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Histological assessment of variations in sexual maturity of cod (*Gadus morhua* L.) at the Flemish Cap (north-west Atlantic)

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A histological method is used to detect the proportion of both adult and recruit females at the beginning of gonad development, in summer, a few months after spawning. At this time it is difficult to make a visual diagnosis of the sexual maturity stage. The proportion of females with postovulatory follicles is used to determine the length and age at maturity in the year of sampling, and the proportion of females in the cortical alveoli and/or vitellogenic stages is used to estimate the same parameter for the following year. Variations in age and length at maturity during the period 1992–1995 were analysed by comparing the respective maturity curves of both the ''previous spawners'' and the ''next-year spawners'' females. A drastic decrease in both the age and the length at maturity is observed in this period and it seems likely that the age at maturity of female cod became fixed at around age 3 at the end of the period analysed. The possibility that this could be a density-dependent response of the stock at the Flemish Cap is discussed.

Key words: cod, Flemish Cap, age-at-maturity, length-at-maturity, maturity curves, cortical alveoli, postovulatory follicles, vitellogenesis.

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Introduction

The Flemish Cap is a traditional fishing area for cod in the north-west Atlantic for the European Union fleet (mainly Spain and Portugal). Cod in this area are managed as a single independent unit stock (Anon., 1996; Cárdenas, 1995) which is the only one still open for fishing in the north-west Atlantic (Anon., 1996). The Flemish Cap cod stock was itself under a fishing moratorium from 1988 to 1990 and currently all the indices show that its abundance is severely reduced (Vázquez, 1996; Vázquez *et al*., 1995; Anon., 1996).

To ascertain the influence of the reduction in abundance of the stock on the length at maturity it is essential to determine the spawning stock biomass. During most of the later stages of the annual reproductive cycle, it is easy to calculate a maturity curve for females using macroscopic features of the ovary (Morrison, 1990) but this becomes very difficult following spawning and at the immediate onset of ripening. This is a problem encountered normally in summer research surveys when the intention is to obtain information on the sexual maturity of spring-spawning species such as cod. In an attempt to overcome this problem, Zamarro *et al*. (1993) designed a method to classify cod ovaries into maturity stages using histological techniques that make it possible to identify recruit and repeat mature females, and to predict the length at maturity for the next breeding season. This paper presents an analysis of recent trends observed in the length and age at maturity of cod at the Flemish Cap.

Material and methods

A total of 715 cod ovaries were collected in July during the European Union survey at the Flemish Cap from 1992 to 1995 (Table 1). Hauls ranged from the shallower parts of the bank (120 m) to depths of 780 m, according to a random stratified design, with a haul duration of 30 min (Vázquez, 1996). Ovaries were collected from females larger than 25 cm. Total length and weight

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Table 1. Number of immature (I) and mature (M) females by length categories (cm), and corresponding percentages of mature females, sampled from 1992 to 1995.

	1992		1993		1994		1995		% mature			
Length	I	M	I	M	I	M	I	M	1992	1993	1994	1995
<30	1	0	—	—	2	0	19	0	0	—	0	0
30–34	2	0	3	0	—	—	44	0	0	0	—	0
35–39	2	0	9	0	5	0	18	5	0	0	0	21.7
40–44	5	1	31	12	23	7	2	26	16.7	27.9	23.3	92.9
45–49	5	0	32	25	12	18	0	36	0	43.9	60.0	100
50–54	5	9	11	12	2	43	0	32	64.3	52.2	95.6	100
55–59	4	25	0	9	0	45	0	13	86.2	100	100	100
60–64	0	20	0	25	0	15	0	4	100	100	100	100
65–69	0	17	0	26	0	5	—	—	100	100	100	100
70–74	0	11	0	6	—	—	0	1	100	100	—	100
>75	0	12	0	13	0	2	0	3	100	100	100	100
Total	24	95	86	128	44	135	83	120				
	119		214		179		203					

of fish were measured and otoliths removed for age determination. Gonads were fixed in 4% buffered formaldehyde (Hunter, 1985) and weighed in the laboratory. One slice, 0.5 cm thick, per ovary was embedded in paraffin and 5 µm sections cut and stained with haematoxylin and eosin. One 5 µm section per ovary was examined with a light microscope to identify different stages of development.

