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Comparative study of plasma physiological indices and oral bacteria of selected crocodiles in Dangana Lake, Niger State, Nigeria.

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Abstract
The need to understand the physiology of organism in its natural habitat had necessitated the study in comparing selected physiological (haematological, biochemical, metabolic waste, electrolytes reserve and enzymatic) indices and oral cavity bacteria isolates of two species of freshwater crocodiles (Nile and slender snouted) sampled from Dangana Lake, Lapai Local Government Area of Niger State, Nigeria with the intent of providing baseline information and stating variations in the monitored parameters of the two species.Eighteen (18) individuals each of the crocodiles' species plasma were sampled for physiological indices. These were conducted in accordance to standard procedures using appropriate standard reagent kits and apparatus. Sterilized swab sticks were used to collect samples from the oral cavity for bacteriological studiesfollowing standard protocols. The mean corpuscular volume and mean corpuscular haemoglobin concentration were the only haematological indices with significant difference ($p<0.05$). No significant difference ($p>0.05$) was observed in all determined biochemical and enzymatic activities; while only sodium activity and bilirubin concentration were significantly different ($p<0.05$) in determined electrolytes reserve concentration and metabolic waste products determined. Staphylococcus aureus, Bacillus subtilis and Pseudomonas aeruginosa were the only bacteria isolates identified from the oral cavity of the two species. The crocodiles have similar physiological indices and nutritional requirements as bacterial flora identified were similar but varies in abundance.

Keyword: Bacteria, Biochemical Indices, Blood, Crocodiles, Dangana Lake

INTRODUCTION
Crocodiles, are aquatic and semi-terrestrial animals, they are predatory in nature with a long evolutionary history dating back two hundred million years (Grigg and Kirshner 2015). They are polyphyodont reptiles found around the continents with varying habitats as they feed on both vertebrates and invertebrates (Lane 1996). All living crocodilians are aquatic ambush predators utilizing camouflage, stealth and eyes adapted for low light situations to their advantage when they hunt along the shore line at night. Their prey preference is dependent on size, age, habitat choice, species of insects, gastropods, shellfish, fish, reptiles, birds, large and small mammals, as well as reports of cannibalism and even predation on humans (Campbell *et al.* 2013;Grigg and Kirshner 2015); however, they have economic

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importance as their skin serves as source of raw materials for shoes, belts, handbags, etc. production. According to Shirley *et al* (2014), the freshwater Nile crocodiles (*Crocodylusniloticus*) is the most iconic and the commonest in Africa and the second largest after the saltwater species (Somma*et al*. 2002) as they are red-listed as vulnerable by International Union for Conservation of Nature (IUCN) (Marais, 2014). Whilst the long-snouted crocodiles (*C. cataphractus)* are more of a riverine distribution throughout the West African region (Sandiezoo 2014). Studies have revealed that hematological indices are related to the blood and blood-forming organs of the body; other physiological indices are used to evaluate the functional capacity of several organs and general health status of the organism (Waugh *et al*. 2001; Adamu and Nwadukwe 2013). These parameters have been used to assess the health status of aquatic organisms such as Pisces (Adamu and Nwadukwe 2013; Adamu*et al*.2018) and Reptilians (Buenviaje*et al*., 1998; Turutoglu*et al*. 2005; Campbell 2006; Padilla *et al*.2011; Charruan*et al*. 2012; Bryant *et al*. 2012; Rajesh *et al*. 2013; La Grange and Mukaratirwa 2014; Warner *et al*.2016). According to Lovely *et al*. (2007) reference for physiological and haematological indices of Nile crocodiles are limited. While Campbell (2006) reported that these indices are important in reptilian diagnostics. Thus, the need to determine and compare selected plasma hematological, biochemical enzymatic, metabolic waste and electrolyte reserve indices as well as oral cavity bacterial isolates of Nile and long-snouted crocodiles sampled in Danganalake, Nigeria, with the objectives to providing their baseline information and comparing these parameters in the two freshwater crocodile species.

