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Growth and Bioaccumulation of Iron in the Body Organs of *Catla catla, Labeo rohita* and *Cirrhina mrigala* during Chronic Exposures

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ABSTRACT

Laboratory tests were conducted, with nine age groups of three fish species, to determine their feed intake, weight gains and feed conversion efficiency (FCE) during 60-days exposure to sub-lethal (1/3 of LC_{50}) iron (Fe) concentrations of 40.05, 23.33 and 40.08 mg L^{-1} for *Catla catla*, *Labeo rohita* and *Cirrhina mrigala*, respectively. This investigation also focuses on the extent of Fe bioaccumulations in the fish organs viz. gills, kidney, liver, skin, muscle and scales during chronic toxicity exposure growth trials. Both treated and control three fish species showed significantly variable responses towards feed intakes, weight increments and FCE values. Feed intake in Fe exposed fish species did not cause significant increase in their body weights. However, control fish exhibited significantly higher feed intake, weight increments and FCE than that of treated fish. Sensitivity of fish to Fe toxicity decreased significantly with age. Significant differences existed among nine fish age groups for the accumulation of Fe in the body organs of three treated fish species. Fish liver and kidney showed significantly higher tendency for the accumulation of Fe. *L. rohita* showed significantly higher tendency for the accumulation of Fe in its body than *C. catla* and *C. mrigala*. Fe contents in gills, kidney and skin increased with fish age also. However, Fe accumulation was predominantly highest in the kidney, followed by that in liver and gills. The accumulation of Fe in fish kidney had significantly direct relationships with both temperature and pH of water. However, water hardness had significantly inverse relationship with the uptake and accumulation of Fe by the fish kidney. © 2010 Friends Science Publishers

Key Words: Fish; Iron; Growth; Bioaccumulation; Fish body organs

INTRODUCTION

The contamination of freshwater with a broad spectrum of pollutants has become a matter of concern all over the world (Voegborlo *et al*., 1999; Vutukuru, 2005; Rauf *et al*., 2009). The natural aquatic bodies have extensively been contaminated with heavy metals released from domestic, industrial and other man-made activities (Conacher *et al*., 1993; Velez & Montoro, 1998; Hayat & Javed, 2008). Heavy metal contamination may cause devastating impacts on the ecological balance of the natural water bodies and hence disturbing the diversity of aquatic organisms (Vosyliene & Jankaite, 2006; Farombi *et al*., 2007; Hayat & Javed, 2008).

Iron (Fe) is an indispensable element for the functioning of organs and tissues of higher animals, including fish, because of its vital role in oxygen transport and cellular respiration. Fe is also one of the most important micronutrients in terms of its effect on functioning of immune system and defense against various infections (Beisel, 1982; Bhaskaram, 1988). Fish can absorb soluble Fe from the water across the gill membrane and intestinal mucosa (Roedar & Roedar, 1968; Sealey *et al*., 1997). However, feed is considered a major source of Fe for fish due to low concentrations of soluble Fe in natural waters (NRC, 1993). The total dietary Fe requirement for optimum growth, feed efficiency, hematological values and immune response of juvenile channel catfish has been determined to about 30 mg/kg diet (Gatlin & Wilson, 1986; Lim *et al*., 1996; Sealey *et al*., 1997). The effect of dietary Fe on resistance of channel catfish to bacterial pathogens is unclear, although Sealey *et al*. (1997) suggested that high levels of dietary Fe (180 mg/kg) may lead to increased susceptibility.

Various studies on fish have demonstrated that heavy metals may alter the physiological activities and biochemical parameters both in tissues and blood (Tort & Torres, 1988; Basa *et al*., 2003). The toxic effects of heavy metals have also been reviewed including bioaccumulation in fish body (Aucoin *et al*., 1999; Rasmussen & Anderson, 2000; Adami *et al*., 2002; Waqar, 2006; Abdullah *et al*., 2007). In nature, aquatic animals are constantly exposed to a wide range of metals. The species and concentration of metals in water are determined by geochemical processes

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and large scale released into the aquatic environment by anthropogenic activities (Wittmann, 1979). Rapid industrial development as well as the use of metals in production processes has resulted in increased discharge of heavy metals, including Fe, into the environment (Koli *et al*., 1977). Therefore, these metals tend to accumulate in the aquatic environment. Since heavy metals are non-biodegradable, they can be bio-accumulated by the fish, either directly from surrounding water or by ingestion of food (Patrick & Loutit, 1978; Kumar & Mathur, 1991). The objective of present investigation was to find out the growth potential and tendency of major carps to bio-accumulate Fe during chronic exposure as no such work is available on these species of fish in Pakistan.

