**ORIGINAL ARTICLE**

# ECHOCARDIOGRAPHIC FEATURES OF APPARENTLY HEALTHY CAPTIVE WHITE-BELLIED SEA EAGLES (*Haliaeetus leucogaster, Gmelin 1788*) (AVES: ACCIPITRIFORMES: ACCIPITRIDAE)

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## ABSTRACT

**Heart diseases, such as myofiber degeneration, pericarditis, myocarditis, and fibrosis, are common in avian species especially in athletic birds such as raptors. However, due to lack of diagnostic tools, proper treatment and management of such cannot be achieved. Ultrasound B-mode and M-mode imaging was done in 12 (eight female, two males, and two unknown) apparently healthy, captive White-bellied Sea Eagles (*Haliaeetus leucogaster)* using a 7.5MHz linear scanner. Acoustic window was determined to be between the left coracoids bone and the first rib. Imaging of the full architecture of the heart was difficult due to the presence of the large air sacs. In B-mode, the heart was recognized due to its rhythmic beat. It shows anechoic lumen for atria and ventricles, hyperechoic thick ventricular walls, and hypoechoic thin atrial walls. Systolic and diastolic measurements for both the walls and lumen were determined in M-mode. Structures such as aortic root and mitral valve were also identified in M-mode. M-mode indices were calculated from the ultrasonograms obtained. Overall, the measurements and indices obtained in the study can be used as reference values for the echocardiographic features of White-bellied Sea Eagles and related species.**

**Keywords:** *echocardiography, Haliaeetus leucogaster, heart, ultrasound, White-bellied Sea Eagle.*

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## INTRODUCTION

Athletic birds, such as raptors, are subject to cardiac diseases which include different areas of the heart and most of the common findings are myofibril degeneration, myocarditis, fibrosis, endocarditis, pericarditis, pericardial effusion, artherosclerosis, and thrombus (Cooper and Pomerance, 1982). Most, if not all, of the cardiac diseases are fatal if not diagnosed and treated. Unfortunately, diagnosis is usually done post-mortem due to the fact that there are few data available about ante-mortem diagnosis in raptors. Most of the cardiac studies are done in companion species such as hawks and psittacines. Echocardiography has been proven to be a useful tool in assessing and diagnosing different conditions of the heart of birds (Jones, 2011).

Echocardiography is a procedure which uses ultrasound in order to produce an image of the heart. Image is produced by the reflection or the bouncing of sound waves through the various structures of the body. Echocardiography has been thoroughly studied in most of domesticated species. There are three main types of echocardiography that are usually used to study the heart: the B-mode, the M-mode, and the Doppler mode. The B-mode is a plane or two-dimensional view of the section of the heart while the M-mode records the movement of one

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section through time. On the other hand, the Doppler mode is the use of gas bubbles in the blood to see the movement of blood inside the heart (Boon 1998). For wild animals, only a few species have recorded data regarding echocardiography. Some of the species studied in the Philippines are tarsiers (Arcamo-Gentica and Acorda, 2014), monitor lizards (Tan *et al.,* 2013) and reticulated pythons (Aguisanda *et al.,* 2011). For avian species, ultrasound studies have been performed in domestic layer chickens (Lorico *et al.,* 2011) and ostriches (Gonzales and Acorda 2012; Bang-asan and Acorda, 2013). Most echocardiographic studies in avian have been conducted in psitaccines, falcons and hawks (Strunk and Wilson, 2003).

The Philippines is rich in avifauna of which 657 species have been identified and with 26 raptors in total (Lepage, 2017). However, due to the increase in land demand as a consequence of increase in human population, there has been a remarkable increase in deforestation. Thus, the natural habitats of avifauna are lost leading to the continuous decline of the population. Conservation efforts have the objectives to maintain populations of certain species, but with wild and exotic animals, health assessment proves to be difficult due to the lack of data, and heart disease may prove to be fatal, leading to wasted efforts and resources in conservation. This study aims to provide echocardiogram measurements and indices of *Haliaeetus leucogaster* that may be proven useful for future diagnosis of different heart diseases. The study may also provide reference techniques and serve as a “model” species for more endangered and vulnerable relatives such as the Philippine eagle (*Pithecophaga jefferyi*).

