OBSERVATIONS ON THE DEVELOPMENT OF *CHILO-SCYLLIUM GRISEUM* M. & H., *PRISTIS CUSPIDATUS* LATH. AND *RHYNCHOBATUS DJIDDENSIS* (FORSK.).

By S. B. SETNA, *Ph.D.* (*Cantab.*), *F.N.I.*, and P. N. SARANGDHAR, *M.Sc.*, *Ph.D.*, *F.Z.S.*, *Department of Fisheries, Bombay.*

[Plate I]

CONTENTS.

INTRODUCTION.

While investigating the breeding habits and life histories of the Bombay elasmobranchs, some striking observations concerning the mode of formation of the egg-cases in the oviparous shark *Chiloscyllium griseum* and the ovo-viviparous forms *Pristis cuspidatus* and *Rhynchobatus djiddensis* were made. The data obtained during these studies, lend support to the part-formation theory of egg-case formation. In the ovo-viviparous forms, the cavities of the egg-capsules were observed to be partitioned by a process of spiral twisting of the inner membrane of the capsules—a process which is probably recorded here for the first time. The embryonic stages of *P. cuspidatus* and *R. djiddensis* described here serve to furnish a more or less complete account of the intra-uterine development in these species. The condition of the parent fishes with successive advancements in pregnancy have also been recorded so as to complete the picture not only from the point of view of the fœtus but also that of the mother.

EGG-CASES AND BREEDING SEASON OF *Chiloscyllium griseum* M. & H.

Egg-cases.—Parent ♀ : Total length—23 inches ; App.wt. 3 lb. ; 10th March, 1945.

The abdomen of the fish was large and distended. Dissection revealed the presence of only the right ovary, which was large and

extended along almost the entire length of the abdominal cavity con-
cealing other viscera. It measured 110 mm. ×50 mm. ×20 mm. and
was studded with 18 large, greenish, yellowish pink, more or less rounded
eggs, having diameters ranging from 20 mm. to 25 mm. There were
also a number of small, light-coloured ova in various stages of develop-
ment. A small strap-shaped, greenish-pink epigonal organ was also
present, merging anteriorly into the substance of the ovary on its dorsal
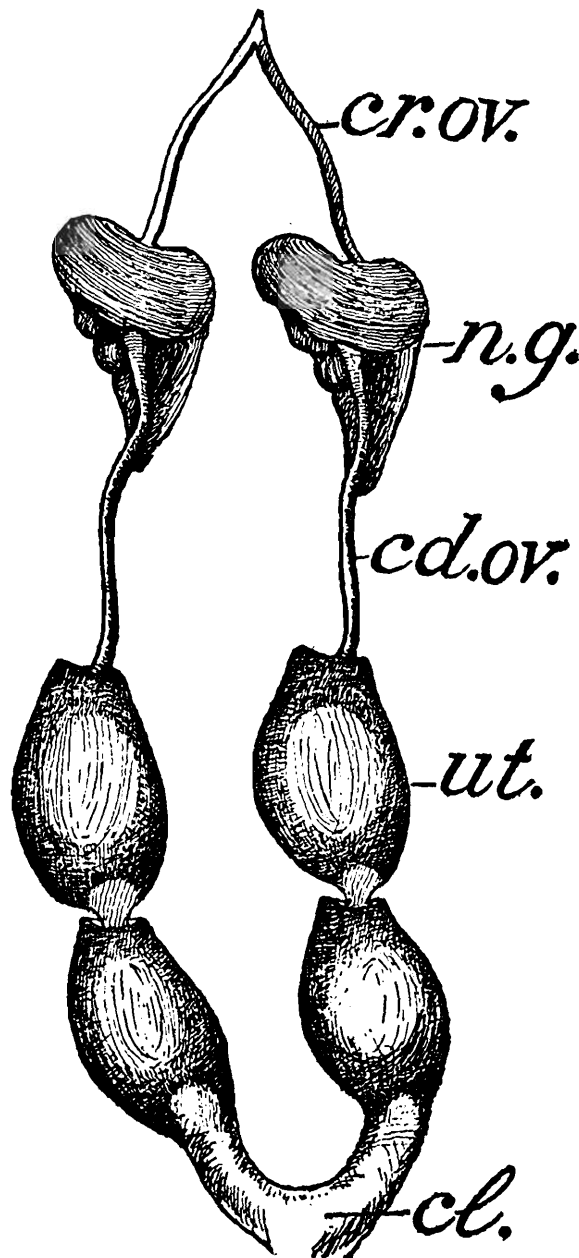
TEXT-FIG 1.—*Chiloscyllium griseum* M. & H.

Oviducts, nidamental glands and uteri with four egg-cases ' in utero.' Note the
similar disposition of nidamental glands and of egg-cases in both the uteri.
cd. ov.=caudal oviduct ; *cl.*=cloaca ; *cr. ov.*=cranial oviduct ; *n.g.*=nidamental
gland ; *ut.*=uterus.

aspect. Each nidamental gland was an asymmetrical, thick, swollen
body, having a short dorsal and an elongated ventral groove, the latter
being situated medially on the right side and laterally on the left. Both
the uteri were thin-walled sacs, each tightly packed with two egg-cases
(Text-fig. 1), the quadrangular contours of which were clearly dis-
cernible through the thin uterine walls.

On opening the uterus the two egg-cases were seen to be placed tandem, in a single cavity roughly divided in the middle into two parts by a thick, horizontal fold or ridge of the uterine mucous membrane, showing that the uterus could accommodate only two egg-cases at a time. The silky shell-membrane cord of the proximal egg-case could be traced up lying in the lumen of the caudal oviduct, while that of the distal lay in the proximal compartment. Both pairs of egg-cases in the two uteri were placed exactly opposite each other and not one was yet extruded, even partially through the cloaca. The egg-cases lay flat in the uteri, presenting a slight bulge ventrally. The longer sides of each egg-case lay parallel to the length of the uterus and the broader end was always the anterior end. The shorter and less curved of the two long sides lay medially in the right uterus while the opposite was the position in the left. Thus, while the orientation of the egg-cases in both the uteri was similar, there was no bilateral symmetry in their disposition. This is to be expected in view of the fact that bilateral symmetry is lacking even in the nidamental glands (Text-fig. 1).

The tough, keratinous egg-cases (Text-fig. 2a) are glossy, dark brown in colour, having darker, thickened edges and angles. Their walls are semitransparent and the oval eggs within are visible even from without.

All the four egg-cases are more or less equal in size, having the following range of dimensions (in mm.) :—

Length of the longer and more curved of the two long sides	68-70
Length of the shorter and less curved of the two long sides	60
Width at the anterior end	20
Width at the posterior end	11
Width in the centre ..	33-35
Maximum thickness ..	17
Size of the egg within the egg-case ..	20-23 × 25-27
Width of the shell-membrane band ..	32
Length of the shell-membrane cord	190

