

THE PRESERVATION AND UTILIZATION OF WILD POPULATIONS OF THE MEDAKA *ORYZIAS LATIPES*

A. Shima¹, A. Shimada¹, J. Komura¹, K. Isa¹, K. Naruse¹, M. Sakaizumi² and N. Egami³

¹ Zoological Institute, Faculty of Science, University of Tokyo, Tokyo 113, ² Tokyo Metropolitan Institute of Medical Sciences, Tokyo 113, ³ National Institute for Environmental Studies, Ibaraki 305, Japan

When the first paper on the allozymic variations in wild populations of the Medaka *Oryzias latipes* was published by Sakaizumi *et al.* (1980), the number of the localities from which the fish were collected was 18. Since then, a continuing effort, primarily by Sakaizumi, has been made to collect the fish from other localities in Japan and China. At present, 95 Japanese and 2 Chinese wild populations have been preserved in the Zoological Institute, Faculty of Science, University of Tokyo. The main purpose of this brief note is to present a map showing the collection sites of the fish (Fig. 1). A very short summary about the work already performed and an introduction of studies under way utilizing these stocks will also be given. The first full listing of the stocks and their origins and allozymic characteristics will appear elsewhere (Shima *et al.*, 1986).

The serial number corresponding to the number on the map, full name, abbreviated name and classification* of each stock are as follows:

1 : Aomori (AO) N*, 2 : Kamikita (KK) N, 3 : Hirosaki (HR) N, 4 : Niigata (NI) N, 5 : Joetsu (JO) N, 6 : Ryotsu (RY) N, 7 : Kosugi (KS) N, 8 : Kaga (KG) N, 9 : Ichinoseki (IC) S/E, 10 : Fujisawa (FS) S/E, 11 : Kesen-numa (KN) S/E, 12 : Iwaki (IK) S/E, 13 : Urizura (UR) S/E, 14 : Mito (MT) S/E, 15 : Tsunozumi (TZ) S/E, 16 : Hokota (HK) S/E, 17 : Shimodate (SD) S/E, 18 : Mibu (MB) S/E, 19 : Hasuda (HS) S/E, 20 : Sakura (SR) S/E, 21 : Odawara (OW) S/E, 22 : Mishima (MM) S/E, 23 : Shizuoka (SZ) S/E, 24 : Iwata (IT) S/E,

25 : Kozakai (KO) S/E, 26 : Suwa (SW) S/E, 27 : Oomachi (OM) S/E, 28 : Nagashima (NS) S/E, 29 : Ueno (UN) S/E, 30 : Tanabe (TB) S/I, 31 : Ayabe (AB) S/I, 32 : Kobe (KB) S/I, 33 : Okayama (OK) S/I, 34 : Tokushima (TI) S/I, 35 : Yasuura (YS) S/I, 36 : Matsuyama (MY) S/I, 37 : Misho (MS) S/-, 38 : Tottori (TT) S/S, 39 : Matsue (ME) S/S, 40 : Hamada (HM) S/S, 41 : Tsuma (TM) S/S, 42 : Hagi (HG) S/S, 43 : Toyoura (TU) S/S, 44 : Kudamatsu (KD) S/I, 45 : Sanyo (SY) S/I, 46 : Hisayama (HY) S/KN, 47 : Izuhara (IH) S/KN, 48 : Kazusa (KZ) S/-, 49 : Reihoku (RH) S/-, 50 : Hiwaki (HW) S/KS, 51 : Kikai (KI) S/R, 52 : Nago (NG) S/R, 53 : Gushikami (GK) S/R, 54 : Tsuruoka (TO) N, 55 : Sabae (SB) N, 56 : Tsuruga (TG) N, 57 : Obama (OB) N, 58 : Kinomoto (KM) S/I, 59 : Maizuru (MZ) N, 60 : Amino (AM) H, 61 : Toyooka (TY) H, 62 : Kochi (KC) S/-, 63 : Kiyotake (KT) S/-, 64 : Izumi (IM) S/KS, 65 : Beppu (BP) S/I, 66 : Nobeoka (NB) S/-, 67 : Oodate (OD) N, 68 : Yokote (YK) N, 69 : Yamagata (YG) N, 70 : Inawashiro (IN) N, 71 : Hakodate (HD) ND, 72 : Arita (AR) S/KN, 73 : Toyota (TA) S/E, 74 : Iida (ID) S/E, 75 : Tokyo (TK) S/E, 76 : Oku (OU) S/I, 77 : Misumi (MI) S/S, 78 : Kukizaki (KE) ND, 79 : Kobuchizawa (KU) S/E, 80 : Yamaguchi (YM) ND, 81 : Hirosaki-1 (HR-1) N, 82 : Yamagata-1 (YG-1) N, 83 : Miyatsu (MA) ND, 84 : Mineyama (MN) ND, 85 : Kumihama (KH) ND, 86 : Takeno (TN) ND, 87 : Kasumi (KA) ND, 88 : Hamasaka (HA) ND, 89 : Iwami (IW) ND, 90 : Fukube (FK) ND, 91 : Kawai (KW) ND, 92 : Hongu (HO) ND, 93 : Shingu (SG) ND, 94 : Takamatsu (TS) ND, 95 : Tanohama (TH) ND, 101 : Shanghai (SH) C, 102 : Beijing (BJ) C.

