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ARTIFICIAL SPAWNING BY STRIPPING IN *DUSSUMIERIA ACUTA*, A MARINE PELAGIC FISH, AND STUDIES ON ITS EARLY DEVELOPMENT

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ABSTRACT

Artificial spawning was successfully conducted in *Dussumieria acuta* Valenciennes (Family: Dussumieriidae), a marine pelagic fish, by stripping the fully ripe female and male. The experiment was conducted on board a privately owned shrimp trawler operated in the Palk Bay, by participating in one of its night fishing trips, on 2nd March, 1973. The stripped eggs and milt were mixed in filtered sea water. Majority of the eggs were fertilized. Detailed microscopic studies were conducted the next day in the laboratory. The first larva hatched out 24 hours after fertilization. The larvae could be reared only upto 48 hours after hatching. The developmental stages of the embryo and the larva are described and discussed. It is suggested that the technique of artificial spawning by stripping and early ranching of the embryos into the sea, if carried out by the fishermen in a variety of commercially important species which it is possible on board the fishing vessels on a large scale, can increase the natural production.

INTRODUCTION

STUDIES on the fish eggs and larvae have been a fascinating subject for fishery scientist in the early half of the twentieth century. As early as 1864, Sars studied the development of the eggs of the cod, haddock and the gurnard. Later the characteristics of the eggs and larvae of most of the commercially important species of the temperate seas and, though a little late, the oriental tropics are now much known. Some of the important works are those of Jones (1937), Devanesan (1937 and 1943), Devanesan and John (1940 and 1941), Devanesan and Varadarajan (1942), Gopinath (1942, 1946 and 1950), Chidambaram and Venkataraman (1946), Jones and Menon (1951) and Vijayaraghavan (1955 and 1957), to mention a few. The contributions by Nair (1946 and 1952), John (1951) and Bapat (1955) are also important since they contain valuable informations on the early development of fish eggs and larvae of the Indian seas.

Quite a few workers have collected and described the planktonic eggs and larvae of the rainbow sardines. Delsman, as early as 1925, studied the embryonic and larval history of *Deussumieria hasseltii* from the Java Sea. Later, Devanesan and Chacko (1944) worked on the bionomics of the same species and described the eggs and larvae. Chacko (1950) could collect the planktonic eggs of *D. hasseltii* from around Krusadai Island in the Gulf of Mannar. Bapat (1955), in a preliminary study on the pelagic fish eggs and larvae of the Gulf of Mannar and the Palk Bay, provisionally refers the eggs he collected and marked as 'Type G' to *Dussumieria* spp. Kuthalingam (1961) described the eggs and larvae of *D. acuta* based on his collection from the offshore area of Madras coast and traced the development upto 56th day after hatching. Mahadevan and Chacko (1962), while studying the biology of *D. hasseltii* from the Gulf of Mannar, have also briefly dealt with its eggs and larvae. In his review on Indian sardines Nair (1973) has summed up the salient points on the development of rainbow sardines.

All these studies on the eggs and larvae are based on the samples collected from the plankton and later identified them as belonging to the respective species depending on certain characteristic features of the larvae. The present study on the early development of *Dussumieria acuta* is based on the artificially induced spawning of the oozing males and females at the time of capture, in a commercial fishing vessel operated in the Palk Bay, on 2nd March 1973 and tracing the developmental stages by rearing the fertilized eggs in the laboratory.

