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# ASPECTS OF THE REPRODUCTIVE BIOLOGY OF THE VELVET **BELLY, ETMOPTERUS SPINAX (ELASMOBRANCHII: SQUALIDAE)**

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ãál Õ وقع اصطياد القروش المستعملة في هذه الدراسة على السواحل التونسية وفي خليج ليون (فرنسا) على قيعان رملية أو وحلية في أعماق تتراوح بين 100 و400 م. الطول الجملي عند أول بلوغ جنسي يكون350 مم عند الذكور وما بين 310 و370 مم عند الاناث

كل الإناث تصبح بالغة بعد400 مم من الطول الجملي. معدل الطول والوزن عند الجنين المتكامل تكون على التوالي126 مم و 6،03 غ. عدد الصغار يكون من 5 إلى 9 في الحملة الواحدة. البويضات تكون أكثر عددا في الغدة اليسرى وعدد الأجنة يكون عادة متساويا في الرحمين

فرنسا -خليج ليون-معدل الطول القرش- بيولوجيا التكاثر : المفاتيح

### **RESUME**

Aspects de la biologie de la reproduction du sagre noir Etmopterus spinax : Des aspects de la biologie de la reproduction du sagre noir, Etmopterus spinax, sont présentés dans cette note. Les spécimens observés sont récoltés au large des côtes tunisiennes et de la côte du Languedoc (France méridionale), sur des fonds sableux et/ou sablo-vaseux allant de 100 à 400 m de profondeur. Les mâles sont adultes au-delà de 380 mm de longueur totale (LT). La phase de maturation des femelles entrent dans une phase de maturation. La plus petite femelle avec des oocytes majeurs prêts à être pondus mesurait 380 mm LT le la plus petite femelle gravide, 400 mm LT. Toutes les femelles adultes ont plus de 400 mm LT. La taille maximale est de 400 mm LT pour chaque sexe. La gestation pourrait durer une année au minimum, mais il serait hypothétique qu'elle puisse s'étendre sur deux années ou plus. La vitellogenèse ne s'effectue pas en parallèle avec la gestation. Toutefois, chez une seule femelle gestante des ovocytes évolutifs ont été observés. La vitellogenèse se poursuit après la parturition qui se situe généralement en été. Les LT et les masses moyennes des foetus à terme sont de 126 mm et de 6, 03 g. La balance chimique de développement (CBD) fondée sur le calcul de la moyenne des masses sèches des ovocytes majeurs et de foetus à terme est de 0,803. Cette faible valeur montre un degré de lecithotrophie chez E. spinax, même si le rôle de la mère durant la gestation n'est pas négligeable. La fécondité est de 5 à 9 individus par portée. Les ovocytes majeurs sont plus nombreux dans l'ovaire gauche. Cependant, chez certains spécimens, ils sont plus nombreux dans le droit. Mots-clés - Elasmobranchii, Squalidae, MED, côtes tunisiennes, côte du Languedoc, biologie de la reproduction.

#### ABSTRACT

Aspects of the reproductive biology of the velvet belly, Etmopterus spinax are presented in this paper. The observed specimens were collected off Tunisian coasts and the coast of Languedoc (southern France), in sandy and/or muddy bottoms from 100 m to 400 m depth. The males were adult over 350 mm total length (TL). Females matured between 310 mm and 370 mm TL. The smallest adult female with ripe oocytes ready to be ovulated and the smallest gravid female were 380 mm and 400 mm TL respectively. All females over 400 mm TL were adults. The maximum size was 460 mm for both sexes. Gestation could be a minimum of one year, but it is hypothesised that it may be as much as two years. Vitellogenesis did not proceed in parallel with gestation. However, in a single pregnant female developing oocytes were observed. Vitellogenesis continued after parturition which occured generally in summer. Average TL and average weight of fullydeveloped fetuses were 126 mm and 6.03 g. A calculated chemical balance of development (CBD) based on mean dryweights of fully-developed fetuses and ripe oocytes was 0.803. This low value is due to a degree of lecithotrophy in E. spinax; even if the mother role during gestation is not negligible. Fecundity ranged from five to nine newborn pups per brood. Ripe oocytes were more numerous in the left organs; generally embryos and fetuses were symmetrically distributed in each uterus, although in some specimens they were more numerous in the left uterus.

