Asian Fisheries Science **22** (2009): 797-804
ISSN: 0116-6514
E-ISSN: 2073-3720
https://doi.org/10.33997/j.afs.2009.22.2.038

Asian Fisheries Society, Selangor, Malaysia

Controlled Breeding and Larval Rearing Techniques of Marine Ornamental Fishes

G. GOPAKUMAR[1]*, BOBY IGNATIUS[2], I. SANTHOSI[1] and N. RAMAMOORTHY[1]

[1]Mandapam Regional Centre of Central Marine Fisheries Research Institute
Mandapam Camp, Tamil Nadu, India. PIN: 623 520
[2]Central Marine Fisheries Research Institute
P.B. No. 1603, Ernakulam North
Kochi, Kerala, India. PIN: 682 018

Abstract

International trade of marine ornamental fishes has been expanding rapidly in recent years, and the fact that nearly 98% of the species traded are collected from reef habitats is of vital concern for the conservation of the fragile coral reef ecosystem. Hence, it is widely accepted that the ultimate answer to a long-term sustainable trade of marine ornamental fishes is only through the development of hatchery production technologies. The techniques for broodstock development, breeding and seed production of three species of damsel fishes *viz.* the three spot damsel, *Dascyllus trimaculatus*, the humbug damsel, *Dascyllus aruanus* and the blue damsel, *Pomacentrus caeruleus*, were developed and standardised, which can be scaled up for commercial level production. Broodstock development was done in one-tonne Fiber Reinforced Plastic (FRP) tanks with biological filter and by feeding with natural feeds. The size range of broodstock fish of *D. trimaculatus, D. aruanus* and *P. caeruleus* were 9-10, 7-8 and 7-9 cm, respectively. The number of eggs per spawning ranged from 5000 to 15000. The interval between two successive spawnings ranged from 3 to 14 days. The eggs were attached either on the sides of the broodstock tank or on the substratum provided in the broodstock tank. Parental care by the male was noted. Hatching occurred on the evening of the fourth day of incubation. The larvae were altricial type with no mouth opening at the time of hatching for *D. trimaculatus* and *D. aruanus*. The larvae of *P. caeruleus* were with mouth opening at the time of hatching. The length range of newly hatched larvae was 1.5-2.5 mm and the range of mouth opening was 150-200 µ.

Larviculture was done in five-tonne capacity FRP tank by employing greenwater technique. Copepod nauplii were used as the starter feed and after about two weeks when the mouth opening of the larvae had reached around 450 µ, newly hatched *Artemia* nauplii were supplemented. The metamorphosis period ranged from 20 to 40 days. Several batches of the three species were hatchery produced, and the technique can be scaled up for commercial level production for ornamental fish trade.

*Corresponding Author. Tel: +91 5942 247 280
Email : drggopakumar@gmail.com

Introduction

It has been reported that nearly 1500 species of marine ornamental fishes are traded globally, and most of these species are associated with coral reefs. Nearly 98% of the marine ornamental fishes marketed are wild collected from coral reefs of tropical countries. The fishing methods, which destroy the fragile corals, and over harvesting of the species in demand are the vital problems associated with the trade. It is widely accepted that the ultimate answer to a long-term sustainable trade of marine ornamental trade can be achieved only through the development of hatchery production technologies (Colette et al. 2003). In this context, it is imperative to develop commercially viable seed production techniques of important species, which are in demand for the long-term sustainability of the trade.

Among the commercially traded families of reef fishes, Pomacentridae dominate, accounting for nearly 43%. The damsels contain approximately 235 species worldwide (Allen 1991). The most widely traded pomacentrids in the international market in the recent past include the humbug damsel (*Dascyllus aruanus)*, the three spot damsel (*Dascyllus trimaculatus*) and the blue damsel (*Pomacentrus caeruleus)*. Methodologies can be scaled up to commercial level production for breeding; seed production of these three species of damsel fishes was developed and several trials of seed production were carried out.

