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ORIGINAL ARTICLE

Assessment of DNA Quantity in *Cirrhinus reba* (Hamilton, 1822) Collected from three Different Sites of Middle Ganga Region

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ABSTRACT
The Reba carp Cirrhinus reba (Hamilton) is one of the most popular and economically important food fish. It is widely distributed in India, Bangladesh, Pakistan, Nepal, Burma and Thailand. Moreover, this species is a most important leading fish resource (year around) of Western U P show the relative catch composition of virtually in tonnes past years. The present study was conducted to assess the quantity of DNA of three different groups (group I, group II, group III) of both male and female Cirrhinus reba using Nanospectrophotometer. Fishes of group I were collected from the water bodies of Kila ParikshitGarh (District Meerut), group II from Garhmukteshwar (district Hapur) and group III from Bijnor district, of Western Uttar Pradesh of India. DNA was isolated and gel electrophoresis was carried out and extracted DNA was analyzed using nanospectrophotometer (Nanophotometer P330; Implen, Germany) to determine the quantity of DNA and its purity level. For the present study both male and female Cirrhinus reba were taken. The value of DNA concentration in group Ist female of Cirrhinus reba was between 60 and 64 ng/µl and of male was between range of 68-76 ng/µl. The value of DNA concentration in group IInd female of Cirrhinus reba was 60 and 63 ng/µl and of male 75- 78 ng/µl. Highest DNA concentration was seen in group IIIrd, in females the concentration was between 76-82 ng/µl and in case of males value was between 86-92 ng/µl.
Keywords: *Fish, Nanophotometer, DNA concentration, Cirrhinus reba.*

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INTRODUCTION

The Reba carp *Cirrhinus reba* (Hamilton, 1822) [1] is one of the most popular and economically important food fish. This species is widely distributed in India, Bangladesh, Pakistan, Nepal, Burma and Thailand. Moreover, this species is a most important leading fish resource (year around) of Western U P show the relative catch composition of virtually in tonnes past years. DNA content of fish tissues are considerable interest for their specificity in relation to food values of fish and evaluating their physiological needs at different periods of life, fish exhibit large variations in their biochemical content from species to species. Biochemical and DNA measurement can provide valuable tool for monitoring the health and condition of fish. The knowledge from such investigations can be used in optimizing and sustaining yield, stock management and conservation of genetic diversity. Many molecular techniques are now available, which allow ecologists and evolutionary biologists to determine the genetic architecture of a wide variety of closely related individuals. In last few years, Nanodrop has established itself as a useful and quick novel method for DNA quantification. For DNA quantification Nanodrop uses fiber optic technology and surface tension, sample is held between a two optical surfaces that define a path length in vertical orientation. Total measurement cycle time, including prepration and removal of the sample is nearly 30 second. The ease of use of this technique not only makes it a feasible option for small volume analysis of DNA but also a practical alternative for all spectrophotometric measurement [2, 3].

The present study was conducted to assess the quantity of DNA of three different groups (group I, group II, group III) of both male and female *Cirrhinus reba* using Nanospectrophotometer. Fishes of group I were

collected from the water bodies of Kila ParikshitGarh (District Meerut), group II from Garhmukteshwar (district Hapur) and group III from Bijnor district, of Western Uttar Pradesh of India.
The present study was conducted on the DNA isolation and determination of DNA concentration of both male and female of *Cirrhinus reba* in three different sites (water bodies) using Nanophotometer.

MATERIAL AND METHODS

In the present study, *Cirrhinus reba* were collected from different water bodies of Western Uttar Pradesh. Various areas of Western U.P. were surveyed. On the basis of survey three sites were selected for sample collection. These were Kila ParikshitGarh (District Meerut), Garhmukteshwar (district Hapur) and Bijnor district. On the basis of survey and site selection (according to water bodies) these were grouped in to three groups. Fishes of group Ist were collected from the water bodies of Kila Parikshit Garh (District Meerut), group IInd from Garhmukteshwar (district Hapur) and group III^{Ird} from Bijnor district, of Western Uttar Pradesh of India. After collection of fish samples muscles, tissue and fin clips were collected and preserved in 95% ethanol for further molecular studies. Species identification was done with the help of standard literature of Day's [4] fauna 1875-78. DNA isolation was done by following the method of Ruzzante *et. al*.,[5] with minor modifications. Gel electrophoresis was carried out. An aliquot (3 µl) of amplicon was checked on 1.5-2% agarose-TBE gels, stained with ethidium bromide and visualized under ultraviolet light. DNA quantification was done by using nanophotometer (Nanophotometer P330; Implen, Germany) using manufacture instruction.

