

Embryonic Development of Saddleback Anemonefish, *Amphiprion polymnus*, Linnaeus (1758)

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ABSTRACT

The breeding pairs of saddleback anemonefish, *Amphiprion polymnus* Linnaeus (1758) were brought from the Gulf of Thailand and reared in the laboratory to observe their spawning behavior, eggs and embryonic development. The spawning was found to be all-year-round with the reproductive cycle between 14-21 days. The eggs were single and adhesive type, capsule shape, and light orange in color, measuring 1.9-2 mm in length and 0.9-1 mm in width containing both large and small fat globules. The process of embryonic development could be divided into 26 stages based on morphological characteristics of the developing embryo. The time elapsed of each embryonic developmental stage was recorded. Hatching took place 148±8 hours after fertilization. This report is the first published record on the morphological development of saddleback anemonefish embryo.

Key words: morphology, stage of embryonic development, saddleback anemonefish

INTRODUCTION

Saddleback anemonefish are tropical marine fish inhabited among the coral reef. They are wide spread in Pacific Ocean, as well as in Australian sea through Solomon Island. Allen (1980) reported that there are two genera of saddleback anemonefish, *Amphiprion* with 25 species and *Premnas* with one species.

In Thailand, there is only one genus of *Amphiprion* with five species: *A. ocellaris* Curvier (1830), *A. alkallopisos* Bleeker (1853), *A. sebea* Bleeker (1853), *A. clarkii* Bennett (1830) and *A. ephippium* Bloch (1790), reported living in the

Andaman Sea, while two different species of the same genus: *A. polymnus* Linnaeus (1758) and *A. perideraion* Bleeker (1855) were found in the Gulf of Thailand (Pathiyasevee, 1994).

At present, anemonefishes are drastically decreased due to their popularity as decorative fish; hence, there are high demands of these fishes for commercial purpose. Attempts have been made to replenish its population and mass-produce them for exportation. Since biological reproduction and their embryonic development are not much known, this study will provide basic information on its stages of development to create the future farming and subsequently, exporting product of Thailand.

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MATERIALS AND METHODS

Ten breeding pairs of saddleback anemonefish, *A. polymnus*, were brought from the Gulf of Thailand and acclimated to the laboratory condition for six months at the Institute of Marine Science, Burapha University, Bangsaen, Chonburi province. They were kept in a tank of 30 × 60 × 40 cm in size. The aerated sea water in the tank was controlled at 25-28 °C, having 30-32 ppt salinity, 0.02 ppm ammonia, 0.01 ppm nitrite, 10 ppm nitrate. The pH varied between 6.5 and 7.5. Egg-laying materials comprised of bivalve shell, bark rock, coral and seaweeds. Broken ceramic plates were also put in the tank as lining for home surrounding, while sea anemones *Heteractis crispera* and *Stichodactyla haddon* were added to mimic the natural environment of this fish. They were fed with brine shrimp, finely chopped fish, shrimp, clams and dry algae flakes *ad libitum*.

Courtship behaviors were observed during the experiment. The samples of fertilized eggs up to hatching were scraped from the lining materials and observed under microscope to determine and identify their stages. Specimens were fixed in 5% neutral buffered formalin.

RESULTS

Courtship behavior

Saddleback anemonefish were found to have nesting habits and closed parental care. Females were generally larger than males. The reproductive cycle was between 14-21 days. During the courtship the pair of breeders isolated themselves from the group and swam side by side. They spent time together before laying egg which was 1-6 months depending on each pair. Both male and female breeders helped building their nest (Figure 1).

Spawning behavior

At spawning time, the abdomen of females



Figure 1 Male and female breeders at the nesting site, showing the size differences as well as their courtship and spawning behavior.

became larger and an extended part of ovipositor, a cone-shaped urogenital papilla, 4-5 mm in length appeared from the cloaca or urogenital sinus. This clinical sign occurred early in the morning. The males also showed a small white urogenital duct, 2 mm in length, extended from the cloaca.

