

Comparative analysis of sectioned-body morphometric characteristics of diploid and triploid marine medaka, *Oryzias dancena*

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Abstract: The sectioned-body morphometric characteristics of the diploid and triploid marine medaka, *Oryzias dancena*, of both sexes were examined to collect basic data on the significant differences between the diploid and triploid fish. Significant differences between the diploid and triploid fish in both sexes were observed in the body circumference anterior to the base of the pelvic fin, the body circumference anterior to the base of the anal fin, the body circumference anterior to the base of the dorsal fin, the area anterior to the base of the pelvic fin, the area anterior to the base of the anal fin, the area anterior to the base of the dorsal fin, the total height anterior to the base of the pelvic fin, the total height anterior to the base of the anal fin, the height anterior to the base of the pelvic fin, the height anterior to the base of the anal fin, the width anterior to the base of the anal fin, the belly thickness II anterior to the base of the anal fin, section shape 2-1, and section shape 4-1 ($p < 0.05$). These measurements were greater in the triploid marine medaka of both sexes than those in the diploid marine medaka of both sexes, and they were also greater in the male diploid and triploid marine medaka than those in the corresponding female fish. Therefore, the sectioned-body morphometric dimensions were greater in the triploid males than those in the triploid females and the diploid fish in this study.

Keywords: diploid, marine medaka (*Oryzias dancena*), sectioned-body morphometric characteristics, triploid

INTRODUCTION

The morphological differences between species or populations are understood and compared with general external form or specific anatomical shapes, including sectioned-body parameters (Gjerde 1989; Gjerde and Schaeffer 1989; Strauss and Bond 1990; Park 2019). Characteristic traits such as size, weight, and sectioned-body parameters are particularly important growth indices. The morphometric characteristics of fish, unlike their meristic (countable) characteristics, can be measured in millimeters.

Although our understanding of the morphometric characteristics of fish is limited because they can be modified by the environment, the general figure of fish is predominantly determined by genetic factors (Park *et al.* 2004; Park 2019). The morphometric characteristics of fish are used in three major ways: to distinguish sex and species and to identify confusing species, such as hybrids; to study figure modifications in groups and species; and to identify and classify biotypic linkages (Park *et al.* 2004).

The marine medaka, *Oryzias dancena* is a euryhaline teleost that can live in both fresh - and seawater (Roberts

1998; Park *et al.* 2016a, 2017; Park 2019). It can spawn 60 days after hatching, so the interval between generations is short (Song *et al.* 2009a, 2009b; Park *et al.* 2011, 2016a, 2017, 2018). Therefore, the species is receiving considerable attention as an experimental animal in aquacultural research. The marine medaka has been shown to have better tolerance for hyperosmotic environments than does the Japanese medaka, *O. latipes*, including increased survival rates in the adult fish and higher hatching rates in the oosperm (Inoue and Takei 2003; Kang *et al.* 2008). The marine medaka is not indigenous to Korea. Recently, detailed information on its biology, especially its early gonadogenesis, sexual differentiation, early ontogenesis, and embryogenesis, is becoming available (Song *et al.* 2009a, 2009b). Nam *et al.* (2010) investigated the tolerance of salinity changes in this species. Park *et al.* (2011, 2017) studied the effects of clove oil and lidocaine-HCl on it, and the results of their study have contributed to the safe laboratory handling, and they carried out the simulated transportation through anesthesia of this species, which is used extensively in fishery and aquaculture research. The study for the sterilization effect of some chemicals on the growth stage of marine medaka was performed by Park *et al.* (2019). Much attention has been directed toward extending the utility of functional triploid and transgenic marine medaka strains for ornamental purposes because they can survive at any spontaneously occurring salinities (Cho *et al.* 2011; Park *et al.* 2016a, 2016b, 2018; Park and Choi 2018; Park and Gil 2018). These studies have demonstrated that the marine medaka has the necessary attributes as an experimental animal (Song *et al.* 2009a, 2009b; Nam *et al.* 2010; Park *et al.* 2011, 2016a, 2016b, 2017, 2018, 2019; Park and Choi 2018; Park and Gil 2018).

Triploidization is a technique used to generate sterile aquatic animals by taking advantage of the incompatible pairing of the three homologous chromosomes during meiosis I (Don and Avtalion 1986; Goo *et al.* 2015; Park *et al.* 2018; Park 2019). This technique has been used to enhance the productivity of several fish species for its assumed ability to increase the yield by channeling the energy required for gonadal development to somatic growth (Tave 1993; Goo *et al.* 2015). Artificial induced triploid fish including spontaneous triploid Cyprinidae were discovered as sterility by previous reports (Gervai *et al.* 1980; Seol *et al.* 2008), but spontaneous triploid Cyprinidae had fertility in previous reports (Al-Sabti *et al.* 1983; Devlin and Nagahama 2002; Kim *et al.* 2002; Goo *et al.* 2018). More importantly, triploidy 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 on exotic fish for a limited purpose, triploidy can serve an effective method of reducing or eliminating the environmental risks posed by genetically modified organisms (Dunham and Devlin 1999; Park *et al.* 2016a, 2016b, 2018; Park and Choi 2018; Park and Gil 2018).

