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THESIS FOR THE DEGREE OF MASTER OF SCIENCE

**Morphometric and fin dimorphism  
between sex in the marine medaka,  
*Oryzias dancena***



Department of Marine Bioscience and Environment

The Graduate School

Korea Maritime University

February 2012

**Morphometric and fin dimorphism  
between sex in the marine medaka,  
*Oryzias dancena***

Advisor: Prof. In-Seok PARK



by

**Hyun Woo Gil**

A dissertation submitted in partial fulfillment of the requirements  
for the degree of

*Master of Science*


In the Department of Marine Bioscience and Environment,  
the Graduate School of Korea Maritime University


February 2012

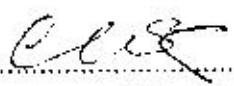
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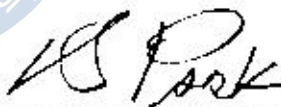
**Morphometric and fin dimorphism between sex  
in the marine medaka, *Oryzias dancena***

A dissertation  
by  
Hyun Woo Gil

Certified by **In-Seok PARK**   
.....  
Thesis Advisor

Accepted by **Bong Seok KIM**   
.....  
Chairman

**Sung Hwoan CHO**   
.....  
Member

**In-Seok PARK**   
.....  
Member

December 2011

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by

Hyun Woo GIL

Submitted to

*The Department of Marine Bioscience and Environment  
Graduate School of Korea Maritime University  
(Supervised by In-Seok PARK, Ph. D.)*

**Abstract**

Sexual dimorphism is the most conspicuous difference between the sexes. This study examines possible sexual dimorphism and the relative growth patterns of morphometric characteristics in the marine medaka, *Oryzias dancena* for their potential to help differentiate between males and females of this species. And I am about to consider more about difference of male and female of marine medaka in morphology seen from anal fin and fin ray as well as dorsal fin. The von Bertalanffy growth parameters estimated by a non-linear regression method were  $L_{\infty}=30.2$  mm,  $K=3.22/\text{year}$ , and  $\tau_0=-0.05$ . All 18 characteristics measured showed a difference between males and females from 70 days after hatching. Five direct distance characteristics included the following: the anterior insertion of the first dorsal fin and the anterior insertion of the first anal fin, 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 dorsal 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. Each of these characteristics were significantly different between sexes (ANCOVA,  $P < 0.05$ ), and the ratio of standard length between sexes showed that males were larger than females for all five characteristics.

Fin length measurements were taken for 21 distances of anal fin and 7 distances of dorsal fin between landmarks. There were all difference for all dorsal fin rays between the males and the females and there is significant difference in 70 days after their hatched when the sexual dimorphism is presented. The significant difference ( $P < 0.05$ ) in fin ray for male and female was more greatly seen as they grow. Male marine medakas showed more rapid growth than females, with longer length, dorsal fins and anal fins. Differences in these characteristics will be useful during experiments when it is necessary to differentiate between sexes of marine medaka.

**Key words:** fin dimorphism, marine medaka (*Oryzias dancena*), morphometric characteristics, sexual dimorphism

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Approved as qualified thesis of Hyun Woo Gil for the degree of Master of Philosophy by the Evaluation Committee in November 2011.

# Korean Abstract

(국문 요약)

## 해산송사리, *Oryzias dancena* 계측형질과 지느러미의 성적이형

김현우

한국해양대학교 대학원  
해양생명환경학과

성적이형(Sexual dimorphism)은 성별의 가장 뚜렷한 형태학상의 차이점을 반영하는 바, 본 연구는 해산송사리, *Oryzias dancena*의 암·수간 상대적인 성장패턴과 성적이형을 규명하고 성적이형이 뚜렷하게 보이는 뒷지느러미와 등지느러미볼 대상으로 형태학적 측면에서 암·수간 비교하였다.

von Bertalanffy 성장 방정식을 Non-linear regression 방법으로 측정한 결과,  $L_{\infty}=30.2$  mm로 나타났고  $K=3.22/\text{year}$ 로 측정되었으며  $t_0=-0.05$ 로 나타났다. 18개의 계측형질에서 암·수간 차이가 부화 후 70일에 모두 나타났으며 그 중 5개의 계측형질(The anterior insertion of the first dorsal fin and the anterior insertion of the first anal fin, 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 dorsal 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)에서 유의한 차이가 나타났다( $P<0.05$ ). 이 5개의 계측형질을 공분산분석을 실시한 결

과, 암수간의 차이가 모두 유의적으로 나타났으며 체장에 대한 계측형질의 비율이 수컷이 암컷보다 더 크게 나타났다( $P<0.05$ ).

지느러미의 형태학적 분석은 총 21개의 뒷지느러미 기조와 7개의 등지느러미 기조를 대상으로 측정하였다. 뒷지느러미와 등지느러미의 암수간 형태적 차이는 성적이행이 나타나는 부화 후 70일에 모든 기조에서 나타났으며 더욱이, 성장함에 따라 뒷지느러미와 등지느러미 기조 크기에서 수컷이 암컷에 비해 더욱 크게 나타났다( $P<0.05$ ). 수컷 해산송사리는 암컷에 비해 더 빠른 성장과 아울러 등지느러미와 뒷지느러미의 길이가 더 크게 나타났다. 본 연구 결과 파악된 형태학적 차이는 해산송사리의 암수 판별에 필수적이고 유용한 정보가 될 것이라 사료된다.



# Introduction

As an experimental fish, the marine medaka, *Oryzias dancena*, is gaining attention as an experimental animal in aquaculture. This fish is a euryhaline teleost that can live in both fresh water and seawater (Robert, 1998). It also has a short interval between generations, with spawning possible only 60 days after hatching (Kim *et al.*, 2009b). Kang *et al.* (2008) and Inoue & Takei (2003) used the marine medaka to study molecular biomarkers and as an experimental fish for adapting to seawater. The marine medaka shows better tolerance than the Japanese medaka, *O. latipes*, in aspects such as survival rates of adult fish and hatched rates of oosperm in hyperosmotic environments (Inoue & Takei, 2003; Kang *et al.*, 2008).

Recently, the Institute of Marine Living Modified Organisms (*i*MLMO, Pukyung National University, Korea) selected this species for a living modified organism evaluation project. In line with this purpose, detailed information on its biology (especially early gonadogenesis, sex differentiation, early ontogenesis and embryogenesis) has begun to be examined (Kim *et al.*, 2009a, 2009b). Nam *et al.* (2010) researched the tolerance capacity to salinity changes in this species and found that the marine medaka is highly capable of hyper-osmoregulation as well as hypo-osmoregulation. The marine medaka shows no mortality during transfer

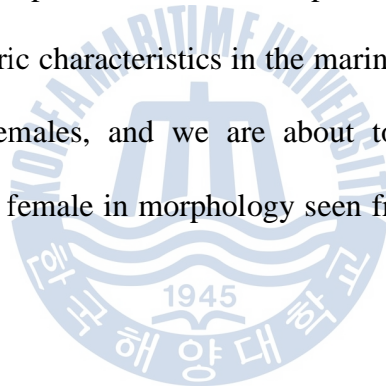
either from 0 ppt freshwater to 40 ppt saltwater or from 70 ppt saltwater to 0 ppt freshwater. Also, no marine medakas in the experimental group in this study (0 ppt ~ 40 ppt) died from stress due to salinity changes (Nam *et al.*, 2010).

Both truss (Strauss & Bookstein, 1982) and classical (Hubb & Lagler, 1947) dimensions are used to describe fish body shape. Truss dimensions consist of a systematically arranged set of distances that are measured between a set of preselected anatomical landmarks. These landmarks are identified based on local morphological features, and they are chosen to divide the body into functional units (Strauss & Bond, 1990). Truss dimensions, which include components of body depth and length along the longitudinal axis, have theoretical advantages over classical morphometric characteristics for discriminating among groups (Park *et al.*, 2007).

Sexual dimorphism is a component of external morphological variation between the sexes, along with features such as the genital papilla, body pigmentation, fin shape (Anderson, 1994). Sexual dimorphism is the most conspicuous difference between the sexes (Kim *et al.*, 2008). Sexual dimorphism occurs in many fishes. Females are usually larger than males of the same age. In some species, however, males are larger than females, e.g., gudgeon, *Gobio gobio* (Mann, 1980), and filefish, *Brachaluteres ulvarum*

(Akagawa *et al.*, 1995). The reason for the size difference is not clear (Katano, 1998). Several authors reported that the evolution of larger body size in male likely results from male-male competition associated with a polygynous mating system (Katano, 1998; Kim *et al.*, 2008). Hence, exploring the nature and extent of sexual dimorphism can aid in understanding social structure and adaptation, as well as species identification.

This study examines possible sexual dimorphism and the relative growth patterns of morphometric characteristics in the marine medaka to distinguish between males and females, and we are about to consider more about difference of male and female in morphology seen from anal fin and fin ray as well as dorsal fin.





# Materials and Methods

## 1. Experimental design

On 12 January 2009, 30 marine medaka, *Oryzias dancena* were obtained from iMLMO at Pukyong National University, Korea. The fish were reared and bred in the Fishery Genetics and Breeding Science Laboratory at Korea Maritime University, Korea. Rearing occurred in a 400 L glass tube consisting of a circulation pump, an aeration system and a temperature control system. Culture water was a mixture of dechlorinated fresh water and seawater. The total culture water salinity was  $3\pm 0.1$  ppt, measured with a refractometer (OxyGuard International, Handy Polaris, Denmark). Dissolved oxygen levels were maintained by air pump, and the water temperature was maintained at  $26\pm 0.5$  °C. Lab conditions allowed for 13 hours of light per day, with darkness for the remaining 11 hours.

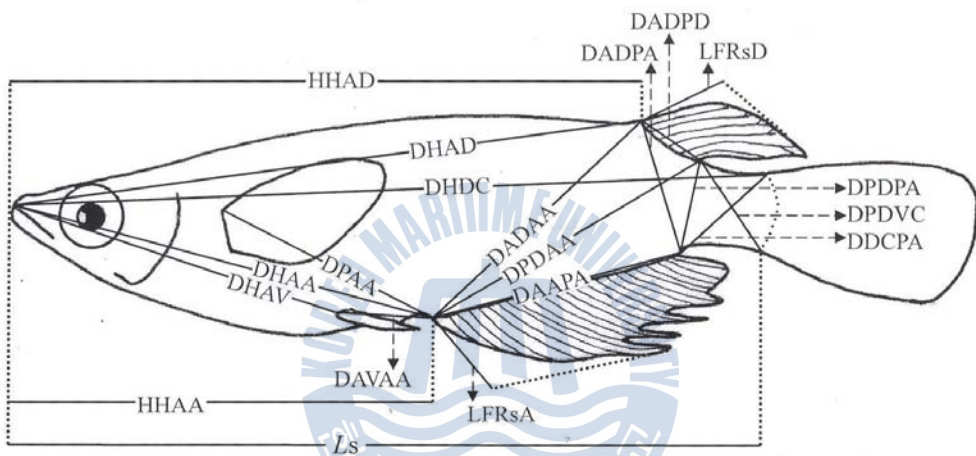
On 10 August 2009, one hundred offspring of the above generation were separated by sex and habituated in 100 L glass aquariums for 3 days. The ratio of males to females was 60:40. The culture water was dechlorinated, and 30% of the water volume in each aquarium was exchanged every day. Brine shrimp, *Artemia sp.*, were collected from the cultured aquarium and provided to the fish every day. Eggs were collected from fish with a standard

length over 25mm. To collect the eggs, 35 male and 15 female marine medakas were placed in each of two aquariums, and 1,000 of their fertilized eggs were collected by net. The fertilized marine medaka eggs were reared in 100 L glass aquariums. The eggs began to hatch after two weeks, and 60 of the newly hatched fry were fixed in 10% neutral formalin.

After hatching, experimental samples were fixed in 10% neutral formalin according to the determined experimental period ( $n=60$ ; 4 day intervals from 40 days after hatching, 10 day intervals from 70 days after hatching and 20 days intervals from 270 days after hatching). To avoid sampling fish with guts that were distended by large quantities of food, fish were starved for 24 h before sampling (Park *et al.*, 2001a).

## **2. Analysis of morphometric dimorphism between sex**

On 24 June 2010, digital pictures were taken for fixed samples of each group using a Nikon D80 camera ( $n=60$ ). A scale bar was inserted within the pictures, which were then printed by laser printer (HP laserjet 1010, Epson, Japan). Using the pictures, standard length measurements were taken to the nearest 0.01 cm using digital vernier calipers (CD-20CP; Mitytoyo, Kawasaki, Japan). Body outline measurements were taken for 19 distances between landmarks for both truss and classical dimensions (Fig. 1, Table 1).



**Fig. 1.** Morphometric measurements between each landmark for the marine medaka *Oryzias dancena* used in this study. For abbreviations, see text.

**Table 1.** Morphometric measurements between each landmark in the marine medaka, *Oryzias dancena* for both truss and classical dimensions

<i>Ls</i>	Standard length
HHAD	Horizontal distance between the most anterior extension of the head and the anterior insertion of the first dorsal fin
HHAA	Horizontal distance between the most anterior extension of the head and the anterior insertion of the first anal fin
DHAD	Direct distance between the most anterior extension of the head and the anterior insertion of the first dorsal fin
DHDC	Direct distance between the most anterior extension of the head and the dorsal base of the caudal fin
DHAA	Direct distance between the most anterior extension of the head and the anterior insertion of the first anal fin
DHAV	Direct distance between the most anterior extension of the head and the anterior insertion of the first ventral fin
DPAA	Direct distance between the dorsal base of the pectoral fin and the anterior insertion of the first anal fin
DADAA	Direct distance between the anterior insertion of the first dorsal fin and the anterior insertion of the first anal fin
DPDAA	Direct distance between the posterior insertion of the last dorsal fin and the anterior insertion of the first anal fin
DAAPA	Direct distance between the anterior insertion of the first anal fin and the posterior insertion of the last anal fin
DADPA	Direct distance between the anterior insertion of the first dorsal fin and the posterior insertion of the last anal fin
DADPD	Direct distance between the anterior insertion of the first dorsal fin and the posterior insertion of the last dorsal fin
DPDPA	Direct distance between the posterior insertion of the last dorsal fin and the posterior insertion of the last anal fin
DPDVC	Direct distance between the posterior insertion of the last dorsal fin and the ventral base of the caudal fin
DDCPA	Direct distance between the dorsal base of the caudal fin and the posterior insertion of the last anal fin
LFRsD	Length of the fin rays of the dorsal fin
LFRsA	Length of the fin rays of the anal fin
DAVAA	Direct distance between the anterior insertion of the first ventral fin and the anterior insertion of the first anal fin

$L_s$ , HHAD, and HHAA indicate horizontal distance measurements, while other distances (DHAD, DHDC, DHAA, DHAV, DPAA, DADAA, etc.) indicate direct distance measurements. On 24 August 2010 and 24 October 2010, the standard length and body outline of samples ( $n=60$ , 330 and 390 days after hatching) were measured using the same method. This study sampled a total of 60 marine medakas, 30 males and 30 females.

