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Interspecific variations in biochemical parameters of *Clarias batrachus* (Linnaeus, 1758) and *Clarias gariepinus* (Burchell, 1822): A comparative study

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Abstract: *Clarias batrachus* (Linnaeus, 1758) commonly called as 'Magur' is Indian native (endogenous) species has similar morphological features as *Clarias gariepinus* (Burchell,1822), which is deadly carnivorous exotic African species introduced to India through various trade means. The indigenous species *C. batrachus* is highly preferred by Indian and other Asiatic consumers because of its high content of arginine and lysine in its flesh and medicinal value. The exotic species *C. gariepinus* has undergone tremendous adaptation to the Indian physicochemical condition resulting in mass substitution of the native species *C. batrachus* from the Indian fish market. The aim of the present study was to investigate and establish reference ranges by comparing biochemical values of two fresh water air breathing species of family Clariidae i.e. *C. batrachus* and *C. gariepinus* from the state Bihar. The study was aimed to investigate serum biochemical parameters like Liver Function Test (LFT) including Serum Glutamic Pyruvate Transaminase (SGPT), Serum Glutamic Oxaloacetate Transaminase (SGOT), Serum Alkaline Phosphatase (ALP), serum bilirubin, and Kidney Function Test (KFT) including serum Total Protein (TP), albumen, globulin, A/G ratio and serum creatinine and blood urea etc. The mean ± SEM and range were established. In the present study, the estimated biochemical values were compared between and within the two species of cat fish using One-Way Analysis of Variance (ANOVA). Nearly all most all parameters differ significantly ($p<0.05$) between *C. batrachus* and *C. gariepinus*. The present study will lead to the proper characterization of LFT and KFT parameters of these two closely allied species of family Clariidae and later on can be used as a sensitive index to monitor various physiological and pathological changes in fish due to xenobiotic stress. It will also help in indicating the health status of the fish and pollution status of the aquatic bodies. Further studies are needed to trace the genetic basis of the significant variation in the biochemical parameters of these two closely allied fresh water air breathing species.

Key words: Biochemical analysis, Blood, *Clarias batrachus*, *Clarias gariepinus*, KFT, LFT, Serum.

INTRODUCTION

Fisheries and aquaculture is a fast-growing sector in India, providing nutrition and food security to large population of country apart from generating income and employment to more than 14.5 million people (The Economic Times, Agriculture, July 04, 2019). Globally, India stands second in culture fisheries production. The fisheries sector plays an important role in the Indian economy and up-liftment of socio-economic status of fishing communities of state and foreign exchange earnings[1]. The fisheries resources in the state of Bihar is chiefly comprised of ponds, tanks, small reservoirs, rivers and water logged areas like ox-bow lakes and chaurs[2-3]. Around 65,000 ha of water areas are covered by ponds and tanks and nearly 35,000 ha of water areas consist of

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ox-bow lakes and chaurs[2]. The family Clariidae represents specialized group of air breathing fishes with high content of arginine and lysine in their flesh, high Vitamin D content, low level of omega-3 fatty acids and a much higher proportion of omega-6 fatty acids, prolific breeding potential and high therapeutic values. They are easy to handle in laboratory, leading to inexpensive and easy rearing demands. In Asiatic countries three species of *Clarias* (family Clariidae) are known to be cultivated by fish farmers as- *Clarias batrachus* (Linnaeus, 1758) in India[4]; *Clarias macrocephalus* in South- East Asia[5] and *Clarias fuscus* (Lucep'de,1803) in Taiwan and Hawaii[6-7].

Clarias batrachus, commonly known as 'magur' is a favourite edible fish with high medicinal value in India and other Asian countries including Bangladesh, Thailand, Vietnam, Malaysia and Indonesia as it is an endogenous Asian species[8-10]. The fish finds its origin in India and later has been shown to spread in the different parts of Globe[11-12]. Even four sub species of *Clarias batrachus* have been reported from South East Asia, Java and India as *C. batrachus; C. aff. batrachus "Indochina"; C. aff. batrachus "Sundaland" and Clarias magur* (Hamilton, 1822)[13]. In India declining trend in the population of the *C. batrachus* is partly contributed by habitat degradation and overfishing, but the major cause is the introduction of a morphologically alike and deadly carnivores African species *Clarias gariepinus* (*Burchell,*1822)[14]. This exotic species have been reported to feed on the native species and also hybridize naturally with them[15]. Due to excessive predation on the native species, this exotic species have generated a potential threat for the survival of *Clarias batrachus* and accordingly the Ministry of agriculture in India put ban on the culture and import of *C. gariepinus* in 1997[16]. But due to prolific breeding ability, excessive acclimatization in the Indian condition and simplicity in rearing, the fisherman in different states of India preferably culture this fish, setting aside the ban and restriction imposed by the ministry of agriculture, Government of India.[17]

