# EARLY DEVELOPMENT AND METAMORPHOSIS OF THE TROPICAL ECHINOID SALMACIS BICOLOR, AGASSIZ.

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Nor much is known about the development of Temnopleurids. Mortensen (1921) describes the larvæ of Temnopleurus toreumaticus, Temnoptrema sculpta, Mespilia globulus and the Echinopluteus larvæ of three other unidentified species of Temnopleurids. The full development has not been traced in any one of these forms. Tennent (1929) gives an account of the very early stages for Salmacis virgulata Alexandri. The anatomy of Salmacis bicolor has been fully worked out and in writing a monograph on it as a type for Indian students it was considered very desirable to incorporate the development of the animal as far as possible. It was therefore decided to rear the form from artificially fertilized eggs. Fertilizations were carried out in February last. The animals were collected from the Madras Harbour, washed in clean sea water, and small quantities of testis and ovary were removed with sterilized instruments. Glass vessels containing sea water brought from about three miles from the shore were kept ready. Over each vessel a piece of silk just dipping into the water was tied through which the sex elements easily passed. Care was taken not to put too many eggs in the vessels as otherwise putrefaction sets in rapidly and development stops.

After fertilization the surface water was replaced by fresh sea water. When the embryos float up, the top water was carefully removed and the larvæ distributed over a large number of jars containing fresh sea water filtered through mull cloth. Every day the surface sea water was removed by means of a funnel and a rubber tube with a piece of bolting silk tied round the mouth of the funnel. Clean fresh sea water without any of the bigger organisms likely to feed on the larvæ was added. The vessels were now kept in flat lead lined trays containing three inches of tap water and by wrapping thin wet cloth round the jars the temperature was maintained at about 25° C. The fact that slight changes in the environmental medium might produce considerable changes in the larval development was kept in mind and only healthy larvæ were picked out and examined.

<sup>&</sup>lt;sup>1</sup> In course of publication.

Now and then, developing larvæ were carefully removed to new jars containing clean sea water. Algæ developed only in the later stages and were fed upon by the growing urchins and it has been quite possible to keep the cultures going for several months. Many growing Salmacis are still living and are a year old. Broken shells and minute debris in the sea water were given to them after metamorphosis. Many of them have the habit of crawling up to the surface feeding on the way and once at the top they gently turn over and drop to the bottom and once again commence climbing up. Most of the urchins have continued to be healthy. Three specimens became sickly and lost their spines—a common phenomenon under natural conditions—and were destroyed.

#### Material and Methods.

The larvæ were studied mostly in the living condition as also from whole mounts. Further they were fixed in Bouin-Duboscq and sectioned by the double embedding method. The stain commonly used was Delafield's Hæmatoxylin.

The development of  $Salmacis\ bicolor\ conforms$  in all essential particulars with the development of  $Echinus\ esculentus\ and\ E.\ miliaris\ described$  by MacBride (1903). It is therefore proposed to give here only an outline of development of this tropical form. Before doing so a resumé of the more important events in the order of their occurrence after fertilization may not be out of place.

Two cells			• •				10 M	inutes.
Four cells	• •	• •				1 Hour	30	,,
Eight cells	• •	• •		• •		1 ,,	50	,,
Sixteen cells			• •	• •		2 ,,	10	,,
Blastula	••			• •	• •	8 Hour	s.	
Gastrula	• •	••	• •	• •	• •	23 Hour	s.	
Pluteus	• •	• •	• •			2 Days		
Appearance of red pigment and amnion. Clear indication of the post-oral and antero-lateral arms.  Meeting of stomodæum with œsophagus. Forma-								
tion of the right and left coeloms. Minute appearance of preoral arms 3 Days.								
The pore can					• •	4 Days		
The cœloms	enlarge.	Differe	ntiation	of the	1eft			
hydrocœl			1	••	••	5th—6		-
Development	or rue bo	stero-dor	sai aims	√ <b>*</b> •	• •	7th—8	ип Da	ıy.

