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The embryonic development of the squid, Illex illecebrosus, in the laboratory.

R. K. O'Dor and N. Balch
Dalhousie University
Halifax, Nova Scotia, Canada B3H 4J1

and

T. Amaratunga, Department of Fisheries and Oceans
Invertebrates and Marine Plants Division
Halifax, Nova Scotia, Canada

INTRODUCTION

The only previously published record of the embryonic development of squid of the genus Illex is that of Naef (1923) for an unknown cephalopod subsequently identified as Illex coindetii (Boletzky et al, 1973). The egg mass collected by Naef also appears to be the only one for this genus ever described from nature. Illex illecebrosus is the subject of an increasingly important fishery in the Northwest Atlantic and its reproductive biology is a key to understanding the life cycle and managing the fishery (NAFO, 1980). The present report is intended primarily as an aid to the identification of egg masses of this squid in the field, but also provides a photographic record of embryonic development for comparison to Naef's work and that of Hayashi (1960) and Hamabe (1962) on Todarodes pacificus, a well studied ommastrephid with a similar life history.

Durward et al (1980) described newly hatched larvae of I. illecebrosus from a large (c.1 m diameter) egg mass spawned in captivity in September, 1978, but attempts to observe the complete developmental sequence in artifically fertilized eggs failed because of microbial contamination. Attempts to observe development in spawned eggs also failed in 1979 (O'Dor et al, MS 1980). The developmental sequence given here is based on both normal and artificially fertilized eggs. The conditions that supported successful development are described and the cause of earlier failures discussed.

MATERIALS AND METHODS

Normal Spawning

The maintenance of I. illecebrosus in the 15 m pool of the Aquatron Laboratory and the induction of precocious maturation have been previously described (O'Dor et al, 1977), as have the resultant large gelatinous egg masses and the larvae that hatch from them (Durward et al, 1980). Table 1 provides a record of all major egg masses produced from 1978 through 1980 with information on ambient temperatures in the pool. In 1979 large fragments of masses were collected in plankton nets with clear plastic sides and positioned in front of observation ports in the pool to allow microscopic examination of eggs with little disturbance. In 1980 a glass bottomed, 4 m by 30 cm diameter caisson was placed over the surface of 60 cm diameter egg mass in situ at the bottom of the pool. A photographic record of individual eggs was kept for 30 days by lowering a close-up camera down the caisson.

Artificially Fertilized Eggs

A variety of techniques for artificial fertilization were tried during 1978 and 1979, but these experiments will not be described in detail because a common fault, revealed by the 1980 experiments, made them uninterpretable.

Two experiments were conducted in 1980. In the first the eggs and spermatophores used were from a mated female that had already produced an egg mass; in the second they came from a mated female near death which had failed to spawn. Eggs were collected from the oviducts and spermatophores from implantation sites inside the mantle cavity. Release of sperm from the spermatophores was induced by chopping several hundred into approximately 1mm fragments in 10 ml of seawater at 10°C. The milky suspension formed after one hour contained 10^7 sperm/ml (Counts were made with a hemocytometer in aliquots killed with 10% formalin). Appropriate aliquots of this suspension were added to bottles, containing 200 ml of seawater and several thousand eggs, to give the desired sperm titer.

In the first experiment, five bottles of egg with a 1:1 serial dilution of sperm giving titers from 7×10^5 to 4×10^4 were incubated overnight at 8°C. Following a microscopic survey to determine fertilization

rates (as indicated by the formation of intrachorionic spaces), several hundred drained eggs from the 4 X 10^4 sperm/ml bottle were poured into a petridish containing 10 ml of penicillin-streptomycin (Gibco Laboratories) 500 units/ml in sterile seawater. After 3 rinses in the same medium individual eggs were drawn gently into a glass capillary tube and placed in 1 ml of the medium in each of 24 wells in sterile Falcon tissue culture plates. Two plates each were then incubated at 8^{o}, 13^{o} and 18^{o}C. The eggs in the original petridish were left at room temperature (~21^{o}C). The eggs in the culture plates were examined and photographed daily on a Zeiss inverted microscope. Medium was also siphoned off daily and replaced.

