Transesophageal echocardiography of cardiac function in Nile crocodiles

A novel tool for assessing complex hemodynamic patterns

Poulsen, Christian F B; Munk, Kim; Wang, Tobias; Damkjaer, Mads

Published in:
Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology

DOI:
10.1016/j.cbpa.2023.111564

Publication date:
2024

Document version:
Final published version

Document license:
CC BY

Citation for pulished version (APA):
Poulsen, C. F. B., Munk, K., Wang, T., & Damkjaer, M. (2024). Transesophageal echocardiography of cardiac function in Nile crocodiles: A novel tool for assessing complex hemodynamic patterns. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *288*, Article 111564. https://doi.org/10.1016/j.cbpa.2023.111564

Comparative Biochemistry and Physiology, Part A 288 (2024) 111564

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Transesophageal echocardiography of cardiac function in Nile crocodiles – A novel tool for assessing complex hemodynamic patterns

Christian F.B. Poulsen [a,b,c], Kim Munk [d], Tobias Wang [e], Mads Damkjaer [b,c,*]

[a] Department of Cardiology, Aalborg University Hospital, Denmark
[b] Department of Paediatrics and Adolescent Medicine, Lillebaelt Hospital, University Hospital of Southern Denmark, Kolding, Denmark
[c] Department of Regional Health Research, University of Southern Denmark, Denmark
[d] Department of Cardiology, Aarhus University Hospital, Aarhus, Denmark
[e] Department of Zoophysiology, Aarhus University, Aarhus, Denmark

ARTICLE INFO

Edited by: Michael Hedrick

Keywords:
Crocodile
Echocardiography
Cardiac imaging
Shunting
Comparative physiology

ABSTRACT

Background: The crocodilian heart is unique among reptiles with its four-chambered structure and complete intracardiac separation of pulmonary and systemic blood flows and pressures. Crocodiles have retained two aortic arches; one from each ventricle, that communicate via Foramen of Panizza, immediately distally from the aortic valves. Moreover, crocodiles can regulate vascular resistance in the pulmonary portion of the right ventricular outflow tract (RVOT). These unique features allow for a complex regulation of shunting between the pulmonary and systemic circulations. Studies on crocodile shunting have predominantly been based on invasive measurements, but here we report on the use of echocardiography.

Methods: Experiments were performed on seven pentobarbital anaesthetized juvenile Nile crocodiles (length and mass of 192 ± 13 cm and 26 ± 5 kg, respectively). Echocardiographic imaging was performed using a transesophageal (TEE) approach. All images were EKG-gated.

Results: We obtain excellent views of cardiac structures and central vasculature through the esophagus. Standard imaging planes were defined for both long- and short axis views of the left ventricle and truncus arteriosus. For the RV, only a short axis view could be obtained. Color Doppler was used to visualize flow. Pulsed waved Doppler for measuring flow profiles across the atrioventricular valves, in the two RVOTs and the left ventricular outflow tract. Shunting across the Foramen of Panizza could be visualized and gated to the EKG.

Conclusion: TEE can be used to image the unique features of the crocodile heart and allow for in-vivo imaging of the complex shunting hemodynamics, including timing of cardiac shunts.

1. Introduction

In contrast to other reptiles, but like birds and mammals, crocodiles have fully divided atria and ventricles, i.e. complete intracardiac separation of oxygenated and deoxygenated blood (Malvin et al., 1995; Webb, 1979). Seymour et al. (Seymour et al., 2004), proposed that this peculiar heart anatomy reflects that extant crocodilians evolved from a endothermic terrestrial ancestor with a high metabolic rate where complete separation of systemic and pulmonary pressures secured adequate oxygen delivery. Then, as crocodilians gradually became aquatic 'sit-and-wait' predators, they reverted to ectothermy, and acquired their unique arrangement of outflow vessels from the heart somewhere along this evolutionary transition (Seymour et al., 2004). Regardless of their evolutionary background, extant crocodiles possess a unique arrangement of their two aortic arches. The right aorta (RAo) emerges from the left ventricle (LV), while the left aorta (LAo) originates from the right ventricle (RV) and carries systemic venous blood. Immediately distal from the hinge point of the aortic valves, an opening between the LAo and the RAo, the Foramen of Panizza (FP), enables shunting of blood between the two aortic arches (Axelsson and Franklin, 2001). The right ventricular outflow tract (RVOT) is subdivided into two distinct regions, a subpulmonary portion giving rise to the pulmonary trunk and a subaortic part from which the LAo arises. Multiple small connective tissue nodules protrude into the subpulmonary conus of the RVOT, collectively forming a structure called the 'cog-tooth valve'. Constriction of the 'cog-tooth valve' increases vascular resistance in the