Different stages were identified following the classification of Morrison (1990) and the criteria of West (1990). Five terms are defined as follows to evaluate reproductive status:

- Primary growth stage. This covers two phases: the chromatin nucleolar phase and the perinucleolar phase. The chromatin nucleolus is the first sign of the primary development of the teleost oocyte, which is very small with a central nucleus containing a large single basophilic nucleolus, surrounded by a thin layer of cytoplasm. As the oocyte grows, both the cytoplasm and the nucleus increase in size and multiple nucleoli appear in the periphery of the nucleoplasm, which is the perinucleolar stage.
- Cortical alveoli stage (CA). The presence of oocytes with cortical alveoli in the periphery of the cytoplasm indicates the onset of the maturation for the following breeding season. With conventional haematoxylin–eosin staining, the alveoli appear as empty spheres. This stage is completed when yolk starts to accumulate.
- Vitellogenic stage (VO). Yolk globules begin to be formed. Further stages of oocyte growth (maturation) have not been observed in the samples analysed.
- Postovulatory follicle (POF). Ruptured empty oocyte envelopes are left in the ovary after ovulation of mature oocytes, indicating that the fish have spawned.
- Atretic oocytes (AO). Vitellogenic oocytes in different phases of development are lost through degeneration (atresia) from the ovary. Although atretic oocytes can appear at any time in the cycle, they are more abundant after spawning.

Immature females were identified when all the oocytes were in the primary growth stage. Mature females, on the other hand, had oocytes in the CA or VO stages, POF and/or AO. *Recruit females* had oocytes in CA or VO stages but not POF or AO, whereas *repeat females* were identified by the presence of POF or AO. According to Zamarro *et al*. (1993), a maturity curve of the previous breeding season can be adjusted from the proportion of females showing POF, while the maturity curve of the next spawning season is given by the proportion of females in CA stage. However, ovaries in VO stage but without CA oocytes could also show a spawner for the next season. In this paper, two maturity curves were generated for every year in the period 1992–1995: one of them using CA and/or VO as the index of the onset of ripening (i.e. the maturity curve of the next-season spawners) and another one using the presence of POF as a measure of the females that had already spawned (maturity curve of the previous spawners). This gives two different estimates of the length and age at 50% maturity.

The proportions of mature females by size and age were fitted to a logistic equation as described by Ashton (1972):

$$\hat{P} = \frac{e^{a + bL}}{1 + e^{a + bL}} \quad (1)$$

and the logit transformation:

$$\ln\frac{\hat{P}}{1-\hat{P}} = a + bL \qquad (2)$$

where $\hat{P}$ is the predicted mature proportion, a and b the estimated coefficients of the logistic equation and L the length (or age).

The maximum-likelihood method was considered to be the most satisfactory methodology for estimating length and age at maturity (Welch and Foucher, 1988; Hosmer and Lemeshow, 1989). However, data from 1995 were adjusted by the least-squares method, because the error estimates of the coefficients could not be computed due to the sharp slope of the maturity curve. It was found that both methods of adjustment yielded similar coefficients, and so it is possible to compare safely the results obtained with the two different adjustment methods. Error variances across the range of the x variable were checked and found to be equal.

The goodness-of-fit was assessed using a chi-square test for maximum likelihood in the period 1992–1994, while for the 1995 data, due to the adjustment method used, the goodness-of-fit was tested by the proportion of variance explained.

Statistica for Windows 5.0 (StatSoft, Inc., 1995) was used to calculate the predicted values and the coefficients.

Size and age at maturity were estimated as the minus ratio of the coefficients (− a/b) by substituting $\hat{P} = 0.5$ in Equation (2).

To evaluate the differences in age and size at maturity between years, the variance of those parameters each year was calculated from the variances and covariance of the maturity curve coefficients (Ashton, 1972):

$$V(L_{50}) = \frac{1}{b^2}\left[V(a) + \frac{a^2}{b^2}V(b) - \frac{2a}{b}\,\mathrm{cov}(a,b)\right]$$

where L_{50} is the age or size at maturity. Assuming that L_{50} estimates are normally distributed, then the Z statistic can be computed as:

$$Z = \frac{\frac{a_1}{b_1} - \frac{a_2}{b_2}}{\sqrt{V_1 + V_2}}$$

where a and b are the logistic regression coefficients and V_i the L_{50} variances of each year compared. Z values can be used to test the null hypothesis of parameter equality (Gunderson, 1977).

