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Effects of seasonal change in Osinmo reservoir on arginase and rhodanese activities in *Clarias gariepinus* Burchell and *Heterotis niloticus* Cuvier

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This work reports the seasonal change and variation in the physico-chemical properties of the water in Osinmo reservoir in Southwestern Nigeria and the effects on tissue distribution of the metabolic enzymes, arginase and rhodanese are the two fish species (*Clarias gariepinus* and *Heterotis niloticus*) collected from the reservoir. This study was carried out to correlate the activities of the enzymes with metabolic status of the fishes and physico-chemical properties of the water. The enzyme activities varied significantly in the different tissues of the *C. gariepinus*. The liver showed the highest mean value of activity and the intestine had the least mean value. In the *H. niloticus*, they showed the highest mean value of activity while the bile showed the lowest mean value. The activities of the two enzymes in the water reservoir were determined at regular intervals over a period of ten months in 2011, spanning both dry and rainy seasons. The activities of the enzymes varied significantly through the two seasons. Arginase was at its peak in June while rhodanese was at its peak in September. The distribution of urea in the reservoir varied significantly in the period studied; the highest mean value was in July while the lowest was in February.

Key words: Water properties, urea; cyanide, enzymes, tissue distribution.

INTRODUCTION

Fish are important vertebrates which contribute as much as 17% of the world's animal protein. Inland fisheries play important role in the provision of protein to Nigerians. The downward trend in fish as food has been attributed partly to environmental, population increase, poor management practices and over-exploitation of water ways (Komolafe and Arawomo, 2008). Free cyanide is the primary toxic agent in the aquatic environment (Eisler, 1991). Numerous accidental spills of sodium cyanide or potassium cyanide (KCN) into rivers and streams have resulted in massive kills of fishes, amphibians, aquatic insects, and aquatic vegetation through discharge of substances generating free hydrogen cyanide (HCN) in the water from hydrolysis or decomposition (Leduc, 1984; Eisler, 1991).

Cyanide adversely affects fish reproduction by reducing the number of eggs spawned, and the viability of the eggs by delaying the process of secondary yolk deposition in the ovary (Lesniak and Ruby, 1982; Ruby et al., 1986). Other adverse effects of cyanide on fish include delayed mortality, pathology, impaired swimming ability and relative performance, susceptibility to predation, disrupted respiration, osmoregulatory disturbances, and altered growth patterns. Cyanide acts rapidly in aquatic environments, does not persist for extended periods, and is highly species selective; organisms usually recover quickly on removal to clean water. The critical sites for cyanide toxicity in freshwater

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organisms include the gills, egg capsules and other sites where gaseous exchange and osmoregulatory processes occur (Eisler, 1991).

In aquatic organisms the most common form of nitrogen waste is ammonia, while land-dwelling organisms convert the toxic ammonia to either urea or uric acid. Urea is found in the urine of mammals and amphibians, as well as some fish. It is noteworthy that tadpoles excrete ammonia but shift to urea production during metamorphosis. Despite the generalization above, the urea pathway has been documented not only in mammals and amphibians but in many other organisms such as birds, invertebrates, insects, plants, yeast, fungi and microorganisms. Environmental conditions have also been reported to be the common stimulus for urea synthesis in some fishes (Campbell and Anderson, 1991; Okonji et al., 2011). Anthropogenic impacts on fish populations in Osinmo and other reservoirs in the Southwestern Nigeria include not only water pollution, but also the extensive fishery, as well as habitat destruction by dam building and river modifications (Lenhardt et al., 2004; Atobatele, 2008; Komolafe and Arawomo, 2008; Okonji et al., 2011). In addition, temperature and water levels are both crucial factors affecting enzyme activities in aquatic environment. Therefore, the present study investigates the effects of seasonal variation in the water properties of Osinmo reservoir and its effect on arginase and rhodanese distribution in the tissues of *Clarias gariepinus* and *Heterotis niloticus*.

