# STUDIES ON THE EARLY LIFE HISTORY OF SARDINELLA SIRM (WALBAUM) FROM VIZHINJAM, SOUTHWEST COAST OF INDIA

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#### ABSTRACT

The early life history of Sardinella sirm has been studied up to the prolarval stage by rearing the fertilized eggs collected from the plankton. The prolarvae attained a maximum length of 3.79 mm around 24 h after hatching. Subsequent larval stages, ranging in size from 4.69 to 30.0 mm, collected from the plankton as well as from the shore seine (Nonna Vala) catches, have been illustrated and described.

#### INTRODUCTION

Only two works are seemed to have been published so far on the early life history of Sardinella sirm. Of these, one is from the Java Sea by Delsman (1926) and the other from the east coast of India by John (1951). These two works, however, do not agree with each other in many respects such as total length, myotome count, pigmentation, formation of the mouth, size of the yolk-mass, etc. Hence, an attempt was made in this study to rear the planktonic eggs in the laboratory and to arrive at the correct identity of the different stages of development by describing them in detail.

### MATERIAL AND METHODS

Although it is preferable to begin with eggs of known parents, as indicated by Ahlstrom and Moser (1979), live eggs obtained from plankton hauls have been utilized for rearing in most of the recent works. This method has proven very useful for constructing life history series. Attempts made to fertilize artificially the eggs were not successful, as the right kind of male was not available at the proper time. On account of this, examination of the naturally fertilized eggs collected from the sea by frequent plankton tows was resorted to in the present study.

Nearly 25% of S. sirm caught from Vizhiajam coastal waters on 19th January 1976 were in ripe and running condition. So plankton samples were taken from the same locality in the early morning hours (4 A.M.) of the following day to collect the naturally fertilized eggs. Plankton was collected from a depth of four to five metres for a period of one hour with an organdie net operated from a catamaran by making oblique hauls. A total of 20 fertilized eggs could thus be collected from the locality.

The eggs were kept in the laboratory in clear finger bowls containing fresh filtered sea water. The water was changed frequently during incubation, and all precautions were taken to keep the eggs free from contamination. The temperature of the water was kept as constant as possible by placing the finger bowls inside a trough of sea water and covering it with a wet towel. Microscopic examinations were made at frequent intervals to observe the changes in the course of development, and to make drawings of the same in fresh condition with the help of a camera lucida. Whenever there was a remarkable change noticed in a particular stage, a sample was fixed in 2% formalin for future detailed study. The embryo and larvae became slightly opaque upon preservation. In some cases shrinkage was noticed especially in the dorsal fin-fold of the larvae. For counting the number of myotomes the larvae were stained in alizarin red and cleared in glycerine. Upon hatching, the larvae were transferred to wide mouthed beakers and the water was kept constantly agitated under a fan to keep the larvae affoat, and to save the larvae from bacterial and ciliate attack.

Subsequent larval stages were collected from plankton and older postlarvae were obtained from shore seine (Nonna Vala) catches. The larvae up to 11.75 mm (T.L.) size described here were collected during January 1976 and the rest were collected during February 1976. The larval stages were classified following Jones (1967). Anon. (1976) was followed for naming the larval pigments. The absence of oil globules, the structure of the yolk-mass and the shape and size of the eggs were in general the characters made use of in the present study for the identification of eggs. The simultaneous occurrence of the spawners was taken as a collateral evidence. In the case of larvae, the features such as number of myotomes, arrangements of muscle fibres, position of anus, nature of alimentary canal and general pigmentation were given due importance as recommended by Bensam (1971b). An intra-ovarian egg taken from an oozing ovary is described so as to confirm the identity of the fertilized eggs.

### THE EARLY LIFE HISTORY

Intraovrian egg: The intraovarian egg (Fig. 1) just before spawning was spherical and completely transparent. The egg membrane was not distinguishable from the yolk and there was no perivitelline space. The yolk was segmented and frothy in nature with innumerable yolk granules. The egg diameter ranged from 0.80 to 1.37 mm with an average size of 1.04 mm. Upon preservation the ovum became opaque and a slight reduction in the size took place.



FIG. 1. Intraovarian egg of S. sirm just before spawning.

