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Variation in DNA Content of Wild and Cultured *Labeo Bata* (Hamilton, 1822) in Different Seasons

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ABSTRACT

The present study was conducted on DNA extraction and determination of DNA content in wild and cultured Labeo bata in different seasons and purity level using Nanophotometer. The results revealed that the value of DNA concentration in wild L. bata in March–May was 63-68 ng/µl; during June- August the concentration was 76-89ng/ µl, September-November 64-70 ng/µl and in case of those samples collected during December – February the DNA concentration was 52-62 ng/µl. The value of DNA concentration in cultured L. bata in March –May was 66-68 ng/ µl, in case of June-August the concentration was 78-98 ng/µl, September – November 78- 88 ng/µl and in case of those samples collected in December- February the DNA concentration was 60-68 ng/µl. The results revealed that the highest concentration was reported in cultured fishes in the month of July-August.

Keywords*: Labeo bata, Nanophotometer, DNA Content, seasonal variation.*

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INTRODUCTION

Labeo bata [8] belong to family Cyprinidae, the carps and minnows of genus *Labeo*. *L. bata* [8] is a freshwater medium sized Indian minor carp, normally attains a length of about 20-25 cm in pond and 30-50 cm in large tanks, reservoir and rivers. *L. bata* is a benthopelagic and potamodromous species, which inhabits rivers. *L. bata* is an important commercial species for aquaculture and is popular for its taste. *Labeo bata* [8] *(syn: Cyprinus acra* Hamilton, 1822, *Cyprinus bata* Hamilton, 1822, *Labeo lissorhynchus* McClelland, 1839,) also known as Minor Carp, Bata, Bata Labeo (Devi and Ali 2001). It is a much sought-after fish with a high value in the market. It has herbivorous feeding habit. It is predominantly a bottom feeder [3]. The natural distribution of *L. bata* is mainly in India, Bangladesh and Nepal [20]. It breeds during July and August [19]. *Labeo bata* is assessed at Least Concern due to its wide distribution and lack of major widespread threats. This species has also been introduced into reservoirs where the species is cultivated. Loss of habitat and overexploitation may be local threats to wild populations.

The fish attained lengths of about 131, 194, 236, 277, 314, 341 and 364 mm at the end of the 1st, 2nd, 3rd, 4th, 5th, 6th and 7th years of life respectively. The body is elongate. Its dorsal profile is more convex than the ventral. The snout slightly projects beyond the mouth, often studded with pores. Pair of small maxillary barbells is hidden inside the labial fold. There is no cartilaginous support to the lips. The dorsal fin originates midway between the snout tip and the anterior base of anal. Pelvic fins originate slightly nearer to the snout tip than to the caudal base [14]. It is bluish or darkish on upper half, silvery below, and the operculum is light orange in colour of fresh specimen. Its food comprises crustaceans and an insect larva in early stages.

Nanodrop is based on fiber optic technology and surface tension, sample is held between a two optical surfaces that define a pathlength in vertical orientation. Total measurement cycle time, including prepration and removal of the sample is nearly 30 second. The ease 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 [9].

Significant progress was made to measure micro volume liquid samples (<1µl) in biotechnology & pharmaceutical applications. In 2005, Nano Drop ND-1000 UV-Vis spectrophotometer (from Nano Drop Technologies) entered the market. This microvolume sample retention system functions by combining fiber optic technology and surface tension properties to capture minute amount of samples. Sample is directly pipette on fiber optic eliminating need of cuvettes and capillaries. Prado *et al* [9] used nanodrop for DNA quantification from different fishes based on nuclear target. Nanodrop technique was also used by Shi *et al*., [17] for DNA quantification in the process of molecular characterization of *Cynoglossus semilaevis.* Determination of DNA concentration of *Clarias gariepinus* was done by nanodrop spectrophotometer [1].

