

Observations on the breeding and development in some batoid fishes

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ABSTRACT

Studies on the breeding and development of guitar fishes, electric rays and sting rays revealed that they are ovoviviparous where there is no placental connection between the young and the mother. In guitar fish, both right and left ovaries are functional along with the uteri whereas in the electric ray *Narcine* and the sting ray *Dasyatis* only the left ovary and uterus are functional. Some of the embryonic characters generally observed are the presence of gill filaments emerging outside the gill clefts and incomplete fusion of pectoral fin with head without forming disc. All *Dasyatis* spp. produce one or two embryos in a litter while the butterfly ray produces a maximum of 6 embryos and the guitar fish *Rhiobatus* up to 16 young ones.

Introduction

Batoid fishes which include saw-fishes, guitar fishes, electric rays, sting rays and skates, exhibit two types of reproduction. The skates, majority of which belong to temperate seas inhabit deep waters, are oviparous. All other members of batoids are ovoviviparous. Literature on the reproduction of batoid fishes is sporadic. Some information is available on the breeding of rays and guitar fishes of Bombay waters (Setna and Sarangdhar, 1949), on the cownose ray, *Rhinoptera javanica* and the butterfly ray, *Gymnura poecilura* (James 1962, 1966) of Mandapam coast. Devadoss (1978, 1982) studied the

maturity, breeding and development of the sting ray, *Dasyatis imbricatus* and the embryonic stage of mottled ray, *Aetomylus maculatus* and Devadoss and Hameed Batcha (1995) made observations on the maturity and embryonic stages of the rare bow-mouth guitar fish *Rhina ancylostoma* from Porto Novo and Madras regions. However, no information on the breeding and development is available on the electric rays and the guitar fish *Rhinobatus*. In the present study, observations were made on breeding and development of some batoid fishes thereby bridging the gap to a certain extent in our knowledge on the embryonic development of these species.

Material and methods

Several specimens of both sexes were examined for this study from April 1985 to March 1997 landed by trawlers at Madras Fisheries Harbour. Specimens were measured at the fisheries harbour. The ovaries and embryos, were removed and preserved in 10 % formaldehyde to be studied in the laboratory. The methods followed here for determining the maturity conditions were those described earlier by Springer (1960) and Devadoss (1978).

Results

Breeding in guitar fish Rhinobatus granulatus Cuvier, 1829

A pair of ovaries and uteri are present and functional. In a mature female fish, large-rounded eggs full of viscous yellow yolk, amidst numerous follicular cells were distinguishable. Mature ova upto 60 mm in diameter were observed during September-March.

Fecundity was estimated from the number of mature ova and also based on the number of embryos present. The number of ova recorded in individual female varied from 3 to 16 (Fig. 1). Embryos were found equally distributed in each uterus. Development of ova in the ovary and embryos in the uteri were observed (Fig. 2) simultaneously indicating the quick succession of ovulation, and fertilization after parturition.

Developmental stages of embryos: During the early embryonic development the fertilised eggs are enclosed in compartmented leathery egg-

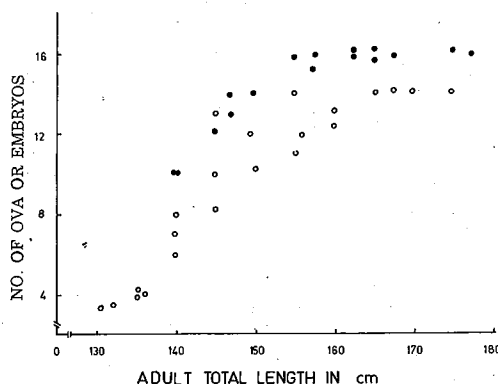


Fig. 1. *Rhinobatus granulatus* : Scatter diagram of number of ova or embryos against TL of the mothers. Solid circles represent ova and open circles embryos.

cases formed of shell membrane. But these compartments get disintegrated after a certain stage of embryonic development. In the early stages, the yolk-sac was large measuring 55 mm in

