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# Seed production and growth of *Neopomacentrus cyanomos* (Bleeker, 1856) in captivity

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## ABSTRACT

Development of the regal demoiselle *Neopomacentrus cyanomos* (Bleeker, 1856) from egg to maturation and spawning stage is described using hatchery reared specimens. Larval rearing of *N. cyanomos* was carried out using zooplankton as the starting feed up to the 10th day post-hatch (dph). Larval and post-larval growth was studied for a period of 340 dph. Caudal fin rays began to develop from 8th dph and the larval body depth increased considerably from 9th dph onwards. Towards the 10th dph, at about 5.7 mm total length (TL) half of the specimens underwent notochord flexion. Larvae exhibited decreased transparency with increased pigmentation of the pre-anal body, characterised by presence of stellate melanophores. Towards 15th dph, the pectoral, pelvic, dorsal, anal and caudal fins were visible with fin rays. The soft dorsal fin started showing pigmentation from 20th dph onwards and the spinous dorsal from 30th dph onwards. Towards 30th day, black pigments were found distributed all over the body. Pigmentation steadily increased from 30th day onwards and the juveniles fully attained the adult pattern of body colouration by about 90-100 days. First spawning occurred on the 340th dph at a size of 64-73 mm TL.

Keywords: Damselfish, Larval rearing, Live feed, *Neopomacentrus cyanomos,* Regal demoiselle

## Introduction

The family Pomacentridae currently comprises 399 species in 28 genera (Eschmeyer and Fong, 2016) and occupies an important position in the marine ornamental fish trade as it accounts for the second largest marine ornamental fish import in the United States (Rhyne *et al*., 2012). Since these fishes have great demand in aquarium trade, attempts have been made to breed them in captivity. The pomacentrid regal demoiselle *Neopomacentrus cyanomos* (Bleeker, 1856), belongs to the subfamily Pomacentrinae. Many morphological and behavioural characteristics show a range of variability within the large (>300 spp.) Pomacentrid family (Allen, 1975; Leis and Rennis, 1983; Thresher, 1984; Kavanagh *et al*., 2003). Most damselfishes have demersal eggs and pelagic larvae (Breder and Rosen, 1966; Leis and Rennis, 1983) and generally short duration of planktonic phase of 2 to 5 weeks (Wellington and Victor, 1989; Kavanagh and Alford, 2003). Since the larval stages of most pomacentrids differ drastically from the adults, study of the larval development is very important for understanding their life history. Gopakumar *et al.* (2009) successfully bred the sapphire devil, *Chrysiptera cyanea* under captive conditions. Though successful breeding of *N. cyanomos* was achieved by Setu *et al.* (2010), detailed information on larval rearing and development of juveniles of *N. cyanomos* in captivity is very meagre and no published information is available on growth of hatchery produced larvae up to maturation and spawning stage of any damsel fish. The present study was carried out to throw light on the larval development and growth up to spawning of hatchery produced larvae of *N. cyanomos*.

## Materials and methods

### *Broodstock development*

Eight numbers of adult *N. cyanomos* (6.5-10 cm length) captured from Vizhinjam along the south-west coast of India by skin diving using scoop net, were transported to the hatchery of Vizhinjam Research Centre of ICAR-Central Marine Fisheries Research Institute, in jerry cans of 20 l capacity in aerated seawater using battery operated aerators. The fishes were stocked in rectangular FRP tank of 500 l capacity provided with biological filter. Uneaten food was siphoned out daily and the water was then replaced with fresh seawater and in addition, seawater was exchanged at the rate of 25% once in a week. Nitrate level of less than 30 ppm, nitrite and ammonia levels of less than 0.01 ppm were maintained in the tanks. Temperature, salinity and pH in the breeding tanks were maintained at 27±02°C, 30-32 ppt and 8 to 8.2 respectively (Anil *et al.,* 2012). Water quality parameters in the breeding tank during the rearing period were estimated

following standard methods. Salinity was measured with a refractometer (ATAGO, Japan) and pH with pH meter (Eutech Instruments, Singapore). Dissolved oxygen $NO_2$, $NO_3$ and $NH_4$ were estimated as per Strickland and Parsons (1972). The fishes were fed 4 times a day as per the schedule: pellet feed at 10.00 hrs; boiled mussel meat at 12.00 hrs and 14.00 hrs and *Artemia* nauplii at 16.00 hrs. The tank was provided with earthen pots and tiles as substratum for laying eggs.

