



## GROWTH PERFORMANCE, REPRODUCTIVE STERILITY AND DISTINCT MORPHOLOGY OF TRIPLOID FRINGED LIPPED CARP, *LABEO FIMBRIATUS*

Pushpa Geetha, S. \* and Jayaprakas, V.

Dept. of Aquatic Biology and Fisheries, University of Kerala, Thiruvananthapuram, Kerala.

\*Corresponding author: [pushpageetha@hotmail.com](mailto:pushpageetha@hotmail.com)

Received on: 20.10.2013, accepted on: 20.12.2013

**Abstract:** The present study was conducted to determine the optimum conditions for the induction of maximum percentage of triploid *Labeo fimbriatus* with high survival rates and to assess the impact of triploidy on survival, growth and reproduction. Optimum heat shock treatment required for triploidy induction in *L. fimbriatus* was 41°C for 3 minutes duration on 2.5 minute old fertilized eggs. By this treatment, 95% triploids were produced. The triploids were as viable as diploids and were externally identifiable at an early stage. Triploidy was confirmed by erythrocyte's nuclear volume measurements and chromosome count. The triploids had 75 chromosomes and diploids had 50 chromosomes. The growth rate of triploids was higher than diploids from juvenile stage onwards. The triploids were 23% heavier than diploid counterparts when grown for one year. All the survived triploids were sterile females. They had reduced gonad and GSI (1.09) than diploids (15.77). The commercial cultivation of triploid *L. fimbriatus* may be more profitable than that of diploids.

**Key words:** Edible fish, survival, chromosome, GSI, commercial cultivation, heat shock

### INTRODUCTION

There has been a constant effort in increasing fish production through different methods. The biotechnological tools are the latest ones being employed for achieving this, through chromosome manipulation. Induced triploidy in fishes has been attracting many aquaculturists and researchers throughout the world. Triploids are often produced with an expectation that they may become sterile and grow faster. The sterility is due to irregular meiotic division of chromosomes resulting in either a failure of gonad development or production of aneuploid gametes. In such fish therefore, energy consumption for sexual maturation may be avoided and more biological effort is directed towards improving flesh quality and somatic growth (Thorgaard and Gall, 1979; Lincoln, 1981; Wolters *et al.*, 1982; Peruzzi *et al.*, 2004). According to Stanley *et al.* (1984), Thorgaard (1986) and Scheerer and Thorgaard (1987), the higher heterozygosity of triploid fish may

enhance growth. Generally creation of triploid fish has been considered as a biotechnological tool to improve growth and yield and to prevent unwanted reproduction when fishes are raised for food or stocked in natural water bodies.

Induction of triploidy has been achieved in many fish species using a variety of techniques. Methods used to date include exposing fertilized eggs to temperature shock - heat or cold (Thorgaard, 1983), chemicals such as colchicine (Smith and Lemoine, 1979), Cytochalasin B (Refstie *et al.*, 1977), high pH multiplied by high calcium (Ueda *et al.*, 1988), Nitrous oxide (Johnstone *et al.*, 1989), combined caffeine-heat shock treatment (Durand *et al.*, 1990), electric shock (Teskeredzic *et al.*, 1993) and hydrostatic pressure shock (Piferrer *et al.*, 1994). Generally physical treatments are most successful and widely used to induce triploidy in fish (Guoxiong *et al.*, 1989; Teskeredzic *et al.*, 1993; Johnson *et al.*, 2004 and Haffray *et al.*, 2005). The

heat shock technique is widely used to ploidy induction, because it is inexpensive, less sophisticated and can be successfully adopted by farmers. However the timing, temperature, and duration of shock must be determined for each species.

*Labeo fimbriatus* (Bloch) is commonly known as fringed-lipped carp, is endemic to peninsular rivers. This fish is commonly used in composite fish culture, but not that much preferred as Indian major carps, as this fish does not grow as fast as major carps. However, it has few advantages such as small head, pleasing appearance and excellent meat quality. *L. fimbriatus* becomes sexually mature in their second year. Usually breeds once in a year. Breeding occurs in running waters during the monsoon season (June – September). This fish can be artificially bred in confined conditions by hypophysation

In an attempt to enhance the growth rate of *L. fimbriatus*, triploidy was induced by heat shocking. The main objectives of the present study was to determine the optimum heat shock treatment which ensure maximum percentage of triploidy and survival and compare the growth rate and maturation of the triploids with their diploid counterparts.

### MATERIALS AND METHODS

#### Breeding and Triploidisation

Sexually matured and healthy brood fish were procured from National Fish Seed Farm, Neyyar Dam, Thiruvananthapuram, during the monsoon season. Breeding was performed by artificial propagation method with carp pituitary hormone injection. Eggs and sperm were collected separately by stripping. Artificial fertilisation was initiated by mixing the eggs and sperm well for one minute and excess milt was washed off. About 2 ml of fertilised eggs were taken in plastic strainers and heat shock was applied. Shock was applied from 1 minute to 3 minutes after fertilisation. The temperature and duration of the shock were 39° to 43°C and 1 to 4 minutes respectively. Eggs of control groups involving no heat shock were also maintained in order to

ascertain the experimental procedure. Hatchability of eggs, number of deformed fry and survival of feeding fry were monitored.

#### Ploidy Assessment

Efficiency of heat shock treatment was ascertained by chromosome counts on 4-5 month old fish from each treatment group. Chromosome slides were prepared according to the method described by Kligerman and Bloom (1977) with slight modifications. Ploidy was also determined by cell and nuclear volume measurements of erythrocyte's (RBC). Student's  $t_{3/4}$  test was conducted to find any significant difference between diploids and triploids in volume measurements of erythrocytes. The morphology of 4 month old *L. fimbriatus* were analysed to visually evaluate successful production of triploids.

#### Growth Analyses

Triploidy induced and normal larvae were reared separately in similar troughs of 50 litre capacity for three weeks. Fifty percentage of water was exchanged daily. After three weeks nursing, the fry were transferred to cement tanks of 500 litre capacity, in equal numbers of 20 each to undertake a comparative study of growth. About 25% of water was replenished once in every week after siphoning out the accumulated detritus and faecal matter. Three replicate samples were maintained for each group. The larvae were fed initially with boiled hens' egg and then with cultured plankton, powdered dry pellets and at one month age they were fed with 35% protein feed pellets to satiation once daily. After two months, the fingerlings were transferred to larger cement tanks of 4000 litres capacity. Total weight and total length measurements were recorded monthly for one year starting at one month. The data were analysed using ANOVA. The percentage composition of the different ingredients in the 35% protein feed is given in Table 3.

#### Gonadal Development and Sex Ratio

Twenty month old fish of both control and ploidy induced were sacrificed and weighed. The sex of each fish was determined by dissection of the

gonads. The gonads were weighed to calculate the GSI using the following formula.

Gonadosomatic Index (GSI) = (Weight of ovary (g) / Weight of Fish (g)) x 100

Development of gonads was assessed using histological procedure. The relationship between gonad weight and body weight was analysed by simple regression analysis.

## RESULTS

### Effect of Heat Shock

Eggs hatched within 20 to 24 hours at 26° to 27°C. The effect of heat shock on triploidy induction, survival and abnormal fry resulting from the heat shock experiment in *L. fimbriatus* is summarised in Table. 1. The optimum heat shock treatment required for the induction of triploidy, in *L. fimbriatus*, was 41°C for 3 minutes duration on 2.5 minutes old eggs. The percentage of hatching was higher in diploids (76) than triploids (67). The triploid fish were almost as viable as diploids (41 versus 42).