Key words - Elasmobranchii, squalidae, MED, tunisian coasts, coast of Languedoc, reproductive biology.

# **INTRODUCTION**

The velvet belly, *Etmopterus spinax*, is recorded off the coasts of Iceland, Scandinavia (Muus and Dahlstrøm 1964-1966), the British Isles (Wheeler 1969), and off the Eastern Atlantic from Belgium (Poll 1947) south the Straits of Gibraltar, Morocco (Collignon and Aloncle 1972), Mauritania (Maurin and Bonnet 1970) to the Gulf of Guinea (Blache et al. 1970).

*E. spinax* also occurs in the two Mediterranean basins (Fisher et al. 1987; (Fredj and Maurin 1987); (Mc Eachran and Branstetter 1986); (Golani 1986).

Hickling (1963) described some aspects the reproductive biology of specimens from the Northern Sea.

In contrast, little is known about the *E. spinax* from the Mediterranean. A limited amount of data was summarized by Tortonese (1956) and Bini (1967). In this paper, we present additional data concerning size at birth, size at sexual maturity, the reproductive cycle and fecundity of specimens from the Tunisian coasts (central Mediterranean) and the coast of Languedoc (southern France) with regard to specimens from the coast of Senegal.

# MATERIALS AND METHODS

Three hundreds and twenty nine specimens of *E. spinax* were collected by bottom trawls in two distincts fishing areas: off northern Tunisian coasts (central Mediterranean) and off the coast of Languedoc (Southern France). These fishing areas are presented in Fig. 1. The distribution of the *E. spinax* captures is summarized in Table I. In addition, 33 embryos and 24 fetuses and two specimens from off the coast of Senegal were also observed.

Embryos still had an umbilical stalk and an external yolksac. In full developed fetuses, the umbilical stalk and yolksac were totally reabsorbed and a scar marked the site of the umbilical stalk. These fetuses possessed a conspicuous internal vitellin vesicle.

Measurements to the nearest millimetre included total length (TL, mm) and claspers-length (CL). TL was measured according to method proposed by Compagno (1984), whilst the CL were measured from the forward rim of the pelvic girdle to the tip of claspers following Collenot (1969). We have counted the developing oocytes while still in the ovaries. We have removed the ripe oocytes ready to be ovulated from the ovaries, the embryos and fetuses from the uteri. Then, these features were counted, measured and weighed.

Males and females were studied separately.

The CL to TL relationship was used to determine the onset of sexual maturity of males. The linear regression equation was expressed in logarithmic coordinates. According to BASS et al. (1975) and to Stevens and Lyle (1989), the claspers of juveniles are short and flexible, and grow in relation to shark TL. In adult males, the claspers were elongated, larger than the pelvic fin, rigid and their cartilages were calcified. These features were verified in velvet belly to avoid underestimation of size of maturity.

Size at sexual maturity of females was determined from the condition of the ovaries and the morphology of the reproductive tract. Three classes of specimens were distinguished: juveniles having small whitish ovaries with oocytes of microscopic size, membranous oviducts and inconspicuous nidamental glands; subadults showing verrucous ovaries with translucent oocytes 2 mm to 3.5 mm in maximum diameter, some of them beginning to vitellogenetic products, accumulate and а differentiated genital tract; adults possessing two functional ovaries with yellow yolked oocytes from 24 to 27 mm in diameter and from 3.1 to 4.5 g in weight and a fully developed genital duct.

