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Applicability of a New Model to NEFSC Bottom Trawl Survey Samples

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Evaluating Otolith Growth Patterns to Discriminate among Subpopulations of Northwest Atlantic Cod (*Gadus morhua*):

Applicability of a New Model to NEFSC Bottom Trawl Survey Samples

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ABSTRACT

US Atlantic cod (*Gadus morhua*) stock structure is complex, with two sympatric spawning subpopulations found in the western Gulf of Maine. Dean et al. (2019) developed a model to distinguish these two cod subpopulations based on measurements of otolith annuli. In this pilot study project, we assess the feasibility of this new model by applying it to independent samples of Atlantic cod otoliths collected aboard the Northeast Fisheries Science Center's bottom trawl surveys. Results of model runs suggest the proportion of the spring spawning cod in the region was lower in the mid-2010s compared to mid-1970s, all relative to the winter spawning cod subpopulation. While the approach appears appropriate and the results are compelling, additional work would be required to ensure observed trends reflect subpopulation composition of the broader western Gulf of Maine Atlantic cod population and to develop appropriate models to examine subpopulation composition across decades.

INTRODUCTION

Currently, US Atlantic cod (*Gadus morhua*) are assessed and regulated as the Gulf of Maine and Georges Bank management units (NEFSC 2017). While there is debate about the spatial boundaries of these units relative to underlying biological stock structure of cod (Zemeckis et al. 2014), there is also an awareness of subpopulation structure within units, which arises from cod groups that spawn at different times of year in an overlapping area of the western Gulf of Maine (Ames 2004; Zemeckis et al. 2014). The presence of both spring (April to July) and winter (October to February) spawning in the western Gulf of Maine has long been known from cod egg and larvae distributions (Berrien and Sibunka 1999). More recently, these 2 spawning periods have been demonstrated to be composed of fish from 2 genotypes (Wirgin et al. 2007; Kovach et al. 2010; Clucas et al. 2019), raising concerns about managing to conserve genotypic diversity as well as population resilience (Zemeckis et al. 2014).

The presence of 2 sympatric subpopulations in the western Gulf of Maine complicates our understanding of stock dynamics and allocation of catch to spawning groups. This dual presence motivated Dean et al. (2019) to develop a method to distinguish between these 2 subpopulations in mixed catches. Using cod collected by bottom trawl as part of an industry-based survey (IBS), many caught in spawning condition and assigned to spawning group, they noted that the first 2 annuli of winter spawning fish were significantly larger than those of spring spawning fish in the western Gulf of Maine. The first annulus is presumably not formed until after the first full over-wintering experience in winter-spawned cod; therefore, the first annulus represents more than 1 year of growth in winter-spawned cod and less than 1 year of growth in spring-spawned cod. Dean et al. (2019) validated a logistic model by using annuli lengths of cod from known spawning periods and used this model to predict the relative contribution of spring and winter spawning subpopulations in an area of the western Gulf of Maine in the last 2 decades.

Here, as a pilot project to assess feasibility of the approach, we applied the logistic model from Dean et al. (2019) to independent measurements of otolith annuli lengths from fish collected by the Northeast Fisheries Science Center (NEFSC) bottom trawl survey during 2 periods: a contemporary (2015-2016) and historic (1974-75) period. Photomicrographs were captured from sectioned otoliths, and, as in Dean et al. (2019), image analysis software was used to measure the length (widest diameter) of each of the first 3 annuli. These measurements were used in the logistic model to predict the relative proportion of spring- and winter-spawned fish. These proportions were compared between the contemporary and historic periods sampled by the NEFSC survey, as well as 2 relatively recent periods (2003-2007, 2016-2019) sampling by the IBS (Dean et al. 2019). Finally, we consider how to apply this method more broadly to the entire archive of NEFSC survey cod samples to address hypotheses about cod population dynamics in response to a changing climate or to discriminate spawning origin among mixed-stock fisheries.

METHODS

Sampling Platform

All otoliths used in this study were collected by the NEFSC, which has been conducting standardized fishery-independent bottom trawl surveys along the northeastern continental shelf of the United States since 1963 (Politis et al. 2014). Per survey protocols, sampling stations were randomly assigned to fixed survey strata (Figure 1), with strata delineations corresponding to depth and latitudinal gradients. All fish and invertebrate species are sorted to lowest possible taxonomic level and measured for size according to standard protocols (e.g., to the nearest cm fork length). Additional biological data (e.g., gonad development status and weight; stomach volume and contents) and samples (e.g., otoliths for age analysis, gonadal tissue for histological analysis) are collected from length-based subsamples for approved special requests that vary with each survey.

Samples included in subpopulation prediction and model comparisons were selected based on survey strata in an attempt to most closely match the area of the western Gulf of Maine covered by the IBS and used to develop the model by Dean et al. (2019). All fish available from offshore strata 26 and 27 and inshore strata 59-66 (Figure 1) were used for both contemporary and historic analyses (Table 1). Fish used in the contemporary analyses were collected during the fall surveys between October 21 and November 5 in 2015 (n = 309) and October 22 and November 1 in 2016 (n = 98). Samples were limited to the fall as the timing of the survey was best suited for corroborating model results based on gonad maturity status: a greater diversity of gonad maturity stages are available in the fall for Atlantic cod than are in the spring. Fish used in the historic analysis were collected during the spring surveys on April 17 and May 5, 1974 (n = 41) and between May 6 and May 11, 1975 (n = 63). The spring survey was used because of the availability of previously captured photomicrographs. Sexes were combined for analyses in both periods, as annuli diameters are not significantly different between sexes (M. Dean, personal communication).

Otolith Imaging and Measuring

All otoliths from the contemporary period had been baked, mounted in resin, and sectioned following the standard protocols of the NEFSC Fishery Biology Program (https://www.nefsc.noaa.gov/fbp/age-man/meth/meth.htm). Grayscale photomicrographs of the sectioned otoliths were subsequently captured under reflected light by using a stereo microscope with mounted digital camera (2.5x objective). For the historic period, 56 previously captured photomicrographs of otoliths that had been embedded in resin and sectioned were used for annuli measurements. The remaining 48 otoliths were baked, embedded in wax, and sectioned, and then photomicrographs were captured. Ages were available from all otoliths used for annuli measurements. Otoliths were excluded from measurements if they were missing from the resin/wax, cracked through the first annulus, or otherwise unreadable.