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MATERIAL AND METHODS

During the course of the studies on the spawning periodicity of *D. acuta*, an attempt was made for artificially induce the fish to spawn by stripping and trace the developmental stages of the embryo. The studies on the bionomics of the species at Mandapam have shown that, it has the peak spawning during February—May. So the experiment was planned and conducted on a new-moon night in March 1973, on board a private shrimp trawler, by participating in one of its night fishing trips in the Palk Bay and by utilizing the minimum facilities available on board the vessel. Trawling was conducted at 10.5 m depth off Mandapam. The equipments carried on board were clean plastic buckets with lids, bolting silk for filtering sea water and plankton net to take plankton hauls to collect eggs and larvae. The first haul of the trawl net came on board at 21.30 hours. On examination of the catch a few live specimens of *D. acuta* of both the sexes in ripe and oozing stages were noticed. Immediately the oozing gametes of males and the females were stripped by gently squeezing on either side of the belly into a bucket containing filtered sea water collected from the fishing area. The milt and the eggs were then mixed thoroughly by adding more filtered sea water into the bucket. The time of mixing the gametes was 21.40 hrs. Further examinations could not be done in the night due to lack of facilities. By the next day morning it was noticed that most of the eggs were fertilized and were floating with tiny embryos inside. The floating embryos were transferred into another bucket containing fresh filtered sea water.

On reaching the laboratory the next day morning at 11.30 hrs, the live embryos were transferred into a clean, round glass trough containing fresh filtered sea water. Regular observations on the embryos and the larvae were made under microscope at fixed intervals, traced the various process in their development and made drawings with the help of camera lucida. The last larva survived upto 48 hours after hatching. The time of fertilization was fixed between 21.45 and 22.00 hrs and the first larva hatched out in the laboratory after 24 hours, at 22.00 hrs the next day. Since no other account on fertilization by stripping on marine fishes is available so far, the success made in this study is the first of its kind in India, that too on a delicate and sensitive pelagic fish such as *D. acuta.*

RESULTS

Description of the egg

The egg is colourless, transparent and spherical with smooth egg membrane and its diameter varied from 1.34 to 1.66 mm. The

yolk is segmented and frothy in the characteristic clupeoid fashion. Single oil globule measuring 0.13 to 0.14 mm in diameter is present at the vegetative pole of the embryo. The proportionate size of the oil globule is 9.22 to 10.16% of the egg diameter. The perivitelline space is narrow and the outline of the embryo is clear (Fig. 3).

All the unfertilized eggs were carefully removed. Diameter of hundred eggs selected at random were measured and the percentage frequency was plotted (Fig. 1). Nearly 86% of the eggs were between 1.45 and 1.57 mm. The range, mean, standard deviation and standard error were calculated and presented graphically in Fig. 2.

Development of the embryo

The embryo after 14 hrs of fertilization is slender with a length slightly more than half of the circumference of the egg. The head is formed and the eyeballs are visible. The rudiment of heart can be traced just behind the head, on the ventral side. The auditory vesicle is faintly seen behind the eye and the myotomes are visible. The yolk is segmented with oil globule situated at the tail end of the embryo (Fig. 3). After 17 hrs of fertilization the embryo is much more elongated and encircles the yolk reaching nearly 3/4 of the circumference (Fig. 4). Development of eyes, heart and auditory vesicles advances. The heart starts pulsating at this stage. The oil globule occupies a position just opposite the head and the yolk is clear and segmented. The 20 hrs old embryo is still longer and becomes 'C' shaped around the yolk. The heart beat is regular and rhythmic. The embryo wriggles at times within the egg case. As development advances, at 23.30 hrs (Fig. 6) the length of the embryo increases slightly more than the circumference of the egg and the tail overlaps the head. The head and body, except at the tail end, closely adhere to the yolk. The yolk is segmented and the oil globule is located at the middle portion of the body, opposite the head. The myotomes are clear, the eye lens formed and the auditory vesicle more prominent. The heart at this stage pulsates at a rate of 132 beats per minute. Rediment of the anus is traceable. The frequency of wriggling movement of the embryo inside the egg capsule increases. At this stage, the independent movement of the tail also takes place. At 24 hrs the embryo is fully developed and the incubation is complete and the larva hatches out.