MATERIALS AND METHODS

Three major carps viz. *C. catla, L. rohita* and *C. mrigala* of nine age groups i.e., 90-to 330-day were grown in glass aquaria, separately, for 60-day under sub-lethal Fe concentrations of 40.05, 23.33 and 40.08 mg L^{-1}, respectively, as determined by Javed and Abdullah (2003). The parameters viz., feed intake, increase in average weight and FEC of the fish were monitored. The fish were acclimated under laboratory conditions in cement tanks for two weeks. Since the values for weight-length coefficient (condition factor) did not show any distinct pathological symptoms, the fish were with good health. After acclimation, fish were exposed to the sub-lethal levels of Fe (as pure chloride compound) in tap water, using a static water system with continuous aeration, under at room temperature. Each growth trial of 60-day was performed, with three replications, in glass aquaria having 50 L water. The exposure mediums were continuously replenished and partly exchanged to maintain the sub-lethal concentrations of Fe for fish species, separately, throughout the 60 day experimental period in each growth trial. Hydro-chemical parameters i.e., temperature, dissolved oxygen, pH, electrical conductivity, total ammonia, chlorides, sodium, potassium and total hardness were monitored on daily basis at 10.00 and 18.00 h by following the methods of APHA (1975).

Concentrations of Fe in the test media were measured by Atomic absorption spectrophotometer (Perkin Elmer Aanalyst 400, USA). During each trial, fish were fed, to satiation, a feed (DE= 2.90 kcal g^{-1}; DP: 35%) daily at 9.00 and 17.00 h daily. The control fish were grown in metal free ground water for comparison. After each trial, fish were dissected and their organs *viz*., gills, kidney, liver, skin, muscle and scales isolated. The fish organs were digested in nitric acid and perchloric acid and Fe concentrations were determined with the methods of SMEWW (1989).

Data were statistically analyzed using ANOVA and Tuckey's Student Newnan-Keul tests. Correlation coefficients were obtained to draw relationships of various parameters under study (Steel *et al*., 1996).

RESULTS

Nine age groups of fish, exposed to Fe concentrations, exhibited significant differences for their feed intakes, which indicated significant variations in weight increments and food conversion efficiency (FCE) values of three fish species. There existed significant variations between treated and control fish for their feed intakes, weight increments and FCE values. Among the nine treated fish age groups, 210-day fish showed higher mean feed intake of 2.87 g, gaving on an average 0.40 g weight increment (Table I). However, the maximum average weight increment of 1.47 g was attained by the 330-day fish with an average feed intake of 1.75 g; thus FCE was 84% higher. The average feed intakes by three control fish species ranged between 4.13±0.77 and 1.09±0.04 g for 330-and 120-day age groups, respectively while fish weight escalations ranged between 3.87±0.05 and 0.65±0.07 g for 330-and 240-day fish groups. The maximum FCE values of 84 and 93.7% were recorded for 330-day treated and control fish, respectively while the same were minimum i.e., 5 and 23.81% for treated and control for 180-and 240-day old fish, respectively. Among three treated fish species, feed intake was significantly higher in *C. mrigala* and *L. rohita* as 2.04 and 2.00 g, respectively, than that of *C. catla* (1.87 g), while all the three control fish species did not show any significant differences. The average weight increments of 0.84 and 1.74 g were highest in *C. mrigala*, followed by *L. rohita* and *C. catla* for both treated and control fish. Among treated fish species, both *C. catla and L. rohita* had significantly higher FCE than *C. mrigala.* However, control *C. mrigala* showed significantly higher FCE of 72.85±3.85%, followed by that of *L. rohita* and *C. catla.* The control fish exhibited significantly higher FCE than those exposed to sub-lethal levels of Fe (Table I).