Growth was observed between 32 and 390 days after hatching, and standard length, weight and length of characteristics were measured. The von Bertalanffy growth equation employed to describe the growth of marine medakas as follows:  $L_t = L_\infty(1 - e^{-K(t-t_0)})$ . In this equation,  $t$  is the age,  $L_t$  is the expected length at age  $t$  years,  $L_\infty$  is the asymptotic maximum length,  $K$  is the von Bertalanffy growth coefficient and  $t_0$  is the theoretical age at zero length. These parameters were estimated from a non-linear regression using the EXCEL Solver Software.

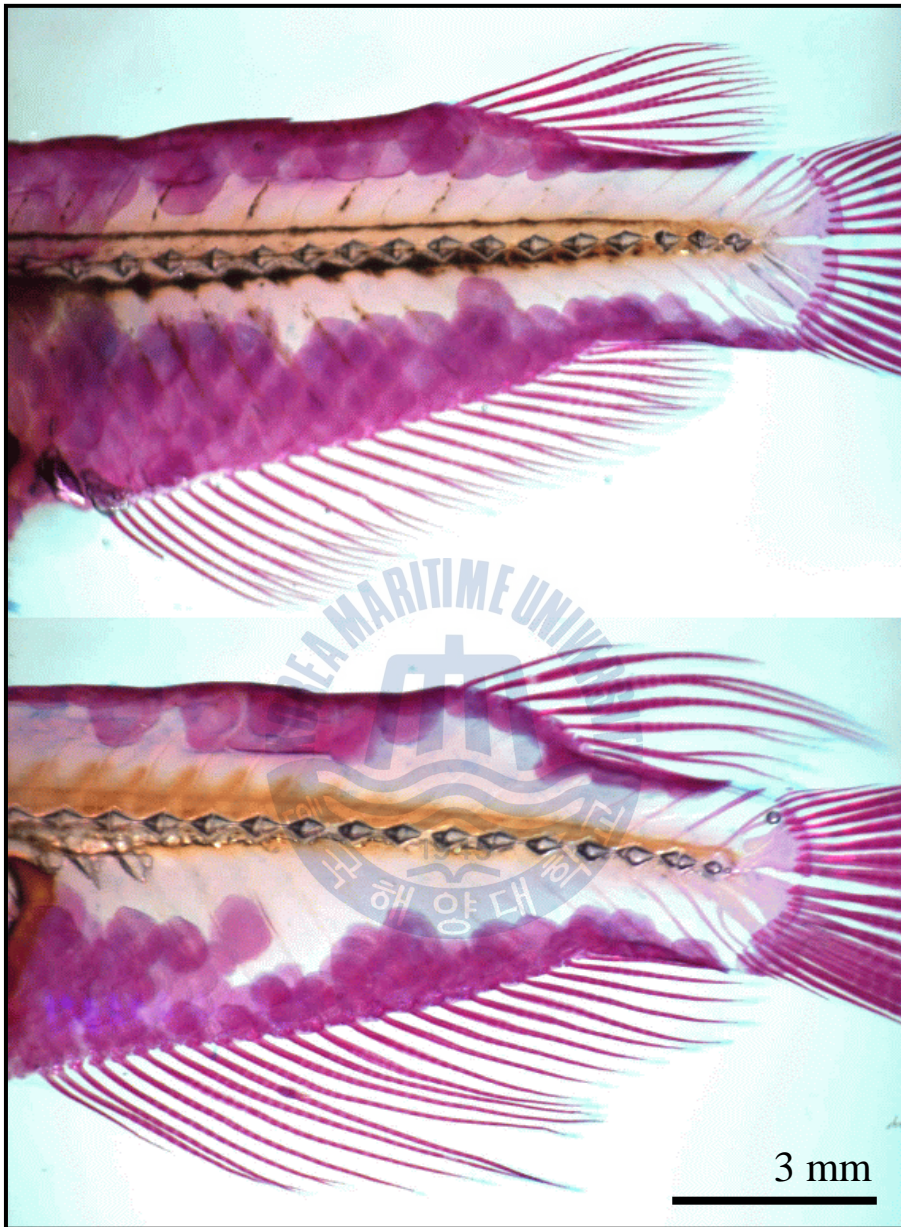
A t-test and an ANCOVA test were both used to determine whether differences between measured male and female parameters were significantly different ( $P < 0.05$ ,  $n=60$ ). The five most significantly different variables were then used for an ANCOVA test ( $n=60$ ) to determine which characteristics were most different between males and females. Differences between the methods were regarded as significant at  $P < 0.05$ .

### 3. Analysis of fin dimorphism between sex

On June 29 2010, anal fin and dorsal fin of fixed samples of each group ( $n = 60$ ) were stained by skeleton staining method (Park *et al.*, 1984). Samples of each group were placed in 5 ml of 0.5% KOH solution and 1-3 ml of a 3% H<sub>2</sub>O<sub>2</sub> solution were added. After 10~20 minutes, all pigmented tissues became transparent. Samples should not be kept in the H<sub>2</sub>O<sub>2</sub> solution longer than necessary and washed the H<sub>2</sub>O<sub>2</sub> solution completely out of the specimens with tap water. This is important because residual H<sub>2</sub>O<sub>2</sub> interferes with staining. Samples were treated with 0.01% alcian blue 8 GX (Sigma, St. Louis, USA) dissolved in 60 ml absolute ethanol and 40 ml glacial acetic acid. Stain for 2 hours. Samples were dehydrated in two changes of absolute ethanol, each 3 hours. Since incomplete dehydration could adversely affect the subsequent alizarine red S staining. Samples were placed in 10 ml of 5% KOH solution to which 5 to 10 drops of 0.1% alizarine red S (Sigma, St. Louis, USA) solution have been added, and samples were stained for 2 hours. Samples were transfer directly to absolute glycerol and change the glycerol every day. Samples were store in 100% glycerol containing small crystals of phenol or thymol to prevent bacterial growth.

On 4 July 2010, stained samples of each group ( $n=60$ , 30 males and 30 females) was take a picture with stereoscopic microscope (Axioskop, Carl

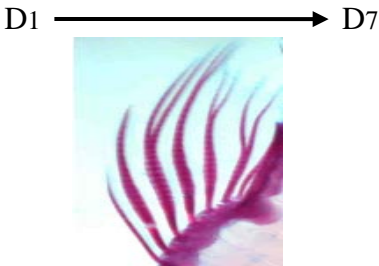

Zeiss, Germany) and microscope camera (Axiocam MR, Carl Zeiss, Germany), and a fin was fixed straight by pincette before take a picture. The pictures were inserted scale bar and printed by laser printer (HP laserjet 1010, Epson, Japan). Fin length measurements were taken for 21 distances of anal fin (A1~A21) and 7 distances of dorsal fin (D1~D7) between beginning and tip (Fig. 2, Table 2). Fin length measurements of pictures were taken to the nearest 0.01 mm using digital vernier calipers (CD-20CP; Mitytoyo, Kawasaki, Japan). All fin length measurements indicates direct distance. On 27 August 2010 and 28 October 2010, the fin length of samples ( $n=60$ , 330 and 390 DAH) from each group were measured using the same method. The differences between males and females were analyzed by t-test ( $P<0.05$ ,  $n=60$ ) using the SPSS statistics package (SPSS 9.0, SPSS Inc., Chicago, IL, USA). Differences between means were regarded as significant at  $P<0.05$ .



**Fig. 2.** Fin length measurements between beginning and tip of the dorsal fin and the anal fin for marine medaka, *Oryzias dancena*. Samples was stained by Alizarine red S. Upper: female, lower: male.



**Table 2.** Fin length measurements between beginning and tip of marine medaka, *Oryzias dancena* for dorsal fin and anal fin

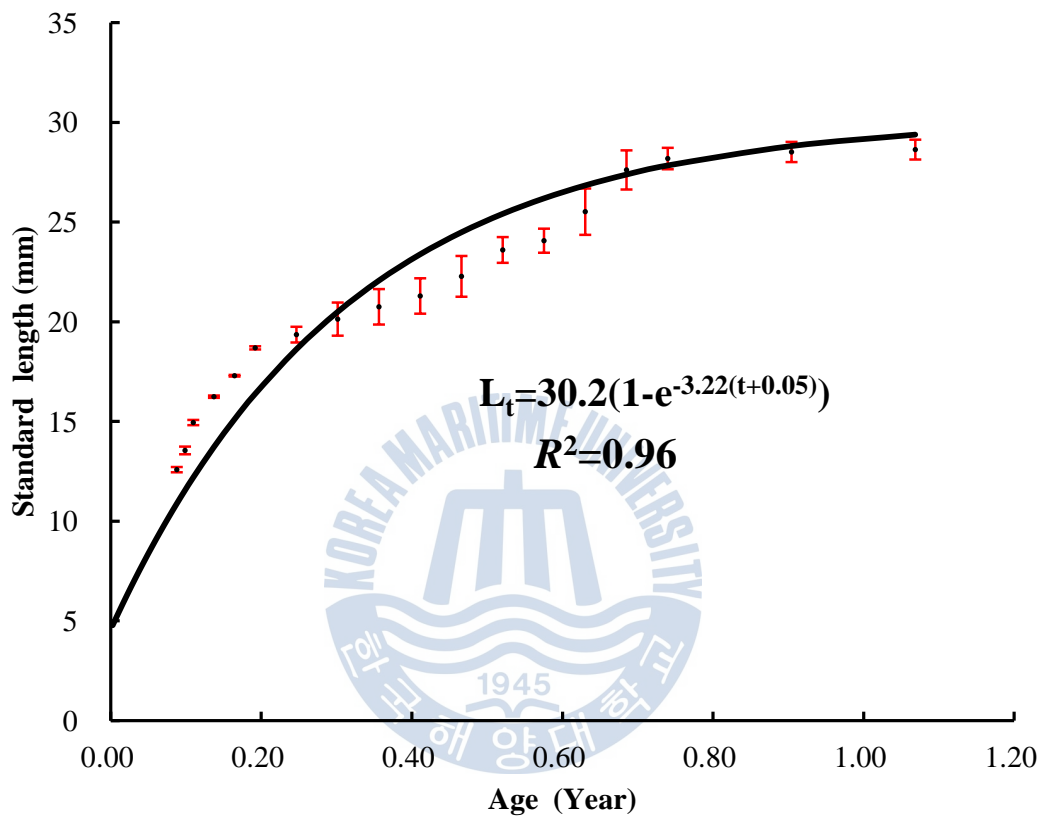
D1	1st fin ray of dorsal fin				
D2	2nd fin ray of dorsal fin				
D3	3rd fin ray of dorsal fin				
D4	4th fin ray of dorsal fin				
D5	5th fin ray of dorsal fin				
D6	6th fin ray of dorsal fin				
D7	7th fin ray of dorsal fin				
					
A1	1st fin ray of anal fin	A8	8th fin ray of anal fin	A15	15th fin ray of anal fin
A2	2nd fin ray of anal fin	A9	9th fin ray of anal fin	A16	16th fin ray of anal fin
A3	3rd fin ray of anal fin	A10	10th fin ray of anal fin	A17	17th fin ray of anal fin
A4	4th fin ray of anal fin	A11	11th fin ray of anal fin	A18	18th fin ray of anal fin
A5	5th fin ray of anal fin	A12	12th fin ray of anal fin	A19	19th fin ray of anal fin
A6	6th fin ray of anal fin	A13	13th fin ray of anal fin	A20	20th fin ray of anal fin
A7	7th fin ray of anal fin	A14	14th fin ray of anal fin	A21	21st fin ray of anal fin

# Results

## 1. Morphometric dimorphism between sex

The von Bertalanffy growth parameters estimated by the non-linear regression method for marine medaka, *Oryzias dancena* are shown in Fig. 3. The von Bertalanffy growth equation was  $L_t = 30.2(1 - e^{-3.22(t-0.05)})$ . The growth coefficient (K) is estimated to be 3.22/year, the asymptotic maximum length ( $L_\infty$ ) is estimated to be 30.2 mm, and the theoretical age at zero length ( $t_0$ ) is estimated to be -0.05. For every measured characteristic, significant differences in growth were found between males and females ( $P < 0.05$ ).

The difference in growth between males and females for all 18 characteristics were analyzed with a t-test, and the results are shown in Table 3. Table 3 shows a difference in the growth between males and females in every characteristic from 70 days after hatching. The ANCOVA test shows differences in 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 (DPDAA), the direct distance between the anterior insertion of the first dorsal fin and the posterior insertion of the last



**Fig. 3.** The von Bertalanffy growth curve by the von Bertalanffy method for the marine medaka, *Oryzias dancena*. Vertical bars indicate one standard deviation.

