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Preliminary study on performance of triploid Thai silver barb, *Puntius gonionotus*

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Abstract

The triploid chromosome condition was induced in Thai silver barb (*Puntius gonionotus*) by application of cold shock (2°C) to eggs at time intervals after activation of 0.5 min with a duration of 10 min which resulted in mean triploidy yield of 72.5% at 9 months of age. Growth rate of the 2–9-month-old, cold shock group (0.1–6.2 g/month) did not differ from that of the control (0.1–5.7 g/month). Gonadal somatic indices (GSI) of presumed triploid males and females were lower than that of control (GSI values of the presumed triploids were 35.0–60.2% and 28.7–75.9% of control males and females, respectively). Spermatogenesis and oogenesis were retarded in triploids. However, all stages of spermatogenic cells were observed in triploid males, including few spermatozoa. Oocytes of triploid females did not undergo vitellogenesis while normal oogenesis was observed in diploids. Nuclear volume of red blood cells (RBCs) of triploid fish was 1.63 times larger than that of diploids.

Keywords: Triploid; Cold shock; *Puntius gonionotus*

1. Introduction

Thai silver barb, *Puntius gonionotus* is one of the most economically important freshwater fish in Thailand. It grows to a marketable size within 8–12 months but matures at 4–6 months of age. Consequently, growth retardation was observed due to reduced feed consumption (Saepithakkiat and Leenanond, 1984) and utilization of energy portions for gonad development and reproduction activities (Cassani and Caton,

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1986). Therefore, culture of sterile fish may enhance growth after maturation and thereby increase yield.

Induction of triploidy was very effective in producing sterile fish, as has been reported in some species of cyprinids such as grass carp, *Ctenopharyngodon idella* (Cassani and Caton, 1986) and common carp, *Cyprinus carpio* (Cherfas et al., 1990). A preliminary study conducted by Na-Nakorn and Legrand (1992) indicated that cold shock of 15°C applied immediately after activation and lasting 10 or 15 min, induced more than 90% triploidy in Thai silver barb, but hatching rate was low. Thus, the cold shock conditions have not been optimized for Thai silver barb and the study on the performance of triploid fish still needs to be assessed.

Therefore, this study was conducted to compare the performance of diploid and triploid fish under culture conditions.

2. Materials and methods

2.1. Brooders and artificial fertilization

Gravid females of Thai silver barb were selected for breeding based on their abdominal swelling and soft bellies. Females were injected with 20 μg LHRHa (Suprefact) + 5 mg Domperidone (Motilium)/kg body weight, and stripping was done 4 h later. Freshly stripped eggs were mixed with milt collected from male Thai silver barb without hormone injection. Water of ambient temperature (28°C) was added immediately when the time was considered 0 min after activation.

2.2. Cold shock

Cold shock was conducted at water temperature of 2°C as reported by Roongratri and Vipudthanumas (1991). The conditions for cold shock were 10-min shock duration, commencing 0.5 min after water activation of eggs, as were obtained from our preliminary experiment.

2.3. Comparison of diploid and triploid performances

Eggs collected from each of four female Thai silver barb were divided into two portions. Each portion was fertilized with sperm collected from the same male. Then, these were either incubated in a hatching jar supplied with water of ambient temperature (28 ± 1°C) or subjected to cold shock. Groups of fry were separately reared in 500-l fiberglass tanks at a stocking rate of 200 fry per tank. For the first 15 days, fry were fed ad libitum three times a day, with whole egg particles prepared by adding 100 ml hot water into a thoroughly mixed whole eggs. From day 16 they were fed ad libitum with artificial powder feed (40% protein) and *Artemia* nauplii twice a day until 1 month of age. Then, artificial pelleted feed (25% protein) was fed throughout the rest of the experiment.

At 2 months of age, three groups of 50 fish each were randomly taken from each tank and randomly stocked in 24 cages of 1.8×3 m fixed in an 800-m^2 earthen pond. Measurements made on 30 fish per tank showed that the initial size of putative triploid and control fish were 0.5 and 0.6 g; 0.4 and 0.4 g; 0.6 and 0.4 g; 0.7 and 0.5 g in full-sib families 1, 2, 3 and 4, respectively, and they were statistically different.

Meanwhile, samples of 50 and 20 fish per family were, respectively, taken from the cold shocked and control groups for determination of chromosome number, following the method described by Kligerman and Bloom (1977).

