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Analysis of DNA Content of Wild and Cultured Labeo Calbasu (Hamilton, 1822) Using Nanophotometer

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ABSTRACT
Labeo calbasu is an important food fish and commonly known as the "calbasu" or "Black Rohu". The present study was conducted on DNA extraction and determination of DNA quantity of both male and female of wild and cultured *Labeo calbasu* using Nanophotometer. DNA isolation was done and gel electrophoresis was carried out. Extracted DNA was analyzed using nanophotometer (Nanophotometer P330; Implen, Germany) to determine the concentration of DNA and its purity level. Several different methods have been employed in the quantification of nuclear DNA over the past 50 years. 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 spectrophotometric measurement. Significant progress was made to measure micro volume liquid samples (<1µl) in biotechnology & pharmaceutical applications. For the present study both male and female of wild *Labeo calbasu* (from middle Ganga Region) and cultured *Labeo calbasu* (from culture ponds and/ hatchery) were taken. The value of DNA concentration in female of wild *Labeo calbasu* was in between 58 -62 ng/µl and of male was in between range of 64- 68 ng/µl. The value of DNA concentration in female of cultured *Labeo calbasu* was 60- 66 ng/µl and of male 68- 74 ng/µl.

KEY WORDS: *DNA Quantity, wild, cultured, Nanophotometer, Labeo calbasu.*

INTRODUCTION
Labeo is a genus of carps in the family Cyprinidae. *Labeo calbasu* locally called Kalibasu or Calbasu or Black rohu, is a freshwater fish species and is the most important carp species next to three Indian Major Carps; *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala*. The synonyms used for this species are *Cyprinus calbas* (Hamilton, 1822), *Labeo calbasu* (Day, 1878), *Labeo calbasu* (Shaw and Shebbeare, 1937). It is a popular food fish and also is admired as a sport fish. Recently this fish species has also made its entry in ornamental fish markets of India and abroad. In last few years, the natural populations of this fish species has seriously declined due to over fishing and other anthropological reasons.

The dorsal profile of Calbasu is more convex than of abdomen.Other identifying features includes; Lips are thick and fringed. Two pairs of barbells present, rostal pair longer than maxillary pair (Rahman, 1989). No pores on snout and eye situated a bit anterior from the half of the head (Bhuiyan, 1964). Caudal peduncle is short an lateral line well marked, scales are moderate in size. There are 20 rows of scales before dorsal fin and 22 rows round the caudal peduncle (Bhuiyan, 1964). Mouth is moderately wide and also inferior. Gill openings of Kalibasu is wide and gill rakers are villiform, short and feeble (Bhuiyan, 1964). Colour of body is dark-black but the ventral part light dark. The ventral surface of the opercular region is white iris coppery. This fish attains a length of nearly 90 cm (Talwar and Jhingran, 1991). Generally it is a bottom feeder. It feeds on vegetable matter, crustaceans, insect larvae etc. *Labeo calbasu* feeds on algae 10%, higher plants 48%, protozoa 12%, crustacean 10%, mollusca 5%, mud and sand 15% (Bhuiyan, 1964). It does inhabit rivers and tributaries. They are also seen in deep pools clear sluggish streams, creeks. It can be reared in ponds and also in tanks (Bhuiyan, 1964 and Rahman, 1989).

Of wide distribution in India, it is one of the major Indian carps. It is an important food fish and at several

places is referred to as the "Black Rohu". It is an important game fish in the tanks where it is stocked and is cultivated along with other species. It thrives better in tanks and lakes than in running waters; can tolerate slightly brackish water also. It does not normally breed in ponds; induced bred by hypophysation. It is essentially a bottom feeder. It attains a length of 90 cm. It can be taken on small fly-spoon (Talwar and Jhingran 1991).

OBJECTIVE

To extract DNA of both male and female of wild and cultured *Labeo calbasu*.

To determine concentration of DNA of both male and female of wild and cultured *Labeo calbasu* using Nanophotometer.

MATERIALS AND METHODS

In the present study, *Labeo calbasu* fishes were collected from different water bodies of Western Uttar Pradesh (Bijnor district, district Hapur and from district Meerut), morphologically identified (with the help of standard literature of Day's fauna 1875-78,1889; Jayaram, 1981) and preserved for molecular studies (for DNA isolation and quantification). Approx. 100mg of muscle tissue and fin clip from 2-5 individuals of each species were preserved in 95% ethanol until used and will be kept in -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/µL. 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 and its purity level. Total DNA quantification was carried by nanophotometetrically taking absorbance of 260 and 280nm. A total of 20 samples of *Labeo calbasu* of wild and cultured fishes were analyzed.

RESULTS

The present study was conducted on the isolation of DNA and determination of DNA concentration of both male and female of wild and cultured *Labeo calbasu* using Nanophotometer.DNA quantification was done by using nanophotometer and all data was noted (Table 1). The value of DNA concentration in female of wild *Labeo calbasu* was between 58-62 ng/µl and of male was between ranges of 64- 68 ng/µl (Fig.3). The value of DNA concentration in female of cultured *Labeo calbasu* was 60 - 66 ng/µl and of male 68- 74 ng/µl (Fig. 4).