ImageJ software (Vers. 1.51k, National Institute of Health, Bethesda, MD) was used to measure the length (0.01 mm) of the first, second, and third annuli on each otolith (Figure 2). Second and third annuli were only measured from fish aged as at least 3 or 4, respectively, to ensure proper measurement of the annulus of interest. Measurements extended to the outermost edge of hyaline growth. Each measurement was assigned a confidence score: 1 = clearly defined margins of annuli, high confidence in accuracy of measurement; 2 = diffuse margins, measurement accuracy to within 100 μm; 3 = extremely diffuse margins or presence of multiple checks, accuracy of measurement unknown. Annuli with a confidence score of 3 (n = 16) were excluded from analyses. Measurements were conducted blindly, without gonad stage, length, or age (previously assigned by the production ager of the NEFSC Fishery Biology Program according to standard protocols https://www.nefsc.noaa.gov/fbp/age-man/cod/cod.htm). Year class (cohort) was assigned by subtracting the assigned age from year of capture.

First Annulus Assignment and Measurement Cross-verification

The age as determined by the image processor (L. Lefebvre) was recorded at the time when annuli measurements were made and compared to the age previously assigned by the NEFSC Atlantic cod production ager (N. Shepherd) for otoliths from the contemporary period. In 58 cases (9% of the samples), the age assigned by the NEFSC production ager was 1 year less than that assigned by the image processor. After conferring with the production ager, these differences were deemed to be in misinterpretation of the settlement check (Pentilla 1988) assigned as a first annulus by the image processor. The diameter of the second annulus was reassigned to the first and the third to the second in these cases. The measurement of the presumed settlement check was retained. First annulus assignment was confirmed by the production ager for all otoliths from the historic period.

To compare first annulus assignment and precision of annuli measurements between the NEFSC and Massachusetts Division of Marine Fisheries (MA DMF), 100 otolith images were sent to the MA DMF. Otoliths from the contemporary period were randomly selected from the cohorts with the greatest sample numbers – 2011, 2012, and 2013. A MA DMF employee independently assigned and measured the first 2 annuli on each image. Measurements were compared to those of the NEFSC, and the coefficient of variation (CV) between each center's first annulus measurement was calculated.

Spawning Subpopulation Prediction and Comparison

We used the best fit model from Dean et al. (2019) to classify individual fish as members of the winter or spring spawning subpopulation for samples from the contemporary period. Details of the model development can be found in Dean et al. (2019). Briefly, the model was a generalized linear mixed model (glmm) with binomial error structure. The response variable was the probability of being spring-spawned, and predictors included the continuous variables of first annulus length (A1) and fractional age (AGE 2; assigned otolith age + the quotient of day of the year of capture and 365) as well as a categorical predictor variable of period (PERIOD; period 1 = 2003-2007; period 2 = 2016-2017; all NEFSC samples from the contemporary period were assigned in the model to period 2). The model was developed by using the glmmTMB package (Brooks et al. 2017) in R (R Core Team 2019) with a training set of measurements from fish of known spawning status (fish with hydrated eggs or flowing sperm considered spawning) collected during the IBS. Individual fish were assigned a probability of being spring-spawned: fish with probabilities >0.50 were considered spring-spawned with all others considered winter-spawned. The subpopulation composition was examined at time of capture (by age) and at age 0 (by cohort). Composition by cohort considered differential mortality rates experienced by spring and winter spawners (spring spawners are hypothesized by Dean et al. 2019 to have lower fishing mortality because of spatial closures). To determine cohort composition, we back calculated each individual by using the slope of the "AGE" term from the glmmTMB model (see Dean et al. 2019 for details).

We also examined results of model runs using the contemporary data with the categorical predictor variable "period" removed (model = "A1 + AGE2") because some of our samples were collected outside these periods (e.g., fall 2015) and "period" was not used in analysis of the historic samples (see below). The model was run twice: first utilizing the full set of contemporary samples available, and second, because all historic samples came from offshore stratum 26 (Table 1), utilizing only samples that were collected in stratum 26 (n = 105).

Model performance for the contemporary period was evaluated by comparing gonad development stage, especially for spawning individuals, to model prediction of spawning season. Gonad development stage (Burnett et al. 1989; I = immature, D = developing, R = ripe, U = ripe and running, S = spent, T = resting) was evaluated macroscopically and assigned at the time of capture on the NEFSC bottom trawl surveys. Fish with stage R and U gonads (n = 122) were considered to be known winter spawners, whereas fish with gonads in other stages of development could not accurately be assigned to a spawning subcomponent. The percentage of known winter spawners accurately assigned by the model to the winter spawning subcomponent was calculated.

Photomicrographs from 104 fish collected in the western Gulf of Maine on the spring bottom trawl surveys of 1974 and 1975 were available for analysis for the historic period (Table 1). We used a model from Dean et al. (2019) to assign subpopulation to each individual fish, with the probability of being spring-spawned as the response and the continuous variable of first annulus length and fractional age as the predictors (model = “A1 + AGE2”). This model did not include the predictor variable “period” used for analysis of contemporary samples because this variable was only valid for fish from cohorts dating from the mid-1990s to 2017. The subpopulation composition was examined at time of capture (by age). Subpopulation at age 0 (by cohort) was not examined with the historic dataset because the extent to which mortality did or did not between the subpopulations during that era was not known. Model performance was evaluated despite the small sample size of known spring spawners (n = 5) in the historic dataset.

In describing patterns of subpopulation composition based on the results of their model, Dean et al. (2019) included a weighting factor for each individual to correct for differences in intensity of sampling spatially (e.g., survey effort) as well as with respect to fish size (e.g., subsampling of catches). We did not include a weighting factor in our analyses, so population-level trends are presented with the caveat that we do not know with certainty whether or not they are representative of the population as a whole.

All analyses were conducted by using RStudio (RStudio Team 2018) and figures were produced by using the packages ggplot2 (Wickham 2016) and cowplot (Wilke 2019).

RESULTS

There were 407 fish from the fall surveys of 2015 and 2016 available for analyses of the contemporary dataset. Fish ranged in length from 24 to 92 cm and 1 to 7 years of age (Table 2). One hundred four fish from the spring surveys of 1974 and 1975 were available for analyses of the historic dataset. Fish ranged in size from 24 to 123 mm and 2 to 11 years of age (Table 3). Respective annuli lengths were similar between survey years within periods (Table 4); however, measurements between periods (contemporary and historic) showed some divergence, with smaller modes and average annuli lengths for the historic period (Table 4; Figure 3).

First Annulus Assignment and Measurement Cross-verification

Assignment of annuli and measurement of annuli lengths were consistent between independent readers at the MA DMF and NEFSC. Agreement on the assignment of the first annulus was 89% (n = 89), with MA DMF assigning the first annulus to the presumed settlement check in 11 images. Of the 89 fish with agreed upon first annuli, 92% (n = 82) had a CV of less than 10% and all had a CV of less than 19%, for annuli lengths. Furthermore, in cases for which the assignment of the first annulus differed, the CV of the settlement check was less than 10%, when compared to the measurement of the first as assigned by MA DMF, suggesting measurement precision was similar between the institutions.