*Planktonic egg:* The fertilized egg collected from the plankton was perfectly spherical and transparent, ranging between 1.57 mm and 1.58 mm in diameter. A clear perivitelline space was present between the yolk and egg membrane. The yolk was segmented and frothy and its diameter ranged from 0.80 to 1.30 mm.

In the developing egg the germinal disc had already disappeared by 11 A.M. on 20-1-1976, giving rise to the embryo with its tail growing out (Fig. 2, A). The embryo was attached to the yolk very firmly except for the caudal region, which was turned over. The yolk was frothy and coarsely segmented, roughly spherical and devoid of oil globules. In the yolk there was a large denser region, somewhat clearly segmented, and a less denser region, where the segmentation was not clear but granules present. At this stage, the optic vesicles of the embryo were clearly seen and the heart was functioning. The tip of the tail was round and the fin-fold was not recognisable.

At 12.30 P.M. the free portion of the tail increased in size to about one-fourth length of embryo (Fig. 2, B). The myotomes had started appearing in the free region of the tail, but it was not possible to count them. Then, the tail acquired a fin-fold and was turned over or curved toward the yolk. The anus made its appearance and the yolk-mass assumed an oval shape.

At 3 P.M. the development of the embryo was almost complete (Fig. 2, C). The embryo grew very long and was curled up inside the egg membrane. The myotomes were found all over the body of the embryo. At this time the free portion of the tail was about one third of the length of the embryo and the yolk-mass assumed a distinct oval shape. The alimentary canal and the

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FIG. 2. The developmental stages of egg and of larva of S. sirm: A-C - the developmental stages of embryo; D - prolarva soon after batching; E - 4-h-old prolarva; F - 16-h-old prolarva; G - 24-h-old prolarva; H'- early postlarva (4.69 mm T.L.); I - late postlarva (5.13 mm T.L.).

vent were visible and the formation of the full complement of the myotomes which extended up to the tip of the tail was apparent. The caudal portion was sharp, the fin-fold became wider, and, at this stage, the embryo frequently changed its position by performing twitching movements inside the egg membrane.

Hatching was observed between 4.30 and 5.00 P.M. Prior to hatching the embryo exhibited active wriggling movements, resulting in the breaking of the egg membrane, and the pro-larva came out by making a few lashing movements. Thus the period of incubation seemed to exceed  $17\frac{1}{2}$  h for this species.

Prolarva immediately after hatching (Fig. 2, D): The newly hatched prolarva measured 3.25 mm in total length. It was inactive and floated in an inclined position with its head facing downward and the yolksac upward. The yolk was segmented and the yolksac was evenly rounded posteriorly. The yolk was divisible into two regions, an anterior smaller portion with segmentation of yolk less clear, indicating absorption of yolk, and a posterior portion with segmentation of yolk very clear. Mouth was not formed at this stage but the head was distinguishable by its rather triangular shape. The eyes were not so prominent but the auditory vesicles started appearing. The anus was placed far behind, the tail portion being about half the length of yolk. The alimentary canal was long, straight and slender. In the preanal region the myotomes were clear, 37 in number; but, in the postanal region, they were discernible only up to half distance, and only 6 myotomes could be counted within that region. The larval fin-fold was continuous and perfectly transparent with a constriction near the anus. A rayed appearance was seen at the caudal end.

Prolarva 4 h after hatching (Fig. 2, E): The larva measured 3.75 mm in total length. There was no indication of a mouth yet. But the head became more roundish. There was a significant reduction in the size of yolk and the yolksac became elliptical in shape. Anteriorly, the dorsal fin-fold was slightly absorbed. The alimentary canal was straight. Twenty-one myotomes with wavy margin could be counted. Crossed nature of the muscle fibres was seen feebly and there was no pigmentation on the body at this stage. The auditory vesicle became prominent.

Prolarva 16 h after hatching (Fig. 2, F): This prolarva measured 3.79 mm in total length. A good amount of yolk had been used up by then. The prolarva swam actively by wriggling movements. Black chromatophores developed on the head, over the anterior region of the body (near the yolksae), over the caudal peduncle and over the region of alimentary canal. The eyes became prominent and the auditory vesicles appeared distinct. The head and mouth attained their shapes and the larval fin-fold was continuous except at the region of anus. The myotomes continued to be indistinct, but the crossed nature of the muscle fibres was more prominent, giving a reticulate appearance.