These egg-cases are somewhat smaller than those examined by Aiyar and Nalini (1938), though the description of the cases agrees closely with that given by these authors. Reference may, however, be made to a few points deserving of special mention. Aiyar and Nalini state that the silky fibres composing the broad band gradually twist into a long cord for attachment to sea weeds, etc., and diagrammatically represent them as if they were equally elongated throughout. We find, however, that the silky band is more or less quadrangular in outline and that its fibres are not equally elongated throughout. The fibres along its upper free border are much more elongated than others and it is these only that chiefly go to form the anchoring cord, although a few fibres along the other free margins of the band also merge into it ultimately. Further, if the orientation of the egg-cases within the uterus, described above and apparently the normal orientation, is considered, the corner slits along the larger of the two long edges are ventral in disposition, while those along the other are dorsal. The slits are, however, extremely narrow and elongated and hardly perceptible in a superficial examination.
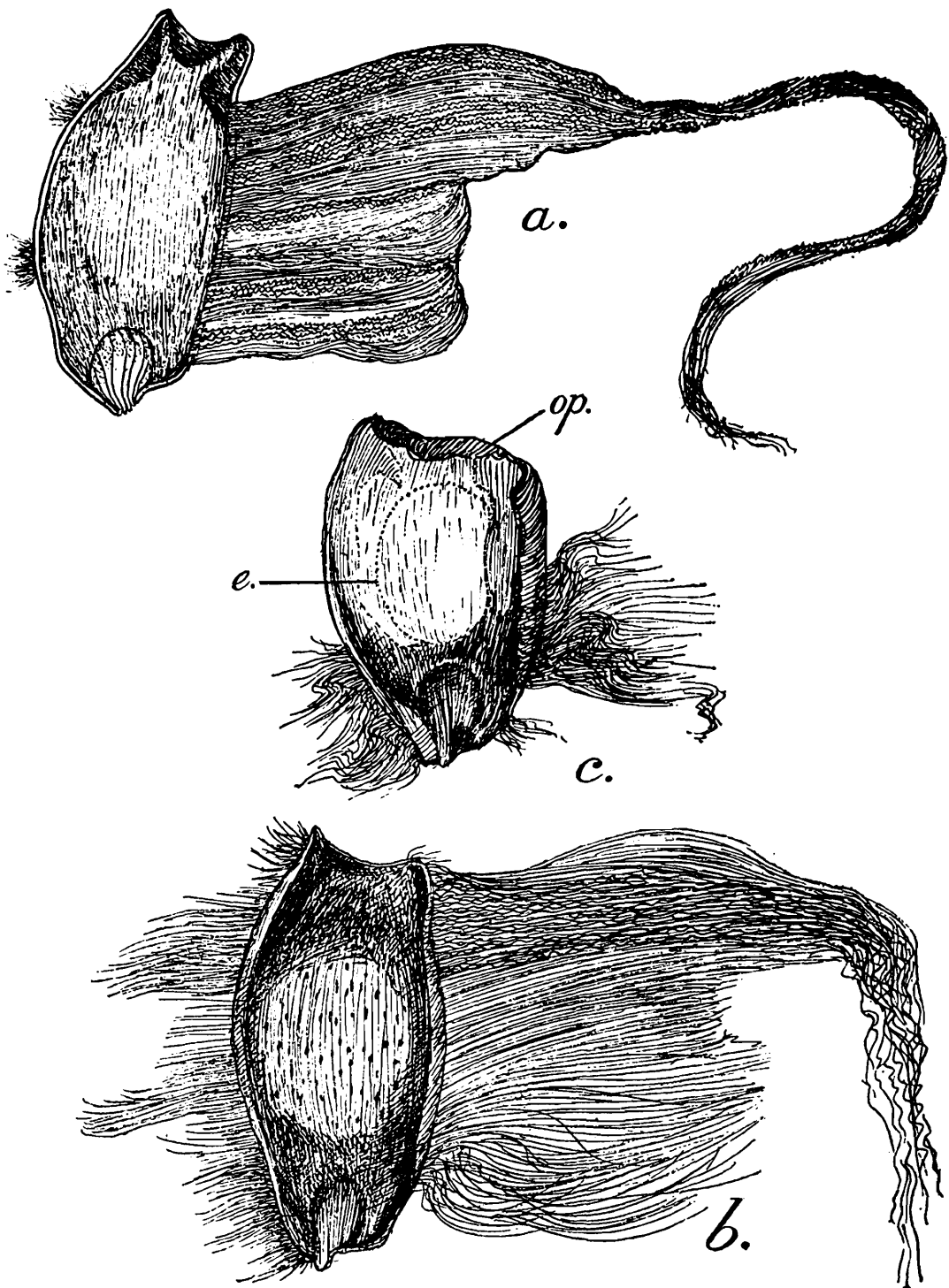
TEXT-FIG. 2.—*Chiloscyllium griseum* M. & H.

a. egg-case with silky band of anchoring fibres ; *b.* recently formed egg-case removed from uterus. Note superficial longitudinal beaded striations ; *c.* incomplete egg-case removed from nidamental gland. It is nearly two-thirds formed and the egg and albumen have already descended into it.
e.=egg ; *op.*=anterior opening.

The cavity of an egg-case on being opened does not extend beyond the superficial slope at either end and is seen to be filled with a quantity of transluscent gelatinous albuminous mass in which is embedded the yellow oval egg. The blastoderms are seen to be scarlet-coloured circular halos about 2 to 2·5 mm. in diameter and situated at one of the poles of the eggs.

The vigorous state of activity of the ovary would make it appear that the female referred to above had just begun its reproductive cycle and that she would produce a number of egg-cases during the oestrous period that she had already entered upon.

Two gravid females, measuring 24″ and 25·5″ in total length and weighing about 4·5 lbs. apiece, were obtained from Sassoon Dock, Bombay, on 20th January 1944. The reproductive organs of both presented the characteristic features described above, but there were a number of significant differences which are noted below.

Each uterus of the female measuring 24″ in length bore a completely formed egg-case having the following as dimensions (in m.m.) :—

Length of the longer side	87
Length of the shorter side	77
Maximum width of the egg-case	38
Size of the fertilized egg within the case	33 × 25

The egg-cases, except for the distinct variation in their size, presented exactly the same features described above.

The left uterus of the other female possessed a completely formed egg-case and there was another incompletely formed egg-case (open at one end) in the nidamental gland of that side. The right nidamental gland also possessed an incompletely formed open egg-case in the process of formation, but the uterus of this side was empty, with flaccid, sagging walls, indicating that its egg-case had just been cast into the sea, while the egg-case on the opposite side was next to be extruded. The completely formed egg-case from the left uterus had the following dimensions (in m.m.) :—

Length of the longer side	83
Length of the shorter side	70
Maximum width of the egg-case	40

This egg-case displayed certain structural peculiarities not noted in those already described. The egg-case was not yet dark or glossy brown in appearance but dull yellow, being coated externally with a dull coloured, rather opaque shelly secretion, which ran into thin, longitudinal streaks that still displayed a beaded appearance (Text-fig. 2b). Both the surfaces of this egg-case also possessed small and delicate silky filaments, which were not quite so densely packed as along the edges. On scraping off this superficial coating, the characteristic dark brown wall of the capsule became exposed. The beaded streaks of the shelly substance revealed the characteristic mode of secretion of the shell glands, described at length by Nalini (1940) during her treatment of the structure of the egg-case.

Another significant point arising from these observations was that this egg-case had just descended from the nidamental gland and would be retained for a time in the uterus till its superfluous secretions disappeared, and till it assumed the dark brown, glossy colour at which stage it would be ready to be cast out.

The most interesting features were, however, presented by the incompletely formed egg-cases in both the nidamental glands of the female (Text-fig. 2c ; Pl. I, fig. 2 left). Both of them were equal in development, similar in orientation, which itself was similar to that of the fully formed egg-cases and they were situated exactly opposite each other. It was obvious that nearly two-thirds of their development had been completed, characteristically, from the posterior end forwards and that anteriorly they were open, the openings being fairly wide and irregular in outline. The fertilised eggs, surrounded by dense masses of transparent, gelatinous albumen, had already entered the egg-cases, and more albumen was seen to be still entering through the open ends. The capsular walls were generally clear and transparent in appearance, but tough in consistency except at the edges of the open anterior ends, where they were still rather soft, parchment-like and plastic. Along both the long edges and in the posterior fourth, they were just assuming the yellowish brown tint and here their consistency was more tough. Evidently, the capsular walls were not equally keratinised all over. In the central region they were clearly transparent and not yet marked by the conspicuous longitudinal beaded striations present in other regions. Except this region, the walls turned whitish opaque in formalin. Further, the posterior third of both the long edges, the posterior angles, and the posterior end of either egg-case already possessed bunches of the soft, silky fibres and these were even present, though sparsely, on both the surfaces of the completely formed posterior portion of the egg-case. It was clear that a portion of the big band of the silky anchoring fibres had already been formed. The colour of the fibres was not, however, the characteristic golden yellow, as in the completely formed egg-cases, but was rather dull. Further, both the posterior corners of the egg-case, where development was almost complete, had formed the slits, with their dispositions similar to those in a completely formed egg-case. The dimensions of these incomplete egg-cases were as follows (in m.m.) :—

Length of the longer edge	..	60
Length of the shorter edge	..	50
Width at the open anterior end		30
Width at the posterior end		10

The walls of the egg-case being gently pressed would cause the jelly like albuminous mass engulfing the egg to project through the open end, and the scarlet-coloured blastoderm in the form of a circular halo about 3 mm. in diameter, would come into view.