*Symbol (s) for classification : *Northern*

Population=N, *Southern Population* ; Eastern Subpop.=S/E, Inland Sea Subpop.=S/I, San-in Subpop.=S/S,Northern Kyusyu Subpop.=S/KN, Southern Kyusyu Subpop.= S/KS,Ryukyuu Subpop.=S/R,Unclassified Subpop.=S/-,*Hybrid Population*=H, *Chinese Population*=C,ND=not yet determined.

Discoveries : The following findings were obtained by an extensive search for allozymic variations at 21 loci and regional differentiation in wild populations of the Medaka. (For details see the original papers.) 1) The wild populations of the Medaka in Japan can be divided into two major groups, the Northern Population and the Southern Population. 2) Genetically, the Northern Population is extremely homogeneous, while the Southern Population is quite varied. These two major groups are strictly isolated from each other. 3) The Southern Population can further be classified into 7 subpopulations. 4) A good correlation was found between the boundary of the Northern and the Southern Populations and the geographic distribution of mountain barriers, indicating that the isolation of the two major groups is essentially due to mountain barriers. 5) The Hybrid Populations between the Northern and the Southern Populations could be found around Wakasa Bay, which is at the western end of the boundary between the two major areas (For the details of the above mentioned results, see Sakaizumi *et al.*, 1980 : Egami *et al.*, 1982 : Sakaizumi *et al.*, 1983 : Sakaizumi, 1984.) . In the search for the origin of the Madaka, 5 species of fishes of the genus *Oryzias* were examined for protein polymorphisms with the result that they can be divided into 3 groups, coinciding with the previous result obtained from karyotype analysis by Uwa and his colleagues (Sakaizumi, 1985). In addition to these allozymic studies, other differences in wild populations were preliminarily examined, including the distribution pattern of skin melanophores, the secondary sexual characteristics and the egg sizes (Egami *et al.*, 1985) and sexual behavior (Shimada *et al.*, 1985).

In progress : Since April 1985, the Zoological

Institute, Faculty of Science, the University of Tokyo, has been in charge of the maintenance of these wild populations under the special financial help of the Ministry of Education, Science, and Culture, Japan. The primary purpose of this project is to preserve these wild stocks as gene resources. However, we believe it also important to develop further studies on the Medaka to characterize it as a potentially useful laboratory animal for research in the field of basic biology. This would include searching for the origin of the species, as well as radiation biology, cancer and aging. The following is a very brief introduction of our work-in-progress which utilizes the Medaka including these wild stocks. 1) Further allozymic studies are being continued by examining alleles of loci other than those previously used. 2) Screening for mutants has begun employing markers such as developmental and behavioral abnormalities, coloration, altered sensitivities to mutagens and carcinogens like ionizing as well as ultraviolet radiations and chemicals, and short and /or long life span (Naruse *et al.*, 1985 : Shimada *et al.*, 1986 : Komura and Shima,1986). 3)We recently succeeded in the fusion of cultured fish cells to human cells and further in transfecting the cultured fish cells with pSV2-*gpt* and obtained the evidence for the expression of the *E. coli gpt* gene in the fish cells (Isa and Shima, 1985 : Isa and Shima, 1986). This is, to the best of our knowledge, a breakthrough in obtaining the transformants of cultured fish cells. The procedures developed here would be applicable for future studies of the Medaka on a DNA basis.