### Ploidy status

Triploidy was confirmed by observing morphological peculiarities, chromosome counts and nuclear volume measurements of erythrocytes.

### Morphological variations

The triploid *L. fimbriatus* could be visually identified from diploids at an early stage of two month old. The triploid fish were distinctly larger than control groups. The triploids had more elongated head and dorsal side of the head was lightly pigmented than the rest of the body. A shallow depression could be seen between the head and trunk of triploids. The diploid and triploid *L. fimbriatus* are shown in Figs. 1 & 2

### Chromosome counting

The metaphase spreads of diploid and triploid *L. fimbriatus* are shown in Figs. 3 and 4. The diploid cells had 50 chromosomes and triploid cells had 75 chromosomes.

### Nuclear volume measurement of erythrocytes

Figures 5 and 6 show the erythrocytes of diploid and triploid *L. fimbriatus*. Triploid erythrocytes

and erythrocyte nuclei were larger than diploid erythrocytes and erythrocyte nuclei. The size differences of erythrocytes between diploid and triploid fishes are presented in Table 2. The differences observed for the cellular as well as nuclear measurements of diploid and triploid *L. fimbriatus* are shown in Fig. 7a, 7b, 7c & 7d. Volume of the triploid nucleus and cell was significantly larger (1.92 and 1.95 times).

### Growth

The triploids were superior to the diploids in growth even during the juvenile stage and this superior growth continued up to the termination of the experiment. Tables 4 and 5 show the comparative growth of diploid and triploid *L. fimbriatus*. Triploids were 23% heavier than diploids at one year old. The comparative growth pattern is presented in Figures 8 and 9. Regression equation relating culture days and percentage length and weight increment of diploid and triploid *L. fimbriatus* are given in Figs. 10, 11, 12 and 13.

### Gonad Maturation and Sex Ratio

In *L. fimbriatus* all triploids were females. The triploid fish had very thin, elongate, paired and fleshy ovaries. The peritoneum was thick and highly vascularised (Fig.14). They had reduced gonado-somatic index (0.56%) compared to diploids (19.74%).

The diploid ovary was elongate, paired and completely filled the abdominal cavity. It was pale yellow in colour. The peritoneum was thin and moderately vascularised. One lobe of the ovary was shorter than the other (Fig.15). The ovaries of diploid and triploid fishes are shown in Fig.16. The comparative GSI between diploid and triploid fishes are presented in Fig.17. Table 6 shows the average gonad weight and gonado-somatic indices for triploids and diploids at 20 months old.

The histological observations of diploid and triploid ovaries showed that the diploid ovary was a ripe one with mature oocytes (Fig.18). Whereas the triploid ovary was an atretic one with extensive connective tissue (Fig. 19).

**Table 1.** Effect of heat shock on triploidy and survival in *Labeo fimbriatus*

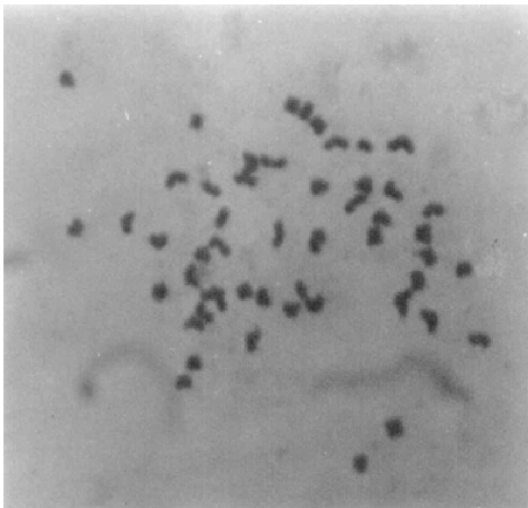
Heat shock	Duration of shock	Time after Fertilization	Hatching	Feeding fry survival	Triploidy	Deformed fry
(°C)	(min.)	(min.)	(%)	(%)	(%)	(%)
27 (Control)	-	-	76	43	0	3
41	3	1.5	57	27	0	7
	3	2	62	33	70	5
	3	2.5	67	42	95	8