Prolarva 24 h after hatching (Fig. 2, G): There was no change in the total length, but a slight decrease in the breadth of the larval fin-fold, particularly at the anterior dorsal portion, was noticeable. A major portion of the yolk was used up, giving a shrunken appearance to the yolksac. Rudimentary

fin rays were seen on the ventral side, a little posterior to the middle, as well as on the caudal region. Posterior to the anus, near the caudal, four big black pigments appeared. The pectoral fin appeared as an ovate structure. Only the myotomes between the anus and half-way to the attachment of yolk to body were countable, and they were 32 in number. The myotomes of the anterior region just below the head and the postanal region were indistinct.

Early postlarva 4.69 mm T.L.: This material was obtained from the plankton collected at Vizhinjam on 22-1-1976 at 4.00 A.M. ie., 3 days after the eggs had been collected. By now, the prolarva had become early postlarva (Fig. 2. H), with the absorption of yolk completed and the median fins without already-formed rays. The fin-fold had shrunken considerably, leaving very little over the developing median fins. The dorsal and anal fins started appearing in this stage. The crown of the head had risen in a crest and the jaws had developed into functional structures. The yolksac was nearly absorbed and the intestine well-differentiated. The black pigments that had been found near the anal and caudal regions were not seen now. But pigments were seen along the ventral region in between the origin of pectoral and anal fins, representing the beginning of the appearance of pre-bladder, post-bladder and sub-guttal pigmentation. The eyes became very dark and the head was light grey in colour. The body gave the appearance of real solidity owing to the presence of clear myotomes and the feebly crossed muscle fibres. At this stage there were 37 preanal myotomes. The postanal myotomes could not be counted correctly because of the overlapping of muscle fibres.

Late postlarva 5.13 mm T.L.: This larva (Fig. 2, I) was collected at 4 A.M. on 23-1-1976 from Vizhinjam with a plankton net from a depth of about 4 metres, four days after the collection of eggs. The head became much more prominent, and the lower jaw protruded slightly beyond the upper jaw giving the head a little pointed appearance. Colour of the cephalic region remained the same as in the previous stage. The opercle was clearly defined and the pectoral fins were triangular in shape. The fin-fold was greatly diminished and only a vestige was found. The caudal fin became triangular, leaving a small area in the middle without rays. The rays had started appearing in the median fins. The pigmentation along the ventral side became more prominent. The prebladder pigmentation appeared in the form of four dots and the sub-guttal in the form of three dots. There was little prominence seen in the development of post-bladder pigments. The pectoral spot and cheek spot appeared in this stage and the throat pigment continued to be in the form of a linear dot. Pigmentation was also noticeable along the base of anal. The myotomes were clearly seen and were 37 in the pre-anal region. The crossed nature of the muscle fibres was superficially seen. The area near the vertebral column was transparent, whereas the dorsal and ventral regions were opaque.

Late postlarva 10 mm T.L.: This larva (Fig. 3) was obtained on 29-1-1976 from a plankton sample collected from the coastal waters of Vizhinjam at 5 m depth. The dorsal and caudal fins had more rays and the anal fin demarcated. Pectorals were rather semicircular in shape with very feeble rays. The larval finfold still persisted, partly as wavy margin. The crossed muscle fibres were visible. The eyes appeared oval, with the long axis vertically oriented. Throat pigmentation was seen as two linear dashes. One pectoral spot was seen on the



FIG. 3. Late postlarva of S. sirm (10 mm T.L.).

right hand side of the larva. Pre-bladder pigmentation was represented cleary as 14 dashes; similarly the post-bladder pigmentation was represented by 14 broad and roundish dots. Sub-guttal pigments were seen along the ventral edge in between the anus and the bladder region as a broken paired line. One broad stellate pigment was present near the end of the gut. Only two pigment dashes were seen on the caudal lobes. 34 preanal and 9 postanal myotomes could be counted. Gill arch was seen when stained with alizarine.

