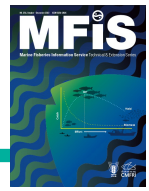

Protocol developed for the hatchery production of marine model fish *Oryzias dancena* (Hamilton, 1822)

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A non-human species that has been widely studied in the laboratory for helping scientists understanding the biological processes is called a 'model organism'. Their inherent characteristics include easy maintenance, anatomical and physiological similarities to humans, high reproductive rate and large offspring number, and a short generation time (the time period from birth to reproduction). Among the fishes, zebra fish (*Danio rerio*) is widely accepted globally as a model organism for research in freshwater providing valuable insights into cell, tissue, organ, and system level. However, for studies involving marine and brackish waters, it is not advisable to superimpose the cues obtained from freshwater model organism, which necessitates the use of a species with marked euryhaline characteristics. *Oryzias dancena* (Hamilton, 1822), with tolerance to varying salinities, are small and easy to bred, and are capable of being maintained in large numbers; therefore offers as an excellent alternate fish model organism for experiments in marine systems.

Oryzias dancena (Hamilton, 1822), belonging to the order Beloniformes and family Adrianichthyidae and popularly known as Indian ricefish or Asian medaka, was successfully bred at Visakhapatnam and Vizhinjam Regional Centres of CMFRI, and three generations were produced. A protocol has been developed for continuous hatchery production of this species. *O. dancena* has a short generation time with the individuals becoming sexually mature and spawning just 60-65 days after hatching. This work was carried out based on the suggestion of the Chairperson of the institute's Research Advisory Committee (RAC), Dr B. Meenakumari so as to meet the requirements of CMFRI for a marine model fish for fish gut microbiome studies. This fish is well studied as a model organism for drug screening, regenerative medicine, genotoxicity studies, pharmacological, genomic and microbiome studies and transgenics in different laboratories across the world and scientists are focussing on developing its breeding technology. In addition to the short generation time, attributes of the medaka that encouraged its laboratory use include small size, external sexual dimorphism, relatively large and clear eggs, longer development time from fertilization till hatching than many other teleosts, ease of maintenance in aquaria, wide availability and reasonable cost (Parenti, 2008). A euryhaline species with strong osmoregulatory capabilities, *O. dancena* is found basically in the coastal and brackishwater areas and is a native of India, Bangladesh, Sri Lanka and Myanmar. With a reported maximum length of only 3.1 centimetres (in the present study, 3.76 cm), this egg-laying teleost is closely related to Japanese killifish or Japanese medaka (*Oryzias latipes*), which is widely used as a model species for biological research. Japanese medaka specimens were sent abroad the US space shuttle 'COLUMBIA' for 15 days and it was the first vertebrate animal species to mate in space in 1994 (Parenti, 2008; In-Seok Park, 2021).

The male individuals of Indian ricefish can be identified easily by the slender body, second dorsal with long filamentous 3rd to 5th rays and the 4th being most extended, some of them tipped with a white marking; and with an elongated and fringed anal fin. In contrast, the female has a broader belly and typical second dorsal and anal fins. They can be easily spotted with recently spawned eggs attached to their bellies when they are

Fig.1. Male and female Oryzias dancena

mature (Fig.1). The distal margin of the anal fin is convex in males, straight or slightly concave in females. The genital papilla in males forms a short tube, while in females it is bilobed.

Live specimens of *O. dancena* were collected from West Bengal (total 356 nos) with the help of Mr Rahul G. Kumar, an aquarium hobbyist and were transported to Visakhapatnam and Vizhinjam by air. They were given prophylactic treatment and stocked in 300 l capacity tanks filled with seawater of salinity 30-32 ppt (PSU) with *in-situ* biological filter at Vizhinjam, and in 1 t FRP tanks filled with freshwater and aeration at Visakhapatnam. Taxonomic identity was confirmed based on morpho-meristic characteristics (Parenti, 2008) and partial sequence information (572bp) of the Cytochrome C Oxidase subunit I (COI) gene. Around 120 numbers of sub-adults which survived in both centres together after 5 days of stocking were fed twice daily with pelleted feed of 300 μm particle size or *Artemia* nauplii (2 nos/ml). These specimens matured within a month of arrival without any hormonal treatment and started spawning. Spawning normally occurs in the early morning hours (~5.00 am), with males darkening in colouration and defending small, temporary territories against one another while attempting to entice females. Egg carrying females are easy to spot with eggs attached to the body. The adhesive eggs are typically expelled as a single mass (28-32 eggs/clutch) and fertilised simultaneously, after which they continue to hang from the genital pore of the female for a period before eventually getting deposited singly or in small clumps among vegetation or other suitable media or they fall off to the bottom in a day or two.