The female then moved closely to the nest and the eggs were laid through the urogenital papilla and stick onto the egg-laying materials. Spawning was confined to the afternoon from 1.00 to 5.00 pm. After a small amount of eggs having been laid, the male moved in to ejaculate the sperm for fertilization. They spawned periodically 5-6 times which lasted 40-120 minutes and completed one spawning session on the same day. The number of eggs per spawning was approximately 400-1,800 eggs depending on the age of the females and the fertility of the couple.

Egg morphology and incubating period

Eggs of saddleback anemonefish were adhesive, covering with clear capsule or transparent chorion with narrow perivitelline space. The eggs were capsule-shaped, measuring 1.9-2.0 mm in length and 0.9-1.0 mm in width (Figure 2). The surface of the egg capsule was smooth. One end of the egg capsule, identified as animal pole, contained some glutinous substance to adhere itself to the

lining materials. Newly-laid eggs had yolk in light orange color with large fat globules and the color was more intense as the eggs became older.

It took 6-7 days for fertilized eggs to hatch and the hatching time often occurred at 7.00-9.00 pm. A female laid the eggs 20-24 times per year. Ninety five percent of the eggs were successfully fertilized. Parental breeders then became pre-occupied with their embryos, cleaning the nest and devouring the unfertilized eggs. The male also played an active role in these activities.

Embryonic development

The embryonic development of the saddleback anemonefish from fertilization to hatching was classified into 26 stages as follows:-

Stage I (Figure 2) - It was a one-cell stage. This stage was specified as zygote or immediately after fertilization, having one uncleaved cell. The cytoplasm of fertilized egg was clear. The animal pole was characterized by its half-circle shape which attached to the egg-laying materials while the vegetal pole contained yolk and different sizes of fat globules dispersed in it for polylecithal egg type.

Stage II (Figure 3) - The first cleavage started by dividing the blastodisc into two blastomeres. This event appeared in the first hour after fertilization with meroblastic type. Two blastomeres were observed at the animal pole containing only half size of the original cell. Their cytoplasm was clear. The fat globules were very small and moved towards vegetal pole.

Stage III (Figure 4) - Four equal blastomeres resulting from the second mitosis appeared on vertical plane. This event happened at one hour and 40 minutes after fertilization. Each blastomere was smaller and was only half size of blastomeres in the previous stage. Fat globules were observed in the yolk.

Stage IV (Figure 5) - Eight blastomeres showed up at two hours after fertilization, resulting from the horizontal mitotic division. The

blastomeres were smaller and equal in size.

Stage V (Figure 6) - Sixteen blastomeres appeared at four hours after fertilization, by another horizontal mitotic division.

Stage VI (Figure 7) - Four and a half hours after fertilization, the blastomeres became overlapping due to the limited confined space of the capsule.

Stage VII (Figure 8) - About five hours after fertilization, blastomeres extended more laterally and arranged themselves as a flat layer called blastoderm or blastodisc.

Stage VIII (Figure 9) - About six hours from fertilization to reach this stage, blastomeres became much smaller but still equal in size.

Stage IX (Figure 10) - The blastomeres were very small at about eight hours from fertilization.

Stage X (Figure 11) - The embryos were called morula having mulberry-like structures, about nine hours after fertilization. The blastomeres were very small since the embryo was still in the capsule.

Stage XI (Figure 12) - The embryo formed a space called blastocoel which was clearly separated from the yolk. It took about 18 hours and a half to reach this stage from fertilization.

Stage XII (Figure 13) - The blastomeres moved downward from the animal pole to cover part of the yolk called epiboly. The blastomeres started moving inward to form three germ layers called gastrulation. It took about 21 hours after fertilization.

Stage XIII (Figure 14) - The outer layer of the embryo formed the longitudinal ridges close to the yolk at about 27 hours and a half from fertilization. The embryo began to form head and neural ectoderm.

Stage XIV (Figure 15) - The longitudinal neural tube was formed and attached to the yolk. The optic buds were observed for the first time. It took about 30 hours after fertilization. The fat globules started to disappear at this stage.