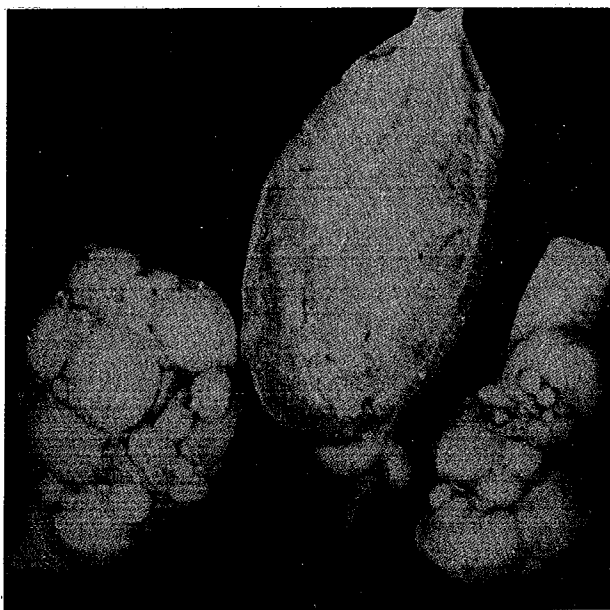


Fig.2. Ovaries and one side uterus of *R. granulatus*.

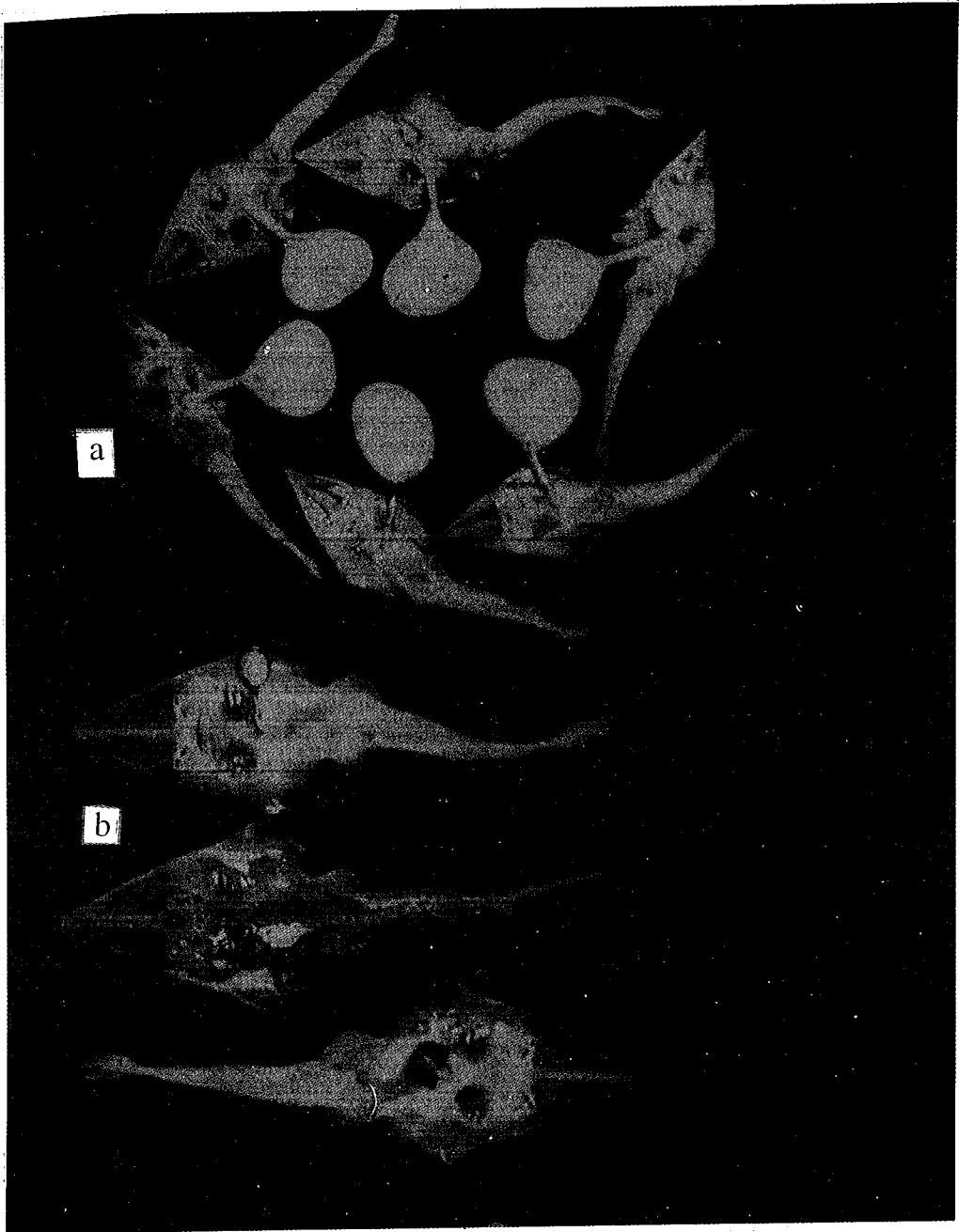


Fig. 3. *Rhinobatus granulatus* : a. Embryos 120 mm in TL with yolk sac, b. Full term embryos 240 mm in TL.