Materials and Methods

Broodstock development

Fishes collected (6-8 nos) by traps were introduced in one-tonne FRP tanks for broodstock development. These tanks were fitted with biological filters to maintain the water quality to the optimum level. The filtration rate was about 200 $l.hour^{-1}$. The range of water quality parameters of the broodstock tanks were as follows:

Temperature –25ºC-29.5ºC	pH – 8.3-8.6
Salinity – 28-31 ppt	Dissolved oxygen – 4.5-5.1 $mL.l^{-1}$

Water in the broodstock tanks was exchanged @30% once in a week. The broodstock tanks were kept under translucent roofing to reduce the light intensity. Feeding of the fishes was done once in a day @ 5-10% of the body weight. Finely chopped fishes, shrimps and molluscan meat were given as feed to the broodstock fishes. Substrata were provided in the broodstock tanks for the attachment of eggs during spawning.

Live feed culture

Live feeds such as microalgae and copepods were cultured separately to maintain required densities of greenwater and zooplankton in the larval rearing tanks to feed the

damsel fish larvae during initial larval phase. Pure cultures of microalgae *Nanochloropsis* sp. were maintained in indoor culture rooms by employing standard methods. These cultures were then scaled up in outdoor algal production facility to the required volume.

Hatching and larval rearing

The substratum with egg clutch was transferred to the larval-rearing tanks containing seawater having the same physicochemical characteristics of the parent tank. A gentle airflow was created over the eggs by placing an air stone near to the egg clutch, and egg clutch was left in darkness. Hatching took place on the night of third day of incubation. In some cases, the eggs were hatched in the broodstock tank, and the newly hatched larvae were introduced into larviculture tanks. Larval rearing was carried out in five-tonne FRP tanks. The inner side of the tank was light blue in colour in order to have a better contrast between the live feed and the surroundings. The range of water quality parameters in the larviculture system were as follows:

Temperature – 27ºC-31.5ºC pH – 7.5-8.6

Salinity – 28-34 ppt; Dissolved oxygen – 4.5-5.1 $mL.L^{1}$

Greenwater technique using microalgae *Nannochloropsis* was adopted for the larval rearing of damselfishes. The adults of two species of copepods *viz.*, *Euterpina acutifrons* and *Pseudodiaptomus serricaudatus* were inoculated into the greenwater at a density of 50 l^{-1}. When the copepods have started their growth phase, as was noted by counting the number of egg-bearing copepods and nauplii per 50 ml, the newly hatched larvae were introduced into these tanks. Approximately 2000 larvae of each species were introduced into the respective tanks. Larviculture was done in water with different cell counts of microalgae and the larval survival was noted on 20 day of post hatch (dph) (in the case of blue damsel on 15 dph). One set of experiment was conducted by employing copepods alone as live feed and another set was conducted by employing copepods and rotifers together as live feed. The density of live feeds in the tanks was examined everyday and adjusted to the desired level by adding from the cultures, maintained separately. The range of values given under each set is based on the results of three trials.

Results

Broodstock development and spawning

All the three species of fishes spawned in captivity after 4-8 months of maintenance in the broodstock tanks. Prior to spawning, the parent fishes actively cleaned the site for attaching the eggs by rubbing it with their pelvic fins and picking off any loose particles or algae with their mouths. During spawning, females attached their eggs on the cleaned site, which were immediately fertilised by the males. Spawning occurred

during the morning hours. The development of egg took place in 3 days at 28˚C. During this period, the parent fishes took care of the eggs by protecting them and by fanning them with the pectoral fins and tail.

D. trimaculatus and *D. aruanus* are dioecious, and the mature fish ranged in size 9-10 cm and 7-8 cm total length (TL), respectively. In a single spawning, 8000-10000 eggs in the case of the former and 12000 to 15000 eggs in the case of latter were present. The eggs were attached either to the sides of the tanks or on the substrata provided inside the broodstock tanks. The average periodicity of spawning was 2 weeks. Parental care by the male was noted. The eggs were oval in shape.

