In memory of Kenjiro Ozato (1938–2006)

Kenjiro Ozato, Emeritus Professor at Nagoya University, passed away on 21 September 2006 at the age of 68. After retiring from the University in March 2002, he became an honorary member of his former laboratory, the Laboratory of Freshwater Fish Stocks at the Bioscience and Biotechnology Center of Nagoya University, where he continued with his research on medaka until he passed away. His career as a researcher was characterized by originality and a pioneering spirit.

He graduated from the Department of Zoology in the Faculty of Science at Kyoto University in March 1962 and studied the development of chick embryos[1,2] under Dr. Tokindo Okada at the Graduate School of Science. Shortly after being awarded the position of Assistant Professor in the Biological Laboratory at Yoshida College at the University in 1967, he visited the Department of Embryology at the Carnegie Institution in the US where he studied cell culture techniques under Dr. James. D. Ebert. Upon returning to Japan, he began working on fish after being prompted in that direction by Dr. Okada who told him that, "The time of fish biology will come in fifteen years". While the field of fish biology was still in its infancy at that time in the 1970s, there is no need to attest how it has developed since then. Kenjiro Ozato was to become a pioneer in this field.

He established an *in vitro* cell culture system for fish pigment cells using goldfish[3,4] and tried to analyze the genetic mechanisms associated with pigment cell transformation in the *Xiphophorus* fish-hybrid melanoma system[5,6]. However, *Xiphophorus* is viviparous, complicating *in vitro* analyses of the genetic mechanisms responsible for the cell transformation. Consequently, he shifted his attention to medaka, an oviparous fish, and attempted to introduce foreign genes into medaka eggs in order to elucidate the functions of genes *in vivo*. In 1986, he successfully demonstrated the expression of the chick δ-crystallin gene in medaka embryos (Fig. 1)[7], the first successful example of transgenic expression in fish and sub-

