups from each

location at older ages indicate that these fish inhabit different habitats over their lifetime and is indicative of ecotype differences.

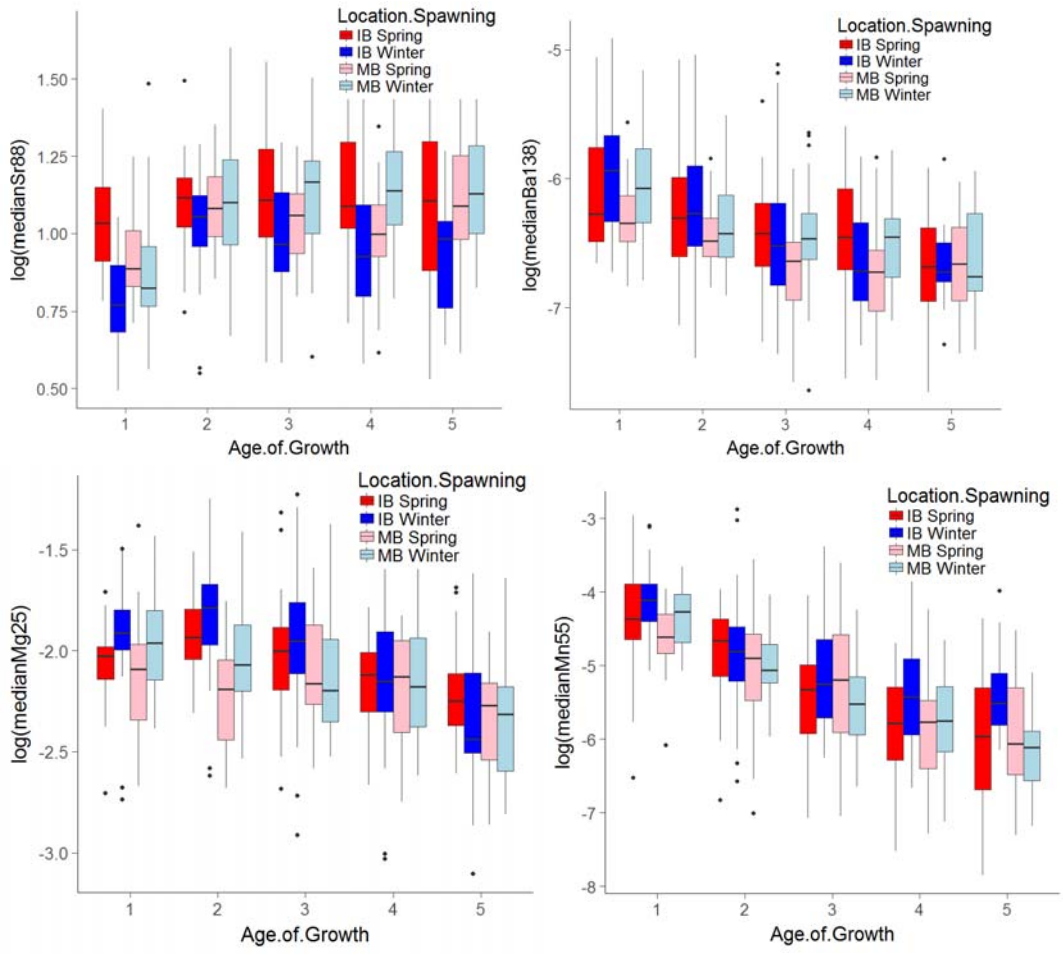


Figure 15. Elemental ratios of Atlantic cod otoliths across ages for fish from winter and spring spawning groups in Ipswich Bay and Massachusetts Bay.

Body Color and Morphometrics

We applied body color and shape analysis to differentiate among spring- and winter-spawning cod and test the hypothesis that these groups represent distinct ecotypes. Life-history strategies can be inferred based on body shape (e.g., Morinville and Rasmussen 2008, Sherwood and Grabowski 2010, Sherwood and Grabowski 2015) and body color of cod is a good indicator of behavior with darker/redder colors indicative of residency in shallower water (Sherwood and Grabowski 2010, Conroy et al. 2017).

Morphometric analysis was completed using digital photos of cod collected in Massachusetts and Ipswich Bays during winter and spring spawning periods. The analysis followed the box-truss network approach described in Cadrin and Friedland (1999) and Sherwood and Grabowski (2010). Specifically, 12 homologous landmarks were identified on the image of cod (Figure 16) and lines connecting these landmarks were drawn in a box-truss design and measured using an image analysis system (ImagePro). Seventeen linear dimensions were used to assess body shape differences/similarities among spawning groups. In addition, body color was analyzed in ImageMSAPro by examining color over a standardized region of the head. Red to green ratio (RGR) is the mean intensity of red pixels divided by the mean intensity of green pixels in this region. Values of RGR can range from below 1.0 (considered olive cod, mostly migrants) to above 2.0 (red cod, highly resident). A threshold of 1.2 has been used to differentiate red and olive cod in the past (Sherwood and Grabowski 2010, Conroy et al. 2017). We did not apply a threshold in this study. Rather we examined differences in mean RGR among spawning groups and explored the impact of including RGR in our morphometric discriminant function analysis (DFA) on spawning group reclassification rates.

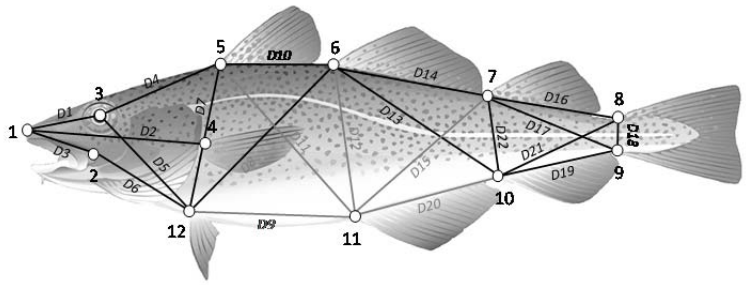


Figure 16. Landmarks and box-truss elements used in morphometric analysis with analyzed linear dimensions highlighted in bold.

There was a significant effect of spawning location on RGR values with higher values seen in Massachusetts Bay compared to Ipswich Bay spawning sites (ANOVA: $F_{1,255} = 163.2$, $p < 0.0001$, Figure 17). Spawning season was not significant. However, there was a significant interaction between spawning location and spawning season (ANOVA: $F_{1,255} = 7.9$, $p < 0.01$) such that the difference in RGR values among locations was highest for winter-spawning cod. It should be noted that despite the strong differences in RGR among locations, the mean difference is subtle compared to other studies that have examined color differences and related behaviors among “red” and “olive” cod (Sherwood and Grabowski 2010, Conroy et al. 2017). We interpret this result as differences in depth preferences among groups from each spawning location. Massachusetts Bay spawners are reported to spawn in shallower water (~ 50m, Armstrong et al. 2013) compared to Ipswich Bay spawners (~ 80-100m, Gurshin et al. 2013, Sherwood et al. 2017). These depth preferences may exist throughout the year which would be consistent with color differences (i.e., darker/redder in shallower water). Although not possible to assess with our existing data, differences in diet may also drive color differences among Massachusetts Bay and Ipswich Bay cod (e.g., Gosse and Wroblewski 2004).

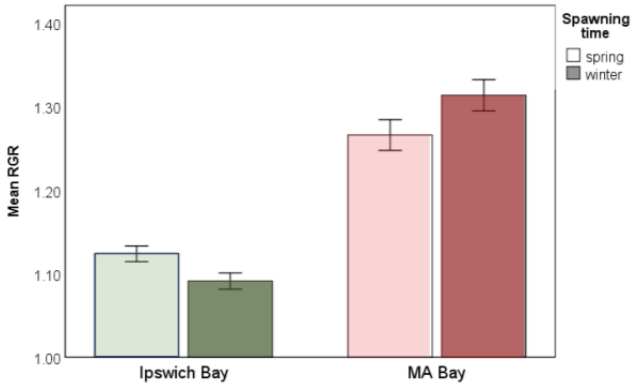


Figure 17. Mean red to green ratio (RGR ± 1 SE) for spawning condition cod from 4 spawning groups. Capture location was significant (ANOVA: $F_{1,255} = 163.1$, $p < 0.0001$) but not spawning time. However, the interaction was (ANOVA: $F_{1,255} = 7.9$, $p < 0.01$).

Discriminant function analysis revealed that the largest discrimination between groups, driven by variation along DF1, existed between Massachusetts and Ipswich Bay cod rather than between spring- and winter-spawning cod, although in both bays spring- and winter-spawning cod were distinguishable along DF2. Overall, 82.3% of cod were correctly reclassified back to their original groupings suggesting that body shape alone is a good means of discriminating between spawning groups. Massachusetts Bay, in particular, had very high reclassification rates (90%). Overall, reclassification rates were even higher (84.3%) when RGR (color) was added as a discriminating variable (Figure 18).

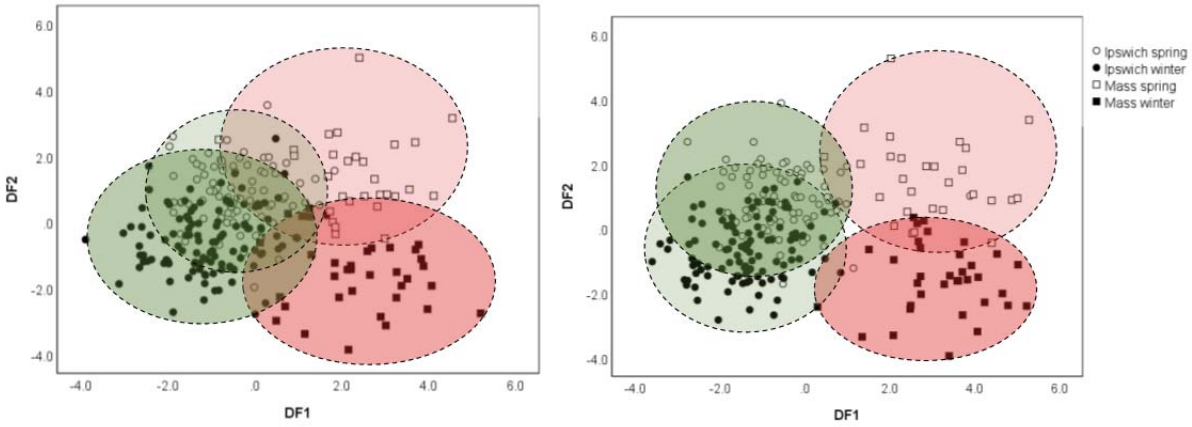


*Figure 18. Results of discriminant function analysis to explore groupings based on 17 body morphometric variables without body color information (left panel) and body morphometric variables with body color information included (right panel). See **Appendix D**: Table 3 for classification results. Colors represent mean RGR for each group from least red (greenest oval) to most red.*

Linear box-truss measurements varied among capture locations and spawning seasons with 15 of the 17 measurements demonstrating significant differences among location and/or season and/or their interaction. Eight of the 17 measurements varied significantly among spawning seasons

with the three strongest differences being body depth variables that were larger in winter compared to spring-spawning cod. Fourteen of the 17 measurements varied significantly among spawning locations with the three strongest variables being head length variables that were longer in Massachusetts Bay than in Ipswich Bay fish. Average reconstructed shapes for cod from different spawning seasons (by location and for both locations) are shown in Figure 19. In this case, measurements linked to landmark #11 were included for illustrative purposes. However, these measurements do not impact the statistical results discussed above. These reconstructions consistently show that spring-spawning cod, regardless of location, are more streamlined than winter-spawning cod. This result suggests that spring-spawning cod are more migratory than winter-spawning cod. This result runs counter to our *a priori* hypothesis based on the scale of movement matching egg/larval dispersal (i.e., Runge et al. 2010). That is, we expected winter-spawning cod to be more migrant (and streamlined) based on the fact that their eggs and larvae are dispersed over wider areas (Runge et al. 2010). In order to "close the loop", winter-spawning cod would have to migrate back to the western Gulf of Maine to spawn once they have matured. This does not appear to be the case and calls into question model assumptions about early-life dispersal patterns.

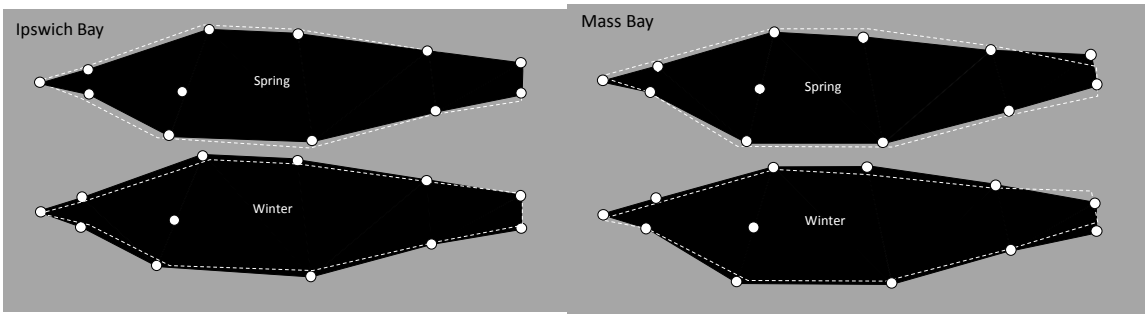


Figure 19. Left panel: Mean reconstructed shape (solid black area) of spring- vs. winter-spawning cod from Ipswich Bay. Dashed white line over/around each shape is outline for the opposite group. Winter-spawning cod from Ipswich Bay are more robust (deeper bodied) than spring-spawning cod. Right panel: Mean reconstructed shape (solid black area) of spring- vs. winter-spawning cod from Massachusetts Bay. Dashed white line over/around each shape is outline for the opposite group. Winter-spawning cod from Massachusetts Bay are more robust (deeper bodied) than spring-spawning cod. White circles are individual land marks (see Figure 16).

Overall, our color and morphometric results suggest differences in migratory strategy among seasonal spawning groups, as well as potential depth related differences associated with geographic location. Cod from Massachusetts Bay, regardless of spawning season, were redder than cod from Ipswich Bay possibly indicating that these cod spend most of their time outside of spawning in shallower waters, since red cod typically associate with shallow water (Sherwood and Grabowski 2010, Conroy et al. 2017). The difference in color was subtle and not as marked as differences noted in directed studies of red cod compared to "olive" cod (Sherwood and Grabowski 2010, Conroy et al. 2017). This suggests that there is a continuum of life-history strategies that may vary from very shallow, resident, kelp-associated cod (~20-30m; very red,

Conroy et al. 2017) to inshore dwelling cod as in Massachusetts Bay (somewhat red, ~40-60m), to slightly deeper living cod as in Ipswich Bay (not very red, ~80-100m), and possibly deeper still (likely highly migratory but not represented here). Indeed, Gosse and Wroblewski (2004) report on a range of color types in Newfoundland and Labrador with variations in assumed movement strategies. Despite differences in color among bays, as well as body shape variables, there appeared to be consistent morphometric differences among seasons regardless of location. Particularly, winter-spawning cod had deeper bodies which would be expected with a more resident lifestyle. This runs counter to previous expectations that winter-spawning cod should be more migrant based on the scale of modeled egg and larval dispersal (Runge et al. 2010).

There appear to be multiple dimensions of life-history strategies among cod groups: a resident/migrant dichotomy that relates to body shape differences (spring vs. winter) and also a depth related contrast as indicated by color (Massachusetts Bay vs. Ipswich Bay). Red cod have been previously associated with highly resident strategies (Conroy et al. 2017). However, our results suggest that more subtle differences in color may indicate depth preference but not necessarily movement strategy since redder Massachusetts Bay cod also had longer heads, which is usually associated with more streamlined bodies and thus higher movement capacity. Further work may examine whether differences in movement and depth preference truly exist between spawning locations and seasons examined in this study.

A detailed summary of the methods and findings for body morphometrics and color analysis is provided in **Appendix C**.

***Hypothesis 3**:* The relative contribution of genetic/ecological units of cod to the fishery has changed over time. We expect that current landings in Gulf of Maine are primarily composed of resident fish (spring spawners) and historical landings are composed of a broader mixture of sources.

Genetics and Genomics

To determine whether the composition of the commercial fishery has changed over time, we analyzed microsatellite genotypes from the four spawning populations, the modern commercial fishery samples, and the archived otolith samples and conducted a mixed stock analysis. For the modern commercial fishery, mixed stock samples were available from 131 individuals sampled during nine separate collections, from statistical areas 513 and 514 in 6 different months (March, May, June, July, December and January). These samples were analysed by month and season. From the archived otoliths, we obtained multilocus genotypes from 232 individuals in two time periods, 1979-1982 and 1989-1992, from statistical areas 513, 514, and 515 (the latter in the latter time period only). These samples were analysed by month, season, time period, and statistical area. Modern commercial fishery and otolith samples were compared to the dataset comprised of 434 genotypes from 2006-2008 and 2014-2015 from Ipswich and Massachusetts Bays in the winter and spring.

For the commercial fishery samples, FST analyses indicated that the collections varied in their compositions, with some collections more genetically similar to the winter populations and others more similar to the spring. These comparisons were roughly consistent by season, however, several collections were genetically distinct from both the winter or spring spawning populations and the two July collections appeared quite divergent, with one clustering with the winter spawning populations and the other with the spring (see **Appendix B**). When the samples were pooled by month and season, a greater proportion of the variation was explained by PC Axis 1 and the patterns became more discernible, with March, December, and January collections clustering with the winter spawning populations and July samples clustering with the spring spawning populations (Figure 20). Fishery samples collected in May appeared rather intermediate and the June collection was divergent from both the winter and spring spawning populations, although more similar along Axis 1 to the spring spawners. Grouping samples by season resulted in greater variation explained by the first two PC axes; however, none of the seasonal collections showed strong similarity to either the winter or spring spawning populations (Figure 21). This suggests that grouping the samples in this way likely obscured some of the variation that was inherent in the individual collections, resulting in a full mixture. Another possibility, suggested especially by the summer collections, is that additional population components are being sampled by the commercial fishery in addition to the winter and spring spawning Gulf of Maine populations (e.g., cod migrating from Georges Bank or other areas).

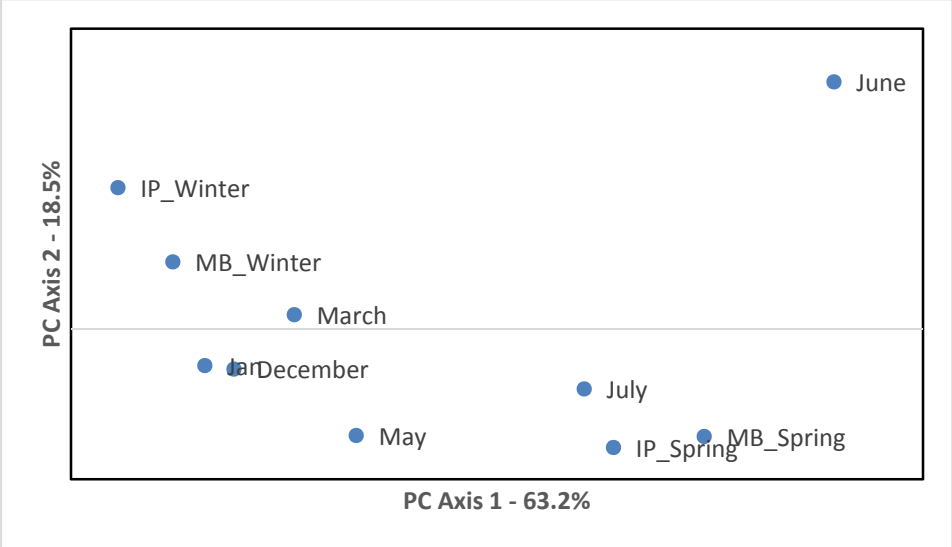


Figure 20. PCA of genetic differentiation measured by pair-wise population FST of cod sampled from the commercial fishery, pooled by month, in comparison with winter and spring spawning populations in Ipswich and Massachusetts Bays.

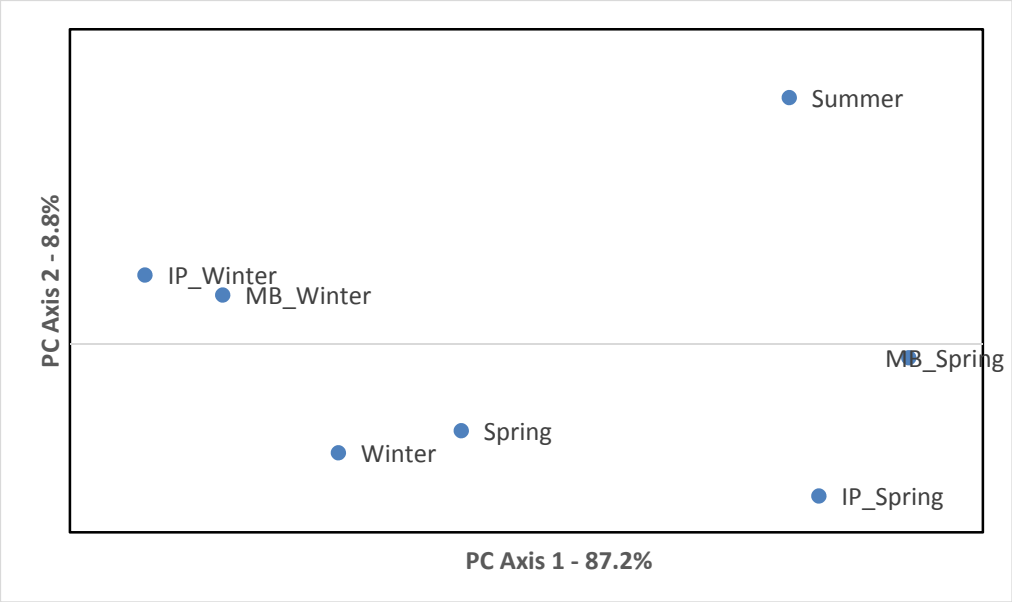
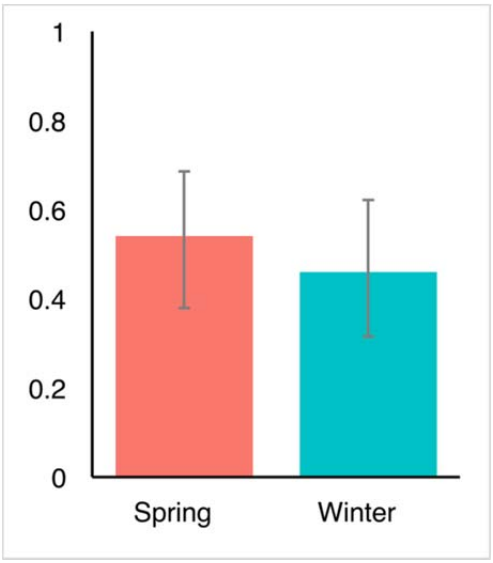
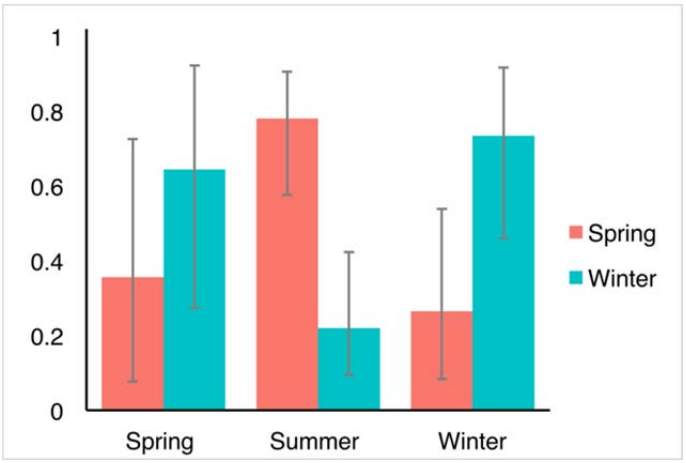


Figure 21. PCA of genetic differentiation measured by pair-wise population FST of cod sampled from the commercial fishery, pooled by season, in comparison with winter and spring spawning populations in Ipswich and Massachusetts Bays.

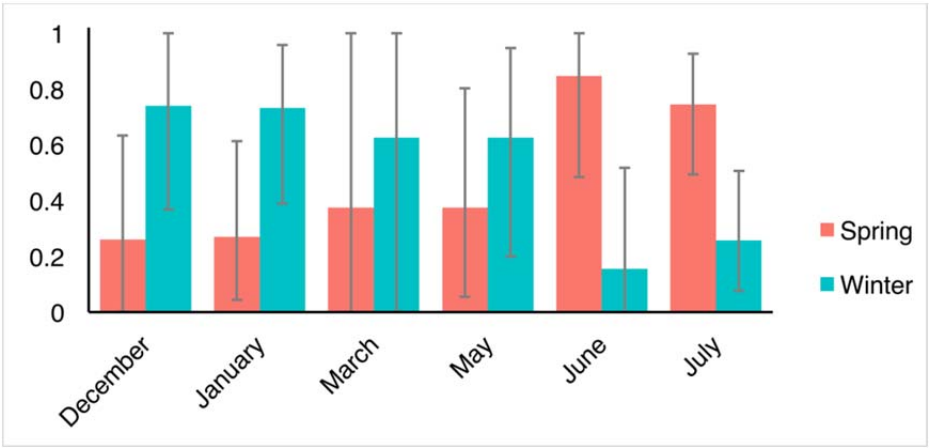
We then conducted mixed stock analyses, using the genotypes from the four spawning populations (winter and spring spawning cod in both Ipswich and Massachusetts bays) as two reporting groups – winter and spring spawning cod. We assigned the commercial fishery samples of unknown population origin to one of the two reporting groups, using a conditional likelihood approach in the mixed stock analysis (MSA) software ONCOR. Analysis of the full mixture (all 131 samples) indicated that the overall the collections were split relatively evenly across the two reporting groups (Figure 22a). When analysed by season, the winter mixture was comprised of 77% winter spawners and the summer mixture was comprised of 80% spring spawners, while the spring mixture (March, April, May) was comprised of a mixture of both winter and spring spawners (Figure 22b). These seasonal patterns were supported in analysis of mixtures by month (Figure 22c). Tests of MSA accuracy (100% simulations) revealed 81-93% accuracy of the reporting group assignments. Realistic fishery simulations were used to provide another test of MSA accuracy; these tests revealed high accuracy for mixtures comprised of 50% each reporting group and slightly lower accuracy for 75%:25% fishery mixtures, with winter assignment accuracy slightly lower than spring (See **Appendix B**).



A.



B.



C.

Figure 22. Proportional assignments to spring and winter spawning populations from mixed stock analyses of commercial fishery mixtures a) overall, b) by season, and c) by month.

For the historical fishery analyses, all five collections of otolith samples were found to be genetically distinct from the four modern spawning populations, with F_{ST} values ranging 0.018 to 0.045, substantially larger than the genetic distances among the modern cod populations. In a PCA, the otolith samples did not cluster together with either the winter or spring spawning populations, and were separated more distinctly from the winter spawning population (Figure 23). The otolith samples were also differentiated from most of the modern fishery sample collections, with the exception of the June and July collections. These results suggest that the composition of the fishery has changed over time and the modern fishery is comprised primarily of cod populations that are not well represented in the historical fishery sample; likewise, the historical fishery was comprised primarily of populations with a genetic signature different from the modern winter and spring spawning populations in Ipswich and Massachusetts Bays.

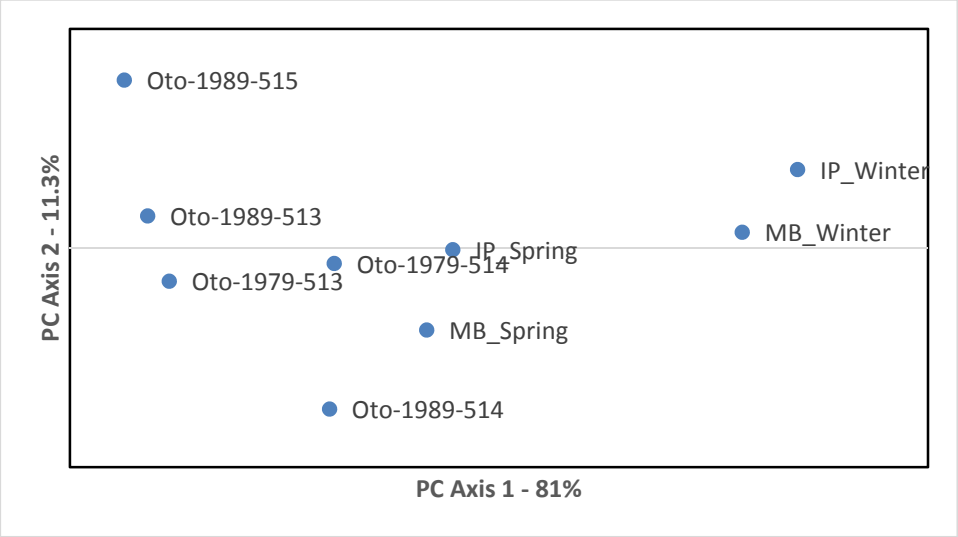
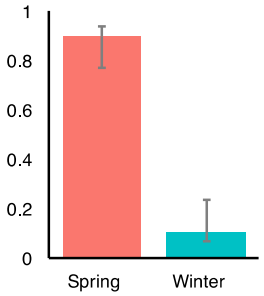
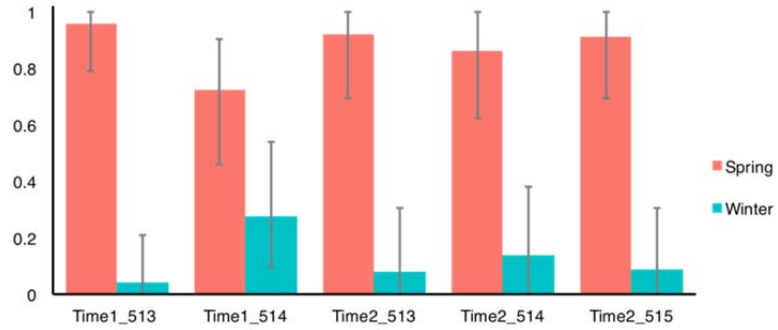


Figure 23. PCA of genetic differentiation (FST) of cod sampled from the historical fishery in two time periods and five statistical areas, in comparison with winter- and spring-spawning populations in Ipswich and Massachusetts Bays. (Historical samples are labeled "oto" by date and statistical area).

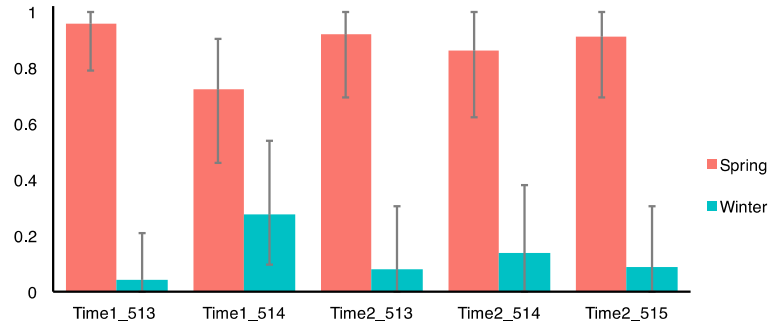
Mixed stock analysis of the historical fishery samples pointed strongly toward a composition more similar to today's spring spawning populations (Figure 24). However, it is important to note that this analysis can only assign individuals to one of the samples in the reporting groups and cannot account for unsampled populations. Therefore, while the MSA tests show that the composition of the historical fishery is different from that of the modern fishery (winter-biased), it is important to note that with MSA we cannot directly test the hypothesis that the historical fishery is comprised of a different population than either the modern winter or spring spawning populations. To evaluate this possibility further, we would need to have additional populations in our baseline and reporting groups. That work was beyond the scope of this study and will be a subject of a follow up study.



