



Journal of
**Fisheries and
Aquatic Science**

ISSN 1816-4927



Academic
Journals Inc.

www.academicjournals.com

Expression Levels of Bone Morphogenetic Protein 2b in Fins of Adult Japanese Medaka (*Oryzias latipes*) Exposed to Sex Steroid Hormones

¹A. Ngamniyom, ²W. Magtoon, ³Y. Nagahama and ¹Y. Sasayama

¹Department of Life Science, Graduate School of Natural Science and Technology, Kanazawa University, Kanazawa, Ishikawa 920-1192, Japan

²Faculty of Science, Srinakharinwirot University, Bangkok 10110, Thailand

³Laboratory of Reproductive Biology, National Institute for Basic Biology, Okazaki, Aichi 444-8585, Japan

Corresponding Author: Dr. Arin Ngamniyom, Department of Life Science, Graduate School of Natural Science and Technology, Kanazawa University, Ishikawa, 920-1192, Japan Tel: 81762646307

ABSTRACT

In teleost fish, bone morphogenetic protein *bmp2b* is important for the development of skeletons, including the development of secondary sex characters of fins. Sex steroid hormones are also necessary for the development of those characters. In this study, *bmp2b* expression levels in fins of adult Japanese medaka (*Oryzias latipes*) after treatment with sex steroid hormones were examined using semi-quantitative RT-PCR. In males, *bmp2b* expression levels in the posterior part of anal fins were increased at 6 days after 11-ketotestosterone (11-KT) treatments. In contrast, *bmp2b* expression levels in the posterior part of anal fins were decreased at 6 days after 17 β -estradiol (E₂) treatments. The *bmp2b* expression levels in the pelvic fin of males were substantially increased at 3 and 6 days after E₂ treatment. No significant difference in the dorsal, anterior part of the anal, pectoral and caudal fins of male was found among time-related courses after 11-KT and E₂ treatments. In females, *bmp2b* expression levels in the dorsal, anterior and posterior part of anal fins were further increased at 3, 12 and 3 days, respectively after 11-KT treatment. Conversely, there was no difference in *bmp2b* expression levels of pectoral, pelvic and caudal fins of females among time-related courses after treatment with 11-KT and E₂. Present results, therefore, suggest that *bmp2b* regulation in the fin may be involved in 11-KT and E₂-mediated regulation depending on the sex-dependent characters of fin morphology in adult Japanese medaka.

Key words: *bmp2b*, 11-KT, E₂, Japanese medaka, fins

INTRODUCTION

Japanese medaka (*Oryzias latipes*, Teleostei) is one of the best model organisms, which is widely used for experiments in various fields (Lynne, 2008) such as developmental biology (Karim *et al.*, 2009) and endocrine (Zhang *et al.*, 2008). In the genus *Oryzias*, the morphologies of the dorsal and anal fins are typical secondary sex characters (Hayashi *et al.*, 2007). In adult fish, the dorsal and anal fins of males are usually longer than those of females (Okada and Yamashita, 1944). Especially, in males, papillar processes are present on anal fins (Oka, 1931; Uwa, 1971). The papillar processes are also formed on the pectoral fin of males (Egemi and Ishii, 1956). In the

pectoral and pelvic fins, however, there is a difference in the length between males and females. The pectoral and pelvic fins of females are longer than those of males (Iwamatsu *et al.*, 2003). In contrast, the caudal fin does not show any difference in length between males and females (Okada and Yamashita, 1944).

It is well known that, in many teleosts, 11-ketotestosterone is a major androgen that plays physiological roles in reproductive function, sexual differentiation and maintenance of secondary sex characters of males (Borg, 1994; Olsson *et al.*, 2005; Leon *et al.*, 2007). As a primary estrogen in teleosts, 17 β -estradiol plays an important role in the maturation of the reproductive organs in females (Nimrod and Benson, 1998; Nilsson *et al.*, 2001; Kagawa *et al.*, 2003).

In all vertebrates, bone morphogenetic proteins are members of transforming growth factor β superfamily and crucial for various developmental processes including bone and cartilage formation (King *et al.*, 1996; Francis-West *et al.*, 1999; Ruggeri *et al.*, 2008). In teleosts, bone morphogenetic protein (*bmp*) 2b plays an important role in fin formation, differentiation and regeneration (Tiso *et al.*, 2002; Crotwell *et al.*, 2004; Smith *et al.*, 2006). In addition, in mammals androgen and estrogen can increase Bmp mRNA levels, suggesting that those hormones enhance bone formation (Thomas *et al.*, 1998; Ide *et al.*, 1997; Yamamoto *et al.*, 2002; Zhou *et al.*, 2003).

As an objective of this study, we examined the *bmp2b* mRNA levels how express in the dorsal, anal, pectoral, pelvic and caudal fins of adult Japanese medaka when exposed to 11-ketotestosterone or 17 β estradiol.

MATERIALS AND METHODS

Fish: Adult Japanese medaka were purchased from a commercial source in Kanazawa City, Ishikawa, Japan. Their standard length was 24-26 mm. Males and females were kept in separate aquariums with controlling 14:10 h light/dark photoperiod cycle at 26 \pm 1 $^{\circ}$ C for 1 week and fed *ad libitum* with TetraMin (Tokyo, Japan). Sex was determined from the morphology of the secondary sex characters of the dorsal and anal fin according to the criteria of (Okada and Yamashita, 1944). This experiment was conducted from September, 2008 through the end of February, 2009.

