SRAS Final Report:

ULTRASONOGRAPHIC FEATURES OF THE UROGENITAL ORGANS IN SUBADULT INDO-PACIFIC CROCODILES (*Crocodylus porosus)* AND PHILIPPINE CROCODILES *(Crocodylus mindorensis)*

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Abstract

Twenty-four (24) apparently healthy subadult *Crocodylus porosus* and *Crocodylus mindorensis* were subjected to ultrasonography to determine the ultrasonographic features of the kidneys, testes, and ovarian follicles, consisting of six animal per sex per species. The kidneys were observed to be elongated, with a generally anechoic parenchyma longitudinally and generally hypoechoic transversely. The testes were observed as thin and fusiform with an anechoic to hypoechoic parenchyma. Three out of the twelve females were observed to have ovarian follicles, which were hypoechoic to isoechoic to the surrounding tissue. Dimension and echo mean values were measured for the respective organs, with the data undergoing statistical analysis using Student T-test and Wilcoxon rank-sum to test for significant variation. Significant differences were present in body size parameters between sexes and species, except for C. *porosus* exhibiting no significant difference in snout to vent length between sexes. Aside from the left kidney length being significantly different between sexes in C. *porosus*, all other parameters between sexes and species yielded no significant difference. This study can be used as a basis for ultrasound examination in both species, with the findings of the appearance, organ dimensions, and echo mean values assumed as normal ultrasonographic features.

Keywords: Indo-Pacific crocodile, kidney, ovarian follicles, testes, Philippine crocodile, ultrasonography

Introduction

The two species of crocodiles that can be found in the Philippines are the endemic Philippine Crocodile, *Crocodylus mindorensis*, and the Indo-Pacific Crocodile, *Crocodylus porosus*. These species are highly threatened in the country due to habitat fragmentation and are being bred in captivity in conservation centers and international zoos (Hinlo *et al*. 2014).

Ultrasonography is an essential tool in the physical assessment of crocodiles, given that the body conformation of the species limits the thorough palpation of internal organs (Martelli, 2019). It is used to evaluate the anatomical size, position, and structure of organs, determine gravidity, detect abnormalities such as inflammation, tumors, developmental irregularities, etc., and perform other anatomical investigations (Urbanova and Halan, 2016).

Previous ultrasound studies of the reproductive tract in crocodiles include its assessment, which were performed by Tucker & Limpus (1997) in *C. johnstoni* and in *Caiman latirostris* by Vac *et al.* (1992), and on ovarian ultrasound and hormone evaluation in *Alligator mississipensis* and *Ca. latirostris* which were performed by Lance *et al*. (2009) and Porthelina *et al*. (2015) respectively. Studies stating the baseline ultrasonographic features of the urogenital organs in reptiles have also been published such as in the monitor lizard (*Varanus marmoratus*) by Prades *et al*. (2013a, 2013b), reticulated python (*Python*

reticulatus) by Enriquez et al. (2011), and in the red-eared slider turtle (*Trachaemys scripta elegans*) by Rodriguez et al. (2018).

This study describes the ultrasonographic features of the kidneys, testes, and ovarian follicles in subadult Indo-Pacific and Philippine Crocodiles in terms of dimensions and echo mean values, comparing these parameters between sexes and species.

Materials and Methods

Ultrasound examination was performed on a total of 24 subadult crocodiles, which were selected from the Palawan Wildlife Rescue and Conservation Center (PWRCC), Puerto Princesa City, Palawan after deeming apparently healthy by physical examination. There were two main groups of the study, by species (*C. porosus* and *C. mindorensis*) each having two subgroups divided by sex. The actual ages of the animals were not available so age was estimated using body length, selecting animals of body lengths which were less than that of mature animals as described in literature (Hinlo, 2010; van Weerd as cited by Schneider, 2014;Webb *et al*., 2010). *C. porosus* crocodiles ranged from 116.00 to 168.00 cm in length, and 4.45 to 18.23 kg in body weight (Table 1). *C. mindorensis* crocodiles ranged from 97.00 to 163.00 cm in length and 2.75 to 20.30 kg in body weight (Table 1). An ultrasound machine (Chison® Digital Color Doppler Ultrasound System ECO 5VET – Chison Medical Technologies Co., Ltd., China) equipped with 6.5 MHz linear array scanner was used to record ultrasound images.