Measurements on weight were made monthly on random samples of 30 fish per cage. Growth rate per month was calculated. At 9 months of age, experimental fish were harvested, 20 fish per replicate were individually weighed, gonads were removed and GSI (gonadal somatic index) was calculated as percentage of gonad weight to total body weight. The fish were filleted and carcass percentage was calculated.

The remaining 13–20 fish per replicate were used for estimating red blood cell (RBC) nuclear volume; individual blood samples were taken from a caudal vein using a heparinized needle. Blood was smeared on a microscopic slide and stained with 10% Giemsa stain (Beck and Biggers, 1983). Approximately 10 cells per individual were investigated under a microscope, and length and width of RBC nuclei were measured. Nuclear volume was calculated as:

$$\text{nuclear volume} = 4/3\pi ab^2 \qquad \text{(Beck and Biggers, 1983)}$$

where $a = \text{RBC length}/2$, and $b = \text{RBC width}/2$.

After blood samples were taken, chromosome numbers of individual fish were determined and the gonads sectioned for histology. Gonadal histology of the fish, whose ploidy level had been identified, was used to characterize histology of either diploid or triploid gonads.

2.4. Data analyses

Analysis of variance in completely randomized design was applied to data on growth rate and survival rate in the experiment on comparison of control and the putative triploid performance. Means were compared using Duncan's new multiple range test. Sex ratio was tested for deviation from a 1:1 ratio using a χ^2 test. Carcass percentage, GSI and RBC nuclear volume of diploids were compared to those of the respective triploids using *t*-test (Steel and Torrie, 1980).

3. Results

3.1. Chromosome number of diploid and triploid Thai silver barb

Chromosome numbers of the diploid and triploid were 50 and 75, respectively. The presumed triploid group of families 1, 2, 3 and 4 consisted of 96%, 91.5%, 100% and 64% triploid fish, respectively.

Table 1
Mean ± s.d. of growth rate (g/month) of triploid fish obtained from different females (T_1–T_4) compared to the respective diploid (D_1–D_4) during 7 months of the experiment and survival rate (%) at harvesting. The fish were stocked at 2 months old

Fish group	Month							Final weight[a] (g)	Survival rate (%)
	0–1	1–2	2–3	3–4	4–5	5–6	6–7		
T_1	3.9 ± 0.1 a	1.9 ± 1.2 a	5.2 ± 1.5 a	4.7 ± 0.6 a	2.0 ± 1.8 a	3.4 ± 0.6 a	1.6 ± 0.6 a	23.3 ± 2.1	73.3 ± 9.2 a
D_1	3.6 ± 0.4 a	3.6 ± 0.9 a	5.3 ± 1.6 a	2.9 ± 2.6 a	1.9 ± 1.1 a	5.7 ± 1.2 a	1.9 ± 1.4 a	25.6 ± 4.8	82.0 ± 2.0 a
T_2	4.1 ± 0.4 a	2.0 ± 0.5 a	3.0 ± 1.5 ab	1.6 ± 1.1 a	1.3 ± 1.3 a	2.3 ± 0.3 a	3.4 ± 1.7 a	18.2 ± 0.4	74.0 ± 13.1 a
D_2	3.7 ± 0.2 a	2.9 ± 1.2 a	4.8 ± 1.6 a	3.2 ± 1.8 a	0.4 ± 0.4 a	2.3 ± 1.1 a	1.6 ± 1.6 a	22.0 ± 1.4	62.7 ± 4.2 a
T_3	4.1 ± 0.5 a	1.5 ± 0.5 a	2.1 ± 0.2 b	2.0 ± 0.6 a	0.5 ± 0.1 a	−0.1 ± 1.4 a	1.4 ± 0.8 a	12.0 ± 2.0	80.0 ± 8.7 a
D_3	4.2 ± 0.5 a	1.8 ± 0.7 a	2.9 ± 0.9 ab	2.2 ± 0.7 a	0.3 ± 0.1 a	0.8 ± 1.8 a	0.1 ± 0.6 a	12.7 ± 1.1	66.7 ± 6.2 a
T_4	4.3 ± 0.2 a	2.9 ± 0.5 a	3.1 ± 0.5 ab	2.9 ± 1.9 a	0.2 ± 0.2 a	1.3 ± 1.3 a	6.2 ± 1.9 a	21.5 ± 3.6	66.0 ± 10.4 a
D_4	3.5 ± 0.4 a	2.8 ± 0.9 a	3.4 ± 0.8 ab	0.3 ± 0.0 a	1.6 ± 0.8 a	1.6 ± 0.5 a	2.7 ± 0.3 a	16.4 ± 0.9	64.0 ± 6.9 a

Triploid (T) and diploid (D) designated with the same numbers were obtained from the same parents.
Means in a column followed by the same letters were not statistically different ($P > 0.05$).
[a] Final weight was not statistically compared due to differences in initial weight.