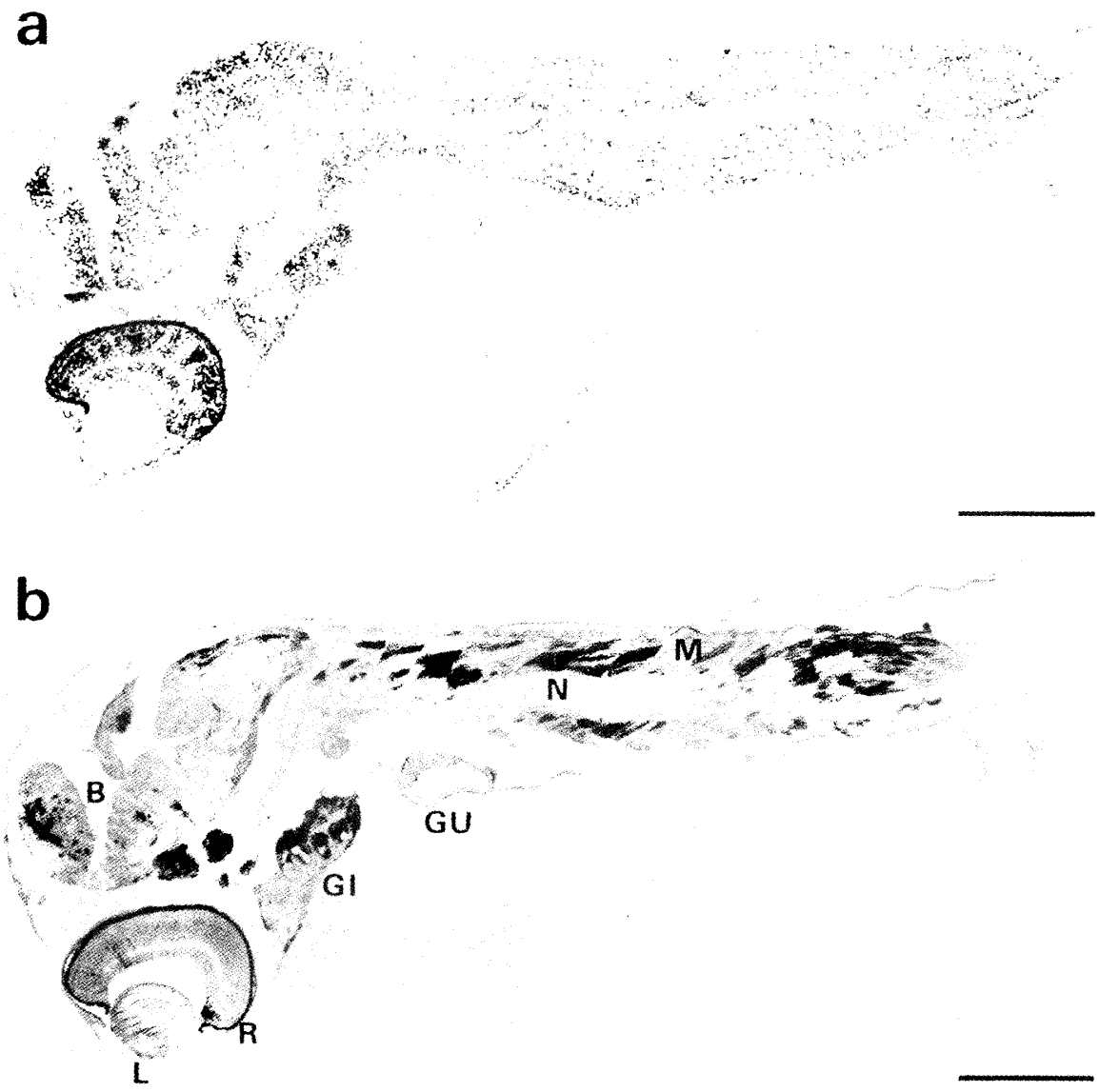

Fig. 1. Histological detection of chicken δ-crystallin gene and its product protein in a transgenic medaka embryo. (a) DNA-DNA *in situ* hybridization. (b) Immunohistological staining of δ-crystallin. B, brain; GI, gill; GU, gut; L, lens; M, muscle; N, notochord; R, retina. The bars represent 500μm. (*Cell Dif.* **19**: 237–244, 1986)

sequently produced a great volume of work in the field of developmental engineering using medaka, particularly in transgenics[8,9] and nuclear transfer[10,11]. He highly evaluated medaka as a vertebrate model that had been established in Japan.

In 1994, he moved to the Laboratory of Freshwater Fish Stocks at the Bioscience Center at Nagoya University. The Center housed collections of natural medaka mutants collected by the late Professor Hideo Tomita[12], and while the collections had been maintained, the culture facilities were outdated and in a state of disrepair. He spent a lot of time to rebuild the facilities (Fig. 2) during the first three years in the University. On the other hand, he began genetic analyses using the collections of medaka. First, he chose a double anal fin mutant, *Da*, for the analysis, which was to become one of the representative mutants showing abnormal morphology in dorso-ventral patterning in the collections [13,14]. In successfully establishing a transparent medaka strain, the see-through medaka[15], in which the body wall is transparent and the internal organs can be observed from the outside which avoids the need for dissection, he was also eager to advance the use the medaka as a disease model. His study on medaka polycystic kidney disease[16] was well received by researchers in a variety of medical fields, and this study was to constitute his final contribution to fish biology.

He also endeavored to introduce medaka to other research fields around the world. He collaborated

Fig. 2. Management of the medaka farm in the Laboratory of Freshwater Fish Stocks at the Bioscience Center.

with investigators assessing the influence of chemical substances on wild animal taxa using medaka[17–20], and in conjunction with the Ministry of Environment, helped convene two international symposiums concerned with medaka research in Nagoya in 2000[21] and 2001[22]. He contributed to research efforts involving the use of medaka in space exploration, and by receiving Asian researchers in his laboratory[23], visiting them in their own countries, and by organizing a workshop at Nagoya University in 1996[24], he sought to help develop Asian scientists. He maintained fond relations with medaka research communities, not only in Japan, but also in other countries in the world, and encouraged and supported them. It was because of his considerable achievements and attitudes toward scientific research, that he was regarded, respected and endeared by researchers both nationally and abroad.

Yuko Wakamatsu

Laboratory of Freshwater Fish Stocks,
Bioscience and Biotechnology Center,
Nagoya University,
Nagoya 464-8601, Japan.

References

1. Ozato, K. (1969) Cell cycle in the primitive streak and the notochord of early chick embryos. *Embryologia*, **10**: 297–311.
2. Ozato, K. (1969) Cell cycle in the primary embryonic induction of chick embryos. *Embryologia*, **10**: 312–317.
3. Ozato, K. (1976): Pigment dispersion in cultured goldfish erythrophores by dubutyryladenosine-3', 5'-monophosphate. *Zool. Mag.*, **85**: 262–264.
4. Ozato, K. (1977) Effects of ACTH, adenyl compounds, and methylxanthines on goldfish erythrophores in culture. *Gen. Comp. Endocrinol.*, **31**: 335–342.
5. Ozato, K. and Y. Wakamatsu (1981) Age-specific incidence of hereditary melanomas in the *Xiphophorus* fish hybrids. *Carcinogenesis*, **2**: 129–133.
6. Ozato, K. and Y. Wakamatsu (1983) Multistep genetic regulation of oncogenes expression in fish hereditary melanoma. *Differentiation*, **24**: 181–190.
7. Ozato, K., H. Kondoh, H. Inohara, T. Iwamatsu, Y. Wakamatsu and T. S. Okada (1986) Production of transgenic fish: introduction and expression of chicken δ-crystallin gene in medaka embryos. *Cell Dif.*, **19**: 237–244.
8. Ozato, K., K. Inoue and Y. Wakamatsu (1992) Medaka as a model of transgenic fish. *Mol. Marine Biol. Biotech.*, **1**: 346–354, 1992.
9. Kinoshita, M., H. Toyohara, M. Sakaguchi, K. Inoue, S. Yamashita, M. Satake, Y. Wakamatsu and K. Ozato (1996) A stable line of transgenic medaka (*Oryzias latipes*) carrying the CAT gene. *Aquaculture*, **143**: 267–276.
10. Wakamatsu, Y., B. Ju, I. Pristyazhnyuk, K. Niwa, T. Ladygina, M. Kinoshita, K. Araki and K. Ozato (2001) Fertile and diploid nuclear transplants derived from embryonic cells of medaka, *Oryzias latipes*. *Proc. Natl. Acad.*

Sci. USA, **98**: 1071–1076.