Triploid fish generally have similar morphological and meristic characteristics, if not identical, to those of diploid fish (Bonar *et al.* 1988; Park *et al.* 2016a, 2018). However, several morphological differences and abnormalities have been associated with triploidy in fish. A variety of deformities have been reported in the triploid pejerrey, *Odontesthes bonariensis* (Strüssmann and Takashima 1993), but it is unclear whether the examined fish were in fact triploid or aneuploid (i.e., having a chromosome number other than a complete multiple of the haploid set). Changes in the scale pattern and a reduction in the scale cover were observed in the triploid common carp, *Cyprinus carpio*, and were attributed to differences in the allelic ratios of genes controlling these traits (Gomelsky *et al.* 1992). Flajšhans *et al.* (1993) described differences in the pelvic fin shapes and lengths between the triploid and diploid tench, *Tinca tinca*, and Tave (1993) observed facial deformities in the triploid big-head carp, *Hypophthalmichthys nobilis* and the grass carp, *Ctenopharyngodon idella*. The most prominent and most frequently described gross morphological difference in triploid fish is probably the development of lower jaw deformities in the triploid Atlantic salmon, *Salmo salar* (Lee and King 1994; McGeachy *et al.* 1996). Although conclusive data are insufficient, this deformity may be linked to its rapid growth rate in seawater (Lee and King 1994).

No previous study of the marine medaka has included a comparative analysis of the morphometric characteristics of the diploid and triploid fish. In this study, we undertook a comparative analysis of the sectioned-body morphometric characteristics of the diploid and triploid marine medaka. The aim of this study is to ascertain whether triploidy induces morphometric changes in marine medaka.

MATERIALS AND METHODS

The experimental group of diploid marine medaka, *Oryzias dancena* used in this study was reared by the method of Park *et al.* (2011) and Goo *et al.* (2015). On 24 September, 2018, 100 fish were quarantined into male and female groups and habituated in 100 L glass aquariums for 3 days.

The sex ratio was 60 males to 40 females. The culture water was dechlorinated and 30% of the water in each aquarium was changed every day. *Artemia* collected from a culture aquarium was provided to the fish every day. Fish with a standard length of >25 mm were used in this experiment; 35 males and 15 females were placed into each of two aquariums, and 1,000 fertilized eggs were immediately collected by net. The fertilized eggs of the diploid experimental group ($n=500$) were reared in a 100 L glass aquarium.

The fertilized eggs of the triploid experimental group ($n=500$) were left for 5 min, and were subjected to cold-shock treatment (4°C) for 60 min to prevent the extrusion of the second polar body (Park *et al.* 2016a, 2016b). The treated eggs were reared in a 100 L glass aquarium. After 2 months, a flowcytometric analysis was performed to estimate the average cellular DNA content of all triploid individuals. After the fish were anesthetized with 100 ppm clove oil, sample tissues were removed from the tail fins of all the fish for analyzing (Park *et al.* 2011). One million tail fin cells were collected and stained with a high-resolution DNA staining kit (Partec GmbH, Germany) in the darkness at room temperature for 15 min (Park *et al.* 2016a, 2016b). The stained samples were analyzed on a Partec PA-II flowcytometer to identify their relative DNA contents. Red blood cells (2.8 pg DNA/nucleus) of the mud loach, *Musgurnus mizolepis* were used as the reference standard. Individuals identified to be triploid were quarantined in a 30 L glass aquarium before the experiment (Park *et al.* 2016a).

On 16 May, 2019 (230 days after hatching), the total sample in each group comprised 60 marine medaka: 30 males and 30 females. Samples of diploid and triploid fish were fixed in 10% neutral formalin. During the experiment, the fish were starved for 24 hrs before sampling to prevent sampling fish with guts that were distended by large quantities of food (Park *et al.* 2016a, 2016b). The body length (BL) of each fish was measured to the nearest 0.1 cm using a digital Vernier caliper (CD-20CP, Japan). The ungutted body weight (UBW) of each sample was measured using an electronic balance (JW-1, Korea). A sectioned-body morphometric analysis was performed in duplicate. The viscera of the samples were removed and the ungutted samples were fixed in 10% neutral formalin solution (100 mL formalin, 6.5 g $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 4.5 g KH_2PO_4 , 900 mL distilled water) for 24 hrs. The gutted body weight (GBW) and visceral weight (VW) of each sample were measured using an electronic balance (JW-1). The condition factor (CF) and visceral index (VI) were calculated with