Late postlarva 11.75 mm T.L.: This specimen (Fig. 4) was obtained from the plankton collected off Vizhinjam on 31-1-1976 at 5 A.M. at 6 m depth. Though this larva was only slightly bigger than the previous larva described above, it



FIG. 4. Late postlarva of S. sirm (11.75 mm T.L.).

showed some significant changes. The larval fin-fold was completely absent in this stage. A blotchy semilunar pigment spot appeared on either side behind the eyes above the tip of vertebral column and also the head pigment above that. A lateral line was also seen in this stage. The bladder found in the earlier stage had disappeared. The shape of eye became more roundish. The head was about 1/6 the length of the larva. The caudal fin was forked. Pectorals, dorsal and anal had grown further. Like in the previous stage, two dash-like throat spots were present with two lateral spots at the base. The pre-bladder pigmentation was made up of 13 dashes and the post-bladder by 22 dots. The stellate pigment was present near the anus; but the caudal streaks were absent. The larva had 33 preanal myotomes and 10 postanal myotomes at this stage.

Late postlarva 20 mm T.L.: This specimen (Fig. 5) was collected from Vizhinjam on 8-2-1976 from a shore seine catch. In this larva the ventral had developed and was seen much before the level of the origin of dorsal. The head had grown further and was about 1/5 of the length of the larva. The



FIG. 5. Late postlarva of S. sirm (20 mm T.L.).

head pigments just appeared on the dorsal and lateral sides. Pigments were also seen on the tip of the jaws and on the lower margin. A blotchy semilunar pigment was seen at the posterior ventral aspect of the mid-brain. Throatspots were seen in the form of three dashes and posterior cross bars. The cheekspot was visible now. The post-bladder pigmentation was seen in between the anus and the ventral fin on the lateral side as 21 dots. The sub-guttal pigmentation was seen below the post-bladder pigmentation. The pre-bladder pigmentation appeared like a broken line. Stellate pigments were seen on the base of anal and caudal fins and also on the caudal peduncle. The maxillary bone was not fully developed and the ventral scute had not yet formed. Thirty-two preanal and 11 postanal myotomes could be seen. The eyes were nearly round and the crossed nature of the muscle fibres had disappeared totally, only the longitudinal fibres were seen.

Late postlarva 25 mm T.L.: This material (Fig. 6) was collected from a shore seine catch off Vizhinjam on 16-2-1976. The eyes were more roundish in this stage. The cheek-spot and throat-spots were prominent; the throat-spot was in the form of two linear dashes. There were 4 opercular-spots present. The lateral



FIG. 6. Late postlarva of S. sirm (25 mm T.L.).

pigmentation was seen on a line indicating the lateral dots characteristic of adult fish, which were 43 in number. The head pigmentation was seen in the form of a circle. Pigmentation on the base of anal and caudal was prominent. Pre- and post-bladder pigmentation and the sub-guttal pigmentation were seen in the form of feeble dots. The body became deeper and more massive and the larva more sardine-like than in the previous stage. The dorsal was seen just behind the level of origin of the ventral and the pre-dorsal and post-dorsal lengths had become almost equal. The head was 1/4 of the total length. Considering the developments in the previous and present stages, the head in the post-larval phase of development appeared to have undergone a more rapid growth than the rest of the body did, reaching the maximum in the present stage. Narial openings were distinctly seen. The maxillary had become more prominent and fin rays were discernible, but scutes had not yet formed. The anus had further moved forward opening below the 29th myotome. About 29 pre-anal and 14 post-anal myotomes could be counted.

Late postlarva 30 mm T.L.: This larva (Fig. 7) was collected on 24-2-1976 from Vizhinjam from a shore seine catch. The body had become more cylindrical, giving a juvenile look. The level of origin of pelvic was a little below the level of origin of dorsal. The maxillary bones were well-developed and the



FIG. 7. Late postlarva of S. sirm (30 mm T.L.)

head was about 1/4 the length of the larva with round eyes. Stellate, small, lateral pigments appeared throughout the body. They were found denser in the posterior region and less denser in the anterior region. The body became almost opaque. There were three faded pigments seen on the opercular border, the upper two were dash-like and the bottom one roundish. The throat-spot was in the form of a linear dash. Below the orbit there were three small pigments. Behind the orbit there were three stellate pigments, of which the central one was big, which might be called a cheek-spot. The caudal streaks had disappeared and nodes were formed in the caudal fin rays. Post-bladder and sub-guttal pigmentations were feebly seen in two rows. Pigments on the anal fin base and caudal peduncle region were prominent. All the fins were well developed with rays. There were 12 pectoral rays, 22 caudal rays, and 14 dorsal rays. Ventral scutes had not appeared and there were about 28 pre-anal and

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15 post-anal myotomes as in the adult. Almost all the larval features had disappeared. Though this stage showed many a juvenile character, the absence of ventral scutes and the difference in disposition of the dorsal and pelvic fin from the juvenile did not permit to classify it as a juvenile.