To investigate embryonic development and the role of the mother during gestation, the chemical balance of development (CBD) was determined. CBD is based on the mean dry weights of fertilized eggs and fully developed fetuses. CBD is the mean dry weight of fully developed fetuses divided by the mean dry weight of fertilized eggs. Based on chemical analyses of the lesser spotted dogfish, *Scyliorhinus canicula*, water content of 50 % in fertilized eggs and 75 % in recently pupped individuals can be taken as standard values (Mellinger and Wrisez 1989). CBD is a tentative estimate.

Correlations were assessed by least-squares regression.

# RESULTS

# Geographic distribution and habitat

Along the Tunisian shore, *E. spinax* is only caught off northern coasts, from the Algerian frontier to the Bank of Esquerquis, (Fig. 1 A), between 150-200 m and 400 m, this latter depth could be considered as a maximum (Quignard and Capape 1971). Two other sharks are found in the same areas, the oviparous blackmouth catshark, *Galeus melastomus* and the viviparous gulper shark, *Centrophorus granulosus*. They were generally captured together during bottom trawlings on areas rather restricted in the Bank of Esquerquis (Capapé, 1989).

The velvet bellies were caught off the coast of Languedoc in two distinct areas (Fig. 1B). In area 1 (80-150 m), the smallspotted catshark, *S canicula* is the most abundant elasmobranch, whereas in area 2, (100 m and 200 m) depth, it is the blackmouth catshark, *G. melastomus*, which is the most abundant.

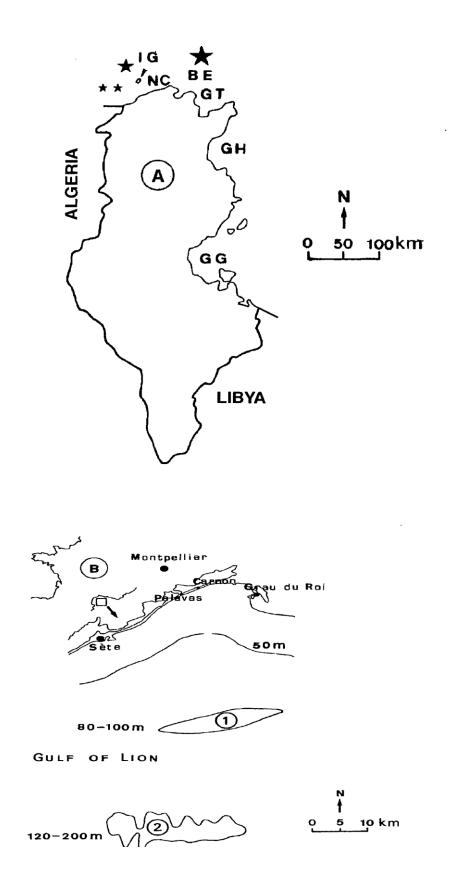


FIG. 1. A. Fishing-areas (black stars) of *Etmopterus spinax* off northern Tunisian coasts (NC). BE: Bank of Esquerquis, GG: Gulf of Gabes, GH: Gulf of Hammammet, GT: Gulf of Tunis, IG: Island of La Galite.
B. Fishing-areas of *Etmopterus spinax* in the Gulf of Lion. 1 and 2 surrounded with a circle indicate respectively the areas where *Scyliorhinus canicula* and *Galeus melastomus* are the dominant elasmobranch species.

# Size at sexual maturity *Males*

In E. spinax, as in other elasmobranchs, there are apparently no seasonal variations in clasper length (Mellinger, 1989). The CL to TL relationship (Fig. 2) shows two inflexions indicating the three stages of sexual development in males. The first stage includes the juveniles and the relationship is: log  $[CL] = 1.416 \log TL - 1.978, n = 27, r = 0.987.$  The second stage concerns the subadults with  $\log [CL] =$ 2.381 log TL - 4.436, n = 16, r = 0.973. The third phase concerns the adults, with  $\log [CL] = 0.625 \log$ TL + 0.067, n = 19, r = 0.968. According to these relations, the claspers grew fastest during the second stage. Juveniles and adults had short, uncalcified and flexible claspers. Those of adults were elongated, calcified, rigid and functional. The third relation shows that they grew allometrically throughout the life. All the males having over 350 mm TL were adult.

