Breeding, early development and larval rearing of cloudy damsel, *Dascyllus carneus* Fischer, 1885

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ABSTRACT

As the demand for marine ornamental fish is ever increasing, the industry largely relies on collections from natural habitat due to insufficient breeding and seed production technologies. Fishes of the family Pomacentridae are popular in marine aquaria throughout the world. Among these, damsel fishes of the genus *Dascyllus* has high demand and are mostly collected from the wild. The present study forms the first-ever report on successful breeding and larval development of Cloudy Damsel (*Dascyllus carneus* Fischer, 1885). Though there are a few reports on breeding of other species of *Dascyllus*, there has been no report on the complete larval development of any of the species in this genus. This forms the first description of early larval development of a *Dascyllus* species. Using the copepod *Parvocalanus crassirostris* as first feed the larval rearing was done. Successful breeding and larval development were achieved from the wild-caught broodstock of *D. carneus* at Vizhinjam Research Centre of ICAR-CMFRI, India. Brood stock from the wild took 4 months to spawn, laid 6500–10,500 eggs per spawning and hatching rate ranged from 90.6 to 98.81%. Newly hatched larvae were the smallest among all the reported larvae of pomacentrid fishes and measured 1.95 ± 0.14 mm in total length. Yolk reserve was completely absorbed within 72 h of hatching. Preflexion stage is from 4 to 10 dph, flexion stage is from 11 to 12 dph and postflexion period is 13–15 dph. Larvae accepted only copepod naupliar stages as first feed and calanoid copepod *P. crassirostris* alone was fed until 25 dph. Larvae settled from planktonic stage in 22–23 days and all the larvae metamorphosed into juveniles by 50 dph. The egg development, larval development and larval pigmentation up to 50 dph has been described. The feeding protocols and feed size preference in relation to their age or mouth gape, the gut contents and mouth gape of the larvae caught from a feed trial with surplus copepods of all stages were analysed at regular intervals. Larvae preferred larger stages of copepods in later stages of their development. Finally larval feeding protocols were modified accordingly and better survival was observed. A final feeding regime has been developed and a strategy for the consistent hatchery production of this species was achieved. Different stages of copepod *P. crassirostris* were used till the settlement of planktonic stage of larvae and *Artemia* nauplii were used from 25 dph. The larvae were completely weaned to artificial diet from 50 dph.

1. Introduction

In recent years, marine ornamental fishes are gaining popularity in global aquarium fish trade and at present > 2000 species of coral reef fishes are there in this trade (Biondo, 2017). Among these, hatchery production has been standardised for only few species of fishes. The freshwater ornamental fish trade almost completely depends on captive bred species. > 90% of economically important marine ornamental fishes are from the wild, exclusively from coral reef ecosystems all around the world (Sweet, 2016; Fotedar and Phillips, 2011).

Members of the family Pomacentridae are popular in marine aquariums throughout the world. In India, within the family Pomacentridae, five species (*Amphiprion sebae, A. clarkii, Pomacentrus caeruleus, Dascyllus trimaculatus* and *Neopomacentrus nemurus*) together contributes more than half the volume (57.9%) of total marine ornamental trade (Prakash et al., 2017).

Till date, 90 species of marine ornamental fishes, 30 of these pomacentrids, have been bred in captivity but the attempts for mass scale production of many species have not yet been standardised. Among those, the early stages in the life of only 15% of pomacentrid species

have been described to any level and the quality and extent of existing descriptions are also not uniform (Danilowicz and Brown, 1992; Murphy et al., 2007; Moorhead and Zeng, 2010; Setu et al., 2010; Madhu and Rema, 2014).

Family Pomacentridae consists of 413 species in 29 genera (Fricke et al., 2018). Damselfishes are common in shallow coral reef areas of tropical and subtropical seas (Allen, 1991). Currently there are 396 recognised species of damselfishes (Cooper and Santini, 2016; Fricke et al., 2018). As of now the genus *Dascyllus* includes 11 species (Getlekha et al., 2016; Fricke et al., 2018) and these are primarily the inhabitants of tropical reefs. *Dascyllus carneus* Fischer, 1885, popularly known as cloudy damsel was selected for broodstock development and breeding trials here. Distribution of the species is limited to the Indian Ocean (Randall and Allen, 1977). This species is being exploited from the wild due to its attractive colour pattern. This is an important species among the marine ornamentals fishes which has a very good demand both in domestic and international markets (Froese and Pauly, 2018) as it is comparatively hardy, attractive and grow well in community tanks. The eggs and larvae of *Dascyllus* spp. are the smallest among pomacentrid fishes (Tanaka, 1999).

Sixteen species of pomacentrids have been successfully bred in captivity by the ICAR-Central Marine Fisheries Research Institute. These are orange clown (*Amphiprion percula*), false clown (*A. ocellaris*), *sebae* clown (*A. sebae*), Maldive's clownfish (*A. nigripes*), red saddleback clownfish (*A. ephippium*), pink skunk (*A. perideraion*), Clark's anemonefish (*A. clarkii*), maroon clown (*Premnas biaculeatus*), blue damsel (*Pomacentrus caeruleus*), peacock damsel (*P. pavo*), three spot damsel (*Dascyllus trimaculatus*), humbug damsel (*D. aruanus*), bluegreen damsel (*Chromis viridis*), yellowtail damsel (*Neopomacentrus nemurus*), filamentous tail damsel (*N. cyanomos*), and sapphire devil damsel (*Chrysiptera cyanea*) (Madhu et al., 2013).

Damselfishes being brilliantly coloured are a highly sought after fish for marine aquarium keeping. Habitat destruction and indiscriminate exploitation of these fishes from wild reduced their natural population in many areas (Sin et al., 1994; Prakash et al., 2017). Sin et al. (1994) emphasized the need for studies on ecology and biology of these fishes and recommended strong action for conservation of the existing stock in the wild. Prakash et al. (2017) pointed out the importance of seed production and breeding of these fishes for reducing the wild collection. *D. carneus* is often reported to be endangered in several localities (Fricke et al., 2009; Chong et al., 2010; Arai, 2015).

Despite the few attempts to breed *D. carneus* by Sreeraj (2002) and Gopakumar et al. (2009b), there has been no information available on the breeding of this species. This is one of the most difficult damsel species for seed production due to its small sized larvae (Gopakumar, pers. comm.) and so far there is no report on larval rearing of this species. The present study is the first-ever report on broodstock development, breeding and successful larval rearing of *Dascyllus carneus* Fischer, 1885. This species has been successfully bred for two generations in hatchery and seed production technology has also been developed.

2. Materials and methods

2.1. Broodstock collection, transportation and acclimatization

Broodstocks of *Dascyllus carneus* were collected from the Tuticorin waters of Tamil Nadu, East Coast of India and were transported to Vizhinjam Research Centre of CMFRI, Thiruvananthapuram, Kerala, India in polyethylene bags with oxygen. Seven fishes collected were in the size range of 4.5–7.3 cm. After the temperature acclimatization, the fishes were stocked in 500 L HDPE tanks with 250 L of sea water with biological filters. The fishes were fed with a mixture of boiled and fresh mussel meat 3 times a day with 10% water exchange daily. The broodstocks maintained under natural photoperiod (12 D: 12 L) inside the hatchery. Water temperature ranged between 27.2 and 29.5 °C, pH 8–8.2 and salinity between 33 and 35 ppt.

2.2. Breeding pair formation and breeding behaviour

Any change in the behaviour of fish were observed and recorded. The responses and interactions of the other individuals were also recorded separately. The breeding behaviour was photographed using a digital camera (Nikon D7100).

2.3. Spawning

The brood stock tanks were provided with small pots made of clay from the initial period of broodstock development. Pots were brown in colour with a hole (10–15 cm) at the bottom. Pots were kept upside down which formed a perfect shelter for the broodstock to settle down. Within few days, the fishes started considering the pot as their territory and also as a substratum for depositing the eggs.

2.4. Nature of the eggs and its development

To study the developmental stages and for taking measurements, eggs were collected carefully from the surface of the pot using scrapers and taken in a small petri dish with fresh sea water. Developmental stages of the eggs were observed and measurements were taken using Leica S8APO stereo zoom microscope with Leica DFC 290 camera and Leica application suite version 4.1.0 software. Developmental stages of eggs were recorded at an interval of 1 h for the first day, 2 h for the second day and for 3 h for the third day.