At the Vizhinjam centre, egg collection was done by placing a clean sponge filter at the bottom of the hatchery tank and carefully removing the attached eggs using sterile tweezers and stocking them in aerated beakers or in separate larval rearing containers of 100 l capacity. The eggs from the bottom of the tank were also collected in 1000 μm nylon mesh by siphoning of bottom water.

At Visakhapatnam, an alternative method for egg collection was successfully attempted. The females

carrying fertilized eggs were collected and shifted to different hatchery tubs for shedding of the egg mass. After shifting, the females and egg mass were carefully observed on a daily basis and once the egg mass was released, the fishes were shifted back to the main tank. This method was found to be better, since the adult fish were cannibalistic and fed on newly hatched larvae. The next batch of egg production was observed in the same females in another 08-10 days.

Fertilised eggs are generally hardy and have hairy outgrowths which help them in getting attached to

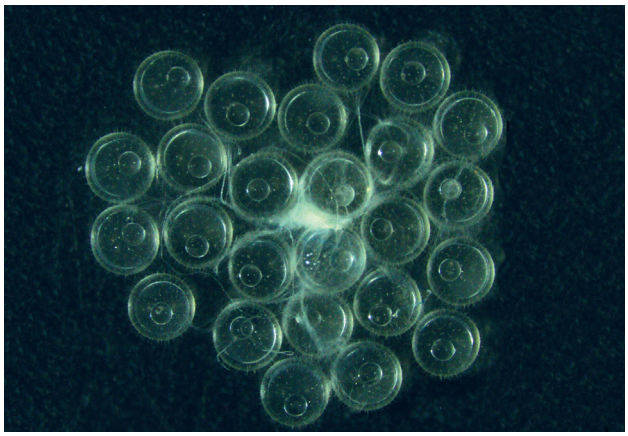

Fig 2. Egg bunch

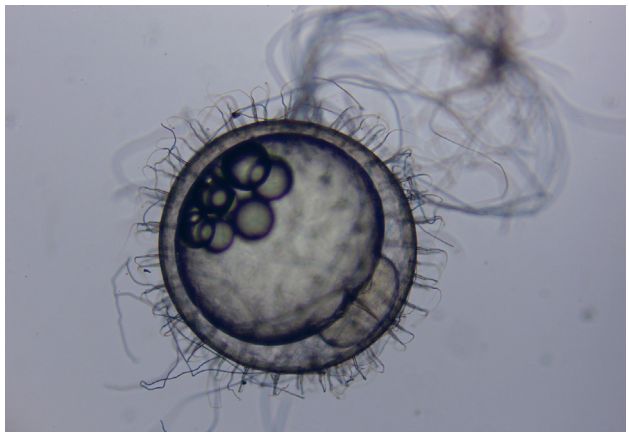

Fig. 3. Egg 1day after spawning (2 cell stage)