Preparation of chemical solutions: In this study, 11-ketotestosterone (11-KT) (Sigma, St. Louis, MO), 17 β -estradiol (E₂) (Wako, Osaka) and dimethyl sulfoxide (DMSO) solutions (Wako, Osaka) were prepared following the methods of (Iwamatsu, 1999; Leon *et al.*, 2007) and (Gonzalez-Doncel *et al.*, 2008) at suitable concentrations and non-lethal toxicity for Japanese medaka.

For the androgen preparation, 1 mg of 11-KT was dissolved in 0.1 mL of DMSO to make a stock solution (10 mg mL⁻¹). In a final concentration, 0.1 mL of the stock solution was diluted with 1,000 mL of aquarium water to 1 μ g mL⁻¹. For the estrogen preparation, 1 mg of E₂ was dissolved in 1 mL of DMSO for a stock solution. The stock solution of E₂ was diluted with aquarium-water to 0.1 μ g mL⁻¹. In each group, three males or four females were immersed in 1,000 mL of aquarium water containing 1 μ g mL⁻¹ of 11-KT or 0.1 μ g mL⁻¹ of E₂ for 3, 6 and 12 days under the above conditions. In the control groups, males or females were treated with only 0.1 mL of DMSO in 1,000 mL of aquarium water without any sex steroid hormones for 12 days. The aquarium-water was changed every three days.

According to the mortality which is shown in Table 1, concentrations of sex steroid hormones seem to be non-lethal and non-acute toxicity for medaka.

Table 1: Effects of various durations of DMSO, 11-ketotestosterone (11-KT) and 17 β -estradiol (E₂) on survival percentages of adult Japanese medaka

Treatment	Time of treatment (days)	No. of adult		No. of survival individuals obtained individuals (%)	
		Male	Female	Male	Female
DMSO (0.1 μ L mL ⁻¹)	12	16	22	16 (100)	22 (100)
11-KT (1 μ g mL ⁻¹)	3	55	66	55 (100)	65 (98)
	6	40	50	39 (98)	50 (100)
	12	25	23	23 (92)	22 (96)
E ₂ (0.1 μ g mL ⁻¹)	3	61	65	60 (98)	65 (100)
	6	46	45	45 (98)	44 (98)
	12	29	24	25 (86)	22 (96)

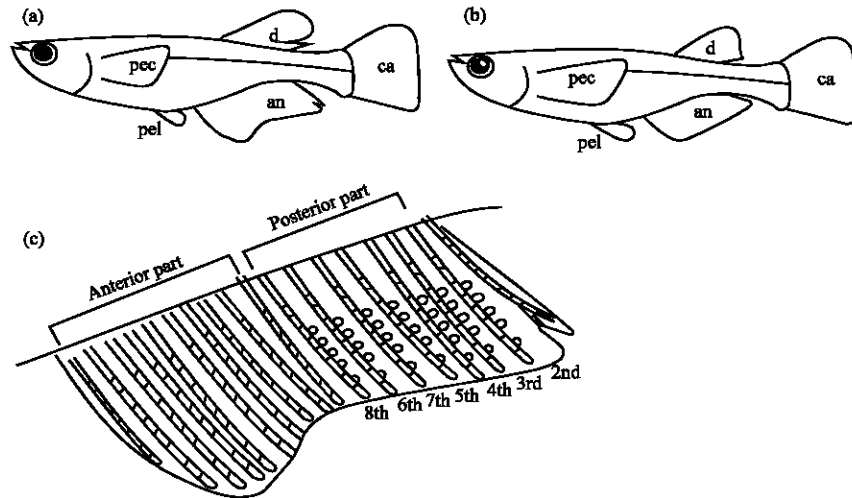


Fig. 1: Diagrammatic illustration of the secondary sex characters of fins in (a) male and (b) female medaka. (c) The posterior part of anal fin in male with papillar processes, comprising the 2nd to the 8th fin rays counted from the posterior end, was separated from the anterior part. an, anal fin; ca, caudal fin; d, dorsal fin; pec, pectoral; fin; pel, pelvic fin

Preparation of fins: Adult males and females were anesthetized with 200 mg L⁻¹ of an ethyl-3-aminobenzoate methanesulphonate (MS-222) solution (Sigma, St. Louis, MO) and placed in a Petri dish. The dorsal, anal, pectoral, pelvic and caudal fins from individual males and females were removed with clean scalpels. In the anal fins, the posterior part with papillar processes, which corresponds to the part from the 2nd to the 8th fin ray counted from the posterior end, was separated from the anterior part according to the criteria of (Iwamatsu *et al.*, 2003) (Fig. 1a, b). Three or four fins were pooled in each tube and a sample of five tubes was collected. Therefore, one experimental group includes fifteen to twenty individuals.

Semi-quantitative RT-PCR: Total RNA from each sample of adult fish was extracted using an isolation reagent Isogen (Wako, Japan) according to the protocol and treated with DNase1 (Takara, Ohtsu) for 30 min at 37°C. Total RNA of 100 ng was reverse-transcribed with AMV reverse transcriptase XL (Takara, Ohtsu) according to the instructions. The solution of 0.5 μ L was used for a PCR template.

