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Archana Durgude
AFDO, Mumbai, Maharashtra, India

Dabir Pathan
Associate Professor, College of Fisheries, Ratnagiri, Maharashtra, India

Nikhil S Sawant
SRF, College of Fisheries, Ratnagiri, Maharashtra, India

Pankaj Patil
Scientist, ICAR-CIBA, Gujarat, India

Gauri Shelar
RA, ICAR-CIFE, Mumbai, Maharashtra, India

Corresponding Author:
Archana Durgude
AFDO, Mumbai, Maharashtra, India

Effect of stocking densities on reproductive performance of black molly, *Poecilia sphenops* in cages

Archana Durgude, Dabir Pathan, Nikhil S Sawant, Pankaj Patil and Gauri Shelar

Abstract
The majority of hobbyists prefer low valued and attractive fish species for keeping in aquarium. Considering the nature, the fishes like molly have potential market. Such fishes are bred and cultured in controlled conditions to satisfy the market demand. Knowledge on optimal stocking density, nutritional requirements, appropriate feeding methods, water quality management and health management are prerequisite for reproduction and culture of ornamental fishes. Thus attempts were made to find out the breeding performance, larval growth and survival of black molly at different stocking densities. In the study, Brood fishes of *Poecilia sphenops* were reared at the different densities of 50, 100, 150, 200 and 250 fishes m^{-2} in cages with sex ratio of 4 females: 1 male for a period of 90 days. Cages were suspended in all- glass tanks. Water level and water volume in each glass tank was maintained up to 25cm and 45L, respectively. Brood fishes were fed with flake feed containing 39.48% protein. The stocking density of 50 brood fishes m^{-2} in cage (0.5 x 0.2 x 0.2 m) for *P. sphenops* is suitable for achieving higher fry production rate.

Keywords: Black molly, breeding cages, stocking density, reproductive performance

Introduction
India has a great biodiversity which includes variety of fishes suitable for ornamental purposes. However, India ranks 31st (2016) in the World ornamental fish trade and contribute 1.6 million US$ [1]. World's ornamental fish trade is approximately about US$ 15 billion and is predicted to increase by 8% with the increasing demands of developed countries [1, 2]. Considering India's freshwater and marine resources it has tremendous scope for development and can come out to be a leading country in ornamental fish trade in following years. Therefore India is called as "sleeping giant". Developing countries are the major producers and suppliers of ornamental fishes globally. More than 60% ornamental fishes are freshwater and remaining are from marine resources. Marine ornamental fishes are usually wild caught while fresh water fishes are bred and grown by many aquarists, farmers and hobbyists. However, most of the ornamental fishes popular among the freshwater aquarists belong to eight families such as *Anabantidae*, *Callichthyidae*, *Characidae*, *Cichlidae*, *Cobitidae*, *Cyprinodontidae*, *Cyprinidae* and *Poeciliidae* (live-bearers) [3].

Ornamental fish keeping and its breeding is an interesting activity providing not only aesthetic pleasure but also employment opportunity both in rural and urban sector. It is environment friendly and socially acceptable activity. It involves low investment with short gestation period. It could be adopted as small-scale back-yard enterprise to export oriented commercial production systems. From the business point of view, the ornamental fish breeding and culture is reaching new heights and has gained accelerated momentum. Thus, more and more people are entering into this flourishing business to earn foreign exchange for the country.

Reproductive success in many fish species is influenced by quality of broodstock, sex ratio, stocking density, age, size, health, water quality, production, nutrition and feeding regime as observed by research workers such as [4-11]. Knowledge on optimal stocking density, nutritional requirements, appropriate feeding methods, optimal water quality and its management along with health management is a pre- requisite for reproduction and culture of ornamental fishes. Therefore, the present study was carried out to find out the effects of stocking density of brood fishes of black molly, *Poecilia sphenops* on larval production in cages.

Materials and Methods

The experiment was conducted in the Wet laboratory of Department of Aquaculture, College of Fisheries, Shirgaon, Ratnagiri. In the present study, *Poecilia sphenops* Valenciennes, 1846 commonly known as black molly was used to study its breeding performance, larval growth and survival.

Sexual dimorphism

Body slender, with long and pointed modified anal fin which serve as the male's copulatory organ, called 'Gonopodium'. Dorsal fin is somewhat larger than that of female fish. Male is smaller than female and grow up to 8 cm [3].