Hatching

The first larva hatched out after 24 hrs of incubation. Before hatching, tilting and wriggling movement of the embryo becomes more frequent. Owing to the lengthening of the embryo it begins to bend at the middle (Fig. 7) and the shape of the yolk becomes slightly nonspherical. Bulging is noticed in the egg membrane near the head of the embryo. At first it develops as a narrow circular groove formed by the thinning of the egg membrane above the head which looks like a ring. Gradually the groove widens and the egg membrane roofing it bulges out forming a hood or cap (Fig. 7). The embryo starts wriggling violently inside the egg case. Simultaneously, the overlapping nature of the head and tail, seen in the advanced stage (23.30 hrs old) is lost and the tail shows signs of moving away from the vicinity of the head. All these changes indicate that hatching of the egg is imminent. Within a short time the hood breaks open and the head of the embryo comes out first followed by yolksac and the tail. The larva swims away by the lashing movement of the tail. The empty egg case is spherical and the bulged out hood remains at the mouth of the case attached to it by means of a short and narrow stalk (Fig. 8).

Newly hatched larva

The newly hatched larva exhibits all clupeoid characteristics, such as the backward position of the anus, the segmented yolk and the peculiar crossed arrangement of the muscle fibre in the myotomes. Immediately after hatching the larva is slightly curved dorsally and the oil globule is situated in mid dorsal side of the yolk. Later the larva straightens itself and the oil globule shifts its position to the posterior end of the yolk. The larva is transparent and measures 2.4 mm in length (Fig. 9). The head is closely applied to the yolk. The eyes are unpigmented and a little behind them are the auditory vesicles. The rudimentary brain is visible. The heart is pulsating regularly. The anus is situated at the posterior end; 48 preanal myotomes are present. The post anal myotomes are not accurately countable. The yolk sac is elongated and slightly tapering posteriorly and have segmented yolk. The finfold is continuous. It starts from the dorsal side of the head and ends at the postero-ventral margin of the yolk. Considerable lengthening of the larva is noticed during the first few hours of development. 3 hours after hatching fine black pigment spots appear along the myotomes and on the upper surface of the head. Rapid changes in the head of the larva takes place and in the 18 hour old larva indications of the gill opening are noticeable below the auditory vesicle. Small pigment spots spread all over the surface of the yolk and also along the gut. At this stage indications of fin rays appear in the caudal region.

24 hour-old larva

24 hours after hatching, the larva attains 4.2 mm length. The unpigmented eye occupies 2/3 of the head. Behind the eye is the enlarged auditory vesicles. The lower jaw starts developing (Fig. 10). The first gill slit has developed just above the anterior part of the heart. The heart is two chambered. The yolk is reduced and a vertical separation is noticed, dividing it into anterior and posterior portions (Fig. 9) The split-up oil globule is scattered in the posterior section of the yolk and a few spreads into the anterior section also. Small black pigment spots spread over the yolk. Posterior portion of the intestine is well formed. The anus is clearly below the 48th myotome. Fine fin rays appear in the caudal fin area. Slight bulging of the finfold is noticeable in the dorsal side, above the anal region and on the ventral side just in front of the anus. Fine black pigment spots are more in these areas, while they are scattered in the rest of the fin fold. Black pigment spots are visible in a row along the myotomes, intestine and a few spots on the dorsal side of the head.

39 hour-old larva

The larva is much more elongated and slender. The yolk is much reduced but not completely resorbed. The oil globule completely disappears. The mouth is wide open (Fig. 11). The lower jaw has developed and ossification starts on both the jaws and rudiments of teeth are visible (Fig. 12). The eye balls cover the major portion of the head. Behind the eye is the first gill slit in the form of a round hole. Two more gill-slits appear behind the first one. On the postero-dorsal side of the heart, below the third and fourth myotomes, is the rudiment of pectoral fin. The fin rays are clear in the caudal region. The black dots in the fin fold almost disappear.