Results

Table 1 shows the number of mature and immature females sampled each year by length. It can be seen that the proportion of large females sampled (larger than 50 cm), progressively decreased over time, reflecting a parallel decrease in the length of cod caught in the surveys. The proportion of mature females by length interval drastically increased in the period analysed, thus for example, in the interval 42–45 cm there was 20% mature females in 1992, while in 1995 there was 100%.

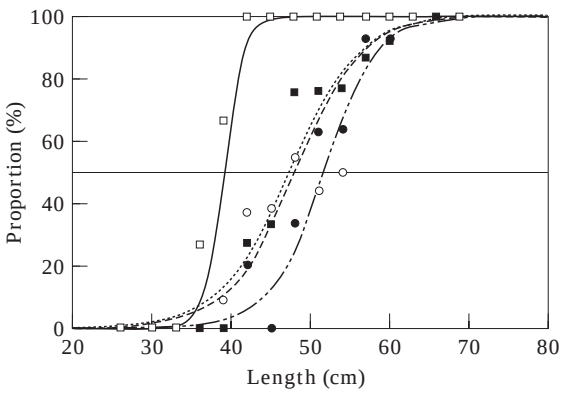

Figure 1. Proportion of females in the cortical alveoli and vitellogenic stages by length from 1992 to 1995. 1992 (●); 1993 (○); 1994 (■); 1995 (□).

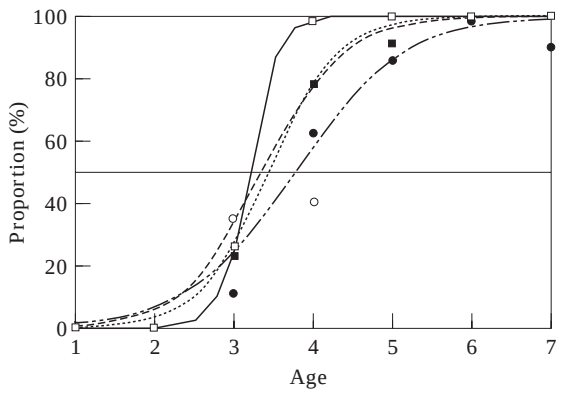

Figure 2. Proportion of females in the cortical alveoli and vitellogenic stages by age from 1992 to 1995. 1992 (●); 1993 (○); 1994 (■); 1995 (□).

Percentages of ovaries in the CA stage by length interval and age, respectively, can be seen in Figure 1 and Figure 2. The number of ovaries in this stage increased gradually with both length and age, showing the proportion of females expected to spawn in the next season. The length at which all females had begun ripening decreased considerably in the period analysed, from 63–65 cm in 1992, to 42–44 in 1995 (Fig. 1). The increase in the proportion of females in the CA stage with length was slight in the earlier years, but became very sharp by the end of the period analysed. The same pattern can be observed in the frequencies by age (Fig. 2), so that in 1995 at age 2 all the females were immature, while at age 4 almost all were mature.

Table 2. Frequency of ovaries with vitellogenic oocytes (VO) by length (cm) in both absolute numbers and percentages, and total percentages (mature females weighted to the number of females sampled by length interval), from 1992 to 1995.

	1992		1993		1994		1995	
Length	n	%	n	%	n	%	n	%
<30	0	0	—	—	0	0	0	0
30–39	0	0	0	0	0	0	0	0
40–49	0	0	3	3	1	2	1	2
50–59	3	7	3	9	2	2	2	4
60–69	10	27	17	33	0	0	0	0
70–79	4	36	3	50	0	0	0	0
>80	5	42	4	31	0	0	1	33
Total		19		20		2		5

