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C. Capapé, O. Guélorget, Y. Vergne, J. P. Quignard. Reproductive biology of the common eagle ray, Myliobatis aquila (Chondrichthyes: Myliobatidae) from the coast cf Languedoc (Southern France, northern Mediterranean). Vie et Milieu / Life & Environment, 2007, pp.125-130. hal-03233955

## HAL Id: hal-03233955 https://hal.sorbonne-universite.fr/hal-03233955v1

Submitted on 25 May 2021

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### REPRODUCTIVE BIOLOGY OF THE COMMON EAGLE RAY MYLIOBATIS AQUILA (CHONDRICHTHYES: MYLIOBATIDAE) FROM THE COAST OF LANGUEDOC (SOUTHERN FRANCE, NORTHERN MEDITERRANEAN)

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CHONDRICHTHYES MYLIOBATIDAE MYLIOBATIS AQUILA REPRODUCTIVE BIOLOGY COAST OF LANGUEDOC MEDITERRANEAN SEA ABSTRACT. – Investigations conducted off the coast of Languedoc (southern France, northern Mediterranean) during 14 years show that common eagle rays *Myliobatis aquila* (Linnaeus, 1758) were caught especially from August to October. The smallest male and female adults were 500 mm and 730 mm disc width (DW), respectively, and weighed 2000 g and 6100 g. The largest male and the largest female were 720 mm and 1140 mm DW, respectively and weighed 5250 g and 29400 g, respectively. There was a significant relationship of total mass versus DW between males and females. Diameter of the largest yolky oocytes ranged from 26 to 32 mm, number of yolky oocytes counted in five females ranged between 8 and 10. Fertilized eggs were enveloped in a diaphanous capsule. Each capsule containing six eggs weighing between 4.5 and 5.3 g (mean:  $4.9 \pm 0.9$ ). Breeding period probably occurred from August to September. The common eagle ray probably reproduced in alternate year, and embryonic development did not exceed one year. Ovarian fecundity and uterine fecundity were rather low, both between 8 and 12.

#### INTRODUCTION

The common eagle ray, Myliobatis aquila (Linnaeus, 1758) presents a large distribution in the Atlantic Ocean and the Mediterranean Sea (Mc Eachran & Capapé 1984). Along northeastern Atlantic shore, the species was reported off Scandinavia (Muus & Dahlstrøm 1964-1966), British Islands (Wheeler 1969), France (Bougis 1959) and Portugal (Albuquerque 1954-1956). South the Strait of Gibraltar, M. aquila occurred off the Atlantic coast of Morocco (Collignon & Aloncle 1972) and Mauritania (Maurin & Bonnet 1970). M. aquila was also recorded off Senegal (Cadenat 1951); in contrast, recent observations did not allow to report its occurrence in the area (Capapé et al. 1995) where the closely related species, the bull ray, Pteromylaeus bovina, is commonly caught (Seck et al. 2002). Fowler (1936) noted that M. aquila inhabits the western coast of Africa until Angola (Smith & Heemstra (1986).

The common eagle ray occurred in the Mediterranean but captures seem to be rather rare (see Capapé 1989). The species was reported in several areas off the southern coast of France (Moreau 1881, Euzet 1960, Quignard *et al.* 1962, Granier 1964, Capapé 1977)

Previously, data on morphology and reproductive biology (Capapé & Quignard 1974, Capapé & Zahnd 1975) and diet (Capapé 1976) were known of specimens from the Tunisian coast and the Adriatic Sea (Jardas *et al.* 2004). Investigations conducted close to 14 years off the coast of Languedoc (Mediterranean shore of France) allow us to collect specimens of *M. aquila* and to provide additional data on some traits of its reproductive biology, such as size at sexual maturity, reproductive cycle and fecundity.

#### MATERIAL AND METHODS

The observed specimens were captured by demersal gill-nets at depths from 30 to 40 m, between Sète and Le Grau-du-Roi, from 1990 to 2004.

Disc width (DW) of the specimens was measured to the near-



Fig. 1. – Map of France pointing out the coast of Languedoc and the captures sites (stars) of *Myliobatis aquila* (redrawn from Capapé *et al.* 2000).

est millimetre following Clark (1926) and mass (TM) to the nearest gram, liver, gonads and the masses of oviducal glands to the nearest decigram. Developing and yolky oocytes, egg cases were measured to the nearest millimetre and their masses to the nearest decigram, when possible.

Clasper length was measured from the forward rim of the pelvic girdle to the tip of the clasper following Collenot (1969).

