ACTA ICHTHYOLOGICA ET PISCATORIA (2014) 44 (3): 221–227 DOI: 10.3750/AIP2014.44.3.06

# INVESTIGATION OF HORMONE RECEPTOR EXPRESSIONS IN THE FINS OF *ORYZIAS WOWORAE* (ACTINOPTERYGII: BELONIFORMES: ADRIANICHTHYIDAE)

Arin NGAMNIYOM [1*], Thayat SRIYAPAI [1], and Peechapack SOMYOONSAP [2]

[1] *Faculty of Environmental Culture and Eco-tourism, Srinakharinwirot University, Bangkok 10110, Thailand*
[2] *Department of Biology, Faculty of Sciences, Srinakharinwirot University, Bangkok 10110, Thailand*

Ngamniyom A., Sriyapai T., Somyoonsap P. 2014. Investigation of hormone receptor expressions in the fins of *Oryzias woworae* (Actinopterygii: Beloniformes: Adrianichthyidae). Acta Ichthyol. Piscat. 44 (3): 221–227.

**Background.** In the genus *Oryzias*, the morphology of the dorsal and anal fin constitute typical secondary sex characteristics that are controlled by sex steroid hormones through hormone receptors. However, the level expressions of hormone receptors in fish fins of this genus have remained to be clarified. To achieve the aims of this study, we completely examined the patterns of hormone receptor expression in all fin types of adult *Oryzias woworae* Parenti et Hadiaty, 2010.
**Materials and methods.** The androgen receptor (AR) α, ARβ, oestrogen receptor (ER) α, and ERβ expression in the dorsal-, anal-, pectoral-, pelvic-, and caudal fins, including the dorsal and ventral edges of the caudal fins, were determined using semi-quantitative RT-PCR.
**Results.** Hormone receptor expression levels were significantly different in the dorsal-, anal-, and caudal fins of males, including the dorsal and ventral edges of their caudal fins, and in the pectoral and pelvic fins, and the dorsal and ventral edges of the caudal fins of females. ARα and ARβ levels in dorsal fins and ARβ levels in anal and caudal fins were higher in males than in females. ERα levels in pectoral fins were higher in males than in females. Conversely, ERβ levels in the pectoral and pelvic fins and in the dorsal and ventral edges of caudal fins were higher in females than in males.
**Conclusion.** These results suggest that AR- and ER-mediated functions may regulate sexual dimorphism, and that characteristics of fin morphology are dependent on androgen and oestrogen regulation in adult *Oryzias woworae*.

**Keywords:** androgen receptor, oestrogen receptor, dorsal fin, anal fin, pectoral fin, pelvic fin, caudal fin

## INTRODUCTION

Among the various teleosts species, the fishes of the genus *Oryzias* are notable for their extensive use as vertebrate model organisms for research in many fields, such as endocrinology, developmental biology, and reproductive biology (Chakraborty et al. 2011, Ismail and Yusof 2011, Zhang et al. 2013). In this genus, the dorsal and anal fins are suggested to be typical secondary sex characteristics. These fins are usually longer in mature males than in females (Parenti 2008). The anal fin of the male plays a role in mating with the female for successful fertilisation (Koseki et al. 2000). Moreover, the dorsal and anal fins have been used as bio-indicators for examination of endocrine disrupting chemicals (Hayashi et al. 2007, Ngamniyom and Panyarachun 2012).

Of the sex steroid hormones, androgens and oestrogens act on target cells via their receptors and mediate several physiological processes in many tissues of teleost fish (Amer et al. 2001, Riley et al. 2002). These hormones play a crucial role in sexual differentiation, development of reproductive organs and maintenance of sexual phenotypes in fish (Pawlowski et al. 2004, Guerrero-Estévez and Moreno-Mendoza 2010, Paul-Prasanth et al. 2013). Androgen receptors (AR) and oestrogen receptors (ER) belong to a large family of nuclear hormone receptors that are ligand-induced transcription factors (Todo et al. 1999, Choi 2007). Two AR isoforms (ARα and ARβ) have been described in teleost fishes (Ogino et al. 2009). ERα, ERβ, and ERγ have been described as the three isoforms of ERs in fishes (Chang et al. 1999, Sabo-Attwood et al. 2004).

