



FISHES OF MADHYA PRADESH WITH SPECIAL REFERENCE TO ZEBRAFISH AS MODEL ORGANISM IN BIOMEDICAL RESEARCHES

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ABSTRACT

Many surveys on fish species found in reservoirs, rivers, ponds or collection centres of Madhya Pradesh (MP) have been done during recent years. Fish are palatable and proteinous food for human beings. India is now at the threshold of blue revolution and it has made a notable progress in the field of inland fisheries. Fishes not only play an important role in the demand of food for humans but they are widely used for various biological experiments. At present, a number of indigenous and exotic fish species are found either naturally or cultured in the reservoirs of MP. One of the very popular freshwater tropical fish, *Danio rerio* (zebrafish, a freshwater tropical fish) has emerged as a major model organism for biomedical research, especially in developmental genetics, neurophysiology, oncology and biomedicine. Like most other biomedical model organisms, the zebrafish is chosen for particular traits that make it convenient for laboratory study. The greatest advantage of the zebrafish as a model system comes from its well-characterized genetics, genetic and developmental techniques and tools, and the availability of well-characterized mutants. The induction of experimental diseases, including cancer using zebrafish as a model organism may be a new and an important field of research.

KEYWORDS: Madhya Pradesh (MP), fish, zebrafish, model organism, biomedical research.

INTRODUCTION

Water is an important constituent of all living organisms and basic needs of human beings. Moreover, fishes live and survive in the water only. Fish are palatable and proteinous food for mankind. It is significant to note that India is at the threshold of blue revolution and it has made notable progress in the field of Inland fisheries. Most of the Indian reservoirs have been utilized for the fish culture. Fishes play an important role in present and future demand of food for human beings. Nowadays, a number of indigenous and exotic fishes are found in the reservoirs, rivers, ponds or collection centres of MP. Some of the important fish genera found in MP include *Catla*, *Channa*, *Cirrihinus*, *Clarias*, *Ctenopharyngodon*, *Cyprinus*, *Heteropneustes*, *Labeo*, *Mastacembelus*, *Mystus*, *Notopterus*, *Ompok*, *Puntius*, *Rasbora*, *Rita*, *Wallago* and *Xenentodon*¹. A case study of Gandhi Sagar reservoir fisheries of MP was done². The pre-impoundment studies on Sardar Sarovar area of Narmada river (west zone) with special reference to fisheries³ and the limnological study of river Narmada with reference to fisheries⁴ have also been done. The report on fish and fisheries of the Tapti river of Betul district of MP has been explored out in the research link¹. The occurrence of zebrafish (*Danio rerio* or *Brachydanio rerio*) was recorded in the year 1961 in Balaghat district of MP⁵.

Fishes not only play an important role in the demand of food for humans but they are widely used as model organisms for various biological experiments. Models are those organisms with a wealth of biological data that make them attractive to study as examples for other species and/or natural phenomena that are more difficult to study directly. Historically, model organisms include a handful of species with extensive genomic research data. Hence, a model organism is a non-human species that is studied to understand particular biological phenomena with the expectation that discoveries made in the organism model will provide insight

into the workings of other organisms⁶. Model organisms are widely used to explore potential causes and treatments for human disease when human experimentation would be unfeasible or considered less ethical⁷. One of the very popular freshwater tropical fish, *D. rerio* (commonly known as zebrafish, zebra fish or zebra danio) has emerged as a major model organism for biomedical research. The zebrafish is a small, robust fish, and so large numbers can be kept easily and cheaply in laboratory, where it breeds all year round^{8,9}. Zebrafish has a nearly transparent body during early development, which provides unique visual access to the animal's internal anatomy. It can be used as a model organism in various biological studies, including the study of specific gene function and roles of signaling pathways. Thus, the induction of experimental diseases, including cancer using zebrafish as a model organism may be a new and an important field of research⁹.

In view of the above facts, this article has been put forth to emphasize the knowledge about the fishes of MP with special reference to usefulness of zebrafish as a model organism in biomedical and other related researches.