Body bulgy with rounded anal fin. The dorsal fin of the female is somewhat smaller than that of male fish. Female is larger than male and grow up to 12 cm [3].

Cage design

Rectangular shaped cage frame (0.5 m X 0.2 m X 0.2 m) made of PVC pipe were used to hold the brood fishes in the breeding tank. The cage bag (nylon cloth of 4 mm mesh size) was provided with top cover to prevent fish escape (Plate 1a and 1b). The cage bag was tied to PVC pipe frame with the help of nylon thread in such a way that the net remained stretched completely. The cages were suspended in experimental tank to maintain a bottom clearance of 5 cm between floor of cage and bottom glass of tank.

Experiment container

Rectangular glass tanks having dimension of 0.6 x 0.3 x 0.3m with 54L capacity were used. During the experiment water level and water volume in each glass tank was maintained up to 25cm and 45L, respectively.

Experimental procedure

The experiment was conducted to evaluate the number of larvae produced per female of *P. sphenops* at different stocking densities. *P. sphenops* females with length in the range from 5.3 to 5.6cm and weight in the range from 2.40 to 2.65g and male length in the range from 4.2 to 4.6cm and weight in the range from 0.84 to 0.89g were stocked in glass aquaria at different stocking densities viz.,

T_1:5 brood fishes cage^{-1},
T_2:10 brood fishes cage^{-1},
T_3: 15 brood fishes cage^{-1}
T_4:20 brood fishes cage^{-1} and
T_5: 25 brood fishes cage^{-1}, equivalent to 50, 100, 150, 200 and 250 fishes m^{-2} respectively with the sex ratio of 4 female: 1 male [12]. The experiment was conducted for a period of 90 days with four replicates for each stocking density following Completely Randomized Design (CRD). Brood fishes were fed with formulated feed (Table 1) two times (10.00 and 17.00h) a day@ 8-10% of body weight.

Cage maintenance and siphoning of tank

The tanks were cleaned with the soft scrubber and about 30% of water was exchanged daily from each tank.

Water quality parameters

Water quality parameters such as temperature, pH, dissolved oxygen, free carbon dioxide, total alkalinity and total hardness were measured by following standard methods [13, 14] and were maintained for 90 days (Table 2).

Growth parameters

At the beginning and end of each experiment, the fish were counted and their individual length and weight were recorded. The average value of weight and length were calculated for analysis of growth parameters. Weight of each fish was taken on mono-pan electric (Sartorius, BS 224S) balance having an accuracy of 0.01 mg. Each fish was kept on blotting paper in order to remove excess water and moisture from the body. Then each blotted fish was taken on plastic paper and weight was recorded.

1. $\text{Length gain (\%)} = \frac{(\text{Final length} - \text{Initial length})}{\text{Initial length}} \times 100$
2. $\text{Weight gain (\%)} = \frac{(\text{Final weigth} - \text{Initial weigth})}{\text{Initial weigth}} \times 100$
3. $\text{Specific Growth Rate (\%)} = \frac{(\text{Log Wt} - \text{Log Wo})}{\text{dt}} \times 100$

Where, W_t = Final weight; W_o = Initial weight; dt = Rearing period in days

Data obtained from the experiments for reproductive performance ie. Number of young ones produced and growth parameters were analysed by one way ANOVA. Significant difference was indicated as $P < 0.05$, Student's Newman Keul (SNK) multiple range tests was used to determine the significant difference between the treatments [15, 16].

Results and Discussion

Number of fry produced per female, length gain (%), weight gain (%), Specific growth rate (SGR %) and survival (%) over a period of 90 days is presented in Table 3.

Black molly is preferred over other relatively costly ornamental fishes because of its low price, hardy nature and compatibility with other ornamental fishes. In the coastal region of Maharashtra and mainly inland areas, it can be bred easily in captive environments. Mass breeding and rearing activities have been recently initiated in this region. Considering its live bearing nature specially designed brooder cages were used to assess the breeding performance of the fish in the present study. In order to know the reproductive performance of the brood fish, male to female ratio 1: 4 was maintained [12].