*Oryzias woworae* Parenti et Hadiaty, 2010 (also known as Daisy's ricefish or Daisy's medaka) is a member of the genus *Oryzias*; it exhibits an autapomorphic colour pattern on its body and is mainly distributed throughout the native freshwater of Sulawesi, Indonesia (Parenti and Hadiaty 2010). This species is also used as

* Correspondence: Dr. Arin Ngamniyom, Srinakharinwirot University, Faculty of Environmental Culture and Eco-tourism, 10110, Bangkok, Thailand, phone: +668-1257-4530, fax: +662-260-1532, e-mail: (AN) *arin@swu.ac.th, ngamniyom.a@gmail.com*.

a non-mammalian vertebrate experimental model with advantageous characteristics: it is easy to maintain, it is reproductively prolific and its secondary sex characteristics are clear. Therefore, in light of this information, we aimed to fill a gap in the existing knowledge concerning the expression of sex hormone receptors in the fins of *Oryzias woworae*. The mRNA expression levels of ARα, ARβ, ERα, and ERβ were examined in the dorsal-, anal-, pectoral-, pelvic-, and caudal fins, including the dorsal and ventral edges of the caudal fins of adult fish using semi-quantitative RT-PCR analysis.

## MATERIALS AND METHODS

**Fish.** Adult *Oryzias woworae* were taken from the fish breeding tanks maintained in our laboratory and originated from the in-house cultures at the Department of Biology of the Faculty of Sciences of Srinakharinwirot University. Males and females were kept in separate aquaria with a photoperiodic control (12 h for light and 12 h for dark cycles), a pH within 7.0–7.4, dissolved oxygen at 8.0–8.3 mg · $L^{-1}$, and a temperature of 26 ± 1ºC for 1 week. These fish were fed ad libitum with a commercial fish feed (Tetra-KilliMin, Tetra, Tokyo, Japan) 2 times per day. Their sex was determined based on the fin morphology. Mature fish were 24–28 mm in standard length. Our experiment was conducted from September 2012 through October 2013.

**Fin preparations.** Fish were anesthetised with 50 mg · $L^{-1}$ of an ethyl-3-aminobenzoate methanesulphonate (MS-222) solution (Sigma, St. Louis, MO, USA) and moved to a Petri dish. The whole dorsal-, anal-, pectoral-, pelvic-, and caudal fins from fish individuals were dissected using a clean scalpel. The dorsal and ventral edges of the caudal fin, marked by orange-red lines, comprised the parts of the fin from the 1st through the 3rd and from the 10th through the 12th fin rays counted from the dorsal or ventral margins; these fin parts were separated from the caudal fins as shown on Fig. 1. Therefore, the caudal fin tissues consisted of the 4th through the 9th fin rays. Total RNA extraction from three fins was adequate for RT-PCR. One tube combined three fin tissue samples; thus, ten tubes were collected from thirty individuals for each gender. In this way, ten replicate samples were collected.

In order to confirm sexes of histological sections, fish gonads were fixed by Bouin's fixative solution for 12 h and were dehydrated through a graded ethanol series and embedded in paraffin. Serial sections with 6-µm thickness were provide by using a Leica RM2125 microtome and stained with hematoxylin and eosin. Histological views of gonads were observed under a light microscope.

**Semi-quantitative RT-PCR.** Total RNA from each sample was extracted using the RNeasy Mini Kit (Qiagen, Hilden, Germany) with DNase I treatment for 30 min at 37ºC, and 100 ng of RNA from each sample was reverse-transcribed with SuperScript III One-Step RT-PCR (Invitrogen, USA). The first strand cDNA solution (0.5 µL) was used as a PCR template.

The primer sets for ARα, ARβ, ERα, ERβ, and β-actin are shown in Table 1, together with the lengths of the corresponding PCR products. Primers of ARα and ARβ were designed on the nucleotide data of Japanese medaka, *Oryzias latipes* (Temminck et Schlegel, 1846) (accession number AB076399 and AB252679, respectively) from GenBank. The cDNA of the housekeeping gene β-actin was amplified as a loading control and reference for each RT reaction. The PCR conditions for the amplification of cDNA were: 95ºC for 30 s for denaturation, 55ºC for 45 s for annealing, and 72ºC for 1 min for extension in each cycle, with a final extension at 72ºC for 10 min. In the RT-PCRs, the linear phases were considered to allow semi-quantitative comparisons of cDNAs for the opti-

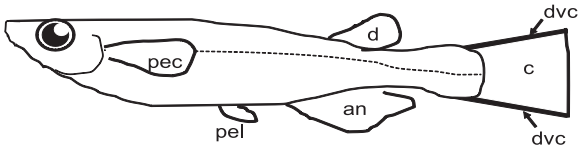



**Fig. 1.** Diagrammatic illustration of fins of the studied *Oryzias woworae*; d = dorsal fin, an = anal fin, pec = pectoral fin, pel = pelvic fin, dvc = dorsal and ventral edges of caudal fin, c = caudal fin