*Live feed culture*

Live feed such as phytoplankton, marine zooplankton including copepods, *Artemia* and mussel larvae were used for the larval rearing experiments. For feeding copepod culture and for maintaining green water in the rearing tanks, stock culture of algae *viz., Nannochloropsis oculata* and *Isochrysis galbana* were maintained in stock culture room at 24°C in 1 to 4 l flasks and then the cultures were upscaled to 20 l carboys for feeding. The copepod *Pseudodiaptomus serricaudatus* was cultured using *N. oculata* and *I. galbana.* In addition to this, marine plankton was collected from the wild using plankton net (200 µ mesh size). The collected samples were then segregated by passing through a sieve of mesh size 500 µ and then through sieve of 20 µ and the organisms which pass through 500 µ and retained in 20 µ were collected and given to the larvae on the 2nd day post-hatch (dph), when the larvae started feeding. Mature mussels were collected from mussel farm and kept ready for artificial spawning so that morula/trochophore stage larvae could be fed to the larvae from 2 dph onwards. Mussels were induced to spawn by thermal stimulation (Loosanoff and Davis, 1963). Four treatments were tried for rearing the larvae and about 400 damsel fish larvae were used for each treatment in a tank holding 500 l of seawater.

All the experiments were conducted adopting green water technique. The cell counts of the green water employed for the experiment ranged from $1.4\times10^5$ - $9\times10^6$ $ml^{-1}$. In the first treatment (T1), the tank contained. *P. serricaudatus* in addition to algae *N. oculata* and *I. galbana*. The number of egg bearing copepod adults and nauplii per 50 ml was maintained at about 26 and 88 respectively. The second treatment tank (T2) contained algae, *N. oculata* as well as *I. galbana* and copepods ranging from 20 - 200 µ in size *viz*., *Acartia spinicauda* (2-3 nos. 10 $ml^{-1}$), *P. serricaudatus* (5 nos. 10 $ml^{-1}$), *Temora turbinata* (4 nos. 10 $ml^{-1}$) and zoea (2 nos. 10 $ml^{-1}$). In the third treatment (T3), morula stage mussel larvae were added to the tank on the second day in such a way that the tank contained morula larvae at the rate of 10 $ml^{-1}$. The 4th treatment (T4) which served as the control contained only algae, *N. oculata* and *I. galbana*.

## Results

*Spawning*

Fishes started spawning after 45 days of stocking in the broodstock tanks and spawning took place in the morning hours (07.00-10.00 hrs). The eggs were capsule-shaped and were found attached to the substratum. Sometimes the fishes were observed to lay eggs on the filters and even on the floor and the sides of the tank. *N. cyanomos* is sexually monomorphic and the male parent exhibited high degree of parental care for the eggs (fanning the eggs with the pectoral and caudal fins, removing the infected eggs with the mouth) (Fig.1a). Since many mature fishes were present in the same broodstock tank, the eggs deposited belonged to different fishes and hence the substratum contained eggs at different stages of incubation. Sometimes two or more clutches were found attached very close to each other. The eggs measured 1.1-1.56 mm in length and 0.5- 0.546 mm in width. Spawning frequency was observed to be 3 - 4 times per month with an interval of about 5 - 8 days. Gradually the frequency increased to 5 - 6 per month with an interval of 4 days. The number of eggs in each clutch ranged from 1536 -1872. Hatching took place in the early evening hours at about 19.00 -19.30 hrs. The larvae were collected from the broodstock tank and transferred to the larval rearing tanks with the help of a small trough by concentrating the larvae to one area using a torch light as the larvae are photopositive in nature.