Most of the live bearers belong to the family *Poecilidae* are non-guarders and do not show parental care and they often tend to eat their young-ones. In order to save young-ones, aquaria need to be densely populated to get suitable hideouts for them. A type of net barrier to separate out brooders and delivered young-ones is a promising way of achieving maximum number of young fishes. Young-ones of guppies produced by using breeding cages [17], breeding net-cages used for sword tail seed production [18]. While the practice of breeding food fishes using hapas for *Oreochromis aureus* [9], for *Oreochromis niloticus* [19]. Wooden netting frame was used for breeding of shol, *Channa striatus* [20]. Thus, the use of cages and hapas can be said as an effective tool of getting young-ones or eggs to their maximum extent.

A due consideration is often given in choosing the sex ratio of a species in the most fish breeding programmes. The food fishes like tilapia, *Oreochromis aureus* required sex ratio of 1:1 [9], and for *Oreochromis niloticus* 1 male: 3 female [19] and 1:1 sex ratio [20]. In ornamental fish breeding, sex ratio from 1: 1 to 1: 10 have used sword tail [18] and guppy fish [17] respectively. Sex ratio of 1 male: 4 Female for breeding of

molly was found to be suitable for larval production [12]. The sex ratio of 1: 4 used in the present study was found to be at par with above mentioned studies.

In the present study among the selected stocking densities the average number of fry produced per female was observed maximum at the lowest stocking density 50 brood fishes m^{-2}. With regard to the breeding performance of guppy [21], observed number of young-ones per female was the maximum at the lowest stocking density of 1 male: 3 female ratio. The maximum reproductive performance in the form of the highest number of young-ones in the lower stocking density of brood fishes of guppy stocked at densities of 15 fish (10 female: 5 male) [22]. Better reproductive performance at lower stocking density i.e. 50 m^{-2} in concrete indoor tank for hybrid tilapia (*O. niloticus* x *O. aureus*) [23]. *Oreochromis aureus* brood stock at the density of 6 fish m^{-2} and the sex ratio of 2 male: 1 female showed maximum seed production [9]. Maximum seed production at lower stocking density [19] and the best performance in terms of spawning at lower density i.e. 6 fish m^{-2} at the sex ratio of 1: 1 for shol, *Channa striatus* [20] was observed. The variation in the results of the above mentioned studies with the present study may be due to the variation in species, stocking densities, feed composition, feed type, feeding frequency and rate, culture period and culture system.

In brood stock management apart from stocking density nutrition is the key aspects which affects the breeding and reproductive performance of fishes. Effect of dietary protein levels on fecundity and fertility was observed for guppy [24, 25], for *Betta splendens* [26], for sword tail [4] and for *Pterophyllum scalare* [11]. Overall observation of these studies indicated that dietary protein level from 30 to 50% is suitable for better breeding performance. Therefore, a semi- purified flake feed was formulated and prepared (39.48%) and fed to molly fish @ 8-10% of body weight day $^{-1}$.

Table 1: Ingredients and proximate composition of flake feed used for the experiment

Feed Ingredients	Proportion Of Each Ingredient In Diet (G $100g^{-1}$)
Fish meat powder	20.0
Casein [b]	25.2
Gelatin [b]	3.0
Cod liver oil	7.9
Dextrin [b]	29.8
Carboxy Methyl Cellulose [b]	1.5
Cellulose [b]	7.6
Vitamin Mixture [c]	3.0
Mineral mixture [d]	2.0
Proximate Composition Of Feed	
Moisture (%)	4.5
Crude Protein (%)	39.48
Crude lipid (%)	4.96
Crude ash (%)	4.4
Crude fiber (%)	3.83
NEF[e]	42.83
Gross energy (MJ kg^{-1})[f]	1893.20