MATERIALS AND METHODS

Study area and collection of fish samples

Osinmo reservoir was created in 2005 by the impoundment of Ataro river and other streams. The reservoir lies between latitude 07° 52.8' N to 07° 53.2' N and Longitude 04° 21.2' E to 04° 21.7' E. The catchment area is about 102 Km^2. The surface area of the reservoir is about 0.78 Km^2 with a mean maximum depth of 3.2 m, cast-net was used once a month to collect fish samples. Water samples were collected forthnight between April 2010 and February, 2011 in Osinmo reservoir. The fish samples were stored in an ice-chest covered with ice before transporting to the laboratory where they were stored at temperature below 0°C until ready for use. The fish species, *C. gariepinus* and *H. niloticus* were identified using the keys by Reed (1967), Paugu et al. (2003) and Adesulu and Sydenham (2007). All reagents used were of analytical grades.

Measurement of physico-chemical parameters

Mercury-in-glass thermometer was used to take water temperature. Dissolved oxygen (DO) was fixed on the field between 8.00 and 9.00 am. The DO was determined by titrating fixed water samples with sodium thiosulphate using starch indicator (N/40) until the sample changed from blue-black to colourless solution (Golterman et al., 1978). The pH of water was measured using the pH meter (Mettler MP200). Total alkalinity of the water was determined by titrating water samples with sulphuric acid standard solution, using a drop of phenolphthalein solution and one sachet of bromocresol green-methyl red as indicator until the sample changed from blue green to pink (Golterman et al., 1978). Water transparency of the reservoir was determined with a Sechi-disc measuring 15 cm in diameter (Quayle, 1988).

Preparation of tissue extract

Prior to extraction, the *C. gariepinus* and *H. niloticus* fish species were slit open and the various tissues of interest (liver, intestine, bile and stomach) were removed and stored at 4°C until required. Tissue extracts were prepared by homogenising 10 g (w/v) of each tissue in 3 volume of homogenisation buffer (phosphate buffer, pH 7.2). The suspensions were centrifuged for 20 min at 4,000 rpm in a Microfield Centrifuge Model 800 D. The supernatants were used as the source of enzyme.

Arginase assays

Arginase activity was determined by the measurement of urea produced by the reaction of Ehrlich's reagent according to the modified method of Kaysen and Strecker (1973). The reaction mixture contained, in final concentration, 1.0 mM Tris-HCl buffer, pH 9.5 containing 1.0 mM $MnCl_2$, 0.1 M arginine solution and 50 µl of the enzyme preparation in a final volume of 1.0 ml. The mixture was incubated for 10 min at 37°C. The reaction was terminated by the addition of 2.5 ml Erhlich reagent (2.0 g of p-dimethylaminobenzaldelyde in 20.0 ml of concentrated hydrochloric acid and made up to 100 ml with distilled water). The optical density reading was taken after 20 min at 450 nm. The urea produced was estimated from the urea curve (graph of optical density against urea concentration). The unit of activity of arginase is defined as the amount of enzyme that will produce one µmol of urea per min at 37°C.

Rhodanese and protein assay

Rhodanese activity was measured during purification and routinely according to the method employed by Agboola and Okonji (2004) using KCN and $Na_2S_2O_3$ as substrates. The activity of the enzyme is expressed in Rhodanese unit (RU). One Rhodanese unit is taken as the amount of enzyme which under the given conditions will produce an optical density reading of 1.08 at 460 nm. Bradford (1976) method was used to measure the protein concentration of the enzyme using Bovine Serum Albumin (BSA) as standard.

Urea concentration of Osinmo reservoir

Urea concentration was determined using Erlich spectrophotometric method. The mixture contained 0.2 mM Tris-HCl buffer pH 7.5, water sample and 20% Erlich reagent. Optical density was read after 25 min at 450 nm. A calibration standard was prepared with 50 µM urea.

Statistical analysis

The results are presented as means ± SD. Data were analyzed by one-way ANOVA using SAS/PC soft ware. Duncan multiple range test was used for paired comparisons. A p-value < 0.05 was considered statistically significant.

RESULTS

The physicochemical parameters of Osinmo reservoir is

Table 1. Physicochemical parameters of Osinmo reservoir (May, 2010-February, 2011).