The dorsal arch appears	• •	• •	• •	8th Day.
Rapid growth of the postero-dorsal arms and the				
dorsal arch	• •	• •	• •	9th—10th Day.
Posterior transverse rod app	iliated	•		
epaulettes appear and the	formation	of the	larval	
skeleton is completed.	Pre-oral	arms	grow	
rapidly	• •			12th—13th Day.
Formation of the first pedicel	llaria	• •	• •	13th—14th Day.
Enlargement of the amnion	• •	• •		15th—16th Day.
Rapid growth of the Echinus	rudiment	• •		16th—19th Day.
Metamorphosis	• •	• •		23rd—24th Day.
Formation of the second coil of the alimentary canal				24th—36th Day.
Development of the adult mo	outh	• •	• •	34th—36th Day.
Young urchin 4-5 mm. in	diameter	. Imm	ature.	
The adult anus opens	• •	• •		3 Months.
Urchin 13 mm. in diameter.	Immatur	e		6 Months.
Urchin 16 mm. across. Imm	ıature	• •	• •	12 Months.

### Early Development.

The eggs are small being 0.1 mm. in size and rather opaque. They sink slowly to the bottom. Fertilization was effected soon and the fertilization membrane separates off but stands very little away from the surface of the egg. The two-celled stage is reached in 10 minutes and the four-celled in 1 hour and 30 minutes. It cannot be said that the rate of development is the same for all the individuals of a culture. For purposes of this study the biggest individuals alone were picked out and examined. The 8-celled stage is reached in 1 hour and 50 minutes and now four of the blastomeres are smaller than the other four. The 16-celled condition is attained in 2 hours and 10 minutes and a central cavity becomes visible.

The 32- and 64-celled stages are soon over and in about 8 hours the multicellular blastula stage is reached (Fig. 1). The cells on one side are slightly more columnar than those on the other. This pole is the blastoporal end. Soon gastrulation commences and is indicated by a few of the cells from this pole pushing themselves inwards. This becomes an actual invagination and in this way a narrow archenteron is formed. This grows forwards rapidly, almost to the anterior end and the process is completed in about 23 hours. While invagination is taking place other changes are in evidence. Mesenchyme cells pass from the outer layer inwards in places and establish a connection with the archenteron. The beginnings of the larval skeleton

are laid down in the form of two triradiate spicules (Fig. 2). Each spicule lies on one side of the archenteron in amongst a group of mesenchyme cells, by which they are actually formed. The occurrence of these two primary spicules is the first indication of the bilateral symmetry which is so characteristic of the pluteus larva. A thickening takes place at the anterior end of the archenteron. This is the commencement of the formation of the This reveals itself in the form of a bag, the anterior coelom. stomodæum now begins to be formed in the form of a shallow pit but does not as yet establish connection with the anterior end of the archenteron. Towards the close of the second day after insemination the larva has definitely assumed the pluteus form. The post-oral arms are the first to appear and the oral lobe has become well established. The stomodæum now fuses with the archenteron and the coelom divides into right and left moities. skeleton (Fig. 4) now consists of the fenestrated rods supporting the newly formed post-oral arms. Body rods are well formed and are thorny while at their lower ends they are produced into antler-like projections. From the junction of the arm-branch and the body rod on each side is given off a comparatively slender rod which first projects inwards and then sharply turns outwards, running to the outer corner of the front edge of the oral lobe from which the antero-lateral arms will soon arise. These rods, the antero-lateral rods, are slender and are comparatively smooth, carrying a few blunt spines only.

The body rods are different in form from those figured by Tennent (1929) for Salmacis virgulata Alexandri in being less thorny.

On the third day reddish pigment granules appear and the œsophagus shows definite signs of muscular contraction (Fig. 7). Two pairs of arms are now present, the post-oral and the antero-lateral. The stomodæum is fully established and the oral lobe is extending forwards. The larva has become humped and the alimentary canal now consists of œsophagus, stomach and a short intestine. The adoral band of cilia is developed in addition to the longitudinal ciliated band.