Where,

a. Fish meat powder: CP-70.09%; CL- 8.58%; moisture – 9.84%; total ash—7.07%; and fiber – 4.44%.
b. Casein: Himedia, USA; Protein- 80-95%; glucose – 0.5%; lactose – 1%; Free acids – 1-1.5%, Water soluble compounds – 3%; Fat content – 0.5-1%.
c. Vitamin: Becosules capsules, Pfizer Ltd. India; Thiamine mononitrate IP-10mg g^{-1}, Riboflavina IP – 10mg g^{-1}, Pyridoxine Hydrochloride IP – 3 mg g^{-1}; Vitamin B12 IP-15 mcg g^{-1}; Niacinamide IP -100mg g^{-1}, Calcium Pantothenate IP – 50mg g^{-1}, Folic acid IP – 1.5mg g^{-1}, Biotin USP – 100mcg g^{-1}, Ascorbic acid IP – 150mg g^{-1}.
d. Mineral: Agrimin, Virbac Animal Health India Pvt Ltd.; Cobalt-50mg kg^{-1}, copper – 1200mg kg^{-1}, Iodine – 325mg kg^{-1}, Iron – 5000mg kg^{-1}, Magnesium – 6000mg kg^{-1}, Manganese – 1500mg Kg^{-1}, Potassium – 100mg kg^{-1}, Selenium – 10mg kg^{-1}, Sodium – 5.9 mg kg^{-1}, Sulphur – 9.220g kg^{-1}, Zinc - 9600 mg kg^{-1}, DL-Methionine - 1920 mg kg^{-1}, L-lysine mono hydrochloride - 4400 mg kg^{-1}, Calcium – 240g kg^{-1}, Phosphorus - 120g kg^{-1}.
e. NFE = (100) – [Crude protein (%) + Crude lipid (%) + Ash (%)]
f. GE = (Protein × 0.24) + (Lipid × 0.40) + (Carbohydrate × 0.17) (kJ/g)

Table 2: Water quality parameters maintained experimental period of 90 days

Water Quality Parameters	Mean observed value
Temperature (°C)	26.99 ± 0.3291
pH	7.81 ± 0.860
Total hardness (mg L^{-1} as $CaCO_3$)	192.29 ± 0.6828
Total alkalinity (mg L^{-1})	79.93 ± 0.3701
Dissolved oxygen (mg L^{-1})	4.96 ± 0.0427
Free carbon dioxide (mg L^{-1})	9.49 ± 0.6028

Values expressed as ± S.E. of mean

Table 3: Average number of fry per tank, average number of fry per female and survival of female of *T. leerii* stocked at different densities for 90 days

Particulars	T_1	T_2	T_3	T_4	T_5
Average number of fry/ Tank	94.75 ± 2.02^a	113 ± 3.39^a	133.5 ± 4.35^b	154.75 ± 4.17^b	174.5 ± 3.43^c
Average number of fry/ Female	23.69 ± 0.50^c	14.12 ± 0.43^b	11.12 ± 0.36^a	9.67 ± 0.26^a	8.72 ± 0.17^a
Survival %	75 ± 5	75 ± 2.8	73.33 ± 2.72	75 ± 2.04	72 ± 1.63

Mean values in similar row with different letters are significantly different (SNK, $P<0.05$)

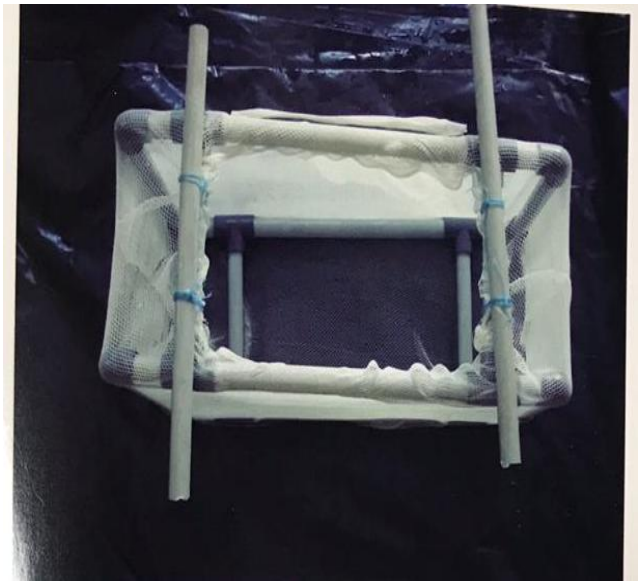

Plate 1a: Cage used for breeding of *P. sphenops* broodfishes

Plate 1b: Rearing of *P. sphenops* broodfishes in cages

Conclusion

Therefore it is concluded that the stocking density of 50 brood fishes m^{-2} in cage (0.5 x 0.2 x 0.2 m) for *P. sphenops* is suitable for achieving higher fry production rate.

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