**Table 1**

List of primer sequences and product lengths

| Primer | Sequence | Product (bp) | Reference |
|---|---|---|---|
| ARα | 5′-GGATGGGGGTGATGGTGTT-3′ | 377 | In this study |
| | 5′-CGACTGGAGGTAGTCCAG-3′ | | |
| ARβ | 5′-GTCAAAGTGGTCAAATGGGC-3′ | 227 | In this study |
| | 5′-CTCATCCGTATGCAGTGCTC-3′ | | |
| ERα | 5′-ATGATGAAAGGCGGTGTGCGCAAGG-3′ | 261 | Hayashi et al. (2007) |
| | 5′-CAACTTCTGACGCGAGCAGAGTATC-3′ | | |
| ERβ | 5′-CTGTTAGATGCCTCGGACCTT-3′ | 204 | Inui et al. (2003) |
| | 5′-GATTGGCTGGCTGGTTTCGTG-3′ | | |
| β-actin | 5′-AGGGAGAAGATGACC-3′ | 472 | Scholz et al. (2004) |
| | 5′-CGCAGGACGCCATACCAA-3′ | | |

mised reactions according to the methods previously described by Ngamniyom et al. (2009). Thus, the cycle number for the hormone receptors was 30, whereas for β-actin, the number of cycles was 20. PCR products were electrophoresed on a 1.5% agarose gel, stained with ethidium bromide, and viewed under a UV transilluminator. The band intensities of the amplification products were quantified using Scion Image Software for Windows (Scion, MD, USA). To obtain relative expression levels, the intensity of the amplified band for each gene in each sample was divided by the corresponding intensity for β-actin (Fig. 2).

**Statistical analysis.** One-way ANOVA followed by Tukey's post-hoc test and the unpaired Student's *t*-test were performed in order to determine significant differences in expression using Statistical Package for the Social Sciences (SPSS) version 20 (SPSS, Chicago IL, USA).

Concerning the animal care, the entire experimental design was approved and permitted by the ethics committee of Srinakharinwirot University's Department of Anatomy in the Faculty of Medicine in accordance with the Guidelines for the Care and Use of Fish in Research, Teaching and Testing (Anonymous 2005).

## RESULTS

**Hormone receptor mRNA levels in different fin types.** In the dorsal fins, the expression levels of ARα, ARβ, and ERα were significantly higher than that of ERβ in males as shown in Fig. 3A. In contrast, no significant difference in hormone receptor expression was found in females. In

