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**Validation of Cod maturity ogive in NAFO 3M. Effect of the reproductive cycle moment
on ogive estimation**

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Abstract

The Flemish Cap (FC) survey is the source of the Atlantic cod maturity data for SSB estimation. FC survey takes place when females are resting and discrimination between spawning active and inactive females is still possible testing the presence of postovulatory follicles, that remains several months after spawning. However, the best moment for discriminate active spawning females is during the spawning season. For 2012 and 2014, the maturity ogives calculated during the spawning season and when females are resting were compared. Assuming a seasonal growth for cod in 3M, the moment in which maturity is estimated, had no effect on the ogives estimation. Thus, it is recommended to continue employing in the 3M cod assessment the maturity ogives produced with the FC survey information.



Introduction

Atlantic cod (*Gadus morhua*, L.) is a species of major commercial interest in the Northwest Atlantic and its spawning strategy is characterized by determinate fecundity. NAFO 3M Cod spawning-stock biomass (SSB) is currently estimated on an annual basis, using a maturity ogive based on microscopic gonad identification. The Flemish Cap (FC) survey (Vázquez et al., 2014) is the source of the maturity data. To achieve the best accuracy on maturity fish data, we should take the samples during the spawning season, when maturity stages are better defined.

FC survey takes place in summer, few months after cod spawning, when females are resting and maturity estimation is more difficult. However, maturity is determined histologically using the proportion of females with ovary structures (postovulatory follicles POFs) that remain in the ovary several months after spawning (Saborido et al., 1998; Witthames et al., 2010). Due to the moment of the reproductive cycle, the maturity data quality could be compromised by the misclassification of resting fish as immature or inactive.

Since the opening in 2010 of cod NAFO 3M fishery, ovary samples of this species have been taken through the Spanish sampling program for commercial fleet. This program has provided us cod ovary samples collected all around the year, including the spawning season, that for NAFO 3M cod takes place in early spring. But as commercial sampling depends on the fishery activity, suitable ovary samples for maturity ogive estimation are only available for 2012 and 2014, when samples were taken at spawning time, and in enough number and adequate size distribution to calculate the maturity ogive. Thus, for those two years, we can compare the ogives calculated using the POF's method with the ogives estimated from females sampled during the spawning time.

The aim of this study is to verify that the ogives calculated outside the spawning period do not show systematic differences with the ogives calculated in the same area and year but in the spawning season.

Our results indicated that, if a seasonal growth is assumed for 3M Cod, the reproductive moment has not a significant effect on the maturity ogive estimation.

Material and methods

Data and ovary samples for this study were collected in 2012 and 2014. Commercial samples were collected during the spawning season, specifically during April 2012 and March 2014, while FC survey samples were taken in June in both years. Table 1 shows the sample size and length range of samples.

In all the surveys (commercial and FC surveys), length-stratified ovary samples were taken from cod females, recording total length, weight and macroscopical maturity stage. Otoliths from all sampled females were also removed. Ovary samples were preserved in 4% buffered formaldehyde. Later in the lab samples were dehydrated, embedded in paraffin, sectioned at 3 μm and stained with haematoxylin and eosin. Histological sections were examined by light microscopy and maturity staged. The presence/absence of different oocyte developmental stages and structures of spawning activity was recorded for each ovary. Following the common maturity scale, proposed in the WKMATCH (ICES, 2013), different maturity stages of the ovary were identified: Immature (1), Maturing (2), Spawning (3), Regressing (4a), Regenerating (4b), Omitted spawning (5), and Abnormal (6). For assessment purposes, the proportion of mature fish is defined as the proportion of fish that spawn in the current year, so mature fish that skip spawning are added to the immature group for ogive

calculation. Thus, ovaries were classified as inactive (including immature and omitted spawning) and active (mature stages; 2 to 4). Immature ovaries were those presenting all oocytes in primary growth stage; inactive-mature ovaries were those from mature females but inactive this reproductive event and mature ovaries were those presenting mature oocytes (cortical alveoli or more advanced stages) or sign of previous spawning activity (POFs).

Fish lengths from March and April samples were adjusted to 1st of July, the reference date used for the post-spawning season. For that purpose, a von Bertalanffy curve by year was fitted to the available length-age pairs of data by nonlinear regression (Table 2). The Infinite parameter was fixed at 140 cm for both years. These equations were used to predict the individual length at post-spawning time. As growth does not necessarily follow the VBGF throughout the year and it can be seasonal (García-Berthou et al., 2012), the predicted length at 1st July was estimated considering two alternative types of growth: a constant VBGF growth through the year and a seasonal VBGF growth in which growth occurs only during 6 months of the year.