Three days old larvæ clearly show the left and right coloms as strands of cells stretching from the side of the œsophagus obliquely backwards (Fig. 6). As yet no cavity is discernible in the strands of cells. The portion of the ciliated band running in front is thickened and provided with slightly stronger cilia. Numerous amæbocytes loaded with pigment have appeared. The larva now corresponds to the stage figured by MacBride (1903) Pl. VII, Fig. 4. Minute indications of the future pre-oral arms have made their appearance (Fig. 6). The early appearance of these arms, which are destined to show themselves more fully only very late, is characteristic. Towards

the close of the third day the ventral transverse rods have become more spiny at their tips. Recurrent rods have become well developed and are becoming spiny. A branch is seen from the base of each. Now the left colom is definitely the larger. During the course of development it was noticed as was done by Shearer, Morgan and Fuchs (1914) that the rate of development varies within wide limits even in the same culture.

The ectodermal invagination, the amnion, which arises and becomes marked out during the 12, 13 and 14th days in *Echinus esculentus* appears in the present form in the shape of a rod of cells on the left side and could be seen slightly prior to the 3rd day of development. This soon separates from the ectoderm during the 3rd and 4th days and becomes converted into a closed vesicle. It is placed slightly in advance of the place which is to be occupied by the hydrocœlic ring at about the 10th day.

In *Echinus* the "amnion" arises as an open ectodermal pit which later becomes closed and so gets converted into the amnion. In *Peronella Lesueri*, Mortensen (1921) has shown that the amnion arises as posterior prolongations of the pharynx and in *Salmacis bicolor* it arises as a solid structure in which a cavity appears later.

The cœlomic sacs have grown large and a distinct space could be seen in both. The left anterior cœlom has further given rise to an ampulla. As yet there is no thinning out to form the stone canal.

The pore canal is seen to start slightly in front of the expansion of the hydrocœl, bends upwards and inwards and opens on the upper surface just above the junction of the gullet and stomach.

5th—12th Day.—On the fifth day the commencement of the supporting rod of the postero-dorsal arms can be seen and by the beginning of the next day the rods have clearly the character of short bladed swords. It is during this day that the left coelom definitely elongates. The pore canal is formed and the madreporic pore is now visible and the left hydroccel is Fig. 9 shows the condition of the coelom on the left clearly laid down. side on the 6th day. The amnion has increased in size, the hydroccel is fully formed and the stone canal is quite clear in front of the hydroccel. posterior cœlom has extended backwards but the two layers are in contact The right anterior coelom does not show all the throughout their extent. divisions mentioned for the left. It has however given rise to the madreporic vesicle. Metschnikoff (1869) described for a corresponding stage in Echinus microtuberculatus a pulsating vesicle (the madreporic vesicle) near the water pore and thought it to be in connection with the anterior cœlom. (1889) describes a similar vesicle but considers it to be independent, having a schizocœlic origin. Gemmil (1919) has described a pulsating vesicle in young Ophiuroids and Murti (1932) states that he observed pulsations in *Echinus miliaris* and in the young of *Ophiocoma*. Several larvæ of *Salmacis* were examined with a view to find out if such pulsations occur, but without success. Dr. Murti who happened to be here and who examined living larvæ of *Salmacis bicolor* could not notice any such pulsations.

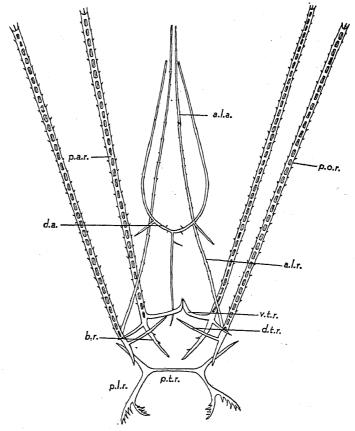
The postero-dorsal arms have clearly shown themselves and grow rapidly during the 7th and 8th days. Early in the 8th day a rudiment of the dorsal arch makes its appearance and by the close of the day has become arched and a median process has been formed (Fig. 11). Two arms of the Y-shaped structure grow and during the course of the day project into the minute elevations which will soon be drawn into the pre-oral arms.

The condition of the cœlom on the 10th day of development is shown in Fig. 13. The two anterior cœloms are very clear. The posterior cœloms are larger and are still separate not having met behind.

After its formation the hydroccel moves backwards along the wall of the stomach and reaches the middle of that organ. The anterior end of the posterior ccelom now grows in front in the form of two arms, finally fusing in front.