*Incubation and development*

Hatching occurred on the 3rd day of incubation, sometimes it progressed to the fourth day. Egg dimensions were unchanged during development. Eggs usually contained single oil droplet, but in some cases multiple oil droplets (Fig. 1b). On the first day, the yolk occupied ¾th of the egg, towards the evening the cytoplasm at the apical region divided to form a blastodisc and as the division progressed the yolk was engulfed by the blastodisc (Fig. 1c). On the second day, the optic vesicle and tail were clearly visible with no sign of eye pigmentation. Yolk occupied $^1/_3{}^{rd}$ of the egg, with a slight yellowish pigmentation and melanophores were found all over the body (Fig.1d). Head contained stellate melanophores and the larval body showed signs of movement. By 3rd day, the yolk sac decreased in size and 3 large stellate melanophores were seen above the stomach. Yellowish colouration was observed above the gut. Eyes were prominent with a bluish tinge, with enlarged head and the body of the embryo occupied almost whole of the capsule. Primordial fin margins appeared and the melanophore pattern changed in such a way that it resembles that of a newly hatched larva (Fig. 1e).

In treatment T1 in which copepods were used, the larvae survived only up to 5 days. Though some larvae were found to feed on copepods as revealed during microscopic

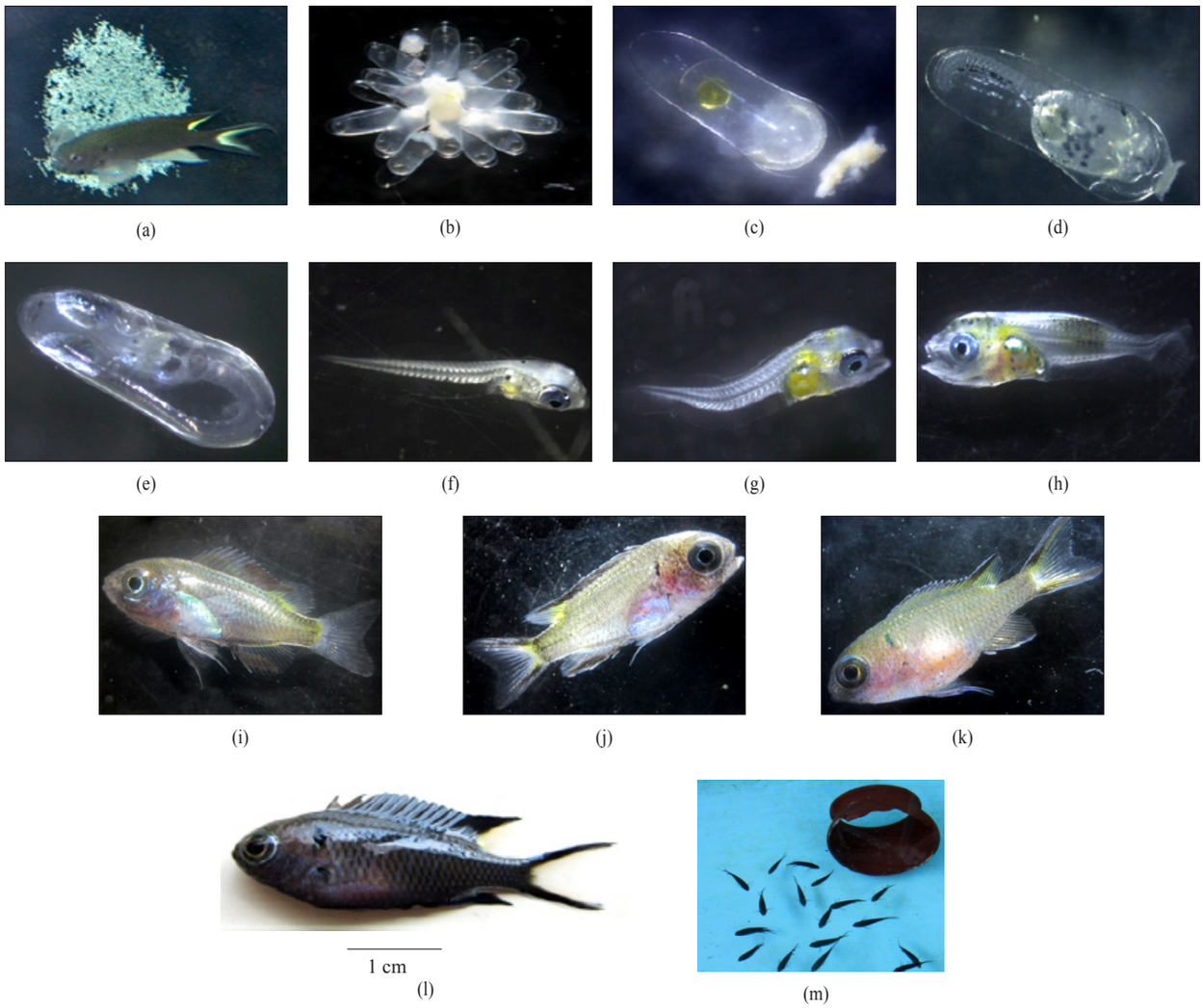


Fig. 1. Developmental stages of *N. cyanomos* under captive conditions (a): Male *N. cyanomos* guarding and aerating eggs, (b): Bunch of freshly laid eggs, (c): 1st day egg, (d): 2nd day egg, (e): 3rd day egg, (f): 1 day old larva, (g): 5 day old larva, (h): 10 day old larva, (i): 30 day old larva, (j): 60 day old juvenile, (k): 75 day old juvenile, (l): 120 day old fish, (m): 300 day old fishes

observation of stomach, no survival was recorded in the tank from the 5th day onwards. The larvae were swimming actively for 3 days but mortality was noticed from the 4th day onwards. In T2, larvae were fed exclusively on zoea and nauplii of copepods *viz., A. spinicauda, P. serricaudatus* and *T. turbinata*. The larvae were found healthy and showed active swimming movements. Though the larvae were seen spread out in the upper region of the water column, their concentration was more near the sides of the tank. This may be due to the high concentration of copepod nauplii near the sides of the tank. High larval survival rate was noted in this treatment and digested remains of copepod nauplii were obtained from the gut of *N. cyanomos*. In the third tank (T3), the larvae were fed on mussel larvae and they survived only for about 3 days and mussel larvae were obtained from the stomach. The control treatment (T4) in which only green water was added, larvae survived only up to 3 days and the stomach was found empty.

