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A study of the sex ratio and fin morphometry of the Thai medaka, *Oryzias minutillus*, inhabiting suburbs of Bangkok, Thailand

Arin Ngamniyom, Wichian Magtoon*, Yoshitaka Nagahama and Yuichi Sasayama*****

*Department of Life Science, Graduate School of Natural Science and Technology, Kanazawa University, Kanazawa, Ishikawa 920-1192, Japan, *Faculty of Science, Srinakharinwirot University, Bangkok 10110, Thailand, **Laboratory of Reproductive Biology, National Institute for Basic Biology, Okazaki, Aichi 444-8585, Japan and ***Division of Biodiversity, Institute of Nature and Environmental Technology, Kanazawa University, Kanazawa, Ishikawa 920-1192, Japan*

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Abstract Thai medaka were collected from 10 localities in the suburbs of Bangkok, Thailand. The sex ratios (male to female) were almost 1:1 in localities 1, 2, 4, 5, 7, and 9 and seemed to be normal. In contrast, in localities 3, 6, 8, and 10, the sex ratios were unbalanced; they were 1:2.9, 1:3.4, 1:3.0, and 1:2.8, respectively. Furthermore, irrespective of the normal or abnormal sex ratios, many individuals whose morphological characteristics did not identify them as males or females were found. The indexes showing the secondary sex characters of the sex-undeterminable individuals, namely, the values (%) of dorsal fin height (HD) divided by standard length (SL) and anal fin height (HA) divided by SL were between the values of normal males and normal females. From the sediment of two ponds in which the sex ratio was unbalanced, a low level of DDT was detected. Although no hermaphrodite gonads were histologically found from those sex-undeterminable individuals, it may be noted that the number of individuals which had testes was almost twice that of those with ovaries. Taking these results into considerations, it is surmised that some feminizing stresses may be triggered in males of the Thai medaka, probably by artificial chemicals.

Introduction

Recently, intersex or hermaphrodite individuals have been reported from wild freshwater environments of various regions of Europe; example include gudgeon (*Gobio gobio*), stickleback (*Gasterosteus aculeatus*), and barbel (*Barbus* sp.) (van Aerle *et al.*, 2001; Gercken and Sordyl, 2002; Vigano *et al.*, 2001, 2006). Bjerregaard *et al.* (2005) indicated that, in Denmark, in the wild roach (*Rutilus rutilus*) inhabiting streams receiving sewage effluent, the rates of intersex individuals are acutely increased. Kavanagh *et al.* (2004) noted that, in Canada, white perch (*Morone americana*) from the lower Great Lakes region had testes including ova in 8 individuals of 16 males examined. Furthermore, Liang *et al.* (2005) found that, in wild armored catfish (*Liposarcus multiradiatus*) inhabiting the Kaoping River of Taiwan, the sex ratio of males to females shifted abnormally to 1 : 2.4. In summary, in various freshwater teleosts, abnormal sex ratios and intersex individuals have been observed worldwide.

Thai medaka, *Oryzias minutillus*, are widely distributed in Thailand (Smith, 1945). The habitats of this species are shallow ponds, ditches, and paddy fields (Magtoon *et al.*, 1992). In general, the sex of species which belong to the genus *Oryzias* can be distinguished by the secondary sex characters of their fins (Okada and Yamashita, 1944). The dorsal and anal fins of the male are usually longer than those of the female.

In this study, the sex ratio and morphometry of the fin characters of a wild population of Thai medaka inhabiting suburbs of Bangkok, Thailand, were examined.

Materials and Methods

All individuals were captured with hand nets in 10 localities of two Provinces of Pathumthani and Ratchaburi near Bangkok, Thailand, on October 2004. Those fish were preserved in a 5% formaldehyde solution.

The distinction of the sex of each individual was conducted according to the criteria of the secondary sex characters of the fins of Japanese

*** Corresponding to: Dr. Yuichi Sasayama, Division of Biodiversity, Institute of Nature and Environmental Technology, Kanazawa University, Kanazawa, Ishikawa 920-1192, Japan. Tel.: 81-76-264-6307, Fax: 81-768-74-1644, Email: sasayama@kenroku.kanazawa-u.ac.jp

medaka (*Oryzias latipes*) (Okada and Yamashita, 1944). The fins were observed under a dissecting microscope (SMZ1000: Nikon Instech Co., Ltd., Japan).

Immature individuals less than 11 mm in standard length were not used in this study. Only adults larger than 11 mm were included. After determination of the sex, the gonads were dissected, re-fixed in Bouin's solution for 12h, and stored in 70% ethanol. Those specimens were dehydrated, embedded in paraffin, and sectioned serially at 6 μm. The sections were stained with hematoxylin and eosin to confirm the sex by histological examination as well.

