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Embryonic Developmental Study of Pink Skunk Clownfish, *Amphiprion perideraion* (Bleeker, 1855)

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Abstract. The present study was conducted to document the embryonic developmental stages of clownfish, *Amphiprion perideraion* in captivity. The water quality parameters such as salinity (30ppt), pH (8.2), temperature (28 ± 1°C), dissolved Oxygen 6.5 ± 0.3, ppmammonia and nitrite (below the detectable levels) were standardized and maintained throughout the study period. The newly spawned eggs were adhesive, telolecithal, and white to yellowish-white-colored yolk. The eggs measured around 2.0-2.1 mm in length and 0.9-1.0 mm in width. The chorion was transparent and embryonic developmental stages could be observed directly through the shell. A large clear oil droplet and several smaller droplets were observed. After one hour, blastodisc appeared at the animal pole followed, various cleavage stages were noted; two, four, eight, sixteen, thirty-two and sixty-four-cells stages, then, morula, blastula, and gastrula were observed. The organogenesis process started after fertilization and later head and notochord formation was evident. The optic vesicles first appeared after 32 hours of fertilization. The turnover stage was passed after 42:10 hours post fertilization (hpf), heart beating and blood circulation started 68:13 hours post fertilization. Lower jaw development started after one hundred twenty-three hours post-fertilization. The embryo reached the pre-hatching stage and hatching was attained after 177 hours of incubation.

Keywords: Pink skunk, *Amphiprion perideraion*, Broodstock management, Embryonic development.

1. Introduction

Tropical marine ornamental fishkeeping is considered as one of the rapidly increasing hobby in the world (Rhyne *et al*., 2012). In order to cater to the ever-increasing demand for marine ornamental fishes, the supply chain relies mostly on the wild specimens caught from the coral reef areas and these wild specimens are an alarming concern for the natural biodiversity in the coral reef areas (Rhyne *et al*., 2012; Prakash *et al*., 2017). So, to fulfil the demand for marine ornamental fish trade in a sustainable way, research is concentrated towards developing the breeding and seed production technology for the marine fish, that having high demand (Moorhead and Zeng, 2010; Gopakumar *et al*., 2011). Keeping this necessity in mind, much effort has been put to understand breeding patterns and behavioural aspects of many tropical and sub-tropical fishes in India and on a global scale, especially groups like Pomacentrids (Gopakumar *et al*., 1999; Ignatius *et al*., 2001; Dhaneesh *et al*., 2009; Ajith Kumar *et al*., 2009; Ghosh *et al*., 2012). Clown fishes are the most longstanding and intensively cultured group of marine ornamentals and are the best-ranked in the aquarium trade.

There are 30 species of clownfish worldwide (Fautin and Allen, 1992), among them 15 are reported from Indian waters (Madhu and Madhu, 2007; Ajith Kumar *et al*., 2012; Raghunathan *et al*., 2014; Dhaneesh *et al.,* 2015). Different species of clownfish are divided into separate clades as skunk clade (*A. akallopisos, A. perideraion, A. pacificus, A. sandaracinos*), Ephippium (*A. frenatus, A. ephippium, A. rubrocinctus, A. melanopus, A. barberi*), Percula (*A. ocellaris, A. percula, P. biaculeatus*), Clarkii (*A. clarkii, A. tricinctus*) and Polymnus (*A. sebae, A. polymnus*) (Litsios and Salamin, 2012). Among these species, most of the attention was given to *Amphiprion percula*, *A. ocellaris, A. frenatus*, *A. clarkii*, *A. sebae, A. ephippium* and *Premnas biaculeatus* and their hybrids. The skunk clade is distinct from other clades of clowns such as *A. ephippium, A. percula, A. clarkii* and *A. polymnus* etc. in terms of their body shape, colour, number and location of the white band on the body (Fautin and Allen, 1992). Very little documentation is available for skunk clades such as orange skunk (*A. akallopisos*), pink skunk (*A. perideraion*), pacificus skunk (*A. pacificus*), and sandracinos (*A. sandracinos*), however in recent days, this group is getting much attention among the hobbyists, hence demand is increasing.

In this situation, the embryological study of *A. perideraion* would improve our knowledge about this species as well as other members of this clade. The objective of this documentation is to understand embryonic development from fertilization to post-hatching. Such studies will help to improve the hatching and rearing protocols of *A. perideraion*.

2. Materials and Methods

2.1 Broodstock Management

Wild-caught *Amphiprion perideraion* fish (n = 10) with an average length of 6 cm was procured from a local trader. Initially, the fish were introduced in a 1000-l fiberglass tank with three earthen pots as hideouts / substrates without any Sea anemones. After pair formation, the potential brooders were transferred to another tank (500 L capacity) provided with an earthen pot and an indigenous biofilter. The successful pairs were obtained and one pair was used for the proposed study. The water quality parameters were maintained at salinity 31 ±1 ppt, pH 8.2 ± 0.1, temperature 28 ± 1 °C, ammonia and nitrite (below the detectable levels), and dissolved Oxygen 6.5 ± 0.3 ppm throughout the rearing period. The tanks were cleaned once in a week. Brooder was fed three times a day with boiled squid meat, and raw fish eggs along with wet feed prepared using fish and shrimp meat, *Acetes* sp and spirulina. The tanks were given 10% water exchange once in a week. At the time of first spawning, the male and female had an average length of 6.2 and 6.5 cm respectively.