*Larval development*

On the first dph, the larvae measured about 2.3-2.6 mm in standard length (SL) (Fig. 1f). Yolk sac was observed to be almost exhausted. Larval body was transparent and had a well developed mouth and pigmented eyes. Eyes were metallic blue in colour. Two stellate melanophores were present on the head. About 13 branched melanophores were present along the ventral midline of the body, the area above the gut contained punctate-reticulate melanophores, gut possessed a light greenish colour. Fin primordia were clearly visible. The

larvae were altricial and possessed a small mouth gape of less than 300 µ. The morphometric measurements are given in Table 1a and b.

Body proportions and pigmentation changed dramatically by 5th dph. Pigmentation of the ventral midline reduced. Caudal fin began to separate from rest of the body. Except for the yellow colour of the gut and slight yellowish tinge on head, the rest of the body was transparent (Fig. 1g). Drastic changes were observed in the larval body by 8th day, the conjoined fins began to separate and caudal rays were visible with opercular region having slight pinkish colouration. Melanophores were present at the region where the dorsal fin joins the body. Black pigment granules appeared in the middle of the body and stellate melanophores started appearing over the dorsal side of the head. By 9th day, larval body increased in width with caudal, dorsal and anal fins very distinct. Except the increase in the number of melanophores, the pigmentation pattern remained more or less the same.

On the 10th day, half of the specimens were in preflexion and half underwent notochord flexion with major changes in body proportions. Pigmentation of deep preanal body was characterised by extensive scattered stellate melanophores. Pigmentation on stomach, operculum and pelvic fins had no noticeable change from the previous stage. More caudal rays developed and the larva showed increased pigmentation and decreased transparency (Fig.1h). A slight yellowish colour was observed in the areas of the body where the melanophores were present.

Larvae developed into sub-adult fishes by 15th day with fully developed fins and fin rays. Pectoral, pelvic, soft and spinous dorsal, anal and caudal fins were visible with fin rays. Pigment granules were found distributed all over the body with transparent snout. Different types of melanophores were present in different parts of the body. Stellate and stellate reticulate granules were present above the eyes. On the snout, stellate reticulate granules were present, stellate granules were also present on the upper jaw. At the point where the caudal peduncle joins the body, stellate melanophores were present which changed into punctate melanophores towards beginning of the anal fin. Elongated punctate melanophores were present over the gut.