Spawning Subpopulation Prediction and Comparison

The first annulus length observed in the contemporary dataset ranged from 1.49 to 2.23 mm (1.98 ± 0.20 [mean ± standard deviation]) and 2.00 to 5.53 mm (2.96 ± 0.57 mm) for predicted spring and winter subpopulations, respectively. The model run including the predictor variable period demonstrated a higher proportion of winter spawners than that of spring spawners for all ages (Figure 4A). Only 18 of the 407 fish (4%) were predicted to be members of the spring subpopulation. The proportion of the spring subpopulation increased with increasing age from 3% of age 2 fish to 11% of age 7 fish (Figure 4A). When examining the cohort-level subpopulation composition (composition at age-0), only the 2012 cohort was predicted to have a noticeable percentage of spring spawners (1%, n = 1; Figure 4B). The model accurately assigned 95% (n = 116) of fish in spawning condition (gonads macroscopically staged as R or U; n = 122) to the winter spawning subpopulation.

When the variable period was removed, the number of fish predicted to be members of the spring subpopulation increased to 47 (12%; Figure 4C). When examining the cohort-level subpopulation composition, 1 fish from the 2008 (11%), 2012 (1%), and 2013 (<1%) cohorts was predicted to be a spring spawner (Figure 4D). Model accuracy decreased when period was removed, with 86% of the fish in spawning condition being accurately assigned to the winter spawning subpopulation. Model results from samples collected only in offshore stratum 26 were similar, with 14 of the 105 fish (13%) predicted to be members of the spring spawning subpopulation at the time of capture.

The first annulus length observed in the historic dataset ranged from 1.35 to 2.52 mm (1.93 ± 0.26 mm) and 2.14 to 3.47 mm (2.82 ± 0.36 mm) for predicted spring and winter subpopulations, respectively. The mean length of the first annulus was significantly smaller in the historic dataset compared to the contemporary dataset for the overall population (Wilcoxon Rank Sum Test; $p<0.001$) as well as within subpopulations (spring, $p < 0.001$; winter, $p = 0.04$) The dominant subpopulation varied between ages (Figure 5), with 51 of the 104 fish (49%) predicted to be members of the spring subpopulation. The model accurately assigned 40% (n = 2) of fish in spawning condition to the spring spawning subpopulation.

DISCUSSION

The spring spawning subpopulation of Atlantic cod currently appears to comprise a minor portion of the cod population in the western Gulf of Maine based on measurements of annuli length in otoliths of fish collected by the NEFSC bottom trawl survey. However, the spring subpopulation may have comprised a larger proportion of the population historically. While the subpopulation mix varied year to year, the reduced proportion of the spring subpopulation observed in our contemporary dataset compared to our historic dataset was in alignment with the findings of Dean et al. (2019). Dean et al. (2019) found that while the population was, on average, composed of less than 20% spring spawners from fish collected 2016-2019 (period 2), as recently as the mid-2000s (period 1), spring spawners accounted for closer to 50% of the overall population.

While the methods appear to be a promising manner to examine current and historic subpopulation composition and the results are interesting, a couple caveats need to be considered.

First, our analyses did not include a weighting factor to account for differences in sampling intensity spatially or by fish size. Therefore, the observed population-level trends may not accurately represent the entirety of the population. Furthermore, both the current study and that of Dean et al. (2019) cover relatively short time periods. It is uncertain whether the subpopulation composition has shifted in recent years from a previously more balanced mix to a population dominated by winter spawners or whether these observed shifts are natural fluctuations occurring over a longer period of time. Despite these uncertainties, the patterns observed are compelling and worthy of further exploration across broader time scales. If the patterns were to hold, the loss of the spring spawning subpopulation could mean a loss of resilience within the Gulf of Maine Atlantic cod stock, particularly for a population in recovery.

The trend of reduced spring spawners in recent years predicted by the model is mirrored in the frequency distributions of the first 3 annuli lengths from Atlantic cod otoliths (Figure 3). The annuli length distributions from the contemporary dataset (Figure 3A) show a unimodal, normal distribution, whereas the distributions from the historic dataset are noisy and may be bimodal (see peaks at approximately 1.9 and 2.9 mm for the first annulus; Figure 3B). The noise evident in the distributions for the historic period may be indicative of a more balanced subpopulation composition compared to the contemporary period: the two peaks in the first annulus correspond with the mean size for predicted spring (1.93 mm) and winter (2.82 mm) spawners, respectively.

Interestingly, the mean annuli lengths were significantly higher in the contemporary dataset compared the historic dataset. Dean et al. (2019) hypothesized that winter-spawned fish have a larger first annulus compared to spring-spawned fish because the first annulus is not formed until the end of the first full winter. Therefore, as we observed, the mode of the first annulus length from the general population would be expected to be higher when the proportion of the winter subpopulation is higher. However, this fails to explain the within subpopulation increase in the mean annuli length from the 1970s to 2015-6 (Figure 3). Perceived growth rates for the Gulf of Maine cod stock have changed in recent years. Historically, cod in the Gulf of Maine were estimated to have slower growth compared to the Georges Bank stock (Begg et al. 1999); however, in the 1990s, the growth rate for the Gulf of Maine stock increased and has remained elevated (Begg et al. 1999; McBride et al., 2021). A winter-spawned fish would superficially appear to have more rapid growth compared to a spring-spawned fish of the same age because of the approximately 6 additional months of growth that winter-spawned fish undergo before laying their first annulus. Considering this, it is unknown how much of the perceived change in growth rates in recent years is due to a true change in growth, as suggested by the increased maximum annuli length, or a superficial change, as a result of the change in the subpopulation composition of the population.

The relative contribution of the spawning subpopulation to the overall population varied year to year in both the contemporary (Figure 4) and historic (Figure 5) periods. The pattern of an increase in the proportion of spring spawning fish with age in the contemporary dataset is potentially due to the lower mortality experienced by the spring subpopulation caused by area closures (as hypothesized by Dean et al. 2019). These closures were not in place in the 1970s, so the year-to-year variability in the historic dataset may represent more realistic temporal variation in the absence of spatial management measures. Such temporal variability may have inferred a degree of resilience to the Gulf of Maine Atlantic cod population. In years where conditions were unfavorable either for spawning (e.g., poor body condition of adults) or larval/juvenile (e.g., a mismatch with productivity) cod of a particular subpopulation, the overall population may have been buffeted by having the other subpopulation spawn/recruit when conditions were more favorable. However, in recent years, there has been near recruitment failure of spring spawning cod in the western Gulf of Maine; even though the spring-spawned fish experience lower morality, and therefore are older/larger among spawners in the area, this subpopulation accounts for less than 2% of total recruitment (Dean et al. 2019). It is probable that the spring subpopulation has reached such a small level that depensation may prevent recovery, though it is possible that environmental conditions also play a role.

