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New observations on the reproductive biology of the pelagic stingray, *Dasyatis violacea* Bonaparte, 1832 (Chondrichthyes: Dasyatidae) from the Mediterranean Sea

Farid HEMIDA [1], Rabea SERIDJI [1], Samira ENNAJAR [2]
Mohamed Nejmeddine BRADAÏ [2], Eric COLLIER [3], Olivier GUÉLORGET [3]
and Christian CAPAPÉ [3]

[1] ***Laboratoire Halieutique, Institut des Sciences de la Nature, Université des Sciences et Techniques Houari Boumedienne, B.P. 32, El Alia, 16111 Bab Ezzouar, Algiers, Algeria***
E-mail: fhemida@yahoo.fr
[2] ***Institut National des Sciences et Technologies de la Mer, Centre de Sfax, BP 1035, 3018 Sfax, Tunisia***
E-mail: mednejmeddine.bradai@instm.rnrt.tn
[3] ***Laboratoire d'Ichtyologie, Case 104, Université Montpellier II, Sciences et Techniques du Languedoc, Montpellier Cedex 05, France***
E-mail: capape@univ-montp2.fr

Captures of the pelagic stingray, Dasyatis violacea, *in three Mediterranean areas, Tunisian coast, Algerian coast and the southern coast of France allow to report about aspects of the reproductive biology of the species. Adult males and females were above 420 mm and 450 mm disk-width (DW), respectively, with the largest male and female being 580 mm DW and 5800 g, and 610 mm DW and 6000 g, respectively. Size at birth was 160-195 mm DW and 191-212 g. Females were not significantly heavier than males. The diameter of fully yolked oocytes ranged between 18-22 mm (mean 19.5±1.10 mm) and weighed 2.0-2.4 g (mean 2.1±0.12 g). Gestation lasted approximately four months. Two litters per year is a probable hypothesis but requires confirmation as embryonic diapause cannot be entirely excluded. A calculated chemical balance of development based on mean dry mass of fully yolked oocytes and of neonates is 47. This high value shows that* D. violacea *is a matrotrophic species. Ovarian fecundity, based on the number of ripe oocytes ready for ovulation, ranged from five to ten. Uterine fecundity, based on the number of eggs and/or embryos, ranged from two to seven. There was no relationship between size and either category of fecundity. Male and female embryos are equally distributed, but in the largest free-swimming specimens, females significantly outnumbered males.*

Key words: Chondrichthyes, Dasyatidae, *Dasyatis violacea*, reproductive biology, Mediterranean Sea

INTRODUCTION

The earliest reports of the pelagic stingray *Dasyatis violacea* were from the Mediterranean Sea. TORTONESE (1956, 1976) suggested that it was an endemic Mediterranean species. The opinion of Tortonese was subsequently revised with records outside the Mediterranean summarized by Mollet (2002) who wrote, "more recent work showed that this species is universal in subtropical and tropical seas, and also occurs at temperate latitudes."

The reproductive biology of *D. violacea* was based on observations of captive specimens (MOLLET *et al.*, 2002). In contrast, the reproductive biology of free-swimming specimens is sketchily known (MOLLET, 2002; MOLLET *et al.*, 2002). In Mediterranean waters, LO BIANCO (1909), RANZI (1932, 1934) and RANZI & ZEZZA (1936) recorded pregnant females in the Bay of Naples containing embryos at different stages of development. RANZI and ZEZZA (1936) suggested that gestation lasts two months and parturition occurs in August-September. RANZI (1932) and TORTONESE (1956, 1976) provided information on size at sexual maturity of males and females. CAPAPÉ (1975) reported the capture of an adult female containing developing oocytes off the northern Tunisian coast.

In this paper, new records of pelagic stingray from three different Mediterranean areas offer an opportunity to enlarge and improve our knowledge on the reproductive biology of *D. violacea* from the Mediterranean Sea.

MATERIAL AND METHODS

Eighty-one pelagic stingrays, including 32 males and 49 females, were observed. Thirty-two specimens were caught in Tunisian waters between 1970 and 2002, 28 off the Algerian coast between 2000 and 2002 and 21 off the southern coast of France between 1988 and 2002 (Fig. 1). The collection of the specimens by month is summarized in Table 1.