48 hour-old larva

The larva is further elongated and still transparent. The yolk is not completely absorbed. It exists as a narrow band along the

ventral side. The auditory vesicle is almost the size of the eye and is slightly oval in shape with ossification in it. The jaws are well developed, prominent and pointed with redimentary teeth, six in the lower jaw and four in the upper jaw (Figs. 13 & 14). Ossification starts in the jaws. The mouth is wide open and it can not be closed. All the gill-slits appear and the first one is slightly elongated and slit-like and the size of the subsequent ones decreases and the fourth one is very small. Rudiments of the gill cover appear extending from the posterio-ventral side of the auditory vesicle to the angle of the jaw, the rudiment of the pectoral fin is clearly visible at this stage. The black spots have disappeared from the finfold and scattered pigment spots appear in the anterior half. A number of regularly arranged black spots along the under side of the gut and a similar series along the upper halves of myotomes are visible.

No larvae survived more than 48 hours after hatching. They died mostly due to severe ciliate attack. Feeding of the larvae with suitable diet was not possible in the limited laboratory facilities.

IMPLICATION OF THE STUDY

This experiment has got a wider implication in the present context. The marine fisheries production in our country in recent years are subjected to wide fluctuations due to fishery dependent or fishery independent factors or both. It is well known that one of the reasons for decline/fluctuation in any fishery resource is recruitment over fishing. It happens when the ripe spawners are fished in large quantities without giving them an opportunity to spawn. This affects the recruitment and replenishment of the stock in subsequent years leading to depletion of the stock in long run.

An alternative suggested to overcome the recruitment overfishing is sea-ranching of fish seeds produced in the hatcheries. This concept, though viable in certain species, is neither practical nor economical in the case of major commercial fish species such as sardines, mackerel, etc. because the establishment of hactchery and culture systems are capital intensive.

Based on the success made in the above experiment on *Dussumieria acuta,* it is suggested that in a number of commercially important fin fish species the artificial spawning by stripping can be conducted on a large scale by fisherman on board the fishing vessel and ranch the fertilized eggs into the sea. Stripping technique has been used effectively in many fresh water fishes such as carps, mrigal, cutla, rohu, etc., in which stripping is done after hypophysation and the embryos are reared in *hapas* and the fry are used as seeds in growout systems (Alikunhi, 1956; Jhingran, 1969; Chaudhuri and Jhingran, 1963; and Khan and Jhingran 1975). In the case of marine fishes, during the peak spawning period of a species, normally both the sexes will be uniformly matured and will be caught in a haul while fishing. Therefore, stripping can be conducted on a large scale on board the fishing vessel. It has been noticed that in most of the fish species the eggs and sperm remain active and viable for quite sometime, even after the fish is dead. This will enable to strip as much fish as possible. The embryos thus obtained can be slowly and gently released back into the sea in the fishing area, its natural environment. Naturally, maximum survival rate can be expected among these embryos. This technique has to be systematically implemented in all the fishing boats in every haul to maximize the quantity of the seeds ranched, so that the loss incurred to the fishery due to removal of the spawners can be compensated to a large extent.