Changing conditions in the Gulf of Maine and along the Northeast US Continental Shelf have shifted the timing and distribution of spring phytoplankton blooms (Friedland et al. 2015), which may have a negative synergistic effect on the ability of the spring subpopulation to recover. Spring blooms occurred earlier every year from 2006 to 2013 in the western Gulf of Maine (Friedland et al. 2015). Early spring blooms were associated with lower zooplankton biovolume (Friedland et al. 2015); the low recruitment of spring spawning cod in recent years may be partially attributed to limited resources for larvae/juveniles. It is possible that conditions in the Gulf of Maine have changed so much that it is no longer favorable habitat for the spring-spawned larvae. If the spring subpopulation is unable to recover, the larger population of cod in the Gulf of Maine may be less resilient to changing conditions.

The model appeared to be an appropriate method of predicting subpopulation composition of Atlantic cod collected in the western Gulf of Maine by the NEFSC bottom trawl survey based on annuli length for the limited years examined. By using the same criteria as Dean et al. (2019) to identify known winter spawners (fish collected in the fall survey with stage R or U gonads; n = 122), only 5% (n = 6) of fish were misclassified. The model performed reasonably well when the variable "period" was removed, though the accuracy of assignment of winter spawners decreased (from 95% to 86%). However, while the model generally appeared to capture the dynamics of the subpopulation composition of cod in the western Gulf of Maine in both contemporary and historic periods, the use of the model is most appropriate for the contemporary dataset. Dean et al. (2019) used annuli measurements from known spawners to create a reference set by which to build and train the glmm. While performance between models that accounted for the period during which fish were collected and those that did not were similar (see Table 3 in Dean et al. 2019), the reference set of known spawners used to build the model were collected between 2003 and 2019. Growth rates within a population vary temporally, whether from environmental conditions such as temperature or food availability (Charnov and Gillooly 2004) or from effects of exploitation (Enberg et al. 2012). The most accurate method to explore the temporal dynamics of Atlantic cod subpopulation dynamics in the western Gulf of Maine would be to build models specific to the period of interest (e.g., decadal).

In order to build accurate models, however, it is necessary to have a reference collection of annuli measurements from cod of known spawning subpopulation. Whether because of a shift in the timing of the survey, a change in sampling protocols to increase sample sizes, a change in environmental conditions, an alteration in subpopulation composition, or a combination of the 4, more fish in spawning condition have been collected from bottom trawl surveys in recent years (2010s) compared to earlier decades (Table 5). Furthermore, maturity data are not available for the archives of Atlantic cod otoliths collected through fishery-dependent means. Unfortunately, the small sample sizes from trawl surveys conducted in the 1970s to 1990s and lack of maturity data for commercially collected samples likely preclude building new models based on otoliths in the NEFSC archives utilizing the same criteria as Dean et al. (2019).

The modeling approach used by Dean et al (2019), and demonstrated here, may be a useful way to explore stock structure of Atlantic cod in areas where the Gulf of Maine and Georges Bank stocks mix. While Atlantic cod have discrete spawning populations throughout their range, mixing may occur during non-spawning times which makes stock assignment of commercial catches in these areas problematic. As previously discussed, historically, the 2 stocks appeared to have different growth rates. Utilizing annuli growth and a similar modeling approach presented by Dean et al. (2019) may be a way to examine the relative contribution of these stocks to fishery landings. However, the development of spatial models would require a subset of fish for which stock (determined through genetic typing, for example) and spawning subpopulation (for Gulf of Maine stock members) is known, data that are not readily available for the archive of NEFSC survey samples.

Heavy fishing pressure and other anthropogenic factors have changed the demographics of Atlantic cod in the Gulf of Maine, and some historic spawning grounds have been severely depleted or entirely fished out (Ames 2004; Alexander et al. 2009). The spring spawning subpopulation in the western Gulf of Maine, while still present in recent years, has been greatly reduced. With the loss of spawning subpopulations comes a loss of resilience of the larger population to cope with continued exploitation and changing environmental conditions. Understanding both the current and historic stock structure of a stock that continues to fail to thrive may lead to population stock assessments that better capture their complex dynamics and could allow for more effective management strategies. Examining subpopulation structure in the western Gulf of Maine from more time periods is necessary in order to determine whether the observed decrease in spring spawners from the 2010s is indeed a unique occurrence or whether stock dynamics are such that similar changes occurred historically. While it may be difficult to build new models based on collections from NEFSC bottom trawl survey, the application of the model used by Dean et al. (2019) to the archive of NEFSC samples may be the best available method to explore the historical composition of the western Gulf of Maine Atlantic cod stock.

IMPLICATIONS FOR CONTINUED INVESTIGATION

This was a pilot project to investigate the efficacy of applying the modeling approach used by Dean et al. (2019) to Atlantic cod collected in the western Gulf of Maine by the NEFSC bottom trawl survey. The modeling approach appeared to be appropriate for use with samples collected by the NEFSC; it appears that the spring spawning subpopulation has decreased in the 2010s compared to older periods, similar to the findings of Dean et al. 2019. Moving beyond the pilot research project outlined here, a full evaluation of subpopulation composition across decades would require additional resources.

First, while the observed trends are compelling, we did not account for differences in sampling effort with respect to spatial coverage (e.g., survey effort) or fish sex or size (e.g., subsampling protocols). Therefore, the observed trends may not be representative of the population as a whole. In order to determine if the trends reflect the overall western Gulf of Maine Atlantic cod population, a weighting factor would need to be applied to each fish. Intensity of sampling spatially varies with each survey. Furthermore, subsampling protocols vary between surveys as well as between hauls within surveys with. These data should be available and relatively easy to retrieve for contemporary samples (2000s forward) but may be difficult or impossible to obtain for older samples. This approach may require a significant investment of time both to retrieve the data and to determine the weighting factor because of the low densities of cod caught per haul.

Second, to accurately compare subpopulation composition across decades would require building new models. In order to build accurate models, a sufficient number of fish in spawning condition would be required. Fewer than 100 fish in spawning condition were collected per decade (46, 86, and 92, respectively) from the 1970s, 1980s, and 1990s by the NEFSC spring and fall bottom trawl surveys in the western Gulf of Maine (Table 5). Determining whether sample sizes were large enough to create new models was beyond the scope of this pilot project. Expanding the survey strata used in analyses would increase the sample sizes of fish in spawning condition but could introduce other complications. Survey strata adjacent to those used in the current analyses are areas where mixing occurs between the Gulf of Maine and Georges Bank stocks. The spawning season for the Georges Bank stock overlaps with that of both winter and spring spawners from the western Gulf of Maine, which could result in dampening the annulus length signal.

Lastly, further analysis of the first annulus assignment would be beneficial in future endeavors. The image processor was an experienced production ager, but the Atlantic cod otoliths were novel, so using the established production ager's first annulus assignment was deemed appropriate for this pilot project. Agreement between agencies (NEFSC and MADMF) and between the image processor and production ager was high (89% and 91%, respectively); however, given a minor increase in agreement between agencies (from 89% to 93%) when the image processor's first annulus assignment was considered suggests it might be beneficial to reexamine settlement check and first annulus assignments. If true first annuli are being labeled settlement checks, the proportion of spring spawning Atlantic cod in the Gulf of Maine may be underestimated.

