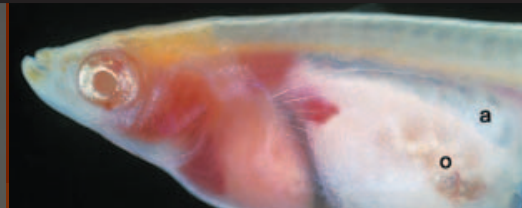
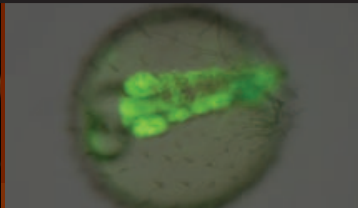
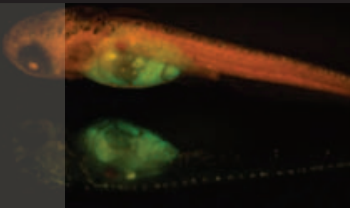




Medaka

Biology, Management, and Experimental Protocols

Editors: Masato Kinoshita, Kenji Murata,
Kiyoshi Naruse, and Minoru Tanaka



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Preface

Medaka, *Oryzias latipes*, is the tiny, fresh water, rice-field fish. In Japan, scientists have used medaka as a model animal, especially since the work of Aida in 1921. Since his work, many Japanese scientists have strived to establish specific strains of medaka and to advance additional experimental methodologies using medaka fish as a model animal. These developments have resulted in the accumulation of basic biological knowledge of medaka, which has contributed to the discovery of new biological facts in both human and other animal systems. They have helped to identify the functional mechanisms of many newly discovered phenomena in areas of both basic and applied research. Furthermore, recent advances in medaka genomics have provided new insights not only into vertebrate genome evolution but also into basic biology, ecological science, medical science, and agricultural science, by comparative analyses with the substantial genomic information that now exists for other vertebrates such as humans, mice, puffer fish, stickleback, and zebrafish. Another recent important activity is a large-scale mutagenesis screening of mutants with specific developmental defects. Currently, more than 500 mutants are available to scientific community (Chapter 10).

There are several principal advantages of using the medaka model for vertebrates over that of the more commonly used zebrafish system (Chapter 1). First, the medaka genome is smaller (ca 800 Mb) (Chapter 9), being about half the size of the zebrafish genome, and one-third that of the human genome. Second, there are highly polymorphic inbred medaka strains derived from genetically distinct wild population (SNP (single nucleotide polymorphism) rate between strains is 4%) and those can be used for both mutagenesis screening, genetic mapping, and Quantitative Trait Loci (QTL) analysis (Chapter 9).

Other advantages include:

1. The database of information on the reproductive biology, fertilization, gamete biology, developmental biology, and molecular/structural biology of molecules in medaka is well developed (Chapter 3, Chapter 9, and <http://shigen.lab.nig.ac.jp/wgr/wgr/siteList.jsp>).
2. Medaka live-fish resources including standard strains, transgenic lines, mutants, and wild stocks from different wild populations; and related species are also available from National BioResource Project Medaka (NBRP Medaka; <http://www.shigen.nig.ac.jp/medaka/>) supported by MEXT, Japan.
3. High-quality draft genome sequences are available from Ensembl, UCSC genome browser, and UT genome browser (Chapter 9 and web information in DVD), and the genomic resources (cDNA and BAC/ fosmid clones) accumulated during medaka EST and genome sequencing projects are also available to the public from NBRP Medaka.

This book's purpose is to familiarize scientists worldwide with the advantages of using medaka in experimental designs, the ease of conducting experiments with medaka, and to stimulate their progress in research by adopting medaka as a model animal.

The book describes and provides basic knowledge on how to use medaka as a model animal. It demonstrates how to successfully raise medaka fish in stable culture conditions, and describes its anatomy and embryonic development. It describes the accumulated technologies using the fish, including use as a molecular tool in the fields of life science, evolution, ecology, and toxicology. It provides an informational bridge to span the varied research disciplines and abilities ranging from undergraduate education through the level of senior researcher. The selection of contributing authors is intended to demonstrate the wide range of biological and molecular tools and systems in which medaka is used. Authors were chosen because of their expertise and demonstrated ability to conduct experiments involving medaka, and because they are recognized pioneers in the use of medaka as the model animal in their scientific fields. The authors were also asked to describe their experimental protocols in detail and the rationale for the chosen protocols in achieving their conceptual goals.