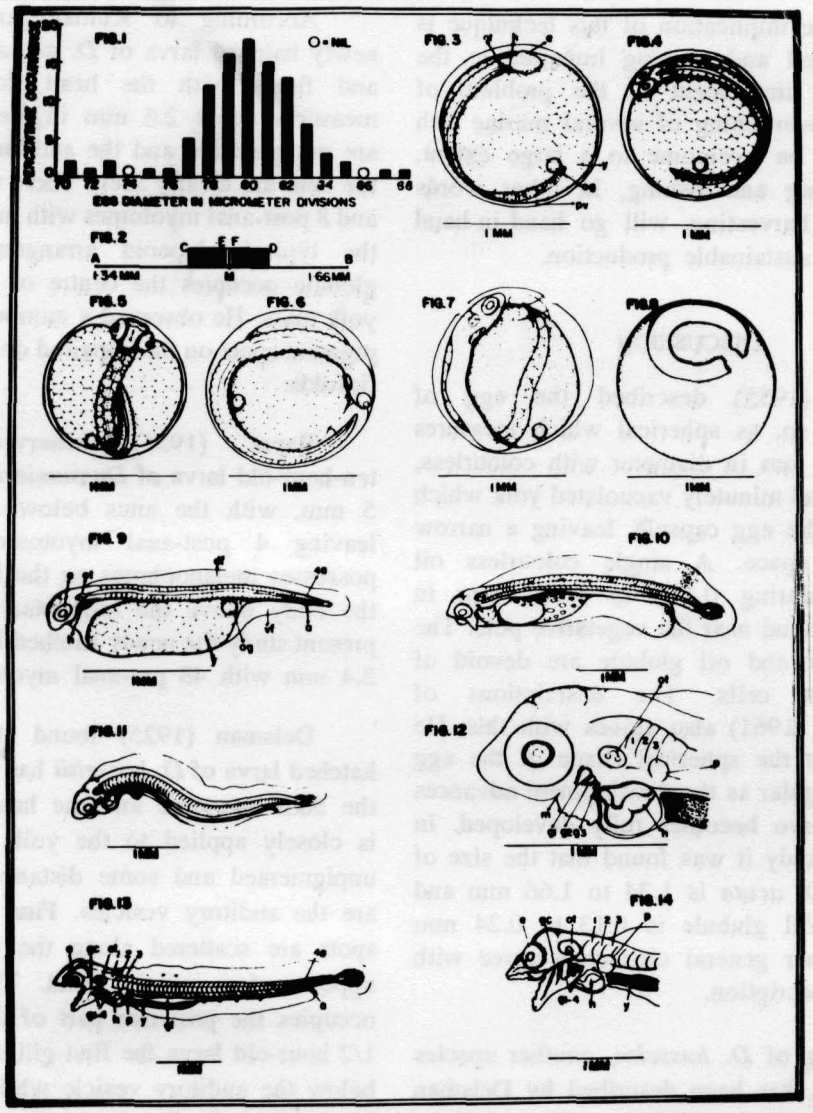

FIG. 1. *Diameter frequency distribution of spawned eggs of* Dussumieria acuta. *(Unfertilized eggs).*

FIG. 2. *Range, mean, standard deviation and standard error of the diameter frequency of the unfertilized eggs. A-B = Range, C-D = Standard deviation, N-F = Standard error and M = Mean.*

FIG. 3. *Fertilized egg of D. acuta with 14 hrs old embryo.*

FIG. 4. *Egg with 17 hrs old embryo.*

FIG. 5. *Another view of the 17 hrs old embryo.*

FIG. 6. *Egg with 23 1/2 hrs old embryo.*

FIG. 7. *Egg with embryo just before hatching.*

FIG. 8. *Empty shell of the egg after hatching.*

FIG. 9. *Newly hatched larva.*

FIG. 10. *Larva 24 hrs after hatching.*

FIG. 11. *Larva 39 hrs after hatching. (larva dead and slightly shrunken).*

FIG. 12. *Enlarged view of the head of the 39 hrs old larva.*

FIG. 13. *Larva 48 hrs after hatching (After Delsman 1925).*

FIG. 14. *Enlarged view of the head of 48 hrs old larva. **a** = anus, **df** = dorsal finfold, **e** = eye, **g1-4** = gill slits, **gc** = gill cover, **gt** = gut, **h** = heart, **m** = mouth, **og** = oil globule, **ot** = auditory vessicle, **p** = rudiment of pectoral fin, **pv** = perivitelline space, **vf** = ventral fin fold, **y** = yolk and **1, 2, 3,....48** = myotomes.*

Once the implication of this technique is fully envisaged and training imparted to the fisherman to implement it, the problem of recruitment overfishing of several marine fish stocks could be overcome to a large extent. Thus, ranching and fishing, in other words seeding and harvesting, will go hand-in-hand leading to a sustainable production.