Water parameter (Mean)	Rainy season	Dry season
Water temperature (°C)	24.83±0.75	27.0±0.56
Dissolved O_2 content (mg/L)	1.97±0.17	2.0±0.33
Transparency (cm)	78.17±3.27	89.83±1.56
pH	7.05±0.18	7.43±0.05
Total alkalinity (mg/L)	77.33±3.29	96.5±3.68

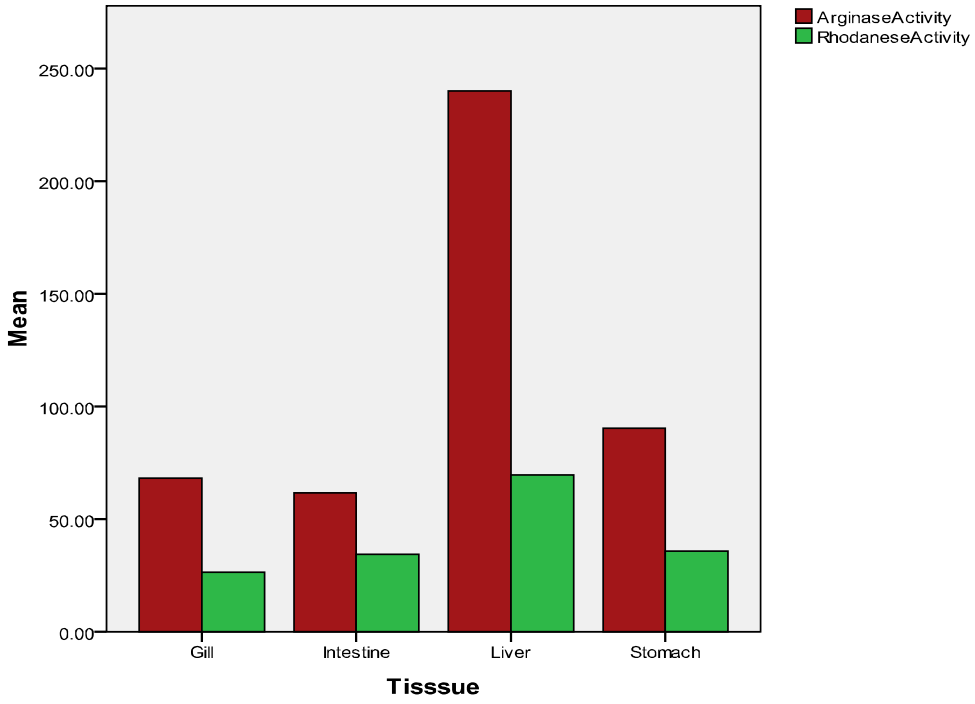

Figure 1. Tissue distribution of arginase and rhodanese enzymes in the tissues of *C. gariepinus*.

presented in Table 1. The dissolved oxygen concentration was fairly low in the rainy season (1.97 ± 0.17) as compared to the dry season (2.0 ± 0.33), while total alkalinity was lower in the rainy season (77.33 ± 3.29) than the dry season (96.5 ± 3.68). The variation in water temperature was high in the dry season as compared to rainy season (27.0 ± 0.56°C and 24.83 ± 0.75 respectively). Similarly, hydrogen ion concentration was moderately high in dry season (7.43 ± 0.05) than in the rainy season (7.05 ± 0.18). High water temperature in the dry season was also observed when compared to the rainy season.

Two different species of fish used in the study were *C. gariepinus* and *H. niloticus.* In Osinmo reservoir, *C. gariepinus* with 20.1% of the population was well represented (Komolafe and Arawomo, 2008) while *H. niloticus* was not observed until the present study. Distribution of the enzyme activities varies significantly in both species of fish. The liver of *C. gariepinus* showed the highest mean value as compared to the intestine with lowest activity (Figure 1). The distribution of the enzyme activities in the *H. niloticus* tissues also showed the liver to have the highest mean value while the bile showed lowest mean value (Figure 2). The enzymes, arginase and rhodanese varied significantly in their distribution throughout the season. Arginase peak was in June, 2010, while Rhodanese activity was at its peak in September, 2010 (Figure 3). The distribution of urea varies significantly in the different months. With the highest mean value in July, 2010 and lowest mean value in February, 2011 (Figure 4).