Primers were all designed on the basis of data of Japanese medaka. Primers for amplification of *bmp2b* nucleotides were designed as 5'-AAGGGCAAAACAACCCAGCAG-3' and 5'-GTTCCATCCCACATCGCTGAA-3' according to the report by Ngamniyom *et al.* (2009). The β -actin mRNA was amplified in each RT reaction as a loading control and reference. Primers for the amplification of β -actin nucleotides were 5'-AGGGAGAAGATGACC-3' and 5'-CGCAGGACGCCATACCAA-3' according to the report by Scholz *et al.* (2004).

Amplification of cDNA was carried out at cycling conditions of 95°C for 30 sec for the denaturation step and 64°C (*bmp2b*) or 58°C (β -actin) for 1 min for the annealing step and 72 °C for 1 min for extension step. The cycle numbers were 30 cycles for *bmp2b* and 20 cycles for β -actin. The PCR products were electrophoresed on 2% agarose gel, immersed in ethidium bromide and visualized on a UV-transilluminator. The amplification rate was quantitated using the software of Scion Image for Windows (Scion, MD, USA). Amplification levels of *bmp2b* for each fin type were divided by the corresponding amplification level of β -actin to obtain relative expression levels. In this study, no effect of 11-KT and E₂ on the levels of β -actin expression in fins were found among time-related courses.

One-way ANOVA with Tukey's multiple comparison tests was used to examine the differences statistically (Zar, 1984). The data were analyzed using a Statistical Package for the Social Sciences (SPSS) for Windows version 13 (SPSS, Chicago, IL, USA).

RESULTS

Effect of 11-ketotestosterone (11-KT) on the level of *bmp2b* mRNA expression in fins: In representative gel electrophoresis, alterations of *bmp2b* mRNA expressions were found in the posterior part of male anal fin at 6 days, anterior part of female anal fin at 12 days and posterior part of female anal fin at 3 days after treatment with 11-KT (Fig. 2b, 3b).

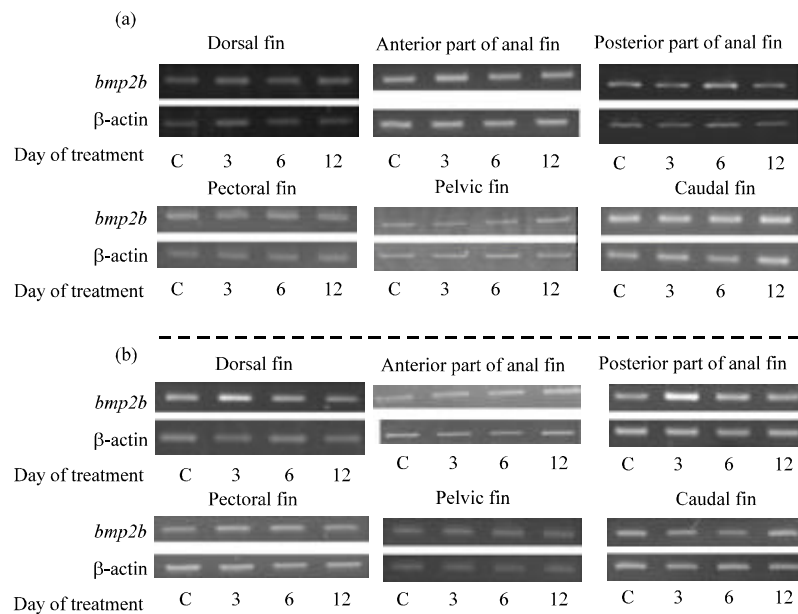


Fig. 2: Gel electrophoresis of RT-PCR analysis of *bmp2b* and β -actin mRNA expression in male and female medaka after treatments of 11-Ketotestosterone. C: Control. (a) male and (b) female

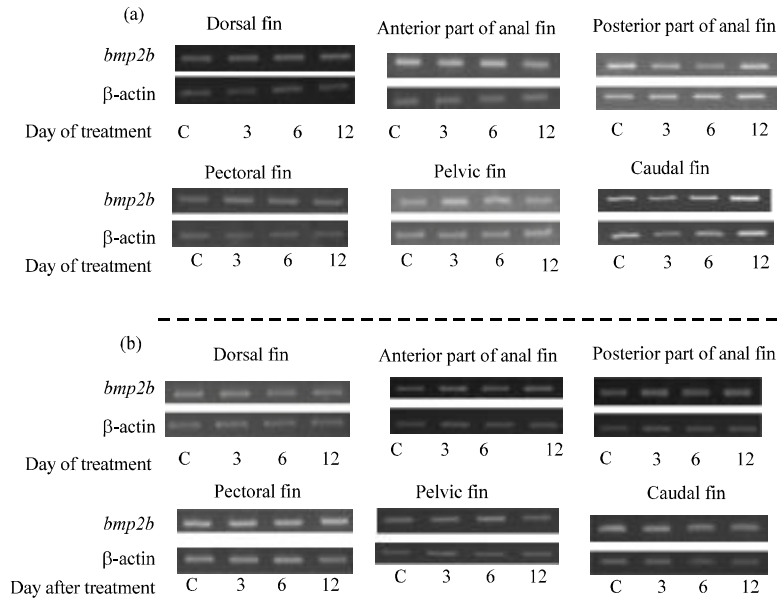


Fig. 3: Gel electrophoresis of RT-PCR analysis of *bmp2b* and β -actin mRNA expression in male and female medaka after treatments of 17 β -estradiol. C: Control. (a) male and (b) female

In male medaka, no significant difference in the expression levels of *bmp2b* mRNA was found in the dorsal, anterior part of anal, pectoral, pelvic and caudal fins by 3, 6 and 12 days between the 11-KT-treated group and the untreated control groups (Fig. 4a). In contrast, the expression levels of *bmp2b* mRNA were significantly increased in the posterior part of anal fins by 6 days after 11-KT treatments (Fig. 4a).