2.2 Eggs Collection and Monitoring of Embryonic Development

Eggs were observed from fertilization until the seventh day of post-hatching. Immediately after fertilization, the eggs were collected from the clutch, using a glass pipette and transferred to a separate glass container with small quantity of seawater from the same brooder tank and given water exchange of 10% once in 30 minutes. Different embryonic stages were monitored and photographed at 5 minutes intervals for the first one hour and every 15 minutes for the next four hours. On the second day samples were collected with 1.5 hours intervals and from the third day to the day of hatching, sampling was done at 3 hours intervals (Yasir and Quin, 2007; Ho *et al*., 2008; Dhaneesh *et al*., 2012; Soman *et al*., 2021). The embryonic stages were documented using, a compound microscope (Magnus, Germany) and photos were taken with a high-definition mobile phone (OPPO Reno 8 T5G).

The length and width of the ova were measured using an ocular micrometer.

3. Results

3.1 Oocyte

The newly spawned eggs were adhesive, telolecithal, and white to yellowish-white in color on day one. Blackish from third day onwards (Fig.1) and silvery just prior to hatch. The embryonic developmental stages of Pink skunk, *A. perideraion* were recorded and summarized in Table 1.

The eggs measured around 2.0-2.1 mm in length and 0.9-1.0 mm in width. The chorion was transparent so various embryonic developmental stages were visible. The egg yolk was yellowish white at the time of spawning and a large clear oil droplet together with a cluster of several smaller droplets could be observed. The fertilized and an unfertilized egg at the time of spawning are shown in Fig.2.

3.2. Blastodisc Formation

Blastodisc started appearing at the animal pole 20 mins post-fertilization, indicating the completion of the activation process (Fig.3).

The perivitelline space was larger at the vegetal pole than at the animal pole. The yolk sac size reduced after the formation of the perivitelline space.

3.3 Cleavage Stage

The embryonic development started just after the fertilization of the ovum by a sperm. The meroblastic type of cleavage was observed, where the cleavage was confined to the superficial layer of the cytoplasm, thus giving rise to a disc of cells on the animal pole called, blastomeres. The embryonic development happened between 0 hours to 24 hours after insemination is considered as the cleavage stage (Fig.4). The cleavage was recorded after one hour thirty-two minutes post-fertilization. The cytoplasm of the embryo was divided into two round blastomeres. The second cleavage took place perpendicular to the first cleavage, resulting with the appearance of four equal-sized blastomeres, hence it is called four-cell stage, which was reported after two hours and ten minutes of post-fertilization. Similarly, 8, 16, 32, and 64-celled stages were observed 2:50, 3:20, 3:50 and 4:25 hours post fertilization (hpf), respectively. The blastodermal cells were smaller than what it appeared in the previous stage.

During this stage, blastomeres reach a count of 64-128, which is considered an early morula stage (Iwamatsu, 2004; Iwamatsu, 2011). After five hours and fifty-two minutes, the blastomeres became smaller in size. In the late morula stage, the blastoderm appears in a bowl shape with densely grown blastomeres.

3.3.1 Blastula

At eight hours post fertilization, the blastoderm became a dense bowl of blastomeres. The blastomeres size was seen to be smaller than that in the previous stage.

3.3.2 Gastrula

The thickened margin of the blastoderm or dorsal lip was observed after ten hours and forty minutes of post-fertilization. The gastrulation process was characterized by different morphogenetic movements for the required occurrence of epiboly. The blastoderm began to expand over the yolk surface.

Mid gastrulation stage was observed after sixteen hours post fertilization, when blastoderm covered 50% area of the yolk. After nineteen hours post fertilization, blastoderm covered 70% area of the yolk and during this stage primitive head was seen. The yolk plug stage was noticed twenty- two hours post-fertilization. Once the blastomeres covered 100% of the yolk area, the gastrulation process was complete and this stage passed after twenty-three hours.

The primordial head started appearing after nineteen hours, when blastomeres covered with (epiboly) 80% yolk area marking the onset of the cephalization process. Rudimentary head and somites started developing twenty- four hours post-fertilization at the animal pole (Fig.5). A black mass was observed at the vegetal pole and a large oil droplet was observed at the animal pole. Primitive eye buds and brain were visible in the developing head during twenty- five hours post fertilization. Chromatophores were visible on the yolk as well as on the head after thirty- two hours of post-fertilization. A black mass was present on the vegetal pole. Rudimentary optic vesicles were clearly visible on the head. Chromatophores were visible on the head as well as on the yolk sac.