diameter and the cord 35 mm when the embryos reach 10-12 cm in total length. The young ones appeared colourless with tufts of gill filaments numbering 12 in each gill cleft. The fins with rays were well developed, the characteristic spines on the dorsal side though visible were soft (Fig. 3a). The eyes were larger than spiracles, teeth not developed. The external gill filaments disappeared by the time the embryos reached 20 cm in TL. Pigmentation on the dorsal side not yet commenced. The dorsal spines have become stiff. The yolk sac was completely absorbed and withdrawn inside when embryos reached 24-25 cm in TL (Fig. 3b). The embryo at this stage appeared to be a faithful replica of the adult. The occurrence of free swimming young during February/March in the shore seine catches in large numbers indicated the time of parturition. These small sized young were recorded upto August (Fig.4).

The sex ratio among the embryos was almost equal. In all, 274 embryos from 25 females were collected of which 126 were males with a sex ratio of one male to 1.3 females. There is no separate compartment for each embryo as seen in grey sharks, but the embryos after hatching stay freely with their yolk sac not being attached to the uterine wall. The embryos lie closely packed one over the other with heads pointing anteriorly towards the head of the mother. The walls of the uteri, folded and glandular, were soft and succulent with a milky fluid. In the advanced stage, the embryos were visible through the thin wall of the distended uterus.

Breeding season : The guitar fish has a protracted breeding season as evidenced by records of full term em-

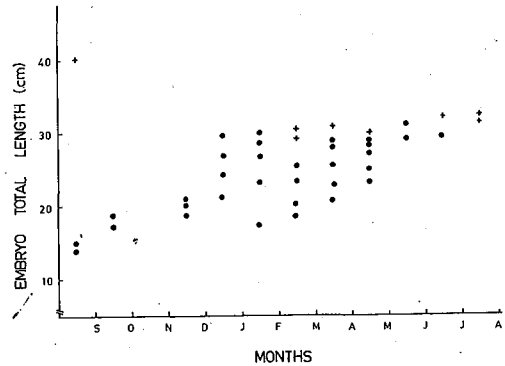


Fig. 4. *Rhinobatus granulatus* : Scatter diagram of mean embryo length against months when the mothers were taken. Crosses represent the smallest free swimming young.

bryos in the uterus and by occurrence of free swimming young ones in the catches from February to September with a peak between February and April. The ovary in fully developed condition with large, mature ova could also be seen besides embryos in the same female. Fertilised eggs were seen encased in less transparent membrane in the uteri during September along with developing ova in the middle stage of 40-45 mm in diameter in the ovary. It is likely that these ova may further develop and become ready for ovulation around February to coincide with the termination of current pregnancy. So the gestation period may be estimated to be around 6 months. The smallest female with developed ovary and large ova measured 120 cm in TL and so this size may be taken as minimum size at maturity.

Breeding in electric ray, *Narcine brunnea* Annandale, 1909

The ovary in *Narcine* is quite different from all the other elasmobranch

fishes in that the ova are elongated and finger like, enclosed in a thick membrane (Fig 5 a.). The number of ova vary in different individuals and correspond to the number of embryos borne by the females. The interior wall of uterus is provided with numerous minute finger like projections or villi all over (Fig. 5 b).

Description of embryos: In Fig. 5c an early stage embryo of 35 mm in total length is compared with an embryo of 51 mm TL (Fig. 5 d & e) The smallest embryo obtained measured 22 mm in TL and differed from the full term embryos in having the pectoral fins separated from the body. The other characteristics are: mouth poorly developed, eyes larger than spiracles, the opening of which partly covered by a semitransparent tissue; electric organs partly developed, not assuming adult shape; gills provided with filaments extending outside from the gill cleft; tail keel absent.

At 25 mm, the muciferous pores started showing early development of mucous canal system. The yolk sac measured about 20 mm in diameter.

At 40-45 mm the mouth became prominent and spiracles fully open; pigmentation on dorsal side commenced; external gill filaments became rudimentary and yolk sac persisting; measured 12 mm in diameter.