In female medaka, the expression levels of *bmp2b* mRNA were significantly higher in the dorsal fins by 3 days after treatment with 11-KT than in the untreated controls (Fig. 4b). In addition, in anal fins, significant increases in the expression levels of *bmp2b* mRNA were found in the anterior part at 12 days and in the posterior part at 3 days after treatment with 11-KT (Fig. 4b). In contrast, the expression levels of *bmp2b* mRNA were not significantly changed in the pectoral, pelvic and caudal fins by 3, 6 and 12 days after treatment with 11-KT (Fig. 4b).

In males and females, there was no phenotype change for any of the fin types after treatment of 11-KT.

Effect of 17 β -estradiol (E_2) on the levels of *bmp2b* mRNA expression in fins: In representative gel electrophoresis, alterations of *bmp2b* mRNA expressions were found in the pelvic fins at 3 and 6 days and posterior part of anal fins of males at 3 days after E_2 treatments (Fig. 2b, 3b).

In male medaka, no significant difference in the expression levels of *bmp2b* mRNA was found between the dorsal, anterior part of anal, pectoral and caudal fins by 3, 6 and 12 days after E_2 treatment and those from the untreated control groups (Fig. 5a). The expression levels of *bmp2b* mRNA were significantly decreased in the posterior part of anal fins by 6 days after E_2 treatments (Fig. 5a). In contrast, the expression levels of *bmp2b* mRNA were significantly increased in the pelvic fins in response to E_2 by 3 and 6 days (Fig. 5a).