5. Turnover Stage

The turnover stage is considered the most critical stage in the process of embryonic development of clownfish. During this stage, the embryo moves its head from the animal pole to the vegetal pole (Fig. 6). If failure happen in this stage, which leads to death of the embryo. The turnover stage started at thirty-three hours sixteen minutes after post fertilization.

The tail was still attached to the yolk sac. The cranium/head and eye development were clearly visible. After forty-two hours post-fertilization, the embryo completely turned itself to the vegetal pole, but the tail was still attached to the yolk sac. The tail became completely free from the yolk sac after forty-four hours post-fertilization and continuous tail movements were observed. The eyes with chromatophores were distinguished and various parts of the brain could be identified.

The heart and eyes of the embryo were clearly visible in organogenesis stage (Fig. 7). The tail of the developing embryo could be seen freely moving inside the egg. Transparent blood was visible and the head was distinct with the presence of brain and notochord. After sixty-eight hours thirteen minutes of post-fertilization, the yolk sac was reduced in size, and blacker melanophores were seen in the eyes. The peritoneal cavity also appeared with more melanophores, red-coloured blood was seen flowing through the veins, heartbeats were 70-75 bpm and a pair of otoliths was seen on each side and the embryo covered whole egg space. A pair of otoliths and eyes with cornea was observed ninety-eight hours and thirty minutes post-fertilization. The heart was beating at 80 bpm and reddish-pink blood was seen flowing. After a hundred- and ten-hour post-fertilization**,** the embryo grew in size and occupied the maximum cavity of the egg. The yolk sac was reduced in size and the eyes were well developed with an eye lens. Blood was seen flowing through the body, the heart-beat rate was 80-90 bpm. Nasal vesicles were observed and notochord was easily distinguished. The body colour was orange-yellow; red-coloured gills were seen under the operculum.

Development of the lower jaw was observed after hundred twenty-three hours post-fertilization. Also, one larger and one smaller otolith was noticed. Movements of the lower jaw, operculum and fins were seen. Well-developed heart beating at 92-100 bpm and notochord was observed. Eyes started turning silvery. One hundred and fifty- three hours post-fertilization-embryo was observed with well-developed heart beating at 150-160 bpm. Upper and lower jaws were visible beside silvery eyes and reduced yolk sac. The embryo covered the entire egg, with continuous movements of the tail, eyes and jaws are seen. After one hundred and seventy- five hours post fertilization, *i.e.*, on the 7^{th} day of spawning, the embryo was ready to hatch with a well-developed lower jaw, but the mouth was not open. The cornea of the eye turned silver-coloured, the head of the embryo was placed at the vegetal pole and the tail at the animal pole.

The embryo covered the whole space of the egg. The full hatching of the egg clutch was achieved at one hundred- and seventy-seven hours post-fertilization.

Fig. 1. *A. perideraion* brooder with egg clutch.

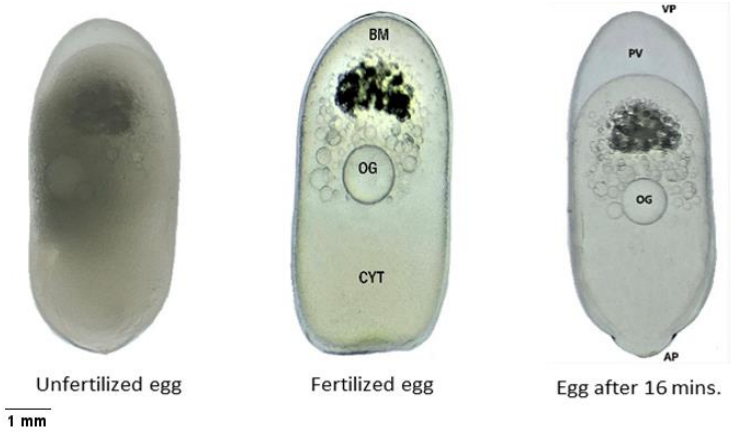

Fig. 2. Fertilized egg collected just after spawning (BM- black mass, OG- oil globule, CYT- cytoplasm, VP- vegetal pole, PV- perivitelline space and AP- animal pole).

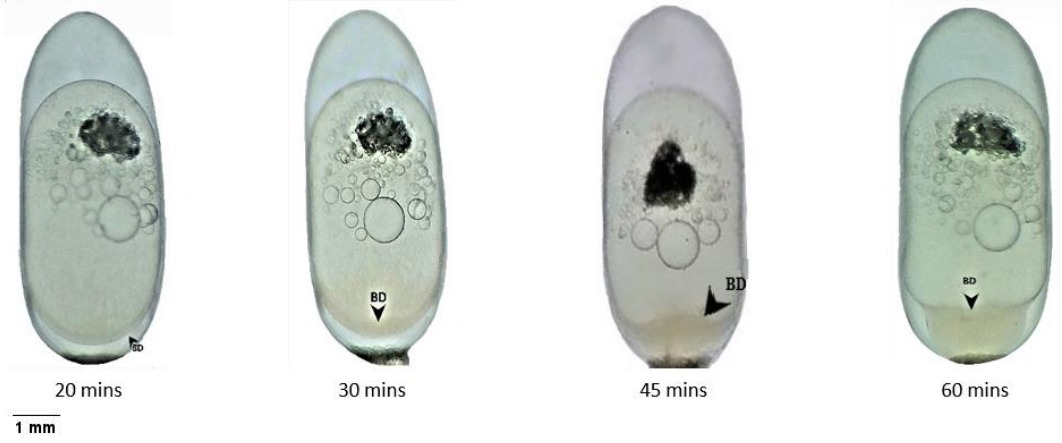

Fig. 3. Blastodisc formation of post fertilized eggs (20 mins, 30 mins, 45 mins and 60 mins).

