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BREEDING AND INDUCED TRIPLOIDY IN THE INDIGENOUS ORNAMAENTAL FISH *PSEUDOSPHROMENUS DAYI* (KOHLER, 1908)

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Abstract: *Pseudosphromenus dayi* is an indigenous ornamental fish and its most obvious diagnostic characters are colouration and colour pattern. They can be easily bred in aquarium. The breeding behaviour involves bubble nest making, circling movements, scratching behaviour, leading-to-nest-behaviour, anabantoid embrace, egg extrusion and placing of eggs into the bubble nest. The one male: one female ratio was found to be the best for the species; spawning lasts for 2-3 hours and the number of eggs laid by the female ranges from 100 to 240. Induction of triploidy in *P. dayi* was achieved by heat shock treatment at 38^{0}C for 3 minutes duration on eggs treated 30 seconds after fertilization. The DNA content of triploids was found to be 55.96±0.8476mg/100g of the tissue.

Key words: *Pseudosphromenus dayi*, breeding, heat shock, triploid

INTRODUCTION

Pseudosphromenus dayi is a belontid fish and a distinctive species of relatively small size. The species which is apparently endemic to Kerala was overlooked by several Indian authors (Jayaram, 1981) or was considered as a synonym of *P. cupanus* (Talwar and Ghingran, 1990). *P. dayi* was originally described as *Polyacanthus cupanus var. dayi* by Köhler (1908) and the type locality of the fish was suggested as southern Kerala (Kottelat, 1994). Many species of anabantids are attractively coloured and regarded as good material for display in aquaria. The mating behaviour of anabantids has been described by Amirthalingam (1939), Kulkarni (1943), Hodges and Behre (1953), Axelrod and Schultz (1983), Innes (1956), Forselius (1957a) and many others. *Pseudosphromenus dayi* belongs to Osphronemidae family and exhibits bubble nest building followed by an interesting breeding and parental behaviour.

The induction of triploidy is the most common type of chromosome manipulation in fish and shellfish. It is applied for a variety of aquaculture purposes, but largely relates to its potential association with improved growth and carcass quality for commercial farming purposes. (Pandian and Koteeswaran, 1998). In fish, triploidy is induced by allowing normal fertilization and then forcing retention of the second polar body (Chourrout, 1980; Lou and Purdom, 1984) by applying temperature (hot or cold) or hydrostatic pressure treatments shortly after fertilization (Dunham, 1990).

MATERIALS AND METHODS

Fishes collected from Vellayani Lake, Kerala were transported to the laboratory and stocked in glass aquaria of 44cm×30cm×30cm size, filled with freshwater (temperature of 28^{0}C and pH- 7) and planted with *Pistia stratiotes* L. They were fed with fish meal based dry feed daily and occasionally with tubifex worms and mosquito larvae to enhance maturation of gonads. Though 100% water was exchanged in the tanks on a daily basis, artificial lights and aeration were not provided. All specimens selected for pairing were above 25mm in standard length to ensure maturity. Various combinations of males and females such as 1 male : 1 female; 1 male : 2 females and 2 males : 1 female were tried. For breeding, conditioned males were introduced in the tanks initially fol

lowed by females. The breeding behaviour of fish was recorded with help of videos and the process was photo documented.

For the induction of triploidy, the eggs of 10th to 30th batches were collected using fine meshed net and immersed in preheated water in a water bath. Shock was applied to the eggs just after fertilization. The temperature and the duration of the shock were 38^{0}C and 40^{0}C and 3 to 5 minutes. After treatment the eggs were immediately returned to water at normal temperature (27^{0}C to 30^{0}C) for incubation. One batch of eggs without treatment was used as control for each treatment. Hatchability of eggs and survival of feeding fry were monitored. The water was exchanged daily and feeding was done four times a day. The larvae were fed initially with boiled hen's egg and then green water, chopped tubifex, chopped earthworm and powdered dry pellets. Ploidy was determined by Diphenylamine method of DNA estimation and by observing morphological differences.