The height and length of the dorsal and anal fins were measured using a digital caliper. The measurement was conducted according to a method of Habbs and Lagler (1958) (Fig. 1).

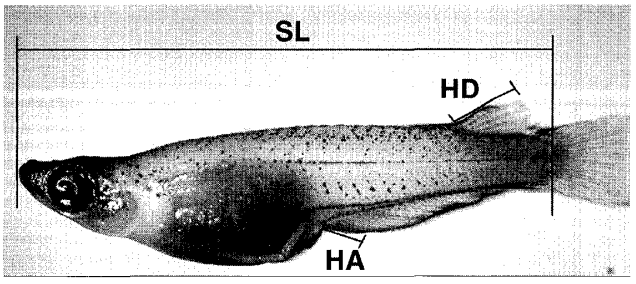

Fig. 1 Measurement of dorsal and anal fins. HA: height of anal fin; HD: height of dorsal fin; SL: standard length.

In this study, to evaluate the secondary sex characters of the male and the female, the following calculation was conducted. The dorsal fin height (HD) was divided by the standard length (SL) and multiplied by 100. This was defined as the HD/SL%. The anal fin height (HA) was divided by the SL and multiplied by 100. This was the HA/SL%. While conducting this examination, sex-undeterminable individuals were found. The HD/SL% and HA/SL% were calculated in these fish as well. The resulting values were compared among males, females, and sex-undeterminable individuals.

The percentages of sex-undeterminable individuals occupying the population were calculated on the basis of the following formula:

$$\text{Percentage of sex-undeterminable} = \frac{\text{Total number of sex-undeterminable}}{\text{Total number of males + females + sex-undeterminable}} \times 100$$

In this study, the DDT concentration was examined in 10 localities using DDT in a soil test kit (EnviroGard^{TM} Test Kit, Strategic Diagnostics Inc., USA).

All data were statistically analyzed using software (SPSS ver. 12 Inc., USA). For examining the significance of differences, the parametric one-way ANOVA, and nonparametric *chi*-squared were adopted.

Results

Sex ratio and percentage of sex-undeterminable individuals

The sex ratios of males to females were normal (almost 1:1) in localities 1, 2, 4, 5, 7 and 9. In contrast, the number of females was significantly larger than that of males in localities 3, 6, 8, and 10 (*Chi*-squared test, $P<0.05$). Sex-undeterminable individuals were found in all localities, irrespective of the normal or abnormal sex ratios (Table 1).

Table 1. Sex ratios of males to females and percentages of sex-undeterminable individuals

	Number of specimens			Sex ratio	Percentage
local.	male	female	sex-undeter.	male:female	Sex-undeter.
1	73	93	26	1.0 : 1.3	13.5
2	27	39	8	1.0 : 1.4	10.8
3	7	20	5	1.0 : 2.9*	15.6
4	80	101	18	1.0 : 1.3	9.1
5	52	58	15	1.0 : 1.1	12.0
6	5	17	6	1.0 : 3.4*	21.4
7	21	16	2	1.3 : 1.0	5.1
8	5	15	4	1.0 : 3.0*	16.7
9	10	9	5	1.1 : 1.0	20.8
10	6	17	5	1.0 : 2.8*	17.9

*Significantly different in the number between males and females ($P<0.05$)

Table 2. HD/SL% and HA/SL% among males, females, and sex-undeterminable individuals

Sex	HD/SL% Mean ± SE	HA/SL% Mean ± SE
males	20.2 ± 0.3	23.1 ± 0.2
sex-undeterminable individuals	15.6 ± 0.1*	18.1 ± 0.2*
females	13.5 ± 0.2	15.7 ± 0.2

* Significantly different from the values of males and females ($P<0.001$)

Fin morphometry

The dorsal fin of males was longer than that of females and reached the caudal peduncle (Fig. 2A, B). The shape of the anal fin of males seemed to be irregularly square (Fig. 2A). The anal fin of females seemed to be more square (Fig. 2B). The dorsal fin of sex-undeterminable individuals was shorter than that of male but longer than that of female (Fig. 2C). The anal fin of sex-undeterminable individuals was similar to that of males in shape but smaller in size (Fig. 2C).

The values of HD/SL % and HA/SL % of males were 20.2 ± 0.3 (Mean ± SE) and 23.1 ± 0.2, respectively (Table 2). The values of HD/SL % and HA/SL % of females were 13.5 ± 0.2 and 15.7 ± 0.2, respectively. Those values in males were significantly larger than those of females (one-way ANOVA test, $P<0.001$). The values of HD/SL % and HA/SL % of sex-undeterminable individuals were 15.6 ± 0.1 and 18.1 ± 0.2, respectively. These values were larger than those of females but smaller than those of males. Both values of sex-undeterminable individuals were significantly different from those of males and females (one-way ANOVA test, $P<0.001$).