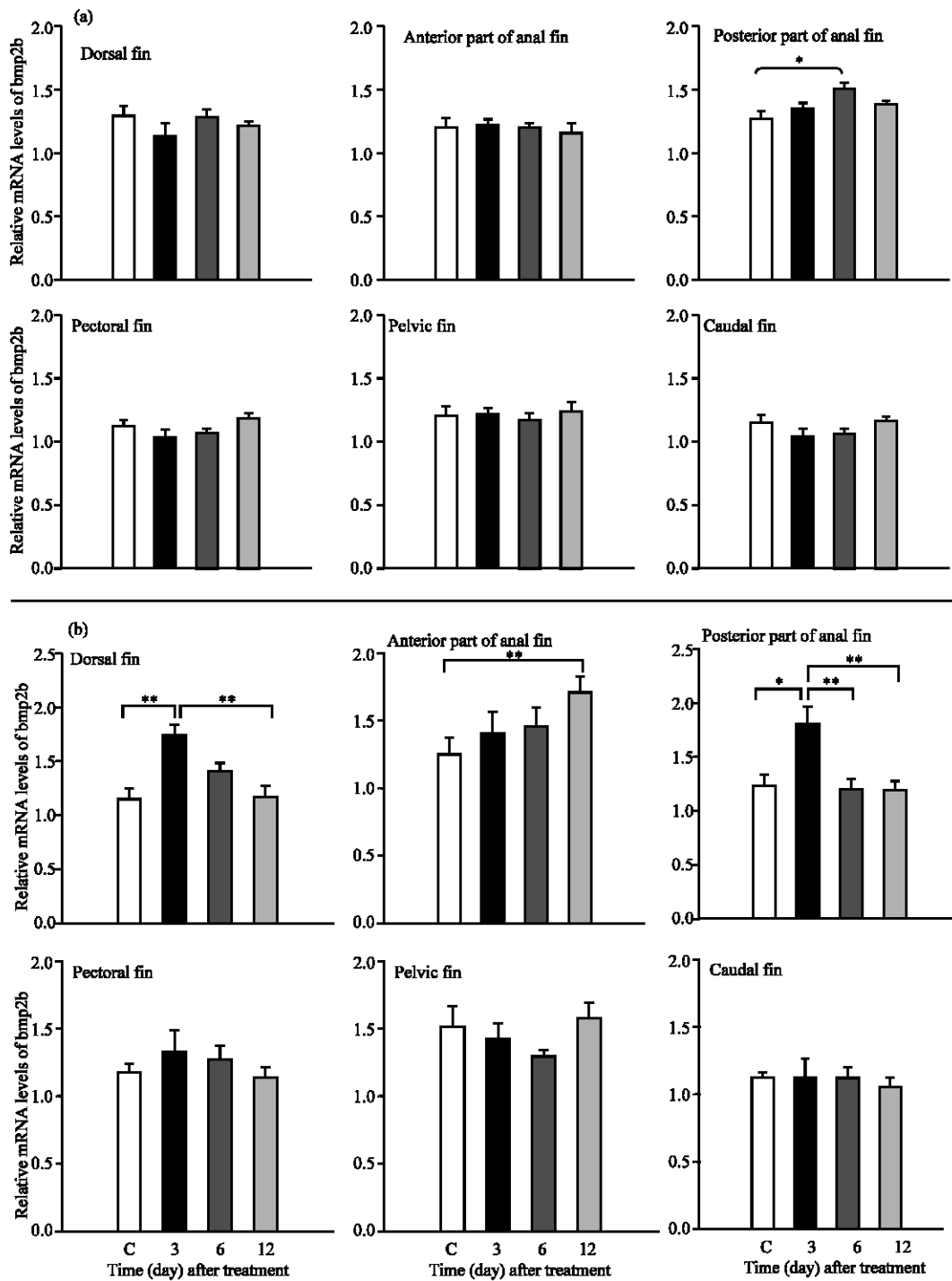


Fig. 4: Time-related effects of 11-ketotestosterone on *bmp2b* mRNA expression levels in fins of (a) male and (b) female medaka. Control groups (C) were treated with DMSO. The expression levels in each fin are relative values compared to the expression levels of β -actin mRNA (Mean \pm SE). Single- and double-asterisk symbols show $p < 0.05$ and $p < 0.01$, respectively (one-way ANOVA followed by Tukey's multiple comparison test). Each experimental group consisted of five samples of medaka fins

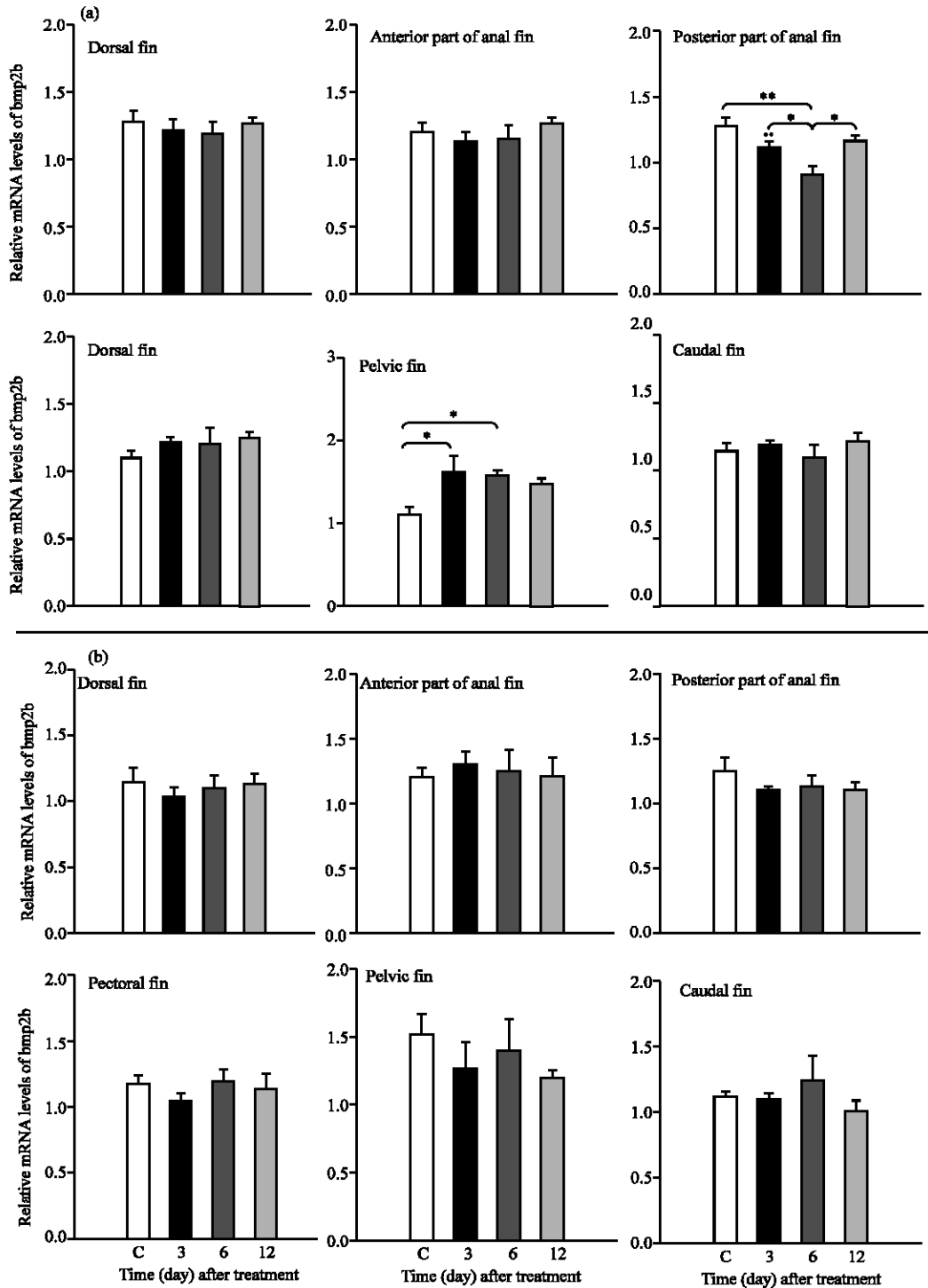


Fig. 5: Time-related effects of 17β -estradiol on *bmp2b* mRNA expression levels in fins of (a) male and (b) female medaka. Control groups (C) were treated with DMSO. The expression levels in each fin are relative values compared to the expression levels of β -actin mRNA (Mean \pm SE). Single- and double-asterisk symbols show $p < 0.05$ and $p < 0.005$, respectively (one-way ANOVA followed by Tukey's multiple comparison test). Each experimental group consisted of five samples of medaka fins

In female medaka, no significant differences in the expression levels of *bmp2b* mRNA were found in the dorsal, anal, pectoral, pelvic and caudal fins after E₂ treatment (Fig. 5b).