Blood parameters analysis has been used widely to determine the systematic relationship among species of fish[18]. Blood parameters have also been considered as indicators of the physiological condition or sub-lethal stress response in fish exposed to internal or external factors[19]. The physiological status of fish can be evaluated authentically by hematological indices. Changes in hematological parameters depend upon the aquatic biotope, fish species, age, and sexual maturity and health status[20].

Blood of fishes comprises 1.3-7% of the total body weight and that is why it is considered as one of the mostactive components that contribute to metabolic processes by ensuring gas exchange between the organism and the environment. They serve to transport all kinds of nutrients, enzymes, hormones, neurotransmitters and all metabolic intermediaries in the different parts of body, apart from helping in transporting nitrogenous wastes outside the body of the fish.

The principle objective of the present study is to characterize a baseline concrete biochemical parameters of *Clarias batrachus* and *Clarias gariepinus,* which can be later used as reference point. It will also generate an authenticated base line data, which can be applied for monitoring of health and disease status, reproduction and ecotoxicological studies. The finding of the present study can be correlated with the advanced mitochondrial genome analysis of these two closely allied species of family *Clariidae.*

MATERIALS AND METHODS

Biological sampling and Acclimatization: Adult specimen of *C. batrachus* ranging from 60-110 gm and size between 4.6"-7.1" and *C.gariepinus* ranging from 120-240gm and size between 5.2"-9.2"were collected from two different fish farms from Purnia, Bihar, India. Fishes were brought to laboratory and disinfected with 0.1% $KMnO_4$ solution. They were segregated as per their size and species in different sized large plexi glass aquaria (capacity 50L, 80 L and 100L) having dechlorinated, aerated tap water at normal temperature and pressure. Fishes were acclimatized to the ideal laboratory condition for 15 days as per standard method[21]. Fishes were fed *ad libitum* and then fresh water was changed regularly in the morning hours of every day.

Collection of Blood Sample: The peripheral blood was collected by puncturing the genital opening with heparin treated 21 gaugex 0.5 inch needle fitted with 2 ml syringe. The collected blood samples were quickly transferred into collection tubes containing EDTA and were gently mixed by carefully turning it upside down. The collection tubes were then kept in the sampling boxes containing ice.

Biochemical Analysis: Blood samples were centrifuged in Remi centrifuge at 3000 rpm for 15 minutes in order to get the serum. Then serum was carefully siphoned out from centrifuged blood of both the species. The serum was used for estimation of Liver Function Test (LFT) and Kidney Function Test (KFT) respectively.

Liver function test: For liver function test, the determination of Serum glutamate pyruvate transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT) was done as per standard protocol[22]. Serum Alkaline Phosphate (ALP) was estimated by specific method[23] and Serum bilirubin was assessed as per standard protocol[24].

Kidney function test: For Kidney function test the determination of Total Protein (TP) was estimated by standard Biurette method[25-26], Serum Albumen (SA), Serum Globulin (SG) &, A/G ratio was estimated by standard BCG Method[27]. Serum creatinine and serum uric acid were estimated by standard Jeff method[28].

Statistical Analysis: For each blood samples, the data of each parameter was analyzed and presented as mean and standard error of mean. One way Analysis of Variance (ANOVA) test was done to observe the variation in different parameters between two species or even within species. All statistical analysis was done using SPSS.

RESULT

A slight morphological variation has been marked in both these species. The body colour of *Clarias batrachus* was dark greyish to greyish black, while in *C. gariepinus* it is silvery greyish black. The demarcating morphological feature was the shape of the occipital, which was broad and blunted in *C batrachus* while narrow and somewhat pointed in *C gariepinus*. Apart from that majority of the morphometric characteristics in both the species were nearly same as per the standard protocol of taxonomic identification of freshwater fish species[29-30].