At birth, the embryos are 65-70 mm in total length. Bigelow and Schroeder (1953) recorded the average length of *Narcine brasiliensis* at birth to be 110-120 mm while McKay (1966) observed 75 mm for *N. westraliensis*.

Fecundity and breeding : The number of young ones produced by this

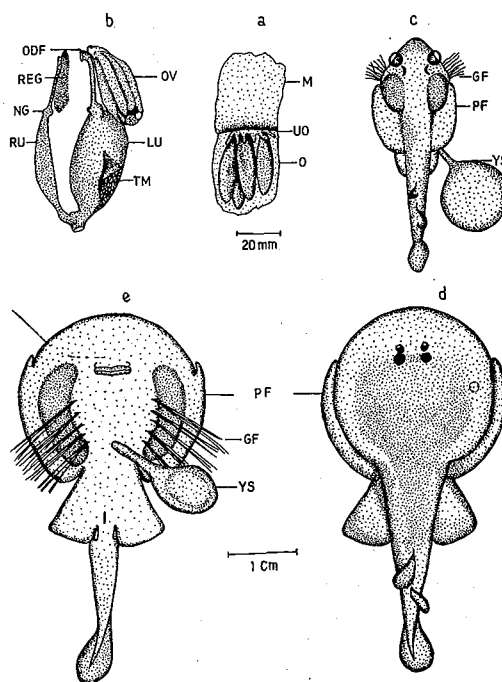


Fig. 5. *Narcine brunnea* : a. reproductive organs – ovary and Uteri. A portion of uterus cut open to show villi or trophonemata on the wall of uterus, b. position of ova after partial removal of sheath, c. embryo early stage 35 mm in TL, d. dorsal view of embryo 51 mm in TL, e. ventral view of the same.

Abbreviations:

C. clasper, ODF. oviducal funnel, CS. caudal spine, OV. ovary, GF. gill filaments, PF. pectoral fin, IYS. internal yolk sac, REG. right epigonal organ, LU. left uterus, RU. right uterus, M. membrane, TM. trophonemata, NG: nidamental gland, UO. undeveloped ova, O. ova, YS. yolk sac.

ray varied from 4 to 10. There was no separate compartment in the uterus for each embryo for development and no placental connection established between the mother and foeti at anytime of the pregnancy. Gravid females of *N.*

brunnea were collected from January to April when intrauterine embryos were ready for release. The ovary in the parent fish at the time of birth of young was not well developed, thus indicating that ovulation was not in quick succession as seen in *Rhinobatus granulatus*. The males possessed seminal fluid in the seminal vesicles during October-November. Hence this may be the period when mating, ovulation and fertilization take place. The period of gestation may be around six months. The smallest mature female measured 180 mm and mature male 170 mm. These lengths may be taken as minimum sizes at maturity.

Breeding in electric ray *Narcine timlei* (Bloch and Schneider, 1801)

Description of ovary, ova and uterus are the same as given for *N. brunnea*. Prasad (1920) counted 198 villi in a square inch of the wall of the preserved uterus of *N. indica* which is a junior synonym of *N. timlei*. The number of ova varied between 4 and 6 in adults. Mature males with fully developed claspers were recorded from specimens of 280 mm TL onwards when seminal fluid was found in the seminal vesicles as well as in the clasper grooves. Females with mature ova were seen from specimens of 300 mm TL onwards.

Description of embryos : The smallest embryo measured 28 mm in TL. The embryo looked different in appearance as the anterior part of the pectoral fin was not fully developed and hence not fused with the head to form the disc as in the adult (Fig. 6a). Further, the knoblike and more or less pointed, mouth small and protruding above surface of skin fold surrounding it; eyes also protruding and larger than spiracles close behind, dorsal fins round, gill

filaments protruding out of gill clefts; yolk sac large and connected to the embryos (Fig 6b).

At 90-100 mm, the embryos looked almost like a faithful replica of the adults. The colour pattern was adult like but the fusion of pectoral fin with snout was yet to be complete as seen in Fig 6c. Eyes equalled spiracles, first dorsal larger than the second dorsal and yolk sac still persisted in small form (Fig. 6d). External gills disappeared. There was no separate compartment for each embryo.