DISCUSSION

Bapat (1955) described the egg of *Dussumieria* sp. as spherical which measures 1.41 to 1.67 mm in diameter with colourless, transparent and minutely vacuolated yolk which almost fills the egg capsule, leaving a narrow perivitelline space. A single colourless oil globule measuring 0.113 to 0.182 mm in diameter is found near the vegetative pole. The embryo, yolk and oil globule are devoid of any pigment cells. The descriptions of Kuthalingam (1961) also agrees with this. He observed that the spherical shape of the egg becomes irregular as the development advances and the embryo becomes fully developed. In the present study it was found that the size of the egg of *D. acuta* is 1.34 to 1.66 mm and that of the oil glubule is 0.13 to 0.24 mm while all other general characters agree with the above description.

The egg of *D. hasseltii,* another species of the genus, has been described by Delsman (1925), Devanesan and Chacko (1944), Chacko (1950) and Mahadevan and Chacko (1962). According to Delsman (1925) the egg diameter varies from 1.45 to 1.55 mm and has a small, colourless oil globule. He observed that the development of the egg is completed in one and a half days. The other workers mentioned above observed that the diameter of the egg is far less (0.88 to 1.15 mm) whereas that of the oil globule is more (0.26 mm).

According to Kuthalingam (1961) the newly hatched larva of *D. acuta* is transparent and floats with the head downwards and measures about 2.6 mm in length. The eyes are unpigmented and the auditory vesicles and the vent are clearly seen. There are 49 pre-anal and 8 post-anal myotomes with muscles showing the typical clupeoid arrangement. The oil globule occupies the centre of the segmented yolk mass. He observed a number of fine black pigment spots on the unpaired dorsal and ventral finfolds.

Bapat (1955) observed that the ten-hour-old larva of *Dussumieria* sp. measures 5 mm, with the anus below 48th myotome leaving 4 post-anal myotomes. The larva possesses melanophores on the head and along the body above the alimentary canal. In the present study the newly hatched larva measured. 2.4 mm with 48 pre-anal myotomes.

Delsman (1925) found that the newly hatched larva of *D. hasseltii* has the anus below the 50th myotome and the head of the larva is closely applied to the yolk. The eyes are unpigmented and some distance behind them are the auditory vesicles. Fine black pigment spots are scattered along the myotomes and upper surface of the head. The oil globule occupies the posterior part of the yolk. In 18 1/2 hour-old larva the first gill opening is seen below the auditory vesicle while the rudiment of the second gill opening is seen behind it. Small pigment spots have spread all over the surface of the yolk and along the gut, but have disappeared from the dorsal finfold. According to Devanesan and Chacko (1944) the newly hatched larva of *D. hasseltii* measures 1.7 mm. They counted 48 pre-anal and 9 post-anal myotomes. The eyes are unpigmented and two rows of brown pigments are present on either side of the larva. They noticed rapid growth

and forward shifting of the anus in the initial hours of development. 18 hour-old larva measures 3 mm in length with the anus below 41st myotome. The rudiments of the pectoral fin appeared when the larva is 3 hour-old. According to Chacko (1950) in the larva of *D. hasseltii* the anus is under 45-48th myotome and fine black spots scatter along the myotomes. Mahadevan and Chacko (1962) found that the hatchlings of *D. hasseltii* measure 1.89 mm with 42 pre-anal and 10 post-anal myotomes. Six hours after hatching fin rays appeared in the caudal region of the finfold.

Kuthalingam (1961) observed that the one-day-old larva of *D. acuta* continues to be transparent and measure 3.1 mm in length. The mouth is not developed and the yolk sac is smaller in size with the oil globule occupying the posterior end of the yolk mass. The eye continues to be unpigmented and the pectoral fin rudiment is seen as a membranous fold. 47 pre-anal myotomes are present in this stage.