In males and females, no phenotype change was found for any of the fin types after treatment of E₂.

DISCUSSION

In the male, *bmp2b* expression was not different in the dorsal, anterior part of anal, pectoral and pelvic fins among time-related courses after treatment of 11-KT. Kawamoto (1969) reported that, in Japanese medaka, no significant difference was found in the length of the dorsal and anal fin of the adult male after treatment with androstenedione, suggesting that an exogenous androgen might be enough for the normal development of fin characters in an adult fish. Therefore, in an adult male, *bmp2b* may not require the effect of an additional androgen for the fin growth of dorsal, anterior part of anal and pectoral fins, where the secondary sex characters ultimately develop.

After treatment with 11-KT, however, *bmp2b* in the posterior part of the anal fins was more highly expressed at 6 days than in the control groups. It is known that, in males, the papillar processes on the anal fin continually develop through the end of the breeding season (May to August) and are increased the number by androgens (Yamamoto, 1975; Shima and Mitani, 2004). This suggests that, in the posterior part of the anal fin, the *bmp2b* may require an effect of exogenous androgen for inducing the development of papillar processes through the breeding seasons.

In caudal fins, *bmp2b* did not respond to 11-KT in the various time-related treatments. This result is reasonable because sex dimorphism is not indicated shown by differences in the length of the caudal fin between the male and female.

Effect of androgens is known to extend the length of the dorsal and anal fins of female Japanese medaka as a secondary sex character of the male (Kawamoto, 1969); however, no male phenotype was found in the dorsal, anterior and posterior part of anal fins of adult female after 11-KT treatment, although *bmp2b* was highly expressed in the dorsal and both parts of anal fins of females at 6 days after 11-KT treatment. Androgen may be required for *bmp2b* expression to act on those fins that are typical secondary sex character. No significant difference in *bmp2b* expression was found in the pectoral and pelvic fins after treatment of 11-KT. This suggests that, in adult female fish, an exogenous androgenic hormone may be not required for *bmp2b* expression in the normal development of at least those fins because estrogen is enough for *bmp2b* expression to act on them.

The development of dorsal and anal fins is suppressed by the administration of estrogen in the juvenile males of Japanese medaka (Iwamatsu, 1999; Tabata *et al.*, 2001). Therefore, *bmp2b* expression, which is necessary for fin development, might be low after estrogen treatment. On the other hand, the expression level of *bmp2b* was similar to that observed in the dorsal, anterior part of anal and pectoral fins of the adult male after treatment with E₂. This result suggests that, in the adult male, *bmp2b* regulation in the dorsal, anterior part of anal and pectoral fins may not be a target for estrogen.

After treatment with E₂, low expression of *bmp2b* in the posterior part of the anal fins was observed at 6 days. Estrogen is also known to suppress the development of papillar processes on the anal fin of males in Japanese medaka (Lin *et al.*, 2004). Therefore, *bmp2b* expression may be decreased by estrogen for inhibiting or retarding the development of papillar processes involving the posterior part of the anal fin.

In the pelvic fin, the *bmp2b* expressions were high at 3 and 6 days after treatment with E₂. It has been reported that, in Japanese medaka, the length of the pelvic fin of an adult male is elongated by the action of estrogen (Niwa, 1969). Therefore, estrogen may increase the expression level of *bmp2* in the pelvic fin to induce the development of the female phenotype in the pelvic fin of the adult male.

In the female, an exogenous estrogen may also be sufficient for the expression of *bmp2b* in the development of at least the fins because the expression level of *bmp2b* in the dorsal, both parts of anal, pectoral and pelvic fins was not different after treatment with E₂. Similarly to the treatment of 11-KT, no significant difference was detected in the *bmp2b* expression levels in the caudal fin after treatment with E₂.

Recently, Ngamniyom *et al.* (2009) reported that in Japanese and Thai medaka androgen receptor (AR) and estrogen receptor (ER) levels were higher in dorsal and anal fins than those in pectoral and caudal fins of male and female, respectively. However, no significant difference in Bmp2b level was found among fin types, suggesting steroid hormones was necessary for adequate *bmp* expression in the normal development of fins. This report of Ngamniyom *et al.* (2009) related to our results that no response to those hormones was also found in Bmp2b levels of the dorsal and anal fins any time-course experiment, after 11-KT treatments in male and E₂ treatments in female. Thus, AR and ER might not require sex steroid hormones for regulating Bmp2b level because AR and ER were excessively expressed for adequate Bmp2b expression in the normal development of at the dorsal and anal fin.