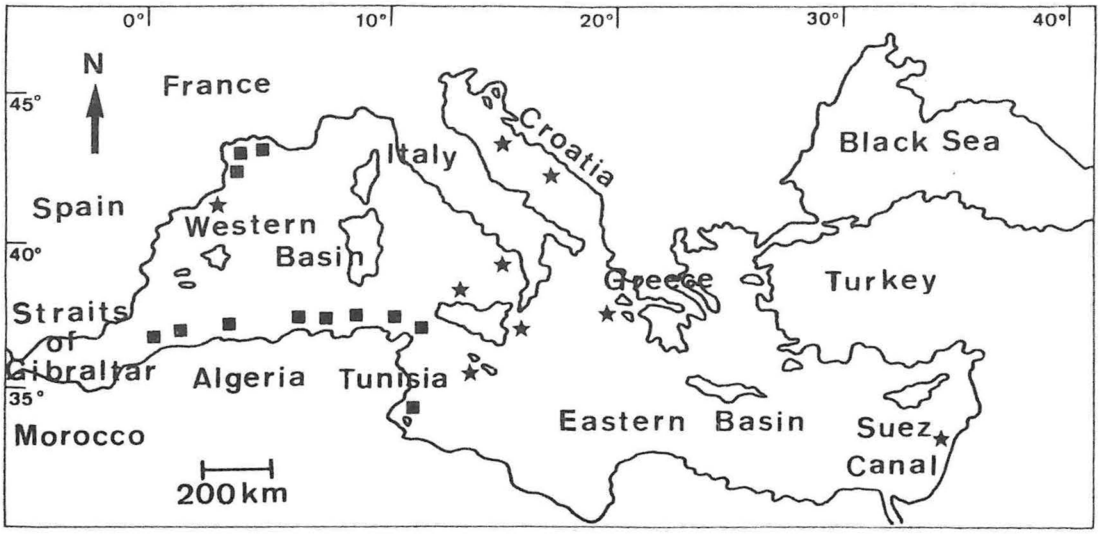

Fig. 1. Map of the Mediterranean Sea showing the fishing places (black squares) where one or more pelagic stingrays from the studied sample were captured

Table 1. Collection of Dasyatis violacea *specimens from three Mediterranean areas, by month*

Month / Area	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Algerian coast					5	1	22						28
Tunisian coast	2	1	3	2	4	5	2	5	3	1	1	3	32
French coast		3		3	2	3	1	1	4	1	2	1	21
Total	2	4	3	5	11	9	25	6	7	2	3	4	81

All the specimens from Algeria were captured by gill nets set on the surface to catch tuna. Specimens from Tunisia were caught by demersal trawl. Among the 21 specimens from the coast of France, 9 were caught by demersal trawling, 9 by surface-set gill nets and three by longlines.

The specimens were measured to the nearest millimetre for disk-width (DW) following CLARK (1926) for skates and to the nearest gram. Measurements also included clasper length (CL, mm) from the rim of the pelvic girdle to the tip of the clasper following COLLENOT (1969), diameter and mass of fully yolked oocytes, mass of eggs, and mass of embryos, which were sexed whenever possible. Fully yolked oocytes, encapsuled eggs and embryos were removed from the females and measured.

The onset of sexual maturity in males was determined from the CL:DW relationship following BASS *et al.* (1975). Males with rigid, elongated and fully calcified claspers were assumed to be mature. Two categories of males were distinguished: juveniles and adults.

The size of females at sexual maturity was determined by the condition of the ovaries and the morphology of the reproductive tract. Three categories of females were distinguished: juveniles, subadults and adults.

To investigate the embryonic development and role of the female parent during gestation, a chemical balance of development (CBD) was determined. CBD is based on the mean dry weight of fertilized eggs and new-born pups. CBD = mass of foetuses x (1.0 - 0.75) /mass of eggs x (1.0 - 0.5) are used to estimate CBD. As water content is assumed to be 50% in eggs and 75% in foetuses, based on chemical analyses of the small spotted catshark, *Scyliorhinus canicula*, by MELLINGER & WRISEZ (1989). CBD is a tentative estimate.