On the 20th day, the larval body was yellowish in colour with black pigmentation at the centre of the dorsal fin. Stellate-reticulate melanophores were present on the head. Stellate melanophores were present on the tail which extended to the two sides of the body. Punctate melanophores were present in the middle part of the body.

Black pigmentation started appearing in the middle of dorsal fin on the 25th day which was noticed at the beginning of the anal fin and black pigment granules were noticed in the body. Upper part of the body as well as the region between the dorsal fin and body showed yellowish colouration while towards the caudal region greenish colouration was noticed. Larval body was golden coloured on the 30th day and black pigment granules were distributed all over the body. Greenish colouration was observed at the beginning of the caudal fin and also between the body and the soft dorsal. Black pigmentation and greenish pigment granules were present on the soft dorsal (Fig.1i). Eyes appeared golden blue in colour with anal and pectoral fin transparent. Tiny spines were visible on the upper half of the body below pectoral fins. By the 45th day, body was slight yellowish in colour and the yellow colour was prominent in the region where the caudal fin joins the body. The spinous rays of dorsal fin were black in colour and the posterior 6 were transparent. Anal fins were transparent but black colouration started appearing in the middle.

On the 60th day, pigment granules were present all over the body. Pectoral fins transparent (Fig. 1j), caudal fin transparent with black pigmentation on both sides, caudal rays 27 and soft dorsal black coloured at the beginning with yellowish tinge at the tips. Lower part of the head below the eye had assumed a bluish colouration by 75th day. Blue pigment granules spread from the gut to the middle part of the anal fin. 1st and 2nd anal spines showed bluish colouration. Dorsal spines with bluish pigmentation towards the middle, in the area where the spinous dorsal joins the body (Fig.1k). Pelvic fins with blue pigment granules, pectoral fins also transparent and eyes yellowish in colouration.

By 90th day, body had slight bluish colour and tips of the scales were dark yellowish. A greenish black dot was observed on the end of the operculum above the pelvic fin. Upper part of the body and spinous dorsal were yellowish grey in colour. Anal fin transparent with blue pigment granules. Pelvic fin was violet coloured on the outer side and transparent with violet pigmentation on the inner region.

Body was greyish black in colour on the 120th day, and a slight yellow colour was observed in the middle region. Pre-opercle had a slight bluish tinge, snout black in colour and slight yellow colour noticed in the middle of the body extending up to the caudal peduncle. Spinous dorsal completely black, anal fin black with blue pigment granules unevenly distributed (Fig. 1l). Black and blue pigment granules noticed in pelvic rays and in the middle part of caudal rays with the proximal part yellow and distal part colourless. The fish weighed about 0.45 g at this stage with a mouth gape of about 0.9 mm and 300 days old fishes are shown in Fig.1m.

Growth progression of the hatchery produced larvae of *N. cyanomos* is given in the Table 1. They grew to an average total length of 68 mm in 340 days (Fig. 2). Growth in length was slow in first 30 days and sharp increment was

Table 1a. Morphometric data (Mean ± SD) of hatchery reared *Neopomacentrus cyanomos*