MATERIALS AND METHODS

Study Area

The lake is located in Dangana Village, Lapai, Niger state, Nigeria. This lake is located within longitude 6°36′29.6′E and latitude 9°02′12.02N with elevation of 159m above the sea level. The vegetation of the area reflects that of Savannah zone, the vegetation is mixed, prominent ones include Malaina (*Gmeilanaarborea*),African Locust beans (*Parkiabiglobosa*), Neem (*Azadirachtaindica*) and other sparsely native trees and grasses. The climate presents two distinct seasons, a rainy season between April and October, and a dry season (November-March) completely devoid of rain.

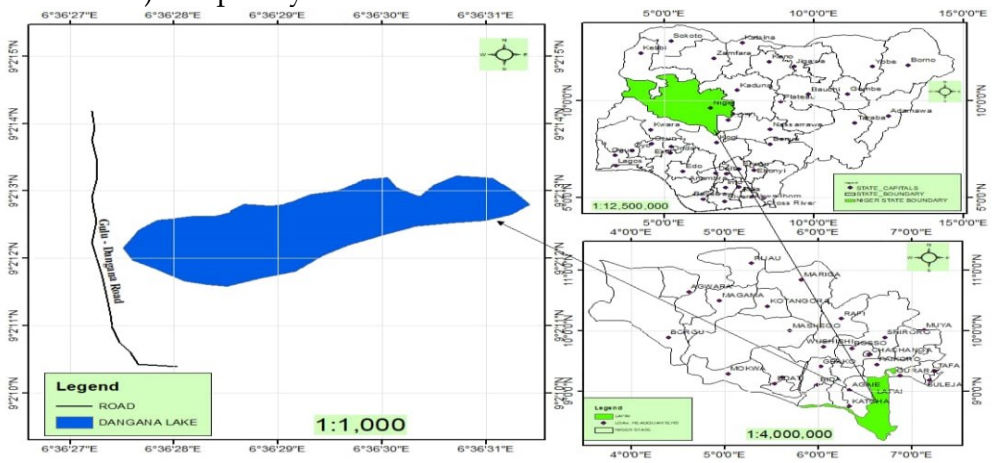

Plate 1: Schematic representation of the study area (Dangana Lake) Lapai, Niger State

Sample Collection

The fisheries in the lake are preserved by traditional policy that prevents fisheries activities in the lake. The chief priest of the water by the permission of the village head is responsible to perform rituals before allowing any fishing activities. The crocodiles were collected by the indigenes using spread nets; identified and tagged. The jaws were taped shut and the

animals were physically restrained, eighteen crocodiles each were randomly selected for the study. Samples of blood and swabs from the oral cavity were collected respectively. Blood samples were collected using 5ml disposable syringe and 21 gauge disposable hypodermic needle from the 11th caudal vertebrae (Olson *et al*. 1992). Sterile swab stick was used to scrub oral cavity for bacteria identification.

Determination of Physiological indices

Blood samples were placed in EDTA tubes for haematological indices while lithium heparin tubes were used for other physiological indices. In other to obtain the plasma, the blood contained in the lithium heparin tubes was centrifuged at 1500 rpm for 10 minutes, thereafter used to determine the presence of the selected physiological indices in the test reptiles.

Haemoglobin (Hgb) was determined based on principle of cyanmethemoglobin method (Tietz*et al*. 1967) using TECO diagnostic, USA. Packed cell volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) were conducted and calculated based on Blaxhall and Daisey (1973). Glucose, triglycerides, cholesterol, inorganic phosphorous, bilirubin and calcium were determined in accordance to Manufacturer's instruction of Cromatest reagent kit (Joaquim Costa, 18, 2ªplanta. 08390 Montgat-Barcelona, Spain). Alkaline phosphatase, Aspartate and Alanine Aminotransferases, urea, creatinine, total protein and uric acid were determined in accordance to Manufacturer instruction of RANDOX reagent kit (55 Diamond Road, Crumlin Co. Antrim, United Kingdom, and BT294QY). Sodium, chloride and potassium in accordance to Manufacturer instruction of TECO reagent kit (TECO diagnostics, 126 N, Lakeview Ave, Anaheim, CA 92807, 1-800-222-9880, USA). Each Absorbance (A) was read against blank with spectrophotometer (20D PEC Medicals U.S.A) at the specified nanometer.