The relative importance of the reproductive time (spawning or post-spawning season) on maturity of cod females was tested means a generalized linear model (GLM) with a logit link. The analysis was done separately for years (2012 and 2014) and types of growth (seasonal or constant through the year).

Results

Maturity stages

As cod is a spring-spawning species, and FC survey takes place at summer, the proportion of females that spawned that year is determined using the persistence of spawning markers (POFs). In 2014 maturity data were available from March, when most fish is spawning but in 2012 data comes from April, at the end of the spawning, when part of the females was already in post-spawning (Figure 1). But for these samples, although maturity has to be estimated using spawning markers as in FC survey, degeneration of the ovary structures is minor, and thus are easily identifiable than two months later, when degeneration made maturity staging more difficult.

Growth estimation

Figure 2 shows the VBGF fitted for cod females in 2012 and 2014 and the range of values for K and t₀. In year 2012, the point estimate of K parameter was 0.128 with a 90% confidence interval of 0.123-0.132 and the point estimate of t₀ was -0.056 and the limits of 90% confidence interval: -0.207-0.077. In year 2014, the point estimate of K parameter was 0.129 with a 90% confidence interval of 0.127-0.131, the point estimate of t₀ was 0.55 and the limits of 90% confidence interval was 0.483-0.609. The obtained VBGF were applied to the individual length of commercial samples to estimate their adjusted-length at the post-spawning reference date.

Maturity at length

Generalized Linear Models (GLMs) with a binomial error distribution and a logit link were fitted to the proportion of fish mature by length class. Estimates of the length at 50% maturity (L₅₀) were derived from the model parameters. For commercial samples the estimated length at 1st of July, following a constant growth and seasonal growth, was used as variable.

In 2012, L_{50} was estimated at 55.5 cm from maturity samples from the post-spawning season (Table 3). A slightly lower value, 54.8 cm, corresponded to spawning samples with length corrected with a seasonal growth. If a constant growth is assumed for cod females, the L_{50} decreases to 52.5 cm.

The L_{50} values in 2014 were lower than in 2012. L_{50} was estimated at 49.3 cm from maturity samples from the post-spawning season with a 90% CI at 47.2-51.4. The analysis with spawning samples, with length corrected following a seasonal growth, provides almost equal estimates: 49.5 cm (47.2-51.1). If a constant growth is assumed for cod females, the L_{50} decreases to 45.9 cm.

Effect of reproductive season on maturity at length

Generalized linear models (GLMs) with binomial error distributions were applied to the proportion of females mature at each length, with the reproductive season as a factor. The GLM results indicated that length was the main variable explaining the variability of the data, with a 74%-75% for 2012 analysis and 65%-64% for 2014 analysis (Table 4; Figure 3).

The reproductive season had a significant effect ($p < 0.01$) in the analysis that assumed a constant growth to adjust the length of spawning samples. The reproductive season variable explained 0.9% and 1% of variability in 2012 and 2014 respectively. Significant *length:reproductive_season* interaction was found for 2012 analysis, indicating that maturity and reproductive season interacted on various levels with length.

When a seasonal growth is considered, the reproductive season is not a significant factor in the maturity determination ($p=0.27$ and $p=0.71$, for 2012 and 2014 respectively) (Figure 3).

Discussion

The purpose of this study was to evaluate the accuracy of the maturity ogives currently used to estimate spawning-stock biomass (SSB) for 3M cod stock. The use of samples from the post-spawning period for the POF method could lead to an underestimation of the size of the reproductive part of the stock. The recommendation of identifying of maturity stage during the spawning season aims to mitigate this risk of misidentification of maturity stages. In this study, we compare the ogives calculated in FC surveys with ogives calculated in spring, when cod was spawning and confusion between immature and mature cod was minimal.

The model results indicated that, assuming a seasonal growth for cod in 3M, the reproductive moment in which maturity is estimated, has no effect on the maturity ogives. Here, we have shown that the length at 50% maturity obtained during the spawning period was not significantly different from the estimated in post-spawning period. In 2012, L_{50} was estimated at 54.8 cm and at 55.5 cm for samples during spawning and post-spawning period respectively. In 2014, values of L_{50} were ever more similar: 49.5 cm and 49.3 cm for spawning and post-spawning period.

Furthermore, assuming a constant growth throughout the year, the differences found, although significant, are small, and could be due to the uncertainty of other factors such as the determination of age. Besides, it should be taken into account that differences found in maturity at length will be smoothed in the maturity at age, and they would probably not have an important impact on the estimation of SSB.