## MATERIALS AND METHODS

The use of the animals for this study was approved by the Institutional Animal Care and Use Committee (IACUC) under the College of Veterinary Medicine, University of the Philippines Los Baños with Protocol number 2018-0016. It was also approved by Department of Environment and Natural Resources – Biodiversity Management Bureau. Twelve (12) mature, apparently healthy, White-bellied Sea Eagles (*Haliaeetus leucogaster)* from the National Wildlife and Rescue Center, Biodiversity Management Bureau, Department of Environment and Natural Resources in Quezon City, Philippines were utilized in the study. History and records of each animal were taken, then identified if male or female based on the size of the animal, talon length, and through ultrasound examination of the genital organs (two confirmed female, six suspected female, two suspected male and two with unknown sex). No anesthetics were administered to the birds during the procedure. The head was covered with a falcon hood to reduce stress. Proper restraint was conducted by the animal handler during the procedure (Heidenreich, 1997). Complete physical examination was done prior to the ultrasound examination. An animal was deemed apparently healthy if it was alert, not emaciated or too fat, and the eyes and nasal opening are clear and free of discharge. Body temperature, which was measured through the cloacal opening, was within normal range. Weight was measured using weighing scale with mean weight of 2.27 kg ± 0.39.

Feathers were plucked, about one inch by one inch, from the left ventral scapular joint area until the area below the sternum. An ultrasound transmission gel (AquaUltra® Basic, Ultragel 2000 Hungary, Ltd., Budapest, Hungary) was applied to both the scanner and skin. An ultrasound machine (Wed-3100V Veterinary ultrasound scanner, Shenzhen Well.D® Medical Electronics Co., Ltd., Shenzhen, China), equipped with two different scanners, a 5.5MHz micro-convex scanner, and 7.5MHz linear scanner was used for the echocardiographic examination.

The left and right parasternal approaches and ventromedian approach were considered in determining the acoustic window. However, the space between the left coracoid and the first rib was used as an acoustic window to identify the structures of the heart. A long axis B-mode image was taken first. M-mode was then obtained through the different areas of the heart at the long axis. All images captured were printed using an ultrasound machine printer (Sony® UP-895MD monochrome video graphic printer) with a high density (110mm x 20 mm) thermal printing paper (Sony® Crop., Tokyo, Japan).

The B-mode image produced was analyzed by describing the shape of the different structures of the heart. Scanner angle was also correlated to the image captured. A photo analysis application (ImageJ®) was used to measure the different M-mode structures such as the systolic and diastolic measurement of the left and right ventricle, left and right ventricular wall, ventricular septum, left atrium diameter, and aortic root diameter. The pattern of the different structures was analyzed as well as the heart rate and systolic interval. The M-mode indices were calculated from the measurements using the following formulas:Stroke Volume: $V_s = 1.72M_b^{0.97}$ where $V_s$ is the stroke volume and $M_b$ is the mass of the animal (Scanes 2015).

Left ventricular diastolic volume: (LVVd)= $(7 \times (LVd)^3) / (2.4 + LVd)$
Left ventricular systolic volume (LVVs) = $(7 \times (LVs)^3) / (2.4 + LVs)$; where in the Left ventricular diastolic volume (LVVd) and left ventricular systolic volume (LVVs), are in centimeters.
Stroke volume (SV) = LVVd – LVVs (Boon 1998)
Where LVd = Left Ventricle Diastolic Diameter
LVs = Left Vetricular Systolic Diameter

The mean and standard deviation for each measurement and index were calculated as well as the weight to heart dimensions ratio. Test for normality was conducted using Shapiro-Wilks test with p-value of less than or equal to 0.05.

## RESULTS AND DISCUSSION

### Acoustic window

The scanner that showed the best resolution was the 7.5 mHz linear array. This scanner was used throughout all the samples to have uniform results. The acoustic window that depicted the image of the heart was between the left first rib and the coracoid bone. The scanner was positioned in a ventrodorsal-craniocaudal direction. The heart was easily recognized because of its rhythmic beating. All the samples produced an image but only partial regions of the heart were observed with huge areas covered by acoustic shadow.

