INDUCTION OF TRIPLOIDY IN *PUNTIUS GONIONOTUS* (BLEEKER) BY COLD SHOCK

Uthairat Na-Nakorn and Emmanuelle Legrand, Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok 10903, Thailand.

ABSTRACT

The experiment on induction of triploidy in *Puntius gonionotus* was done by using cold shock technique. Eggs stripped from 2 females were fertilized with sperm from different males and subsequently dipped in cold water (15°C) at 0 and 5 minutes after fertilization with shock durations of 15 and 30 minutes. It was revealed that only cold shock at 0 minute after fertilization with shock durations of 15 and 30 minutes produced triploid fry with the average percentages of 95.45 and 90.45 respectively. Their average hatching rates decreased considerably to 40.96 and 33.87% as well as survival rates which were 46.53 and 16.01% respective to shock durations of 15 and 30 minutes. At maturity gonads of triploid fish tremendously reduced in size when compared with their diploid counterpart. Histological study showed few previtellogenic oocytes and spermatozoa in ovaries and testes respectively.

INTRODUCTION

Inferior growth rate of male has been widely accepted in tawes, *Puntius gonionotus* (Bleeker) which may be a result of its early maturing (4 months compares to 8 months in female). In normal rearing condition female reaches marketable size (500–700 grams) within 8–12 months while male grows to only 300 grams in the same period. Thus, the production of sterile triploid, which could potentially have better growth than diploid at time of sexual maturation, may provide practical solution to this problem.

This experiment was intended to learn the effects of cold shock on induction of triploidy in tawes as well as the gonadal development of triploid fish at normal time of maturation.

MATERIALS AND METHODS

Fertilization and cold shock

Gravid females were injected crude pituitary extract collected from silver carp (1.5 kgs donor/1 kg brooders). No injection was made for male. At 6 hours after injection eggs were stripped and separated into groups of 300 and inseminated with equal amounts of milt. Water–activation was done for about 30 seconds and rinsed prior to cold shock.

The cold shock was carried out at 0 and 5 minutes after water activation. Eggs in a dipnet were submersed in cool water (15°C) for either 15 or 30 minutes and subsequently transferred to a hatching jar at 28 ± 2°C. A control group was incubated at 28 ± 2°C throughout the hatching period.

The experiment was carried out in 2 replicates using different females. Hatching rates was determined and expressed as percentage of hatching rate of control. Larvae were separately reared in fine mesh cages (30 × 50 cm^2).

They were fed boiled egg yolk plus *Chlorella* for 10 days which were gradually substituted with rice bran. Survival rate at 1 month was determined and expressed as percentage of survival rate of control.

Determination of ploidy

Approximately ten fish were randomly taken from each replicate and level of ploidy was determined by chromosome counts in gill tissue prepared according to the method described by Kligerman and Bloom (1977.)

Study of gonadal development

Fish from control group and the groups that produced triploid were reared separately in nylon net cages in earthen pond (10 fish/1m^2 – cage, 2 cages/ group). They were fed ad libitum commercial feed (15 % crude protein). Chromosome study was carried out in 10-month-old fish. Gonads were fixed in Carnoy fluid, dehydrated and embedded in paraffin. Section of 5 μm were stained with haematoxylin eosin.

Statistical methods

Analysis of variance in hatching rate, survival rate at 1 month and incidence of triploid were performed using one way ANOVA. Effects of each factor were analyzed. Means were compared using Duncan's new multiple range test.

RESULTS

Cold shock initiated immediately after fertilization resulted in lower hatching rates compared to cold shock initiated 5 minutes after fertilization (Table 1). Shock durations of 15 and 30 minutes did not affect hatching success. Triploid fish were observed only in the groups immediately subjected to cold shock after fertilization with shock durations of 15 or 30 minutes. Survival rates of all the treatment groups were not statistically different (Table 1).

Chromosome study

Chromosome spread of diploid (2n = 50) and triploid fish (2n = 75) were shown in figure 1. Only the groups exposed to cold shock at 0 minute after fertilization produced triploid fry. Incidence of triploid did not differ significantly for shock durations of 15 and 30 minutes.