FISHES FOUND IN MP

Table 1 elucidates the different fish species obtained in 2008 from Tighra collection centre as well as Tighra fresh water reservoir, Gwalior (MP). Total 33 fish species were identified from Tighra collection centre, Gwalior. These included 1 species of *Catla* (*C. catla*), 2 species of *Cirrhinus* (*C. mrigala* and *C. reba*), 5 species of *Labeo* (*L. rohita*, *L. calbasu*, *L. fimbriatus*, *L. bata* and *L. gonius*), 1 species of *Rasbora* (*R. daniconius*), 4 species of *Mystus* (*M. seenghala*, *M. bleekeri*, *M. cavasius* and *M. vittatus*), 4 species of *Puntius* (*P. chola*, *P. ticto*, *P. sarana* and *P. sophore*), 1 species of *Xenododon* (*X. cancila*), 3 species of *Channa* (*C. marulius*, *C. punctata* and *C. striatus*), 1 species of *Clarias* (*C. batrachus*), 1 species of *Heteropneustes* (*H. fossilis*), 2 species of *Notopterus* (*N. Chitala* and *N. notopterus*), 2

species of *Ompak* (*O. bimaculatus* and *O. pabo*), 1 species of *Rita* (*R. rita*), 1 species of *Wallago* (*W. attu*), 1 species of *Ctenopharyngodon* (*C. idella*), 1 species of *Cyprinus* (*C. carpio*), 1 species of *Hypophthalmichthys* (*H. molitrix*) and 1 species of *Mastacembelus* (*M. armatus*). Table 1 also includes a total of 32 fish species belonging to 5 orders, 9 families and 33 genera obtained in the Tighra reservoir. Furthermore, 20 fish species from Tapti river (Betul district) and 70 fish species from Chambal river (western MP) have been obtained¹. Similarly, 39 fish species after the impoundment of Gandhi Sagar reservoir² and 84 fish species in Sardar Sarovar dam of Narmada river³ have been reported. In one more study⁴, many species of fish have been recorded in Narmada river of Barwani (west Nimar) area. The species of *Catla*, *Cirrihinus*, *Labeo*, and *Mastacembelus* and *Mystus* have been found in highest percentage throughout the year.

ZEBRAFISH AS SUITABLE MODEL ORGANISM

The zebrafish (*Danio rerio*) is an important model organism in developmental genetics, neurophysiology and biomedicine. It is a small, shoaling cyprinid, native to the flood-plains of the Indian subcontinent, where it is found in shallow, slow-flowing water. Zebrafish belongs to the family of freshwater fishes, Cyprinidae, the most species rich vertebrate family¹⁰. The name *Danio* derives from the Bengali name “dhani”, meaning “of the rice field”¹¹. The two genera, viz., *Devario* and *Danio* of zebrafish are ecologically quite distinct, *Devario* spp. occurring in hill streams with clear running water, while *Danio* spp. are confined to lowland areas, typically inhabiting slow-flowing, turbid rivers and pools. Other danionin species found with zebrafish are *Danio dangila*, *D. meghalayensis*, *Devario devario*, *Devario assamensis* and *D. aequipinnatus*. The zebrafish are characterized by small, slender shape (up to about 4 cm long, or <120 mm total length), the presence of a ‘danionin notch’ in the ventro-medial margin of the dentary and a distinctive colour pattern based on alternating dark and light horizontal stripes, which may be broken up into blotches or bars. In the other words, the zebrafish have luminous bluish-black and silvery-gold horizontal stripes or dark-blue and silvery longitudinal stripes. The natural range of the zebrafish is centred around the Ganges and Brahmaputra river basins in north-eastern India, although in the past specimens have also been collected in the Indus, Cauvery, Pennar, Godavari and Mahanadi river basins. In addition, it has been reported as occurring in the Krishna river basin. On the other hand, the zebrafish originate from eastern Asia, and distributed throughout south and south-east Asia, their highest species diversity in north-eastern India (including Rajasthan, Gujarat, Andhra Pradesh, West Bengal, Assam, Meghalaya and Orissa), Pakistan, Bangladesh, Nepal, Burma (Myanmar) and Sri Lanka^{5,12}. It is commonly found in most types of water ranging from streams to stagnant ponds and rice fields. The zebrafish has also been accidentally introduced into a number of different waters around the world and it can today also be found in countries such as USA, Australia and Colombia¹².

The zebrafish is popular in home aquariums and particularly abundant in silt-bottomed, well-vegetated pools and rice paddies adjacent to slow moving streams at a range of elevations. The zebrafish is a friendly fish, and is very hardy and adaptable which makes it an ideal beginner fish. The zebrafish is a very popular fish and we can find it in just about any aquarium shop around the world. They are breed in huge numbers for the aquarium market. The zebra fish has been a popular fish for a long time and became popular early

due to its hardiness, cold resistance and easiness to breed. There are a long row of different variants of this fish available in addition to the regular form. These forms include classical forms like long finned zebrafish, albino zebrafish, golden zebrafish, sandy zebrafish and leopard danios (earlier known as *Danio frankeri*), but also modern genetically engineered zebrafish that glows in the dark. The glowing variant is available in red, yellow and green and is often sold under the name ‘Glowfish’ (the name is a trademark for these patented fish). Glowfish was not designed for the aquarium market but is proving popular and might lead to more design species being developed for the hobby in the future. The zebrafishes are very popular among scientists as they are a perfect model organism for vertebrates and can be used as a complement to mice and rats in research. The zebrafish has proven very useful for research and the study of this fish has formed the basis for many new promising medical technologies¹².