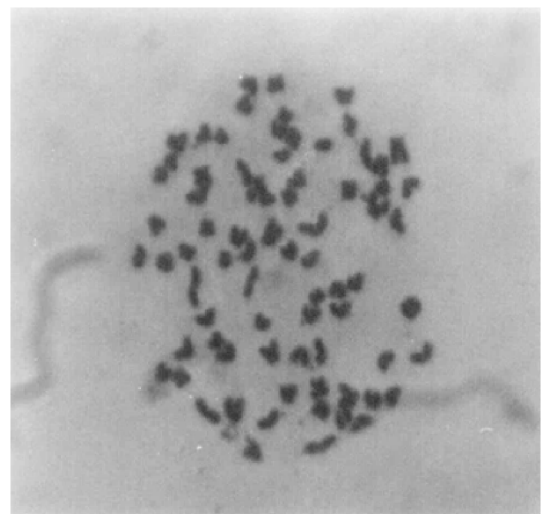
**Fig. 1.** Diploid *L.fimbriatus*



**Fig. 2.** Triploid *L. fimbriatus*



**Fig. 3.** Diploid



**Fig. 4.** Triploid

**Chromosomes**

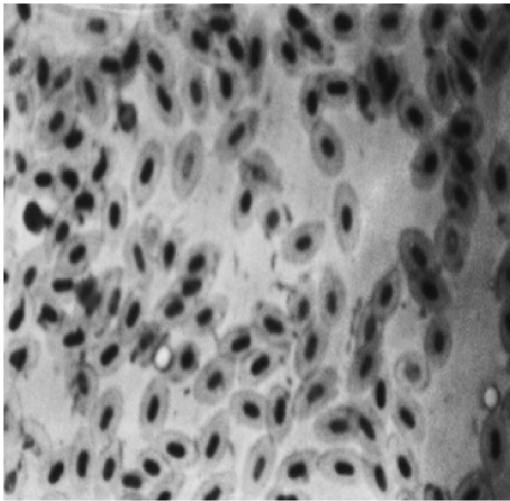


Fig. 5. Diploid

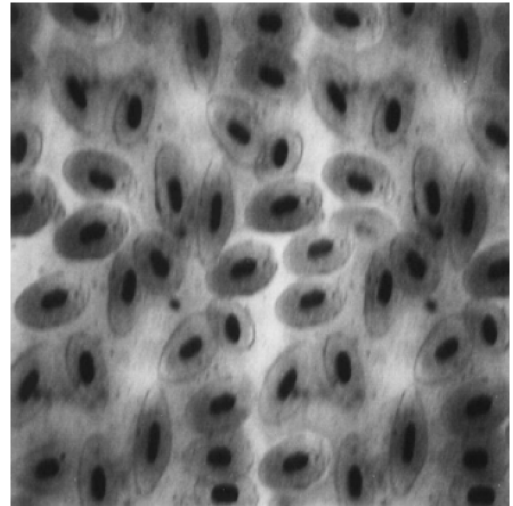
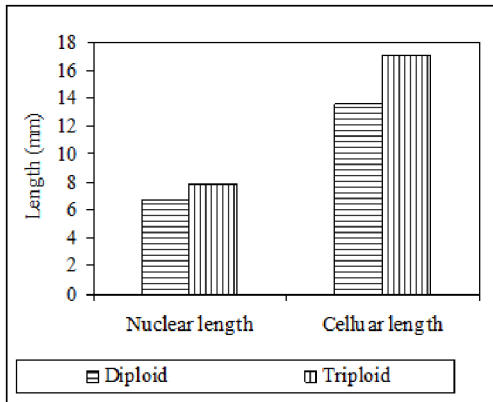
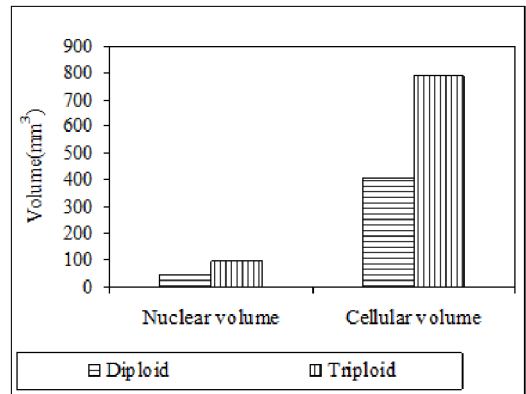


Fig. 6. Triploid

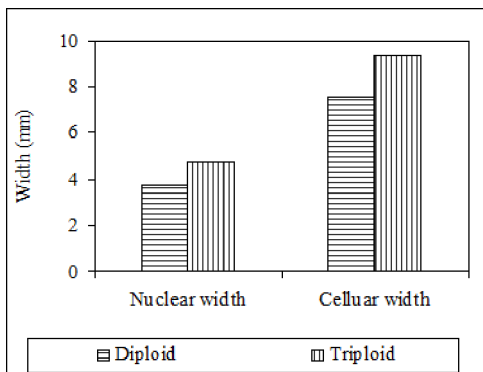
Erythrocytes



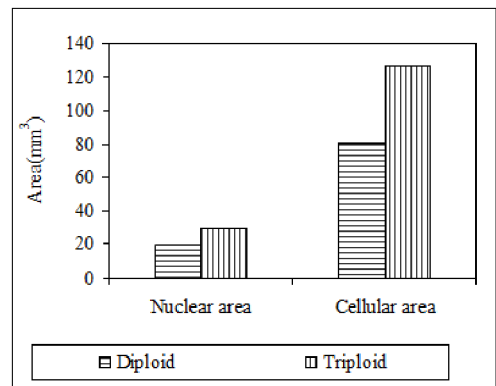
7a



7b



7c



7d

Fig. 7. Comparison of nuclear and cellular measurements of triploid and diploid *L. fimbriatus*

## Growth performance of triploid *Labeo fimbriatus*

**Table 2.** Comparative cellular and nuclear measurements of diploid and triploid *L.fimbriatus*

Sl. No.	Parameters	Diploid		Triploid		t value
		Mean	± SD	Mean	± SD	
1	Nuclear length (µm)	6.77	0.32	7.84	0.32	- 7.425**
2	Cellular length (µm)	13.53	0.65	17.07	0.32	- 15.429**
3	Nuclear width (µm)	3.7	0.32	4.76	0.32	- 7.356**
4	Cellular width (µm)	7.54	0.32	9.38	0.32	- 12.728**
5	Nuclear volume (µm <sup>3</sup> )	49.07	9.76	94.09	17.98	- 6.959**
6	Cellular volume (µm <sup>3</sup> )	404.48	50.65	788.99	72.31	- 13.774**
7	Nuclear area (µm <sup>2</sup> )	19.69	2.54	29.41	3.33	- 7.343**
8	Cellular area (µm <sup>2</sup> )	80.2	7.05	125.85	6.86	- 16.053**

\*\* P < 0.01

**Table 3.** Proportion of feed ingredients and proximate composition of feed

Ingredients	Proportion (g)	Protein content (%)	Proximate composition of diet (Dry weight basis)	
			Parameter	Percentage
Rice bran	17.82	1.23	Moisture	14.59
			Protein	34.96
Tapioca flour	17.82	0.37	Fat	5.9
			Carbohydrate	10.13
Groundnut oil cake	32.18	15.44	Fiber	4.2
			Ash	2.7

**Table 4.** Growth of diploid and triploid *L.fimbriatus*

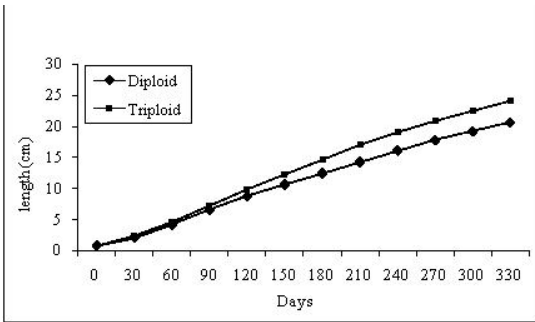
Fish	genotype	Culture period (days)												
		0	30	60	90	120	150	180	210	240	270	300	330	
Length (cm)	Diploid	Mean	0.81	2.09	4.17	6.56	8.76	10.7	12.52	14.34	16.2	17.82	19.25	20.72
		6SD	0.14	0.33	0.49	0.57	0.56	0.53	0.68	0.8	0.88	1	1.05	1.11
	Triploid	Mean	0.79	2.31	4.62	7.25	9.81	12.28	14.76	17.05	19	20.9	22.51	24.15
		6SD	0.14	0.29	0.38	0.44	0.53	0.67	0.78	1.03	1.25	1.39	1.52	1.66
Weight (g)	Diploid	Mean	0.5	1.26	3.74	7.89	15.79	28.11	45.31	67.96	97.1	133.2	174.21	221.87
		6SD	0.01	0.17	0.52	0.72	1.18	1.6	2.13	2.9	4.65	5.6	8.92	12.92
	Triploid	Mean	0.5	1.26	4.28	10.9	22	38.12	59.82	87.57	121	163.46	214.07	273.7
		6SD	0.02	0.19	0.54	1.44	2.51	3.76	5.19	6.75	8.35	10.79	14.01	17.75

**Table 5.** Percentage increment of *L.fimbriatus*

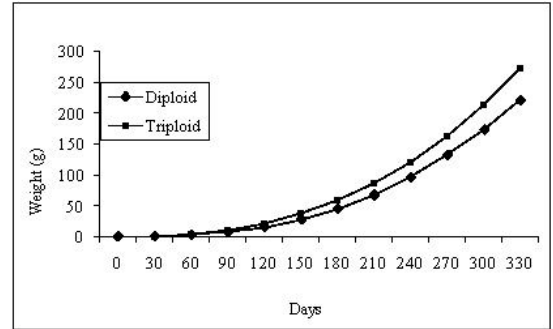
Parameter	Fish Genotype	Culture period (Days)										
		30	60	90	120	150	180	210	240	270	300	330
Length (%)	Diploid	158	415	710	981	1221	1446	1670	1896	2100	2277	2458
	Triploid	192	485	818	1142	1454	1768	2058	2308	2546	2749	2957
F value		14.03**	8.01*	5.44*	5.19*	6.46*	9.37**	10.47**	9.23**	8.92**	8.00*	39.63**
Weight (%)	Diploid	152	648	1478	3058	5522	8962	13492	19312	26540	34742	44274
	Triploid	152	756	2080	4300	7524	11864	17414	24140	32592	42714	54640
F value		0	5.74*	38.72**	55.38**	66.23**	74.00**	78.68**	70.55**	68.55**	63.67**	55.72**