There are some differences between the mammalian and avian heart. First is that the aortic arch lies at the right of the median plane in avian species, instead of left, like in mammals. Furthermore, avian species have a renal portal system and the presence of two cranial vena cava (Konig *et al.,* 2009). The heart is also positioned at the midline and both sides are partially surrounded by the liver. In the present study, the heart was observed at the midline, with both sides surrounded by the liver. The aortic root lies in the middle, rather than covering the left atrium like in mammals. The liver was also used as an acoustic landmark to determine where the heart is. Ventricular images usually showed part of the liver as well.

Unlike most mammals, in which echocardiography can be accessed through the intercostal space, most of the avian species do not produce a standard image of the heart (Boon 1998). This is mainly because of the anatomical structures such as small intercostal spaces, pneumatic bones, lungs, and air sacs. Such structures hinder a clean production of images of an ultrasound machine. Thus, Pees and Krautwald-Junghanns (2005) address such hindrances and provided two access points, the ventromedian approach and the parasternal approach. Both access points require advance ultrasound machines with high frame rate (>100 frames/sec) and a scanner with small coupling surfaces (microcurved), with a frequency of 7.5MHz or higher. This approach was optimum to birds weighing 1000g or less. Larger birds, such as those used in the study, require deeper penetration of ultrasound waves thus, lowering the frequency affecting the resolution of the image. Furthermore, abdominal air sacs were larger compared to flightless birds such as chickens, greatly reducing the acoustic window. As a result, both approaches were ineffective during the study, producing large acoustic shadows.

Another echocardiographic study, conducted in chickens, produced an image through the pectoral muscles with a scanner frequency of 7.5 MHz (Lorico *et al.,* 2011). This approach was ineffective in this study, producing image of the pectoral muscle but unable to penetrate the keel bones. This may be due to the anatomical difference of keel of flightless birds, which is long and narrow, while athletic avians have a more prominent and deeper keel (Konig *et al.,* 2009). Though the space between the left coracoid bone and the first rib produced an image of the heart, large acoustic shadows cover some areas. The coverage of the acoustic shadowing was not uniform throughout the samples. This may be due to the size of the animal, the scanner positioning, and positioning of the clavicular air sac. Other options include a more invasive approach such as intraesophageal which requires sedation and a specialized scanner (Beaufrere *et al.*, 2010), but these were not available in the present study.

### B-mode

The weight, systolic interval, and heart rate are summarized in Table 1. The atrium was observed in seven (7) samples and can be easily recognized in B-mode due to its two crescent-shaped apical borders connected at the middle (Figure 1). Its lumen is anechoic with walls that are thin and isoechoic. The shape of the ventricles was hard to recognize in B-mode as acoustic shadowing covered both the apical and the basal ends. However, in one sample, it is presented as an elongated structure with thick hyperechoic walls and anechoic lumen. In M-mode, the ventricles were recognized due to their thick hyperechoic walls with anechoic lumen (Figure 3). Though the general standard for the lumen should be fully anechoic, some

Table 1. Weight, Systolic Interval and Heart Rate in White-bellied Sea Eagle

| Parameter | Number of Samples (n) | Mean (±SD) |
|---|---|---|
| Mass (g) | 12 | 2.27 ± 0.39 |
| Systolic Interval (sec) | 12 | 0.45 ± 0.11 |
| Heart Rate (beats/ min) | 12 | 141 ± 34 |

