

## Rays and Skates.

### No. 2.—Description of Embryos.

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With 11 Figures in the Text.

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#### THE MATERIAL.

IN the years 1921 and 1922 a series of experiments on the rearing and hatching of the species of Rays and Skates occurring on the English side of the western end of the English Channel was carried out at the Laboratory of the Marine Biological Association, Plymouth. The egg-capsules were collected from fish landed at the Barbican at Plymouth and from fish captured by the Research steamers, *Oithona* and *Salpa*. The egg-capsules were kept in the circulation of sea-water in the tanks at the Laboratory, and for six species the period of incubation under these artificial conditions was determined.

The experiment of introducing mature-spawning females into the large aquarium tanks was tried successfully. Two species—*Raia brachyura* and *Raia clavata*—thus provided a large number of eggs which, being extruded naturally, had a much better chance of developing than these taken from fish landed at the fish market, the capsules of which had probably been subjected to much handling before transference to the tanks could be effected.

The six species hatched out in the tanks were kept in captivity to describe the specific characters of each species at hatching, and to determine the extent of the changes before the adult characters are assumed.

In a previous publication ("Rays and Skates," No. 1, Clark, 1922) the experiment in rearing and hatching, and the characteristic features of the young forms immediately after hatching, were described in some detail.

The greater part of the large material, however, was set apart for a special study of the developmental stages, and capsules were opened periodically so as to provide a consecutive series illustrating the changes taking place within the capsule. This material was fixed and preserved according to standard methods, so that it might be available for future histological and embryological work.

The descriptions of these changes taking place within the capsules have been given, however, not only as a guide for future histological and embryological work, but to extend the specific descriptions to the embryonic stages.

The sequence of developmental changes appear to be similar for the species occurring at Plymouth, as has been ascertained by examination

### GRAPH 1.

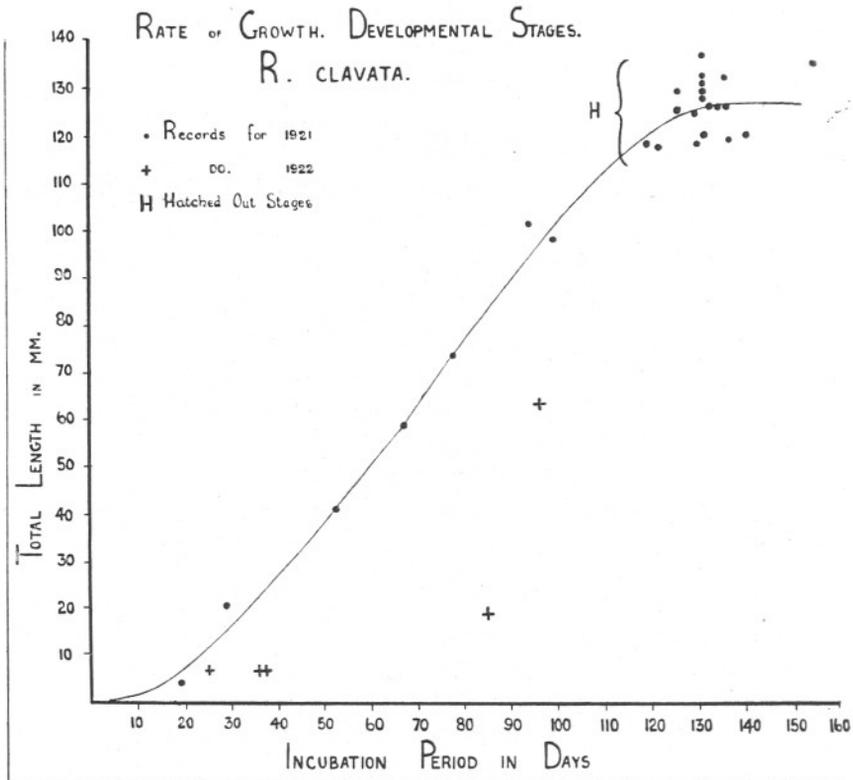


FIG. 1.—Rate of growth. Developmental stages. *R. clavata*.

made at different times during the incubation period. The material is most complete for *Raja brachyura*, and this species has therefore been described in detail. The descriptions of the embryos are given also in tabular form for easier reference.

In "Rays and Skates," No. 1, where the incubation periods for the different species have been given (Clark, 1922, p. 587), it will be seen that there are considerable differences. For example, *Raja clavata*, which

has a mean total length of 126 mm. on hatching, may take 121 to 154 days, while *Raia brachyura*, requiring 189 to 219 days to hatch out, may attain a mean size of 178 mm. The growth rates of these two species throughout the period of incubation are illustrated in the accompanying graphs (Figs. 1 and 2).

The two graphs show similar features after the tail has been straightened out and there is free rhythmical movement. Growth in length (tip of snout to end of tail) is progressive until the approach of hatching. The

GRAPH 2. RATE OF GROWTH. DEVELOPMENTAL STAGES.  
R. BRACHYURA.

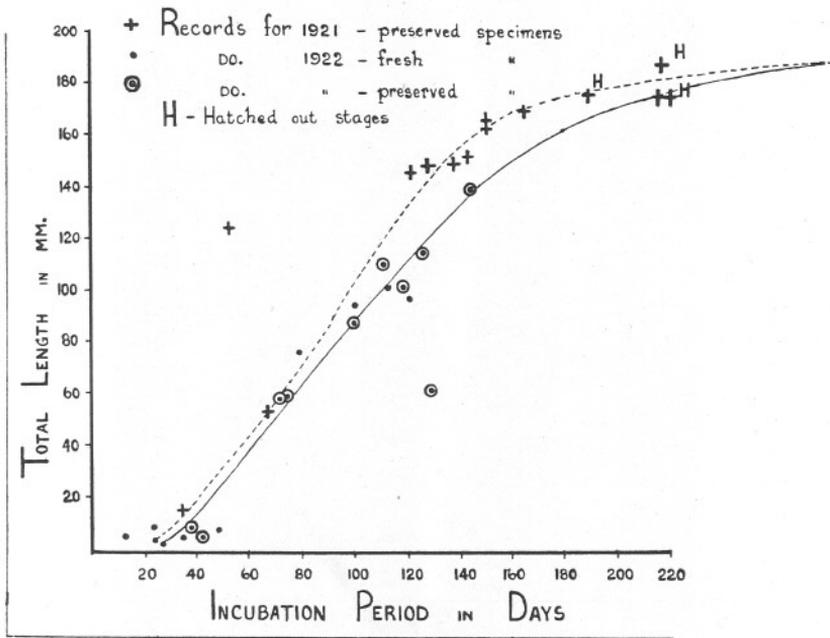


FIG. 2.—Rate of growth. Developmental stages. *R. brachyura*.

graphs in both cases appear to indicate that there is a slowing down at this stage. In "Rays and Skates," No. 1, Clark, 1922, p. 611, the absorption of the post-dorsal part of the tail after hatching has been described, and it is therefore probable that this process of absorption has already begun before the embryo emerges.