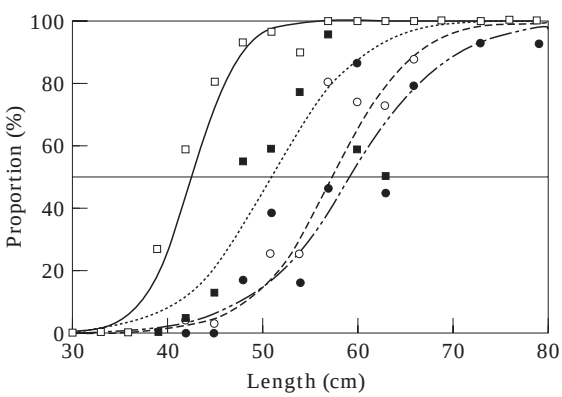

Figure 3. Proportion of females with postovulatory follicles by length from 1992 to 1995. 1992 (●); 1993 (○); 1994 (■); 1995 (□).

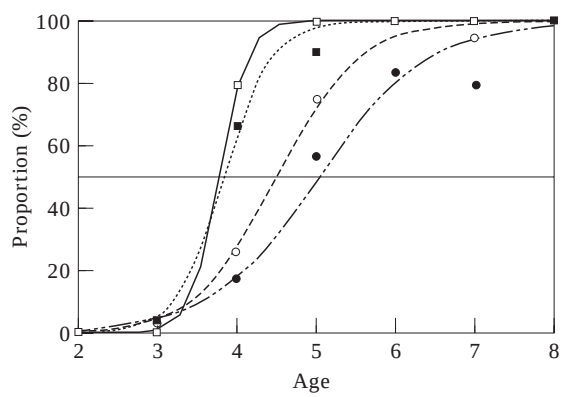

Figure 4. Proportion of females with postovulatory follicles by age from 1992 to 1995. 1992 (●); 1993 (○); 1994 (■); 1995 (□).

The proportion of ovaries with vitellogenic oocytes (VO) was always very low (Table 2). All the vitellogenic oocytes found in the ovaries were at an early development phase. In most cases both CA and VO were found in the same ovary. Only five ovaries, two in 1993 and three in 1994, had VO without CA oocytes.

The proportion of ovaries with POF by length interval and age are shown in Figure 3 and Figure 4, respectively. The percentage of spawned females increased with both size and age in each year. However, the size and age at which all females had spawned decreased year by year. So, while in 1992 the length at which all females had spawned was about 70 cm, in 1995 it was about 58 cm, although at 50 cm most females had spawned (Fig. 3). The minimum size and age at which females spawned also decreased year by year, from approximately 50 cm in 1992 to 40 cm in 1995. In 1995, none of the females at age 3 had spawned while all of them had spawned at age 5 (Fig. 4).

Repeat and recruit females were identified by the presence of POF. The percentage of first-spawners decreased drastically from 1992 to 1995. While in 1992 and 1993 it was almost half of the reproductive population (43 and 48% respectively), in 1994 it had declined to 31% and to 20% in 1995.

The proportion of recent post-spawning females (ovaries with POF or AO but without CA or VO) changed a lot from year to year. However, a common feature in the period analysed is the post-spawning females were mostly the younger individuals.

Two maturity curves were generated by both length and age for each year. In the first curve, corresponding to the females that would spawn in the next year, the presence of CA and VO was used to identify mature females. For the second, corresponding to the females that had spawned in the current year, the frequency of females with POF was used as a criterion. Maturity curves by length are shown in Figures 1 and 3, and by age in Figures 2 and 4. Table 3 shows the parameters of the fitted curves. The fitting was significant for all the curves, except for the one for the previous spawners in 1995.

Difference in length at maturity between previous-spawners in 1995 and next-spawners in 1994 is negative,

Table 3. Analysis of the female cod age and length at maturity at the Flemish Cap from 1992 to 1995. a and b = coefficients of the logistic curve, t = t-values with the degrees of freedom in parentheses*; χ^2 = chi-square of goodness of fit, for the period 1992 to 1994†; Prop. Var. = proportion of variance explained; 50% = age or length at 50% maturity (− a/b).