Gonadal development

After 10 months only 10 fish from the diploid group and 8 triploid fish survived. Triploidy was confirmed by chromosome study. Surprisingly all diploid were males and produced milt that can easily be stripped out. Their gonads were fully developed (Fig. 2a). Investigation of gonads of triploids revealed that sex differentiation occured, 6 triploids were male and two of them were female. Sizes of triploid gonads reduced tremendously (Fig. 2b and 2c).

Testis of male diploid fish contained every stage of gamete especially

spermatozoa which occupied most of testes area (Fig 3). In triploid testes few spermatozoa were found. Spermatogenic cells at early stages, namely spermatocytes and spermatids, were observed (Fig 4). Ovaries of triploid females contained previtellogenic oocytes and few oogonia.

DISCUSSION

The results indicated that cold shock (15°C) initiated 0 minute after fertilization and lasted for 15 or 30 minutes was effective in inducing triploidy in tawes. Cold shock applied at 5 minutes after fertilization could not induce triploidy, because eggs have finished extrusion of second polar body before cold shock was applied (Richter et al., 1987) or water absorbed during water hardening might prevent the eggs from directly exposed to low temperature, thereby lowered the efficiency of cold shock. The exact time after fertilization must be studied in order to increase hatching rates of triploids.

Hatching rates of eggs exposed to cold shock at 5 minutes after fertilization were higher than that of the control group, because in estimating hatching rates of treated groups the eggs broken during application of cold shock were discarded. These eggs might be low quality ones which lowered the hatching rate of the control groups.

Survival rates of the treated groups were relatively low ; most of larvae died at yolk absorption. This indicated that temperature of 15°C may not be suitable for triploid induction of tawes. Higher temperature must be studied.

Sampling error was accounted for occurance of all-male controls. Ovaries of triploid fish contained no oocytes that succeeded in passing through the first and second meiotic divisions while both meiotic divisions were succeeded in male triploid. Similar results were obtained in Atlantic salmon, *Salmo salar* in which triploid males produced mature gametes while meiotic division did not occur in female gonads (Benfey and Sutterlin, 1984). Different type of sterility has been reported in triploid channel catfish, *Ictalurus punctatus* and African catfish, *Clarias gariepinus*, in which gonadal development of both sexes was reported to be totally suppressed. (Wolters et al., 1982 ; Richter et al., 1987).

Further studies are needed concerning growth, feed conversion ratio and dressing percentage of triploid tawes to evaluate its commercial values compares to the diploid ones.

Table 1 Hatching and survival rates and incidence of triploid of tawes (% of control) treated with cold shock (1.5 °C) at 0 and 5 minutes after fertilization with shock durations of 15 and 30 minutes

Time after fertilization (min)	shock duration (min)	hatching rate (%)	survival rate (%)	triploid (%)
0	15	40.96 ± 4.75 a	46.53 ± 1.93 a	95.45 ± 4.54 a
	30	33.87 ± 9.53 a	16.01 ± 0.90 a	90.45 ± 0.45 a
5	15	115.16 ± 24.37 b	18.33 ± 7.56 a	0 b
	30	102.65 ± 6.65 b	27.52 ± 11.70 a	0 b
Control		70.25 ± 5.75	67.25 ± 2.25	

Note : – Means in the same column followed by same alphabets are not significantly different ($P > 0.05$)