Capture was by manual restraint. This involved putting a rubber band over the snout to keep the mouth shut and the eyes were covered with self-adherent wrap tape and a towel. Each animal was placed in dorsal recumbency on top of a table with a cushioned surface. The head, legs, and tail were secured all throughout the ultrasound examination. Ultrasound settings were set at 68 FPS, gain at 130 dB, and power at 15 KHz. The kidneys were visualized using ultrasonography by placing the linear scanner with copious amounts of ultrasound gel on the caudal part of the abdomen. The testes and ovaries were observed by placing the scanner on the caudolateral part of the abdomen. Longitudinal views were used to observe for the full length of the organs and transverse views for the width, diameter, and circumference.

The recorded ultrasound images were then analyzed using Adobe Photoshop CC version 19.1.4 (Adobe Systems Incorporated 1990-2018, USA). The dimensions were measured from the image analysis function, whereas the echo mean values were measured using the histogram feature. The generated data was then analysed, testing for normality and standard deviation, using a level of significance of $\alpha = 0.05$. Shapiro-Wilk test was used to test normality, and Levene's test was used to test homogeneity of variance. Significant variation was tested using student T-test for data with normal distribution and homogenous variances ($p>0.05$), and Wilcoxon rank-sum test for data with non-normal distribution and non-homogenous variances ($p<0.05$).

Results and Discussion

Visualization of the kidneys was done by placing the scanner ventrally on the caudal aspect of the abdomen, with the left and right kidneys observed on each respective side (Figures 1 and 2). The kidneys appeared elongated, with varying levels of echogenicity throughout all samples. The kidney parenchyma was generally anechoic with mildly hypoechoic regions when viewed longitudinally. The reptilian kidneys bear renal tissue with no cortical or medullary regions and do not possess a renal pelvis or renal pyramids (O'Malley, 2018; Holz, 2020). The fatty stroma in crocodilian kidneys, as described by Knotek

et al., (2018) may have factored in during observation of regions with greater echogenicity. Observation of the kidney transversely revealed a much more uniform parenchyma, generally hypoechoic with some with anechoic parts. Prades *et al*. (2013a, 2013b) and Rodriguez *et al.* (2018) both observed reptilian kidneys as having a generally hypoechoic parenchyma; however, Martelli (2019) describes the crocodilian kidneys as isoechoic to the surrounding fat.

Statistical analysis of the data (Table 1) showed that male *C. porosus* had a significantly longer left kidney than females, but no other kidney parameter including between the two species was different. In previous ultrasound studies, there was also no significant difference found when comparing the dimensions and echo mean values of the kidneys between sexes in water monitor lizards (Prades *et al*., 2013a and 2013b) and in red-eared slider turtles (Rodriguez *et al*., 2018).