Results of Liver Function Test

The value of various parameters of Liver function test has been represented in Table- I.

Table I: Various parameters of Liver Function Test of *Clarias batrachus* and *Clarias gariepinus*

Parameters	Sample size	*Clarias batrachus*		*Clarias gariepinus*		Calculated F value	Table F -value
		Mean	SEM	Mean	SEM		
Serum Glutamate Pyruvate Transaminase (SGPT = U/L)	20	92.6	12.58	47	7.99	9.35	5.31
Serum Glutamate Oxaloacetate Transaminase (SGOT = U/L)	20	167.6	24.31	34.8	7.28	27.3	5.31
Serum Alkaline Phosphate (ALP = U/L)	20	18.8	3.55	61.8	5.40	44.14	5.31
Serum Bilirubin (mg/dl)	20	2.02	0.53	1.39	0.22	1.15	5.31

Figures in each parameters have been shown as Mean ± SEM, the sample size in each in case is n = 20, significant at p < 0.05. One way analysis of Variance was done. Calculated value has been compared with table value.

Results of Kidney Function Test

The value of various parameters of Kidney function test has been represented in Table – II.

Table II: Various parameters of Kidney Function Test of *Clarias batrachus* and *Clarias gariepinus.*

Parameters	Sample size	*Clarias batrachus*		*Clarias gariepinus*		Calculated F value	Table F -value
		Mean	SEM	Mean	SEM		
Total Protein (gm/dl)	20	3.91	0.38	3.20	0.16	2.82	5.31
Serum Albumen (gm/dl))	20	2.02	0.53	1.39	0.22	1.15	5.31
Serum Globulin (gm/dl)	20	1.40	0.19	2.10	0.17	6.90	5.31
Serum Creatinine (mg/dl)	20	0.50	0.083	0.62	0.037	1.71	5.31
Serum Uric Acid (mg/dl)	20	2.46	0.30	2.99	0.18	2.15	5.31

Figures in each parameters have been shown as Mean ± SEM, the sample size in each in case is n = 20, significant at p < 0.05. One way analysis of Variance was done. Calculated value has been compared with table value

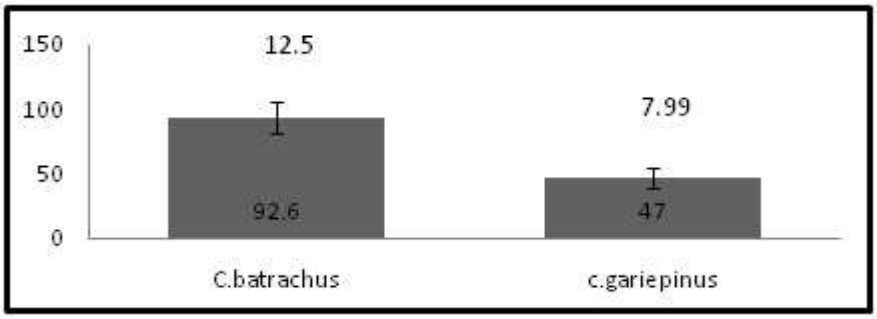

Fig.1: Histogram showing interspecific variation in SGPT (U/L) of *Clarias batrachus* and *Clarias gariepinus*

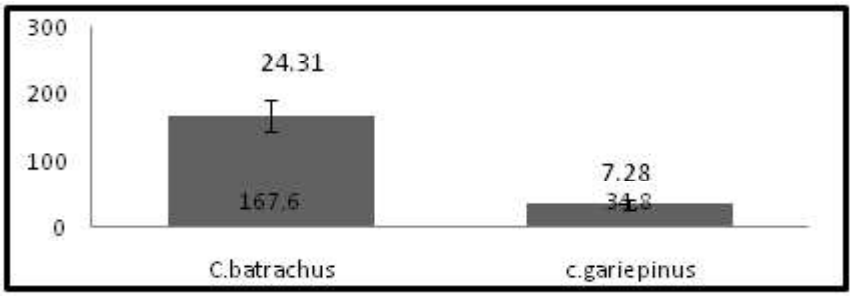

Fig.2: Histogram showing interspecific variation in SGOT (U/L) of *Clarias batrachus* and *Clarias gariepinus*