Other factors that could be affecting the results of this study and that should be included in a further analysis are the effect of selectivity of commercial and survey gears on maturity at length, the low coverage of small sized fish in commercial samples, and the different depth strata covers by FC survey and commercial sampling.

Based on the small differences observed and until more conclusive studies were available, it is recommended to continue employing in the assessment of 3M cod the maturity ogives produced with the FC survey information.

References

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Tables

Table 1. Maturity information by date of collection and reproductive season for cod females.

MonthYear/Reproductive season (source)	n	Length range (cm)
April2012 / Spawning (commercial sampling)	333	31-127
July2012 / Post-spawning (scientific survey)	342	25-125
March2014 / Spawning (commercial sampling)	330	30-104
July2014 / Post-spawning (scientific survey)	409	25-126

Table 2. The data used to fit von Bertalanffy growth equations for female cod in 2012 and 2014.

Year	Samples Source	Laboratory Age determination	n	Length range	Age range
2012	Survey	IIM (Vigo)	299	25-125	1-14
2014	Survey	IIM (Vigo)	1002	11-126	0-13

Table 3. Maturity estimates: length at 50% maturity (L_{50}), in cm TL, with confidence intervals (L_{50Low} , L_{50Upp}) for female cod. Length data of commercial samples were adjusted to 1st July following constant growth and seasonal growth.

Year	Reproductive Season	Growth	L_{50}	L_{50Low}	L_{50Upp}
2012	Spawning	Seasonal	54.8	53.0	56.8
	Spawning	Constant	52.5	50.6	54.5
	Post-spawning	Not applicable	55.5	53.6	57.5
2014	Spawning	Seasonal	49.5	47.2	51.1
	Spawning	Constant	45.9	43.5	47.6
	Post-spawning	Not applicable	49.3	47.2	51.4

Table 4. GLM analysis of the effect reproductive season on probability of being mature being length the covariate: deviance analysis. (df: degrees of freedom; *** p < 0.001; **p < 0.01; * p < 0.05, ns: p > 0.05).

	2012				2014			
Seasonal Growth	df	Deviance	Residual deviance	P (Chisq)	df	Deviance	Residual deviance	P (Chisq)
+length	1	583.1	197.7	< 0.0001 ***	1	468.6	255.1	< 0.0001 ***
+reproductive_season	1	1.2	196.5	0.273 ns	1	0.1	255.0	0.705 ns
+length:reproductive_season	1	7.0	189.6	0.008 **	1	1.4	253.6	0.232 ns
Constant Growth	df	Deviance	Residual deviance	P (Chisq)	df	Deviance	Residual deviance	P (Chisq)
+length	1	578.0	202.9	< 0.0001 ***	1	461.6	262.1	< 0.0001 ***
+reproductive_season	1	7.0	195.9	0.008 **	1	7.5	254.6	0.006 **
+length:reproductive_season	1	6.3	189.6	0.01 *	1	1.0	253.6	0.306 ns