Bacterial Isolation

Bacteria isolation was determined by standard phenotypic techniques. The spread and pour plate methods were adopted (Cheesbrough, 2000). Nutrient Agar (NA) (BIOTEC laboratory Ltd, Suffolk IP5 3RG United kingdom) media was used and prepared in accordance to the Manufacturer's instruction. Bacteria colonies were counted using colony machine (Model R250000614 manufactured by Stuart Scientific Co. Ltd., Great Britain). Pure isolates were characterized using biochemical test as described by Cheesbrough, (2000).

Data Analysis

All data were presented as mean ± standard error, data were analyzed using analysis of variance, after which individual mean were compared using Sidak's multiple comparisons test. In all cases, difference were considered statistically significant at either $p<0.01$ or $p>0.05$. All statistical analyses were performed using either Graphpad prism @ software version 6.0 San Diego, C.A or Microsoft Excel, version 2003.

RESULTS

Physiological Indices

The mean and standard error of the determined haematological indices are presented in Fig 1. The highest PCV (31.00±1.00%), haemoglobin (33.06±6.51g/dl) values were recorded in Nile crocodile as slender snout species recorded the highest values in RBC ($91.61\pm0.04x10^4$/cm) and MCHC (95.41±2.63%). However, PCV and Haemoglobin were not significantly ($p>0.05$) higher in Nile crocodiles but other monitored haematological indices were significantly ($p<0.05$) lower in slender snout species.

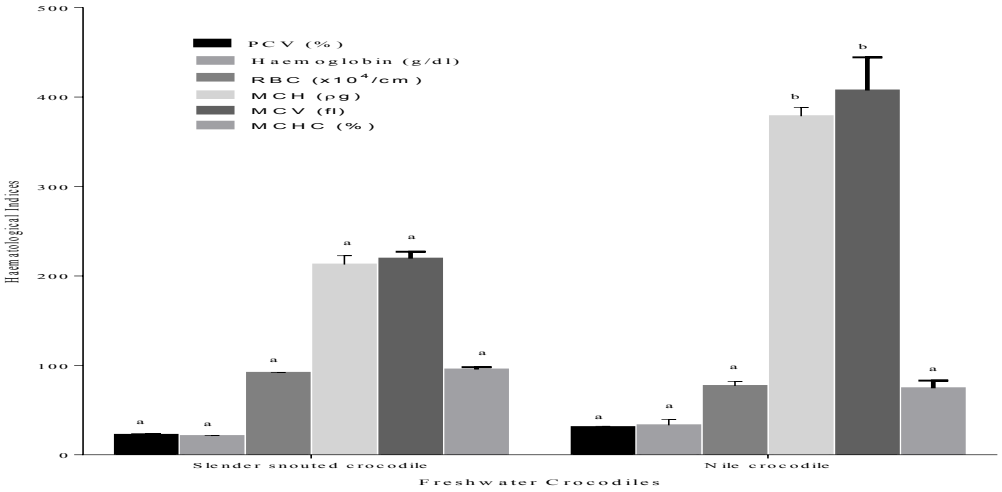

Fig 1: Hematological parameters of Freshwater crocodiles sampled fromDangana Lake. Each similar bar superscript represents p>*0.05* but different superscript represents p<*0.05*.

The determined biochemical indices revealed that only cholesterol was significantly (p<0.05) higher in Nile crocodile (Fig 2). The highest glucose (80.11±0.97mg/dl), triglycerides (185.47±12.50mg/dl) and cholesterol (357.10±44.26mg/dl) and least total protein (19.05±0.42g/dl) values were recorded in Nile crocodile.

