

**ASIAN MEDAKAFISHES: IDEAL MODELS FOR ANALYSES
OF SEAWATER ADAPTABILITY**

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Introduction

The genus *Oryzias* is a group of small fishes that consists of more than 14 diverse species including Japanese medaka (*O. latipes*), a well-known laboratory animal (Roberts, 1998). This genus is expected to offer good models for studying mechanisms of osmotic adaptation because it contains freshwater (FW) and seawater (SW) species. In this study, we compared SW adaptability of four species, *O. javanicus*, *O. dancena*, *O. latipes* and *O. marmoratus* inhabiting different osmotic environments (Table 1). SW adaptability was evaluated using several indices including survival of adult fish after transfer from FW to SW and fertilization and hatching rates in SW (Inoue and Takei, 2002).

Survival of adult fish after transfer from FW to SW

In the first experiment, the survival rate of adult fish after direct transfer from FW to SW or 50%SW was compared. *O. javanicus* and *O. dancena* revealed high adaptability to sudden increase in the ambient osmotic pressure and survived the transfer to SW. In contrast, all *O. marmoratus* and *O. latipes* transferred to SW died within 2 hr and 9.5 hr, respectively. *O. marmoratus* was not adaptable even to 50% SW; all fish died within 25 hr after transfer. On the other hand, *O. latipes* survived in 50% SW. It was also found that prior acclimation of *O. latipes* in 50% SW for 24 hr allowed them to survive in SW. This result suggests that *O. latipes* is potentially euryhaline but only lacks the adaptability to a sudden increase of the ambient osmotic pressure. This characteristic is especially interesting for studying osmoregulatory mechanisms because it becomes possible, using this species, to study the functions of fast-acting osmoregulatory factors such as natriuretic peptides that may act immediately after encountering the environment with different salinity (Takei and Hirose, 2002).

Table 1. Adaptability to seawater in four *Oryzias* species

Species	<i>Oryzias marmoratus</i>	<i>Oryzias latipes</i>	<i>Oryzias dancena</i>	<i>Oryzias javanicus</i>
Geographic Distribution	Lake Towuti in Sulawesi Island	Japan, Korea and China	Pakistan–Thailand	Malaysian Peninsula–Indonesia
Habitat	FW	FW, BW	BW, FW	BW, SW
Immediate adaptation to SW	–	–	++	++
Slow adaptation to SW	–	++	++	++
Spawning in SW	N.D.	++	++	++
Fertilization in SW	N.D.	+	++	++
Sperm motility in SW	±	±	++	++
Embryonic development in SW	–	+	++	++
Hatching in SW	–	++	+	++

++, possible; +, possible in low rate; ±, hardly possible; –, impossible; N.D., no data.

Fertilization rates in FW and SW

O. latipes spawned eggs everyday while other three species spawned several times a week in FW. More than 90% eggs of all four species were found to be fertilized. *O. latipes*, *O. dancena* and *O. javanicus* also spawned eggs in SW as frequently as in FW. Most eggs of *O. dancena* and *O. javanicus* spawned in SW were fertilized as in FW. However, 84% of *O. latipes* eggs spawned in SW remained unfertilized. By microscopic observation, we found that the sperm of *O. javanicus* and *O. dancena* is motile both in FW and SW. In contrast, the sperm of *O. latipes* and *O. marmoratus* is motile in FW but hardly motile in SW. It is well known that the sperms of SW fish are activated when they are exposed to water with osmotic pressure or concentration of ions such as K^+ higher than the seminal plasma, while those of FW fish are activated by lower osmotic pressure or ion concentration (Billard and Cosson, 1992). The sperm of *O. dancena* and *O. javanicus*, which is motile both in FW and in SW, is quite unique and offers a new model to study sperm activation mechanisms.

Hatching rates in FW and SW

The normally fertilized eggs of the four species spawned in FW were incubated in SW or FW and the embryonic development and hatching were monitored. The hatching rate in SW was the highest in *O. javanicus* (84%), followed by *O. latipes* and *O. dancena* (60%), and the lowest in *O. marmoratus* (0%). The low hatching rate of *O. marmoratus* and *O. latipes* embryos in SW was due to the death of embryos before reaching the hatching stage. In contrast, embryos of *O. dancena* developed successfully to hatching stage but failed to hatch out. One possible cause of the low hatching ability of *O. dancena* in SW is that hatching enzyme does not function in high salinity. Molecular cloning of cDNAs encoding hatching enzymes of *O. javanicus* and *O. dancena* is now in progress to compare enzyme activities under the different salt concentration.

Future studies

O. latipes is known to have various advantages for experimental purposes, e.g., its small size, frequent spawning, and short generation time, etc. We found that other three species, share such advantages with *O. latipes*. Thus, it seems possible to apply to these species experimental approaches including foreign gene transfer (Ozato *et al.*, 1989). We expect that the application of such experimental techniques to various *Oryzias* species (e.g., overexpression of natriuretic peptide genes in *O. latipes*, transfer of *O. javanicus* hatching enzyme genes into *O. dancena*, etc.) would bring us new

information to understand how fish has developed diverse mechanisms to adapt to hyper- and hypo-osmotic environments.

References

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