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Table 1. Sample sizes by survey year and stratum for Atlantic cod (*Gadus morhua*) otoliths used in annuli measurement analyses collected on spring (1974-75) and fall (2015-16) Northeast Fisheries Science Center bottom trawl surveys.

Stratum		**Year**			
		1974	**1975**	**2015**	**2016**
Offshore	**26**	41	63	20	84
	27	0	0	10	1
Inshore	**61**	0	0	142	1
	64	0	0	20	3
	65	0	0	67	7
	66	0	0	49	2

Table 2. Summary of Atlantic cod (*Gadus morhua*) samples from the contemporary period and used for the prediction of subpopulation assignment based on otolith annuli lengths. All fish were collected on the fall bottom trawl survey of 2015 and 2016 from the western Gulf of Maine. Values in parentheses represent average ± standard deviation. Gonad stages follow Burnett et al. (1989): I = immature, D = developing, R = ripe, U = ripe and running, S = spent, T = resting.

Gonad stage	**Number**			**Length (cm)**			**Age (years)**		
	Female	Male	*Overall*	Female	Male	*Overall*	Female	Male	*Overall*
I	29	29	*58*	25-59 (38.55 ± 8.86)	24-58 (40.41 ± 10.24)	*24-59* *(39.48 ± 9.54)*	1-3 (1.7 ± 0.7)	1-3 (1.9 ± 0.7)	*1-3* *(1.8 ± 0.7)*
D	45	72	*117*	42-92 (71.24 ± 13.5)	29-80 (58.53 ± 10.27)	*29-92* *(63.42 ± 13.13)*	2-7 (4.1 ± 1.3)	1-6 (3.7 ± 1)	*1-7* *(3.8 ± 1.2)*
R	10	63	*73*	60-91 (74.1 ± 8.45)	45-84 (66.11 ± 8.89)	*45-91* *(67.21 ± 9.2)*	3-7 (4.2 ± 1.4)	2-7 (4.2 ± 1.4)	*2-7* *(4.2 ± 1.4)*
U	0	49	*49*	na	51-84 (69.9 ± 7.81)	*51-84* *(69.9 ± 7.81)*	n/a	2-7 (4.6 ± 1.4)	*2-7* *(4.6 ± 1.4)*
S	0	10	*10*	na	45-71 (56.6 ± 8.95)	*45-71* *(56.6 ± 8.95)*	n/a	2-4 (2.7 ± 0.7)	*2-4* *(2.7 ± 0.7)*
T	65	35	*100*	38-81 (55.66 ± 10.49)	28-79 (49.54 ± 11.42)	*28-81* *(53.52 ± 11.16)*	2-6 (2.9 ± 1)	2-5 (2.5 ± 0.8)	*2-6* *(2.8 ± 0.9)*

Table 3. Summary of Atlantic cod (*Gadus morhua*) samples collected in the historic period and used for the prediction of subpopulation assignment based on otolith annuli lengths. All fish were collected on the spring bottom trawl survey of 1974 and 1975 from the western Gulf of Maine. Values in parentheses represent average ± standard deviation. Gonad stages follow Burnett et al. (1989): I = immature, D = developing, R = ripe, U = ripe and running, S = spent, T = resting. UNK = unknown.

Gonad stage	**Number**				**Length (cm)**			**Age (years)**			
	Female	Male	UNK	*Overall*	Female	Male	*Overall*	Female	Male	UNK	*Overall*
I	39	19	0	*57*	24-61 (41.49 ± 9.73)	24-54 (38.28 ± 9.04)	*24-61 (40.47 ± 9.55)*	2-4 (3.1 ± 0.7)	2-4 (2.8 ± 0.6)	n/a	*2-4 (3.0 ± 0.7)*
D	3	0	0	*3*	83-91 (88.33 ± 4.62)	n/a	*83-91 (88.33 ± 4.62)*	7-11 (9.3 ± 2.1)	n/a	n/a	*7-11 (9.3 ± 2.1)*
R	0	5	0	*5*	n/a	56-82 (71.00 ± 12.08)	*56-82 (71.00 ± 12.08)*	n/a	4-6 (4.8 ± 0.8)	n/a	*4-6 (4.8 ± 0.8)*
U	0	0	0	*0*	n/a	n/a	*n/a*	n/a	n/a	n/a	*n/a*
S	3	1	0	*4*	81-123 (101.00 ± 21.07)	89	*81-123 (98.00 ± 18.22)*	5-11 (7.3 ± 3.2)	6	n/a	*5-11 (7.0 ± 2.7)*
T	17	5	0	*22*	32-103 (62.65 ± 23.85)	35-71 (52.4 ± 15.90)	*32-103 (60.32 ± 22.38)*	2-9 (4.8 ± 2.3)	2-5 (3.6 ± 1.1)	n/a	*2-9 (4.5 ± 2.2)*
UNK	0	0	12	*13*	n/a	n/a	*n/a*	n/a	na	2-5 (3.3 ± 0.8)	*2-5 (3.3 ± 0.8)*

Table 4. Sample sizes (N) and summary statistics (units = mm) of lengths of the first 3 annuli from Atlantic cod (*Gadus morhua*) otoliths collected in 2015-16 (contemporary period) and 1974-75 (historic period) on Northeast Fisheries Science Center bottom trawl surveys. Avg = average length; SD = standard deviation; IQR = interquartile range. Italic text represents the N and summary statistics for each period.

		Contemporary			**Historic**		
		2015	**2016**	***overall***	**1974**	**1975**	***overall***
1st annulus	N	309	98	*406*	41	63	*104*
	avg	2.89	2.97	*2.91*	2.29	2.44	*2.38*
	SD	0.60	0.55	*5.90*	0.50	0.57	*0.55*
	IQR	0.77	0.83	*0.75*	0.63	0.93	*0.98*
	mode	2.43	2.55	*2.43*	2.09	2.75	*2.02*
2nd annulus	N	285	98	*383*	40	62	*102*
	avg	5.31	5.21	*5.28*	4.10	3.98	*4.02*
	SD	0.65	0.63	*0.65*	0.72	0.75	*0.74*
	IQR	0.81	0.92	*0.86*	0.83	0.98	*0.89*
	mode	5.45	5.49	*5.45*	3.63	3.33	*4.21*
3rd annulus	N	198	85	*268*	34	47	*81*
	avg	6.60	6.47	*6.56*	5.48	5.15	*5.29*
	SD	0.62	0.53	*0.60*	0.83	0.86	*0.86*
	IQR	0.87	0.68	*0.80*	1.06	1.15	*1.22*
	mode	6.32	6.49	*6.49*	5.34	4.67	*5.30*

Table 5. Atlantic cod (*Gadus morhua*) otoliths from fish collected aboard the Northeast Fisheries Science Center fall and spring bottom trawl surveys from 1970 to 2018. Only samples for which age data were present and that were collected in offshore strata 26-27 and inshore strata 59-66 were included. Samples were grouped by gonad development stage (in parentheses; I = immature; D = developing; R = ripe; U = ripe and running; S = spent; T = resting). Italic text represents total counts.