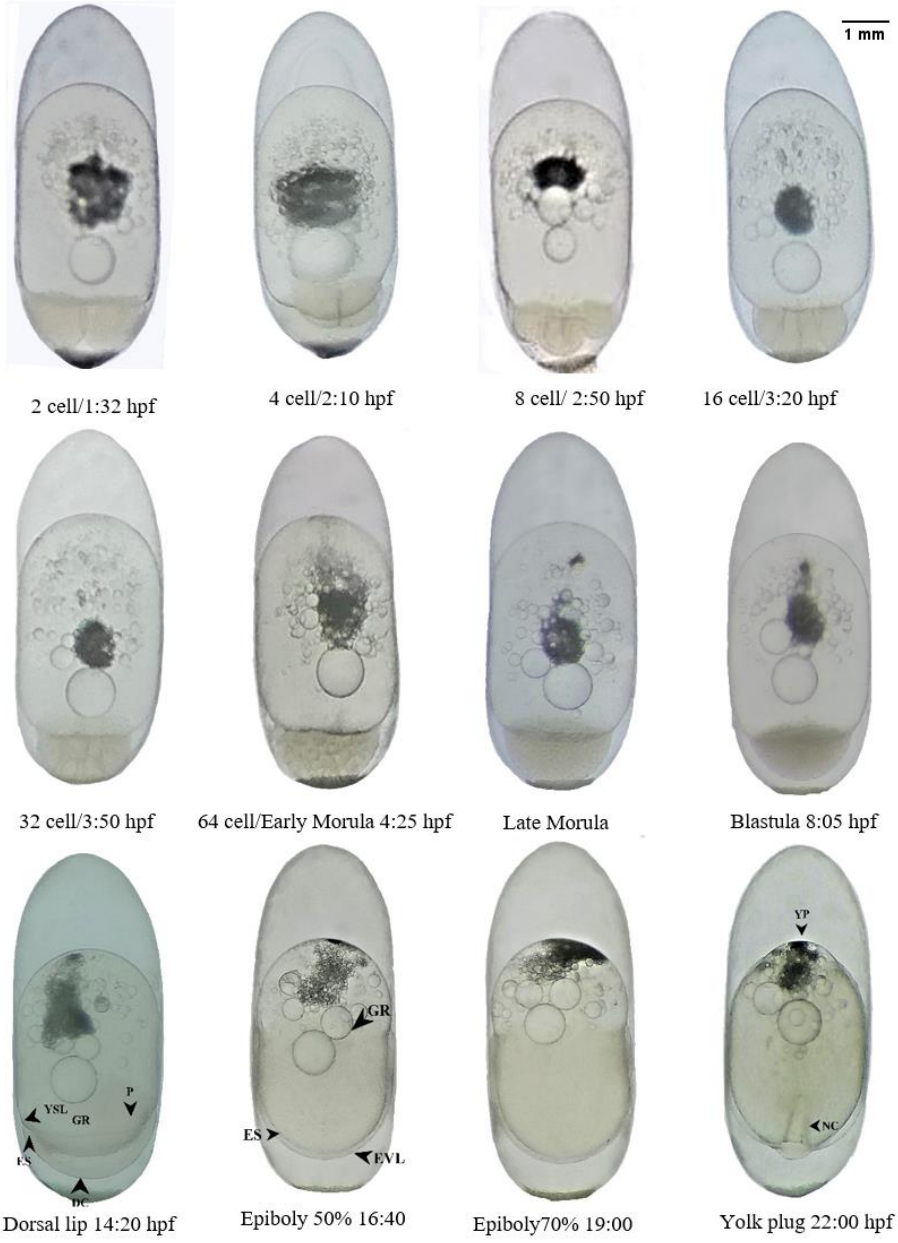

Fig. 4. Different stages of cleavage in the developing egg of *A. perideraion* (GR- Germ ring, EVL- Envelope layer, hpf- Hours post-fertilization, NC- Notochord, P- Periblast, YP- Yolk plug, YSL- Yolk syncytial layer).