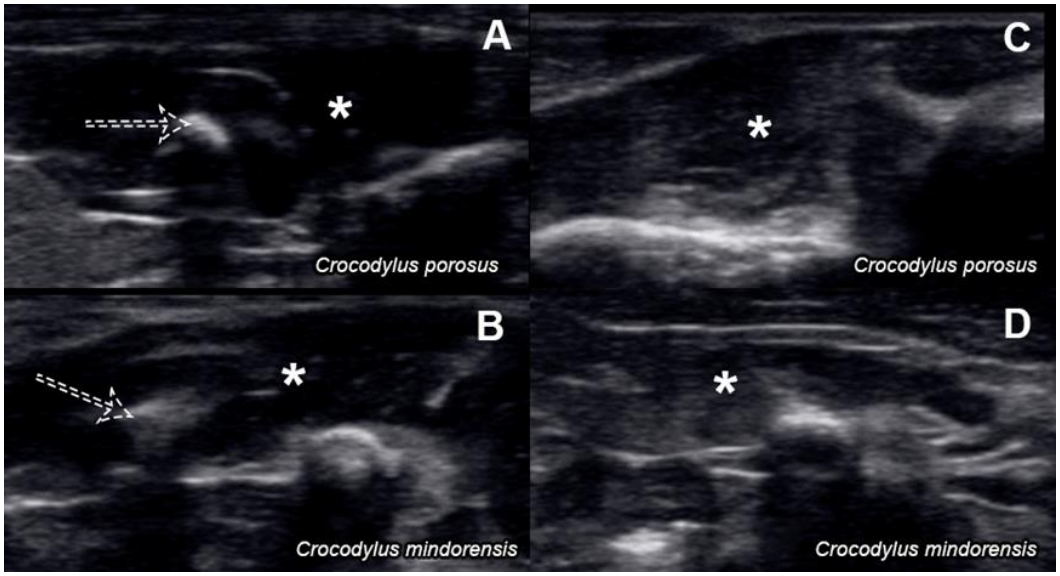


Figure 1. Longitudinal views (A, B) of the left kidneys of *Crocodylus porosus* and *Crocodylus mindorensis* showing a generally anechoic parenchyma (*) with regions of varying echogenicity (arrows). Transverse views (C,D) show a homogenous parenchyma of generally hypoechoic echogenicity (*).

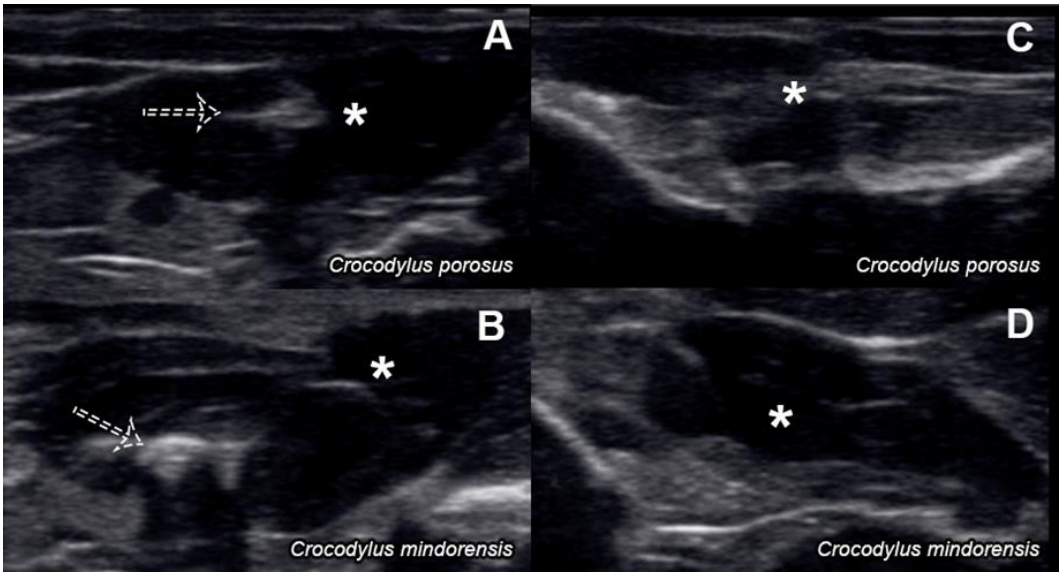


Figure 2. Longitudinal views (A, B) of the right kidneys of *Crocodylus porosus* and *Crocodylus mindorensis* exhibit generally anechoic parenchyma (*) with areas of greater echogenicity (arrows). Transverse views of the right kidneys (C,D) exhibit parenchyma (*) with both anechoic and hypoechoic to hyperechoic regions.

Table 1. Mean ± SD dimensions (cm) and echo mean values of the left and right kidneys of *Crocodylus porosus* and *Crocodylus mindorensis.* Means in the same row with the same superscripts are significantly different ($p<0.05$). L – longitudinal ; T- transverse

