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Breeding, larval rearing and growth of black *Amphiprion ocellaris* (Cuvier, 1830) under captivity

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ABSTRACT

Broodstock development, breeding, spawning, larval rearing and growth of black *Amphiprion ocellaris* in captivity are described in this study. The black ocellaris, a magnificent colour morph of *A. ocellaris* was bred in captivity at Vizhinjam Research Centre of ICAR-Central Marine Fisheries Research Institute (ICAR-CMFRI). Two adults, approximately 4-6 cm in total length (TL) were stocked in FRP tanks of 300 l capacity, under optimum water quality. The broodfishes were fed a combination of boiled mussel meat, boiled squid and semimoist feed (protein - 40 %, lipid - 9.5 %, fibre - 2% and moisture - 31%) twice daily at a rate of 3-5% of their body weight. After a period of 1.7 years, the fishes started showing courtship behaviour and initiated spawning. The oocytes were of 3-3.3 mm in length and 1.1-1.9 mm in width. Approximately 200-300 eggs spawned the first time and the number of eggs gradually increased in subsequent spawnings. Spawning was obtained at an interval of 12-14 days providing an average of two spawnings per month. The incubation period of the eggs were 7-8 days and the fertilised eggs hatched on the 8th day, soon after sunset, generally between 19.00 and 20.00 hrs. Different strains of cultured plankton like *Isochrysis galbana*, *Nanochloropsis oculata*, L-type rotifer *Brachionus plicatilis*, calanoid copepod *Acartia southwelli* and Artemia were used as feed for larvae. The newly hatched larvae with a total length of 5±0.16 mm metamorphosed to juveniles on the 40th day (18.87±0.07 mm). Black ocellaris is a highly priced clownfish and can be used to crossbreed with the normal orange coloured ocellaris clown fish to produce different colour variants.

Keywords: Black *Amphiprion ocellaris,* Breeding, Broodstock development, Larval Rearing, Live feed, Variants

Introduction

The ornamental fish trade is a global multi-million dollar industry with a world export value of US$ 362 million and corresponding imports valued at US$ 362 million (FAO, 2014). The production and trade of freshwater and marine ornamental fish provides an income to the developing countries where more than 90% of the freshwater ornamentals are captive bred and only 25 species of marine fishes are commercially produced. However, efforts are being made by different researchers to breed and rear some of the high value marine ornamental species (Thresher, 1984; Riley and Holt, 1993; Holt, 2003; Olivotto *et al*., 2005; Olivotto *et al*., 2006).

The marine ornamental fish industry has grown significantly over the past decades, the value of global exports increased from US$181 million to US$372 million during 2001-2011 and the total trade in live marine ornamentals is estimated at around US$44 million annually (Pierluigi, 2010). Out of the 1000 species of coral reef fishes traded (Green, 2003), only 51 have been cultured in captivity for the aquarium trade (Arvedlund *et al*., 2000). Rearing of aquarium fish in captivity can help production of hardier species, which grow far better in captivity and survive longer (Ogawa and Brown, 2001; Olivier, 2003). Pomacentrid fishes (369 known species), which include clownfishes and damselfishes, are the most popular aquarium fishes inhabiting tropical and subtropical seas in Indo-Pacific regions (FAO, 2004; Randall, 2005; Fishbase, 2008). Clownfishes are very popular among aquarists due to their bright colour, interesting display behaviour and their ability to adapt to captive conditions (Wilkerson, 1998). Clownfishes are a highly diverse group including 30 species with two intergeneric hybrids *Amphiprion* and *Premnas* (Allen *et al*., 2008, 2010). The family Pomacentridae is probably the most represented of all coral reef fish families, comprising a significant portion of the fish biomass in coral reefs (Williams and Hatcher, 1983).

Rotifers and brine shrimp are the most widely used live food items in marine fish culture as they can be cultured easily in large quantities at high densities (Leu, 1994, 1997; Leu and Chou, 1996; Holt, 2003; Olivotto *et al*., 2003; Leue *et al*., 2005; Olivotto *et al*., 2005; Olivotto *et al*., 2006). However, most of the ornamental species

are usually characterised by a small mouth (Leu *et al*., 2009) and therefore, rotifers and brine shrimp are not always the best first food for the hatchlings (Holt, 2003).