The accumulation of Fe in nine age groups of fish varied significantly. All the three fish species performed differently regarding their ability to accumulate Fe in their body organs. The 330 day fish showed significantly higher tendency for the accumulation of Fe than rest of the age groups. Among the three fish species, *L. rohita* showed significantly higher Fe accumulation than *C. catla* and *C. mrigala*. The accumulation of Fe in fish organs varied significantly also. Fish kidney and liver exhibited significantly higher tendency for the accumulation of Fe. However, Fe concentrations in fish kidney and liver were statistically at par (Table II).

Table III shows age related relationships of fish organs with metal exposure concentrations for the uptake and accumulation of Fe. The accumulation of Fe in fish gills, kidney and skin showed direct relationships with fish age. However, the correlation for all the organs, except kidney, was non-significant. The accumulation of Fe in fish gills, skin and muscle showed direct relationships with the metal concentrations of the test mediums. However, the correlation between Fe and its uptake by the gills was

Table I: Growth responses of fish exposed to sub-lethal concentrations of water-borne iron

	Average feed intake by three fish species (g)		Average weight increments in three fish species (g)		Average feed conversion efficiency of three fish species (%)	
	Treated	**Control**	**Treated**	**Control**	**Treated**	**Control**
Fish Age						
90-day	1.32±0.03	1.56±0.05	1.11±0.24	1.37±0.06	84.09	87.82
120-day	1.21±0.21	1.09±0.04	0.38±0.08	0.96±0.02	31.40	88.07
150-day	1.76±0.23	1.36±0.11	0.29±0.02	1.05±0.03	16.48	77.21
180-day	2.40±0.11	3.28±0.63	0.12±0.01	1.10±0.04	05.00	33.54
210-day	2.87±0.23	3.97±0.39	0.40±0.04	1.32±0.11	13.94	33.25
240-day	2.01±0.22	2.73±0.55	0.23±0.06	0.65±0.07	11.44	23.81
270-day	1.87±0.37	2.85±0.42	0.21±0.03	1.01±0.01	11.23	35.44
300-day	2.56±0.33	3.42±0.31	1.46±0.15	2.98±0.03	57.03	87.13
330-day	1.75±0.40	4.13±0.77	1.47±0.14	3.87±0.05	84.00	93.70
Overall Means	**1.97±0.55 b**	**2.71±1.13 a**	**0.63±0.55 b**	**1.59±1.08 a**	**34.96±31.85 b**	**62.22±26.91 a**
(Means with similar letters within columns for a single parameter are statistically similar at p<0.05)						
Fish Species						
C. catla	1.87±0.08 b	2.67±0.32 a	0.52±0.02 c	1.70± 0.01 a	38.11±2.01 a	55.60±3.08 c
L. rohita	2.00±0.10 a	2.78±0.41 a	0.65±0.03 b	1.33± 0.03 b	37.25±3.33 a	58.21±2.04 b
C. mrigala	2.04±0.12 a	2.69±0.22 a	0.84±0.04 a	1.74±0.08 a	29.52±2.00 b	72.85±3.85 a

(Means with similar letters in a single column are statistically similar at p < 0.05)

Table II: Fish age, organs and species specific iron (µg g^{-1}) accumulation during growth trials

	Fish age								
	90-day	**120-day**	**150-day**	**180-day**	**210-day**	**240-day**	**270-day**	**300-day**	**330-day**
Treated Fish	181.96 ±11.26 d	330.66 ±28.95 c	352.15 ±30.11 c	356.71 ±34.59 c	542.07 ±18.66 b	509.80 ± 11.25 b	514.00 ±8.33 b	511.33 ± 12.20 b	706.07 ± 21.23 a
Control Fish	68.35 ±1.25 d	67.22 ±2.35 d	73.51 ±1.28 c	72.59 ±2.05 c	80.21 ±2.95 b	85.39 ±1.69 b	84.67 ± 2.15 b	94.53 ±1.86 a	102.37 ± 2.05 a

Fish Organs	**Gills**	**Kidney**	**Liver**	**Skin**	**Muscle**	**Scales**
Treated Fish	218.22 ± 21.65 b	1176.10 ± 45.22 a	1238.44 ± 10.10 a	233.10 ± 14.35 b	81.26 ± 11.21 c	199.43 ± 9.18 b
Control Fish	121.54 ± 3.85 a	115.61 ± 4.65 a	120.33 ± 3.02 a	82.54 ± 2.00 b	52.25 ± 1.95 c	18.60 ± 2.00 d