	Immature (I)		**Pre-spawning (D)**		**Spawning (R, U)**		**Post-spawning (S, T)**		***Total***	
Decade	**Spring**	**Fall**	**Spring**	**Fall**	**Spring**	**Fall**	**Spring**	**Fall**	***Spring***	***Fall***
1970s	270	530	82	144	28	18	187	288	*567*	*980*
1980s	582	588	174	215	60	26	530	421	*1346*	*1250*
1990s	485	599	190	107	80	12	542	475	*1297*	*1193*
2000s	974	835	218	400	130	62	996	664	*2318*	*1961*
2010s	779	358	216	468	137	303	1198	448	*2330*	*1577*
Total	*3090*	*2910*	*880*	*1334*	*435*	*421*	*3453*	*2296*	*7858*	*6961*

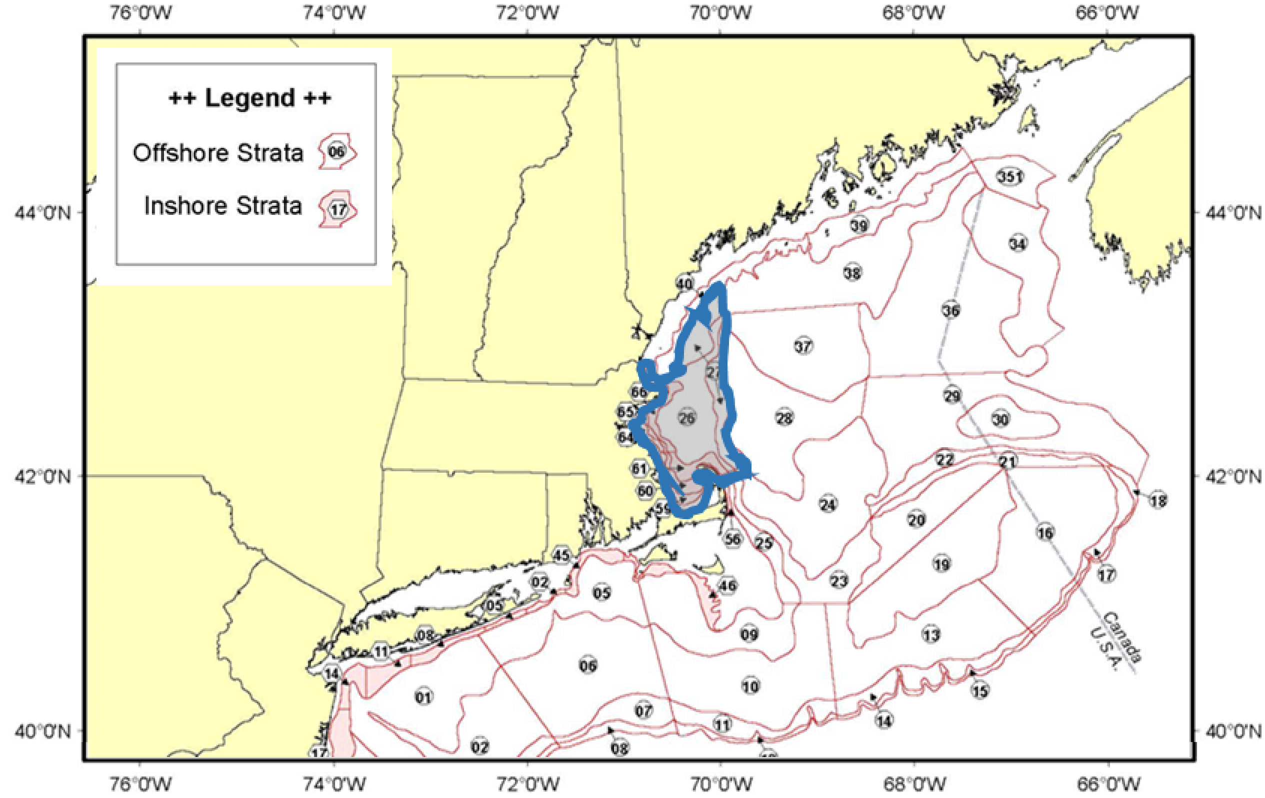

Figure 1. Locations of Northeast Fisheries Science Center (NEFSC) bottom trawl survey strata. Analyses were limited to the western Gulf of Maine (outlined in blue), which included samples collected in inshore strata 59-66 and offshore strata 26-27. Figure is modified from that provided by J. Kircun (NEFSC).