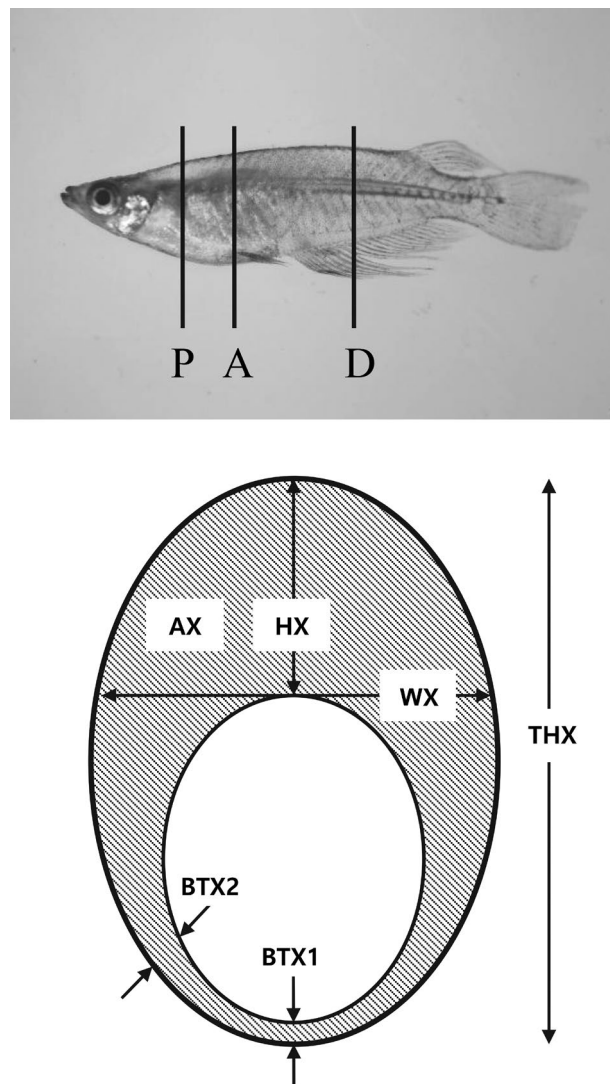


Fig. 1. Total height (THX), height (HX), width (WX), area (AX), and belly thickness (BTX I and BTX II) measured in marine medaka, *Oryzias dancena*, on a cross-section slice taken just anterior to the base of the pelvic fin ($X=P$), just anterior to the base of the anal fin ($X=A$), and just anterior to the base of the dorsal fin ($X=D$).

the following equations: $\text{CF} = (\text{UBW}/\text{BL}^3) \times 100$, and $\text{VI} = (\text{VW}/\text{GBW}) \times 100$, respectively. The samples were then refixed in Bouin's solution for a further 24 hrs. All fixed tissues were routinely dehydrated in ethanol, equilibrated in xylene, and embedded in paraffin according to standard histological techniques. Sectioned samples from each group were cut across the P, A, and D lines, as shown in Fig. 1. Transverse sections were then cut at $6\ \mu\text{m}$ intervals and routinely stained with Mayer's hematoxylin and eosin Y-phloxine B before observation under a high-powered mi-

Table 1. Means of the phenotypic traits of the diploid and triploid marine medaka, *Oryzias dancena*, and results of *t*-tests for differences between the sexes¹⁾

Trait	Diploid			Triploid		
	Female	Male	<i>t</i> -test	Female	Male	<i>t</i> -test
Body length (mm)	24.6	26.8	**	26.1	28.4	**
Ungutted body weight (mg)	364.1	458.1	**	679.9	584.4	**
Gutted body weight (mg)	301.5	427.7	**	602.8	542.2	**
Condition factor	2.44	2.37	*	3.82	2.54	**
Viscera weight (mg)	67.3	29.7	**	76.5	35.2	**
Viscera index	22.32	6.47	**	12.70	6.49	**

¹⁾Condition factor = (ungutted body weight/body length³) × 100, Viscera index = (viscera weight/gutted body weight) × 100.

*: denotes significant at $p=0.05$, **: denotes significant at $p=0.01$.

croscope (Carl Zeiss, Germany). The Axioskop 4.1 image analysis software (Carl Zeiss, Germany) was used to measure the sectioned-body morphometric dimensions.