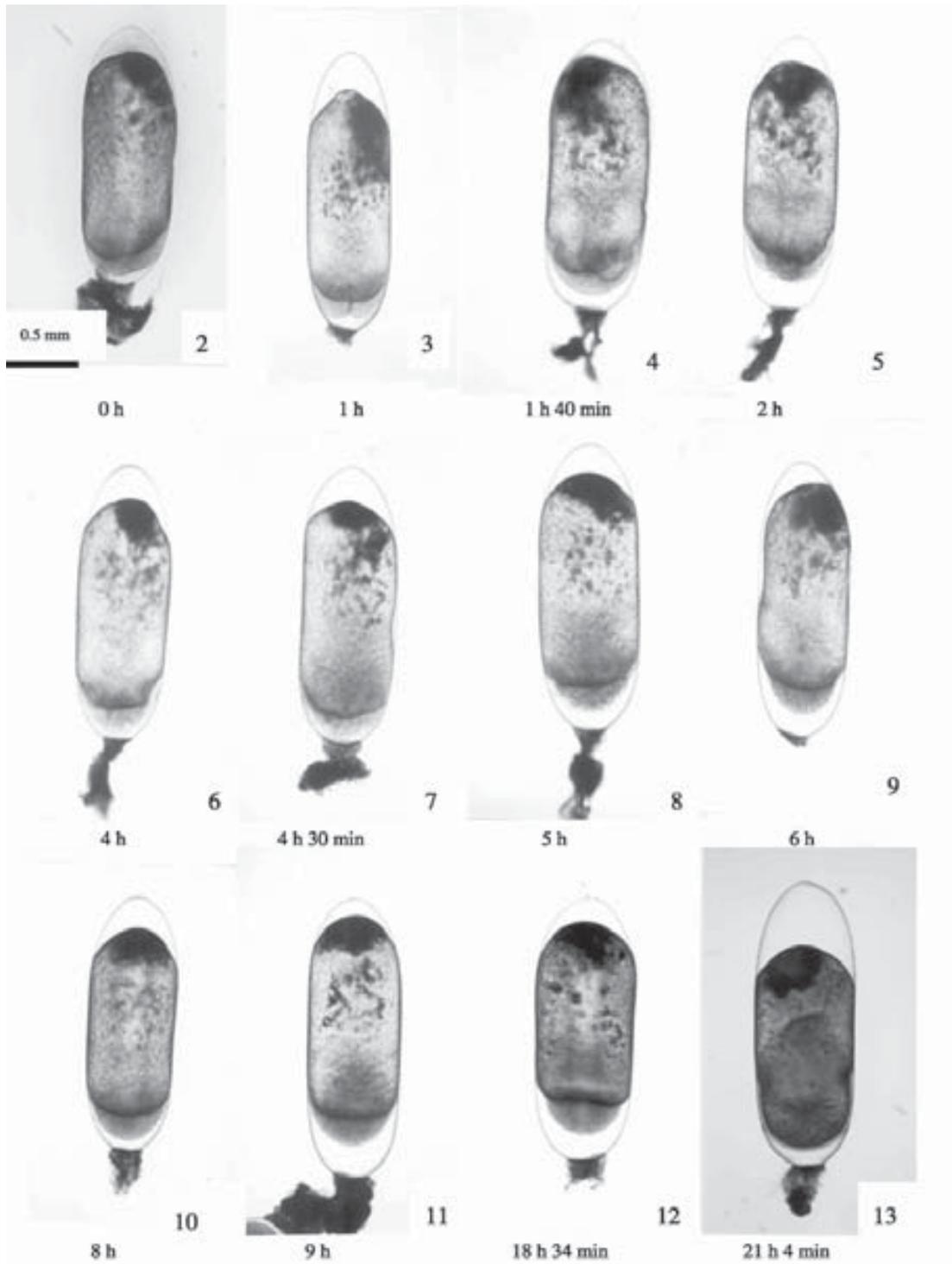


Figure 2-13 The embryonic development of *Amphiprion polymnus* stages 1 to 12.

Stage XV (Figure 16) - The head with two optic buds was separated from the body. The mouth was evident. The body was transparent having no muscular structure. It took about 34 hours after fertilization.

