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Preliminary Study on Triploid of Yellowtail

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Abstract: The possibility of a large-scaled cold shock induction of triploidy, viability of triploid fish and growth performance were investigated in yellowtail *Seriola quinqueradiata*. Induction of triploidy by a large-scaled cold shock (approximately 0.1 million fertilized eggs) was successful in our preliminary test, and approximately 40 thousand hatched larvae were obtained. Some of the hatched larvae were tested for polyploidy, the results of which indicated that fish treated with two different cold shocks (two trials) were 100% triploid. Then, the growth performance of the triploid and diploid fish was investigated in both mixed and separate rearing. At the age of 100 and 122 days, the body weights of the triploid and untreated control fish were not significantly different in both mixed and separate rearing in two trials. However, as they grew larger, the growth rate of the untreated control was significantly higher than that of the triploid fish in both rearing conditions. Thus, the triploid fish at early development (at least half a year) showed a delay in growth. Also, the triploid fish showed longer term viability. In future studies, we will investigate growth performance at larger size and infertility (gonadal developmental stage) at the age of maturation.

Key words: *Seriora quinqueradiata*, triploid, cold shock, growth performance

Introduction

Yellowtail (*Seriola quinqueradiata*) is one of the most important species for aquaculture in Japan. Under culture conditions, however, mature yellowtail are remarkably reduced in body weight after the spawning season (mainly summer). This is a serious concern for the market. Thus, there is an interest in the production of sterile yellowtail. This can be achieved by chromosome set manipulation techniques (Thorgaard, 1983; Felip *et al*., 2001), including the production of triploids (e.g. Purdom, 1972; Garrido-Ramos *et al*., 1996; Holmefjord and Refstie, 1997; Felip *et al*., 1997; Piferrer *et al*., 2000). Recently, the basis for triploid induction by a cold shock treatment was established for yellowtail at the small-scale (Shimada unpublished data; Nagoya *et al*., unpublished data). It showed that cold shocks for 5 – 20 min duration at 0 – 5 °C within 5 min after fertilization resulted in approximately > 80% triploid rate.

Our goal is to achieve industrial use of triploidy in yellowtail, but there remain several issues to resolve, such as developing a large-scaled cold shock technique and determining viability, growth performance and infertility of triploid fish, and also making a basic law system for triploid fish culture in

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a sea cage. To determine viability of triploid fish in a long-term culture, a large-scaled cold shock method is required to produce a lot of fertilized triploid eggs. The objectives of the present study are (1) to examine the efficacy of large-scaled cold shock induction of triploidy, (2) to determine viability of triploid fish in long-term rearing, and (3) to compare a growth performance of diploid and triploid fish.

Materials and methods

Artificial fertilizations and cold shock treatments

Artificial fertilization of yellowtail was performed at two facilities, Kamiura Laboratory and Komame Laboratory of National Research Institute of Aquaculture, in February (trial 1) and April (trial 2) 2015, respectively. Fertilized eggs were treated by cold shock 3 min after fertilization in 15 L containers equipped with nets at the bottom. No temperature difference was observed between the container and surrounding water bath. The conditions of the cold shock treatment were 5 min duration at 0 °C at Kamiura Laboratory, and 20 min duration at 5 °C at Komame Laboratory, and the sea water temperature was regulated by sea water ice and / or a cooling equipment (AZ-280X, Iwaki Co. Ltd., Tokyo).

Rearing and growth of fish

Treated and untreated fertilized eggs were separately maintained in 1000 L tanks until they hatched. Approximately 5,000 hatched larvae were reared in a 500 L tank with flow-through sea water. Tanks were maintained in a 4000 L water-bath to minimize variance of water temperature among them (water temperature > 20 °C). Fish were fed the L-type rotifer *Brachionus plicatilis* from day 2 to 25, *Artemia* spp. from day 21 to 35 with DHA and EPA enrichment (Hyper-gross, Mrinetech Co. Ltd., Aichi), and dry pellets after day 30. Fish were size segregated and transferred to 1500 L tanks during days 35 to 40. On day 100 or 122 in each trial, body weights of all fish were measured after anesthesia with 2-phenoxyethanol (Wako Co. Ltd., Osaka). Then, the body weights in the mixed rearing were measured on day 151, 185, 212, 245 and 275, whereas those in the separate rearing were measured only on day 150 and 186 because all triploids died in an accident on day 186. In addition, all fish in the mixed rearing were injected with a syringe on day 122 with a PIT tag placed into the ventral part of the body cavity.