## Females

As in males, three categories of females were distinguished (Table II). Females begin sexual maturation at 310 mm TL. The first mature female with ripe oocytes and the first female gravid were 380 mm TL and 400 mm TL respectively. All the female adults were over 380 mm TL. Among the adults, 50 were non-pregnant with ripe oocytes, 8 were pregnant. Among these latter, 4 had developing embryos and 4 other fully developed fetuses in their uteri

# Reproduction

*E. spinax* is an aplacental viviparous species with two uteri and both left and right ovaries are functionnal.

Table III summarizes data concerning the adult females.

Females with ripe oocytes ready to be ovulated were observed in August and in October; their uteri were in a resting phase.

Females with embryos at different stages of their development were caught from April to October; their ovaries had attretic oocytes.

Females with fully developed fetuses occured in September and in October. Among these latter, a single specimen exhibited developing oocytes, their diameter ranged from 10 to 12 mm; the ovaries of the other females showed attretic oocytes.

Table I. Distribution of male and female free-living *E. spinax* caught from off the Tunisian northern coasts and in the coast of Languedoc (southern France).

Area	Tunisian coasts			coast of L	coast of Languedoc		General
Sex Category	Males	Females	Total	Males	Females	Total	Total
Juveniles	38	45	83	14	13	27	110
Subadults	46	55	101	7	8	15	116
Adults	37	44	81	8	14	22	103
Total	121	144	265	29	35	64	329

Table II. Distinctive features and sizes (TL, mm) in three categories of female Etmopterus spinax.

Category	n	Oocytes	Oviducts	Range (TL, mm)
Juveniles	58	Microscopic	membraneous	160-300
Subadults	63	< 5 mm	fully-developed	310-370
Adults	58	24 - 27 mm	fully-developed	> 380

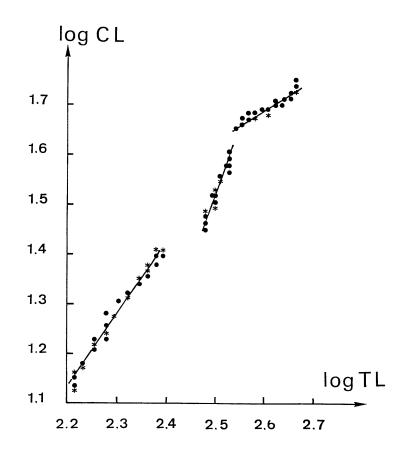


FIG. 2. Clasper length (CL) *versus* total length (TL) in male *E. spinax*, expressed in decimal logarithmic co-ordinates. Black circles and asterisks indicate the specimens caught off the Tunisian coasts and in the Gulf of Lion respectively.

### Size and weight at birth

Fully developed fetuses ranged measured119 mm-133 mm TL (mean 126, s.e.m. 0.61); their weight ranged from 5.3 g to 6.7 g (mean 6.03, s.e.m. 0.46). The smallest free-living specimens measured 112-126 mm (mean 117.50, s.e.m. 0.45) and weighed 5.3 g to 6.8 g (mean 6.05, s.e.m. 0.42) respectively.

### Chemical balance of development

The average weights of ripe oocytes and of full developed fetuses were 3.75 g and 6.05 g respectively (Table IV). The computed CBD, based on dry weights calculated for *E. spinax* was 0.803.

### Fecundity

Ovarian fecundity based on number of oocytes

counted in 24 females ranged from 9 to 17 (mean 13.54, s.e.m. 2.65). This type of fecundity is higher than uterin fecundity based on number of embryos and/or fetuses counted in 8 females which ranged from 5 to 9 (mean 6.83, s.e.m. 1.60). Ovarian fecundity increased with size of females and it is correlated with females TL, according to regression

equation: number of oocytes = 0.70 TL - 16.209; n: 24; r = 0.673. This is not the case for uterine fecundity.