2.5. Hatching and larval rearing

After incubation for 55 h inside the breeding tank, pots with the eggs were carefully transferred to the pre-prepared larval rearing tanks. While transferring clay pots from broodstock tanks to larval rearing tanks, egg patches on the pots were photographed using a digital camera (Nikon D7100) and the number of eggs were counted from the photographs. After hatching, the pot was removed, unhatched and opaque eggs were counted and the hatching percentage was calculated. The larval hatching and rearing were carried out in rectangular, dark brown coloured tanks of size $1 \times 1.95 \times 0.5$ m. (975 L capacity) with 500 L of green water. Gentle regulated aeration was given around the pot using small aeration stones to mimic the fanning by parents. No water exchange was done up to 10 days and after that, everyday 10% of water was removed using narrow tubes from the bottom and 12% of fresh seawater was added back with minimal disturbance till 25 dph and later 15% water was exchanged daily. Physico-chemical parameters such as atmospheric temperature, water temperature, salinity, dissolved oxygen, total ammoniacal nitrogen (TAN), NH_3-N, NH_4-N and pH were monitored regularly (APHA, 1998).

2.6. Live feed culture

From the stock culture of algae maintained at ICAR- CMFRI, mass culture of *Isochrysis galbana*, *Chlorella marina*, *Nannochloropsis oculata* and *N. salina* were produced in circular tanks of 1 t capacity. Algal cultures were prepared using recommended doses of fertilizers in FRP tanks. Algae cultured were harvested regularly and used for making green water for larval rearing and also for live feeds like copepods and rotifers. *Artemia* nauplii were also allowed to feed algae for a short period before using this as a feed for fish larvae.

Mass culture of rotifer (*Brachionus plicatilis*) was maintained at high density and fed with a mixture of algae *I. galbana*, *N. oculata*, *N. salina* and *C. marina* in circular tanks of 1 t capacity. *Artemia* cysts were also kept ready for use. Among the stock culture of copepods maintained at Vizhinjam Research Centre of ICAR-CMFRI (Santhosh et al., 2018) the mass cultures of calanoid copepod *Parvocalanus crassirostris* were initiated in 1 t FRP tanks, 5 days before the larval rearing (Anzeer et al., 2018). The copepods were fed with an equal mixture of microalgae *I.*

galbana, C. marina and *N. salina*.

2.7. Feeding trials

2.7.1. Trials for identification of feed preference

All larval rearing trials were conducted using green water technique. *N. oculata* and *I. galbana* were used in the ratio of 1:1 for the preparation of green water. The final algal density in all the larval rearing tanks was maintained at 1×10^5 cells mL^{-1}. The experiment was carried out in three sets of 500 L HDPE tanks in three replicates containing 300 L of green water. For each tank, 300 newly hatched larvae were stocked. The experiment continued for 15 days and the survival has been noted at 5 days intervals on each trial. Live feeds were introduced on 2 dph in all the tanks. In this experiment, one set of tanks were inoculated with rotifer *B. plicatilis* at the rate of 10 ind. mL^{-1} alone as live feed. In second set of tanks, calanoid copepod *P. crassirostris* nauplii were introduced from mass culture tanks at the rate of 10 ind. mL^{-1}. In third set of tanks, larvae were fed with a combination of *B. plicatilis* (5 ind. mL^{-1}) and nauplii of *P. crassirostris* (5 ind. mL^{-1}). Water quality parameters were maintained at optimum levels in all tanks throughout the study. The results were statistically analysed using SPPS Statistics 20.

2.7.2. Assessment of mouth gape and size of larval feed

Since survival was noted only in the trials with *P. crassirostris*, it is assumed that the larvae will survive only if we provide suitable copepod as live feed. An experiment was carried out to identify the size preference in relation to growth of larvae or mouth gape. In this trial, 750 newly hatched larvae from the subsequent batch were introduced into a FRP tank of 1 t capacity having 0.75 t green water. The copepod *P. crassirostris* were introduced into the tank with adult copepod density maintained at a rate of 1 ind. mL^{-1}, copepodites 2 ind. mL^{-1} and nauplii 4 ind. mL^{-1} throughout the trial by adding the required quantity of copepod stages after the daily population assessment at morning and evening by serial filtration (Anzeer et al., 2018). Larval mouth gape and feed preference were observed by periodic sampling. The feed size preference at each stage of the larval development was tracked by observing the gut contents of the larvae from 3rd day onwards up to 25 dph. Daily 10 ind. of larvae were anesthetized using sodium bicarbonate buffered MS-222, dissected and observed under Leica S8APO stereo zoom microscope and photographed under Zeiss AXIO Lab A1 compound microscope with Zeiss AxioCamERc5s camera with ZEN 2 lite software. From the photographs, size of copepods which were present in the gut was measured and the stages of the copepods were identified from the body parts and appendages.

Mouth gape was assessed using the lateral view method described by Shirota (1970) using Pythagorean Theorem, assuming that the jaws represent two sides of a right angled triangle and the hypotenuse, the expected mouth gape. The length from anterior-most point of the pre-maxilla to the posterior edge of the maxilla was considered as the upper jaw length. The length between anterior-most part of the mandible to its posterior edge were measured as the lower jaw length. The mouth gape in relation to age of larvae is graphically represented.

2.8. Protocols for larval rearing of D. carneus

Four subsequent trials were carried out using the larvae from subsequent batches to finalize the larval rearing protocols. 750 newly hatched larvae from the subsequent batch were introduced into a FRP tank of 1 t capacity having 0.75 t green water. *P. crassirostris* of required size were used at each stage of larvae. Sufficient numbers of different stages of copepods were made available in the rearing tanks by the process of serial filtration using different combinations of sieves of size 35 μm, 60 μm, 100 μm and 170 μm. During the filtration, for all copepodites and adult stages, the sieve with smaller mesh (35 μm) was used to collect all the smaller stages back to the mass culture tank (Anzeer

et al., 2018). Copepod density 2 ind. mL^{-1} was maintained till 25 dph. *Artemia* nauplii (enriched by feeding *N. oculata* and *I. galbana*) were fed at a rate of 0.4 to 0.5 ind. mL^{-1} from 25 dph to 50 dph. Pelleted feed of 500 μm size started from 33 dph onwards.

2.9. Larval development

Larval developmental stages were photographed and measurements were taken using a Leica S8APO stereo zoom microscope attached with Leica DFC 290 camera and software Leica application suite version 4.1.0 and Zeiss AXIO Lab A1 compound microscope attached with Axio Cam ERc5s with ZEN 2 lite software. To study the larval developments, 5 larvae were taken from the rearing tanks and were anesthetized using sodium bicarbonate buffered MS-222. For assessing the growth, linear measurements like total length, standard length, head length, body depth and eye diameter were also recorded. Observations on post hatch larvae for its development and changes in the pigmentation continued until complete metamorphosis into juveniles (40–50 dph). Photographs of newly hatched larvae were taken at an interval of 24 h up to 17 dph, from 20 dph to 50 dph at an interval of 5 days. Late larval stages and juveniles were photographed using digital cameras (Nikon D7100 & Nikon D90).

2.10. Nursery rearing

The fully metamorphosed larvae were gently transferred after acclimatization into 500 L HDPE tanks with 250 L filtered seawater with a biological filter in the middle. Each tank was stocked with 20 juveniles and were fed daily 2 times using artificial pellets and boiled mussel meat.

3. Results and discussion

3.1. Breeding pair formation and breeding behaviour

After 4 months of rearing, fishes began to show breeding behaviour. Males started showing territorial behaviour and pair formation. Dominant male starts to chase other males and immature females. This behaviour is the primary indication of sexual maturity. As the male becomes extremely aggressive, it is ideal to keep only one male and female combination in the breeding tanks. *D. carneus* has been reported as functional, diandric protogynous hermaphrodite. Only the largest fish in the colony becomes functional male and second largest fish becomes functional female in the wild. (Asoh and Yoshikawa, 2003). Though smaller mature males were reported to coexist in wild, Asoh and Yoshikawa (2003) did not relate their presence in the colony to a chance of sneak fertilization. In case of its congeners like *D. reticulatus* (Schwarz and Smith, 1990) and *D. aruanus* (Fricke and Holzberg, 1974; Coates, 1982; Cole, 2002) mature smaller males were never noticed in the colony.