RESULTS

For the present study both male and female *Cirrhinus reba* were taken. The value of DNA concentration in group Ist female of *Cirrhinus reba* was between 60- 64 ng/µl and of male was between 68- 76 ng/µl. The value of DNA concentration in group IInd female of *Cirrhinus reba* was 60 and 63 ng/µl and of male 75- 78 ng/µl. Highest DNA concentration was seen in group IIIrd, in females the concentration was between 76-82 ng/µl(Table 1) and in case of males the value of DNA content was between 86-92 ng/µl (Table 3).
Of all three groups in female fish the highest concentration was seen in female fish of group IIIrd from Bijnor district (Fig. 1) whereas in male fish the highest concentration in male fish was also in group IIIrd from Bijnor district (Fig. 2). In compassion of male and female fish of group Ist those samples collected from District Meerut the highest content was in male fish (Fig. 3); in group IInd of all samples collected from district Hapur in male fish; and in group IIIrd of Bijnor district the highest content was seen in also in male fish. The data obtained from this study was statistically analysed showed significant (Table2, Table 4).

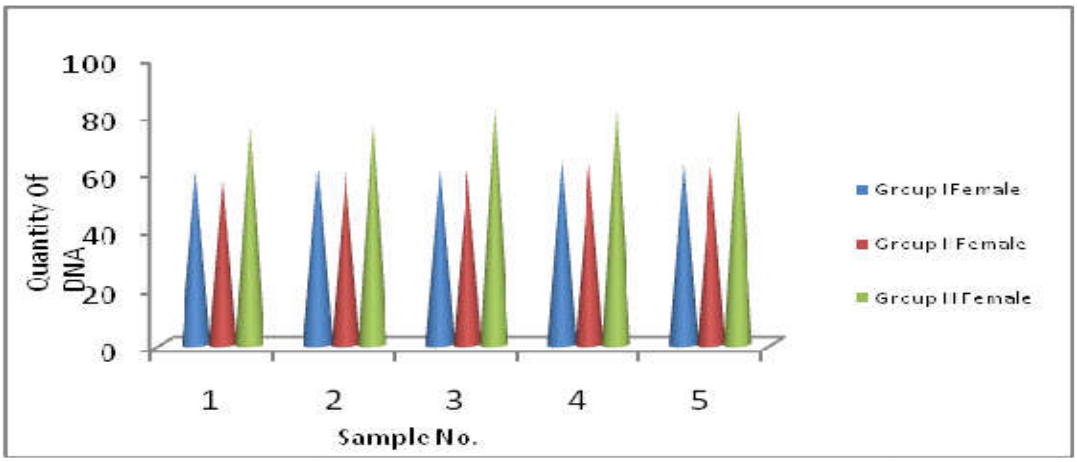

Fig1: Comparative Quantity of DNA content in female *Cirrhinus reba* in ng/µl in all three groups.

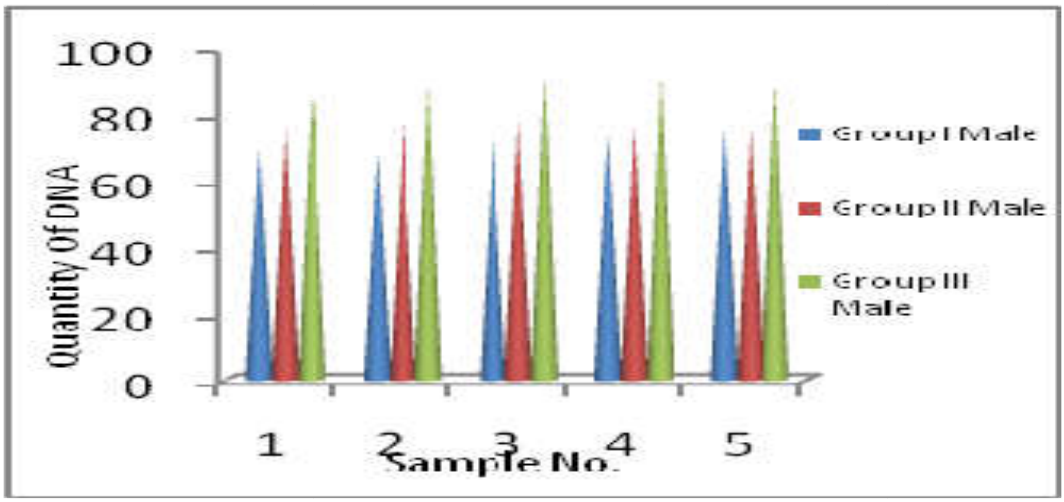

Fig.2: Comparative Quantity of DNA content in male *Cirrhinus reba* in Nanogram/ µl/individual in all three groups.