Fig.3: Histogram showing interspecific variation in ALP (U/L) of *Clarias batrachus* and *Clarias gariepinus*

Fig.4: Histogram showing interspecific variation in Serum bilirubin (mg/dl) of *Clarias batrachus* and *Clarias gariepinus*

Fig. 5: Histogram showing interspecific variation in Total protein (gm/dl) of *Clarias batrachus* and *Clarias gariepinus*

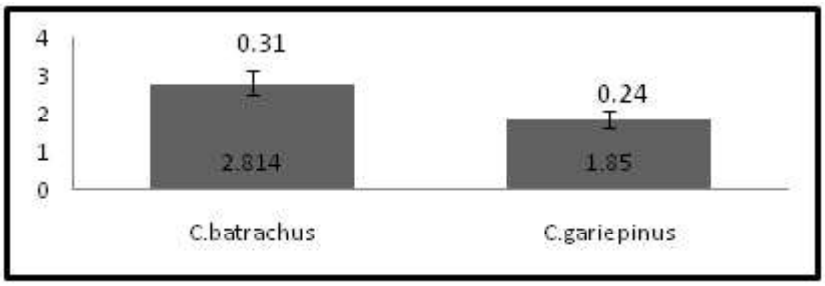

Fig. 6: Histogram showing variation in Serum albumin (gm/dl) of *Clarias batrachus* and *Clarias gariepinus*

Fig.7: Histogram showing variation in Serum globulin (gm/dl) of *Clarias batrachus* and *Clarias gariepinus*

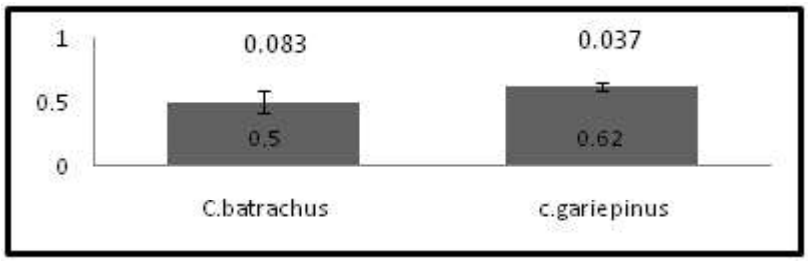

Fig. 8: Histogram showing variation in Serum creatinine (mg/dl) *of Clarias batrachus* and *Clarias gariepinus*

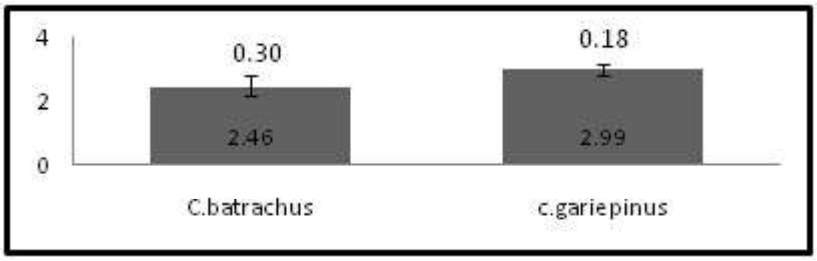

Fig. 9: Histogram showing variation in Serum uric acid (mg/dl) of *Clarias batrachus* and *Clarias gariepinus*

DISCUSSION

The central theme of the present investigation is to establish a baseline reference value of biochemical parameters of two closely allied species of family Clariidae i.e. *Clarias batrachus,* the indigenous species and *Clarias gariepinus*, the exotic species with special reference to Liver function test and kidney function test profile. The present study also deals with the biochemical variations in between these two species.