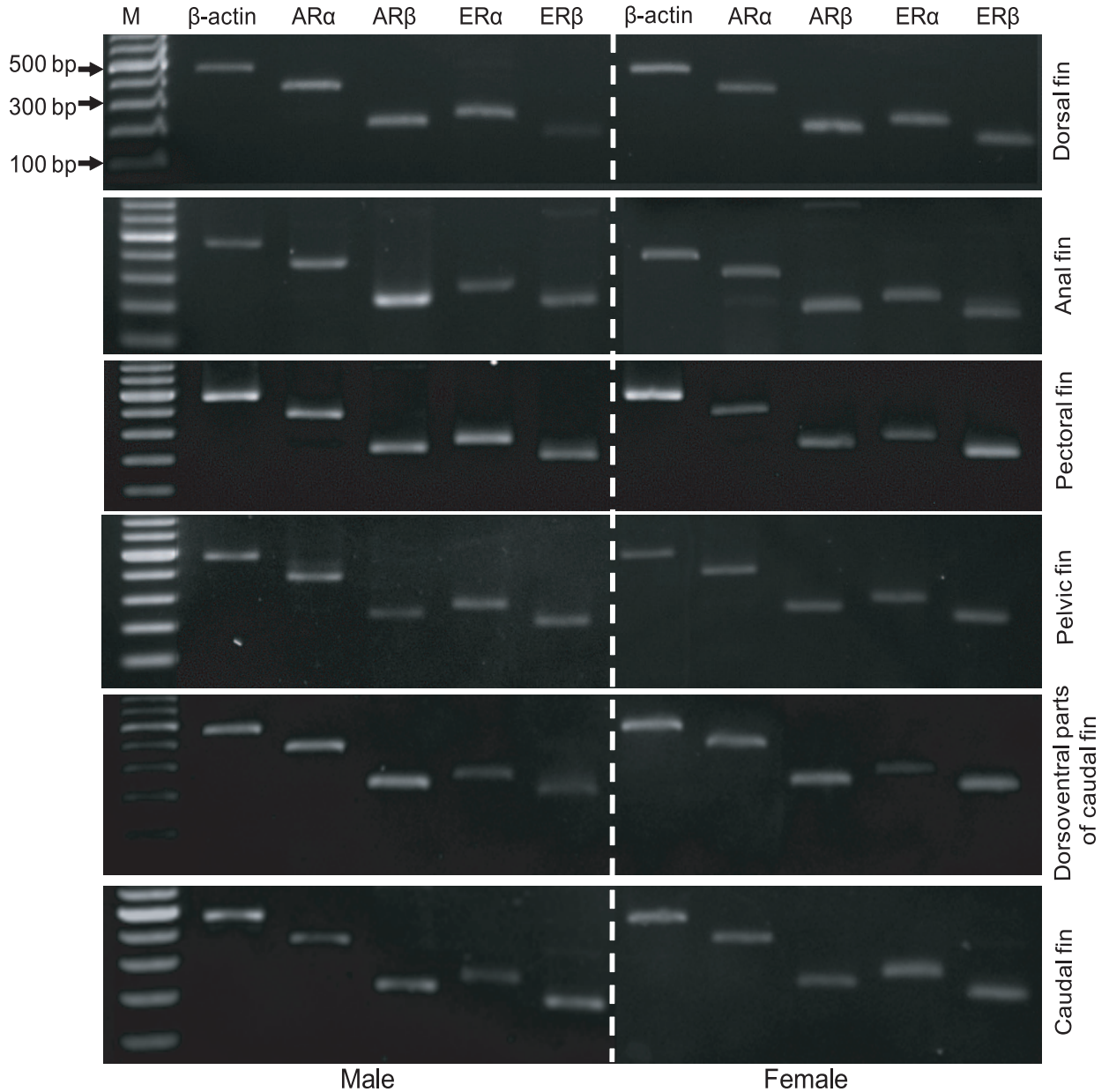


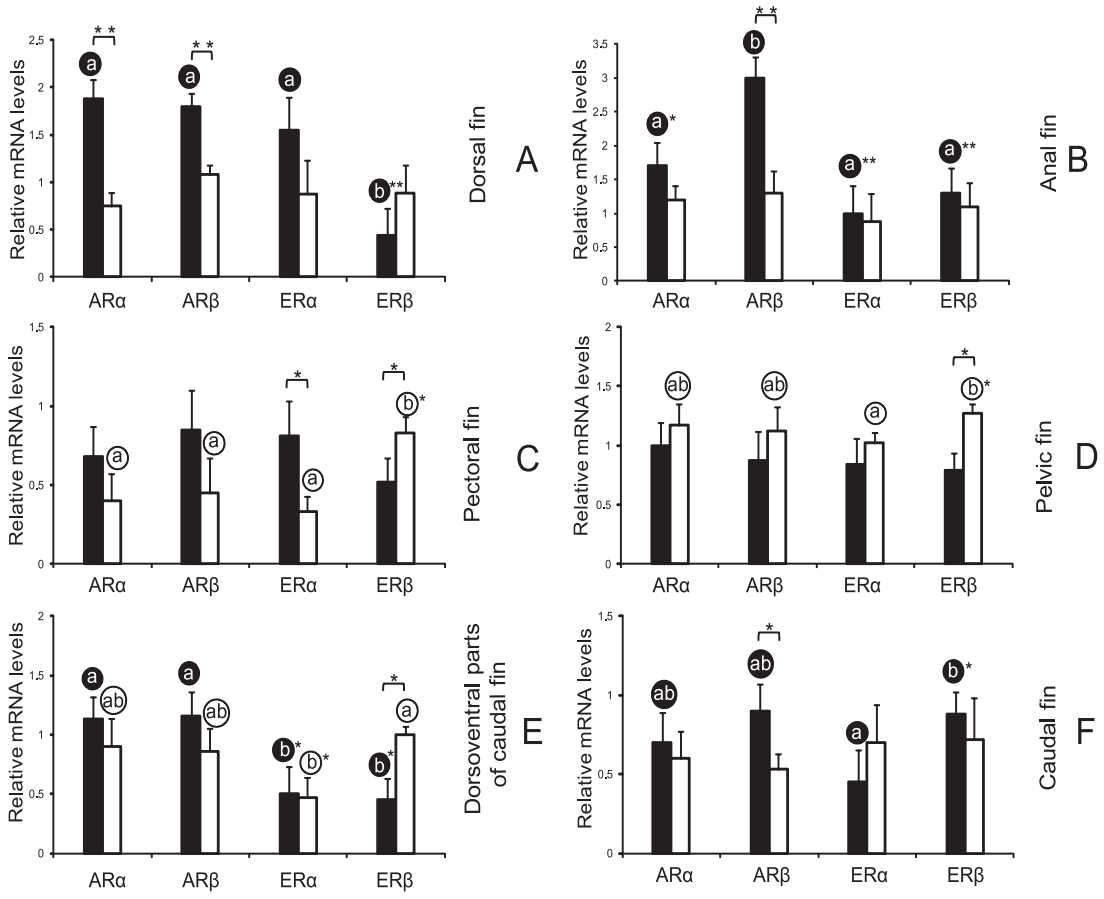

**Fig. 2.** Gel electrophoresis of the products of semi-quantitative RT-PCR to analyse ARα, ARβ, ERα, and ERβ expression in the dorsal-, anal-, pectoral-, pelvic-, and caudal fins and in the dorsal and ventral edges of the caudal fins of male and female *Oryzias woworae*; M = molecular marker of DNA (100 bp ladder)

the anal fins, the ARβ levels were significantly higher than the ARα, ERα, and ERβ levels in males. No significant difference was found among these hormone receptor mRNA levels in females (Fig. 3B). In the pectoral fins, the hormone receptor mRNA levels were not different in males. In contrast, ERβ levels in females were significantly higher than those of ARα, ARβ, and ERα (Fig. 3C). In the pelvic fins, no significant difference was observed among hormone receptors in males. Conversely, in females, ERβ levels were significantly higher than ERα levels (Fig. 3D). In the dorsal and ventral edges of the caudal fins, ARα and ARβ levels were highly significantly different from ERα and ERβ levels in males. ERβ levels were significantly higher than ERα levels in females (Fig. 3E). In the caudal fins, ERβ levels were significantly higher than ERα levels in males. However, in females, no significant difference was found for any of the hormone receptor levels (Fig. 3F).

**Comparison of hormone receptor mRNA levels in each fin type between males and females.