Fish Species	***C. catla***	***L. rohita***	***C. mrigala***
Treated Fish	392.06 ± 23.19 b	720.80 ± 20.55 a	460.42 ± 43.58 b
Control Fish	82.25 ± 2.66 c	91.86 ± 4.35 b	118.66 ± 7.22 a

Means with similar letters in a single column are statistically similar at p<0.05

Table III: Relationships of fish organs with age and exposure concentrations for the uptake and accumulation of iron

	Fish age	Iron exposure concentration
Gills	0. 321^{NS}	0. 521*
Kidney	0. 597*	- 0. 311^{NS}
Liver	- 0. 211^{NS}	- 0. 338^{NS}
Skin	0.333^{NS}	0. 187^{NS}
Muscle	- 0. 259^{NS}	0. 285^{NS}
Scales	- 0.301^{NS}	- 0. 872**

^{NS} non-significant; * significant at p<0.05; ** significant at p<0.01

significant (p<0.05). The accumulation of Fe in fish scales showed inverse (p<0.01) relationship with Fe contents in the culture media.

Results showed no differences among the physico-chemical variables *viz*., temperature, pH, dissolved oxygen, electrical conductivity, total ammonia, chlorides, sodium, potassium and total hardness of both treated and control test media studied during growth of fish species at sub-lethal Fe levels. However, these variables showed statistical differences (p<0.01) among the trials conducted for nine fish age groups (Table IV). The relationships in all the physico-chemical variables and the uptake/ accumulation of Fe by the fish organs were obtained. The accumulation of Fe in fish kidney had direct relationships with both temperature and pH of the test media while water hardness had inverse relationship for fish kidney (Table V).

DISCUSSION

Iron plays a key physiological role in all aspects of animal's life; however, it causes deleterious effects on living organisms at supra-optimal concentrations (Davies, 1991; Misra & Mani, 1992). The present studies revealed that the feed intakes by all the three fish species exposed to sub-lethal Fe concentrations did not cause significant impact on the growth performance of fish due to low FCE. The fish grown in metal free water (control) had higher overall weights and FCE. The form of Fe frequently found, in solution, in ground water is the ferrous (Fe^{2+}) ion (Hem, 1989) and ferrous compounds are assumed to be toxic. The availability of Fe in aqueous solution is affected by environmental conditions, particularly pH, redox potential and temperature (Wetzel, 1983; Hem, 1989). Iron is readily

Table IV: Characteristics of water during growth trials with three fish species

Species treated	Temperature (°C)	Dissolved oxygen (mg L^{-1})	pH	Electrical conductivity (ms cm^{-1})	Total NH_3 (mg L^{-1})	Chloride (mg L^{-1})	Sodium (mg L^{-1})	Potassium (mg L^{-1})	Total hardness (mg L^{-1})
Treated									
C. catla	22.13±7.03 a	6.57±0.98 a	7.81±0.40 a	1.71±0.21 a	1.50±1.23 a	252.14±3.74 a	319.53±1.06 a	10.74±0.48 a	277.25±1.82 a
L. rohita	21.77±6.84 a	6.66±1.08 a	7.79±0.37 a	1.68±0.21 a	1.29±0.64 a	252.40±4.67 a	328.74±1.07 a	10.41±0.37 a	276.64±2.06 a
C. mrigala	21.85±6.81 a	6.84±1.04 a	7.71±0.36 a	1.68±0.21 a	1.36±0.66 a	251.14±7.65 a	329.75±1.05 a	10.32±0.29 a	271.52±1.95 a
Control									
C. catla	21.78±6.83 a	6.57±1.13 a	7.92±0.43 a	1.71±0.20 a	1.24±0.78 a	243.06±6.30 a	326.05±1.07 a	10.28±0.62 a	257.18±3.18 a
L. rohita	21.58±6.79 a	6.56±1.15 a	7.91±0.41 a	1.71±0.20 a	1.24±0.80 a	243.87±8.40 a	325.34±1.05 a	10.20±0.61 a	256.79±2.81 a
C. mrigala	21.66±6.76 a	6.55±1.12 a	7.90±0.42 a	1.71±0.20 a	1.19±0.77 a	243.83±7.43 a	325.06±1.06 a	10.25±0.56 a	244.65±4.67 a
Fish Age									
90-day	22.78 d	6.49 c	7.44 e	1.68 d	2.52 a	255.20 a	209.6 e	10.30 cd	289.50 a
120-day	15.36 f	7.52 b	7.54 de	1.45 f	1.17 cd	249.40 bc	210.10 e	10.74 ab	244.70 de
150-day	12.33 h	8.51 a	7.70 c	1.39 g	0.38 e	247.10 c	211.10 e	10.21 cd	274.70 abc
180-day	13.67 g	7.59 b	7.66 cd	1.50 e	0.80 de	246.40 c	248.50 d	10.25 cd	294.60 a
210-day	20.29 e	6.72 c	7.61 cd	1.74 c	1.04 cd	254.20 a	379.10 c	9.97 d	286.00 ab
240-day	23.86 c	5.47 e	7.63 cd	1.79 b	0.39 e	240.70 c	379.80 c	10.52 bc	266.00 bc
270-day	28.94 b	5.33 e	8.21 b	1.90 a	2.07 ab	238.50 d	424.00 b	11.08 a	257.90 cd
300-day	29.79 a	5.95 d	8.54 a	1.90 a	1.96 b	240.50 d	476.10 a	10.32 bcd	229.90 e
330-day	29.16 b	6.04 d	8.24 b	1.92 a	1.36 c	251.70 ab	393.40 c	9.90 d	232.80 e