\* P<0.05

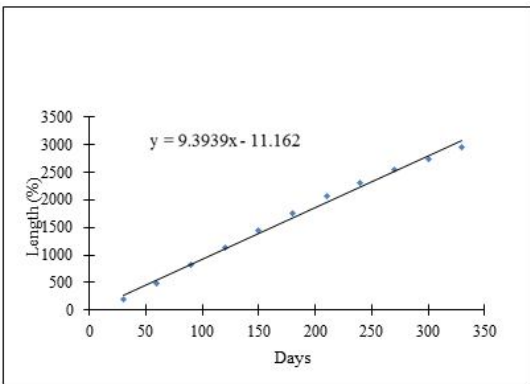
\*\* P<0.01



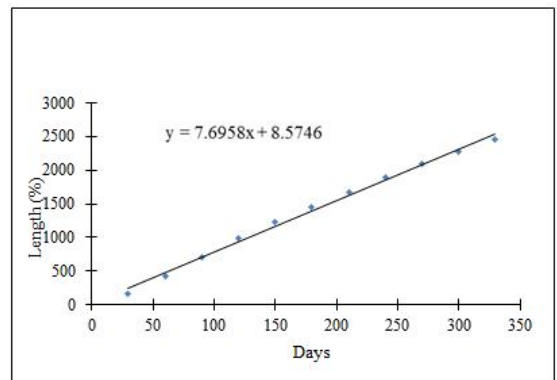
**Fig. 8 .** Mean length of diploid and triploid *L. fimbriatus*



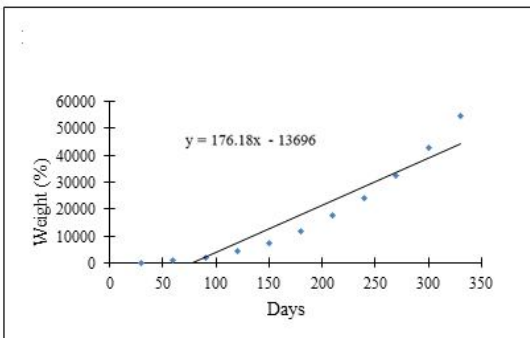
**Fig. 9.** Mean weight of diploid and triploid *L. fimbriatus*



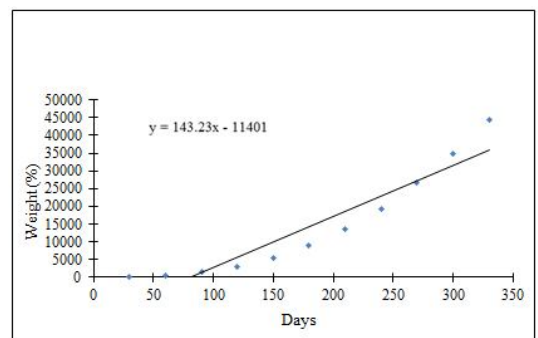
**Fig. 10.** Regression between days and percentage length increment of triploid *L. fimbriatus*



**Fig. 11.** Regression between days and percentage length increment of diploid *L. fimbriatus*



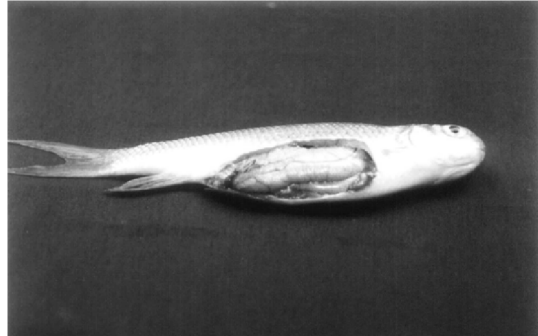
**Fig. 12.** Regression between days and percentage weight gain of triploid *L. fimbriatus*



**Fig. 13.** Regression between days and percentage weight gain of diploid *L. fimbriatus*



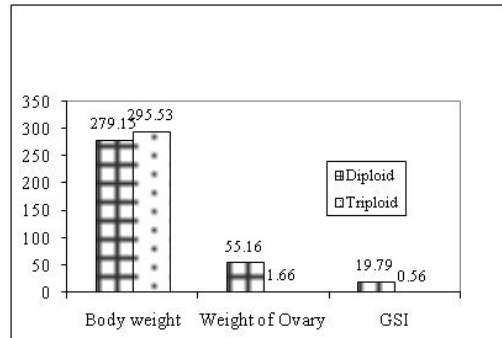
**Fig. 14.** Triploid *L.fimbriatus* with visceral cavity cut open to show the ovary



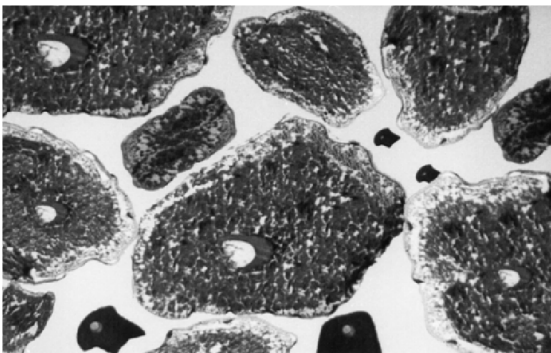
**Fig. 15.** Diploid *L.fimbriatus* female with visceral cavity cut open to show the ovary



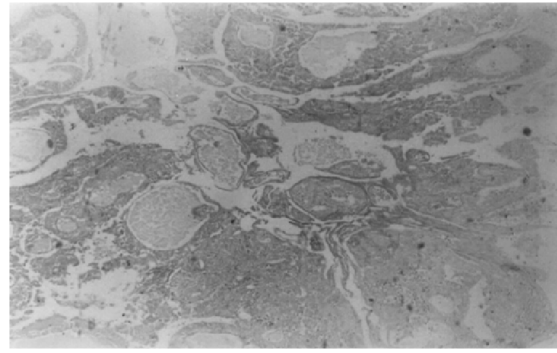
**Fig. 16.** Ovaries of diploid and triploid *L. fimbriatus*



**Fig. 17.** Weight of body and ovary and Gonado-Somatic Index of *L. fimbriatus*



**Fig. 18.** Cross section of diploid ovary



**Fig. 19.** Cross section of triploid ovary

## DISCUSSION

The present study demonstrates that heat shock treatment is an effective method for the production of triploidy in *L. fimbriatus*. The optimum heat shock treatment was 41°C for 3 minutes duration on 2.5 minute old eggs. The highest triploid induction rate was 95%.

Although heat shock is 100% effective in certain species like *O. mossambicus* (Pandian and Varadaraj, 1988a) and *B. rerio* (Kavumpurath and Pandian, 1990), in the present study heat shock induced only 95% triploidy in *L. fimbriatus*. As reported by Lou and Purdom (1984b), the difference in the percentage of triploidy may be related to egg quality or to the susceptibility of



**Table 6.** Gonado-Somatic Index (GSI) of *L. fimbriatus*

	Diploid	Triploid
Body weight	279.15	295.53
±SD	65.1	57.34
Weight of Ovary	55.16	1.66
±SD	12.55	0.36
Gonadosomatic Index	19.79	0.56
±SD	0.55	0.05

eggs of different origin to triploidization treatment.