		Length				Age			
		Next spawners		Previous spawners		Next spawners		Previous spawners	
		a	b	a	b	a	b	a	b
1992	Estimate	− 15.2062	0.2941	− 11.3216	0.1907	− 5.6564	1.5009	− 7.3486	1.4625
	Std Err	3.5068	0.0642	2.2353	0.0373	1.3625	0.3201	1.2508	0.2397
	t (117)	− 4.3362	4.5795	− 5.0650	5.1109	− 4.1513	4.6891	− 5.8749	6.1018
	χ^2	63.867		54.383		43.408		59.960	
	50%	**51.69**		59.38		3.76		5.02	
1993	Estimate	− 11.4401	0.2382	+ 13.7404	0.2389	− 6.6263	1.9754	− 8.8793	1.9824
	Std Err	1.7443	0.0367	1.6654	0.0296	1.0958	0.3392	1.0759	0.2488
	t (212)	− 6.5585	6.4875	− 8.2506	8.0578	− 6.0468	5.8232	− 8.2532	7.9692
	χ^2	117.86		152.78		105.15		153.77	
	50%	**48.02**		57.53		3.35		4.48	
1994	Estimate	− 10.7806	0.2275	− 11.1939	0.2185	− 8.0517	2.3387	− 13.0169	3.4118
	Std Err	1.7973	0.0362	1.7942	0.0346	1.4126	0.3803	2.4262	0.6181
	t (177)	− 5.9981	6.2874	− 6.2390	6.3112	− 5.6998	6.1502	− 5.3652	5.5200
	χ^2	65.649		66.209		54.211		77.407	
	50%	**47.39**		51.23		3.44		3.82	
1995	Estimate	− 32.0419	0.8201	− 17.4532	0.4092	− 17.8600	5.6061	− 21.0785	5.6189
	Std Err	4.2903	0.1083	2.5184	0.0592	5.0458	1.6723	11.6880	2.9311
	t (201)	− 7.4685	7.5731	− 6.9302	6.9151	− 3.5396	3.3523	− 1.8034[a]	1.9170[a]
	Prop. Var.	89.619		72.493		88.944		69.873	
	50%	**39.07**		42.65		3.18		3.75	

*$p < 0.001$ in all cases escept where indicated with [a].
†$p < 0.001$ in all cases.

Table 4. Z values of the comparative analysis of the length and age at maturity between years.

	Next spawners			Previous spawners		
	1992	1993	1994	1992	1993	1994
Length						
1993	2.6241**	—	—	1.2083	—	—
1994	2.9169**	0.5210	—	5.6316**	5.1312**	—
1995	10.7781**	10.7637**	8.6734**	12.8673**	14.1735**	9.3869**
Age						
1993	1.8887	—	—	2.6000**	—	—
1994	1.5029	0.6796	—	6.7536**	4.8535**	—
1995	2.8224**	1.5046	2.4011*	7.1525**	5.3750**	0.8012

while a positive difference should be expected. In addition, differences in age-at-maturity between both estimates are less than one year in all time series analysed.

Table 4 shows the Z values of the comparison of length and age at maturity between years. In most cases, the differences were significant. In the next-season spawner curves, the differences in length were significant for all comparisons, except between 1993 and 1994. However, the differences in the age at maturity were only significant between 1992 and 1995 ($p < 0.01$) and between 1994 and 1995 ($p < 0.05$). In the ''previous-spawners'' age at maturity, the differences were all significant except between 1992 and 1993 in the curves by length and between 1994 and 1995 in the curves by age.

Discussion

The identification of mature cod females in summer, a few months after the spawning is only accurate using histological techniques. However, a constraint of the method is that the results of a relatively small sample have to be extended to a large population.

Since the proportions of females in CA and vitellogenic stages will vary with the spawning time and the survey time, it is necessary to use both stages to assess the maturity for the next breeding season. Four to eight months are required in north-east Arctic cod between the end of the spawning and the start of the vitellogenesis (Kjesbu, 1991). On the other hand, CA seems to be a more suitable criterion than other pre-vitellogenic stages, since most of the adult females are in this stage by the sampling month. However, oocytes in the CA stage show a seasonal occurrence declining rapidly in number as vitellogenesis proceeds. A progressive decline in the proportion of females in vitellogenesis is observed during the period analysed, probably because spawning was completed later in recent years. Several reasons can explain the delay in spawning time, i.e. changes in the environment, as well as in the age structure of the spawning stock.