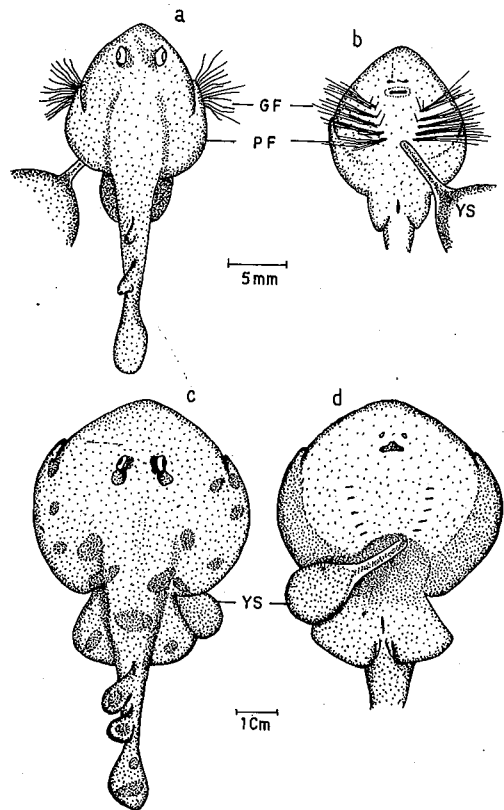


Fig. 6. *Narcine timlei* : Early stage embryo 28 mm in TL. a. dorsal view, b. ventral view, c. embryo advanced stage 10 mm in TL dorsad view, d. ventral view of the same embryo.

Breeding season : Intrauterine embryos were collected from August to February but full term embryos were recorded from October to February. This period is considered as the parturition time for this species, as free swimming young (105-110 mm size) were also recorded in the collections. Ovary in the adult was not fully developed at the time of birth of young ones and there is no evidence to show that this electric ray may spawn immediately after parturition.

The non-gravid females (without embryos) were found to possess developed ova during May-June. Female specimens contained 'spindle' about the size of 60 mm occupying the entire length of uterine chamber in June-July. Based on the observations the gestation period may be calculated as 5-6 months.

Breeding in sting ray, *Dasyatis zugei* (Muller and Henle, 1841)

The ovary and uterus on the left side

only are functional, the reproductive organs on the right side being rudimentary. Specimens of 240 mm length and above were mature. The number of young ones produced was limited to one or two only. Along with developing embryos, ova were also found developing in the ovary. Full term embryos measuring 85-100 mm across the disc were recorded from January to May. The breeding data are given in Table 1. No placenta or compartment for each embryo could be seen during development, but the wall of uterus was provided with numerous villi or trophonemata. The time of parturition appears to be protracted. Setna and Sarangdhar (1949) recorded gravid females from February to April in the Mumbai waters.

The embryos collected were all fully developed with yolk sac hanging on the ventral side (Fig. 7 a&b). Tufts of gill filaments seen in early stages disappeared later. The yolk from yolk sac was

TABLE 1. *D. zugei*, summary of the breeding data of females

Month	Adult size across disc(mm)	No. of embryo/litter	Remarks
Jan.	225		Maturing virgin ova are small upto 10 mm. Only two distinguished out of general egg stock
	255	2	Full term, but yolksac seen as small rudimentary organ
Feb.	265	2	Full-term ova upto 15mm
	262	-	Ova measure 15-18 mm; young released
	220	-	Virgin mature ova 12, 13 mm
Mar.	240	-	Only one large ovum of 18 mm out of general egg-stock
Apr.	260	2	Fully developed young ones about to be released; came out on pressure by hand
May	285	-	Young ones released
Dec.	248	2	Mid-term embryos; ova upto 6 mm

used up and found stored inside the stomach of the embryo. This condition known as 'yolk stomach' is common in ovoviviparous elasmobranchs. The embryos over and above 60 or 65 mm size were found in this condition.

Breeding season : Parturition in this species is extended from January to May and the young ones measured 100 mm at birth. The simultaneous development of large ova and embryos suggests that spawning and fertilization probably followed immediately after the birth of young ones.

***Dasyatis sephen* (Forsskal, 1775)**

This ray produces one or two embryos per litter at a time. In Fig. 8 a&b an embryo of mid term stage measuring 156 mm across disc is shown with yolk sac partly used up. Embryos in earlier stages were provided with tufts of gill filaments which disappeared when they reached 120 mm across the disc and only rudimentary gill filaments were sometimes seen at this size. Other characters were : tail longer; disc longer than width and pigmentation not yet started. Pigmentation visible from 200 mm onwards. Teeth cut in embryos at

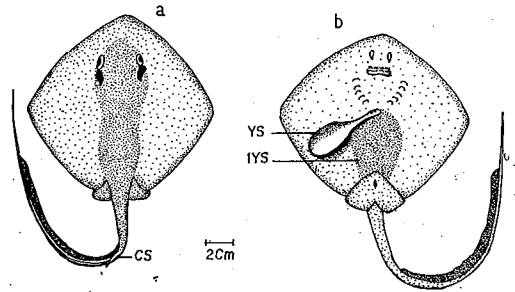


Fig. 8. *Dasyatis sephen* : Mid term embryo 156 mm across disc. a. Dorsal view, b. ventral view.

the time of parturition when the embryos measured around 250 mm indicating that this species may start feeding immediately after parturition.