12th to 15th Day.—The larva is provided with three pairs of well developed arms and keeps swimming constantly with the arms directed upwards. Now a rapid basal broadening of the arms, especially of the post-oral and postero-dorsal, takes place so that they are definite leaf-like structures, as has been noted also for Mespilia globulus. They are not inactive but are capable of a great deal of movement. They can close on the central part and open out and they do this pretty frequently though not rhythmically. As seems to be the case in Echinopluteus transversus first supposed to be a Cidaroid larva by Mortensen (1921) and later referred by him to Diadema setosum (1931) a well-developed muscular system is present. of the posterior transverse rod appear now and its development is fairly advanced by the 13th day. The pre-oral arms grow rapidly and their full length is reached by the end of the 13th day, and their skeletal supports keep closely with their growth. The larvæ have grown comparatively heavy and it is becoming increasingly difficult for them to keep afloat. Now the anterior ciliated epaulettes begin to appear on the 12th day and complete separation from the main band has taken place by the end of 13th. All arms are broad at base and could be seen to open and shut. There is a remarkable similarity between this stage and the 15th day larva of Mespilia globulus, figured by Mortensen (1921). The median rod of the dorsal arch has

extended considerably backwards and two side branches could be seen. By the 13th day the development of the larval skeleton may be said to be complete. Text-Fig. 1 shows the final condition of the skeleton as it exists in



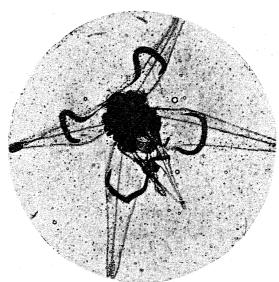
TEXT-FIG. 1.

the 13-day old larva. The dorsal arch is fully developed. The median process is long and slightly bent, and bears no thorns. There are well-developed lateral processes and these carry two or three minute horns. The posterior transverse rod has become well developed and on each side there are two branches, one simple and going upwards obliquely, and the other, the lower one soon bifurcates into two antier-like branches. The post-oral and the postero-dorsal rods are, as usual, finely fenestrated and are thorny. The antero-lateral rods are slender and are covered by minute, rather blunt, spinous processes. The pre-oral rods into which the dorsal arch is continued are more or less smooth. It must however be stated that even before this stage is reached a certain absorption of the skeleton has been going on. The body rods have become less prominent and many of their spines have disappeared. The recurrent rods have also vanished. After the 14th day very little change takes place in skeleton formation except that the posterior

median branch of the dorsal arch seems to grow a little longer and the preoral rods give off just beyond their origin a single branch on each side. Posterior lateral earlike processes have commenced to form.

The fully formed larval skeleton shows great similarity with that figured by Mortensen (1921) for his "Temnopleurid larvæ species a" taken from Jolo. His larva undoubtedly belongs to Salmacis as Tennent (1929) has already suggested. But there are certain differences in the details of the skeleton between the two forms under comparison, so that it becomes impossible to consider his "Temnopleurid species a" as belonging to Salmacis bicolor. It would be interesting to know if the latter is common near Misaki on the Japanese coast where Mortensen collected the plankton material from which he picked out his Temnopleurid larvæ.

Just after the completion of the formation of the ciliated epaulettes the beginnings of the first pedicellaria can be seen at the hind end. At first it is a mere knot in which a large number of mesodermal cells accumulate. In a few hours this structure shows division into three parts, each being a jaw. Very soon, before the end of the 13th day and in some cultures on the 14th day, the Ophiocephalus type stands revealed. During the course of development two larvæ were noticed to have developed a double hydrocæl. A microphotograph of this is given. Such abnormal larvæ have been noticed by others also in other forms.



Text-Fig. 2.

Further development and metamorphosis.—On the 15th day the larva has not changed much though the hydroccel ring has grown a little larger and the amnion rudiment bigger. Groups of mesodermal cells are seen collecting on

either side of the already formed pedicellariæ. The larvæ feed voraciously and are very active. Even large-sized Coscinodiscus are swallowed with ease.

