KOREAN JOURNAL OF ICHTHYOLOGY, Vol. 28, No. 4, 215-222, December 2016
ISSN: 1225-8598 (Print), 2288-3371 (Online)

Received: October 19, 2016
Revised: November 17, 2016
Accepted: December 13, 2016

Cytogenetic Study of Diploid and Triploid Marine Medaka, *Oryzias dancena*

By In-Seok Park, Hyun Woo Gil, Tae Ho Lee, Yoon Kwon Nam[1], Min Gyun Ko[1] and Dong Soo Kim[1,]*

Division of Marine Bioscience, College of Ocean Science and Technology, Korea Maritime and Ocean University, Busan 49112, Republic of Korea
[1]Department of Marine and Aquaculture, Pukyung National University, Busan 48513, Republic of Korea

ABSTRACT Triploidy was induced in the marine medaka, *Oryzias dancena* by cold shock treatment (0°C) of fertilized eggs for 30, 45, or 60 min, applied two minutes after fertilization. The triploid genotype was induced by each of the thermal shock regimes tested. The best result was obtained when the eggs were treated for 45 min, which induced triploidy in all the resulting fish. Triploidy was confirmed using chromosomal and flow cytometer analyses, and erythrocyte measurements. The surface areas and volumes of the erythrocytes of triploid fish were significantly larger than those of diploid fish, and their chromosome number (3N = 72) was 1.5 times greater that for the diploids (2N = 48). Based on a flow cytometer analysis, the triploid fish had approximately 1.5 times the cellular DNA content (2.40 pg/cell) of the diploid specimens (1.61 pg/cell). Data from this study provide the basis for the development of unique models for studying reproductive confinement in transgenic fish.

Key words: Cold shock, DNA contents, flowcytometry, marine medaka, triploid

INTRODUCTION

The marine medaka, *Oryzias dancena* is a truly euryhaline teleost fish, having a great capacity for hypo- and hyper-osmoregulation. Most of its physiological attributes are similar across a wide spectrum of salinities, ranging from fresh water to normal seawater (Inoue and Takei, 2003; Kang *et al*., 2008; Cho *et al*., 2010). Until now, this species was selected by the Institute of Marine Living Modified Organisms (*i*MLMO) for a living modified organism evaluation project. Consistent with this purpose, detailed information on its biology, especially its early gonadogenesis, sexual differentiation, early ontogenesis, embryogenesis and and exceptional capacity for hyperosmoregulation and hypoosmoregulation, and is becoming available (Kim *et al*., 2009a, b). In addition, Kim *et al*. (2009a) suggested that this species has a short interval between generations with spawning possible only 60 days after hatching. Much attention has been directed at extending the utility of functional transgenic marine medaka strains for ornamental purposes, because they can be used at most naturally occurring salinities (Cho *et al*., 2011). In addition, in a recent study of transgenic marine medaka containing the myosin light chain-2 (*mlc2f*) promoter, the expression of a vivid red fluorescent color in their fast skeletal muscles suggested great potential for these as novel ornamental fish for both freshwater and seawater aquaria (Cho *et al*., 2012).

However, the practical application of transgenic fish has raised public and scientific concern about the ecological risks involved, especially those associated with the adverse consequences for natural gene pools, which can be genetically contaminated if unwanted transgenic animals are released (Maclean and Laight, 2000; Devlin *et al*., 2006). For these reasons, much recent scientific research has focused on risk assessment in relation to transgenic fish, with particular emphasis on the repro-

*Corresponding author: Dong Soo Kim Tel: 82-51-629-7066, Fax: 82-51-629-7067, E-mail: dongskim@pknu.ac.kr

ductive confinement of transgenic stocks (Wong and Van Eenennaam, 2008; Thresher *et al*., 2009; Hu *et al*., 2010). Triploidization involving blocking of the second meiotic division has been proposed as one approach to the generation of transgenic fish having depressed reproductive capacities (Piferrer *et al*., 2009). To date, transgenic triploid fish have been reported among several growth hormone-transgenic fish strains, and the effects of triploidy on the functions and reproductive attributes of transgenic fish are known to be species specific (Razak *et al*., 1999; Nam *et al*., 2001; Devlin *et al*., 2004; Yu *et al*., 2011).

Triploidization is a technique used to generate sterile aquatic animals by taking advantage of the incompatibility in pairing the three homologous chromosomes during meiosis I (Don and Avtalion, 1986). This technique has also been used to enhance the productivity of several fish species because of its assumed ability to increase yield by channeling the energy required from gonadal development to somatic growth (Tave, 1993). More importantly, it generates fish that are unable to breed and contribute to the local gene pool if they were to accidentally escape from confinement. By conferring sterility of exotic fish for a limited purpose, triploidy can serve as an effective method for reducing or eliminating the environmental risks of genetically modified organisms (Kim *et al*., 1994). Triploidy was confirmed by the 1.5-fold increase in nuclear volume, cellular DNA content and chromosome number as estimated by erythrocyte counting, respectively (Seol *et al*., 2008).