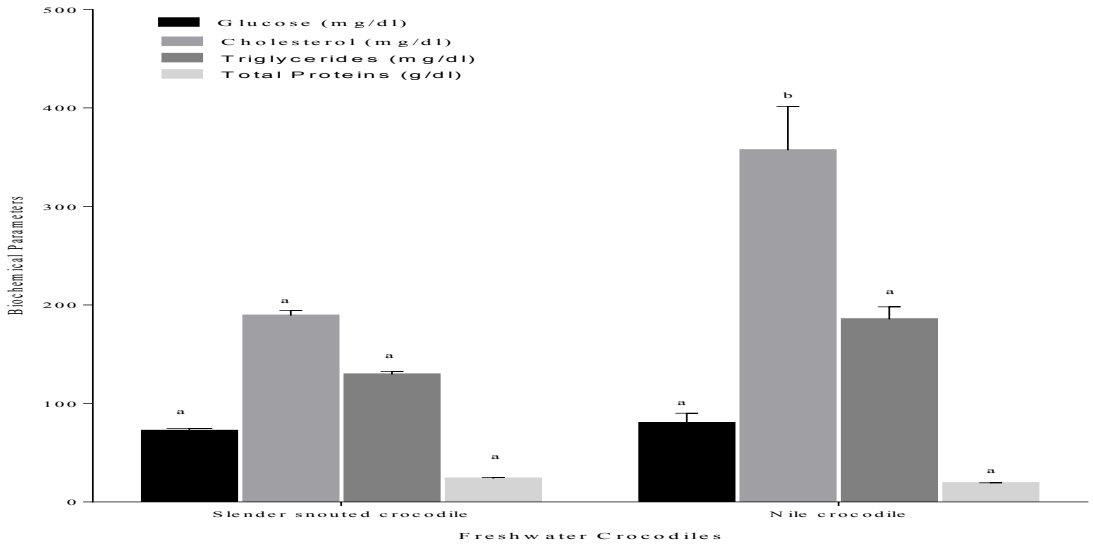

Fig 2: Biochemical parameters of Freshwater crocodiles sampled from Dangana Lake. Each similar bar superscript represents p>0.05 but different superscript represents p<0.05.

The determined electrolytes reserve concentrations were not significantly (p>*0.05*) different with the exception of sodium that was significantly (p<*0.05*) higher in Nile crocodile species as presented in Fig 3. The highest sodium, calcium and inorganic phosphorous values of 134.57±2.57mEq/l, 18.78±0.52mg/dl and 7.14±0.25mg/dl respectively were recorded in Nile crocodiles whilst chloride value of 20.61±2.41mg/dl was the least in the species.

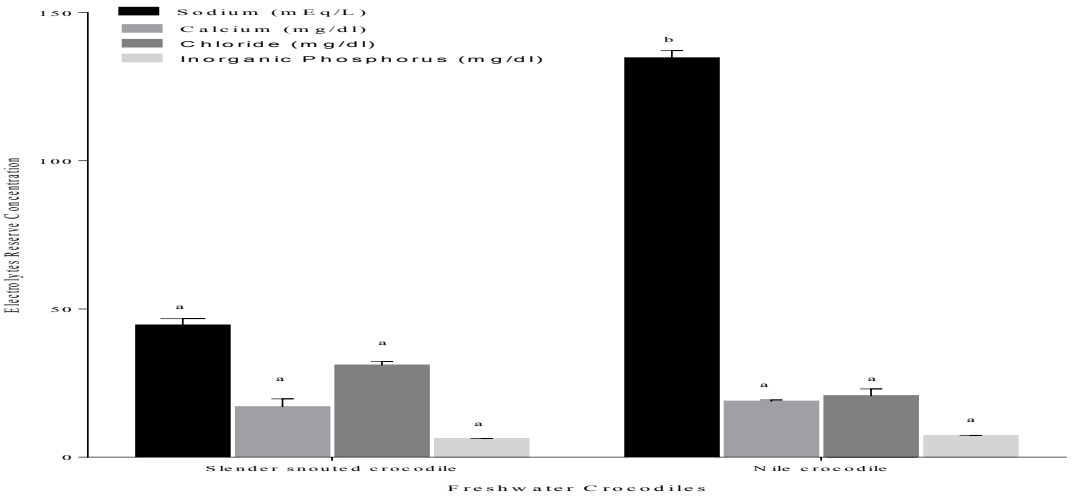

Fig 3: Electrolyte reserve concentrations of Freshwater crocodiles sampled from Dangana Lake. Each similar bar superscript represents $p>0.05$ but different superscript represents $p<0.05$.

The only significantly ($p<0.05$) different metabolic waste products was bilirubin (Fig 4). However, the highest creatinine and urea activities of 3.48±0.36mg/dl and 5.14±0.75mg/dl were recorded in Nile crocodile. Whilst highest values of uric acid and bilirubin of 94.39±2.52mg/dl and 149.89±5.60mg/dl was recorded in slender snout crocodile.