The sectioned-body morphometric dimensions examined were body circumference anterior to the base of the pelvic fin (CIP), body circumference anterior to the base of the anal fin (CIA), body circumference anterior to the base of the dorsal fin (CID), area anterior to the base of the pelvic fin (AP), area anterior to the base of the anal fin (AA), area anterior to the base of the dorsal fin (AD), total height anterior to the base of the pelvic fin (THP), total height anterior to the base of the anal fin (THA), total height anterior to the base of the dorsal fin (THD), width anterior to the base of the pelvic fin (WP), width anterior to the base of the anal fin (WA), width anterior to the base of the dorsal fin (WD), height anterior to the base of the pelvic fin (HP), height anterior to the base of the anal fin (HA), belly thickness I anterior to the base of the pelvic fin (BTP I), belly thickness II anterior to the base of the pelvic fin (BTP II), belly thickness I anterior to the base of the anal fin (BTA I), belly thickness II anterior to the base of the anal fin (BTA II), average belly thickness (ABT), body shape (BS), and section shape (SS). The data were analyzed with one-way ANOVA with the SPSS statistical package (SPSS 9.0, SPSS Inc., USA). Means were separated with Duncan's multiple range test and considered significantly different at $p < 0.05$ (Duncan 1955).

RESULTS

The measurements for the growth and phenotypic traits of the male and female fish in the diploid and triploid ma-

rine medaka, *Oryzias dancena* are presented in Table 1. BL, UBW, and GBW for the males in each ploidy group were greater than those for the females in the corresponding ploidy groups ($p < 0.05$). CF differed significantly between the males and females in the diploid and triploid groups ($p < 0.05$); VW and VI were significantly different across all groups (between the males and females of each ploidy and between the diploid and triploid fish; $p < 0.05$). The measurements for the growth and phenotypic traits of the diploid and triploid fish of each sex are presented in Table 1. BL, UBW, and GBW in both sexes of the triploid group were greater than BL, UBW, and GBW in the corresponding sexes of the diploid group ($p < 0.05$). CF differed significantly between the males and females in both the diploid and triploid groups ($p < 0.05$). VW and VI were significantly different between the males and females in each ploidy group and between the diploid and triploid fish ($p < 0.05$).

The sectioned-body morphometric dimensions of triploid marine medaka were the greater than those of diploid marine medaka (Fig. 2). As shown in Tables 2 and 3, the morphometric dimensions CIP, CIA, CID, AP, AA, AD, THP, THA, HP, WP, WA, BTP I, ABT, SS 1-1, SS 1-2, SS 3-2, and SS 4-1 were greater in the males than in the females in both ploidy groups ($p < 0.05$). The morphometric dimensions BS 1, BS 2, SS 2-1, and SS 3-1 were significantly greater in the females than in the males in both ploidy groups ($p < 0.05$). The morphometric dimensions BTP II, HA, BTA I, BTA II, THD, WD, BS 3, and SS 3-2 did not differ significantly between males and females, and between diploid and triploid fish ($p > 0.05$). The morphometric dimensions CIP, CIA, CID, AP, AA, AD, THP, THA, HP, HA, WA, BTA II, SS 2-1, and SS 4-1 were