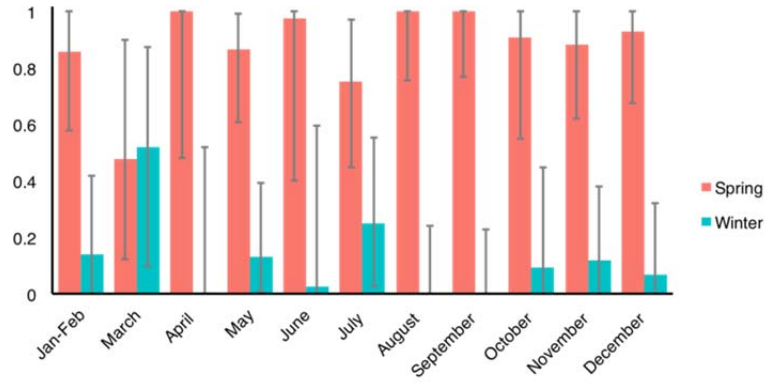
A.



B.



C.



D.

Figure 24. Proportional assignments to spring and winter spawning populations from mixed stock analyses of commercial fishery mixtures a) overall, b) time and statistical area, c) by season, and d) by month.

We further evaluated the change in the fishery over time by testing for patterns in the genetic data. Comparison of genetic diversity in the modern and historical samples indicated little difference, except a slight reduction in the genetic diversity of the modern spring spawning population (see **Appendix B**). This result further suggests that the shift in genetic composition observed between the historical and modern fishery samples is not due to a loss of genetic diversity within the same populations, but rather more consistent with a shift in population structure and potentially a loss of population segments. Examination of allele frequency differences over time further supported this hypothesis of a shift in genetic structure over time. Specifically we identified 7 alleles with allele frequency differences >10% in the historical samples compared to the modern samples. These 7 alleles were all found in the key loci driving genetic divergence between the winter and spring spawning populations (Gmo132, C123, C94, and PanI). Of those 7 alleles, the 5 that are decreasing in frequency are all alleles that are found at a higher frequency in the modern spring spawners, while the 2 alleles that are increasing are found in a higher frequency in the winter spawners (Figure 25). This finding suggests a shift in allele composition away from the spring spawning ecotype (cold-adapted; migratory) and toward the winter spawning ecotype (warmer water adapted; resident).

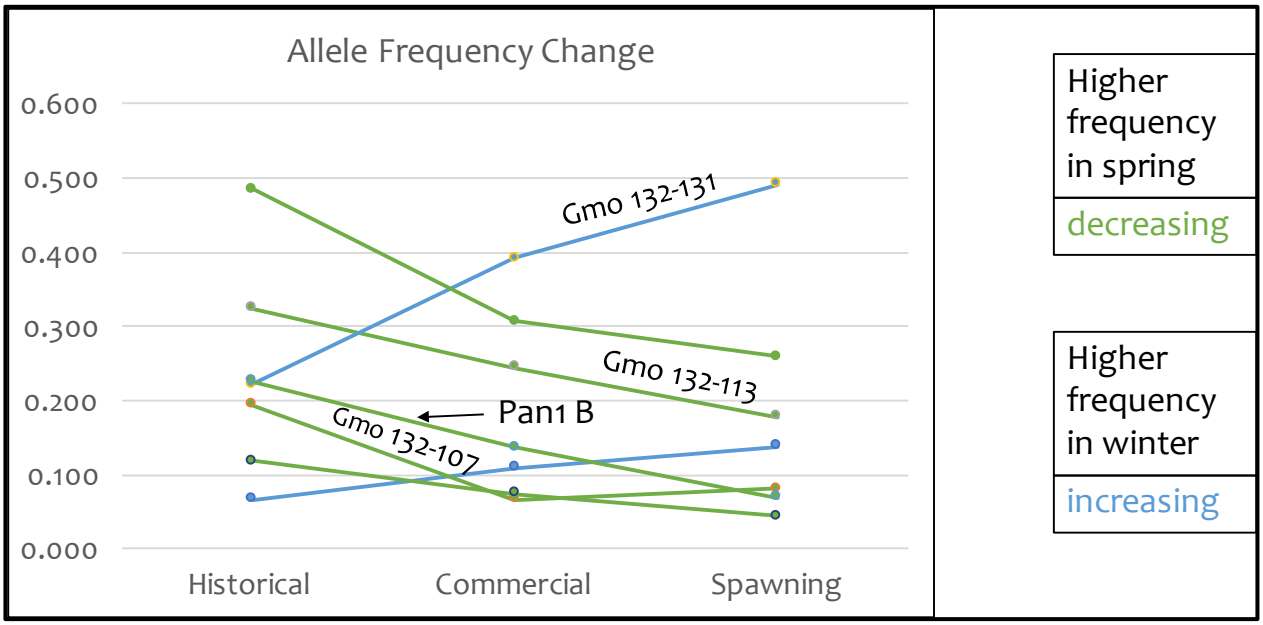


Figure 25. Changes in allele frequencies (Y axis) over time from the historical to modern cod populations (X axis) for seven alleles that drive the divergence between modern spring and winter spawning cod populations. Alleles that that are decreasing in frequency over time are shown in green (these alleles are more prevalent in spring spawners), and alleles that are increasing over time are shown in blue (these are more prevent in winter spawners). The observed shift in allele frequencies supports a shift away from the cold-water adapted spring-spawning ecotype toward the warmer water adapted winter-spawning ecotype.

Otolith Chemistry

Mixed stock analysis was conducted to classify fish from modern and historical fishery collections to either the winter or spring spawning group based on their age one (i.e., core) otolith chemistry. The baseline used for classification was the age one chemistry of modern collections of known winter and spring spawners. Here we used random forest classification approach for which the baseline classification accuracy of spawners to their known origin was 73%. Random forest classification of mixed stock fishery samples from the modern fishery (n = 187; 2015-2016) indicated that 57% of the fish sampled were winter spawning fish (Figure 26). Across years (2015-2016), the proportion of winter spawners in the sample ranged from 55 to 65% (Figure 26). Across seasons, the proportion of winter spawners in the sample was consistent at ~60%, with the exception of summer which was dominated by spring spawners (58%, Figure 26). Across statistical areas, the proportion of winter spawners in the sample varied from 36% in 513 to 64% in 514 (Figure 26). However, it is important to note that the sample size for statistical area 513 was considerable lower than 514 (513: n = 36, 514: n = 151).

As we did with modern commercial fish collections, the age one otolith chemistry of fish from historical fishery collections were used to classify individuals to either the winter or spring spawning group using modern spawning fish as a baseline sample. This assumes that the modern sample is representative of historical winter and spring spawners. Classification of historical fishery samples indicate a lower proportion of winter fish in the early 1980s (42% winter spawners, n = 117) and 1990s (38% winter spawners, n = 153; Figure 27). Across years, the proportion of winter spawners in the sample ranged from 14 to 48% (Figure 27). Across statistical areas, the proportion of winter spawners in the sample ranged from 38% in 513 to 43% in 514, and 39% in 515 (Figure 27).

Together, mixed stock analysis of modern and historical fishery collections suggest an increase in the proportion of winter spawners in fishery samples during the recent time period. It is important to note the small sample size and that these trends may not be representative of the overall mixed stock composition of the fishery over time. Furthermore, the results are contingent on the modern baseline being suitable for mixed stock assignment of fish to spawning time from historical collections.

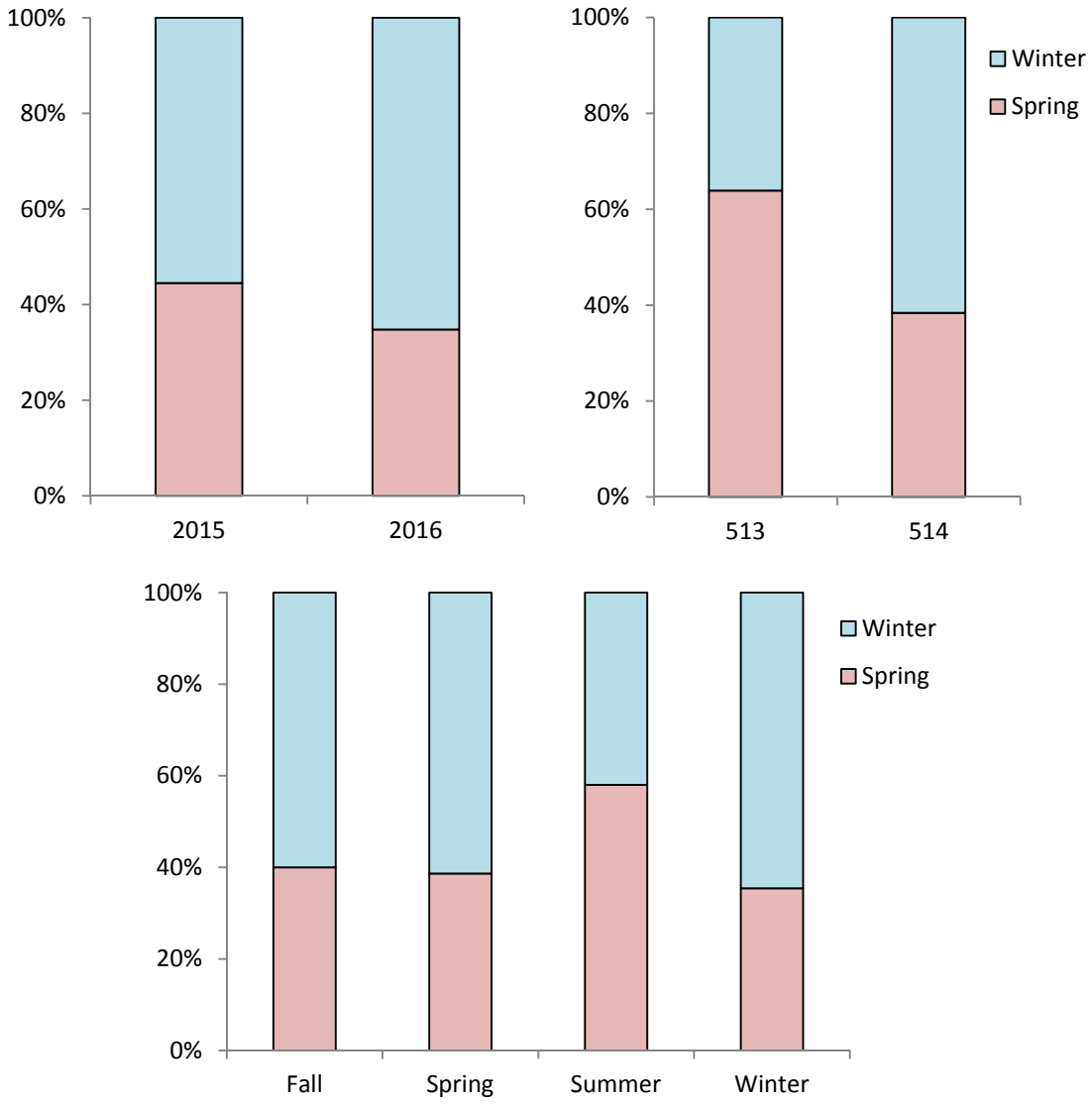


Figure 26. Stock composition of fishery collected 2015-2016 Atlantic cod over time using random forest classification approach.

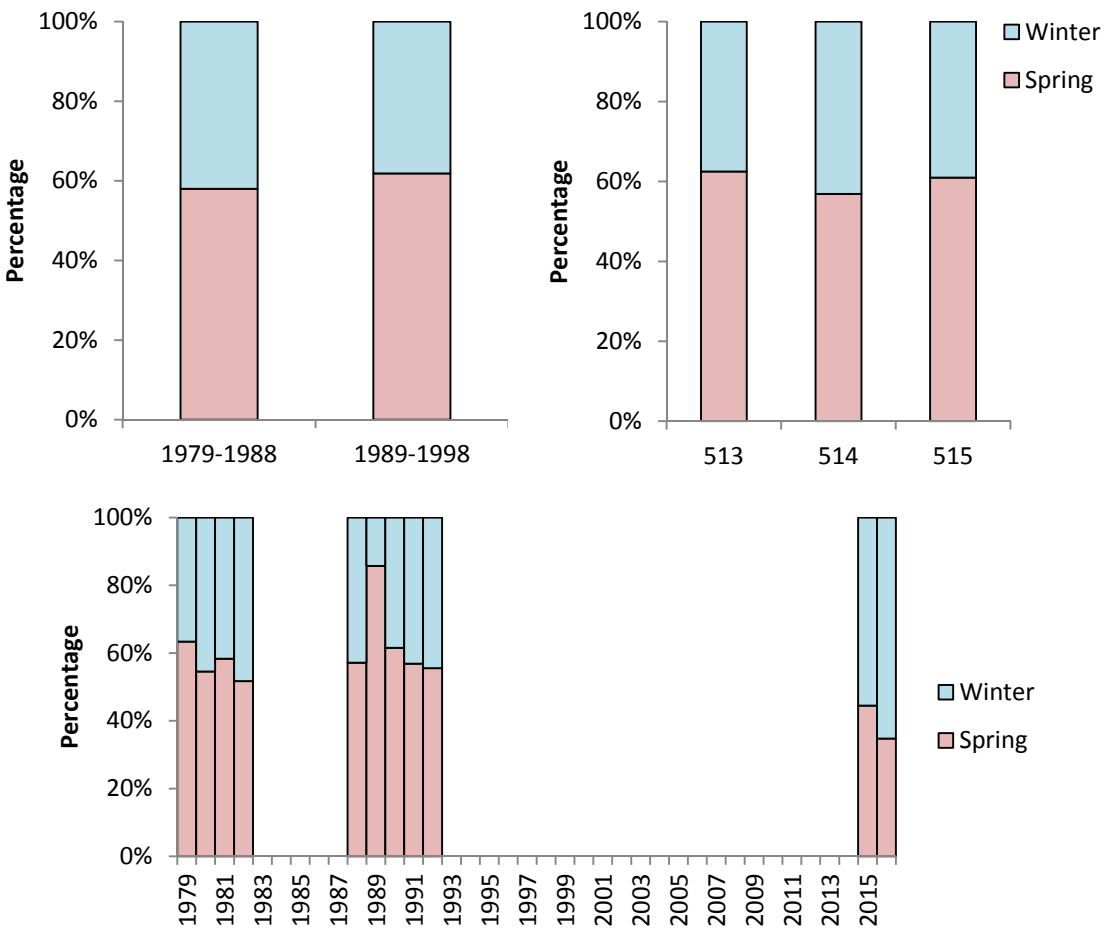


Figure 27. Stock composition of fishery collected Atlantic cod in 1980s and 1990s using random forest classification approach.

E. Interdisciplinary Synthesis of Findings

In addition to reviewing results from each discipline (genetics, genomics, otolith chemistry, otolith structure, body morphometrics, and color) with respect to the stated hypotheses, we have also applied an interdisciplinary approach to synthesize information from multiple techniques (following Cadrin et al. 2015). The unique perspective offered from each discipline along with the sensitivity of specific characters for detecting diverse ecological types were considered to identify congruent results and to reconcile apparent differences.

Hypothesis 1*:* The genetic differences previously identified between winter and spring spawners in the Gulf of Maine are temporally stable. We expect that genetic differences identified between winter and spring spawning cod collected during this project will be the same as those found in previous sampling (2003-2008).

Genetic analysis of winter and spring spawning cod from this study (2014-2015) indicated genetic divergence. Combined with our prior work (Kovach et al. 2010), this shows stability of this structure between 2003-present day, suggesting a consistent, long-term stability in population structure. Our analysis also suggests that fine scale differences occur between Ipswich and Massachusetts Bays within season, particularly for the winter spawning groups. The temporal comparisons suggest some variability in these fine-scale patterns, or perhaps that larger sample sizes and higher resolution genetic markers are needed to track these fine-scale differences consistently over time. Notably, the differences in season and spatial location are significantly greater than the small temporal differences.

Hypothesis 2*:* Winter and spring spawning fish represent distinct ecological units with spring spawning fish adopting a resident life history strategy and residing in inshore regions and winter spawning fish adopting a migratory life history strategy and exhibiting broader dispersal/habitat use. Adaptive (functional) genetic differences will be consistent with these life history differences.

Genome-wide SNPs and microsatellite datasets revealed that genetic differentiation is driven largely by markers putatively under selection, indicating that adaptive differences are important in separating the winter and spring-spawning cod. Identification of outlier loci associated with regions of the cod genome known to be associated with ecotype differences, including migratory/resident and temperature-driven adaptations, provide support for the hypothesis that winter and spring spawning cod have important ecological and life history differences. Furthermore, we found robust differences in otolith growth and chemistry between winter and spring spawners, with more subtle differences based on location. Significant differences in otolith growth and chemistry suggest that winter and spring spawning groups from each location experience different habitats over their lifetime and is suggestive of ecotype differences. Body color and morphometric results suggest differences in migratory strategy among seasonal spawning groups, as well as potential depth related differences associated with geographic

location. Taken in sum, the finding from all three techniques are corroborative and consistently support our hypothesis that the winter and spring populations of cod are distinct ecological units with adaptive life history differences. Morphometric analysis contradicts our original prediction about the specific winter and spring ecotypes and indicates that winter spawners have features associated with a more resident life history (deeper bodied and shorter head) than spring spawners.

Hypothesis 3*:* The relative contribution of genetic/ecological units of cod to the fishery has changed over time. We expect that current landings in Gulf of Maine are primarily composed of resident fish (spring spawners) and historical landings are composed of a broader mixture of sources.

Mixed stock analyses of fishery samples based on genotypes indicated that modern commercial fishery samples of unknown population origin were split relatively evenly between winter and spring spawners. Mixed stock analysis of the historical fishery samples based on genotypes pointed strongly toward a composition more similar to today's spring spawning populations. However, it is important to note that this analysis can only assign individuals to one of the samples in the reporting groups and cannot account for unsampled populations. Therefore, while the MSA tests show that the composition of the historical fishery is different from that of the modern fishery (winter-biased), it is important to note that with MSA we cannot directly test the hypothesis that the historical fishery is comprised of a different population than either the modern winter or spring spawning populations. To evaluate this possibility further, we would need to have additional populations in our baseline and reporting groups. That work was beyond the scope of this study and will be a subject of a follow up study. However, while unsampled baseline populations cannot be accounted for, the genetic results are suggestive of additional genetic diversity comprising the historical fishery, because the otolith collections did cluster similarly with the modern winter and spring spawning baseline.

Similar results were obtained with otolith chemisitry analysis. Specially, otolith chemistry analysis revealed the modern fishery samples were composed of more winter spawning fish (57%) than spring. Classification of historical fishery samples indicate a lower proportion of winter fish in the early 1980s (42% winter spawners) and 1990s (38% winter spawners). Together, mixed stock analysis of modern and historical fishery collections suggest an increase in the proportion of winter spawners in fishery samples during the recent time period which aligns with genetic information. However, the same caveats noted for the genetic technique apply to mixed stock analysis based on otolith chemistry in that we cannot discount that there are unsampled baseline populations present in historical sample of the fishery. Furthermore, it is important to note for both techniques that due to the relatively small sample size, compared to the scale of fisheries landings, these trends may not be representative of the overall mixed stock composition of the fishery over time.

A. Products and Presentations

Presentations

Clucas, G.V., N. Lou, L. Kerr, G. Sherwood, S. Cadrin, D. Zemeckis, D. Goethel, Z. Whitener, N. Therkildsen, and A. Kovach. 2018. Population structure of Atlantic cod in the Gulf of Maine. (poster) 51st Population Genetics Group Meeting of the Genetics Society, Bristol, UK, January 3-6 2018.

Kovach, A.I. Molecular Signatures of Biocomplexity and Resilience in Atlantic Cod across Space and Time. University of Connecticut Department of Ecology & Evolution Seminar Series, October 12, 2017.

Kerr, L.A. Ecological diversity of Atlantic cod in the Gulf of Maine. GMRI Seminar Series. June 2015.

Kerr, L.A. Ecological diversity of Atlantic cod in the Gulf of Maine. Fish Tank Seminar Series with stakeholders. November 2015.

Kerr, L.A. The current and future status of Gulf of Maine cod. Bates Seminar Series. February, 2016.

Kerr, L.A. The current and future status of Gulf of Maine cod. Bowdoin Seminar Series. March 2016.

Kerr, L.A. The current and future status of Gulf of Maine cod. University of New Hampshire Seminar Series. April 2016.

Kerr L., Whitener Z., Sherwood G., Cadrin S., Goethel D., Kovach A., Zemeckis D. 2015. Otolith chemistry as a natural marker of stock identity and habitat use of Atlantic cod in the Gulf of Maine. ICES Annual Science Conference. (September 2015, Copenhagen Denmark).

Kerr L., Kovach A., Sherwood G., Cadrin S., Goethel D., Whitener Z., Zemeckis D. Ecological diversity of Atlantic cod in the Gulf of Maine. 2016. Benthic Ecology Society Meeting. (March 16-19, 2016, Portland, Maine).

Kerr L., Whitener Z., Sherwood G., Cadrin S., Goethel D., Kovach A., Zemeckis D. 2016. Reconstructing environmental histories of Atlantic cod in the Gulf of Maine. 4th International Sclerochronology Meeting held (June 5-9, 2016, Portland, Maine).

Kerr L. ; Cadrin S. , Goethel D.; Kovach A., , Sherwood G.; Whitener Z.; Zemeckis D. 2017. Ecological diversity of Atlantic cod in the Gulf of Maine. Canadian Conference for Fisheries Research. (January 2017, Montreal, Canada).

Kerr L., Cadrin S., Goethel D., Kovach A., Sherwood G.; Whitener Z., Zemeckis D. 2017. Reconstructing growth and environmental histories of Atlantic cod in the Gulf of Maine. American Fisheries Society Annual Meeting (August, 2017 Tampa, Florida)

Kerr L., Cadrin S., Goethel D., Kovach A., Sherwood G.; Whitener Z., Zemeckis D. Biocomplexity of Atlantic cod in the Gulf of Maine and its role in resiliency of a fishery. 2017. ICES Annual Science Meeting. (September, 2017, Fort Lauderdale, Florida).

Publications in Prep

Clucas, G.V., L. Kerr, G. Sherwood, S. Cadrin, D. Zemeckis, D. Goethel, Z. Whitener, and A.I. Kovach. *In prep.* Genome-wide Differentiation and Adaptation of Northwest Atlantic cod Ecotypes in Gulf of Maine and Georges Bank.

Kovach, A.I., L. Kerr, G. Sherwood, D. Zemeckis, D. Goethel, Z. Whitener, S. Cadrin. *In prep.* Changes in composition of the Atlantic cod fishery over time as revealed by genetic analyses.

Kerr L., Whitener Z., Sherwood G., Kovach A., Cadrin S., Goethel D., Zemeckis D. *In prep.* Otolith chemistry as a natural marker of stock identity and habitat use of Atlantic cod in the Gulf of Maine.

B. Literature Cited

Armstrong, M. P., Dean, M. J., Hoffman, W. S., Zemeckis, D. R., Nies, T. A., Pierce, D. E., Diodati, P. J., and McKiernan, D. J. 2013. The application of small scale fishery closures to protect Atlantic cod spawning aggregations in the inshore Gulf of Maine. Fisheries Research 141: 62-69.

Burnett, J., O'Brien, L. , Mayo, R. K. , Darde, J. A. , Bohan, and M.. 1989. Finfish Maturity Sampling and Classification Schemes Used during Northeast Fisheries Science Center Bottom Trawl Surveys, 1963-1989. NOAA Technical Memorandum NMFS-F/NEC-76. https://www.nefsc.noaa.gov/publications/tm/pdfs/tmfnec76.pdf.

Cadrin, S. X., and Friedland, K. D. 1999. The utility of image processing techniques for morphometric analysis and stock identification. Fisheries Research 43: 129-139.

Cadrin, S.X., Kerr, L.A., Mariani, S. 2015. Interdisciplinary evaluation of spatial population structure for definition of fishery management units. In: Stock Identification Methods (Eds. S. Cadrin, L. Kerr, S. Mariani). 2nd Edition. Elsevier. 566 pp.

Conroy, C. W. Calvert, J., Sherwood, G. D., and Grabowski, J. H. 2017. Distinct responses of sympatric migrant and resident Atlantic cod phenotypes to temperature at a remote Gulf of Maine seamount. ICES Journal of Marine Science fsx101.

Delghandi, M., Stenveik, J., Nilsen, F., Wesmajervi, M. S., Kjersti, A., Fjalestad, T., and Damsgard, B. 2008. dentification and characterisation of nine new gene-associated microsatellite markers for Atlantic cod (Gadus morhua L.). Conservation Genetics 9:747-749.

Department of Commerce. 2014. Northeast Groundfish Fishery; Fishing Year 2014; Emergency Gulf of Maine Cod Management Measures. Federal Register, Volume 79(219), pages 67362-67371.

Department of Commerce. 2015. Magnuson-Stevens Fishery Conservation and Management Act Provisions; Fisheries of the Northeastern United States; Northeast Groundfish Fishery; Framework Adjustment 53. Federal Register 80(84), 25110-25143.

Gosse, K.R., and Wroblewski, J.S. 2004. Variant colourations of Atlantic cod (*Gadus morhua*) in Newfoundland and Labrador nearshore waters. ICES Journal of Marine Science 61: 752-759.

Gurshin, C.W.D., Howell, W.H., and Jech, J.M. 2013. Synoptic acoustic and trawl surveys of spring-spawning Atlantic cod in the Gulf of Maine cod spawning protection area. Fisheries Research 141: 44-61.

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

Morinville, G.R., and J.B. Rasmussen. 2008. Distinguishing between juvenile anadromous and resident brook trout (*Salvelinus fontinalis*) using morphology. Environmental Biology of Fishes 81: 171-184.

Northeast Fisheries Science Center. 2013. 55th Northeast Regional Stock Assessment Workshop (55th SAW) Assessment Report. US Department of Commerce, Northeast Fish Science Center Reference Doc. 13-11; 845 p. Available from: National Marine Fisheries Service, 166 Water Street, Woods Hole, MA 02543-1026, or online at https://www.nefsc.noaa.gov/nefsc/publications/.

Runge, J.A., Kovach, A.I., Churchill, J.H., Kerr, L.A., Morrison, J.R., Beardsley, R.C., Berlinsky, D.L., Chen, C., Cadrin, S.X., Davis, C.S., Ford, K.H., Grabowski, J.H., Howell, W.H., Ji, R., Jones, R.J., Pershing, A.J., Record, N.R., Thomas, A.C., Sherwood, G.D., Tallack, S.M.L., and Townsend, D.W. 2010. Understanding climate impacts on recruitment and spatial dynamics of Atlantic cod in the Gulf of Maine: integration of observations and modelling. Progress in Oceanography 87: 251-263.

Sherwood, G.D., Grabowski, J.H. 2010. Exploring the life-history implications of colour variation in offshore Gulf of Maine cod (*Gadus morhua*). ICES Journal of Marine Science 67: 1640-1649.

Sherwood, G.D., and Grabowski, J.H. 2015. A comparison of cod life-history parameters inside and outside of four year-round groundfish closed areas in New England, USA. ICES Journal of Marine Science 73: 316-328.

Sherwood, G.D., Kerr, L.A., Gurshin, C.W.D., Howell, W.H. Goethel, D. 2017. Synoptic acoustic and trawl survey of winter-spawning cod in Ipswich Bay, western Gulf of Maine. Final Report to Northeast Consortium 2014/2015 NEFMC Groundfish Research Program. 60 p.

Stanley, R.R.E., Bradbury, I.R., DiBacco, C., Snelgrove, P.W.R., Thorroled, S.R., Killen, S.S. 2015. Environmentally mediated trends in otolith composition of juvenile Atlantic cod (Gadus morhua ICES Journal of Marine Science. 72 (8): 2350–2363.

Appendix A

The large volume of data and data sources that contributed to this project have warranted including an appendix to fully describe all of the samples available for analysis. The following tables create a more complete description of the cod samples than could be afforded in the text of the report.

Table A. Sex of all collected cod samples. Gonads of immature samples were not sufficiently developed to determine sex. Samples that did not have sex recorded are identified as unknown.

Sample Type	Sex				Total
	Immature	Female	Male	Unknown	
Spawning	1	329	530	2	**862**
Commercial		149	173	2	**324**
Historical				303	**303**
Total	**1**	**478**	**703**	**307**	**1489**

Table B. Summary of 80 sampling trips for spawning fish collections. This table denotes the numbers of spawning fish for each spawning time (determined by gonad development) collected at each location. Fish with insufficient gonadal development for determining spawning time are listed as unknown.

Vessel	Date Landed	Spawning Time						Total
		Spring		Winter		Unknown		
		Ipswich Bay	MA Bay	Ipswich Bay	MA Bay	Ipswich Bay	MA Bay	
R/V Alosa	5/3/2010		4					4
	5/7/2010		3					3
	5/11/2010		4					4
	5/18/2010		2				1	3
	5/21/2010		2				3	5
	5/24/2010		1					1
	5/26/2010		5				1	6
	6/3/2010		2				1	3
	6/8/2010		4					4
	6/18/2010		1					1
	6/19/2010		1					1
	6/23/2010		3					3
	7/2/2010		5					5
	7/7/2010						1	1
	7/16/2010		2					2
	7/20/2010						1	1
	4/14/2011		1				1	2
	4/19/2011		2					2
	4/22/2011		8					8
	4/26/2011		2					2
	5/5/2011		2					2
	5/20/2011		7					7
	5/26/2011		6					6
	6/1/2011		4					4
	6/6/2011		1					1
	6/8/2011		3					3
	6/10/2011		4					4
	6/13/2011		3					3
	6/15/2011		4					4
	6/22/2011		2					2
	7/19/2011		2					2

	12/5/2011			3		3
	4/3/2012		2			2
	4/11/2012		1		1	2
	4/17/2012		4			4
	4/26/2012		3			3
	4/30/2012		2			2
	5/7/2012		4			4
	5/10/2012		6			6
	5/12/2012		1			1
	5/18/2012		5		1	6
	5/21/2012		3			3
	5/24/2012		2			2
	5/31/2012		6			6
	6/7/2012		4			4
	6/15/2012		14			14
	4/25/2013		1			1
	5/2/2013		5		1	6
	5/14/2013		35		5	40
	5/22/2013		68		1	69
	5/31/2013		13		3	16
	6/5/2013		8		1	9
	5/15/2014		9		1	10
	5/30/2014		14			14
	6/10/2014		19		1	20
	6/17/2014		4		6	10
	Total		**323**	**3**	**30**	**356**
F/V Ambjorg & Julie	3/30/2009				1	1
	Total				**1**	**1**
F/V Barbara L Peters	11/23/2014			22	2	24
	12/5/2014			23	1	24
	Total			**45**	**3**	**48**
F/V Ellen Diane	12/22/2014		73	2		75
	4/16/2015	42		9		51
	5/14/2015	47				47
	10/21/2015		6	13		19
	11/25/2015		33	7		40
	Total	**89**	**112**	**31**		**232**
R/V Mystique	12/4/2013			2		2
	12/14/2013			30	10	40

Lady	12/17/2013				20		2	22
	1/10/2014				19			19
	11/22/2014				15		5	20
	12/12/2014				24		1	25
	Total				**110**		**18**	**128**
F/V Odyssey	6/12/2012		3					3
	6/18/2012		3					3
	Total		**6**					**6**
Unknown Vessels	6/18/2012		3					3
	12/6/2011				14		5	19
	12/7/2011				12		11	23
	12/12/2011				17			17
	12/17/2011				6			6
	12/22/2011				3			3
	6/12/2012		1		2			3
	Total		**4**		**54**		**16**	**74**
F/V Yankee Rose	12/19/2013				14		3	17
	Total				**14**		**3**	**17**
Total		**89**	**333**	**112**	**226**	**31**	**71**	**862**

Table C. Summary of age and sex distribution of spawning fish samples from Ipswich Bay and Massachusetts Bay by designated spawning time.