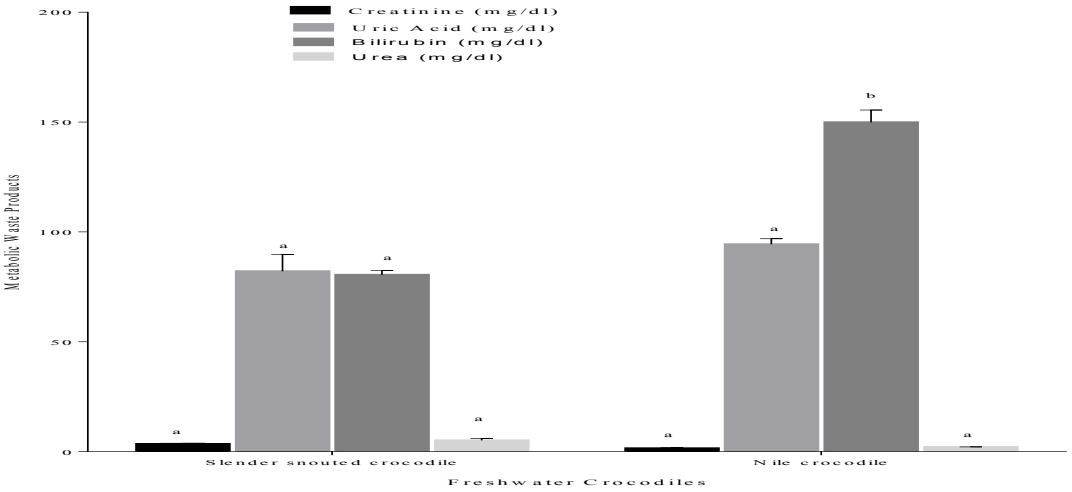

Fig 4: Metabolic waste products of Freshwater crocodiles sampled from Dangana Lake. Each similar bar superscript represents $p>0.05$ but different superscript represents $p<0.05$.

The three monitored enzymatic activities were not significantly ($p<0.05$) different (Fig 5).

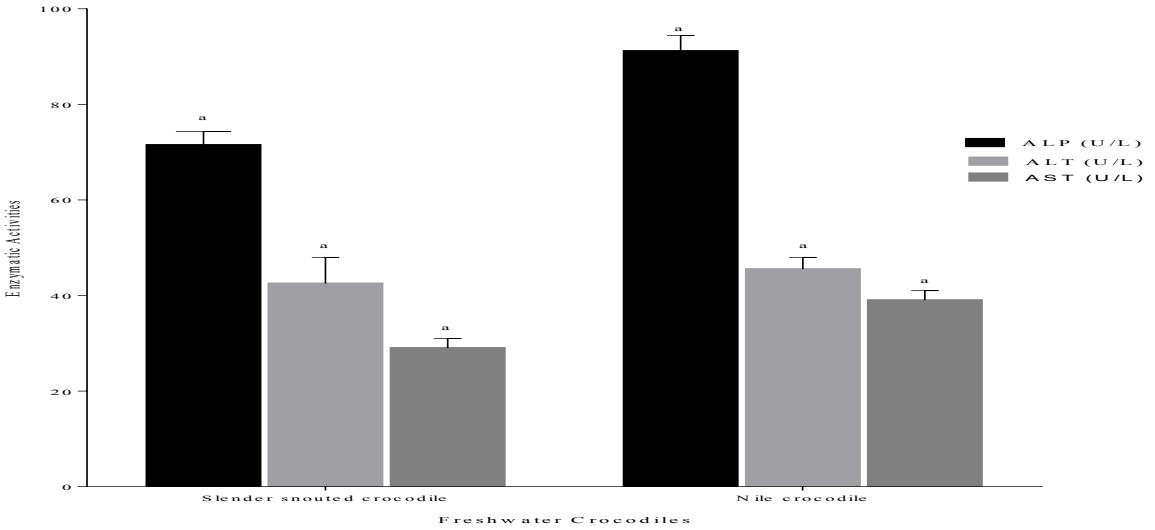

Fig 5: Enzymatic activities of Nile crocodile and long-snouted crocodile sampled from Dangana Lake. Each similar bar superscript represents $p>0.05$ but different superscript represents $p<0.05$.