– Control was not included in the statistical analysis

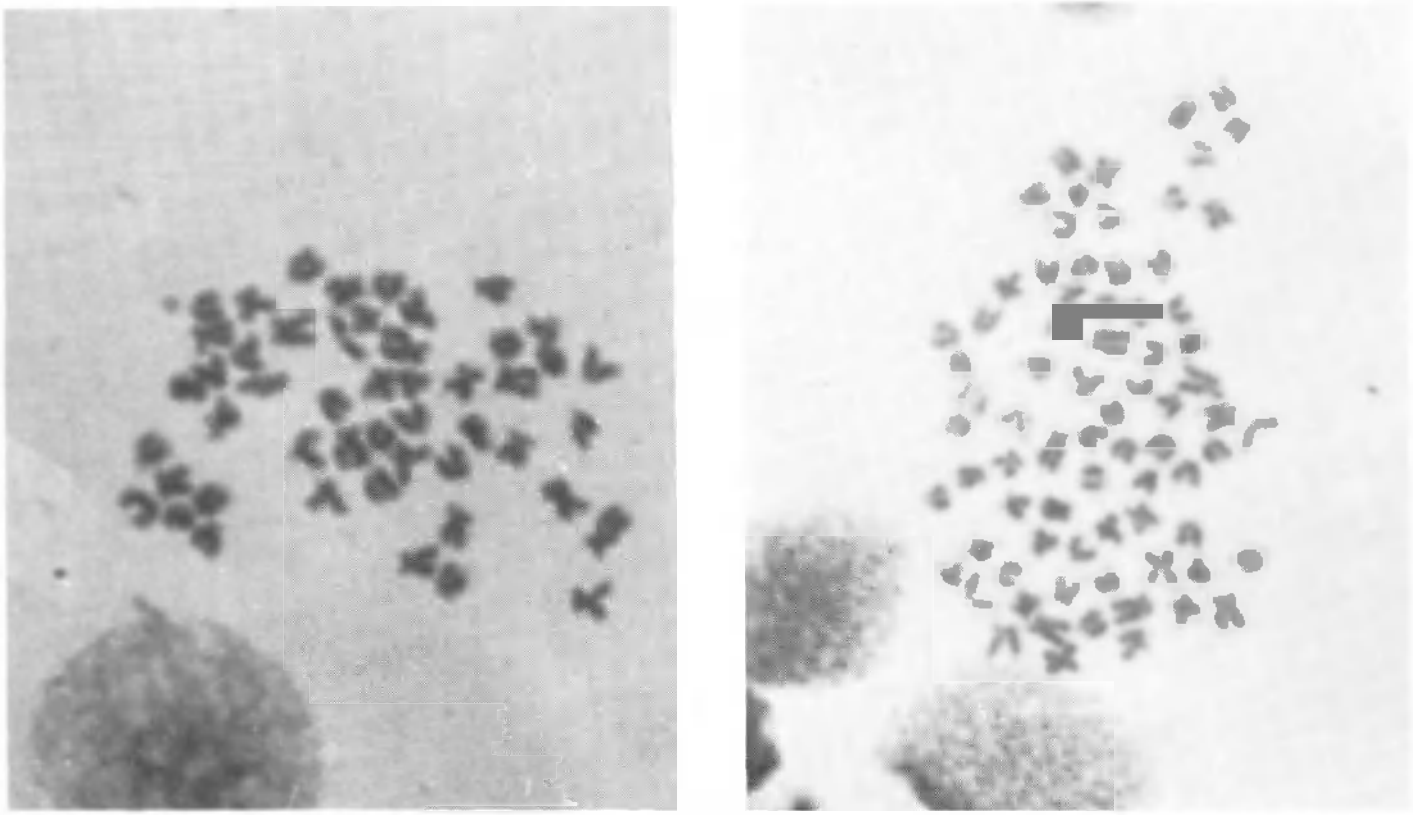
REFERENCES

Benfey, T.J. and A.M. Sutterlin. 1984. Triploidy induced by heat shock and hydrostatic pressure in land locked Atlantic salmon (*Salmo salar* L.). Aquaculture, 36 : 359–367.

Kligerman, A.D. and S.E. Bloom. 1977. Rapid chromosome preparations from solid tissues of fishes. J. Fish. Res. Board Can., 34 : 266–269.