3.2. Growth rate and survival rate

Average weight of the experiment fish at harvesting was 12–25 g. Growth rates of the putative triploid and control were very low and ranged between -0.1 ± 1.4 to 6.2 ± 1.9 and 0.1 ± 0.6 to 5.7 ± 1 g/month, respectively (Table 1). Survival rates of diploids ranged from $62.7 \pm 4.2\%$ to $82 \pm 2\%$, while that of the putative triploid were $66 \pm 10.4\%$ to $80 \pm 8.7\%$ (Table 1). No significant differences between the two groups were observed on growth rate and survival rate at 9 months.

3.3. Carcass percentage

Carcass percentages of the control and putative triploid fish of the same family did not differ, although differences were shown between control fish of different families (Table 2). More fat mass was found in the body cavity of the putative triploid than in the control fish.

3.4. Sex ratio

Sex ratio, based on histology of gonads, showed that the ratio of female:male control fish and putative triploid fish from families 1 and 4 did not differ from a 1:1 ratio. Surprisingly, the putative triploids of family 2 consisted of more males than females (sex ratio, 1 female:5.7 males), and family 3 consisted of all males (Table 2).

Table 2
Mean $\pm$ s.d. of RBC volume, carcass percentage, sex ratio and GSI (at 9 months old) of triploid fish (T_1–T_4) obtained from four different females compared to diploid fish (D_1–D_4) from the same four females. Data were analyzed using ANOVA in completely randomized design, excepted sex ratios that were tested for deviation from 1:1 ratio using a λ^2 test. GSI values were compared between diploid and triploid fish of the same sex obtained from the same female (data were obtained from 20 fish per group excepted where indicated)

Fish group	RBC volume (μm^3)	Carcass (%)	Sex ratio (female:male)	Number of fish examined for sex ratio	GSI	
					Female	Male
T_1	25.8 ± 1.2 a	57.6 ± 4.6 a	1:1.9^{ns}	20	$0.4 \pm 0.8^{*}$	$0.3 \pm 0.2^{*}$
D_1	15.4 ± 1.1 b	58.7 ± 3.3 ad	1:1.5^{ns}	15	1.5 ± 1.1	0.8 ± 0.7
T_2	24.5 ± 0.8 a	55.0 ± 3.5 abc	1:5.7**	20	$1.0 \pm 0.1^{*}$	$0.5 \pm 0.1^{*}$
D_2	15.5 ± 0.9 b	52.9 ± 3.6 bce	1:1.2^{ns}	13	2.1 ± 0.8	0.8 ± 0.4
T_3	25.3 ± 1.1 a	53.3 ± 3.4 bde	0:1**	20	–	$0.3 \pm 0.2^{*}$
D_3	15.3 ± 0.6 b	52.5 ± 5.7 bce	1:1.9^{ns}	20	1.5 ± 0.3	0.6 ± 0.4
T_4	25.5 ± 0.8 a	53.5 ± 3.1 bde	1:1.5^{ns}	20	0.6 ± 0.6	$0.3 \pm 0.1^{*}$
D_4	15.6 ± 0.9 b	50.9 ± 2.4 e	1:1.2^{ns}	13	0.8 ± 0.5	0.6 ± 0.4

Means in a column followed by the same letters are not statistically different ($P > 0.05$).
^{ns} not deviated from a 1:1 ratio.
* Significantly differed from the diploid counterpart.
** Significantly deviated from a 1:1 ratio ($P < 0.01$).

3.5. GSI

The GSI of the putative triploid males and females was smaller than those of the respective control (35.0–60.2% and 28.7–75.9% of the control males and females) except those of family 4, where GSI of control females and putative triploid female were not different (Table 2).