** In the dorsal fins, ARα and ARβ expression levels were significantly higher in males than in females. No significant differences in ERα and ERβ levels were found between males and females (Fig. 3A). In the anal fins, only ARα levels were highly significantly different between males and females (Fig. 3B). In the pectoral fins, ERα levels were significantly higher in males than in females, but ERβ levels were significantly lower in males than in females. ARα and ARβ levels were not significantly different between males and females (Fig. 3C). In the pelvic fins and dorsal and ventral edges of caudal fins, ERβ levels were significantly higher in females than in males. No significant differences of ARα, ARβ and ERα levels were found between both sexes (Figs. 3 D–E). In the caudal fins, ERβ was more highly expressed than ERα in males. In contrast, no significant difference was found in females between hormone receptor levels. ARβ levels were significantly higher in males than in females (Fig. 3F).

## DISCUSSION

The ARα, ARβ, ERα, and ERβ were expressed in all fin tissues in both sexes. The AR levels were high in the




**Figs. 3.** Expression levels of ARα, ARβ, ERα, and ERβ in the dorsal-, anal-, pectoral-, pelvic-, and caudal fins and in the dorsal and ventral edges of the caudal fins of male (black bar) and female (white bar) *Oryzias woworae*; The expression levels in each fin are relative values compared to the β-actin mRNA level (mean + standard deviation); Top square brackets indicate significant differences between males and females of each type of fins using the unpaired Student's *t*-test (* = $P < 0.05$ and ** = $P < 0.01$); The dissimilar letters in black circle (l) indicate significant differences between fin types of males and in white circle (¡) between fin types of females with one-way ANOVA followed by Tukey's post-hoc test (* = $P < 0.05$ and ** = $P < 0.01$)

