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STUDY ON EMBRYONIC DEVELOPMENT AND EARLY GROWTH OF TRIPLOID AND GYNOGENETIC DIPLOID LEFT-EYED FLOUNDER, *PARALICHTHYS OLIVACEUS*(T. et S.)*

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Abstract The early effects of chromosomal manipulation of eggs and sperm on the yields of triploid and gynogenetic diploid larvae of *Paralichthys olivaceus* were investigated. Triploidy was achieved by cold shocking fertilized eggs at 0 - 2℃ for 45 minutes duration 5 minutes after fertilization, and the induced triploidy rates were 31.2% - 50% and the relative hatching rates were 53.3% - 99%. Gynogenetic diploids were obtained when eggs were inseminated with irradiated sperm and cold shocked at 0 - 2℃ for 45 minutes duration 5 minutes after fertilization. The induced gynogenetic diploid rates and the relative hatching rates were 94% - 96% and 48.5% - 68.5% respectively. The embryonic development of the triploid experimental group and of the gynogenetic diploid experimental group was delayed at first compared with the control group. But from the gastrula stage, it was not delayed anymore. There were no significant differences in the growth of the triploid experimental group larvae and the control group larvae, and in the growth of the gynogenetic diploid experimental group larvae and the control group larvae according to Student's t-test ($\alpha = 0.05$). The relationship between the early growth of the triploid experimental group larvae and that of gynogenetic diploid experimental group larvae was also studied.

Key words: triploidy, gynogenetic diploid, embryonic development, early growth, *P. olivaceus*

INTRODUCTION

Triploidy induction and gynogenetic diploid induction as main parts of chromosomal manipulation have been playing more and more important roles in modern fish breeding. Most triploid fish are sterile and their reduced gonadal development results in improvement of growth rate and food conversion (Qin et al., 1998), so inducing triploidy in fish can be a useful means for enhancing their growth. The artificially induced gynogenetic diploid is useful for rapid establishment of inbred lines and production of all-female progeny since gynogenesis results in progeny with all-maternal inheritance with no genetic contributions of spermatozoa (Fujioka, 1998). Therefore many scientists are interested in the above and related work (Felip et al., 1999; Lu and Chen, 1993; Ihssen et al., 1990).

Left-eyed flounder, *Paralichthys olivaceus* is a highly popular marine food fish and is recently becoming more and more important cultivated fish in the northern coastal region of China due to the high demand for it in the international and domestic market. With the aim to improve growth rate and shorten the fish's culture period, chromosomal manipulation was investigated and tried.

This paper mainly compares the embryonic development and larval growth of the artificial triploid experimental group and control group, and of the gynogenetic diploid experimental group and control group, of the studied species *Paralichthys olivaceus*.

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MATERIALS AND METHODS

Broodstock

The studies were conducted in the laboratory of the Institute of Oceanology, Shidao Fishfarm and Longxu Island Fishfarm from May to August during 1994 – 1996. Broodstock *P. olivaceus* were obtained from the coastal waters of Shandong and were cultured under artificial conditions temporally or long term.

Treatment

Induction of triploidy: Five minutes after fertilization, cold shock which results in second polar body retention that yields triploid fish (3N), was administered by immersing the fertilized eggs in 0 – 2℃ seawater for 45 minutes, after which the eggs were returned to normal seawater (16 – 18℃, salinity 30).

Induction of diploid gynogenesis: Fertilized (with UV-irradiated sperm) eggs were incubated in normal seawater. Then most of them were taken out and cold shocked (as described above) to induce gynogenetic diploidy. The rest comprised the haploid control group without cold shock.

Control group: Normal eggs and sperm were fertilized and the fertilized eggs were reared in normal seawater.

Ploidy determination

Gastrula stage chromosome samples of the haploid, gynogenetic diploid, triploid experimental groups and also those of the control group obtained by using the air-dried, Giemsa stain method were observed under microscope as described in the literature (You and Liu, 1995) to obtain the gynogenetic diploid rate and triploidy rate in the experimental groups.

Observation and comparison of embryonic development

The embryonic development in the three groups was regularly observed and recorded under stereomicroscope after treatment. The hatching rate in each group was also calculated.

Observation and comparison of early growth

After hatching, the larvae were put into seawater. The culture conditions such as larva density, feeding schedule, water flow rate, management, etc. were kept similar. The total length of different group larvae was regularly recorded by measuring 30 individuals of each group. Student's t-tests were used to test whether the relations between total length of the triploid experimental group larvae and that of control group larvae, between that of the gynogenetic diploid experimental group larvae and control group larvae, and between that of the triploid group larvae and gynogenetic diploid experimental group larvae were significant or not. Differences were considered significant when $P < 0.05$ (Felip et al., 1999).

RESULTS AND DISCUSSION

Embryonic development

It was very obvious that cold shock could delay early stage embryonic development . The cell cleavage of fertilized eggs in the experimental groups was slower than that of the control group as shown in Table 1. Cold shock also adversely influenced the outside morphology and cleavage of the

fertilized eggs. Some eggs had wrinkled membranes and abnormal cleavages resulting in different sized cells, 3-celled and 5-celled irregular shape embryos. But with embryonic development, the difference among experimental groups and the control group became less and less. At the late gastrula stage, development speed became the same, the membranes became smooth and were no longer wrinkled. The deformed cleavage eggs did not develop furthermore and died. The early embryonic development stage of the triploid experimental group and the gynogenetic diploid experimental group were similarly delayed because of the same cold shock treatment.