The onset of sexual maturity was determined in males from the condition and the length of claspers following Bass *et al.* (1973), Stevens & Mc Loughlin (1991) and Watson & Smale (1998).

In both males and females, specimens were divided in three categories: juveniles, sub-adults and adults.

The claspers of juveniles are shorter than pelvic fin length, flexible and not calcified, those of sub-adults are greater than pelvic fin length, flexible but slightly calcified. In adults, claspers are elongated, longer than pelvic fins length, rigid and calcified. Some aspects of the testes and other reproductive organs are given following Capapé *et al.* (1990) and Bridge *et al.* (1998), Hamlett *et al.* (1999) and Callard *et al.* (2005). Concomitantly, males developed supra-orbitary tubercles as they grew. Capapé & Quignard (1974) described similar patterns in *M. aquila* from the Tunisian coast.

Size at sexual maturity was determined in females from the condition of ovaries and the morphology of the reproductive tract (Capapé *et al.* 1990).

To investigate the embryonic development and the role of the mother during gestation, a chemical balance of development (CBD) was determined. It is based on the mean dry mass of fertilized eggs and fully developed embryos and can be computed as the mean dry mass of fully developed embryos divided by the mean dry mass of yolked oocytes or eggs. Water content of 50% in ripe oocytes and 75% in fully developed embryos were standard values, based on chemical analyses of the small spotted catshark *Scyliorhinus canicula*, by Mellinger & Wrisez (1989). The chemical balance of development is a tentative estimate of the degree of nutriotional support provided by the mother aside yolk reserves.



Fig. 2. – Clasper length (CL) vs Disc Width (DW) in male Myliobatis aquila.

Tests for significance (p < 0.05) were performed using ANOVA, Student t-test and the chi-square test. The linear regression was expressed in decimal logarithmic coordinates. Correlations were assessed by least-square regression. In the relationship mass versus total length, comparisons of curves were carried out by ANCOVA.

#### RESULTS

In all, 73 common eagle rays were observed, 41 were males and 32 females. The monthly collection of the observed specimens is summarized in Table 1.

#### Size at sexual maturity

#### Males

Juvenile stage comprised 16 specimens, the disc width ranged between 210 and 490 mm and they weighed between 166 and 1495 g. Juveniles were generally caught between August and October, one specimen was caught in March, one in May (see Table I). Testes and genital ducts were thread-like and inconspicuously developed. The surface of the orbits was entirely smooth in all specimens.

Eight sub-adults were collected, seven between August and October and a single specimen in May (see Table I). The smallest sub-adult observed was 460 mm DW and weighed 1600 g, the largest 520 mm DW and weighed 1780 g. The testes had not spermatocysts externally visible, no sperm was observed in the seminal vesicles. The superior surface of the orbit exhibited a conspicuous protuberance.

In adult males, the claspers were elongated, calcified and rigid. They were larger than the pelvic fins. The testes were well developed and had spermatocysts externally visible. The genital duct was twisted and sperm was observed in the seminal vesicles. The smallest adult male was 500 mm DW and weighed 2000 g, the largest was 720 mm DW and weighed 5250 g, while the heaviest specimen weighed 5460 g and was 710 mm DW. All males above 540 mm DW were adult. Seventeen adult males were collected, 2 in March, 4 in May and 11 from August to October (Table I). The adults had cone shaped supra-orbitary tubercles that were well developed.

#### Females

Juvenile females ranged between 240 and 470 mm DW and weighed between 190 and 1405 g. They had whitish and membranous ovaries, thread-like oviducts and inconspicuous oviducal glands. Sixteen juveniles were collected, fourteen from August to October, one in March and one in May (see Table I).

Eight sub-adults were captured, 2 specimens in May and 6 from August to October. They exhibited white