dorsal and anal fins in males of *Oryzias woworae*; this finding was in agreement with a previous report by Ngamniyom et al. (2009) in which AR expression was found to be high in the dorsal and anal fins of male Japanese medaka and Thai medaka, *Oryzias minutillus* Smith, 1945. With regard to other species, high expression of AR was found in the long gonopodium of the anal fin, a male secondary sex characteristic, in eastern mosquitofish, *Gambusia holbrooki* Girard, 1859 (see Ogino et al. 2004). From a molecular viewpoint, our finding suggests, that the AR expression levels in the dorsal and anal fins were congruent with their secondary sex characteristics and may involve androgenic control in the male *O. woworae*. In the pectoral and pelvic fins, the ERβ levels were also higher in females than in males. The ERβ was predominantly expressed compared with ERα in females. Iwamatsu et al. (2003) reported that the pectoral and pelvic fins exhibited sexual dimorphism in their length in Japanese medaka. However, the pectoral and pelvic fins were rather shorter in males than in females. It has been reported that the administration of oestrogen to Japanese medaka can increase the length of pelvic fins in males and females (Niwa 1959). In *Oryzias woworae*—similar to the Japanese medaka—the pairs of pectoral and pelvic fins are larger in females than in males (Parenti and Hadiaty 2010). These results suggest that the oestrogenic effect may regulate the female characteristics, and ERβ may act as the main ER mediating the growth of pectoral and pelvic fins of *O. woworae*. ERα expression levels in the pectoral fins were dramatically higher in males than in females. This result was incongruent with fin phenotypes, because it is thought that oestrogen might drive feminine hormones controlling the secondary characteristics of females. A well known fact in teleosts fishes is that oestrogens not only play crucial roles in reproductive functions of females but also are important in the reproductive functions of males, including the development of secondary sex characteristics and behaviour (Muramatsu and Inoue 2000, Chakraborty et al. 2011, Cheung et al. 2013). This result suggests that the pectoral fins of males may be the target for oestrogens, even though oestrogens affect that coordinate functions via ERα in these fins have so far been discussed as being related to sex characteristics.

Taking our results into consideration, the ARα and ARβ isoforms differed in their pattern of expression between fins and between sexes. ARβ expression was dominant and differed in the dorsal and anal fins of males. However, there is insufficient evidence to hypothesise that the dorsal and anal fins of males are specific target tissues for androgen activity in ARβ-mediated regulation, because ARα and ARβ have not been investigated *in situ* in the fins of teleosts.

The chromatophores in the Japanese medaka are well developed on the male caudal fins and can be regulated by administration of androgenic steroids (Arai and Egami 1961); these chromatophores are reduced in colour by inhibition with oestrogen. In *Oryzias woworae*, the orange-red pigments are found to be more prominent in males than in females on the upper and lower sides of the caudal fins (Parenti and Hadiaty 2010). In addition, black spots along a caudal fin became more numerous in the male than in the female. In the dorsal and ventral edges of caudal fins, ARα and ARβ were highly expressed in male *O. woworae*. ERβ levels were predominant over ERα levels in females. ERβ and ARβ exhibited a sexual dimorphism in expression levels in the dorsal and ventral edges and middle parts of caudal fins, respectively. Androgenic control may create and maintain the brilliant colouration of males on the dorsal and ventral edges of caudal fins. Oestrogens may suppress the pigment colouration through ERβ in those parts of caudal fins in females. In the caudal fins, the occurrence of the predominant ARβ in males more so than in females suggests that androgen may regulate pigmentation in males. For both ER isoforms, there was no difference in gene expression levels between the genders, suggesting that oestrogen signalling may be sufficient for feminising the caudal fins.

In our study, the signals of ARs in females were similar to those observed in males, although their relative expression levels were lower in females. In teleosts, androgens are well known to be necessary for oestrogen synthesis through the aromatising of androgens into oestrogens via the aromatase enzyme (Piferrer et al. 1993, Zhang et al. 2012). This suggests that androgen may be required for oestrogen to function in teleost fishes.

In the presently reported study, we characterised the expression patterns of sex steroid hormone receptors across median and paired fin tissues. These patterns correlated with secondary sex characteristics in both sexes of mature *Oryzias woworae*. It is hypothesised that androgen and oestrogen regulation may confer sex-dependent characteristics of fin morphology, suggesting involvement of AR- and ER-mediated regulation in *O. woworae*. The precise molecular biology *in situ*, however, has yet to be elucidated in the fins of fish.

## REFERENCES

**Amer M.A., Miura T., Miura C., Yamauchi K.** 2001. Involvement of sex steroid hormones in the early stages of spermatogenesis in Japanese huchen (*Hucho perryi*). Biology of Reproduction **65** (4): 1057–1066. DOI: 10.1095/biolreprod65.4.1057

**Anonymous** 2005. Guidelines on: the care and use of fish in research, teaching and testing. Canadian Council on Animal Care, Ottawa, Canada.

**Arai R., Egami N.** 1961. Occurrence of leucophores on the caudal fin of the fish (*Oryzias latipes*) following administration of androgenic steroids. Annotationes Zoologicae Japonenses **34** (4): 185–192.