**Table 3.** Results of the Student's t-test for differences in 18 characteristics between male and female of marine medaka, *Oryzias dancena* by days after hatched

Day	HHAD	HHAA	DHAD	DHDC	DHAA	DHAV	DPAA	DADAA	DPDAA
70	2.22E-12***	7.56E-10***	1.25E-12***	6.34E-12***	6.09E-14***	7.94E-11***	1.83E-10***	1.26E-26***	1.46E-16***
90	3.11E-19***	2.32E-19***	5.70E-18***	1.33E-18***	4.21E-18***	1.23E-19***	2.87E-19***	5.60E-27***	2.63E-21***
110	4.59E-44***	3.57E-46***	2.94E-40***	4.96E-45***	4.16E-39***	2.79E-37***	1.33E-43***	2.09E-33***	3.41E-43***
130	1.13E-43***	2.40E-42***	8.13E-45***	4.79E-44***	2.89E-42***	1.05E-40***	2.57E-41***	2.38E-44***	6.85E-45***
150	4.33E-42***	1.20E-46***	3.58E-47***	2.58E-48***	1.70E-46***	1.15E-42***	1.28E-42***	4.24E-42***	6.32E-53***
170	8.06E-38***	4.69E-38***	1.17E-37***	9.98E-40***	6.19E-40***	9.63E-38***	1.32E-39***	4.65E-35***	2.05E-42***
190	1.93E-35***	1.32E-34***	1.64E-32***	1.35E-34***	2.13E-33***	2.40E-29***	2.94E-31***	2.21E-40***	8.82E-42***
210	6.48E-37***	3.25E-36***	1.23E-33***	5.45E-36***	3.65E-26***	3.03E-30***	1.66E-32***	9.00E-49***	1.40E-41***
230	6.61E-43***	1.56E-41***	4.36E-38***	4.57E-42***	5.23E-38***	1.86E-39***	7.61E-41***	7.34E-44***	3.29E-49***
250	1.30E-34***	4.35E-33***	1.74E-31***	9.14E-37***	3.14E-35***	1.65E-37***	3.70E-34***	1.40E-45***	1.69E-41***
270	1.29E-26***	7.99E-31***	6.31E-33***	2.22E-37***	4.76E-32***	4.86E-34***	5.92E-29***	3.00E-49***	1.12E-47***
330	2.27E-32***	2.70E-40***	9.31E-30***	1.98E-40***	4.05E-33***	2.78E-27***	3.35E-36***	5.71E-52***	1.43E-45***
390	2.62E-31***	2.66E-39***	9.78E-31***	1.91E-37***	3.09E-32***	4.90E-26***	6.90E-34***	1.56E-49***	5.19E-46***

For abbreviations see text. The sample numbers of males and females are 30 and 30, respectively. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; NS, not significant.

**Table 3. Extended**

Day	DAAPA	DADPA	DADPD	DPDPA	DPDVC	DDCPA	LFrsD	LFrsA	DAVAA
70	1.70E-12***	6.38E-06***	1.52E-05***	0.0141***	5.01E-05***	0.0006***	1.06E-18***	2.36E-08***	7.95E-05***
90	8.79E-18***	7.15E-18***	5.52E-15***	5.66E-15***	1.15E-16***	4.01E-14***	4.24E-24***	5.33E-14***	1.20E-16***
110	2.18E-45***	8.39E-30***	4.16E-31***	1.68E-26***	1.51E-27***	2.44E-26***	6.34E-47***	5.08E-27***	6.13E-28***
130	3.09E-39***	7.47E-38***	1.19E-26***	1.84E-24***	9.81E-33***	4.37E-25***	1.37E-49***	2.08E-27***	2.94E-34***
150	1.25E-44***	3.40E-40***	3.59E-27***	5.93E-33***	2.05E-35***	9.96E-37***	2.89E-41***	4.96E-27***	1.36E-37***
170	7.11E-32***	5.47E-29***	1.30E-27***	1.30E-32***	1.80E-26***	1.73E-33***	1.26E-37***	4.54E-24***	7.70E-27***
190	1.95E-27***	1.30E-38***	2.07E-23***	8.98E-27***	1.17E-28***	1.47E-29***	3.43E-33***	4.23E-27***	1.24E-28***
210	1.06E-28***	3.43E-30***	1.94E-23***	4.92E-25***	1.36E-29***	2.35E-26***	5.73E-25***	2.00E-34***	4.84E-28***
230	5.39E-34***	3.76E-33***	4.55E-33***	3.27E-34***	9.11E-34***	3.46E-34***	2.24E-28***	3.67E-34***	1.89E-33***
250	5.93E-30***	7.48E-32***	1.13E-29***	7.24E-30***	2.10E-22***	2.55E-31***	6.32E-28***	3.65E-36***	5.78E-23***
270	1.33E-27***	2.49E-29***	1.30E-18***	1.77E-17***	1.12E-24***	8.57E-18***	2.90E-26***	5.50E-33***	1.27E-24***
330	1.70E-30***	7.90E-30***	3.38E-28***	1.92E-18***	2.34E-29***	1.92E-18***	3.30E-26***	4.58E-34***	1.07E-24***
390	7.80E-30***	2.73E-29***	4.48E-27***	1.02E-17***	8.79E-28***	3.86E-18***	1.28E-26***	4.68E-35***	5.23E-23***

anal fin (DADPA), the length of the fin rays of the dorsal fin (LFRsD) and the length of the fin rays of the anal fin (LFRsA). The results of the covariance analysis can be found in Table 4.

Five measured characteristics showed significant differences between sexes ( $P < 0.05$ ). When relating the characteristics to the standard length of the fishes, males had significantly larger measurements than females for all five characteristics (Figs. 3, 4, 5, 6 and 7). For DADAA, the difference in the slope of the equation between males and females was larger than in the slopes for DPDAA or DADPA. In other words, between the three characteristics, DADAA had most the significant difference between males and females. For LFRsA, the difference in the slope of the equation between males and females was larger than those in LFRsD ( $P < 0.05$ ), with both dorsal fin and anal fin lengths being longer in males than females. Naked eye observations also confirmed these findings (Fig. 8).

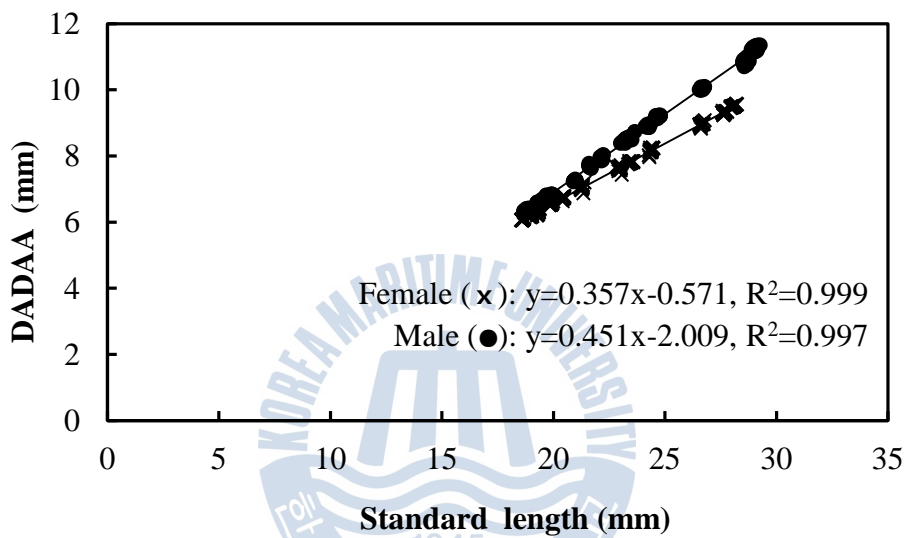
## **2. Fin dimorphism between sex**

The difference in each fin ray length of male and female dorsal fin is shown in Table 5. There were all difference from dorsal fin 1 (D1) to D7 for all fin rays from males and females and there is significant difference in 70 days after hatched (DAH) when the sexual dimorphism is presented.

**Table 4.** Test for differences of regression coefficients between males and females by 5 characteristics of the marine medaka, *Oryzias dancena*

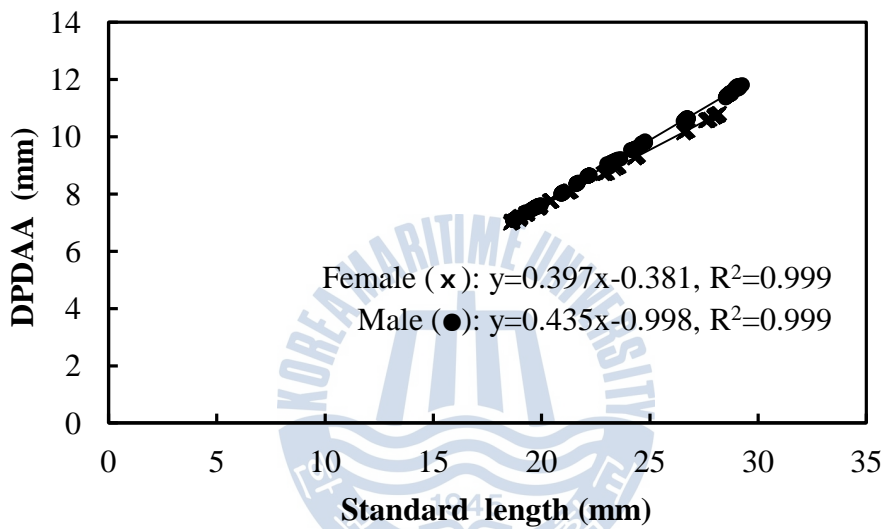
Statistics	DADAA	DPDAA	DADPA	LFRsD	LFRsA
$b_m$	0.3705	0.3953	0.1743	0.1023	0.1177
$b_f$	0.3335	0.3811	0.1621	0.0866	0.0961
residual $SS_m$	54.54	13.35	29.30	18.27	37.62
residual $SS_f$	4.07	0.73	3.20	6.26	2.69
residual $DF_m$	538	538	538	538	538
residual $DF_f$	538	538	538	538	538
$s_{m-f}$	0.0007	0.0003	0.0005	0.0005	0.0006
t	53.0692	41.4923	23.6211	34.7538	37.2436
v	1076	1076	1076	1076	1076
$t_{0.05(2),1076}$	1.9600	1.9600	1.9600	1.9600	1.9600
P	<0.05	<0.05	<0.05	<0.05	<0.05

For abbreviations, see text.

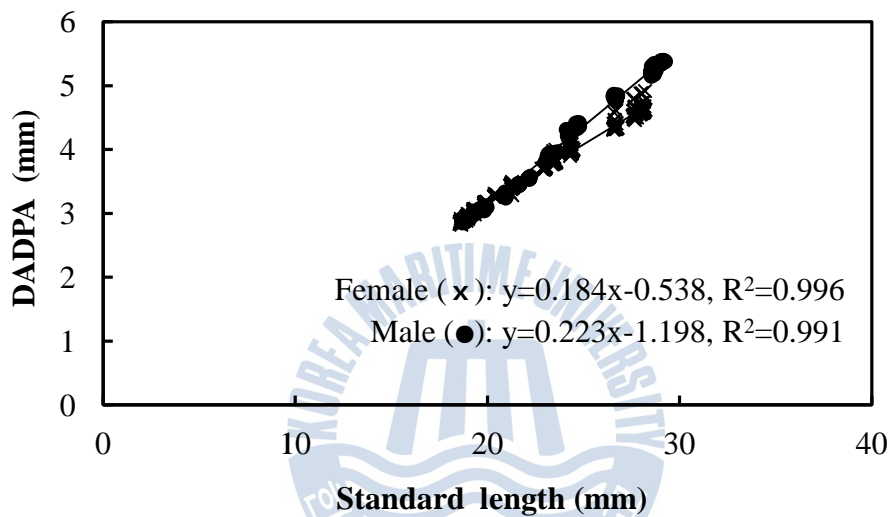


**Fig. 4.** The relationship between standard length and direct distance between the anterior insertion of the first dorsal fin and the anterior insertion of the first anal fin (DADAA) of the female (x) and male (●) marine medaka, *Oryzias dancena*.

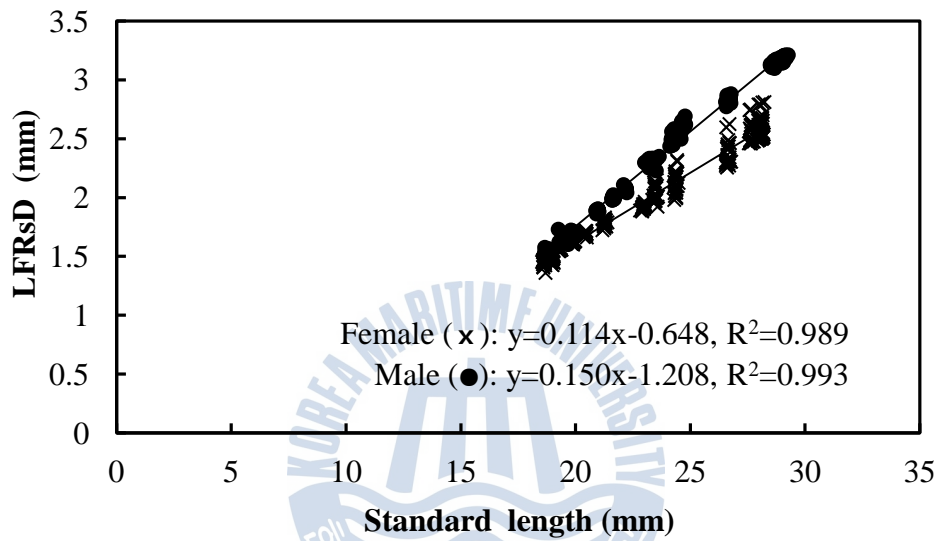




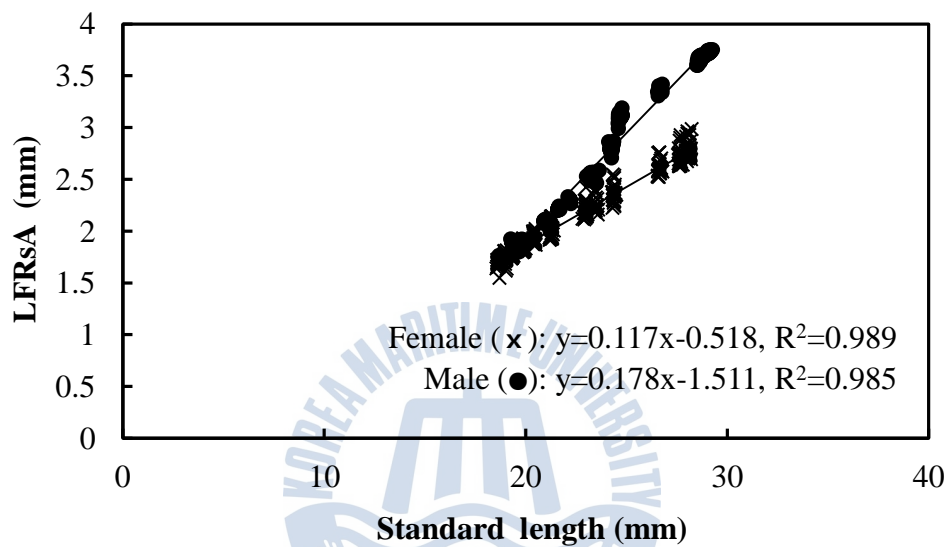
**Fig. 5.** The relationship between standard length and direct distance between the posterior insertion of the last dorsal fin and the anterior insertion of the first anal fin (DPDAA) of the female (x) and male (●) marine medaka, *Oryzias dancena*.