Stage XVI (Figure 17) - The embryo began to move itself. At this stage the embryo increased in size. The body was still attached to the yolk. The lens, tail bud, and melanophores located in the head and on the surface of the yolk were evident. It took about 42 hours after fertilization.

Stage XVII (Figure 18) - The embryo completely turned itself over while body length distinctly increased. The tail became separated from the yolk, although the body still attached to the yolk. At this stage, the heart began to beat and the skeletal muscles were found along the body length. The melanophores were increased in the head region. The embryo took about 48 hours from fertilized egg to be at this stage.

Stage XVIII (Figure 19) - The small otolith of the inner ear was evident and the tail began to move. Young fin was progressively elongated and became parallel to the body length. It took about 55 hours from fertilized egg to reach this stage.

Stage XIX (Figure 20) - The yolk was decreasing while the embryo was growing. The head and tail were clearly separated from the yolk. Three primary brain vesicles: forebrain, midbrain and hindbrain were clearly seen in the head region. The large eyes contained brown pigments and their lens were prominent. High pigmentation was seen in the head, but less pigment was in the tail region. The axis of vertebral column was not clear. Red blood cells running in the vessels could be observed indicating the functioning of circulatory system of the embryo. It took about 64 hours from fertilized egg to be at this stage. The head and tail of the embryo extended to attach to the capsule.

Stage XX (Figure 21) - The forming organs became enlarged. The abdomen was larger and covered the yolk. It took about 75 hours and a half from fertilized egg to be at this stage.

Stage XXI (Figure 22) - The head was enlarged containing the prominent big eyes and brown pigments. The melanophores were evenly distributed in the whole body. It took about 93 hours from fertilized egg to be at this stage.

Stage XXII (Figure 23) - The embryo was further enlarged and filled most of the space in the capsule. It moved and rotated more often. The pectoral fin was quite large. It took about 97 hours from fertilized egg to reach this stage.

Stage XXIII (Figure 24) - The yolk sac became quite small and covered by the abdomen of the embryo. The melanophores were distributed throughout the body. It took about 109 hours from fertilized egg to reach this stage.

Stage XXIV (Figure 25) - The head occupied one-third of the capsule space. The embryo moved itself more often. It took about 119 hours from fertilized egg to reach this stage.

Stage XXV (Figure 26) - The embryo began to hatch by moving itself vigorously to break the capsule. The breaking point was at the caudal half of the capsule where the body and tail were found. It took at least 127 hours after fertilization before hatching took place.

Stage XXVI (Figure 27) - The embryo was free from the capsule to become a larva. The dorsal fin, caudal fin and anal fin were continuous in a longitudinal line. The abdominal fin was not evident at this stage. It took about 148 hours after fertilization to become a larva. The total length of larva was about 39 mm.

DISCUSSION

The courtship and spawning behavior of saddleback anemonefish is similar to other anemonefish as described by Delsman (1930), Allen (1972, 1980, 1991), Ross (1978), and Pathiyasevee (1994).

The eggs of saddleback anemonefish are classified as polylecithal egg. They were 1.9-2.0 mm in length and 0.9-1.0 mm in width. The