In the second experiment two bottles of egg were prepared as before using 4 X 10^4 sperm/ml and incubated overnight at 7 and 17^{o}C. After the same rinsing procedure eggs from each temperature group were separated into three petridishes. A dissecting microscope was used to select eggs with a distinct intrachorionic space (See Fig. 1b) for transfer from one dish to well plates. Plates of eggs fertilized at each temperature were then incubated at each temperature yielding four treatments: 7-7, 7-17,17-7, and 17-17. The plates were examined and photographed daily, but the medium was not changed. One other petridish containing several thousand rinsed but unselected eggs from each group was incubated at each temperature; these were examined infrequently to minimize handling and temperature fluctuations during examination. Estimates of total fertilized and developing eggs were made by placing dishes over a grid under a dissecting microscope, and counting about 500 eggs in random squares.

RESULTS

Fig. 1 presents an essentially complete record of the embryonic development of I. illecebrosous based on observation of early stages from artificially fertilized eggs and later stages from the normal egg mass observed in 1978. After fertilization the egg membrane withdraws from the chorion near the micropyle to form an intrachorionic space (Fig. 1b). Early meroblastic cleavage at the animal pole (Fig. 1c) is followed by the formation of a multilayered blastoderm which is clearly visible after 20 h at 17^{o}C (Fig. 1d). The blastoderm grows down in a distinct front (Fig. 1e) and the entire egg surface is cellulated after about 50 h. By 72 h (Fig. 1f) the thickened placodes which will form the eyes are visible near the vegetal

pole. The next distinctive feature to appear is the mantle (Fig. 1g) which begins to form at the animal pole, grows down over the egg as a sheet (just as the blastoderm did earlier), and stops about three fifths of the way down. Orange pigmented chromatophores are visible on the mantle almost as soon as it begins to form (Fig. 1g), and mantle contractions start just before it reaches the equator (Fig. 1h). Red pigmentation of the eye appears at the same time as the chromatophore pigment. Arm primordia are distinct by the sixth day (Fig. 1i). No details of further organ development will be given because there is no assurance that development after the chorions had burst (See below.) was normal, but in the naturally developing eggs all major organs are present and most fairly advanced by the eighth day (Fig. 1j). In the descriptions of individual experiments that follow, the figured stages will be referred to alphabetically for convenience.

Artificially Fertilized Eggs

In experiment 1 over 80% of eggs incubated with highest sperm titer were fertilized; however, after 24 h a high proportion of such eggs formed a distinct zone beneath the chorion about 0.1 mm thick over their entire surface and showed no further development. Over night incubation (about 18 h) at $8^{\circ}C$ with sperm titers below 10^5 sperm/ml produced few such eggs, but also yielded fertilization rates of less than 8%.

Little quantitative information came from the first attempt to follow development at varying temperatures. Because of the low proportion of fertilized eggs and damage from excessive handling only one egg at $18^{\circ}C$ was followed for 3 days to Stage f and one egg at $13^{\circ}C$ for 2 days to Stage e. No eggs advanced as far as Stage d at $8^{\circ}C$. The most successful part of this experiment was the petridish left at room temperature in which about twenty eggs developed to Stage g after 5 days showing chromatophore development and mantle contractions. This demonstrated that reasonably advanced development could occur and that daily changes of medium (which caused most of the damage in the plates) were not necessary to prevent bacterial and fungal contamination.

The design of the second experiment took advantage of these observtions and both individual eggs (left in the same medium) in plates and petridishes with several thousand eggs were used. This allowed repeated observations of the same egg and estimates of successful development in large numbers of eggs. Table II summarizes the numerical observations.