Table V: Relationships among fish organs and physico-chemical variables of the test mediums for the uptake and accumulation of iron

Variables	Gills	Kidney	Liver	Skin	Muscle	Scales	Temp.	D. O.	pH	E.C.	NH_3	Chloride	Sodium	Potassium
Kidney	0.0483													
Liver	0.1812	0.1433												
Skin	0.5235	0.0325	0.2011											
Muscle	0.8796	-0.0119	0.0908	0.6077										
Scales	-0.0148	0.2872	0.6302	0.0843	-0.0303									
W. Temp.	-0.0528	**0.3495**	-0.2695	0.1442	-0.0151	-0.1945								
D. O.	0.0688	-0.2211	0.3500	-0.2087	0.0163	0.2770	-0.8829							
pH	-0.0486	**0.4107**	0.0210	0.1652	-0.1562	-0.0354	0.7469	-0.5416						
E. C.	-0.3096	0.4174	-0.2723	0.2033	0.0534	-0.2192	0.9576	-0.8678	0.7379					
NH_3	-0.1312	-0.1272	-0.3263	0.0091	0.0081	-0.2445	0.4510	-0.4143	0.0920	0.3319				
Chlorides	0.3268	-0.0623	0.0674	-0.1699	0.3362	0.0188	-0.2199	0.1917	-0.4710	-0.1756	-0.0970			
Sodium	0.1245	0.4172	-0.1602	0.2578	0.0564	-0.1699	0.8231	-0.7543	0.7919	0.8902	0.0616	-0.2898		
Potassium	0.0608	-0.3062	0.0630	0.1146	0.0582	-0.0830	-0.3383	0.1719	--0.0953	-0.3148	-0.2245	-0.0688	-0.1629	
T. Hardness	-0.0260	**-0.4154**	0.0544	-0.1543	-0.0228	0.1307	-0.8418	0.6332	-0.7723	-0.8018	-0.2278	0.2551	-0.7819	0.3830

(Critical value (1-tail) ± 0.3238)

W. Temp. = water temperature; D. O. = dissolved oxygen; E.C. = electrical conductivity; NH_3 = total ammonia; T. Hardness = total hardness