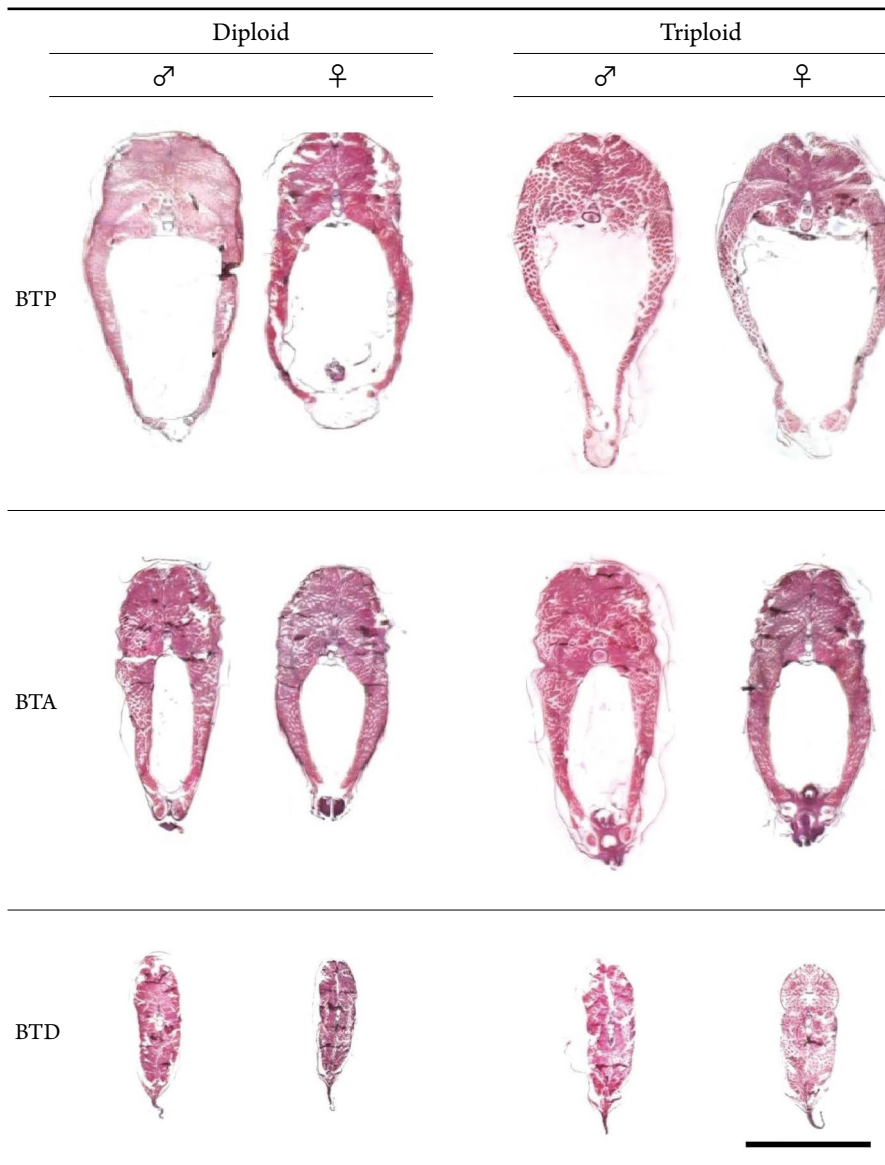


Fig. 2. Typical sectioned morphology of belly thickness in diploid and triploid marine medaka, *Oryzias dancena* (female: ♀; male: ♂), just anterior to the base of the anal fin (BTA), just anterior to the base of dorsal fin (BTD), and just posterior to the base of pelvic fin (BTP) (HE-staining). The bar indicates 2 cm.

greater for both sexes in the triploid group than for the corresponding sexes in the diploid group ($p < 0.05$), whereas morphometric dimensions BS 2 and BS 3 were significantly greater in both sexes of the diploid group than in the triploid group (Tables 2 and 3; $p < 0.05$).

The morphometric dimensions that did not differ significantly between the male and female fish of the same ploidy group and between the diploid and triploid fish were WP, ABT, BTP I, BTP II, BTA I, SS 3-1, SS 3-2, and SS 3-3

($p > 0.05$). Among the female fish, THD, WD, and SS 1-2 were greater in the triploid group than in the diploid group. However, in the males, THD, WD, and SS 1-2 did not differ significantly between the diploid and triploid fish. SS 1-1 was greater in the diploid females than in the triploid females, whereas SS 1-1 did not differ significantly between the diploid and triploid male fish. Among the male fish, BS 1 was greater in the diploid group than in the triploid group, whereas BS1 did not differ significantly between the

Table 2. Means of the phenotypic traits of diploid and triploid marine medaka, *Oryzias dancena*, and results of *t*-tests for differences between the sexes¹⁾

Trait	Diploid			Triploid		
	Female	Male	<i>t</i> -test	Female	Male	<i>t</i> -test
Body circumference at						
pelvic fins (CIP) (cm)	9.98	10.57	**	10.18	10.64	**
anal fin (CIA) (cm)	7.54	8.84	**	7.67	8.91	**
dorsal fin (CID) (cm)	5.15	6.24	*	6.28	6.31	*
Section at pelvic fins						
area (AP) (cm ²)	8.45	10.25	**	8.67	11.42	**
total height (THP) (cm)	3.24	3.35	*	3.26	3.38	*
height (HP) (cm)	1.16	1.20	*	1.17	1.21	*
width (WP) (cm)	1.91	2.02	*	2.51	2.72	*
belly thickness I (BTP I) (mm)	0.02	0.03	*	0.02	0.03	*
belly thickness II (BTP II) (mm)	0.91	0.93	NS	0.93	0.95	NS
Section at anal fin						
area (AA) (cm ²)	7.24	8.15	*	7.26	8.20	*
total height (THA) (cm)	2.62	2.71	*	2.64	2.75	*
height (HA) (cm)	1.25	1.26	NS	1.27	1.27	NS
width (WA) (cm)	1.38	1.44	*	1.39	1.46	*
belly thickness I (BTA I) (mm)	5.12	5.12	NS	5.12	5.12	NS
belly thickness II (BTA II) (mm)	3.06	3.06	NS	3.07	3.07	NS
Section at dorsal fin						
area (AD) (cm ²)	5.18	6.01	*	5.19	6.04	*
total height (THD) (cm)	1.95	2.01	NS	1.97	2.04	NS
width (WD) (cm)	0.72	0.74	NS	0.73	0.74	NS
Average belly thickness (ABT) (mm)	0.47	0.48	*	0.47	0.48	*

¹⁾ABT: average belly thickness, (BTP I + BTP II)/2.