The fertilization rate at 17°C was more than double that at 8°C, and nearly all of the fertilized eggs showed considerable blastoderm development (Stage d) after 18 h. At 17°C about a third of these eggs continued normal development for 4 to 5 days, but by the sixth day most had burst their chorions which had failed to expand. Fig.1i shows such an embryo with its head still encased in a chorion. This embryo is fairly well advanced showing mantle contractions, chromatophores, eye pigmentation and arm rudiments, but it lacks sucker primordia visible in Fig. 1j, a normally developing squid in an expanded chorion taken from an egg mass. The ruptured chorion in Fig. 1i clearly occurred prematurely as even squid as far advanced as Fig. 1k were found with intact, expanded chorions in the egg mass. All premature hatchlings developed abnormal mantles which tended to balloon out, but most organs continued to develop; by the tenth day such features of normal hatchlings as a pigmented ink sack and fully formed eyes were present. Eggs fertilized at 7°C developed in the same way when transferred to 17°C (Although the success rate was lower, Table II) However, eggs retained at 7°C for 24 days showed no development. Eggs which had reached Stage d during the fertilization period at 17°C degenerated in 2 to 3 days; constrictions formed in the eggs, and the cells of the blastoderm rounded up and became dissociated.

Normally Spawned Eggs

The above results prompted a re-examination of temperature regimes in the pool at the times when egg masses were present. The only egg mass in which normal development was seen (Fig. 1 j and k) was spawned at 13°C and developed at an average temperature of 14°C (26 IX 1978) to produce normal hatchlings after 11 days (some early hatching occurred after 6 to 8 days with hatchlings resembling Fig. 1j; Durward et al, 1980). All other egg masses, including several that were carefully observed in 1979 and failed to develop, were exposed to temperatures of 10°C or less.

Observations of the 1980 egg mass, spawned at 9°C, began within a few hours of spawning, and it was photographed every second day for 30 days. At first observation, the transparent mass contained many cloudy areas which expanded during the first day and then disappeared. Subsequently close-up photographs revealed numerous spermatophores in the mass, and it is probable that the expanding clouds were sperm dispersing from these through the gel. The female that produced this mass also provided the eggs and sperm (from

spermatophores remaining inside the mantle) for the first in vitro experiment described above, so that this mass unquestionably contained viable sperm and eggs. The photographs showed two distinct classes of eggs: 1) unfertilized eggs (about 30%), which remained transparent even after 30 days and did not expand, and 2) other, presumably fertilized, eggs which turned white inside and eventually expanded. The 30 day photographic survey of the mass revealed no eggs in recognizably advanced stages. By day thirty the gel had completely collapsed, apparently from microbial action and from the effects of a variety of small worms and crustaceans that colonized it. Eggs recovered after the collapse were examined microscopically and resembled those kept in vitro at 7°C except that many had expanded chorions.

DISCUSSION

Embryologically, the best known cephalopods are the Myopsida and particularly Loligo pealei for which Arnold (1971, 1979) has established a complete staging scheme. Unfortunatley, his 30 stages cannot be transferred directly to I. illecebrosus or to other ommastrephids like Todarodes pacificus which have similar development patterns (Hayashi, 1960; Hamabe, 1962). The smaller ommastrephid eggs develop more rapidly (11 days at 13°C and probably about 8 days, at 21°C for I. illecebrosus compared to 27 days at 15°C for L. pealei), and they do not form an external yolk sac. There are also many subtle differences in timing: pigmentation of the eye and chromatophores begins very early in I. illecebrosus (See Fig. 1), but is not seen until Stage 26 in L. pealei.

The studies of I. illecebrosus are still too sketchy to justify erection of a staging scheme, but Fig. 1 should be adequate to identify ommostrephid eggs and to estimate the age of the eggs even under field conditions. The problem of distinguishing between the eggs of the several species of Ommastrephidae occuring in the range of I. illecebrosus is similar to the problem of identifying larvae (Roper and Lu, 1979). If photophores develop early enough in the embryos of genera other than Illex (Ommastrephes, Ornithoteuthis) they may be a key charateristic in egg identification as they are in larvae (Roper and Lu, 1979). Within the genus Illex there are no known characters that can be distinguished at these early life stages, but comparisons of field material to the extensive set of reference material available from these experiments may make characterization to species possible.