P. caeruleus is protogynous and polygamous. The mature fish ranged in total length from 7 to 9 cm. Approximately 5000-6000 eggs were present in a single spawning. The eggs were attached on the substrata provided inside the broodstock tanks. The average periodicity of spawning ranged between 3 and 12 days. Parental care by the male was noted. The eggs were oval in shape.

Larval rearing

D. trimaculatus & *D. aruanus:* Larvae were altricial type with no mouth opening at the time of hatching. The average length of newly hatched larvae was 2.5 mm and 2.4mm, respectively. The larvae were transferred to five-ton capacity round FRP tanks in which mixed culture of two species of copepods *viz.*, *P. serricaudatus* and *E. acutifrons* were maintained in greenwater. Mouth opening was formed on the second day, and the gape measured around 150 µm in *D. trimaculates* and 160 µm in *D. aruanus*. The larvae started feeding from the third day of hatching. The results of the larviculture systems experimented with copepods as live feed and the combination of copepods and rotifer as live feeds are given in Tables 1-4. The highest number of egg bearing copepods and nauplii in the larviculture system and the maximum larval survival was noted when the cell count of the greenwater was maintained at a range of 1 x 10^5 cells-6 x 10^5 cells. mL^{-1}. After 20 days when the average size of the larvae had reached around 4 mm with average mouth gape of around 450 µm, freshly hatched *Artemia* nauplii were fed *ad libitum*. Thereafter, no mortality was noted. The larvae started metamorphosing from 35^{th} day of hatching and all the larvae metamorphosed by 40^{th} day. The just metamorphosed young one measured from 12 to 13 mm in length. In the case of *D. aruamus*, the metamorphosis started from 25 dph and completed by 31 dph, young ones measured 8.0-8.5 mm in length.

Table 1. Larviculture of *D. trimaculatus* with copepods

Sl. No.	Range of cell count of green water cells mL^{-1}	Range of egg-bearing copepods nos. mL^{-50}	Range of nauplii nos. mL^{-50}	Larval survival (20 dph)
1	$1x10^4$ - $6x10^4$	1-2	2-4	0-1%
2	$1x10^5$ - $6x10^5$	7-97	35-203	3-4%
3	$1x10^6$ - $6x10^6$	2-4	2-6	0-2%

Table 2. Larviculture of *D. trimaculatus* with combination feed of copepods and rotifers

Sl. No.	Range of cell count of green water cells mL^{-1}	Range of egg-bearing copepods nos.mL^{-50}	Range of nauplii nos. mL^{-50}	Range of rotifers nos. mL^{-1}	Larval survival (20 dph)
1	$1x10^4$ - $6x10^4$	Nil	Nil	2-8	Nil
2	$1x10^5$ - $6x10^5$	Nil	Nil	4-14	Nil
3	$1x10^6$ - $6x10^6$	Nil	Nil	8-20	Nil

Table 3. Larviculture of *D. aruanus* with copepods as live feed

Sl. No.	Range of cell count of green water cells mL^{-1}	Range of egg bearing copepods nos. mL^{-50}	Range of nauplii nos. mL^{-50}	Larval survival (20 dph)
1	$1x10^4$ - $6x10^4$	1-2	2-5	0-1%
2	$1x10^5$ - $6x10^5$	1-109	3-273	3-8%
3	$1x10^6$ - $6x10^6$	1-4	2-8	0-3%

Table 4. Larviculture of *D. aruanus* with copepods and rotifers as live feed

Sl. No.	Range of cell count of green water cells mL^{-1}	Range of egg-bearing copepods nos.mL^{-50}	Range of nauplii nos. mL^{-50}	Range of rotifers nos. mL^{-1}	Larval survival (20 dph)
1	$1x10^4$ - $6x10^4$	Nil	Nil	2-10	Nil
2	$1x10^5$ - $6x10^5$	Nil	Nil	6-12	Nil
3	$1x10^6$ - $6x10^6$	0-1	Nil	10-20	Nil