These observations on the egg-cases in the process of formation facilitate an understanding of the mode of formation of the egg-case in *Chiloscyllium griseum.*

Mode of formation of egg-case.—Beard (1890), Hobson (1930), and Metten (1939) are of the view that the lower half or more of the egg-case is formed before the egg arrives in the nidamental gland and that the closure of the purse is effected only after the entry of the egg in the partially formed egg-case. Widakowich (1906) and Nalini (1940), on the other hand, are of the opinion that the casing material is secreted over the egg only when it actually arrives in the nidamental gland. This process would seem to suggest that the egg-case is secreted as a whole, at one and the same time, that its formation is completed uniformly and simultaneously on all sides, and that there is no preformation of a part of the egg-case before the arrival of the egg in the nidamental gland. Observations on the egg-cases obtained by us point to a lack of uniformity in the composition of the structure of the capsular wall in different regions and clearly suggest a non-synchronous activity of the shell-glands in the different zones of the nidamental glands. These features, together with the very incomplete nature of the egg-cases encountered by us, thus support the former view regarding the formation of egg-cases in oviparous forms. The incomplete egg-cases obtained by us represent obviously a stage slightly later than that of the unique egg-case described by Hobson in the skate *Raia radiata.* Nalini's criticism (*loc. cit.,* p. 209) of Hobson's observations on the half-formed egg-case and of his view about the part-formation of the egg-case is not sound, when it is reasoned that the different ends of the dorsal and ventral walls of the already secreted portion of the. egg-case are not subjected to the same pressure throughout and that the different stages in the completion of the egg-case formation (*viz.,* the secretion of the posterior half of the egg-case, the entry into it of the egg surrounded by the albumen and the secretion of the remaining portion of the egg-case) succeed one another so quickly as not to permit of a differential harden-ing of the uniting regions, such as might lead to the ' suture ' and a break in the superficial consistency of the egg-case as imagined by Nalini. Further, our observations regarding the presence already of the silky, anchoring fibres at the posterior ends of the long edges of the incomplete egg-case, and the evident revelation of part-formation of even the anchoring filamentous band, finally dispose of Nalini's view that these filaments are formed, all at the same time and last of all, after the complete formation of the egg-case.

The encountering of such partially formed egg-cases is, indeed, a sheer matter of chance and it is reasonable to believe that a more intensive fishing during the breeding season may reveal the normality of the phenomenon of part-formation of the egg-cases, thus ruling out the possibility of abnormality as implied by Nalini (*op. cit.*), on the grounds that such encounters are only solitary, as at present.

Breeding season.—It will be seen from the following table that though the ova are developed in December, the egg-cases do not appear till January. Females with egg-cases were recorded from January to March and thereafter the ovaries were found to be reduced in size and uteri with flaccid walls. It may, therefore, be surmised that the breeding season lasts from January to March.

Table showing the lengths of parent fishes and the dimensions of their egg-cases from the Bombay, Malabar and Madras areas.

| Length of fish. Inches.	Date of capture.	Size of the egg-case. Max. length. mm.	Max. width. mm.	Locality.	Observer.	Remarks.
23	10-3-43	70	35	Bombay	Authors	4 egg-cases; right ovary full of ripe ova.
23	5-5-43		.	Do. .	Do.	⎫ No egg - c a s e s
24	5-5-43			Do	Do. .	⎬ ovaries reduced in size; uteri with ⎭ flaccid walls.
25	15-12-43			Do.	Do.	No e g g - c a s e s only the right ovary with round greenish ova, about 5 mm. in diameter.
24	20-1-44	87	38	Do.	Do.	2 e g g - c a s e s right ovary in vigorous state of activity.
25·5	20-1-44	83	40	Do.	Do. .	1 complete egg-case and 2 incomplete egg-cases in nidamental glands.
[26·5	6-3-44	86	38	Do.	Do.	2 egg-cases.
25	6-3-44			Do.	Do. .	No egg - c a s e s; right ovary with sm a l l-s i z e d eggs. Only one egg 24 mm.× 20 mm.
[21·5	Dec. '37	60	25	Malabar Coast.	Aiyar and Nalini.	2 e g g - c a s e s; o v a r y v e r y active.
[22·5	Dec. '37	.	.	Do.	Do.	No egg - c a s e s; ovary active.
26·5 (5 Speci-mens.)	Feb. and Mar. 1937-38.	80	40	Madras	Do. .	⎫ One, two or three egg-cases in each uterus and
29	31-1-38	80	40	Do.	Do. .	⎬ ovary in a high state of ⎭ activity.
	Jan. and Feb. 1913.	78	36	Do.	Sundara Raj	
	Do.	70	32	Do.	Do.	

The foregoing table makes it clear that, as on the Madras coast, the species spawns in the Bombay waters from January to March or even April. Further, in view of the fact that the incubation period in this form is about 2½ to 3 months (Aiyar and Nalini, 1938), the latter conclusion is further supported by the fact that free swimming, recently hatched young ones about 12·5 cm. to 17·5 cm. in total lengths (often unbanded) are available in Bombay during April, May, June and even a s late as early July.

Another important fact emerging from a scrutiny of the table is that fishes of nearly the same size, namely, 23″ to 26·5″ in total length, and from the same locality (Bombay) are capable of producing egg-cases of conspicuously different sizes, 70 to 87 mm. long and 35 to 38 mm. broad (Pl. I, fig. 1). This inequality of size of the egg-cases from the same locality does not therefore support Aiyar and Nalini's view that there are definite regional differences in the size of the egg-capsules of this species from the Madras and Malabar coasts, a view which would accord with Clark's (1922) deductions regarding similar differences in the Mediterranean and Plymouth capsules of the same European species of skates. A comparison of the lengths of the parent fishes and of the sizes of their egg-cases from all the three regions would rather suggest that the differences are due to individual variations only and that there are hardly any distinctive differences in the size of the mature fish from the two coastal regions of the same Indian waters, differences such as are suggested by Clark (*op. cit.*) for the same European species in waters so widely separated as the Mediterranean Sea and the English Channel. It may also be noted that the quality and quantity of food available to the mother during the breeding season at a particular locality may have- considerable influence on the growth of the intra-uterine phases of the young.

EMBRYONIC STAGES OF *Pristis cuspidatus* LATH.

Embryonic 33 mm. stage (Shark-like Stage).—Parent ♀ : Total length including saw—8 feet 2 inches ; App. wt.—300 lb. ; 15th March, 1943.