The book's format is designed to capture the thoughts and methods of researchers using medaka as the model animal, and to make this expertise accessible to students, beginning researchers, and senior researchers who would like to incorporate medaka fish as the model animal in their own works. For this reason the reader is initially guided through the necessary background information, and then presented with step-by-step specifics for each protocol described. The information includes reagents, instrumentation, and other essential requirements. It is anticipated that this highly practical format will permit the reader to bring new concepts into personal practice in a more efficient manner.

The use of medaka fish as a model animal requires experimental insight and an ability for practical troubleshooting of experimental designs. Of equal importance is an overall appreciation of the power and limits of using medaka fish as the model animal. To assist the readers to visualize and understand the medaka fish and the research protocols, we have provided a DVD as an appendix. For example, we have provided illustrations and a digital movie of the entire process of microinjection, a procedure that is difficult to understand just by simply reading the protocol.

The preparation of this book would not have been possible without the dedication of the contributing authors and all the people who have supported and encouraged the authors. The staff at Wiley-Blackwell publishers, Mr. Justin Jeffryes and Ms. Shelby Hayes Allen, have demonstrated great patience with our efforts, and have provided excellent guidance and assistance. Finally, we also express our thanks to the people who assisted to accomplish this project.

Reference

1. Aida, T. (1921) On the inheritance of color in a fresh-water fish *Aplocheilus latipes* Temminck and Schlegel, with special reference to sex-linked inheritance. *Genetics* 6, 554–573.

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Medaka

Biology, Management, and Experimental Protocols

Chapter 1

History and Features of Medaka

Medaka, *Oryzias latipes*, is a small egg-laying secondary freshwater fish native to East Asian countries, primarily Japan, Korea, Taiwan, and China. This fish is a member of the atherinomorpha taxon Beloniformes. Other members of order Beloniformes are halfbeaks and garfish, many of which are marine fish. This suggests that the common ancestors of medaka and relatives were marine fish and some species of this group adapted to the freshwater environment. This is one possible reason why there are several species adapted to freshwater or seawater within the same genus (Inoue and Takei, 2003).

1.1 History

Medaka has been reared as an ornamental fish since the Edo period. Figure 1-1 shows the Ukiyoe painting called “Medaka Scooping,” published in 1767–1768. Two girls are scooping medaka and putting them in a small glass tank. In 1835, Motohisa Mori described three medaka strains: wild type, orange-red type, and white type (see also Figure 1-7). The orange-red type strain has a mutation at the *b* locus and the white-type strain is a double mutant at the *b* and *r* loci. As it is not feasible to isolate a double mutant from wild type medaka, it is likely that relatively large numbers of the orange-red type strain were cultured at the end of Edo period.

Medaka was first described in Siebold’s *Fauna Japonica*, and originally assigned to the genus *Poecilia* by Temminck and Schlegel in 1846. After this description, medaka has been a favorite experimental animal for researchers in Japan and other countries. Several important achievements have been made with the color mutants of medaka described by Motohisa Mori. Aida (1921) found the sex-limited inheritance of the *r* locus that controls the expression of orange pigment cells (xanthophores). Yamamoto (1953, 1975) established the d-rR strain and demonstrated the artificial induction of sex reversal with estrogen and androgen to fish of the d-rR strain. The d-rR strain showed body-color dimorphism, with the male orange-red and the female white. Because the *R* allele is on the Y chromosome and the *r* allele is on the X chromosome, genetic sex can be distinguished by body color. Studies of medaka sex determination and differentiation finally resulted in the identification of the primary sex-determination gene, *DMY* (Matsuda et al., 2002; Nanda et al., 2002). This gene is the second “primary sex determination gene” isolated in vertebrates, and is the functional equivalent of the *Sry* gene in mammals. The establishment of an efficient method for making transgenic medaka was also an important achievement (Ozato et al., 1986), and the establishment of several inbred lines from genetically different natural populations is unique to medaka (Hyodo-Taguchi and Egami, 1985). From around 2000, several important studies to establish genetic/genomic resources have been archived. A large-scale Expressed Sequence Tag (EST) analysis was done by Kimura et al. (2004). There