Tests for significance ($P < 0.05$) were performed by analysis of variance, STUDENT t test and c^2 test. Correlations were assessed by least-squares regression. The statistical difference between the log mass versus the log DW of males and females were checked by ANCOVA.

Whenever possible, water surface temperatures were taken at the fishing site from a depth of approximately 6-10 m, on the continental shelf.

RESULTS

Size at sexual maturity

Males

Male development was divided into two stages: juvenile and adult (Fig. 2). The juvenile stage included 11 males between 160 and 400 mm DW. Adults included 21 specimens ranging 420-580 mm DW and weighing 2100-5800 g. Two neonates of 160 and 190 mm DW had an internal vitellin vesicle and an unhealed scar.

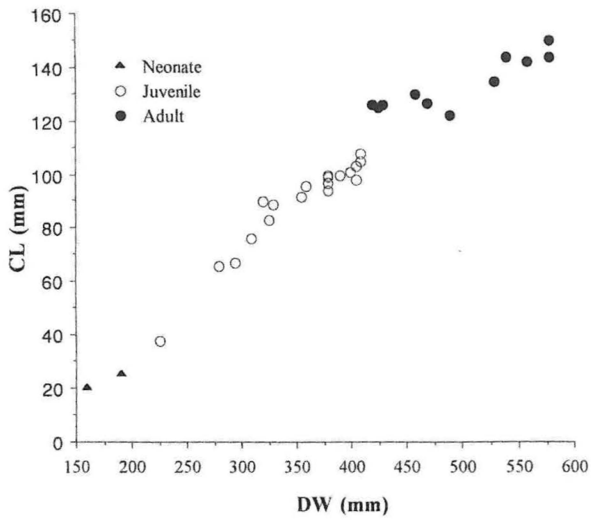

Fig. 2. Clasper-length (CL) vs disk-width (DW) in male Dasyatis violacea

Females

The females were divided into three categories: juvenile, subadult and adult. There were ten juveniles with a DW of 195-390 mm, whitish ovaries with microscopic oocytes and indistinct oviductal glands. The smallest juvenile was a neonate. Ten subadult females had a DW of 400-430 mm, translucent oocytes and a differentiated genital duct. The 29 adults ranged 450-610 mm DW and 2400-6000 g. They had functional ovaries with batches of developing and fully yolked oocytes. The smallest adult with fully yolked oocytes ready to be ovulated and the smallest gravid female were 450 mm DW. The uterus of the gravid female contained encapsuled eggs.

Reproduction

In juvenile females two ovaries were observed. Their mass was similar. In the adult specimens, only the right ovary was vestigial. However, in two females only, it persisted but produced fewer oocytes than the left one. In the adults, only the left uterus was functional. The ovary produced oocyte batches similar in size and weight. Only one of these batches developed into fully yolked oocytes, the other degenerated. Fourteen oocytes were measured. Their diameter ranged from 18 to 22 mm (mean 19.5±1.10 mm) and their mass from 2.0 to 2.4 g (mean 2.1±0.12 mm). Fertilized eggs were enveloped with a transparent and brownish capsule that ended with a short expansion on both sides. This egg case was fragile. Sometimes the eggs spilled when handled, therefore, it was difficult to measure their diameter although every egg was weighed if possible. The mass of 12 eggs ranged between 2.0 and 2.2 g (mean 2.1±0.08 g). The masses of fully yolked oocytes and eggs did not significantly differ.

Table 2 shows that ten females with encapsuled eggs in the uterus occurred from December to May, and one in September. Females with active vitellogenesis and embryos in different stages of development were caught between May and July, and between September and November. Oocytes grew with embryonic development. Embryos, 72-73 mm DW, in two females had an external vitelline vesicle that was almost resorbed and a conspicuous internal vitelline vesicle. An adult female was caught off Six-Fours-les-Plages (southern France), it expelled six new-born pups which were provided to us. Two free-swimming neonates were caught off the Tunisian coast in September and one in December.