There are numerous studies in the literature which have investigated various aspects of triploid fish identification methodology including analysis of chromosome sets (Thorgaard, 1986), the microfluorimetry of nuclear DNA content (Komaru *et al*., 1988), the nuclear DNA content by flowcytometry (Allen and Stanley, 1978), the measurement of erythrocyte and nuclear size (Thorgaard, 1986; Park and Kim, 1994; Park *et al*., 1994), the distinction of nucleolar number (Philips *et al*., 1986), the measurement of cell number (Park and Park, 1994), and the measurement of cell and nuclear size in different tissues (Aliah *et al*., 1990). Park and Kim (2000) reported that characteristics of the some tissues of retina, optic tectum and trunk kidney in triploid and diploid hybrid between female mud loach, *Misgurnus mizolepis* and male cyprinid loach, *M. andguillicaudatus*.

The objectives of this study were: 1) to develop the optimal conditions for induction of the triploid genotype in marine medaka; and 2) to evaluate the cytogenetic of the triploid form of this species.

MATERIALS AND METHODS

The specimens of marine medaka, *Oryzias dancena* used in this study were from a laboratory stock maintained at the Institute of Marine Living Modified Organisms (*i*MLMO), Pukyong National University, Busan, Korea. The general maintenance of the experimental fish was according to the method of Song *et al*. (2009). Breeding occurred in brackish water (5 psu), as described by Cho *et al*. (2010). The breeding conditions included a temperature of 25 ± 1°C and a 16 h light : 8 h dark cycle. Triploid and diploid specimens were fed brine shrimp (Artemia nauplii; INVE, Salt Lake City, Utah, USA) and microparticle feed (150∼500 μm diameter; Ewha Oil Co., Busan, Korea).

Fertilized eggs were obtained each day by mating male and female broodfish in a glass tank containing 30 L of well-aerated water. The eggs were collected from females immediately following fertilization. At 2 min after fertilization the fertilized eggs were subjected to a cold shock treatment at 0°C for 30, 45, or 60 min. Following treatment the eggs were placed in a 25°C incubator until they hatched. The hatching success and the incidence of abnormal larvae were assessed based on stereoscopic microscope (C-DS; Nikon Co., Tokyo, Japan) examination of at least 23 eggs per group. The values for these parameters in the experimental treatments were expressed as percentages of treated eggs.

Flow cytometer analysis was undertaken in the Fishery Genetics and Breeding Sciences Laboratory, Korea Maritime and Ocean University, Korea. To assess the incidence of triploidy using flow cytometry, 22 individuals were randomly chosen from each treatment and control group. The caudal fin was excised from each fish using scissors, and the cells were dissociated in nucleic acid extraction buffer, and stained for 15 min in DNA staining buffer (CyStain DNA 2 step; Partec, Germany). To determine their ploidy, the cells were analyzed using a Ploidy Analzer II flow cytometer (Partec, Germany). The DNA content per cell was measured based on a reference standard prepared from the caudal fin of a diploid mud loach (*Misgurnus mizolepis*; 2.8 pg/cell) (Nam *et al*., 1999).

Chromosome analysis was performed using a direct method involving kidney cells (Kim *et al*., 1995). Metaphase spreads were prepared from randomly selected diploid and triploid fish (n = 10) that had been identified using flow cytometry, as described above. At least 12 countable metaphases were examined per slide, and distinct metaphase chromosomes were photographed using a digital camera (ARTCAM-300MI; Artray Co., Tokyo,

Japan) attached to an optical microscope.

For the erythrocyte and hormone measurements, six diploid and six triploid fish (three of each gender per ploidy group) were selected. Blood samples were taken by cutting the caudal fin and collecting the blood, or by inserting a heparin-coated syringe (Sigma, USA) into the heart and withdrawing blood. The major and minor axes of each cell and nucleus of at least 30 erythrocytes per fish were measured using a micrometer. The surface area ($1/4 \times ab\pi$) and volume ($4/3 \times \pi(a/2) \times (b/2)^2$) were calculated; in these formulae, 'a' is the major axis of the cell or nucleus, and 'b' is the minor axis of the cell or nucleus (Lemoine and Smith, 1980; Park and Kim, 2000).

The hatching success, incidence of abnormal larvae, and early survival rates were assessed using ANOVA followed by Duncan's multiple range test at the significance level $P=0.05$. Differences in erythrocyte size between the diploid and triploid groups were assessed using the Student's t-test. Difference was considered to be significant when $P<0.05$.

RESULTS AND DISCUSSION

Cold shock treatment (0°C) reduced the hatching success of embryos of marine medaka, *Oryzias dancena* (Table 1). The hatching rate was significantly different between the control and treatment groups, and decreased significantly ($P<0.05$) with increased treatment period. The occurrence of abnormal larvae in the treatment groups was slightly higher than in the control group, but was similar among the treatment groups, where it ranged from 6.5% to 7.5% ($P<0.05$). The incidence of triploidy was 90.9% in the 30 min treatment group, and 100% in the 45 and 60 min treatment groups (Table 1). The production rate was the highest in the treatment involving cold shock for 45 min, applied 2 min after fertilization.