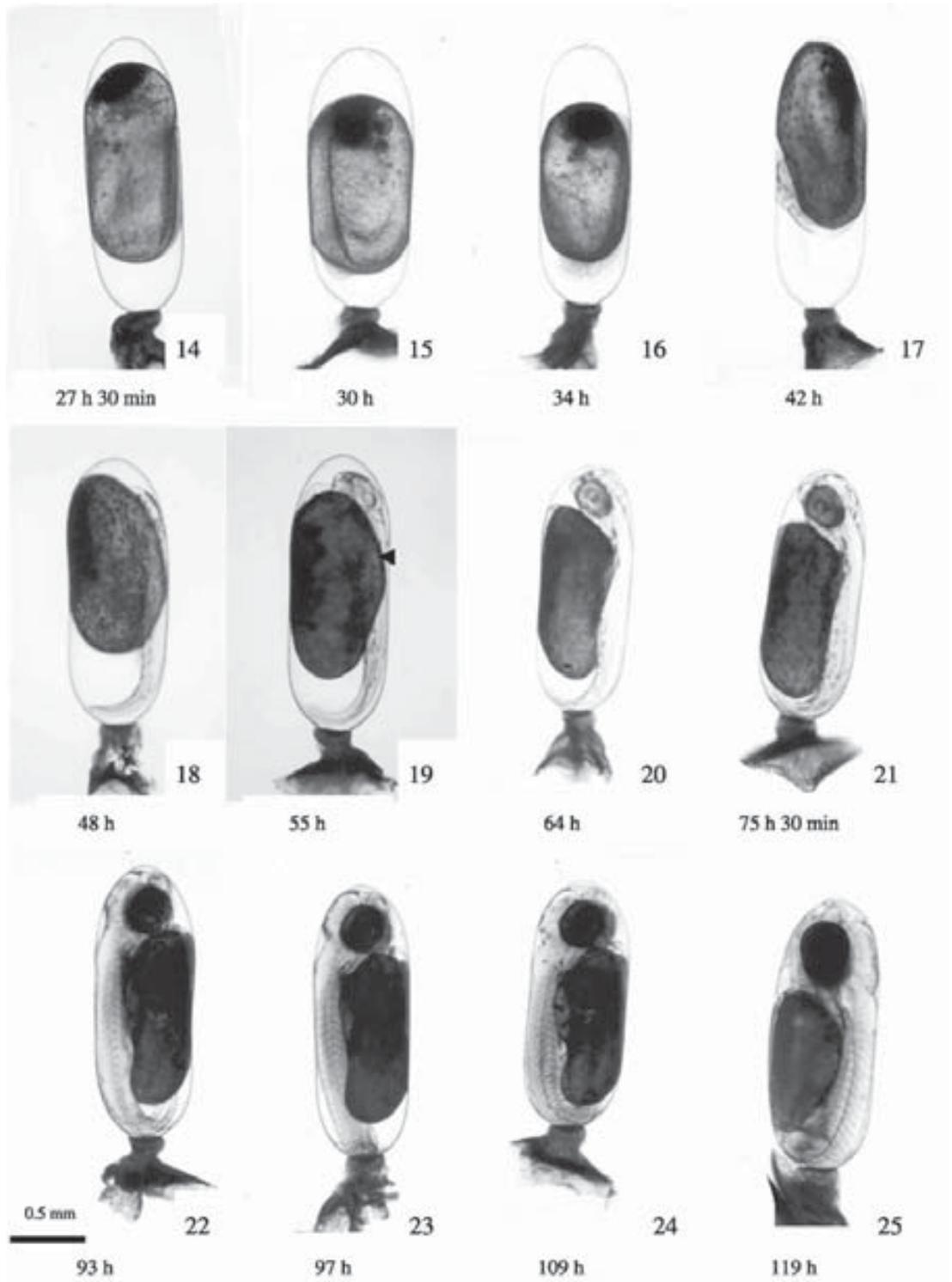


Figure 14-25 The embryonic development of *Amphiprion polymnus* stages 13 to 24.