Age	Sex	Ipswich Bay			MA Bay			Total
		Spring	Winter	Unknown	Spring	Winter	Unknown	
2	**Female**		5	1		1	1	**8**
	Male	3	3			7		**13**
3	**Female**	2	27	7		5	3	**44**
	Male	18	18	2	1	7	1	**47**
4	**Female**	12	21	9	2	3	1	**48**
	Male	15	33	6	5	11		**70**
5	**Female**	6	3	3	1	7	1	**21**
	Male	7	1	1	12	8	1	**30**
	Unknown		1					**1**
6	**Female**	2		1		1		**4**
	Male	3			10	2		**15**
7	**Female**	4				1		**5**
	Male	1		1	1			**3**
8	**Female**	1			1			**2**
	Male	1				1		**2**
9	**Male**				2			**2**
Unknown	**Immature**						1	**1**
	Female	1			93	54	49	**197**
	Male	13			205	118	12	**348**
	Unknown						1	**1**
Total		**89**	**112**	**31**	**333**	**226**	**70**	**862**

Table D. Some fish that we captured during surveys of spawning aggregations for other projects were used in this project and included in the commercial sample dataset in order to increase that sample size. These fish are representative of historical fishing practices that target spawning aggregations. Spawners added to the commercial sample were collected by the R/V Alosa in Massachusetts Bay and the R/V Ellen Diane in Ipswich Bay.

Vessel	Date	Spawners Added to Commercial Data		Total
		Yes	No	
F/V Ellen Diane	3/25/2015		8	**8**
	5/18/2015		60	**60**
	6/4/2015		25	**25**
	7/7/2015		50	**50**
	7/9/2015		16	**16**
	11/16/2015	19		**19**
	11/22/2015	26	2	**28**
	12/1/2015	5	1	**6**
F/V Justice	1/7/2016	20		**20**
	1/8/2016	5		**5**
F/V Sarah Ann	12/1/2015	4	1	**5**
R/V Alosa	12/18/2015		80	**80**
R/V Michael Craven	12/1/2015	2		**2**
Total		**81**	**243**	**324**

Table E. Summary of 12 sampling trips for commercial fish collections, samples tallied by location of capture.

Vessel	Season	Date	Location		Total
			Ipswich Bay	MA Bay	
F/V Ellen Diane	Spring	3/25/2015	8		**8**
		5/18/2015		60	**60**
	Summer	6/4/2015	25		**25**
		7/7/2015	50		**50**
		7/9/2015		16	**16**
	Fall	11/16/15	19		**19**
		11/22/2015	28		**28**
	Winter	12/1/2015	6		**6**
F/V Justice	Winter	1/7/2016		20	**20**
		1/8/2016		5	**5**
F/V Sarah Ann	Winter	12/1/2015		5	**5**
R/V Alosa	Winter	12/18/2015		80	**80**
R/V Michael Craven	Winter	12/1/2015		2	**2**
Total			**136**	**188**	**324**

Table F. Summary of 12 sampling trip for commercial fish collections, samples tallied by statistical area of capture.

Vessel	Season	Date	Statistical Area		Total
			513	514	
F/V Ellen Diane	Spring	3/25/2015	1	7	**8**
		5/18/2015		60	**60**
	Summer	6/4/2015	8	17	**25**
		7/7/2015	43	7	**50**
		7/9/2015		16	**16**
	Fall	11/16/15		19	**19**
		11/22/2015	1	27	**28**
	Winter	12/1/2015		6	**6**
F/V Justice	Winter	1/7/2016		20	**20**
		1/8/2016		5	**5**
F/V Sarah Ann	Winter	12/1/2015		5	**5**
R/V Alosa	Winter	12/18/2015		80	**80**
R/V Michael Craven	Winter	12/1/2015		2	**2**
Total			**53**	**271**	**324**

Table G. Summary of age and sex distribution of commercial fish samples from Ipswich Bay and Massachusetts Bay.

Age	Sex	Location		Total
		Ipswich Bay	MA Bay	
2	Female	1		**1**
	Male	3	4	**7**
3	Female	5	16	**21**
	Male	12	18	**30**
4	Female	22	33	**55**
	Male	26	29	**55**
5	Female	10	10	**20**
	Male	24	8	**32**
6	Female	5	1	**6**
	Male	10	4	**14**
7	Female	7		**7**
	Male	5		**5**
8	Male	5		**5**
Unknown	Female		39	**39**
	Male	1	24	**25**
	Unknown		2	**2**
Total		**136**	**188**	**324**

Appendix B – Additional Details of Genetics & Genomics Analyses

Table B1. Pair-wise population FSTs for comparisons of all commercial fishery collections with winter and spring spawning populations in the Gulf of Maine. Significantly differentiated comparisons are shown in bold.

IP_Sp	MB_Sp	IP_Win	MB_Win	CF_May	CF_June	CF_July1	CF_July2	CF_Mar	CF_Dec1	CF_Dec2	CF_Jan1	CF_Jan2	
0													IP_Sp
-0.0037	0												MB_Sp
0.0101	0.0089	0											IP_Win
0.008	0.0062	0.0018	0										MB_Win
0.0072	0.0019	0.0102	0	0									CF_May
0.0029	0.0062	**0.0214**	**0.0217**	**0.0206**	0								CF_June
0.0089	0.0035	**0.0292**	**0.0177**	0.0078	0.0012	0							CF_July1
-0.0021	0.0001	0.0047	0.0026	0.0014	**0.0221**	**0.012**	0						CF_July2
-0.0046	-0.006	-0.0027	-0.0032	0.0009	0.0039	**0.0099**	0.004	0					CF_Mar
0.0195	0.0028	0.0028	0.0075	0.0062	**0.0309**	**0.0299**	0.0012	0.018	0				CF_Dec1
0.0102	0.0023	0.0067	0.0042	0.0022	**0.0178**	0.0083	0.0023	-0.0269	0.0139	0			CF_Dec2
0.001	0.0042	0.0015	0.0015	0.0051	**0.0255**	**0.0218**	-0.0049	-0.0031	0.0068	0.0027	0		CF_Jan1
0.0054	**0.0118**	**0.0259**	**0.0281**	0.0224	**0.0175**	**0.0302**	0.0041	0.0264	-0.0309	0.0187	0.0094	0	CF_Jan2

Table B2. Results of accuracy testing for mixed stock analyses. Results of 100% simulations for mixture sample sizes of 50, 100, and 200 fish.

Mixture sample size = 50

	POPULATION ESTIMATES			REPORTING GROUP ESTIMATES		
	AVG	ST DEV	(95 PERCENT INT)	AVG	ST DEV	(95 PERCENT INT)
IP_Spring	0.3760	0.1307	(0.1130, 0.6033)	0.8355	0.0825	(0.6657, 0.9713)
MB_Spring	0.4885	0.1183	(0.2179, 0.7228)	0.9296	0.0529	(0.8184, 0.9998)
IP_Winter	0.2768	0.1179	(0.0520, 0.4732)	0.8981	0.0553	(0.7811, 0.9998)
MB_Winter	0.4684	0.1158	(0.2208, 0.6444)	0.8495	0.0803	(0.6895, 0.9799)

Mixture Sample size = 100

	POPULATION ESTIMATES			REPORTING GROUP ESTIMATES		
	AVG	ST DEV	(95 PERCENT INT)	AVG	ST DEV	(95 PERCENT INT)
IP_Spring	0.3562	0.0929	(0.1713, 0.5067)	0.8233	0.0590	(0.7113, 0.9272)
MB_Spring	0.4682	0.0879	(0.2733, 0.6162)	0.9369	0.0439	(0.8337, 0.9984)
IP_Winter	0.2721	0.0745	(0.1109, 0.3921)	0.9062	0.0498	(0.8018, 0.9792)
MB_Winter	0.4647	0.0840	(0.3141, 0.6229)	0.8485	0.0589	(0.7345, 0.9330)

Mixture Sample size = 200

	POPULATION ESTIMATES			REPORTING GROUP ESTIMATES		
	AVG	ST DEV	(95 PERCENT INT)	AVG	ST DEV	(95 PERCENT INT)
IP_Spring	0.3685	0.0582	(0.2444, 0.4611)	0.8225	0.0503	(0.7229, 0.8969)
MB_Spring	0.4914	0.0548	(0.3882, 0.5789)	0.9385	0.0276	(0.8790, 0.9838)
IP_Winter	0.2860	0.0579	(0.1844, 0.3921)	0.9037	0.0396	(0.8049, 0.9640)
MB_Winter	0.4781	0.0593	(0.3840, 0.5803)	0.8569	0.0393	(0.7820, 0.9294)

Table B3. Results of realistic fishery simulations for mixed stock analysis accuracy testing. Results are shown for 3 different mixture compositions (50-50, 75% winter, and 75% spring), for mixture sample sizes of 200. Similar results were obtained with smaller sample sizes, with slightly larger confidence intervals (data not shown).

50-50 Simulation

		ESTIMATES		
	ACTUAL VALUE	AVG	ST DEV	(95 PERCENT INT)
IP_Spring	0.2500	0.2533	0.0551	(0.1378, 0.3396)
MB_Spring	0.2500	0.2453	0.0485	(0.1560, 0.3350)
IP_Winter	0.2500	0.1739	0.0456	(0.0921, 0.2753)
MB_Winter	0.2500	0.3276	0.0589	(0.2179, 0.4211)
GROUPS				
Spring	0.5000	0.4986	0.0522	(0.3985, 0.5945)
Winter	0.5000	0.5014	0.0522	(0.3934, 0.5998)

75%Winter-25% Spring

		ESTIMATES		
	ACTUAL VALUE	AVG	ST DEV	(95 PERCENT INT)
IP_Spring	0.1000	0.1866	0.0504	(0.0833, 0.2802)
MB_Spring	0.1500	0.1376	0.0435	(0.0574, 0.2257)
IP_Winter	0.2500	0.2639	0.0505	(0.1782, 0.3430)
MB_Winter	0.5000	0.4118	0.0614	(0.2882, 0.5170)
GROUPS				
Spring	0.2500	0.3242	0.0560	(0.2257, 0.4233)
Winter	0.7500	0.6758	0.0560	(0.5657, 0.7677)

75% Spring- 25% Winter

		ESTIMATES		
	ACTUAL VALUE	AVG	ST DEV	(95 PERCENT INT)
IP_Spring	0.2500	0.3528	0.0688	(0.2401, 0.4802)
MB_Spring	0.5000	0.3603	0.0589	(0.2612, 0.4629)
IP_Winter	0.1000	0.0967	0.0363	(0.0324, 0.1648)

MB_Winter	0.1500	0.1901 0.0447 (0.1178, 0.2638)
GROUPS		
Spring	0.7500	0.7132 0.0444 (0.6360, 0.8033)
Winter	0.2500	0.2868 0.0444 (0.1923, 0.3639)

Table B4. Comparison of genetic diversity over time. Allelic richness values for each modern cod spawning population from Ipswich and Massachusetts bays in winter and spring and for each otolith collection. These data do not suggest a loss of genetic diversity in modern populations compared to historical ones, although the allelic richness of the two modern spring populations is lower than the two winter populations and lower than all five otolith collections, except one.

	Allelic Richness	
	Value	**SD**
Ipswich Bay - Spring	**9.9**	**1.6**
Mass. Bay - Spring	**9.1**	**1.3**
Ipswich Bay - Winter	**10.8**	**1.5**
Mass. Bay - Winter	**10.7**	**1.6**
OtoTime1-513	**10.8**	**1.5**
OtoTime1-514	**9.3**	**1.2**
OtoTime2-513	**10.3**	**1.6**
OtoTime2-514	**10.8**	**1.8**
OtoTime2-515	**10.0**	**1.3**

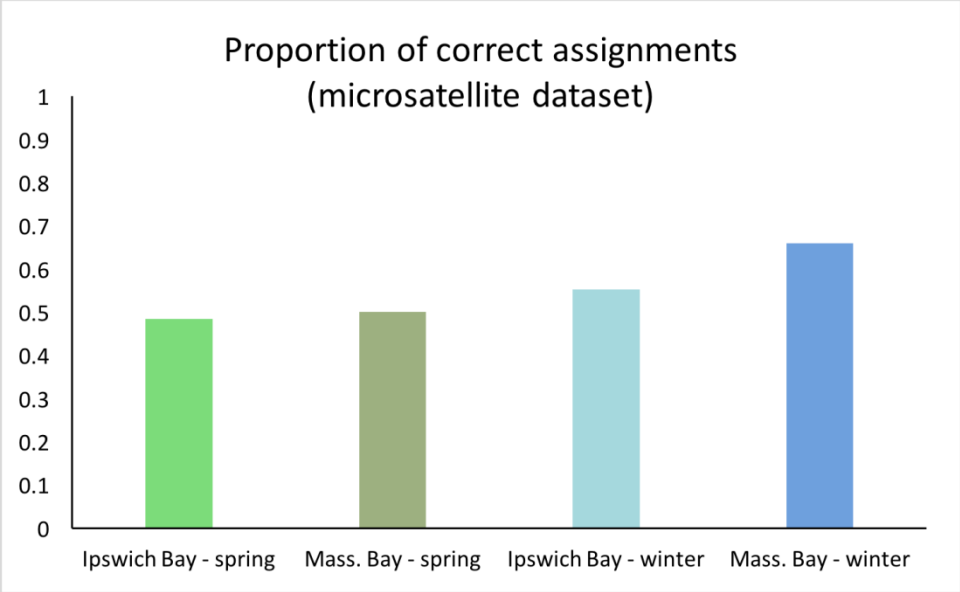


Fig. B1. Proportion of correct assignments of cod to the specific season and bay (i.e. seasonal and geographic distinction) based on the microsatellite data. These results indicate relatively low resolution to assign individuals accurately to their specific bay of origin, compared to the much higher resolution assignments to seasonal spawning group (winter vs. spring).

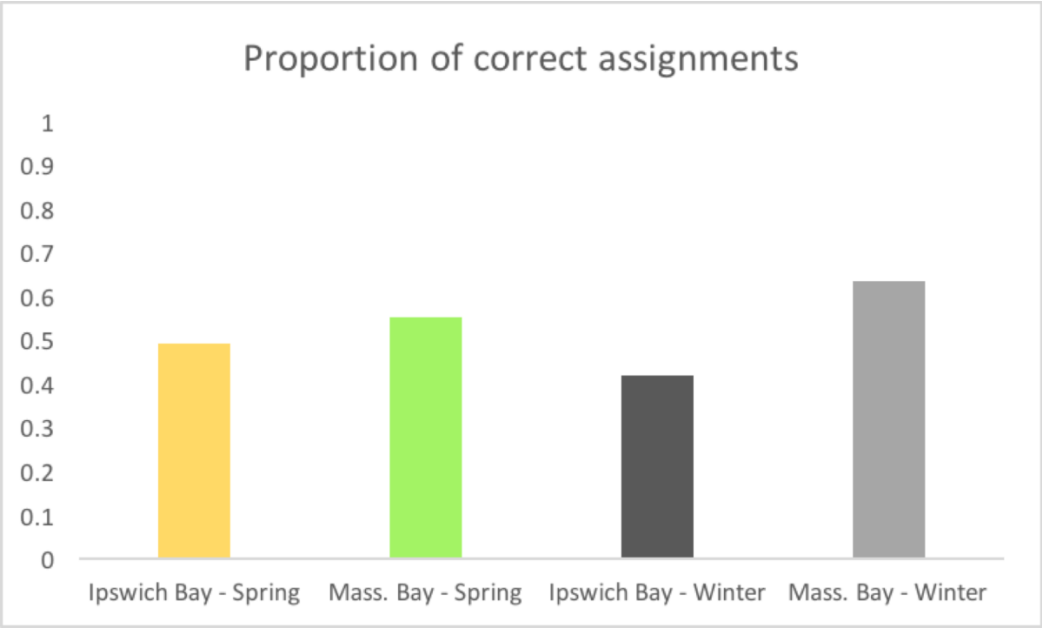


Fig. B2. Proportion of correct assignments of cod to season and bay, based on the combined microsatellite dataset of Kovach et al. 2010 with the dataset in this study (this combined dataset is the baseline for the mixed stock analyses, below).

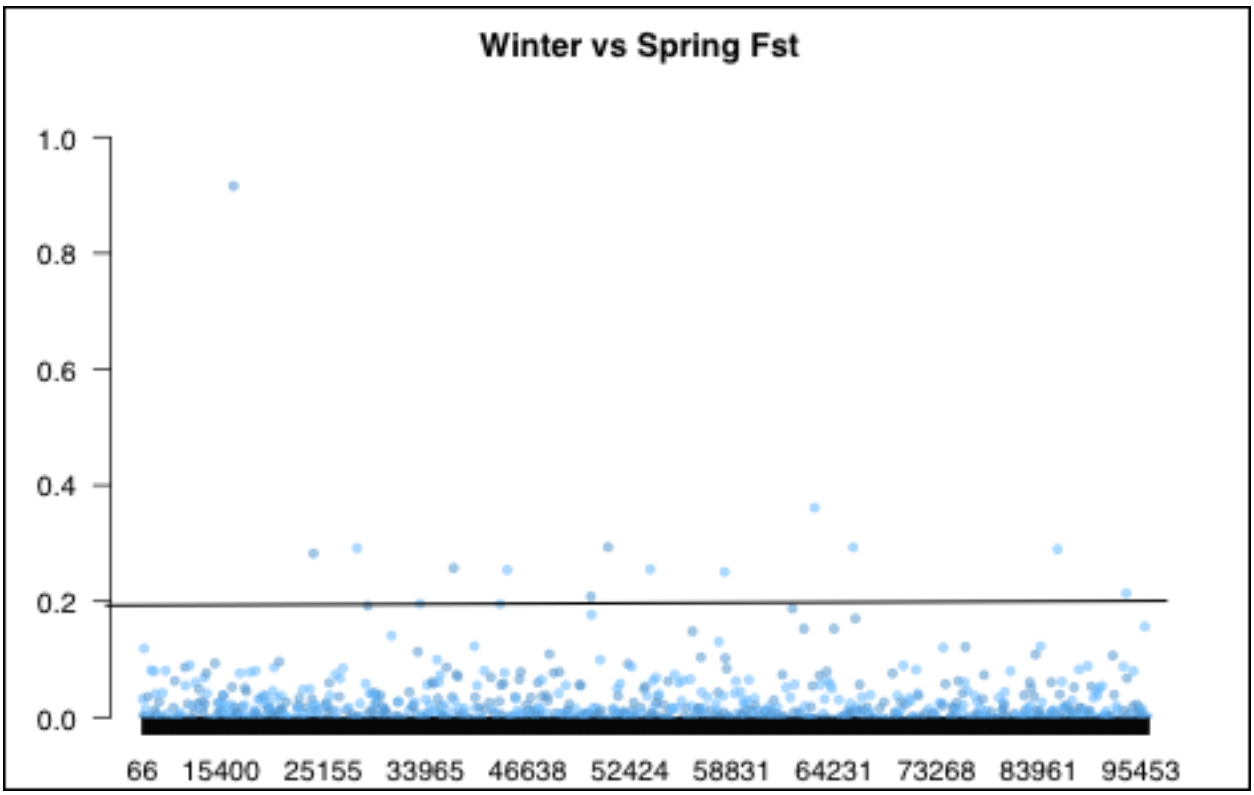


Fig. B3. Distribution of the FST values (Y axis) of all SNPs (Single Nucleotide Polymorphisms) identified in this study. The black horizontal line represents the 95% percentile of all FSTs (0.195). SNPS that were above this 95% cut-off value were considered outliers, and thereby potential candidate markers for genes under the influence of natural selection (adaptive loci).

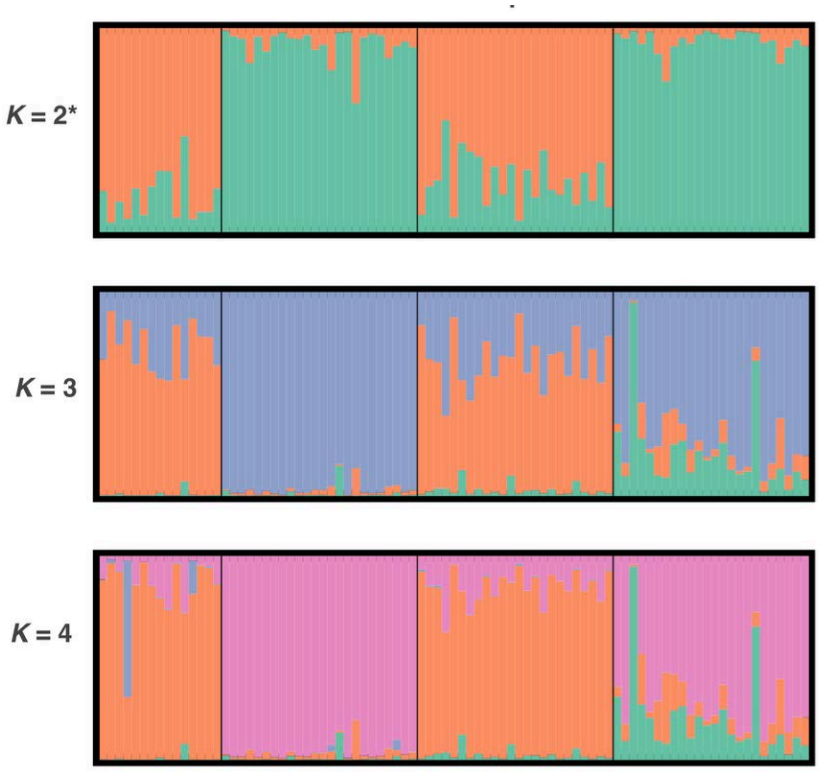


Fig. B4. Results of Bayesian clustering analyses using program STRUCTURE for the 4 cod populations, from left to right: Ipswich Bay spring, Ipswich Bay winter, Massachusetts Bay spring, and Massachusetts Bay winter. Each vertical bar represents the genetic ancestry of an individual fish. The different colors indicate genetically distinct groupings. Results are displayed from top to bottom for 2, 3, and 4 populations; the solution for 2 populations (top graph) received the most support from the data. These results show that the populations cluster primarily by season (similarity of the two spring pops and the winter pops is much more pronounced than the similarity of the two samples from the same spawning locations). Finer scale spatial differentiation is also apparent (at k = 3 and 4), especially between the two winter populations, with a unique signal in the Massachusetts Bay winter.

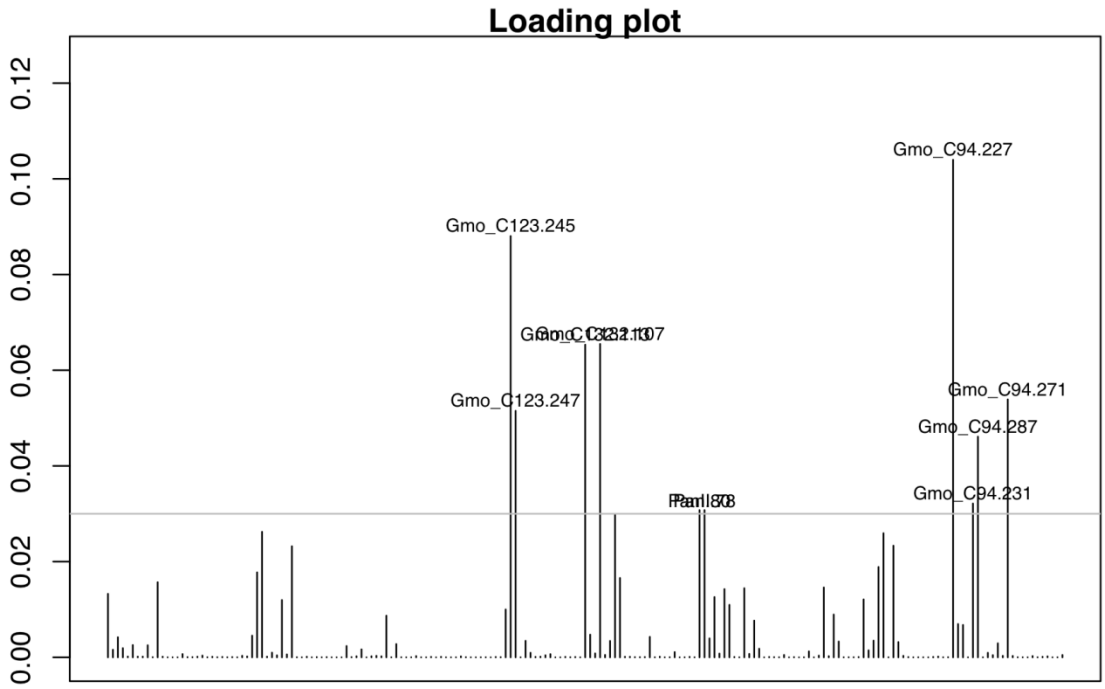


Fig. B5. Loading plots of microsatellite alleles used in this study, indicating which loci were most informative (had highest loadings showing greatest differentiation of populations, FST on the Y axis). Alleles from four loci had significantly higher loadings: GmoC123, GmoC94, Pan I, and Gmo132. These loci are suspected to be non-neutral – i.e., linked to genes under natural selection.

APPENDIX C: Details of otolith chemistry and structure analysis.

Introduction

Atlantic cod is an iconic species in New England, renowned as the motivation for the first settlement of these shores and an integral part of the social and economic fabric of our coastal communities. Cod is a highly desired source of seafood and historically was a greater component of the New England groundfish fishery and economy. Today, however, the fishery for cod is essentially closed and persists only as a bycatch fishery. The most recent assessment of stock status for Gulf of Maine cod indicates that the stock is overfished and overfishing is occurring (NEFSC 2017). Complex spatial structure and population diversity of Atlantic cod are known to be key factors contributing to the resilience and persistence of this fishery resource (Frank and Brickman 2000, Hutchinson 2008, Kerr et al. 2014). Currently, these features are compromised in US waters due to historical patterns of exploitation that resulted in extirpation of local spawning components (Ames et al. 2004). This phenomenon may be an underlying factor limiting the capacity of cod populations to rebuild. However, there also appears to have been a fundamental shift in the productivity of Atlantic cod due to climate change in the region and the resource may no longer have the capacity to rebuild to historic levels, even with extreme reductions in harvesting activities (Pershing et al. 2015). Managing the recovery of cod populations will require an improved understanding of existing population structure and connectivity of cod in US waters, as well as the unique attributes (e.g., spatial behavior and habitat preferences) of these remaining groups of fish and how they might respond to a changing climate.

Genetic analysis of Atlantic cod (Lage et al. 2004, Wirgin et al., 2007, Kovach et al. 2010) has revealed stock complexity that is not aligned with the Gulf of Maine and Georges Bank management units of cod (Figure 1). Three spawning complexes were identified: 1) a northern spawning complex, which spawns in inshore Gulf of Maine waters (off of western Maine to Massachusetts Bay) in the spring; 2) a southern spawning complex, which spawns in inshore and nearshore water from the Gulf of Maine to southern New England (from Ipswich Bay to southern New England waters, including the Great South Channel) in the winter and early spring; and 3) a population that spawns offshore on northeast peak of Georges Bank in the early spring. Information on cod movement patterns obtained from a large-scale tagging effort support this paradigm of population structure informed by genetics (Tallack et al. 2009, Zemeckis et al. 2014). The presence of two temporally distinct spawning populations (winter and spring) within the Gulf of Maine stock area that are genetically distinct (Kovach et al. 2010) was unexpected, based on previously held assumptions about connectivity of marine fish, and may reflect differences in the scale of habitat use and spatial behavior of these groups of fish indicative of alternative life history types.

There is a considerable body of work that has established the utility of otolith chemistry as a useful natural marker of fish stock structure (including alternative life history types) and tracer of fine-scale habitat use of fish (Campana 2005; Elsdon et al. 2008; Kerr and Campana 2014). Otoliths are composed of calcium carbonate that accretes throughout the lifetime of the fish, preserving a detailed record of the chemistry of the environment experienced by an individual fish (Campana 1999). This application depends on geographic variation in water chemistry (e.g., coastal vs. offshore gradients) or other factors (e.g., temperature, salinity) that influence the chemistry of the otolith such that fish that inhabit different environments exhibit differences in their otolith chemical composition (Thresher 1999; Secor et al. 2001; Campana 2005; Kerr et al. 2007). In the past, the assumed homogeneity of the marine environment and its water chemistry was thought to preclude application of otolith chemistry in this manner. However, recent research has revealed significant otolith chemistry differences in coastal and marine fish, enabling the study of fine-scale population structure (e.g., Warner et al. 2005; Jónsdóttir et al. 2007; Svedäng et al. 2010; Thorrison et al. 2011). Of specific relevance is work by Campana and Gagne (1995) and D'Avignon and Rose (2012) that identified significant differences in the elemental concentration of Atlantic cod otoliths collected from spawning grounds in the northwest Atlantic using elemental fingerprints (i.e. the unique otolith chemistry signature that characterizes the fish stock). The presence of different elemental fingerprints among groups of fish implies different environmental histories and consequently can serve as an indicator of stock identity or life history type (Campana 2005; Kerr and Campana 2014).

The goal of this research was to characterize the ecological diversity (i.e., habitat use and spatial behavior) of two major spawning complexes of Atlantic cod in the Gulf of Maine and evaluate how the Gulf of Maine cod fishery interacts with these groups today and in recent history. To test the hypothesis that winter and spring spawning fish represent distinct ecological units, we applied otolith chemistry to winter and spring cod collected in spawning condition in recent years. To evaluate the relative contribution of these ecological units of cod to the fishery over time, we analyzed the otolith chemistry of cod sampled as part of the commercial fishery today and historically and compared values to our characterization of winter and spring cod.

Methods

Overall, 588 Atlantic cod samples collected in the Gulf of Maine were analyzed for their otolith chemistry (Table 1). Three types of Atlantic cod samples were collected to address our research questions: 1) spawning fish collections (2012-2016), 2) modern fishery collections (2015-2016), and 3) historical fishery collections over two time periods (1979-1982 and 1988-1992) from three statistical areas (513, 514, and 515).

Spawning fish collections

Atlantic cod were collected in two areas (Ipswich Bay and Massachusetts Bay) and at two spawning times (spring and winter) to represent distinct cod spawning complexes (Figure 2). A total of 131 fish were sampled across five years (Table 2). Winter spawners were collected in Ipswich Bay in December of 2014 and 2015 and in Massachusetts Bay on seven dates, ranging from November to January in 2012 to 2015. Spring spawners we collected in Ipswich Bay in May (2014 - 2015) and in Massachusetts Bay on six dates from May to June (2012-2015).

All of the Ipswich Bay samples were collected at known spawning locations in collaboration with our fishing industry partner, Captain David Goethel, from his vessel, the *F/V Ellen Diane* (n = 73). Collections were made using a commercial groundfish bottom otter trawl deployed for approximately 60-minute tows at three knots, although the specific tow durations were determined by the captain based on the bottom characteristics and concentrations of fish determined using a commercial fishfinder. Spawning cod samples from Massachusetts Bay were collected aboard Massachusetts Division of Marine Fisheries' research vessel *R/V Alosa* or contracted commercial fishing vessels using bottom otter trawl, rod and reel, or longline gear (n = 58). Length to the nearest centimeter, sex, and maturity stage (Morse 1977 unpublished, described in Burnett et al. 1989) were recorded for all fish. Weight to the nearest gram was also recorded for Ipswich Bay fish.