Triplodization is considered to be a potential method for preventing unwanted reproduction in fish (Piferrer *et al*., 2009). The hatching rates for the eggs of marine medaka were lower in the temperature shock treatment groups than in the control group ($P<0.05$), with the exception of eggs treated for 30 min ($P>0.05$). A reduction in the hatching rate following a temperature shock has been reported in previous studies (Kavumpurath and Pandian, 1990; Da Silva *et al*., 2007; Karami *et al*., 2010). The occurrence of abnormal larvae in the treatment groups was also significantly different from the control group ($P<0.05$). The lower hatching rate and greater incidence of abnormal larvae in triploid fish compared with that of diploid fish may be related to the adverse effects of thermal shock on membrane fluidity, RNA/DNA synthesis, cell morphology, and protein function (Hildebrandt *et al*., 2002; Al-Fageeh and Smales, 2006). Our cold shock treatment protocol for the induction of triploidy in marine medaka was 100% effective, but the hatching rate was lower for eggs treated for 60 min than for those treated for 45 min. Therefore, the optimal conditions for the induction of triploidy in marine medaka in this study was a cold shock treatment at 0°C for 45 min. The yield of triploidy in this study was very similar to that previously reported in other fish species (Felip *et al*., 1999; Piferrer *et al*., 2000; da Silva *et al*., 2007).

The number of chromosomes in the diploid and triploid marine medaka were 48 and 72, respectively (Fig. 1). The karyotype of diploids comprised 24 acrocentric chromosome pairs, while the triploids contained three sets of 24 acrocentric chromosomes. Two nucleoli were observed in the metaphase stage of diploids, and three were observed in the triploids. The chromosome number in the cells of triploid fish was 1.5 times greater than that of the diploid fish. The flow cytometry assessment showed that triploidy was successfully induced in all treatment groups. The DNA content of the diploid and triploid fish is shown in

Table 1. Effects of cold shock (0°C) treatment 2 mins after fertilization at fertilized eggs of marine medaka, *Oryzias dancena*

Duration of shock (min)	Number of eggs used	Hatching rate (%)*1	Abnormal rate of juvenile (%)	Induction rate of triploidy (%)*2	Early survival (%)*3	Production rate of triploid (%)*4
No treatment	164	91.7 ± 1.8^{a}	1.7 ± 1.7^{b}	0	97.8 ± 0.8^{a}	0
30	315	82.2 ± 1.6^{ab}	7.5 ± 1.2^{a}	90.9	94.8 ± 3.7^{a}	88.1 ± 1.4^{a}
45	419	71.4 ± 5.9^{bc}	6.5 ± 1.1^{a}	100	95.1 ± 2.6^{a}	97.2 ± 1.2^{b}
60	391	61.0 ± 12.9^{c}	7.0 ± 1.1^{a}	100	93.8 ± 4.5^{a}	95.9 ± 1.7^{b}

This experiment was performed in triplicate.
*1Hatching rates of each group were analyzed until 24 hours after first hatching observed.
*2Induction rates of triploid were analyzed at 50 days after hatched. Each value is mean percentage of triplicate experiments. Induction rates of triploid were analyzed in survived experimental animals.
*3Early survival rates of each group were analyzed at 96 hours after hatched.
*4Production rate of triploid = [(early survival rate of treated group)/(early survival rate of control group)] × (induction rate of triploid).