By the 19th day the second and the third pedicellariæ are very clear in relation with the first formed pedicellaria and a rudiment of the fourth could be seen on the right side. All the four pedicellariæ are of the Ophiocephalus type. During 15-19 days ear-shaped postero-lateral processes supported by branches from the post-transverse rod have become well developed and have become prominent. The left side commences to bulge out considerably, the stomach being pushed to the right. In the absorption of the arms no regular order can be traced. Sometimes the reduction seems to be more pronounced in the post-oral arms, sometimes the postero-dorsal. It may however generally be stated that the first formed arms go first to be followed by the later formed arms. Some of the arms show gradual diminution in length as a sign of the approaching metamorphosis. In this respect there is great variation, some larvæ showing more rapid shortening of the arms than the others. Fig. 17 shows the parts on the left side when the larva is 21 days old and is very similar to the Fig. 43, Plate XII, given by MacBride (1903) for Echinus esculentus 30 days old. The amnion has become spacious and several tentacles project into it. Nerve fibrils can be seen just above the dental sac and the epineural spaces are fully established. It is interesting to note that in the figures given by MacBride the inner layer of the colom is always separated by jelly from the alimentary wall, but here there is no jelly and the layer closely adheres to the wall of the digestive tract. The hydroccelic ring has sent out primary tentacles which could easily be seen in entire specimens as also in sections. Some of them are provided with suckers also. Yellow pigment bodies are found in considerable numbers in relation with the postero-dorsal and post-oral arms. They seem to surround the skeletal rods of these arms and especially accumulate around the tips of the arm rods and probably are the cause of their rapid destruction. The larva now finds it difficult to float and sinks now and again to the bottom. The arms undergo rapid reduction and when the larvæ are 23 days old they are greatly reduced. The epaulettes still continue to be active though their work is almost over. The Echinus rudiment now occupies the left side of the larva. The amœbocytes have become very active. The epaulettes now undergo rapid reduction and before the close of the 23rd day have disappeared almost completely. Sections of larvæ of this stage display a great amount of histolysis. The alimentary canal which hitherto had walls of one cell thickness now shows considerable thickening. The cells divide rapidly and the pharynx and portions of the alimentary canal get thrown into folds. About this time the larval mouth closes as also the larval anus.

Spines have appeared and are of two types, four thorned or crowned dorsal spines and normal ventral spines. The latter clearly show the two sheaths of muscles forming the two layers, the ball and the socket joint and the main spine. The paired tube feet are already provided with sucker plates. On the ventral side the elements of the jaw apparatus are being laid down and on the 32nd day the entire lantern apparatus has reached a stage corresponding more or less closely to the stage figured by Devanesan (1922) in Fig. 5, Plate XIV, for Echinus miliaris. The teeth, the jaws, the rotulæ and alveoli are clear. Mouth opening cannot be seen. About a fortnight later sagittal sections of young Salmacis 2 mm. in diameter and 42 days old show the mouth opening clearly. The metamorphosis is very rapid occupying about 8 hours. Healthy metamorphosing specimens were watched and it was noticed that the process took place very quickly. At 8-30 A.M. of the 24th day after fertilization in a healthy larva the amnion was projecting out almost to breaking point. It burst at 10 and one hour later two tentacles had pushed themselves out, and by 12 noon the amnion had shrunk away and at 4 P.M. all the primary tube feet were showing and the urchin was actively moving on its tube feet. Growth hereafter is slow and all individuals do not show the same rate of growth. Section of a newly metamorphosed larva shows the lower alimentary coil only and the larval œsophagus could be seen on one side. This state of things lasts for a fortnight more and now the larval gullet has become absorbed and the upper coil of the alimentary canal has developed by extension of the first primary coil. The large type of globiferous pedicellariæ characteristic of the species was noticed for the first time about 12 days after metamorphosis. Subsequent growth is by no means uniform even in the same jars. From the same culture 12 specimens, all 90 days old, were taken and measured and the following table gives the details of the measurement:-

Diameter of Test.	Size with Spines
$3  \mathrm{mm}.$	7 mm.
3 ,,	7 ,,
3 ,, •	7 ,,
3 ,,	7.5,
4 ,,	8 ,,
4 ,,	8 ,,
4 ,,	8 ,,
$4\cdot 5$ ,,	8 ,,
$4\cdot 5$ ,,	11 ,,
$4\cdot 5$ ,,	11 ,,
7 ,,	15 ,,
10 ,,	20 ,,

Anus was observed to be formed like a slit when the urchin shows a test measurement of 4.5.