Considering the early embryological development, heat shock treated eggs exhibited comparatively higher mortalities relative to their diploid controls. The percentage of hatching was higher in diploids (76) than triploids (67). Major losses irrespective of treatment occurred between hatching and feeding stages as in the case of rainbow trout (Chourrout, 1980; Chourrout and Quillet, 1982) and *Bettasplendens* (Kavumpurath and Pandian, 1992). This may be due to the deleterious effects of heat shock on early embryological development. From feeding stage onwards survival was similar in both ploidies. Similar observations were reported by many workers (Gervai *et al.*, 1980; Krasznai *et al.*, 1984; Benfey and Sutterlin, 1984a; Oliva-Teles and Kaushik, 1990; Simon *et al.*, 1993; Carter *et al.*, 1994; Withler and Clarke, 1998; Sheehan *et al.*, 1999; Piferrer *et al.*, 2003; Cal *et al.*, 2006; Opstad *et al.*, 2013).

Most triploid induction techniques result in some diploids mixed with triploids. A number of techniques exists for separating triploid and diploid fishes *viz*, flow cytometric measurement of fish erythrocyte DNA (Allen and Stanley, 1979; Thorgaard *et al.*, 1982; Allen, 1983), Coulter counter estimation of erythrocyte nuclear size (Wattendorf, 1984), spectrofluometry of erythrocyte DNA (Schwarzbaum *et al.*, 1992) and measurement of erythrocyte nuclear volume by light microscopy (Wolters *et al.*, 1982a; Wattendorf, 1984). However, these methods generally are impractical for screening large numbers of fish. But the presence of any morphological markers in triploids would be

ideal for screening large numbers of fish. In the present study the triploid *L. fimbriatus* were larger than their diploid counterparts and exhibited distinct morphological features. An experienced person can easily distinguish the triploids from diploids by observing such morphological peculiarities. The ability to distinguish between the diploid and triploid *L. fimbriatus* based on external morphology may provide an easy method for determining the effectiveness of induced triploidy in hatcheries.

The change in external morphology due to triploidy has also been noted in several fishes. In stickleback, triploids had larger tail and shorter trunk than its diploid siblings (Swarup, 1959b). Cassani and Caton (1985) and Bonar *et al.*, 1998 reported that grass carp larger than 300 mm total length could be separated visually with an accuracy of 95%. In Atlantic salmon (*Salmosalar*), protruding lower jaw was found to be the abnormality associated with triploidy (Sutterlin *et al.*, 1987). Altered body shape is reported as a morphometric indicator of triploidy in Indian catfish, *H. fossilis* (Tiwary *et al.*, 1999). The ratio between standard length and body depth (SL/BD) was found to be a precise indicator of triploidy in this fish. According to Ihssen *et al.* (1990), the morphological difference would have to be due to a dosage or co-dominant effect, because the diploids and triploids share the same alleles.

The diploid and triploid chromosome numbers of *L. fimbriatus* were 50 and 75 respectively. Presence of one additional set of chromosomes confirmed the successful induction of triploidy in *L. fimbriatus*. The model number of diploid chromosomes in Cyprinidae is 50 (Manna and

## Growth performance of triploid *Labeo fimbriatus*

KhudaBuksh, 1977a). The diploid chromosome number of *L. fimbriatus* corresponds this model number of 50.

The triploid *L. fimbriatus* had larger erythrocytes than that of their diploid siblings. Volume of the triploid nucleus and cell was significantly larger (1.92 and 1.95 times). The increased nuclear volume also confirmed triploidy. These results agree well with previous studies by other workers dealing with other fish species. (Wolters *et al.*, 1982a; Small and Benfy, 1987; Kavumpurath and Pandian, 1990; Arai *et al.*, 1993; Kim *et al.*, 1994; Tiwary *et al.*, 1999; Svobodova *et al.*, 1998; Felip *et al.*, 1999).

Growth in the triploid *L. fimbriatus* was found to be faster than the normal diploids even during the juvenile stage and this superior growth rate continued up to the termination of one year growth study. The triploids were 23% heavier than diploids at the end of one year growth. This result is in good agreement with results for other species. Purdom (1972) reported that triploid plaice, *Pleuronectes platessa* grew faster than the diploid sibs. Similar observations were also reported by Wolters *et al.* (1982a) in channel catfish (*Ictalurus punctatus*), Cassani and Caton (1985) in grass carp (*Ctenopharyngodon idella*), Suzuki *et al.* (1985) in loach (*Misgurnus anguillicaudatus*), Thorgaard (1986) in rainbow trout (*Onchorhynchus mykiss*), Tave (1993) in big head carp (*Hypophthalmichthys nobilis*), Galbreath *et al.* (1994) in Atlantic salmon (*Salmosalar*), Islam *et al.* (1994) in rohu (*Labeorohita*), Tiwary *et al.* (1997) in catfish (*Heteropneustes fossilis*), Reddy *et al.* (1998) in carp (*Cyprinus carpio*) and Taranger *et al.* (2003) in Atlantic salmon (*Salmo salar*), and Cal *et al.* (2006) in turbot (*Scophthalmus maximus*). According to Hawkins *et al.* (1998) the higher growth of triploids not only resulted from reduced reproductive output, but also from higher net growth efficiencies that were associated with greater genetic polymorphism and resulted energy requirements for protein turnover renewal as seen for heterosis generally. The most striking observation of the present study was that all the triploids produced were

females and sterile. Triploids had rudimentary ovary and reduced gonadosomatic index (GSI). The lack of gonad development in triploids observed is consistent with results for other species like Asian cat fish (Fast *et al.*, 1995), sea bass (Felip *et al.*, 1999), sun shine bass (Kerbyet *et al.*, 2002) yellow tail flounder (Manning *et al.*, 2004) and turbot (Cal *et al.*, 2006). According to Nakamura *et al.* (1987), the sterility observed in female triploids may be in addition to mechanical difficulties involved in chromosome separation at meiosis I due to triploidy, the reduced levels of ovarian steroid hormones could also account for female sterility.

The commercial production of triploid fish might be more profitable than that of normal or monosex culture (Allen and Stanley, 1979; Wolters *et al.*, 1982a; Donaldson and Hunter, 1982; Don and Avtalion, 1986, 1988; Vejaratpimol and Pewnim, 1990; Peruzzi *et al.*, 2004). Moreover the sexuality of fish has great significance in fish breeding because there are differences in growth rate, behaviour pattern, breeding time, body colour, shape or size between male and female in each cultured species. There is no commercial advantage in producing male triploid fish if testis growth is unaffected by triploidy. On the other hand, the aquaculture production of all female triploid populations is recognised as being of potential advantage for many species of fish where sexual maturation is not desired. Therefore the production of all female triploids may be useful for *L. fimbriatus* culture. All female triploids have been produced generally by inducing triploidy in eggs that had been fertilised with monosex female sperm (sperm from masculinised females) (Lincoln and Scott, 1983; Okada, 1985; Galbreath and Thorgaard, 1995) or by the direct feminization of triploid embryo by direct oestrogen treatment shortly after hatching (Varadaraj and Pandian, 1990; Piferrer *et al.*, 1994). Whereas in the present study, the heat shock treatment alone produced all female populations.