DISCUSSION

It is well documented that humans have greatly altered

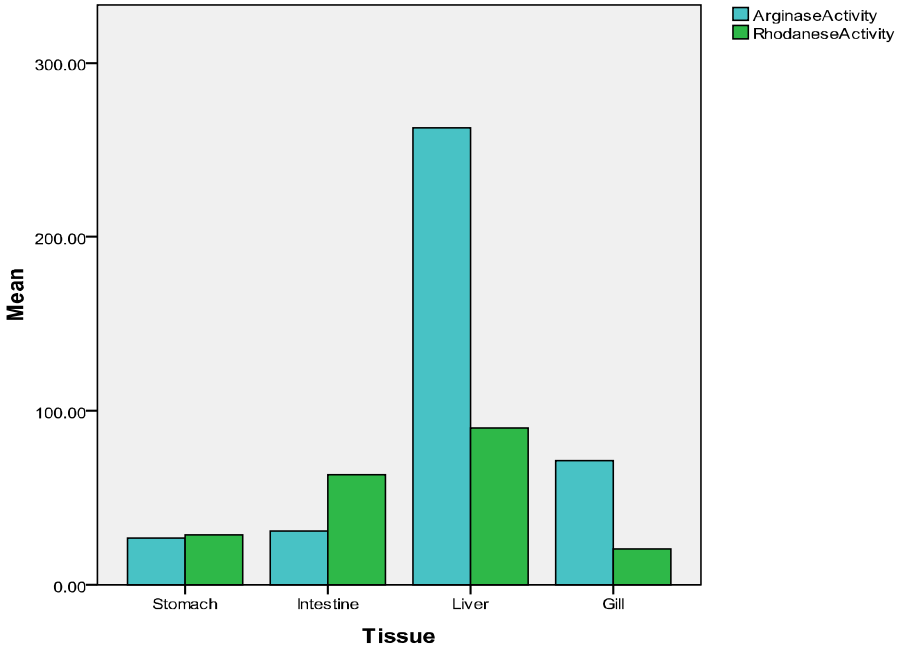

Figure 2. Tissue distribution of arginase and rhodanese enzymes in the tissues of *H. niloticus.*

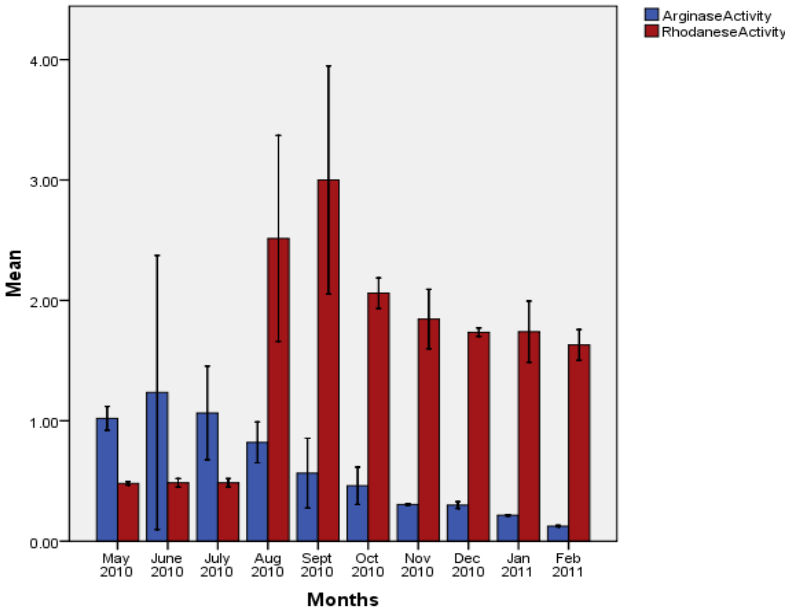

Figure 3. Distribution of arginase and rhodanese in water of Osinmo reservoir between May, 2010 and March, 2011.

predatory fish communities worldwide, especially through industrialized commercial and recreational fisheries (Christensen et al., 2003; Worm et al., 2005; Stallings, 2009). Coastal regions and areas that are home to large and growing proportion of the world's population are undergoing environmental decline. The problem is particularly acute in developing countries. The reasons for environmental decline are complex, but population factors play a significant role. Contaminants and activities that destroy habitats and ecosystems also contribute to

