THE INTERNATIONAL JOURNAL OF SCIENCE & TECHNOLEDGE

Studies on Growth and Development of Hatchery Produced Juveniles of *Amphiprion Clarkii* (Bennett, 1830)

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Abstract:
Growth and development of Clark's anemone fish, Amphiprion clarkii from hatchlings to about 130th day old juveniles was described from captive - spawned and hatchery - reared specimens. On the 1st day of hatching the body of the larvae was transparent and all the fins were fused together to form a single fin fold. The hatchlings measured 4.0-4.3mm in standard length. On the 10th day all the fins were visible and body colouration had begun to develop, the larvae then measured 6.3-6.5mm in standard length. The banding began to appear from the 15- 17th day and on the 25th day the head and middle band were clearly visible. On the 30th day the caudal band was clearly visible, adult pigmentation had begun to appear from the 30th day onwards, the larva then measured 9.5-10.9 mm in standard length. On the 100th day the larvae exhibited bright yellow pigmentation and had developed the characteristic black colouration of the adult from the 130th day onwards. On the 130th day the larvae measured about 38 mm in standard length.

Keywords: *Amphiprion clarkii, Chromatophores, Pigmentation, Clownfish*

1. Introduction

The family Pomacentridae (Perciformes) is one of the most diverse groups of reef fishes, and is distributed mainly in the tropical and temperate seas of the Indo-Pacific (Allen, 1991; Nelson, 2006; Allen and Erdmann, 2012). The tropical marine anemone fishes are important in the trade for ornamental fish (Wilkerson, 1998) and are a popular subject of research (Fautin, 1991). Over the last 20 years, mariculture centers and scientific laboratories have started rearing these fishes in large quantities (McLarney, 1985; Miyagawa, 1989; Hoff, 1993; Young, 1996; Job et al., 1997). The members of the family Pomacentridae, commonly known as damselfishes and anemone fishes include 29 genera and 350 species under four subfamilies: Amphiprioninae, Chrominae, Lepidozyginae and Pomacentrinae (Allen, 1991). Amphiprion and Premnas have the shortest larval stages ranging from 7-14 days (Thresher et al., 1989), so they can be reared successfully and will reach saleable size easily. Anemonefishes are often found in groups of up to five or more individuals and exhibit strict dominance hierarchy or a "pecking order" that is usually related to size (Allen, 1991). Conspecific Amphiprion, of similar size, sometimes fought in captivity until one or both combatants were killed or seriously injured; although this behaviour was never observed under natural conditions (Allen, 1972). Development of early life stage, from fertilization to embryo formation among Teleost fish, generally follows the same pattern (Falk-Petersen, 2005). The young stages of many Pomacentrid species are brightly coloured and in certain cases may be very different from the adults (Allen, 1991). Therefore a comprehensive study of the larval development may be very essential in understanding the life history of Pomacentrids.

Amphirion clarkii is popularly known as Clark's anemone fish or yellowtail clownfish, it has a wide distribution throughout West Coast of Indian Ocean. A.clarkii has been known to associate with 8 different sea anemones. They usually associate with Cryptodendrum adhaesivum, Stichodactyla mertensii, S. gigantea and Entacmaea quadricolor and occasionally with Macrodactyla doreensis and S.haddoni and rarely with Heteractis malu and H.magnifican (Rema and Madhu, 2007). This is indeed advantageous since it can be kept along with a number of specimens in the aquarium tank. A. clarkii is black in colour with three white bars, one on the head behind the eye the other one on middle of the body above the anus and the third one on the caudal peduncle. The snout is orange in colour. The dorsal and anal fins are black, the pectoral, pelvic and caudal fins are yellowish in colour. They are of high demand in international trade due to their attractive coloration. Another attractive feature of this species is the high larval survival compared to other species in the hatchery (Personal observation). (Swagath Gosh et al., 2012) had successfully reared A.clarkii using brackish water, but the information on larval development and growth of juveniles under captive conditions is very scanty.