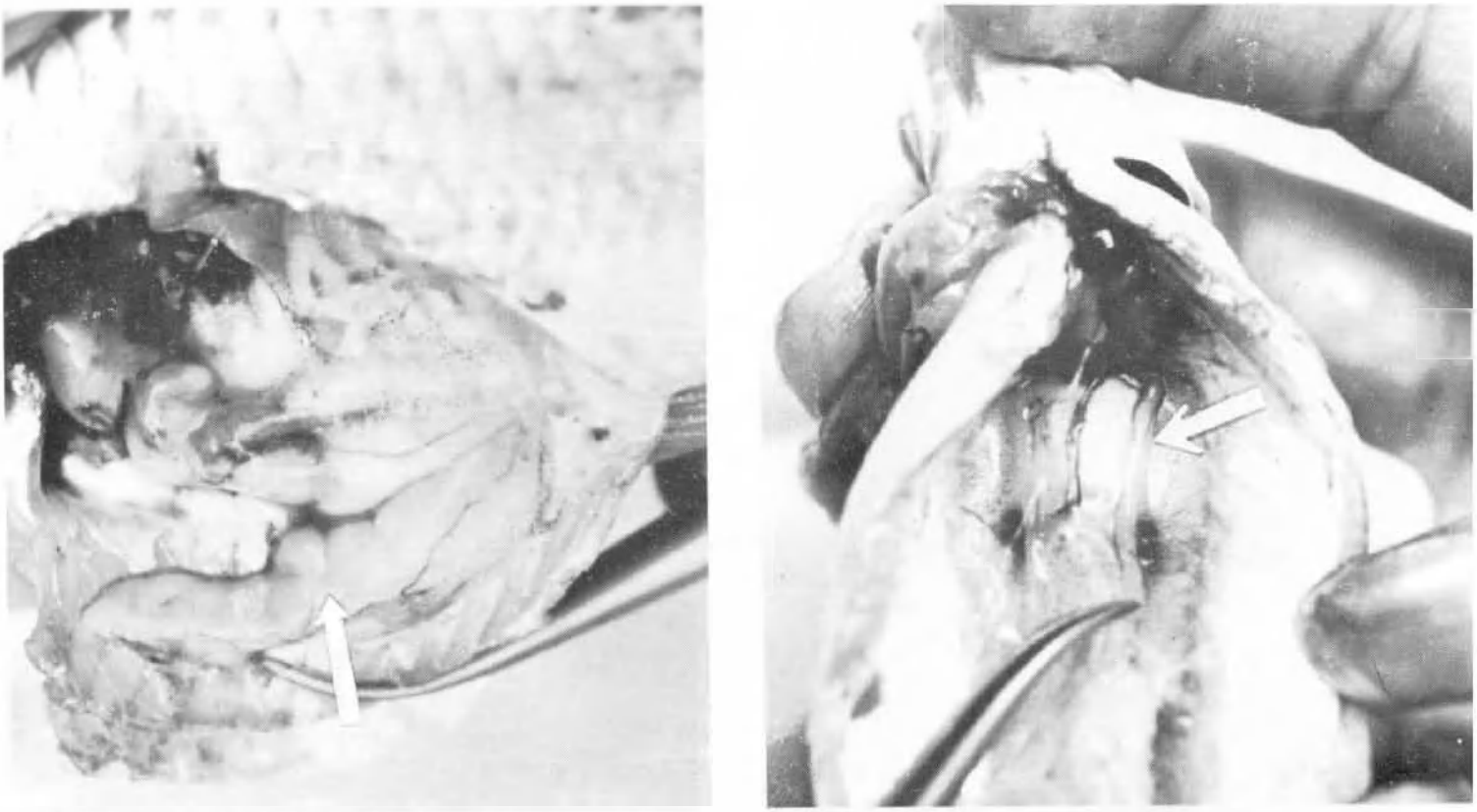
Richter, C.J.J., A.M. Henken, R.H. Eding, J.H. Van Doessurm, and P. De Boar. 1987. Induction of triploidy by cold-shocking eggs and performance of triploids of the African catfish, *Clarias gariepinus* (Burchell, 1822).

Wolters, W.R., G.S. Libey and C.L. Chrisman. 1982. Effect of triploidy on growth and gonad development of channel catfish. Trans. Am. Fish. Soc. 111 : 102–105.