---

[*] Corresponding author at: Department of Paediatrics, Lillebaelt Hospital, University Hospital of Southern Denmark, Sygehusvej 24, DK-6000 Kolding, Denmark.
E-mail address: mads.damkjaer2@rsyd.dk (M. Damkjaer).
@Tobias_Wang_AU (T. Wang), @DamkjaerMads (M. Damkjaer).

https://doi.org/10.1016/j.cbpa.2023.111564
Received 22 September 2023; Received in revised form 12 December 2023; Accepted 18 December 2023

subpulmonary part of the RVOT. This in turn will impede pulmonary blood flow, and thus pulmonary venous return to the left atrium and thereby left ventricular outflow to the RAo. Constriction of the 'cog-tooth valve' can also induce flow from the right ventricle into the LAo if right ventricular pressure exceeds systemic pressure, and this oxygen-poor blood may enter the RAo through the FP. Depending on the level of vascular resistance in the 'cog-tooth valve', pulmonary to systemic shunting can increase from insignificant to a situation where the systemic circulation is driven exclusively by deoxygenated blood pumped via the right ventricle. Both the 'cog-tooth valve' and FP have been shown to be regulated by the autonomic nervous system (see (Axelsson, 2001; Grigg, 1991) for reviews).

Many of the studies on shunting patterns in crocodilians have generally been performed on anaesthetized animals, subjected to sternotomy to expose the heart and central vasculature for instrumentation (Syme et al., 2002; Jones and Shelton, 1993), studies on explanted hearts (Axelsson and Franklin, 2001; Franklin and Axelsson, 2000), or long-term studies following surgical removal of the right-to-left cardiac shunt (Eme et al., 2010; Eme et al., 2009). Echocardiography is an excellent tool for visualizing cardiovascular structure and function. It offers high temporal and spatial resolution. Echocardiography is not just capable of studying shunting. It also provides a comprehensive view of cardiovascular physiology. It allows for the visualization and quantification of blood flow across chamber junctions, arterial valves, and the assessment of parameters such as magnitude/volume, number of phases (early/active), and peak blood flow velocity (Friedberg, 2016). Furthermore, echocardiography can detect the presence and magnitude of regurgitation, providing insights into the functional integrity of cardiac valves (Frommelt, 2016; Mertens and Friedberg, 2016). These measurements are particularly significant in reptiles, where cardiovascular physiology can vary widely across species (Burggren, 2015). For instance, flow dynamics across chamber junctions can reveal insights into the reptilian heart's adaptability to different physiological demands. Such detailed cardiovascular assessments, made possible by echocardiography, pave the way for a deeper understanding of reptilian physiology and its evolutionary implications. However, echocardiographic descriptions rely on the ability to obtain standardized images of the heart in defined imaging planes, e.g. parasternal long axis or apical four chamber views. Such descriptions, have to our knowledge, not been made in crocodilians. Crocodiles have several unique anatomical features. These suggest that echocardiographic imaging might be challenging on these animals. Their thick scutes might impede the penetration of ultrasound signals into deeper tissue, and the ribs and gastralia might cause image artefacts preventing visualization of the heart. We hypothesized that with either transthoracic echocardiography (TTE), transesophageal echocardiography (TEE) or a combination of the two methods it would be possible to define standardized imaging planes, and visualize central anatomy and function of the crocodile heart through the heart cycle. Conventional TTE was attempted, as will be shown below, but the heart was essentially impossible to visualize due to the impedance offered by various tissues. Additionally, we hypothesized that the high temporal resolution of echocardiography would allow for studying the timing and direction of the FP shunt in relation to the cardiac cycle.