| Days post-hatch (dph) | Total length (mm) | Standard length (mm) | Head length (mm) | Head width (mm) | Body width (mm) | Eye diameter (mm) |
|---|---|---|---|---|---|---|
| 1 | 2.44±0.11 | 2.28±0.16 | 0.55±0.05 | 0.52±0.08 | 0.2 | 0.24±0.05 |
| 2 | 2.52±0.08 | 2.36±0.13 | 0.62±0.08 | 0.44±0.05 | 0.24±0.05 | 0.24±0.05 |
| 5 | 3.1±0.12 | 2.9±0.12 | 0.82±0.08 | 0.62±0.08 | 0.73±0.07 | 0.31±0.04 |
| 10 | 5.7±0.10 | 4.78±0.20 | 1.5±0.10 | 1.52±0.04 | 1.45±0.05 | 0.65±0.05 |
| 15 | 8.18±0.29 | 6.56±0.25 | 2.26±0.25 | 2.16±0.25 | 2.44±0.21 | 1±0.12 |
| 20 | 10.76±0.81 | 8.12±0.36 | 2.98±0.20 | 2.66±0.15 | 2.76±0.22 | 1.22±0.08 |
| 25 | 13.96±0.61 | 11.14±0.47 | 3.82±0.20 | 3.4±0.37 | 3.66±0.22 | 1.46±0.09 |
| 30 | 18.6±0.82 | 14.6±0.82 | 5.04±0.21 | 4.42±0.30 | 4.6±0.33 | 1.8±0.20 |
| 45 | 20±1.22 | 17±1.22 | 5.38±0.16 | 5.84±0.22 | 5.6±0.27 | 2.18±0.16 |
| 60 | 21.1±1.14 | 17.8±0.76 | 5.6±0.19 | 6.04±0.29 | 6.28±0.57 | 2.32±0.16 |
| 75 | 23.4±0.82 | 19.4±0.82 | 6.08±0.16 | 6.58±0.22 | 7.46±0.21 | 2.72±0.08 |
| 90 | 26±1.00 | 21.8±2.05 | 6.68±0.16 | 7.44±0.13 | 8.36±0.15 | 2.78±0.26 |
| 120 | 31.82±1.66 | 25.24±1.61 | 6.94±0.13 | 7.98±0.08 | 8.92±0.22 | 2.96±0.17 |
| 180 | 46.2±2.2 | 33±3.4 | 9.46±0.4 | 9.98±0.9 | 11.34±0.6 | 3.22±0.2 |
| 240 | 53.8±4.7 | 39.6±2.1 | 11.4±0.9 | 11±1.2 | 14.3±1.5 | 4.32±0.6 |
| 300 | 61.8±6.9 | 44.2±3.8 | 13.3±0.8 | 14.9±0.9 | 18±1.0 | 4.8±0.6 |
| 340 | 68±3.4 | 48.4±2.5 | 15±1.2 | 16.2±1.5 | 19.6±2.1 | 5.6±0.5 |

Table 1b. Morphometric data (Mean±SD) of hatchery reared *Neopomacentrus cyanomos*

| Days post-hatch (dph) | Dorsal fin base length (mm) | Pectoral fin length (mm) | Pelvic fin length (mm) | Anal fin length (mm) | Caudal peduncle depth (mm) |
|---|---|---|---|---|---|
| 75 | 6.02±0.3 | 3.36±0.4 | 3.62±0.4 | 2.68±0.1 | 1.48±0.2 |
| 90 | 10.66±0.8 | 4.28±0.3 | 6.38±0.2 | 3.74±0.2 | 2.52±0.1 |
| 120 | 12.62±0.4 | 6.84±0.3 | 6.78±0.3 | 5.06±0.4 | 3.24±0.3 |

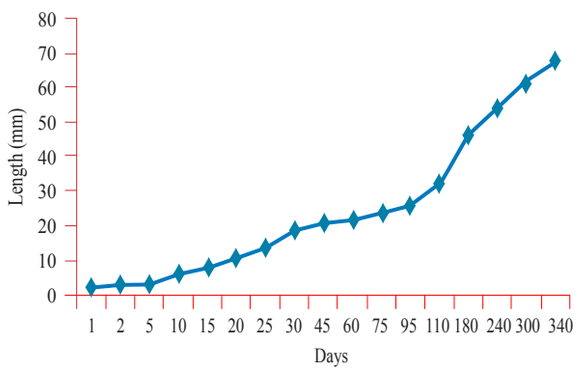


Fig. 2. Growth in terms of length of hatchery produced *N. cyanomos*

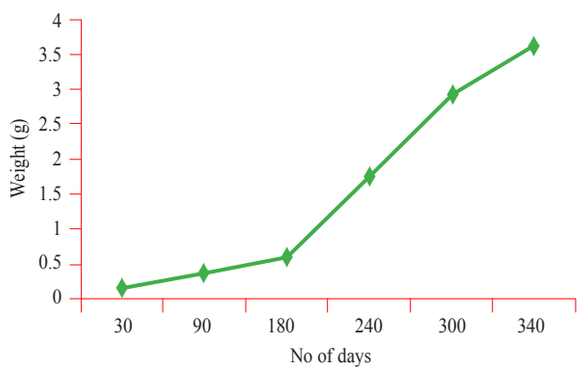


Fig. 3. Growth in terms of weight of hatchery produced *N. cyanomos*

noticed after three months. From 30 days onwards, weight of the fish increased gradually upto 180 dph, thereafter sharp increase in weight was observed, till 340 dph (Fig. 3). In the second treatment in which larvae survived beyond 5$^{th}$ day, the average survival at the end of 100 days was 12.2%, thereafter the mortality was almost negligible. The first spawning of the hatchery produced fishes was observed 340 dph at a size of about 64-73 mm.