#### Discussion.

A comparison of the various Temnopleurid larvæ described so far may be of interest here. Tennent's (1929) 55-hour old larva of Salmacis virgulata Alexandri is very similar to the 9-day old larva of Temnopleurus toreumaticus described by Mortensen (1921). What is more remarkable is that it should be so similar to the 10-day old larva Salmacis bicolor considering that temperature conditions in Madras are so similar to those of the Torres Straits. Evidently there appears to be great disparity in the rate of development even in closely allied forms in spite of uniformity of temperature conditions, a fact which has already been noticed in other cases by Mortensen (1921). He seems to consider that the difference in yolk and temperature cannot account for the deviation that actually exists in the rate of development.

A comparison of the skeletal rods of some of the described Temnopleurid larvæ brings out the following facts. The body rods of Salmacis bicolor larva at two and a half days age are similar to those figured by Mortensen for Temnopleurus toreumaticus but are less thorny and Tennent's figure of the skeleton of Salmacis virgulata Alexandri larva 29 hours old shows that in the Australian form it is more robust. In Mespilia globulus the body rods when best developed appear very much more thorny than in the other Temnopleurid larvæ. The anterior lateral rods of the Madras form are slightly more thorny than in T. toreumaticus and the dorsal arch definitely makes its appearance on the 8th day of development. The skeletal rods in the larva of Temnotrema sculpta when two days old appear to be more slender than the skeleton at a corresponding stage in Salmacis bicolor and Salmacis virgulata Alexandri. Regarding the posterior transverse rod its development has been described so far in Mespilia globulus, "Echinopluteus of Temnopleurid (?) species a", "Echinopluteus of Temnopleurid (?) species b", "Echinopluteus of Temnopleurid (?) species c" and Temnopleurus toreuma-The transverse rod of the present form is totally unlike that figured by Mortensen (1921) for Temnopleurid species b and c but agrees closely with that figured for species "a" though it is distinctly smaller.

A comparison of the full larval skeleton of Temnopleurid species "a" figured by Mortensen and a corresponding stage skeleton of Salmacis bicolor shows that he is dealing with a species of Salmacis as Tennent has already suggested. But one cannot be equally sure whether his suggestion that "Echinopluteus of Temnopleurid species c" is also a Salmacis larva is correct. The arms of the Madras form are different in shape from those of

Temnopleurid species "c". Further there is very great deviation in the nature of the dorsal arch and the posterior transverse rod. It is more probable, as Mortensen himself has suggested, that this form belongs to a genus other than the larvæ described as species "a" and "b".

Basing his statement on the development of *Temnopleurus toreumaticus*, *Temnotrema sculpta* and *Mespilia globulus* and the Echinopluteus larvæ of a few unidentified Temnopleurids Mortensen came to the conclusion that the larval family Temnopleuridæ appears to be characterised by the absence of a basket structure, the body rods dividing into branches in the first stage and in the second stage by the development of a posterior transverse rod with two short branched postero-lateral rods and the formation of 4 epaulettes. The development of *Salmacis bicolor* confirms his opinion in every respect.

An attempt was made to collect from the Madras Harbour specimens of small size with a view to getting some idea of the rate of growth and also to find out if specimens of the same size as those reared in the laboratory are mature. This attempt has not met with success as small urchins of less than 3-4 cms. have not been met with though hundreds of large forms have been obtained. In studying the gonadic spicules of Salmacis bicolor the author (1934) experienced the same difficulty and it seems probable that the young forms undergo their development in the deeper parts of the harbour and that they creep up only after attaining maturity. Orton and others have noticed a similar phenomenon for Echinus esculentus. Mortensen (1921) also mentions that for Echinarchinius excentricus he could not get small sized individuals in the place where adults were collected in large numbers.