The black ocellaris clownfish *Amphiprion ocellaris* is a member of the family Pomacentridae. Among all the species of clownfishes, *A. ocellaris* is the best known to aquarium traders due to its colour pattern, interesting behaviour and robustness. From 1997 to 2002, *A. ocellaris* contributed 15.6% of marine ornamental fishes exported (in total numbers) worldwide and over 25% into European countries (Wabnitz *et al*., 2003). *A. ocellaris* can be found individually or more commonly in pairs or small groups within the anemone such as *Heteractis magnifica* or *Stichodactyla mertensii* (Myers, 1999) as part of a symbiotic relationship. *A. ocellaris* are found in different colours depending on where they are located, Black *A. ocellaris* with its jet-black body and three white stripes on each side are commonly found near northern Australia (Williams and Hatcher, 1983), south-east Asia, and Japan (Allen, 1997). It is one of the most beautiful and hardy tropical marine aquarium fishes with great demand in the international market.

Among the marine ornamental fishes, the first success was achieved in captive breeding and seed production of clownfishes, as their larviculture protocols are comparatively easy (Hoff, 1996). Ornamental fish breeding programmes tasted success in India when the breeding of the seahorse *Hippocampus kuda* was carried out successfully(Anil *et al.*, 1999), followed by the development of hatchery techniques of many species of the clownfishes - *A. chrysogaster* (Gopakumar *et al.,* 1999), *A. seba* (Ignatius *et al.,* 2001), *Premnas biaculeatus* (Madhu *et al.,* 2006, Anil *et al*., 2010), *A. ocellaris* (Kumar and Balasubramanian, 2009), *A. percula* (Madhu and Rema, 2011), *A. nigripes* (Anil *et al.*, 2012), *A. akallopisos* (Dhaneesh *et al.,* 2009) and *A. ephippium* (Rohini Krishna *et al.,* 2018). The key factor for the successful larviculture of marine finfishes is appropriate size and nutritional quality of the live feeds employed (Gopakumar and Santhosh, 2009). The aim of the present study was to assess captive spawning, larval development and growth of black *A. ocellaris* and to test the effect of different livefeed on growth and survival.

Materials and methods

Broodstock maintenance

Five sub-adults of black *A. ocellaris*, approximately 4-6 cm in total length (TL), were procured from ornamental fish traders and were transported to the research laboratory in a polythene bag with oxygen. After temperature acclimatisation, the fishes were stocked in two FRP tanks (two in one tank and three in the other) of 500 l seawater capacity and the tanks were fitted with a biological filter. The temperature in the breeding tanks was maintained between 27.4-30°C, salinity 33-35 ppt, pH 8-8.2 and NO_2-N and NH_3-N <0.03 mg l^{-1}. The broodfish were fed a combination of boiled mussel meat (*Perna perna=P. indica*), boiled squid [*Uroteuthis (Photololigo) duvaucelii=Loligo duvauceli*] and semi-moist feed (protein 40%, lipid 9.5%, fibre 2% and moisture 31%) twice daily at the rate of 3-5% of their body weight. For maintaining the water quality in the broodstock tank, about 10% of water was exchanged daily. Excreta and excess feed were siphoned off before topping up with fresh seawater. Photoperiod schedule during the pre-conditioning and spawning period was maintained at 12L:12D, natural light inside the hatchery which has transparent roofing. The fishes were observed two times a day (at 09.30-10.30 hrs and 16.00-17.00 hrs) and any change in the behaviour indicating courtship or spawning was monitored and recorded. Sea anemone *H. magnifica* was provided in the broodstock tank to accelerate the symbiotic relationship and to induce selection of a nest site adjacent to the sea anemone for deposition of eggs. The broodstock tank was provided with earthen pots for egg deposition. The pots were checked every morning at 09.30 hrs for egg clutches. When egg clutches were found, the embryonic development was inspected under a Stereo zoom microscope (Zeiss Discovery.V8) attached with Zeiss AxioCam ICc5 with ZEN software.

Live feed culture

Micro algae (*Isochrysis galbana* and *Nanochloropsis oculata*), L-type rotifer *Brachionus plicatilis*, calanoid copepod *Acartia southwelli* and brine shrimp *Artemia* were maintained and cultured for the rearing of black *A. ocellaris* larvae. The culture of microalgae *I. galbana* and *N. oculata* were maintained in stock room at 24ºC in 250 ml to 4 l flasks and was upscaled to 20 l carboy for feeding the copepod and for maintaining green water in the rearing tank. The calanoid copepod *A. southwelli* was cultured using an equal mixture of microalgae *I. galbana* and *N. oculata*. Copepod nauplii were separated from copepodites and adults using a different sized mesh (40-250 µm) for feeding the larvae. The rotifer *B. plicatilis* (lorica length 70-239 µm) were cultured using a combination of microalgae *I. galbana* and *N. oculata*. Rotifers were enriched using Algamac 2000 (Aquafauna Bio-Marine, USA), 8 h before they were fed to the larvae. The enriched rotifers were rinsed three times or properly washed in clean seawater (salinity 33 ppt) to remove the media remnants. *Artemia* nauplii were produced by hatching commercially available cysts (Microfeast® Artemia, USA) and enriched with microalgae for a short period before using it as feed for the fish larvae.