oxidized to the Fe^{3+} from Fe^{2+} in the neutral to slightly acidic pH. However, both forms of Fe are toxic to the fish. (Forstner & Wittmann, 1979). Naz *et al*. (2009) reported significant effect of chronic Pb stress on the growth performance of *C. catla*, *L. rohita* and *C. mrigala*. Vincent *et al*. (1996) reported metal stress induced disturbances resulted in reduced fish metabolic rate and hence reduced the growth (Sanowski, 2003). Therefore, nutritional status, fish size and growth rate are considered, while comparing whole-body as well as tissue specific heavy metal concentration data for bio-monitoring and risk assessment (Javed, 2003). All the treated and control fish species showed statistical differences for their responses towards feed intakes, weight increments and FCE values. The significant variations in feed intake, weight escalations and FCE of fish, among nine age groups, indicating age related impact of Fe stress on fish growth. Rainbow trout exposed to half the lethal concentration of copper showed initial loss of appetite but compensated during 39 day experiment so that their growth rate almost equaled to that of control. Furthermore, compensation was faster at low ration than at higher one (Lett *et al*., 1986). Thus, Fe toxicity would not only influence the fish appetite but also acclimation and FCE that ultimately affected the growth performance of fish.

Present investigation that metal concentrations in fish organs increased significantly with exposure of water-borne Fe for nine age groups of fish. The responses of three fish species to accumulate Fe in their body organs varied significantly. *L. rohita* exhibited greater tendency for Fe accumulation followed by *C. mrigala* and *C. catla*, although the difference between *C. catla* and *C.* was non-significant. Pelgrom *et al*. (1994) reported that exposure of immature tilapia to copper (0 to 400 µg L^{-1}) and cadmium (0 to 155 µg L^{-1}) resulted in increased whole body metals contents. Liver and kidney appeared to accumulate significantly higher Fe. However, the ability of fish kidney to concentrate Fe did not differ significantly when compared with liver. Metal contamination in yellow Perch and major carps has been shown to vary according to water contamination and exposure period (Rajotte & Couture, 2002; Hayat & Javed,

2008). Therefore, a first line of evidence for assessing the potential metal effects on fish health appears in examining whether the metal would accumulate in the target tissues during chronic exposure. Hansen *et al*. (2002) reported that growth performance and bioaccumulation of copper in rainbow trout was both time and dose dependant along with reduced sensitivity to heavy metal with the passage of time.

Sub-lethal Fe concentrations caused significant increase of Fe in fish body with age probably because of its limited ability to store metals as exposure persisted. A positive correlation between increase in tissue metallic ions and fish tolerance has been observed. The concentration of Fe in 330-day fish increased significantly showing an exponential function of the exposure time when the storage capacity limits the liver and kidney. Furthermore, the accumulation of these metals in fish muscle was also stimulated during metal exposure (Cinier *et al*., 1997). The accumulation of Fe in fish gills, kidney and skin showed direct relationships with fish age, whilst the correlation of these organs was non-significant, except kidney. The accumulation of Fe in fish gills, skin and muscle showed direct relationships with metallic ions of the test mediums. Javid *et al*. (2007) reported that among the major carps, *C. catla* showed significantly more susceptibility to lead toxicity while *L. rohita* had significantly higher tendency to accumulate metallic ions in its body. The liver, in its role as a storage and detoxification organ, also accumulated high levels of Fe during present investigation. However, muscle and skin accumulated significantly less Fe. Nussey *et al*. (2000) reported the dependence of metal bioaccumulation in *L. umbratus* on size, gender, age and season. Therefore, smaller fish had higher body loads of metals.

The accumulation of Fe in fish kidney showed direct relationships with both temperature and pH of water. However, decline in water hardness caused significant increase of Fe in kidney. Erickson *et al.* (1996) reported that increased hardness of water caused metal toxicity in *Pimephales promelas* to decrease. The major modifying physico-chemical factors of metal's toxicity are hardness and pH of water (Svecevicius, 1999; Javed, 2003). Water borne metals generally exhibit their highest toxicity to aquatic organisms in soft water of low pH and low dissolved organic carbon (Nogami *et al*., 2000). This is because the hardness cations (Mg & Ca)) compete with heavy metal cations for binding sites within the organism.

In conclusion, chronic exposure of Fe to the fish did not only influence the fish appetite but acclimation and FCE that ultimately affected the growth performance of fish. Sensitivity of fish to Fe toxicity decreased significantly with age. The significant variations in feed intake, weight escalations and FCE of fish, among nine age groups, indicating age related impact of Fe stress on fish growth. The accumulation of Fe in fish body organs varied significantly. *L. rohita* showed significantly higher tendency for Fe accumulation. Fish liver and kidney exhibited significantly higher tendency while muscle and skin accumulated lesser Fe.

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