Fuchs and Morgan (1914) state that species of *Echinus* can become sexually mature when relatively small and below their full normal size. They obtained ripe eggs from *Echinus miliaris* 1 cm. across the spines. *Echinus esculentus* and *Echinus acutus* have been found by them to be ripe females when merely a fraction of their natural size. They seem to consider that *Echinus* species which they raised under laboratory conditions were not placed under unfavourable situation of growth judging from a comparison of forms raised in the laboratory and those of similar size from the sea. *Echinus esculentus*, which grows to a larger size after a year's growth under laboratory conditions, showed a diameter of test 1.5 cm. and a height of 6 mm. while the Madras form though smaller as an adult showed a growth of 1.5 cm. ×9 mm. after a year, a fact which goes to show that *Salmacis bicolor* had not been growing under any unfavourable conditions in the laboratory.

Another point which might be mentioned here concerns the breeding habits of tropical marine animals. Mortensen (1921) is not prepared to accept the view expressed by Semper (1883) and endorsed by Orton (1922) that in the tropics marine animals are continuously breeding. Pending publication of a paper in which this problem has been tackled in a systematic manner I wish merely to record here that I was able to obtain fully ripe individuals throughout the year from the Madras Harbour and artificial fertilization was invariably successful. The temperature of the water varies from 24° 4° C. to 30° C. during the year.

### Summary.

- 1. The full development of Salmacis bicolor, a tropical form has been traced both in regard to the external characters and several internal features.
  - 2. The amnion has been shown to arise very early in development.
  - 3. The development of the larval skeleton has been traced.
  - 4. The metamorphosis and the post-larval stages have been described.
  - 5. The absence of sexual periodicity in Salmacis bicolor has been proved.

## Acknowledgment.

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#### LIST OF ILLUSTRATIONS.

- Fig. 1.—Blastula. 10 hours old.  $\times$  125.
- Fig. 2.—Early Gastrula, with primary spicules. 23 hours old.  $\times$  200.
- Fig. 3.—Late Gastrula, optical section. × 250.
- Fig. 4.—Skeleton of pluteus, early stage.  $\times$  250.
- Fig. 5.—Side view of pluteus. × 125.
- Fig. 6.—Larva, three days old, dorsal view. X 125.
- Fig. 7.—Another larva of the same age, ventral view.  $\times$  125.
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- Fig. 10.—Larval skeleton at the end of 7th day.  $\times$  125.
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#### KEY TO LETTERING.

a.cil.ep.	Anterior ciliated epaulette.	am.c.	Amniotic cavity.
a.l.a.	Antero-lateral arms.	am.f.	Amniotic fold.
a.l.r.	Antero-lateral rod.	amp.	Ampulla.
$a. \omega s.$	Adult œsophagus.	arch.	Archenteron.
ad. $stom.$	Adult stomodæum.	a.z.t.	Azygos tube foot.
am.	Amnion.	b.r.	Body rod.

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cœ.	Cœlom.	p.sp.	Primary spicule.
d.	Tooth.	p.d.r.	Postero-dorsal rod.
d.a.	Dorsal arch.	ped.	Pedicellaria.
d.s.	Dental sac.	pig.	Pigment.
d.t.r.	Dorsal transverse rod.	p.l.p.	Postero-lateral processes.
ер.с.	Epineural cavity.	p.o.a.	Post-oral arm.
ер.f.	Epineural fold.	p.o.r.	Post-oral rod.
hy.	Hydrocœl.	pr.o.a.	Pre-oral arm.
int.	Intestine.	q.sp.	Quadrangular spine.
in. sp.	Inferior spiral.	r.a.c.	Right anterior cœlom.
ip. mus.	Interpyramidal muscle.	r.nerv.	Radial nerve.
l.a.c.	Left anterior colom.	r.p.c.	Right posterior cœlom.
l.hy.	Left hydrocæl.	r.r.	Recurrent rod.
$l.\alpha s.$	Larval œsophagus.	sp.	Spine.
	Left posterior cœlom.	su. sp.	Superior spiral.
l.p.c.	Mesenchyme.	st.	Stomach.
mes.	Primary water pore.	st.c.	Stone canal.
m.p.	Monales	t.f.	Tube feet.
musc.	Muscles Nerve fibrils.	v.t.r.	Ventral transverse rod.
nerve.		w.v.r.	Water vascular ring.
o.sp.	Ordinary spicule Pore canal.	W	
b.c.	Pore canal.		