From a consideration of the measurements of total length and the distance from the tip of the snout to the origin of the 1st dorsal fin, it is evident that there is a definite differential rate of growth of the parts—pre-dorsal and post-dorsal. After a total length of 58 mm. the actual

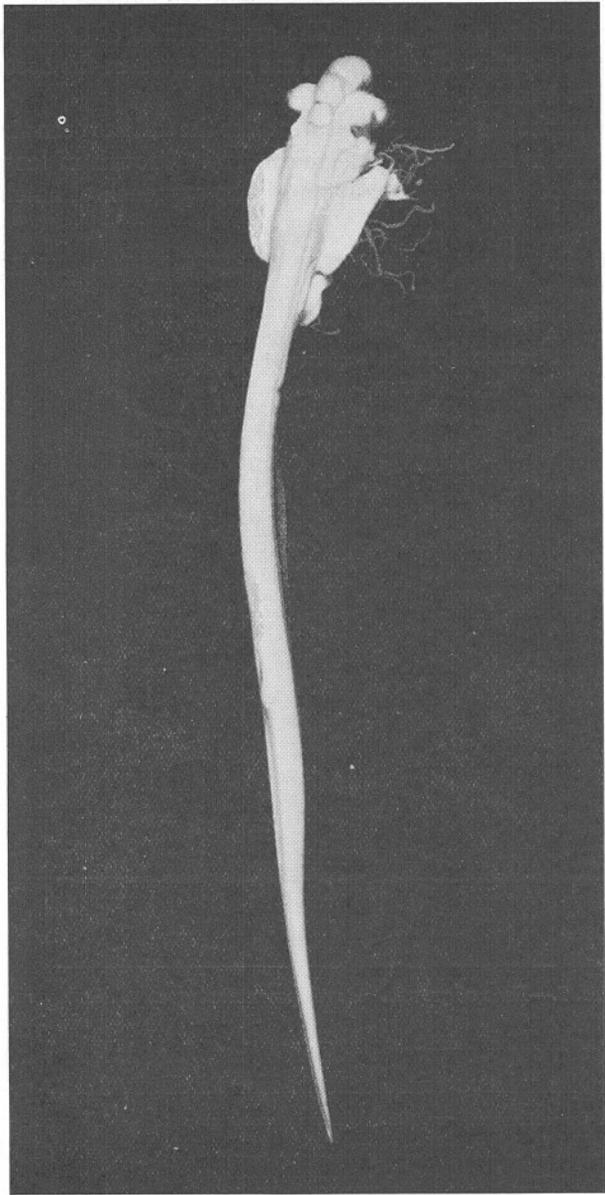


FIG. 3.—*Raia brachyura* embryo, No. 8. Dorsal view.  $\times 2.47$ .

length of the post-dorsal remains practically stationary. On the other hand, the pre-dorsal length shows a progressive growth. The relative position of the dorsal fins during development may be shown by the following percentages:—

<i>Embryo total length</i>	58.5	59	88	110	115	139	146	147	148	150	162	169 mm.
<i>% Length of predorsal</i>	47	47	54.5	60	61.7	64	68.1	65	69.5	70.6	70.3	73.9

DESCRIPTIONS OF EMBRYOS OF *RAIA BRACHYURA* Lafont.

Most of the egg-capsules from which the following stages were taken were spawned in the tanks of Plymouth Aquarium by a large Blonde (*R. brachyura*), which was secured alive for other experimental purposes (*vide* No. 1 of this Series, Clark, 1922, p. 582). Close and constant observation was kept on the appearance and deposition of the capsules, many of which were marked as they protruded from the fish, so that the period of incubation may be taken as fairly accurate.

The embryos were anæsthetised with drops of 90 per cent alcohol, fixed usually in saturated corrosive sublimate, or occasionally in osmic-corrosive, corrosive-acetic, picro-formol-acetic, and corrosive formol, and preserved in 80 per cent alcohol.

1. Segmentation stage. Duration, 4 days (28.5.22–1.6.22). The germinal disc was 5–6 mm. in diameter, and the blastoderm showed a single furrow, cf. Dean "On the Development of Chimaera," Fig. 20.
2. Segmentation stage. Duration, 1 day (19.5.22). The diameter of the germinal ring was 6 × 6.5 mm., and of the blastoderm 4 × 4.5 mm. The disc was nearly circular. This stage corresponds to Balfour's Plate 1, Figure 5. It is more advanced than No. 1, and the earlier cleavages must have taken place while the egg was hung up in the oviduct.
3. Early embryo. Duration, 6 days (1st–7th.6.22). The embryo appeared as a scar, and showed the beginning of the medullary groove. It is equivalent to Balfour's Stage B and Scammon's Figure 4.
4. Early embryo. Length, 3.5 mm. Duration, 24 days (21.4–17.5.22). There is a median constriction, and the medullary folds are closely set, but still separate. This stage is comparable with Scammon's Figures 11 and 12, and lies between Balfour's Stages E and F.
5. Embryo. Length, 4–4.5 mm. Duration, 43 days (30.3–11.5.22). This embryo showed no lateral movement. The head is bent at