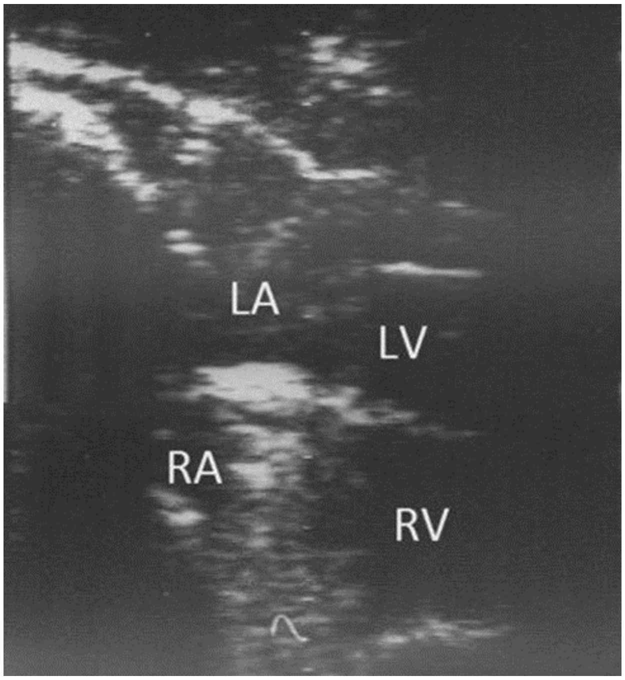

Figure 1. B-mode longitudinal scan showing four chambers of the heart of White-bellied Sea Eagle. LA: Left Atrium; RA: Right Atrium; LV: Left Ventricle; RV: Right Ventricle

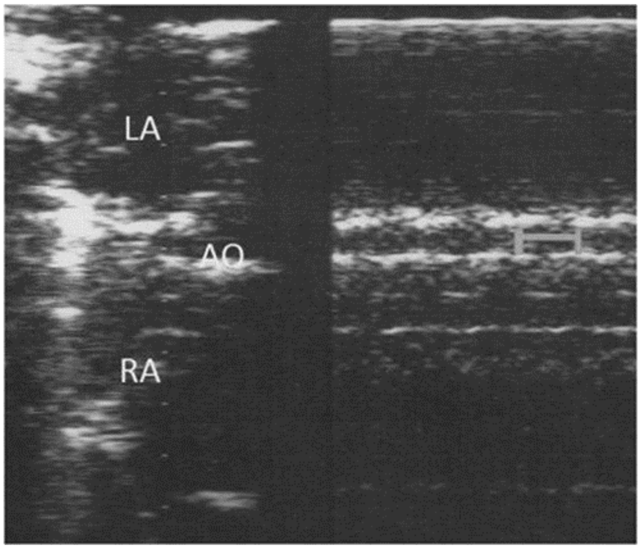

Figure 2. BM mode showing the left and right atrium and the aortic valve.LA- Left atrium, RA-right atrium, AO – Aorta, White line- Aortic Valve opening and closing

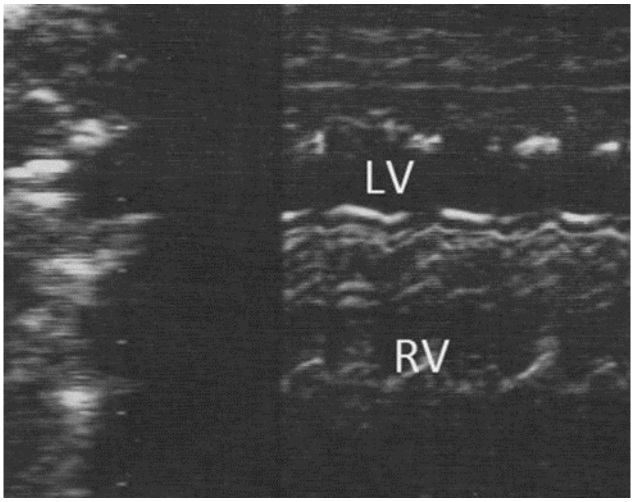

Figure 3. BM-mode at the level of ventricles LV – Left Ventricle, RV – Right Ventricle

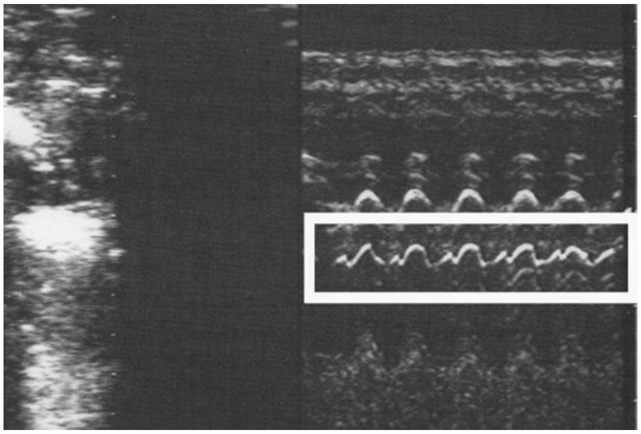

Figure 4. Left Ventricular section showing the Mitral Valve (White box)