The use of POF to determine the proportion of females that spawned in the current year is only possible if the duration of this stage in the ovary is known. In warm water species, where reabsorption of the POF is well-documented (Hunter and Goldberg, 1980; Hunter and Macewicz, 1985), it is known that post-ovulatory follicles remain in the ovary for only a few days. The available information on this subject in cod is scarce, although Woodhead and Woodhead (1965) indicate that the POF can be recognized in the ovaries four to five months after spawning and even longer according to Zamarro *et al.* (1993). Though old POF are easy to confuse with atretic oocytes, according to our results, the POF are still clearly identifiable by the sampling month, i.e. about three to four months after spawning.

Size and age at maturity have drastically declined in the period analysed. The maximum-likelihood method is considered to be a robust procedure to avoid bias in the estimates related to the decrease in abundance of larger and older females over this period (Welch and Foucher, 1988). Age at maturity for the ''next'' and ''previous spawners'' within each year should differ by one year. However larger differences appeared in 1990 and 1992. Zamarro *et al.* (1993) proposed two possible explanations: a difference between cohorts in the age at maturity or presence of non-reproductive adult females. Although non-reproductive adult females have been reported at the Flemish Cap (Walsh *et al.*, 1986) and in other areas (Oganesyan, 1993; Morgan and Brattey, 1996), they have only rarely been observed in the material analysed here, namely one female in 1992 and another one in 1990. A more likely explanation is that females became mature younger than expected. According to the results, the main reduction of age at maturity would have occurred in the early 1990s, as has also been reported by Morgan and Brattey (1996) in the Newfoundland shelf cod. Difference in age-at-maturity estimates between years was always less than 1, especially between 1994 and 1995, and also length-at-maturity differences in those years were negative. This might be due to a systematic overestimation for ''next spawners'', if a portion of the younger females had not begun ripening by the sampling time, as according to the results, it seems likely that the repeat spawners begin ripening earlier than do the first spawner ones.

Size and age at maturity are highly plastic parameters that change under external pressure, particularly with a decrease in the population abundance (Adams, 1980; Gunderson, 1980; Wootton, 1990). Examples of this are plentiful, both in cod (Beacham, 1983; Jørgensen, 1990; Morgan and Brattey, 1996) and in many other commercial fish stocks in the North Atlantic. The Flemish Cap cod total biomass declined from 103 644 t in 1989 to only 8815 t in 1995 (Vásquez, 1996). In the same way, catch at age showed a parallel decrease, especially in the oldest individuals: 22.5% of the females were adult in 1990, compared with only 3% in 1992 and 7% in 1993. The sharp knife edge curves in both ''next'' and ''previous spawners'' seem to indicate that the Flemish Cap cod has reached its biological limit for the onset of the maturation, which would be located at around age 3. A physiological limit exists for the age (and length) at which individuals of a species are able to become sexually mature (Trippel, 1995). In the more southerly cod stock in the north-west Atlantic (Georges Bank stock), females are presently maturing at age 2 (Trippel *et al.*, 1995). Cod in northerly populations mature at older ages than do those in southern areas (Morgan *et al.*, 1993), thus it seems likely that age 3 could be the physiological limit for age at maturity in the Flemish Cap cod stock.

It might be argued that, if the indicated reduction were a response to a decline in the population abundance, then an accelerated growth under a lower stock density would be expected and individuals would mature at younger ages without changing length at maturity. However, the majority of the documented shifts in the reproductive parameters affect both the age and length at maturity (Pinhorn, 1969; Beacham, 1983) and this is also the case of the Flemish Cap cod. This point is apparently inconsistent with the compensatory theory underlying density-dependent effects.

The reduction of age at maturity has been a positive response to low fish densities, which could generate an increase of the spawning stock. However, the implications of this increase in the stock dynamics must be carefully considered. The reproductive potential of one age-truncated stock, whose spawning biomass is based on young females, is not equivalent to the same spawning biomass of large females, since it is known that older females have a longer spawning season, higher fecundity and produce larger eggs than do the younger ones (Buzeta and Waiwood, 1982; Kjesbu, 1989; Kjesbu *et al.*, 1991). It seems clear that offspring of older cod are

more likely to survive. This is an important point in the Flemish Cap cod stock since in 1990, 61% of the spawning stock was 5 years and older, but by 1995, 80% was 3 and 4 years old.

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