Figures

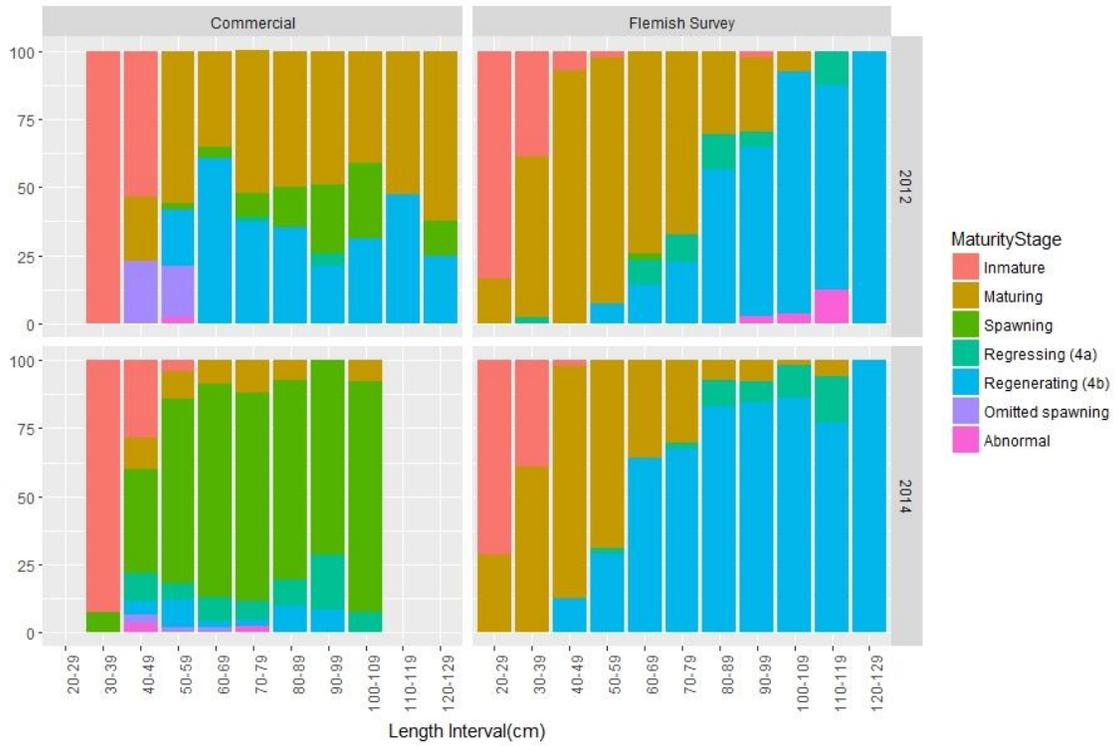


Fig 1. Maturity stages by length interval for the commercial (left) and FC survey samples (right) in 2012 and 2014.

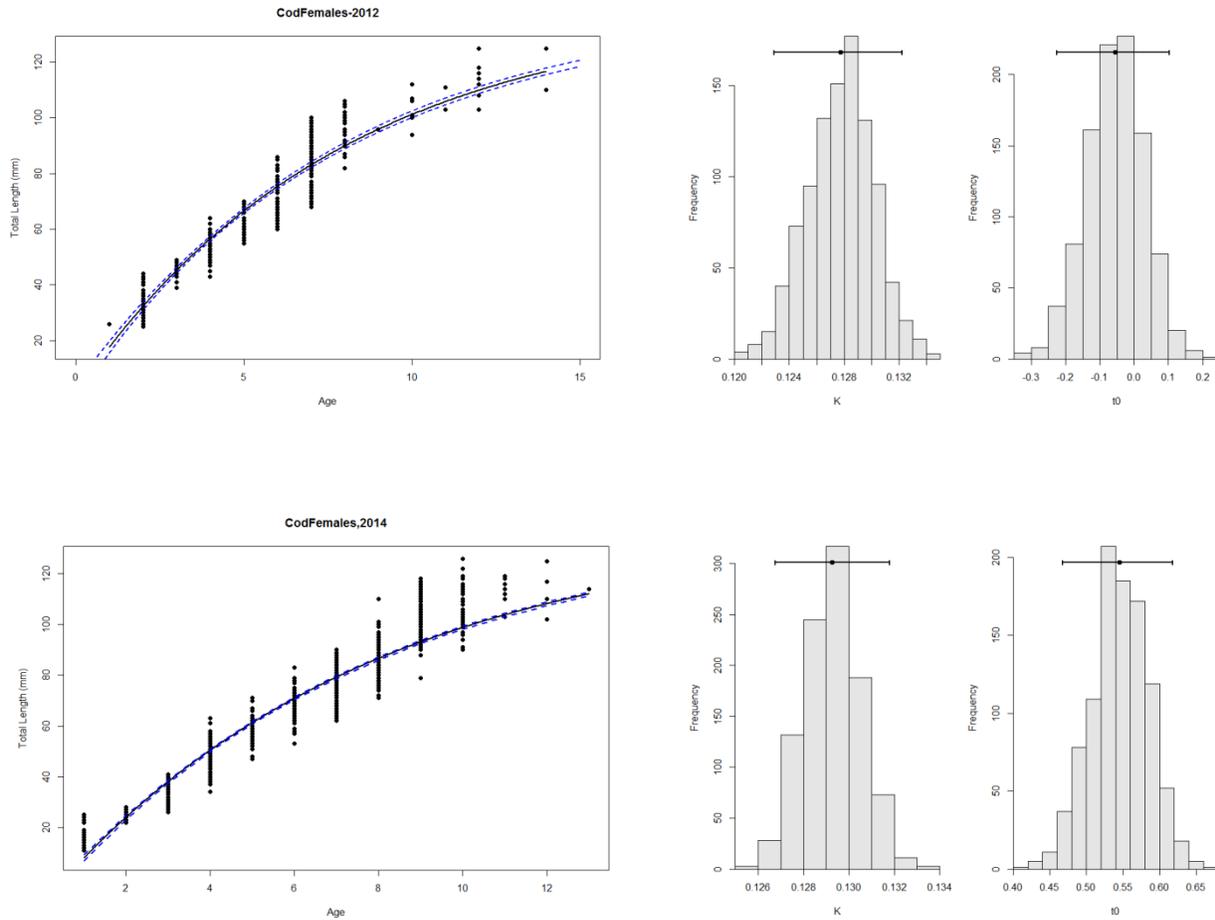


Fig. 2. The estimated growth curves for female cod in 2012 and 2014 are shown on the left. Point estimates and 90% confidence intervals are presented by the black line and dashed blue lines, respectively. On the right, histograms of parameters (K , t_0) values estimated by bootstrap.