Finally, with regard to the significance of the preservation and utilization of wild stocks, studies by two groups are quite suggestive. The first is a work by Moriwaki and his associates on the genetic status of Japanese wild mice (Moriwaki *et al.*, 1981 : Yonekawa *et al.*, 1982 : Miyashita *et al.*, 1985). The second is to design sound conservation programs in Arizona for an endangered fish, *Poeciliopsis occidentalis*, utilizing knowledge of the distribution of genetic variation (Vrijenhoek *et al.*, 1985).

ACKNOWLEDGEMENTS

This research project has been supported by the Ministry of Education, Science, and Culture, Japan. We are grateful to Dr. Kazuo Moriwaki of the National Institute of Genetics for his invaluable advice and encouragement. Thanks are also due to Miss Izumi Sakura for her excellent laboratory assistance and preparation of the map.

REFERENCES

- Egami, N., Sakaizumi, M., Mitani, H., Shimada, Y., Kyono-Hamaguchi, Y. and Kirita, A. (1982) *Medaka*, **1**, 3-4.
- Egami, N., Nakaya, M., Sakaizumi, M., Shimada, A., and Naruse, K. (1985) *Zool. Sci.*, **2**, 996.
- Isa, K. and Shima, A. (1985) *Zool. Sci.*, **2**, 895.
- Isa, K. and Shima, A. (1986) *J. Radiat. Res.*, **27**, in press.
- Komura, J. and Shima, A. (1986) *J. Radiat. Res.*, **27**, in press.
- Miyashita, N., Suzuki, K. and Moriwaki, K. (1985) *Japan. J. Cancer Res. (Gann)*, **76**, 1141-1145.
- Moriwaki, K., Shiroishi, T., Yonekawa, H., Miyashita, N. and Sagai, T. (1981) In : *Teratocarcinoma and Embryonic Cell Interactions*. eds. T. Muramatsu, G. Gachelin, A. A. Moscona and Y. Ikawa (Japan Sci. Soc. Press and Academic Press Japan, Tokyo) pp. 157-175.
- Naruse, K., Komura, J., and Shima, A. (1985) *Zool. Sci.*, **2**, 895.
- Sakaizumi, M., Moriwaki, K., and Egami, N. (1980) *Proc. Japan Acad.*, **56** Series B, 448-451.
- Sakaizumi, M., Moriwaki, K., and Egami, N. (1983) *Copeia*, 311-318.
- Sakaizumi, M. (1984) *Zool. Sci.*, **1**, 795-800.
- Sakaizumi, M. (1985) *Copeia*, 521-522.
- Shima, A., Shimada, A., Sakaizumi, M., and Egami, N. (1986) *J. Fac. Sci., Univ. Tokyo*, in press.
- Shimada, A., Egami, N., and Sakaizumi, M. (1985) *Zool. Sci.*, **2**, 1001.
- Shimada, A., Shima, A., Egami, N. and Sakaizumi, M. (1986) *J. Radiat. Res.*, **27**, in press.
- Vrijenhoek, R.C., Douglas, M.E. and Meffe, G. K. (1985) *Science*, **229**, 400-402.
- Yonekawa, H., Moriwaki, K., Gotoh, O., Miyashita, N., Migita, S., Bonhomme, F., Hjorth, P., Petras, M.L. and Tagashira, Y. (1982) *Differentiation*, **22**, 222-226.