In *D. hasseltii* Devanesan and Chacko (1944) found that the one-day old larva measures 3.12 mm and rudiment of the lower jaw appears. After 27 hours the length of the larva is 3.24 mm with two gill-slits and the upper jaw is differentiated. 39 pre-anal and 18 post-anal myotomes are counted. The eyes are unpigmented. 45 hours after hatching the larva attains 3.28 mm in length and the pectoral fins are well developed. The yolk disappears completely and the mouth is wide open. The eyes are pigmented, the pigmentation in the finfolds disappears and there are two rows of black pigment cells on the sides of the larva. Mahadevan and Chacko (1962) found that in *D. hasseltii* the yolk sac is completely absorbed after 32 hours when the larva measures 3.4 mm in length. They counted 48 pre-anal and 7 post-anal myotomes.

In the 48 hour-old larva of *D. hasselltii* Delsman (1925) observed all the gill-slits broken through with very wide openings. The lower jaw is well developed and the rudiments of the teeth begin to appear. The yolk is almost fully absorbed in this stage. There are 48 pre-anal myotomes. A few scattered pigment spots are present in the anterior half of the dorsal finfold. Immediately in front of the pectoral fin is the typical pigment spot and two small spots are invariably found on the interorbital space. According to him the most striking features of the head of the larvae of *Dussumieria* spp. is the wide gaping mouth with pointed jaws having strongly developed teeth and in this respect the larvae resemble the eel larvae. Apart from these, the larvae could be easily distinguished from other clupeiod larvae by the elongated, slender and transparent appearance. He found that the yolk sac is fully absorbed in about 2 1/2 days when the eyes become black. He could not keep the larvae alive for more than three days. However, he described a few advanced stages in the development of rainbow sardine obtained from the plankton collections. Devanesan and Chacko (1944) found the 53 hour-old larva of *D. hasseltii* measuring 3.46 mm and possesses a wide mouth showing a strong dentition on the lower jaw. They noticed three gill-slits in the stage with clear pigmented eyes.

According to Kuthalingam (1961) the 3 day-old larva of *D. acuta* continues to be transparent and measures 7.2 mm. The yolk sac is completely absorbed and the eyes have become black. The auditory vesicles are enlarged. Four gills are seen and the mouth and alimentary tract are well developed. The 12 day-old larva continued to be transparent and measures 14.2 mm. The head is distinct and the mouth is well formed. There are 46 pre-anal and 11 post-anal myotomes. The gills are well developed and the rudiments of teeth

are seen in this stage. The pigmentation on the dorsal side is very faint while on the ventral side it disappears completely. Two groups of black pigment patches are seen, one above the eye and the other above the auditory vesicles. Rays are well developed on the caudal fin. The 21 day-old larva, according to him, measures 20.2 mm with 44 pre-anal and 13 post-anal myotomes. The fin rays appears in the dorsal and anal regions. He considered the 32 day-old larva, measuring 33.3 mm, as post-larva. At this stage the dorsal and anal fins are extremely soft and transparent. The pectoral fins are also well developed. The post-larva at 56th day measures 48.2 mm and the gills are well developed. "These young fishes", says Kuthalingam (1961), "have a beautiful green colour with a light blue shade along the upper margin of the opercle and along the back of the body. The caudal fin is blue-green in colour and the upper surface of the head and eye is emerald green. The pectoral, ventral and anal fins are white and almost transparent".

Studies on the life history of *D. acuta* by Kuthalingam (1961) is based on the planktonic eggs from the tow-net collection made in the offshore area of the Madras Harbour and he succeeded in rearing them in the laboratory to the juvenile stage (fiftysixth-day-old larva) by providing "concentrate of fresh plankton" as food. Nair (1973) commented on this stating "it is extremely interesting that he (Kuthalingam) reared them to the juvenile stages in the laboratory without any difficulty when the other workers under similar conditions have failed to rear them beyond the third day". In the present experiment the larvae could be reared only for two days and the last one died after 48 hours after hatching.

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