***P. caeruleus**:* The newly hatched larvae measured approximately 1.2 mm with an average mouth gape of 200 µ. The larvae were transferred to five-tonne capacity FRP tanks in which greenwater was developed and a mixed culture of copepods (*P. serricaudatus* and *E. acutifrons*) was maintained. The results of the larviculture trials with copepods and combination of copepods and rotifer as live feeds are given in Tables 5 and 6, respectively. The highest number of egg bearing copepods and nauplii in the larviculture system and the maximum larval survival was noted when the cell count of the greenwater was maintained at a range of 1 x 10^5 cells-6 x 10^5 cells mL^{-1}. After 15 days, freshly hatched *Artemia* nauplii were also supplemented. Thereafter, no mortality was noted. The larvae started metamorphosing from the 17th day and by 21st day all of them metamorphosed. The average length of just metamorphosed juvenile was 21 mm.

Table 5. Larviculture of *P. caeruleus* with copepods as live feed

Sl. No.	Range of cell count of green water cells mL^{-1}	Range of egg-bearing copepods nos.mL^{-50}	Range of nauplii nos. mL^{-50}	Larval survival (20 dph)
1	1 x 10^4 - 6 x 10^4	0-2	0-2	0-1%
2	1 x 10^5 - 6 x 10^5	7-41	23-132	3-4%
3	1 x 10^6 - 6 x 10^6	2-4	1-4	0-2%

Table 6. Larviculture of *P. caeruleus* with combination of copepods and rotifers as live feed

Sl. No.	Range of cell count of green water cells mL^{-1}	Range of egg-bearing copepods nos.mL^{-50}	Range of nauplii nos. mL^{-50}	Range of rotifers nos. mL^{-1}	Larval survival (20 dph)
1	1 x 10^4 - 6 x 10^4	Nil	Nil	5-10	Nil
2	1 x 10^5 - 6 x 10^5	Nil	Nil	8-18	Nil
3	1 x 10^6 - 6 x 10^6	0-1	Nil	10-18	Nil

Discussion

The global marine ornamental fish trade has been increasing and hence in recent years, research and development on breeding and seed production of marine ornamental fishes has also gained momentum. It is well understood that the key factors for the successful larviculture of marine finfishes depend chiefly on the appropriate size and nutritional quality of live feeds employed. Among the marine ornamental fishes, the first success was achieved in the breeding and seed production of clownfishes, as their larviculture protocols are comparatively easy (Hoff 1996). In India also the first success

was in the development of hatchery techniques of clownfishes (Gopakumar et al. 2001; Ignatius et al. 2001; Madhu and Rema 2002). Experimental success was also obtained in the breeding and larval rearing of damselfishes (Gopakumar et al. 2002). Olivetto et al. (2003) reported successful larval rearing of the pomacentrid *Chrysiptera parasema*. The most critical aspect of larviculture of pomacentrids other than clownfishes is the underdeveloped state of larvae at hatching and the consequent problems of starter feed. The three species of damselfishes studied were with altricial type of larvae and the mouth gape of newly hatched larvae ranged from 150 to 200 µ. In trials on feeding with the available strain of the rotifer *Brachionus rotundiformis* as starter feed, the larvae survival was not successful. The co-feeding of the selected two species of copepods *viz.*, *P. serricaudatus* and *E. acutifrons* in greenwater along with larvae yielded positive results. The small size of the first naupliar stages of the copepods employed and the availability of different sizes of nauplii during the initial phase of larviculture would have sustained the first exogenous feeding of the larvae. The initial stages of nauplii noted in the larviculture system measured from 60 to 80 µ, which is suited for the first feeding of the larvae. The high EPA, DHA and ARA content of copepods also would have facilitated the larval survival and growth (Stottrup 2003).