2. Materials and Methods

In June 2014, 5 sub adult to adult fishes were brought from Mandapam. The fishes were stocked in rectangular FRP tanks of 500 L capacity. The tanks were fitted with a biological filter for maintaining the water quality. Uneaten food was siphoned out twice daily, the siphoned out water was then replaced with fresh seawater, in addition to this sea water was exchanged at the rate of 25 % once in a week. During the rearing period water quality parameters such as water temperature, pH, salinity, NH_3, nitrate and phosphate were measured using standard methods. Salinity and pH in the breeding tanks were maintained at 32-34 ppt and 8 to 8.2 respectively by replacement of sea water whenever required. Temperature during this period was 27±02 °C. The fishes were fed 4 times a day. The feeding schedule followed was pellet feed at 10.00 am, boiled mussel meat at 12.00 pm and 2.00 pm and Artemia naupli at 4.00 pm. Of the 5 fishes put in the broodstock tanks two fishes showed pairing behaviour and started behaving aggressively to the other fishes, so the other three fishes were moved to separate broodstock tanks of this two fishes succumbed to their injuries, the remaining two fishes began laying eggs on August 2014. The fishes were provided with earthen pots and tiles for laying eggs.

2.1. Live Feed Culture

Live feed such as phytoplankton, rotifers, Artemia, were used for the larval rearing experiments. For feeding rotifer culture and for maintaining green water in the rearing tanks, stock culture of algae such as Nanochloropsis oculata and Isochrysis galbana were maintained in stock culture room at 24 ^{0}C in 500- 4000 ml flasks and then the cultures were upscaled to 20 litre carboys for feeding. Rotifer, B. rotundiformis and Brachionus plicatilis were cultured by feeding mixed culture of N. oculata and I.galbana in equal proportions. Artemia nauplii were produced by hatching commercially available artemia cysts (Microfeast® Artemia, U.S.A.).

2.2. Larval Rearing

Algal density was kept between $1x10^5 – 3x10^5$ cells ml^{-1} in the larval rearing tanks. Larvae were stocked at the rate of 2 nos. L^{-1} and 100 larvae were stocked in 50 l of water. For studying larval survival and growth three replicates were provided. The larvae were fed on rotifers from 1st to 10th day of hatch, from 1st to 5th day on B.rotundiformis and from 5th to 10th day on B.plicatilis at the rate of 8 nos.ml^{-1}. Artemia nauplii were given at the rate of 2nos. ml^{-1} from 7 dph and slowly increased to 4 and 6 nos. ml^{-1} on 15 and 20 dph respectively. Particulate feed was started from the 25th day onwards using ground pellet feed of size from 200 to 500 micron. They were given boiled mussel meat from the 30th day onwards. Morphological changes were noted and measurements were taken on every 5th day from the 1st to 30th day of hatching. After that measurements were taken every 10 days upto the 100th day, then measurements were taken after 30 days ie, on the 130th day. Each time 5 individuals were taken for making the measurements.

3. Results

3.1. Hatching and Parental Care

Spawning took place between 6-9 am. The eggs were found attached to the substratum and they were capsule shaped. The male parent exhibited high degree of parental care for the eggs, such as fanning the eggs with the pectoral and caudal fins and removing the infected eggs with its mouth. The eggs measured 2.3-2.4mm in length and 0.9mm in width. The spawning frequency was observed from 25-08-2014 to 25-03-2015. The spawning frequency was found to be 3-4 times per month. The eggs hatched on the 7th day of incubation. Hatching took place in the early evening hours at about 7.00-8.00 pm. Since the larvae are photopositive, they were attracted by a torch and collected using a small trough and then transferred to the larval rearing tanks.

The newly hatched larvae measured about 4.18 - 4.3 mm total length and 3.8- 4.1 mm in standard length. The larvae were transparent except for the presence of yellow pigmentation on the gut and the middle of the body. A row of stellate melanophores were also observed in the ventral midline. Black pigment granules were also distributed throughout the body. The dorsal, pelvic and anal fins were fused together to form a single fin fold, which was present along the 3/4th of the body (Figure 1).

On the 10th day light yellow colouration was visible along 3/4th of the body and black chromatophores were distributed along the yellow coloured region. The pectoral, pelvic, anal and dorsal fins were clearly visible with distinct fin rays. The fins were transparent without any pigmentation. Teeth had begun to form in the upper and lower jaws (Figure 2). On the 15th day larva was bright yellow in colour except in the head region and had black pigment granules scattered all over the body. The opercular region was pinkish in colour. The belly region was silvery grey. Fins were clear without any pigmentation. A faint head band was visible in some larvae on the 15th day, banding began to appear in most larvae from the 15-17th day onwards. (Figure 3).