Several different methods have been employed in the quantification of nuclear DNA over the past 50 years. Some of the earliest studies involved bulk biochemical DNA extraction techniques to estimate the total DNA content of a preparation, which was then divided by an estimate of the number of nuclei present. Developed in the late 1970s primarily as a means of detecting the anomalous DNA contents of cancer cells, flow cytometry has since become a mainstay of genome size research. This method involves treating samples of nuclei in suspension with a DNA-specific fluorochrome (e.g., propidium iodide or DAPI), and measuring their fluorescence against that of a known standard included in the sample. This is accomplished by passing the stained nuclei through the path of a laser of a specific wavelength, which stimulates the emission of light by the fluorochrome. The technique is rapid and accurate, but it has some important limitations related to the large number of nuclei required for analysis and the need to place them in suspension. This method is also typically limited to the inclusion of only one (or sometimes two) standard (s), and problems with staining can therefore be difficult to detect. Finally, the fluorochromes used in flow cytometry are base-pair specific, such that differences in GC/AT-content can affect the measurements if only one stain is used.

Fortunately, advances in computing and imaging technology have facilitated the development of a method which maintains the advantages of Feulgen densitometry (e.g., permanent and easily prepared specimens, tissue-specific measurements, multiple standards, low cost) without the immense time consumption of traditional densitometric techniques. The method, nanodrop spectrophotometry, is the one used in the present study.

The NanoDrop microvolume sample retention system functions by combining fiber optic technology and natural surface tension properties to capture and retain minute amounts of sample independent of traditional containment apparatus such as cuvettes or capillaries. Furthermore, the system employs shorter path lengths, which result in a broad range of nucleic acid concentration measurements, essentially eliminating the need to perform dilutions. Reducing the volume of sample required for spectroscopic analysis also facilitates the inclusion of additional quality control steps throughout many molecular workflows, increasing efficiency and ultimately leading to greater confidence in downstream results.

OBJECTIVE OF THE STUDY

- The present study was conducted on the DNA isolation and determination of DNA concentration of *Labeo bata* in different seasons using Nanophotometer, and also to find out the change in DNA content in different seasons.
- To compare DNA content in wild and cultured *L. bata* and to analysis the level of DNA content of both wild and cultured fish using two different DNA isolation methods: traditional method [16] and Kit method (Hi media HiPur A™ mammalian genomic DNA purification Kit).

MATERIALS AND METHODS

In the present study, *Labeo bata* fishes were collected from different water bodies of Western Uttar Pradesh, morphologically identified [4-5, 8] and preserved for molecular studies (for DNA isolation and quantification). Approx. 100mg of white 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 Hi media Hi Pur A ™ mammalian genomic DNA purification Kit and also by following the method of Ruzzante *et. al*., [16] 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 40 samples of *Labeo bata* of wild and cultured fishes were analyzed.

RESULTS

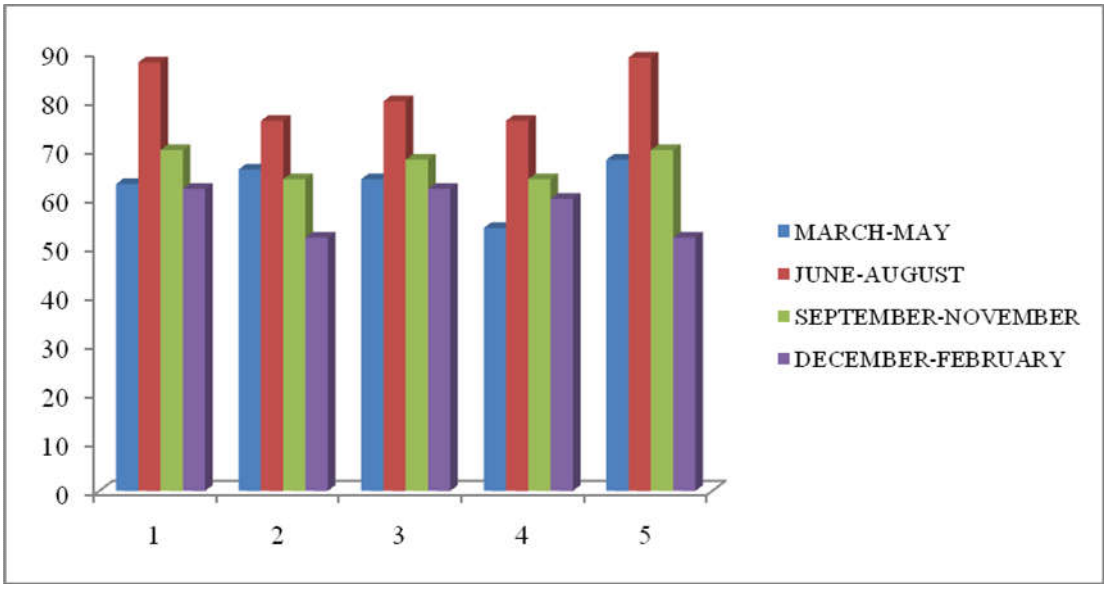
The results revealed that the value of DNA concentration in wild *L. bata* in March–May was 63-68 ng/µl during June- August the concentration was 76-89ng/ µl, September- November 64-70 ng/µl and in case of those samples collected during December – February the DNA concentration was 52-62 ng/µl (Table 1).