Cleaning the substratum, sudden changes in the behaviour and dancing movements of the male indicate breeding behaviour (Hattori and Casadeva, 2016). If the female is ready, it becomes conspicuously darker in colour. In general, courtship behaviour start by 'signal jump' (Tanaka, 1999) of male *i.e.,* upward and downward swimming movement of male to attract its mate (Fig. 1a). Circular movements around the substratum and vigorous cleaning is also noticed during this phase (Fig. 1b). Frequent crossing movement and rubbing side by side is the clear indication for attracting the pair (Fig. 1c, d). Pairing and resting side by side (Fig. 1e) was observed after completion of pair formation. Both male and female positioned and move side by side in the same direction was observed during spawning (Fig. 1f). Randall and Allen (1977) reported similar courtship behaviour in *D. carneus* from islands of western Indian Ocean. Reproductive behaviour of *D. carneus* is very similar to that of *D. aruanus*, reported by Chlupaty (1957), Fishelson (1964) and Sale (1970) and *D. albisella* by Stevenson (1963) and Asoh
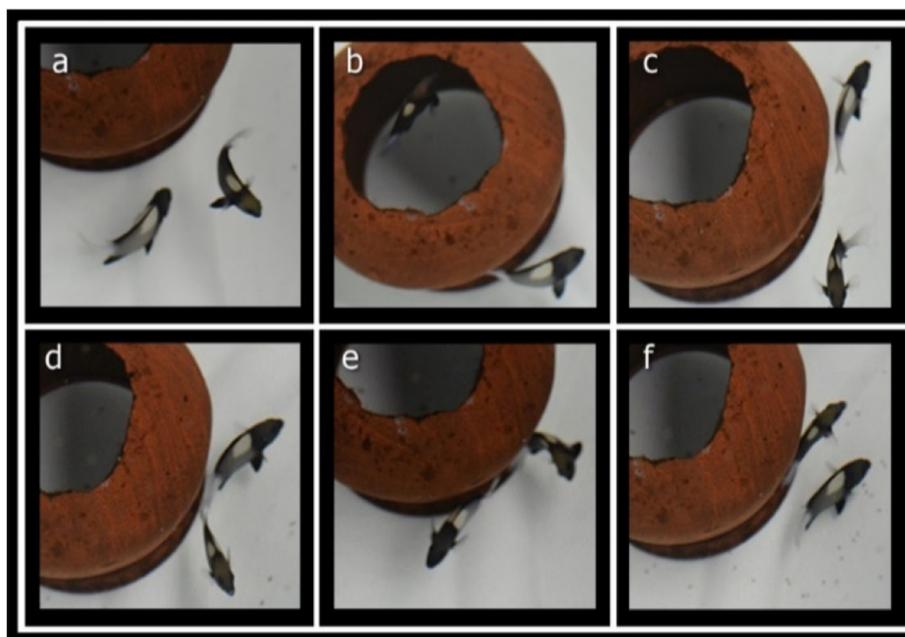
Fig. 1. Breeding behaviour of *D. carneus* in breeding tank around the artificial substratum (pots) kept for spawning; (a) 'signal jump' (upward and downward movement of male), (b) male making substratum ready, (c, d) crossing movements, (e) initiated pairing, (f) positioned for spawning.

and Yoshikawa, (2003). Tanaka (1999) also reported similar breeding behaviour for *D. aruanus, D. trimaculatus, D. melanurus* and *D. reticulatus* in aquaria. According to Wickler (1967), the breeding behavior within the genus has been found to be similar and unique.Dominant males selected for breeding trials were 50–65 mm in standard length, 20–25 mm in body depth and 8–9.7 g in weight. Females selected were 35–45 mm in standard length, 25–27 mm in body depth and 5.0–5.5 g in weight. *D. carneus* needs a substratum like earthen pot for spawning. Males spent most of their time for cleaning the pot for attaching the eggs.

3.2. Spawning

Observations were made from different breeding pairs. All spawning occurred early in the morning around 5 am and in some cases, it extended up to 10 am. This is very common in other pomacentrid fishes also (Suzuki et al., 1985; Olivotto et al., 2003; Gopakumar et al., 2009b; Rohini Krishna et al., 2016). Eggs were attached to the outer surface of the pot evenly as a single layer (Fig. 2). Male protected the territory and guarded the eggs, fanning was done by both male and female but in case of female it was not frequent. During the incubation, both the parents were aggressive and tried to attack even the siphoning tubes. Number of eggs per spawning varies from 6500 to 10,500. Sreeraj (2002), Gopakumar et al. (2009b) and Randall and Allen (1977) reported 5000–6000 eggs per spawning for *D. carneus*. Cobb (1975) reported the spawning of *D. carneus* in aquarium with a range of 5900–8260 eggs in each spawning. Under hatchery conditions, spawning of *D. carneus* was observed in almost all the months and peak spawning frequency was seen from September to June. Breeding pair continuously spawned for 2 months at an interval of 7–15 days. Sreeraj (2002) reported continuous spawning of *D. carneus* mostly at an interval of 12 days. In *D. albisella* spawning was reported throughout the year with a peak during the month of June by Stevenson (1963) and June to September by Asoh and Yoshikawa (2003). Tanaka (1999) reported continuous spawning for 7–10 months in four species of damsel fishes. The interval of consecutive spawning was reported as 8–15 days for *D. aruanus,* 10–13 days for *D. trimaculatus*, 5–7 days for *D. melanurus* and 9–13 days for *D. reticulatus*. Average spawning periodicity of *D. aruanus* and *D. trimaculatus* was reported as 14 days by Gopakumar

Fig. 2. Eggs of *D. carneus* on substratum (clay pot) before hatching.

et al. (2009a).

3.3. Nature of the eggs and its development

Eggs (Fig. 3a-s) were elliptical and were almost uniform size in each spawning. Marked variations in size of eggs were observed in the same breeding pair over the total breeding season. The egg size was smaller in the beginning and towards the end of breeding cycle and egg size was largest towards the middle of the breeding cycle. Size of largest eggs recorded were 754.30 ± 10.81 µm in length and 534.01 ± 24.06 µm width. Size of smallest eggs recorded were 669.77 ± 15.08 µm in length and 490.04 ± 13.6 µm in width. Sreeraj (2002) reported eggs of
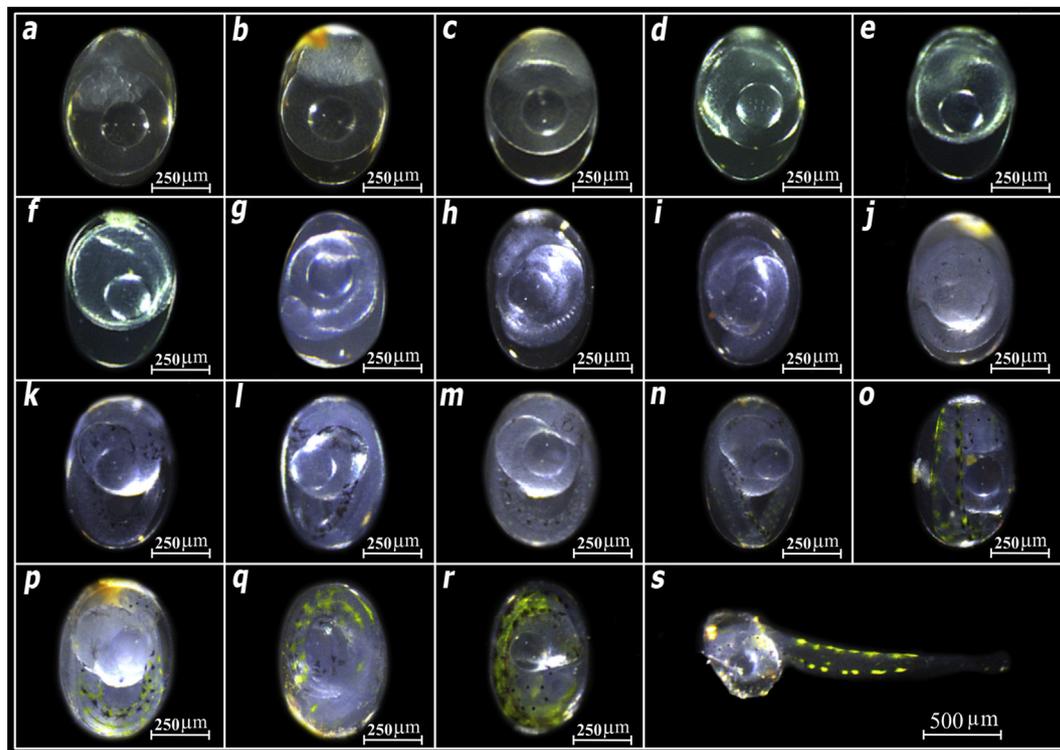
Fig. 3. Embryonic development of eggs of *D. carneus* till hatching. Scale bar as indicated in respective figures. (a) 1 hpf - 32 celled stage, (b) 2.5 hpf - morula stage, (c) 3 hpf - blastula stage, (d) 6 hpf - gastrula stage, (e) 9 hpf - blastopore closing, (f) 10 hpf - development of embryonic axis, (g)15 hpf - larval somites started appearing, (h) 19 hpf - non pigmented primitive eye, (i) 20 hpf - scattered yellowish-green pigments appeared, (j) 24 hpf - melanophores became more visible and tail detachment initiated, (k) 30 hpf - melanophores expanded to the stellated stage, (l), 32 hpf - larval movements initiated. (m) 35 hpf - yellowish-green pigments became conspicuous, (n) 41 hpf - tail and trunk got thickened, (o) 48 hpf - green pigmentation and melanophores became more prominent, By (p) 55 hpf - embryo started to wriggle inside egg shell, (q) 58 hpf - tail twitching movements initiated, (r) 60 hpf - ready to hatch and the larvae started moving vigorously (s) 61 hpf - Egg shell broke near the tail and the head remains inside the shell for a while. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