Figure 1-1. Ukiyoe painting “Medaka Scooping” by Harunobu Suzuki, 1767–1768. Two girls are scooping medaka and putting them into a small glass tank. Courtesy of <http://www.japanism.net/>.

are now over 210,000 cDNA/EST sequences deposited in the public DNA database (DDBJ/EMBL/Genbank), and summarized up to about 39,000 unique sequences. A genome-wide linkage map was also established (Naruse et al., 2000, 2004a). The medaka genome sequencing project commenced in 2002 and the draft genome sequence was published by Kasahara et al. (2007). All genetic/genomic data are now open to the public through the UT genome browser (<http://medaka.utgenome.org/>), Ensembl genome browser (http://www.ensembl.org/Oryzias_latipes/index.html), and UCSC genome browser (<http://genome.ucsc.edu/cgi-bin/hgGateway>). In addition to these genome resources, mutagenesis screening for the isolation of mutants with a specific phenotype during embryonic development has been conducted by several groups. About 500 mutants with specific phenotypes have been established, and projects to identify the causal gene of the mutants have been conducted in several laboratories

(Furutani-Seiki et al., 2004; Yokoi et al., 2007). Since these studies and activities, medaka has become a representative model for vertebrates.

1.2 Phylogeny

1.2.1 Phylogeny and distribution of medaka and relatives

Teleostei is the most diversified group in the vertebrates, comprising over 26,800 species in 4278 genera, 448 families, and 40 orders (Nelson, 2006). This group includes other model organisms, such as zebrafish (*Danio rerio*), pufferfish (*Takifugu rebripes* and *Tetraodon nigroviridis*), and the three-spined stickleback (*Gasterosteus aculeatus*). Among these fishes, zebrafish (Cypriniformes) belong to the basal teleostean lineage (Ostariophysii) (Inoue et al., 2003). In contrast, the medaka (Beloniformes), pufferfish (Tetraodontiformes), and stickleback (Gasterosteiformes) are members of the higher teleosts (Percomorpha) (Miya et al., 2005; Figure 1-2). The approximate divergence times of the medaka are estimated to be 485 million years ago (mya) with mammals, 324 mya with zebrafish, and 191 mya with the lineage leading to pufferfish and the stickleback (Yamanoue et al., 2006).

The family Adrianichthyidae is a small group native to Asia, containing four genera, *Oryzias* with 20 species, *Adrianichthys* with two species, *Horaichthys* with one species, and *Xenopoecilus* with three species. Before the 1980s, this family was placed in the order Cyprinodontiformes. However, Rosen and Parenti (1981) indicated a monophyly of Adrianichthyidae within the order Beloniformes, based on characters of the gill arch skeleton hyoid apparatus. Nelson (1994, 2006) agreed with this relationship and placed Adrianichthyids within Beloniformes. A recent molecular phylogeny based on entire mitochondrial DNA sequences also supports a monophyly of the group containing the medaka and other beloniform fishes (Miya et al., 2005). Medaka is currently regarded as a member of Beloniformes.

Most fishes in family Adrianichthyidae are confined to freshwater, such as brooks, ponds, canals, paddy fields, and lakes, but some species are found in brackish- and seawater along the coast. Fishes in this family have a wide distribution, from India to Japan, and south along the Indo-Australian archipelago across Wallace's line to Timor, Sulawesi, and Luzon (Table 1-1). Above all, *Oryzias dancena*, *O. javanicus*, and

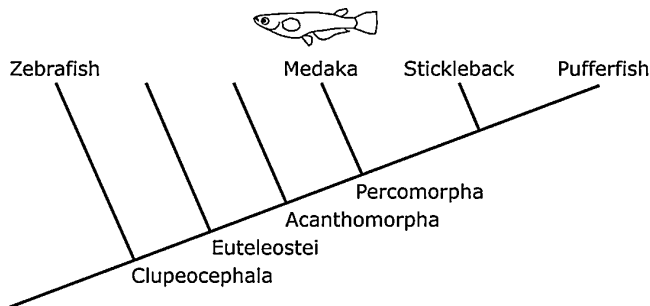


Figure 1-2. A simplified phylogenetic tree of Teleostei. The lineage leading to zebrafish is the most basally diverged group, while medaka, stickleback, and pufferfishes are members of Percomorpha.