Fig. 2. Clasper-length (CL) vs disk-width (DW) in male Dasyatis violacea

Observation no.	*Number of females*	*Month of catch*	*Fishing area*	*Surface water temperature*	*Ovarian activity*	*Diameter of oocytes (mm)*	*Uterine content*	Embryos DW (mm)
1	2	Jan	Tunisian coast	15°C	Resting	-	Egg cases	-
2	1	Feb	Tunisian coast	13°C	Resting	-	Egg cases	-
3	3	Mar	Tunisian coast	15°C	Resting	-	Egg cases	-
4	2	Apr	Tunisian coast	18°C	Resting	-	Egg cases	-
5	2	May	Tunisian coast	18.5°C	Resting	-	Egg cases	-
6	1	May	Tunisian coast	18.5°C	Vitellogenesis	10-12	Embryos	50-59
7	2	Jun	Tunisian coast	20°C	Vitellogenesis	15-16	?	-
8	1	Jul	Tunisian coast	22°C	Vitellogenesis	?	-	-
9	1	Jul	Tunisian coast	26°C	?	?	Uterine liquid	?
10	1	Aug.	French coast	22°C	?	?	Full-term embryos (?)	?
11	1	Sep.	Tunisian coast	22°C	Resting	-	Egg cases	-
12	1	Sep	Tunisian coast	22°C	Vitellogenesis	10-12	Embryos	?
13	1	Oct.	French coast	19°C	Vitellogenesis	20-24	-	-
14	1	Oct.	Tunisian coast	22°C	Vitellogenesis	21-22	-	-
15	1	Oct.	Tunisian coast	18°C	Vitellogenesis	?	Embryos	22-27
16	2	Nov.	Tunisian coast	18°C	Vitellogenesis	12-25	Embryos	72-73
17	1	Dec.	Tunisian coast	15°C	Resting	-	Eggs	-

Mass-growth and disk-width relationships

Observations of the smallest specimens, three newborn pups, suggest that birth in Mediterranean pelagic stingrays occurs at 160-195 mm (mean 181.6±18.9 mm) and 191-212 g (mean 201.3±10.5 g). Since the mean mass of the 14 largest ripe oocytes was 2.1±0.1 g, the CBD for *D. violacea* is 47.

The relationship between total mass (TM) and disk-width (DW) did not significantly differ between males and females. The relationships were for males: log TM = 2.552 log DW - 3.386; $r = 0.990$; $n = 17$ and for females: log TM = 2.828 log DW - 4.082; $r = 0.977$; $n = 27$.

Fecundity

Most of the developing oocyte batches were entering in degenerescence. Ovarian fecundity was, therefore, based on the number of fully yolked oocytes, ready for ovulation, counted in eight females ranging 460-600 mm DW. The number of such oocytes ranged from five to ten. There was no relationship between the size of the female and ovarian fecundity.

Uterine fecundity was based on the number of eggs and/or developing embryos in 14 females ranging 450-580 mm DW. The number of eggs and developing embryos ranged from two to seven. As with ovarian fecundity, there was no relationship between the female size and uterine fecundity.

Sex ratio

Among the sexed embryos, there were seven males and seven females (Table 3). Juvenile and subadult females were significantly more abundant than juvenile and subadult males. It is also the case for adults. Table 4 shows that among specimens less than 400 mm DW, males were significantly more abundant than females but in specimens over 400 mm DW, females were significantly more abundant than males, especially from 450 mm DW.

Table 3. Dasyatis violacea *sex ratio in each category of specimens and in the total sample*

Category	Number of females	Number of males	Ratio (F : M)
Embryos	7	7	1 : 1
Juveniles + Subadults	20	11	1.8: 1
Adults	29	21	2.6: 1
Total	56	39	1.4 : 1

Table 4. Size of male and female Dasyatis violacea *in the sample. Sex-ratio versus size ranges in the sample*

Size (DW, mm)	< 350	350-400	400-450	450-500	500-550	> 550	Total
Males	9	7	9	3	3	1	32
Females	7	3	10	12	8	9	49
Sex-ratio (F:M)	1/ 1.3	1./2.3	1.1/1	4/1	2.6/1	9/1	1.5/1

DISCUSSION AND CONCLUSION

Earlier and recent studies report that *D. violacea* occur most often in the western Mediterranean basin but seem to be restricted in some areas. However, captures of specimens of both sexes varying in size and females with embryos in varying stages of development show that the species develops and reproduces despite fishing pressure in these areas (BRADAÏ, 2000; HEMIDA & LABIDI, 2001; ORSI RELINI *et al.*, 2002).