In majority of fish species the sex ratio among triploids equals controls. For example in tilapia, *O. mossambicus*, the sex ratio of triploids is 1:1

(Pandian and Varadaraj, 1989). Similarly in common carp, *Cyprinus carpio* (Cherfas *et al.*, 1994) and in sea bass, *Dicentrarchus labrax* (Felix *et al.*, 1998) the sex ratio of triploids is not different from diploids. At the same time there are a few reports about all male triploids. In bitterling, *Rhodeusocellatus* all triploids were males. Similar result is reported by Kavumpurath and Pandian (1990) in fighter fish, *Brachydanio rerio* and Warrillow *et al.* (1977) in brook trout, *Salvelinus fontinalis*. Whereas Byamungu *et al.* (2001) reported that in blue tilapia (*Oreochromis aureus*) the triploids were predominantly females (80%). The reason for the total absence of males in the present study is unknown. As reported by Streisinger *et al.* (1981), this may be due to variation in the expression of sex determining mechanism among the different ploidy levels.

In the present study the triploid *L. fimbriatus* had much more fat deposits around the digestive tract. Such large fat deposits were also observed by many workers (Thorgaard and Gall, 1979; Chevassus *et al.*, 1983; Lincoln and Scott, 1984; Johnson *et al.*, 1986). But Sheehan *et al.* (1999) found no such fat deposits in triploid rainbow trout. According to Johnson *et al.* (1986), large fat deposition in triploid female may be due to the failure of lipid withdrawal from body reserves for vitellogenesis, which normally takes place during breeding season in normal diploid fish.

The growth performance of triploids is a critical factor in determining their utility in aquaculture. From the present study it is evident that the triploid *L. fimbriatus* were sterile and their sterility allowed better growth performance. The triploids were heavier than their diploid counterparts under controlled conditions. If they were reared in the natural habitat they might exhibit much better growth. Furthermore, triploid *L. fimbriatus* would have less wastage in processing because they have smaller heads. Therefore, triploids of Fringed-lipped carp, *L. fimbriatus* may be of great value to fish farmers.

In conclusion, induction of triploidy by heat shock treatment can be recommended as a technique for efficient *L. fimbriatus* culture.

## REFERENCES

- Allen, S.K.Jr., 1983. Flow cytometry: Assaying experimental polyploid fish and shellfish. *Aquaculture*, 33: 317-328.
- Allen, S.K. Jr. and Stanley, T.G. 1979. Polyploid mosaics induced by Cytochalasin B in land locked Atlantic salmon, *Salmosalar*. *Trans. Am. Fish. Soc.*, 108: 462-466.
- Benfey, T.J. and Sutterlin, A.M. 1984. Growth and gonadal development in triploid land locked Atlantic salmon, *Salmosalar*. *Can.J. Fish. Aquat. Sci.*, 41: 1387-1392.
- Bonar, S.A., Thomas, G.L. and Pauley. 1988. Evaluation of the separation of triploid and diploid grass carp, *Ctenopharyngodon idella*, by external morphology. *J. Fish Biol.*, 33: 895-898
- Byamungu, N., Darras, V.M. and Kuhn, E.R. 2001. Growth of heat shock induced triploids of blue tilapia (*Oreochromis aureus*), reared in tanks and ponds in Eastern Congo; Feeding regimes and compensatory growth responses of triploid females. *Aquaculture*, 198 (1-2): 109-122.
- Cal, R.M., Vidal, S., Gómez, C., Álvarez-Blázquez, Martínez, P. and Piferrer, F. 2006. Growth and gonadal development in diploid and triploid turbot (*Scophthalmus maximus*). *Aquaculture*, 251: 99-108.
- Carter, C.G., McCarthy, I.D. and Houlihan, D.F. 1994. Food consumption, feeding behaviour and growth of triploid and diploid Atlantic salmon, *Salmo salar* L. *Can. J. Zool.*, 72(4): 609-617.
- Cassani, J.R. and Caton, W.E., 1985. A method of inducing triploidy in grass carp and growth variations of diploid and triploid Grass carp. *Proc. 19 Ann. Meet. Aquat. Plat Control. Res. Prog. Misc. Paper.*, A85(4): 165-167.
- Cherfas, N.B., Gomelsky, B., Ben-Dom, N., Peretz, Y. and Hulata, G. 1994. Assessment of triploid common carp, *Cyprinus carpio* L. for culture. *Aquaculture*, 127(1): 11-18.
- Chevassus, B., Guyomard, R., Chourrout, D. and Quillet, E. 1983. Production of viable hybrids by triploidization. *Genet. Sel. Evol.*, 15(4): 519-532.
- Chourrout, D. 1980. Thermal induction of diploid gynogenesis and triploidy in the eggs of the Rainbow trout *Salmo gairdneri*. *Reprod. Nutr. Develop.*, 20: 727-733.
- Chourrout, D. and Quillet, E. 1982. Induced gynogenesis in the rainbow trout: Sex and survival