Polyploidy of fish

To determine the polyploidy of treated and

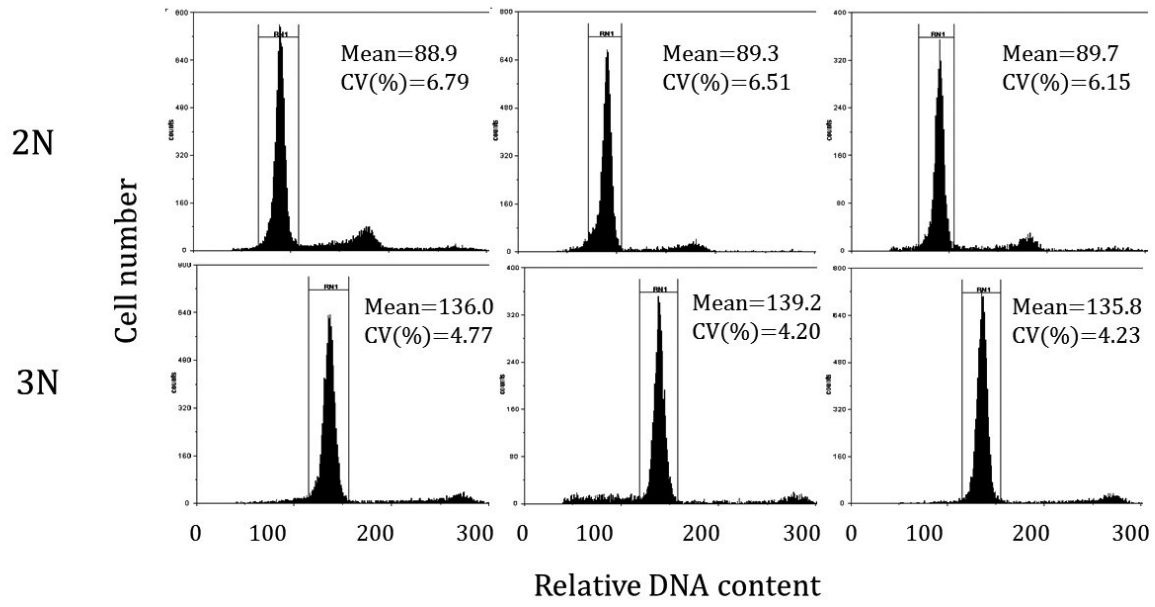

Fig. 1. Examples of polyploidy determination for untreated control (upper) and cold shock treated yellowtail (lower). 2 N and 3 N indicate diploid and triploid, respectively. The mean DNA contents of 3N are approximately 1.5 times larger than those for 2N.

untreated larvae, a total of 49 individuals were sampled on day 0 and fixed and preserved in 100% ethanol at 4 °C. These individuals were subjected to flow cytometry (Partec GmbH, Germany) for relative DNA content of the whole body cells in order to determine their ploidy status as shown in Fig. 1.

Statistics

The data were expressed as the mean ± standard error of the means (SEM), and analyzed by an independent *t*-test.

Results

Cold shock treatments and ploidy status

Large-scaled cold shocks were performed on approximately 0.1 million fertilized eggs in trial 1 (separate rearing) and 2 (mixed rearing). Hatching rates in cold shock treatments for 5 min at 0 °C and 20 min at 5 °C, were 18.5% and 36.9%, respectively, whereas those in untreated controls were 78.2 and 22.9%. The triploid rates in cold shock treatments were 100% (N = 8 and 12), whereas those in untreated controls were 0 and 11.8% (N = 15 and 17) on day 0. In addition, we confirmed ploidy status of all individuals using their fin clips at the final measurement. In trial 1, 106 out of 121 treated fish were triploid (87.6%), but all untreated control fish were diploid (100%). In trial 2, treated and untreated control fish were triploid (11 / 11 fish) and diploid (15 / 15 fish), respectively.