On the 20th day the black pigment granules increased in number such that they masked the yellow colour of the body, the yellow colour had begun to spread to the dorsal and anal fins, black pigment granules were also visible on the fins. Gut was dark reddish in colour (Figure 4). On the 25th day two bands on the larval body were clearly visible, the first one on the head, behind the eye the second one from the beginning of the anal fin to the middle of the dorsal fin. The spinous dorsal and soft dorsal fins have distinctly separated. Black chromatophores were distributed all over the body, yellow pigmentation was visible in between the black pigment granules (Figure 5).

On the 30th day the larva had transformed into a subadult fish with black pigment granules distributed on the whole body, the spinous dorsal, pectoral and anal fins were light yellowish in colour and also possessed black pigment granules, in addition to the head and middle band present on the 25th day a caudal band had appeared on the caudal peduncle. The head band is thicker than the other two bands. The middle band extends towards the soft dorsal whereas the caudal band extends towards the caudal fin so the dorsal and

caudal fin appears half yellow and half white. Black pigment granules were distributed throughout the body. The head was yellowish in colour (Figure 6).

On the 40th day, black pigment granules were distributed all over the body, the pectoral and pelvic fins were yellow, the anal fin was yellowish but had black pigment granules near the region where it joins the body, the dorsal and the caudal fins have yellow colour with black pigment granules. The head band had increased in thickness. On the 50th day the head and the middle band had a black outline on both sides, this black outline was restricted to one side in the case of the caudal band. The black pigmentation of the fins had decreased notably and now all the fins have bright yellow colouration with very less number of black pigment granules (Figure 7). On the 60th day the head band was found to be tapering and the upper jaw and lower jaw were transparent (Figure 8). On the 100th day the larvae exhibited bright yellow pigmentation and the black pigment granules surrounding the head band was found extending towards the eye .The caudal fin also contained high number of black pigment granules (Figure 9). On the 130th day the larva developed the characteristic black colouration of the adult with black coloration on the pectoral, caudal and anal fins (Figure 10).

Growth of A.clarkii is summarised in the table shown below (Table 1). The newly hatched larvae measured 4.0-4.3 mm in total length and 3.8-4.1 mm in standard length. The body width ranged from 0.9-1.2mm. On the second day the larvae measured 5.4-6.1 mm in standard length, the body width didn't show much increase on the 5th day, it ranged from 1.2- 1.4 mm. On the 10th day the standard length ranged between 6.3-6.5 mm, it then increased to 9.5-10.9 mm on the 30th day and to 12.4-13.0 mm on the 50th day. On the 100th day the larvae had a standard length of 28-32 mm which increased to 33-38 mm on the 130th day. By this time the larvae had reached a marketable size.