Size at sexual maturity and maximum size slightly differ between males and females. Females are not significantly heavier than males. RANZI (1932) and TORTONESE (1976) reported that, in the Bay of Naples, males and females mature at 375 mm DW and 500 mm DW, respectively. WILSON & BECKETT (1970) reported that females off the western Atlantic begin to mature at 400-500 mm DW and grow to a larger size than males. MOLLET *et al.* (2002), referring to data from the California coast collected by NEER (in press), wrote: "All males (411-565 mm) were mature and it was difficult to determine the maturity of females with a size range of 410-753 mm."

Records of fully developed foetuses and neonates from the Tunisian coast show large variation in size and weight with a mean DW of 182 mm and mean mass of 201 g for newborn pups. In the Bay of Naples, the average mass of neonates was 175 g (LO BIANCO, 1909) and birth occurred at 128 mm DW and 118 g (RANZI 1932). MOLLET *et al.* (2002) reported records of new term litters in captivity with a DW range of 140-240 mm and mass range of 77-378 g.

Mean mass for Tunisian eggs, 2.08 g, was slightly heavier than reported by RANZI (1932) who calculated 1.9 g as the mean mass for eggs in specimens caught off Naples. For pelagic stingrays in captivity, MOLLET *et al.* (2002) reported egg masses of 1.2-1.4 g in one aborted egg case and a mean of 0.99 g in a second aborted egg case with 7 eggs. These changes could be due to the size of females. The egg mass increase with the females size in the common torpedo, *Torpedo torpedo*, from Tunisian waters (QUIGNARD & CAPAPÉ, 1974) and the Senegalese coast (CAPAPÉ *et al.*, 2000).

Ovarian activity in Table 2 shows that fertilized eggs did not develop and remained undamaged from December to May in *D. violacea*. The eggs might have been infertile and failed to develop. However, during this period, the temperature of the surface water was rather low, from 13 to 18°C maximum. This suggests an embryonic diapause. MOLLET *et al.* (2002) were aware of the possibility of a diapause in pelagic stingray in captivity, but also suggested storage of sperm for several months as another hypothesis. They consider that a diapause can occur if environmental conditions are unfavourable, which is not the case in captivity, where the tank water temperature is 20°C. An embryonic diapause was described for the first time by LESSA (1982) in *Rhinobatos horkelii* from Brazilian waters. It was not observed in *R. cemiculus* and *R. rhinobatos* from the Lagoon of Bibans, close to the Gulf of Gabès in southern Tunisian (CAPAPÉ & ZAOUALI, 1994; CAPAPÉ *et al.*, 1997) but it was suspected in *R. rhinobatos* from the coast of Senegal by CAPAPÉ *et al.* (1999).

SIMPFENDORFER (1992) reported a similar pattern for the Australian sharpnose shark, *Rhizoprionodon taylori*. He suggested that ova development was suppressed for several months and that embryonic development resumed when environmental conditions for gestation were favourable. SIMPFENDORFER (1992) cited reports of embryonic diapause for the stingray, *Dasyatis say*, from Florida. Embryonic diapause was not described in Tunisian *Dasyatis* spp.