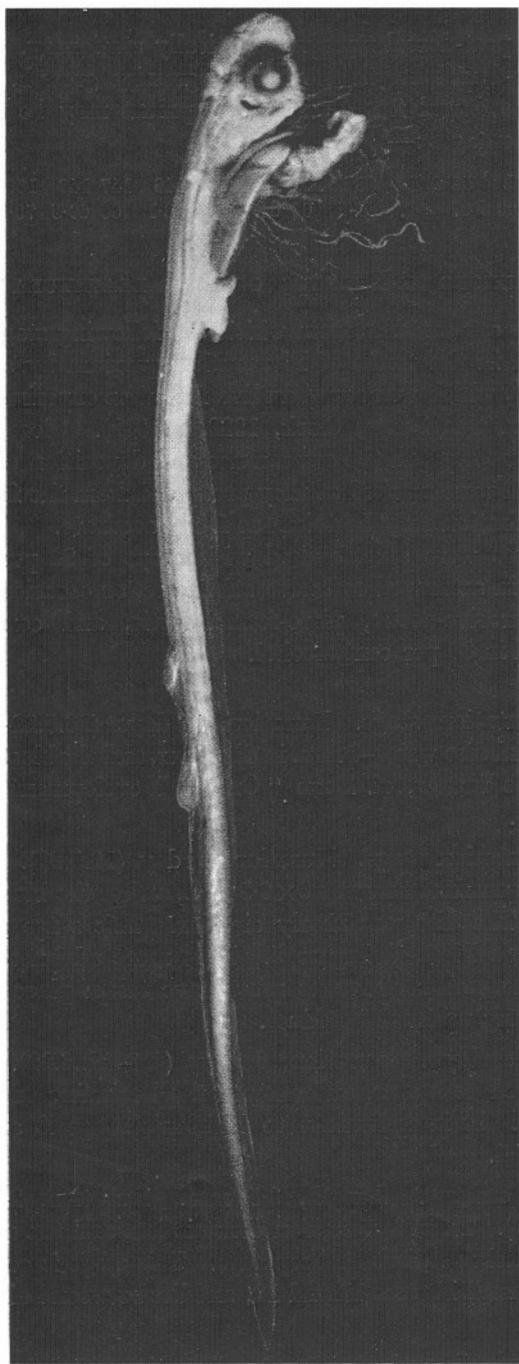


FIG. 4.—*Raia brachyura* embryo, No. 9. Lateral view.  $\times 2.98$ .

less than  $90^\circ$  from the body. The blastoderm shows a pitted structure. It is near to Dean's Figures 38, 41A, and 41B ("Development of *Chimæra*") and to Scammon's Figure 15.

6. Length, 8 mm. (fresh). Duration, 38 days (19.4-27.5.22). The tail was free for part of its length, and the embryo showed rhythmical lateral movement. The end of the tail is slightly knobbed. It appears to correspond to Scammon's Figures 21 and 22. There is a distinct advance in development from No. 5. The tail is longer and free for the greater part of its length, while the lateral movement is a distinct departure.
7. Length, 7.5 mm. (fresh). Duration, 48 days (24.3-11.5.22). The head of this embryo is at right angles to the body. The lateral movement was quite pronounced on extraction from the capsule. Three clefts were visible. It seems to be slightly less advanced than the previous embryo (No. 6) and corresponds to Scammon's Figure 19.
8. Length, 58.5 mm. (preserved). Duration, 72 days (17.6-28.8.22). There is a considerable gap between this stage (Fig. 3) and embryo No. 7, which was due to accident. The gap, however, can be partially bridged by similar material for another species, *R. clavata*, which will be treated in a future contribution. Both dorsal fins are present. The caudal, from the end of the 2nd dorsal fin to the tip of the tail, measured 23 mm. The distance from the beginning of the 1st dorsal to the tip of the tail was 31 mm. The anal fin membrane is continuous except for a concavity, 15-16 mm. from the tip of the tail, which gives it a double appearance. The anterior half is much deeper than the posterior. There is also a membrane extending from the 2nd dorsal to the caudal tip. The mid-brain projects considerably beyond the snout. The branchial filaments are long.
9. Length, 59 mm. (preserved). Duration, 74 days (15.6-28.8.22). This embryo (Fig. 4) is exactly similar to No. 8. The caudal from end of 2nd dorsal to tip of tail measured 23 mm., and the distance from the beginning of the 1st dorsal to the caudal tip 31 mm. There is a slight constriction in the anal membrane at 18 mm. from the tip of the tail. The pelvics are well developed, and the branchial filaments are long. The snout and pectoral are united.
- 9A. Embryo of *R. clavata*. Length, 63.5 mm. (preserved). Duration, 96 days (12.7-16.10.22). This embryo (Fig. 5) is included here to illustrate a further stage in development than the preceding No. 9. The division of the anal membrane is well shown, while

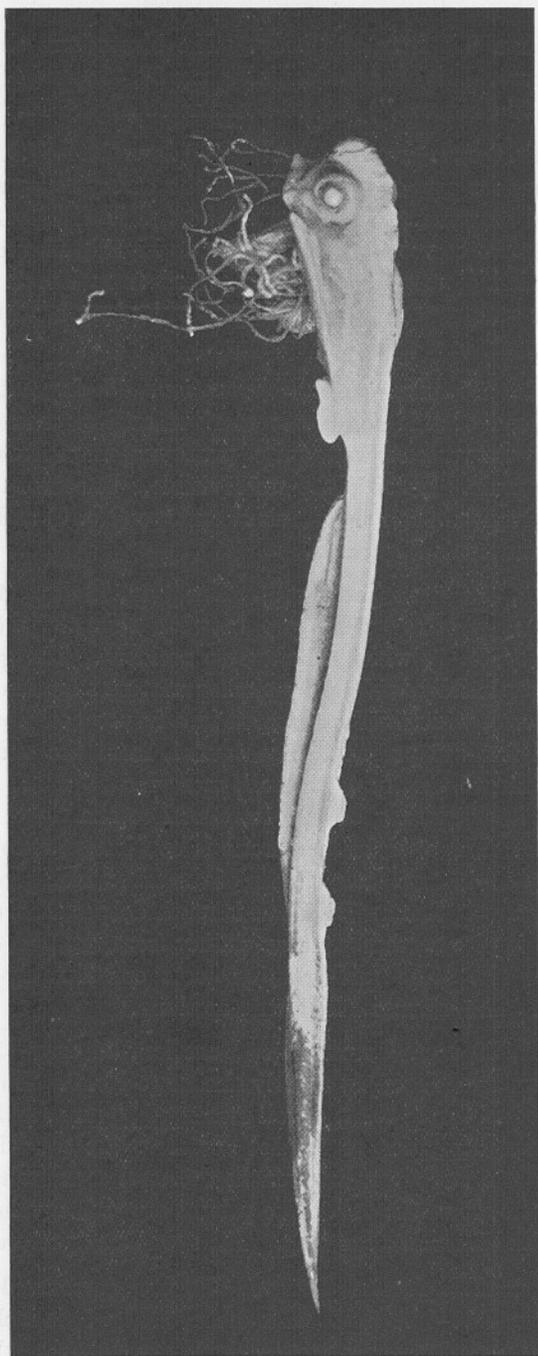


FIG. 5.—*Raia clavata* embryo, No. 9A. Lateral view.  $\times 2.45$ .

the snout has advanced considerably. The snout and disc are beginning to unite. The length of the caudal fin from the end of the 2nd dorsal is 20 mm., and the distance from the beginning of the 1st dorsal is 28.5 mm. The mid-brain still projects beyond the tip of the snout.