Modern commercial fishery collections

A total of 187 fish were collected as representative samples of the modern groundfish fishery. We focused sampling in two statistical areas (513 and 514), areas from which the majority of commercial fishing landings have come from in the past decade, including >75% landings in 2011 (NEFSC 2013; Figure 3, Table 3). The originally proposed sampling plan for collecting cod from the modern commercial fishery involved sampling fish caught as part of normal harvesting by the *F/V Ellen Diane*. However, the Gulf of Maine Cod and Haddock 2014 Interim and Emergency Actions enacted by the National Marine Fisheries Service halted the directed fishery for cod in the months before sampling was to be conducted (Department of Commerce 2014 and 2015). As such, we acquired a Scientific Research Letter of Acknowledgement from the NMFS to allow Capt. Goethel and the *F/V Ellen Diane* to conduct sampling activities that mimicked normal commercial fishing operations in 2015 and 2016. Some fish representing the modern commercial fishery were in spawning condition and were included in this project due to the fact that commercial fishermen target spawning aggregations in both Ipswich Bay and Massachusetts Bay when closures are not in effect (Table 4).

Historical commercial fishery otolith collections

A total of 270 otoliths were collected from the NMFS archives at Woods Hole. These otoliths were port-sampled from commercial vessels during two time periods, 1979-1982 and 1988-1992. Samples were selected to represent and characterize commercial landings in the past, with focus on statistical areas that comprised at least 75% of the commercial landings during the 1979-1981 (statistical areas 513 and 514) and the 1989-1991 (statistical areas 513, 514, and 515) time periods (NEFSC 2013; Table 5). Landing date, statistical area, and length data were available for all samples.

Otolith ageing and structural analysis

Otoliths were removed from fish, cleaned of adhering tissue, and stored dry. One sagittal otolith from each fish was embedded in epoxy resin (Buehler EpoHeat 2 Epoxy Resin) and sectioned with a Buehler IsoMet low speed saw to 1.0 mm thickness. Otolith thin sections were mounted on glass slides using SPI Crystalbond 509 adhesive polished with fine-grit polishing paper (P600, 1200) and alumina powder (Buehler MicroPolish II: 0.3 micron) until annuli were clearly visible. Thin sections were photographed using an image analysis system (MicroPublisher 3.3 RTV camera mounted on a Nikon SMZ800 dissecting microscope at 2x magnification). If needed, further image enhancement was conducted with Image-Pro Premier 9.2 software. Each section was aged twice from photos, and if there was disagreement between the first and second age assignment, a third read was conducted and accepted if in agreement with a prior age assignment (Figure 4). Using photos, measurements of annual growth were conducted using ImagePro Premier 9.3 (Media Cybernetics, Rockville, MD) software.

Otolith elemental analysis

In preparation for elemental analysis, otolith sections were cleaned by rinsing with deionized water three times and air-dried for 24 h under a laminar-flow hood. Elemental analysis was conducted using the Thermo Elemental 2 ICP-MS coupled to a New Wave Research UP 193 nm excimer laser ablation system at Woods Hole Oceanographic Institution Plasma Facility. Otolith sections were pre-ablated to remove possible surface contamination from otolith sections (35-µm spot diameter, 25 µm s^{-1} scan speed). Otoliths of spawning fish were ablated along the longest growth axis in a transect from the edge of otolith through the core to the opposite edge of year one otolith growth. The laser was run at a 30 µm spot diameter, 10 µm s^{-1} scan speed, and 10% power. Otoliths of modern commercial fishery collected fish were ablated across the expanse of the core, defined here as age one growth. Otoliths were analyzed for a suite of isotopes, including ^{25}Mg, ^{48}Ca, ^{55}Mn, ^{88}Sr, ^{138}Ba, ^{114}Cd, ^{68}Zn, and ^{63}Cu. The intensity of isotopes in each sample was measured as counts per second (cps). An instrument blank (1% nitric acid; HNO_3) and a standard (MACS-3 calcium carbonate reference material; USGS 2013) were run before

each set of two otolith samples.

Otolith Chemistry Data Reduction

Raw data (isotope intensity in counts per second) were filtered to remove non-sample or erroneous portions of blank, standard, and sample runs. Stable portions of standard and blank runs were characterized as mean isotope intensities. Background corrections for samples were based on the calibration blank run before the sample in sequence. Isotope intensities (cps) of individual samples were blank-corrected by subtracting isotope intensities of the instrument blank that preceded the sample in sequence:

$$I_i^{Oto} = I_i^{Rawoto} - I_i^{Bl}$$

where $I_i^{Rawoto}$is the raw isotope intensity output (cps) of the otolith sample and I_i^{Bl}in the isotope intensity output of the blank.

The MACS-3 calcium carbonate reference material (USGS 2013) was used to convert background-corrected intensity counts to concentrations (µg g^{-1}) and correct for instrument drift. Calcium was used as an internal standard to compensate for signal variation caused by differences in mass of ablated material and all elements were expressed as molar ratios relative to ^{48}Ca. Concentration of the isotopes in otolith samples was calculated as:

$$C_i^{Oto} = C_{IS}^{Oto} * \frac{I_i^{Oto}}{I_{IS}^{Oto}} * \frac{C_i^{STD}}{C_{IS}^{STD}} * \frac{I_{IS}^{STD}}{I_i^R}$$

where

C_i^{Oto} : concentration of isotope *i* in the sample
C_{IS}^{Oto} :concentration of internal standard in the sample
I_i^{Oto} :background-corrected signal (cps) intensities for isotope *i* in the sample
I_{IS}^{Oto} :background-corrected signal (cps) intensities for internal standard in the sample
C_{IS}^{STD} :concentration of internal standard in reference standard
C_i^{STD}:concentration of isotope *i* in reference standard
I_{IS}^{STD} :background-corrected signal (cps) intensities for the internal standard in MAC-3 standard
I_i^{STD} :background-corrected signal (cps) intensities for isotope *i* in reference standard

Results are expressed as absolute concentrations of elemental molar ratios with respect to calcium: Element:Ca ratios, expressed as units of mmol/mol or µmol/mol. Not all elements were suitable for post-processing analysis. To be included the elemental concentration had to above the LOD for 80% of the samples. ^{114}Cd was below detection limits >80% of the samples.

Otolith Ablation Measurements

Following ablation, photos of each otolith were taken with ImagePro software to measure the distance along the trough produced by laser ablation. Ablation transects were aligned with annuli distances from photographs to calculate Element:Ca ratios for each year of life for each fish. Point measurements were related to the annulus or interannular material they sampled and chronologies of isotope ratios.

Statistical Analysis

The median and coefficient of variation of isotope ratios were calculated for year-1 and whole otolith growth for individual fish. Values were compared across spawning time (winter, spring) and location of collection (Massachusetts Bay, Ipswich Bay) using a two-way MANOVA and univariate response were examine using ANOVA. Diagnostics were examined to test the assumptions of these models. Multivariate linear discriminant analysis with jackknifed (i.e., leave one out) predictions was used to test the classification success of individuals to their respective origin based on isotope ratios (age one and whole). We conducted stepwise linear discriminant function analyses, using different combinations of elements, to identify the combination that provided the best classification success. Data were inspected for outliers and residuals were evaluated to determine if they conform to a multivariate normal distribution.

All statistical analysis was conducted using the R statistical programming environment (R Development Core Team 2017).

Results

Spawning Fish

Demographics

Overall, the age of Atlantic cod in our sample of spawning fish ranged from 2 to 9, with winter spawners ranging from 2 to 7 and spring spawners from 3 to 9 (Table 6, Figure 5). Mean age was significantly different based on time of spawning ($p < 0.001$), but did not differ by capture location or by the interaction of location and spawning time (Table 7). Overall, spring spawners tended to be older than winter spawners, which show a younger and more truncated age structure (Table 6). The length of Atlantic cod in our sample ranged from 36 to 105 cm. Mean length was significantly different based on location ($p = 0.004$), but did not differ by spawning time or the interaction of location and spawning time (Table 7, Figure 6). The overall sex ratio of winter spawners was close to 50:50 and spring spawners was selected to be relatively similar (Table 8).

Otolith Structure

The relative growth of otolith annuli is known to be related to growth of fish. Changes in fish and otolith growth are expected to occur ontogenetically, but will also vary spatially and temporally in response to the different ocean conditions experienced. Large differences in the width of the first annuli of winter and spring spawners have been recognized in winter and spring spawners in Massachusetts Bay (M. Dean pers comm.). In this study, we identified significant differences in otolith growth of winter and spring spawning cod across ages one to five, with the exception of age 3 growth (Table 9, 10, Figure 7). Significant differences in cod otolith growth were also evident between locations and the interaction of location and spawning time for age one and two (Table 9, 10, Figure 7). The largest differences in growth were evident in the width of the age one annulus between winter and spring spawning cod (Table 11, Figures 7, 8). Differences in age one otolith growth are related to the spawning phenology of cod and how we define the first annulus of an otolith. We count one opaque and translucent zone as a year and, because of their time of spawning, winter spawned fish experience a longer growing period (e.g. December to January) compared to spring spawners (e.g. May to January) during what we call age one. We also expect that winter and spring spawners experience very different early growth conditions due to starting life at different time periods in seasonally variable Gulf of Maine waters which also likely influences differences in early growth.

Applying a discriminant function analysis classification approach with jackknife prediction to otolith growth information we were able to assign winter and spring spawners to their known spawning group with reasonable classification accuracy using increment width across all ages (~66%), but we achieved considerably higher classification accuracy when only relying on age one otolith increment width (78%). Classification accuracy of fish to location (54%) and spawning time at location (44%) based on age one otolith growth was considerably lower (Table 12).

Our sample of spawning fish encompassed several recent year classes, with reasonable samples sizes in the years 2008 to 2012 for the purpose of comparison of age one increment widths across years. Significant differences in age one growth were found based on spawning group, year class, and the interaction of spawning group and year class (Table 13). No significant differences were found based on capture location or the interaction of this term. Winter spawners exhibited increasingly higher growth over this short time period compared to spring spawners (Figure 9).

Otolith Core Chemistry

Significant differences in the median values of the suite of elemental ratios measured in age one growth of cod otolith were evident for the main factors of spawning time and capture location, as well as the interaction of these factors (Table 14, Figure 10, 11). An examination of the individual response of isotopes indicated significant differences in Sr:Ca, Mg:Ca, Ba:Ca, and

Mn:Ca based on spawning time (Table 15). Spring spawners had lower Ba:Ca, Mg:Ca, and Mn:Ca values and higher Sr:Ca values compared to winter spawners (Table 16). Based on laboratory-based associations between these elemental ratios in cod otoliths with water temperature (Stanley et al. 2015), we can infer that spring spawners experienced a colder thermal environment during the period age one year of life. In addition, significant differences were identified in Mn:Ca and Cu:Ca based on capture location and significant differences in Sr:Ca and Cu:Ca based on the interaction of these factors (Table 15). Significant differences in the CV of Sr:Ca and Ba:Ca ratios in age one growth of cod otoliths were also evident between fish based on the spawning time of fish (Table 17).

Linear discriminate function analysis with jackknife prediction was used to classify fish based on age one otolith chemistry to spawning time (winter and spring), location (Ipswich and Massachusetts Bay), and the interaction of these factors (spawning time*capture location). Stepwise linear discriminate function analysis was used to select the parameters providing the most discrimination based on scale of classification. Classification success of Atlantic cod to spawning time was relatively high at 74%, compared to classification rates to capture location (48%), and to spawning times within each location (46%, Table 18, Figure 12).

Whole Otolith Chemistry

Significant differences in the combined chemistry of whole cod otolith growth were evident for the main factors of spawning time ($p<0.001$) and the interaction of these factors ($p < 0.001$) based on a two-way MANOVA (Table 19). Examination of elemental ratios indicated significant differences Sr:Ca, Ba:Ca, Mg:Ca, Mn:Ca, and Cu:Ca based on spawning time, and significant differences in Sr:Ca, Ba:Ca, Mg:Ca, and Mn:Ca based on location, and a significant interaction for Cu:Ca (Table 20). Significant differences in CV elemental ratios were also identified with respect to spawning time and capture location MANOVA (Table 21). Significant univariate differences in the CV of Sr:Ca and Ba:Ca ratios in whole growth of cod otoliths were evident between fish based on the spawning time and between capture location for Cu:Ca (Table 22).

Linear discriminate function analysis with jackknife prediction was used to classify fish based on whole otolith chemistry to spawning time (winter and spring), and to location (Ipswich and Massachusetts Bays). Classification success of Atlantic cod to spawning time was higher (70%), than classification rate to capture location (65%), or classification rate to spawning times within each location (44%, Table 23). Classification accuracy of spawners based on their whole otolith chemistry was slightly lower than the classification rate based on age one otolith chemistry alone.

Otolith Chemistry at Age

Otolith chemistry was summarized across each age of growth in the otolith that had sufficient sample representation (ages 1-5) and was compared across spawning times and locations. All elemental ratios (Sr:Ca, Ba:Ca, Mg:Ca, Mn:Ca) demonstrated differences across age, indicative in ontogenetic changes in habitat use (Tables 24, Figures 13, 14). Significant differences in otolith chemistry at age were identified between spawning groups based on Sr:Ca, and differences in chemistry at age between capture locations was identified based on Mg:Ca, and Mn:Ca demonstrated differences at age between spawning time and location (Table 24). Further examination of otolith elemental ratio differences at age revealed differences in Sr:Ca values for fish age 3-5, in Mn:Ca values for age 3-4, and in Ba:Ca values for fish age 4 between spawning times within location (Table 25). The fact that differences extend beyond the first year of life supports the hypothesis that winter and spring spawning groups from each location experience different habitats over their lifetime aligns with ecotype differences.

Modern Commercial Fish

Mixed stock analysis was conducted Core otolith chemistry of fish from modern and historical fishery collections were used to classify individuals to either the winter or spring spawning group using modern spawning fish as a baseline sample. The baseline used for classification was the age-one chemistry of modern collections of known winter and spring spawners. Baseline classification accuracy based on a random forest approach was 73%. Random forest classification of mixed stock fishery samples from the modern fishery (n = 187; 2015-2016) indicate that 57% of the fish sampled were winter spawning fish (Figure 15). Across years (2015-2016), the proportion of winter spawners in the sample ranged from 55 to 65% (Figure 15). Across seasons, the proportion of winter spawners in the sample was consistent at approximately 60%, with the exception of summer which was dominated by spring spawners (58%, Figure 15). Across statistical areas, we see a more pronounced difference, with fish caught in area 513 being dominated by spring spawners (64%) and 514 dominated by winter spawners (62%).

Historical Commercial Fish

Similar to modern commercial fish collections, the core otolith chemistry of fish from historical fishery collections were used to classify individuals to either the winter or spring spawning group using modern spawning fish as a baseline sample. This assumes that the modern sample is representative of historical winter and spring spawners. Classification of historical fishery samples indicate a lower proportion of winter fish in the early 1980s (42% winter spawners, n=117) and 1990s (38% winter spawners, n =153; Figure 16). Across years, the proportion of winter spawners in the sample ranged from 14 to 48% (Figure 16). Across statistical areas,

the proportion of winter spawners in the sample ranged from 56% in 514 to 62 in 513 (Figure 16).

Conclusions

Overall, we found robust differences in otolith growth and chemistry between winter and spring spawners, with more subtle differences based on location. Age one differences in growth and chemistry offered the best classification accuracy for the purpose of stock identification. Significant differences in otolith growth and chemistry suggest that winter and spring spawning groups from each location experience different habitats over their lifetime and is suggestive of ecotype differences.

Otolith chemistry analysis revealed the modern fishery samples were composed of more winter spawning fish (57%) than spring. Classification of historical fishery samples indicate a lower proportion of winter fish in the early 1980s (42% winter spawners) and 1990s (38% winter spawners). Together, mixed stock analysis of modern and historical fishery collections suggest an increase in the proportion of winter spawners in fishery samples during the recent time period. It is important to note the small sample size and that these trends may not be representative of the overall mixed stock composition of the fishery over time. Furthermore, the results are contingent on the modern baseline being suitable for mixed stock assignment of fish to spawning time from historical collections.

References

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

Burnett, J., O'Brien, L., Mayo, R. K., Darde, J., Bohan, M. (1989). Finfish maturity sampling and classification schemes used dueing Northeast Fisheries Center bottom trawl surveys, 1963-1989. NOAA Technical Memorandum NMFS-F/NEC-76, iii + 14p.

Campana, S. E.& Gagne, J. A. (1995). Cod stock discrimination using ICPMS elemental assays of otoliths. In *Recent Developments in Fish Otolith Research* (Secor, D. H., Dean J. M. &. Campana S.E, eds.), 671-691. University of South Carolina Press. Columbia, S.C.

Campana, S.E. (1999). Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Marine Ecology Progress Series* **188**, 263-297.

Campana, S. E. (2005). Otolith elemental composition as a natural marker of fish stocks. In *Stock Identification Methods* (Cadrin, S. X., Friedland, K. D. & Waldman, J. R., eds.), 227-245. New York: Academic Press.

D'Avignon, G. & Rose, G. A. (2013). Otolith elemental fingerprints distinguish Atlantic cod Spawning areas in Newfoundland and Labrador. *Fisheries Research* **147**, 1-9.

Department of Commerce (2014). Northeast Groundfish Fishery; Fishing Year 2014; Emergency Gulf of Maine Cod Management Measures. Federal Register, 79, (219), 67362-67371.

Department of Commerce (2015). Magnuson-Stevens Fishery Conservation and Management Act Provisions; Fisheries of the Northeastern United States; Northeast Groundfish Fishery; Framework Adjustment 53. Federal Register 80(84), 25110-25143.

Elsdon, T. S., Wells, B. K., Campana, S. E., Gillanders, B. M., Jones, C. M., Limburg, K. E., Secor, D.H., Thorrold, S.R., Walther, B.D. (2008). Otolith chemistry to describe movements and life-history parameters of fishes: hypotheses, assumptions, limitations, and inferences. *Oceanography and Marine Biology Annual Review* **46**, 297-330.

Frank, K. T. & Brickman, D. (2000). Allele effects and compensatory population dynamics within a stock complex. *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 513-517.

Hutchinson, W. F. (2008). The dangers of ignoring stock complexity in fishery management: the case of the North Sea cod. *Biology Letters* **4**, 693-695.

Jónsdóttir, I. G., Marteinsdottir, G., Campana, S. E. (2007). Contribution of different spawning components to the mixed stock fishery for cod in Icelandic waters. *ICES Journal of Marine Science* **64**, 1749-1759.

Kerr, L. A., Secor, D. H., Kraus, R. T. (2007). Stable isotope (δ13C and δ18O) composition of

otoliths as a proxy for environmental salinity experienced by an estuarine fish. *Marine Ecology Progress Series* **348**, 245-253.

Kerr, L. A. and Campana, S. E. 2014. Chemical composition of fish hard parts as a natural marker of fish stocks. In: Cadrin, S. X., Kerr, L. A., and Mariani, S. (ed). Stock Identification Methods. 2nd ed. Academic Press, San Diego, CA. P. 205–234.

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

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

NEFSC (Northeast Fisheries Science Center) (2013). 55th Northeast Regional Stock Assessment Workshop (55th SAW) Assessment Summary Report. US Dept Commer, Northeast Fisheries Science Center Reference Document. 13-01; 41 p. Available from: National Marine Fisheries Service, 166 Water Street, Woods Hole, MA 02543-1026, or online athttp://www.nefsc.noaa.gov/publications/.

NEFSC (Northeast Fisheries Science Center) (2017). Operational Assessment of 19 Northeast Groundfish Stocks, Updated Through 2016. US Department Commerce, Northeast Fish Science Center Reference Document 17-17; 259 p. doi: 10.7289/V5/RD-NEFSC-17-17.

Pershing, A. J., Alexander, M. A., Hernandez, C. M., Kerr, L. A., Le Bris, A., Mills, K. E., Nye, J. A., Record, N. R., Scannell, H. A., Scott, J. D., Sherwood, G. D., Thomas, A. C. (2015). Slow adaptation in the face of rapid warming leads to collapse of the Gulf of Maine cod fishery. *Science* **350** (6262), 809-812.

Secor, D. H., Rooker, J. R., Zlokovitz, E., Zdanowicz, V. S. (2001). Identification or riverine, estuarine, and coastal contingents of Hudson River striped bass based upon otolith elemental fingerpringts. *Marine Ecology Progress Series* **211**, 245-253.

Svedäng, H., André, C., Jonsson, P., Elfman, M., Limburg, K. E. (2010). Migratory behaviour and otolith chemistry suggest fine-scale sub-population structure within a genetically homogenous Atlantic Cod population. *Environmental Biology of Fishes* **89**(3-4), 383-397.

Tallack, S. (ed.) (2009). Proceedings from a workshop to identify future research priorities for cod tagging in the Gulf of Maine, 12 February 2009. Northeast Fisheries Science Center Reference Document 09-09, Woods Hole, MA.

Thorisson, K., Jonsdottir, I. G., Marteinsdottir, G., Campana, S. E. (2011). The use of otolith

chemistry to determine the juvenile source of spawning cod in Icelandic waters. *ICES Journal of Marine Science* **68**, 98-106.

Thresher, R. E. (1999). Elemental composition of otoliths as a stock delineator in fishes. *Fisheries Research* **43**, 165-204.

USGS 2013. MACS-3 standard values

Warner, R. R., Swearer, S. E., Caselle, J. E., Sheehy, M. S. & Paradis, G. L. (2005). Natal trace elemental signatures in the otoliths of an open-coast fish. *Limnology and Oceanography* **50**, 1529-542.

Wirgin, I., Kovach, A., I., Maceda, L., Roy, N. K., Waldman, J., Berlinsky, D. L. (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.

Zemeckis, D. R., Martins, D., Kerr, L. A., Cadrin, S. X. (2014). Stock identification of Atlantic cod (*Gadus morhua*) in US waters: an interdisciplinary approach. *ICES Journal of Marine Science* **71(6)**, 1490-1506.

Table 1. Atlantic cod samples analyzed for otolith chemistry grouped by sample type (spawning, modern commercial and historical commercial), statistical area, and time period.

Sample Type	**Location/Stat Area**	**Time Period**			**Total**
		1972-1982	1988-1992	2012-2016	
Spawning	513			10	10
	514			121	121
	Total			131	131
Modern Commercial	513			32	32
	514			155	155
	Total			187	187
Historical Commercial	513	60	56		116
	514	56	56		112
	515		42		42
	Total	116	154		270
Total		116	154	318	588

Table 2. Spawning cod otoliths analyzed from spring and winter spawning aggregations in Ipswich and Massachusetts Bays between 2012 and 2016.

Location	**Season**		**Total**
	Spring	Winter	
Ipswich Bay	34	34	68
Mass. Bay	30	33	63
Total	64	67	131

Table 3. Some fish that we captured during surveys of spawning aggregations for other projects were used in this project and included in the commercial sample dataset in order to increase that sample size. These fish are representative of historical fishing practices that target spawning aggregations.

Vessel	Date	Spawners Added to Commercial Dataset		Total
		Yes	No	
F/V Ellen Diane	3/25/2015		8	8
	5/18/2015		60	60
	6/4/2015		25	25
	7/7/2015		50	50
	7/9/2015		16	16
	11/16/2015	19		19
	11/22/2015	26	2	28
	12/1/2015	5	1	6
F/V Justice	1/7/2016	19		19
	1/8/2016	5		5
F/V Sarah Ann	12/1/2015	4	1	5
R/V Alosa	12/18/2015		80	80
R/V Michael Craven	12/1/2015	2		2
Total		80	107	187

Table 4. Modern commercial fishery samples collected from statistical areas 513 and 514 from 2015 to 2016.

Statistical Area	Season				Total
	Winter	Spring	Summer	Fall	
513			31	1	32
514	49	44	18	44	155
Total	49	44	49	45	187

Table 4. Otoliths from the Northeast Fisheries Science Center otolith archive that were sampled to represent catches of Atlantic cod during two time periods (1972-1982 and 1988-1992) in the historical commercial fishery.

Time Period	Statistical Area			Total
	513	514	515	
1979-1982	60	56		116
1988-1992	56	56	42	154
Total	116	112	42	270

Table 6. Count of spawning cod samples by age, season, and location.

Age	Spawning Season				Total
	Spring		Winter		
	Ipswich Bay	Mass. Bay	Ipswich Bay	Mass. Bay	
2			3	5	8
3	3	1	16	8	28
4	10	7	13	8	38
5	10	11	1	10	32
6	4	8	1	1	14
7	5	1		1	7
8	2	1			3
9		1			1
Total	34	30	34	33	131

Table 7. Results of two-way ANOVAs for age and length differences related to spawning time and capture location.

Response Variable	Predictor Variables	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age	Spawning time	1	4.20	4.20	54.81	0.00
	Capture location	1	0.17	0.17	2.25	0.14
	Spawning time:Capture location	1	0.01	0.01	0.16	0.69
	Residuals	126	9.67	0.08		
Length	Spawning time	1	0.00	0.00	0.04	0.85
	Capture location	1	0.25	0.25	8.40	0.00
	Spawning time:Capture location	1	0.10	0.09	3.13	0.08
	Residuals	127	3.84	0.03		

Table 8. Sex ratio of winter and spring spawners caught in Ipswich and Massachusetts Bay.

Sex	Spring		Winter		Total
	Ipswich Bay	Mass. Bay	Ipswich Bay	Mass. Bay	
Female	15	4	17	17	53
Male	19	26	17	16	78
Total	34	30	34	33	131

Table 9. Results of ANOVA for otolith growth differences at age related to spawning time and capture location.

Age	Factor	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Age 1	Spawning.time	1	6.683	6.683	51.13	0.00	***
	Capture.Location	1	1.026	1.026	7.85	0.01	**
	Spawning.time:Capture.Location	1	0.524	0.524	4.01	0.05	*
	Residuals	124	16.207	0.131			
Age 2	Spawning.time	1	1.289	1.2893	14.94	0.00	***
	Capture.Location	1	0.578	0.5783	6.70	0.01	*
	Spawning.time:Capture.Location	1	0.651	0.6511	7.55	0.01	**
	Residuals	127	10.958	0.0863			
Age 3	Spawning.time	1	0.067	0.06658	0.82	0.37	
	Capture.Location	1	0.129	0.12944	1.60	0.21	
	Spawning.time:Capture.Location	1	0.067	0.06708	0.83	0.37	
	Residuals	131	10.618	0.08106			
Age 4	Spawning.time	1	0.563	0.5634	11.43	0.00	***
	Capture.Location	1	0.158	0.1578	3.20	0.08	
	Spawning.time:Capture.Location	1	0.077	0.0773	1.57	0.21	
	Residuals	119	5.864	0.0493			
Age 5	Spawning.time	1	0.1156	0.11563	3.95	0.05	*
	Capture.Location	1	0.0093	0.00934	0.32	0.57	
	Spawning.time:Capture.Location	1	0.0265	0.02654	0.91	0.34	
	Residuals	88	2.5758	0.02927			

Table 10. Otolith growth differences at age for fish from different spawning times (winter, spring) and capture locations (IB: Ipswich Bay, MB: Massachusetts Bay).

Age	Spawning Time	Capture Location	N	Length	SD
1	S	IB	33	0.86	0.37
1	S	MB	33	0.80	0.37
1	W	IB	34	1.43	0.37
1	W	MB	28	1.12	0.34
2	S	IB	32	1.16	0.30
2	S	MB	33	0.88	0.22
2	W	IB	34	1.21	0.29
2	W	MB	32	1.22	0.36
3	S	IB	34	0.82	0.34
3	S	MB	35	0.80	0.25
3	W	IB	34	0.82	0.27
3	W	MB	32	0.71	0.28
4	S	IB	32	0.55	0.25
4	S	MB	35	0.58	0.24
4	W	IB	30	0.37	0.18
4	W	MB	26	0.50	0.21
5	S	IB	30	0.38	0.22
5	S	MB	31	0.38	0.17
5	W	IB	12	0.26	0.08
5	W	MB	19	0.33	0.13

Table 11. Otolith growth differences (age one) for fish across year classes from different spawning times (winter, spring) and capture locations ().

Year	Spawning Time	N	Length	SD
2008	S	14	0.86	0.24
2008	W	9	0.98	0.35
2009	S	8	0.78	0.33
2009	W	10	1.07	0.24
2010	S	12	0.90	0.34
2010	W	13	1.33	0.29
2011	S	8	0.75	0.36
2011	W	23	1.33	0.23

Table 12. Results of linear discriminant analysis with jackknifed (i.e., leave one out) predictions to test the classification success of individuals to their respective origin based on age-one otolith growth. (IB: Ipswich Bay, MB: Massachusetts Bay.)

Classification Scale	Classification Accuracy				
	Overall	Spring	Winter		
Spawning time (n = 2)	78%	74%	82%		
	Overall	IB	MB		
Capture location (n = 2)	54%	67%	41%		
	Overall	IB Spring	IB Winter	MB Spring	MB Winter
Spawning time*Capture location (n = 4)	44%	24%	94%	45%	7%

Table 13. Results of ANOVA for year one otolith width differences related to spawning time, capture location, and year class.

Factor	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Spawning.time	1	3.60	3.60	43.41	0.00	***
Year.Class	1	0.44	0.44	5.30	0.02	*
Capture.Location	1	0.00	0.00	0.00	0.95	
Spawning.time:Year.Class	1	0.56	0.56	6.76	0.01	*
Spawning.time:Capture.Location	1	0.09	0.09	1.09	0.30	
Year.Class:Capture.Location	1	0.08	0.08	0.92	0.34	
Spawning.time:Year.Class:Capture.Location	1	0.10	0.10	1.18	0.28	
Residuals	89	7.38	0.08			

Table 14. Manova results for median chemistry (Sr:Ca, Ba:Ca, Mg:Ca, Mn:Ca, Cu:Ca) of year-1 otolith growth of Atlantic cod based on timing of spawning (winter, spring) and location (Ipswich Bay and Massachusetts Bay).

Factor	Df	Pillai	approx F	num Df	Den Df	Pr(>F)
Capture location	1	0.13352	3.7292	5	121	**0.003562**
Spawning time	1	0.24942	8.0419	5	121	**1.41E-06**
Capture location:Spawning time	1	0.19374	5.8152	5	121	**7.55E-05**
Residuals	125					

Table 15. Anova results for median element:calcium chemistry of age-one otolith growth of Atlantic cod.

Element	Factor	Df	Sum Squares	Mean Square Error	F value	Pr(>F)
Sr:Ca	Capture location	1	0.17	0.17	1.07	0.30
	Spawning time	1	6.06	6.06	37.59	**0.00**
	Capture location:Spawning time	1	2.23	2.23	13.83	**0.00**
	Residuals	123	19.82	0.16		
Ba:Ca	Capture location	1	0.59	0.59	3.22	0.08
	Spawning time	1	1.36	1.36	7.43	**0.01**
	Capture location:Spawning time	1	0.07	0.07	0.37	0.54
	Residuals	125	22.93	0.18		
Mg:Ca	Capture location	1	0.17	0.17	3.72	0.06
	Spawning time	1	1.23	1.23	27.42	**0.00**
	Capture location:Spawning time	1	0.00	0.00	0.01	0.92
	Residuals	122	5.45	0.04		
Mn:Ca	Capture location	1	1.52	1.52	6.84	**0.01**
	Spawning time	1	0.85	0.85	3.80	0.05
	Capture location:Spawning time	1	0.24	0.24	1.06	0.31
	Residuals	122	27.14	0.22		
Cu:Ca	Capture location	1	2.83	2.83	8.45	**0.00**
	Spawning time	1	0.14	0.14	0.40	0.53
	Capture location:Spawning time	1	4.44	4.44	13.26	**0.00**
	Residuals	122	40.89	0.34		

Table 16. Otolith chemistry (age one) for fish from different spawning times (winter, spring) and capture locations (IB: Ipswich Bay, MB: Massachusetts Bay).