Table 2. Left and right atrial measurements in White-bellied Sea Eagle

| Parameter | Number of samples (n) | Mean ± SD |
|---|---|---|
| Left Atrium | | |
| Wall Thickness Systolic (mm) | 7 | 1.04 ± 0.35 |
| Wall Thickness Diastolic (mm) | 7 | 0.61 ± 0.22 |
| Lumen Width Systolic (mm) | 7 | 5.96 ± 2.91 |
| Lumen Width Diastolic (mm) | 7 | 6.60 ± 3.2 |
| Lumen Length (mm) | 2 | 6.71 |
| Right Atrium | | |
| Wall Thickness Systolic (mm) | 4 | 0.87 ± 0.12 |
| Wall Thickness Diastolic (mm | 4 | 0.69 ± 0.10 |
| Lumen Width Systolic (mm) | 5 | 7.32 ± 1.40 |
| Lumen Width Diastolic (mm) | 5 | 8.75 ± 1.97 |
| Lumen Length (mm) | 2 | 6.21 |

Table 3. Left and right ventricular measurements in White-bellied Sea Eagle

| Parameter | Number of samples (n) | Mean ± SD |
|---|---|---|
| Left Ventricle | | |
| Wall Thickness Systolic (mm) | 5 | 1.69 ± 0.57 |
| Wall Thickness Diastolic (mm) | 5 | 1.30 ± 0.34 |
| Lumen Width Systolic (mm) | 6 | 4.62 ± 1.16 |
| Lumen Width Diastolic (mm) | 6 | 5.29 ± 1.18 |
| Width Fractional Shortening (%) | 6 | 13.23 ± 3.78 |
| Right Ventricle | | |
| Wall Thickness Systolic (mm) | 4 | 1.26 ± 0.18 |
| Wall Thickness Diastolic (mm) | 4 | 0.84 ± 0.11 |
| Lumen Width Systolic (mm) | 5 | 4.31 ± 1.38 |
| Lumen Width Diastolic (mm) | 5 | 4.86 ± 1.44 |

Table 4. Aortic Diameter and Interventricular septum measurements in White-bellied Sea Eagle.

| Parameter | Number of Samples (n) | Mean ± SD |
|---|---|---|
| Aortic Diameter | | |
| Systolic (mm) | 2 | 2.24 ± 1.22 |
| Diastolic (mm) | 2 | 3.41 ± 1.41 |
| Atrioventricular septum | | |
| Systolic (mm) | 9 | 1.43 ± 0.24 |
| Diastolic (mm) | 9 | 0.93 ± 0.27 |
| Systolic thickening (%) | 9 | 60.1 ± 31.29 |

Table 5. Echocardiographic indices in White-bellied Sea Eagles.

| Parameter | Number of Samples (n) | Mean ± SD |
|---|---|---|
| Left Ventricular Systolic Volume (mL) | 6 | 0.27 ± 0.16 |
| Left ventricular Diastolic Volume (mL) | 6 | 0.39 ± 0.20 |
| Stroke Volume (mL) | | 0.12 ± 0.05 |
| Ventricular Wall Systolic Thickening (%) | 5 | 29.46 ± 14.52 |
| Left Ventricular Ejection Time (sec) | 1 | 0.552 |
| Ejection Fraction (%) | 6 | 32.8 ± 8.4 |
| Velocity of circumferential fiber shortening (sec) | 1 | 0.23 |

scans show acoustic noises presented as hypoechoic structures (Boon 1998). Wall thickness is generally used to differentiate the ventricles and atrium.

