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Histological Assessment of Sexual Maturity
of the Flemish Cap Cod (Division 3M) in 1995

by

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Introduction

Since 1990 an histological method has been used to determine the length and age at maturity of the Flemish Cap female cod in summer, when the EC-research survey is carried out. It consists in using the cod ovaries that are in the *cortical alveoli* stage as an index of the onset of the oocyte development for the next breeding season, whereas the presence of postovulatory follicles in the ovaries show the females that already spawned (Zamarro et al., 1993). The cortical alveoli are the first structures to appear during the phase of oocyte growth (Wallace and Selman 1981), and thus are the first sign of the start of the female ripening before the beginning of the vitellogenesis. The postovulatory follicles are the structures left in the ovary after oocyte ovulation, once its maturation is finished. It is indicative of a previous spawning, but the use of this structure to determine the proportion of females that have spawned requires to know its duration in the ovary. The spawning season of the Flemish Cap cod is known to be short and the earliest in relation to other areas of the Northwest Atlantic (Myers et al., 1993), as it is concentrated around March. This method allow to accurately detect the proportion of both adult and primiparous females at the very beginning of the gonad development, as it happens in summer, few months after spawning, when it is very difficult to make a visual diagnosis of the sexual maturity stage (Morrison 1990). In this paper we use the proportion of females with postovulatory follicles in 1995 (July) to determine the length and age at maturity in this year and the proportion of females in the *cortical alveoli* stage to estimate the same parameter for 1996.

Material and methods

A total of 201 cod ovaries were sampled during the summer survey in Flemish Cap (Vázquez 1996). Total length and weight were recorded for each individual and otoliths removed for age determination. Gonads were immediately fixed in 4% buffered formalin (Hunter 1985). Pieces of 0.5 cm thick were embedded in paraffin based on conventional histological processing and 5 µ sections stained with Harris hematoxyline and eosine-floxine.

Different stages were identified in the sections according to the classification of West (1990) and Morrison (1990). Immature females were identified since 100% of the oocytes were in the circumnuclear ring stage; instead, mature females had oocytes in cortical alveoli (CA), vitellogenesis, postovulatory follicles (POF) or degenerating (atresic) oocytes. Primiparous females were identified since they have no postspawning structures such as POF or atresic oocytes. Recent postspawning stage is identified by the presence of postovulatory follicles and atresic oocytes, without any oocyte in the *cortical alveoli* or any further stage of development.

The proportion of mature female by size and age was adjusted to a logistic equation as described by Ashton (1972) :

$$\hat{P} = \frac{e^{\alpha + \beta L}}{1 + e^{\alpha + \beta L}} \qquad (1)$$

and the logit transformation:

$$\ln \frac{\hat{P}}{1 - \hat{P}} = \alpha + \beta L \qquad (2)$$

where

$\hat{P}$ = predicted mature proportion

α and β are the coefficients of the logistic equation

L is the length (or age).

BMDP LR (Dixon *et al.*, 1990) was used to calculate the predicted values and the coefficients. Thus, the size and age at maturity could be estimated as the minus ratio of coefficients ($-\alpha / \beta$) by substituting $\hat{P} = 0.5$ in equation 2.

Two maturity curves were generated: one using CA as the index of the onset of ripening for 1996, and another one using the presence of POF as a guide for spawned females in summer 1995.

Results

Table 1 and table 2 show the number of mature and immature females sampled by length and age respectively. A 57 % of the females sampled were either primiparous or multiparous adult females, while 43 % were immature. The frequencies of females with *cortical alveoli*, postovulatory follicles, vitellogenic oocytes and in postspawning stage by length intervals and age are presented in table 3 and 4 respectively. All the ovaries of adult females sampled have oocytes in the CA stage. There are no female in recent postspawning stage, since oocytes in CA are already present in all the ovaries with POF or atresic oocytes in July 1995. A total of 94 of the sampled female had spawned in 1995, as indicated by the presence of POF and atresic oocytes in the ovary. The proportion of females with this kind of postspawning structures increases with length and age and gets 100 % of females larger than 57 cm (age 5 +). As occurred in 1994, the number of females with oocytes in the vitellogenic stage was very low in summer (only 3 individuals).

The maturity curves obtained by length and by age are shown in Figures 1 and 2 respectively. Each include two curves, one corresponding to the spawners in 1995, using the frequencies of POF to identify mature females and the other corresponding to the next year spawners, identified by the presence of CA. Length and age at 50% maturity obtained from the CA curves is 39.07 cm and 3.1 years respectively (table 5). The same parameter obtained from POF frequencies is 42.65 cm and 3.9 years respectively. Like in 1994, there is a difference less than one years between the two estimates of the age at maturity, what is an unusual situation compared with the results of previous years (table 5). During the period from 1990 to 1995 a decrease in both the age and the length at maturity is observed.