Element	Location	Spawning time	N	Mean	sd
Sr:Ca	IB	Spring	34	2.8823	0.4854
	MB	Spring	27	2.5488	0.3746
	IB	Winter	34	2.1985	0.3070
	MB	Winter	32	2.3972	0.4130
Ba:Ca	IB	Spring	35	0.0026	0.0015
	MB	Spring	27	0.0019	0.0006
	IB	Winter	34	0.0029	0.0015
	MB	Winter	33	0.0026	0.0011
Mg:Ca	IB	Spring	35	0.1280	0.0213
	MB	Spring	26	0.1193	0.0293
	IB	Winter	32	0.1565	0.0279
	MB	Winter	33	0.1446	0.0359
Mn:Ca	IB	Spring	33	0.0169	0.0105
	MB	Spring	26	0.0110	0.0037
	IB	Winter	34	0.0176	0.0094
	MB	Winter	33	0.0144	0.0053
Cu:Ca	IB	Spring	34	0.0069	0.0039
	MB	Spring	26	0.0084	0.0062
	IB	Winter	33	0.0115	0.0106
	MB	Winter	33	0.0053	0.0025

Table 17. Anova results for coefficient of variation (CV) element:calcium chemistry of age-one otolith growth of Atlantic cod.

Element	Factor	Df	Sum Squares	Mean Square Error	F value	Pr(>F)
Sr:Ca CV	Capture location	1	13.00	12.60	0.43	0.51
	Spawning time	1	406.00	405.70	13.80	0**.000307**
	Capture location:Spawning time	1	11.00	11.10	0.38	0.54
	Residuals	124	3646.00	29.40		
Ba:Ca CV	Capture location	1	298.00	298.00	0.98	0.33
	Spawning time	1	5240.00	5240.00	17.18	**6.26e-05**
	Capture location:Spawning time	1	659.00	659.00	2.16	0.14
	Residuals	124	37827.00	305.00		
Mg:Ca CV	Capture location	1	0.24	0.24	1.18	0.28
	Spawning time	1	0.25	0.25	1.23	0.27
	Capture location:Spawning time	1	0.15	0.15	0.74	0.39
	Residuals	124	25.25	0.20		
Mn:Ca CV	Capture location	1	0.09	0.09	0.45	0.51
	Spawning time	1	0.00	0.00	0.02	0.90
	Capture location:Spawning time	1	0.10	0.10	0.50	0.48
	Residuals	124	24.29	0.20		
Cu:Ca CV	Capture location	1	4.47	4.47	15.02	0.00
	Spawning time	1	0.07	0.07	0.24	0.62
	Capture location:Spawning time	1	2.01	2.01	6.75	0.01
	Residuals					

Table 18 Results of multivariate linear discriminant analysis with jackknifed (i.e., leave one out) predictions to test the classification success of individuals to their respective origin based on elemental ratios (year-1)

Classification Scale	Model Structure	Classification Accuracy				
		Overall	Spring	Winter		
Spawning time (n = 2)	Spawning.time~medianSr88+log(medianBa138)+ log(medianMg25)+log(medianMn55)	74%	68%	79%		
		Overall	IB	MB		
Capture location (n = 2)	Capture.Location~medianSr88+log(medianMn55)+ log(medianCu63)	48%	56%	40%		
		Overall	IB Spring	IB Winter	MB Spring	MB Winter
Spawning time*Capture location (n = 4)	Location.Spawning~log(medianMg25)+ log(medianMn55)+medianSr88+log(medianBa138) +log(medianCu63)	46%	49%	62%	30%	39%

Table 19. Manova results for median chemistry (Sr:Ca, Ba:Ca, Mg:Ca, Mn:Ca, Cu:Ca) of whole otolith growth of Atlantic cod based on timing of spawning (winter, spring) and location (Ipswich Bay and Massachusetts Bay).

Factor	Df	Pillai	approx F	num Df	den Df	Pr(>F)
Capture location	1	0.21967	6.7561	5	120	**1.39E-05**
Spawning time	1	0.21226	6.467	5	120	**2.34E-05**
Capture location:Spawning time	1	0.27916	9.2945	5	120	**1.67E-07**
Residuals	125					

Table 20. Anova results for median element:calcium chemistry of whole otolith growth of Atlantic cod.

Element	Factor	Df	Sum Squares	Mean Square Error	F value	Pr(>F)
Sr:Ca	Capture location	1	0.08	0.08	0.62	0.43
	Spawning time	1	1.32	1.32	10.24	**0.00**
	Capture location:Spawning time	1	3.04	3.04	23.64	**0.00**
	Residuals	123	15.83	0.13		
Ba:Ca	Capture location	1	0.79	0.79	7.17	**0.01**
	Spawning time	1	0.78	0.77	7.06	**0.01**
	Capture location:Spawning time	1	0.21	0.21	1.91	0.17
	Residuals	123	13.51	0.11		
Mg:Ca	Capture location	1	0.76	0.76	18.93	**0.00**
	Spawning time	1	0.82	0.82	20.30	**0.00**
	Capture location:Spawning time	1	0.00	0.00	0.06	0.81
	Residuals	122	4.93	0.04		
Mn:Ca	Capture location	1	0.94	0.93	4.74	**0.03**
	Spawning time	1	1.03	1.03	5.21	**0.02**
	Capture location:Spawning time	1	0.11	0.11	0.57	0.45
	Residuals	121	23.85	0.20		
Cu:Ca	Capture location	1	7.96	7.96	19.64	**0.00**
	Spawning time	1	0.33	0.33	0.80	0.37
	Capture location:Spawning time	1	4.20	4.20	10.37	**0.00**
	Residuals	123	49.81	0.41		

Table 21: Manova results for CV elemental ratios (Sr:Ca, Ba:Ca, Mg:Ca, Mn:Ca, Cu:Ca) of whole otolith growth of Atlantic cod based on timing of spawning (winter, spring) and location (Ipswich Bay and Massachusetts Bay).

Factor	Df	Pillai	approx F	num Df	den Df	Pr(>F)
Capture location	1	0.1134	3.1208	5	122	**0.01097**
Spawning time	1	0.1806	5.3778	5	122	**0.00017**
Capture location:Spawning time	1	0.04692	1.2011	5	122	0.31272
Residuals	126					

Table 22. Anova results for CV element:calcium chemistry of whole otolith growth of Atlantic cod.

Element	Factor	Df	Sum Squares	Mean Square Error	F value	Pr(>F)
Sr:Ca	Capture location	1	0.001	0.0011	0.025	0.87463
	Spawning time	1	0.316	0.31558	7.161	**0.00847**
	Capture location:Spawning time	1	0.011	0.0114	0.259	0.61202
	Residuals	123	5.421	0.04407		
Ba:Ca	Capture location	1	0.239	0.2393	1.33	0.25109
	Spawning time	1	2.332	2.3321	12.961	**0.00046**
	Capture location:Spawning time	1	0.003	0.0027	0.015	0.90277
	Residuals	121	21.771	0.1799		
Mg:Ca	Capture location	1	0.047	0.04708	0.53	0.468
	Spawning time	1	0.032	0.03155	0.355	0.552
	Capture location:Spawning time	1	0.146	0.14628	1.646	0.202
	Residuals	121	10.753	0.08887		
Mn:Ca	Capture location	1	0.034	0.03364	0.327	0.5684
	Spawning time	1	0.303	0.3029	2.945	0.0887
	Capture location:Spawning time	1	0.106	0.10599	1.03	0.312
	Residuals	123	12.651	0.10285		
Cu:Ca	Capture location	1	3.68	3.676	12.311	**0.00063**
	Spawning time	1	0.12	0.125	0.418	0.51927
	Capture location:Spawning time	1	0.42	0.416	1.393	0.24014
	Residuals	125	37.32	0.299		

Table 23 Results of multivariate linear discriminant analysis with jackknifed (i.e., leave one out) predictions to test the classification success of individuals to their respective origin based on elemental ratios (whole otolith).

Classification Scale	Model Structure	Classification Accuracy				
		Overall	Spring	Winter		
Spawning time (n = 2)	Spawning.time~medianSr88+log(medianBa138)+ log(medianMg25)+log(medianMn55)	70%	68%	72%		
		Overall	IB	MB		
Capture location (n = 2)	Capture.Location~log(medianCu63)	65%	72%	58%		
		Overall	IB Spring	IB Winter	MB Spring	MB Winter
Spawning time*Capture location (n = 4)	Location.Spawning~medianSr88+log(medianCu63)	44%	44%	74%	3%	49%

Table 24. Anova results for element:calcium chemistry of otolith growth of Atlantic cod as a function of age, spawning time, and capture location.

Element	Factor	Df	Sum Squares	Mean Square Error	F value	Pr(>F)
Ba:Ca	Age	4	23.33	5.83	31.27	**< 2e-16**
	Spawning time	1	1.51	1.51	8.09	**0.00**
	Capture location	1	1.55	1.55	8.33	**0.00**
	Age:Spawning time	4	0.49	0.12	0.66	0.62
	Age:Capture location	4	0.23	0.06	0.31	0.87
	Spawning time:Capture location	1	0.91	0.91	4.89	**0.03**
	Age:Spawning time:Capture location	4	1.14	0.29	1.53	0.19
	Residuals	562	104.84	0.19		
Sr:Ca	Age	4	2.61	0.65	18.67	**0.00**
	Spawning time	1	0.57	0.57	16.33	**0.00**
	Capture location	1	0.19	0.19	5.49	**0.02**
	Age:Spawning time	4	0.47	0.12	3.36	**0.01**
	Age:Capture location	4	0.11	0.03	0.81	0.52
	Spawning time:Capture location	1	1.44	1.44	41.28	**0.00**
	Age:Spawning time:Capture location	4	0.21	0.05	1.48	0.21
	Residuals	563	19.68	0.04		
Mg:Ca	Age	4	6.73	1.68	20.57	**0.00**
	Spawning time	1	0.81	0.81	9.96	**0.00**
	Capture location	1	1.59	1.59	19.48	**0.00**
	Age:Spawning time	4	0.73	0.18	2.23	0.06
	Age:Capture location	4	0.92	0.23	2.80	**0.03**
	Spawning time:Capture location	1	0.07	0.07	0.89	0.35
	Age:Spawning time:Capture location	4	0.18	0.05	0.56	0.69
	Residuals	561	45.85	0.08		
Mn:Ca	Age	4	177.12	44.28	94.61	**< 2e-16**
	Spawning time	1	2.04	2.04	4.35	**0.04**
	Capture location	1	6.01	6.01	12.83	**0.00**
	Age:Spawning time	4	1.71	0.43	0.92	0.46
	Age:Capture location	4	0.61	0.15	0.32	0.86
	Spawning time:Capture location	1	2.53	2.53	5.41	**0.02**
	Age:Spawning time:Capture location	4	4.89	1.22	2.61	**0.03**

	Residuals	555	259.75	0.47		
Cu:Ca	Age	4	14.03	3.51	7.63	0.00
	Spawning time	1	2.71	2.71	5.89	0.02
	Capture location	1	27.07	27.07	58.86	0.00
	Age:Spawning time	4	1.20	0.30	0.65	0.62
	Age:Capture location	4	0.90	0.22	0.49	0.75
	Spawning time:Capture location	1	18.43	18.43	40.08	**0.00**
	Age:Spawning time:Capture location	4	0.37	0.09	0.20	0.94
	Residuals	552	253.83	0.46		

Figure 25. Results of ANOVA for otolith growth differences at age related to spawning time and capture location.

Element	Age	Factor	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Sr:Ca	Age 2	Spawning.time	1	0.04	0.04	1.45	0.23
		Capture.Location	1	0.06	0.06	1.96	0.16
		Spawning.time:Capture.Location	1	0.04	0.04	1.37	0.24
		Residuals	125	3.52	0.03		
	Age 3	Spawning.time	1	0.02	0.02	0.80	0.37
		Capture.Location	1	0.06	0.06	2.02	0.16
		Spawning.time:Capture.Location	1	0.45	0.45	14.56	**0.00**
		Residuals	124	3.80	0.03		
	Age 4	Spawning.time	1	0.03	0.03	0.69	0.41
		Capture.Location	1	0.07	0.07	1.76	0.19
		Spawning.time:Capture.Location	1	0.58	0.58	14.78	**0.00**
		Residuals	105	4.12	0.04		
	Age 5	Spawning.time	1	0.01	0.01	0.23	0.64
		Capture.Location	1	0.13	0.13	2.42	0.12
		Spawning.time:Capture.Location	1	0.23	0.22	4.14	**0.05**
		Residuals	83	4.51	0.05		
Ba:Ca	Age 2	Spawning.time	1	0.13	0.13	0.76	0.39
		Capture.Location	1	0.54	0.54	3.10	0.08
		Spawning.time:Capture.Location	1	0.00	0.00	0.01	0.94
		Residuals	125	21.83	0.17		
	Age 3	Spawning.time	1	0.35	0.35	1.72	0.19
		Capture.Location	1	0.47	0.47	2.31	0.13
		Spawning.time:Capture.Location	1	0.48	0.48	2.35	0.13
		Residuals	124	25.35	0.20		
	Age 4	Spawning.time	1	0.02	0.02	0.13	0.72
		Capture.Location	1	0.06	0.06	0.32	0.57
		Spawning.time:Capture.Location	1	1.48	1.48	8.05	0.01
		Residuals	105	19.30	0.18		
	Age 5	Spawning.time	1	0.07	0.07	0.46	0.50
		Capture.Location	1	0.00	0.00	0.00	0.96
		Spawning.time:Capture.Location	1	0.03	0.03	0.18	0.68
		Residuals	82	13.05	0.16		
Mg:Ca	Age 2	Spawning.time	1	0.73	0.73	10.50	0.00
		Capture.Location	1	1.70	1.70	24.51	0.00
		Spawning.time:Capture.Location	1	0.13	0.12	1.79	0.18
		Residuals	125	8.69	0.07		
	Age 3	Spawning.time	1	0.03	0.03	0.29	0.59

	Capture.Location	1	0.53	0.53	5.65	0.02
	Spawning.time:Capture.Location	1	0.04	0.04	0.37	0.54
	Residuals	125	11.74	0.09		
Age 4	Spawning.time	1	0.05	0.05	0.46	0.50
	Capture.Location	1	0.00	0.00	0.01	0.95
	Spawning.time:Capture.Location	1	0.04	0.04	0.38	0.54
	Residuals	104	10.63	0.10		
Age 5	Spawning.time	1	0.06	0.06	0.64	0.43
	Capture.Location	1	0.11	0.11	1.18	0.28
	Spawning.time:Capture.Location	1	0.06	0.06	0.61	0.44
	Residuals	82	7.43	0.09		

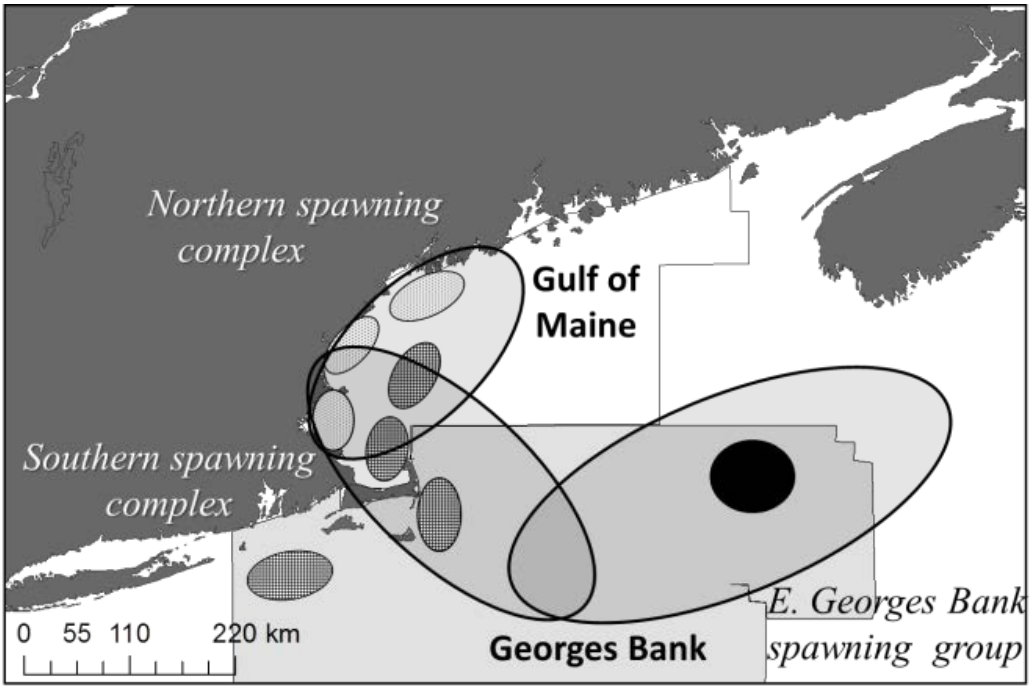


Figure 1. Genetic groupings of Atlantic cod (solid ovals) and distribution of spawning complexes based on tagging (large ovals, based on work by Kovach et al. 2010.

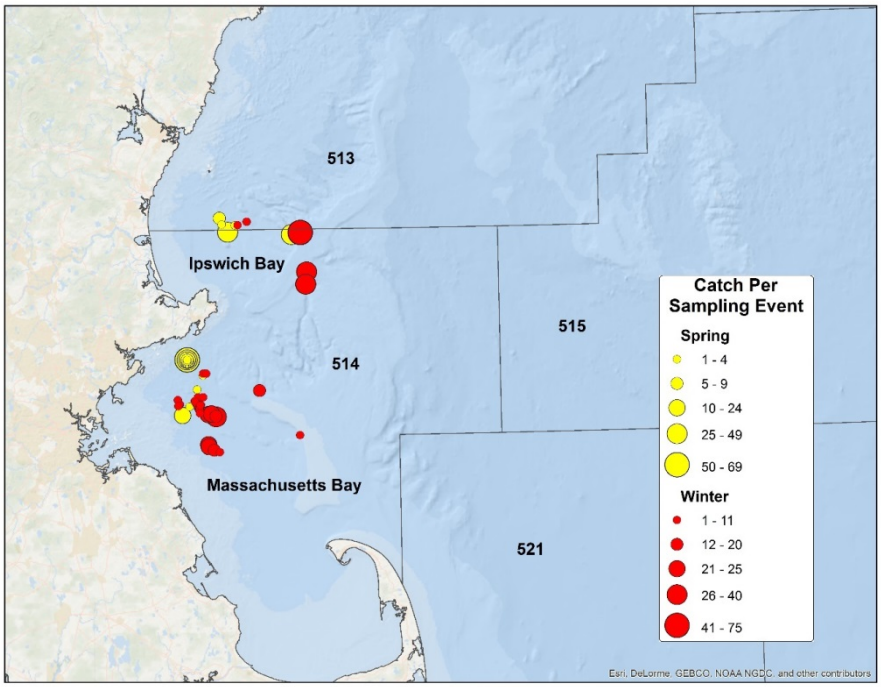


Figure 2. Map illustrating sampling locations and timing of spawning Atlantic cod samples. Each bubble represents numbers of fish sampled per discrete sampling event.

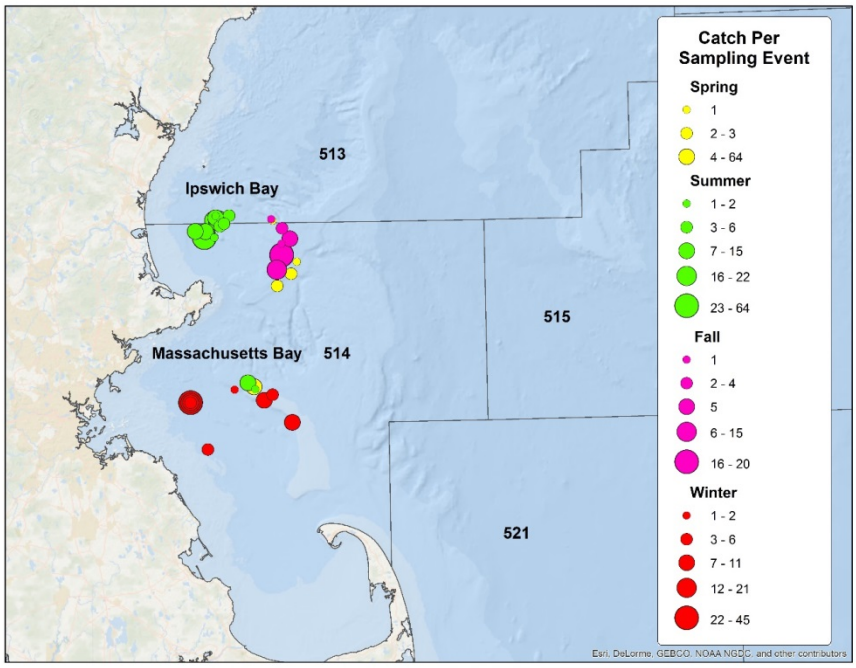


Figure 3. Map illustrating the locations of capture of Atlantic cod representing modern fishery samples by season (2015 to 2016).

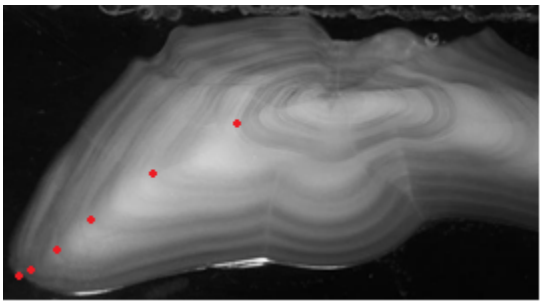


Figure 4. Example of Atlantic cod otolith photo taken for ageing. The age of this individual was estimated at 6.

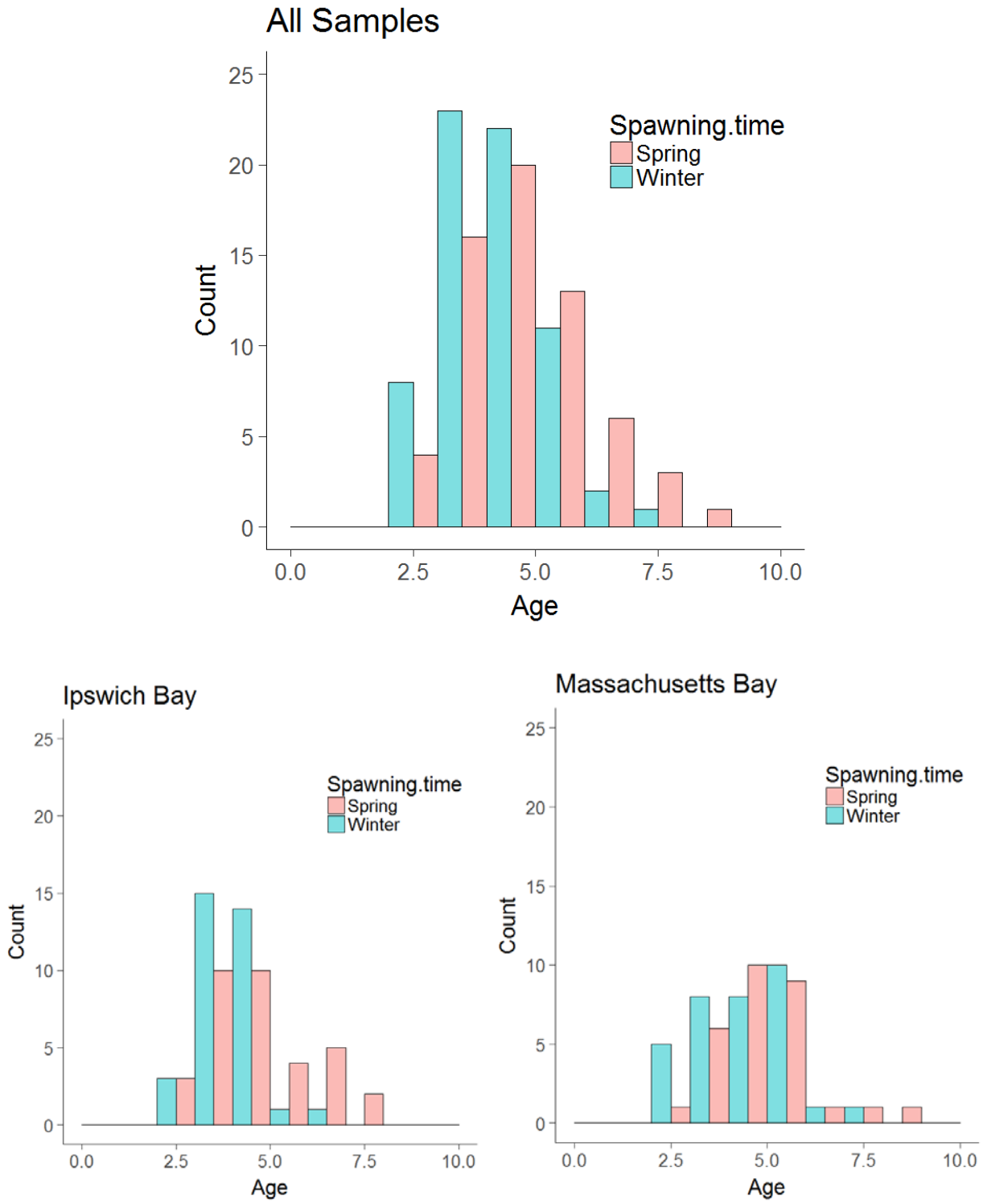


Figure 5. Age distribution of Atlantic cod across all samples (top panel) and by location and spawning time (bottom panels).

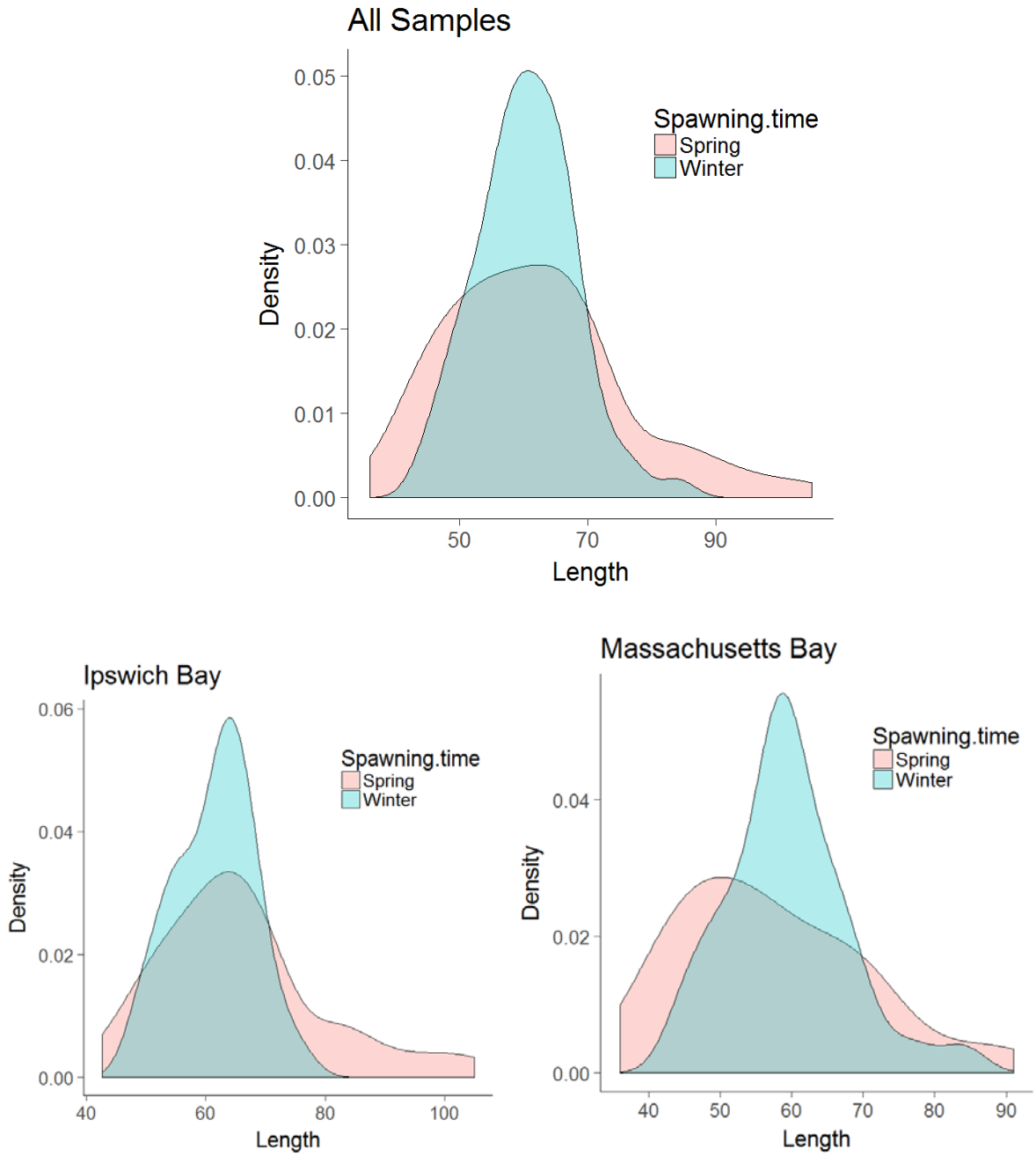


Figure 6. Length distributions across all samples (top panel) and by location and spawning time (bottom panels).

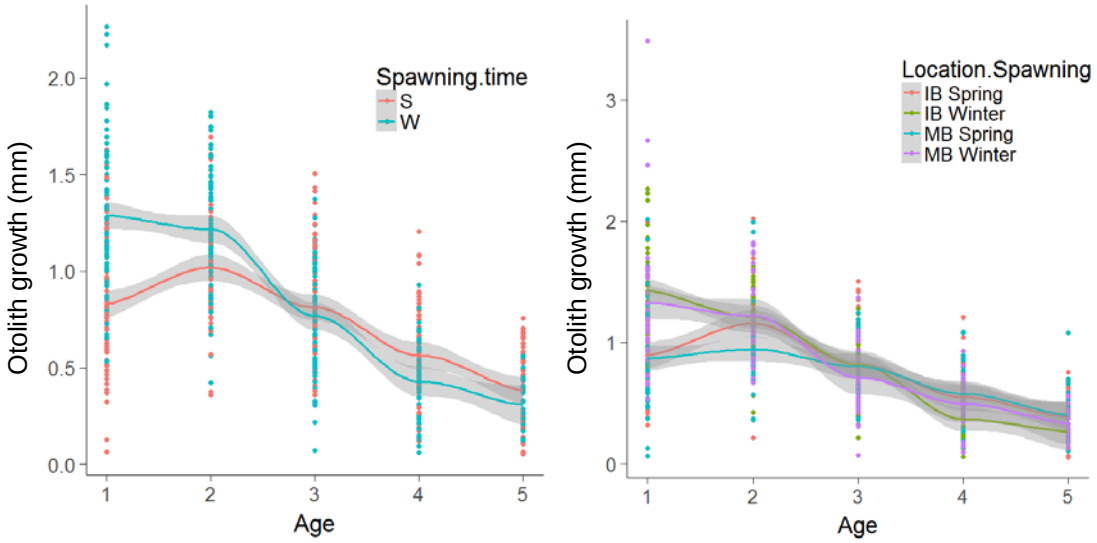


Figure 7. Annual growth increments widths of winter and spring spawning cod fit with Lowess smoothing function.

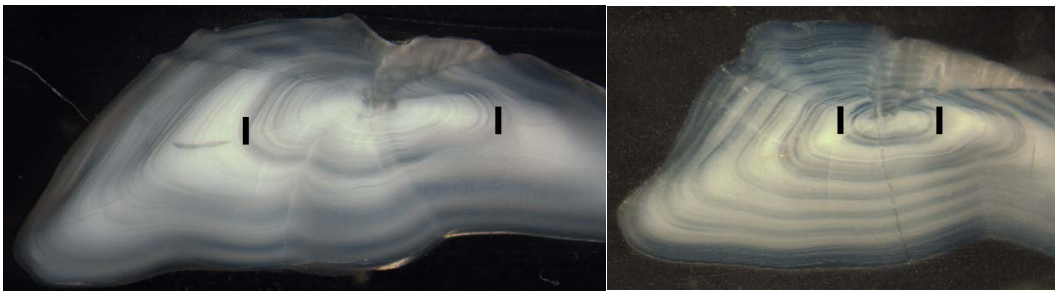


Figure 8. Left photo: Image of winter spawner depicting large year-1 annulus. Right photo: Image of spring spawner with comparatively small year-1 annulus. Black bars denote width of first annulus.