Standard image identification of heart structures is generally done using B-mode. However, due to large interference of the air sacs, complete images of the heart were not possible, and specifying the chamber was challenging. The space between the left first rib and the coracoid bone would commonly reveal the apical structures of the heart such as the left and right atrium as shown (Figure 1). Measuring parameters in M-mode, as suggested by Boon (1998), is to do different cross section images of the heart at different levels such as at the level of the atrium, the ventricle, the valves, and the aortic root and measuring the maximum diameter at each level. Using a linear array scanner contributed to the limitation of rotation of the scanner, and as a consequence, a cross section image was not viable. On the other hand, alternate ways on measuring M-mode parameters were suggested through the use of the longitudinal section and moving the cursor across the plane (Krautwald and Pees 2005).

### M-mode

The results of systolic and diastolic measurement of the atrium and ventricular walls and lumen are shown in Tables 2 and 3. The image of the mitral valve is obtained from one of the samples (Figure 4). This was easily recognized due to its "M" shaped pattern due to the dual filling process (Boon 1998). However, the E point to the septal separation or EPSS, cannot be measured as the ventricular septum was not well defined in the image. The aortic root was observed in two samples, in which one shows a definite pattern of the aortic valve (Figure 2). This image was used to measure the left ventricular ejection time by measuring the opening and closing of the valve which resulted to 0.552 second. A summary of the values of the aortic root and the ventricular septum is shown in Table 4.

The mean systolic interval of the twelve (12) samples was 0.45 second leading to an average heart rate of 141 bpm. Heart rate largely varies with species and its mass in which larger birds have slower heart rates (Scanes 2015). Substituting the mean weight of the samples to Seymour and Baylock's (2000) formula of heart rate ($HR=178.5M\text{-}_{b}^{-282}$) would provide a heart rate of 142.2 bpm. This was extremely close to the actual average heart rate of 141 bpm therefore, the formula was proven to be accurate in White-bellied Sea Eagle.

The M-mode parameters including the left ventricular diastolic volume, left ventricular systolic volume, stroke volume, and ejection fraction were calculated accordingly and results are shown in Table 5. The velocity of circumferential fiber shortening was calculated using the mean values of the left ventricular systolic and diastolic lumen and the left ventricular ejection time of the single sample.

Among the results the standard deviation of the aortic root, the left ventricular ejection time, and the length of both atria were not calculated as the imaging was only taken from only one sample. Using Shapiro-Wilk test, the fractional shortening of the wall of the right atrium and the systolic measurement of the septum was observed to be not normally distributed.

Comparing the result of measurement with that of Pees and Krautwald-Junghanns (2005), the ventricular lumen of the White-bellied Sea Eagle is relatively smaller than the diurnal raptors of under 1000 grams. This may be due to limited access, where measurement is generally taken at more apical portion rather than mid area of the ventricles. On the other hand, comparing with domestic chicken, which have a base width of 13.9±1.0 cm at the age of 10 weeks (Lorico *et al.* 2011), the size of the heart of the eagle is relatively smaller.

The formula of stroke volume, $V_s= 1.72M_b^{0.97}$ shows a stroke volume of 3.81ml

(Scanes 2015). Whereas, the result using the formula, Stroke volume (SV) = LVVd – LVVs (Boon 1998) result to 0.12ml, producing a different result to the mass base formula. The formula suggested by Boon (1998), was generally tested in mammalian species, and the factors may greatly differ in the avian species and the fact that the ventricular measurement was taken at a more apical area.

Visualization of the heart through echocardiography in large raptors was challenging. The acoustic window was determined to be at the space between the left first rib and the coracoid bone. Maximum visualization and resolution were achieved using a 7.5MHz linear scanner. Further studies can be done to differentiate the mean measurement of males and females through definite sexing techniques such as genetic sexing. Echocardiography of eagles suffering from diseases may also be done.

Overall, measurements and calculated patterns obtained may serve as reference value for the echocardiography of White-bellied Sea Eagles and related species.

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## STATEMENT ON COMPETING INTEREST

The authors have no competing interest to declare.

## AUTHOR'S CONTRIBUTION

EALT and JAA for conceptualization and methodology. JDR and JAA for collection and analysis of data. JDR and EALT for resources, project administration and manuscript writing.

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