Table 1. Seasonal Variation in DNA content of wild *Labeo bata* .

Sample No.	Concentration of DNA (ng/µl) in *Labeo bata*			
	MARCH-MAY	JUNE-AUGUST	SEPTEMBER-NOVEMBER	DECEMBER-FEBRUARY
1	63	88	70	61
2	66	75	64	52
3	64	80	68	62
4	54	76	65	60
5	68	89	70	52

The comparative quantity of all samples revealed that the highest concentration was observed in June-August (Fig. 1). However DNA content was constant in the month of September.

Fig.1. Comparative Quantity of DNA content of wild Labeo *bata* in Nanogram/ µl in all twelve month.



The value of DNA concentration in cultured *L. bata* in March –May was 66-68 ng/ µl, in case of June-August the concentration was 78-98 ng/µl, September – November 78- 88 ng/µl and in case of those samples collected in December- February the DNA concentration was 60-68 ng/µl. The results revealed that the highest concentration was reported in cultured fishes in the month of July-August (Table 2, Fig. 2).

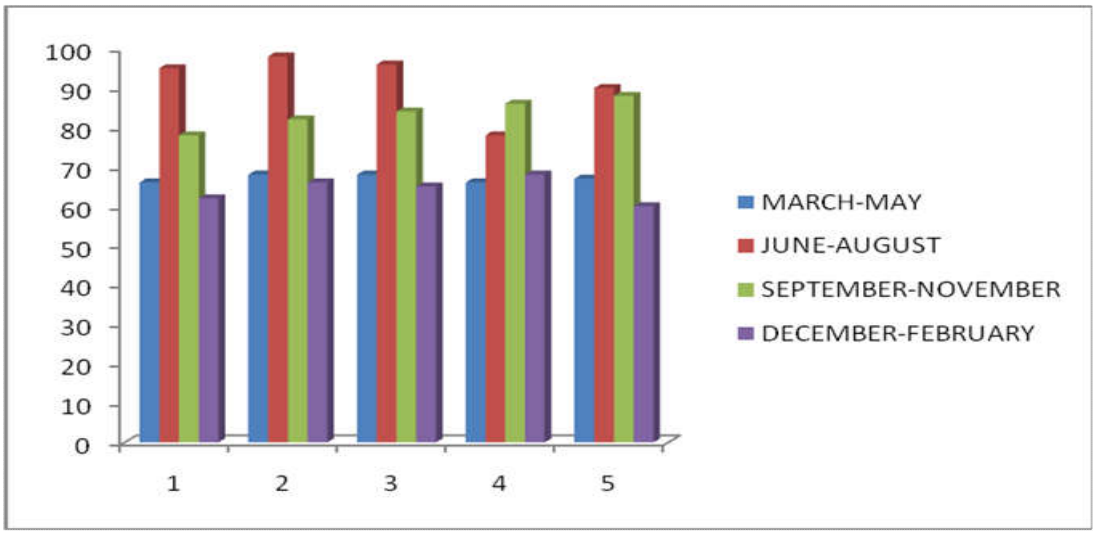
Table 2. Seasonal Variation in DNA content of cultured *Labeo bata*.