Feeding experiment

The larvae were stocked in three 100 l glass tanks at a stocking density of 100 larvae per tank. The sides of the glass tanks were covered with a black sheet to reduce the reflection of light. Three feeding trials were conducted, the first with copepod *A. southwelli* @ 1-10 nos ml^{-1} (Treatment I), the second with enriched rotifer *B. plicatilis* (lorica length 70-239 µm) @ 5-6 nos. ml^{-1}) (Treatment II) and the third with unenriched rotifer (Treatment III). Seawater used for the larval rearing tank was filtered using a series of 10, 5 and 2 µm cartridge filters and mild aeration was given throughout the rearing period. The green water technique was employed for the larval rearing and algal cell density was maintained at 1×10^4 cells ml^{-1} for the experiment. The microalgae *N. oculata* and *I. galbana* in the ratio 1:1 were added to all the three tanks. Larvae in each treatment were given corresponding feed items upto 8 days post-hatch (dph) and enriched *Artemia* nauplii was given 9 dph onwards. Boiled and finely chopped mussel meat was given to the growing fry from 25 dph until the termination of the experimental rearing period at 60 dph. Debris and dead larvae were siphoned out from the tank daily. About 15% of the water exchanged from 1st day onwards and was done two times per day. Treatments were repeated three times to arrive at the results. Details of feeding schedule and larval rearing are given in Table 1.

Results

Courtship and spawning

Two fishes in the tank stocked with three fishes started showing courtship after a period of 1 year and seven months and the third fish was subsequently removed from the tank. After about two months, the male started cleaning a particular area to deposit eggs. When spawning was about to occur, the male started chasing the female to a secluded area to induce the process. Spawning occurred in the morning between 07.00 to 09.30 hrs. Female made several passes over the nest before laying capsule shaped eggs on the cleaned substratum in a nearly rounded or oval patch. This was followed by fertilisation. The spawning lasts for 1 to 1.5 h. The adhesive eggs were covered with a transparent chorion and a narrow perivitelline space. The eggs measured 3-3.3 mm in length and 1.1-1.9 mm in width. Approximately 40-200 eggs were spawned at a time and the number of eggs gradually increased in subsequent spawnings. Spawnings were obtained at an interval of 12-14 days giving an average of two spawnings per month. The newly laid eggs were orange to pale yellowish in colour and contained many tightly packed oil globules; with the yolk colour becoming more intense with time. At the initial stage, the eggs were macrolecithal and telolecithal and cytoplasm was not visible due to yolk content. As the embryo developed, colour of the

Table 1. Feeding schedule of hatchery-reared black *A. ocellaris*

Days post-hatch (dph)	1	2	3	4	5	6	7	8	9	10	12	14	16	18	20	25	30	35	40	45	50	55	60
Treatment I																							
Nanochloropsis oculata and *Iscochrysis galbana* (1:1)																							
Acartia southwelli nauplii (1-10 per ml)																							
Artemia nauplii (1-3 per ml)																							
Boiled mussel meat																							
Treatment II																							
Nanochloropsis oculata and *Iscochrysis galbana* (1:1)																							
Brachionus plicatilis (5-6 per ml) enriched																							
Artemia nauplii (1-3 per ml)																							
Boiled mussel meat																							
Treatment III																							
Nanochloropsis oculata and *Iscochrysis galbana* (1:1)																							
Brachionus plicatilis (5-6 per ml) unenriched																							
Artemia nauplii (1-3 per ml)																							
Boiled mussel meat																							
Water Management																							
Water exchange (~15%)																							
Bottom siphoning(~daily)																							
Water addition (~15%)																							

eggs turned to black during the 3rd to 6th days and later turned to silvery on the 7th day of incubation. At this stage, the glowing eyes of the developing larvae inside the egg capsule were clearly visible. The photograph depicting the egg development of *A.ocellaris* is shown in Fig. 1. The fertilised eggs hatched within 7-8 days, soon after sunset, between 19.00 and 20.00 hrs. Since the larvae are photopositive, they were attracted by light and the hatched out larvae were gently collected with a beaker and transferred to the larval rearing tanks. Males exhibited more eggs care and were involved in fanning the eggs with pectoral and caudal fins and removing the infertile or damaged eggs. Once the eggs hatched, the larvae were found not to depend on the parents.