Observations 6 and 10 in Table 2 suggest that gestation lasts four months, or five months when taking into account observation 6 and the neonates recorded in September. Gestation lasted approximately two months for specimens caught in the Bay of Naples (LO BIANCO, 1909; RANZI, 1932), as for specimens collected off California and placed in captivity (MOLLET *et al.*, 2002). CAPAPÉ (1976, 1993) noted that gestation lasts four months in the common stingray, *D.*

pastinaca, and the thorny stingray, *D. centroura*, from the Tunisian coast, but that the number of litters per year remains unknown. CAPAPÉ & ZAOUALI (1995) reported a three-month gestation in the blue stingray, *D. chrysonota* (= *D. marmorata*), from the same areas. Nevertheless, they added that it is quite impossible to know the number of litters per year. In Table 2, it appears that a new reproductive cycle probably starts in September and that embryos are at mid-term of development in November (record 16), but no observation allows us to know when the females expelled their foetuses. As in other Mediterranean dasyatid species, the number of litters per year remains questionable in *D. violacea*, but MOLLET *et al.* (2002) wrote that in captivity "pelagic stingrays could have two litters per year." The role of mating in the number of litters per year cannot be neglected. In aquaria, mating activity was observed by MOLLET *et al.* (2002) who reported that "successful mating in captivity must have occurred because litters were observed in June 1997 and 2000."

Dasyatis violacea exhibited a vitellogenesis proceeding simultaneously with gestation. Therefore, the next batch of fully yolked oocytes was almost ready to be ovulated at the time of parturition. This phenomenon was reported in various viviparous elasmobranch species such as squalids (DODD, 1983; MELLINGER, 1989), dasyatids and rhinobatids (CAPAPÉ, 1986).

Ovarian fecundity is slightly higher than the uterine fecundity. This is due to the fact that some fully yolked oocytes were not ovulated and entered atresia. Moreover, pregnant females probably aborted during capture or handling and lost part of their brood. This phenomenon was observed in *Dasyatis* spp. and is commonly reported in viviparous elasmobranchs (MELLINGER, 1989).

The length of the reproductive cycle of *D. violacea* from wild environments is difficult to determine. It depends on various parameters, a possible embryonic diapause, the length of sperm storage, concomitant vitellogenesis and gestation and mating.

In matrotrophic elasmobranchs, the contribution of mother-derived organic molecules is very important (WOURMS, 1981; HAMLETT & WOURMS, 1984; HAMLETT *et al.* 1985a,b,c,d,e; HAMLETT, 1987, 1989; HAMLETT *et al.*, 1993a,b; FISHELSON & BARANES, 1998; HAMLETT *et al.*, 2002). These species produce an egg mass that is clearly less than the mass of newborn pups. Matrotrophy is characteristic of dasyatids, rhinopterids and gymnurids (WOURMS, 1981; MELLINGER, 1989; CAPAPÉ *et al.*, 1992; HAMLETT *et al.*, 1996; SECK *et al.*, 2002). In pure lecithotrophic species (*sensu* WOURMS, 1977, 1981) that produce heavier eggs, the role of the mother during gestation is reduced to a minimum. In lecithotrophic species, the mother only protects embryonic development and provides inorganic nutriments to the embryos (MELLINGER, 1989; HAMLETT *et al.*, 1998). This was observed in squatinids (CAPAPÉ *et al.*, 1990; CAPAPÉ *et al*, .2002) and centrophorids (RANZI, 1932; GUALLART & VICENT, 2001).

The CBD calculated in our study (47) is the highest value ever observed in elasmobranchs, higher than those reported by CAPAPÉ *et al.* (1992) for the butterfly ray, *Gymnura altavela*, from Tunisian waters (30.6) and by SECK *et al.* (2002) for the bull ray, *Pteromylaeus bovinus* from the coast of Senegal (31.12). *D. violacea* is a matrotrophic species following the definition of WOURMS (1977, 1988). By contrast, very low CBDs were obtained for *Squatina squatina* and *S. oculata* from the Tunisian coast (0.5; CAPAPÉ *et al.*, 1990) and *S. oculata* from the Senegalese coast (0.73; CAPAPÉ *et al.*, 2002). A CBD of 0.5 was also observed in *C. granulosus* by RANZI (1932).

