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DNA Quantification of Wild and Cultured *Cirrhinus mrigala* (Hamilton, 1822) Collected from Different Sites of Western Uttar Pradesh, India

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Abstract: The mrigal carp *Cirrhinus mrigala* is an economically important food fish and also an important aquaculture freshwater species. DNA content of fish tissues are of considerable interest for their specificity in relation to food values of fish and evaluating their physiological requirements at different periods of life. Fish exhibit large variations in their biochemical contents from species to species. The present study was conducted on DNA extraction and determination of DNA quantity of both male and female of wild and cultured *Cirrhinus mrigala* using Nanophotometer. DNA extraction was done and extracted DNA was analyzed using nanophotometer to determine the concentration of DNA. For the present study both male and female of wild *Cirrhinus mrigala* (from middle Ganga Region) and cultured *Cirrhinus mrigala* (from culture ponds and/ hatchery) were taken from different sites of Western Uttar Pradesh. The value of DNA concentration in female of wild *Cirrhinus mrigala* was between 62 - 66 ng/ μ l and of male was between 64- 78 ng/ μ l. The value of DNA concentration in female of cultured *Cirrhinus mrigala* was between 60 - 88 ng/ μ l and of male was between 70- 78 ng/ μ l.

Keywords: DNA extraction, DNA quantification, nanophotometer, *Cirrhinus mrigala*

Introduction

The *Cirrhinus mrigala* (also known as the mrigal and locally as Nain in western Uttar Pradesh, India) is a species of ray-finned fish in the genus *Cirrhinus*. Mrigal is the benthopelagic and potamodromous plankton feeder. It inhabits fast flowing streams and rivers, but can tolerate high levels of salinity. It reaches a maximum length of 1 m (3.3 ft)(Froese *et al.*, 2014). Mrigal is popular as a food fish and an important aquaculture

freshwater species throughout South Asia. This species is widely farmed as a component of a polyculture system of three Indian major carps, along with rohu and catla.

The introduction of aquaculture across India started in the early 1940s and between 1950s and 1960s in other Asian countries. The mrigal fails to breed naturally in ponds, thus induced breeding is done. The Indian carps are considered as a delicacy compared to any

other exotic carp species cultured in Asia, and sell for higher price (Rema *et al.*, 2011). It is found in northern India, Pakistan, and Bangladesh (Dahanukar, 2010). Spawning occurs in marginal areas of the water bodies with a depth of 50 to 100 centimetres over a sand or clay substrate.

The cultured mrigal carp has been cited as *Cirrhinus mrigala* and is so still treated by FAO, whereas sources such as FishBase, IUCN Red List and Eschmeyer's Catalog of Fishes now consider *C. cirrhosus* and *C. mrigala* as distinct species (Dahanukar, 2010). Reported annual aquafarming production numbers of mrigal carp since the early 1990s varied between 250,000 and 550,000 tonnes, with no clear trend. India and Bangladesh are the largest producers (FAO, 2014). In Pakistan, this fish is known by the name of "Morakhi" or "Moree".

The present study was conducted on the DNA isolation and determination of DNA concentration of both male and female of *Cirrhinus mrigala* using Nanophotometer and also to find out variation in DNA contents of wild and cultured fish.

Materials and Methods

In the present study, the fish *Cirrhinus mrigala* (Middle Ganga region, culture fish from hatchery and ponds) were collected from different water bodies of Western Uttar Pradesh (Bijnor district, district Hapur and from district Meerut), morphologically identified (with the help of Day's fauna 1875-78,1889) and preserved for molecular studies (for DNA isolation and quantification). Approximately 100 mg of muscle tissue and fin clip from 2-5 individuals of each species were preserved in 95% ethanol until used and

kept at -20 C for molecular analysis. Voucher specimens were preserved in 10% formalin solution. DNA isolation was done by following the method of Ruzzante *et al.* (1996) with minor modifications. The DNA was diluted to a final concentration of 100ng/ μ L. Gel electrophoresis was carried out by 1.5-2% agarose gel. The extracted DNA was further analyzed using Nanodrop spectrophotometer (Nanophotometer P330; Implen, Germany) to determine the concentration of DNA. Total DNA quantification was carried by nanophotometrically (absorbance 260 and 280 nm). A total of 20 samples of *Cirrhinus mrigala* of wild and cultured fishes were analyzed.