Growth performance of triploid and diploid fish

Growth performances of diploid and triploid fish are shown in Fig. 2. In the separate rearing, triploid and diploid fish were not significantly different in body weight on day 100 (df = 240, t = - 1.636 and p = 0.1032), but at older ages diploid fish showed significantly larger body size than triploid fish (day150; df = 204, t = 4.454 and p < 0.0001, day 186; df = 94, t = 5.659 and p < 0.0001, Fig. 2A). This phenomenon was also observed in the mixed rearing (df = 24, day 122; t = 0.646 and p = 0.5246, day 151; t = 2.057 and p = 0.0507, day 185; t = 2.829 and p = 0.0093, day 212; t = 2.547 and p = 0.0177, day 245; t = 2.210 and p = 0.0369, and day 275; t = 2.285 and p = 0.0315).

Discussion

The most salient finding of this study was that a large-scaled triploid induction (0.1 million fertilized

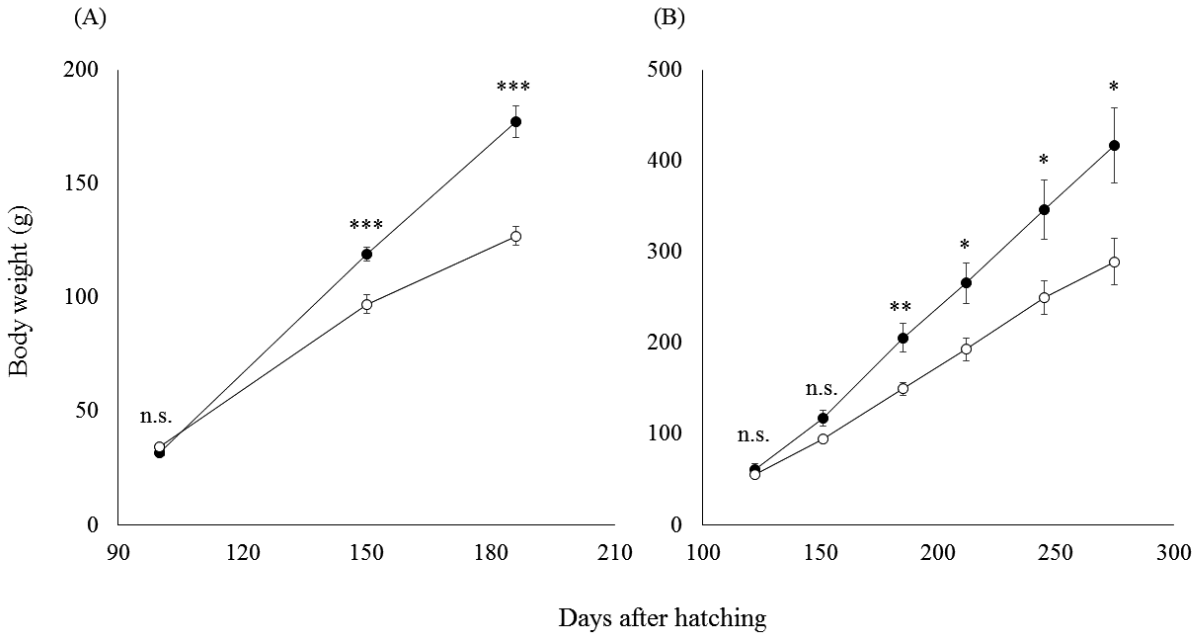

Fig. 2. Growth performance of triploid (open circle) and diploid (black circle) under separate rearing (A) and mixed rearing (B) conditions. All statistics were done by independent *t*-test. Symbols of n.s., *, ** and *** mean not significant, P < 0.05, P < 0.01 and P < 0.001, respectively.

eggs) was possible and triploid fish were viable in long-term rearing in yellowtail. However, a growth performance of triploid fish was lower than that of diploid fish in both separate and mixed rearing. In what follows, we will discuss these results as well as some methodological issues in detail.