This species appeared to have an extended breeding season from October to April with peak during March-April. Chaudhuri (1916) who collected embryos in Chilka Lake opined that the species had no definite breeding season.

***Dasyatis jenkinsii* (Annandale, 1909)**

The adults grow upto a metre across the disc. The relationship between the clasper length and disc width showed that males reach maturity when they attain a size of 400 mm (Fig. 9). The clasper length increases fast with the growth of the adult and reaches 110-124 mm when the animal attains 375 mm across the disc and thereafter the growth slackens at a size of 400 mm. After this the growth rate is not appreciable and this indicates the size at which males attain maturity. Female specimens from 450 mm and above contained two developing ova 24-25 mm in diameter amidst small whitish follicular cells and the uterus on the posterior side was enlarged.

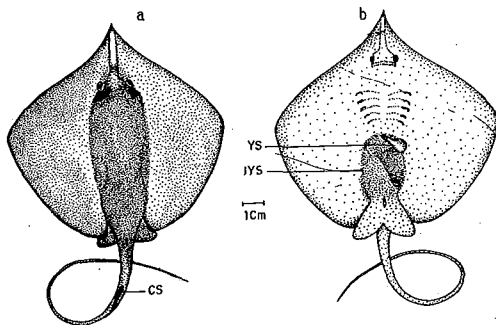


Fig. 7. *Dasyatis zugei* : Full term embryo 95 mm across disc. a. dorsal view, b. ventral view.

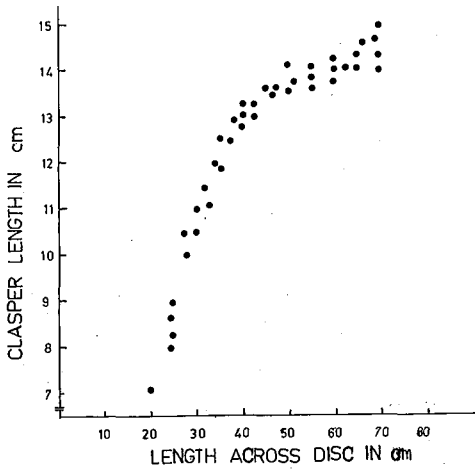


Fig. 9. *Dasyatis jenkinsii* : Relation between TL across disc and clasper length.

Breeding season : Eggs were found inside the uterus during March-May period. Breeding data given in Table 2 suggested a gestation period of about 10-12 months. Embryos in full term stage (180-190 mm) were collected from

TABLE 2. *D. jenkinsii*. Summary of the breeding data of females

Month	Adult size across disc(mm)	No. of embryo/litter	Remarks
Jan.	885,760	-	Young ones released; ova range 27-30 mm in diameter
	450,470,485	-	Virgin mature; 2 large ova 24-25 mm in diameter
	890,700,750	2	Full term embryos 2 in each specimen; ova 27-30 mm
Feb.	600,610	-	Young ones released; ova 35 and 37 mm in diameter
	840	2	Ova 36-37 in diameter (mm)
	622	-	Ova 35-36 in diameter (mm)
Mar.	845	-	2 ova traced inside uterus; others small, less yellow in colour
	815	2	Embryos full term; ova 27-28 mm
	675	-	2 ova 30, 32 mm; bigger in size, other small and many
Apr.	840	2	Embryos full-term; ova 28-29 mm in diameter
	670,650	-	Young ones released; ova 35 mm in diameter
May	1000	-	Young ones released; no ova seen
Sep.	550	2	Embryos mid-term; ova very small, upto 15 mm
Oct.	680	2	Nearly full term; ova 16-18 mm
Nov.	675	2	Nearly full term; ova 20-22 mm

December to March. A full term embryo of *D. jenkinsii* is illustrated in Fig. 10. Upto two embryos in a litter were observed. Free swimming young ones of 200-220 mm size seen in the estuaries and backwaters, suggest these as breeding grounds. Ova measuring 24-37 mm in diameter for the succeeding were observed along with the embryo from January to April.