Results

For the present study both male and female of wild *Cirrhinus mrigala* (from middle Ganga Region) and cultured *Cirrhinus mrigala* (from culture ponds and hatchery) were taken from different sites of Western Uttar Pradesh. The concentration of DNA in all samples of wild and cultured fish was represented as nanogram/ μ l (Table 1).

The value of DNA concentration in female of wild *Cirrhinus mrigala* was between 62 - 66 ng/ μ l and of male was between 64- 78 ng/ μ l (Fig. 1). The value of DNA concentration in female of cultured *Cirrhinus mrigala* was between 60 - 88 ng/ μ l and of male was between 70- 78 ng/ μ l (Fig. 2). A slight variation was seen in DNA content of male and female fish samples.

Discussion

Nanodrop spectrophotometry is an extremely powerful technology that allows Quantification of DNA, RNA (A260) and

Table 1: Quantity of DNA (nanogram/ μ l) in samples of *C. mrigala*

Sample No.	Wild Female	Wild Male	Cultured Female	Cultured Male
1	62	66	86	78
2	66	64	65	70
3	63	66	60	74
4	62	68	88	72
5	64	78	67	74

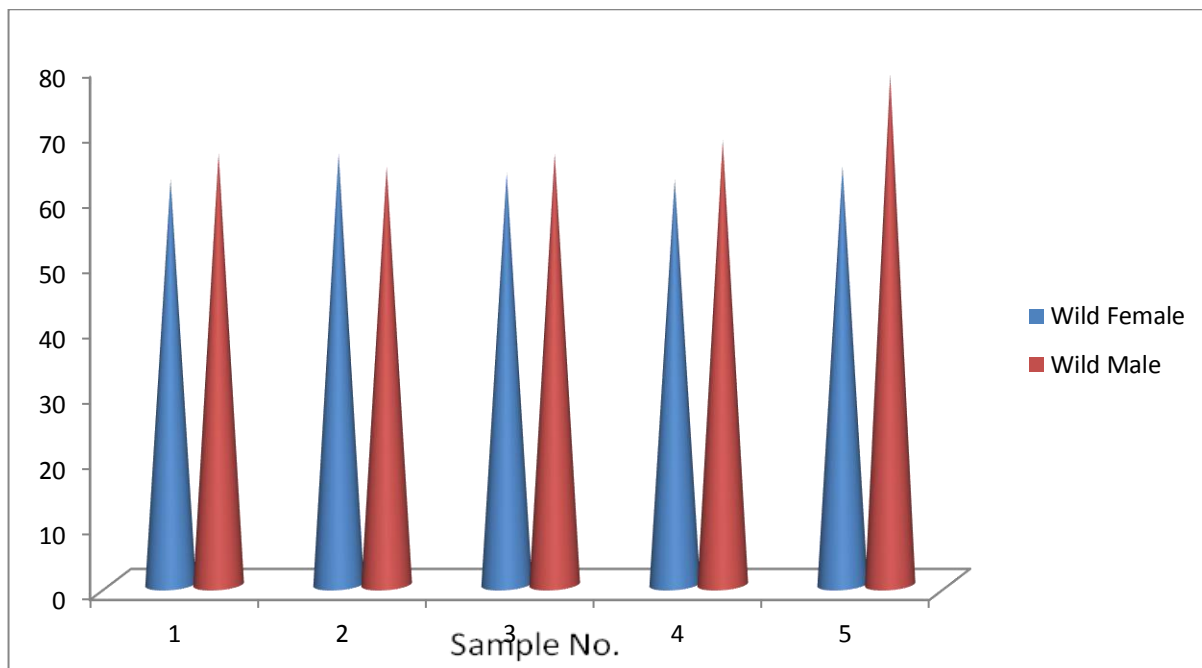


Fig.1: Quantity of DNA content (nanogram/ μ l) in wild *Cirrhinus mrigala*

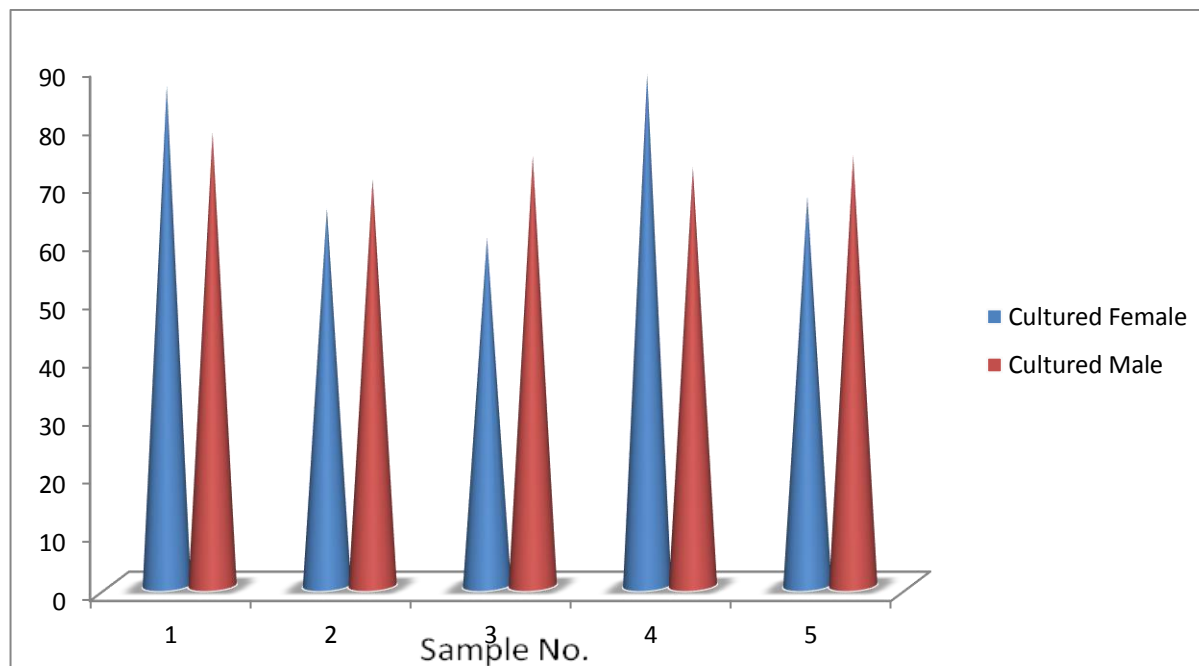


Fig. 2: Quantity of DNA content (nanogram/ μ l) in cultured *Cirrhinus mrigala*

protein (A280) concentrations and sample purity (260/280 ratio) over a large concentration range of 2 - 15,000 ng/L double standards DNA (Pratima *et al.*, 2014). The present study revealed that the value of DNA concentration in female of wild *Cirrhinus mrigala* was between 62 - 66 ng/ μ l and of male was between 64- 78 ng/ μ l. The value of DNA concentration in female of cultured *Cirrhinus mrigala* was between 60 - 88 ng/ μ l and of male was between 70- 78 ng/ μ l.