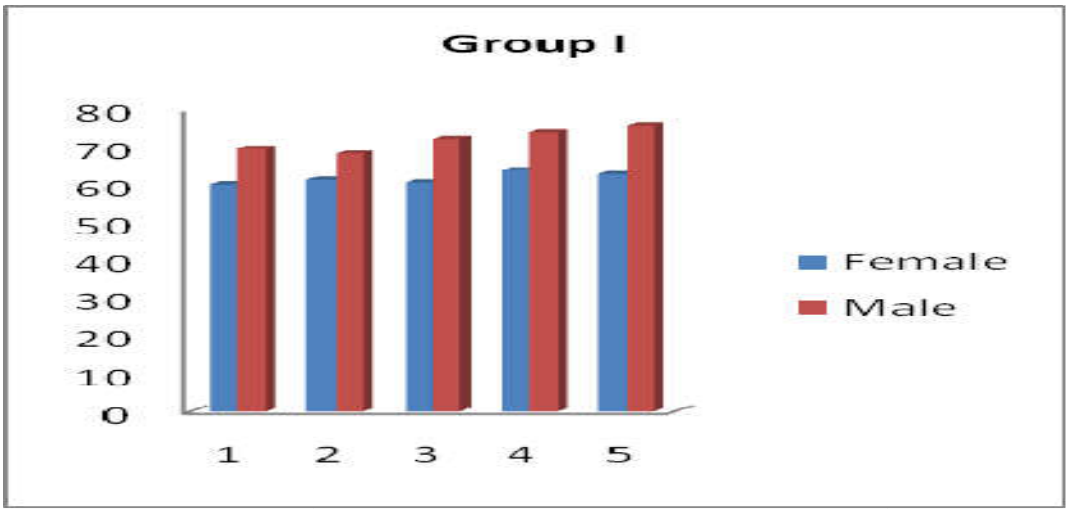

Fig. 3: Comparative DNA quantification of female and male fish in group I.

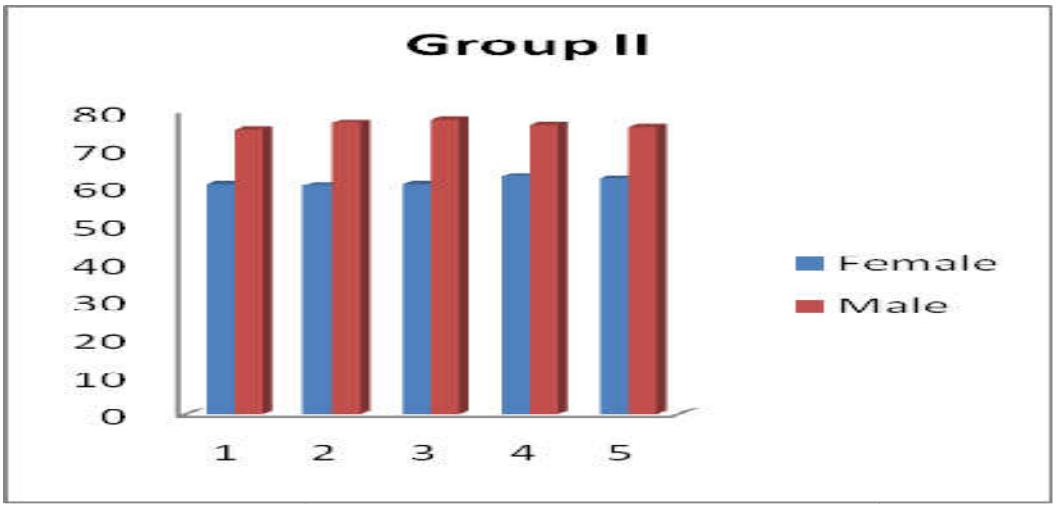

Fig. 4: Comparative DNA quantification of female and male fish in group II.