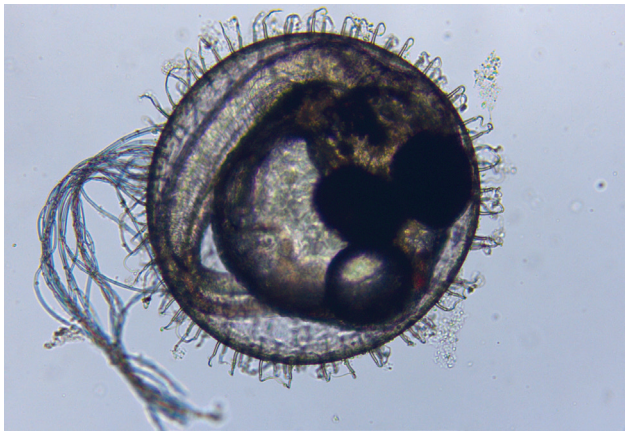

Fig 4. Egg 10 days after spawning

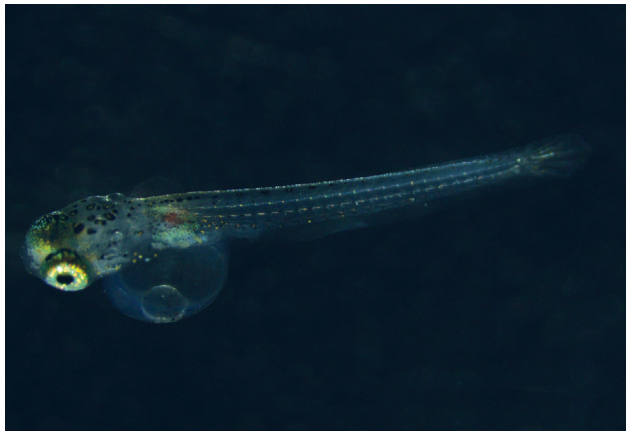

Fig. 5. Hatchling

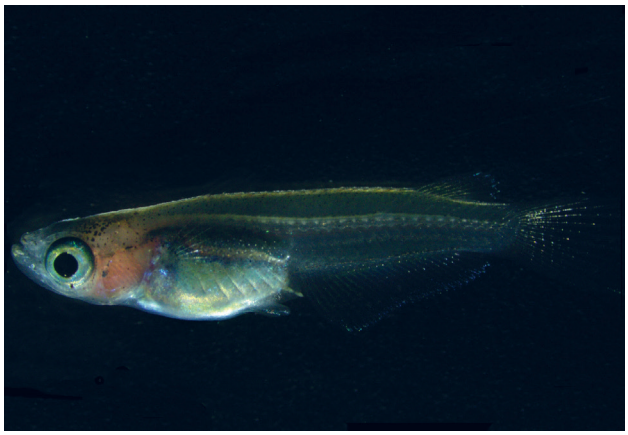

Fig.6. 20 DPH hatchling

Fig. 7. Hatchery produced O.dancena juveniles

Fig. 8 & 9. Hatchery produced F3 generation of O. dancena

suitable substrata. Both at Vizhinjam and Visakhapatnam Centres, eggs hatched in 10-11 days (both in salinity 31‰ or in freshwater; water temperature 29±0.6^{0}C) and the hatchlings were fed on *Artemia* nauplii or microencapsulated feed. The hatched larvae averaged 4.50 mm in total length (TL). The yolk sacs of the larvae were almost completely absorbed in 3-4 days after hatching and at 20 days after hatching, the larvae were 7.25 mm TL and had reached the juvenile stage. Mean survival rates of the hatched larvae up to 7 days post-hatching (dph) were >97% in salinity levels ranging from 0 to 31‰. The hatchlings sexually matured and first ovulation occurred in F1 individuals about 9 weeks (57-63 days) after hatching and at 22.6mm TL (mean) in different batches in both the centres. Totally, three generations (F3) of *O. dancena* were produced from different batches to date (Fig. 2-9).

Cho *et al*. (2010) conducted a study on the effects of different salinity conditions on the spawning performance, embryonic development, and early viability of *O. dancena* and observed that the embryonic development and early viability were influenced by higher and lower salinity levels (both in freshwater and > 35‰). Their study concluded that the optimal salinity condition for the normal embryonic and larval development and viability of the species is around 15-27 ppt (PSU). Such information is valuable for the long term maintenance and aquaculture practices involving this species. However, in the present study, no casualties were observed when the species was reared and bred (up to F3 generation) in two salinities–0 ppt and 31 ppt (PSU).

ICAR-CMFRI is planning to expand the laboratory rearing, mass propagation and maintenance of the living stocks of this valuable marine model fish species with a view to develop as a 'national facility' in its centres (Vizhinjam & Visakhapatnam), by rearing *O. dancena* in saline waters of 25-30ppt (PSU). Embryos, larvae, juveniles and adults at any stage of development could thus be produced year round and can be used for various genetic, genomic and pharmacological studies, not only for our own in-house experiments, but also for supplying to other Indian research and academic institutions. In this regard, efforts have been initiated recently by the institute for the round the year seed production of one more putative marine model species–the miniature Indian ricefish, *Oryzias setnai* (=*Horaichthys setnai* Kulkarni) in addition to *O .dancena*.

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