Table1. Quantity of DNA in all 20 samples of *Labeo calbasu* in Nanogram/ µl/individual

Sample No.	Wild Female	Wild Male	Cultured Female	Cultured Male
1	60	66	66	68
2	58	64	65	70
3	61	66	60	74
4	62	68	66	72
5	59	64	62	74

Nucleic acids play a major role in growth and development. The amount of deoxyribonucleic acid (DNA), the carrier of genetic information, is stable under changing environmental situations and has been used as an indicator of biomass (Dortch et al. 1983) and cell number (Regnault and Luquet 1974). The study revealed that the highest content of DNA was seen in female fishes of wild and also of cultured**.** However slight change was seen in male fish. The comparative result revealed that the highest content was in cultured fishes, where all optimal conditions were maintained and also in female fishes of cultured fishes.

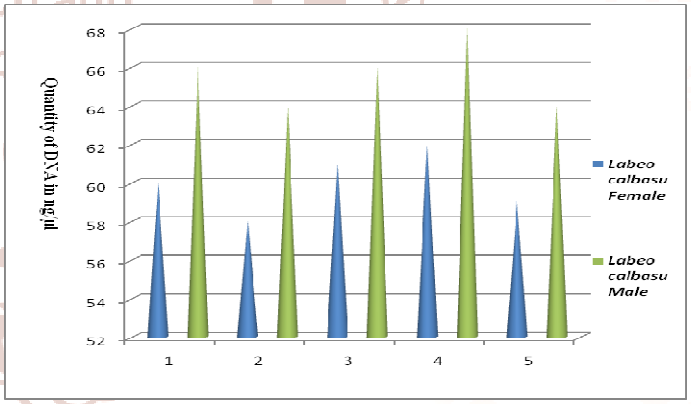

Fig. 1 Quantity of DNA content in Wild *L. calbasu* in Nanogram/ µl/individual

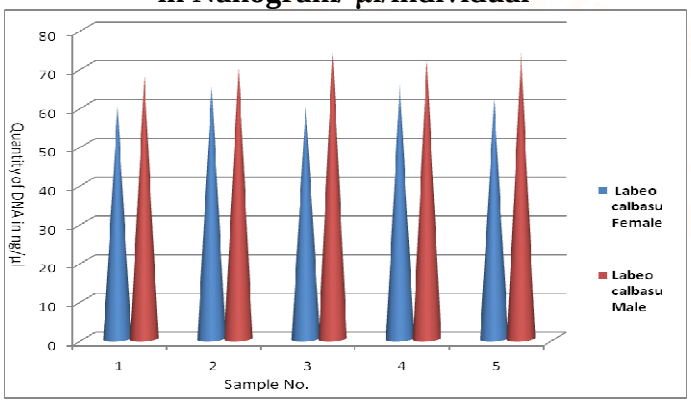

Fig. 2 Quantity of DNA content in Cultured *L. calbasu* in Nanogram/ µl/individual

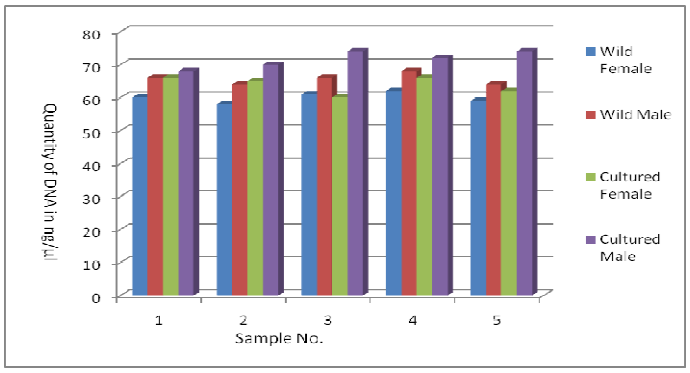

Fig. 3 Comparative DNA Quantification in nanogram/ µl in all 20 samples of both male and female *L. calbasu*

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.

DISCUSSION

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 (Pratima *et. al*,. 2014). The value of DNA concentration in female of wild *Labeo calbasu* was between 58-62 ng/µl and of male was between ranges of 64- 68 ng/µl. The value of DNA concentration in female of cultured *Labeo calbasu* was 60 - 66 ng/µl and of male 68- 74 ng/µl. 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.* In the project report submitted by Aderibigbe Adedunni (2014) to the department of aquaculture and fisheries management, University of agriculture, Nigeria determination of DNA concentration of *Clarias gariepinus* was done by nanodrop. 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 *C. batrachus* was in between 59 and 61 ng/µl and of male was in between range of 70- 76 ng/µl. The value of DNA concentration in female of *C. gariepinus* was 60 and 63 ng/µl and of male 75- 78 ng/µl. Highest DNA concentration was seen in hybrid individuals, in females the concentration was in between 74-76 ng/µl and in case of males value was between 88-91 ng/µl. Similar study on diminution level of RNA/DNA ratio in tissue of *Labeo rohita* by exposure to some endocrine disrupting compounds was also done by Verma *et al.*, 2016.

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