Embryonic development

The fertilised eggs were spherical in shape and adhesive; each egg contained one large oil globule and yolk mass with clear cytoplasm. The chorion was transparent and the egg could be seen through the shell. A border between the animal pole and vegetal pole developed due to the accumulation of cytoplasm at the animal pole, when the eggs attached to the substrate. The perivitelline space was small yet transparent, especially at the animal pole. The blastodisc was barely seen during this stage. On the 2nd day cleavage completely stopped and oil globules moved to the opposite end from the animal pole. Pigmentation started to appear on the yolk with the prominence of stellate melanophore. Head demarcation, brain formation and tail were clearly visible. On the 3rd day the embryo showed clearly visible head, mytosomes and tail. The entire body of the embryo was covered with the melanophores. The mouth, eye cup and the lenses were evident. The body was transparent with no muscular structure. The head and tail of the embryo had distinctly separated from the yolk. The eye started to develop with pigments and the heart became visible. The yolk volume was found to reduce and the pigments on the head and the yolk regions increased. The heart started beating and blood circulation was observed within the whole body. On the 4th day, the tail became separated from the yolk and moved freely but the body was still attached to the yolk. The head of the embryo increased in size showing prominent eyes and brown pigmentation. The caudal and anal fins started to develop. The embryo was growing larger and showed movements. On the 5th day the mouth and eye were evident, blood was circulating through vessels and the size of the yolk sac reduced. Microscopic observations were made to understand the utilisation of yolk and the movement of embryo inside the egg capsule. On the 6th day, the embryo further enlarged and occupied most of the space in the capsule. Gill rakers and muscles developed, gut cavity was very clear as also a prominent eye with pigmentation and silvery colour. Fins were fully developed and clearly visible. Head was now almost one third of the egg capsule. On the 7th day, the yolk sac became quite small and the capsule was fully occupied by the embryo. Fins and eyes were well developed and the egg clutch turned silvery in colour. The eyes were shining, rotating and showed jerking movements. Glowing and shining eye is an indication that they are about to hatch. The embryo began to hatch by vigorously moving inside the eggs to break the capsule. At about 19.00 to 20.00 hrs (155 h after fertilisation) of the same day, the embryos

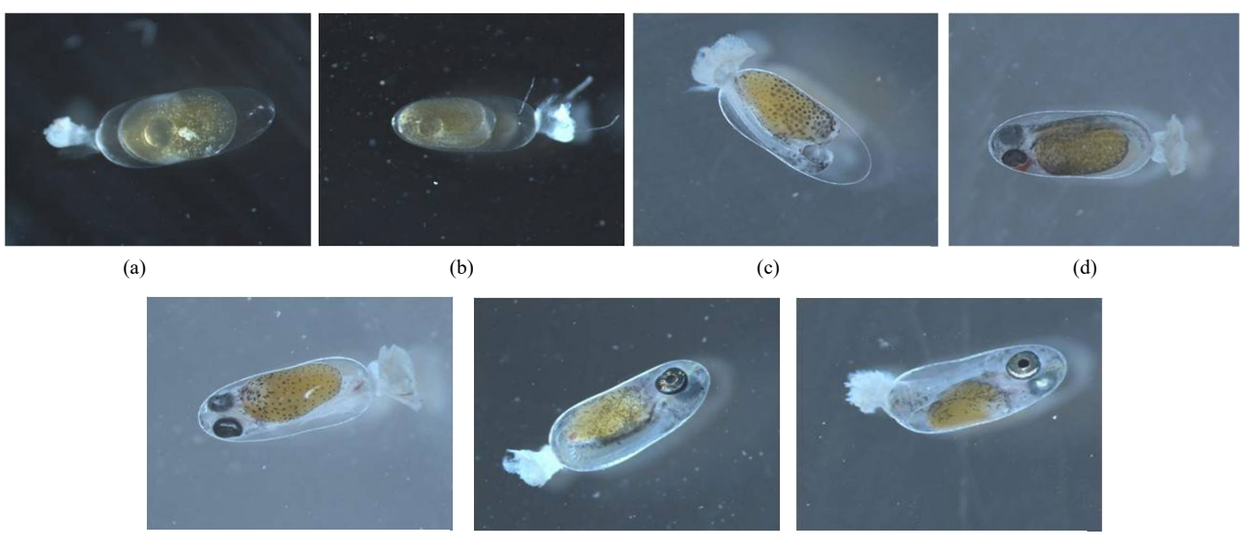

Fig. 1. Embryonic development of black *A. ocellaris* at different days after spawning. (a) Day 1; (b) Day 2; (c) Day 3; (d) Day 4; (e) Day 5; (f) Day 6 and (g) Day 7

hatched free from the capsules and became larvae. The embryonic period was characterised by the exclusive utilisation of endogenous nutrition from yolk.