**Chakraborty T., Katsu Y., Zhou L.Y., Miyagawa S., Nagahama Y., Iguchi T.** 2011. Estrogen receptors in medaka (*Oryzias latipes*) and estrogenic environmental contaminants: An in vitro–in vivo correlation. Journal of Steroid Biochemistry and Molecular Biology **123** (3–5): 115–121. DOI: 10.1016/j.jsbmb.2010.11.015

**Chang X., Kobayashi T., Todo T., Ikeuchi T., Yoshiura M., Kajiura-Kobayashi H., Morrey C., Nagahama Y.** 1999.

Molecular cloning of estrogen receptors α and β in the ovary of a teleost fish, the tilapia (*Oreochromis niloticus*). Zoological Science **16** (4): 653–658. DOI: 10.2108/zsj.16.653

**Cheung N.K.M., Cheung A.C.K., Ye R.R., Ge W., Giesy J.P., Au D.W.T.** 2013. Expression profile of oestrogen receptors and oestrogen-related receptors is organ specific and sex dependent: the Japanese medaka *Oryzias latipes* model. Journal of Fish Biology **83** (2): 295–310. DOI: 10.1111/jfb.12164

**Choi C.Y.** 2007. Effects of 17β-estradiol on estrogen receptor α and β mRNA expression in tissues of the olive flounder (*Paralichthys olivaceus*). Zoological science **24** (8): 824–828. DOI: 10.2108/zsj.24.824

**Guerrero-Estévez S., Moreno-Mendoza N.** 2010. Sexual determination and differentiation in teleost fish. Reviews in Fish Biology and Fisheries **20** (1): 101–121. DOI: 10.1007/s11160-009-9123-4

**Hayashi H., Nishimoto A., Oshima N., Iwamuro S.** 2007. Expression of the estrogen receptor alpha gene in the anal fin of Japanese medaka, *Oryzias latipes*, by environmental concentrations of bisphenol A. Journal of Toxicological Sciences **32** (1): 91–96. DOI: 10.2131/jts.32.91

**Inui M., Adachi T., Takenaka S., Inui H., Nakazawa M., Ueda M., Watanabe H., Mori C., Iguchi T., Miyatake K.** 2003. Effect of UV screens and preservatives on vitellogenin and choriogenin production in male medaka (*Oryzias latipes*). Toxicology **194** (1–2): 43–50. DOI: 10.1016/S0300-483X(03)00340-8

**Ismail A., Yusof S.** 2011. Effect of mercury and cadmium on early life stages of Java medaka (*Oryzias javanicus*): A potential tropical test fish. Marine Pollution Bulletin **63** (5–12): 347–349. DOI: 10.1016/j.marpolbul.2011.02.014

**Iwamatsu T., Nakamura H., Ozato K., Wakamatsu Y.** 2003. Normal growth of the "see-through" medaka. Zoological Science **20** (5): 607–615. DOI: 10.2108/zsj.20.607

**Koseki Y., Takata K., Maekawa K.** 2000. The role of the anal fin in fertilization success in male medaka, *Oryzias latipes*. Fisheries Science **66** (4): 633–635. DOI: 10.1046/j.1444-2906.2000.00103.x

**Muramatsu M., Inoue S.** 2000. Estrogen receptors: How do they control reproductive and nonreproductive functions? Biochemical and Biophysical Research Communications **270** (1): 1–10. DOI: 10.1006/bbrc.2000.2214

**Ngamniyom A., Magtoon W., Nagahama Y., Sasayama Y.** 2009. Expression levels of hormone receptors and bone morphogenic protein in fins of medaka. Zoological Science **26** (1): 74–79. DOI: 10.2108/zsj.26.74

**Ngamniyom A., Panyarachun B.** 2012. Effects of the herbicide pendimethalin on hormone receptor expressions and dorsal fin biometrics in Thai Medaka, *Oryzias minutillus* (Actinopterygii: Beloniformes: Adrianichthyidae). Acta Ichthyologica et Piscatoria **42** (3): 239–246. DOI: 10.3750/AIP2011.42.3.08

**Niwa H.S.** 1959. Inhibitory effect of male hormone on growth and regeneration of the pelvic fin in the medaka, *Oryzias latipes*. Embryologia **4** (4): 349–358. DOI: 10.1111/j.1440-169X.1959.tb00260.x