10. Length, 94 mm. (fresh), 88 mm. (preserved). Duration, 100 days (13.5-21.8.22). This embryo (Fig. 6) is much further advanced than No. 9. The disc and snout are united, but the union is not quite complete. There is still a notch anteriorly. The following are the more important measurements:—

Width of disc . . . . .	20 mm.
Length of disc . . . . .	19 mm.
End of 2nd dorsal to tip of tail . . . . .	28 mm.
Beginning of 1st ,, ,, ,, . . . . .	40 mm.

There is a concavity in the anal fin membrane below the dorsals and the depth of the membrane is greater anteriorly than posteriorly.

The mouth and nasal openings are as in the adult. The teeth have not broken through.

11. Length, 100 mm. Duration, 119 days (1.5-28.8.22). This embryo (Fig. 7) is slightly more advanced than No. 10. The snout and pectorals are completely united. The frontal angle of the mid-brain is not so pronounced as in the previous embryo.

Width of disc . . . . .	19 mm.
Length of disc . . . . .	18 mm.
End of 2nd dorsal to tip of tail . . . . .	21 + x mm.
Beginning of 1st ,, ,, ,, . . . . .	36 + x mm.

The tip of the caudal was very delicate, and was accidentally lost.

12. Length, 110 mm. Sex ♂. Duration, 111 days (9.5-28.8.22). The claspers are well defined. There is a well-defined skin flap along the sides of the tail longitudinally. The anal membrane is much reduced in depth (Fig. 8).

Width of disc . . . . .	28.5 mm.
Length of disc . . . . .	27 mm.
End of 2nd dorsal to tip of tail . . . . .	22 mm.
Beginning of 1st ,, ,, ,, . . . . .	44 mm.

The position of the spines along the median ridge of the body and tail and on the sides of the tail is indicated by small knobs. The branchial filaments are still long, and the teeth are not visible.

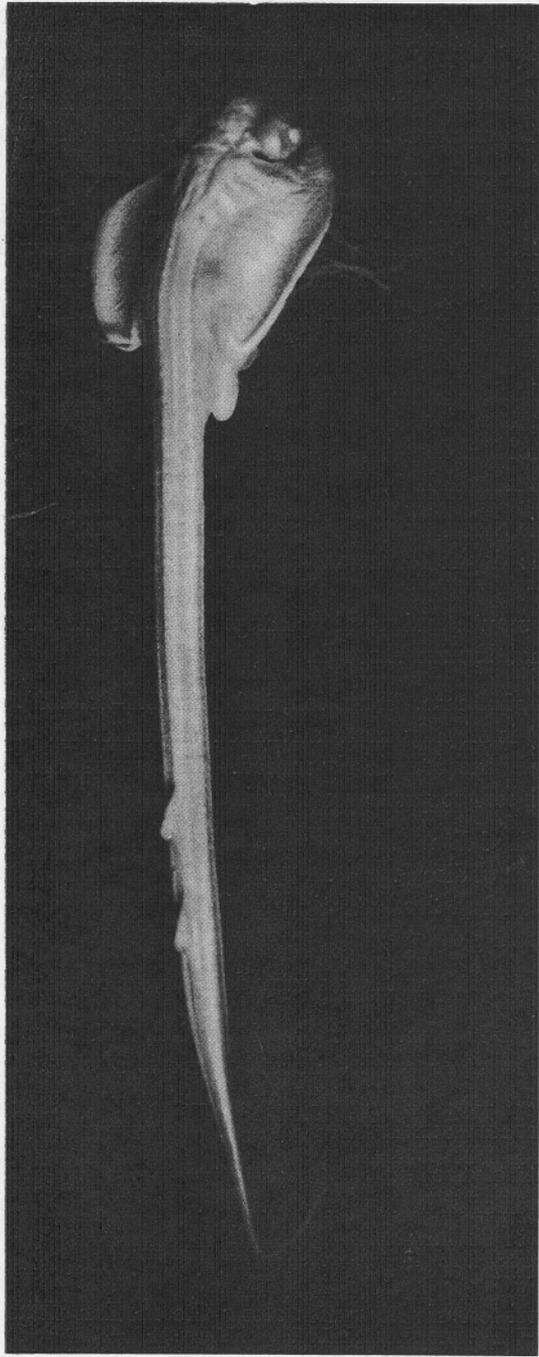


FIG. 6.—*Raia brachyura* embryo, No. 10. Dorso-lateral view.  $\times 1.92$ .

There is complete union of the snout and the pectorals. This embryo is further advanced than No. 11, and this is shown chiefly in the development of the spiny armature of the skin.

13. Length, 115 mm. Sex ♂. Duration, 128 days (12.6-16.10.22).

Width of disc . . . . .	37 mm.
Length of disc . . . . .	34 mm.
End of 2nd dorsal to tip of tail . . . . .	27 mm.
Origin of 1st " " " . . . . .	44 mm.

This embryo is exactly similar to No. 12. There is just a slight advance in development. The teeth are now defined. A pair of endolymphatics appear as open tubes on a level with the posterior margin of the spiracle.

14. Length, 115 mm. Sex ♀. Duration, 153 days (21.6-9.11.22).

This embryo (Fig. 9) was extracted on 15th October, 1922, for cinematograph purposes. It was kept alive for over 25 days in sea-water and isolated in one of the tanks. The period within the capsule amounted to 128 days. It is reproduced here to show the quick absorption of the elongated caudal and the reduction of the branchial filaments. The yolk sac is still large, and measures 32×22 mm.