## Growth performance of triploid *Labeo fimbriatus*

- of progenies. Production of all triploid populations. *Theor. Appl. Genet.*, 63: 201-205.
- Don, J. and Avtalion, R.R. 1986. Induction of triploidy in *Oreochromis aureus* by heat shock. *Theor. Appl. Genet.*, 72: 186-192.
- Don, J. and Avtalion, R.R. 1988. Comparative study on the induction of triploidy in tilapias using cold and heat shock techniques. *J. Fish. Biol.*, 32: 665-672.
- Donaldson, E.M. and Hunter, G.A. 1982. Sex control in fish with particular reference to salmonids. *Can. J. Fish. Aquat. Sci.*, 39: 99-110.
- Durand, P., Wada, K.T. and Komaru, A. 1990. Triploidy induction by Caffeine-heat shock treatment in the Japanese pearl oyster *Pinctada fucata martensii*. *Bull. Jap.Soc.,Sci. Fish.*,56(9): 1423-1425.
- Fast, A.W.,Pewnim, T.,Keawtabtim, R., Saijit, R. and Vejaratpimol, R. 1995. Comparative growth of diploid and triploid Asian catfish (*Clarias macrocephalus*) in Thailand. *J. World Aquaculture*, 26: 390-395.
- Felip, A., Zanuy, S., Carillo, M. and Piferrer, F. 1998. Study of the treatment conditions leading to the mass production of triploid and gynogenetic Sea bass. Genetics and breeding of Mediterranean aquaculture species. *Proceedings of the seminar of the CIHEAM*, 34: 123-129.
- Felip, A., Zanuy, S., Carillo, M and Piferrer, F. 1999. Growth and gonadal development in triploid Sea bass *Dicentrarchus labrax* L. *Aquaculture*, 173(1-4): 387-397.
- Galbreath, P.F., St. Jean, W., Anderson, V. and Thorgaard, G.H. 1994. Freshwater performance of all female diploid and triploid Atlantic salmon. *Aquaculture*, 128: 41-49.
- Galbreath, P.F. and Thorgaard, G.H. 1994. Viability and freshwater performance of Atlantic salmon, *Salmo salar* X Brown trout, *Salmo trutta* triploid hybrids. *Can. J. Fish. Aquat. Sci.*, 51: 16-24.
- Galbreath, P.F. and Thorgaard, G.H. 1995. Saltwater performance of all female triploid Atlantic salmon. *Aquaculture*, 138(1-4): 77-85.
- Gervai, T., Peter, S., Nagy, A., Horvath, L. and Casnyi, 1980. Induced triploidy in Carp, *Cyprinus carpio* L. *J. Fish. Biol.*, 17: 667-671.
- Guoxiong, C., Solar, I.I. and Donaldson. 1989. Comparison of heat and hydrostatic pressure shocks to induce triploidy in steelhead trout (*Oncorhynchus mykiss*). *Can. Tech. Rep. Fish. Aquat. Sci.*, 1718: 1-11.
- Haffray, P., Bruant,J.S., Facquer, J.M. and Fostier, A. 2005. Gonad development, growth, survival and quality traits in triploids of protandrus hermaphrodite gilthead seabream *Sparus aurata*(L). *Aquaculture*, 247; (1-4) : 107-117.
- Hawkins, A.J.S., Herard, A., Heral, M. and Zouror, E. 1998. Assessment of aquacultural advantages following the cytogenetic induction of polyploids in commercially important shellfish. MAST Conference, Lisbon, Project Synopses, 5: 22-27.
- Kerby, J.H., James, M., Everson, J.M., Harrell, R.M., Geiger, J.G., Starling, C.C. and Revels, H. 2002. Performance comparisons between diploid and triploid sunshine bass in fresh water ponds. *Aquaculture*, 211(1-4): 91- 108.
- Kraszani, Z., Marian, T. and Kovacs, G. 1984. Production of triploid European catfish *Silurus glanis* by cold shock. *Aquacult. Hung.*, 4: 25-32.
- Ihssen, P.E., Mc Kay, L.R., Mc Millan, J. and Phillips R.B. 1990. Ploidy manipulation and gynogenesis in fishes: Cytogenetic and fisheries applications. *Trans. Am. Fish. Soc.*, 119: 698-717.
- Islam, M.S. Shah, M.S., Rahman, M.A. and Chowdhary, H.A. 1994. Induction of triploidy in Rohu, *Labeo rohita*, by heat shock treatment and comparative growth with normal diploid. *J. Aquacult. Trop.*, 9(4): 299-310.
- Johnson, O.W., Dickhiff, W.W. and Uter, F.M. 1986. Comparative growth and development of diploid and triploid Coho salmon, *Oncorhynchus kisutch*. *Aquaculture*, 57: 329-336.
- Johnson, R.M., Shrimpton, J.M., Heath, J.W. and Heath, D.D. 2004. Ploidyinduction methodology and interaction effects on the performance of diploid and triploid chinook salmon *Oncorhynchus tshawytscha*. *Aquaculture*, 234: 123-137.
- Johnstone, R., Knott, R.M., Macdonald, A.G. and Walsingham, M.V. 1989. Triploidy induction in recently fertilized Atlantic salmon ova using anesthetics. *Aquaculture*, 78: 229-236.
- Kavumpurath, S. and Pandian, T.J. 1990. Induction of triploidy in the Zebra fish, *Brachydanio rerio*. *Aquacult. Fish. Manag.*, 21(3): 299-306.
- Kavumpurath, S. and Pandian, T.J. 1992. Effects of induced triploidy on aggressive display in the

- fighter fish, *Betta splendens*. *Regan. Aquacult. Fish. Manage.*, 23(3): 281-290.
- Kerby, J.H., James, M., Everson, J.M., Harrell, R.M., Geiger, J.G., Starling, C.C. and Revels, H. 2002. Performance comparisons between diploid and triploid sunshine bass in fresh water ponds. *Aquaculture*, 211(1-4): 91- 108.
- Kim, D. S., Jo, Y. Y. and Lee, T.Y. 1994. Induction of triploidy in Mud loach, *Misgurnus mezolepis* and its effect on gonad development and growth. *Aquaculture*, 120 (3-4): 263-270.
- Kligerman, A.D. and Bloom, S.E. 1977. Rapid chromosome preparations from solid tissues of fish. *J. Fish. Res. Board Can.*, 34: 266-269.
- Kraszani, Z., Marian, T. and Kovacs, G. 1984. Production of triploid European catfish *Silurus glanis* by cold shock. *Aquacult. Hung.*, 4: 25-32.
- Lincoln, R.F. 1981b. Sexual maturation in triploid male Plaice *Pleuronectes platessa* and plaice × Flounder *Platichthys flesus* hybrids. *J. Fish. Biol.*, 19: 415-426.
- Lincoln, R.F. and Scott, A.P. 1983. Production of all female triploid Rainbow trout. *Aquaculture*, 30: 385-390.
- Lincoln, R.F. and Scott, A.P. 1984. Sexual maturation on triploid Rainbow trout, *Salmo gairdneri* Richardson. *J. Fish. Biol.*, 25: 385-392.
- Lou, Y.D. and Purdom, C.E. 1984. Polyploidy induced by hydrostatic pressure in Rainbow trout, *Salmo gairdneri* Richardson. *J. Fish. Biol.*, 24:345-351.
- Manna, G.K. and Khuda-Buksh, A.R., 1977. Karyomorphology in eleven species of Cyprinid fishes and the cytological evaluation of the family. *Nucleus*, 20: 119-127.
- Manning, A.J., Burton, M.P.M., and Crim, L.W. 2004. Original reproductive evaluation of triploid yellowtail flounder. *Aquaculture*, 242(1-4):625-640.
- Nakamura, M., Nagahama, Y., Iwahashi, M. and Kojima, M., 1987. Reproduction of triploid Rainbow trout *Salmo gairdneri*. University of Tokyo Ocean Research Laboratory Oh Tsu Chi Marine Biological Laboratory Report, 12: 167-171.
- Okada, H. 1985. Studies on the artificial sea control in rainbow trout, *Salmo gairdneri*. *Report of the Hokkaido Fish Hatchery*, 40: 1-49 (Canadian Translation of Fisheries and Aquatic Sciences 5329: 105).
- Oliva – Teles, A. and Kaushik, S.J. 1990. Growth and nutrient utilization by O<sup>+</sup> and 1<sup>+</sup> triploid Rainbow trout, *Oncorhynchus mykiss*. *J. Fish Biol.*, 37(1): 125-138.
- Opstad, I., Fjellidal, P.G., ØrjanKarlsen, Q., Thorsen, A., Tom, J., Hansen, T.J. and Taranger, G.L. 2013. The effect of triploidization of Atlantic cod (*Gadus morhua* L.) on survival, growth and deformities during early stages. *Aquaculture*, 388–391:54-59.
- Pandian, T.J. and Varadaraj, K. 1988. Techniques for producing all male and all triploid *Oreochromis mossambicus*. In. *Proc. Int. Symp. on Tilapia in Aquaculture. ICLARM Con. Proc.*, 15: 243-249.
- Pandian, T.J. and Varadaraj, K. 1989. Sterile female triploid in *Oreochromis mossambicus*. *Bull. Aqua. Assoc. Canada*, 88: 134-136.
- Peruzzi, S., Chatain, B., Saillant, E., Haffray, P., Menu, B., Falguiere, J.C. 2004. Production of meiotic gynogenetic and triploid seabass, *Dicentrarchus labrax*. 1. Performances, maturation and carcass quality. *Aquaculture*, 230 (1-4): 41-64.
- Piferrer, F., Benfey, T.J. and Donaldson, E.M. 1994. Gonadal morphology of normal and sex-reversed triploid and gynogenetic diploid Coho salmon, *Oncorhynchus kisutch*. *J. Fish Biol.*, 45: 451-553.
- Piferrer, F., Cal, R.M., Gomez, C., Bouza, C. and Marti, P. 2003. Induction of triploidy in the turbot (*Scaphthalmus maximus*). II. Effects of cold shock, timing and induction of triploidy in a large volume of eggs. *Aquaculture*, 220 (1-4): 821-831.
- Purdom, C.E. 1972. Induced polyploidy in Plaice *Pleuronectes platessa* and its hybrid with the Flounder *Platichthys flesus*. *Heredity*, 29: 11-29.
- Reddy, P.V.G.K., Mahapatra, K.D., Saha, J.N. and Jana, R.K. 1998. Effect of induced triploidy on the growth of Common carp, *Cyprinus carpio*. *J. Aqua. Trop.*, 13(1): 65-72.
- Refstie, T., Vassvik, V. and Gjedrem, T. 1977. Induction of polyploidy in salmonids by Cytochalasin B. *Aquaculture*, 10: 65-74.
- Scheerer, P.D. and Thorgaard, G.H. 1987. Performance and developmental stability of triploid Tiger trout, Brown trout × Brook trout. *Trans. Am. Fish. Soc.*, 116: 92-97.
- Schwarzbaum, P.J., Valcarcel, A. and Maggese, M.C. 1992. Ploidy of South American catfish *Rhamdiasapo* determined by spectrofluorometrically. *J. Aquacult. Trop.*, 7(2): 151-156.