**Fig. 6.** The relationship between standard length and direct distance between the anterior insertion of the first dorsal fin and the posterior insertion of the last anal fin (DADPA) of the female (x) and male (●) marine medaka, *Oryzias dancena*.



**Fig. 7.** The relationship between standard length and length of the fin rays of the dorsal fin (LFRsD) of the female (x) and male (●) marine medaka, *Oryzias dancena*.



**Fig. 8.** The relationship between standard length and length of the fin rays of the anal fin (LFRsA) of the female (x) and male (●) marine medaka, *Oryzias dancena*.



**Fig. 9.** External morphology of the marine medaka, *Oryzias dancena*. Samples in this picture had grown 270 days after hatching. Upper: male; lower: female.

**Table 5.** Results of Student's t-test for differences in 7 dorsal fin distance between male(♂) and female(♀) of marine medaka, *Oryzias dancena* by days after hatched

Days after hatched	32	36	40	50	60	70	90	110	130
D1 (♂)	1.06±0.33 <sup>a</sup>	1.12±0.51 <sup>a</sup>	1.17±0.24 <sup>a</sup>	1.47±0.51 <sup>a</sup>	1.77±0.33 <sup>a</sup>	1.80±0.43 <sup>a</sup>	1.91±0.52 <sup>a</sup>	2.04±0.43 <sup>a</sup>	2.17±0.31 <sup>a</sup>
D1 (♀)	1.02±0.48 <sup>b</sup>	1.09±0.43 <sup>b</sup>	1.14±0.34 <sup>b</sup>	1.38±0.49 <sup>b</sup>	1.58±0.48 <sup>b</sup>	1.60±0.42 <sup>b</sup>	1.63±0.61 <sup>b</sup>	1.81±0.49 <sup>b</sup>	1.92±0.48 <sup>b</sup>
D2 (♂)	1.44±0.28 <sup>a</sup>	1.51±0.55 <sup>a</sup>	1.56±0.33 <sup>a</sup>	2.09±0.58 <sup>a</sup>	2.56±0.51 <sup>a</sup>	2.66±0.46 <sup>a</sup>	2.88±0.64 <sup>a</sup>	3.04±0.44 <sup>a</sup>	3.26±0.55 <sup>a</sup>
D2 (♀)	1.44±0.49 <sup>b</sup>	1.51±0.57 <sup>b</sup>	1.54±0.36 <sup>b</sup>	1.84±0.59 <sup>b</sup>	2.20±0.55 <sup>b</sup>	2.26±0.51 <sup>b</sup>	2.31±0.66 <sup>b</sup>	2.72±0.55 <sup>b</sup>	2.73±0.54 <sup>b</sup>
D3 (♂)	1.43±0.56 <sup>a</sup>	1.60±0.49 <sup>a</sup>	1.64±0.38 <sup>a</sup>	2.34±0.48 <sup>a</sup>	2.73±0.39 <sup>a</sup>	3.01±0.53 <sup>a</sup>	3.22±0.51 <sup>a</sup>	3.42±0.59 <sup>a</sup>	3.54±0.53 <sup>a</sup>
D3 (♀)	1.43±0.59 <sup>b</sup>	1.60±0.44 <sup>b</sup>	1.63±0.33 <sup>b</sup>	1.94±0.51 <sup>b</sup>	2.20±0.47 <sup>b</sup>	2.23±0.55 <sup>b</sup>	2.57±0.58 <sup>b</sup>	2.77±0.55 <sup>b</sup>	2.80±0.59 <sup>b</sup>
D4 (♂)	1.46±0.51 <sup>a</sup>	1.60±0.47 <sup>a</sup>	1.60±0.29 <sup>a</sup>	2.22±0.47 <sup>a</sup>	2.61±0.44 <sup>a</sup>	2.72±0.59 <sup>a</sup>	2.90±0.49 <sup>a</sup>	3.15±0.56 <sup>a</sup>	3.40±0.69 <sup>a</sup>
D4 (♀)	1.38±0.44 <sup>b</sup>	1.44±0.38 <sup>b</sup>	1.48±0.40 <sup>b</sup>	1.79±0.42 <sup>b</sup>	2.11±0.43 <sup>b</sup>	2.13±0.54 <sup>b</sup>	2.19±0.46 <sup>b</sup>	2.58±0.48 <sup>b</sup>	2.61±0.61 <sup>b</sup>
D5 (♂)	1.18±0.39 <sup>a</sup>	1.34±0.31 <sup>a</sup>	1.38±0.30 <sup>a</sup>	1.76±0.44 <sup>a</sup>	2.03±0.46 <sup>a</sup>	2.16±0.57 <sup>a</sup>	2.30±0.45 <sup>a</sup>	2.41±0.61 <sup>a</sup>	2.49±0.48 <sup>a</sup>
D5 (♀)	1.17±0.58 <sup>b</sup>	1.22±0.34 <sup>b</sup>	1.25±0.35 <sup>b</sup>	1.47±0.48 <sup>b</sup>	1.75±0.49 <sup>b</sup>	1.78±0.49 <sup>b</sup>	1.84±0.44 <sup>b</sup>	2.16±0.54 <sup>b</sup>	2.19±0.43 <sup>b</sup>
D6 (♂)	0.69±0.10 <sup>a</sup>	1.02±0.23 <sup>a</sup>	1.07±0.34 <sup>a</sup>	1.34±0.38 <sup>a</sup>	1.66±0.51 <sup>a</sup>	1.70±0.61 <sup>a</sup>	1.87±0.55 <sup>a</sup>	1.96±0.68 <sup>a</sup>	2.15±0.44 <sup>a</sup>
D6 (♀)	0.69±0.11 <sup>b</sup>	0.95±0.19 <sup>b</sup>	0.96±0.21 <sup>b</sup>	1.20±0.28 <sup>b</sup>	1.44±0.55 <sup>b</sup>	1.45±0.66 <sup>b</sup>	1.47±0.55 <sup>b</sup>	1.78±0.66 <sup>b</sup>	1.84±0.47 <sup>b</sup>
D7 (♂)	0.48±0.11 <sup>a</sup>	0.66±0.12 <sup>a</sup>	0.74±0.20 <sup>a</sup>	0.95±0.24 <sup>a</sup>	1.34±0.49 <sup>a</sup>	1.43±0.41 <sup>a</sup>	1.51±0.59 <sup>a</sup>	1.60±0.49 <sup>a</sup>	1.69±0.48 <sup>a</sup>
D7 (♀)	0.48±0.08 <sup>b</sup>	0.66±0.14 <sup>b</sup>	0.74±0.25 <sup>b</sup>	1.06±0.23 <sup>b</sup>	1.16±0.32 <sup>b</sup>	1.25±0.52 <sup>b</sup>	1.32±0.43 <sup>b</sup>	1.32±0.46 <sup>b</sup>	1.34±0.43 <sup>b</sup>

For abbreviations see table 2. The sample numbers of males and females are 30 and 30, respectively.  $P < 0.05$ .

**Table 5. Extended**

Days after hatched	150	170	190	210	230	250	270	330	390
D1 (♂)	2.17±0.43 <sup>a</sup>	2.23±0.54 <sup>a</sup>	2.48±0.47 <sup>a</sup>	2.68±0.61 <sup>a</sup>	2.85±0.72 <sup>a</sup>	3.16±0.72 <sup>a</sup>	3.24±0.70 <sup>a</sup>	3.25±0.70 <sup>a</sup>	3.26±0.51 <sup>a</sup>
D1 (♀)	2.00±0.48 <sup>b</sup>	2.01±0.58 <sup>b</sup>	2.18±0.49 <sup>b</sup>	2.24±0.64 <sup>b</sup>	2.53±0.61 <sup>b</sup>	2.57±0.69 <sup>b</sup>	2.62±0.77 <sup>b</sup>	2.70±0.86 <sup>b</sup>	2.72±0.55 <sup>b</sup>
D2 (♂)	3.27±0.67 <sup>a</sup>	3.28±0.60 <sup>a</sup>	3.69±0.52 <sup>a</sup>	3.87±0.59 <sup>a</sup>	4.27±0.58 <sup>a</sup>	4.75±0.78 <sup>a</sup>	4.80±0.67 <sup>a</sup>	4.85±0.72 <sup>a</sup>	4.87±0.69 <sup>a</sup>
D2 (♀)	2.85±0.62 <sup>b</sup>	2.94±0.50 <sup>b</sup>	3.13±0.53 <sup>b</sup>	3.21±0.58 <sup>b</sup>	3.63±0.51 <sup>b</sup>	3.86±0.64 <sup>b</sup>	4.07±0.51 <sup>b</sup>	4.08±0.58 <sup>b</sup>	4.11±0.78 <sup>b</sup>
D3 (♂)	3.65±0.51 <sup>a</sup>	3.98±0.58 <sup>a</sup>	4.56±0.66 <sup>a</sup>	4.73±0.69 <sup>a</sup>	5.07±0.59 <sup>a</sup>	5.71±0.66 <sup>a</sup>	5.86±0.79 <sup>a</sup>	6.26±0.72 <sup>a</sup>	6.26±0.80 <sup>a</sup>
D3 (♀)	2.90±0.53 <sup>b</sup>	2.96±0.40 <sup>b</sup>	3.17±0.56 <sup>b</sup>	3.23±0.48 <sup>b</sup>	3.64±0.76 <sup>b</sup>	3.76±0.77 <sup>b</sup>	4.01±0.80 <sup>b</sup>	4.61±0.79 <sup>b</sup>	4.61±0.89 <sup>b</sup>
D4 (♂)	3.48±0.48 <sup>a</sup>	3.48±0.49 <sup>a</sup>	4.05±0.60 <sup>a</sup>	4.21±0.58 <sup>a</sup>	4.73±0.71 <sup>a</sup>	5.26±0.51 <sup>a</sup>	5.70±0.70 <sup>a</sup>	5.86±0.74 <sup>a</sup>	5.87±0.78 <sup>a</sup>
D4 (♀)	2.65±0.55 <sup>b</sup>	2.80±0.52 <sup>b</sup>	2.92±0.61 <sup>b</sup>	3.04±0.55 <sup>b</sup>	3.43±0.77 <sup>b</sup>	3.56±0.78 <sup>b</sup>	4.10±0.60 <sup>b</sup>	4.40±0.79 <sup>b</sup>	4.40±0.90 <sup>b</sup>
D5 (♂)	2.52±0.43 <sup>a</sup>	2.55±0.57 <sup>a</sup>	2.95±0.49 <sup>a</sup>	3.36±0.57 <sup>a</sup>	3.51±0.72 <sup>a</sup>	3.87±0.59 <sup>a</sup>	4.15±0.68 <sup>a</sup>	4.28±0.73 <sup>a</sup>	4.29±0.88 <sup>a</sup>
D5 (♀)	2.29±0.65 <sup>b</sup>	2.31±0.39 <sup>b</sup>	2.53±0.51 <sup>b</sup>	2.68±0.69 <sup>b</sup>	3.02±0.41 <sup>b</sup>	3.04±0.80 <sup>b</sup>	3.18±0.66 <sup>b</sup>	3.26±0.62 <sup>b</sup>	3.27±0.77 <sup>b</sup>
D6 (♂)	2.15±0.48 <sup>a</sup>	2.16±0.51 <sup>a</sup>	2.53±0.55 <sup>a</sup>	2.72±0.61 <sup>a</sup>	2.93±0.68 <sup>a</sup>	3.23±0.78 <sup>a</sup>	3.26±0.79 <sup>a</sup>	3.37±0.78 <sup>a</sup>	3.38±0.76 <sup>a</sup>
D6 (♀)	1.87±0.61 <sup>b</sup>	1.90±0.54 <sup>b</sup>	2.06±0.56 <sup>b</sup>	2.11±0.70 <sup>b</sup>	2.38±0.79 <sup>b</sup>	2.48±0.87 <sup>b</sup>	2.61±0.73 <sup>b</sup>	2.83±0.62 <sup>b</sup>	2.84±0.71 <sup>b</sup>
D7 (♂)	1.70±0.44 <sup>a</sup>	1.76±0.55 <sup>a</sup>	1.99±0.55 <sup>a</sup>	2.07±0.68 <sup>a</sup>	2.44±0.53 <sup>a</sup>	2.68±0.79 <sup>a</sup>	2.85±0.74 <sup>a</sup>	2.95±0.64 <sup>a</sup>	2.96±0.79 <sup>a</sup>
D7 (♀)	1.42±0.47 <sup>b</sup>	1.48±0.51 <sup>b</sup>	1.59±0.50 <sup>b</sup>	1.61±0.51 <sup>b</sup>	1.74±0.76 <sup>b</sup>	1.92±0.75 <sup>b</sup>	2.07±0.51 <sup>b</sup>	2.21±0.87 <sup>b</sup>	2.21±0.77 <sup>b</sup>

The significant difference in fin ray for male and female was more greatly seen as they grow. The consistent growth until 70 DAH from the fin rays of all dorsal fins then the rapid growth since 170 DAH and the flat growth since 250 DAH were seen. The difference of each fin ray from anal fin of male and female is seen in Tables 6 and 7. There was difference for male and female in all fin rays from anal fin 1 (A1) to A22. There was a significant difference since 70 DAH when the sexual dimorphism appears and they become more significant difference as they grow ( $P<0.05$ ). The consistent growth until 70 DAH from the fin rays of all dorsal fin then the rapid growth since 170 DAH and the flat growth since 210 DAH were seen.