Table 1 Comparison of embryonic development of the left-eyed flounder *P. olivaceus* in different groups (Temp. 18 - 20℃)

Stage	Duration after fertilization (Hour: Minute)		
	Control group	Triploid exp. group	Gynogenetic diploid exp. group
2-celled	1:15	1:45	1:50
4-celled	1:45	2:15	2:20
8-celled	2:15	2:45	2:50
16-celled	2:45	3:10	3:15
32-celled	3:20	3:45	3:50
Multi-celled	4:00	4:25	4:30
Blastula	5:45	6:10	6:10
Early gastrula	12:30	12:40	12:40
Late gastrula	19:15	19:15	19:15
Tail bud	34:00	34:00	34:00
Embryonic development	40:00	40:00	40:00
Beginning of hatching	48:00 or so	48:00 or so	48:00 or so

Induced rate and relative hatching rate

The induced ploidy rate and hatching rate in the haploid control, diploid gynogenesis experimental, triploid experimental, and control groups are listed in Table 2. Because different batches of eggs had different quality, the relative hatching rate of the control group was used to compare the results of different batches. In Table 2, the haploid hatching rate was the lowest because the haploids became deformed at the tail bud stage, and then died before or after hatching. All the newly hatched haploid larvae had short and curved caudal vertebrate and distorted yolk sac —— the so-called "haploid syndrome". But why was the relative hatching rate in the triploid experimental group higher than that in the gynogenetic diploid experimental group? It could be explained that triploidy induction needs only one cold-shock and diploid gynogensis induction needs two-step shocks: UV irradiation shock for producing genetically inactivated sperm and cold-shock for doubling the chromosomal set; both shocks can affect the normal development of fertilized fish eggs and then decrease the hatching rate of the eggs. The induction of the gynogenetic experimental group also included 2-step shocks; initially by UV-shock yielding 96% - 98% haploids, then by cold-shock yielding 98% diploids; so that the total induced rates of the gynogenetic diploid experimental group were 94% - 96% (shown and explained in detail in Liu Jing et al., 1999).

Table 2 The induced rate and relative hatching rate of the left-eyed flounder *P. olivaceus* in different groups (Temp. 16 - 18℃)

	Control	Haploid control	Gynogenetic diploid exp.	Triploid exp.
Induced rate (%) of	-	96 - 98	94 - 96	31.2 - 50.0
Relative hatching rate (%)	100	38.1 - 68.0	48.5 - 68.5	53.3 - 99.0

Early stage growth

Fig.1 shows the growth curves of three groups (Temp.: 18 – 25℃). Separate tests by student's t-test ($\alpha = 0.05$) showed these were no statistically significant total length differences between the gynogenetic diploid experimental and control group ($0.2 < P < 0.3$), and between the triploid experimental and control group ($0.2 < P < 0.3$) up to 30 days after fertilization.

The growth of triplods and gynogenetic diploids were compared with that of the control in many papers. The results were quite different. Wolters et al. (1982) reported that after the age of 8 months, triploid channel catfish *Ictalurus punctatus* had higher mean weight than the diploid fish. Faster growth of triploid females was also observed in *Olija Tilapia*, loach (*Misgurnus anguillicardatus*) and rainbow trout (*Oncorhynchus mykiss*) after the normal age of sexual maturation (Suzuki et al., 1985; Thorgaad, 1986); although most studies showed that the growth of triploid fish was similar to that of the diploid fish. Cherfas et al. (1994) found that triploid common carp did not exhibit any growth advantage over the diploid. In the genus *Clarias*, triploid *C. mecrocephalus* was much larger and heavier than the diploid at 8 months of age (Fast et al., 1995), while triploid *C. gariepinus* did not grow faster than the diploid (Henken et al., 1987). Lower growth rates of the gynogenetic diploid in comparison with that of control were reported for loach (Suzuki et al., 1985). Thorgaad (1986) thought these variable results might depend on the biological characteristics of a species, as well as on experimental conditions such as ambient temperature and feed quality.

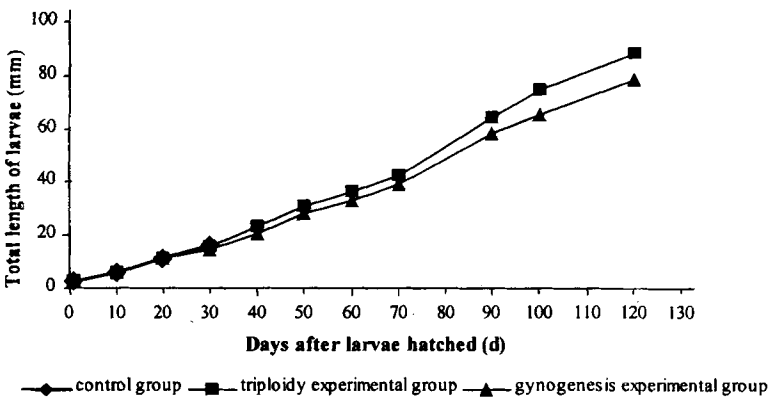

Fig.1 Early growth curves of triploid, gynogenetic diploid experimental and control groups

The present study's result showing that left-eyed flounder triploid and gynogenetic diploid experimental groups grew as fast as the control at the early growth stage, accords with that of Tabata and Gorie (1988), who observed that there were similar growth rates in the triploidy and control groups, and in the gynogenetic diploid and control groups in hirame, *Pralichthys olivaceus*. The relationship between the early growth of the triploidy and gynogenetic diploid experimental group was different. According to t-test, there was no significant growth difference between these two groups from day 1 to day 50 after fertilization ($0.05 < P < 0.1$). But from day 60, there was significant difference between them ($0.001 < P < 0.01$), when the triploid experimental group grew obviously faster than the gynogenetic diploid experimental group.

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