D. carneus having length 670 ± 0.002 μm and width 450 ± 0.0015 μm from the same locality. Gopakumar et al. (2009b) reported egg size of *D. carneus* from 625 to 650 μm. In general, eggs of fishes of the genus *Dascyllus* are smaller than most of the other genera in the family Pomacentridae. Tanaka (1999) reported that the size of egg of *D. aruanus* ranged from 700 to 780 μm in length and width ranged from 480 to 530 μm, *D. trimaculatus* length from 600 to 650 μm and width from 450 to 480 μm, *D. melanurus*, length ranged between 650 and 680 μm and width from 430 to 450 μm and for *D. reticulatus* length varied from 630 to 650 μm and width from 480 to 500 μm. The eggs in *D. albisella* were of 800 μm in length and 400 μm in width (Stevenson, 1963).

Morphological details of eggs of *D. carneus* closely resemble the description of eggs of other *Dascyllus* spp. reported by Tanaka (1999). The eggs were transparent and slowly become translucent during the late hours of incubation. The egg case was flexible and smooth in appearance. A peripheral stalk by which the eggs get attached to the substratum was present at the animal pole and the stalk was pale yellow in colour. The egg clutches comprised of eggs at different stages, which may be due to the variation in the time of fertilization. Better survival was observed in larvae developed from larger eggs even though; the size range of eggs varies considerably.

The eggs reached 32 celled stage (Fig. 3a) within 2 h post fertilization (hpf). Morula stage (Fig. 3b) observed by 2.5 hpf and blastula stage (Fig. 3c) has started after 3 hpf. Eggs developed into the gastrula stage by 6 hpf. Gastrula stage (Fig. 3d) ended by 9 hpf, blastoderm started covering the yolk sac and blastopore closed by the end of 9 hpf (Fig. 3e). Development of embryonic axis (Fig. 3f) was observed at 10 hpf. By 15 hpf, the larval somites started appearing (Fig. 3g).

Development of head region and a non-pigmented primitive eye were observed at 19 hpf (Fig. 3h). Scattered yellowish-green pigments appeared from 20 hpf (Fig. 3i). By 24 hpf (Fig. 3j), melanophores became more visible and tail detachment initiated. Further, melanophores expanded to the stellated stage and spread over the yolk by 30 hpf (Fig. 3k). At 32 hpf (Fig. 3l), stellate and expanded melanophores extended along the ventral region of notochord and larval movements initiated. Yellowish-green pigments became conspicuous by 35 hpf (Fig. 3m). Up to 41 hpf (Fig. 3n), the embryo growth continued, tail and trunk got thickened and green pigments appeared clearer. After this stage, larval movements inside the egg became more vigorous. By 48 hpf (Fig. 3o), the dorsal and ventral margins of embryos trunk showed extensive green pigmentation and melanophores also expanded. By 55 hpf (Fig. 3p) embryo occupied the entire area of the egg, started to wriggle inside the shell. Twitching movements of tail was noticed from 58 hpf (Fig. 3q). By 60 hpf (Fig. 3r), the eggs were ready to hatch and the larvae now started to move inside eggshell vigorously. Sreeraj (2002) reported similar events in development of eggs of *D. carneus* in captivity. The embryonic development of *D. aruanus*, *D. trimaculatus*, *D. melanurus* and *D. reticulatus* reported were similar to that of *D. carneus* but the time periods for various events were lengthier in all species except *D. aruanus* reported by Tanaka (1999).

3.4. Hatching

In the larval rearing tanks, hatching occurred in the late evening after sunset on 61–65 h of incubation. Egg shell broke near the tail and the head remained inside the shell for a while (Fig. 3s). Almost similar incubation period was reported by Sreeraj (2002) for *D. carneus* but
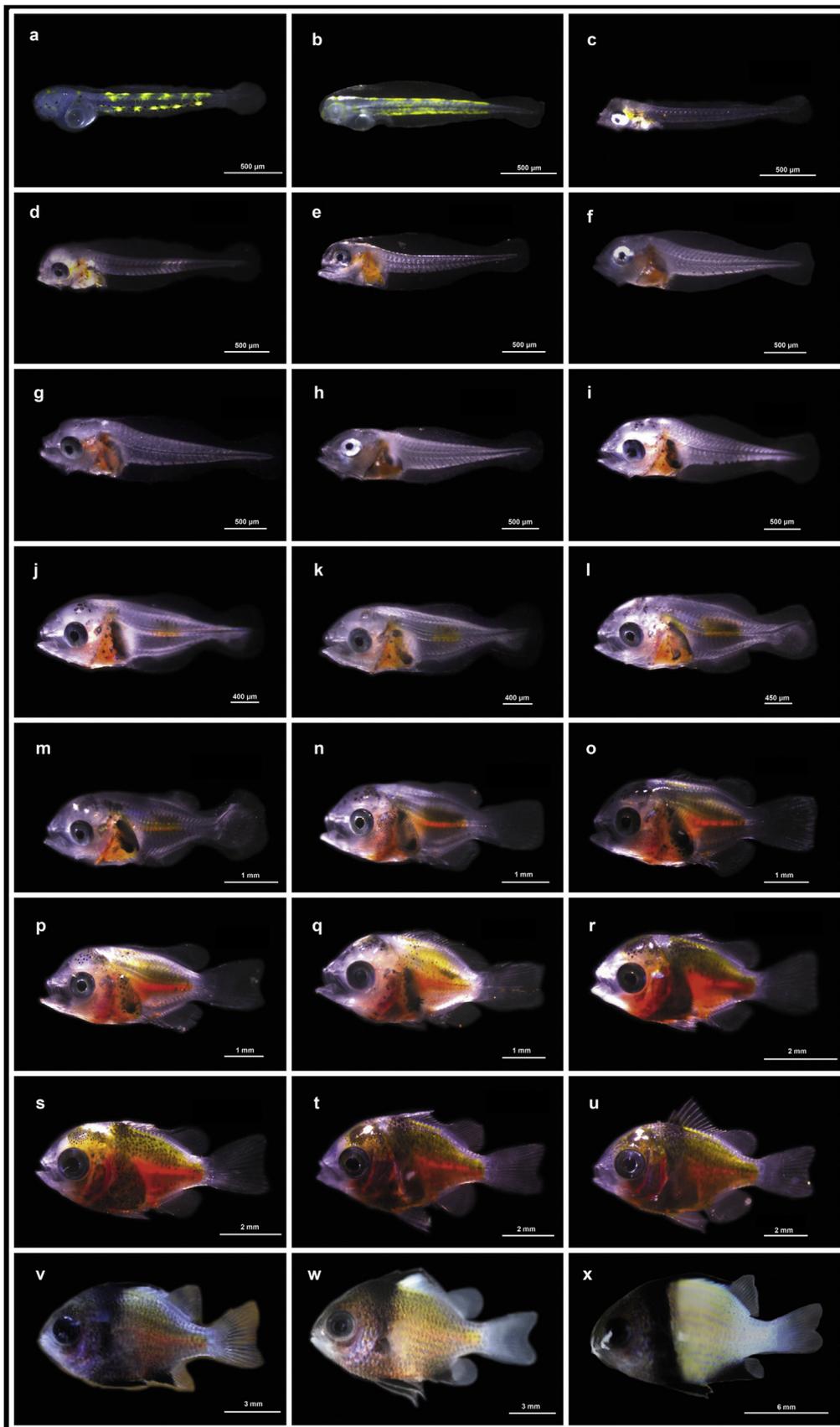
Fig. 4. The early larval development of *D. carneus*. Shown are multiple individuals taken from the same batch of larvae. Scale bar as indicated in respective figures. (a) A newly hatched larva, (b)1 dph larva, (c) 2 dph larva, (d) 3 dph larva, (e) 4 dph larva, preflexion stage begins (f) 5 dph larva, (g) 6 dph larva, (h) 7 dph larva, (i) 8 dph larvae, (j) 9 dph larva, (k) 10 dph larva, (l)11 dph larva showing flexion stage, (m)12 dph larva showing flexion stage, (n)13 dph larva postflexion stage begins, (o)14 dph larva, (p)15 dph larva; postflexion completed, (q) 17 dph larva, in transition stage, (r) 20 dph larva, (s) 25 dph larva, (t) 30 dph larva in juvenile stage and squamation begins (u) 35 dph larva, (v) 40 dph larva (w) 45 dph larva, juveniles (x) 50 dph fully formed juvenile.