Discussion

The age at maturity in 1995 obtained using POF is 3.9 years, the same as in 1994 (Saborido-Rey and Junquera 1995). The same parameter obtained from CA, that is the age at maturity for the next year spawners, shows a slight reduction from 3.4 years in 1994 to 3.2 years in 1995. Conversely, the decreasing trend in the length at maturity already noted in 1994, continues in 1995 and it is very pronounced, since if compared with 1994 a reduction of 9 and 8 cm are observed in the estimates based on POF and CA respectively. The fact of this great reduction in the length at maturity while the age at maturity remain almost invariant is not attributable to a change in the ageing criteria and could indicate that the growth parameters of the stock are changing as well as the reproductive ones.

It is remarkable that the maturity curve in 1995 based on POF have a knife edge shape. Almost all the females became mature between the ages 3 (0 % spawned in July 1995) and 4 (80 % spawned). At age 5, 100 % of the females were mature. Those data do not properly fitted the logistic model and consequently the age at 50 % maturity is probably not a suitable parameter in this case. The information obtained in the summer 1995 gives a further support to the fact already pointed in 1994 (Saborido-Rey and Junquera, 1995) that likely in those recent years the age at maturity of female cod is fixed around age 3. This age apparently determine a biological limit for the onset of the maturation process in this species, as it suddenly begin there, without presence of any mature female at earlier ages, and from the next age group (4 +) virtually all the females already are mature.

From the maturity curves obtained using POF it can be concluded that in the period 1990 to 1995 the age at maturity decreased from about age 5 to about age 4 (3.9 years). A similar reduction is obtained using CA. Although the proportion of ovaries with CA is a useful tool to predict a maturity curve one year in advance, the use of this kind of structure has the problem of the interanual variations in the date of mass spawning of the stock, that can lead to incongruent results from year to year (less than one year between both estimates, with POF and with AC), as it was observed in 1994 (Saborido-Rey and Junquera, 1995). The presence of CA in the cod ovaries is seasonal (Zamarro et al. 1993). They appear soon after the spawning (probably in less than two

months) and so its frequency would be underestimated in the summer samples whenever the spawning shifts from March toward the summer. Taking into account that the small cod are supposed to spawn later (Morrison 1980), an overestimation of both the age and length at maturity could be systematically expected when the CA are used to generate maturity curves in the Flemish Cap area, specially considering that the stock is at the moment mainly composed by small fish (Vázquez 1995; Vázquez 1996). In 1995, the absence of females in recent postspawning indicate that the spawning occurred not as late as in 1994, when a high quantity of females were in postspawning stage in July (Saborido-Rey and Junquera, 1995). However the spawning season seems still delayed compared with the period 1990-1992, when a high number of the females were already in the vitellogenesis stage in July (Zamarro et al. 1993; González and Larrañeta 1994), while negligible proportions were found both in 1994 and 1995 in the same month.

The possible effect of the decrease in the cod stock abundance, especially in the largest individuals, in Flemish Cap on the reduction of the age at maturity during the period analysed, has already be pointed (Saborido-Rey and Junquera 1995). The 1995 summer research survey in this area indicated that the cod biomass is notably reduced compared with 1994. The 1991 year class, which was the most important one in those previous years, virtually disappeared and the year classes of 1993 and 1994 are very weak. Consequently if the mentioned reduction in the age at maturity is a density-dependent response, this feature can be expected to be maintained in the next years.

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Table 1.- Number of adult and immature females by length
sampled in Flemish Cap in summer 1995.

Length	Immature	Mature	TOTAL
< 27	40	0	4
27 - 30	15	0	15
31 - 33	35	0	35
34 - 36	13	0	13
37 - 39	12	3	15
40 - 42	7	8	15
43 - 45	0	17	17
46 - 48	0	15	15
49 - 51	0	27	27
52 - 54	0	23	23
55 - 57	0	9	9
58 - 60	0	6	6
61 - 63	0	3	3
64 - 66	0	1	1
70 - 72	0	1	1
> 73	0	2	2
TOTAL	**86**	**115**	**201**

Table 2.- Number of adult and immature female cod
by age sampled in Flemish Cap in summer 1995.

Age	Immature	Mature	TOTAL
2	2	0	2
3	63	0	63
4	19	4	23
5	2	93	95
6	0	15	15
7	0	1	1
9	0	1	1
TOTAL	**86**	**115**	**201**

Table 3.- Number of females with ovaries in the *cortical alveoli* stage (CA),
postovulatory follicles (POF), vitellogenic oocytes (OV) and postspawning (PS) stage by
length sampled in summer 1995.