The separation which the anal and dorsal fins of fin rays becomes two fin rays was seen from both male and female and the trend of separation had differences in male and female which is presented in Tables 8 and 9. For female, all fin rays of dorsal fin since 70 DAH showed separation and the separated fin rays gradually grew as time flows ( $P<0.05$ ). For male, they did not show separation until the 190 DAH and it was witnessed on 190 DAH and after for A1-4 and A19-21 fin rays. The separated fin rays grew as time flow ( $P<0.05$ ).



**Table 6.** Results of Student's t-test for differences in A1~A10 fin distance of anal fin between male (♂) and female (♀) of marine medaka, *Oryzias dancena* by days after hatched

Days after hatched	32	36	40	50	60	70	90	110	130
A1 (♂)	0.81±0.11 <sup>a</sup>	0.96±0.24 <sup>a</sup>	0.97±0.21 <sup>a</sup>	1.18±0.31 <sup>a</sup>	1.40±0.52 <sup>a</sup>	1.48±0.57 <sup>a</sup>	1.57±0.55 <sup>a</sup>	1.66±0.70 <sup>a</sup>	1.71±0.81 <sup>a</sup>
A1 (♀)	0.74±0.15 <sup>b</sup>	0.83±0.28 <sup>b</sup>	0.83±0.22 <sup>b</sup>	1.01±0.35 <sup>b</sup>	1.24±0.57 <sup>b</sup>	1.25±0.58 <sup>b</sup>	1.26±0.60 <sup>b</sup>	1.50±0.50 <sup>b</sup>	1.50±0.82 <sup>b</sup>
A2 (♂)	1.50±0.28 <sup>a</sup>	1.57±0.35 <sup>a</sup>	1.59±0.25 <sup>a</sup>	1.88±0.47 <sup>a</sup>	2.23±0.59 <sup>a</sup>	2.29±0.59 <sup>a</sup>	2.44±0.65 <sup>a</sup>	2.60±0.55 <sup>a</sup>	2.78±0.85 <sup>a</sup>
A2 (♀)	1.33±0.34 <sup>b</sup>	1.38±0.39 <sup>b</sup>	1.41±0.54 <sup>b</sup>	1.71±0.55 <sup>b</sup>	1.96±0.54 <sup>b</sup>	1.97±0.58 <sup>b</sup>	2.01±0.48 <sup>b</sup>	2.37±0.54 <sup>b</sup>	2.43±0.74 <sup>b</sup>
A3 (♂)	1.63±0.31 <sup>a</sup>	1.70±0.36 <sup>a</sup>	1.75±0.51 <sup>a</sup>	2.10±0.52 <sup>a</sup>	2.42±0.55 <sup>a</sup>	2.53±0.52 <sup>a</sup>	2.70±0.69 <sup>a</sup>	2.85±0.74 <sup>a</sup>	3.02±0.78 <sup>a</sup>
A3 (♀)	1.40±0.45 <sup>b</sup>	1.46±0.37 <sup>b</sup>	1.50±0.43 <sup>b</sup>	1.80±0.51 <sup>b</sup>	2.05±0.60 <sup>b</sup>	2.08±0.61 <sup>b</sup>	2.11±0.66 <sup>b</sup>	2.51±0.48 <sup>b</sup>	2.54±0.86 <sup>b</sup>
A4 (♂)	1.64±0.35 <sup>a</sup>	1.75±0.33 <sup>a</sup>	1.80±0.39 <sup>a</sup>	2.26±0.34 <sup>a</sup>	2.61±0.61 <sup>a</sup>	2.71±0.62 <sup>a</sup>	2.88±0.66 <sup>a</sup>	3.02±0.82 <sup>a</sup>	3.23±0.85 <sup>a</sup>
A4 (♀)	1.37±0.42 <sup>b</sup>	1.56±0.27 <sup>b</sup>	1.57±0.34 <sup>b</sup>	1.87±0.49 <sup>b</sup>	2.12±0.66 <sup>b</sup>	2.16±0.63 <sup>b</sup>	2.20±0.64 <sup>b</sup>	2.58±0.58 <sup>b</sup>	2.61±0.82 <sup>b</sup>
A5 (♂)	1.72±0.47 <sup>a</sup>	1.88±0.34 <sup>a</sup>	1.91±0.33 <sup>a</sup>	2.27±0.39 <sup>a</sup>	2.66±0.47 <sup>a</sup>	2.93±0.68 <sup>a</sup>	3.15±0.81 <sup>a</sup>	3.34±0.87 <sup>a</sup>	3.64±0.73 <sup>a</sup>
A5 (♀)	1.38±0.52 <sup>b</sup>	1.52±0.44 <sup>b</sup>	1.57±0.45 <sup>ba</sup>	1.90±0.44 <sup>b</sup>	2.14±0.68 <sup>b</sup>	2.19±0.66 <sup>b</sup>	2.22±0.74 <sup>b</sup>	2.60±0.48 <sup>b</sup>	2.64±0.89 <sup>b</sup>
A6 (♂)	1.55±0.53 <sup>a</sup>	1.81±0.40 <sup>a</sup>	1.96±0.41 <sup>a</sup>	2.32±0.47 <sup>a</sup>	2.90±0.61 <sup>a</sup>	3.05±0.69 <sup>a</sup>	3.38±0.71 <sup>a</sup>	3.74±0.87 <sup>a</sup>	4.01±0.80 <sup>a</sup>
A6 (♀)	1.33±0.48 <sup>b</sup>	1.44±0.42 <sup>b</sup>	1.52±0.39 <sup>b</sup>	1.83±0.43 <sup>b</sup>	2.11±0.67 <sup>b</sup>	2.12±0.61 <sup>b</sup>	2.15±0.67 <sup>b</sup>	2.58±0.88 <sup>b</sup>	2.61±0.79 <sup>b</sup>
A7 (♂)	1.55±0.39 <sup>a</sup>	1.79±0.49 <sup>a</sup>	1.85±0.28 <sup>a</sup>	2.29±0.38 <sup>a</sup>	2.85±0.48 <sup>a</sup>	3.32±0.65 <sup>a</sup>	3.78±0.48 <sup>a</sup>	4.17±0.77 <sup>a</sup>	4.45±0.75 <sup>a</sup>
A7 (♀)	1.25±0.54 <sup>b</sup>	1.35±0.41 <sup>b</sup>	1.38±0.39 <sup>b</sup>	1.69±0.49 <sup>b</sup>	1.96±0.62 <sup>b</sup>	1.97±0.64 <sup>b</sup>	1.99±0.35 <sup>b</sup>	2.40±0.66 <sup>b</sup>	2.48±0.78 <sup>b</sup>
A8 (♂)	1.53±0.24 <sup>a</sup>	1.78±0.47 <sup>a</sup>	1.80±0.37 <sup>a</sup>	2.19±0.42 <sup>a</sup>	2.81±0.43 <sup>a</sup>	3.37±0.48 <sup>a</sup>	3.68±0.41 <sup>a</sup>	4.00±0.64 <sup>a</sup>	4.56±0.47 <sup>a</sup>
A8 (♀)	1.17±0.35 <sup>b</sup>	1.27±0.33 <sup>b</sup>	1.32±0.34 <sup>b</sup>	1.58±0.45 <sup>b</sup>	1.81±0.48 <sup>b</sup>	1.82±0.58 <sup>b</sup>	1.86±0.51 <sup>b</sup>	2.26±0.62 <sup>b</sup>	2.36±0.71 <sup>b</sup>
A9 (♂)	1.45±0.30 <sup>a</sup>	1.65±0.31 <sup>a</sup>	1.73±0.54 <sup>a</sup>	2.05±0.47 <sup>a</sup>	2.79±0.42 <sup>a</sup>	2.97±0.55 <sup>a</sup>	3.15±0.61 <sup>a</sup>	3.53±0.67 <sup>a</sup>	3.85±0.55 <sup>a</sup>
A9 (♀)	1.12±0.33 <sup>b</sup>	1.17±0.42 <sup>b</sup>	1.21±0.35 <sup>b</sup>	1.46±0.49 <sup>b</sup>	1.73±0.55 <sup>b</sup>	1.73±0.58 <sup>b</sup>	1.75±0.52 <sup>b</sup>	2.08±0.62 <sup>b</sup>	2.20±0.43 <sup>b</sup>
A10 (♂)	1.37±0.28 <sup>a</sup>	1.50±0.45 <sup>a</sup>	1.64±0.60 <sup>a</sup>	1.96±0.55 <sup>a</sup>	2.39±0.48 <sup>a</sup>	2.48±0.59 <sup>a</sup>	2.87±0.58 <sup>a</sup>	3.18±0.63 <sup>a</sup>	3.48±0.48 <sup>a</sup>
A10 (♀)	1.11±0.38 <sup>b</sup>	1.15±0.49 <sup>b</sup>	1.19±0.38 <sup>b</sup>	1.46±0.50 <sup>b</sup>	1.68±0.59 <sup>b</sup>	1.69±0.51 <sup>b</sup>	1.74±0.58 <sup>b</sup>	2.06±0.66 <sup>b</sup>	2.18±0.54 <sup>b</sup>

For abbreviations see table 2. The sample numbers of males and females are 30 and 30, respectively.  $P < 0.05$ .

**Table 6.** Extended

Days after hatched	150	170	190	210	230	250	270	330	390
A1 (♂)	1.75±0.48 <sup>a</sup>	1.76±0.88 <sup>a</sup>	1.96±0.84 <sup>a</sup>	2.04±0.97 <sup>a</sup>	2.28±0.85 <sup>a</sup>	2.52±0.78 <sup>a</sup>	2.53±0.78 <sup>a</sup>	2.54±0.91 <sup>a</sup>	2.54±0.99 <sup>a</sup>
A1 (♀)	1.54±0.58 <sup>b</sup>	1.60±0.78 <sup>b</sup>	1.75±0.86 <sup>b</sup>	1.87±0.84 <sup>b</sup>	2.11±0.91 <sup>b</sup>	2.18±0.87 <sup>b</sup>	2.28±0.85 <sup>b</sup>	2.29±0.82 <sup>b</sup>	2.29±0.84 <sup>b</sup>
A2 (♂)	2.78±0.84 <sup>a</sup>	2.79±0.79 <sup>a</sup>	3.14±0.83 <sup>a</sup>	3.31±0.78 <sup>a</sup>	3.46±0.87 <sup>a</sup>	3.66±0.95 <sup>a</sup>	3.74±0.77 <sup>a</sup>	3.80±0.75 <sup>a</sup>	3.81±0.92 <sup>a</sup>
A2 (♀)	2.48±0.87 <sup>b</sup>	2.52±0.94 <sup>ba</sup>	2.71±0.81 <sup>b</sup>	2.86±0.94 <sup>b</sup>	2.95±0.83 <sup>b</sup>	3.14±0.86 <sup>b</sup>	3.20±0.89 <sup>b</sup>	3.22±0.79 <sup>b</sup>	3.23±0.89 <sup>b</sup>
A3 (♂)	3.02±0.89 <sup>a</sup>	3.06±0.87 <sup>a</sup>	3.50±0.84 <sup>a</sup>	3.73±0.77 <sup>a</sup>	4.04±0.85 <sup>a</sup>	4.65±0.87 <sup>a</sup>	4.74±0.97 <sup>a</sup>	4.81±0.77 <sup>a</sup>	4.81±0.94 <sup>a</sup>
A3 (♀)	2.63±0.90 <sup>b</sup>	2.67±0.78 <sup>b</sup>	2.89±0.89 <sup>b</sup>	3.02±0.89 <sup>b</sup>	3.54±0.77 <sup>b</sup>	3.62±0.94 <sup>b</sup>	3.82±0.91 <sup>b</sup>	3.85±0.85 <sup>b</sup>	3.85±0.81 <sup>b</sup>
A4 (♂)	3.29±0.99 <sup>a</sup>	3.30±0.88 <sup>a</sup>	3.74±0.94 <sup>a</sup>	3.89±0.71 <sup>a</sup>	4.00±0.76 <sup>a</sup>	4.14±0.92 <sup>a</sup>	4.22±0.99 <sup>a</sup>	4.39±0.91 <sup>a</sup>	4.40±0.97 <sup>a</sup>
A4 (♀)	2.72±0.81 <sup>b</sup>	2.74±0.70 <sup>b</sup>	3.05±0.99 <sup>b</sup>	3.17±0.82 <sup>b</sup>	3.65±0.94 <sup>b</sup>	3.72±0.87 <sup>b</sup>	3.88±0.75 <sup>b</sup>	3.92±0.93 <sup>b</sup>	3.92±0.99 <sup>b</sup>
A5 (♂)	3.66±0.98 <sup>a</sup>	3.66±0.80 <sup>a</sup>	4.08±0.90 <sup>a</sup>	4.26±0.81 <sup>a</sup>	4.86±0.78 <sup>a</sup>	5.40±0.86 <sup>a</sup>	5.72±0.95 <sup>a</sup>	5.80±0.97 <sup>a</sup>	5.80±0.94 <sup>a</sup>
A5 (♀)	2.76±0.88 <sup>b</sup>	2.80±0.73 <sup>b</sup>	3.03±0.91 <sup>b</sup>	3.15±0.89 <sup>b</sup>	3.64±0.78 <sup>b</sup>	3.72±0.94 <sup>b</sup>	3.89±0.93 <sup>b</sup>	3.97±0.90 <sup>b</sup>	3.97±0.84 <sup>b</sup>
A6 (♂)	4.06±0.99 <sup>a</sup>	4.07±0.82 <sup>a</sup>	4.59±0.97 <sup>a</sup>	4.87±0.78 <sup>a</sup>	5.55±0.95 <sup>a</sup>	6.30±0.87 <sup>a</sup>	6.53±0.94 <sup>a</sup>	6.70±0.87 <sup>a</sup>	6.70±0.97 <sup>a</sup>
A6 (♀)	2.65±0.91 <sup>b</sup>	2.78±0.86 <sup>b</sup>	3.01±0.84 <sup>b</sup>	3.12±0.91 <sup>b</sup>	3.51±0.75 <sup>b</sup>	3.83±0.78 <sup>b</sup>	4.32±0.78 <sup>b</sup>	4.50±0.71 <sup>b</sup>	4.50±0.81 <sup>b</sup>
A7 (♂)	4.47±0.93 <sup>a</sup>	4.47±0.85 <sup>a</sup>	5.02±0.87 <sup>a</sup>	5.19±0.95 <sup>a</sup>	6.33±0.97 <sup>a</sup>	7.00±0.99 <sup>a</sup>	7.65±0.87 <sup>a</sup>	7.80±0.91 <sup>a</sup>	7.80±0.86 <sup>a</sup>
A7 (♀)	2.53±0.92 <sup>b</sup>	2.64±0.81 <sup>b</sup>	2.79±0.89 <sup>b</sup>	2.90±0.92 <sup>b</sup>	3.48±0.78 <sup>b</sup>	3.61±0.78 <sup>b</sup>	3.92±0.88 <sup>b</sup>	4.00±0.83 <sup>b</sup>	4.00±0.77 <sup>b</sup>
A8 (♂)	4.90±0.94 <sup>a</sup>	5.28±0.84 <sup>a</sup>	5.85±0.98 <sup>a</sup>	6.40±0.97 <sup>a</sup>	7.40±0.88 <sup>a</sup>	7.56±0.92 <sup>a</sup>	7.61±0.91 <sup>a</sup>	7.88±0.78 <sup>a</sup>	7.89±0.88 <sup>a</sup>
A8 (♀)	2.39±0.96 <sup>b</sup>	2.44±0.87 <sup>b</sup>	2.70±0.91 <sup>b</sup>	2.76±0.96 <sup>b</sup>	3.24±0.85 <sup>b</sup>	3.50±0.88 <sup>b</sup>	3.75±0.81 <sup>b</sup>	3.86±0.86 <sup>b</sup>	3.86±0.91 <sup>b</sup>
A9 (♂)	3.94±0.91 <sup>a</sup>	4.28±0.86 <sup>a</sup>	4.94±0.90 <sup>a</sup>	5.12±0.78 <sup>a</sup>	6.38±0.97 <sup>a</sup>	7.34±0.94 <sup>a</sup>	8.03±0.83 <sup>a</sup>	8.19±0.94 <sup>a</sup>	8.19±0.90 <sup>a</sup>
A9 (♀)	2.26±0.87 <sup>b</sup>	2.34±0.88 <sup>b</sup>	2.57±0.80 <sup>b</sup>	2.77±0.77 <sup>b</sup>	3.20±0.86 <sup>b</sup>	3.36±0.85 <sup>b</sup>	3.51±0.89 <sup>b</sup>	3.59±0.95 <sup>b</sup>	3.59±0.87 <sup>b</sup>
A10 (♂)	3.56±0.88 <sup>a</sup>	3.72±0.94 <sup>a</sup>	4.29±0.87 <sup>a</sup>	4.54±0.94 <sup>a</sup>	5.94±0.78 <sup>a</sup>	6.62±0.97 <sup>a</sup>	6.93±0.98 <sup>a</sup>	7.00±0.88 <sup>a</sup>	7.00±0.77 <sup>a</sup>
A10 (♀)	2.25±0.97 <sup>b</sup>	2.26±0.84 <sup>b</sup>	2.50±0.94 <sup>b</sup>	2.59±0.71 <sup>b</sup>	3.11±0.88 <sup>b</sup>	3.19±0.92 <sup>b</sup>	3.35±0.78 <sup>b</sup>	3.50±0.84 <sup>b</sup>	3.50±0.98 <sup>b</sup>