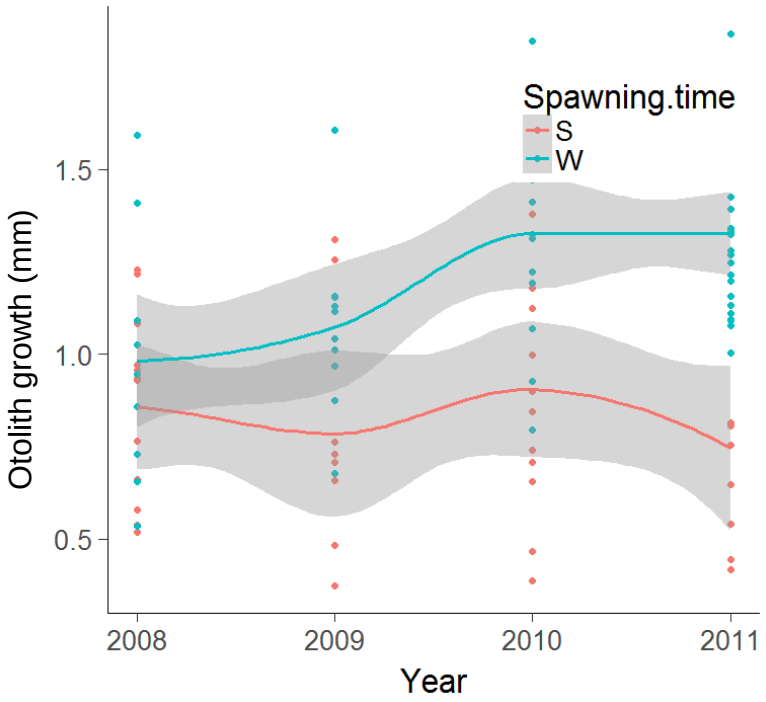


Figure 9 Age one otolith growth over time for winter and spring spawning fish.

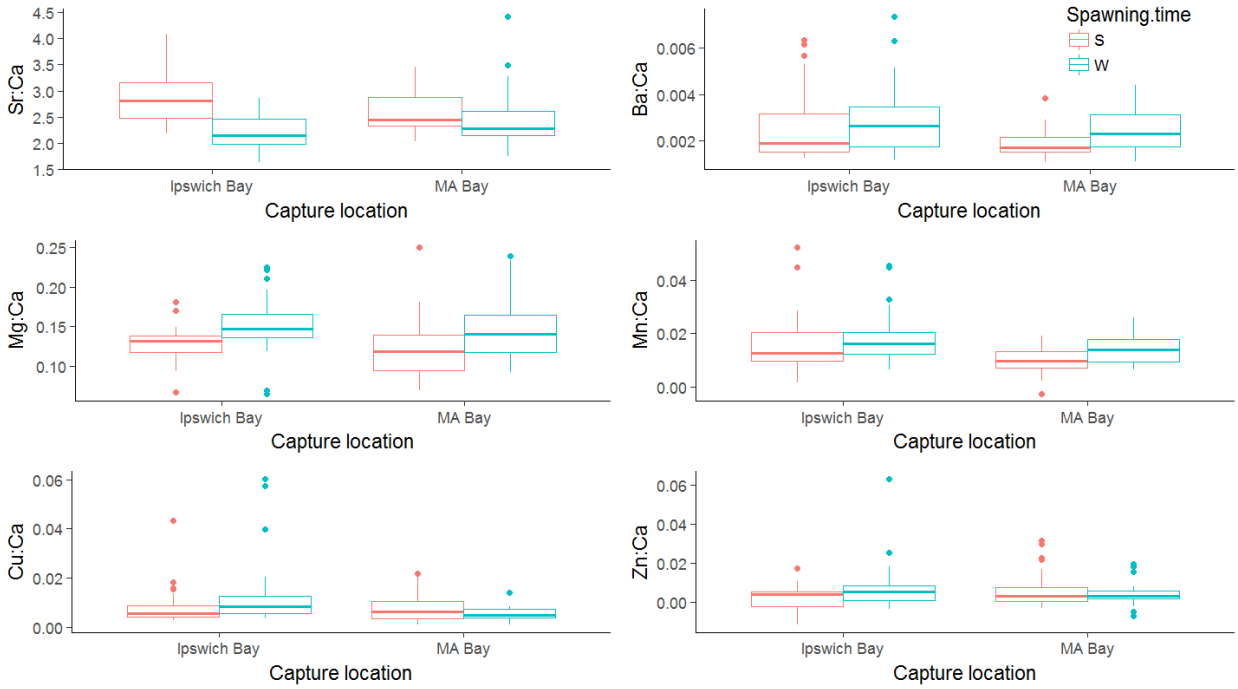


Figure 10. Median isotope values for otolith cores of Atlantic cod winter and spring spawners caught in Ipswich and Massachusetts Bay.

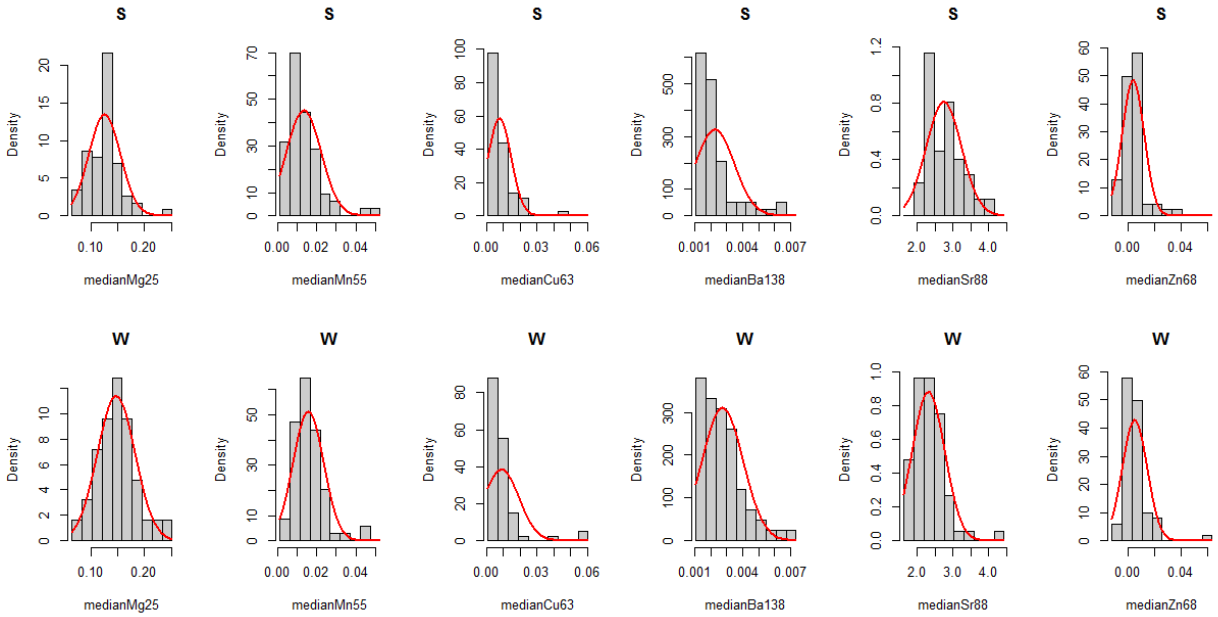


Figure 11. Element:Calcium ratios for otolith cores of Atlantic cod winter and spring spawners caught in Ipswich and Massachusetts Bay.

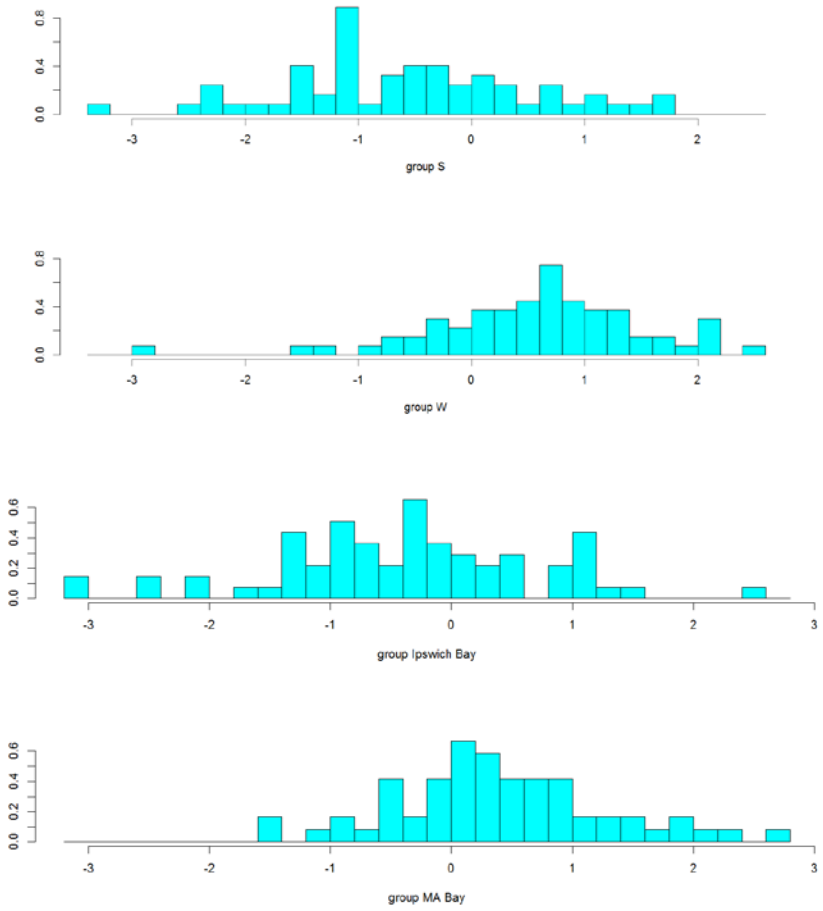


Figure 12. Discriminant function scores for winter and spring spawning cod based on core otolith chemistry.

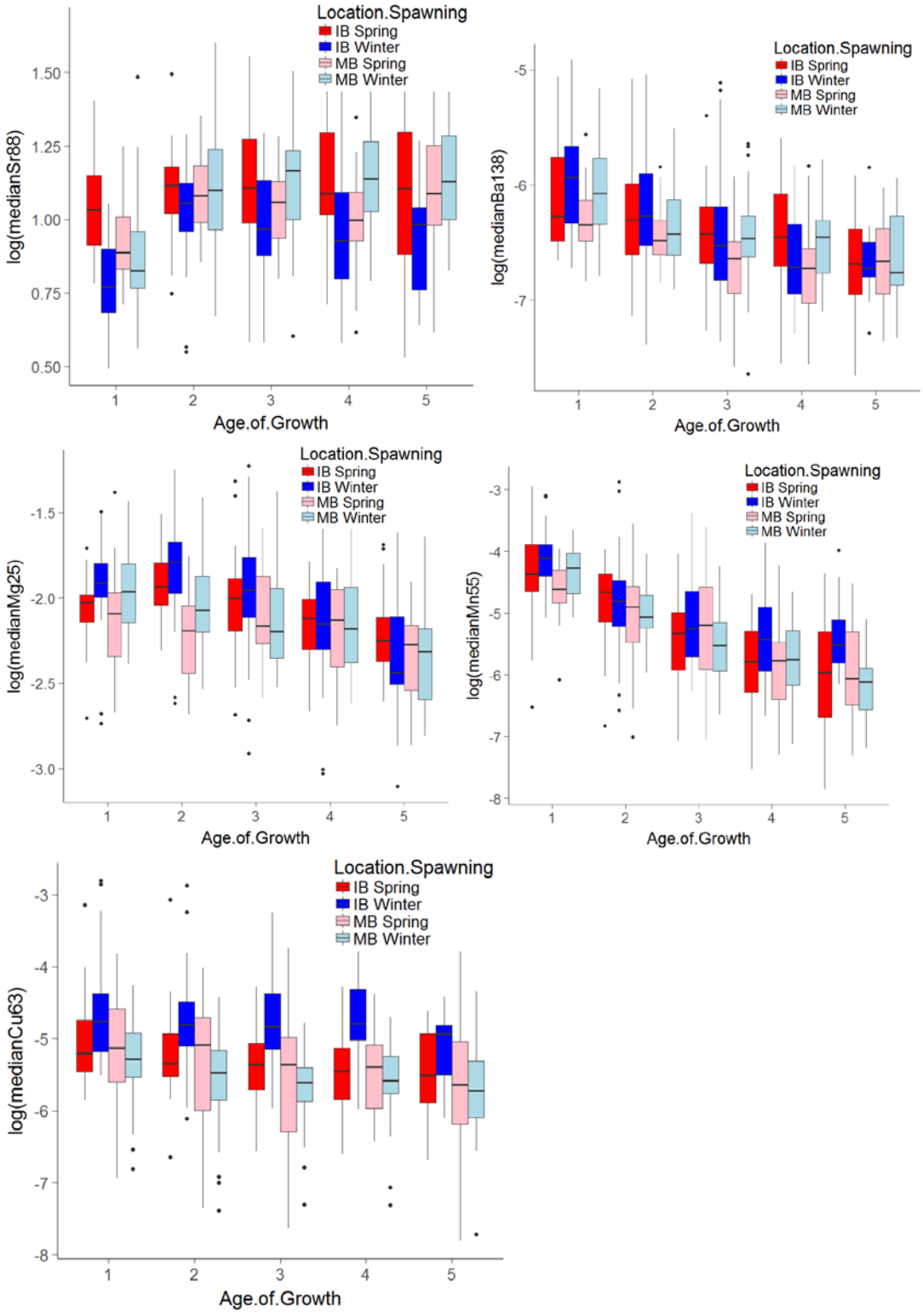


Figure 13. Otolith chemistry at age in relation to spawning time and capture location.

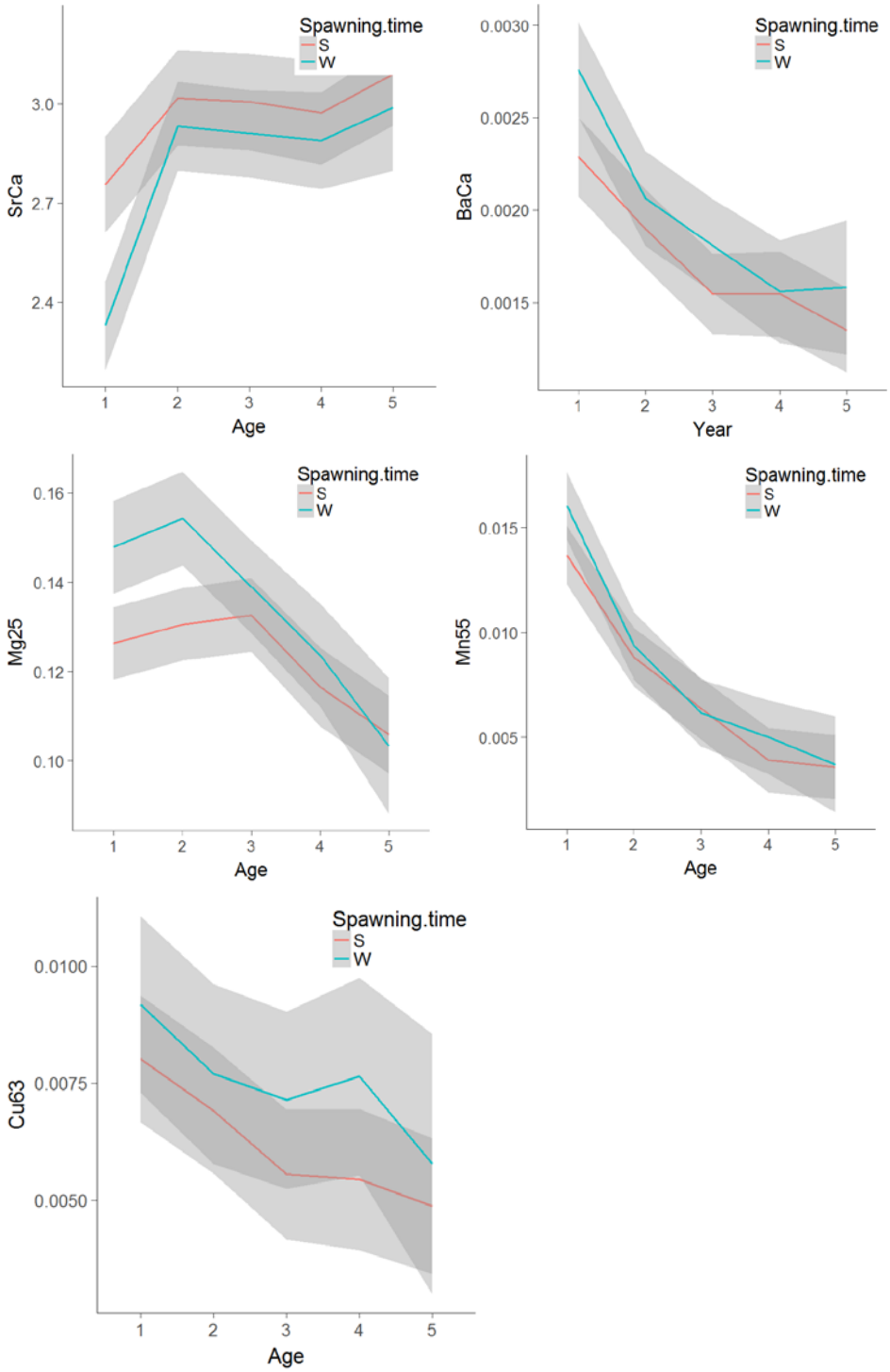


Figure 14. Lowess model fits of otolith chemistry at age in relation to spawning time and capture location across ages.

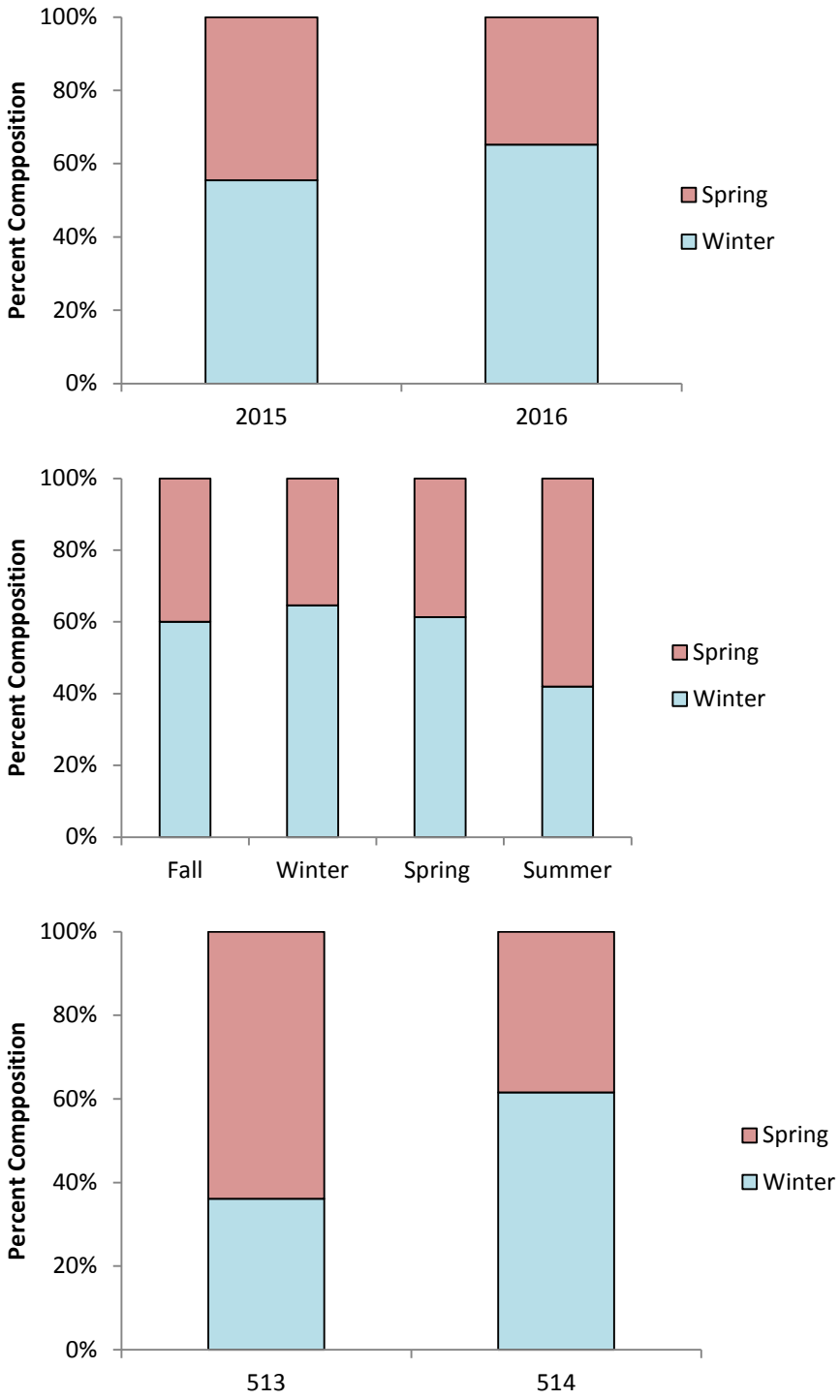


Figure 15. Stock composition of fishery collected 2015-2016 Atlantic cod over time using random forest classification approach.

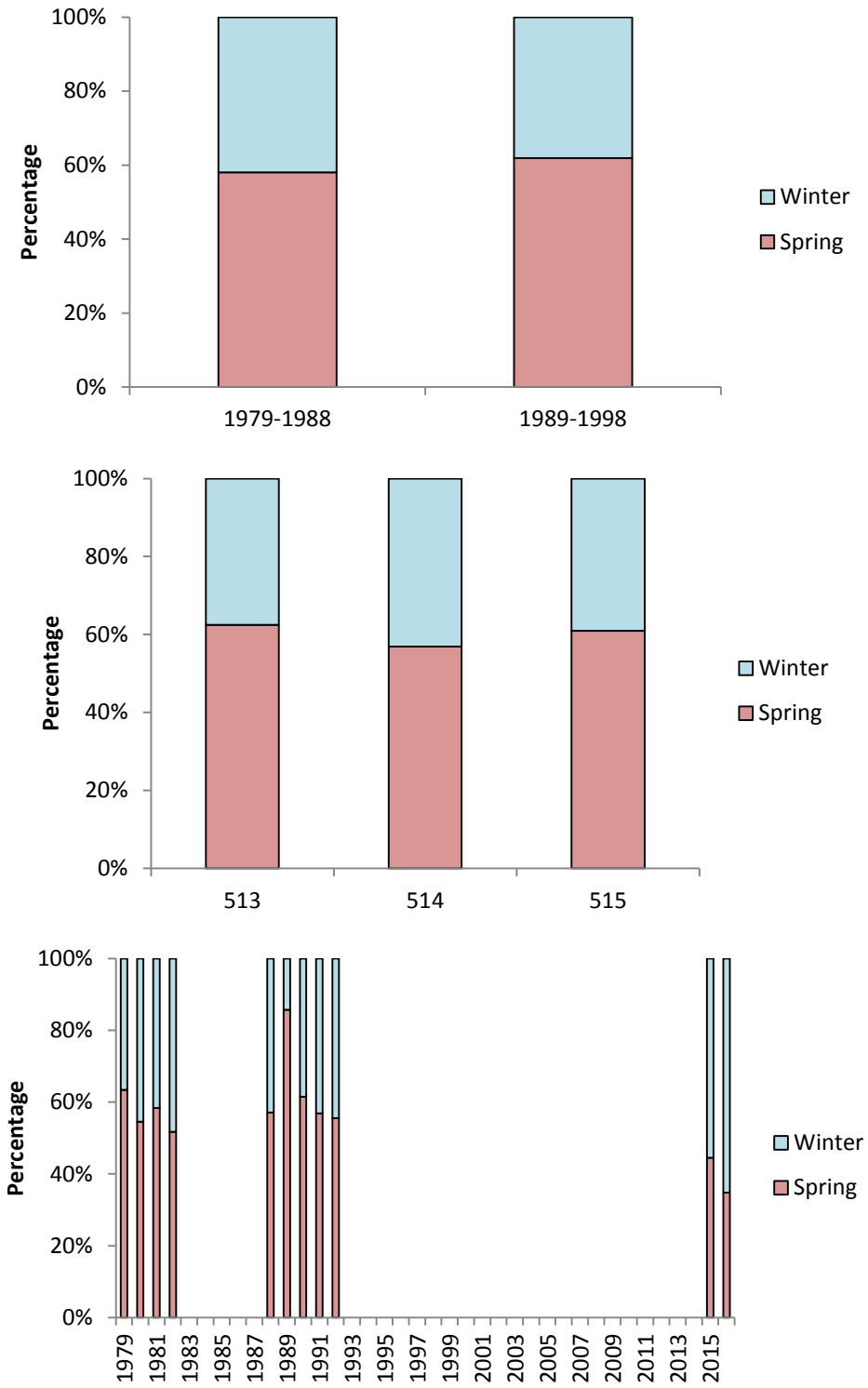


Figure 16. Stock composition of fishery collected Atlantic cod in 1980s and 1990s using random forest classification approach.

APPENDIX D: Details of body morphometric and color analysis

Introduction

Morphometrics has long been used in conjunction with other techniques to aid in identification of stock structure in fish (Meng and Stocker 1984, Haddon and Willis 1995, Begg and Waldman 1999, Cadrin and Silva 2005). The basic idea with morphometrics (body shape analysis) is that genetics and/or environment lead to subtle differences in body shape among populations (Marcil et al. 2006). For example, some populations may be adapted for more migratory behavior than others which may be more sedentary and this may lead to differences in body shape (e.g., migrants should be more streamlined than residents; Morinville and Rasmussen 2008). Differences in diet among regions may also drive differences in body shape. For example, Sherwood and Grabowski (2010) found that red cod at Cashes Ledge (central GOM) have more robust body shapes than normal cod which is likely a result of their sedentary behavior, but also could be related to a more crustacean dominated diet (i.e., large crabs and lobsters).

Here, we applied body shape analysis to differentiate among spring- and winter-spawning cod to test the hypothesis that these groups represent distinct spawning populations (in addition to otolith chemistry and genetic analyses). An advantage of morphometric analysis is that differences in life-history strategies can be inferred based on body shape differences (e.g., Morinville and Rasmussen 2008, Sherwood and Grabowski 2010, Sherwood and Grabowski 2015). It has been previously hypothesized that winter-spawning cod are more migratory than spring-spawning cod because their eggs and larvae are advected offshore during the spawning season (due to offshore prevailing winds, Runge et al. 2010). Spring-spawning cod, on the other hand, spawn at a time when winds and currents favor local retention of eggs and larvae in nearshore areas of the western Gulf of Maine (Runge et al. 2010). Migratory behavior of adult cod is expected to match the modeled dispersal patterns of eggs and larvae; that is, high dispersal of eggs and larvae in winter should match a migratory lifestyle later in life, whereas local retention of eggs and larvae in the spring should precede a relatively sedentary lifestyle in adults. Thus, we hypothesize that winter and spring spawning cod, experiencing different environmental conditions, at least in early life, should be distinguishable based on body shape and that these differences should reflect a resident/migrant dichotomy (e.g., Morinville and Rasmussen 2008) with spring spawners (residents) having more robust bodies than winter spawners (migrants).

Methods

Morphometric analysis was completed using digital photos of cod that were obtained from multiple sources. These included our own sampling for this project for spawning cod, another NEC-funded project to examine winter-spawning cod in Ipswich Bay (at “the Cove”, Sherwood et al. 2017) and from photos provided by D. Zemeckis as part of ongoing surveys on Massachusetts Bay spawning cod. While photos of cod were available from the western Gulf of Maine for periods and locations outside of spawning (e.g., to mimic the fishery for otolith and genetic analyses), we limited our morphometric comparisons to cod that were captured at known spawning locations including the Winter Cod Conservation Zone (WCCZ, Massachusetts Bay), the Spring Cod Conservation Zone (SCCZ, Massachusetts Bay), the Gulf of Maine Cod

Spawning Protection Area (GOMCSPA, Ipswich Bay), and the Cove (Outer Ipswich Bay) during spawning seasons (Nov – Jan for winter and April – June for spring). We also limited our samples to cod that were in or near to spawning condition. These included cod that were classified as ripe, ripe/running and spent based on macroscopic examination of gonads. Overall, we examined body shape for a total of 260 cod from the 4 spawning groups: Mass Bay spring (SCCZ), Mass Bay winter (WCCZ), Ipswich Bay spring (GOMCSPA), and Ipswich Bay winter (the Cove). Kocovsky et al. (2009) examined the influence of sample size on the stability of multivariate analyses of truss-based morphometrics (see next) and found that robust results were obtained when the sample size exceeded the number of truss elements by a factor or 3.5-8.0; in our analysis this ratio was 15.

In all photos, cod were laid out as flat as possible on a measuring board so that all fins and features were easily visible in the resulting photograph. The analysis followed the box-truss network approach described in Cadrin and Friedland (1999) and Sherwood and Grabowski (2010). Specifically, 12 homologous landmarks were identified on the image of cod (Figure 1) and lines connecting these landmarks were drawn in a box-truss design and measured using an image analysis system (Image Pro). This resulted in 22 linear dimensions connecting landmarks. However, for all analyses we excluded landmark #11 (insertion point of first anal fin) and all dimensions connected to this landmark since this landmark and its related measurements may be influenced by spawning condition and feeding (i.e., a full or empty belly). While most fish were at or near spawning condition, with the exception of spent fish, the biggest risk of including this landmark was possible differences in condition (e.g., stomach fullness) that may exist between winter- and spring-spawning cod that may be seasonal in nature and not related to population differences. The remaining 17 linear dimensions were natural log transformed to normalize distributions. A principle components analysis (PCA) was then conducted on these transformed values (SPSS 16.0). The first axis from the PCA represents length. Thus, to remove the influence of length on our results, all linear dimensions were regressed against the first PCA axis (PCA1), although this may not account for the possible effect of ontogeny on body shape. Finally, residual linear box-truss distances (from PCA1 relationships) were used in a discriminant function analysis (DFA, SPSS 16.0) to assess body shape differences/similarities among spawning groups.

We also considered color in our analyses. Color in cod is a good indicator of behavior with darker/redder colors indicative of residency in shallower water (Sherwood and Grabowski 2010, Conroy et al. 2017). Color was analyzed in Image Pro by examining color over a standardized region of the head (an ellipse contained within the operculum). Red to green ratio (RGR) is the mean intensity of red pixels divided by the mean intensity of green pixels in this region. Values of RGR can range from below 1.0 (considered olive cod, mostly migrants) to above 2.0 (red cod, highly resident). A threshold of 1.2 has been used to differentiate red and olive cod in the past (Sherwood and Grabowski 2010, Conroy et al. 2017). We did not apply a threshold in this study. Rather we examined differences in mean RGR among spawning groups, and also explored the impact of including RGR in our morphometric DFA on spawning group reclassification rates.