Both ovaries were present, each containing a number of round yellow ova about 22 mm. in diameter. Each epigonal organ, about a foot long, was a thick, strap-shaped, greyish, pinkish band merging anteriorly into the substance of the ovary on its dorsal aspect. Each nidamental gland (Text. fig. 3a) was a large, horizontally ovoid body, made up of thick dorsal and ventral bands of tough, greenish grey spongy matter, thickly covered over with connective tissue. These bands overlapped each other laterally and thus caused the rather thickened lateral prominences. Each gland measured 50 mm. × 30 mm. × 10 mm. The uteri occupied the major portion of the abdominal cavity. Each measured 10″×5″×1·75″ and possessed firm muscular walls. On opening the uterus, its entire cavity was seen to be compactly filled by a completely whole snuff coloured, rather opaque egg-capsule enclosing embryos. On removing the egg-capsule the peculiar character of the uterine mucous membrane became apparent. Except for a short zone at the distal end of the uterus, the entire mucous membrane was raised into a number of prominent, long, close-set plaits or frills running obliquely, each frill
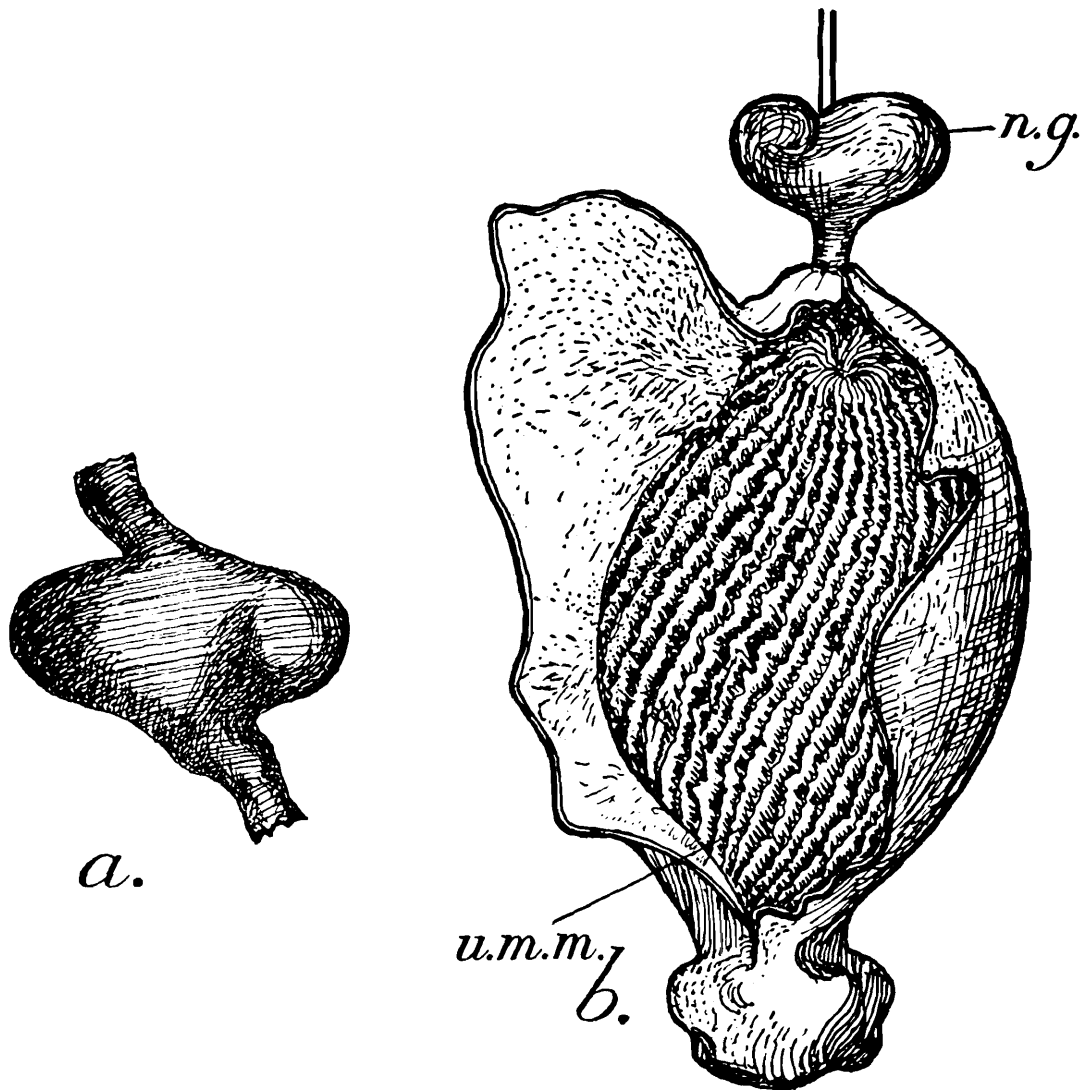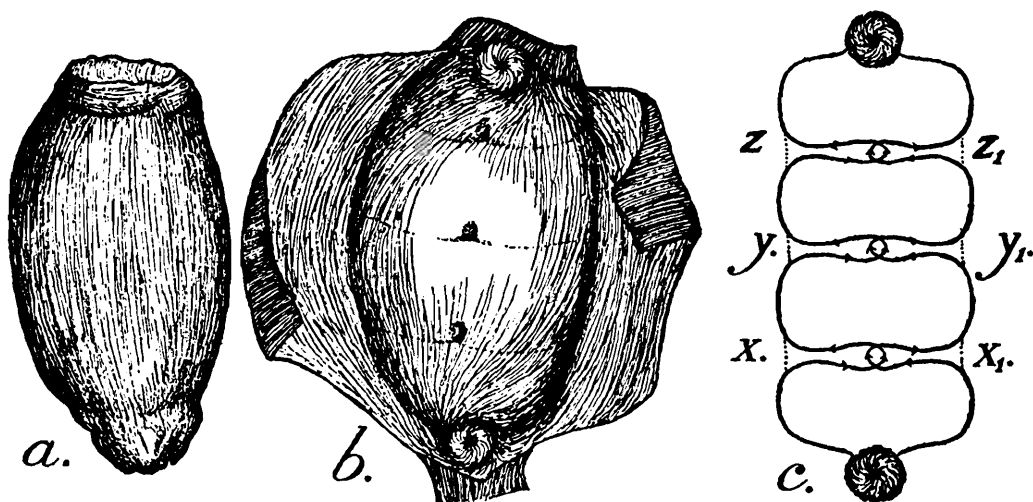
Text-fig. 3.—*Pristis cuspidatus* (Lath.) (33 mm. stage).

a. nidamental gland ; *b.* uterus opened to show the plaited and frilled character of
   the uterine mucous membrane.
n.g.=nidamental gland ; *u.m.m.*=uterine mucous membrane.

being about a centimeter broad. The frills were plain and membran-
ous and moderately vascular (Text-fig. 3*b*). Examination of the egg-cap
sule (Text-fig. 4*a*), which measured as much as the gravid uterus, showed
that its outline was quadrangular, and its contour similar to that of the
four-cornered egg-case of *Chiloscyllium griseum*. The capsular wall
was not, however, tough and horny as in a typical egg-case of an ovipar-
ous form, but quite soft and pliant. Slight resilience and firmness and
an irregular rugosity were apparent, however, in two band-like areas at
either end, where the walls of the sac fused to close the sac. The posterior
end of the sac was narrower than, and not as thick as, the anterior.
Both these ends were seen to be plugged internally with a profuse
quantity of transparent, gelatinous albuminous mass. Except for the
two thick, rather resilient bands mentioned above, the wall of the egg-
capsule was generally thin, soft and fragile. Its appearance externally
was bright and glossy, and internally dull and whitish yellow. Examina-
tion of the structure of the capsular wall revealed an extremely thin
and delicate brown membrane, reinforced internally by a uniform deposi-
tion of dull-coloured shelly secretion that imparted thickness to the wall.

The secretion formed a uniform coating on the internal surface of the capsular wall. It was not, however, hyaline in appearance, but displayed a conspicuous longitudinally fibrose configuration, suggesting that it was formed as the result of coalescence of the thin, narrow, longitudinally running streaks of shelly secretions, whose plasticity in the fresh condition helped in the formation of an entire sheet. Such a mode of secretion of the shelly matter in this form conforms with that in the oviparious form *Chiloscyllium griseum*, although, however, it may be stated that the longitudinal striations in the present case did not, at this stage, display a beaded appearance as in the latter.

Striking peculiarities were noted in the internal structure of the egg-capsule. The wall of the egg-capsule was seen to be lined internally, throughout, by a very thin, delicate membrane which was colouless, transparent and gelatinous in consistency. It closely adhered to the capsular wall on its internal surface, but there was no organic connection between the two and the former could easily be peeled off from the latter without any rupture of the tissues. This internal membranous layer greatly resembled the delicate and hyaline shell membrane of a typical viviparous selachoid, except for the yellow colour and iridiscence of the latter. Its disposition in the egg-capsule under consideration could well be compared with that of the thin and soft shell membrane on the inside of the shell of a hen's egg. A further striking peculiarity was, that the cavity of this internal membranous pouch was not whole, but was divided into compartments placed transversely one behind the other and each lodging a tiny embryo with its huge, massive, yolk-sac (Text-fig. 4b). There were four compartments and four embryos in each of the egg-cases of the specimen under consideration. Ed Graeffe has recorded the presence of such compartments in the egg-cases of the ovo-viviparous selachoid *Acanthias vulgaris* B. P.