3.6. Histology of gonads

Testes of the control fish were swollen, milky white and fully developed. Cross-section showed seminiferous tubules filled with spermatozoa. Few spermatids were also observed. Testes of the putative triploid fish were small, pinkish and thread-like. Histologically, the spermatogenesis in the putative triploids was retarded, as characterized by small vacuolated seminiferous tubules. Germ cells of every spermatogenic stage were found, including small number of spermatozoa in the putative triploid fish (Fig. 1).

Ovaries of control fish contained numerous pre-vitellogenic oocytes characterized by pinkish nuclei and deep-purple cytoplasm. However, certain amount of oocytes had undergone vitellogenesis, revealing normal oogenesis in the control fish ovaries. On the contrary, no oocytes had undergone vitellogenesis in the putative triploid ovaries indicating the sterility of females of this group (Fig. 2).

3.7. RBC nuclear volume and percentage of triploid at harvesting

Nuclear volume of RBC of every triploid group was larger than that of their full-sib diploid (Table 2). RBC nuclei of triploids were approximately 1.63 times larger than diploid.

Percentage of triploid fish in families 1–4 were 100%, 95%, 100% and 60%, respectively.

4. Discussion

The fish used in this experiment grew slower than previously reported. Normally, the average weight of 9-month-old Thai silver barb cultured in cages is about 50 g. High stocking density and low temperature during the winter months (20 ± 5°C), when the experiment was conducted, may account for the slow growth rate in our study. However, we believe that the unfavorable culture condition did not alter the general result. Cassani and Caton (1986) reported that when diploid and triploid grass carp were separately cultured in both favorable and unfavorable environments the same relative growth of the diploid and triploid fish was observed.

Sexual maturation of Thai silver barb commences at ages 4–6 months (Saepithakkiat and Leenanond, 1984). Growth difference before maturation was not observed between the control and the putative triploid fish. This suggests no metabolic advantage resulting from an extra chromosome-set of the triploids. Similar results of growth trials were