In addition to providing a record of embryonic development the experimental results allow some inferences to be made about conditions necessary for normal egg development in this species. Three factors which appear to be important are sperm titers, the gel produced by the nidamental glands, and temperature.

In the artificial fertilization experiments high sperm titers produced a high proportion of eggs which were fertilized but developed abnormally in a consistent pattern. The proportion of such eggs decreased with decreasing sperm titers, and at the lowest titers they were rarely seen. However; at the lower titers less than 20% of the eggs were fertilized. The simplest explanation for these results would be that the eggs are susceptible to polyspermy which results in pathological development. No histological confirmation of this was made, but polyspermy has been reported, in many molluscan eggs (Webber, 1977; Fretter and Graham, 1962). While Arnold (1971) reports that he has never seen polyspermy in the eggs of Loligo pealei he does note that many sperm may enter the intrachorionic space and that there is no known mechanism to prevent it in cephalopods. The possiblity of polyspermy should be considered in any attempt at artifical fertilization of ommastrephid eggs.

The gel of an egg mass probably has a critical function in development related to chorion expansion. Expansion was seen to occur in both developing and degenerating eggs associated with gel, and to be absent in both types of eggs without gel. This evidence is not conclusive; but, it does reinforce similar observations in L. pealei (Arnold, 1971) and T. pacificus (Hayashi, 1960). The relatively tenuous egg mass of I. illecebrosus presumably must remain intact for several days to allow normal development, and free floating eggs would be unlikely to hatch successfully. The gel may also influence sperm motility and thereby regulate fertilization.

The factor which appears to have suppressed development of eggs in masses in the pool and in earlier experiments is temperature. Observations to date have shown no development of eggs at temperatures below 13°C. Fertilization at 7°C was followed by normal development when the temperature was raised, but there was a suggestion from both artifically and naturally fertilized eggs that, once begun, development may be permanently disrupted by exposure to low temperatures. The minimum temperature for development lies between 7 and 13°C and is probably above 10°C. Previous records of

embryonic development in ommastrephids are all at temperatures above 10°C (Hayashi, 1960; Hamabe, 1962); Boletzky et al (1973) report that eggs of I. coindetii develop normally at 15°C, but fail at 10°C.

Although adult I. illecebrosus can thrive at low temperatures, their offspring seem to require warmer waters. The migration of these squid from the fishing grounds in November is presumably in search of a suitable spawning site. The Gulf Stream is the closest site where water of a suitable temperature is available at this tim of year. It may not be useable, however, since spawning of captive squid has ays occurred on the bottom. If this is the case in nature, Newfoundland squid face a trip at least as long as the 2000 km migration of T. pacificus in search of bottom water 13°C during the winter months.

SUMMARY

1. The embryonic development of Illex illecebrosus is described and the major stages illustrated based on both artificially fertilized and naturally spawned eggs. The basic pattern of development resembles that of Todarodes pacificus.
2. The gelatinous egg mass appears to have important functions related to the avoidance of polyspermy and to chorionic expansion during later development stages.
3. Fertilization can occur at temperatures as low as 7°C, but normal development appears to require minimum temperatures in the range of 10 to 13°C. Development time is temperature dependent, ranging from 11 to 8 days at temperatures from 13 to 21°C.

REFERENCES

Arnold, J.M. 91974) Embryonic development, pp. 24-49, In: Arnold, J.M,, W.C. Summers, D.L. Gilbert, R.S. Manalis, N.W. Daw and R.J. Lasek (eds), A Guide to Laboratory Use of Squid, Loligo pealei, Marine Biological Laboratory, Woods Hole, Mass. U.S.A.

Arnold, J.M. (1971) Cephalopods, pp. 265-311, In: Reverberi, G. (ed.), Experimental Embryology of Marine and Frest-water Invertebrates. North-Holland Publishing Co. Amsterdam.

Boletzky, S.V., L. Rowe and L. Aroles (1973). Spawning and development of the eggs, in the laboratory, of Illex coindetii (Mollusca: Cephalopoda). Veliger 15: 257-258.