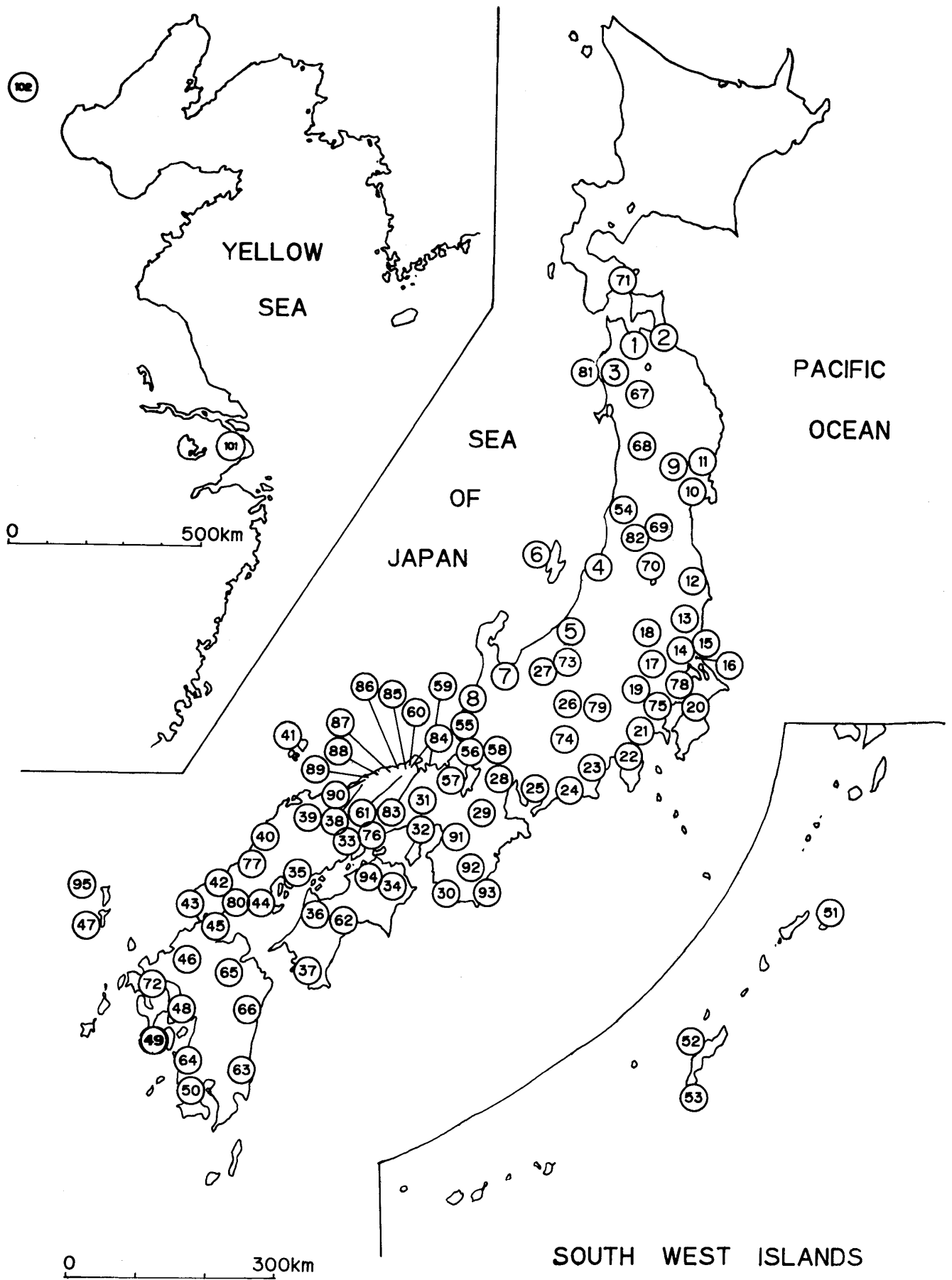


Fig.1. Collection localities of wild Medaka. Numbers refer to the text.