Results and Discussion

Figure 2 shows mean RGR values by spawning group. There was a significant effect of spawning location on RGR values with higher values seen in Massachusetts Bay compared to Ipswich Bay spawning sites (ANOVA: $F_{1,255} = 163.2$, $p < 0.0001$). Spawning season was not significant. However, there was a significant interaction between spawning location and spawning season (ANOVA: $F_{1,255} = 7.9$, $p < 0.01$) such that the difference in RGR values among locations was highest for winter-spawning cod. Individual RGR values are plotted against length by spawning group in Figure 3. In this case there was an effect of length on RGR values (decreasing with length; ANCOVA: $F_{1,255} = 45.6$, $p < 0.0001$, $R^2 = 0.54$) and an effect of spawning group (ANCOVA: $F_{3,255} = 68.4$, $p < 0.0001$, $R^2 = 0.54$). It should be noted that despite the strong differences in RGR among locations, the mean difference is subtle compared to other studies that have examined color differences and related behaviors among "red" and "olive" cod (Sherwood and Grabowski 2010, Conroy et al. 2017). We interpret this result as differences in depth preferences among groups from each spawning location. Massachusetts Bay spawners spawn in shallower water (~ 50m, Armstrong et al. 2013) compared to Ipswich Bay spawners (~ 80-100m, Gurshin et al. 2013, Sherwood et al. 2017). These depth preferences may exist throughout the year which would be consistent with color differences (i.e., darker/redder in shallower water). Although not possible to assess with our existing data, differences in diet may also drive color differences among Massachusetts Bay and Ipswich Bay cod (e.g., Gosse and Wroblewski 2004). Isotope analysis as in Sherwood and Rose (2005) may help to address this question.

Results of our discriminant function analysis are shown in Figure 4. Interestingly, the largest discrimination between groups, driven by variation along DF1, existed between Massachusetts and Ipswich Bay cod rather than between spring- and winter-spawning cod, although in both bays spring- and winter-spawning cod were distinguishable along DF2. Overall, 82.3% of cod were correctly reclassified back to their original groupings suggesting that body shape alone is a good means of discriminating between spawning groups (Table 1). Massachusetts Bay, in particular, had very high reclassification rates (90%). Overall, reclassification rates were even higher (84.3%) when RGR (color) was added as a discriminating variable (Table 2, Figure 5).

Figure 6 (multipaneled) shows how each linear box-truss measurement varied among capture locations and spawning seasons (see Table 3 for ANOVA statistics). 15 of the 17 measurements varied significantly among location and/or season and/or their interaction. 8 of the 17 measurements varied significantly among spawning seasons. The three strongest variables that differed among seasons were D8, D22 and D7 (in order of F values). These are all body depth variables and they were larger in winter- versus spring-spawning cod. 14 of the 17 measurements varied significantly among spawning locations. The three strongest variables that differed among locations were D4, D1 and D6. These were all head length variables and were longer in Massachusetts Bay than in Ipswich Bay. Average reconstructed shapes for cod from different spawning seasons (by location and for both locations) are shown in Figures 7-9. In this case, measurements linked to landmark #11 were included for illustrative purposes. However, these measurements do not impact the statistical results discussed above. These reconstructions

consistently show that spring-spawning cod, regardless of location, are more streamlined than winter-spawning cod. This result suggests that spring-spawning cod are more migratory than winter-spawning cod. This result runs counter to our *a priori* hypothesis based on the scale of movement matching egg/larval dispersal (i.e., Runge et al. 2010). That is, we expected winter-spawning cod to be more migrant (and streamlined) based on the fact that their eggs and larvae are dispersed over wider areas (Runge et al. 2010). In order to "close the loop", winter-spawning cod would have to migrate back to the western Gulf of Maine to spawn once they've matured. This does not appear to be the case and calls into question model assumptions about early-life dispersal patterns.

Overall, our morphometric results suggest the existence of at least 4 distinct spawning groups in the western Gulf of Maine, all of which appear to have different behavioral strategies. Cod from Massachusetts Bay, regardless of spawning season, were redder than cod from Ipswich Bay possibly indicating that these cod spend most of their time outside of spawning in shallower waters, since red cod typically associate with shallow water (Sherwood and Grabowski 2010, Conroy et al. 2017). The difference in color was subtle and not as marked as differences noted in directed studies of red cod compared to "olive" cod (Sherwood and Grabowski 2010, Conroy et al. 2017). This suggests that there is a continuum of life-history strategies that may vary from very shallow, resident, kelp-associated cod (~20-30m; very red, Conroy et al. 2017) to inshore dwelling cod as in Massachusetts Bay (somewhat red, ~40-60m), to slightly deeper living cod as in Ipswich Bay (not very red, ~80-100m), and possibly deeper still (likely highly migratory but not represented here). Indeed, Gosse and Wroblewski (2004) report on a range of color types in Newfoundland and Labrador with variations in assumed movement strategies. Despite differences in color among bays, as well as body shape variables (Table 2, Figure 6), there appeared to be consistent morphometric differences among seasons regardless of location. Particularly, winter-spawning cod had deeper bodies which would be expected with a more resident lifestyle. This runs counter to previous expectations that winter-spawning cod should be more migrant based on the scale of modeled egg and larval dispersal (Runge et al. 2010).

There appear to multiple dimensions of life-history strategies among cod groups. There is a resident/migrant dichotomy that relates to body shape differences (spring vs. winter) and there is also a depth related contrast as indicated by color (Massachusetts Bay vs. Ipswich Bay). Red cod have been previously associated with highly resident strategies (Conroy et al. 2017). However, our results suggest that more subtle differences in color may indicate depth preference but not necessarily movement strategy since redder Massachusetts Bay cod also had longer heads, which is usually associated with more streamlined bodies and thus higher movement capacity. Further work may examine whether differences in movement and depth preference truly exist between spawning locations and seasons examined in this study.

References

Armstrong, M.P., Dean, M.J., Hoffman, W.S., Zemeckis, D.R., Nies, T.A., Pierce, D.E., Diodati, P.J., and McKiernan, D.J. 2013. The application of small scale fishery closures to protect Atlantic cod spawning aggregations in the inshore Gulf of Maine. Fisheries Research 141: 62-69.

Begg, G.A., and J.R. Walman. 1999. An holistic approach to fish stock identification. Fisheries Research 43: 35-44.

Cadrin, S.X., and Friedland, K.D. 1999. The utility of image processing techniques for morphometric analysis and stock identification. Fisheries Research 43: 129-139.

Cadrin, S.X., and V.M. Silva. 2005. Morphometric variation of yellowtail flounder. ICES Journal of Marine Science 62: 683-694.

Conroy, C.W. Calvert, J., Sherwood, G.D., and Grabowski, J.H. 2017. Distinct responses of sympatric migrant and resident Atlantic cod phenotypes to temperature at a remote Gulf of Maine seamount. ICES Journal of Marine Science fsx101.

Gosse, K.R., and Wroblewski, J.S. 2004. Variant colourations of Atlantic cod (*Gadus morhua*) in Newfoundland and Labrador nearshore waters. ICES Journal of Marine Science 61: 752-759.

Gurshin, C.W.D., Howell, W.H., and Jech, J.M. 2013. Synoptic acoustic and trawl surveys of spring-spawning Atlantic cod in the Gulf of Maine cod spawning protection area. Fisheries Research 141: 44-61.

Haddon, M., and T.J. Willis. 1995. Morphometric and meristic comparison of orange roughy (*Hoplostethus atlanticus*: Trachichthyidae) from the Puysegur Bank and Lord Howe Rise, New Zealand, and its implications for stock structure. Marine Biology 123: 19-27.

Kocovsky, P.M., J.V. Adams, and C.R. Bronte. 2009. The effect of sample size on the stability of principal components analysis of truss-based fish morphometrics. Transactions of the American Fisheries Society 138: 487-496.

Marcil, J., D.P. Swain, and J.A. Hutchings. 2006. Genetic and environmental components of phenotypic variation in body shape among populations of Atlantic cod (*Gadus morhua* L.). Biological Journal of the Linnean Society 88: 351-365.

Meng, H.J., and M. Stocker. 1984. An evaluation of morphometrics and meristics for stock separation of Pacific herring (*Clupea harengus* pallasi). Canadian Journal of Fisheries and Aquatic Sciences 41: 414-422.

Morinville, G.R., and J.B. Rasmussen. 2008. Distinguishing between juvenile anadromous and resident brook trout (*Salvelinus fontinalis*) using morphology. Environmental Biology of Fishes 81: 171-184.

Runge, J.A., Kovach, A.I., Churchill, J.H., Kerr, L.A., Morrison, J.R., Beardsley, R.C., Berlinsky, D.L., Chen, C., Cadrin, S.X., Davis, C.S., Ford, K.H., Grabowski, J.H., Howell, W.H., Ji, R., Jones, R.J., Pershing, A.J., Record, N.R., Thomas, A.C., Sherwood, G.D., Tallack, S.M.L., and Townsend, D.W. 2010. Understanding climate impacts on recruitment and spatial dynamics

of Atlantic cod in the Gulf of Maine: integration of observations and modelling. Progress in Oceanography 87: 251-263.

Sherwood, G.D., and Rose, G.A. 2005. Stable isotope analysis of some representative fish and invertebrates of the Newfoundland and Labrador continental shelf food web. Estuarine Coastal and Shelf Science 63: 537-549.

Sherwood, G.D., Grabowski, J.H. 2010. Exploring the life-history implications of colour variation in offshore Gulf of Maine cod (*Gadus morhua*). ICES Journal of Marine Science 67: 1640-1649.

Sherwood, G.D., and Grabowski, J.H. 2015. A comparison of cod life-history parameters inside and outside of four year-round groundfish closed areas in New England, USA. ICES Journal of Marine Science 73: 316-328.

Sherwood, G.D., Kerr, L.A., Gurshin, C.W.D., Howell, W.H. Goethel, D. 2017. Synoptic acoustic and trawl survey of winter-spawning cod in Ipswich Bay, western Gulf of Maine. Final Report to Northeast Consortium 2014/2015 NEFMC Groundfish Research Program. 60 p.

Table 1. Anova results for effect of capture location, spawning time and their interaction on morphometric variables (residuals). See Figure 6 for direction and magnitude of effects.

	Effect						
	Capture location		Spawning time		Interaction		
Measure	F	Sig	F	Sig	F	Sig	N
D1	**120.23**	**0.000**	**8.97**	**0.003**	**4.63**	**0.032**	260
D2	**56.16**	**0.000**	**26.03**	**0.000**	2.16	0.143	260
D3	**14.12**	**0.000**	3.55	0.061	2.55	0.112	260
D4	**140.76**	**0.000**	1.38	0.240	0.21	0.648	260
D5	**49.28**	**0.000**	**9.80**	**0.002**	0.12	0.727	260
D6	**73.23**	**0.000**	**16.06**	**0.000**	0.52	0.473	260
D7	**16.90**	**0.000**	**36.50**	**0.000**	0.38	0.536	260
D8	**45.71**	**0.000**	**88.59**	**0.000**	**7.10**	**0.008**	260
D10	**10.27**	**0.002**	**16.52**	**0.000**	0.00	0.953	260
D13	0.81	0.368	0.04	0.841	0.77	0.381	260
D14	**22.38**	**0.000**	1.22	0.271	0.33	0.567	260
D16	3.29	0.071	2.15	0.144	0.02	0.902	260
D17	**17.63**	**0.000**	0.00	0.970	1.53	0.217	260
D18	**51.09**	**0.000**	0.00	0.964	**24.02**	**0.000**	260
D19	0.61	0.436	2.88	0.091	**10.61**	**0.001**	260
D21	**29.06**	**0.000**	0.93	0.336	**7.08**	**0.008**	260
D22	**7.03**	**0.009**	**54.56**	**0.000**	0.53	0.466	260

Table 2. Results of discriminant function analysis using 17 body morphometric variables to predict membership in 4 spawning groups (Ipswich Bay spring IS, Ipswich Bay winter IW, Mass Bay spring MS, and Mass Bay winter MW). Overall, 82.3% of cases were correctly reclassified to their respective groups.

			Predicted group membership			
		Spawning group	IS	IW	MS	MW
Original groupings	Count	IS	**68**	14	1	1
		IW	19	**90**	2	1
		MS	2	0	**26**	1
		MW	3	0	2	**30**
	%	IS	**81**	17	1	1
		IW	17	**80**	2	1
		MS	7	0	**90**	3
		MW	9	0	6	**86**

Table 3. Results of discriminant function analysis using 17 body morphometric variables and RGR to predict membership in 4 spawning groups (Ipswich Bay spring IS, Ipswich Bay winter IW, Mass Bay spring MS, and Mass Bay winter MW). Overall, 84.3% of cases were correctly reclassified to their respective groups.

			Predicted group membership			
		Spawning group	IS	IW	MS	MW
Original groupings	Count	IS	**71**	11	1	1
		IW	20	**88**	0	0
		MS	1	0	**26**	1
		MW	0	1	4	**30**
	%	IS	**84**	13	1	1
		IW	18	**82**	0	0
		MS	4	0	**93**	4
		MW	0	3	11	**86**

Figure Captions

Figure 1. Landmarks and box-truss elements used in morphometric analysis. Distances including landmark #11 (grey lines) were not included in statistical analyses but are represented in Figures 7-9.

Figure 2. Mean red to green ratio (RGR ± 1 SE) for spawning condition cod from 4 spawning groups. Capture location was significant (ANOVA: $F_{1,255} = 163.1$, $p < 0.0001$) but not spawning time. However, the interaction was (ANOVA: $F_{1,255} = 7.9$, $p < 0.01$).

Figure 3. Red to green ratio (RGR) as a function of length and spawning group. Both variables significantly predict RGR (ANCOVA: $R^2 = 0.53$, $p < 0.0001$ for both effects and interaction).

Figure 4. Results of discriminant function analysis to explore morphometric groupings based on 17 body morphometric variables. See Table 2 for classification results. Colors represent mean RGR for each group from least red (greenest oval) to most red.

Figure 5. Results of discriminant function analysis to explore morphometric groupings based on 17 body morphometric variables and RGR. See Table 3 for classification results. Colors represent mean RGR for each group from least red (greenest oval) to most red.

Figure 6. (multifaceted) Bar graphs of mean residuals (± 1 SE) from relationships between PCA1 and individual distance measurements (i.e., length-corrected distances) for 4 spawning groups of cod. The individual measurement associated with each graph is shown on the right. See Table 1 for significance levels.

Figure 7. Mean reconstructed shape (solid black area) of spring- vs. winter-spawning cod from Ipswich Bay. Dashed white line over/around each shape is outline for the opposite group. Winter-spawning cod from Ipswich Bay are more robust (deeper bodied) than spring-spawning cod. White circles are individual land marks (see Figure 1).

Figure 8. Mean reconstructed shape (solid black area) of spring- vs. winter-spawning cod from Massachusetts Bay. Dashed white line over/around each shape is outline for the opposite group. Winter-spawning cod from Massachusetts Bay are more robust (deeper bodied) than spring-spawning cod. White circles are individual land marks (see Figure 1).

Figure 9. Mean reconstructed shape (solid black area) of spring- vs. winter-spawning cod from both spawning areas. Dashed white line over/around each shape is outline for the opposite group. Winter-spawning cod, in general, are more robust (deeper bodied) than spring-spawning cod. White circles are individual land marks (see Figure 1).

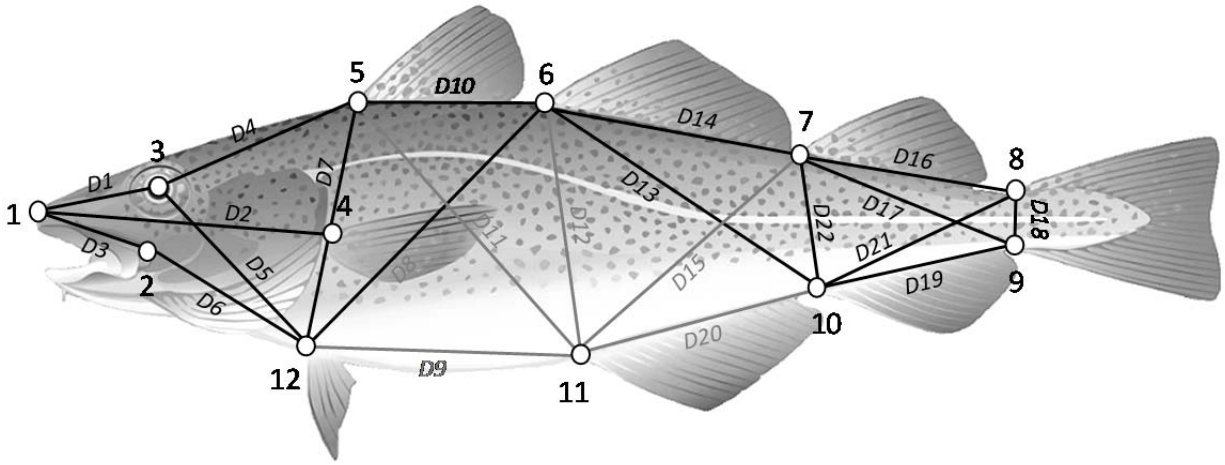

1
2
3
4
5
6
7
8
9
10
11
12
D1
D2
D3
D4
D5
D6
D7
D8
D9
D10
D11
D12
D13
D14
D15
D16
D17
D18
D19
D20
D21
D22

Figure 1.

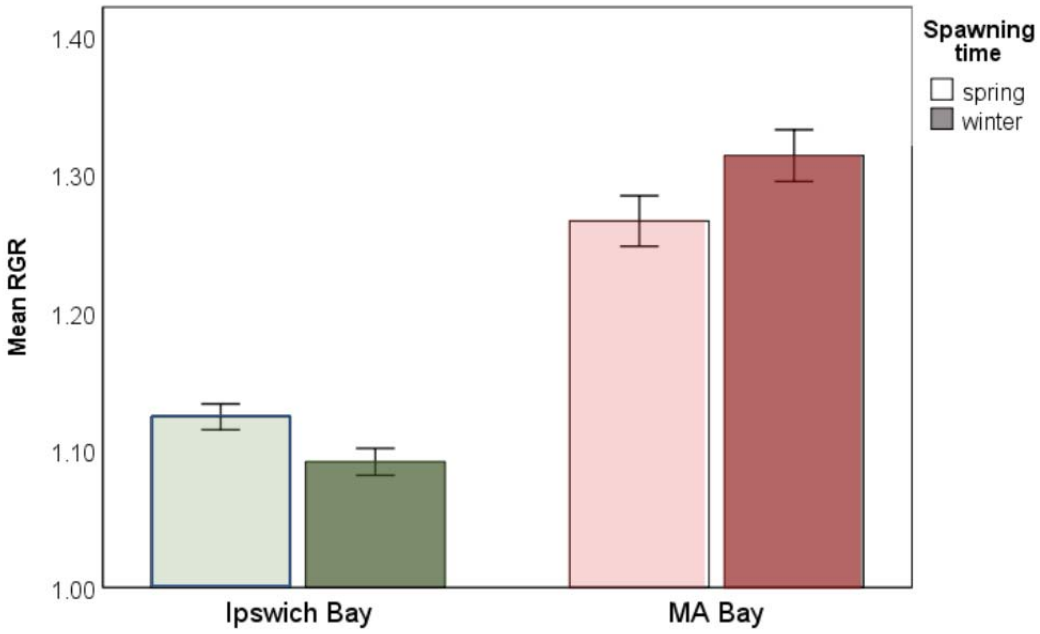

Mean RGR
1.40
1.30
1.20
1.10
1.00
Ipswich Bay
MA Bay
Spawning time
spring
winter

Figure 2.

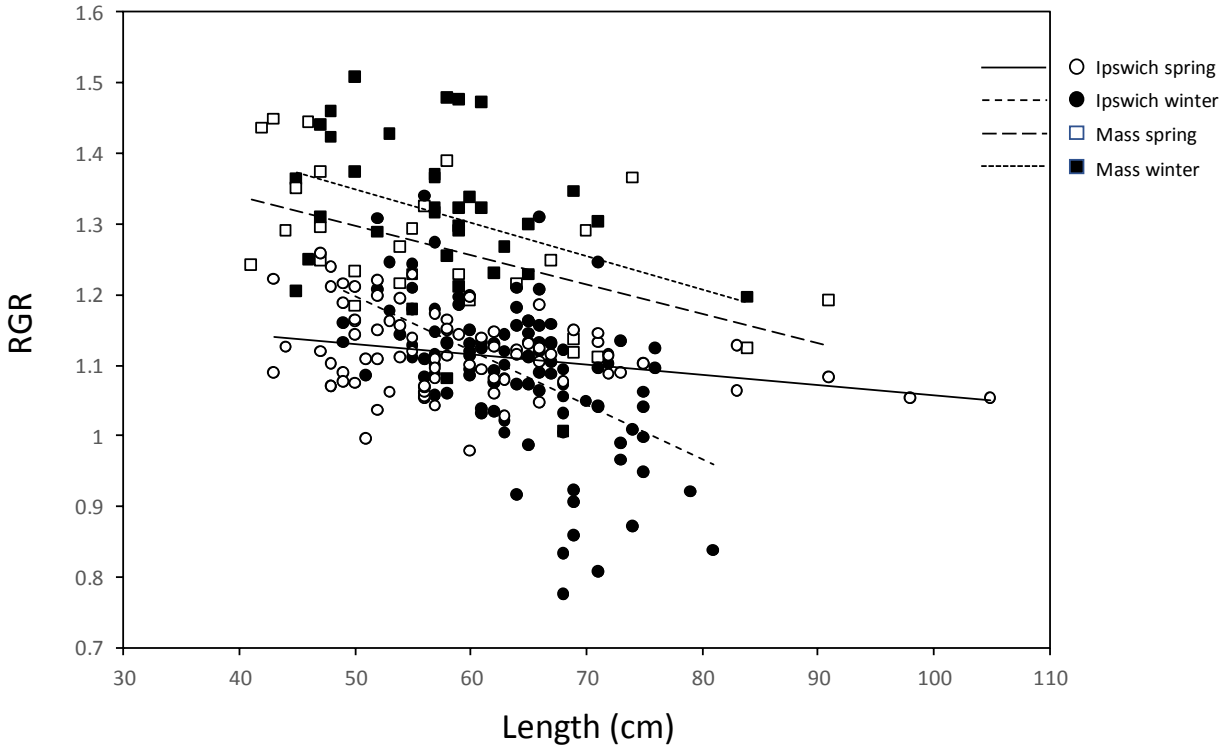

RGR
Length (cm)
Ipswich spring
Ipswich winter
Mass spring
Mass winter
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
30
40
50
60
70
80
90
100
110

Figure 3.

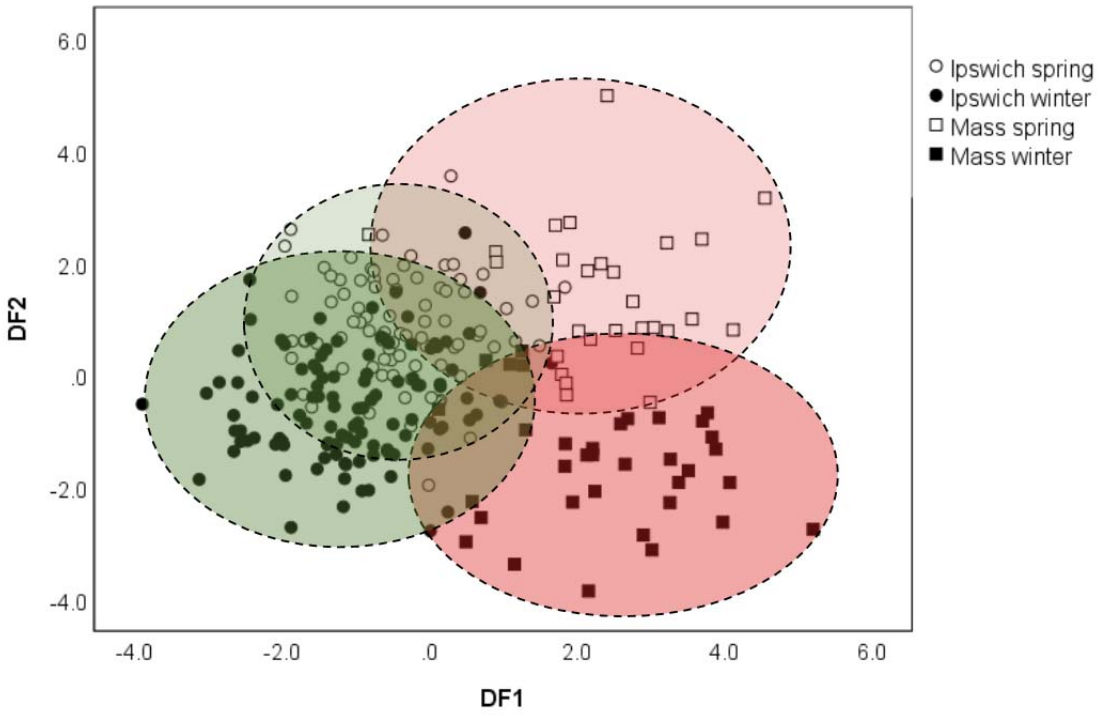

6.0
4.0
2.0
.0
-2.0
-4.0
DF2
-4.0
-2.0
.0
2.0
4.0
6.0
DF1
Ipswich spring
Ipswich winter
Mass spring
Mass winter

Figure 4.

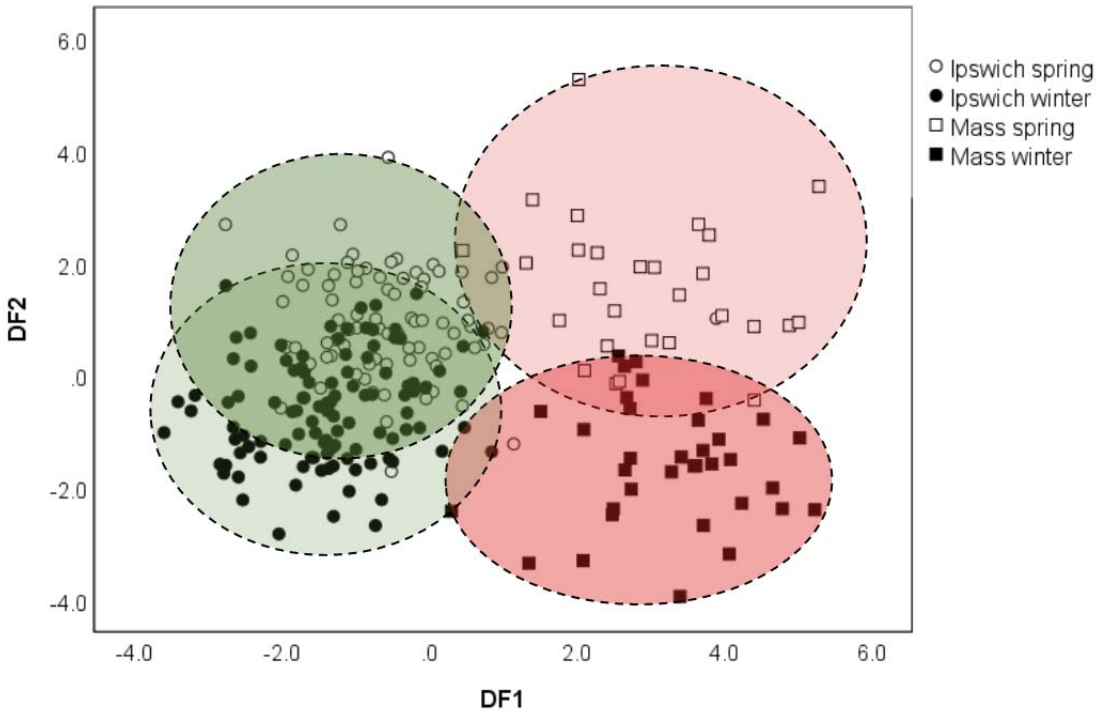

Ipswich spring
Ipswich winter
Mass spring
Mass winter
DF2
DF1
6.0
4.0
2.0
.0
-2.0
-4.0
-4.0
-2.0
.0
2.0
4.0
6.0

Figure 5.

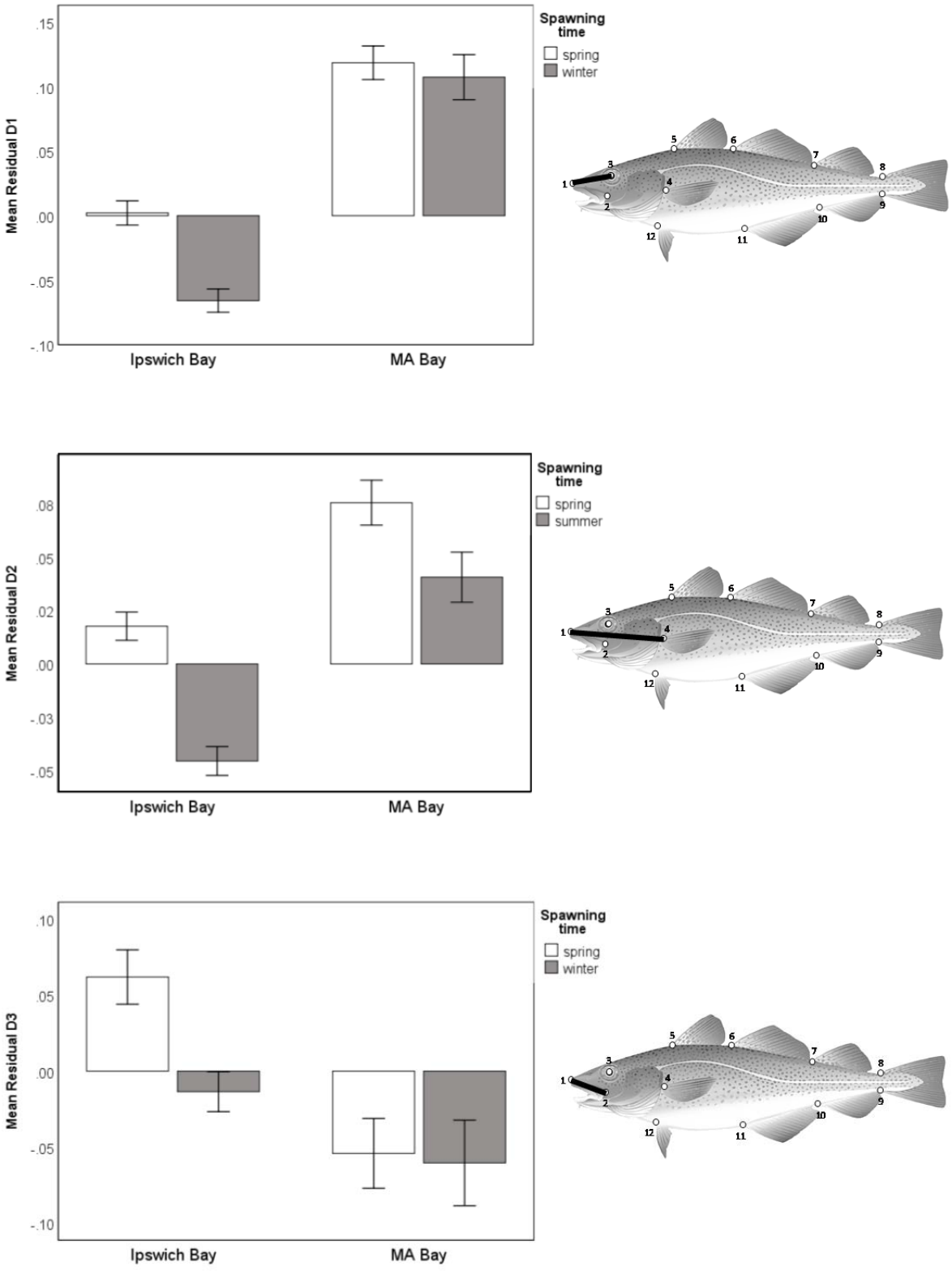

Mean Residual D1
.15
.10
.05
.00
-.05
-.10
Ipswich Bay
MA Bay
Spawning time
spring
winter
Mean Residual D2
.08
.05
.02
.00
-.03
-.05
Ipswich Bay
MA Bay
Spawning time
spring
summer
Mean Residual D3
.10
.05
.00
-.05
-.10
Ipswich Bay
MA Bay
Spawning time
spring
winter

Figure 6.