a b

Figure 1 Metaphase chromosome of *Puntius gonionotus*
a) diploid (2n = 50)
b) triploid (3n = 75)



a

b

c

Figure 2 Gonads of 10-month-old *Puntius gonionotus*

a) diploid male

b) triploid male

c) triploid female

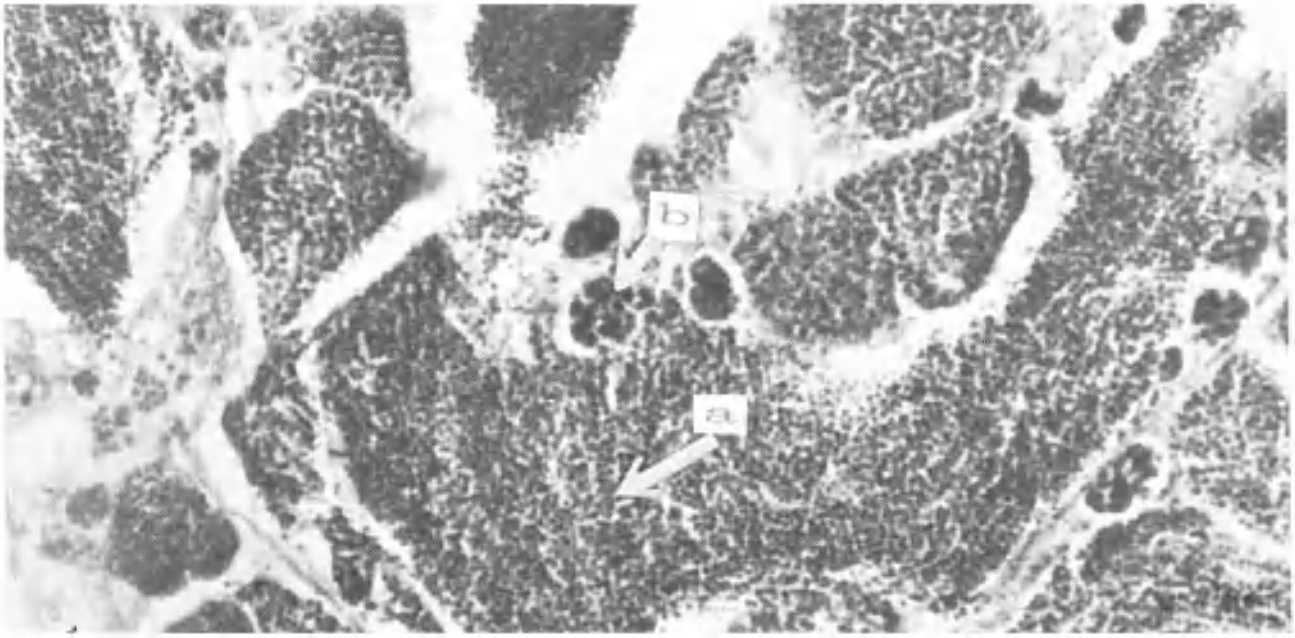


Figure 3 Cross section of diploid testes filled with spermatozoa (a) and few spermatids (b) (x 400)