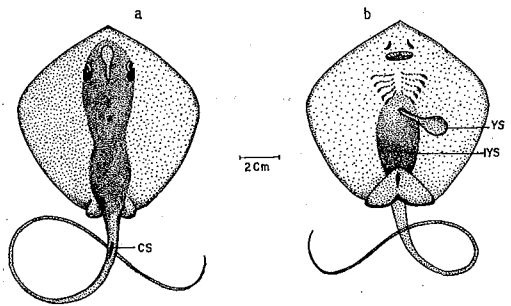


Fig. 10. *Dasyatis jenkinsii* : Full term embryo 72 mm across disc. a. dorsal view, b. ventral view.

***Gymnura poecilura* (Shaw, 1804)**

The smallest female carrying young ones measured 405 mm across disc. Gravid females having developing young ones were observed throughout the year with a peak season during February - June. Setna and Sarangdhar (1949) also reported a prolonged season for this species along Bombay coast.

The embryos collected were all full term and exact replica of the adult. The embryos with yolk sac measured 110-140 mm, the yolk sac on an average measured 15 mm in diameter and the stalk 8 mm in length; eyes larger, nine clear cross bands visible on tail but incomplete on ventral side, spines on tail not developed; pigment chocolate brown dorsally with scattered small rounded spots and creamy white ventrally. An embryo of 115 mm is illustrated in Fig. 11 a&b.

In early development, the embryos are provided with minute external gill filaments which disappear later. When the yolk from the yolk sac is used up, the embryos depend on the secretion from the uterus of the mother for further growth. To facilitate this, the wall of uterus is produced into numerous finger like villi. The embryos were observed to lie with pectoral fins folded like a tube with the tail tucked

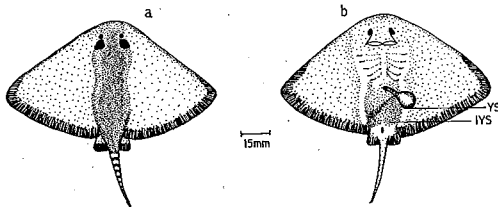


Figure 11. *Gymnura poecilura* : Advanced stage embryo 115 mm across disc. a. dorsal view, b. ventral view.

in. In advanced pregnancy the embryos could be removed with slight pressure on the abdomen. The embryos measured 150 mm when the yolk sac was completely with-drawn and continued to remain within the uterus without yolk sac till they reached 230 mm to be released outside. Setna and Sarangdhar (1949) reported 250 mm size at birth along the Bombay coast.

Sex ratio: The number of intra ovarian embryos observed from 40 gravid females was 148 with an average of 3-4 embryos per litter. There was a slight dominance of females over males numbering 86 and 62 respectively. The same trend was noticed in Mandapam waters also (James, 1966).

***Aetomylus nichofii* (Schneider, 1801)**

The developing embryos measuring 140-235 mm were recorded from August to April (Table 3). Full term embryos ready for parturition were collected from December to April. One to three embryos were recorded in each litter. The tufts of gill filaments present externally in the early stage disappeared in embryos at 130mm. An embryo of 140 mm across disc is shown in Fig. 12 a&b. The yolk sac and connected stalk persisted; pigmentation and blue bands on dorsal side started developing and teeth present. The embryos, even

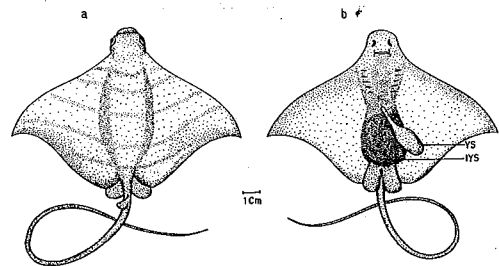


Fig. 12. *Aetomylus nichofii* : Full term embryo 140 mm across disc. a. dorsal view, b. ventral view.

after complete withdrawal of yolk sac, remain in the uterus as seen in all rays described earlier. The characteristic rolling of embryos (tube like) was also observed. From Table 3, it is clear that this ray has an extended breeding season, liberating embryos during December to April. The size at birth of embryos was 220-235 mm across disc.