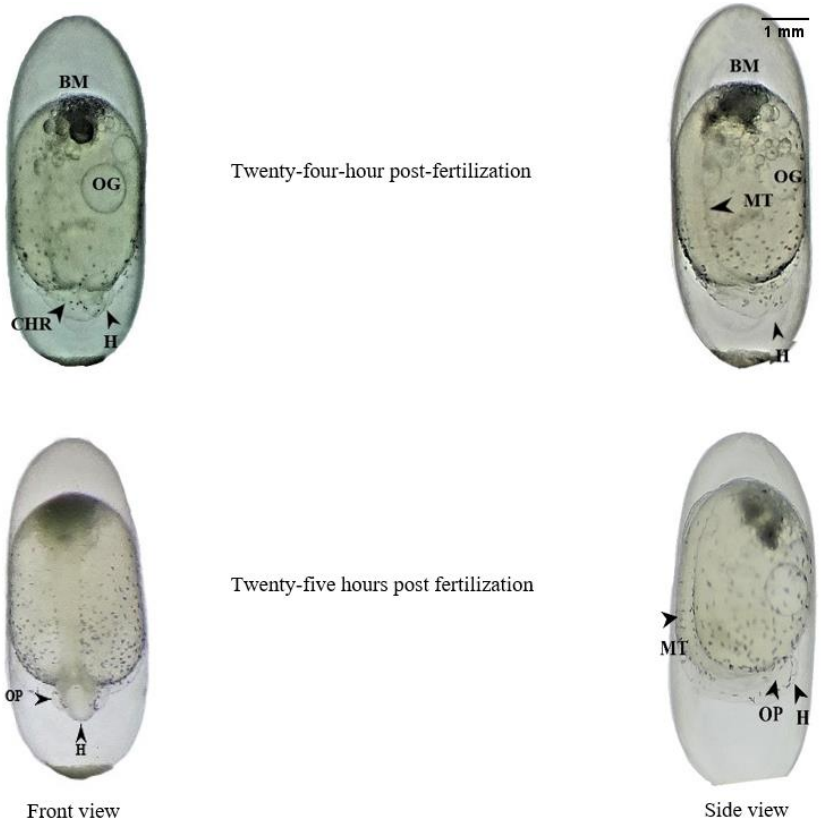

Fig. 5. Head development of the pink skunk embryo (BM- Black mass, CHR- Chromatophores, H- Head, MT- Myotomes, OG- Oil globule, OP-Optical vesicle).

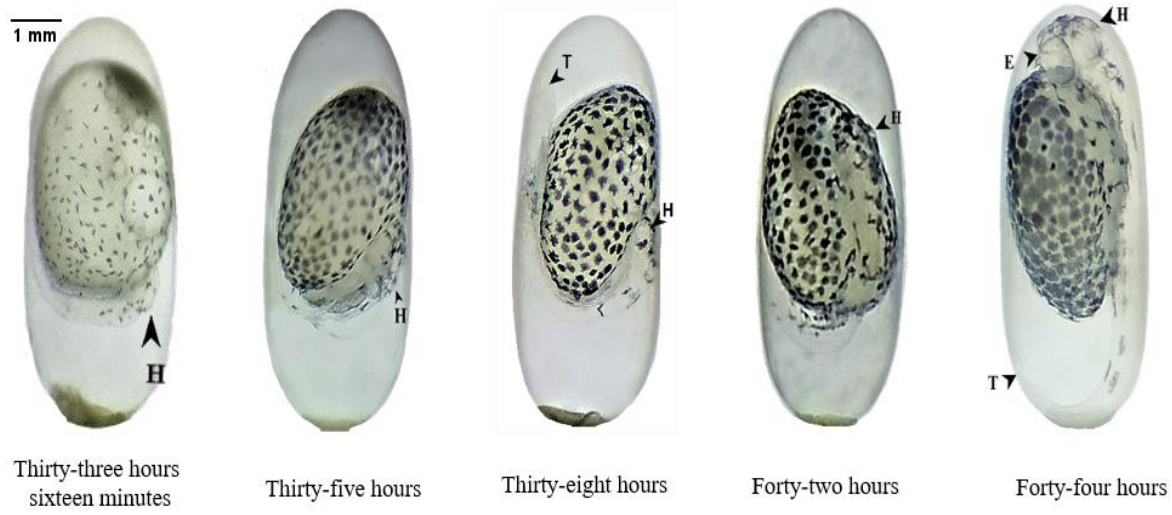

Fig. 6. Various stages during head turnover in embryonic development (E- Eye, H- Head, T- Tail).