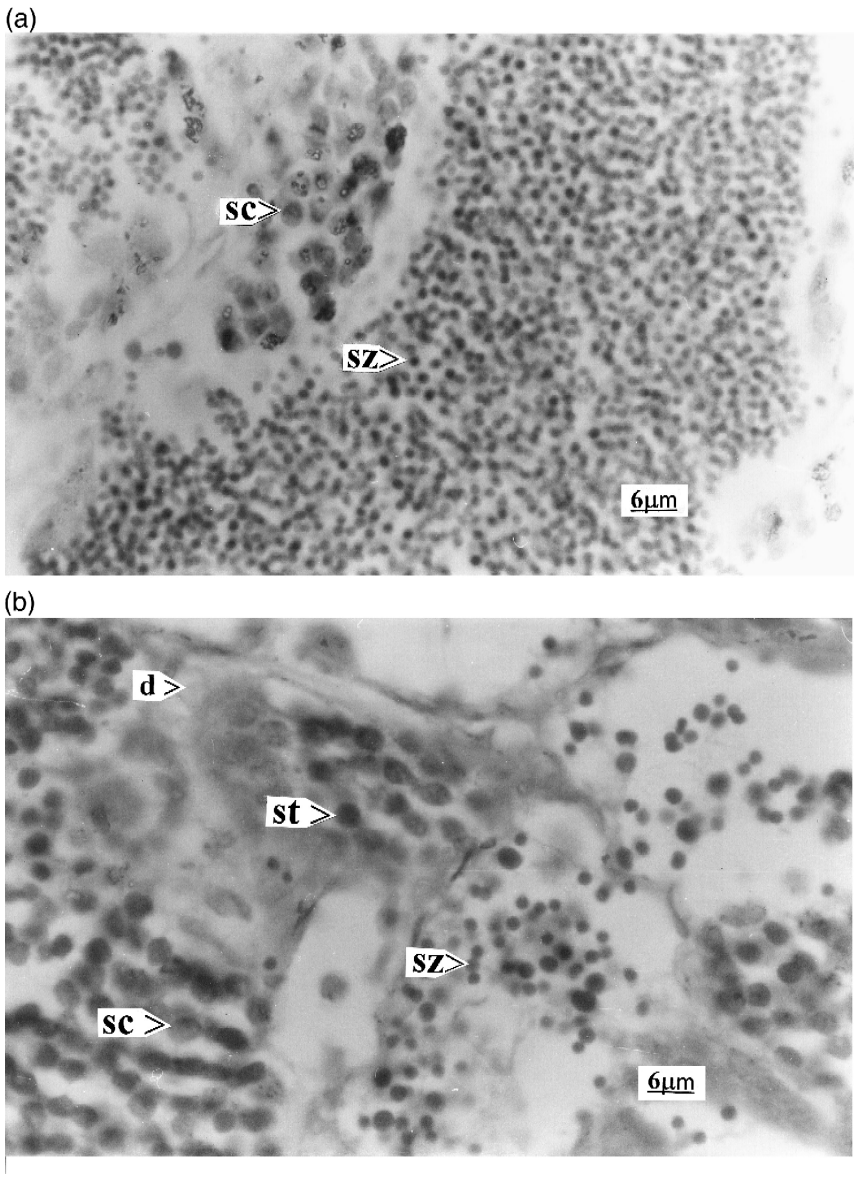

Fig. 1. Cross-section of testes of diploid (a) and triploid (b) *P. gonionotus* at 9 months old. A diploid testis is dominated with spermatozoa (sz). A few spermatocytes (sc) were also observed. While in a triploid testis, spermatogenic cells of early stages are more abundant (st = spermatids, sc = spermatocytes) and degenerated area is observed (d = degenerated area).

reported in many fish species such as African catfish, *Clarias gariepinus* (Henken et al., 1987), channel catfish, *Ictalurus punctatus* (Wolters et al., 1987), and where difference between growth rate before sexual maturation of triploids and diploids was not observed.