**Table 7.** Results of Student's t-test for differences in A11~A21 fin distance of anal fin between male (♂) and female (♀) of marine medaka, *Oryzias dancena* by days after hatched

Days after hatched	32	36	40	50	60	70	90	110	130
A11 (♂)	1.30±0.34 <sup>a</sup>	1.49±0.31 <sup>a</sup>	1.51±0.43 <sup>a</sup>	1.83±0.51 <sup>a</sup>	2.26±0.45 <sup>a</sup>	2.32±0.58 <sup>a</sup>	2.50±0.58 <sup>a</sup>	2.76±0.58 <sup>a</sup>	3.02±0.64 <sup>a</sup>
A11 (♀)	1.07±0.44 <sup>b</sup>	1.16±0.36 <sup>b</sup>	1.18±0.33 <sup>b</sup>	1.40±0.24 <sup>b</sup>	1.70±0.44 <sup>b</sup>	1.71±0.65 <sup>b</sup>	1.78±0.61 <sup>b</sup>	2.14±0.64 <sup>b</sup>	2.16±0.57 <sup>b</sup>
A12 (♂)	1.26±0.42 <sup>a</sup>	1.43±0.34 <sup>a</sup>	1.46±0.48 <sup>a</sup>	1.76±0.35 <sup>a</sup>	2.12±0.53 <sup>a</sup>	2.17±0.54 <sup>a</sup>	2.33±0.42 <sup>a</sup>	2.51±0.68 <sup>a</sup>	2.79±0.68 <sup>a</sup>
A12 (♀)	1.05±0.32 <sup>b</sup>	1.17±0.35 <sup>b</sup>	1.18±0.32 <sup>b</sup>	1.43±0.44 <sup>b</sup>	1.64±0.59 <sup>b</sup>	1.68±0.52 <sup>b</sup>	1.70±0.61 <sup>b</sup>	2.01±0.71 <sup>b</sup>	2.02±0.55 <sup>b</sup>
A13 (♂)	1.22±0.25 <sup>a</sup>	1.34±0.26 <sup>a</sup>	1.40±0.31 <sup>a</sup>	1.69±0.45 <sup>a</sup>	2.03±0.55 <sup>a</sup>	2.09±0.61 <sup>a</sup>	2.23±0.54 <sup>a</sup>	2.41±0.64 <sup>a</sup>	2.67±0.66 <sup>a</sup>
A13 (♀)	1.07±0.21 <sup>b</sup>	1.11±0.31 <sup>b</sup>	1.14±0.33 <sup>b</sup>	1.36±0.48 <sup>b</sup>	1.62±0.47 <sup>b</sup>	1.66±0.56 <sup>b</sup>	1.68±0.47 <sup>b</sup>	1.95±0.61 <sup>b</sup>	1.96±0.67 <sup>b</sup>
A14 (♂)	1.18±0.39 <sup>a</sup>	1.32±0.28 <sup>a</sup>	1.35±0.36 <sup>a</sup>	1.68±0.31 <sup>a</sup>	1.94±0.55 <sup>a</sup>	2.10±0.68 <sup>a</sup>	2.22±0.59 <sup>a</sup>	2.38±0.51 <sup>a</sup>	2.58±0.61 <sup>a</sup>
A14 (♀)	1.04±0.34 <sup>b</sup>	1.11±0.32 <sup>b</sup>	1.16±0.38 <sup>b</sup>	1.38±0.45 <sup>b</sup>	1.65±0.51 <sup>b</sup>	1.65±0.59 <sup>b</sup>	1.68±0.61 <sup>b</sup>	1.97±0.67 <sup>b</sup>	1.98±0.62 <sup>b</sup>
A15 (♂)	1.14±0.31 <sup>a</sup>	1.31±0.31 <sup>a</sup>	1.31±0.37 <sup>a</sup>	1.58±0.48 <sup>a</sup>	1.86±0.50 <sup>a</sup>	1.98±0.57 <sup>a</sup>	2.11±0.60 <sup>a</sup>	2.37±0.54 <sup>a</sup>	2.54±0.64 <sup>a</sup>
A15 (♀)	1.05±0.28 <sup>b</sup>	1.09±0.35 <sup>b</sup>	1.13±0.34 <sup>b</sup>	1.38±0.38 <sup>b</sup>	1.59±0.56 <sup>b</sup>	1.60±0.61 <sup>b</sup>	1.62±0.48 <sup>b</sup>	1.89±0.68 <sup>b</sup>	1.93±0.62 <sup>b</sup>
A16 (♂)	1.09±0.29 <sup>a</sup>	1.26±0.45 <sup>a</sup>	1.27±0.33 <sup>a</sup>	1.63±0.49 <sup>a</sup>	1.95±0.46 <sup>a</sup>	2.00±0.53 <sup>a</sup>	2.13±0.63 <sup>a</sup>	2.24±0.48 <sup>a</sup>	2.52±0.66 <sup>a</sup>
A16 (♀)	1.05±0.36 <sup>b</sup>	1.09±0.31 <sup>b</sup>	1.10±0.41 <sup>b</sup>	1.34±0.34 <sup>b</sup>	1.57±0.57 <sup>b</sup>	1.58±0.61 <sup>b</sup>	1.59±0.48 <sup>b</sup>	1.90±0.69 <sup>b</sup>	1.92±0.65 <sup>b</sup>
A17 (♂)	1.06±0.34 <sup>a</sup>	1.22±0.38 <sup>a</sup>	1.22±0.31 <sup>a</sup>	1.59±0.33 <sup>a</sup>	1.84±0.48 <sup>a</sup>	1.96±0.54 <sup>a</sup>	2.17±0.68 <sup>a</sup>	2.35±0.77 <sup>a</sup>	2.50±0.60 <sup>a</sup>
A17 (♀)	1.00±0.28 <sup>b</sup>	1.07±0.38 <sup>b</sup>	1.08±0.31 <sup>b</sup>	1.30±0.44 <sup>b</sup>	1.53±0.54 <sup>b</sup>	1.59±0.55 <sup>b</sup>	1.65±0.51 <sup>b</sup>	1.87±0.72 <sup>b</sup>	1.90±0.58 <sup>b</sup>
A18 (♂)	1.05±0.34 <sup>a</sup>	1.09±0.33 <sup>a</sup>	1.14±0.42 <sup>a</sup>	1.37±0.48 <sup>a</sup>	1.78±0.51 <sup>a</sup>	1.86±0.61 <sup>a</sup>	2.22±0.61 <sup>a</sup>	2.36±0.70 <sup>a</sup>	2.52±0.68 <sup>a</sup>
A18 (♀)	0.96±0.29 <sup>b</sup>	1.00±0.34 <sup>b</sup>	1.04±0.31 <sup>b</sup>	1.24±0.47 <sup>b</sup>	1.46±0.49 <sup>b</sup>	1.48±0.50 <sup>b</sup>	1.50±0.55 <sup>b</sup>	1.75±0.48 <sup>b</sup>	1.82±0.65 <sup>b</sup>
A19 (♂)	0.94±0.33 <sup>a</sup>	1.04±0.38 <sup>a</sup>	1.05±0.29 <sup>a</sup>	1.29±0.30 <sup>a</sup>	1.67±0.42 <sup>a</sup>	1.74±0.56 <sup>a</sup>	2.02±0.69 <sup>a</sup>	2.19±0.65 <sup>a</sup>	2.31±0.59 <sup>a</sup>
A19 (♀)	0.83±0.25 <sup>b</sup>	0.91±0.36 <sup>b</sup>	0.91±0.27 <sup>b</sup>	1.15±0.40 <sup>b</sup>	1.34±0.51 <sup>b</sup>	1.35±0.50 <sup>b</sup>	1.41±0.54 <sup>b</sup>	1.63±0.52 <sup>b</sup>	1.65±0.55 <sup>b</sup>
A20 (♂)	0.81±0.35 <sup>a</sup>	0.86±0.31 <sup>a</sup>	0.86±0.23 <sup>a</sup>	1.18±0.47 <sup>a</sup>	1.39±0.49 <sup>a</sup>	1.44±0.43 <sup>a</sup>	1.63±0.61 <sup>a</sup>	1.77±0.59 <sup>a</sup>	1.90±0.58 <sup>a</sup>
A20 (♀)	0.80±0.33 <sup>b</sup>	0.83±0.34 <sup>b</sup>	0.86±0.28 <sup>b</sup>	1.01±0.44 <sup>b</sup>	1.18±0.53 <sup>b</sup>	1.19±0.21 <sup>b</sup>	1.21±0.51 <sup>b</sup>	1.47±0.42 <sup>b</sup>	1.49±0.57 <sup>b</sup>
A21 (♂)	0.58±0.22 <sup>a</sup>	0.67±0.13 <sup>a</sup>	0.82±0.34 <sup>a</sup>	0.99±0.39 <sup>a</sup>	1.17±0.56 <sup>a</sup>	1.25±0.48 <sup>a</sup>	1.41±0.53 <sup>a</sup>	1.51±0.68 <sup>a</sup>	1.61±0.50 <sup>a</sup>
A21 (♀)	0.53±0.20 <sup>b</sup>	0.58±0.14 <sup>b</sup>	0.69±0.18 <sup>b</sup>	0.88±0.45 <sup>b</sup>	1.02±0.57 <sup>b</sup>	1.03±0.45 <sup>b</sup>	1.04±0.57 <sup>b</sup>	1.26±0.45 <sup>b</sup>	1.26±0.59 <sup>b</sup>

For abbreviations see table 2. The sample numbers of males and females are 30 and 30, respectively.  $P < 0.05$ .