## DISCUSSION

*E. spinax* is a little shark with a typical Atlantomediterranean distribution. It lives on continental shelves and penetrates to about 2000 m (Compagno 1984; Mc Eachran and Branstetter 1984). Off Tunisian northern coasts and in the Gulf of Lion, it inhabits with other sharks especially *G. melastomus*. These sharks may be slow moving and sedentary, suggesting that interspecific competition for food may occur.

Sexual maturity is attained at a smaller size by males (350 mm) than females (380 mm) and we recorded a maximal size of 460 mm for both sexes in this study. Similar observations observations have been reported by Hickling (1963) and Wheeler (1969) for specimens caught from off the British Isles. Both

authors recorded maturity at a smaller size in males (330 mm) and females (360 mm). Hickling (1963) noted that most of the velvet bellies in commercial

samples were between 350 and 420 mm and the largest 520 mm. Moreover, two

Table III. Classification of female *Etmopterus spinax* according to their sexual condition.

Month of catch	Category	TL (mm)	n	Oocytes	Uterine contents
May	Juveniles	110-160	11	microscopic	-
Aug	Juveniles	160-300	15	microscopic	-
Sep	Juveniles	120-200	11	microscopic	-
Nov	Juveniles	190-270	18	microscopic	-
Dec	Juveniles	170-290	3	microscopic	-
Aug	Subadults	310-370	9	microscopic	-
Sep	Subadults	300-320	3	translucide	-
Nov	Subadults	300-330	33	translucide	
Dec	Subadults	300-360	16	translucide	-
Jan	Subadults	330	2	translucide	-
Aug	Adults	380-460	18	ripe	-
Nov	Adults	390-460	32	ripe	-
Apr	Adult	400	1	translucide	embryos
May	Adult	420	1	translucide	embryos
Aug	Adult	430	1	translucide	embryos
October	Adult	420	1	translucide	embryos
September	Adult	400	1	developing	fetuses
September	Adult	410	1	degenerating	fetuses
October	Adult	410-430	2	degenerating	fetuses

Table IV. Fresh weights (g) of ripe oocytes and of full developed fetuses in gravid female *Etmopterus spinax*.

Features	n	Range	Mean	SEM
Ripe oocytes	108	3.1-4.4	3.75	0.35
Fetuses	24	5.3-6.8	6.05	0.42

adult males from the Bay of Biscay (Eastern Atlantic) measured 350 mm and 360 mm (Capapé unpublish. data). An adult male of 380 mm and an adult female of 420 mm have been captured off the

coast of Senegal (Capapé et al. 2001).

Our data on fully developed fetuses and on smallest free living *E. spinax* allow us to state that size at birth occured between 112 and 133 mm TL, weight at birth was between 5.3 and 6.8 g. These data agree

with Hickling (1963) for specimens from the British Isles and Relini-Orsi and Wurtz (1977)

for those from the Ligurian Sea (Italy).

All these observations do not show important intraspecific variations in size at sexual maturity, maximal size and size at birth between *E. spinax* from the Mediterranean and the Eastern Atlantic whatever the areas.

This is not the case among oviparous elasmobranchs species, such as the smallspotted catshark, *S. canicula*, it appears that the Mediterranean specimens are smaller than the Atlantic's ones

because they mature at a smaller size (Mellinger 1966, 1989); (Collenot 1969); (Capapé et al. 1991). Leloup and Olivereau (1951) stated that these intraspecific variations are related to environmental influences, especially light and temperarure, on growth. In contrast, Dodd (1983) added: 'Nothing is known of environmental influences on reproduction in viviparous species; however, the precision of the timing of their annual and biennal cycles indicates that such influences may play an important role in reproduction.'. These influences are unknown on deep sea viviparous sharks as *E. spinax* and related species, but also in pelagic viviparous sharks (Mellinger 1989).