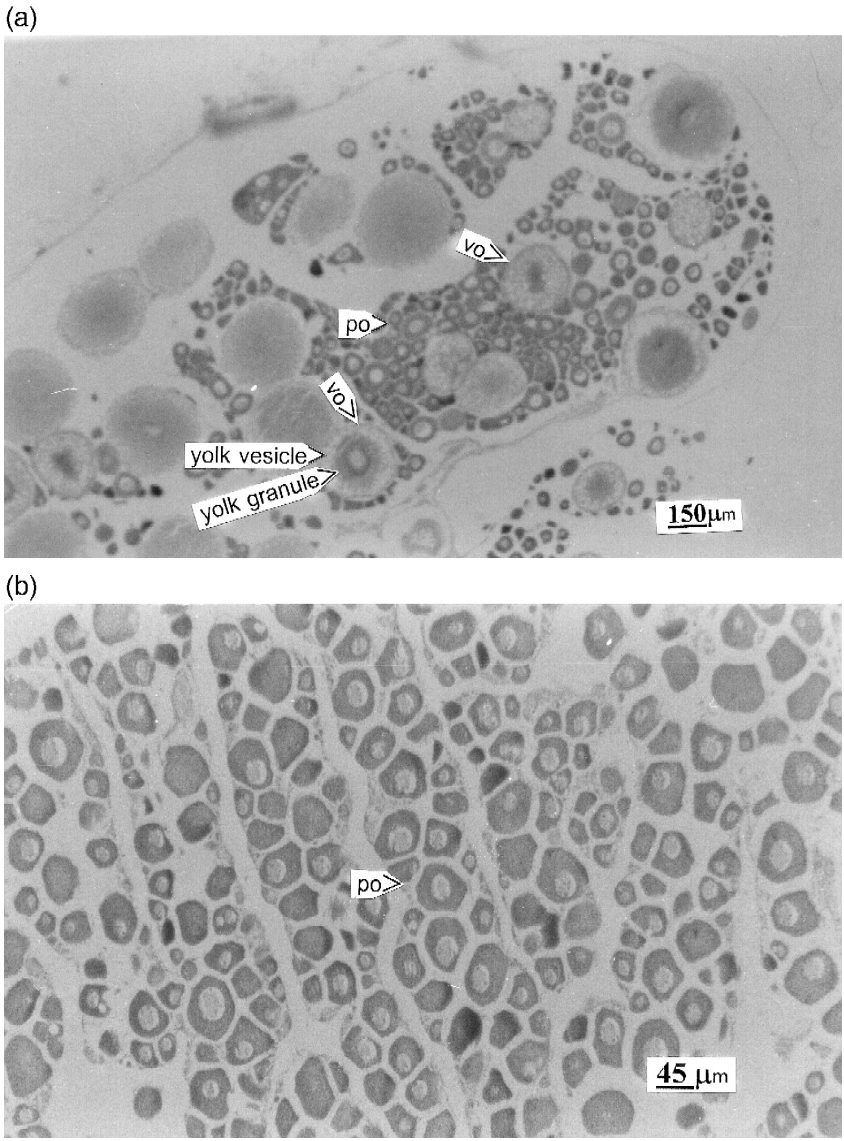

Fig. 2. Cross-section of ovaries of diploid (a) and triploid (b) *P. gonionotus* at 9 months old. A diploid ovary is dominated by pre-vitellogenic oocytes (po), few oocytes undergoing vitellogenesis (vo) are also observed. In a triploid ovary, only pre-vitellogenic oocytes are found.

Moreover, juvenile triploids of common carp (Taniguchi et al., 1986) and Thai walking catfish, *C. macrocephalus* (Na-Nakorn and Lakhaanantakun, 1993) even grew slower than diploid fish of the same species.

Triploid females were sterile as indicated by the absence of vitellogenic oocytes in the ovaries of the putative triploid group, while oogenesis was normal in the control

ovaries. On the contrary, male fish of the putative triploid group were not completely sterile as indicated by the presence of spermatozoa in their testes. Our results agreed with those previously reported by Na-Nakorn and Legrand (1992). Fertilizing ability of the spermatozoa was not evaluated. They may normally function as in male triploid grass carp (Thompson et al., 1987) and male triploid rainbow trout (Lincoln and Scott, 1983), whereas, triploid females of these two species were also completely sterile. However, although spermatozoa were observed in triploid testes of common carp, fertilization resulted in unviable fry that might have been due to aneuploidy (Cherfas et al., 1994).

Theoretically, growth advantage of triploid fish is expected after sexual maturation, when somatic growth of diploid fish is usually suppressed by the reproductive process. However, such advantage was observed in only a few fish species such as channel catfish (Wolters et al., 1982). Better growth rates of triploid female over the diploid control were observed at the age of sexual maturation in rainbow trout (Thorgaard, 1986). In the present study, although triploid females were completely sterile and triploid males showed obvious retardation of testes, growth of the presumed triploid group did not exceed that of the control diploid. This might support the suggestion of Cherfas et al. (1994), who observed inferior growth of triploid common carp to the corresponding diploid control, that triploidy might have some negative physiological effects on growth rate. Equal or inferior growth performances of sterile triploids to diploids has been reported in several fish species such as grass carp (Cassani and Caton, 1986), African catfish, (Henken et al., 1987), Asian catfish (Rustidja et al., 1992) and Thai walking catfish (Na-Nakorn and Lakhaanantakun, 1993).

However, in the present study, results of performance in the growth experiment were preliminary and it is possible that the environmental effects, statistical error and experimental design (in which sex and ploidy were not separately treated) have made the difference of growth performance between ploidy less clear.

Carcass percentages of diploid and triploid were not different. Our results contradicted those reported by Lincoln and Scott (1983) in rainbow trout, *Oncorhynchus mykiss* and African catfish (Henken et al., 1987). Dress-out percentage of triploid female rainbow trout and mixed-sex African catfish was higher than diploid control by 6.5% and 2%, respectively, due to more fat deposits. However, we also observed more fat deposited in the body cavity of triploids but it was removed when carcass percentage was determined.

Different carcass percentages were observed between diploid of different families which might indicate genetic control of this trait in Thai silver barb.

Deviation from 1:1 sex ratio was observed in triploid fish from two families while the diploid groups had normal sex ratio of 1:1. Altering sex ratio in favor of males due to triploidy was previously reported in rainbow trout (Solar et al., 1984) and common carp (Cherfas et al., 1994). Differential mortality between sex was a possible explanation of the abnormal sex ratio (Cherfas et al., 1994). In our case, it is unlikely that triploidy led to the lower survival rate of females because percentage of triploid fish at harvesting did not decrease. However, we do not have any appropriate explanation.

RBC nuclear volume of triploid fish was approximately 1.63 times larger than diploids, as would be expected with an extra set of chromosomes. This provided an

alternative technique to assess ploidy level without killing the experimental fish. Moreover, this technique was cheap and fast as compared to the actual chromosome count technique.

Although triploid Thai silver barb might not be useful for aquaculture, the information concerning sex ratio might be valuable for further studies on sex determining system of this species.

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