**Ogino Y., Katoh H., Kuraku S., Yamada G.** 2009. Evolutionary history and functional characterization of androgen receptor genes in jawed vertebrates. Endocrinology **150** (12): 5415–5427. DOI: 10.1210/en.2009-0523

**Ogino Y., Katoh H., Yamada G.** 2004. Androgen dependent development of a modified anal fin, gonopodium, as a model to understand the mechanism of secondary sexual character expression in vertebrates. FEBS Letters **575** (1–3): 119–126. DOI: 10.1016/j.febslet.2004.08.046

**Parenti L.R.** 2008. A phylogenetic analysis and taxonomic revision of ricefishes, *Oryzias* and relatives (Beloniformes, Adrianichthyidae). Zoological Journal of the Linnean Society **154** (3): 494–610. DOI: 10.1111/j.1096-3642.2008.00417.x

**Parenti L.R., Hadiaty R.K.** 2010. A new, remarkably colorful, small ricefish of the genus *Oryzias* (Beloniformes, Adrianichthyidae) from Sulawesi, Indonesia. Copeia **2010** (2): 268–273. DOI: 10.1643/CI-09-108

**Pawlowski S., Sauer A., Shears J.A, Tyler C.R., Braunbeck T.** 2004. Androgenic and estrogenic effects of the synthetic androgen 17α-methyltestosterone on sexual development and reproductive performance in the fathead minnow (*Pimephales promelas*) determined using the gonadal recrudescence assay. Aquatic Toxicology **68** (3): 277–291. DOI: 10.1016/j.aquatox.2004.03.018

**Paul-Prasanth B., Bhandari R.K., Kobayashi T., Horiguchi R., Kobayashi Y., Nakamoto M., Shibata Y., Sakai F., Nakamura M., Nagahama Y.** 2013. Estrogen oversees the maintenance of the female genetic program in terminally differentiated gonochorists. Scientific Reports **3**: 2862. DOI: 10.1038/srep02862

**Piferrer F., Baker I.J., Donaldson E.M.** 1993. Effects of natural, synthetic, aromatizable, and nonaromatizable androgens in inducing male sex differentiation in genotypic female chinook salmon (*Oncorhynchus tshawytscha*). General and Comparative Endocrinology **91** (1): 59–65. DOI: 10.1006/gcen.1993.1104

**Riley L.G., Hirano T., Grau E.G.** 2002. Disparate effects of gonadal steroid hormones on plasma and liver mRNA levels of insulin-like growth factor-I and vitellogenin in the tilapia, *Oreochromis mossambicus*. Fish Physiology and Biochemistry **26** (3): 223–230. DOI: 10.1023/A:1026209502696

**Sabo-Attwood T., Kroll K.J., Denslow N.D.** 2004. Differential expression of largemouth bass (*Micropterus salmoides*) estrogen receptor isotypes alpha, beta, and gamma by estradiol. Molecular and Cellular Endocrinology **218** (1–2): 107–118. DOI: 10.1016/j.mce.2003.12.007

**Scholz S., Kordes C., Hamann J., Gutzeit H.O.** 2004. Induction of vitellogenin in vivo and in vitro in the model teleost medaka (*Oryzias latipes*): Comparison of gene expression and protein levels. Marine Environmental Research **57** (3): 235–244. DOI: 10.1016/S0141-1136(03)00082-5

**Todo T., Ikeuchi T., Kobayashi T., Nagahama Y.** 1999. Fish androgen receptor: cDNA cloning, steroid activation of transcription in transfected mammalian cells, and tissue mRNA

levels. Biochemical and Biophysical Research Communications **254** (2): 378–383. DOI: 10.1006/bbrc.1998.9919

**Zhang Y., Zhang S., Zhou W., Ye X., Ge W., Cheng C.H.K., Lin H., Zhang W., Zhang L.** 2012. Androgen rather than estrogen up-regulates brain-type cytochrome P450 aromatase (*cyp19a1b*) gene via tissue-specific promoters in the hermaphrodite teleost ricefield eel *Monopterus albus*. Molecular and Cellular Endocrinology **350** (1): 125–35. DOI: 10.1016/j.mce.2011.12.001

**Zhang J., Huang J.-Q., Li Y.-Y., Wang N., Sun J., Wang T.-Z., Chen S.-L.** 2013. Histological observations of medaka (*Oryzias latipes*) gonad sexual differentiation and development. Zoological Research **34** (5): 464–470. DOI: 10.11813/j.issn.0254-5853.2013.5.0464 [In Chinese with English abstract.]

Received: 2 January 2014
Accepted: 12 June 2014
Published electronically: 15 October 2014