Gonads of sex-undeterminable individuals

The testes or ovaries of sex-undeterminable individuals were not conspicuously different histologically from those of normal males or females. Testes including ova were not observed. In these fish, however, the number of individuals with testes was larger than that of those with ovaries (Table 3) in spite of the fact that, in localities 3, 6, 8, and 10, the number of females was larger than that of males (Table 1).

DDT concentrations in the soil of ponds

DDT was detected in localities 3 and 6 (each 0.2 ppm), in which the sex ratios were abnormal.

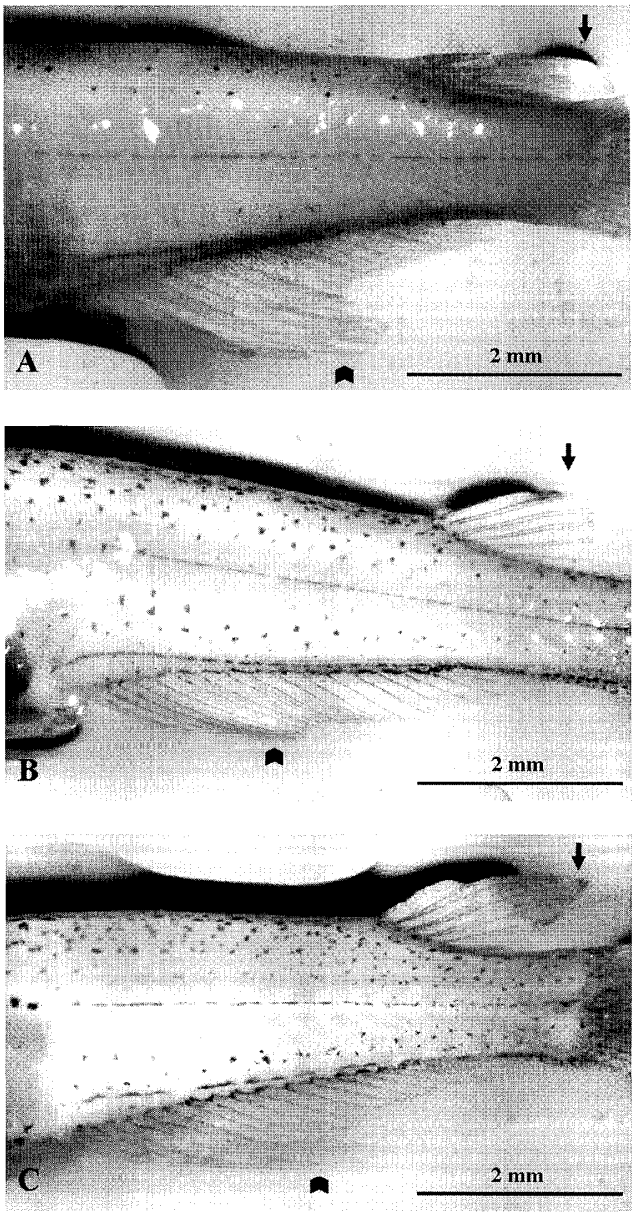

Fig. 2 Characteristics of the dorsal fin (arrow) and anal fin (arrow head). A, male; B, female; and C, sex-undeterminable.

Discussion

In mosquito fish (*Gambusia affinis*) inhabiting Hawksbury-Nepean River, Australia, the anal fin of males collected from a discharge point of

Table 3. Number of gonads determined as testes or ovaries in the sex-undeterminable individuals.

local.	Number of sex-undeter.	Gonads determined as testes	ovaries	Proportion of testes to ovaries
1	26	16	10	1.6 : 1.0
2	8	5	3	1.7 : 1.0
3	5	4	1	4.0 : 1.0
4	18	10	8	1.3 : 1.0
5	15	6	9	1.0 : 1.5
6	6	6	0	—
7	2	1	1	1.0 : 1.0
8	3	3	0	—
9	5	3	2	1.5 : 1.0
10	5	5	0	—
Total	93	59	34	

a sewage treatment plant has been reported to be reduced in length (Batty and Lim, 1999). In this species, the anal fin is a secondary sex character and plays a role as a gonopodium. It is also known that in another species of mosquito fish (*Gambusia holbrooki*), the length of the fourth anal fin ray in males and females caught at secondary sewage treatment was somewhat more elongated than in normal ones (Leusch *et al.*, 2006). In Japanese medaka (*Oryzias latipes*), an abnormal anal fin which appears to be female-like has been observed in males exposed to 100 ppb of *p*-nonylphenol (Tabata *et al.*, 2001). These facts suggest that the secondary sex characters expressed on the fins are easily affected by artificial chemicals.