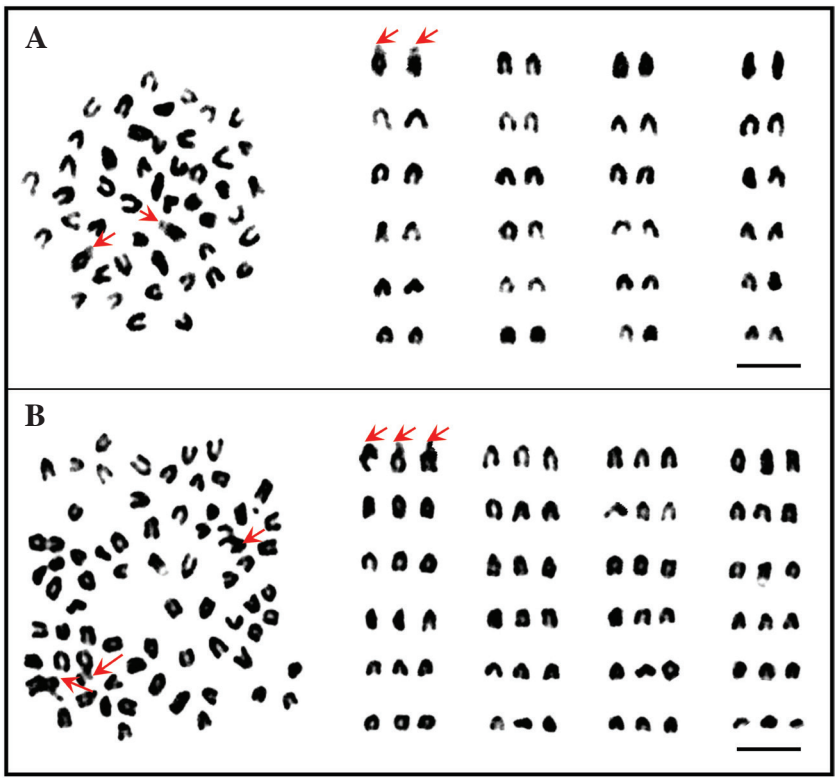

Fig. 1. Metaphase chromosome spreads: (A) diploid 2n = 48 and (B) triploid 3n = 72 marine medaka, *Oryzias dancena*. Scale bars indicate 10 μm. Arrows are larger acrocentrics marker for indentification of diploid and triploid.

Table 2. DNA contents of diploid and triploid in marine medaka, *Oryzias dancena*[*1]

Sample No.	DNA contents (pg/nucleus)	
	Diploid	Triploid
1	1.61	2.46
2	1.65	2.43
3	1.64	2.46
4	1.62	2.44
5	1.65	2.45
6	1.63	2.47
7	1.64	2.45
8	1.64	2.45
9	1.64	2.42
10	1.66	2.46
Mean ± SD[*2]	1.64 ± 0.019	2.45 ± 0.026

[*1]DNA contents of mud loach, *Misgurnus mizolepis* (2.81 pg/nucleus) were used standard references of diploid and triploid Korean rose bitterling's DNA contents measurement (Nam *et al*., 1999).
[*2]The values are means ± standard deviation of each group.

Table 2. The mean quantity of DNA in the diploid and triploid groups was 1.64 ± 0.019 pg/nucleus and 2.45 ± 0.026 pg/nucleus, respectively. The DNA content of the triploid group was a factor of 1.5 higher than that of the diploid group (Table 2 and Fig. 2).

Flow cytometer and chromosomal analyses are accurate methods for assessing the ploidy of fish. Estimating the average DNA content per cell in triploid fish cells, and observations of the metaphase chromosome stage clearly showed that an extra haploid chromosome set was present relative to normal diploid nuclei; the average cellular DNA content (2.40 pg/cell) and modal chromosome number (3n = 72) were factors of 1.5 greater than the diploid values; these values are typical for induced triploidy in fish (Kavumpurath and Pandian, 1990; Kim *et al*., 1994; Felip *et al*., 1999; Piferrer *et al*., 2000; Karami *et al*., 2010). Two and three sets of 24 acrocentric chromosomes were observed in the karyotype of diploid and triploid marine medaka, respectively. The karyotype of Japanese medaka, *O. latipes*, is the same as that of marine medaka. However, Uwa and Ojima (1981) showed that the karyotype of Japanese medaka comprises 48 chromosomes consisting of 2 metacentric pairs, 8 submetacentric pairs, 1 subtelocentric pair, and 13 acrocentric pairs. Therefore, karyotype analysis can be used to distinguish the marine and Japanese medaka. In addition, no heteromorphic sex chromosomes were observed in the diploid and triploid marine medaka in this study, which is consistent with a previous study that indicated the karyotype of diploid marine medaka does not include heteromorphic sex chro-

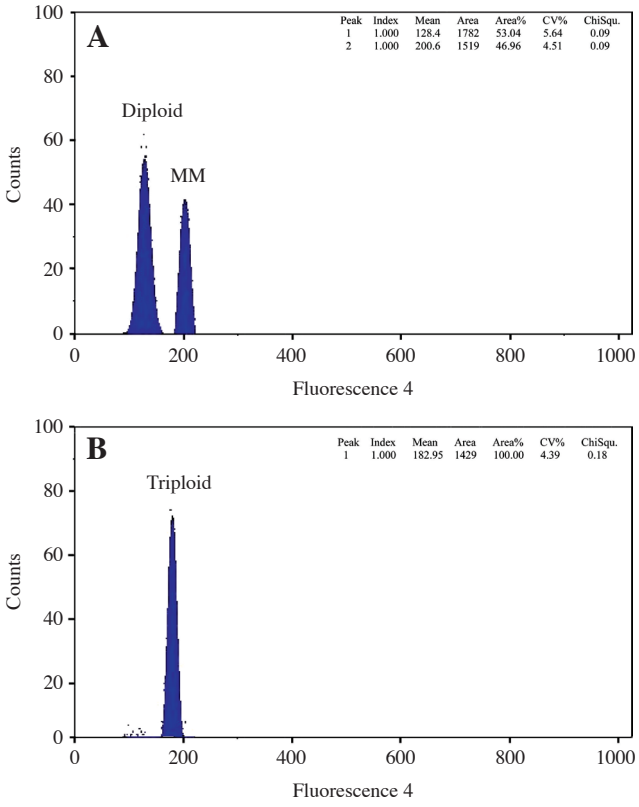

Fig. 2. DNA histogram of diploid and triploid marine medaka, *Oryzias dancena*. A: diploid marine medaka (Diploid) and mud loach, *Misgurnus mizolepis* (MM, standard); B: triploid marine medaka (Triploid). Each cell cycle fraction with background correction is indicated. Fluorescence 4 is ray of red light.

mosomes (Uwa *et al*., 1983). Therefore, the gender of each ploidy in this species can be investigated by analyzing sexual dimorphism.