Cobb (1975) reported the incubation period as 48 h in a temperature range of 26–30 °C. In case of *D. aruanus, D. trimaculatus, D. melanurus* and *D. reticulatus* reported by Tanaka (1999), the hatching periods were 65, 77, 86, and 87 h respectively.

Hatching rates observed during present study was 90.6 to 98.81%. Danilowicz and Brown (1992) reported hatching rates as 100% for *D. albisella* and 80% for *D. aruanus*. The larvae were pelagic and spread all over the water column. Very low level aeration was provided to avoid entrapment of larvae in the air bubbles and surface of water because the larvae at pre-larval stages were very weak and tend to stick on bubbles and water surface.

3.5. Larval development

Newly hatched larvae (Fig. 4a) were small, pelagic and distributed evenly in the water column. Total length was 1.95 ± 0.14 mm; standard length 1.75 ± 0.04 mm and it is the smallest larvae reported among *Dascyllus* spp. Body depth was measured as 0.18 ± 0.01 mm and optic vesicle diameter was 0.15 ± 0.005 mm. The total length of newly hatched larvae of *D. carneus* was much smaller (1.4 mm) as reported by Cobb (1975). Newly hatched larvae of other *Dascyllus* species of damsel fishes like *D. aruanus, D. trimaculatus, D. melanurus* and *D. reticulatus* were in the range of 2.08 to 2.50 mm (Tanaka, 1999) for *D. albisella* it was 2.5 mm (Stevenson, 1963) and for *D. albisella* and *D. aruanus* it was 2.2 mm (Danilowicz and Brown, 1992). In 1 dph larvae (Fig. 4b), the total length was measured as 2.28 ± 0.03 mm, standard length measured as 1.86 ± 0.03 mm, head length 0.36 ± 0.02 mm, body depth 0.16 ± 0.002 mm and eye diameter 0.17 ± 0.003 mm. Yolk sac and oil globule were measured 0.12 ± 0.01 mm and 0.2 ± 0.01 mm respectively. Tanaka, (1999) reported length range of 1 dph larvae from 2.38 to 2.63 mm for *D. aruanus, D. trimaculatus, D. melanurus* and *D. reticulatus*. Average total length of 2 dph larvae (Fig. 4c) was 2.27 ± 0.2 mm, standard length 1.95 ± 0.002 mm; head length 0.38 ± 0.01 mm, eye diameter, 0.2 ± 0.01 mm and body depth 0.22 ± 0.01 mm. Mouth opening was noted 28 h after hatching and eyes became functional. Yolk sac measured 0.10 ± 0.01 mm. The 3 dph larvae (Fig. 4d) measured 2.43 ± 0.04 mm in total length, 2.3 ± 0.02 mm in standard length, 0.45 ± 0.0172 mm in head length, 0.23 ± 0.01 mm in body depth and eye diameter was 0.19 ± 0.004 mm. The external body colouration started appearing. Unlike the present case, Tanaka, (1999) reported a reduction Tanaka (1999) reported a reduction in length range (2.0–2.18) for *D. aruanus, D. trimaculatus, D. melanurus* and *D. reticulatus* in 2nd and 3rd day. In *D. carneus*, yolk and oil globule were completely absorbed by third day as in the case of most of the other damsel species reported (Alshuth et al., 1998; Tanaka, 1999).

Preflexion stage of larvae was observed from 4 dph to 10 dph. The 4 dph larvae (Fig. 4e) were 2.47 ± 0.02 mm in total length, 2.33 ± 0.03 mm in standard length, head length of 0.5 ± 0.020 mm, 25 ± 0.01 mm in body depth and eye diameter was 0.23 ± 0.01 mm. Tanaka (1999) reported a reduction in length range (2.012–2.15) in all four species *D. aruanus, D. trimaculatus, D. melanurus* and *D. reticulatus* consecutively in fourth day also. In 5 dph larvae (Fig. 4f), total length measured was 2.53 ± 0.02 mm, standard length 2.37 ± 0.01 mm, head length 0.63 ± 0.02 mm, body depth 0.3 ± 0.02 mm, eye diameter 0.22 ± 02 mm indicating marked increase in all measurements except eye diameter. Tanaka (1999) reported increase of total length from 2.28 to 2.48 mm in *D. trimaculatus* of similar age group. The 6 dph larvae (Fig. 4g) measured 2.69 ± 0.03 mm in total length, 2.55 ± 0.06 mm in standard length, 0.78 ± 0.02 mm in head length, 0.36 ± 0.01 mm in body depth and eye diameter was 0.28 ± 0.02 mm indicating marked increase in all measurements than 5 dph. Tanaka (1999) reported that the larval development of *D. aruanus, D. reticulatus* and *D. trimaculatus* up to 5 days and *D. melanurus* up to 4 days only. There has been no report about the larval description of any of the *Dascyllus* spp. beyond 5 dph. The 7 dph larvae (Fig. 4h) measured

2.97 ± 0.02 mm in total length, 2.73 ± 0.04 mm in standard length, 0.83 ± 0.02 mm in head length, 0.43 ± 0.02 mm in body depth and eye diameter was 0.29 ± 0.06 mm. The fin fold at the sagittal plane slightly reduced at the dorsal area. The 8 dph larvae (Fig. 4i) measured 3.09 ± 0.07 mm in total length, 2.85 ± 0.01 mm in standard length, 0.91 ± 0.02 mm in head length, 0.8 ± 0.01 mm in body depth and eye diameter was 0.35 ± 0.01 mm. Orange pigmentation became more prominent in abdominal area. The 9 dph larvae (Fig. 4j) measured 3.31 ± 0.04 mm in total length, 3.13 ± 0.09 mm in standard length, 1.05 ± 0.05 mm in head length, 0.96 ± 0.06 mm in body depth and eye diameter was 0.37 ± 0.01 mm. Caudal peduncle depth was 0.19 ± 0.002 mm. Hypural plates began to develop. Orange pigments were randomly distributed externally at the lateral middle regions of the notochord. The 10 dph larvae (Fig. 4k) measured 3.49 ± 0.09 mm in total length, 3.18 ± 0.01 mm in standard length, 1.08 ± 0.02 mm in head length, 1.07 ± 0.02 mm in body depth and eye diameter was 0.42 ± 0.01 mm. Caudal rays developed further, sagittal fin fold reduced and the caudal peduncle region began to develop.

Flexion stage of larvae was from 11 dph to 12 dph. Larvae of 11 day (Fig. 4l) measured 3.76 ± 0.05 mm in total length, 3.46 ± 0.06 mm in standard length, 1.24 ± 0.02 mm in head length 1.23 ± 0.04 mm in body depth caudal peduncle depth 0.33 ± 0.01 mm and eye diameter was 0.43 ± 0.01 mm. In the flexion stage, caudal fin rays, dorsal fin anlage and anal fin anlage were further developed. Caudal peduncle region started developing and ventral fin buds were clearly visible. The 12 dph larvae (Fig. 4m) measured 4.15 ± 0.1 mm in total length, 3.6 ± 0.21 mm in standard length, 1.22 ± 0.19 mm in head length 1.31 ± 0.03 mm in body depth, caudal peduncle depth 0.39 ± 0.005 mm and eye diameter was 0.47 ± 0.004 mm. The dorsal melanophores at the head region began to develop further dorsally. The mid lateral region of the body showed stellate expanded melanophores and orange pigments. The notochord tip was in maximum upward position. Caudal fin rays became more visible. The fin fold through the sagittal plane disappeared. Dorsal fin rays began to develop.