RESULTS AND DISCUSSION

Breeding

Pseudosphromenus dayi can be bred easily in the aquarium tank. It shows breeding colouration and courtship behaviour. The first cue indicating readiness to spawn was the nest building behaviour which is almost invariably performed by the male (Fig.1). The bubble nest is usually anchored to water plants especially *Pistia*. If no aquatic plants are available in the aquarium, the nest is built in a corner of the tank. Mills and Vevers (1989) reported that in the case of *P. dayi* the male will build the bubble nest either under a rock cave or at the surface under floating plants. During the present study it was observed that an intensification of colouration had set in indicating readiness to spawn. Similar observations were also made by Padmanabhan (1955) and Sneha *et al*. (2013) in the case of *P. cupanus.*

On the completion of nest building, the male turns its attention to the female beginning with scratching behaviour. A noteworthy observation with regard to the initiation of mating behaviour is that positive initiative is taken by the male. On spotting the female, the male starts chasing and 'leading-to the-nest' behaviour. Mating behaviour begins with the circling movements (Fig.2), leading to 'anabantoid embrace' which results in the extrusion of eggs. Spawning occurs underneath the nest in the typical anabantoid embrace, with the male wrapping itself around the female as eggs and sperm are released simultaneously (Figs.3&4). The female rotates through 180^0 and lies ventral side up. The pair remains motionless in this posture and this is typical "anabantoid embrace". After 2 to 3 seconds, when the pressure is relaxed they slowly sink down to the bottom resulting in the extrusion of a batch of eggs (Fig.5). At the time the eggs are released which were simultaneously fertilized by milt from the male. The fertilized eggs sink down in the water and are immediately picked by the male and blown into the nest (Fig.6).

The first few enfolding in *P. dayi* do not lead to the extrusion of eggs and have been termed 'pseudo spawning' (Forselius, 1957b and Pal and Southwick, 1965). The duration of the mating varies from 2-3 hours. Each enfolding during the mating act has a mean duration of 3 seconds. The interval between successive enfolding ranged from 8-12 minutes. The number of eggs released during a single spawning varied from 100-240. The spawning interval ranged from 2-5 days depending on the temperature; during rainy seasons *i.e*., when temperature was low, spawning was noticed only on alternate days. The spawning frequency depends on the condition of the brooders, the temperature and the food given. Live feeds such as mosquito larvae, tubifex worms and mussel meat enhanced gonadal maturation and spawning (Tamaru *et.al.,* 1997 and Chong *et al.,* 2002). *P. dayi* is a perennial breeder but spawning frequency is more in rainy seasons when temperature is low. It was observed that during the present study the same pair could breed successively at an interval as low as two days.

Even though various combinations of sex ratios were tried during the present study, it was found that one male for one female ratio formed the best breeding pair; similar observations were also made in the case of *P. cupanus* (Sneha *et al.,* 2013). Indira 1986 reported more successful breeding in *P. cupanus* when males were introduced prior to

Fig. 1. Making of Bubble nest by male fish

Fig. 2. Circling movements

Fig. 3. Holding of female by the male

Fig. 4. Anabantid embrace

Fig. 5. Egg extrusion

Fig. 6. Placing of the eggs in bubble nest by male fish

females in the breeding tank. Following these observations in the present study, males were introduced into the breeding tank prior to the females and there was 100% breeding success. The breeding was noticed only during the day time. In the present study males were found to be more active in parental care by guarding the eggs in the nest as well as placing the dropping eggs back to the nest. These observations are in agreement with the breeding behaviour of *P. cupanus* (Jones, 1940; Padmanabhan, 1955; Pal and Southwick, 1965; Indira, 1986; Sneha *et al.*, 2013).

Induction of triploidy

Triploidy was induced to check the variation in the morphology, external appearance and body colouration of triploids compared to their diploid counterparts**.** Induced triploidy of fishes has generated great interest among aqua culturists and the techniques of triploidization by heat shock method has been applied successfully in many species of fishes (Johnstone, 1985 and Varadaraj and Pandian, 1988). The effect of heat on triploidy induction and survival from the heat shock experiment in *P. dayi* is shown in Table1&2. In *P. dayi,* the optimum heat shock treatment based on highest percentage of hatching (46%) and fry survival (8%) after one month was 38^{0}C for 3 minutes duration on eggs treated 30 seconds after fertilization. The optimum duration of treatment was 3 minutes which showed higher survival rate than that of 5 minutes duration.

The eggs hatched out within 32 hours after extrusion. The larval rearing tanks were provided with continuous gentle aeration and water exchange (50% daily). The larvae were fed with egg custard in the first week followed by phytoplankton concentrated from pure culture tanks for the second and third weeks. After the third week the larvae were fed with *Artemia nauplii.* The one month old larvae were fed with mosquito larvae. Afterwards the larvae were fed with chopped tubifex worms, chopped earthworm and powdered flakes of supplementary diet. The amount of DNA in diploid fishes was found to be 34.93 ± 0.3917mg/100g of the tissue and that of triploid fishes were found to be 55.96 ± 0.8476mg/100g of the tissue (Table.3). The induction of triploidy may also cause changes in external morphology and colouration. Mortality of heat shock treated eggs was comparatively higher than that of the control groups. In *P. dayi* major losses irrespective of treatment occurred between hatching and feeding stage as in the case of *Salmo giardneri* (Chorrout, 1980; Chorrout and Quillet, 1982) and *Betta splendens* (Kavumpurath and Pandian, 1992).