Width of disc . . . . .	49 mm.
Length of disc . . . . .	42 mm.
End of 2nd dorsal to tip of tail . . . . .	11 mm.
Origin of 1st " " " . . . . .	31 mm.

Pigment is generally distributed over the upper surface, and the spines and spinulæ are well defined. The rapid advance in growth may be compared with embryos Nos. 12 and 13.

15. Length, 139 mm. Sex ♂. Duration, 144 days (11.5-2.10.22).

Width of disc . . . . .	53 mm.
Length of disc . . . . .	43.5 mm.
End of 2nd dorsal to tip of tail . . . . .	30 mm.
Origin of 1st " " " . . . . .	50 mm.

This embryo (Fig. 10) is more advanced than Nos. 12 and 13. The disc is much broader in proportion to the total length and the branchial filaments are shorter. The claspers and the teeth are well defined. Pigment is beginning to appear on the upper surface. The median spines and the spinulæ on the anterior margin are indicated as knobs. The anal membrane is disappearing. It has quite vanished on the area below the dorsal fins, but it is still present anteriorly and posteriorly to this blank space as a thin

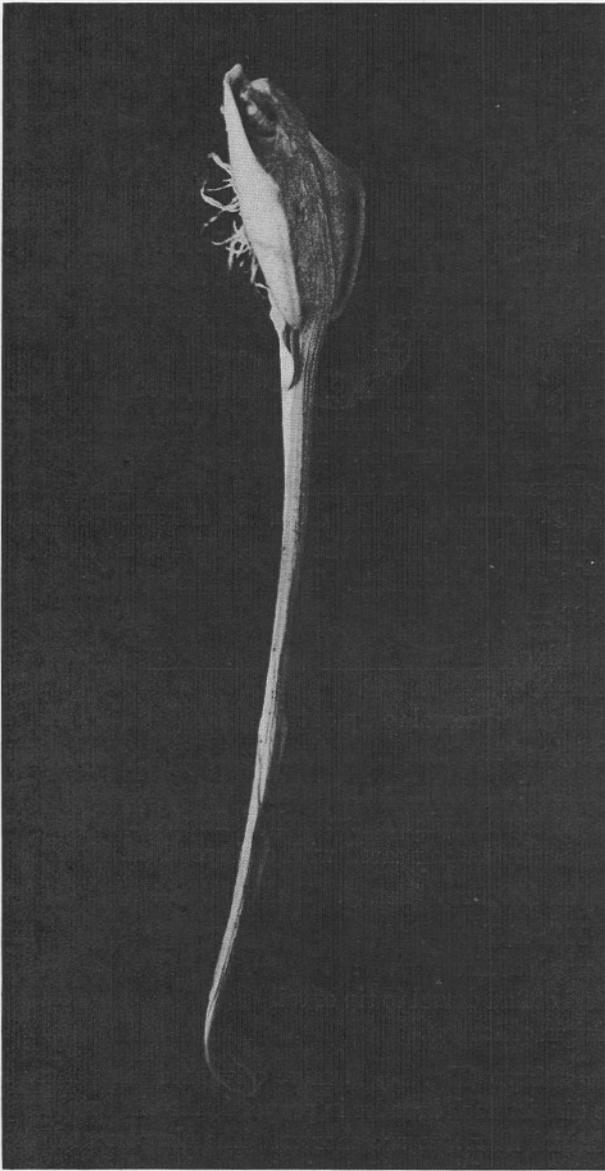


FIG. 7.—*Raia brachyura* embryo, No. 11. Dorso-lateral view.  $\times 1.82$ .

low ridge. The membrane behind the 2nd dorsal is continuous to the tip of the caudal.

16. Length, 146 mm. Sex ♀. Duration, 123 days (29.5-29.9.21). This embryo (Fig. 11) shows a big advance from No. 15. The disc is more circular in outline, and the snout is blunter. The branchial filaments are much reduced, and are seen to project only from the third and fifth gill clefts.

Width of disc . . . . .	65 mm.
Length of disc . . . . .	55 mm.
End of 2nd dorsal to tip of tail . . . . .	25.5 mm.
Origin of 1st ,, ,, ,, . . . . .	46.5 mm.

Pigment is well shown on the upper surface. The spines and spinulæ are strongly indicated. As yet, the points have not pierced the epiderm. A pair of open endolymphatic tubes and the ends of the mucous canals are well defined. There is a pronounced skin flap extending along each side of the tail. The membrane behind the 2nd dorsal fin is still continuous. The anal membrane which has vanished below the dorsal area persists anteriorly and posteriorly, but is not very pronounced.

17. Length, 147 mm. Sex ♀. Duration, 130 days (23.5-30.9.21). This embryo is identical with the preceding No. 16, but the branchial filaments are overgrown by the gill arches.

Width of disc . . . . .	62 mm.
Length of disc . . . . .	52 mm.
End of 2nd dorsal to tip of tail . . . . .	29.5 mm.
Origin of 1st ,, ,, ,, . . . . .	51.5 mm.

18. Length, 148 mm. Sex ♀. Duration, 138 days (1.7-16.11.21). This embryo is similar to No. 17, but the disc is broader and the caudal shorter.

Width of disc . . . . .	70 mm.
Length of disc . . . . .	53.5 mm.
End of 2nd dorsal to tip of tail . . . . .	25 mm.
Origin of 1st ,, ,, ,, . . . . .	45 mm.

The branchial filaments are absorbed. The tips of the median tail spines have pierced the epiderm. The yolk sac is still large and measures 32×26 mm.

19. Length, 150 mm. Sex ♀. Duration, 143 days (26.6-16.11.21).

Width of disc . . . . .	71 mm.
Length of disc . . . . .	56 mm.
End of 2nd dorsal to tip of tail . . . . .	23 mm.
Origin of 1st ,, ,, ,, . . . . .	44 mm.