TEXT-FIG. 4.—*Pristis cuspidatus* (Lath.) (33 mm. stage).

a. egg-case ; *b.* egg-case opened to show arrangement of compartments within the egg-case ; *c.* schematic representation of compartment-formation in a typical ovo-viviparous egg-case. Note that complete fusion of layers at xx, yy, zz, except in the region of the central twists results in the formation of bilaminated inter-compartmental partitions producing the effect shown in *b.*

The mode of formation of these compartments was unique (Text-fig. 4*b, c*), being not observed by us in any other elasmobranch so far studied and is, we believe, recorded here for the first time Both at the anterior and posterior end of each compartment, a sharp spiral twist was notice-able in the compartmental walls, the twists at the extreme anterior and posterior ends being traced into rounded, spirally formed rather opaque discs of the gelatinous membranous walls. Careful examination of these spiral twists and the partitions intervening between the compart-ments revealed the mode of formation of the latter. The soft membranous walls of each compartment crossed each other, centrally towards the anterior end of the compartment, where they whirled spirally so as to effect a closure of that compartment and then extended untwisted but crossed so as to form the walls of the next compartment. At the anterior end of that compartment, they would again cross each other, twist spirally and so close it and proceed further to form the walls of the next compart-ment ; and so on till the end. The intervening partitions were thus formed as the result of fusion of the crossing layers and extended through-out the width of the egg-case. Evidently, the partitions had double layers while in other regions, the wall of this internal pouch consisted of one layer only (Text-fig. 4*c*). In this mode of formation of the com-partments, it may be imagined that, if the spiral twists were uncoiled, the compartments would disappear and the entire cavity of the pouch be turned into one spacious compartment.

The outsize of the egg-case (which is very nearly that of the uterus itself) containing, as it did, four large eggs, in comparison with that of the nidamental gland, leads us to the view that the egg-case is formed in parts and is not secreted as a whole, as is believed by Nalini (1940) in the oviparous form *C. griseum*. It would appear that in a typical ovo-viviparous form such as *P. cuspidatus*, the posterior portion of the egg-case and a major portion of the hindmost compartment are formed first (complete formation so far as the detailed structural composition is concerned), the latter receives the fertilized egg wrapped in albumen and then closes by the spiral-twisting process described above. This portion of the egg-case would slip down into the uterus, while simul-taneously, the second compartment and a corresponding portion of the egg-case would be secreted in continuity with that of the previous one The second compartment, on receiving the second zygote would close and the partially formed egg-case slip further down. The process would continue till finally the last zygote would enter the last compartment (the foremost), to be closed finally and sealed with a profuse quantity of gelatinous albumen. Such a mode of formation of the egg-case would naturally imply its formation from the posterior end forwards, so that the zygote near the cloacal end of the egg-case would be the first to be received and the zygote at the other extremity, the last to enter the shelly capsule.

This view about the part-formation of the egg-capsule in the species under consideration appears to be the only plausible one in view of the marked disparity in the sizes of the whole egg-capsule and the nidamen-tal gland and is strikingly similar to the theory of part-formation of the egg-case in the oviparous form *C. griseum*.

The following are the dimensions of an embryo (in mm.) :—

Total length	.. 32-34
Length of umbilical cord	15
Diameter of yolk-sac	125

The embryos (Text-fig. 5a) are typically shark-like at this stage and there is not even a trace of the characteristic ' sawfish '-like rostrum. The snout is, at this stage, only a soft and blunt downward extension of the soft and swollen head region.   On the ventral aspect of this snout, the nostrillar pits show themselves as mere notches on the lateral borders, immediately in front of the mouth.   The eyes are prominent, with round, opaque lenses and the ocular pigment has not yet appeared. Immediately behind the eyes and in front of the first gill-opening, is the diamond-shaped spiracle, which is devoid of spiracular filaments. The visceral region is still in a premature condition of development.
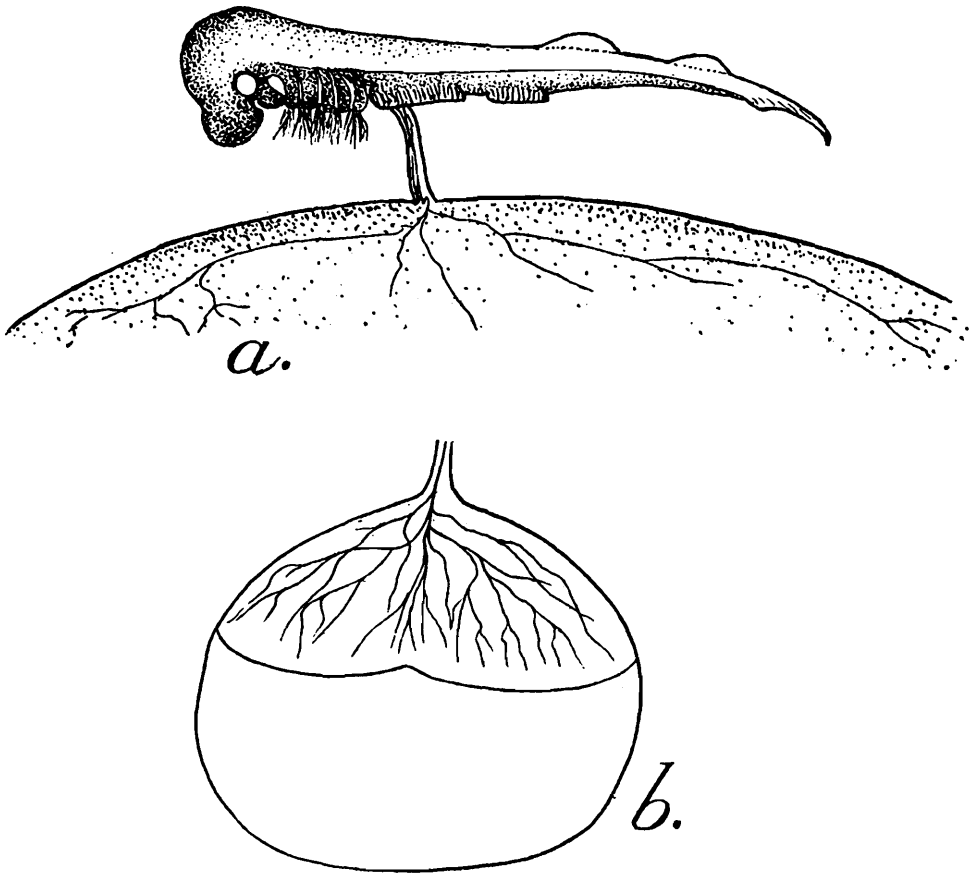
Text-fig. 5.—*Pristis cuspidatus* (Lath.) (33 mm. stage).

a. 33 mm. embryo with yolk-sac only partly depicted.   Note the rudimentary gill region ; b. reduced view of the yolk-sac showing vascularisation only half-way down its walls.

It is not yet compact nor incorporated completely in the embryonic body.   The visceral arches. are free, still unlined by the lateral and ventral body walls.   They extend as separate more or less vertically disposed   dorso-ventral loops, from the angles of which, bunches of

external branchial filaments issue forth. Short and rudimentary internal gill-filaments have just made their appearance as slight projections of the posterior mucous lining of each of the visceral arches. All the fins have made their appearance in their respective adult positions, but are in a rudimentary state of development, being merely soft, curved flaps supported internally by delicate fin rays. The pectoral fin originates slightly behind the last gill-arch. It is an elongated, curved flap with a broad base and no free anterior portion. Nor is it yet brought into relation with the gill-region. Slightly behind the posterior termination of the pectoral fin originates the pelvic fin. It is about half to two-thirds the size of the pectorals. The first dorsal fin arises above the posterior third of the pelvic base and is nearly equal in size to the second dorsal which is located behind the former at a distance of about the basal length of either. The vertical fin membrane has not yet vanished completely. The caudal fin is typically lanceo'ate and the sub-caudal lobe is not yet clearly marked out. It is seen to terminate in a small and delicate curved piece. The sexes of the embroys are not yet distinguished externally. Nor has the process of general pigmentation started and the embryos are generally cream-coloured, being crimson only in regions of high vascular activity.

The yolk-sac is a huge, massive bag full of pasty, yellow yolk, connected with the embryo by a short thick umbilical cord. Its walls, which are extremely thin, are, at this stage,' vascularised only half way down their extent, the lower half being still without blood-capillaries (Text-fig. 5*b*). A vascular ring, formed by the union of the curved extensions of the umbilical vessels, runs round the sac equatorially, bisecting as it were the globe. The blood-capillaries of the vascular regions are wavy in outline. The umbilical cord consists, as in Selachoids, of an artery, a vein and a very fine capillary like yolk-duct, all enclosed in a thick, gelatinous sheath of connective tissue, reflected from the upper pole of the sac. The latter does not, however, possess any appendicula nor any superficial markings.