Previous studies have shown that in most tropical fishes the extrusion of second polar body can be inhibited 2-5 minutes following insemination, by heat shocking the eggs at 40^{0}C to 42^{0}C (Penman *et al.,* 1987; Varadaraj and Pandian, 1988). In species like *O. mossambicus* (Pandian and Varadaraj, 1988) and *B. rerio* (Kavumpurath and Pandian, 1990) heat shock is effective in inducing 100% triploidy. However, in the present study, heat shock produced 83% triploidy in *P. dayi.* (Table.4).The high level of triploidy achieved in the experiments conducted here confirms the effectiveness of heat shock treatment in suppressing meiosis II in *P. dayi*.

Fig. 7. Diploid fish

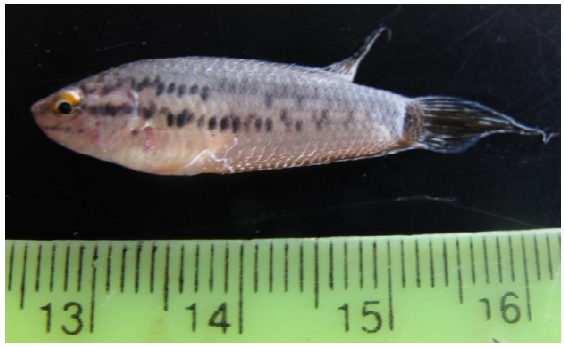

Fig. 8. Triploid fish

Table 1. Details of 3 minutes heat shock treatment for the induction of triploidy in *P. dayi*

SL No	Temperature (°C)	Number of eggs	Time after fertilization (Sec)	Hatch (%)	Fry survival		
					One week	Two week	One month
1	Control(28°C)	60	30	52	24	18	11
	Treated (38°C)	60	30	18	16	14	4
2	Control(27°C)	60	30	47	22	20	14
	Treated (37°C)	60	30	20	20	18	5
3	Control(29°C)	60	30	58	19	13	10
	Treated (39°C)	60	30	28	24	18	5
4	Control(28°C)	60	30	56	27	19	14
	Treated (38°C)	60	30	28	23	17	3
5	Control(28°C)	60	30	49	23	15	11
	Treated (38°C)	60	30	25	20	19	5

Table 2. Details of 5 minutes heat shock treatment for the induction of triploidy in *P. dayi*

SL No	Temperature (°C)	Number of eggs	Time after fertilization (Sec)	Hatch (%)	Fry survival		
					One week	Two week	One month
1	Control(28°C)	60	30	52	24	18	11
	Treated (38°C)	60	30	24	5	5	0
2	Control(27°C)	60	30	47	22	20	14
	Treated (37°C)	60	30	19	9	2	0
3	Control(29°C)	60	30	58	19	13	10
	Treated (39°C)	60	30	25	12	9	1
4	Control(28°C)	60	30	56	27	19	14
	Treated (38°C)	60	30	17	8	4	0
5	Control(28°C)	60	30	49	23	15	11
	Treated (38°C)	60	30	21	5	1	0

Table 3. Amount of DNA estimated using Diphenylamine method in diploid and triploid *P. dayi*

SL.No	DNA content in diploid fish (mg/100g)	DNA content in triploid fish (Mg/100g)
1	34.5	55.6
1'	34.5	55.6
2	34.9	55.7
2'	35.4	57.14
3	35.2	55.71
3'	35.2	55.71
4	34.5	54.4
4'	34.5	55.8
5	35.3	57
5'	35.3	57
AVERAGE	34.93 ± 0.3917	55.966 ± 0.8476

Table 4. Summary of successful triploidy inductions verified by DNA analysis

Duration of heat shock	Temperature	Total number of fry survived		Total Number of triploid fry
		After one month	After four months	
3 minutes	Treated (38^{0}C)	4	1	6 Nos. (83% Triploidy)
	Treated (37^{0}C)	5	2	
	Treated (39^{0}C)	5	2	
	Treated (38^{0}C)	3	0	
	Treated (38^{0}C)	5	1	
5 minutes		No survivors		

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