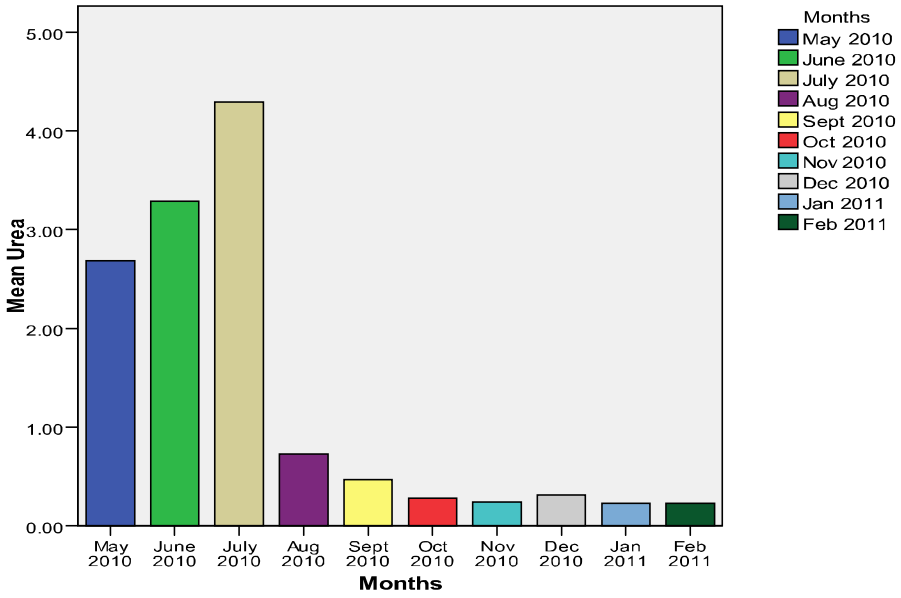

Figure 4. Distribution of urea in water of Osinmo reservoir between May, 2010 and February, 2011.

the loss of fresh water and marine fishes on which many people rely for food and income. Human anthropogenic activities relating to cyanide in an environment include industrial processes, laboratories, fumigation operations, cyanogenic drugs, fires and chemical warfare operations (Marrs and Ballantyne, 1987). Cyanides are also present in many industrial wastewaters, especially those of electroplaters; manufacturers of paint, aluminum, and plastics; metal finishers; metallurgists; coal gasification processes; certain mine operations; and petroleum refiners (Towill et al., 1978; Way, 1984; Eisler, 1991). Maintaining a healthy environment is critical because most of the world's fish produce their young inshore and feed on organisms in both fresh and marine waters.

Physicochemical parameters of Osinmo reservoir showed variations during the season as a result of suspended particulate matters brought into the reservoir by flood. Water temperature varied with a mean of 24.0°C ± 0.75 and 27.0°C ± 0.56 in rainy and dry seasons respectively. The means dissolved oxygen was 1.97 ± 0.17 mg/L during rainy season and 2.0 ± 0.33 mg/L in the dry season. Akinbuwa (2008) and Komolafe and Arawomo (2008) also observed variation in Osinmo and Opa reservoir. Oke (1998) also recorded a dissolved oxygen concentration below 5 mg/ml in Owena reservoir. Low dissolved oxygen production in the present study could be due to phytoplankton bloom and decomposition of allochthonou organic materials in the reservoir as reported by Okayi (2003). Water transparency was higher in dry season (89.83 ± 1.56 cm) than in the rainy season. Low transparency of the reservoir in rainy season might be attributed to flood resulting to increase in turbidity. The pH of 7.05 ± 0.18 and 7.43 ± 0.05 observed in rainy and dry seasons respectively were moderately alkaline and within the range of pH known for most lakes and streams of the world (Welch, 1952). The mean total alkalinity of 96.5 ± 3.68 mg/l and 77.33 ± 3.29 mg/l in rain and dry seasons respectively were high compared to 66.15 ± 15 and 63.68 ± 1.29 mg/ml reported to Aiba reservoir (Atobatele and Ugwumba, 2008). Wide range in total alkalinity had been attributed to season, location, plankton population and the nature of the bottom deposits.

C. gariepinus and *H. niloticus* represents two of species of fish in Osinmo reservoir. *C. gariepinus* was well represented in the population by 5.8% (Komolafe and Arawomo, 2008), while *H. niloticus* was no observed until present study. Influx of water from adjoining streams into the reservoir affected the quality of water and enzyme distribution in the fishes during the season. The presence of arginase in the reservoir water and its distribution in *C. gariepinus* and *H. niloticus* is an indication of the acidity and alkalinity of the reservoir. Arginase, an enzyme known to catalyse the conversion of arginine to urea becomes more effective in aquatic organisms, especially fresh water fishes when their environment becomes polluted and made more alkaline (Campbell and Anderson, 1991; Mommsen and Walsh, 1992; Wood, 1993). During the rainy season, allochotonous materials flushed into the reservoir and affected its status (Eisler, 1991; Okonji et al., 2010, 2011). Saha and Ratha (2007) had also reported *Heteropneustes fossilis* and *C. batrachus* to be hardy and capable of living in derelict water bodies and tolerating temporary water deprivation.