Overall, the erythrocytes of the induced triploids were larger than those of the diploids (Fig. 3). The ratio of the major axis to the minor axis of erythrocytes for the diploids and induced triploids was 1.40 and 1.27, respectively, and the surface area to volume ratio (based on the major and minor axes) was 1.77 and 2.26, respectively. Similarly, the ratio of the major axis to the minor axis of the nucleus of erythrocytes for the diploids and induced triploids was 1.25 and 1.19, respectively, and the corresponding surface area to volume ratio (based on the major and minor axes), was 1.49 and 1.79, respectively (Table 3). The measurements showed that the surface area and volume of both the erythrocyte cells and nuclei of triploid marine medaka were larger than those of the diploid fish.

As expected, the triploids of marine medaka had significantly larger erythrocyte cell and nuclear dimensions

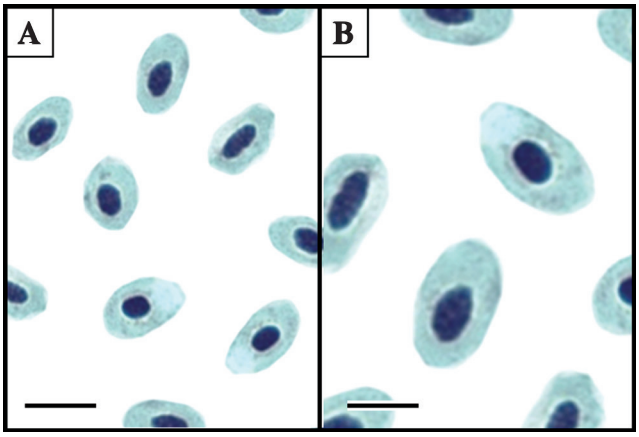

Fig. 3. Erythrocyte in diploid (A) and induced triploid (B) marine medaka, *Oryzias dancena*. Bars indicate 50 μm.

Table 3. Erythrocyte size of marine medaka, *Oryzias dancena* between diploid and triploid*[1]

	Diploid	Triploid	Ratio of triploid to diploid
Linear length (μm)			
Cell			
Major axis	8.29 ± 0.33^{a}	11.61 ± 0.61^{b}	1.40 : 1
Minor axis	5.28 ± 0.12^{a}	6.71 ± 0.30^{b}	1.27 : 1
Nucleus			
Major axis	5.06 ± 0.05	6.32 ± 0.36^{b}	1.25 : 1
Minor axis	2.66 ± 0.07^{a}	3.17 ± 0.13^{b}	1.19 : 1
Area (μm^2)*[2]			
Cell	34.43 ± 1.79^{a}	61.06 ± 3.21^{b}	1.77 : 1
Nucleus	10.55 ± 0.30^{a}	15.72 ± 1.29^{b}	1.49 : 1
Volume (μm^3)*[3]			
Cell	121.90 ± 8.64^{a}	276.07 ± 21.35^{b}	2.26 : 1
Nucleus	18.82 ± 1.16^{a}	33.70 ± 3.90^{b}	1.79 : 1

*[1]Six samples for each ploidy were used in this experiment. Thirty erythrocytes for each sample were analyzed. Mean ± S.D. Means in superscript letter are significantly different.
*[2]Area = $(a \cdot b \cdot \pi)/4$; a: major axis of cell and nucleus; b: minor axis of cell and nucleus (Seol *et al*., 2008).
*[3]Volume was calculated by formulas of Sezaki *et al*. (1988).
Volume = $4/3\pi \cdot (a/2) \cdot (b/2)^2$; a: major axis of cell and nucleus; b: minor axis of cell and nucleus.

than the diploids. For both the cells and nuclei, the increase in length was more pronounced in the major axis than in the minor axis, which is a feature commonly observed in induced triploid fish (Kavumpurath and Pandian, 1990; Kim *et al*., 1994; Peruzzi *et al*., 2005; Gao *et al*., 2007). In a previous study, Seol *et al*. (2008) reported that the erythrocyte count for diploids was higher than that for triploids, and they found that in triploids the nucleus of red blood cells had major and minor axes that were factors of 1.33 and 1.26 larger than in the diploids, re-

spectively. Differences in erythrocyte size is commonly used as the sole criterion for determining the ploidy in fish (Benfey, 1999).