Prado *et al.* (2012) used nanodrop method for DNA quantification from different fishes based on nuclear target. Nanodrop technique was also used by Shi *et al.* (2015) for DNA quantification in the process of molecular characterization of *Cynoglossus semilaevis*. Determination of DNA concentration of *Clarias gariepinus* was done by nanodrop Aderibigbe (Adedunni, 2014). DNA quantification of male and female *Clarias batrachus*, *Clarias gariepinus* and *Clarias* hybrids was also done

by Shobhna (2017). She observed the value of DNA concentration in female of *Clarias batrachus* was between 59 and 61 ng/ μ l and of male between 70- 76 ng/ μ l. The value of DNA concentration in female of *Clarias gariepinus* was 60 and 63 ng/ μ l and of male 75- 78 ng/ μ l. Highest DNA concentration was seen in hybrid individuals, in females the concentration was between 74-76 ng/ μ l and in males value was between 88-91 ng/ μ l. Similar study on diminution level of RNA/DNA ratio in tissue of *Labeo rohita* by exposure to some endocrine disrupting compounds was also reported by Verma *et al.* (2016). Comparing data from DNA content of cultured fish from the data of wild fish we conclude that DNA content is useful in monitoring the physiological condition of the fish. DNA content in wild fish is slightly low (in most estimations) as compared to the cultured fish which may be attributed to the capture of wild fish which were brought to the

laboratory and may be that these fish had low levels of physiological activity/metabolic activity.

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