4 **Table 1-1.** Geographic distribution of adrianchthyid fishes.

Species	Distribution	Reference
<i>Adrianchthys kroyti</i> Weber, 1913	Lake Poso in Sulawesi	Parenti and Soeroto (2004)
<i>Adrianchthys roseni</i> Parenti & Soeroto, 2004	Lake Poso in Sulawesi	Parenti and Soeroto (2004)
<i>Horatichthys setnai</i> Kulkarni, 1940	W India	Talwar and Jhingran (1991)
<i>Oryzias carmaticus</i> Jerdon, 1849	E India and Bangladesh	Roberts (1998)
<i>Oryzias celebensis</i> Weber, 1894	S Sulawesi	Parenti and Soeroto (2004)
<i>Oryzias curvinotus</i> Nichols & Pope, 1927	N Vietnam, Hainan, and S China	Uwa and Parenti (1988)
<i>Oryzias dancena</i> Hamilton, 1822	E India, a Bangladesh, Myanmar, and N Malaya	Roberts (1998)
<i>Oryzias haugiangensis</i> Roberts, 1998	S Vietnam	Roberts (1998)
<i>Oryzias hubbsi</i> Roberts, 1998	W Java	Roberts (1998)
<i>Oryzias javanicus</i> Bleeker, 1854	Java, Sumatra, Malaya, Borneo, Sulawesi, and Lombok	Kottelat et al. (1993) and Roberts (1998)
<i>Oryzias latipes</i> Temminck & Schlegel, 1846	Japan, Korea, Taiwan, and China	Uwa and Parenti (1988)
<i>Oryzias luzonensis</i> Herre & Ablan, 1934	N Luzon	Formacion and Uwa (1985)
<i>Oryzias marmoratus</i> Aurich, 1935	Lakes Towuti, Mahalona, and Wawontoa in Sulawesi	Kottelat (1990b)
<i>Oryzias matanensis</i> Aurich, 1935	Lake Matano in Sulawesi	Kottelat (1990b)
<i>Oryzias mekongensis</i> Uwa & Magtoon, 1986	NE Thailand	Roberts (1998)
<i>Oryzias minutillus</i> Smith, 1945	Thailand	Roberts (1998)
<i>Oryzias nebulosus</i> Parenti & Soeroto, 2004	Lake Poso in Sulawesi	Parenti and Soeroto (2004)
<i>Oryzias nigrimas</i> Kottelat, 1990a	Lake Poso in Sulawesi	Parenti and Soeroto (2004)
<i>Oryzias orthognathus</i> Kottelat, 1990a	Lake Poso in Sulawesi	Parenti and Soeroto (2004)
<i>Oryzias pectoralis</i> Roberts, 1998	Laos	Roberts (1998)
<i>Oryzias profundicola</i> Kottelat, 1990b	Lake Towuti in Sulawesi	Kottelat (1990b)
<i>Oryzias timorensis</i> Weber & de Beaufort, 1922	Timor	Uwa and Parenti (1988)
<i>Oryzias uwai</i> Roberts, 1998	Myanmar	Roberts (1998)
<i>Xenopoeilus oophorus</i> Kottelat, 1990a	Lake Poso in Sulawesi	Parenti and Soeroto (2004)
<i>Xenopoeilus poptae</i> Weber & de Beaufort, 1922	Lake Poso in Sulawesi	Parenti and Soeroto (2004)
<i>Xenopoeilus sarasinorum</i> Popta, 1905	Lake Lindu in Sulawesi	Parenti and Soeroto (2004)