The blood of the fish is highly sensitive and truly reflects the health status of the fish. It is widely used to study the vital biological process taking place in a fish species[31]. Fish blood is being consistently been used in toxicological researches and environmental monitoring as a probable indicator of pathophysiological changes in effective fishery management[32]. Liver function test includes a battery of serological tests done for the initial detection and management of liver diseases. LFT serves three basic purposes- **a.** Assessing the potential of liver to transport organic anions and to metabolize drugs (including LFT parameters like serum bilirubin, urine bilirubin, urobilinogen etc.; **b.** Assessing the injury to hepatocytes (including LFT parameters like aminotransferases, alkaline phosphatase, glutamyltranspeptidase, 5 nucleotidase, leucineaminopeptidase etc.). **c.** Assessing the biosynthetic capacity of the liver (including LFT parameters like serum protein, albumen, prealbumen, serum ceruloplasmin, procollagen III peptide, α 1 antitrypsin, α feto protein etc.). In the present investigation SGPT, SGOT ALP and serum bilirubin has been considered under LFT profile.

Transamination represents one of the principal metabolic pathway for the synthesis and deamination of amino acids. Glutamic pyruvic transaminase (SGPT) or alanine amino transferases (ALT) catalyse the transfer of an amino group from L-alanine to α-ketoglutarate, the products of this reversible transamination reaction being pyruvate and L-glutamate. It helps the liver in converting food into energy. In the present study the level of SGPT in control *C. batrachus* and *C. gariepinus* have been recorded as 92.6±12.58 and 47±7.99 respectively. The calculated F value is 9.35, which is much higher than the table value *i.e.* 5.31. It clearly depicts that the liver metabolism and related hepatic impairment is higher in *C. gariepinus*. The transaminase level in different fishes has been earlier reported[33-34].

Aspartate Transaminase (AST) or aspartate amino transferase, also known as AspAT/ASAT/AAT or (serum) glutamic oxaloacetic transaminase (GOT, SGOT), is a pyridoxal phosphate (PLP)-dependent transaminase enzyme that catalyzes the reversible transfer of an α-amino group between aspartate and glutamate and, as such, is an important enzyme in amino acid metabolism. The level of SGOT in control *C. batrachus* and *C. gariepinus* have been recorded as 167.6 ± 24.31 and 34.8 ± 7.28 respectively. The calculated F value is 27.3, which is much higher than the table value i.e. 5.31. The difference is highly significant ($p<0.05$).

ALP is an enzyme found throughout the body, but it is mostly found in the liver, bones, kidneys, and digestive system. It plays an integral role in metabolism within the liver and development within the skeleton. When the liver is damaged, ALP may leak into the blood stream. High levels of ALP can indicate liver disease or bone disorders. The level of ALP in control *C. batrachus* and *C. gariepinus* have been recorded as 18.8±3.55 and 61.8±5.40 respectively. The calculated F value is 44.14, which is much higher than the table value i.e .5.31. The difference is highly significant ($p<0.05$).

Bilirubin (BR) is a yellow compound that occurs in the normal catabolic pathway that breaks down heme in vertebrates. This catabolism is a necessary process in the body's clearance of waste products that arise from the destruction of aged or abnormal red blood cells[35]. First the haemoglobin gets stripped of the heme molecule which thereafter passes through various processes of porphyrin catabolism, depending on the part of the body in which the breakdown occurs. The production of biliverdin from heme is the first major step in the catabolic pathway, after which the enzyme biliverdin reductase performs the second step, producing bilirubin from biliverdin[36-37]. The level of serum bilirubin (BR) in control *C. batrachus* and *C. gariepinus* have been recorded as 2.02±0.53 and 1.39±0.22 respectively. The calculated F value is 1.15, which is much lower than the table value i.e. 5.31.The difference is not significant at ($p<0.05$).

The Kidney Function Test (KFT) provides valuable information about the functional status of kidney and physiology of excretion and acid base balance. In general, nephrotoxicity can be generated by pre renal, renal and post renal causes. Serum total protein is estimated for

monitoring gross changes in protein levels marked in various pathological conditions. The accuracy of the test lies in association with the liver function test and protein electrophoresis. The level of serum total protein (TP) in control *C. batrachus* and *C. gariepinus* have been recorded as 3.91±0.38 and 3.20±0.16 respectively. The calculated F value is 2.82, which is much lower than the table value i.e.5.31. The difference is not significant at ($p<0.05$).The reference value of serum total protein in different group of teleost have been reported time to time as 2.02±2.37gm/dl in *Cyprinus carpio*[38], 4.55±0.13 gm/dl in *Clarias gariepinus*[39] and 3.99±0.99 gm/dl & 5.97±0.56 gm/dl in *Clarias batrachus*[40-41]. Plasma protein is the protein component of the blood and it increases with starvation and any other physiological stress[42]. Plasma protein gives an index of the health status of the brood fish[43] and serves as indicator of nutritional status[44].