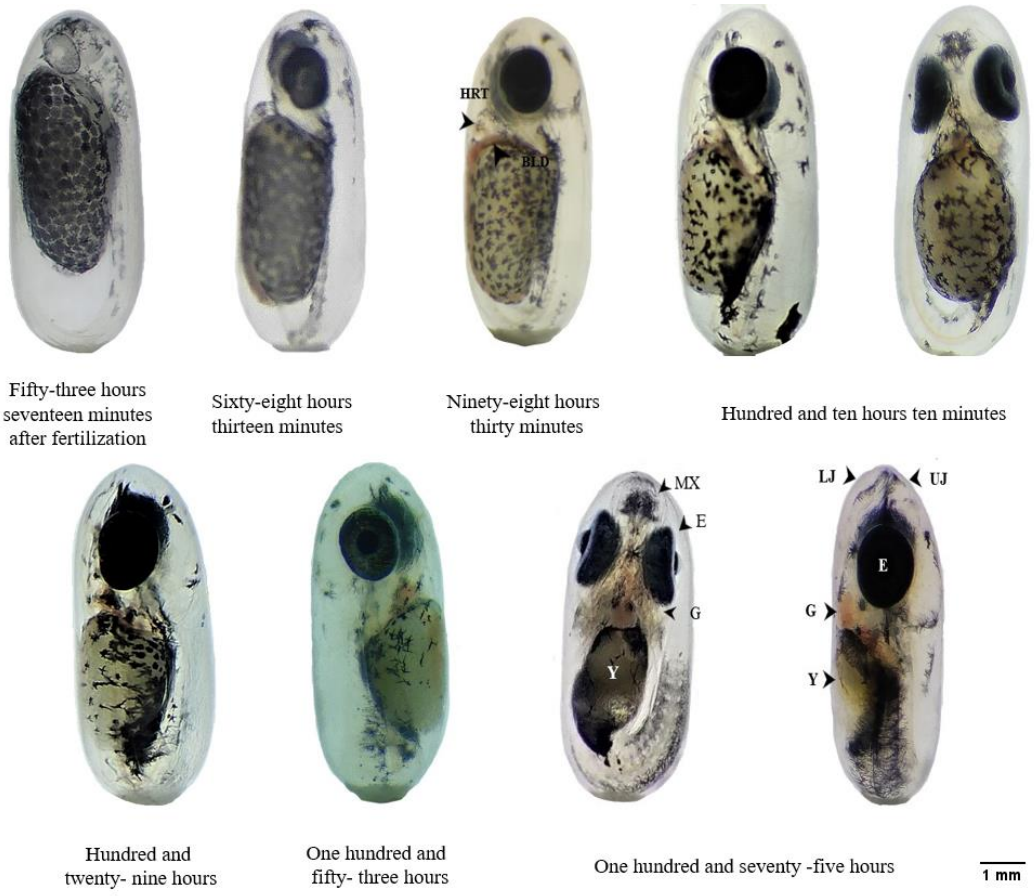

Fig. 7. Embryonic development during the organogenesis stage (E- Eye, G- Gills, H- Heart, Mx- Maxilla, LJ- Lower jaw, UJ- Upper jaw, Y- Yolk).

4. Discussion

The newly spawned eggs were adhesive, telolecithal, white to yellowish white in color and slightly curvature at the center of the yolk as mentioned by Yasir and Quin, 2007 in *A. ocellaris*. The eggs' color is considered to depend upon the brooder's age and nutrition (Nanthinidevi *et al*., 2016). Ho *et al.* (2008) reported that the eggs of *A. perideraion* were orange-coloured, whereas Wilkerson (2001) noticed *A. perideraion* laying pinkish eggs. During this experiment, yellowish-white coloured eggs were observed. The embryonic development during 0 to 24 hours post-fertilization is considered as the cleavage phase (Yasir and Quin, 2007; Ho *et al*., 2008; Dhaneesh *et al*., 2012; Ghosh *et al*., 2012). The perivitelline space was formed just after the spawning and which was to be smaller on the animal pole compared to that on the vegetal pole. Within a few hours of fertilization, the egg undergoes repeated mitotic cell divisions, which increases the number of cells without any addition in its volume. In *A. perideraion* as well as in other clownfishes such as *A. ocellaris* (Yasir and Quin, 2007; Soman *et al*., 2021**)**, *A. percula* (Dhaneesh *et al*., 2009), *A. nigripes* (Ghosh *et al*., 2012), *P. biaculeatus* (Madhu *et al*., 2012) a meroblastic or discoidal type of cleavage was noticed.

The blastodisc was seen at the animal pole of the egg, where all the cleavage stages were noticed. During the first cleavage stage, the blastodisc was divided into two equal-sized

blastomeres; and this stage was observed after one hour and twenty-two minutes of fertilization. Four equal-sized blastomeres appeared after two hours ten minutes post fertilization, during the second cleavage which occurred perpendicular to the first cleavage. Followed by the 8, 16 and 32 cells stages were noticed within the interval of 30-35 minutes. The late morula stage was recorded at five hours fifty-two minutes of fertilization and at this stage, the blastomeres were very small in size, compared to the previous stages. Ho *et al*. (2008) recorded 4, 8, 16 and 32 cells in *A. perideraion* at 1. 32, 2.00, 2.25 and 3.00 hrs at 27±1 °C, post fertilization, respectively.

The morula stage was completed at five hours fifty-two minutes after post-fertilization and the blastoderm turned into a blastula with a dense bowl of blastomeres. During this stage blastomeres size was seen to be smaller than that in the previous stage. The blastula stage was recorded at eight hours five minutes after post fertilization, whereas the blastula stage for *A. nigripes* was recorded at 10 hrs post-fertilization at 28±1 °C (Ghosh *et al*., 2012) and late blastula for *P. biaculeatus* at 05 hrs post-fertilization at 29±1 °C (Madhu *et al*., 2012).