NS: denotes not significant at $p=0.05$, *: denotes significant at $p=0.05$, **: denotes significant at $p=0.01$.

Values for female (means of 40 fish \pm SD), values for male (means of 60 fish \pm SD).

diploid and triploid female fish.

DISCUSSION

A variety of characteristics of the sectioned surface are critical elements in the marketing of fish for consumer preferences with respect to the size and shape of the sectioned surface in gutted, sectioned, smoked, and non-gutted fish (Park *et al.* 2002). In addition, sectioned-body morphometric characteristics can be used as indices to establish growth and nutritional status (Park and Zhang 1994; Park *et al.* 2002). Park *et al.* (2002) investigated the effects of starvation on some nutritional parameters in the Chinese minnow, *Rhynchoypris oxycephalus*. Their results suggest

that the nutritional parameters used in the study are useful indices of nutritional status in the Chinese minnow. In this study, BL, UBW, GBW, CF, VW, VI and some phenotypic traits of triploid marine medaka were significantly higher than those of diploid ($p < 0.05$). The results of Park and Zhang (1994) were different from the results of this study. The morphometric differences between diploid and induced triploid cherry salmon, *Oncorhynchus masou* were investigated by Park and Zhang (1994). According to Park and Zhang (1994), BL and BW of the diploid cherry salmon were greater than those of the induced triploid cherry salmon, and the dressing percentage, gonadal weight, gonadal index, VI, and liver index were also greater in the diploid cherry salmon. However, the induced triploid cherry salmon showed higher values for one belly thickness trait

Table 3. Means of the phenotypic traits of diploid and triploid marine medaka, *Oryzias dancena*, and results of *t*-tests for differences between the sexes¹⁾

Trait	Diploid			Triploid		
	Female	Male	<i>t</i> -test	Female	Male	<i>t</i> -test
Body shape (BS)						
BS 1	8.74	8.36	**	8.74	8.13	**
BS 2	3.58	3.40	*	3.41	3.20	*
BS 3	0.89	0.90	NS	0.88	0.85	*
Section shape (SS)						
SS 1-1	46.59	57.14	**	46.06	57.14	**
SS 1-2	25.00	26.37	*	25.84	26.37	*
SS 2-1	39.02	38.46	*	41.46	40.38	*
SS 3-1	21.95	19.23	**	21.95	19.23	**
SS 3-2	17.07	17.31	NS	17.07	17.31	NS
SS 3-3	7.32	5.77	**	7.32	5.77	**
SS 4-1	0.994	1.358	**	1.054	1.419	**

¹⁾BS 1: body shape 1, (CID/BL) × 100; BS 2: body shape 2, (THD/BL) × 100; BS 3: body shape 3, (WD/BL) × 100; SS 1-1: section shape 1-1, (WP/THP) × 100; SS 1-2: section shape 1-2, (VD/THD) × 100; SS 2-1: section shape 2-1, (HP/WP) × 100; SS 3-1: section shape 3-1, (ABT/WP) × 100; SS 3-2: section shape 3-2, (BTP I/WP) × 100; SS 3-3: section shape 3-3, (BTP II/WP) × 100; SS 4-1: section shape 4-1, $[0.5(\pi \times HP \times 0.5 \times WP)/AP] \times 100$.

NS: denotes not significant at $p=0.05$, *: denotes significant at $p=0.05$, **: denotes significant at $p=0.01$.

Values for female (means of 40 fish ± SD), values for male (means of 60 fish ± SD).

and for some section shapes. The differences in body traits were attributable to the sterility induced in the triploid cherry salmon (Park and Zhang 1994). In a previous study, improved growth traits have been observed in triploid fish of several species, and attributed to their presumed capacity to channel the energy required for sexual maturation into somatic growth (Tave 1993; Park and Zhang 1994; Park *et al.* 2018).

Im *et al.* (2016) reported that the sexual dimorphism in the diploid marine medaka, *Oryzias dancena* appears as a difference in growth 70 days after hatching. Among the traits involved, the direct distance between the anterior insertion of the first dorsal fin and the anterior insertion of the first anal fin (DADAA), the direct distance between the posterior insertion of the last dorsal fin and the anterior insertion of the first anal fin, the direct distance between the anterior insertion of the first anal fin and the posterior insertion of the last anal fin, the length of the fin rays of the dorsal fin, and the length of the fin rays of the anal fin were clearly different, and the male characteristics were greater than the corresponding female characteristics. In diploid and triploid marine medaka, BL, UBW, GBW, CF, VW, VI and most of phenotypic traits in male marine medaka were significantly higher than those in female ($p < 0.05$). Especially, body shape and section shape values of male were mostly bigger than those of female. In summary, the mor-

phometric characteristics of the marine medaka differed significantly between male and female fish, with the male marine medaka having more rapid growth than the female, greater length, bigger body shape, longer dorsal fins, and longer anal fins.