			Crocodylus porosus			*Crocodylus mindorensis*		
			Male	Female	Ave	Male	Female	Ave
Right Kidney	Length (cm)		4.83 ± 1.53	4.75 ± 0.63	4.79 ± 1.12	5.28 ± 1.47	4.61 ± 0.57	4.95 ± 1.12
	Width (cm)		4.49 ± 0.26	4.65 ± 0.68	4.58 ± 0.52	4.74 ± 0.77	4.50 ± 0.95	4.62 ± 0.83
	Echo mean value	L	1.28 ± 1.67	10.17 ± 7.03	2.47 ± 4.37	8.46 ± 9.77	7.57 ± 5.93	8.01 ± 7.72
		T	16.94 ± 20.82	3.67 ± 5.98	17.94 ± 18.22	12.26 ± 12.45	7.65 ± 5.55	9.95 ± 9.50
Left Kidney	Length (cm)		5.38[a] ± 1.86	4.26[a] ± 0.06	4.82 ± 1.38	5.26 ± 1.12	4.32 ± 0.47	4.79 ± 0.96
	Width (cm)		4.77 ± 0.55	4.36 ± 0.27	4.55 ± 0.45	4.99 ± 0.92	4.00 ± 0.38	4.50 ± 0.85
	Echo mean value	L	6.93 ± 5.81	3.98 ± 5.27	5.45 ± 5.51	3.66 ± 5.03	4.10 ± 4.14	3.88 ± 4.40
		T	16.79 ± 11.32	16.09 ± 13.30	16.41 ± 11.82	9.03 ± 9.18	11.80 ± 7.67	10.41 ± 8.19

Ultrasonography of the testes was performed by placing the scanner on the caudolateral aspect of the abdomen, with the left and right testis viewed on their respective sides (Figure 4). The testes were observed to be thin, elongated and fusiform in shape, with an anechoic to hypoechoic parenchyma. This is similar with the findings in other reptile species, which describes the testes as being homogenously hypoechoic (Enriquez *et al*., 2011; Prades *et al*., 2013b). The testes in the red-eared slider turtles, however, were hyperechoic. Testis lengths were greater in larger animals, such as observed in varanids (Prades et al., 2013b).

Table 3 summarizes the mean length and echo mean values of the left and right testes with their respective standard deviations in all male samples. No significant difference was found in the left and right testis lengths and echo mean values between species.

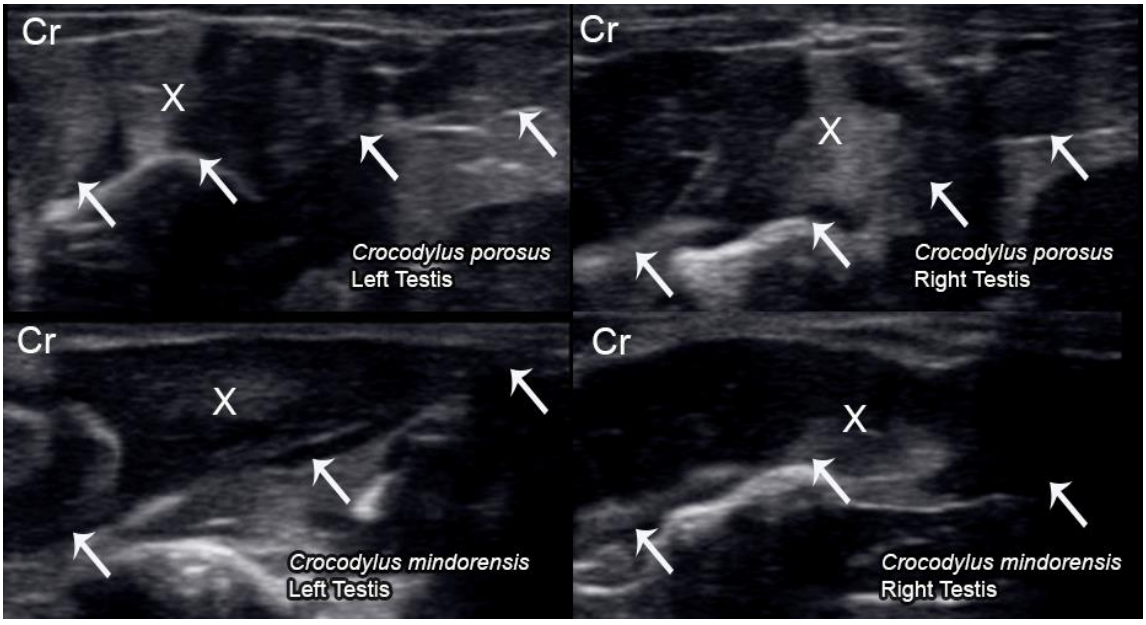


Figure 4. Longitudinal view of the left and right testis of *Crocodylus porosus* and *Crocodylus mindorensis*, exhibiting an elongated fusiform shape along its length (white arrows) with a parenchyma (X) that is generally hypoechoic to isoechoic to surrounding tissue.