**Table 7. Extended**

Days after hatched	150	170	190	210	230	250	270	330	390
A11 (♂)	3.14±0.51 <sup>a</sup>	3.15±0.62 <sup>a</sup>	3.83±0.61 <sup>a</sup>	4.10±0.66 <sup>a</sup>	5.08±0.77 <sup>a</sup>	5.91±0.89 <sup>a</sup>	6.61±0.94 <sup>a</sup>	6.89±0.77 <sup>a</sup>	6.89±0.91 <sup>a</sup>
A11 (♀)	2.23±0.68 <sup>b</sup>	2.25±0.68 <sup>b</sup>	2.44±0.77 <sup>b</sup>	2.55±0.78 <sup>b</sup>	2.89±0.68 <sup>b</sup>	2.93±0.87 <sup>b</sup>	3.14±0.96 <sup>b</sup>	3.34±0.85 <sup>b</sup>	3.34±0.87 <sup>b</sup>
A12 (♂)	2.85±0.81 <sup>a</sup>	2.88±0.61 <sup>a</sup>	3.45±0.68 <sup>a</sup>	3.84±0.71 <sup>a</sup>	4.94±0.87 <sup>a</sup>	5.45±0.99 <sup>a</sup>	6.45±0.98 <sup>a</sup>	6.67±0.98 <sup>a</sup>	6.67±0.95 <sup>a</sup>
A12 (♀)	2.15±0.46 <sup>b</sup>	2.17±0.64 <sup>b</sup>	2.41±0.78 <sup>b</sup>	2.47±0.72 <sup>b</sup>	2.83±0.54 <sup>b</sup>	2.89±0.91 <sup>b</sup>	3.17±0.99 <sup>b</sup>	3.44±0.66 <sup>b</sup>	3.44±0.95 <sup>b</sup>
A13 (♂)	2.70±0.71 <sup>a</sup>	2.74±0.66 <sup>a</sup>	3.21±0.88 <sup>a</sup>	3.34±0.73 <sup>a</sup>	3.82±0.89 <sup>a</sup>	4.28±0.64 <sup>a</sup>	4.54±0.97 <sup>a</sup>	4.60±0.77 <sup>a</sup>	4.62±0.86 <sup>a</sup>
A13 (♀)	2.08±0.65 <sup>b</sup>	2.09±0.65 <sup>b</sup>	2.38±0.87 <sup>b</sup>	2.41±0.70 <sup>b</sup>	2.79±0.62 <sup>b</sup>	2.82±0.78 <sup>b</sup>	3.03±0.89 <sup>b</sup>	3.45±0.57 <sup>b</sup>	3.45±0.87 <sup>b</sup>
A14 (♂)	2.64±0.66 <sup>a</sup>	2.86±0.78 <sup>a</sup>	3.09±0.87 <sup>a</sup>	3.22±0.61 <sup>a</sup>	3.46±0.94 <sup>a</sup>	3.74±0.99 <sup>a</sup>	3.88±0.87 <sup>a</sup>	3.92±0.68 <sup>a</sup>	3.93±0.94 <sup>a</sup>
A14 (♀)	2.05±0.86 <sup>b</sup>	2.11±0.65 <sup>b</sup>	2.30±0.88 <sup>b</sup>	2.39±0.66 <sup>b</sup>	2.75±0.62 <sup>b</sup>	2.78±0.68 <sup>b</sup>	2.88±0.65 <sup>b</sup>	2.91±0.78 <sup>b</sup>	2.92±0.95 <sup>b</sup>
A15 (♂)	2.58±0.67 <sup>a</sup>	2.62±0.75 <sup>a</sup>	3.00±0.68 <sup>a</sup>	3.24±0.68 <sup>a</sup>	3.40±0.99 <sup>a</sup>	3.54±0.99 <sup>a</sup>	3.61±0.94 <sup>a</sup>	3.78±0.69 <sup>a</sup>	3.80±0.99 <sup>a</sup>
A15 (♀)	1.93±0.66 <sup>b</sup>	2.03±0.77 <sup>b</sup>	2.22±0.77 <sup>b</sup>	2.27±0.78 <sup>b</sup>	2.43±0.54 <sup>b</sup>	2.51±0.61 <sup>b</sup>	2.71±0.94 <sup>b</sup>	2.80±0.96 <sup>b</sup>	2.83±0.96 <sup>b</sup>
A16 (♂)	2.56±0.60 <sup>a</sup>	2.58±0.70 <sup>a</sup>	2.89±0.69 <sup>a</sup>	2.98±0.81 <sup>a</sup>	3.50±0.91 <sup>a</sup>	3.73±0.94 <sup>a</sup>	3.80±0.64 <sup>a</sup>	3.85±0.91 <sup>a</sup>	3.86±0.76 <sup>a</sup>
A16 (♀)	1.96±0.58 <sup>b</sup>	2.02±0.65 <sup>b</sup>	2.15±0.72 <sup>b</sup>	2.21±0.73 <sup>b</sup>	2.41±0.62 <sup>b</sup>	2.63±0.67 <sup>b</sup>	2.65±0.68 <sup>b</sup>	2.70±0.87 <sup>b</sup>	2.71±0.86 <sup>b</sup>
A17 (♂)	2.62±0.59 <sup>a</sup>	2.74±0.61 <sup>a</sup>	2.99±0.68 <sup>a</sup>	3.24±0.70 <sup>a</sup>	3.43±0.87 <sup>a</sup>	3.65±0.91 <sup>a</sup>	3.76±0.87 <sup>a</sup>	3.80±0.99 <sup>a</sup>	3.82±0.94 <sup>a</sup>
A17 (♀)	1.92±0.64 <sup>b</sup>	2.01±0.76 <sup>b</sup>	2.08±0.71 <sup>b</sup>	2.13±0.65 <sup>b</sup>	2.32±0.62 <sup>b</sup>	2.48±0.88 <sup>b</sup>	2.65±0.89 <sup>b</sup>	2.70±0.88 <sup>b</sup>	2.71±0.86 <sup>b</sup>
A18 (♂)	2.52±0.69 <sup>a</sup>	2.56±0.66 <sup>a</sup>	2.83±0.78 <sup>a</sup>	3.15±0.64 <sup>a</sup>	3.34±0.88 <sup>a</sup>	3.57±0.72 <sup>a</sup>	3.60±0.94 <sup>a</sup>	3.62±0.99 <sup>a</sup>	3.63±0.96 <sup>a</sup>
A18 (♀)	1.85±0.68 <sup>b</sup>	1.92±0.78 <sup>b</sup>	2.03±0.86 <sup>b</sup>	2.07±0.63 <sup>b</sup>	2.34±0.64 <sup>b</sup>	2.61±0.64 <sup>b</sup>	2.70±0.67 <sup>b</sup>	2.75±0.87 <sup>b</sup>	2.76±0.87 <sup>b</sup>
A19 (♂)	2.33±0.77 <sup>a</sup>	2.33±0.66 <sup>a</sup>	2.60±0.61 <sup>a</sup>	2.98±0.69 <sup>a</sup>	3.23±0.69 <sup>a</sup>	3.49±0.86 <sup>a</sup>	3.50±0.74 <sup>a</sup>	3.51±0.91 <sup>a</sup>	3.52±0.94 <sup>a</sup>
A19 (♀)	1.71±0.57 <sup>b</sup>	1.74±0.61 <sup>b</sup>	1.86±0.69 <sup>b</sup>	1.99±0.78 <sup>b</sup>	2.27±0.65 <sup>b</sup>	2.40±0.24 <sup>b</sup>	2.55±0.78 <sup>b</sup>	2.61±0.78 <sup>b</sup>	2.61±0.87 <sup>b</sup>
A20 (♂)	1.91±0.67 <sup>a</sup>	1.94±0.62 <sup>a</sup>	2.16±0.62 <sup>a</sup>	2.31±0.79 <sup>a</sup>	2.81±0.67 <sup>a</sup>	3.19±0.89 <sup>a</sup>	3.34±0.67 <sup>a</sup>	3.35±0.92 <sup>a</sup>	3.35±0.84 <sup>a</sup>
A20 (♀)	1.53±0.69 <sup>b</sup>	1.56±0.78 <sup>b</sup>	1.73±0.74 <sup>b</sup>	1.77±0.80 <sup>b</sup>	1.99±0.77 <sup>b</sup>	2.03±0.64 <sup>b</sup>	2.16±0.98 <sup>b</sup>	2.20±0.95 <sup>b</sup>	2.20±0.64 <sup>b</sup>
A21 (♂)	1.69±0.61 <sup>a</sup>	1.76±0.80 <sup>a</sup>	2.02±0.67 <sup>a</sup>	2.10±0.87 <sup>a</sup>	2.59±0.69 <sup>a</sup>	2.87±0.78 <sup>a</sup>	2.87±0.98 <sup>a</sup>	2.90±0.94 <sup>a</sup>	2.90±0.87 <sup>a</sup>
A21 (♀)	1.29±0.62 <sup>b</sup>	1.32±0.71 <sup>b</sup>	1.45±0.78 <sup>b</sup>	1.48±0.53 <sup>b</sup>	1.68±0.59 <sup>b</sup>	1.73±0.69 <sup>b</sup>	1.84±0.77 <sup>b</sup>	1.85±0.68 <sup>b</sup>	1.85±0.77 <sup>b</sup>

**Table 8.** Results of Student's t-test for differences in A1~A10 separated fin distance of anal fin between male (♂) and female(♀) of marine medaka, *Oryzias dancena* by days after hatched

Days after hatched	60	70	90	110	130	150	170
A1 (♂)	N	N	N	N	N	N	N
A1 (♀)	N	N	N	N	N	N	N
A2 (♂)	N	N	N	N	N	N	N
A2 (♀)	N	N	N	N	N	N	N
A3 (♂)	N	N	N	N	N	N	N
A3 (♀)	N	0.13±0.03	0.25±0.01	0.28±0.03	0.32±0.01	0.33±0.03	0.33±0.03
A4 (♂)	N	N	N	N	N	N	N
A4 (♀)	N	0.16±0.01	0.37±0.02	0.38±0.04	0.39±0.02	0.47±0.10	0.62±0.05
A5 (♂)	N	N	N	N	N	N	N
A5 (♀)	0.19±0.05	0.28±0.07	0.39±0.03	0.47±0.01	0.48±0.06	0.51±0.09	0.52±0.06
A6 (♂)	N	N	N	N	N	N	N
A6 (♀)	0.24±0.08	0.33±0.02	0.36±0.05	0.44±0.03	0.51±0.08	0.52±0.08	0.62±0.08
A7 (♂)	N	N	N	N	N	N	N
A7 (♀)	0.22±0.09	0.23±0.06	0.23±0.04	0.43±0.06	0.46±0.12	0.47±0.04	0.50±0.07
A8 (♂)	N	N	N	N	N	N	N
A8 (♀)	0.19±0.05	0.19±0.05	0.25±0.06	0.30±0.04	0.38±0.03	0.39±0.03	0.41±0.05
A9 (♂)	N	N	N	N	N	N	N
A9 (♀)	0.18±0.05	0.22±0.12	0.27±0.08	0.42±0.01	0.43±0.06	0.45±0.12	0.51±0.03
A10 (♂)	N	N	N	N	N	N	N
A10 (♀)	0.22±0.04	0.24±0.10	0.31±0.01	0.38±0.04	0.41±0.08	0.43±0.11	0.50±0.10

For abbreviations see table 2. The sample numbers of males and females are 30 and 30, respectively. N: non-separation,  $P < 0.05$ .

**Table 8.** Extended

Days after hatched	190	210	230	250	270	330	390
A1 (♂)	N	N	N	N	N	0.03±0.11 <sup>a</sup>	0.03±0.11 <sup>a</sup>
A1 (♀)	N	N	N	N	N	0.01±0.11 <sup>a</sup>	0.01±0.11 <sup>a</sup>
A2 (♂)	N	N	N	N	N	0.03±0.01 <sup>a</sup>	0.03±0.01 <sup>a</sup>
A2 (♀)	N	N	N	N	N	0.01±0.00 <sup>a</sup>	0.01±0.00 <sup>a</sup>
A3 (♂)	N	N	0.51±0.12 <sup>a</sup>	0.60±0.09 <sup>a</sup>	0.68±0.19 <sup>a</sup>	0.73±0.11 <sup>a</sup>	0.73±0.14 <sup>a</sup>
A3 (♀)	0.50±0.11	0.52±0.09	0.63±0.13 <sup>b</sup>	0.72±0.15 <sup>b</sup>	0.73±0.20 <sup>b</sup>	0.75±0.13 <sup>a</sup>	0.75±0.19 <sup>a</sup>
A4 (♂)	N	N	0.69±0.11 <sup>a</sup>	0.86±0.17 <sup>a</sup>	0.98±0.21 <sup>a</sup>	0.98±0.22 <sup>a</sup>	0.98±0.18 <sup>a</sup>
A4 (♀)	0.63±0.12	0.66±0.11	0.71±0.15 <sup>a</sup>	0.77±0.16 <sup>b</sup>	0.77±0.16 <sup>b</sup>	0.87±0.20 <sup>b</sup>	0.87±0.13 <sup>b</sup>
A5 (♂)	N	N	N	N	N	N	N
A5 (♀)	0.64±0.13	0.70±0.12	0.83±0.16	0.83±0.17	0.89±0.15	1.12±0.15	1.12±0.14
A6 (♂)	N	N	N	N	N	N	N
A6 (♀)	0.68±0.09	0.70±0.10	0.85±0.17	0.88±0.19	0.92±0.18	1.20±0.19	1.20±0.18
A7 (♂)	N	N	N	N	N	N	N
A7 (♀)	0.62±0.08	0.64±0.09	0.74±0.18	0.76±0.21	0.90±0.17	1.03±0.16	1.03±0.17
A8 (♂)	N	N	N	N	N	N	N
A8 (♀)	0.53±0.11	0.58±0.07	0.67±0.15	0.71±0.20	0.83±0.13	1.10±0.18	1.10±0.19
A9 (♂)	N	N	N	N	N	N	N
A9 (♀)	0.57±0.14	0.59±0.13	0.71±0.14	0.78±0.19	0.82±0.14	0.99±0.22	0.99±0.21
A10 (♂)	N	N	N	N	N	N	N
A10 (♀)	0.58±0.10	0.61±0.12	0.69±0.10	0.70±0.13	0.82±0.15	0.95±0.23	0.95±0.20

**Table 9.** Results of Student's t-test for differences in A11~A21 separated fin distance of anal fin between male (♂) and female (♀) of marine medaka, *Oryzias dancena* by days after hatched

Days after hatched	60	70	90	110	130	150	170
A11 (♂)	N	N	N	N	N	N	N
A11 (♀)	0.21±0.11	0.27±0.05	0.27±0.11	0.40±0.11	0.48±0.11	0.49±0.11	0.51±0.11
A12 (♂)	N	N	N	N	N	N	N
A12 (♀)	0.25±0.05	0.25±0.09	0.26±0.09	0.32±0.11	0.46±0.11	0.49±0.11	0.57±0.11
A13 (♂)	N	N	N	N	N	N	N
A13 (♀)	0.19±0.07	0.24±0.11	0.28±0.08	0.37±0.11	0.42±0.11	0.46±0.11	0.53±0.11
A14 (♂)	N	N	N	N	N	N	N
A14 (♀)	0.21±0.09	0.22±0.07	0.29±0.06	0.35±0.11	0.39±0.13	0.48±0.13	0.53±0.12
A15 (♂)	N	N	N	N	N	N	N
A15 (♀)	0.20±0.05	0.25±0.10	0.26±0.07	0.33±0.16	0.42±0.12	0.55±0.14	0.56±0.11
A16 (♂)	N	N	N	N	N	N	N
A16 (♀)	0.20±0.06	0.24±0.05	0.31±0.12	0.39±0.14	0.44±0.11	0.52±0.16	0.55±0.11
A17 (♂)	N	N	N	N	N	N	N
A17 (♀)	0.18±0.07	0.30±0.08	0.31±0.10	0.40±0.11	0.50±0.10	0.53±0.12	0.55±0.16
A18 (♂)	N	N	N	N	N	N	N
A18 (♀)	0.24±0.04	0.37±0.07	0.38±0.09	0.46±0.15	0.55±0.13	0.57±0.14	0.62±0.15
A19 (♂)	N	N	N	N	N	N	N
A19 (♀)	0.32±0.01	0.36±0.06	0.37±0.07	0.44±0.10	0.56±0.12	0.58±0.15	0.64±0.11
A20 (♂)	N	N	N	N	N	N	N
A20 (♀)	0.37±0.09	0.44±0.12	0.48±0.11	0.62±0.19	0.67±0.18	0.71±0.17	0.73±0.17
A21 (♂)	N	N	N	N	N	N	N
A21 (♀)	N	0.46±0.11	0.47±0.13	0.48±0.12	0.48±0.13	0.64±0.15	0.69±0.16

For abbreviations see table 2. The sample numbers of males and females are 30 and 30, respectively. N: non-separation,  $P < 0.05$ .






