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HEAT SHOCK INDUCTION OF TRIPLOIDY IN THE INDIGENOUS ORNAMENTAL FISH, *PSEUDOSPHROMENUS CUPANUS* (CUVIER 1831)

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Abstract: The Spike tail Paradise Fish, *Pseudosphromenus cupanus* (Cuvier), a hardy indigenous ornamental fish inhabiting a wide variety of inland aquatic ecosystems in India,can be bred easily in the aquarium tanks. As a typical anabantoid fish it exhibits bubble nest building and interesting breeding behaviour. Induction of triploidy in *P. cupanus* was achieved by heat shock treatment for 3 minutes duration on eggs treated 30 seconds after fertilization, gives 82% hatchingand 8% fry survival. The DNA contentof the triploids was found to be 53.7mg/100g of the tissue.

Key words: diphenylamine method, fry survival, DNA estimation

INTRODUCTION

The Spike tail Paradise Fish, *Pseudosphromenus cupanus* (Cuvier) (Family Osphronemidae) is distributed in the freshwater and brackishwater bodies of India, Sri Lanka, Bangladesh, Myanmar, Malaysia and Indonesia (Froese and Pauly, 2013). This anabantoid or labyrinth fish (order: Perciformes and suborder: Anabantoidei) is distributed in a wide variety of inland aquatic ecosystems as it possesses accessory respiratory organ (labyrinthiform organ) (Jayaram, 2010; Abraham, 2011). Primarily carnivorous in nature, this species is effective in controlling mosquito larvae (Shen *et al.,* 1991) and are exported from India as an ornamental fish (Jayalal and Ramachandran, 2012). Ploidy manipulation of vertebrates by the deletion or addition of whole haploid set of chromosomes dates back to the early part of 19th Century.The first report on induction of triploidy in fish was published by Svardson (1945). Svardson (1945) also experimentally induced triploidy in Atlantic salmon x Brown trout embryos with the cold shock technique that had proven successful in amphibians. The application of heat shock was successful in the ploidy manipulation of warm water fishes like channel catfish (Bidwell *et al.,* 1985) and tilapia (Chourrout and Itskowitch, 1983; Don and Avtalion, 1986; Penman *et al*., 1987; Pandian and Varadaraj, 1987).Genetically manipulated stock shows fast growth rate, better colouration even they will be more attractive than the original stock (Pushpa geetha, 2002).

MATERIALS AND METHODS

Fishes for the study were collected from the Vamanapuram river, Thiruvananthapuram district, Kerala using mosquito net and brought to the laboratory. *P. cupanus* can be easily bred in the glass aquaria. After each mating a group of eggs was laid. The eggs of 6th to 21st batches each consists of 10 to 24 eggs, were collected by using fine meshed hand nets and immersed in pre-heated water in a water bath. Shock was applied to the eggs just after fertilization. The temperature and duration of the shock were 38°C to 40°C and 3 to 5 minutes. After treatment, the eggs were immediately returned to water at normal temperature (27°C to 30°C) for

incubation. One batch of eggs without heat shock treatment was used as control for each experiment. The eggs hatched out about 32 hours at 28°C. Newly hatched larvae measured about 2 to 2.5mm in length. Yolk was completely absorbed by the third day and then the larvae started feeding.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.

Extraction of tissues for DNA and RNA estimation

Homogenize weighed fresh tissue in 3 or 5 ml of 0.85% saline. Two ml of 10% TCA was added and centrifuged after 30 minutes in ice bath. The supernatant was decanted and the residue was washed with ethanol: ether (3:1) three times.Each time the supernatant was decanted and the residue was resuspended in 3ml of 5% TCA. And is kept in a water bath for 15 minutes at 90°C and then centrifuged at 3000 rpm for 15 minutes. The supernatant was used for the estimation of DNA.One ml of extract was taken and mixed with 5ml of diphenylamine reagent. Then heated in boiling water bath for 10 minutes and was then cooled to room temperature and was read absorbance at 95nm. When the DNA is treated with diphenylamine under acidic condition, a blue compound is formed with sharp absorption maxima at 595

Concentration of DNA = (Test OD/Standard OD) X (Conc of standard/Volume of test) X Dilution Factor.

O. D = optical density

RESULTS

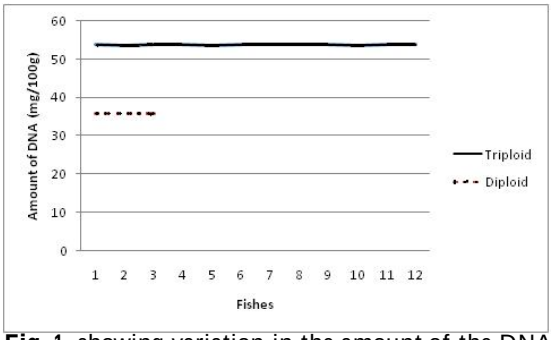

Fig. 1. showing variation in the amount of the DNA content of diploid and triploid *P.cupanus*

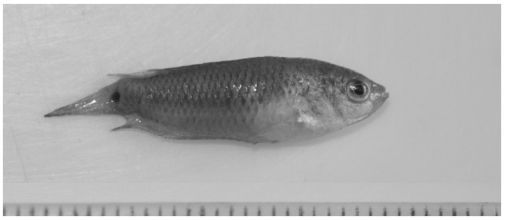

Fig. 2. Diploid *P.cupanus*after three months.