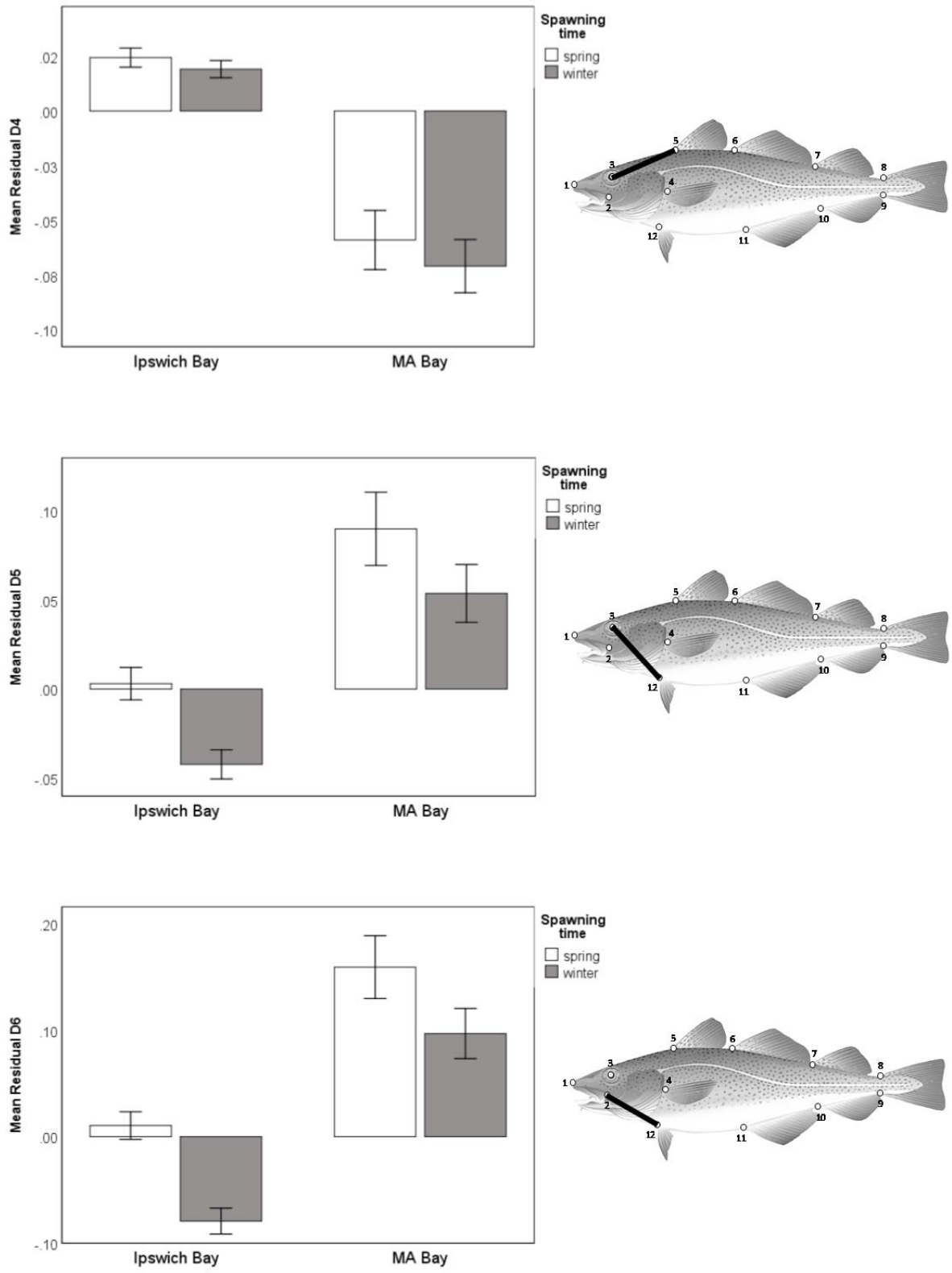
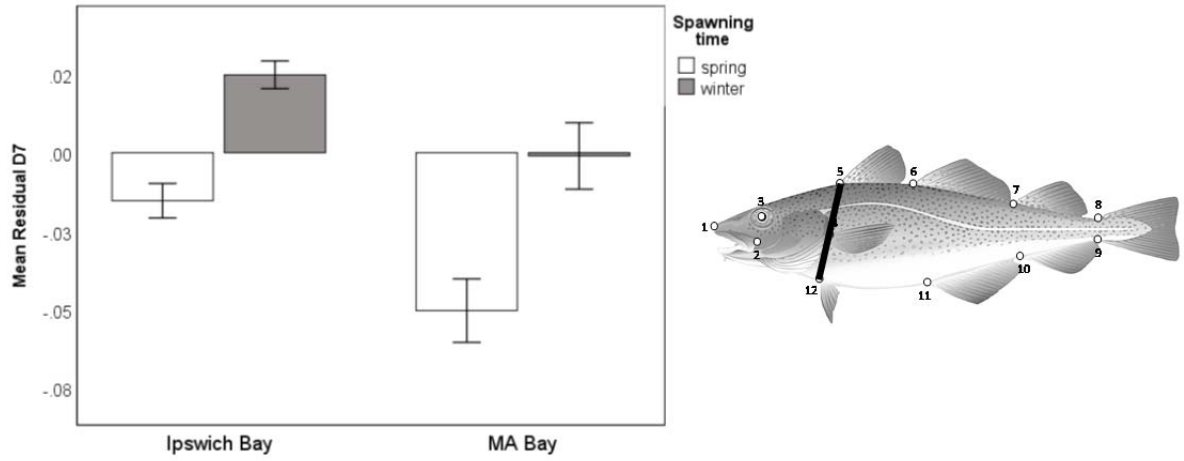
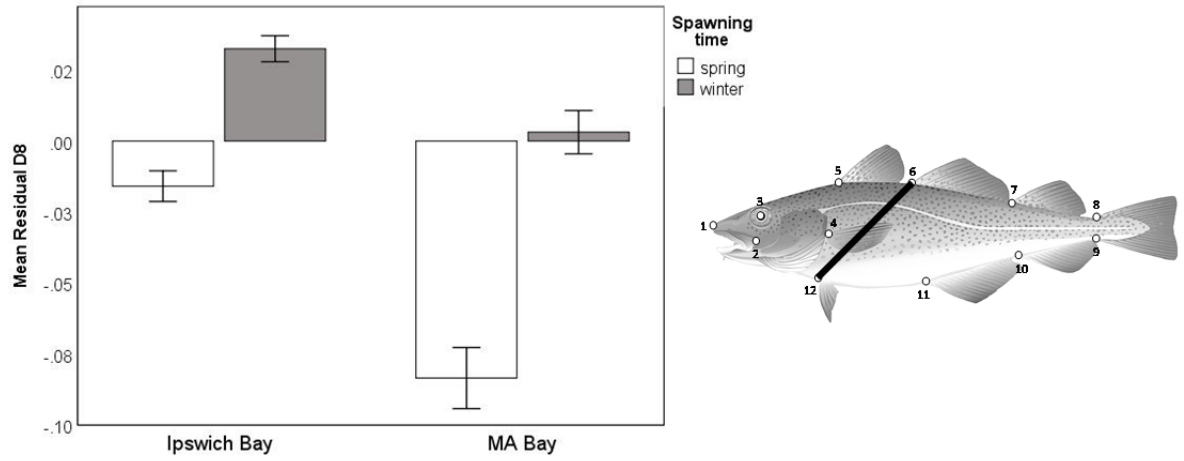


Figure 6. (cont.)





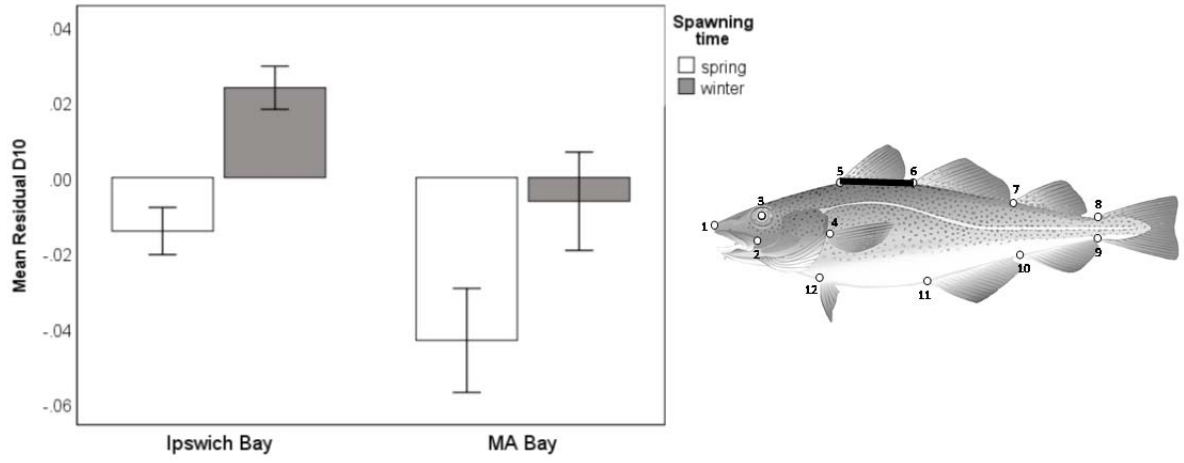
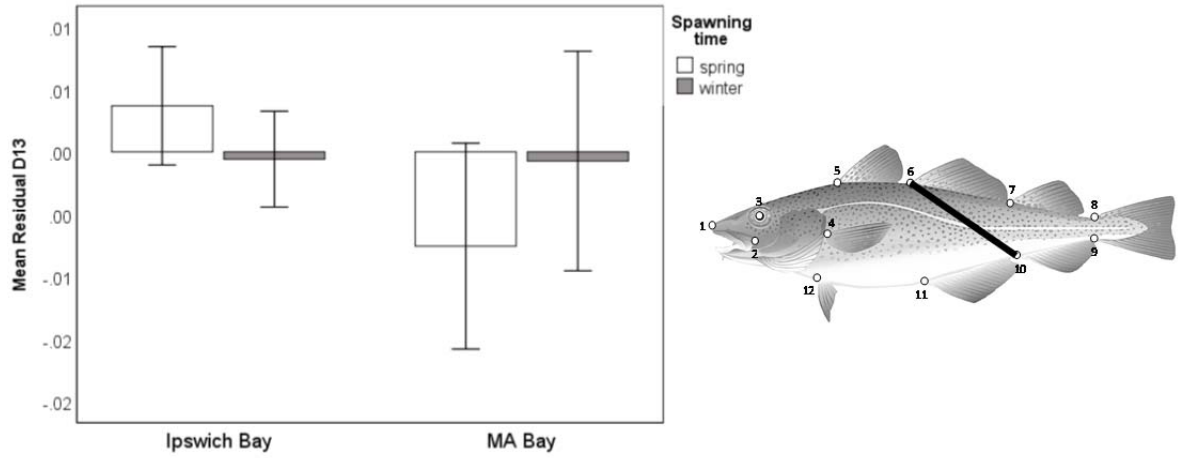
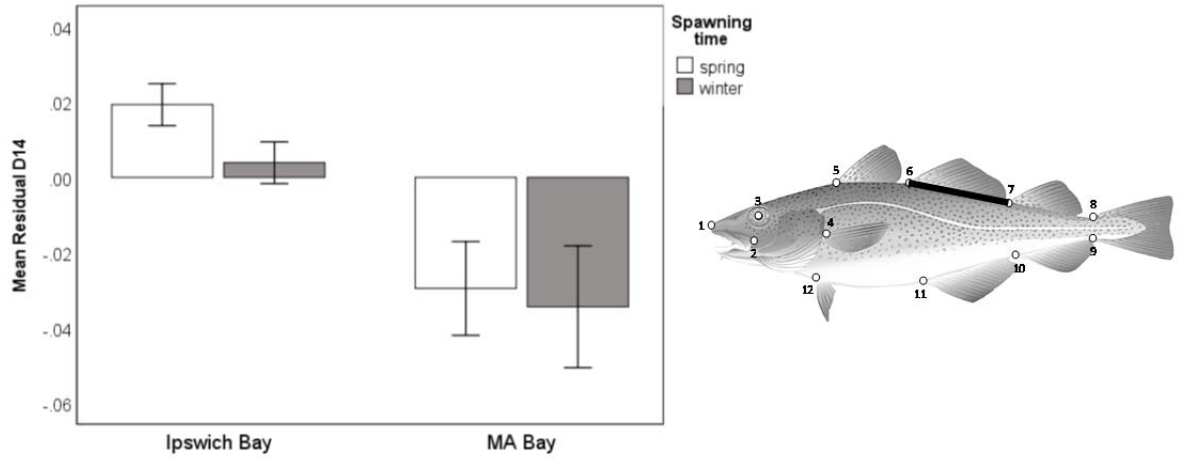


Figure 6. (cont.)





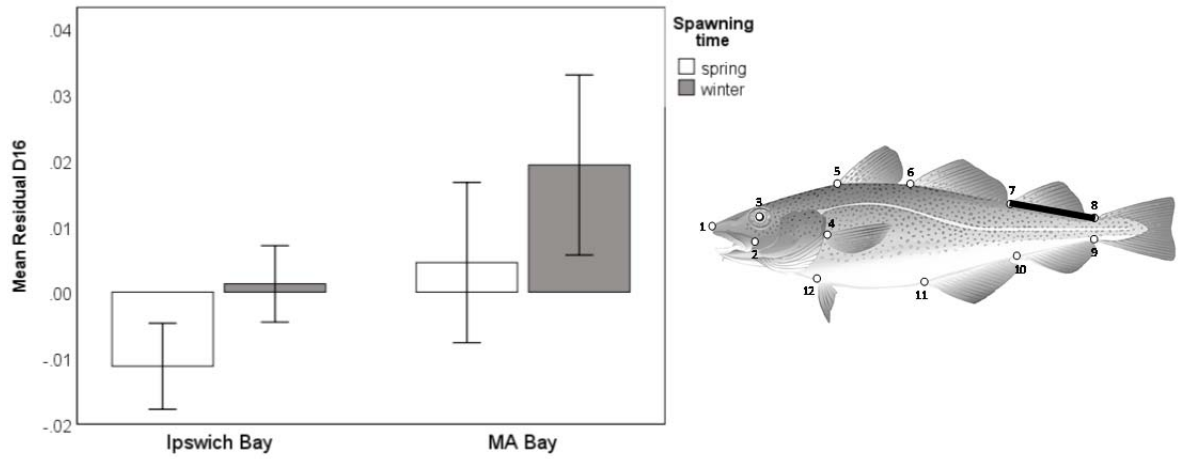
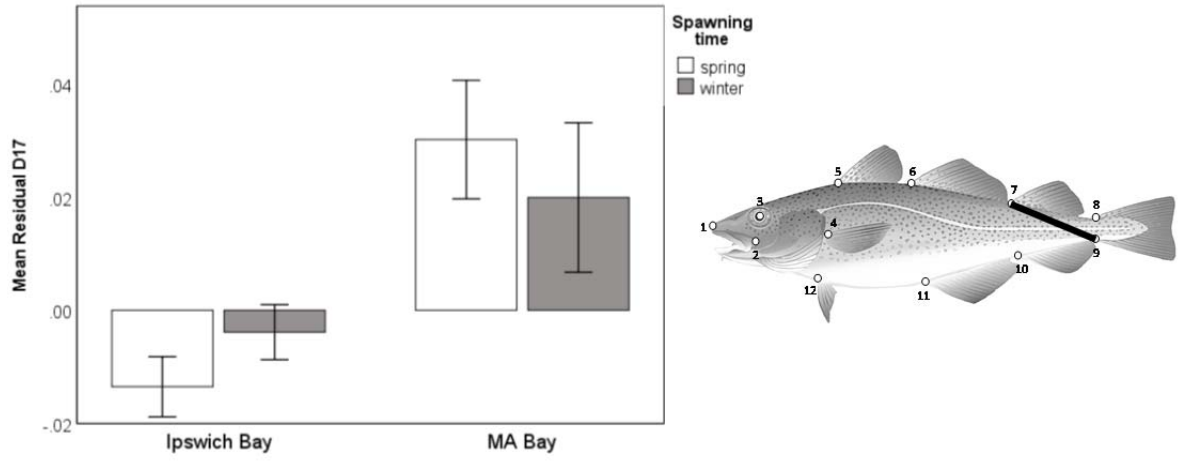
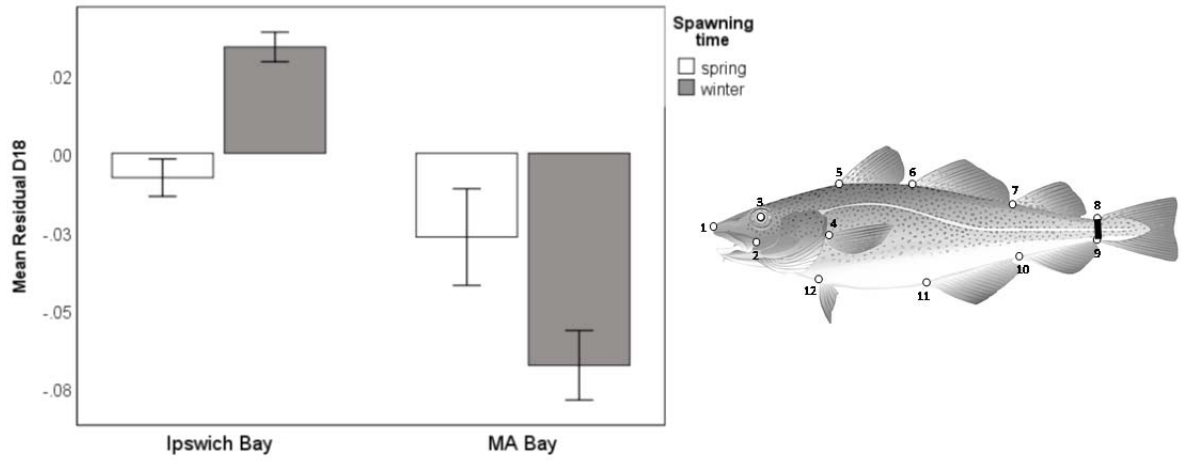


Figure 6. (cont.)





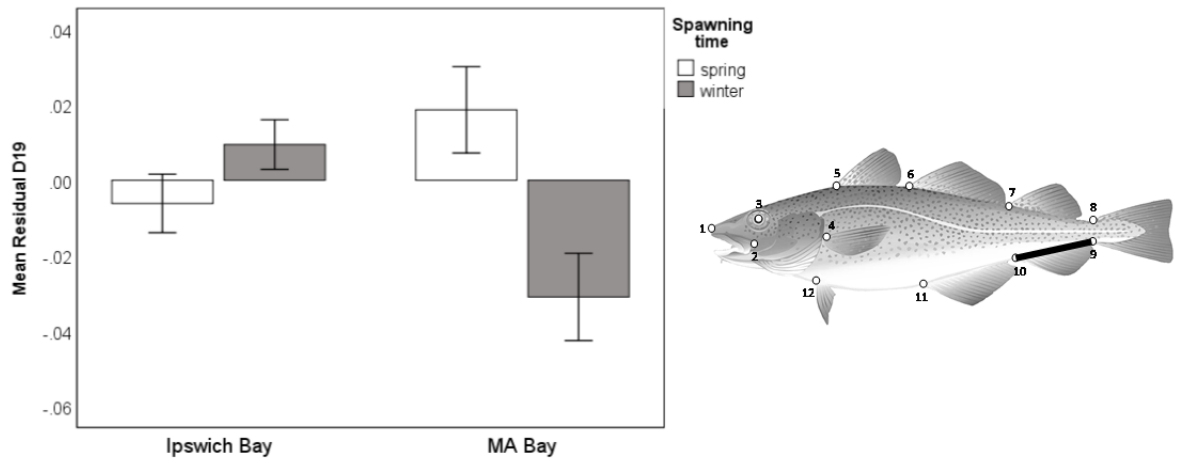


Figure 6. (cont.)

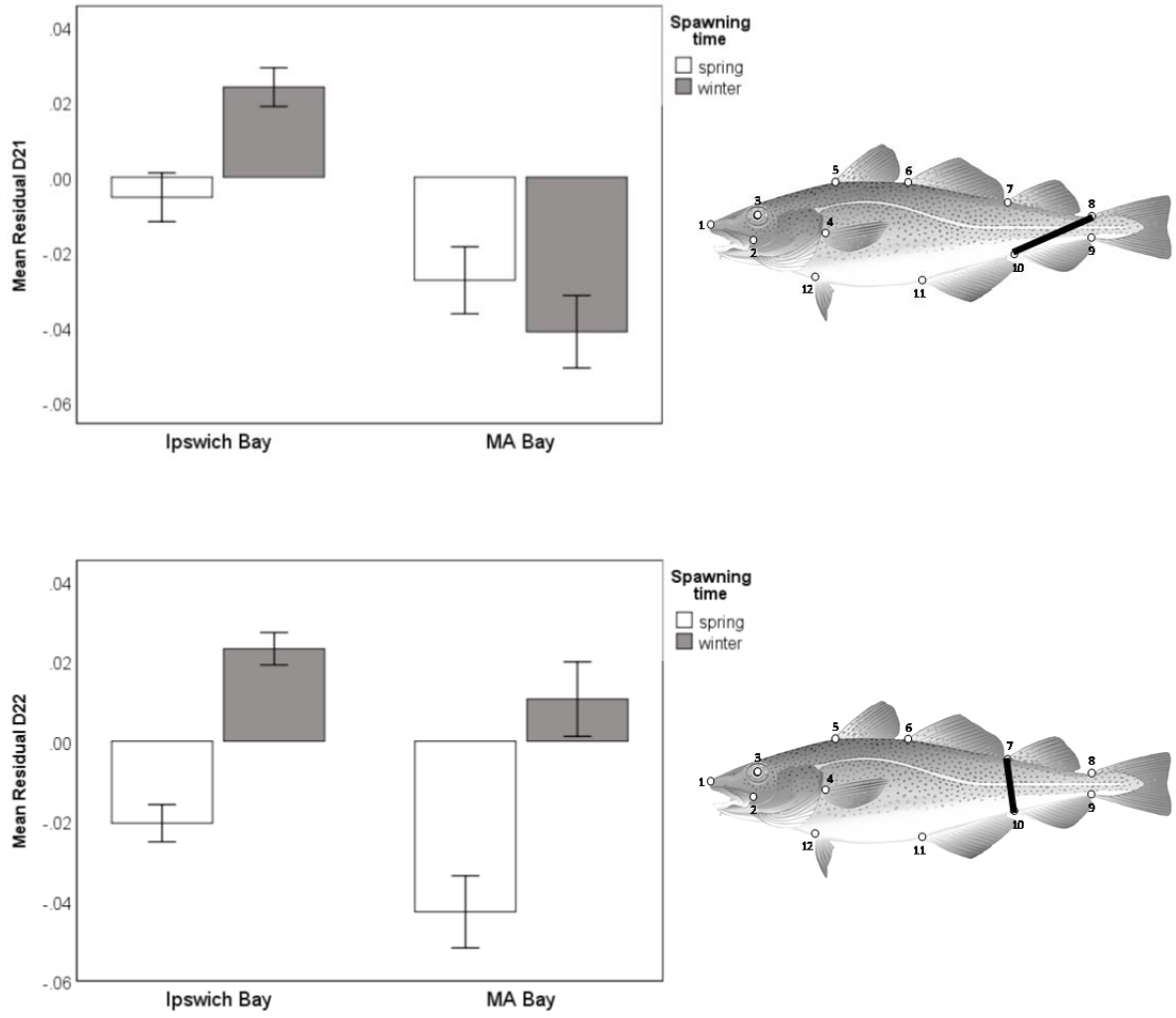


Figure 6. (cont.)

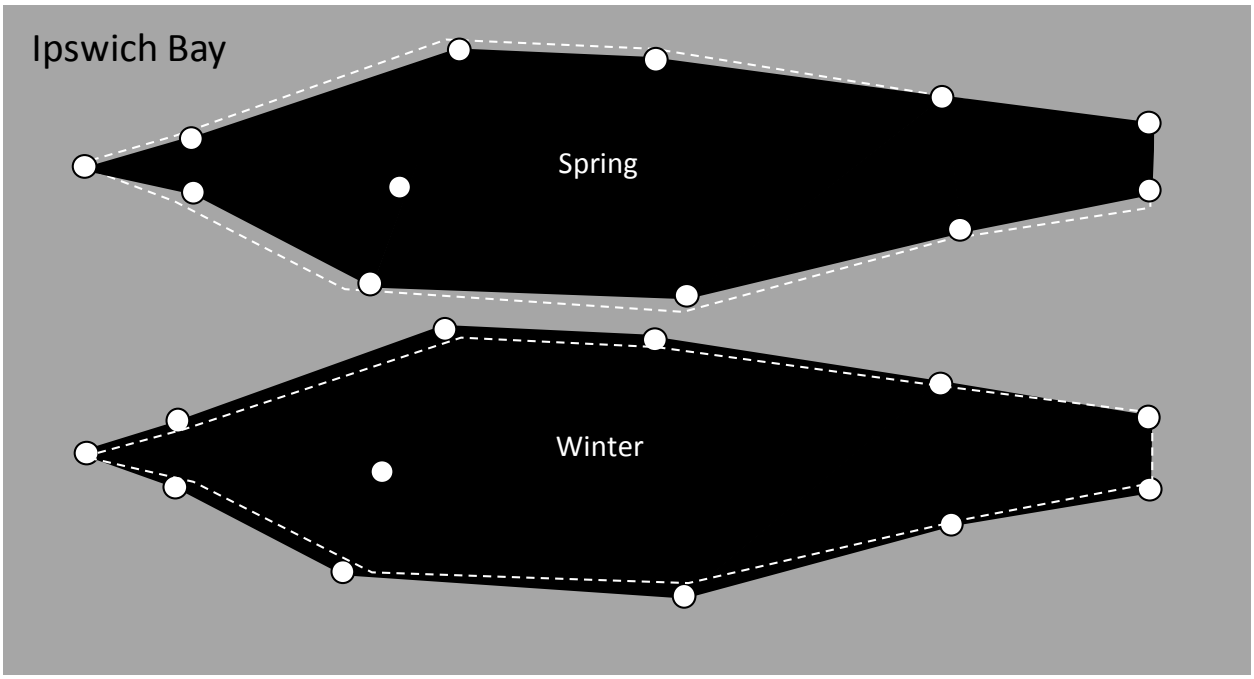


Figure 7.

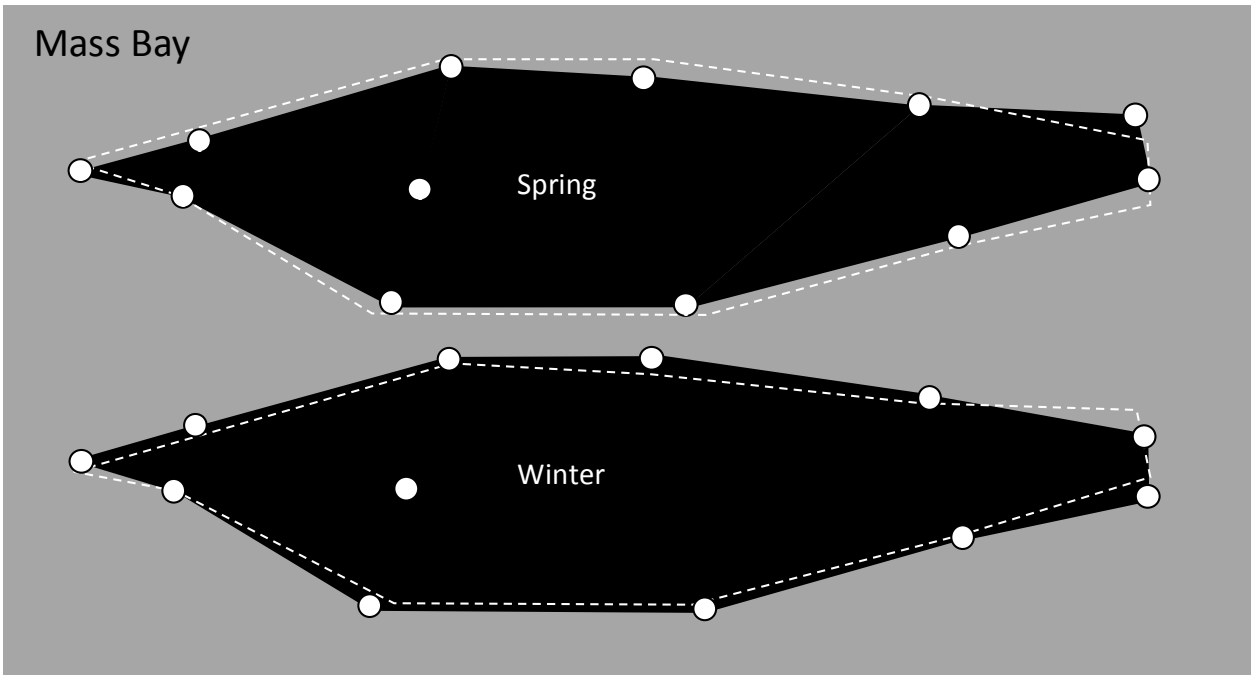


Figure 8.

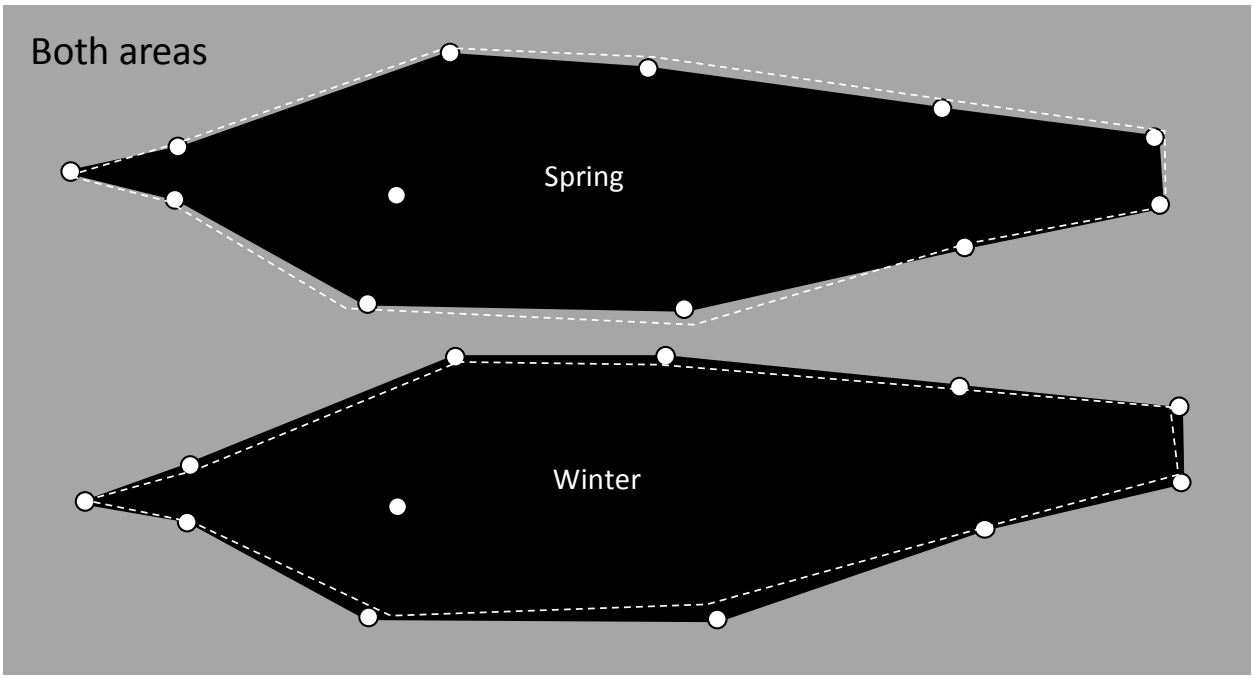


Figure 9.