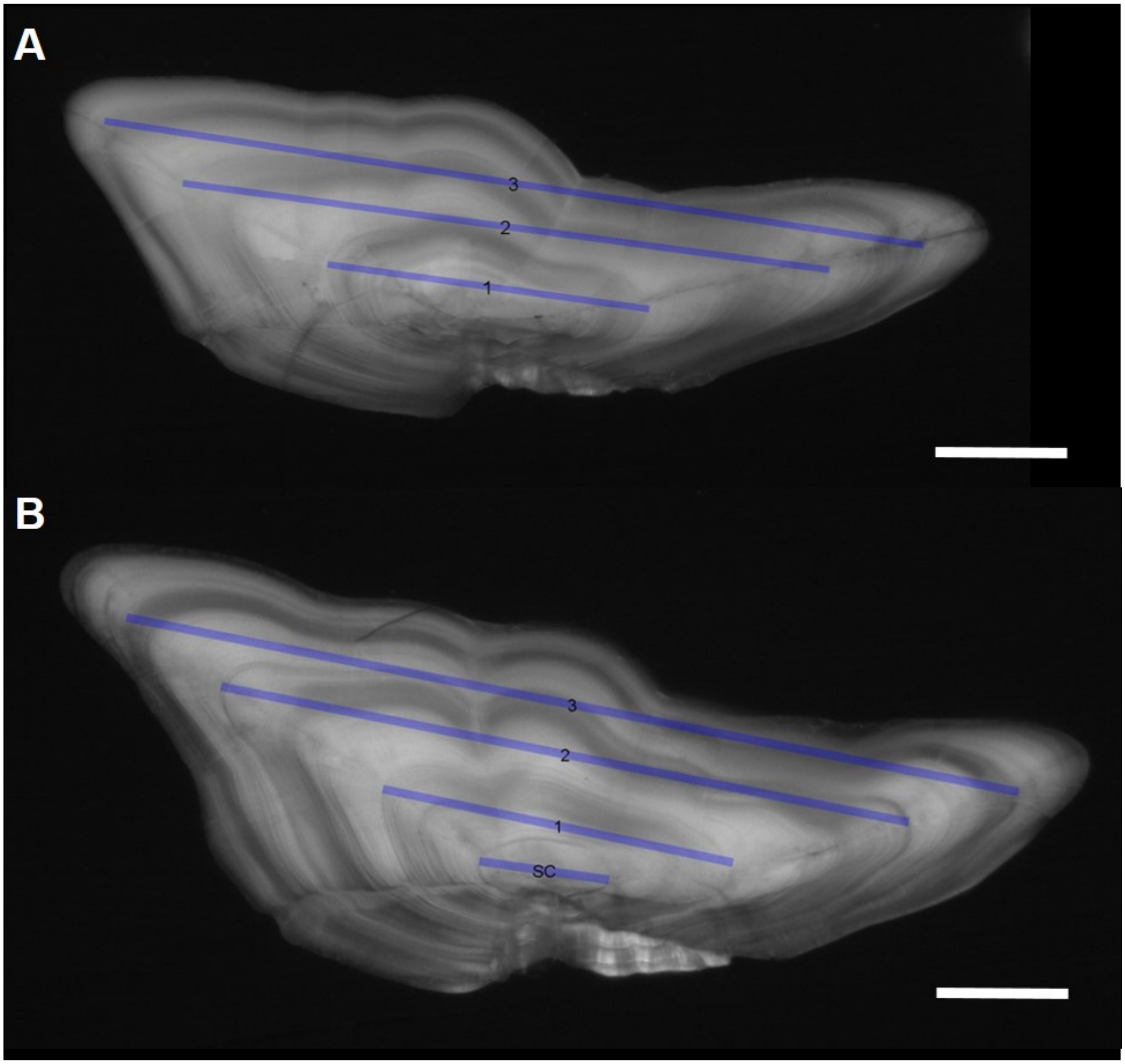

Figure 2. Photomicrographs of Atlantic (*Gadus morhua*) cod otoliths with an overlay of the length measurements of the first (1), second (2), and third (3) annuli lengths. (A) No obvious settlement check. (B) Settlement check (SC) present. Scale bars = 1 mm.

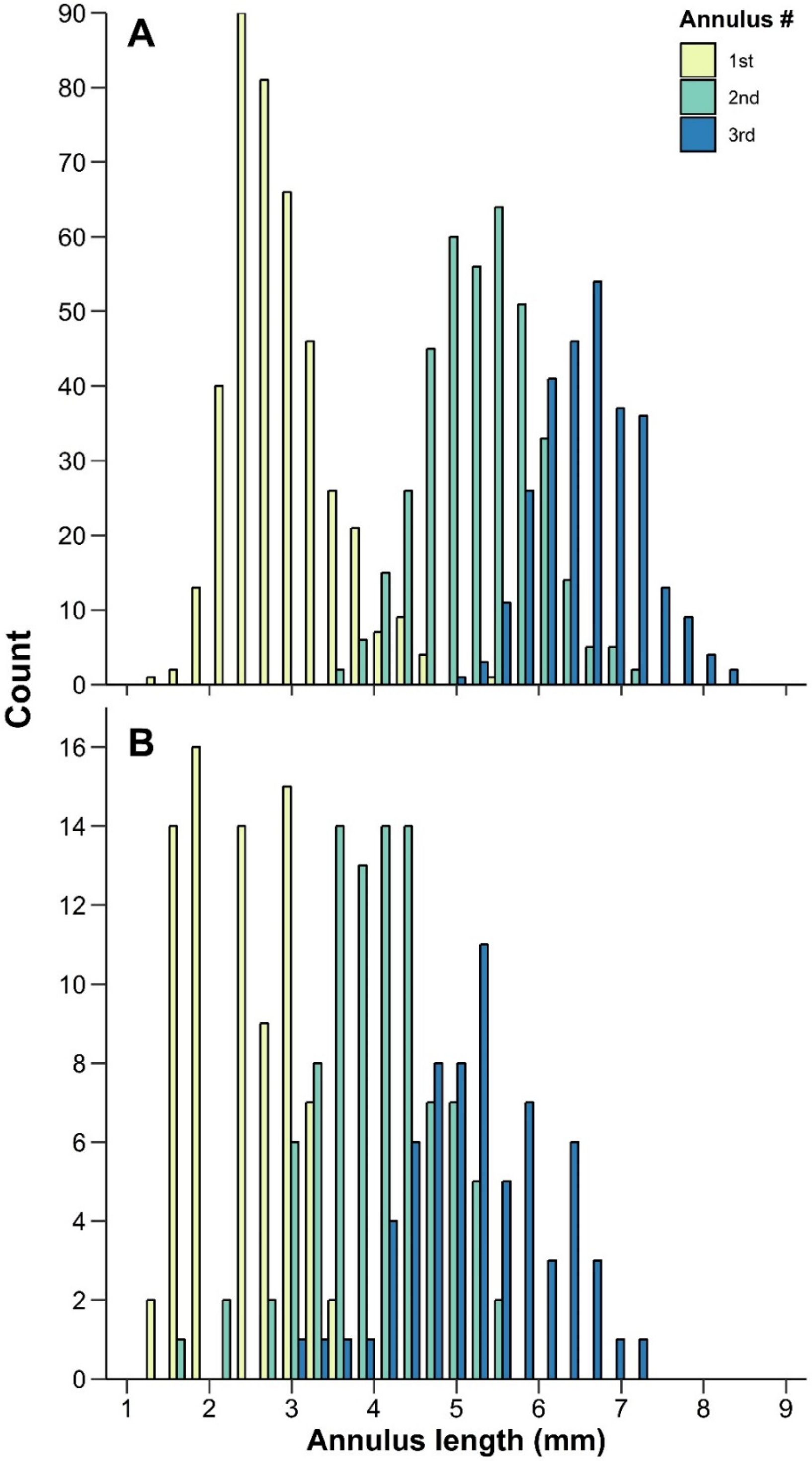

Figure 3. Frequency distribution of the first 3 annuli lengths measured from photomicrographs of Atlantic cod (*Gadus morhua*) otoliths collected during the (A) 2015 and 2016 fall and (B) 1974 and 1975 spring Northeast Fisheries Science Center bottom trawl surveys.