In the previous study, all the morphometric dimensions of the triploid fish were greater than those of the diploid fish (Park *et al.* 2016a). The triploid marine medaka also showed sexual dimorphism, which was similar to that of the diploid marine medaka. Therefore, triploid marine medaka had more rapid growth rates with sexual dimorphism than diploid marine medaka including a longer dorsal fin and a longer anal fin (Park *et al.* 2016a). In this study, the sectioned-body morphometric dimensions of the male triploid fish were the greatest in those of all observed (Fig. 2). This phenomenon is caused by a combination of the sterility induced from the triploid with the inherent sexual dimorphism of the triploid fish (Im *et al.* 2016; Park *et al.* 2016a, 2018). The specific dimensions examined in this study have been shown to be effective indicators for fish growth conditions. The differences in these characteristics should also be useful in distinguishing both sex and ploidy of the marine medaka. These results extend our knowledge of the morphometric changes that occur in diploid and triploid and should be useful for application to the sterility of this species.

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REFERENCES

- Al-Sabti K, B Kurelec and N Fijan. 1983. Spontaneous triploidy and tetraploidy in the common carp (*Cyprinus carpio* L). *Veterinarski Arhiv* 53:217–223.
- Bonar SA, GL Thomas and GB Pauley. 1988. Evaluation of the separation of triploid and diploid grass carp (*Ctenopharyngodon idella*) (Valenciennes), by external morphology. *J. Fish. Biol.* 33:895–898.
- Cho YS, SY Lee, YK Kim, DS Kim and YK 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.
- Devlin RH and Y Nagahama. 2002. Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. *Aquaculture* 208:191–364.
- Don J and RR Avtalion. 1986. The induction of triploidy in *Oreochromis aureus* by heat shock. *Theor. Appl. Genet.* 72:186–192.
- Duncan DB. 1955. Multiple range and multiple F tests. *Biometrics* 11:1–42.
- Dunham RA and RH Devlin. 1999. Comparison of traditional breeding and transgenesis in farmed fish with implications for growth enhancement and fitness. pp. 209–229. In: *Transgenic Animals in Agriculture*, (Murray JD, GB Anderson, AM Oberbauer and MN McGloughlin eds.). CAB International, New York.
- Flajšhans M, O Linhart and P Kvasnika. 1993. Genetic studies of tench (*Tinca tinca* L.): induced triploidy and tetraploidy and first performance data. *Aquaculture* 113:301–312.
- Gervai J, S Péter, A Nagy, L Horváth and V Csányui. 1980. Induced triploidy in carp, *Cyprinus carpio* L. *J. Fish Biol.* 17: 667–671.
- Gjerde B. 1989. Body traits in rainbow trout: Phenotypic means and standard deviation and sex effects. *Aquaculture* 80:7–24.
- Gjerde B and LR Schaeffer. 1989. Body traits in rainbow trout. II. estimates of heritabilities and phenotypic and genetic correlations. *Aquaculture* 80:25–44.
- Gomelsky BI, OV Emelyanova and AV Recoubratsky. 1992. Application of the scale cover gene (N) to identification of type of gynogenesis and determination of ploidy in common carp. *Aquaculture* 106:233–237.
- Goo IB, JH Im, HW Gil, SG Lim and IS Park. 2015. Comparison of cell and nuclear size difference between diploid induced triploid in marine medaka, *Oryzias dancena*. *Dev. Reprod.* 19:127–134.
- Goo IB, SG Lim, HW Gil, IS Park and CY Choi. 2018. Cytogenetic characteristics of Cyprinidae between diploid and spontaneous triploid in major river of Korea. *JMLS* 3:9–21.
- Im JH, HW Gil, TH Lee, HJ Kong, CM Ahn, BS Kim, DS Kim, CI Zhang and IS Park. 2016. Morphometric characteristics and fin dimorphism between male and female on the marine medaka, *Oryzias dancena*. *Dev. Reprod.* 20:331–347.
- Inoue K and Y Takei. 2003. Asian medaka fishes offer new models for studying mechanisms of seawater adaptation. *Comp. Biochem. Physiol. B* 136:635–645.
- Kim EO, JY Lee, YK Nam, JK Noh, SY Lee and DS Kim. 2002. Genetic identification on natural population of triploid crucian carp, *Carassius auratus* in Korea. *J. Korean Fish. Soc.* 35:589–594.
- Kang CK, SC Tsai, TH Lee and PP 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. A* 151:566–575.
- Lee P and H King. 1994. Effects of reduced dietary energy on the incidence of jaw deformity in Tasmanian Atlantic salmon. pp. 61–69. In: *Reports from the SALTAS 1993-94*. SALTAS, Hobart, Tasmania, Australia.
- McGeachy SA, FM O'Flynn, TJ Benfey and GW Friars. 1996. Seawater performance of triploid Atlantic salmon in New Brunswick aquaculture. *Bull. Aquacult. Assoc. Can.* 96:24–28.
- Nam YK, YS Cho, SY Lee and DS Kim. 2010. Tolerance capacity to salinity changes in adult and larva of *Oryzias dancena*, a euryhaline medaka. *Korean J. Ichthyol.* 22:9–16.
- Park IS. 2019. A comparative analysis of cell cycles in diploid and induced triploid tissues in marine medaka (*Oryzias dancena*). *Korean J. Environ. Biol.* 37:735–740.
- Park IS and CI Zhang. 1994. Morphometrical differences between diploid and induced triploid cherry salmon, *Oncorhynchus masou*. *Korean J. Ichthyol.* 6:206–221.
- Park IS and CY Choi. 2018. Occurrence of amitosis-like nuclear division in erythrocytes of induced triploid Far Eastern cat-