Larval development and survival

Newly hatched larvae were very active, photopositive and remained at the bottom of the tank for a few seconds before becoming free swimming. After hatching, photoperiod of 24 h light was maintained for 20 days for better survival and growth. The larvae measured about 3 to 4.5 mm in total length with a small yolk sac, transparent body and single fin fold; the mouth size was about 160 to 200 µm. Yolk sac got absorbed within 5 to 7 h post-hatch. On 1 dph, the total length of the larvae ranged from 4.84 to 5.27 mm, with an average of 5.00±0.16 mm and scattered melanophores were observed on the body. Its intensity increased both on the line along the vertebrae and near the gut area. Well developed sensory and olfactory systems enabled the larva to detect and ingest exogenous food after hatching. On 5 dph, the larvae measured 7.12 to 7.41 mm with an average total length of 7.26±0.11 mm. Pigmentation started prominently after 5 dph, fin fold gradually disappeared and separate anal, dorsal and pelvic fin rays were seen. On 8 dph, one white band started developing in the opercular region; the larvae were predominantly orange in colour and were actively swimming in the water column. On 10 dph, they had one anterior white band near to the eye and a second small white band in middle of body just after the dorsal fin, later the bands became prominent. The pectoral, pelvic, anal and dorsal fins were clearly visible with distinct fin rays. On 10 dph, average total length of larvae was about 9.71±0.41 mm. On 15 dph, fins were distinct, anterior portion of the body looked light orange to yellowish and had 2 clear white bands. The average total length of the larvae was about 10.75±0.16 mm and the size of the mouth opening ranged from 920 to 960 µm. The larvae started changing habitat from pelagic to benthic after 15 dph and the juvelines started expressing the black colouration on the body; white bands became prominent in the opercular and the middle part of the body. Black colouration started appearing on the pelvic fins and the orange colouration began to spread in the lower part of the dorsal and anal fins. On 20 dph larvae, the 3rd white band became visible, black pigmentation started and it extended posteriorly. Mouth, opercular region, part of the anal fins and dorsal fins had orange colouration. On 25 dph larvae, the total length measured about 14.04 to 14.23 mm with an average of 14.18±0.10 mm. All the three white bands slowly broadened with the growth of the fish and the body colour of the fish started changing from orange to black. On 35 dph, average total length of larvae was about 17.45±0.11 mm and all the three white stripes were completely visible. On 50 dph, the juveniles measured 20.51-21.2 mm TL. The body colouration gradually changed from orange to black except in the mouth region. All the juveniles together formed a shoal in the tank and attained marketable size by the end of the 2nd month of rearing. On 90 dph, the juveniles were 30-33 mm TL with an average length of 31.84±1.07 mm and had a faint orange colour on the face. The observations in this study clearly indicated that black ocellaris begin their life as orange coloured and switch to black colour as they mature. It took roughly a year to gradually develop to their final jet-black colouration. The larval development of *A. ocellaris* is depicted in Fig. 2. Morphometric data of hatchery reared *A. ocellaris* is given in Table 2 and growth in terms of total length is graphically represented in Fig. 3.