The thickened margin of the blastoderm or dorsal lip and the yolk syncytial layer (YSL) was observed after fourteen hours and twenty minutes post-fertilization. Epiboly starts, when blastomeres cover a one-third area of the yolk/egg and this stage was recorded sixteen hrs post-fertilization. Blastomeres covered 50% of the yolk at sixteen hrs forty min, epiboly 70% after nineteen hrs; and gastrulation completes or 100% epiboly achieved after twenty-two hrs post-fertilization. In the case of *A. nigripes,* the start of epiboly was recorded after 19 hrs 30 min (Ghosh *et al*., 2012); for *A. akallopisos* and it was started at 18 hrs and completed at 21 hrs 50 min (Dhaneesh *et al*., 2012), whereas the start of the epiboly in *A. perideraion* was mentioned at 16 hrs 45 min (Ho *et al*., 2008). During the yolk plug stage, a primordial head could be observed in the developing embryo. After 24 hrs of fertilization, primitive eye buds, head and notochord were seen with the start of organogenesis. The beginning of the process of organogenesis was recorded at 23 hrs 10 min for *A. perideraion* 27±1°c (Ho *et al*., 2008) and 23 hrs 40 min for *A. nigripes* at 28±1°c (Ghosh *et al*., 2012). The development rate of fertilized eggs varies depending on the brooder's health, temperature and dissolved oxygen content of water (Soman *et al*., 2021), which was noticed in the present study also.

The most critical stage considered in embryological development is the turnover stage, when the embryo turns its head from the animal pole to the vegetal pole. This stage was completed at 44 hrs post-fertilization. The completion of the turnover stage was reported at 42 hrs and 15 min after post-fertilization in *A. akallopisos* (Dhaneesh *et al*., 2012) the same stage was noticed after 46 hrs 35 min post-fertilization in maroon clown (Madhu *et al*., 2012) and observed after 52 hrs post-fertilization in *A. perideraion* (Ho *et al*., 2008). Red blood cells were first seen in sixty-eight hrs thirteen min post-fertilization. Elliptical, pinkish blood cells were observed ninety-six hrs post-fertilization in *A. ocellaris* (Yasir and Quin, 2007), blood circulation was noticed fifty-five hrs after post-fertilization in *P. biaculeatus* (Madhu *et al*., 2012) and documented blood circulation at fifty-five hrs ten min in *A. percula* (Dhaneesh et al., 2009) and fifty-five hrs post-fertilization in *A. akallopisos* (Dhaneesh *et al*., 2012). A pair of otoliths was observed ninety-eight hours and thirty minutes after post-fertilization. One hundred and fifty-three hours after fertilization-embryo with a well-developed heart beating at 150-160 bpm, upper and lower jaws and silvery eyes were observed. Hatching was noticed after one hundred seventy seven hrs post-fertilization. The hatching efficiency,

incubation period and yolk utilization depend to a greater extent upon the water temperature. Hatching was noticed after one hundred sixty-two hrs at 30°C and one hundred seventy hrs at 26°C in *A. ocellaris* (Soman *et al*., 2021).

5. Conclusion

The time required for every important embryological developmental stage from cleavage to hatching in *A. perideraion* has been documented in this study. The comparison of organogenesis highlights variation among specimens from the same species, *A. perideraion* (Ho *et al*., 2008) and closely related species such as *A. akallopisos* (Orange skunk) and *A. nigripes* (Maldives anemonefish) from the Amphiprion genus. It is found that like other clown fishes, *A. perideraion* lays demersal, adhesive, telolecithal eggs. The progression of various developmental stages in *A. perideraion* is more closely related to other species from skunk clade *A. akallopisos* in comparison with other clownfish species. The overall understanding of the embryology and ontogeny of *A. perideraion* may help to improve the breeding protocol of the species and other closely related species from the skunk clade.

Table 1. Observations during the embryonic development of Pink skunk, *Amphiprion perideraion*.