Embryonic 53 mm. stage (Proboscid stage) :—Parent ♀ : Total length including saw—9 feet, 3 inches ; App. wt.—400 lb. ; 4th February, 1944.

Each ovary of the above female was 6″×2·2″×0·5″ in size, studded with small, yellowish pink ova, the largest being about the size of a grape. (12 mm.). The other reproductive organs presented features more or less similar to those described before. Each uterus in the present case measured 9″×4·5″×2·3″ Both the uteri contained completely whole, brown egg-capsules enclosing embryos with large massive yolk-sacs. Superficially, these egg-capsules resembled, in toto, the ones described in the earlier stage, and the characteristic internal compartmental arrangement was found to persist even at this stage of pregnancy. The disposition of the embryos in the compartments was, however, slightly oblique now and the inter-compartmental partitions were partially hidden from view. There were three compartments and three embryos in each egg-case. The uterine mucous membrane was besmeared with a sticky cream-coloured fluid secreted by the uterine glands. Either

all the six embryos were female or their sexes had not yet been differentiated externally. They were all of nearly the same dimensions namely—

Total length from tip of head to tip of caudal fin	53 mm.
Length of caudal fin	7 „
Length of rostral prolongation from its root	13 „
Width of rostral prolongation at base ..	3 „
„ „ „ further up	2 „
Length of umbilical cord	80 „
Diameter of yolk-sac ..	100-105 „
Length of branchial filament .	15 „

The embryo (Text-fig. 6*a*, *b*) is soft, ivory-coloured, devoid of any pigment and scales. The presence of a prominent rostral prolongation or proboscis has already imparted to it the " sawfish " character, but unlike as in the adult, the rostrum is still soft and without the "lateral teeth. " Even rudiments of these have not appeared at this stage. The rostrum is rather wide at the base and in view of the still downward extension of the snout region (not anterior such as would be as the result of dorso-ventral flattening of the head and snout region) it has a curved and dorsiflexed disposition, and not antero-posterior as in the adult condition. The head region is swollen and rounded and not flattened dorso-ventrally. The eyes are small, resembling studs and are directed antero-laterally:
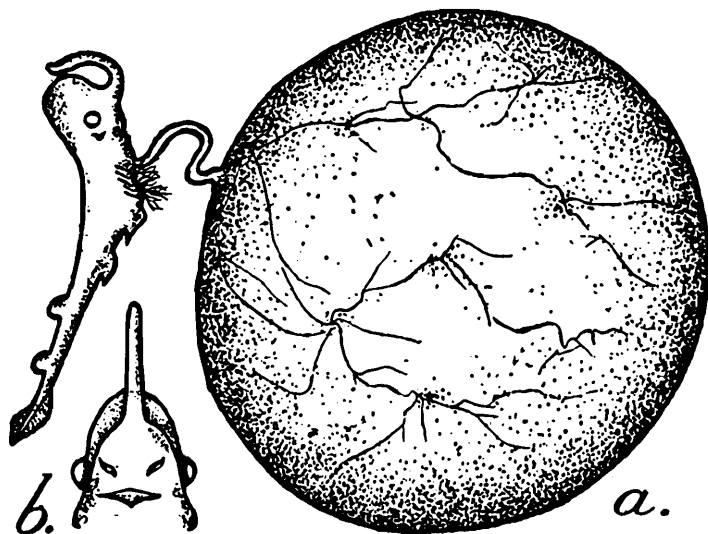
TEXT-FIG. 6.—*Pristis cuspidatus* (Lath.) (53 mm. stage).

a. 53 mm. embryo with umbilical cord and yolk-sac. Note that its vascularisation is total at this stage ; *b.* ventral view of rostral and head regions.

The lens has a ring of pigment all round. The spiracle, directed postero-laterally and now in the form of an oblique slit, is situated behind the eye at a distance of half the orbital diameter. It has a thick anterior valve, but there are no spiracular filaments. Ventrally, the depressions of the nostrils, with characteristic valves, are distinguishable just in front of the fairly wide mouth opening. The visceral region is now well incorporated in the body. The gill-openings are V-shaped and extend latero-ventrally, not having yet assumed a complete ventral

disposition. Through each issues out a bunch of crowded, crimson-colour-ed, blunt-tipped branchial filaments. *Fins.*—In accordance with the still lateral disposition of the gill slits, the pectoral fins too are not yet completely fused with the lateral body-wall to complete the 'disc' They are ventro-lateral in their disposition and nearly the anterior third of each is by now free and in the form of a narrow constricted knob. In the recess between this free anterior part of the pectoral fin and the sides of the body are located the last two or three gill-slits. The fore-most point of the pectoral fin reaches as far as the second gill slit. Both the dorsal fins are equal in size and similar in shape. The first originates slightly ahead of the posterior end of the pelvic base. The pelvic fins are located almost in the middle of the body excluding the rostrum. The caudal fin is lanceolate in shape and has already the characteristic lateral keels. The subcaudal lobe is not distinguishable even at this stage.

The yolk in the yolk-sac is still viscous and has not been liquified to any great extent. The vascularisation of the yolk-sac walls is, however, total at this stage. The arrangement of the blood capillaries on its basal wall is peculiar. Here, they are arranged radially and converge towards the centre to form a central, circular vascular ring of a fairly big size. From this ring start two prominent vessels, an artery and a vein which rise up the walls of the sac to ultimately enter the umbilical cord. The upper pole of the yolk-sac tapers gradually to form the yolk-duct which, too, passes up into the umbilical cord. But for its greater length, the latter is exactly similar to the cord described in the previous stage. The connections of the umbilical vessels within the embryonic body are characteristic of the elasmobranchs in general, while the yolk-duct joins the upper end of the colon on its dorsal aspect without the intervention of an internal yolk-sac.

The reproductive conditions of a female measuring 8' 1" in total length and landed on 2nd March, 1944 corresponded exactly with those noted for the stages previously described. As in the 53 mm. stage, each egg-case in the present instance also bore three compartments and three embryos. The latter measured 43 mm. in total length and re-presented obviously a stage of embryonic development, intermediate between the 33 mm. and 53 mm. stages. As in the 53 mm. stage, a definite rostrum has been formed, but it is only 3 mm. long, hardly extending beyond the head region. Also, like the 53 mm. stage, two strips of ocular pigment are present even at this stage. The gill region of the embryos is, however, similar to that of the 33 mm. stage, not being yet incorporated within the body and bounded by ventral and lateral body walls. The foremost point of the pectoral fin reaches at this stage, as far as the fourth visceral arch. The vascularisation of the yolk-sac has progressed only two-thirds down its vertical walls and even in this respect the condition is intermediate between the 33 mm. and the 53 mm. stages.

Advanced stages.—Southwell (1910) has described the gravid condi-tion of a female sawfish (*P. cuspidatus*), 15·5' in length, captured off the Ceylonese Coast. He and later Hussakof (1912) have described the 14" embryonic stage obtained from the above mentioned female. The

internal anatomy of these embryos has been described by Southwell and Baini Prashad (1919). Obviously, these embryos had, at this stage, reached a fairly advanced stage of development and resembled a fully-grown sawfish except for the fact that they still possessed yolk-sacs and umbilical cords and that the 'teeth' on their saws had not yet cut through the membrane enveloping them   The fact that their yolk-sacs measured 2·75″ in diameter and evidently still contained some yolk suggests, however, that they would have to remain some more time in the wombs before birth.

The stage described by us below represents a much more advanced condition which could be regarded as only slightly prior to the condition of parturition.

Parent ♀ : Total length including saw—8 feet 11 inches; App. wt.—
        400 lb.; 6th March, 1944.

The ovaries of this female were small and contained small ova, the biggest about a centimeter in diameter and containing yellow yolk. The left uterus was empty and contained a few fragmentary pieces of the shell membrane (egg-case), while the right contained three foetuses, besides a small quantity of the disintegrating shelly substance.   Two of the foetuses were male and one female.   The embryos of the left uterus had probably been discharged into the sea.