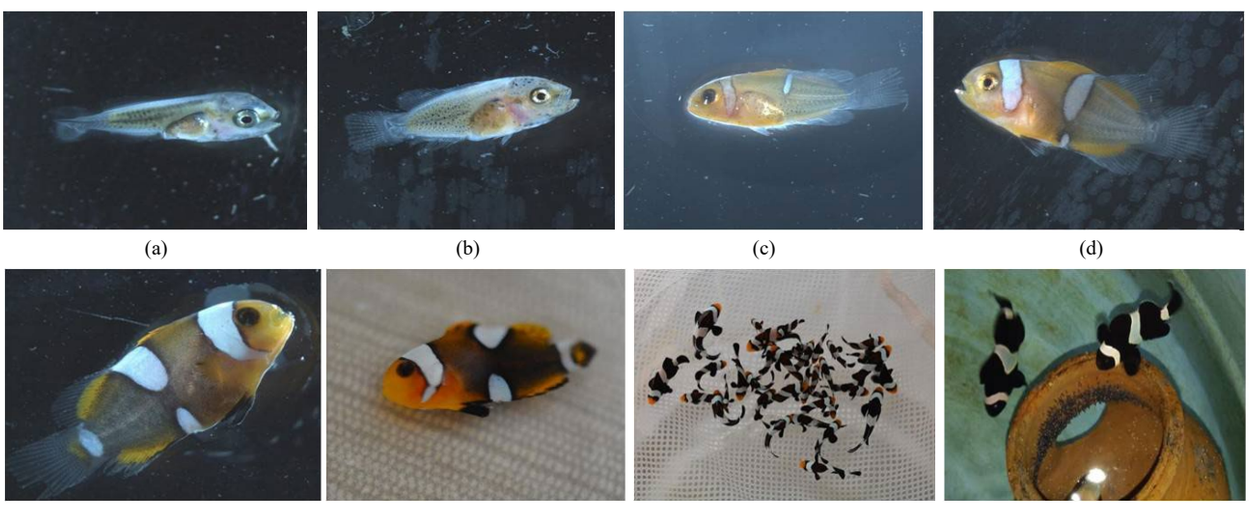

Fig. 2. Larval development of black *A. ocellaris* after hatching. (a) 1st day post hatch (1 dph); (b) 5 dph; (c) 10 dph; (d) 15 dph; (e) 25 dph; (f) 35 dph; (g) 90 dph and (h) Brood pair guarding the eggs

Table 2. Morphometric data of hatchery reared black *A. ocellaris*

Days post hatch (dph)	Total length (mm)	Standard length (mm)	Head length (mm)	Head width (mm)	Body width (mm)	Eye diameter (mm)
4.71±0.10	5.00±0.16	4.71±0.10	1.35±0.04	1.22±0.07	0.09±0.07	0.49±0.04
Range	4.84-5.27	4.61-4.87	1.30-1.42	1.11-1.34	0.98-1.19	0.47-0.58
5	7.26±0.11	6.06±0.15	1.99±0.11	1.89±0.05	2.14±0.10	0.69±0.01
Range	7.12-7.41	5.90-6.30	1.90-2.20	1.85-1.93	0.09±0.07	0.49±0.04
10	9.71±0.41	7.85±0.25	2.71±0.14	2.54±0.11	3.00±0.19	0.78±0.02
Range	9.18-10.23	7.46-8.09	2.5-2.80	2.41-2.69	2.82-3.30	0.76-0.81
15	10.75±0.16	8.65±0.27	3.18±0.12	3.00±0.15	3.35±0.16	0.83±0.17
Range	10.53-10.97	8.25-8.94	3.01-3.34	2.76-3.15	3.16-357	0.81-0.85
20	12.42±0.29	9.48±0.14	3.47±0.11	3.24±0.06	4.39±0.20	0.95±0.01
Range	12.06-12.72	9.34-9.67	3.39-3.65	3.18-3.35	4.04-4.58	0.93-0.96
25	14.18±0.10	11.34±0.12	3.66±0.11	3.49±0.08	4.9±0.13	0.97±0.01
Range	14.04-14.23	11.18-11.51	3.49-3.79	3.4-3.63	4.68-5.03	0.95-0.99
30	16.17±0.29	13.5±0.26	4.28±0.14	4.04±0.13	5.07±0.19	1.61±0.13
Range	15.83-16.53	13.24-13.94	4.13-4.5	3.89-4.21	4.89-5.34	1.49-1.84
35	17.45±0.11	15.04±0.15	4.55±0.172	4.2±0.08	5.84±0.10	1.85±0.07
Range	17.29-17.59	14.92-15.3	4.38-4.78	4.12-4.34	5.71-6.00	1.78-1.95
40	18.87±0.07	16.32±0.12	5.16±0.05	4.74±0.05	6.45±0.06	2.08±0.08
Range	18.79-18.98	16.2-16.52	5.11-5.25	4.69-4.82	6.37-6.53	1.99-2.17
50	20.82±0.26	18.81±0.32	5.45±0.04	5.11±0.05	7.36±0.04	2.28±0.08
Range	20.51-21.2	18.57-19.36	5.39-5.50	5.03-5.19	7.29-7.41	2.19-2.38
60	23.69±0.31	21.66±0.41	5.79±0.09	6.1±0.11	7.89±0.05	2.49±0.03
Range	23.21-24.0	21.16-22.13	5.69-5.91	5.03-5.19	7.84-7.96	2.45-2.53
70	26.22±0.15	24.23±0.18	6.43±0.04	6.74±0.06	8.48±0.05	2.64±0.029
Range	26.00-26.41	24.00-24.49	6.39-6.50	6.67-6.82	8.42-8.53	2.60-2.67
80	28.32±0.08	26.37±0.07	7.27±0.02	7.45±0.05	9.42±0.06	2.81±0.03
Range	28.20-28.43	26.30-26.49	7.24-7.30	7.39-7.52	9.34-9.50	2.77-2.85
90	31.84±1.07	29.92±1.03	8.14±0.05	8.26±0.03	10.37±0.05	2.9±0.02
Range	30.20-33.0	28.4-31.1	8.09-8.2	8.23-8.3	10.31-10.45	2.88-2.95
100	34.5±2.57	32.45±2.35	8.67±0.08	9.36±0.08	11.86±0.09	3.07±0.10
Range	30.3-37.0	28.93-35.12	8.59-8.8	9.29-9.5	11.7-11.96	2.96-3.21