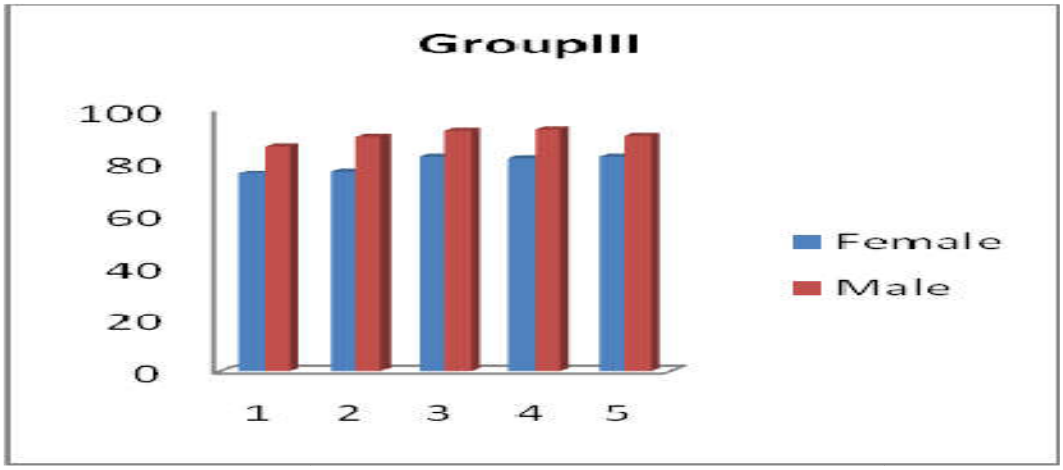

Fig 5: Comparative DNA quantification of female and male fish in group III.

DISCUSSION

The present study was conducted to assess the quantity of DNA of three different groups (group I, group II, group III) of both male and female *Cirrhinus reba* using Nanospectrophotometer. Fishes of group Ist were collected from the water bodies of Kila ParikshitGarh (district Meerut), group IInd from Garhmukteshwar (district Hapur) and group IIIrd from Bijnor district, of Western Uttar Pradesh of India. DNA was isolated by using Ruzzante *et. al*.,[5]. Gel electrophoresis was carried out and extracted DNA was analyzed using nanospectrophotometer (Nanophotometer P330; Implen, Germany) to determine the quantity of DNA and its purity level. The value of DNA concentration in group Ist female of *Cirrhinus reba* was between 60 and 64 ng/µl and of male was between range of 68- 76 ng/µl. The value of DNA concentration in group IInd female of *Cirrhinus reba* was 60 and 63 ng/µl and of male 75- 78 ng/µl. Highest DNA concentration was seen in group IIIrd, in females the concentration was between 76-82 ng/µl and in case of males value was between 86-92 ng/µl. Nanodrop spectrophotometry is an extremely powerful technology that allows Quantification of DNA, RNA (A260) and protein (A280) concentrations and sample purity (260/280 ratio) over a large concentration range of 2 - 15,000 ng/L double standards DNA. Prado *et. al.,*[3] used nanodrop for DNA quantification from different fishes based on nuclear target. Nanodrop technique was also used by Shi *et. al.,* [6] for DNA quantification in the process of molecular characterization of *Cynoglossus semilaevis.* In the project report submitted by Aderibigbe Adedunnijanet[2] to the department of aquaculture and fisheries management, University of agriculture, Nigeria determination of DNA concentration of *Clarias gariepinus* was done by nanodrop spectrophotometer. Preliminary study on diminution level; of RNA/DNA ratio in tissue of *Labeo rohita* by exposure to some endocrine disrupting compounds was done by Verma *et. al*.,[7]. DNA quantification of Male and Female *Clarias batrachus, Clarias gariepinus* and *Clarias* hybrids was also done by Shobhna [8].

Table 1: Comparative Quantity of DNA content in female *Cirrhinus reba* in Nanogram/ µl in all three groups.

Sample No.	Group I Female	Group II Female	Group III Female
1	60	61	75
2	61	60	76
3	60	61	82
4	64	63	81
5	63	62	82

Table 2. ANOVA results of female *Cirrhinus reba* in Nanogram/ µl in all three groups.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	907.1717	2	453.5858	129.8226	2.31-07	4.256495
Within Groups	31.445	9	3.493889			
Total	938.6167	11				

Table3.Comparative Quantity of DNA content in male *Cirrhinus reba* in Nanogram/ µl in all three groups.

Sample	Group I	Group II	Group III
No.	Male	Male	Male
1	69	75	86
2	68	77	89
3	72	78	91
4	74	76	92
5	76	76	90

Table 4. ANOVA results of male *Cirrhinus reba* in Nanogram/ µl in all three groups.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	729.5	2	364.75	84.68881	1.46-06	4.256495
Within Groups	38.7625	9	4.306944			
Total	768.2625	11				

CONCLUSION

Fish has long been valued as a source of high quality animal protein, relatively cheap, for human nutrition. Currently, fish accounts for more than, or close to 50% of the total animal protein consumed in most countries of the world. DNA measurement can provide valuable tool for monitoring the health and condition of fish. The knowledge from such investigations can be used in optimizing and sustaining yield, stock management and conservation of genetic diversity.

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