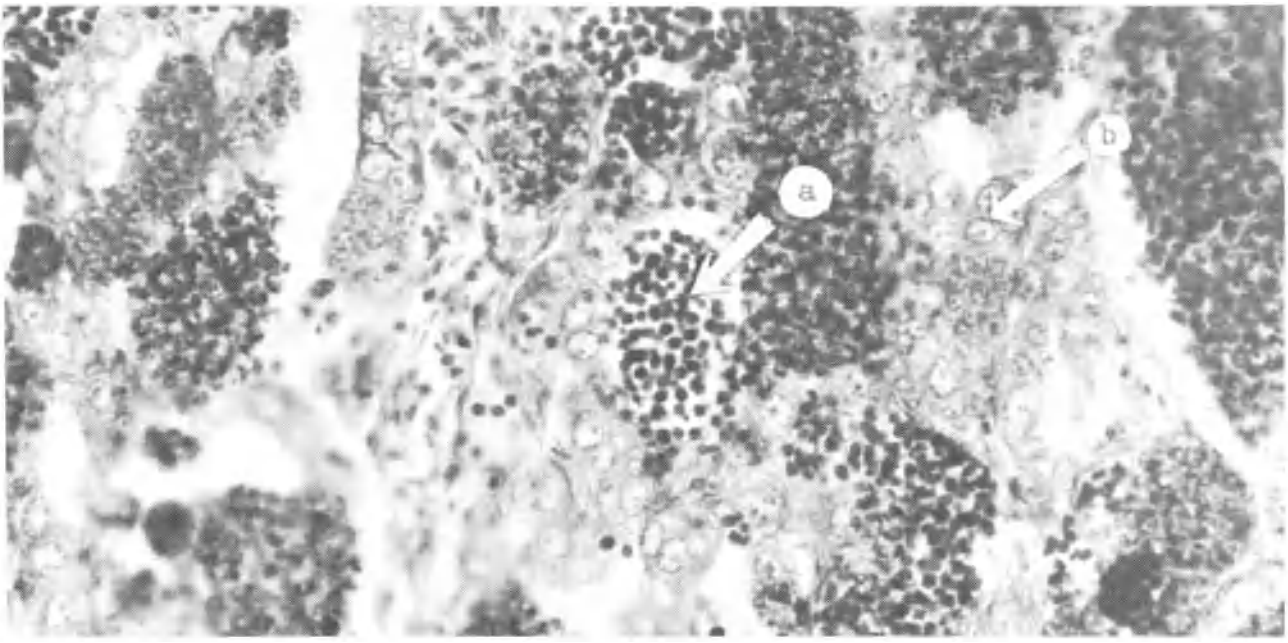


Figure 4 Cross section of triploid testes contained spermatozoa (a) and spermatids (b) (x 400)

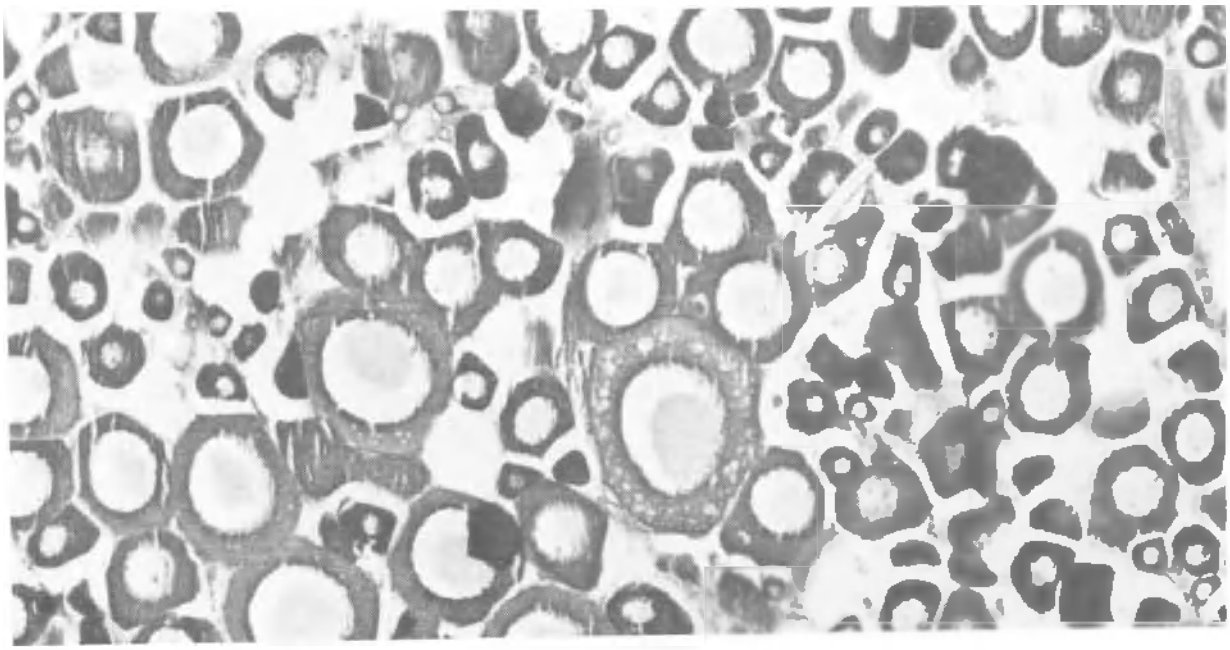


Figure 5 Cross section of triploid ovary contained previtellogenic oocytes (arrow) (x 100)

Printed The Agricultural Cooperative Federation of Thailand Ltd.