Feeding experiment

In treatment I with copepod *A. southwelli* nauplii as initial feed, the larvae had better survival and faster growth as compared the other two treatments. In treatment II, with enriched rotifer *B. plicatilis* as first feed, the larvae showed considerably lower survival as compared to Treatment 1

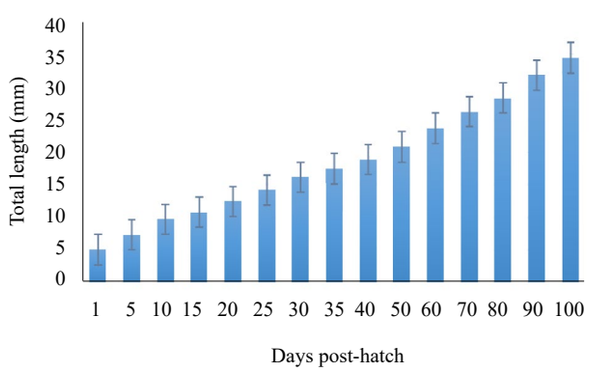

Fig. 3. Growth in terms of total length of hatchery produced black *A. ocellaris*

and there was no significant difference in growth rates, where higher mortality rates were observed on day 7 and 11 dph. In Treatment III, larvae fed with unenriched rotifers showed higher mortality while the growth rate was similar to that in Treatment II. Better melanophore formation and pigmentation on the larval body was quite evident when larvae were fed with *A. southwelli* nauplii. The larval survival rate in the feeding experiment at the end of 60 dph with different live feeds was 73, 55 and 40% for *A. southwelli* nauplii, enriched rotifer *B. plicatilis* and unenriched rotifers respectively. So, the feeding regime or protocol followed in Treatment I is better for larval rearing of black *A. ocellaris.* Percentage of survival for 25 days of larval rearing is shown in Fig. 4. On one dph, 95% survival was observed and larval mortality rates were observed till 18 dph. There was no mortality afterwards and average percentage survival till 25 dph was 60%.

Discussion

Clownfishes usually live in groups consisting of a monogamous breeding pair and a few juveniles (Allen

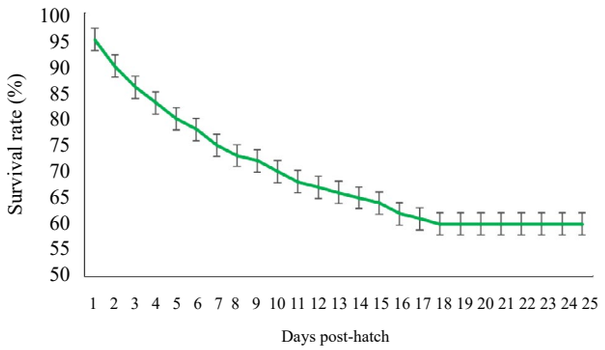

Fig. 4. Survival rate of black *A. ocellaris* during larval rearing

1975; Fricke 1974; 1979, Fricke and Fricke 1977; Moyer and Nakazono, 1978; Ross, 1978; Hattori, 1991). They are protandrous, monogamous and sex change seems to be controlled socially (Moyer and Nakazono, 1978; Hattori, 1991). It has been suggested that sequential hermaphroditism in reef habitats improves adaptation, increases survival rates and enhances reproduction (Fricke and Fricke, 1977). In the present study, for broodstock maturation and breeding, a pair of fishes, where the larger one was female, was maintained. Successful breeding and larval rearing of fishes depends on the nutritional quality of broodstock diet and larval feeds, in addition to water quality. Broodstock diet is one of the key factors which determines gonadal maturation, successful spawning, larval growth and survival. In the present study, brood fishes were fed a combination of boiled mussel meat, boiled squid and semi-moist feed twice daily at a rate of 3-5% of their body weight, for better growth and condition. If the broodstock fish are not duly fed, the results are usually directly reflected in the number of eggs laid, fertilisation rate, hatch rate and the quality of hatched larvae. After 1 year and 9 months of rearing, the fishes laid eggs on earthen pots provided in the tank in the morning hours. Normal *A. ocellaris* spawns much early in their life (Madhu *et al.,* 2012). The present sudy indicates slightly delayed spawning activity in black ocellaris. Reports of Alava and Gomes (1989) regarding *A. clarkii* and Malpass (1996) in the case of *A. percula* revealed that the eggs were laid always on clean and stable substratum during morning hours in contrast to spawning during evening hours as in the case of pelagic spawners like *Lethrinus lentjan* (Anil *et al.*, 2019). The placement of the sea anemone *H. magnifica* in the broodstock tank ensured the added benefit of compounds released by the anemone that help to protect the eggs or even chemically induce immunity that clownish gain through their symbiotic relationship with the anemone (Madhu *et al*., 2012). In the present study, photoperiod of 12 h light and 12 h dark were maintained with light intensity of 2000-5000 lux, using the natural lighting available in the hatchery. It is reported that clownfish species occurring in tropical waters spawn throughout the year (Allen, 1972; Ross, 1978; Alava and Gomes, 1989; Hoff, 1996). By the simulated temperatures and lighting regimes during broodstock rearing, the reproductive activity can be manipulated (Kohler *et al*., 1994). In the present study, black *A. ocellaris* was found to breed twice in a month under captive conditions, with a fecundity of 200 to 300 number per spawning.