Length	AC	POF	OV	PS	TOTAL
< 27	0	0	0	0	4
27 - 30	0	0	0	0	15
31 - 33	0	0	0	0	35
34 - 36	0	0	0	0	13
37 - 39	3	0	0	0	15
40 - 42	8	4	0	0	15
43 - 45	17	10	1	0	17
46 - 48	15	12	0	0	15
49 - 51	27	25	0	0	27
52 - 54	23	22	1	0	23
55 - 57	9	8	1	0	9
58 - 60	6	6	0	0	6
61 - 63	3	3	0	0	3
64 - 66	1	1	0	0	1
70 - 72	1	1	0	0	1
> 72	2	2	0	0	2
TOTAL	**115**	**94**	**3**	**0**	**201**

Table 4.- Number of females with ovaries in the *cortical alveoli* stage (CA), postovulatory follicles (POF), vitellogenic oocytes (OV) and postspawning (PS) stage by age, sampled in summer 1995.

Age	AC	POF	OV	PS	TOTAL
1	0	0	0	0	2
2	0	0	0	0	63
3	4	0	0	0	23
4	93	76	3	0	95
5	15	15	0	0	15
6	1	1	0	0	1
7	1	1	0	0	1
9	1	1	0	0	1
TOTAL	115	94	3	0	1

Table 5.- Age and length at 50 % maturity of female cod obtained from the maturity curves based on postovulatory follicles (POF) and cortical alveoli (CA) frequencies, from July 1990 to July 1995. (From Zamarro et al. 1993, Gonzalez and Larrañeta 1994 and Saborido-Rey and Junquera 1995).

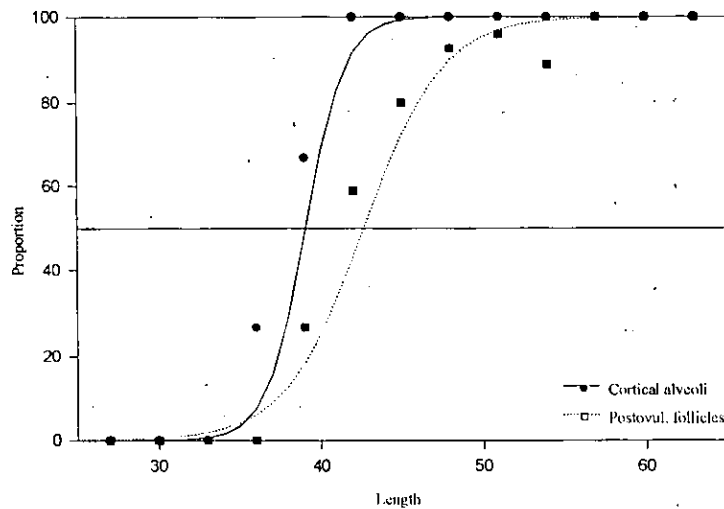|	AGE		LENGTH	
Year	POF	AC	POF	AC
1990	5.6	3.8	61	?
1992	4.7	3.7	59.1	51.9
1993	5	4	64	50
1994	3.9	3.4	51.2	47.8
1995	3.9	3.2	42.6	39.1

Fig. 1- Proportion of mature female by length obtained using the frequency of cortical alveoli and of postovulatory follicles in July 1995.
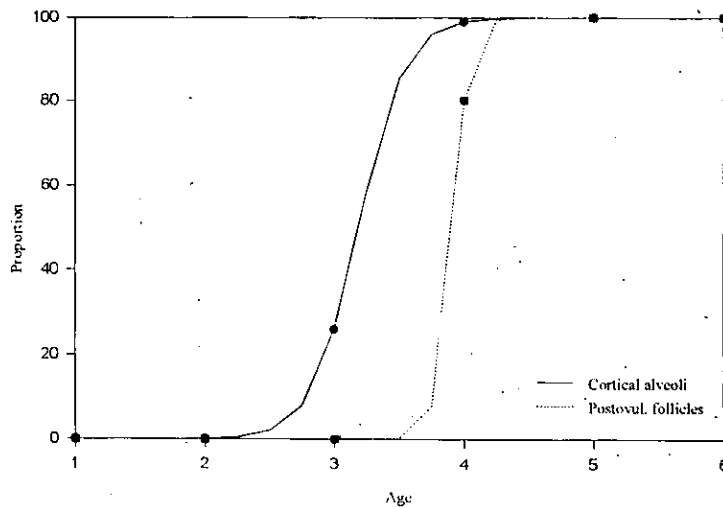

Fig. 2- Proportion of mature female by age obtained using the frequency of cortical alveoli and of portovulatory follicles in July 1995.