The maintenance of copepods in multiplicative phase in the larviculture system is the crucial factor for the survival of the larvae. An optimum cell count of greenwater was found to be required for the larval rearing which is found to be $1 \times 10^5 - 6 \times 10^5$ mL^{-1}. The cell count range of $1 \times 10^4 - 6 \times 10^4$ cells. mL^{-1} would have been too low for the multiplication of the copepods. The cell count range of 1×10^6-6×10^6 appears to be too high as it would have affected the filter feeding of the copepods. Hence, the cell count range $1 \times 10^5 - 6 \times 10^5$ cells mL^{-1} appears to be optimum for multiplication as was indicated by the maximum number of egg-bearing copepods and nauplii. The naupliar count alone cannot be taken as an indicator of multiplication due to the fact that most of the newly hatched nauplii will be fed by the larvae. The better survival of the larvae can be directly attributed to the availability of freshly hatched nauplii, which was indicated by the abundance of egg-bearing copepods and nauplii in the larviculture system. It is believed that survival rates could be further enhanced if the copepods in the larviculture system could be kept at optimum production level. The optimum cell count of 1×10^5-6×10^5 mL^1 for greenwater was maintained in the larval system by adding fresh cultures of phytoplankton after checking the cell density.

The larviculture trials with copepods and rotifers as live feeds were not successful. The rotifers multiplied rapidly by parthenogenesis and filled the system. The copepods being sexually reproducing could not keep pace with the rotifer multiplication and were rapidly eliminated from the system. The larvae of the species experimented were unable to accept rotifers as starter feed which resulted in total mortality of the larvae. It is also noted that the critical phase of larviculture was over by 15-20 dph. After 15-20 dph, the

mouth gape had reached around 450 µ and can be fed with freshly hatched *Artemia* nauplii. The absence of any mortality from this stage onwards indicated that if the larvae could be fed with suitable feed initially, the larviculture of these species could be accomplished easily with conventional live feeds.

References

Allen, G.R. 1991. Damselfishes of the world. Mergus Publishers, Melle, Germany, 27 pp.

Colette, W., M. Taylor, E. Green and T. Razak. 2003. From ocean to aquarium: a global trade in marine ornamental species. UNEP World conservation and monitoring centre (WCMC), 65 pp.

Gopakumar, G., Rani Mary George and S. Jasmine. 2001. Hatchery production of the clown fish *Amphiprion chrysogaster*. In: Perspectives in mariculture (ed. N.G. Menon and P.P. Pillai), pp. 305-310. Marine Biological Association of India, Kochi, India.

Gopakumar, G., G. Sreeraj, T.T. Ajithkumar, T.N. Sukumaran, B. Raju, C. Unnikrishnan, P. Hillary and V.P. Benziger. 2002. Breeding and larval rearing of three species of damselfishes (Family: Pomacentridae). Marine Fisheries Information Service 171:3-5.

Hoff Jr, H.F. 1996. Conditioning, spawning and rearing of fish with emphasis on marine clownfish. Aquaculture Consultants Inc., Dade City, Florida, USA.

Ignatius, B., G. Rathore, D. Kandasamy and A.C.C. Victor. 2001. Spawning and larval rearing techniques for tropical clownfish *Amphiprion sebae* under captive conditions. Journal of Aquaculture in the Tropics 16(3):653-662.

Madhu, K. and M. Rema. 2002. Successful breeding of common clown fish under captive conditions in Andaman and Nicobar Islands. Fishing Chimes 22(9):16-17.

Olivetto, I., M. Cardinali, L. Barbaresi, F. Maradonna and O. Carnevali. 2003. Coral reef fish breeding: the secrets of each species. Aquaculture 224:69-78

Stottrup, J.G. 2003. Production and nutritional value of copepods. In: Live feeds in marine aquaculture (ed. J.G. Stottrup and L.A. McEvoy), pp. 145-205. Blackwell Publishing company, Oxford, UK.

Received: 07 December 2007; Accepted: 13 March 2009