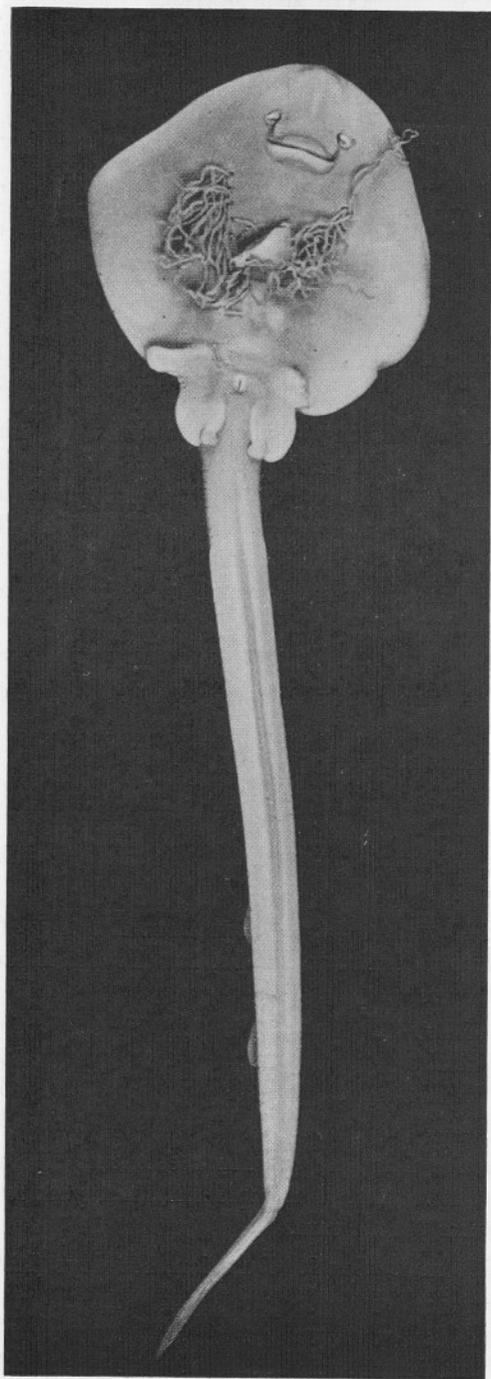


FIG. 8.—*Raia brachyura* embryo, No. 12. Ventral view.  $\times 1.62$ .

The tips of the median tail spines in front of the first dorsal are through the epiderm. The diameter of the yolk sac measures 29×26 mm. The anal membrane has practically vanished anterior to the position of the 1st dorsal, but is still present posteriorly behind the dorsal fin area, otherwise the embryo is similar to No. 18.

20. Length, 162 mm. Sex ♂. Duration, 150 days (4.6-1.11.21).

Width of disc . . . . .	87 mm.
Length of disc . . . . .	66 mm.
End of 2nd dorsal to tip of tail . . . . .	27 mm.
Origin of 1st " " " . . . . .	48 mm.

The teeth are more developed than in No. 19, and the anterior margin of the disc is broadly undulated. The tip of the snout does not project. Pigmentation is well developed on the upper surface. The spines on the median ridge have pierced the epiderm.

21. Length, 169 mm. Sex ♀. Duration, 165 days (4.6-16.11.21).

Width of disc . . . . .	95 mm.
Length of disc . . . . .	68 mm.
End of 2nd dorsal to tip of tail . . . . .	22 mm.
Origin of 1st " " " . . . . .	44 mm.

This embryo is much further advanced than No. 20. The tips of all the spines and spinulæ are clearly through the epiderm. The ends of the mucous canals are well marked. This stage is exactly similar to Fig. 9, Clark, 1922, p. 609. The tip of the snout is slightly recessed. The teeth are obtusely pointed. The anal membrane has vanished.

TABULAR DATA OF

DEVELOPMENTAL STAGES OF *R. BRACHYURA*. YEAR 1921-22.

Period of Incubation, 29th April, 1921, to 3rd February, 1922.

Incubation period.		Days' duration.	Total length of embryo.	Remarks.
Date begun.	Date ended.			
9/6/21	14/7	35	15	
23/5/21	14/7	52	123	Capsule kept in shallow table trough exposed to direct sunlight.

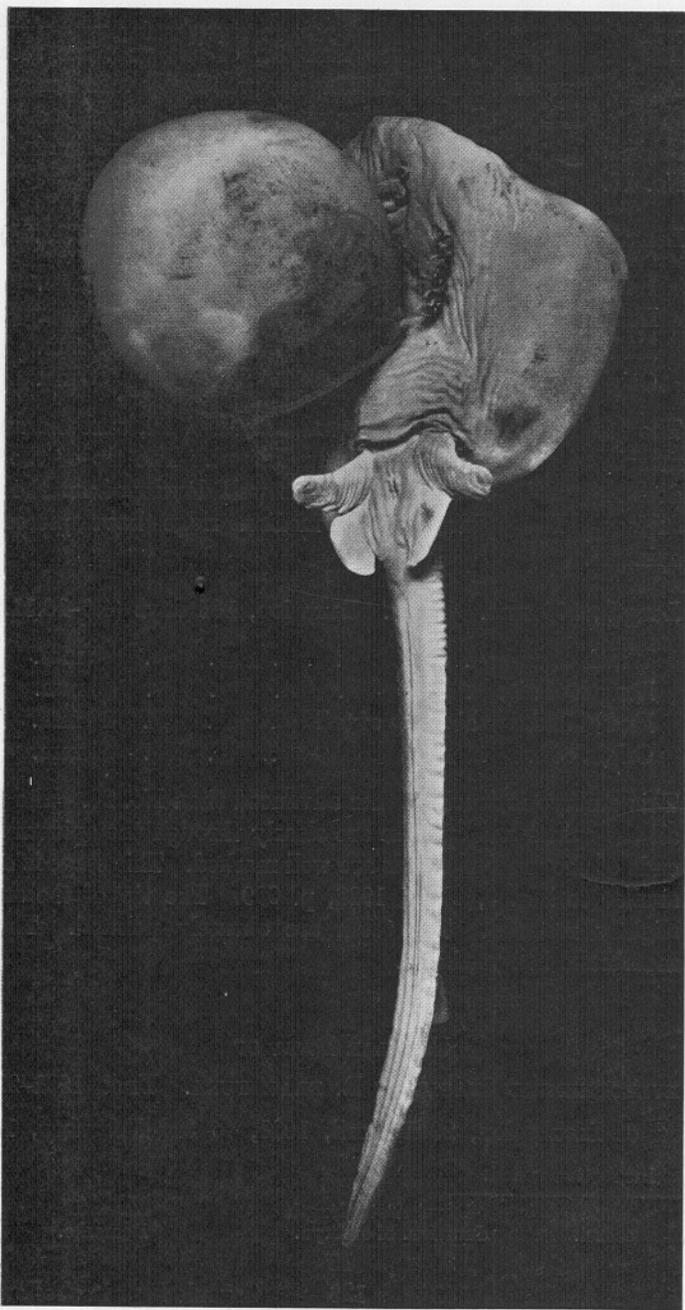


FIG. 9.—*Raia brachyura* embryo, No. 14. Ventral view.  $\times 1.33$ .