In teleost fish, it is known that the gonadal steroids, including androgen and estrogen, also exert positive or negative feedback and are associated with the regulation of hormone receptors on the metabolism of gonadotrophs (Borg, 1994; Linard *et al.*, 1998; Khan *et al.*, 1999; Larsson *et al.*, 2002). After treatment with sex steroid hormones, the expression level of *bmp2b* was normal in the posterior part of the anal and pelvic fins of the male at 12 days and in the dorsal and anal fins of the female at 6 days. Therefore, androgen and estrogen regulation on the expression of *bmp2b* in fins are also involved in positive or negative feedback control, suggesting that the excess expressions of *bmp2b* may recover to a normal expression by the negative feedback control of androgen and estrogen for the adequate expression of *bmp2b* in the development of fins. However, it is not clear whether androgen and estrogen regulation on *bmp2b* are mediated by androgen and estrogen receptors.

The present study demonstrated that *bmp2b* expressions were affected by 11-KT and E₂ depending on the sex and characters of the fin morphology in Japanese medaka. This is the first study in fish to investigate *bmp2b* regulation in response to sex steroid hormones across five tissues of fin. In addition, this study provides evidence for 11-KT- and E₂-mediated regulation on *bmp2b* expression in the secondary sex characters of fins.

REFERENCES

- Borg, B., 1994. Androgens in teleost fish. *Comp. Biochem. Physiol. C.*, 109: 219-245.
- Crotwell, P.L., A.R. Sommervold and P.M. Mabee, 2004. Expression of Bmp2a and Bmp2b in late-stage zebrafish median fin development. *Gene. Expr. Patterns.*, 5: 291-296.
- Egemi, N. and S. Ishii, 1956. Sexual differences in the shape of some bones in the fish, *Oryzias latipes*. *J. Fac. Sci. Tokyo Univ.*, 7: 563-571.
- Francis-West P.H., J. Parish, K. Lee and C.W. Archer, 1999. BMP/GDF-signalling interactions during synovial joint development. *Cell Tissue Res.*, 296: 111-119.

- Gonzalez-Doncel, M., M.S. Okihiro, C.F. Torija, J.V. Tarazona and D.E. Hinton, 2008. An artificial fertilization method with the Japanese medaka: Implications in early life stage bioassays and solvent toxicity. *Ecotoxicol. Environ. Saf.*, 69: 95-103.
- Hayashi, H., A. Nishimoto, N. Oshima and S. Iwamuro, 2007. Expression of the estrogen receptor alpha gene in the anal fin of Japanese medaka (*Oryzias latipes*) by environmental concentrations of bisphenol A. *J. Toxicol. Sci.*, 32: 91-96.
- Ide, H., T. Yoshida, N. Matsumoto, K. Aoki, Y. Osada, T. Sugimura and M. Terada, 1997. Growth regulation of human prostate cancer cells by bone morphogenetic protein-2. *Cancer Res.*, 57: 5022-5027.
- Iwamatsu, T., 1999. Convenient method for sex reversal in a freshwater teleost, the Medaka. *J. Exp. Zool.*, 283: 210-214.
- Iwamatsu, T., H. Nagamura, K. Ozato and Y. Wakamatsu, 2003. Normal growth of the see-through medaka. *Zool. Sci.*, 20: 607-615.
- Kagawa, H., K. Gen, K. Okuzawa and H. Tanaka, 2003. Effects of luteinizing hormone and follicle-stimulating hormone and insulin-like growth factor-I on aromatase activity and P450 aromatase gene expression in the ovarian follicles of red seabream, *Pagrus major*. *Biol. Reprod.*, 65: 1186-1194.
- Karim, M., P. Daniele, M. Arul, P.D. Simone and E. Marc, 2009. Medaka proteome study: Use of cross-species matching and expressed sequence tag data for protein identification. *J. Proteomics Bioinform.*, 2: 67-77.
- Kawamoto, N., 1969. Effects of androstenedione, 19-norethynyltestosterone, progesterone and medaka, *Oryzias latipes*. *Dev. Growth Differ.*, 11: 89-103.
- Khan, I.A., M.B. Hawkins and P. Thomas, 1999. Gonadal stage dependent effects of gonadal steroids on gonadotropin 2 secretion in the atlantic croaker (*Micropogonias undulatus*). *Biol. Reprod.*, 61: 834-841.
- King, J.A., E.E. Strom, P.C. Marker, R.J. Dileone and D.M. Kingsley, 1996. The role of BMPs and GDFs in development of region-specific skeletal structures. *Ann. N. Y. Acad. Sci.*, 885: 70-79.
- Larsson, D.G., T.S. Sperry and P. Thomas, 2002. Regulation of androgen receptors in atlantic croaker brains by testosterone and estradiol. *Gen. Comp. Endocrinol.*, 128: 224-230.
- Leon, A., S.J. The, L.C. Hall and F.C. The, 2007. Androgen disruption of early development in Qurt strain medaka (*Oryzias latipes*). *Aquat. Toxicol.*, 82: 195-203.
- Lin, B.L., S. Hagino, M. Kagoshima, S. Ashida and T. Iwamatsu *et al.*, 2004. An effective quantitative method (node count method) for evaluation of endocrine-disrupting chemicals based on secondary sexual characteristics of medaka, *Oryzias latipes*. *J. Jpn. Soc. Water Environ.*, 27: 47-51.
- Linard, B., T. Bailhache, A. Cheguillaume, I. Anglade, O. Kah, P. Jégo and C. Saligaut, 1998. Control of gonadotropin release in trout estrogen receptor-containing dopaminergic neurons and tyrosine hydroxylase expression. *Ann. N. Y. Acad. Sci.*, 839: 422-424.
- Lynne, R.P., 2008. A phylogenetic analysis and taxonomic revision of ricefishes, *Oryzias* and relatives (Belontiiformes, Adrianichthyidae). *Zool. J. Linn. Soc.*, 154: 494-610.
- Ngamniyom, A., W. Magtoon, Y. Nagahama and Y. Sasayama, 2009. Expression levels of hormone receptors and bone morphogenetic protein in fins of medaka. *Zool. Sci.*, 26: 74-79.
- Nilsson, S., S. Makela, E. Treuter, M. Tujague and J. Thomsen *et al.*, 2001. Mechanisms of estrogen action. *Physiol. Rev.*, 81: 1535-1565.