Days	Total length(mm)	Standard length(mm)	Head length(mm)	Head width(mm)	Body width(mm)	Eye diameter (mm)
1	4.18± 0.12	3.96± 0.11	1.07±0.12	0.99±0.14	0.92±0.19	0.42±0.03
Range	4.0-4.3	3.8-4.1	0.9-1.2	0.8-1.15	0.9-1.2	0.3-0.5
5	5.84±0.30	4.8±0.25	1.6±0.03	1.49±0.11	1.33±0.08	0.61±0.09
Range	5.4-6.1	4.4-5.0	1.3-1.8	1.35-1.6	1.2-1.4	0.5-0.7
10	6.41±0.09	5.09±0.30	1.74±0.25	1.63±0.08	1.56±0.04	0.77±0.04
Range	6.3-6.5	4.9-5.4	1.5-2.0	1.5-1.7	1.5-1.6	0.7-0.8
15	7.36±0.69	5.9±0.56	2.16±0.31	1.94±0.15	1.9±0.37	0.85±0.05
Range	6.7-8.2	5.4-6.5	1.9-2.5	1.8-2.1	1.6-2.3	0.8-0.9
20	8.54±0.21	6.54±0.11	2.53±0.14	2.23±0.05	2.56±0.17	0.91±0.07
Range	8.3-8.8	6.4-6.7	2.3-2.65	2.1-2.35	2.3-2.7	0.85-1.0
30	10.1±0.62	7.44±0.44	2.86±0.30	2.96±0.38	3.14±0.19	1.14±0.19
Range	9.5-10.9	7.5-8.0	2.5-3.2	2.6-3.3	3.0-3.4	1.0-1.4
40	12.68±0.27	9.4±0.30	3.76±0.24	3.56±0.24	3.5±0.12	1.46±0.05
Range	12.4-13.0	9.1-9.8	3.5-4.0	3.3-3.8	3.4-3.7	1.4-1.5
50	13.98±0.31	9.56±0.40	4.08±0.23	3.94±0.27	4.37±0.23	1.48±0.13
Range	13.5-14.3	9.1-10	3.7-4.3	3.5-4.2	4.0-4.6	1.3-1.6
60	16.12±0.39	12.24±0.49	4.5±0.20	4.24±0.19	4.8±0.20	1.71±0.15
Range	15.5-16.5	11.5-12.7	4.2-4.7	4.0-4.5	4.5-4.9	1.6-1.85
70	17.8±1.64	14.6±2.70	5.14±0.30	5.32±0.08	5.44±0.43	1.99±0.12
Range	16-20	13-18	4.8-5.5	5.2-5.4	5.2-6.0	1.8-2.15
80	21.2±3.63	17.6±2.88	5.64±0.43	5.92±0.58	6.52±0.96	2.04±0.17
Range	17-25	13-20	5.4-6.0	5.2-6.4	5.2-7.3	1.8-2.2
90	26.8±0.57	20±0.61	6.53±0.12	6.61±0.10	8.02±0.54	2.49±0.07
Range	26-27.5	19.5-21	6.4-6.7	6.45-6.7	7.5-8.7	2.4-2.6
100	30±1.58	23.4±1.14	8±0.38	8.8±0.27	9.86±0.27	2.79±0.09
Range	28-32	22-25	7.5-8.5	8.4-9.1	9.5-10.2	2.7-2.9
130	35.8±1.92	28.4±1.52	9.48±0.40	9.52±0.35	10.66±0.32	3.24±0.19
Range	33-38	26-29	9.0-10	9.1-10	10.2-11.0	3.0-3.25

Table 1: Summarised morphometric data on growth of hatchery produced and captive reared A.clarkii (mean ±standard deviation & range)

3.2. Larval development of A.clarkii

Figure 1: Newly hatched larvae of A.clarkii

Figure 2: 10th day larvae of A.clarkii

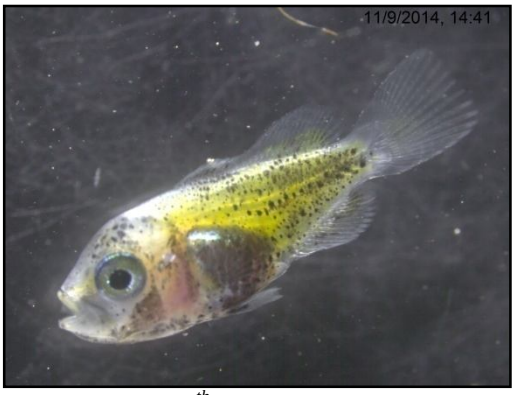

Figure 3: 15th day larvae of A.clarkii

Figure 4: 20th day larvae of A.clarkii

Figure 5: 25th day larvae of A.clarkii

Figure 6: 30th day larvae of A.clarkii

Figure 7: 50th day larvae of A.clarkii

Figure 8: 60th day larvae of A.clarkii

Figure 9: 100th day larvae of A.clarkii

Figure 10: 130th day larvae of A.clarkii

4. Discussion

It was observed that the egg laying took place in the morning hours, similar observations were made in A. chrysogaster (Gopakumar et al., 1999), A. sebae (Ignatius et al., 2001) and in A. ocellaris (Madhu and Rema, 2010) and A. nigripes (Anil et al., 2010). The eggs of A. clarkii measured 2.3-2.4mm in length and 0.9mm in width. In Amphiprion akallopisos the eggs measured 2.0–2.1 mm in length and 0.9-1.0 mm in width (Daneesh et al., 2012). In A. clarkii the spawning frequency was found to be 3 times per month, whereas it was reported to be 2 per month in P.biaculeatus (Madhu et al., 2010) and in A.clarkii reared in brackish water (Swagath Gosh et al., 2012). The higher spawning frequency observed in the present study maybe due to the better water quality management and feeding. In A. clarkii the eggs hatched on the 7th day of incubation at a temperature of 27±02 °C this was in contrast with Amphiprion akallopisos which took 7- 9 days to hatch at a temperature of 27 ± 1^0C (Daneesh et al., 2012).