11. Bubenshchikova, E., B. Ju, I. Pristyazhnyuk, K. Niwa, E. Kaftanovskaya, M. Kinoshita, K. Ozato and Y. Wakamatsu (2005) Generation of fertile and diploid fish, medaka (*Oryzias latipes*), from nuclear transplantation of blastula and four-somite-stage embryonic cells into nonenucleated unfertilized eggs. *Cloning and Stem Cells*, **7**: 255–264.
12. Tomita, H. (1992) The lists of the mutants and strains of the medaka, common gambusia, silver crucian carp, goldfish, and golden venus fish maintained in the Laboratory of Freshwater Fish Stocks, Nagoya University. *Fish Biol. J. Medaka*, **4**: 45–47.
13. Tamiya, G., Y. Wakamatsu and K. Ozato (1997) An embryological study of ventralization of dorsal structures in the tail of medaka (*Oryzias latipes) Da* mutants. *Dev. Growth Dif.*, **39**: 531– 538.
14. Ohtsuka, M., N. Kikuchi, H. Yokoi, M. Kinoshita, Y. Wakamatsu, K. Ozato, J. Wittbrodt, H. Takeda, H. Inoko and M. Kimura (2004) Possible roles of zic1 and zic4, identified within the medaka Double anal fin (*Da*) locus, in dorsoventral patterning of the trunk-tail region (related to phenotypes of the *Da* mutant). *Mech. Dev.* **121**: 873–882.
15. Wakamatsu, Y., S. Pristyazhnyuk, M. Kinoshita, M. Tanaka and K. Ozato (2001) The see-through medaka: A fish model that is transparent throughout life. *Proc. Natl. Acad. Sci. USA*, **98**: 10046– 10050.
16. Mochizuki, E., K. Fukuta, T. Tada, T. Harada, N. Watanabe, S. Matsuo, H. Hashimoto, K. Ozato and Y. Wakamatsu (2005) Fish mesonephric model of polycystic kidney disease in medaka (*Oryzias latipes*) pc mutant. *Kidney Intl.*, **68**: 23–34.
17. Wakamatsu, Y. and K. Ozato (2002) Medaka (*Oryzias latipes*) as a fish model for endocrine-disrupting substance testing. *Environ. Sci.*, **9**: 419–426.
18. Scholz, S., K. Kurauchi, M. Kinoshita, Y. Oshima, K. Ozato, K. Schmirmer and Y. Wakamatsu (2005) Analysis of effects by quantification of green fluorescent protein in juvenile fish of the ChgH-GFP transgenic medaka. *Environ. Toxicol. Chem.*, **24**: 2553–2561.
19. Hano, T., Y. Oshima, M. Kinoshita, M. Tanaka, N. Mishima, T. Ohyama, T. Yanagawa, Y. Wakamatsu, K. Ozato and T. Honjo (2007) Quantitative bioimaging analysis of gonads in olvas-GFP/ST-II YI medaka (transgenic *Oryzias latipes*) exposed to ethinylestradiol. *Environ Sci. Tech.*, **41**: 1473–1479.
20. Kashiwada, S., K. Goka, H. Shiraishi, K. Arizono, K. Ozato, Y. Wakamatsu and D. E. Hinton (2007) Age-dependent i*n situ* hepatic and gill CYP1A ativity in the see-through medaka (*Oryzias latipes*), *Comp. Biochem. Physiol. C: Toxicol. Pharmacol.*, **145**: 96–102.
21. International Symposium *Endocrine-Disrupting Substance Testing in Medaka*, March 17–20, 2000, Nagoya, Japan.
22. International Workshop on *Standardization of Endocrine-Disrupting Testing in Medaka*, June 6–8, 2001, Nagoya, Japan.
23. Tsai, H.-J., S.-H. Wang, K. Inoue, S. Takagi, M. Kimura, Y. Wakamatsu and K. Ozato (1995) Initiation of the transgenic *lacZ* gene expression in medaka (*Oryzias latipes*) embryos. *Mol. Marine Biol. Biotech.*, **4**: 1–9.
24. Japan Society for the Promotion of Science Asian Science Seminar, *Reproductive Biology and Biotechnology in Aquatic Animals-Asia '96*, March 16–28, 1996, Nagoya University.