- Nimrod, A. and W. Benson, 1998. Reproduction and development of Japanese medaka following an early life stage exposure to xenoestrogens. *Aquat. Toxicol.*, 44: 141-156.
- Niwa, H., 1969. Inhibitory effect of male hormone on growth and regeneration of the pelvic fin in the medaka, *Oryzias latipes*. *Embryologia*, 4: 349-358.
- Oka, T.B., 1931. On the processes on the fin-rays of the male of *Oryzias latipes* and other sex characters of this fish. *J. Fac. Sci. Imp. Univ. Tokyo Sec.*, 2: 209-218.
- Okada, Y.K. and H. Yamashita, 1944. Experimental investigation of the manifestation of secondary sexual characters in fish, using the medaka (*Oryzias latipes*) as material. *J. Fac. Sci. Tokyo Univ.*, 6: 387-437.
- Olsson, P., A.H. Berg, J.V. Hofsten, B. Grahn and A. Hellqvist *et al.*, 2005. Molecular cloning and characterization of a nuclear androgen receptor activated by 11-ketotestosterone. *Reprod. Biol. Endocrinol.*, 3: 37-53.
- Ruggeri, B., M. Ubaldi., A. Lourdusamy, L. Soverchia and R. Ciccocioppo *et al.*, 2008. Variation of the genetic expression pattern after exposure to estradiol-17 β and 4-nonylphenol in male zebrafish (*Danio rerio*). *Gen. Comp. Endocrinol.*, 158: 138-144.
- Scholz, S., C. Kordes , J. Hamann and H.O. Gutzeit, 2004. Induction of vitellogenin in vivo and in vitro in the model teleost medaka (*Oryzias latipes*): Comparison of gene expression and protein levels. *Mar. Environ. Res.*, 157: 235-244.
- Shima, A. and H Mitani, 2004. Medaka as a research organism: Past, present and future. *Mech. Develop.*, 121: 599-604.
- Smith, A., F. Avaron, D. Guay, B.K. Padhi and M.A. Akimenko, 2006. Inhibition of BMP signaling during zebrafish fin regeneration disrupts fin growth and scleroblasts differentiation and function. *Dev. Biol.*, 299: 438-454.
- Tabata, A., S. Kashiwada, Y. Ohnishi, H. Shikawa, N. Miyamoto, M. Itoh and Y. Magara, 2001. Estrogenic influences of estradiol-17 beta, p-nonylphenol and bis- phenol-A on Japanese medaka (*Oryzias latipes*) at detected environmental concentrations. *Water Sci. Technol.*, 43: 109-116.
- Thomas, R., W.A. Anderson, V. Raman and A.H. Reddi, 1998. Androgen-dependent gene expression of bone morphogenetic protein 7 in mouse prostate. *Prostate*, 37: 236-245.
- Tiso, N., A. Filippi, S. Pauls, M. Bortolussi and F. Argenton, 2002. BMP signalling regulates anteroposterior endoderm patterning in zebrafish. *Mech. Dev.*, 118: 29-37.
- Uwa, H., 1971. The synthesis of collagen during the development of anal-fin processes in ethisterone-treated females of *Oryzias latipes*. *Dev. Growth Differ.*, 13: 119-124.
- Yamamoto, T., 1975. Medaka (killifish), Biology and stains. Keigaku Publishing Co, Q Tokyo, pp: 17-29.
- Yamamoto, T., F. Saatcioglu and T. Matsuda, 2002. Cross-talk between bone morphogenic proteins and estrogen receptor signaling. *Endocrinol.*, 143: 2635-2642.
- Zar, J.H., 1984. *Biostatistical Analysis*. 2nd Edn., Prentice-Hall, Englewood Cliffs NJ, pp:186-190.
- Zhang, Z.B., J.Y. Hu, S.X. Sai, Y.B. Zhao, C. Huang and X.J. Tian, 2008. Gene cloning, sequence analysis and tissue expression of estrogen-related receptor alpha (Erralpha) in Japanese medaka and its transcriptional responses after differential EDCs exposure. *Huan. Jing. Ke. Xue.*, 29: 3153-3158.
- Zhou, S., G. Turgeman, S.E. Harris, D.C. Leitman, B.S. Komm, P. Bodine and D. Gazit, 2003. Estrogens active bone morphogenetic protein-2 gene transcription in mouse mesenchymal stem cells. *Mol. Endocrinol.*, 17: 56-66.