## Discussion

*N. cyanomos* was found to spawn in the morning during the present study, as reported by Gopakumar *et al*. (2009) in the damsel fish *Chrysiptera cyanea.* But this is in contrast to the spawning events in *Amblyglyphidodon leucogaster* which occur throughout the day from first light to sunset with peak spawning activity at sunrise (Goulet, 1995). *N. cyanomos* eggs were transparent and capsule shaped, 1.1-1.56 mm in length and 0.5- 0.546 mm in width. Larger eggs were reported by Kavanaugh (2000) in *Acanthochromis polycanthus* which measured 3.7-4.3 mm in length and 1.4 to 1.5 mm in width. In the present study, the eggs were found attached to the substratum in a sideways orientation and on the first day had single/multiple oil globules. Similar observations were made in *Abudefduf saxatilis* by Wellington and Victor (1989). In the present study, hatching took place on the 3$^{rd}$ day of incubation whereas in *A. polycanthus* hatching takes place 15-17 days post-fertilization at 27-28$^0$C (Kavanagh, 2000). This could be attributed to the small egg size of *N. cyanomos.* In the case

of other pomacentrids like *Amphiprion nigripes*, the eggs are larger in size (2.4-2.6 mm in length and 0.9 -10 mm in width) and the incubation period was 6 - 7 days (Anil *et al.*, 2012).

Larval rearing of pomacentrids is indeed complicated as larvae are altricial and possess small mouth gape less than 300 µ and live feeds like rotifers generally given to the larvae of most marine fishes are not suitable feed for damsel fishes. Maintenance of zooplankton culture in the larval rearing tank along with green water technique helped to overcome the problem of feeding initiation. Green water containing microalgae such as *Chlorella* and *Nannochloropsis* are added to stabilise the water quality by functioning as a nutrient sink (Jobs *et al.,* 1997). Among the four treatments attempted, success was achieved when zooplankton was used as feed from the second day onwards. Gopakumar *et al.* (2009) successfully reared *C. cyanea* larvae on copepods. In the larval development of *A. saxatilis,* larvae were sequentially fed oyster trochophores, rotifers and copepods supplemented with *Artemia* after 10 days (Alshuth *et al.,*1998). During the present study, phytoplankton could be replaced successfully by *P. serricaudatus* from the 10$^{th}$ day onwards and from the 15$^{th}$ day onwards freshly hatched *Artemia* nauplii were given to the larvae. No mortality was reported after the 15$^{th}$ day. Pellet feed and boiled mussel meat was given to the larvae from the 30$^{th}$ day onwards.

The first sign of pigmentation started appearing in the larval body from 10$^{th}$ day onwards at SL of about 4.6-5.0 mm whereas in *A. polycanthus* colour change occurred at SL of about 25-35 mm (Kavanagh, 2000). Towards 15$^{th}$ day, the larvae developed in to juvenile fish with fully developed fins and fin rays but the adult colouration was not yet attained. Pigmentation began to appear in the spinous dorsal fin on the 20$^{th}$ day and then on the soft dorsal on the 30$^{th}$ day. The pigmentation on the anal fin was visible only on the 30$^{th}$ day. In *N. cyanomos,* transformation of juveniles into a miniature adult fish occurred mainly during 28 - 32 days (14 -16 mm SL). Yellowtail damselfish transformed at about the same size (~17 mm SL), but at greater age (between 50 and 79 dph (Potthoff *et al.,* 1987). Juveniles of *N. cyanomos* can be transferred to the nursery tanks from the 30$^{th}$ day onwards after reaching TL of about 18-20 mm. The larvae fully attained the adult pattern of pigmentation by about 90-100 days. The first spawning occurred at about 340 days after hatching at a size of about 64-73 mm and average weight of 3.6 g. Therefore fishes of above 60 mm from the rearing tanks could be used for broodstock development. The present study thus delineated the growth and development of *N. cyanomos* under captive conditions.

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