|         |             | Months |      |      |      |     |      |      |      |      |      |      |      |       |
|---------|-------------|--------|------|------|------|-----|------|------|------|------|------|------|------|-------|
| Sex     | Category    | Jan.   | Feb. | Mar. | Apr. | May | Jun. | Jul. | Aug. | Sep. | Oct. | Nov. | Dec. | Total |
| Males   |             |        |      |      |      |     |      |      |      |      |      |      |      |       |
|         | Juveniles   | -      | -    | 1    | -    | 1   | -    | -    | 5    | 6    | 4    | -    | -    | 16    |
|         | Sub-adults  | -      | -    | -    | -    | 1   | -    | -    | 2    | 4    | 1    | -    | -    | 8     |
|         | Adults      | -      | -    | 2    | -    | 4   | -    | -    | 3    | 2    | 6    | -    | -    | 17    |
|         | Total       | -      | -    | 3    | -    | 6   | -    | -    | 10   | 12   | 11   | -    | -    | 41    |
| Females |             |        |      |      |      |     |      |      |      |      |      |      |      |       |
|         | Juveniles   | -      | -    | 1    | -    | -   | -    | -    | 2    | 11   | 2    | -    | -    | 16    |
|         | Sub-adults  | -      | -    | 2    | -    | -   | -    | -    | 2    | 1    | 3    | -    | -    | 8     |
|         | Adults      | 1      | -    | 1    | -    | -   | -    | -    | 2    | 3    | 1    | -    | -    | 8     |
|         | Total       | 1      | -    | 4    | -    | 6   | -    | -    | 6    | 15   | 6    | -    | -    | 32    |
|         | Grand total | 2      | -    | 7    | -    | 12  | -    | -    | 16   | 27   | 17   | -    | -    | 73    |

Table I. – Monthly collection of the observed Myliobatis aquila captured off the coast of Languedoc.

translucent follicles and a well-differentiated genital duct. The oviducal glands were visible and slightly rounded. The smallest sub-adult was 360 mm DW and weighed 862 g; the largest specimen was 700 mm DW and weighed 4850 g.

Eight adults were collected, a single specimen in May and seven from August to October (see Table I). They had both ovaries and uteri functional. Ovaries contained batches of yolky oocytes and the genital ducts were fully developed. The smallest adult was 730 mm DW and weighed 6100 g; the largest was 1140 mm and weighed 29400 g.

#### **Reproductive status of females**

Two females observed in January and March had developing oocytes in both ovaries, their uteri were thick, musculous and contained uterine fluid probably secreted by short villi which covered the internal wall. The five females caught in August and September developed batches of oocytes similar in size and mass, a batch comprised yolky oocytes which were between 26 and 32 mm in diameter, both uteri were enlarged and contained uterine fluid, the uterine villi were well-developed and formed the trophonemata which plays an important role during gestation in stingrays (see Hamlett et al. 2005). The number of yolky oocytes counted in these five females ranged from 8 to 10. The female captured in October contained fertilized eggs enveloped by a diaphanous and yellowish capsule ending by a short filament on both sides (Fig. 3). Each capsule contained six eggs weighing between 4.5 and 5.3 g (mean:  $4.9 \pm 0.3$ ). The ovaries of the female exhibited batches of atretic oocytes. From May to September, of the 12 smallest freeswimming specimens, 8 were females and 4 males. They ranged between 210 and 290 mm DW (mean: 265.8  $\pm$ 

25.4) and weighed between 166 and 375 g (mean: 276.5  $\pm$  60.1). They exhibited an unhealed scar on their ventral surface. They probably were neonates. This suggests that gestation lasted one year maximum.

#### Size and mass relationships

The relationships between DW and total mass (TM) showed significant differences between males and females (F = 7.9: p = 0.006). The relationships were: for males log TM =  $3.0 \log DW - 4.8$ ; r = 0.98; n = 41 and for females: log TM =  $3.3 \log DW - 5.6$ ; r = 0.98; n = 32.

The relationships between DW and liver mass (LM) significantly differed between males and females



Fig. 3. – Egg-case containing fertilized eggs in *Myliobatis* aquila (white bar = 50 mm).

| Category   | Males | Females | Males:<br>Females |  |  |
|------------|-------|---------|-------------------|--|--|
| Juveniles  | 16    | 16      | 1/1               |  |  |
| Sub-adults | 8     | 8       | 1/1               |  |  |
| Adults     | 17    | 8       | 2.2/1             |  |  |
| Total      | 41    | 32      | 1.3/1             |  |  |

Table II. – *Myliobatis aquila* sex ratio for each category of specimens and for the total sample.

(F = 5.5: p = 0.02). The relationships for males were log LM =  $3.2 \log DW - 6.6$ ; r = 0.95; n = 41 and for females log LM =  $3.7 \log DW - 7.8$ ; r = 0.99; n = 32.

Mean fresh masses of twelve encapsuled eggs and of twelve neonate free-swimming specimens were 4.9 g ( $\pm$  0.3) and 276.5 ( $\pm$  60.1) respectively. CBD based on mean dry masses for *M. aquila* was 28.

#### Sex-ratio

Both juvenile and sub-adult males and females were equally distributed in the sample (Table II), while adult males significantly outnumbered the female ones, and in the total sample male outnumbered females, however these differences were not significant.