Humans and zebrafish having shared waterways around the river Ganges for tens of thousands of years¹³. Like most other biomedical model organisms, the zebrafish has chosen for particular traits that make it convenient for laboratory study, not for a broad understanding of the organism in its native environment¹⁴. Several sources agree that zebrafish are found in rivers, small streams and other channels, stagnant or slow-moving pools near streams, and rice paddies¹⁵⁻¹⁶. Zebrafish appear to be primarily a floodplain species, inhabiting shallow ponds and ditches or the slower reaches of streams. They are an abundant species and are among the smallest fish species in the assemblages in which they occur. They feed throughout the water column and tend to be confined to shallow margins of water bodies. Zebrafish are particularly abundant in silt-bottomed, well-vegetated pools and rice paddies adjacent to slow moving streams at a range of elevations¹⁷. The breeding season of zebrafish is between April and August⁵, presumably varying somewhat by latitude, elevation and prevailing climatic conditions. Egg laying is thought to occur in small pools adjacent to streams¹⁵. The generation time of zebrafish is short, typically 3-4 months, making it suitable for experiment. Its development is rapid, with precursors to all major organs developing within 36 h and larvae displaying food seeking and active avoidance behaviours within 5 days post-fertilization, i.e., 2-3 days after hatching¹⁸. The mean life span of domesticated zebrafish is 42 months, with the oldest fish surviving for 66 months¹⁹. The growth rate of zebrafish is most rapid during first 3 months following hatching, after which it starts to decrease, reaching zero by about 18 months. Zebrafish are omnivorous; their natural diet consists mainly of zooplankton and insects; though phytoplankton, filamentous algae and vascular plants, spores and invertebrate eggs, fish scales, arachnids, detritus, sand and mud have also been reported from gut content analysis¹⁵. Zebrafish themselves are known to feed on mosquito larvae and, presumably, other insects, although the precise species are not known⁵.

Zebrafish are used to study development, toxicology and toxicopathology²⁰. The zebrafish is known for its rapid development in the laboratory. The greatest advantage of the zebrafish as a model system comes from its well-characterized genetics, genetic and developmental techniques and tools, and the availability of well-characterized mutants. It has been explained²¹ that the experimental carcinogenesis using fish species as alternative models may be a new and an important field of research. Expansion of synthetic chemical

producing industries during the 1940's coincided with a number of pollution-associated fish neoplasia epizootics, with PAHs as significant components of contaminated sediments in several cases. Epizootics of primarily liver and skin neoplasia in benthic species near coastal urban or industrial areas indicated the sensitivity of fish species to known mammalian carcinogens. The potential for the application of research findings to both human and environmental health issues make fish species attractive and valuable alternative models in the carcinogenesis and toxicity research. The strength of zebrafish as a model organism is that as a vertebrate it is more comparable to humans than invertebrate model species such as *Drosophila*²², while being more tractable to genetic and embryological manipulation than mammalian model species such as mice, in which such procedures are both more complicated and costly. The zebrafish is increasingly important in biomedical research, particularly as a model of human disease and for the screening of therapeutic drugs²³. Over 400 labs worldwide now routinely use the zebrafish in several researches, and there is an increasing interest in its use as a model for understanding the genetic basis of behaviour²⁴. It has been further reported that reported that the zebrafish (*D. rerio*) is a premiere model organism to study the vertebrate development. It may also be a powerful model for the study of human diseases because many cellular processes are conserved throughout vertebrate evolution, including corresponding disease genes²⁵. The zebrafish has been increasingly recognized as a promising animal model for cancer research. Zebrafish tumours can be generated by treatment with chemical carcinogens or by genetic approaches. The liver has been a main target organ for tumorigenesis after carcinogen treatment while many other tissue-specific tumours have been generated by tissue-specific expression of proven oncogenes. Both chemical and transgenic approaches were used to generate liver tumours. They observed a remarkable similarity in the molecular hallmarks during human liver tumorigenesis and carcinogen-induced zebrafish liver tumour, thus validating the zebrafish model for human cancer studies. These workers established the transgenic zebrafish models for liver cancers, including hepatocellular carcinoma (a type of liver cancer) which will be further used to understand the molecular and genetic mechanisms of liver carcinogenesis as well as for anticancer drug discovery^{8,26}. Therefore, the zebrafish has been recognized as a suitable model for different experimental studies⁸⁻⁹.