Sample No.	Concentration of DNA (ng/µl) in *Labeo bata*			
	MARCH-MAY	JUNE-AUGUST	SEPTEMBER-NOVEMBER	DECEMBER-FEBRUARY
1	66	95	78	62
2	68	98	82	66
3	67	96	84	65
4	66	78	86	68
5	67	90	88	60

The results also revealed that there was no significant change in DNA content when compared to both DNA isolation methods: traditional method [16] and Kit method (Hi media HiPur A™ mammalian genomic DNA purification Kit). A slightly fluctuation was seen in both methods.

The data obtained from this study also suggested that the DNA content was lowest in month of March-May, the level of DNA content was rapidly increase in those samples collected during June-August, during September -November a slightly decrease content was observed. The study suggested that the highest content was seen in June – August, which is also the breeding season of *L. bata.* However the lowest content was in March – May and in winter season (December- February)

Fig.2. Comparative Quantity of DNA content of cultured Labeo *bata* in Nanogram/ µl in all twelve month.



DISCUSSION

In the present study DNA concentration of both wild and cultured *Labeo bata were calculated* using Nanophotometer. Prado *et al*., [9] used nanodrop method for DNA quantification from different fishes based on nuclear target. 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 [10]. The present study was conducted on the isolation of DNA and determination of DNA concentration of wild and cultured *Labeo bata* using Nanophotometer and also to analyse the effect of environmental conditions in DNA Concentration. DNA was isolated and extracted DNA was analyzed using nanophotometer to determine the concentration of DNA and its purity level. The results revealed that the value of DNA concentration in wild *L. bata* in March–May was 63-68 ng/µl during June- August the concentration was 76-89ng/ µl, September- November 64-70 ng/µl and in case of those samples collected during December – February the DNA concentration was 52-62 ng/µl. The value of DNA concentration in cultured *L. bata* in March – May was 66-68 ng/ µl, in case of June-August the concentration was 78-98 ng/µl, September – November 78- 88 ng/µl and in case of those samples collected in December- February the DNA concentration was 60-68 ng/µl. The results revealed that the highest concentration was reported in cultured fishes in the month of July-August. Similar study on Labeo angra was also done by Rana and Jain [11] and results revealed that the value of DNA concentration in wild *L. angra* in March–May was 64-68ng/ µl during June-August the concentration was 66-78 ng/µl, September-November 64-75 ng/µl and in case of those samples collected during December–February the DNA concentration was 54-68 ng/µl. The value of DNA concentration in cultured *L. angra* in March –May was 54-70ng/ µl, in case of June-August the concentration was 90-105 ng/µl, September – November 78- 89 ng/µl and in case of those samples collected in December- February the DNA concentration was 66-75 ng/µl.

Study on DNA quantification of *Labeo calbasu* was done by Rana and Jain [12] and their results revealed that 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.

DNA quantification of *Cirrhinus mrigala* was also done by Rana and Jain [13] and their results revealed that the value of DNA concentration in female of wild *Cirrhinus mrigala* was between 62 - 66 ng/µl and of male was between 64- 78 ng/µl. The value of DNA concentration in female of cultured *Cirrhinus mrigala* was between 60 - 88 ng/µl and of male was between 70- 78 ng/µl.

Similar studies were also done by Adedunni in 2014 on *Clarias gariepinus* by nanodrop spectrophotometer and Nanodrop technique was also used by Shi *et. al.,* [17] for DNA quantification in

the process of molecular characterization of *Cynoglossus semilaevis*, Verma *et. al.,* in [21]. DNA quantification of Male and Female *Clarias batrachus, Clarias gariepinus* and *Clarias* hybrids was also done by Shobhna [18].

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