Table 1. Details of the heat shock treatment for the induction of Triploidy in *P. cupanus*

	Temperature (æ%C)	Number of eggs	Duration of treatment(Min)		Time after fertilization (Sec)		Hatch (%)		Fry survival (%)					
									One week		Two week		One Month	
1	Control (30æ%C)	100		- -	- -		95		46		25		18	
	Treated (40æ%C)	100	3	5	30	30	70	50	40	20	20	5	2	1
2	Control (28æ%C)	100		- -	- -		96		45		30		20	
	Treated (38æ%C)	100	3	5	30	30	64	30	32	10	17	2	3	0
3	Control (30æ%C)	100	—		—		93		42		21		19	
	Treated (40æ%C)	100	3	5	30	30	82	35	41	9	18	4	8	0
4	Control (30æ%C)	100	—			—		92		55		35		24
	Treated (40æ%C)	100	3	5	30	30	79	26	36	13	15	5	7	1
5	Control (30æ%C)	100	—		—		95		50		35		27	
	Treated (40æ%C)	100	3	5	30	30	81	20	41	9	12	0	7	0

Table 2. Confirmation of Triploidy by DNA Analysis in *P.cupanus*

Duration of heat shock	Number of fry survived		Triploid Fish
	After 1 month	After 3 months	
3 minutes	2	2	12 Nos
	3	3	
	8	4	
	7	3	
	7	4	
	1	0	
		Total- 16	

The number of fry survived after three months was 16 and out of this 12 were confirmed as triploid using diphenylamine method of DNA analysis. This showed 75% triploidy.

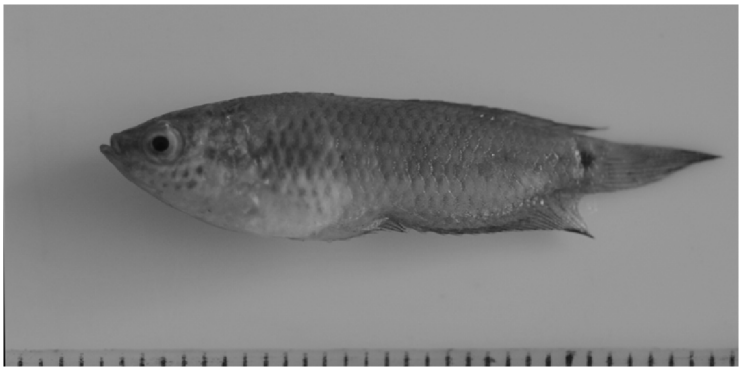

Fig. 3. Triploid *P. cupanus* after three months

DISCUSSION

The Spike tail Paradise Fish *Pseudosphromenus cupanus* belongs to the Family Osphronemidae, in which many of the members are bubble nest builders and eggs are extruded inbatches. 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 (Valenti,1975; Chourrout, 1980;Lincoln and Scott,1984;Diter *et al*.,1993; Islam *et al*.,1994; Linhart and Flajshans,1995; Ramos *et al.,*1996 and Azari *et al.,*1997).Previous studies have shown that in most tropical fish the extrusion of second polar body can be inhibited 2-5minutes following insemination, by heat shocking the eggs at 40°C to 42°C (Penman *et al*., 1987).In species like *O.mossambicus (*Pandian and Varadaraj, 1988)and *B. rerio* (Kavumpurath and Pandian, 1990) heat shock is 100% effective in inducing triploidy. However, in the present study, heat shock produced 75% triploidy in *P.cupanus*. The difference in the percentage of triploidy may be related to egg quality or to the susceptibility of eggs of different origin to triploidization treatment .

Mortality of heat shock treated eggs was comparatively higher than that of the control groups. This may be due to the deleterious effect of heat shock on early embryological development .In *P.cupanus* major losses irrespective of treatment occurred between

Table 3. The amount of DNA estimated using diphenylamine method in diploid and triploid *P.cupanus*

SL.NO	DNA content in diploidfish (mg/100g)	DNA content in triploidfish (mg/100g)
1	35.9	53.8
1'	35.8	53.7
2	35.8	53.6
2'	35.9	53.7
3	35.7	53.8
3'	35.8	53.8
4	-	53.7
4'	-	53.8
5	-	53.6
5'	-	53.5
6	-	53.6
6'	-	53.8
7	-	53.7
7'	-	53.7
8	-	53.8
8'	-	53.9
9	-	53.9
9'	-	53.7
10	-	53.6
10'	-	53.7
11	-	53.8
11'	-	53.9
12	-	53.6
12'	-	53.8
Average	35.8	53.7

The amount of DNA in the diploid fishes were found to be 35.8 mg/100 g of the tissue and that of triploid fishes were found to be 53.7 mg/100 g of the tissue.

hatching and feeding stage as in the case of rainbow trout (Chourrout, 1980; Chourrout and Quillet 1982) and *Betta splendens* (Kavumpurath and Pandian, 1992).The morphological abnormalities due to triploidy were also observed in several fishes. In stickle back; triploids had larger tail and shorter trunk than its diploid siblings (Swarup, 1959). The induction of triploidy may also cause changes in external morphology and colouration. The triploid *T.trichopterus* was more beautiful than diploid females and as beautiful as diploid males. They had longer dorsal fins, bright colour and flat belly than diploid females (Pushpageetha, 2002).During the present study a dark spot near the opercular region was noticed in the triploid *P.cupanus.*

CONCLUSION

Triploidy was induced to check the variation in the morphology, external appearance and body colouration of triploids compared its diploid counterparts.Induction of triploidy in *P. cupanus* was achieved by heat shock treatment with highest percentage hatching (82%) and frysurvival(8%) was at 40°C for 3 minutes duration on eggs treated 30 seconds after fertilization. The eggs treated for 5 minute duration showed low survival rate . A sudden rise of 10ºC in the incubation temperature was found to be successful in inducing triploidy in *P. cupanus*. The duration of heat shock treatment of the fertilized eggswas found to be 3 minutes. The amount of DNA of the diploid was found to be 35.8mg/100g of the tissue and that of triploids was found to be 53.7mg/100g of the tissue

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