Several studies have been made on their ureogenic adaptations, ureogenic metabolic machinery and regulation under different physiological and environmental conditions. Both species (*H. fossilis* and *C. batrachus*) are potentially ureogenic teleosts expressing the complete repertoire of ornithine-urea cycle (OUC) enzymes, not only in hepatic tissue but also in certain non-hepatic tissues. They have developed peculiar ureogenic machinery and the induction of ureogenesis during adaptation to deal with the various stressful conditions such as exposure to high environmental ammonia, water deprivation and highly alkaline environment (Saha and Ratha, 2007).

Rhodanese also known as thiosulphate-cyanide sulphurtransferase is widely distributed in the body (Westley, 1980; Agboola and Okonji, 2004), but activity levels in mammals are highest in the mitochondrial fraction of liver. Rhodanese detoxifies cyanide to a less toxic thiocyanate. Its activity was found to be high in rainy season at Osinmo reservoir. This high activity could be explained based on the premise that the enzyme can be induced in the presence of cyanide (Nakajima et al., 2008). Leduc (1984) reported the increase in the concentration of cyanide in larger rivers with peaks more frequent during the summer because of cyanide production by plants and low in winter owing to dilution by high runoff.

The dissolved oxygen concentration of Osinmo reservoir obtained in the rain season was lower compared to the dry season. Reports have shown that cyanide is more toxic to freshwater fish under conditions of low dissolved oxygen (Doudoroff, 1976; Towill et al., 1978; Smith et al., 1979; EPA, 1980; Leduc, 1984). The pH levels within the range 6.8 to 8.3 had little effect on cyanide toxicity but enhanced toxicity at acidic pH (Smith et al., 1979; EPA, 1980; Leduc et al., 1982; Leduc, 1984). The present pH result throughout the season stable. However, changes in the dissolved oxygen, high concentration of cyanide affects survival of different fish species at different developmental stages. This could explain the low population of *H. niloticus* in the Osinmo reservoir. This is also supported by the report that juveniles and adults fishes were the most sensitive life stages tested and embryos and sac fry where the most resistant (Smith et al., 1978; Leduc, 1984; Eisler, 1991). On the distribution of arginase and rhodanese enzymes in different tissues of the fish species (*C. gariepinus* and *H. niloticus*), the activities of these enzymes were found to be more in the liver. This is not uncommon going by the function of liver in metabolism and in particular, detoxification. These results provide further evidence of the importance of the two enzymes in the survival of the fishes. Raymond (1998) has shown the importance of trimethylamine Oxide (TMAO) and urea in some cold water fishes, his result demonstrated that the synthetic machinery for these osmolytes are present in the liver. Similarly, Wai et al. (2003) reported that marine elasmobranchs (sharks, skates and rays) which are common in tropical waters, exhibit osmoconforming hypoionic regulation with body fluid osmolalities equal to or slightly higher than the environment. They also found that marine elasmobranchs possess an active ornithine-urea cycle synthesizing urea through carbamoy-lphosphate synthetase III primarily for osmoregulation (Ballantyne, 1997; Anderson, 2001; Yancey, 2001; Wai et al., 2003).

In conclusion, the results from this investigation on urea, arginase and rhodanese activities could be used as good indicators of fish population in the water systems. This could be supported by Lenhardt et al. (2004) report that urea and creatinine were used as indicators of state of fish population in some water systems. They were also reported to be indicators of gill and kidney dysfunction, respectively. As human population density increases, presence of large-bodied fishes declines, and fish communities become dominated by a few smaller-bodied species with complete disappearance of several large-bodied fishes suggesting ecological and local extinctions (Christensen et al., 2003). Fish populations and other aquatic resources are also likely to be affected by changes in seasonal flow regimes, total flows, water levels and water quality. These changes affect the health of aquatic ecosystems, with impacts on productivity, species diversity, and species distribution.

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