Durward R.D., E. Vessey, R.K. O'Dor and T. Amaratunga (1980). Reproduction in squid, Illex illecebrosus: First observations in captivity and implications for the life cycle. ICNAF Sel. Papers No. 6: 7-13.

Fretter, V. and Graham, A. (1962) British Prosobranch Molluscs. pp. 342-345. Ray Society, London.

Hamabe, M. (1962) Embryological studies of the common squid, Ommastrephas sloani pacificus STEENSTRUP, in the Southwestern waters of the Sea of Japan. Bull, Jap, Sea Reg. Fish. Res. Lab. 10: 1-45.

Hayashi, S. (1960) Development of the squid, Ommastrephes sloani pacificus (Steenstrup). Bull, Fac. Fish. Nagasaki Univ. 9: 43-48.

Naef, A. (1923) Die cephalopoden. Fauna Flora Golfo Napl. 35th Monogr. Vol. 1: 1-148; Vol. 2: 149-863.

NAFO Redbook Scientific Council Rept. 1979-80, p. 101.

O'Dor R.K., R.D. Durward and N. Balch (1977) Maintenance and maturation of squid (Illex illecebrosus) in a 15 meter circular pool. Biol. Bull. 153: 322-335

O'Dor R.K., E. Vessey and T. Amaratunga (1980) Factors affecting fecundity and larval distribution in the squid, Illex illecebrosus. NAFO SCR Doc. Ser. No. N070, 9 pp.

Roper, C. and C.C. Lu (1979) Rhynchoteuthion larvae of ommastrephid squids of the western North Atlantic, with the first discription of larvae and juveniles of Illex illecebrosus. Proc. biol. Soc. Wash. 91 (4): 1030-1059

Webber, H.H., (1977) Gastropoda: Prosobranchia. p. 62. In: Giese, A.C. and J.S. Pearse (Eds.) Reproduction of Marine Invertebrates Vol. IV. Molluscs: Gastropods and Cephalopods. Academic Press. N.Y.

TABLE I: Record of Egg Masses and Temperatures Observed in the Pool.

Date Noted	Temperature at Spawning (°C)	mean and Range* for next 2 weeks (°C)
12 XI 1980	9.0	8.1 (9.0-7.5)
20 X 1979	7.2	8.7 (7.2-9.6)
9 XI	10.1	10.3 (10.0-10.5)
10 XI	10.3	10.3 (10.0-10.5)
13 XI	10.5	10.2 (10.5-9.6)
14 XI	10.5	10.1 (10.5-9.5)
16 XI	10.5	10.0 (10.5-9.5)
21 XI	10.0	9.6 (10.0-9.1)
25 XI	9.7	9.2 (9.7-8.6)
13 IX 1978	15.6	12.3 (15.6-9.7)
26 IX	12.9	14.0 (12.9-14.7)
9 X	14.0	12.3 (14.1-10.0)

* Ranges are arranged to indicate the general trend over the period, but are not necessarily for first and last days.

TABLE II: Percentage of Eggs in Each Temperature Group Reaching Various Development Stages.*

	7-7°C (%)	7-17°C (%)	17-7°C (%)	17-17°C (%)
Fertilization	6	8	18	20
Stage d (18h)	0	4	16	18
Stage g (90h)	0	0.5	0	7

* Egg were incubated with sperm for 18 hours at the first temperature indicated and then transferred to the second for the rest of the experiment.