### DISCUSSION

One of the direct methods of identifying the planktonic egg of sardines is by tallying the size of its yolk — rather than the size of the egg — with that of the yolk in the ripe ovum, because, though, with development, the egg becomes larger, the size of the yolk remains constant (Bensam 1971a). It may be noted in this connection that in the ripe ovum the membrane was not distinguishable and the perivitelline space was absent. The egg membrane would become visible only after the ovum came into contact with water, when it would swell itself pulling from the yolk and leaving a perivitelline space; the egg thus would become larger and larger with advancing development without the yolk taking part in the process (Miller 1952). It was based on this criterion that the present eggs had been mainly identified as that of Sardinella sirm. The same criterion was followed by the author to identify the fertilized egg of S. longiceps also (Lazarus 1985). The yolk-diameter range of the planktonic eggs of S. sirm (0.80-1.30 mm) came within the diameter range (0.80-1.37 mm) of the intra-ovarian eggs. Moreover, the shape of the egg, the segmented nature of the yolk devoid of oil globules and the wide perivitelline space of the present material agreed well with the descriptions given by Delsman (1926) and John (1951). According to Delsman (1926), the diameter of the egg varied from 1.42 to 1.63 mm, whereas John (1951) recorded a greater diametre, 2.12 mm. The diameter of the fertilized egg (1.57-1.58 mm) described here was within the range given by Delsman (1926).

The sizes of the newly hatched larva recorded by Delsman (1926) and John (1951) also did not agree. Delsman (1926) recorded a length of only 3.21 mm, while John (1951) recorded a length of 6.5 mm. This vast difference in the length of the newly hatched larva could be due to the wrong assessment of hatching time given by John (1951). Delsman (1926) gave the hatching time as 3:30 to 5:15 P.M., whereas John (1951) has given it as 'next morng-ing', stating that hatching did not take place till 6 P.M. of the preveious day. From this it is evident that John (1951) had not followed the development of his material from 6 P.M. to the next morning.

As in the present study, the earlier authors had also collected the eggs in the early morning hours. At the time of collection itself the eggs were in an advanced stage of development, that is, they were with embryo and not with germinal disc. In the present study, hatching was noticed between 4:30 and 5.00 P.M. Delsman (1931) stated that most marine fishes probably spawn a few hours before mid-night.

John (1951) observed the formation of the mouth as an anterior terminal slit and the rudiment of the pectoral fin as a clear circular structure in the newly hatched larva. But these characters were observed by Delsman (1926) and in the present study in 12-h and 14-h-old larvae. The size of the yolkmass in the newly hatched larva as illustrated by John (1951) was considerably smaller than that described by Delsman (1926) and the present author. These would clearly support the view that the description of the so called newly hatched larva by John (1951) may have been of an older larva.

John (1951) also observed, in his newly hatched larva, certain black spots on the body, of which the one at the tip of upper jaw he believed as characteristic of this stage only, because it disappeared on the same day. He also observed minute, stellate, black chromatophores in the interorbital region and a row of very minute black dots along the alimentary canal. Further, he found on the dorsal and ventral sides of body in the tail region a few black dots and considered this pigmentation to be characteristic of this species. Delsman (1926), on the other hand, did not record any pigmentation in the newly hatched larva. He could observe pigment cells only after 12 h. Similarly, in the present study also the pigment cells could not be observed either in the newly hatched larva or in the 4-h-old larva. But, black chromatophores were observed on the head, anterior region of the body (near the yolksac), at the caudal peduncle and over the region of alimentary canal in the larvae of about 16-h-old only. Thus, apparently, larvae described by John (1951) were not newly hatched ones but several hours old. Nair (1960) also expressed a similar opinion about the larvae described by John (1951). So it may be concluded that the larvae described by John (1951) as the newly hatched ones must be at least 12-h-old. And so, the 12-h-old and 24-h-old larvae described by John (1951) could be in reality 24-h- and 36-h-old larvae, respectively.