Observations on egg colouration and embryoic development in back *A. ocellaris* in the present study are similar to that reported in *Amphiprion ephippium* (Krishna *et al*., 2018), *Amphiprion nigripes* (Anil *et al*., 2012), *P. biaculatus* (Madhu *et al.*, 2012) and *A. ocellaris* (Rema *et al.*, 2012). However, hatching in these species was reported on the 6th day of incubation while in the present study, the fertilised eggs hatched within 7-8 days, soon after sunset. Larvae starts changing from pelagic to benthic from 15 dph onwards. In false clown *A. ocellaris*, the larvae metamorphosed to juveniles during 15 to 17 dph and settled in the sea anemones on 17 to 20 dph (Madhu *et al*., 2012).

The successful larviculture of marine finfishes depend chiefly on the appropriate size and nutritional quality of live feeds. Better result in terms of growth and survival was observed in copepod fed larvae when compared with rotifer and enriched rotifer fed larvae. Copepods become inevitable in a marine hatchery because they are the only suitably sized prey for small larvae of many ornamental fish species and the only live feed that supports the rearing of many species which have altricial type of larvae. Copepod nauplii offer a diverse size spectra and nutrition that can meet the specialised needs of small and fast growing fish larvae. The high eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (ARA) contents of copepods also facilitate larval survival and growth (Ritesh *et al.*, 2017; Gomathi *et al.*, 2020). In the present study, naupliar stage of copepod *A. southwelli* measures about 50 to 60 µm which is well accepted by black *A. ocellaris* larvae having initial mouth size of 160 µm. A number of studies have shown that the inclusion of copepod nauplii into the early larval diet significantly improves the survival and growth of groupers (Hussain and Higuchi, 1980; Toledo *et al*., 1999) and snappers (Singhagraiwan and Doi, 1993). Experiments with larvae of the striped trumpeter *Latris lineata* showed that the use of copepods improved survival (Morehead *et al*., 2005). *Lutjanus campechanus* larvae fed with nauplii of *Parvocalanus* sp. exhibited significantly greater survival to day 7 after hatching (50.3%) and were larger in size (Shields *et al.,* 2005). Survival of seabass larvae fed with *Acartia clausi* was the highest (58.13%) against 39.93%

and 41.62% in larvae fed with rotifer and *Artemia* nauplii respectively (Rajkumar and Kumaraguru, 2006). As per the earlier studies, marine copepods like *Tigriopus* sp. are rich in highly unsaturated fatty acids (HUFA) content as compared to other live feeds (Watanabe, 1993). Better survival of the larvae of *Pseudanthias marcia* (Anil *et al.*, 2018), *L. lentjan* (Anil *et al.*, 2019), *Ephinephelus coioides* (Rithesh *et al.*, 2018) and *Dascyllus carneus* (Anzeer *et al.,* 2019) were also reported with copepods as the first feed. Copepods have high protein content (44-52%) and a good amino acid profile with the exception of methionine and histidine and also contain high level of digestive enzymes, which may play an important role in larval nutrition (Delbare *et al.,* 1996).

Marine ornamentals are always a visual treat in aquaria. They attracted the attention of researchers due to overexploitation and their threatened status in the wild. Breeding of marine ornamentals started worldwide by hobbyist breeders, which had a positive influence on the sustainability of the resources. Black ocellaris has great demand in the domestic and international market due to its rarity in most areas. This is a highly priced clownfish and can be crossbred with normal orange coloured *A. ocellaris* to produce different colour variants. To increase the production, trade and sustainability of this resource, standardisation of breeding and larval rearing protocols is of utmost importance and the present study provides valuable information for developing these protocols.

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