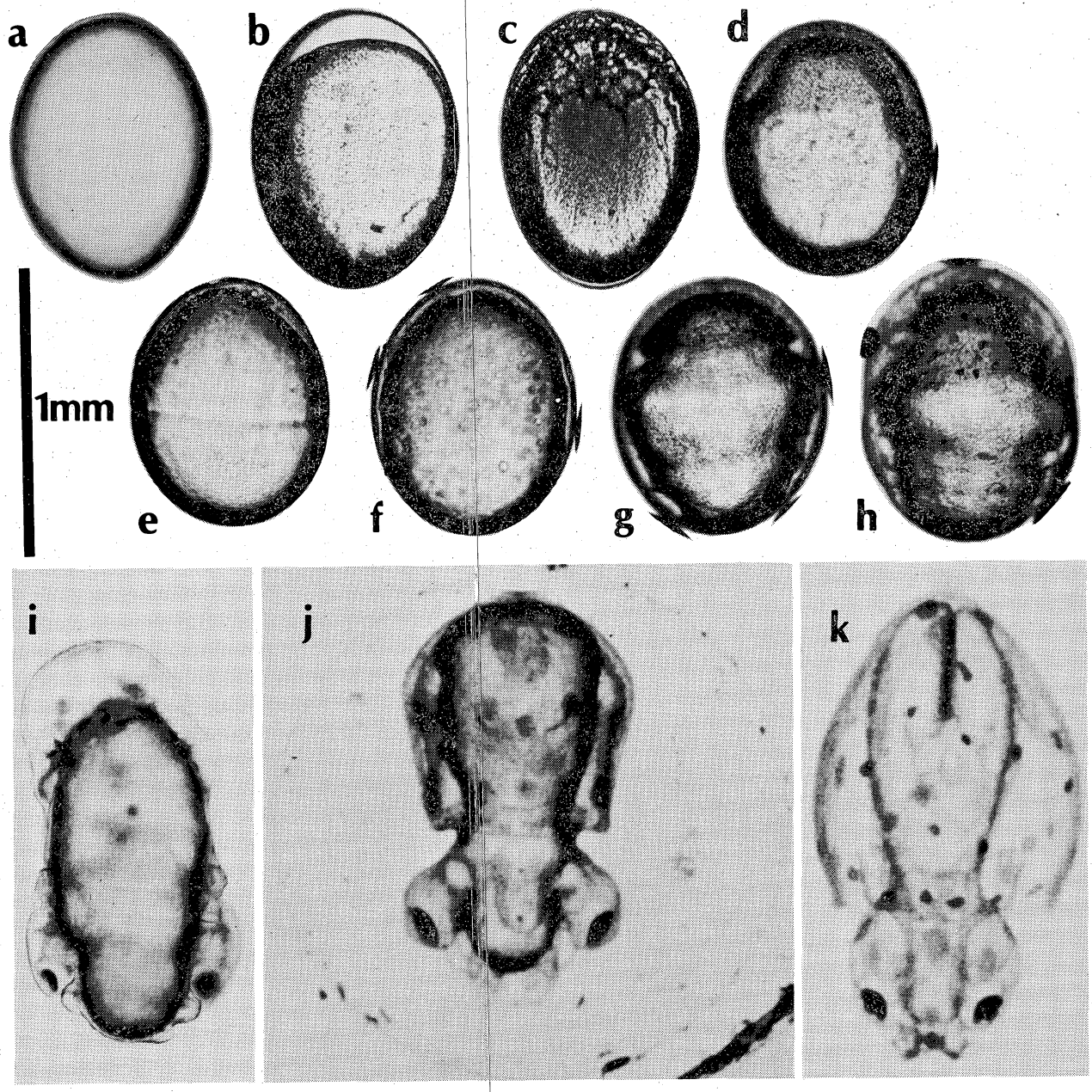

Fig. 1. Development of artificially (a to i) and naturally (j,k) fertilized eggs of Illex illecebrosus.
(a) Unfertilized egg; (b) Fertilized egg; (c) Sixth cleavage after 6h at 17°C; (d) Granular bastoderm covers 1/3 of egg, 18h; (e) blastoderm covers 2/3 of egg, 50h; (f) Complete cellulation, Organ primordia visable as thickened placodes, 72h; (g) Pigmented optic vesicle. Mantle begins downward growth . Chromatophores visible and moving, 90h; (h) Mantle grows to mid-line, shows frequent pulsations. Patches of cilia move fluid around embryo, 114h; (i) Premature hatch from an unexpanded chorion. Arm rudiments present, 145h; (j) Normally developing embryo in expanded chorion after 8 days at 13°C. Sucker and fin primordia present. Funnel fused; (k) Fully formed larva ready to hatch, 11 days. Funnel enlarged. Internal yolk gone from head region.