The feeding schedule followed for larval rearing was same as that followed by Anil et al. (2012) for A.nigripes with an exception that in the case of A.clarkii the larvae were fed on rotifers B.rotundiformis from 1st to 5th day and on B.plicatilis from 5th to 10th day at the rate of 8 nos.ml^{-1}, whereas in case of A.nigripes the larvae were given rotifer B.plicatilis from the 1st to 15th day at a rate of 10 nos. ml^{-1}. Hoff (1996) recommended 300 to 600 rotifers per larvae per day for a period of 5-10 days to rear clownfish larvae.

A.clarkii hatchlings had a total length of 4.0-4.3 mm with functional eyes, whereas the total length of the hatchlings were 3.9 mm in case of A.nigripes (Anil et al., 2012). The larvae were transparent and the dorsal, pelvic and anal fins were fused together to form a single fin fold, which was present along the 3/4th of the body. This was similar to the observation made by Anil et al. (2012) in A.nigripes except that in A.clarkii yellow pigmentation was present on the gut and the middle of the body. On the 10th day the fins were clearly visible in A.clarkii, this were similar to the findings of Sreeraj (2002) in A. sebae. Banding began to appear in the larvae in 15-17th day onwards, this was similar to the observations made Swagath Gosh et al. (2012). But Ignatius et al. (2001) reported that in A.seba the two white bands appeared on the body on the 12th day. On the 20th day the black pigment granules increased in number and black pigment granules were also visible on the fins whereas in case of A.nigripes scattered dark pigment granules were observed on the body. On the 25th day the head band and the middle band were clearly visible, the caudal band was clearly visible on the 30th day, but in case A.ocellaris all the three body bars become opaque and fully developed from 14th -30th day (Bertschy, 1979). Another notable difference between the banding pattern of the two species is that in A.clarkii by the 30th day the middle band had extended to the middle portion of the soft dorsal fin and the caudal band has extended to the outer surface of the caudal fin, but in the case of A.ocellaris the middle band had begun to appear from the 12th to 18th day and it extended to the middle of the dorsal fin whereas the caudal band did not extend into the caudal fin.

On the 40th day the pectoral, pelvic, dorsal and anal fins displayed light pigmentation and the caudal fins were yellow in colour with black pigment granules whereas in case of A.ocellaris early stages of pigmentation was visible only on the caudal fins by the 40th day. In A.clarkii on the 50th day the head and the middle band had a black outline on both sides, this black outline was restricted to one side in the case of the caudal band, whereas in A.ocellaris heavy black lines develop along bars from 40- 58 days. In A.clarkii on the 100th day the larvae exhibited bright yellow pigmentation and on the 130th day the larva developed the characteristic black colouration of the adult. They reached the saleable size of 3.8cm in about 4 months, whereas Dhaneesh et al. (2012) reported that A. akallopisos reach a marketable size of 3 cm within 3 months of rearing and according to Anil et al. (2012) A.nigripes reached a saleable size of 25 – 30 mm within 4 months.

5. Acknowledgements

We gratefully acknowledge the Research Grants from the Kerala State Council for Science Technology and Environment for the work.We would like to extend our warm gratitude to Dr. A. Gopalakrishnan, the Director, Central Marine Fisheries Research Institute,Kochi, for the permission granted and facilities provided. We thank Dr.Rani Mary George,Scientist In Charge,Vizhinjam Research Centre of CMFRI for the support provided during the work. We thank Dr.P.ManoharanPillai, Associate Professor(Rtd), NSS College,Kottiyam for all encouragement given to complete this paper work.We would like to thank all the staff members of Vizhinjam Research Centre of CMFRI for all support.

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