The foetuses had the following dimensions, in inches (Pl. I, fig. 3) :—

Total length including saw	21·5
Length of saw	6·6
Toothed portion of the saw	5·4
Maximum width of the saw	1·2
Width of the saw at lower tooth-less portion	0·75
Width across pectoral expansion	6·5
Yolk-sac	1·2 × 0·6
Yolk-stalk	2·5
Biggest lateral tooth on saw	8 mm. × 2·5 mm. at base.

But for the presence of short yolk-stalks and reduced yolk-sacs and the fact that the rostral teeth (quite hard and sharp) have not even yet cut through the thick and tough membrane enveloping them, the foetuses closely resemble the adult fish. The characteristic dorsal pigmentation is now present, but the body is not yet covered over uniformly or extensively with the dermal denticles. These latter have developed only in certain regions such as both the surfaces of the toothed portion of the saw, the anterior borders of the pectorals, pelvics and the dorsals, the upper border of the caudal and the anterior border of the subcaudal lobe. The yolk-sac contains very little yolk and its blood capillaries have nearly atrophied. Nor is there any blood vessel in the yolk-stalk. There is, however, a fairly big internal yolk-sac containing a quantity of thin and liquified yellow yolk and it appears that the foetus would be borne in the uterus for yet some time before expulsion.

B

Our observations on the dimensions of the newly born young ones of this species (with very much reduced, stublike, yolk-stalks and sacs hardly half a centimeter long) reveal that the foetuses are born when from 17″—24″ in total length. It would appear that the rostral teeth are cut through the membrane only when the foetuses are free, as otherwise, parturition would indeed be a difficult process for the parent because of the sharp and hard teeth in even the prenatal stages.

EMBRYONIC STAGES OF *Rhynchobatus djiddensis* (FORSK.).

Embryonic 45 mm. stage (Shark-like stage).—Parent ♀ : Total length 9 feet 9 inches ; App. wt.—400 lb. ; August, 1943.

Both ovaries of the female were in a vigorous state of activity being packed with variously sized, round eggs, measuring from 1·5 cm. to 3·75 cm. in diameter. The smaller eggs were yellowish, cherry red in colour, while the bigger ones were yellow, with an extremely delicate network of blood capillaries spread on their membranes. The nidamental glands presented features exactly similar to those of *P. cuspidatus.* Both uteri were gravid, each measuring 9″×5·4″×2·5″ The walls of the uteri were firm and elastic. Each uterus on being cut open was seen to be compactly filled with a turgid, completely whole shell-membrane sac exactly similar to the egg-capsules of *P. cuspidatus.* Its cavity too, when slit open, was seen to be partitioned in a manner similar to that in the 33 mm. stage of *P. cuspidatus.* There were three compartments and three embryos in the egg-case of the right uterus and four in the left. Either all the embryos were female or the sexes were not yet distinguished externally.

On removing the embryos and the shelly egg-cases, the uterine mucous membrane was seen to bear all over its face longitudinally directed plaits and folds very similar to those in *P. cuspidatus.*

The following are the dimensions of embryos (in mm.) :—

Total length	..	44-48
Length of caudal fin		7
Length of umbilical cord	..	.. 38
Diameter of yolk-sac		125
Length of branchial filaments	..	17-18
Length of snout projection beyond the head region		3
Interorbital distance		8-9

The head region of the embryo (Text-fig. 7a, b) is soft and swollen, oval in outline and bears two big and round studlike eyes laterally. Anteriorly, it descends almost vertically downwards to merge into a short, bluntly triangular soft snout. The latter bears ventrally two prominent nostrils immediately in front of the widely open, triangular mouth. The anterior and posterior nasal valves of the adult condition are present even at this stage, though in a rudimentary condition. The ovoid spiracles, with thick anterior valves are situated immediately behind the eyes, but on a lower level. They are without any spiracular filaments. The gill region is still in a primitive state of development and very similar to that of the 33 mm. stage of *Pristis cuspidatus.* It
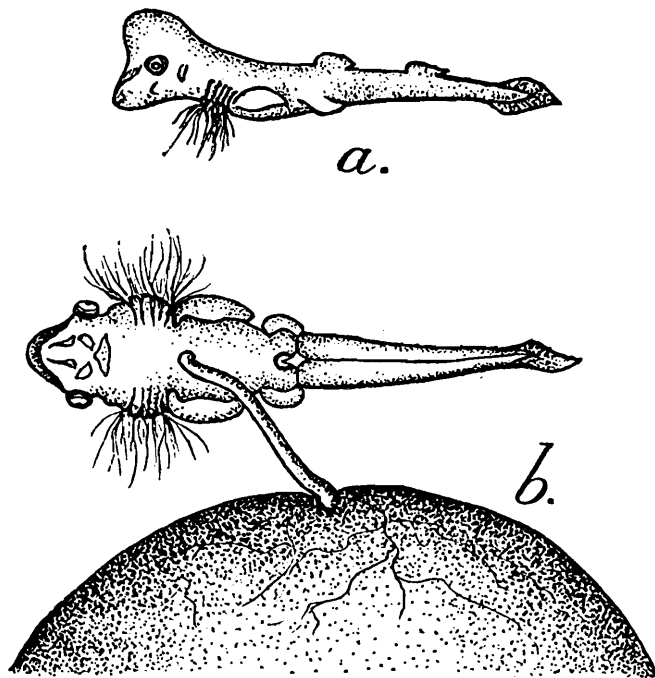
TEXT-FIG. 7.—*Rhynchobatus djiddensis* (Forsk.).

a. profile of 45 mm. embryo ; b. ventral view of the same with umbilical cord and yolk-sac (depicted partially).

is not yet compacted and incorporated within the body and not yet bounded by ventral and lateral body walls. The visceral arches are free and bunches of branchial filaments issue out from their angles. All the fins are developed in their respective adult positions, though differing considerably from their respective adult shapes. The dorsals are nearly semi-circular, the caudal is lanceolate and the pelvics are reniform. The condition of the pectorals at this stage deserves special mention. They are elongated antero-posteriorly, have broad basal attachments and their narrow anterior prolongations are free and not yet fused with the sides of the body to give rise to the ' disc ' In the recesses between these and the sides of the body lie the hindmost gill-slits with their bunches of branchial filaments. It is only with sub-sequent growth and a total ventral movement of the gill-slits that these ends of the pectorals would coalesce with the sides of the body to form the disc typical of the Batoids. The embryo, is at this stage, ivory yellow in colour, suffused with crimson in regions of high vascular activity. The process of pigmentation has not yet begun, nor have scales appeared and the embryo is quite soft. The trunk is crossed ventrally by a single blood vessel in the median line.

The yolk-sac is a huge, rounded, massive structure full of viscous yellow yolk. The walls of the yolk-sac are very thin and highly vascular, the blood capillaries mounting up its walls in a zigzag manner and running more or less parallel to each other. Basally, however, they run radially to join a thick vascular ring placed centrally. The yolk-sac capillaries ultimately join to form the umbilical vessels, which pass up into the umbilical cord. The latter also contains, besides the vessels, the yolk-duct. The cord has a gelatinous, connective tissue sheath, which is seen to be reflected from the apical walls of the yolk-sac. The

connections of the umbilical vessels within the body of the embryo are typical and the yolk-duct joins the colon without the intervention of a yolk-sac at this stage of development.

The embryonic stage described above corresponds in many respects with the 36 mm. stage of *Rhinobatus columnae* described by Southwell and Prashad (1919) and is also similar to the 43 mm. stage of *Pristis cuspidatus* dealt with by us before.

However, a few points of contrast between the 45 mm. stage of *R. djiddensis* and the 43 mm. stage of *P. cuspidatus* invite comment.

The bodies of the 45 mm. embryos of *R. djiddensis* are stouter and more thickest than those of the 43 mm. embryos of *P. cuspidatus*. Also, while in the embryos of *P. cuspidatus* a definite rostral prolongation is present, already imparting to it the typical " Pristis " character, the snout region in *R. djiddensis* has hardly flattened dorso-ventrally so as to assume its specific shovel-shaped charcater. Further, while at this stage in *R. djiddensis*, the vascularisation of the yolk-sac is complete, that in the yolk-sac of *P. cuspidatus* has progressed only two-thirds down its vertical walls, it being total at only the 53 mm. stage.