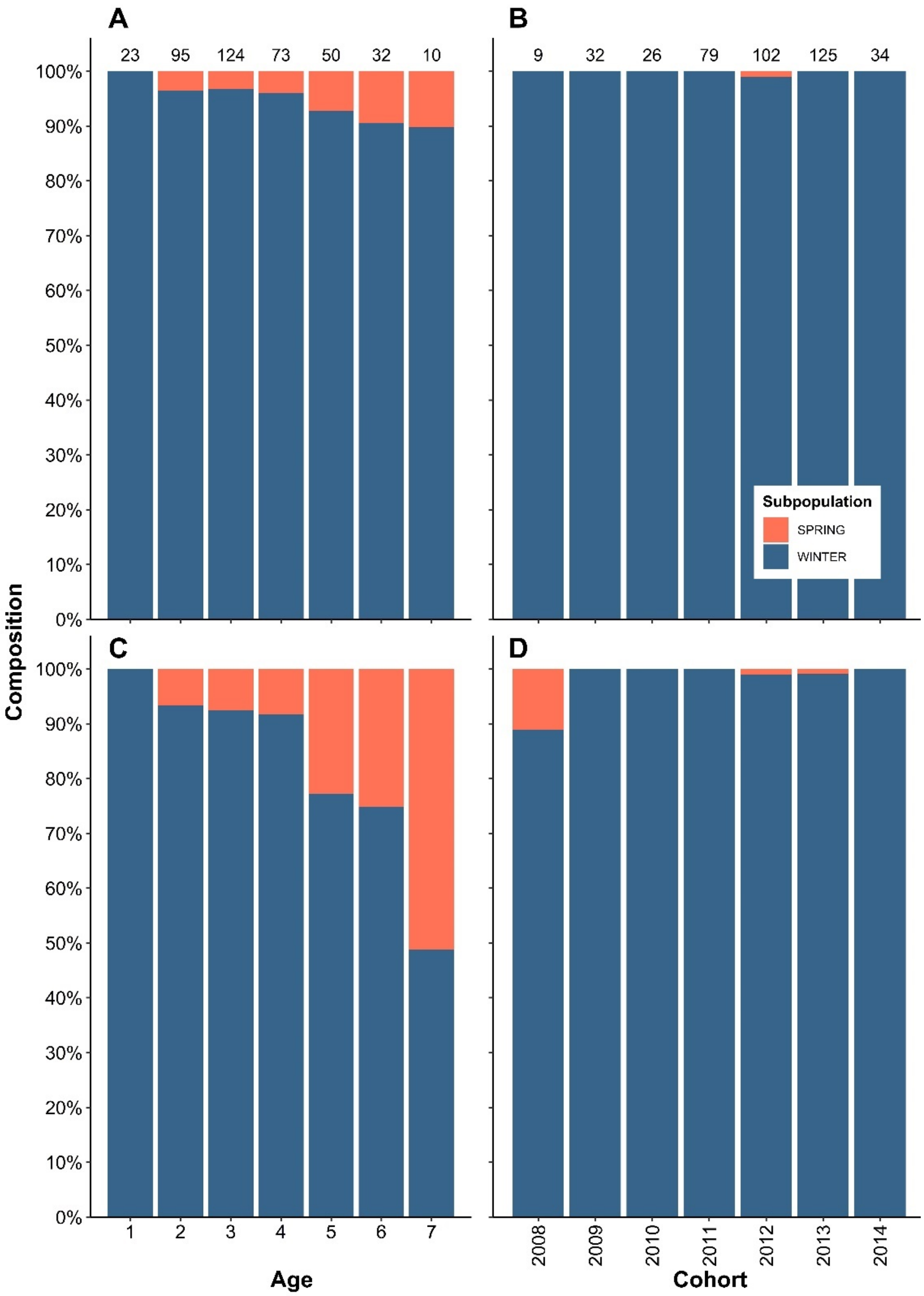

Figure 4. Spawning subpopulation prediction by age (A and C) and cohort (at age 0, B and D) for Atlantic cod (*Gadus morhua*) collected in the fall of 2015-2016 by the Northeast Fisheries Science Center bottom trawl survey. Prediction by cohort assumes differential mortality between the 2 subpopulations because of seasonal area closures. (A and B) Results from model runs including a predictor variable "period" (C and D). Results from model runs without the predictor variable period. Sample sizes are denoted above the bars.

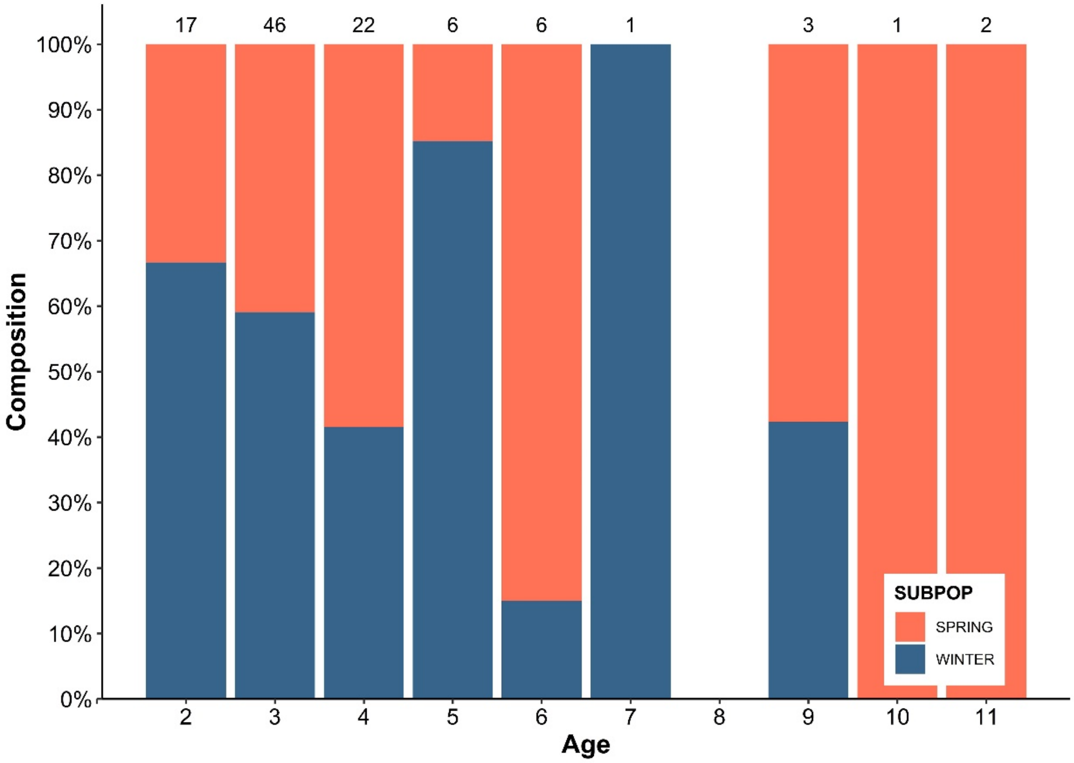

Figure 5. Spawning subpopulation (SUBPOP) prediction by age for Atlantic cod (*Gadus morhua*) collected in spring of 1974-1975 by the Northeast Fisheries Science Center bottom trawl survey. Sample sizes are denoted above the bars.

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