Postflexion stage of the larvae was from 13 dph to 15 dph. Larvae (Fig. 4n) measured 4.53 ± 0.08 mm in total length, 3.77 ± 0.11 mm in standard length, 1.59 ± 0.03 mm in head length 1.65 ± 0.03 mm in body depth and eye diameter was 0.47 ± 0.01 mm. Dorsal fin rays and spines started to develop. Caudal peduncle has formed. Caudal fin rays ossification intensified. Anal fin rays and anal spine also further developed. The 14 dph larvae (Fig. 4o) measured 4.92 ± 0.03 mm in total length, 3.93 ± 0.06 mm in standard length, 1.82 ± 0.02 mm in head length 1.85 ± 0.01 mm in body depth and eye diameter was 0.62 ± 0.03 mm. The ossification of caudal fin rays further progressed. The dorsal spines further developed at the anterior region. Anal fin developed and anal fin rays were clearly visible. On 15 dph, larvae (Fig. 4p) measured 5.07 ± 0.05 mm in total length, 4.01 ± 0.09 mm in standard length, 1.89 ± 0.02 mm in head length 1.92 ± 0.005 mm in body depth and eye diameter was 0.65 ± 0.05 mm. Deep orange pigmentation was noted almost throughout the body.

Transition stage of the larvae was from 16 dph to 25 dph. On 17 day, larvae (Fig. 4q) measured 5.38 ± 0.05 mm in total length, 4.29 ± 0.03 mm in standard length, 2.04 ± 0.04 mm in head length 1.96 ± 0.05 mm in body depth and eye diameter was 0.68 ± 0.01 mm. Dorsal spines, dorsal fin rays, anal fin rays, ventral fin, caudal peduncle and caudal fin rays were well developed. The 20 dph larvae (Fig. 4r) measured 6.07 ± 0.06 mm in total length, 4.63 ± 0.01 mm in standard length, 2.17 ± 0.02 mm in head length, 2.3 ± 0.01 mm in body depth and eye diameter was 0.89 ± 0.03 mm. The base colour of larvae became olive green. All fin rays were well ossified at this stage. The 25 dph larvae (Fig. 4s) measured 7.34 ± 0.1 mm in total length, 5.57 ± 0.05 mm in standard length, 2.45 ± 0.02 mm in head length, 3 ± 0.005 mm in body depth and eye diameter was 1.08 ± 0.02 mm. The upper half of the larva became olive green in colour decorated with more numbers of contracted punctate melanophores and the lower half was, more or less orangish in

colour. Dorsal, ventral, anal and caudal fins were well developed. Caudal fin edge started becoming concave indicating the development of forked tip.

Juvenile stage started from 30 dph. The larvae (Fig. 4t) measured 8.42 ± 0.1 mm in total length, 6.58 ± 0.11 mm in standard length, 3.26 ± 0.11 mm in head length, 3.59 ± 0.02 mm, in body depth and eye diameter was 1.24 ± 0.02 mm. Pigmentation remained almost same as the larvae of 25 dph but the colour gradation thickens as it results in fading of melanophores. Dorsal spines are well developed. Squamation also started. The 35 dph larvae (Fig. 4u) measured 9.55 ± 0.1 mm in total length, 7.41 ± 0.22 mm in standard length, 3.34 ± 0.02 mm in head length, 3.93 ± 0.04 mm in body depth and eye diameter was 1.42 ± 0.03 mm. Body was deep red coloured with a greenish tint dorsally above the lateral line. All the fins and fin rays were well developed and similar to that of the adults.

The 40 dph larvae (Fig. 4v) continued metamorphosis. Body became darker in the anterior half with pale yellow and orange colour in the posterior half. Most of scales were with one or more small fluorescent blue dots. Lips became blue and basal portion of body scales were slightly dusky. The black bar from base of the dorsal fin to base of pelvic fin became more prominent. Caudal fin was light yellow in colour. The anterior part of the dorsal fin, pelvic fin and anterior part of anal fin were black in colour. The 45 dph larvae (Fig. 4w) measured 12 ± 0.12 mm in total length, standard length 10.9 ± 0.2 mm and body depth 6.45 ± 0.13 mm. Eye diameter was 1.67 ± 0.8 mm. Head length was measured as 3.87 ± 0.02 mm. Pelvic fin length was 1.29 ± 0.11 mm. Dorsal fin length was 2.58 ± 0.1 mm. The metamorphosis of all the larvae of *D. carneus* into juvenile was completed by 50 dph. At this stage, juveniles (Fig. 4x) were light yellowish in colour with a dark band behind the head. Numerous diffused blackish and deep blue coloured patches and spots were visible on the head. Almost all lighter scales were with fluorescent blue dots or lines. Danilowicz and Brown (1992) reported metamorphosis of larvae of *D. albisella* from 23 to 45 days and *D. aruanus* from 30 to 50 day in different feeding trials. Gopakumar et al. (2009a) reported metamorphosis of damsel fishes like *D. trimaculatus* as 35–40 days, *D. aruanus* as 25–31 days, *Pomacentrus caeruleus* as 17–21 days, *Chromis viridis* as 30–49 days, *N. nemurus* as 16–21 days. In regal demoiselle (*Neopomacentrus cyanomos*), metamorphosis was completed from 28 to 32 days (Setu et al., 2010; Rohini Krishna et al., 2016). In sergeant major (*Abudefduf saxatilis*), metamorphosis was completed by 32 days (Alshuth et al., 1998). Metamorphosis of *C. atripectoralis* larvae was completed in 21–25 days (Kavanagh and Alford, 2003) and of *P. amboinensis* in 20–22 days (Kavanagh and Alford, 2003; Murphy et al., 2007). Gopakumar et al. (2002) reported the period of metamorphosis in larvae of *N. nemurus, P. caeruleus* and *N. filamentosus* as 30 to 40 days.

Knowledge on early development of larvae is essential for confirmation of critical periods of first feeding, mortality, malformation and nutritional needs and environmental requirements. Critical periods often overlap with major events in ontogeny such as initiation of flexion (preflexion), flexion period, transition stage and final metamorphosis (Kovac and Copp, 1999; Battaglene and Cobcroft, 2007; Somarakis and Nikolioudakis, 2010). In case of *D. carneus* also critical days of survival observed was observed in 3–4 dph, 9–10 dph and 14–15 dph. Linear growth measurement chart of larvae for total length, standard length, head length, body depth and eye diameter up to 35 dph is represented in Fig. 5. Trends in all the measurements except eye diameter changes its phase after all the three critical periods. Growth was maximum after postflexion stage (15 dph). Steady increase in length was noticed in daily growth graph of *D. carneus* from the postflexion period onwards and continued beyond juvenile stage. Somarakis and Nikolioudakis (2010) indicated notochord flexion stage as a 'developmental milestone in the early life history' of fishes. In this stage, the organogenesis and differentiation was maximum and faster growth is essential to have early settlement, schooling and metamorphosis. Steady and fast growth is observed in *D. carneus* also after postflexion stage *i.e.,* after 15 dph
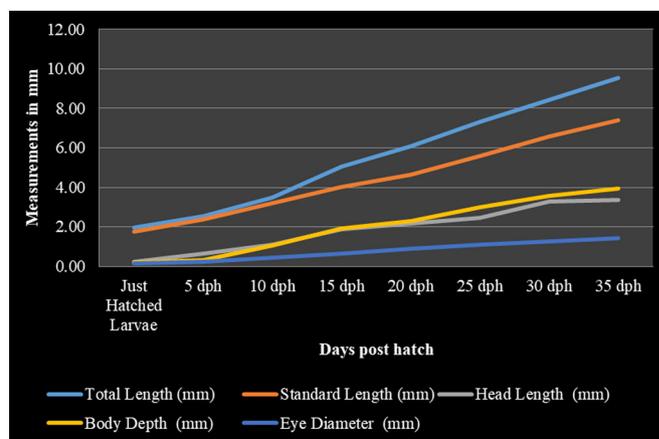

Fig. 5. Increase in linear measurements of *D. carneus* larvae (*n* = 5) during larval rearing.

and beyond the juvenile stage.