Despite the greater size of their erythrocytes, triploid fish typically have lower hematocrit values (i.e. lower cell numbers) than diploid fish, because of the compensatory increase in cell volume in polyploidy (Benfey, 1999; Peruzzi *et al*., 2005; Gao *et al*., 2007). Therefore, it will be useful to investigate whether respiratory performance and energy metabolism differ between diploid and induced triploid marine medaka (Stillwell and Benfey, 1996; Hyndman *et al*., 2003; Lemieux *et al*., 2003; Shrimpton *et al*., 2007). So, further studies will be necessary to comparative study of respiratory ability between diploid and triploid marine medaka. In particular, longer-term observations of growth and maturation is necessary in triploid marine medaka.

ACKNOWLEDGMENTS

The comments of the anonymous reviewers greatly improved the quality of this manuscript. All experiments in this study complied with the current laws of Korea (the Law Regarding Experimental Animals, No. 9932). This study was conducted as part of LMO safety management studies for ocean and fisheries in 2015 supported by the Ministry of Maritime Affairs and Fisheries of Korea.

REFERENCES

Al-Fageeh, M.B. and C.M. Smales. 2006. Control and regulation of the cellular responses to cold shock: the responses in yeast and mammalian systems. Biochem. J., 397: 247-259.

Aliah, R.S., K. Yamaoka., Y. Inada and N. Taniguchi. 1990. Effects of triploidy on tissue structure of some organs in ayu. Nip. Suis. G., 56: 569-575.

Allen, S.K. and J.G. Jr. Stanley. 1978. Reproductive sterility in polyploidy brook trout (*Salvelinus fontinalis*). Trans. Amer. Fish. Soc., 107: 473-478.

Benfey, T.J. 1999. The physiology and behavior of triploid fishes. Rev. Fish. Sci., 7: 39-67.

Cho, Y.S., S.Y. Lee, D.S. Kim and Y.K. Nam. 2010. Tolerance capacity to salinity changes in adult and larva of *Oryzias dancena*, a euryhaline medaka. Kor. J. Ichthyol., 22: 9-16.

Cho, Y.S., S.Y. Lee, Y.K. Kim, D.S. Kim and Y.K. Nam. 2011. Functional ability of cytoskeletal β-actin regulator to drive constitutive and ubiquitous expression of a fluorescent reporter throughout the life cycle of transgenic marine medaka, *Oryzias dancena*. Transgenic Res., 20: 1333-1355.

Cho, Y.S., S.Y. Lee, D.S. Kim and Y.K. Nam. 2012. Characterization of stablefluorescent transgenic marine medaka (*Oryzias dancena*) lines carrying red fluorescent protein gene driven by myosin light chain 2 promoter. Transgenic Res., 22: 849-859.

Da Silva, F.S.D., R.G. Moreira, C.R. Orozco-Zapata and A.W. Silva Hilsdorf. 2007. Triploidy induction by cold shock in the South American catfish, *Rhamdia quelen* (Siluriformes) (Quoy and Gaimard, 1824). Aquaculture, 272: 110-114.

Devlin, R.H., C.A. Biagi and T.Y. Yesaki. 2004. Growth, viability and genetic characteristics of GH transgenic coho salmon strains. Aquaculture, 236: 607-632.

Devlin, R.H., L.F. Sundström and W.M. Muir. 2006. Interface of biotechnology and ecology for environmental risk assessments of transgenic fish. Trends Biotechnol., 24: 89-97.

Don, J. and R.R. Avtalion. 1986. The induction of triploidy in *Oreochromis aureus* by heat shock. Theor. Appl. Genet., 72: 186-192.

Felip, A., S. Zanuy, M. Carrillo and F. Piferrer. 1999. Growth and gonadal development in triploid sea bass (*Dicentrarchus labrax* L.) during the first two years of age. Aquaculture, 173: 389-399.

Gao, Z., W. Wang, K. Abbas, X. Zhou, Y. Yang, J.S. Diana, H. Wang, H. Wang, Y. Li and Y. Sun. 2007. Haematological characterization of loach, *Misgurnus anguillicaudatus*: Comparison among diploid, triploid and tetraploid specimens. Comp. Biochem. Physiol., Part A 147: 1001-1008.

Hildebrandt, B., P. Wust, O. Alhers, A. Dieing, G. Sreenivasa, T. Kerner, R. Felix and H. Riess. 2002. The cellular and molecular basis of hyperthermia. Crit. Rev. Oncol. Hematol., 43: 33-56.

Hu, S.Y., P.Y. Lin, C.H. Liao, H.Y. Gong, G.H. Lin, K. Kawakami and J.L. Wu. 2010. Nitroreductase-mediated gonadal dysgenesis for infertility control of genetically modified zebrafish. Mar. Biotechnol., 12: 569-578.