#### DISCUSSION

*Myliobatis aquila* was mostly captured between August and October off the Languedocian coast. This phenomenon was probably due to the fact that pregnant females approached inshore waters in order to find favourable environmental conditions to expelle the near term embryos. However, at present, our data are still insufficient to state whether the Languedocian shore is a specialized area for elasmobranch species (*sensu* Castro, 1993). However, sampling could not be totally excluded: at this period of the year, fishermen targeted areas where *M. aquila* occurred in order to find preys and to reproduce.

Off the coast of Languedoc, size at sexual maturity was reached by males between 500 and 540 mm DW, and by females at 730 mm DW. The largest male and the largest female were 720 and 1140 mm DW, respectively. Sexual dimorphism in size was also observed among *M. aquila* from the Tunisian coast (Capapé & Quignard 1974). Nevertheless, in this latter area both males and females matured at a smaller size than off the coast of Languedoc. Tunisian male matured between 360 and 410 mm DW, and females between 480 and 580 mm DW. The largest Tunisian male was 550 mm DW and the largest female 830 mm DW, respectively. Common eagle

rays from the coast of Languedoc were larger than those caught off the Tunisian coast. This suggests that two different populations occurred in each area. Difference in size related to environmental parameters was described for oviparous species (Leloup & Olivereau 1951), but this hypothesis needs further observation with special regards to viviparous species (Dodd 1983, Capapé *et al.* 2001). Seck *et al.* (2002) noted that Pteromylaeus bovinus from the coast of Senegal were smaller than specimens from the Tunisian coast (Capapé & Quignard 1975).

Development of supra-orbitary tubercles were similar from both areas, such as for pre-orbitary tubercles described by Capapé & Quignard (1975) and Seck *et al.* (2002) in *P. bovinus* from both Tunisian and Senegalese coasts.

Captures of adult females and neonates between August and October suggests that breeding period occurred at this period of the year such as for Tunisian *M. aquila* (see Capapé & Quignard 1974). In Italian seas, parturition occurred in November (Lo Bianco 1909) and breeding period from September to February (Tortonese 1956).

These observations suggest that embryonic development lasted at least eleven-twelve months off the coast of Languedoc. Capapé & Quignard (1974) did not provide information on reproductive cycle from Tunisian *M. aquila*. Our sample did not allow us to state if vitellogenesis proceeded in parallel with embryonic development or if it was delayed such as in *P. bovinus* from both Tunisian and Senegalese coasts (Capapé & Quignard 1975, Seck *et al.* 2002).

CBD of 28 calculated for *M. aquila* is slightly lower than CBD of 31 calculated for P. bovinus from the coast of Senegal (Seck et al. 2002), but these values confirmed the role of mother during gestation and they were previously defined as matrotrophic species (sensu Wourms 1977, 1981, Wourms et al. 1988). Recent investigations (see Hamlett et al. 2005) pointed out the role of trophonemata having cells abundantly rich in lipid secretions and 'the term of uterolactation was introduced to refer a production of lipid rich uterine histotroph' according to Hamlett et al. 1996. So, elasmobranch species developing a trophonemata during gestation such as dasyatids and myliobatids are defined as histotrophic species sensu Hamlett et al. (2005). Relationship liver mass vs total mass showed significant differences between males and females, this suggests that liver play an important role in reproductive life cycle of the latter. Rossouw (1987) noted: "Relative liver size was demonstrated by several authors to increase with total length and body weight". Moreover, Bone & Roberts (1969) and Baldridge (1970, 1972) showed the role of liver in buoyancy for some elasmobranch species, especially for both deep sea and pelagic elasmobranch species, such as Myliobatis aquila, in some period of its life. Rossouw (1987) and Braccini & Chiaramonte (2002) suggested 'that bottom dwelling elasmobranchs required little static lift and hence the liver would not play such a very significant role in buyoancy'. Liver accumulated hepatic lipids for metabolic functions such as gonadic products, this phenomenon is more evident in females during vitellogenesis.

Both ovarian and uterine fecundities were higher in *M. aquila* from the coast of Languedoc, 8-10, 16 and 12, respectively than in *M. aquila* from the Tunisian coast, 6-7 and 3-5, respectively, were higher than those of Tunisian *M. aquila*, 5-7 and 3-5, respectively; this may be due to the fact that specimens off the coast of Languedoc were larger than the Tunisian specimens.

Males and females were equally distributed in the sample, except for adults. However the sample is not sufficiently supported to state on distribution on the species in the area, it only shows a decline of *M. aquila* in the area.

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Received February 9, 2006 Accepted January 24, 2007