Serum albumin is principally synthesized in the liver and maintains the osmotic pressure in the blood. It helps in transportation of lipids and general metabolism in fishes[45]. It also forms the part of amino acid pool. The rise in albumen concentration in animals due to loss through urine or faeces or through break down may result in impaired synthesis[46]. The level of serum albumin in control *C. batrachus* and *C. gariepinus* have been recorded as 2.02±0.53 and 1.39±0.22 respectively. The calculated F value is 1.15, which is much lower than the table value i.e.5.31. The difference is not significant at ($p<0.05$). The present finding is in agreement with earlier recorded reference value of serum albumen in different teleost as 1.083±0.82 gm/dl in *Clarias gariepinus*[39], 1.0±0.79 gm/dl in *Clarias batrachus*[41].

The globulin fraction includes hundreds of serum proteins including carrier proteins, enzymes, complement, and immunoglobulins. Most of these are synthesized in the liver, although the immunoglobulins are synthesized by plasma cells. Increase in the globulin fraction usually result from an increase in immunoglobulins and in some pathological condition. Malnutrition and congenital immune deficiency can cause a decrease in total globulins due to decreased synthesis, and nephrotic syndrome can cause a decrease due to protein loss through the kidney. The level of serum globulin in control *C. batrachus* and *C. gariepinus* have been recorded as 1.40±0.19gm/dl and 2.10±0.17gm/dl respectively. The calculated F value is 6.90, which is much higher than the table value i.e.5.31. It is significant at $p<0.05$.

Serum creatinine is an important indicator of kidney health because it is an easily measured byproduct of muscle metabolism that is excreted unchanged by the kidneys. Creatinine itself is produced via a biological system involving creatine, phosphocreatine and adenosine triphosphate (ATP) Creatine is synthesized primarily in the liver from the methylation of glycol-cyamine. It is then transported through blood to the other organs, muscle and brain, where through phosphorylation, it becomes the high-energy compound phosphocreatine. Creatine conversion to phosphocreatine is catalyzed by creatine kinase and in due course of reaction creatinine is formed spontaneously. The level of serum creatinine in control *C.batrachus* and *C. gariepinus* have been recorded as 0.50±0.083 mg/dl and 0.62±0.037mg/dl respectively. The calculated F value is 1.71, which is much lower than the table value *i.e.* 5.31. It is not significant at $p<0.05$.

Uric acid is the ultimate catabolite of purine metabolism. It is a weak organic acid that under physiologic conditions exists mainly as a monosodium salt. Serum uric acid reflects the interactions of four major processes:dietary purine intake, endogenous purine metabolism, urinary urate excretion, and intestinal uricolysis. Many additional factors, including exercise, diet, drugs, and state of hydration, may result in transient fluctuations of uric acid levels. The level of serum uric acid in control *C. batrachus* and *C. gariepinus* have been recorded as 2.46±0.30 mg/dl and 2.99±0.18mg/dl respectively. The calculated F value is 2.15, which is much lower than the table value i.e. 5.31 rendering it not significant at $p<0.05$.

CONCLUSION

The present study has generated the reference value of various biochemical parameters associated with Liver function test and Kidney function test of two closely allied species of cat fishes *Clarias batrachus* and *Clarias gariepinus* (Family:*Clariidae*). Statistical ANOVA test clearly reveals that apart from serum bilirubin all the parameters of LFT *viz.* SGPT, SGOT and ALP *etc.* significantly ($p<0.05$) vary between *C. batrachus* and *C. gariepinus.* Amongst KFT parameters except serum globulin (which is highly significant at $p<0.05$), all

parameters show non-significant difference between these two species. The biochemical parameters are considered as the basic valuable tool to monitor fish health and production parameters of the aquatic bodies. The base line reference data can be further used for studying any kind of xenobiotic stress in fish. It will help in making effective conservation strategies and successful productive management of the aquatic bodies. The findings of the present study can be further correlated with the advanced mitochondrial genome analysis of these two closely allied species of family *Clariidae.*

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