The primitive, lateral disposition of the visceral region and the in-completeness of the ' disc ' in the 45 mm. stage of *R. djiddensis* and in the 33 mm., 43 mm. and 53 mm. stages of *P. cuspidatus* impart to these stages a typical ' shark-like ' character and definitely reveal their shark-like ancestry and the assumption of the Batoid condition as purely of a secondary nature.

Embryonic 150 mm. stage—Parent ♀ : Total length—8 feet 8 inches ; App! wt.-200 lb. ; 18th March, 1942.

The reproductive organs of this female presented features quite similar to the ones described previously except for the fact that the ovarian eggs in the present case ranged from 2·5 cm. to 6·25 cm. in diameter. Also the embryos in each of the uteri were no longer enclosed in the shelly egg-capsules referred to in the previous stage but lay naked in its cavity, there being five embryos in either uterus. Disintegrating fragments of the capsule could, however, be discerned in the uterine cavity and the uterine mucosa was besmeared all over with a pinkish creamy liquid not noted in the earlier stage described before. Three of the embryos in the right uterus were male and two female while in the left one was male and four female. One of these had the following dimensions (in mm.) :—

Total length	150
Maximum width across the pectoral expansions	55
Length of snout in front of interorbital line	30
Interorbital distance	11
Length of the umbilical cord	62·5
Diameter of yolk-sac	106
Length of branchial filaments	1·8 to 2·5
Length of clasper	4

The embryo (Text-fig. 8 ; Pl. I, fig. 4, *upper right corner*) has by now assumed the characteristic ray-like appearance and in external morphology resembles the adult in most respects.   The following differences may, however, be noted.   The embryo is still ivory yellow in colour and the process of pigmentation has not yet commenced.   The dorsal surface of the trunk is, however, marked by four dark crimson lines running parallel to each other.   The skin is soft and there is no scale or tubercle formation yet.   The gill region is slightly inflated and bunches of long, thread-like filaments still issue out of the gill slits.   The spiracles, too, are very narrow and not yet fully opened out.
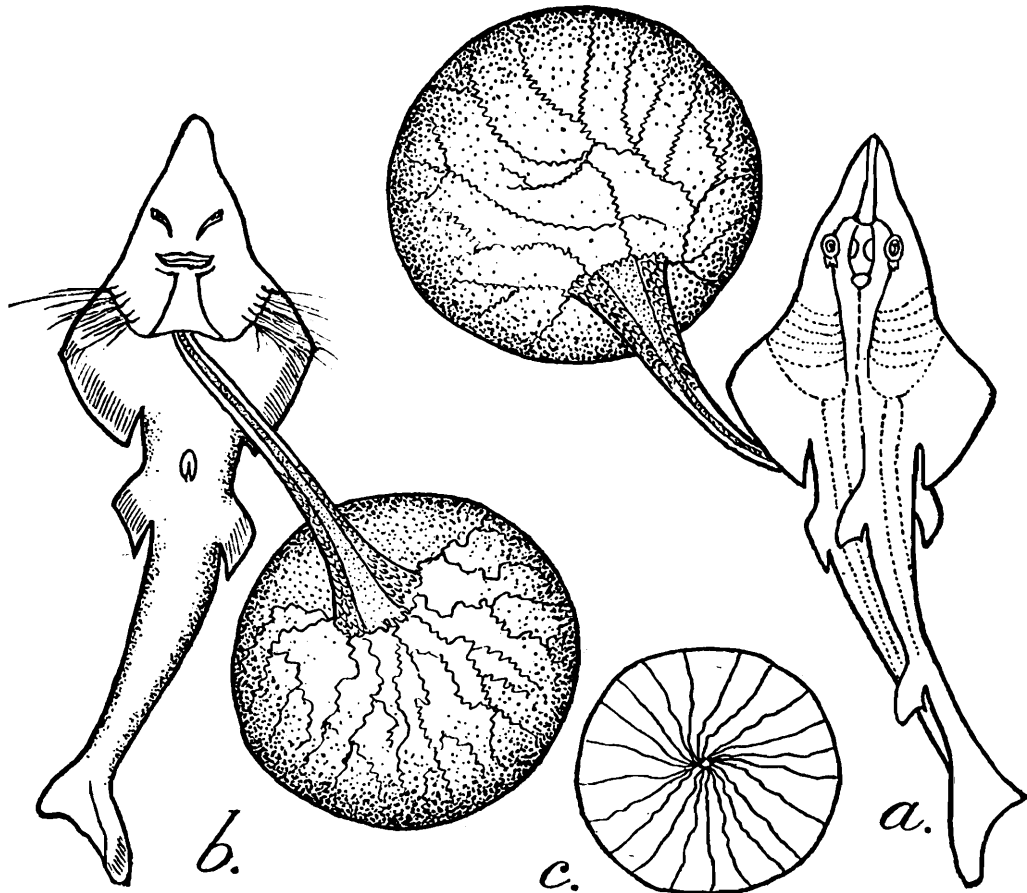
TEXT-FIG.   8.—*Rhynchobatus djiddensis* (Forsk.) (150 mm. stage).

a. dorsal view of embryo with umbilical cord and yolk-sac ; *b.* ventral view of the same ; *c.* radial arrangement of blood capillaries on the basal wall of the yolk-sac.

It may be pointed out here that very small yellow fragments of the disintegrating shelly capsule were seen attached to some of the branchial filaments and these could be traced right inside the gill slits also.   It is probable that they served some nutritive function and as such were being injested by the embryo.

The yolk-sac and umbilical cord at this stage are essentially similar to those described in the earlier stage except for the fact that the gelatinous investing sheath of the umbilical cord displays, at this stage, scale-like markings which are seen to extend from the commencement of the cord right up to its mid-length.   The large quantity of yolk in the yolk-sac even at this stage of development suggests that the embryo would grow yet considerably before being born.

Examination of several gravid females at various stages of pregnancy landed at Sassoon Dock, Bombay, from July to October revealed, during the course of embryonic development, the following interesting features.

Embryos ranging from 95 mm. to 157 mm. in total length are alike in development and exactly similar to the 150 mm. stage described above. These stages may well be designated as ' intermediate ' stages of development.

Advanced Stages.—From the 200 mm. stage onwards the embryos may be said to have entered upon advanced stages of development. The progressive characterisation in different stages is described below.

In an embryo at the 200 mm. stage, the branchial filaments are no longer present. The process of pigmentation has started already and the black pectoral spot has appeared. There are no other pigment spots, however, nor has the general grey pigmentation developed. The mid-dorsal and, to a very slight extent, the dorso-pectoral spinous rudiments are apparent at this stage.

At the 235 mm. stage the dorsal surface is slightly tinged with grey and four diffused round or oval white spots are discernible around the black pectoral spot. Faint black pigment spots have also appeared in the orbital regions. The rudimentary markings of the mid-dorsal and dorso-pectoral spines are quite conspicuous now (Pl. I, fig. 4, *middle*).

At the 287 mm. stage, the process of pigmentation has advanced considerably and the dorsal surface tends to become more and more grey. The diffused dark spot and band in the orbital region are very conspicuous now and, in addition to the pectoral spots, round or oblong white spots are also present on the pectoral roots and sides of the body, extending below the first dorsal fin. Further, in addition to the mid-dorsal and dorso-pectoral spinous rows, the orbital row has also appeared. The spiny rudiments at this stage are elongate, slender, yellowish streaks not projecting above the general body surface.

At the 437 mm. stage, the foetuses are more or less perfect replicas of the parent, except for the fact that they still possess umbilical cords and yolk-sacs which are definitely reduced in size. All the dorsal spinelets, too, are grey and slender and just project above the general body surface. There is a huge internal yolk-sac present at this stage (Pl. I, fig. 4, *left*).

A characteristic worthy of note is that every successive advancement in the various stages of development described above is accompanied " pari passu " with a progressive reduction in the size of the yolk-sac, the diameter of the yolk-sac in an embryo of 45 mm. being 125 mm. and that in the yolk-sac of an embryo measuring 437 mm. being 50 mm. only.

The presence of some amount of yolk in the yolk-sac of a foetus 437 mm. in total length and the attainment of a total length of 450 mm. by the smallest free-swimming young one of this species examined by us justify the assumption that the young ones of this species, at birth, measure from 18" to at the most 24" in total length.

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