Hyndman, C.A., J.D. Kieffer and T.J. Benfey. 2003. Physiology and survival of triploid brook trout following exhaustive exercise in warm water. Aquaculture, 22: 629-643.

Inoue, K. and Y. Takei. 2003. Asian medaka fishes offer new models for studying mechanisms of seawater adaptation. Comp. Biochem. Physiol., Part B 136: 635-645.

Kang, C.K., S.C. Tsai, T.H. Lee and P.P. Hwang. 2008. Differential expression of branchial Na^+/K^+-ATPase of two medaka species, *Oryzias latipes* and *Oryzias dancena*, with different salinity tolerances acclimated to fresh water, brackish water and seawater. Comp. Biochem. Physiol., Part A 151: 566-575.

Karami, A., A. Christianus, Z. Ishak, S.C. Courtenay, M.A. Syed, M. Noor Azlina and H. Noorshinah. 2010. Effect of triplodization on juvenile African catfish (*Clarias gariepinus*). Aquacult. Int., 18: 851-858.

Kavumpurath, S. and T.J. Pandian. 1990. Induction of triploidy in the zebrafish, *Brackydanio rerio* (Hamilton). Aquacult. Fish. Manage., 21: 299-306.

Kim, D.S., J.Y. Jo and T.Y. Lee. 1994. Induction of triploidy in mud loach (*Misgurnus mizolepis*) and its effect on gonad development and growth. Aquaculture, 120: 263-270.

Kim, D.S., Y.K. Nam, I.-C. Bang and H.Y. Song. 2009a. Early gonadogenesis and sex differentiation of marine medaka, *Oryzias dancena* (Beloniformes; Teleostei). Kor. J. Ichthyol., 21: 141-148. (in Korean with an English abstract)

Kim, D.S., Y.K. Nam, I.-C. Bang and H.Y. Song. 2009b. Embryogenesis and early ontogenesis of a marine medaka, *Oryzias dancena*. Kor. J. Ichthyol., 21: 227-238. (in Korean with an English abstract)

Kim, D.S., Y.K. Nam and I.-S. Park. 1995. Survival and karyological analysis of reciprocal diploid and triploid hybrids between mud loach (*Misgurunus mizolepis*) and cyprinid loach (*Misgurunus anguillicaudatus*). Aquaculture, 135: 257-266.

Komaru, A., Y. Uchimura, H. Ieyama and K.T. Wada. 1988. Detection of induced triploid scallop (*Chlamys nobilis*) by DNA microflurometry with DAPI staining. Aquaculture, 69: 201-209.

Lemieux, H., N.R.L. François and P.U. Blier. 2003. The early ontogeny of digestive and metabolic enzyme activities in two commercial strains of arctic charr (*Salvelinus alpinus* L.). J. Exp. Zool., 299A: 151-160.

Lemoine, H.L. and L.T. Jr. Smith. 1980. Polyploidy induced in brook trout by cold shock. Trans. Amer. Fisher Soc., 109: 626-631.

Maclean, N. and R.J. Laight. 2000. Transgenic fish: an evaluation of benefits and risks. Fish and Fisher, 1: 146-172.

Nam, Y.K., H.J. Cho, Y.S. Cho, J.K. Noh, C.G. Kim and D.S. Kim. 2001. Accelerated growth, gigantism and likely sterility in autotransgenic triploid mud loach *Misgurnus mizolepis*. J World Aquacult. Soc., 32: 353-363.

Nam, Y.K., G.C. Choi and D.S. Kim. 1999. Blocking of the 1st cleavage in mud loach (*Misgurnus mizolepis*). Kor. J. Aquacult., 12: 167-173.

Park, I.-S. and D.S. Kim. 2000. Comparison of some tissues in diploid and induced triploid hybrid between mud loach, *Misgurnus mizolepis* and cyprinid loach, *M. anguillicaudatus*. Dev. Reprod, 4: 19-28.

Park, I.-S. and H.B. Kim. 1994. Induction of triploid cherry salmon, *Oncorhyncus masou*. J. Aquacult., 7: 207-223.

Park, I.-S. and K.Y. Park. 1994. Haematological and physiological characteristics of diploid and triploid in cherry salmon (*Oncorhynchus masou*). J. Aquacult., 7: 193-201.

Peruzzi, S., S. Varsamos, B. Chatain, C. Fauvel, B. Menu, J.C. Falguière, A. Sévère and G. Flik. 2005. Haematological and physiological characteristics of diploid and triploid sea bass, *Dicentrarchus labrax* L. Aquaculture, 244: 359-367.

Phillips, R.B., K.D. Zajicek, P.E. Ihssen and O. Johnson. 1986. Application of silver staining to the identification of triploid fish cells. Aquaculture, 54: 313-319.

Piferrer, F., A. Beaumont, J.C. Falguière, M. Flajšhans, P. Haffray and L. Colombo. 2009. Polyploid fish and shellfish: Production, biology and applications to aquaculture for performance improvement and genetic containment. Aquaculture, 293: 125-156.