On the other hand, a high level of DTT (10-360 ng/g lipid weight) was concentrated in catfish (*Protosus canius*) collected from the Bangkok region in Thailand (Kannan *et al.*, 1995). It is a fact that organochlorine pesticides, such as DDT, have been used in paddy fields and ponds in parks for a long time in Thailand (Boonyatumanond *et al.*, 2002). Furthermore, in 55 of 90 wells, DDT has been detected in three provinces, including Ratchaburi Province, in Thailand (Thapinta and Hudak, 2003). In this study, DDT was detected in 2 localities, although the level of pollution was rather low.

Vigano *et al.* (2001), Barnhoorn *et al.* (2004), and Hinck *et al.* (2006) reported the presence of hermaphrodites in barbell (*Barbus plebefus*), sharptooth catfish (*Clarias gariepinus*), and smallmouth bass (*Micropterus dolomieu*), respectively, because oocytes were observed to be scattered in their testicular tissue. In this study, however, a histological examination of the gonads of these individuals did not yield any indication that the sex-undeterminable individuals were hermaphrodites. In this study, although the sex ratio was unbalanced in some localities, it is not clear whether the number of sex-undeterminable individuals was directly related to the imbalance or not because there was a locality in which the number of sex-undeterminable individuals was high but the sex ratio was normal. In locality 9, however, there is a possibility that the sex ratio may show an abnormality in the future.

It may be worth to note that, in sex-undeterminable fish, the rate of testes to ovaries was almost twice. Taking all these data into consideration, it is surmised that some feminizing stresses may be triggered in males of the Thai medaka, probably caused by artificial chemicals.

References

Barnhoorn, I.E., M.S. Bornman, G.M. Pieterse and J.H. Vuren (2004) *Environ. Toxicol.*, **19**: 603–608.

Batty, J. and R. Lim (1999) *Arch. Environ. Contam. Toxicol.*, **36**: 301–307.

Bjerregaard, L.B., B. Korsgaard and P. Bjerregaard (2005) *Ecotoxicol. Environ. Saf.*, **26**: 1–8.

Boonyatumanond, R., A. Jaksakul, P. Puncharoen and M.S. Tabucanon (2002) *Environ. Pollut.*, **119**: 245–252.

Gercken, J. and H. Sordyl (2002) *Mar. Environ. Res.*, **54**: 651–655.

Hinck, J.E., C.J. Schmitt, V.S. Blazer, N.D. Denslow, T.M. Bartish, P.J. Anderson, J.J.

Coyle, G.M. Dethloff and E. Tillitt (2006) *Sci. Total Environ.*, **366**: 549–578.

Hubbs, C.J. and K.F. Lagler (1958) *Bull. Cranbrook Ins. Sci.*, **26**: 1–213.

Kannan, K., S. Tanabe and R. Tatsukawa (1995) *Environ. Sci. Tech.*, **29**: 2673–2683.

Kavanagh, R.J., G.C. Balch, Y. Kiparissis, A.J. Niimi, J. Sherry, C. Tinson and C.D. Metcalfe (2004) *Environ. Health Perspect.*, **112**: 898–902.

Leusch, F.D., H.F. Chapman, G.W. Kay, S.R. Gooneratne and L.A. Tremblay (2006) *Arch. Environ. Contam. Toxicol.*, **50**: 562–74.

Liang, S.-H., H.-P. Wu and B.-S. Shieh (2005) *Zool. Studies*, **44**: 252–259.

Magtoon, W., N. Nadee, T. Higashitani, K. Takata and H. Uwa (1992) *J. Fish Biol.*, **41**: 489-497.

Okada, Y. and H. Yamashita (1944) *J. Fac. Sci. Tokyo Univ.*, **6**: 383–437.

Smith, H. (1945) *Bull. U.S. Nat. Mus.*, **188**: 1-662.

Thapinta, A. and P.F. Hudak (2003) *Environment Inter.*, **29**: 87–93.

Tabata, A., S. Kashiwada, Y. Ohnishi, H. shikawa, N. Miyamoto, M. Itoh and Y. Magara (2001) *Water Sci. Technol.*, **43**: 109–116.

van Aerle, R., T.M. Nolan, S. Jobling, L.B. Christiansen, J.P. Sumpter and C.R. Tyler (2001) *Environ. Toxicol. Chem.*, **20**: 2841–2847.

Vigano, L., A. Arillo, S. Bottero, A. Massari and A. Mandich (2001) *Sci. Total Environ.*, **26**: 189–194.

Vigano, L., A. Mandich, E. Benfenati, R. Bertolotti, S. Bottero, E. Porazzi and E. Agradi (2006) *Arch. Environ. Contam. Toxicol.*, **51**: 641–651.

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