Table 2. Mean±SD length and echo mean values of the left and right testis of *Crocodylus porosus* and *Crocodylus mindorensis*.

	Crocodylus porosus		*Crocodylus mindorensis*	
	Right Testis	Left Testis	Right Testis	Left Testis
Length (cm)	4.04 ± 1.14	4.06 ± 1.01	4.88 ± 1.23	4.29 ± 1.01
Echo mean value	7.25 ± 3.26	23.36 ± 15.81	14.30 ± 8.39	13.04 ± 11.09

The ovarian follicles were visualized by placing the scanner on the lateral aspect of the caudal abdomen, similar to that of the testes. Ovarian follicles were observed as hypoechoic to isoechoic round structures (Figure 5). Only three females possessed ovarian follicles that were observable via ultrasound. The largest female in the study had the largest observable ovarian follicle, although having a larger body size did not entail the presence of an observable ovarian follicle. This is due to an observable ovarian follicle in an animal with a smaller body size. Statistical analysis was not performed due to the small number of samples with ovarian follicles. The dimensions and echo mean values of the observed ovarian follicles are listed in Table 3.

There have been previous studies conducted describing ovarian follicles in mature reptiles. According to Prades *et. al* (2013a), pre-vitellogenic follicles can be observed as round anechoic structures, while vitellogenic follicles can be seen as hypoechoic structures. Tucker and Limpus (1997) describes pre-vitellogenic ovarian follicles in *C. johnstoni* in size, with small-sized follicles being 1-4 mm in diameter, and intermediate-sized follicles being 7-17 mm in diameter. Their study also describes vitellogenic and pre-ovulatory follicles as round echogenic structures ranging from 22-38 mm and 32-38 mm, respectively. Based on echogenicity, the hypoechoic to isoechoic appearance of the observed ovarian follicles in this study warrants their classification as vitellogenic follicles; however, their sizes --as comparable to the ovarian follicles in C. *johnstoni*—fit the description of pre-vitellogenic follicles. It is also worth noting that these females are not yet at mature sizes as per literature.



Figure 5. Longitudinal view of the observed ovarian follicles (outlined) having a parenchyma that is hypoechoic to isoechoic to the tissue surrounding the follicle. The largest female crocodile in the study (C) had the largest observed ovarian follicle with the greatest echogenicity. Ovarian follicle B follows as the second largest despite the animal being smaller in size than that of A.

Table 3. Dimensions and echo mean values of the ovarian follicles of *Crocodylus porosus* and *Crocodylus mindorensis*.

	Crocodylus porosus	*Crocodylus mindorensis*	
	F06	F01	F06
Height (cm)	0.93	0.96	1.13
Width (cm)	0.79	0.71	1.38
Circumference (cm)	3.14	3.06	5.05
Echo mean value	37.41	68.74	83.70

Conclusion and Recommendations

The ultrasonographic appearance of the kidneys in both species are similar: generally anechoic to hypoechoic longitudinally and more isoechoic transversely. Male *C. porosus* had a significantly longer left kidney compared to female *C. porosus*, but otherwise no other differences were noted. The testes in both species have an anechoic to hypoechoic parenchyma with no significant difference in terms of length and echo mean values between species. The ovarian follicles were observed to be hypoechoic to isoechoic. The dimensions were directly proportional to the body size of the animal, although body size does not ensure the presence of an observable ovarian follicle. The ovarian follicles were not able to be classified as pre-vitellogenic or vitellogenic due to characteristics conflicting with literature and may require further studies.

This is the first study describing the ultrasonographic features of the urogenital organs of subadult *C. porosus* and *C. mindorensis* as to the author's knowledge. With this, ultrasonographic examination in the species may be conducted with the results of this study as normal, as all samples were examined to be apparently healthy animals.

Both *C. porosus* and *C. mindorensis* have not been thoroughly studied and have the potential for more research involving anatomy and ultrasonography. Reproductive assessment can be performed to further identify sexual maturity in both species

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