Piferrer, F., R.M. Cal, B. Álvarez-Blázquez, L. Sánchez and P. Martinez. 2000. Induction of triploidy in the turbot (*Scophthalmus maximus*) I. Ploidy determination and the effects of cold shocks. Aquaculture, 188: 79-90.

Razak, S.A., G.L. Hwang, M.A. Rahman and N. Maclean. 1999. Growth performance and gonadal development of growth enhanced transgenic tilapia *Oreochromis niloticus* (L.) following heat-shock-induced triploidy. Mar. Biotechnol., 1: 533-544.

Seol, D.W., S.Y. Im, W.J. Hur, M.O. Park, D.S. Kim, J.Y. Jo and I.-S. Park. 2008. Haematological parameters and respiratory function in diploid and triploid Far Eastern catfish, *Silurus asotus*. Genes and Genomics, 30: 205-213.

Sezaki, K., S. Watanabe and K. Hashimoto. 1988. Haematolocical parameters and erythrocyte enzyme activities associated with increase in ploidy status of the spinous loach, *Cobitis biwae* Jordan and Snyder. J. Fish Biol., 32: 149-150.

Shrimpton, J.M., A.M.C. Sentlinger, J.W. Heath, R.H. Devlin and D.D. Heath. 2007. Biochemical and molecular differences in diploid and triploid ocean-type chinook salmon (*Oncorhynchus tshawytscha*) smolts. Fish Physiol. Biochem., 33: 259-268.

Song, H.Y., I.C. Bang, Y.K. Nam and D.S. Kim. 2009. Embryogenesis and early ontogenesis of a marine medaka, *Oryzias dancena*. Kor. J. Ichthyol., 21: 227-238.

Stillwell, E.J. and T.J. Benfey. 1996. Hemoglobin level, metabolic rate, opercular abduction rate and swimming efficiency in female triploid brook trout (*Salvelinus fontinalis*). Fish Physiol. Biochem., 15: 377-383.

Tave, D. 1993. Growth of triploid and diploid bighead carp (*Hypophthalmichthys nobilis*). J. Appl. Aquacult., 2: 13-25.

Thorgaard, G.H. 1986. Ploidy manipulation and performance. Aquaculture, 57: 57-64.

Thresher, R., P. Grewe, J.G. Patil, S. Whyard, C.M. Templeton, A. Chaimongol, C.M. Hardy, L.A. Hinds and R. Dunham. 2009. Development of repressible sterility to prevent the establishment of feral populations of exotic and genetically modified animals. Aquaculture, 290: 104-109.

Uwa, H., T. Iwamatsu and O.P. Saxena. 1983. Karyotype and cellular DNA content of the Indian ricefish, *Oryzias melastigma*. Proc. Jpn. Acad., 59B: 43-47.

Uwa, H. and Y. Ojima. 1981. Detailed and banding karyotype analysis of the medaka, *Oryzias latipes* in cultured cells. Proc. Jpn. Acad., 57B: 39-43.

Wong, A.C. and A.L. Van Eenennaam. 2008. Transgenic approaches for the reproductive containment of genetically engineered fish. Aquaculture, 275: 1-12.

Yu, F., J. Xiao, X.Y. Liang, S.J. Liu, G.J. Zhou, K.K. Luo, Y. Liu, W. Hu, Y.P. Wang and Z.Y. Zhu. 2011. Rapid growth and sterility of growth hormone gene transgenic triploid carp. Chn. Sci. Bullet., 56: 1679-1684.

해산송사리, *Oryzias dancena* 유도 3배체의 세포유전학적 연구

박인석 · 길현우 · 이태호 · 남윤권[1] *· 고민균*[1] *· 김동수*[1]

한국해양대학교 해양생명과학부, [1]부경대학교 해양바이오신소재공학과

요 약 : 본 연구는 3배체 해산송사리, *Oryzias dancena*를 생산하기 위해서 다양한 조건에서 실험을 실시하였다. 저온처리(0°C)에서 30, 45, 60분간 처리하여 3배체를 유도 생산하였다. 여러 유도조건 결과, 45분간 처리하였을 때 가장 높은 3배체 생산율이 나타났다. 3배체 판별은 Chromosome 관찰, flow cytometer 분석 및 적혈구 측정을 통해서 판별하였다. 3배체 해산송사리의 적혈구 표면적과 부피는 2배체 해산송사리보다 크게 나타났고, 3배체 Chromosome number는 72개, 2배체는 48개가 관찰되었다. Flow cytometer 분석에서도 3배체가 2.40 pg/cell 그리고 2배체가 1.61 pg/cell 측정되어 DNA contents도 3배체가 2배체보다 1.5배 정도 크게 관찰되었다. 본 연구 결과는 불임 형질전환 어류를 위한 실험동물로의 3배체 해산송사리의 유용성 및 가치성을 제공한다.

찾아보기 낱말 : 저온처리, DNA 함량, 유세포분석기, 해산송사리, 3배체