Aetobatus narinari (Euphrasen, 1790)

Gravid females in good numbers were landed during April to May. Full term embryos of 180-250 mm across the disc were obtained upto 3 embryos per litter. No placenta was formed in this species also. An embryo measuring 230 mm across the disc (Fig. 13 a&b) possessed yolk sac 28 mm in diameter and yolk stalk 21 mm. Eyes smaller than spiracles; tail thrice longer than disc, tail spine just organised into a thick fleshy structure but not protruding out. The young ones without yolk sac remained in the uterus for some

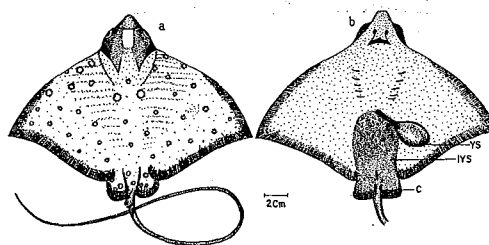


Figure 13. *Aetobatus narinari* : Embryo of 230 mm across disc. a. dorsal view, b. ventral view.

more time till they were released outside.

Discussion

Elasmobranchs have a highly evolved reproductive system by having internal fertilization, bringing out the young and thus eliminating larval stages. Differences exist in the distribution of the organs of reproduction among various groups. In batoids, while testes are always paired, the ovaries do not develop in pairs in all cases. In guitar fish,

TABLE 3. Summary of the breeding data of the females *A. nichofili*

Month	Adult size across disc(mm)	No. of embryo/litter	Remarks
Aug.	620	2	Full-term embryos, 176-180 mm size; ovisac still present; trophonematous villi present
	750	1	Full-term embryos, 165-172 mm size; ovisac still present, trophonematous villi present
Sep.	960	3	Full-term embryos, 180-195 mm
Oct.	950	2	Mid-term embryos, 140-145 mm size, rudimentary gill filaments still persistent
Nov.	850	3	Full-term embryos; 175-180 mm
Jan.	940	2	Parturition stage yolk sac completely withdrawn, range 230-235 mm
Feb.	702	1	Embryo 228 mm size; parturition stage
Mar.	980	3	Embryos 232-235 mm size; parturition stage
Apr.	920	2	Embryos 230-235 mm size; parturition stage

saw fish and the numb fish *Narke* both the ovaries on left and right sides are fully functional. In the sting ray, the eagle ray and the electric ray *Narcine* the ovary on the left side only is functional. Both uteri are functional in the guitar fish, the numbfish *Narke* and the butterfly ray *Gymnura*, while only the left uterus is functional in the case of electric ray *Narcine*, sting ray and eagle ray.

Ovoviviparous reproduction has been observed in all the species studied here. The capsules instead of being laid out straightaway, are being kept inside the uterus for development. In some cases more than one egg may be enclosed in a capsule called "spindle". The embryos emerge from the capsule with yolk sac hanging on the ventral side. There is no placental connection and a separate chamber for developing embryos. As the yolk gets used up, the yolk sac and the stalk are withdrawn into the stomach of the embryo. Hence there is no possibility of embryos being born with umbilical scar. The embryos remain further inside the uterus until they emerge outside. In some cases the embryos after hatching are fed by secretions from the uterine wall. The secreting surface vary from folds or frills as in sawfish and guitar fish (Setna and Sarangdhar, 1949; Wallace, 1967) to numerous finger like projections known as villi as in all sting rays (Prasad, 1920; Devadoss, 1978). Wallace (1967) observed that in Butterfly ray, *Gymnura natalensis* the villi are so pronounced as to enter the gillslits of the embryos and the nutrient secreting glands practically function within the stomach of the embryo. All these suggest that dependence on uterine secretion is a necessity for further growth of the foetus.

In all the species of batoid group, excluding the electric rays, the mature ova (ovarian cycle) and the developing embryos in advanced stage (uterine cycle) co-exist. Ford (1921) and Devadoss (1978) inferred that copulation, ovulation and fertilization take place almost immediately after the termination of the current pregnancy. Such a phenomenon was not reported anywhere in respect of pelagic sharks, where the ovarian cycle and then the uterine cycle function in sequence thereby providing enough resting time for each cycle to become active again for the subsequent breeding. But in the case of animals studied presently, there is not much resting time between each cycle. This phenomenon of ovarian and uterine cycles functioning simultaneously may in all probability be an adaptation on the part of these species (which have the capacity to produce only a few offsprings) to beget more young ones in a short span of time.

Acknowledgment

The author wishes to thank Dr. M. Devaraj, Director, CMFRI and Dr. P. S. B. R. James, the former Director for their constant encouragements and the facilities extended during the period of work.

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