Hickling (1963) wrote: There is not evidence that the period of gestation extends more than a year, as is the case with the much larger Spur-Dog *Squalus acanthias*.' In the Bay of Naples, Lo Bianco (1909) observed, at the same period of the year, two females, the first with fertilized eggs, the second with fully-developed fetuses. Other authors (Tortonese 1956); (Wheeler 1969) noted that the reproductive cycle of the velvet belly continues probably one year. However a two year gestation period cannot be excluded. It is generally the case in other squalids: *Squalus acanthias* from off British waters (Holden and Meadows 1964); (Jensen 1966), *S. blainvillei* and *C. granulosus* from off the Tunisian coasts (Quignard 1971); (Capapé 1985).

Squalids and other viviparous elasmobranchs exhibit a vitellogenesis proceeding simultaneously with gestation. Therefore, the next batch of oocytes is practically ready to be ovulated at the time of parturition (Dodd 1983); (mellinger 1989). However, in E. spinax from off the British coasts, Hickling (1963) reported that during pregnancy the ovary returned to the inactive state in which only small eggs were visible in the ovary. This phenomenon was also observed in other squalid species as Centroscymnus spp. according to Yano and Tanaka (1988), C. granulosus and O. centrina from the Mediterranean coasts (Capapé 1985); (Capapé et al. 1999) and also in torpedinids (Ouignard and Capapé 1974); (Mellinger 1981). This is also the case for Mediterranean velvet bellies. The female E. spinax produce several batches of oocytes, among these batches one only gives ripe oocytes which are ovulated, the other degenerate. The pregnant female with developing oocytes could be an uncommon phenomenon. However, it was also observed in some specimens of torpedinids caught off the coast of Languedoc (Capapé, unpublish. data).

The total duration of vitellogenesis is unknown in *E. spinax*. Therefore, its reproductive cycle period remains difficult to delineate. Gestation period lasts a maximum of two years as in other squalids. A long gestation period is rather the case in lecithotrophic species. Wourms et al. (1988) emphazised that in these species transfer of stored nutriments from

vitellin vesicle to embryos generally requiers a long period.

The CBD of the velvet belly, 0.803, has a relative low value. However, it is a little higher than *C. granulosus* and squatinids where Ranzi (1932) and Capapé et al. (1990) have calculated 0.5, and than *Torpedo* (0.8 according to Ranzi, 1932). Thus this confirms that *E. spinax* is a lecithotrophic species according to the definition of Wourms (1981). The contribution of mother during embryonic development seems to be not negligible, even if it is as important as in the gulper shark according to the recent observations of Guallart and Vicent (2001).

Fertilized eggs have never been found, and the embryos observed are always free in the uteri. The egg-capsule probably lack in *E. spinax* as in the other squalid species cited above.

Risso (1826) cited that E. spinax caught from off Nice which give birth in summer from 15 to 20 newborns per litter. Lo Bianco (1909) counted 4 embryos per uterus in females from the Bay of Naples. Hickling (1963) reported 14 ripe oocytes, 12 fertilized eggs and 6 developing embryos in females from off the British coasts. Compagno (1984) wrote that E. spinax fecundity ranged from 6 to 20 newborns per breeding act. The velvet bellies from off the Tunisian coasts had a fecundity per litter ranged between 5 and 9. Our observations are closely related to those previously reported. Uterine fecundity is probably underestimated because some individuals lose their brood during trawlings. The ovarian fecundity is higher than uterine fecundity It is probably overestimated because some ripe oocytes are not ovulated and became atretic. However, *E. spinax* is not a prolific elasmobranch as other related species of genus Squalus (Dodd 1983); (Mellinger 1989) and of genus Centrophorus. The gulper shark gives only birth to one newborn every two years (Capapé 1985).

In the majority of squaloid sharks, both ovaries and uteri are functional (Borcea 1906); (Mellinger 1989). However, in genus *Centrophorus*, only the right side is functional (Ranzi 1934). In genus *Squalus* and *E. spinax*, the left side is functional but less developed than the right (Holden and Meadows 1964). Mellinger (1971) stated that this morphologic characteristic is related to development of liver lobes. This could explain the disparity in numbers of oocytes in ovaries, of embryos or fetuses in uteri according to side.

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