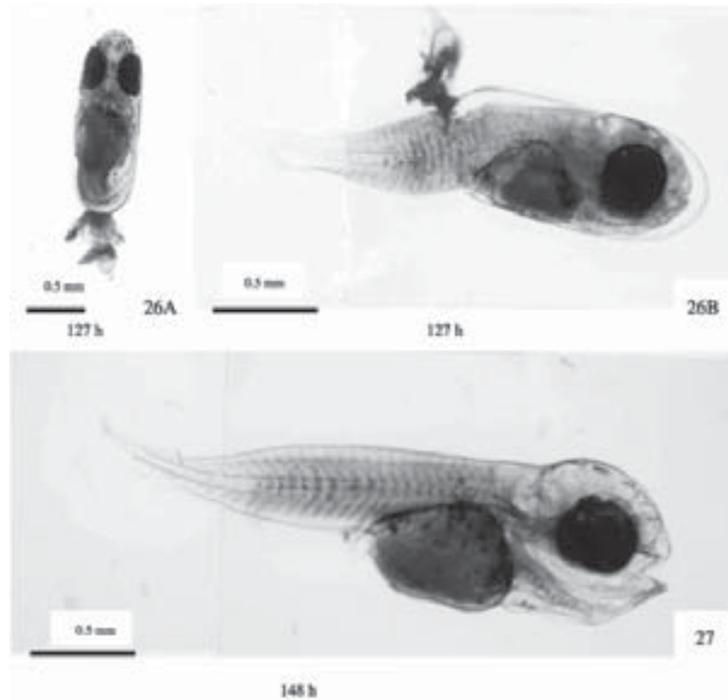


Figure 26-27 The embryonic development of *Amphiprion polymnus* stages 25 to 26.

developmental rate of the fertilized egg is profoundly varied with temperature. It varies also with oxygen content of water (Yamamoto, 1975). Complete hatching took place over 148 ± 8 hours or about 6 days and 4 hours after fertilization. The cleavage pattern of saddleback anemonefish is meroblastic and equal, the same as those of other anemonefish (Delsman, 1930; Allen, 1972, 1980; Ross, 1978; Pathiyasevee, 1994).

In *Amphiprion polymnus*, the yolk had different sizes of fat globules dispersed in the vegetal pole. The dispersion of these fat globules of anemonefish was different from those of Medaka, *Oryzias latipes* where they were mainly found located in the peripheral area of cytoplasm and became aggregated into large globules near the vegetal pole when mitosis began (Iwamatsu, 1994).

Delsman (1930) studied *Amphiprion percula* eggs and larvae from the Java Sea. He

described the morphological characteristic correlated roughly with time after fertilization but did not divide them into stages. The eggs of *A. percula* measured 2.2 mm in length which is a little larger than *Amphiprion polymnus*. The fertilized eggs of *A. percula* took a week to hatch which is about one and a half day longer than what we found in *A. polymnus*.

There were variation in time and stages of embryonic development among different genus and species of fishes. Several factors, e.g. photoperiod were also known to affect their growth and development (Arvedlund, 2000). Morphological development of saddleback anemonefish gave us a better idea on their developmental changes and provided useful information which led to the planning for their nutritional need as well as management for commercial production of this colorful and valuable fish.

Table 1 Stages in embryonic development of saddleback anemonefish, *Amphiprion polymnus*.

Stages	Time elapsed from spawning	Developmental characteristics
Stage I	0 h	Immediately after fertilization, one-cell stage
Stage II	1 h	2 blastomeres
Stage III	1 h 40 min	4 blastomeres
Stage IV	2 h	8 blastomeres
Stage V	4 h	16 blastomeres
Stage VI	4 h 32 min	32 blastomeres
Stage VII	5 h	64 blastomeres
Stage VIII	6 h	128 blastomeres
Stage IX	8 h	256 blastomeres
Stage X	9 h	Morula stage
Stage XI	18 h 34 min	Blastula stage
Stage XII	21 h 4 min	Gastrula stage
Stage XIII	27 h 30 min	Early neurula stage
Stage XIV	30 h	Late neurula stage
Stage XV	34 h	The head was separated from the body, the mouth was evident
Stage XVI	42 h	Formation of lens, tail bud
Stage XVII	48 h	The embryo was turned over completely, the heart began to beat
Stage XVIII	55 h	The small otolith of the inner ear was evident, the tail began to move
Stage XIX	64 h	Three primary brain vesicles: forebrain, midbrain and hindbrain were clearly seen
Stage XX	75 h 30 min	The forming organs were enlarged
Stage XXI	93 h	The head was distinctly enlarged with prominent big eyes and brown pigment
Stage XXII	97 h	The embryo was enlarged and filled most of the space in the capsule
Stage XXIII	109 h	The yolk sac was reduced in size
Stage XXIV	119 h	Movement of the embryo
Stage XXV	127 h	Hatching occurred
Stage XXVI	148 h	Embryo became a larva

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