A large-scaled cold shock treatment

In a large-scaled cold shock for fertilized eggs, temperatures in the equipped container and surrounding water bath were the same, suggesting that the equipment used may be applied to larger volumes of fertilized eggs.

Triploid viability

In this study, hatching rates of cold shock treated eggs were 18.5 and 36.9% in trial 1 and 2, respectively, whereas those of untreated control were 78.2 and 22.9 %. Usually, survival of triploids is about 70 to 80% of that of the controls (Felip *et al*., 2001). However, the present study showed varying results ranging from 24% to 161% of the hatching rates of untreated controls. Most probably, we still need to improve the maintenance method of fertilized eggs in order to resolve the surprising result.

Triploid fish were observed in untreated controls (trial 2). So far, it is reported in a case study that over ripening of eggs after ovulation increases triploid rates in *Anguilla japonica* (Nomura *et al*., 2013). Therefore, over ripening might be one of the reasons for occurrence of triploids in untreated controls of yellowtail.

Growth performance of triploid

The present study demonstrated that triploid yellowtail had a growth delay compared to diploid yellowtail. Similar results have been reported in several other fish species (Felip *et al*., 2001). In contrast, Johnstone *et al*. (1991) reported that non-maturing triploid females showed better growth compared with sexually maturing diploid female fish. In turbot, Cal *et al*. (2006) reported that body weight in diploid and triploid fish started to differentiate at first maturity, and the differences expanded in later age. In our knowledge, we have observed that most triploid female yellowtail showed sexual maturation because the sex determination system of yellowtail is ZZ-ZW type (Fujii *et al*., 2010) and had no abnormal oocytes (Yamaguchi *et al*., unpublished data) as in the case of tilapia (Razak *et al*., 1999). Also, in the aspect of rearing technique Oppedal *et al*. (2003) reported that triploid salmon tended to display enhanced weight gain compared to diploid ones when given continuous lighting conditions. Thus, by improving rearing techniques and/or controlling fish maturation, the growth of triploid yellowtail might be enhanced comparable to the existing production system in diploid yellowtail.

Conclusion

To summarize, the results of this study demonstrated the feasibility of large-scale production of triploid yellowtail and their use in aquaculture. However, in a comparison between separate and mixed rearing, we found that growth performance of triploid fish was lower than that of diploid fish. Thus, we need to develop appropriate rearing methods for triploid yellowtail.

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Diploid and triploid in channel catfish *Ictalurus punctatus* were reared in indoor tanks. Triploids were significantly heavier than diploids at 8 months of age and older. Triploid female and male had smaller gonads with altered histology. Triploids converted feed more efficiently, and may provide greater profits in commercial culture than diploids.

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Red blood cell size was measured in brook trout (*Salvelinus fontinalis*) from Phillips Hatchery in Maine to investigate naturally occurring polyploid sterility. In eight brook trout in which gonads were lacking or undeveloped, the red blood cells were large, suggesting polyploidy. The average size of the red blood cells in other sterile fish fit into the normal range but all of the eight fish appeared to have some red blood cells that were polyploid. All polyploids appeared to be mosaics, containing diploid, triploid, or pentaploid cells. The cause of the polyploidy was not determined but may have been caused by the inadvertent exposure of the eggs to low temperatures after fertilization.

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Induced triploidy is widely accepted as the most effective method for producing sterile fish for aquaculture and fisheries management. Artificially produced triploids generally differ from conspecific diploids in three fundamental ways: they are more heterozygous, they have larger but fewer cells in most tissues and organs, and their gonadal development is disrupted to some extent. Despite these basic biological differences, triploids are similar in most respects to diploids when examined at the whole animal level. The only clear differences relate to the effects of impaired gametogenesis on the

reproductive physiology and behavior of triploids, especially in females. Other apparent differences include reduced aggressiveness, occasional specific morphological abnormalities, and inferior performance when reared under suboptimal conditions. The causes of these latter two problems are poorly understood but must be addressed if triploids are to be used more extensively.