In our sample, the size of the litter was between two and seven, corroborating LO BIANCO (1909) and RANZI (1932) who reported 5-6 neonates per litter. MOLLET (2002) reported 2-7 neonates per litter in pelagic stingrays caught off California and kept in captivity at MBA, Steinhart, or Sea World, and 4-9 neonates for rays caught off Japan and kept at Osaka or

KAMPA. MOLLET *et al.* (2002) considered two litters per year and consequently a maximum of 18 neonates per year.

Among the observed embryos, males and females were equally distributed, but among the free-swimming specimens, females outnumbered males, especially among large specimens. This was also the case for specimens from the coast of California and records reported from captive pelagic stingrays (MOLLET, 2002; MOLLET *et al.*, 2002). This suggests a higher male mortality in free-swimming specimens. However, changes in pelagic stingray sex ratio in adults could be due to sexual segregation related to reproduction in agreement with MOLLET's opinion (2002).

All the adult females were gravid, and among them some specimens carried developing embryos, probably at mid-term of gestation. Female elasmobranchs generally approach warmer waters near coasts (pupping areas) to expel their fully developed foetuses in the best environmental conditions.

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Nova opažanja o reprodukciji pelagijske raže žutulje, *Dasyatis violacea* Bonaparte, 1832 (Chondrichthyes: Dasyatidae) u Mediteranu

Farid HEMIDA [1], Rabea SERIDJI [1], Samira ENNAJAR [2]
Mohamed Nejmeddine BRADAÏ [2], Eric COLLIER [3], Olivier GUÉLORGET[3]
i Christian CAPAPÉ [3]

[1] *Laboratorij za ribarstvo, Institut prirodnih znanosti, Sveučilište znanosti i tehnike Houari Boumedienne, P.P. 32, El Alia, 16111 Bab Ezzouar, Algiers, Alžir*
E-mail: fhemida@yahoo.fr

[2] *Nacionalni institut znanosti i tehnologije mora, Centar u Sfax-u, P.P. 1035, 3018 Sfax, Tunis*
E-mail: mednejmeddine.bradai@instm.rnrt.tn

[3] *Laboratorij za ihtiologiju, P.P. 104, Sveučilište u Montpellier-u II, Znanosti i tehnika Languedoc-a, Montpellier, cedex 05, Francuska*
E-mail: capape@univ-montp2.fr

SAŽETAK

Primjerci pelagijske raže žutulje, *Dasyatis violacea,* uhvaćeni u tri sredozemna područja - na tuniskoj, alžirskoj obali i na južnoj obali Francuske - omogućili su izvješće o novim nalazima u svezi reprodukcije ove vrste. Širina diska (DW) kod odraslih mužjaka i ženki iznosila je 420 do 450 mm, dok je najvećem mužjaku širina diska iznosila 580 mm (DW) i težina 5800 g, najveća ženka je bila teška 6000 g s širinom diska (DW) od 610 mm. Veličina novorođenih je iznosila 160-195 mm širine diska DW i 191-212 g težine. Ženke nisu bile znatno teže od mužjaka. Promjer zrelih oocita kretao se između 18-22 mm (srednja vrijednost 19,5±1,10 mm), dok je težina varirala od 2,0 do 2,4 g (srednja vrijednost 2,1±0,12 g). Gravidnost je trajala otprilike četiri mjeseca.

Postavljena je hipoteza o dva okota godišnje koju, međutim, treba potvrditi jer nije isključeno da postoji embrijska dijapauza. Izračunata kemijska ravnoteža razvoja, bazirana na prosječnoj suhoj težini potpuno zrelih oocita i novorođenih, bio je 47.

Ovako velika vrijednost ukazuje na to da je *D. violacea* čisto matrotrofna vrsta. Ovarijski fekunditet, na osnovu broja zrelih oocita spremnih za ovulaciju, kretao se od 5 do 10. Uterini fekunditet, na osnovu broja zrelih jaja i/ili embrija, iznosio je od 2 do 7. Nije utvrđen odnos između veličine i kategorije plodnosti. Embriji mužjaka i ženki bili su jednoliko raspodijeljeni, ali među najvećim slobodnim primjercima prevladavali su mužjaci.

Ključne riječi: Chondrichthyes, Dasyatidae, *Dasyatis violacea*, reprodukcija, Mediteran