29/4/21	5/7	67	52	Albumen absorbed. Water drained off through slits in horns of capsule.
29/5/21	29/9	122	145	♀ Pigmented. Spines below epiderm. Branchial filaments visible in anterior gill clefts.
23/5/21	29/9	128	147	♀ Pigment. Spines not through epiderm. No branchial filaments visible.
1/7/21	16/11	138	148	♀ Yolk sac 32×26 mm. Median tail spines with tips through epiderm. Branchial filaments absorbed.
26/6/21	16/11	143	150	♀ Branchial filaments absorbed. Median tail spines through epiderm.
4/6/21	1/11	150	165	♂ Pigment spots. Median tail spines with tips through epiderm.
4/6/21	10/11	165	169	♀ ca. Hatching. All tail spines and most of interior spinulæ with tips through epiderm.
Mid-June-	21/12/21	ca. 189	175	♀ Hatched.
End June-	31/1/22	ca. 215	175	♂ Hatched.
1/7	3/2/22	217	187-5	♂ Hatched.
Mid-June-	20/1/22	ca. 220	174	♂ Hatched.

All the egg-capsules were secured from fish which were landed on the Barbican at Plymouth. They were first marked and then placed in the experimental tanks at the Laboratory of the Marine Biological Association. Reference to the average monthly temperatures of the sea-water in circulation in these tanks will be found in "Rays and Skates," No. 1, Clark, 1922.

TABULAR DATA OF  
DEVELOPMENTAL STAGES OF R. BRACHYURA. YEAR 1922.

Incubation period.			Embryo.	Remarks.
Date begun.	Date ended.	Days' duration.	Total length in mm.	
19/5	19/5	0	—	Germinal ring 5-6 mm. in diameter. Initial cleavage. Single furrow. Barbican capsule.

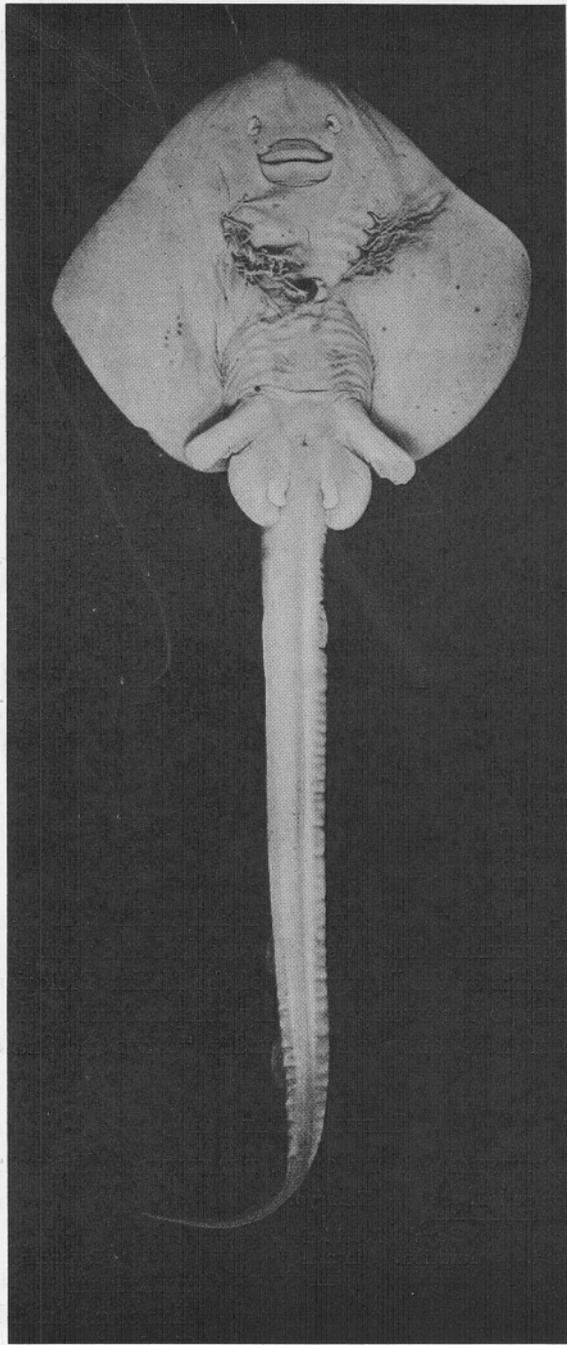


FIG. 10.—*Raia brachyura* embryo, No. 15. Ventral view.  $\times 1.21$ .

19/5	19/5	0	—	Capsule taken from living fish when first observed protruding. No visible sign of cleavage in blastoderm.
25/5	25/5	0	—	Capsule taken from living fish. Germinal ring 6×5 mm. Cleavage not observed.
31/5	1/6	1	—	Diameter of germinal ring 5-6 mm.
16/5	17/5	1	—	Tank spawned. Germinal disc showing. Segmentation stage, <i>vide</i> Balfour, Pl. I, Fig. 4.
29/5	1/6	2	—	Germinal disc 5-6 mm. in diameter with single furrow on the blastoderm.
23/5	25/5	2	—	Capsule first observed in living fish on 23/5. Also seen on 24/5. Deposited naturally. Germinal disc. Cleavage of blastoderm not observed.
28/5	1/6	3	—	Tank spawned. Germinal disc 5-6 mm. Blastoderm with single furrow.
13/5	17/5	4	—	Tank spawned. Germinal disc 7×5 mm. Segmentation stage, <i>vide</i> Balfour, Pl. I, Figs. 4-5.
1/6	7/6	6	—	Early embryo. Beginning of medullary groove. Balfour, Stage B.
5/5	17/5	12	—	Taken from living fish. Segmentation stage. Late cleavage, <i>vide</i> Balfour, Pl. I, Fig. 5.
21/4	17/5	24	3-5	Tank spawned. Embryo, <i>vide</i> Balfour, Stage F.
12/4	17/5	35	4-5	No lateral movement in embryo. Balfour, Stage G.