## Growth performance of triploid *Labeo fimbriatus*

- Sheehan, R.J., Shasteen, S.P., Suresh, A.V., Kapuscinski, A.R. and Seeb, J.E. 1999. Better growth of all female diploid and triploid Rainbow trout. *Trans. Am. Fish. Soc.*, 128(3): 491-498.
- Simon, D.C., Scaeler, C.G. and Dillon, J.C. 1993. Field performance of diploid and triploid Rainbow trout in South Dakota ponds. *N. Am. J. Fish. Manage.*, 13: 134-140.
- Small, S.A. and Benfey, T.J. 1987. Cell size in triploid Salmon. *J. Exp. Zool.*, 241: 339-342.
- Smith, L.T. and Lemoine, H.L. 1979. Colchicine induced polyploidy in brook trout. *Prog. Fish. Cult.*, 41: 86-88.
- Stanley, J.G., Hidu, H and Allen, S.K.Jr. 1984. Growth of American oysters increased by polyploidy induced by blocking meiosis I but not meiosis II. *Aquaculture*, 37: 147-155.
- Streisinger, G., Walker, C. Dower, N., Knanber, D. and Singer, F. 1981. Production of clones of homozygous diploid Zebra fish. *Brachydanio rerio*. *Nature*, 29: 293-296.
- Svobodova, Z., Kolarova, J. and Flajshans, M. 1998. The first findings of the differences in complete blood count between diploid and triploid Tench, *Tinca tinca* L. *Acta Veterinaria-Brno.*, 67(4): 243-248.
- Sutterlin, A.M., Holder, T. and Benfey, T.T. 1987. Early survival rates and subsequent morphological abnormalities in landlocked, anadromous and hybrid (landlocked x anadromous) diploid and triploid Atlantic salmon. *Aquaculture*, 64(2): 157-164.
- Suzuki, R., Nakanishi, T. and Oshiro, T. 1985. Survival, growth and sterility of induced triploid Cyprinid loach *Mirgarnus anguillicaudatus*. *Bull. Jpn. Soc. Scum Fish.*, 51: 889-894.
- Swarup, H. 1959. Effect of triploidy on the body size general organization and cellular structure in *Gasterosteus aculeatus* L. *J. Genet.*, 56: 143-155
- Tave, D. 1993. Growth of triploid and diploid Big head carp, *Hypophthalmichthys nobilis*. *J. Appl. Aquacult.*, 2(2): 13-25.
- Taranger, G.L., Oppedal, F. and Hansen, T. 2003. Growth performance and sexual maturation in diploid and triploid Atlantic salmon (*Salmo salar* L.) in sea water tanks exposed to continuous light or simulated natural photoperiod. *Aquaculture*, 215 (1-4): 145-162.
- Teskeredzic, E.E., Donaldson, Z., Teskeredzic, Z., Solar, I.I. and McLean, E. 1993. Comparison of hydrostatic pressure and thermal shocks to induce triploidy in coho salmon (*Oncorhynchus kisutch*). *Aquaculture*, 117:47-55.
- Thorgaard, G.H. 1983. Chromosome set manipulation and sex control in fish. *Fish Physiology IX (Part B)*(Eds. W.S. Har, D.J., Randell, E.M. Donaldson), Academic Press, New York, pp. 405-434.
- Thorgaard, G.H. 1986. Ploidy manipulations and performance. *Aquaculture*, 57: 57-64.
- Thorgaard, G.H. and Gall, G.A.E. 1979. Adult triploids in Rainbow trout family. *Genetics*, 93: 961-973.
- Thorgaard, G.H., Rabinovitch, P.S., Shen, M.W., Gall, G.A.E., Propp, J. and Utter, F.M. 1982. Triploid Rainbow trout identified by flow cytometry. *Aquaculture*, 29: 305-309.
- Tiwary, B.K., Kirubagaran, R. and Ray, R.K. 1999. Altered body shape as a morphometric indicator of triploidy in Indian catfish *Heteropneustes fossilis* (Bloch). *Aquacult. Res.*, 30(11-12): 907-910.
- Ueda, T. Sato, R. and Kabayashi, J. 1988. Triploid Rainbow trout induced by high pH – high calcium. *Jap.Soc. Sci. Fish.*, 54(11): 2045.
- Varadaraj, K. and Pandian, T.J. 1988. Induction of triploids in *Oreochromis mossambicus* by thermal hydrostatic pressure and chemical shocks. *Proceedings of the Aquaculture International Congress and Expo*: 531-535.
- Vejaratpimol, R. and Pewnim, T. 1990. Induction of triploidy in *Clarias macrocephalus* by cold shock. *Proceeding of the second Asian Fisheries Forum*: 531-534.
- Warrillow, J.A., Josephson, D.C., Young, W.D. and Krueger, C.C.A. 1997. Difference in sexual maturity and fall emigration between diploid and triploid Brook trout, *Salvelinus fontinalis* in an Adiraondack lake. *Can. J. Fish. Aquat. Sci.*, 54(8): 1808-1812.
- Wattendorf, K.J. 1984. Genetic monitoring Grass carp/hybrid/grass. Reports for 1980-1984: No 13 Gainesville: State of Florida Game and Fresh Water Fish Commission.
- Withler, R.W. and Clarke, W.C. 1998. Effect of triploidy on growth and survival of pre-smolt and post-smolt Coho salmon *Oncorhynchus kisutch*. *Aquaculture*, 168: 413-422.
- Wolters, W.R., Chrisman, C.L. and Libey, C.S. 1982. Erythrocyte nuclear measurements of diploid and triploid Channel catfish, *Ictalurus punctatus*. *J. Fish. Biol.*, 20: 253-258.