Bacterial Isolates

Nile crocodile mouth recorded higher bacterial colony count in all dilution factors used (Table 1). *Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa* were three bacteria isolates identified in the oral cavity of the crocodiles (Table 2).

Table 1: Mean and Standard error bacteria colony count in the oral cavity of two crocodile species sampled from Dangana Lake, Niger State.

Crocodile	Dilution factor	Mean ± SE (Range) x10^6Cfu/ml
Nile	10^{-5}	94.00±2.30 (90-98)
	10^{-6}	78.67±1.45 (76-81)
	10^{-7}	74.67±1.76 (72-78)
Slender snout	10^{-5}	88.00±1.15 (86-90)
	10^{-6}	92.67±1.76 (90-96)
	10^{-7}	75.33±1.76 (72-78)

Table 2. Biochemical characteristics of bacteria isolates sampled from the oral cavity of sampled crocodile species from Dangana Lake

Crocodiles	Gram reaction	Gram shape	Catalase	Coagulase	Indole	Methyl red	Citrate	Sucrose	Glucose	Fructose	Lactose	Probable Organism.
Nile	+	Cocci	+	+	-	+	-	AG	AG	AG	AG	*Staphylococcus aureus*
	-	Rod	+	+	+	-	+	A	AG	AG	AG	*Bacillus subtilis.*
	-	Rod	+	+	+	-	+	A	AG	AG	AG	*Pseudomonas aeruginosa.*
Slender snout	-	Rod	+	+	+	-	+	A	AG	AG	AG	*Bacillus subtilis.*
	-	Rod	+	+	+	-	+	A	AG	AG	AG	*Pseudomonas aeruginosa.*
	+	Cocci	+	+	-	+	-	AG	AG	AG	AG	*Staphylococcus aureus*

+ = Positive; - = Negative; A= Acid production; AG= Acid and gas production

DISCUSSION

Hematological parameters are those parameters that are related to the blood and blood-forming organs (Waugh *et al*. 2001). Normal hematologic values for reptiles (including crocodilians), as determined by different laboratories, vary significantly due to difference in blood sampling, handling, analytic techniques, differences in the environmental conditions of the reptiles habitat, physiologic status of the reptiles, its age, gender, nutrition and use of anesthetics (Campbell 2006). Haematology and serum biochemical profile can facilitate the diagnosis and treatment of disease in reptile species (Bryant *et al*. 2012). Frye (1991) reported that normal values of PCV in healthy reptiles ranged from 20% to 35% thus agreement with that reported in this study. This study revealed that the determined physiological indices were within the range of earlier reported studies (Campbell 1996; Watson 1999; Kohler, 2006; La Grange and Mukaatirwa 2014). However, with little variation in that of snout slender crocodile.

Aquaticorganism particularly those from tropical regions may harbour pathogenic bacteria, which form part of natural micro-flora of aquatic environment (Eze *et al*.2011).More oral cavity bacteria isolates were reported by Charruau *et al* (2012) as compared to this study. *Staphylococcus aureus*is not commonly reported in oral cavity, however, it was recorded in this study as in the study conducted by Charruau *et al*(2012). According to Huchzermeyer (2003), few bacteria causes' specific diseases in crocodiles with fewer being crocodile specific. As all the crocodiles captured during the study were apparently healthy, thus assumed that the bacteria isolates may be normal flora of the crocodiles. However, some of the isolates may be pathogenic when the reptiles are subjected to stress (Huchzermeyer 2003).The presence of this organism is not surprising since Shinkafi and Ukwaja (2010) also reported that fish live in habitat full of microorganisms and confirmed that bacterial flora associated with Nigerian water culture include *Bacillus* sp., *Staphylococcus* sp., *Micrococcus* sp. and others.

CONCLUSION

Aside the mean corpuscular volume, mean corpuscular haemoglobin concentration, sodium activity and bilirubin concentration; there were no variations in the monitored physiological indices whilst the bacterial flora were similar in both species. It is thereof an indication that the two crocodiles have similar physiological indices and nutritional requirements.

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