3.6. Optimisation of feeding

3.6.1. Identification of feed preference

Since there was no information available on larval rearing of this species, the first few trials were undertaken to identify feed preferences of the larvae. Larval survival was obtained in both the trials where nauplii of copepod *P. crassirostris* were used as first feed. One-sample Chi-Square test indicated significant difference (*p* < .01) among the survival on 5th day, 10th day and 15th day. No survival was observed beyond 5th day in the tanks where only rotifers were used for feeding. Maximum survival was obtained in the tank where only copepods were used as first feed. Results are graphically represented (Fig. 6). Larvae of *D. aruanus* and *D. trimaculatus* also could not consume rotifer for first feeding (Gopakumar et al., 2009b). In the trials with a combination of nauplii of *P. crassirostris* and rotifer, the survival rate at 5th day (6.78%) and 10th day (6%) was very low compared to those fed with copepods alone (51.67% and 19.89%). By 15th day there was no survival in tanks were the combination of feeds were used. This is because of the faster multiplication of unconsumed rotifer in the tanks which was also reported by Gopakumar et al. (2009b) during larval rearing of *D. aruanus* and *D. trimaculatus*. At the end of 15 days trial, final survival noticed as 2.56% in trials with *P. crassirostris* and beyond this period no mortality was observed. Analysis of the results clearly indicated that presence of copepods is important for the survival and growth of larvae of *D.*
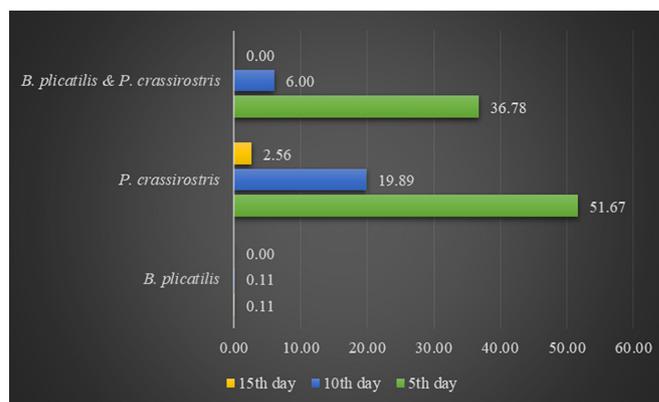

Fig. 6. Percentage survival of *D. carneus* larvae (*n* = 300) using different live feeds for 15 days; a combination of rotifer *Brachionus plicatilis* (5 mL⁻¹) and copepod *Parvocalanus crassirostris* (5 mL⁻¹), nauplii of *P. crassirostris* alone (10 mL⁻¹) and *B. plicatilis* alone (10 mL⁻¹).
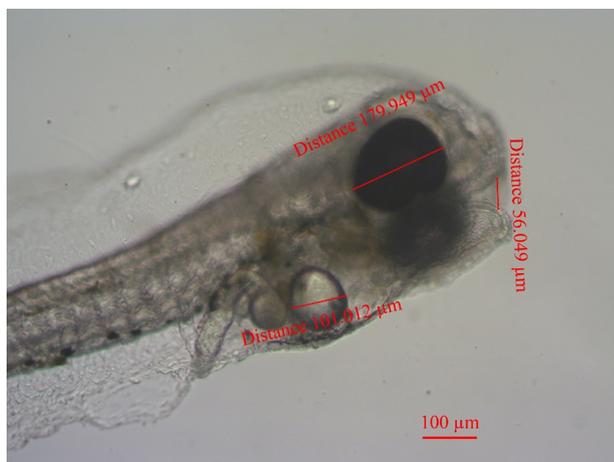
Fig. 7. Larval mouth opening of *D. carneus* at 28th hour (1.16 dph).

carneus. It is also confirmed that copepods are essential as first feed for larvae. Brough et al. (2018) clearly indicated that the larvae of *D. carneus* are difficult to rear. This is true in case of almost all species of *Dascyllus* and survival was noticed only when the first feed used was copepod nauplii (Danilowicz and Brown, 1992: Gopakumar et al., 2009b). Trials with mixed feeding of copepod and rotifer failed to get survival after 15 days indicating that the rotifer could not support the feeding of larvae in later stages also. *D. aruanus* and *D. trimaculatus* larvae survived only when copepod alone was used as their first feed (Gopakumar et al., 2009a).

Copepods have a highly relevant and rich biochemical profile suited for the proper development of most of the marine fish larvae and highly unsaturated fatty acids such as DHA and EPA are present in the most appropriate ratios in copepods suitable for fish larval development (Sun and Fleeger, 1995; Stottrup and Norsker, 1997; Sargent et al., 1997; Olivotto et al., 2008). Consumption of these essential fatty acids can reduce the chances of morphological abnormalities of fish larvae (Satoh et al., 1989; Satoh and Takeuchi, 2009). Moreover, the characteristic moving pattern 'pause and move' of copepod nauplii makes them more vulnerable prey for initial feeding. Copepods can improve health, reduce abnormalities in growth, increase stress tolerance, enhance development and improve pigmentation and growth of fish larvae (Bell

et al., 2003; Copeman et al., 2002; Olivotto et al., 2006a, 2006b, 2008; Vagelli, 2004).

3.6.2. Mouth gape assessment

Mouth opening (Fig. 7) was noticed at the age of 1.16 dph (28th hour and mouth gape was measured as 0.1 ± 0.04 mm which subsequently increased into 1.08 ± 0.08 mm by 17 dph. The measurements were graphically represented (Fig. 8). Larval mouth gape is an important measure of prey size (Shirota, 1970; Munk, 1997; Planas and Cunha, 1999; Ostergaard et al., 2005; Yúfera and Darias, 2007; Carassou et al., 2009). Shirota (1970) reported that the prey size would go up to 50% of the mouth gape, if the larvae could fully expand its lower and upper jaws. Larval fishes having small mouth size, feed mainly on phytoplankton, protozoa and the nauplii of small copepods. Among these, copepod nauplii forms the major food for first feeding of many marine fish larvae (Llopiz and Cowen, 2009; Sampey et al., 2007; Burgess and Callan, 2018).

3.6.3. Prey size observations

Larval mouth gape was measured from the beginning itself. Mouth gape measured was 0.10 mm at the time of opening. The yolk sac got fully absorbed by 3 dph. Gut content on 5th day indicated undigested nauplii of size 83.78–93.3 μm in length and 35.42–45.39 μm in width. The gut contents of 8 dph larvae consisted of both late naupliar stages and copepodites. Late naupliar stages measured around 116.12 μm and copepodites measures between 291.26 and 112.11 μm in length and width varied in between 31.13 μm and 89.62 μm. The gut contents of 11th and 14th days were mainly composed of late copepodite stages which were measured in between 420.25 and 530.42 μm in length and 130.23–156.24 μm in width. At 17th day, remnants of adult copepods were present in the gut contents. The total length measured as 605.96–630.5 μm and width measured as 158.36–165.12 μm. Mouth gape is the common measure of indicator for prey size of fish and fish larvae (Schmitt and Holbrook, 1984: Bremigan and Stein, 1994; De Vries et al., 1998; Gill, 2003; Scharf et al., 2000). It is very clear that the larvae preferred bigger sized copepods at later stages of development. Though, there were sufficient numbers of naupliar stages in the tank, the larvae preferred large sized copepods towards later stages of their development. Burgess and Callan (2018) indicated that the yellow tang (*Zebrasoma flavescens*) larvae preferred large sized copepods in the later stages of the larval life from 6 dph onwards. However, Jackson and Lenz (2016) indicated the escape reflex of adult *Parvocalanus*
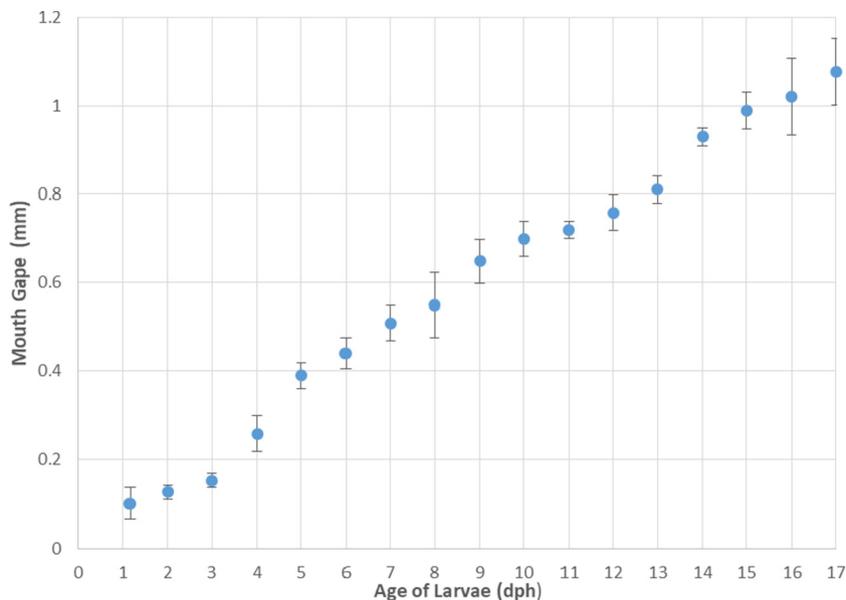

Fig. 8. Daily increment in mouth gape of *D. carneus* ($n = 10$) during initial larval rearing.