Delsman (1926) found the total length of the larva at different ages as follows: Newly hatched one 3.21 mm, 12-h-old one 5.11 mm, 24-h-old one 5.67 mm and 3-day-old one 5 mm. Thus in his study an apparent decrease in length could be observed in the 24-h-old to 3-day-old larvae. In the present observation, however, the length of the larva was 3.25 mm when freshly hatched, 3.75 mm when 4-h old, 3.79 mm when 16- as well as 24 h old, 4.69 mm when 2-day old, 5.13 mm when 3-day old and 6.75 mm when 4-day old. Though the growth rate was found to be less (1.54 mm) in the first day, the larvae reached a total length of 5.13 mm on the 3rd day, that is, 0.13 mm more than Delsman's material. Since the 2-, 3- and 4-day-old larvae were from the plankton, they had obviously a growth rate faster than that of Delsman's material, which he had reared in the labogatory up till the 3rd day.

According to John (1951), the larva possessed 37 preanal and 6 postanal myotomes. Delsman (1926) reported 38 preanal myotomes in the larvae

till it became 24-h-old and 37 in the 3-day-old larvae. But, in the present material 37 preanal myotomes were found both in the newly hatched larvae and in 2- (4.69 mm) and (5.13 mm) 3-day-old larvae. It was not possible to count the post-anal myotomes correctly in the above mentioned stages. However, it was possible to count it in the later stages (10 to 30 mm sizes). The number of preanal and postanal vertebrae in Sardinella sirm as given in earlier works (Delsman 1926 and Anon. 1976) ranged from 28 to 29 and 14 to 15, respectively, with a total of 43 vertebrae. A few adult specimens from Vizhinjam examined by the author also had 43 (28 + 15) vertebrae. It may be noted that one of the characteristic phenomenon during the development of clupeoid larvae is the forward movement of the anus, resulting in decrease in the number of preanal myotomes, a phenomenon evident in the present case. The 37 preanal myotomes found in the newly hatched larva (3.25 mm) had reduced to 34 in the 10.0 mm larva, which further reduced to 33 in the 11.75 mm larva, 32 in the 20.00 mm larva and 29 and 28 in the 25.00 mm and 30.00 mm larvae, respectively. Similarly a corresponding increase was seen in the postanal set of myotomes which were countable with accuracy from 10.00 mm size onwards. In the 10.00 mm larva there were 9 post-anal myotomes, which increased to 15 when the larva reached 30 mm length passing through 10 myotomes in 11.75 mm larva, 11 and 14 in 20.00 and 25.00 mm larvae, respectively. Bensam (1973) also observed a similar change in the number of preanal and postanal myotomes of 18.70 mm (34 + 12) and 20.25 mm larvae (34 + 14) of Sardinella dayi. In an earlier observation on Sardinella jussieu (Lacep.) (Bensam 1971a), a decrease of preanal myotomes from 39 (1.28 mm size) to 29 (22.24 mm size) and an increase of postanal myotomes from 12 (4.28 mm size) to 16 (22.24 mm size) were noted.

The larval sardine can thus be sorted out based on the very rearward position of the anus. The gut-length:length ratio is relatively high in sardine larvae. The larvae of whitebait (*Stolephorus* spp), though are looking similar to the above, have their anus in a more forward position. The larvae of the white sardine, *Kowala coval*, and the rainbow sardine (*Dussumeieria* spp) also have a very rearward position of the anus. Though in the former the total myotome number is about 42 the pigmentation is light. In the latter the myotome number is about 57 and the shape of the larvae is distinctive by the more pointed snout.

The occurrence of spawners of Sardinella sirm on the previous day, the presence of wide perivitelline space in the egg, the segmented nature of yolk without oil globule, the posteriorly rounded yolk in the newly hatched larva, the elongated shape of the larva with the backwardly situated anus, the crossed arrangement of the muscle fibres, and the number of preanal and postanal myotomes indicated clearly that the eggs and larvae described here belonged to S. sirm. The characters described by Delsman (1926) further confirms the identity.