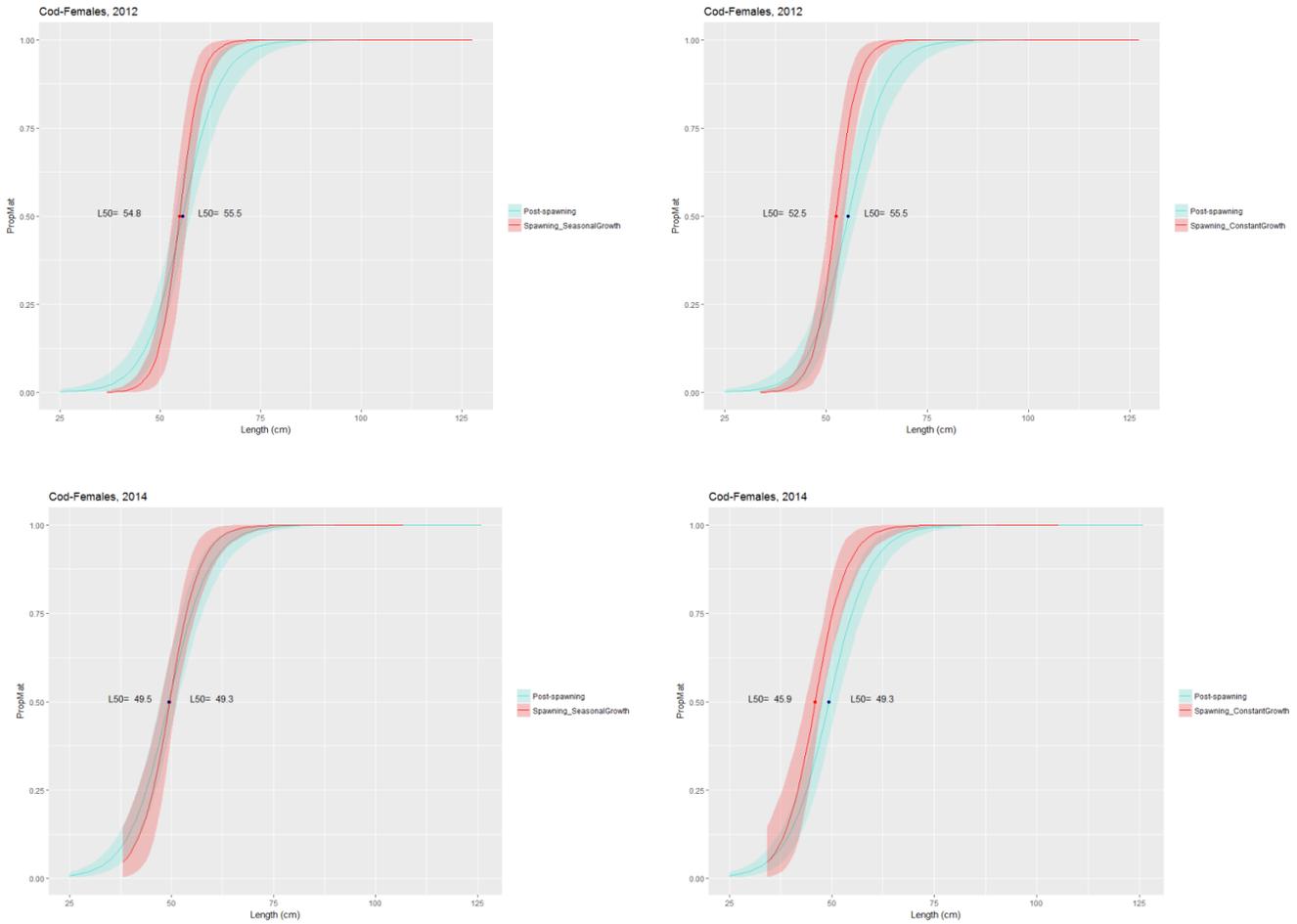


Fig. 3. Comparison of maturity ogives obtained from different sampling moment related to reproductive season. On the left, the comparison of post-spawning samples and spawning samples adjusted with a seasonal growth are presented for 2012 (up) and 2014 (bottom). On the right, the comparison of post-spawning samples and spawning samples adjusted with a constant growth are presented.