Table 1
Physico-chemical parameters during larval rearing.

Physico-chemical parameters	Value (mean ± SD)
Salinity (ppt)	35.43 ± 0.53
Water temperature (°C)	28.35 ± 1.15
Dissolved oxygen (mg L^{-1})	7.5 ± 0.5
Total ammoniacal nitrogen (TAN) (mg L^{-1})	0.37 ± 0.05
NH$_3$-N (mg L^{-1})	0.024 ± 0.007
NH$_4$-N (mg L^{-1})	34 ± 0.04
pH	7.88 ± 0.38

Fig. 10. Juveniles of *D. carneus* 75 dph.

crassirostris is more in comparison to larval strike of *Amphiprion ocellaris*. In addition to mouth gape of larvae, sensory detection and escape reflex of copepod influenced the capture success of larvae at later stages. When the feed was surplus, the larvae preferred bigger sized copepods as feed proportional to their development.

3.6.4. Larval rearing protocol

During the larval rearing, water temperature measured was 28.35 ± 1.15 °C, salinity was maintained at 35.43 ± 0.53 ppt. pH range was 7.88 ± 0.38. Dissolved oxygen varied between 6 and 8 mg L^{-1}. Total ammoniacal nitrogen (TAN) measured was 0.37 ± 0.05 mg L^{-1}, NH3-N measured was 0.024 ± 0.007 mg L^{-1} and NH$_4$-N was in the range of 0.34 ± 0.04 mg L^{-1} (Table 1).

When the larval mouth opened by 28th hour (1.16 dph) with commencement of first feeding, the naupliar stages were filtered out using 60 μm and 35 μm sieve combination. The naupliar stages retained in 35 μm sieve were fed to the larvae up to 8 dph. Copepod density was counted daily and compensated with copepods of required stage from mass culture tanks. The copepodite stages were obtained by serial filtration through a combination of sieves of 100 μm and 60 μm till 10 dph. Similarly, adult copepods were separated using sieve of 170 μm and fed to larvae till 25 dph so that all the larvae surpass the planktonic stage (22 − 23) with least mortality. Once and after the settlement of all the larvae, *Artemia* was used as feed and this continued till all the larvae metamorphosed into juveniles (50 dph). Artificial diet of 500 μm size was used from 33 dph and fishes were completely weaned to artificial feed from 50 dph.

Average survival rate obtained in the optimization of feeding trial was 2.56%, which is the first record on successful larval rearing of *D. carneus*. For *D. albisella* and *D. aruanus*, Danilowicz and Brown (1992) observed survival of 0.1–41.2% using co-culture of algae, rotifer and plankton. Gopakumar and Santhosi (2009) got 3–8% survival at 25th day for *D. aruanus* and 3–4% survival for *D. trimaculatus* using mixed copepod as feed. Gopakumar et al. (2009a), obtained survival rates ranging between 10 and 15% using copepods and rotifers in *D. aruanus* and *D. trimaculatus*. In the present study, only copepods were used till 25 dph and later supplemented with *Artemia* nauplii enriched with microalgae till completion of metamorphosis (50 dph). In the light of studies conducted on mouth gape assessment and prey preference,

specific feeding practice with required size of copepods need to be adopted for getting better survival in the larval rearing of *D. carneus*.

Better survival rates (3.4 to 8.7%) were obtained from successive larval rearing conducted by feeding serial combination of stages of copepods from nauplii to adult as indicated in Fig. 9. Artificial feed was used from 33 dph onwards. Critical period of initial larval survival in *D. carneus* were 3 to 7 dph. Systematic feeding regime was followed in relation to mouth gape suggested a gradual shift of preference in feeding copepods from nauplii at 2 dph to adult at 15 dph. Overlapping regimes were followed in case of copepods and *Artemia* from 25 dph onwards and *Artemia* and artificial feeds from 33 dph and the larvae were completely weaned to artificial diet from 50 dph. The larval rearing protocol has been finalized and summarized in (Fig. 9). Adequate supply of copepods of required size identified by the present experiment holds the key for improving production of cloudy damsel larvae. Both 75 days (Fig. 10) and 150 days (Fig. 11) old juveniles fully resembles adult in colouration and behaviour.

3.7. Size at first maturity

The fishes matured and showed courtship behaviour at 160–182 days in captivity and subsequently started spawning within 10–15 days. The SL and weight of fishes which spawned for the first time was 33 mm and 3 g for male and 28 mm and 2.8 g for female respectively. In the wild, individuals with maturing ovary ranged from 17.7 to 49.2 mm in SL, with mature ovary from 24 to 52 mm and with functional testis ranged from 31.4 to 56.6 mm (Asoh and Yoshikawa, 2003). *D. carneus* was observed to mature and breed at a much smaller size than all its congeners which fully agrees with the observations of Randall and Allen (1977).

4. Conclusion

Development of larval rearing protocols of marine ornamental fishes shall definitely reduce their exploitation from natural ecosystem especially for trade. The popular ornamental fishes of the Genus *Dascyllus*,
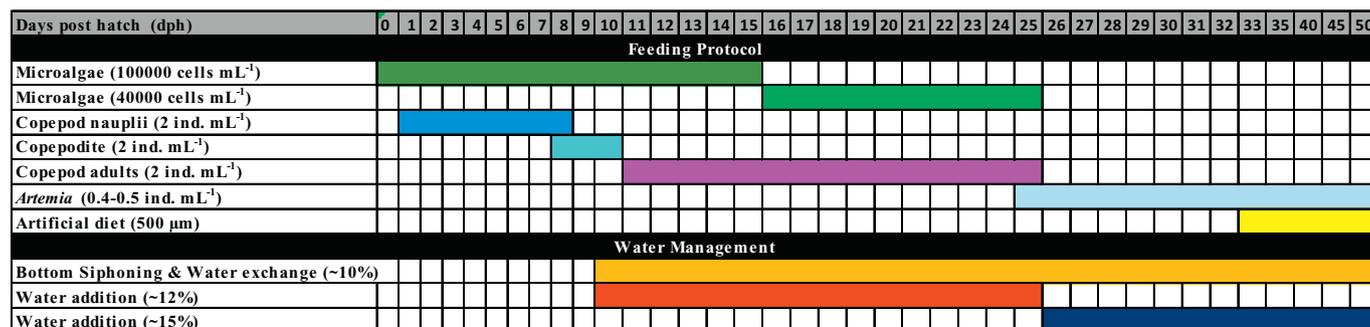
Days post hatch (dph)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	35	40	45	50
Feeding Protocol																																						
Microalgae (100000 cells mL^{-1})																																						
Microalgae (40000 cells mL^{-1})																																						
Copepod nauplii (2 ind. mL^{-1})																																						
Copepodite (2 ind. mL^{-1})																																						
Copepod adults (2 ind. mL^{-1})																																						
Artemia (0.4-0.5 ind. mL^{-1})																																						
Artificial diet (500 μm)																																						
Water Management																																						
Bottom Siphoning & Water exchange (~10%)																																						
Water addition (~12%)																																						
Water addition (~15%)																																						

Fig. 9. Standardised feeding and water management protocol developed for larval rearing of *D. carneus*.

Fig. 11. Juveniles of *D. carneus* 150 dph.

has been found to be one of the most difficult group for seed production due to its smaller eggs and altricial type of larvae. This is the first-ever successful work on broodstock development, breeding, description of developmental stages and larval rearing of cloudy damsel *Dascyllus carneus* under captivity. The present study proved the efficiency of calanoid copepod *Parvocalanus crassirostris* in successful captive rearing of *D. carneus*. Larval gut content in relation to mouth gape helped in determining feed size preferences of larval stages and the larval rearing protocols were finalized accordingly.

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