- fish, *Silurus asotus* and marine medaka, *Oryzias dancena*. JFMSE 30:1519–1527.
- Park IS and HW Gil. 2018. Comparative analysis of fluctuating asymmetry between ploidy and sex in marine medaka, *Oryzias dancena*. Dev. Reprod. 22:275–281.
- Park IS, HW Gil and DS Kim. 2018. Morphometric characteristics of diploid and triploid marine medaka, *Oryzias dancena*. Dev. Reprod. 22:183–192.
- Park IS, HW Gil, TH Lee, YK Nam and DS Kim. 2016a. Comparative study of growth and gonad maturation in diploid and triploid marine medaka, *Oryzias dancena*. Dev. Reprod. 20:305–314.
- Park IS, HW Gil, TH Lee, YK Nam, MG Ko and DS Kim. 2016b. Cytogenetic study of diploid and triploid marine medaka, *Oryzias dancena*. Korean J. Ichthyol. 28:215–222.
- Park IS, HW Gil, TH Lee, YK Nam, SG Lim and DS Kim. 2017. Effects of clove oil and lidocaine-HCl anesthesia on water parameter during simulated transportation in the marine medaka, *Oryzias dancena*. Dev. Reprod. 21:19–33.
- Park IS, JH Im and JW Hur. 2004. Morphometric characteristics of catfish (Siluridae) in Korea. Korean J. Ichthyol. 16:223–228.
- Park IS, JH Im, CH Jeong, JK Noh, YH Kim and YH Lee. 2002. Effect of starvation on some nutritional parameters in *Rhynchocypris oxycephalus* (Sauvage and Dabry). 2. Characteristics of the morphometric change in the sectioned body. Korean J. Ichthyol. 14:11–18.
- Park IS, SJ Park, HW Gil, YK Nam and DS Kim. 2011. Anesthetic effects of clove oil and lidocaine-HCl on marine medaka, *Oryzias dancena*. Lab Anim. 40:45–51.
- Park IS, SW Baek and KH Moon. 2019. The sterilization effect of methylene blue, formalin, and iodine on egg and adult stage of marine medaka, *Oryzias dancena*. Dev. Reprod. 23:199–211.
- Roberts TR. 1998. Systematic observations on tropical Asian medakas or ricefishes of genus *Oryzias*, with descriptions of four new species. Ichthyol. Res. 45:213–224.
- Seol DW, SY Im, WJ Hur, MO Park, DS Kim, JY Jo and IS Park. 2008. Haematological parameters and respiratory function in diploid and triploid far eastern catfish, *Silurus asotus*. Genes Genom. 30:205–213.
- Song HY, YK Nam, IC Bang and DS Kim. 2009a. Early gonadogenesis and sex differentiation of marine medaka, *Oryzias dancena* (Belontiiformes; Teleostei). Korean J. Ichthyol. 21:141–148.
- Song HY, YK Nam, IC Bang and DS Kim. 2009b. Embryogenesis and early ontogenesis of a marine medaka (*Oryzias dancena*). Korean J. Ichthyol. 21:227–238.
- Strauss RE and CE Bond. 1990. Taxonomic methods, morphology. pp. 125–130. In: Methods for Fishery Biology (Schreck CB and PB Moyle eds.). American Fishery Society, Bethesda, MD.
- Strüssmann CA and F Takashima. 1993. Hepatocyte nuclear size and nutritional condition of larval pejerrey (*Odontesthes bonariensis*) (Cuvier et Valenciennes). J. Fish Biol. 36:59–65.
- Tave D. 1993. Growth of triploid and diploid bighead carp (*Hypophthalmichthys nobilis*). J. Appl. Aquacult. 2:13–25.