**Table 9.** Extended

Days after hatched	190	210	230	250	270	330	390
A11 (♂)	N	N	N	N	N	N	N
A11 (♀)	0.54±0.19	0.58±0.15	0.71±0.13	0.78±0.12	0.88±0.19	1.01±0.17	1.01±0.16
A12 (♂)	N	N	N	N	N	N	N
A12 (♀)	0.64±0.18	0.66±0.16	0.77±0.19	0.80±0.11	0.89±0.17	1.03±0.17	1.03±0.17
A13 (♂)	N	N	N	N	N	N	N
A13 (♀)	0.61±0.11	0.63±0.15	0.71±0.18	0.72±0.18	0.78±0.11	1.02±0.19	1.02±0.11
A14 (♂)	N	N	N	N	N	N	N
A14 (♀)	0.58±0.18	0.62±0.11	0.75±0.17	0.79±0.17	1.17±0.19	1.37±0.17	1.37±0.18
A15 (♂)	N	N	N	N	N	N	N
A15 (♀)	0.62±0.17	0.66±0.16	0.74±0.18	0.74±0.17	0.83±0.14	1.10±0.18	1.10±0.17
A16 (♂)	N	N	N	N	N	N	N
A16 (♀)	0.64±0.16	0.66±0.17	0.81±0.18	0.81±0.18	0.88±0.17	1.09±0.19	1.09±0.11
A17 (♂)	N	N	N	N	N	N	N
A17 (♀)	0.65±0.13	0.69±0.18	0.81±0.11	0.86±0.16	0.90±0.11	1.03±0.17	1.03±0.16
A18 (♂)	N	N	N	N	N	N	N
A18 (♀)	0.70±0.19	0.73±0.19	0.83±0.11	0.86±0.11	1.23±0.11	1.44±0.11	1.44±0.11
A19 (♂)	0.92±0.19 <sup>a</sup>	1.08±0.20 <sup>a</sup>	1.29±0.23 <sup>a</sup>	1.39±0.21 <sup>a</sup>	1.40±0.25 <sup>a</sup>	1.41±0.27 <sup>a</sup>	1.41±0.29 <sup>a</sup>
A19 (♀)	0.74±0.14 <sup>a</sup>	0.76±0.19 <sup>b</sup>	0.91±0.14 <sup>b</sup>	0.95±0.19 <sup>b</sup>	1.19±0.20 <sup>b</sup>	1.20±0.22 <sup>b</sup>	1.20±0.19 <sup>b</sup>
A20 (♂)	0.91±0.14 <sup>a</sup>	1.11±0.27 <sup>a</sup>	1.21±0.30 <sup>a</sup>	1.28±0.22 <sup>a</sup>	1.47±0.29 <sup>a</sup>	1.56±0.28 <sup>a</sup>	1.56±0.11 <sup>a</sup>
A20 (♀)	0.81±0.12 <sup>b</sup>	0.82±0.20 <sup>b</sup>	0.94±0.13 <sup>b</sup>	1.04±0.12 <sup>b</sup>	1.10±0.18 <sup>b</sup>	1.37±0.17 <sup>b</sup>	1.37±0.15 <sup>b</sup>
A21 (♂)	0.84±0.11 <sup>a</sup>	0.89±0.17 <sup>a</sup>	0.92±0.14 <sup>a</sup>	0.95±0.13 <sup>a</sup>	1.22±0.15 <sup>a</sup>	1.23±0.18 <sup>a</sup>	1.23±0.31 <sup>a</sup>
A21 (♀)	0.75±0.12 <sup>b</sup>	0.83±0.19 <sup>b</sup>	0.86±0.18 <sup>b</sup>	0.88±0.15 <sup>b</sup>	0.93±0.14 <sup>b</sup>	1.41±0.14 <sup>b</sup>	1.41±0.13 <sup>b</sup>



The separation of dorsal fin had more special phenomenon than the result of anal fin and it is re-separation of fin rays with separation which randomly happened. The five type of re-separation (Table 10) and its appearance frequency were analyzed in Table 11. In case of D1, there was no separation for male and female, and for D2, male had type 1 and type 3 while female had type 1 so the re-separation barely happened. In case of D3, there was hardly any re-separation for male and low frequency of type 2 and 3 and the result was similar for both male and female. In case of D4, male and female showed opposite result that similar frequency for type 1 and 4 in female while male had high type 1 frequency. The result for D5 showed the vice versa result of D4 for male and female that high-similar frequency of type 1 and 4 for male and high frequency of type 4 for female. The result D6 showed similar trend as D5 and D7 showed similar high frequency of type 1 for male and female.

**Table 10.** The re-separation type of dorsal fin on marine medaka, *Oryzias dancena*

	Type				
	1	2	3	4	5
Re-separation type					

**Table 11.** The re-separation frequency of dorsal fin on marine medaka, *Oryzias dancena* between male and female

Re-separation type	D1	D2	D3	D4	D5	D6	D7
	<b>Male</b>						
1	0	55	70	80	35	30	55
2	0	0	5	0	10	0	20
3	0	40	25	20	20	20	0
4	0	5	0	0	35	40	0
5	100	0	0	0	0	5	25
	<b>Female</b>						
1	0	95	55	40	15	5	55
2	0	0	10	15	10	15	20
3	0	5	15	5	5	0	0
4	0	0	20	40	70	80	30
5	100	0	0	0	0	0	0

The sample numbers of males and females are 30 and 30, respectively. Data were converted into a percentage.

## Discussion

Every measured morphometric characteristic of marine medaka, *Oryzias dancena* showed a difference in growth 70 days after the incubation. Among these, direct distance between the anterior insertion of the first dorsal fin and the anterior insertion of the first anal fin (DADAA), direct distance between the posterior insertion of the last dorsal fin and the anterior insertion of the first anal fin (DPDAA), direct distance between the anterior insertion of the first dorsal fin and the posterior insertion of the last anal fin (DADPA), length of the fin rays of the dorsal fin (LFRsD), and length of the fin rays of the anal fin (LFRsA) had an apparent difference, and male characteristics were larger than female characteristics. Morphometrics were measured year round, and no difference in the growth was detected. Some experiments reported the differences in measured characteristics depending on whether measurements were conducted during the spawning season. In the sexual dimorphism test of Korean chub, *Zacco koreanus*, 12 characteristics had a difference in males and females among the 37 characteristics measured in the spawning period, while only 1 characteristic had a difference between sexes in the non-spawning period (Kim *et al.*, 2008).

Among the characteristics with apparent differences, differences in classical dimensions were not found. Classical dimensions were applied in

the majority of studies measuring characteristics of fish during the past 30 years. Such classical dimensions focus on the length, width and height of fish, as well as mainly focusing on the axis of the fish body, including the tail and head part (Strauss & Bond, 1990; Park *et al.*, 2004). The results of this study showed differences in classical dimensions between sexes of marine medaka, but no clear and apparent difference was detected.

Apparent differences between sexes included the truss dimensions DADAA, DPDAA and DADPA. The truss dimension is a method to divide the fish body in functional units to investigate parts of appearance (Strauss & Bond, 1990). This method complements the vertical measuring characteristics when characteristics are measured in classical dimensions by measuring across the body shape, and it also determines the body in network type (Park *et al.*, 2004). The male marine medaka has a larger standard length and appearance than the female with respect to the middle of tail that is connected to the body of fish. The truss dimension is applied to determine the characteristics of *Rhynchocypris oxycephalus* when they are starved or satiated (Park *et al.*, 2004). In this experiment, there was a change from the main body and rear body to the tail, which indicates that different food supplies were provided in the different environments inhabited by *R. oxycephalus*. Conversely, because no difference is shown between hungry

and satiated *R. oxycephalus* under the head, this measurement can be used as an index of *Rhynchocypris* (Park *et al.*, 2001a). Additionally, in a Korean chub sexual dimorphism experiment, the female had a higher index in direct distance between the insertion of the dorsal fin and the insertion of the anal fin (IDF-IAF), and the female also had a greater height than the male (Kim *et al.*, 2008).

The dorsal fin and anal fin characteristics of the marine medaka showed significant differences between males and females. In particular, the results for the dorsal fin were similar to those of Park *et al.* (2001b). The result of an investigation measuring characteristics between sexes of the cocktail wrasse, *Pteragogus aurigarius*, by Park *et al.* (2001b) showed that there was a significant difference in the length of the first fin ray of the dorsal fin (LFDF1) and the length of the second fin ray of the dorsal fin (LFDF2) by sexes. In male cocktail wrasses, changes in the first and second spiny rays of the dorsal fin are more pronounced in the breeding season and are not present in sexually immature fish. In male marine medakas, however, the dorsal fin and anal fin are always larger than in the female. In other words, marine medakas do not show changed ratios of the dorsal fin rays and the anal fin rays in spawning season and breeding season because marine medakas spawn all year. As mentioned in Park *et al.* (2010), during spawning,

the male marine medaka stacks his anal fin, the anal fin of the female marine medaka, his dorsal fin and then her dorsal fin. The body of the female is covered by the dorsal and anal fins of the male. Spawning then begins after covering is completed (Park *et al.*, 2010). For this reason, the dorsal fin length and anal fin length of the male is higher than that for the female.

Sexual dimorphism is a component of external morphological variation between the sexes along with features such as the genital papilla, body pigmentation and fin shape (Kim & Kim, 2001). Sexual dimorphism is the most conspicuous difference between the sexes (Kim *et al.*, 2008), and it is observed in many fish species. Females are usually larger than males of the same age. However, in some species like gudgeon, *Gobio gobio* and filefish, *Brachaluteres ulvarum*, males are larger than females (Mann, 1980; Akagawa *et al.*, 1995). The reason for this size difference is not clear (Katano, 1998). Several authors have reported that the evolution of larger body size in male likely results from male-male competition associated with a polygynous mating system (Katano, 1998; Kim *et al.*, 2008). Hence, exploring the nature and extent of sexual dimorphism can aid in the understanding of the social structure and adaptation of a species while also enabling more accurate species identification.

The characteristics of a dorsal fin and anal fin of marine medaka showed significant differences between males and females. As mentioned by Kim *et al.* (2009b), sexual dimorphism of marine medaka was possible to easily classify through forms of the dorsal fin and the anal fin. The result of Kim *et al.* (2009b) was similar to those of this research. In addition, the results of dorsal fin were similar to those of Park *et al.* (2001b). The result of the investigation of measuring characters between sexes of cocktail wrasse, *Pteragogus aurigarius* by Park *et al.* (2001b) showed that there had a significant difference in the length of the first fin ray of the dorsal fin and length of the second fin ray of the dorsal fin by sexes. In male cocktail wrasse, changes in the first and second spiny rays of the dorsal fin are more pronounced in the breeding season and are not present in sexually immature fish. In male marine medaka, however, the dorsal fin and anal fin are always larger than female.

In some species in which the males provide parental care, *Cottus amblystomopsis* (Berg, 1932) and *C. hangiongensis* (Goto, 1984), the pelvic and pectoral fin lengths and the mouth size are distinctly greater in males than in females. During the spawning season, males of these species attract females to spawn, and subsequently defend the nest from intruders and fan the eggs with their pectoral or pelvic fins. In contrast, male *Zacco* leave the



nest after spawning without providing parental care, and many satellites (both males and females) prey upon the eggs at the instant of spawning (Katano, 1998). The pectoral and pelvic fins (including the anal fin) do not seem to play a functional role in the protection of eggs through fanning (Kim *et al.*, 2008).

In other words, marine medaka didn't change tendency of the dorsal fin rays and the anal fin rays in spawning season and breeding season, because marine medaka has all year round spawn. As mentioned by Park *et al.* (2010), for spawning, the male marine medaka stacked his anal fin, the female marine medaka's anal fin, his dorsal fin and her dorsal fin. The female's body was covered by the male's dorsal and anal fins. Spawning began after covering was completed (Park *et al.*, 2010). For this reason, we discussed that dorsal fin length and anal fin length of the male is higher than those of the female.

In this study, when classical dimensions and truss dimensions were measured, the male marine medaka had more rapid growth than the female, with longer length, longer dorsal fins, and longer anal fins. Moreover, the structural difference of male and female was clearly seen separation and re-separation of fin rays. Differences in these characteristics will be useful during experiments when it is necessary to differentiate between sexes of

marine medaka. These measurements and characteristics can also be used as an index to classify Cyprinodontidae. Based on the results of this study, further inquiry might determine the difference in the external measurement characteristics between artificially induced diploid and triploid marine medaka.



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