Stage	Hours after fertilization (Hrs. : min)	Observations
1	0	Eggs were demersal, adhesive, and telolecithal, yolk was white to yellowish white in color, with a large oil droplet in the middle and a black mass was placed just above numerous small oil droplets
2	1	**Blastodisc** appears at the animal pole signalling the completion of the activation process. The perivitelline space is larger at the vegetal pole than at the animal pole
3	1:32	**Two cell stage** meroblastic type of cleavage was observed. The blastodisc divided into two equal-sized blastomeres
4	2:10	**In the four-cell stage,** the cleavage took place perpendicular to the previous one resulting in four equal-sized blastomeres
5	2:50	**Eight cell stage**
6	3:20	**Sixteen cell stage**
7	3:50	**Thirty-two cell stage**
8	4:25	**Sixty-four cell** stage or early morula
9	5:52	**Late morula,** the blastomeres became smaller in size as they increase in numbers. The blastoderm appears in a bowl shape with densely grown blastomeres
10	8:05	**Blastula,** the blastoderm develops into a flattened bowl with smaller densely packed blastomeres
11	10:40	The thickened margin of the blastoderm also known as the **dorsal lip** observed
12	14:20	The blastoderm started expanding over the yolk sac. Germ ring is evident.
13	16:40	Mid gastrulation or epiboly 50% blastoderm covers nearly half the area of the yolk
14	19:00	Blastoderm covers 70% area of the yolk, the primordial head can be seen
15	22:00	Yolk plug stage
16	24:00	The rudimentary head and somites start developing at the animal pole. A black mass is observed at the vegetal pole and a large oil droplet is observed at the Animal Pole
17	32:00	Chromatophores are visible on the yolk as well as on the head. Developing eye buds are visible on the head
18	42:00	**In the turnover stage**, the embryo completely turns itself to the vegetal pole but the tail is still attached to the yolk sac.
19	53:17	The mouth and eye buds can be distinguished. Melanophores are visible in the eyes.
20	68:13	Black melanophores can be seen in the eyes. Red pink blood cells can be seen in eyes with a cornea, embryo covering the whole egg space, heart beats are at 70-75 bpm, a pair of otoliths can be seen on each side
21	98:30	A pair of otoliths can be observed, as well as eyes with cornea.
22	110:00	The eyes are well developed with an eye lens. The heart rate is 80-90 bpm. Nasal vesicles can be observed. Orange-yellow body colour can be seen. Red colour gills can be seen under the operculum.
23	123:00	Developing lower jaw, also one larger and one smaller otolith can be noticed. Movements of the lower jaw, operculum and fins can be seen. Well-developed heart beating @ 92-100 bpm. Eyes started turning silvery.

24	153:00	Embryo with well-developed heart beating at 150-160 bpm. The embryo covers an entire egg, and continuous movements of the tail, eyes and jaws can be seen.
25	175:00	Well-developed lower jaw but the mouth still closed. The cornea of the eye turned silver coloured, the head of the embryo placed at the vegetal pole and the tail at the animal pole
26	177:00	The full hatching of the egg clutch was achieved

Declarations

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***Contributions*:** MN & DNB conducted experiments and wrote the manuscript. DPR revised the manuscript. TTAK designed the work and edited the manuscript. UKS provided overall guidance. All the authors contributed to reviewing the manuscript and approved the last version of the manuscript.

***Ethical Statement*:** Not applicable or not required for this study

***Data Availability Statement*:** The data can be made available upon reasonable request to the authors.

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دراسة النمو الجنيني لسمكة المهرج الظربان الوردي، ***Amphiprion perideraion*** (بليكر، 1855)

ميهير نخوة [1 و2]، و إن بي دهانيثي [1]، و بي آر ديفيا [1]، و تي تي أجيث كومار [1*] و أوتام كومار ساركار [1]

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المستخلص. أجريت الدراسة الحالية لتوثيق مراحل النمو الجنيني لسمكة المهرج *Amphiprion perideraion* في الأسر. تم توحيد معايير جودة المياه مثل الملوحة (30 جزء لكل تريليون)، ودرجة الحموضة (8.2)، ودرجة الحرارة (28 ± 1 درجة مئوية)، والأكسجين المذاب 6.5 ± 0.3 جزء في المليون، والأمونيا والنتريت (أقل من المستويات القابلة للاكتشاف)، والحفاظ عليها طوال فترة الدراسة. كان البيض الذي تم تفريخه حديثًا عبارة عن صفار لاصق، وتيلوسيتال، وأبيض إلى أبيض مصفر. يبلغ طول البيض حوالي 2.0–2.1 ملم وعرضه 0.9–1.0 ملم. كانت المشيماء شفافة ويمكن ملاحظة مراحل النمو الجنينية مباشرة من خلال القشرة. ولوحظت قطرة زيت كبيرة واضحة وعدة قطرات أصغر. بعد ساعة واحدة، ظهر القرص الأريمي في عمود الحيوان، ولوحظت مراحل انقسام مختلفة؛ تمت ملاحظة المراحل المكونة من مرحلتين، وأربع وثمانية، وستة عشر، واثنين وثلاثين، وأربعة وستين خلية، ثم التوتية والأريمة والمعيدة. بدأت عملية تكوين الأعضاء بعد الإخصاب، وبعد ذلك أصبح تكوين الرأس والحبل الظهري واضحًا. ظهرت الحويصلات البصرية لأول مرة بعد 32 ساعة من الإخصاب. تم تجاوز مرحلة الدوران بعد 42:10 ساعة بعد الإخصاب (hpf)، وبدأ نبض القلب والدورة الدموية بعد 68:13 ساعة بعد الإخصاب. يبدأ نمو الفك السفلي بعد مائة وثلاث وعشرين ساعة بعد الإخصاب. وصل الجنين إلى مرحلة ما قبل الفقس، وتم الوصول إلى الفقس بعد 177 ساعة من الحضانة.

الكلمات المفتاحية: الظربان الوردي، أمفيبريون بيريديريون، إدارة الأمهات، التطور الجنيني.