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Table 1: Fishes found in reservoirs / rivers / collection centres of Madhya Pradesh¹

Order	Family	Generic (Biological) name	Common name
Cypriniformes (Carp)	Cyprinidae (Minnows or carps)	<i>Catla catla</i> (Hamilton)	Indian carp, Indian major carps, Bhakur
		<i>Cirrhinus mrigala</i> (Hamilton)	Indian carp, Mrigal carp
		<i>Cirrhinus reba</i> (Hamilton)	Reba carp
		<i>Ctenopharyngodon idella</i>	Grass carp
		<i>Cyprinus carpio</i> (Linnaeus)	Common carp, Soneri masha
		<i>Hypophthalmichthys molitrix</i>	Silver carp, Belli-gende
		<i>Labeo bata</i> (Hamilton)	Bata, Bata labeo, Minor carp, Tambti
		<i>Labeo calbasu</i> (Hamilton)	Kalbasu, Black rohu
		<i>Labeo gonius</i> (Hamilton)	Khursa, Kuria labeo, Kuri
		<i>Labeo fimbriatus</i> (Hamilton)	Fringed-lipped peninsula carp, Belji, Kijan
		<i>Labeo rohita</i> (Hamilton)	Rohu, <i>Rohi</i> , Indian major carp
		<i>Puntius chola</i> (Hamilton)	Swamp barb, Chola barb
		<i>Puntius sarana</i> (Hamilton)	Olive barb
		<i>Puntius sophor</i> (Hamilton)	Pool barb
		<i>Puntius ticto</i> (Hamilton)	Ticto barb, Two-spot barb
		<i>Rasbora daniconius</i> (Ham.)	Slender rasbora, Dhera, Danikono
Siluriformes (Catfishes)	Siluridae (Sheatfishes)	<i>Ompak bimaculatus</i> (Bloch)	Chechera, Gugli, Jalkapoor, Papta, Puffta, Indian butter catfish
		<i>Ompak pabo</i> (Bloch)	Pabo catfish, Pabo, Kaliwanz
		<i>Wallago attu</i> (Bloch & Schneider)	Boal, Boalee, Paran, Valai, Barali
	Bagridae (Bagrid catfishes)	<i>Mystus bleekeri</i> (Day)	Singorah, Tengra, Palwa, Keongar, Singhala
		<i>Mystus cavasius</i> (Hamilton)	Gangetic mystus, Kavasi, Gulia, Singarah, Shingta
		<i>Mystus seenghala</i> (Sykes)	---
		<i>Mystus vittatus</i> (Bloch)	Lal tingara, Striped dwarf catfish, Kuggur
		<i>Rita rita</i> (Hamilton)	Rita, Ritha, Choua, Hunna
	Clariidae (Air-breathing catfishes)	<i>Clarias batrachus</i> (Linnaeus)	Walking catfish, Magur, Mangri
	Heteropneustidae (Saccobranchidae) (Stinging catfishes or fossil catfishes)	<i>Heteropneustes fossilis</i> (Bloch)	Asian stinging catfish, Fossil catfish, Liver catfish, Singhi
Beloniformes (Needle fishes)	Belonidae (Needlefishes)	<i>Xenentodon cancila</i> (Hamilton)	Freshwater garfish, Asian freshwater needlefish, Kankely
Perciformes (Perches, perch-like)	Channidae (Snakeheads, Asian snakehead fishes)	<i>Channa marulius</i> (Hamilton)	Gajal, Giant snakehead, Bullseye snakehead, Pumuri, Bohr
		<i>Channa punctata</i> (Bloch)	Spotted snakehead, Taki, Lata, Phool-dhok
		<i>Channa striatus</i> (Bloch)	Snakehead, Air-breathing fish
Mastacembeleformes Synbranchiformes (Swamp eels)	Mastacembelidae (Spiny eels)	<i>Mastacembelus armatus</i> (Scopoli)	Tire track eel, Zig-Zag eel, White-spotted spiny eel
Osteoglossiformes (Bony-tongued fishes)	Notopteridae (Featherbacks or knifefishes)	<i>Notopterus chitala</i> (Hamilton)	Clown featherback, Clown Knifefish
		<i>Notopterus notopterus</i> (Pallas)	Bronze featherback