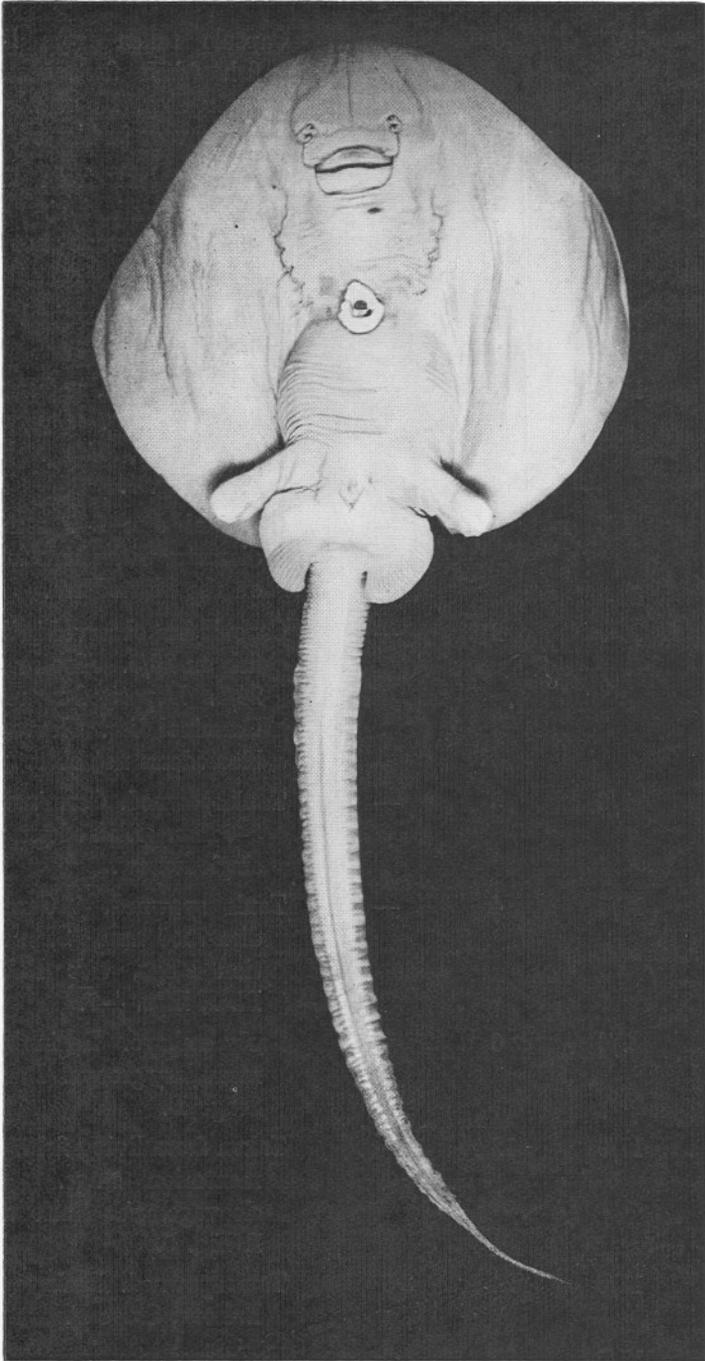


FIG. 11.—*Raia brachyura* embryo, No. 16. Ventral view.  $\times 1.11$ .

30/3	11/5	43	4-4.5	No lateral movement in embryo. Blastoderm much pitted, <i>vide</i> Dean, Fig. 38. "Chimæroid Fishes and their Development."
24/3	11/5	48	7.5 (fresh)	(5 mm. preserved measurement.) Deep furrow encircling yolk. Embryo showing lateral movement, <i>vide</i> Balfour, Stage I, 3 gill clefts.
17/6	28/8	72	58.5	Tank spawned capsule. (58.5 mm. = preserved length.) Snout and pectorals NOT united. Mouth subterminal. No filaments from spiracle.
15/6	28/8	74	59	Do.
3/6	21/8	79	76 (fresh)	Capsule tank spawned. Albumen absorbed. Snout and pectorals NOT united.
13/5	21/8	100	94 (fresh)	88 mm. preserved measurement. Capsule tank spawned. Albumen absorbed. Snout and pectorals UNITED, not completed. Vascular system well developed. Branchial filaments long. Width of disc 21 mm.
9/5	28/8	111	110	Preserved length. Capsule tank spawned. Sex ♂. Snout and disc united completely. Branchial filaments absorbed. Not visible externally. Width of disc, 28.5 mm.
1/5	21/8	112	100	Snout and disc united. Branchial filaments long. Vascular system well devel-

				oped. Water drained off through slits on horns on capsule being removed from tank. Width of disc 21 mm.
1/5	28/8	119	100	Preserved length. Snout and disc united. Branchial filaments long.
22/4	21/8	121	97	Measurement on fresh embryo. Albumen absorbed. Snout and disc united.
12/6	16/10	126	114	Preserved length. Sex ♂. Embryo inactive when extracted. Quick movement on transfer to outside seawater, and more so with initial stimulus of anaesthetic. Branchial filaments considerably reduced, but still visible. On removal of capsule from tank, internal water drained off readily through slits on horns.
9/6	15/10	128	?	Embryo extracted for cinematograph. Yolk large. Vascular system well developed. Disc and snout united. Branchial filaments reduced. This embryo was kept alive in a separate tank for a short period. The tail shortened very quickly, and the branchial filaments were similarly greatly reduced, <i>vide</i> Fig. 7.
11/5	2/10	144	139	Preserved length. Sex ♂. Pigment spots on upper surface. Branchial filaments still moderately long.

## LITERATURE CITED.

- BALFOUR, F. M. 1878. A Monograph on the Development of Elasmobranch Fishes. London, Macmillan and Co.
- CLARK, R. S. 1922. Rays and Skates (*Raiiae*), No. 1. Egg-capsules and Young. Journ. Mar. Biol. Assoc., N.S., Vol. XII, No. 4.
- DEAN, BASHFORD. 1906. Chimæroid Fishes and their Development. Carnegie Institute of Washington. Publication No. 32.
- SCAMMON, R. E. 1911. Normal Plates of the Development of *Squalus acanthias* with an Introduction by Charles S. Minot. Normentafeln zur Entwicklungsgeschichte der Wirbeltiere. Jena. This publication contains a very exhaustive Bibliography under subject headings.