2. Methods

2.1. Animal handling and anesthesia

For the experiments, seven Nile crocodiles were used (*Crocodylus niloticus*) with a body length and body mass of 192 ± 13 (range; 220–149) cm 26 ± 5 kg (range; 45–12), respectively. All animals were purchased from a local breeding facility and transported to the experimental facility. They were then placed in a large pond for over four weeks prior to the commencement of the experiments. The experimental protocol was approved by the Animal Ethics Committee at the University of Pretoria, Gauteng Province, South Africa (project number v072–13).

Firstly, crocodiles were caught with a noose, then blindfolded and restrained. Anesthesia was induced by an injection of pentobarbital (10 mg/kg) into the dorsal occipital venous sinus. Anesthetic depth was monitored by checking corneal reflexes. In a few animals it was necessary to give a supplementary dose of 5 mg/kg pentobarbital, and spontaneous respiration ceased six out of seven animals on this regime. In these animals a cuffed endotracheal tube (ID 7 mm) was inserted allowing for ancillary manual ventilation with room air using a Hudson demand valve (Hudson RCI, Teleflex Medical, Morrisville, North Carolina 27,560, USA).

2.2. Blood pressure monitoring

The brachial artery on left forelimb was exposed by blunt dissection to insert a vascular sheath that could accommodate a tip-transducer catheter (5F Micro-Tip SPC 350, Millar Instruments, Houston, TX, USA). The catheter was advanced centrally into the left subclavian for continuous monitoring of arterial blood pressure (ABP). Signals from the pressure transducer were recorded with a Biopac MP100 data acquisition system (Biopac Systems, Goleta, CA, USA) at 200 Hz, and heart rate (HR) was derived from the pulsatile pressure signal.

2.3. Echocardiographic studies

We used a commercially available ultrasound system (Vivid I, GE Healthcare Horten, Norway) for all imaging. Initially, a transthoracic ultrasound approach was tested with both a 3.5 and 5 MHz phased array transducer (M4S). The combination of thick dermal scutes and ribs/gastralia made it impossible to obtain any echocardiographic views of the heart via either TTE or transabdominal imaging. An injection with a local anesthetic (lidocaine, 20 mg/ml) was given around the scutes directly above the presumed location of the apex cordis. A few minutes after lidocaine injection the scutes were surgically removed to expose the underlying thoracic wall. The thickness and composition of the crocodile's thoracic/abdominal wall made it difficult for the ultrasound waves to penetrate effectively, preventing clear visualization of the heart. This led us to explore TEE as an alternative, which, as detailed in the subsequent sections, provided clearer and more informative images of the crocodylian heart.

With the animals sedated in a dorsal position, a rigid polyvinyl chloride tube (diameter 5 cm) was secured in the crocodiles mouth, and a transesophageal transducer (GE 6 T-RS, Tip size 12 × 14 mm and length 45 mm, Probe Bandwidth: 2.2–8 MHz) was the inserted through the esophagus to a position directly posterior to the heart. Using this approach, we were able to obtain cross sectional images from the apex of the ventricles, through to the basis of the ventricles, atria and the truncus arteriosus. Standard imaging views of the outlet tracts were defined, i.e. probe positions and angles from which the heart could be imaged to obtain long and short axis views of the relevant cardiac structures. Color Doppler was used to visualize blood flow. Pulsed wave ultrasound was used to measure flow velocity profiles in the right-sided aorta, in the pulmonary artery, and through the mitral valve. The EKG was recorded by attaching EKG electrodes from the ultrasound system to cannulas (BD Microlance, 18G, Becton Dickinson) inserted at 3 points around the heart. All images were gated to the EKG by the ultrasound system.

At the end of the experiments, animals were terminated by intravenous injection of a pentobarbital overdose. The hearts were the removed and fixated in a 4% formaldehyde solution.

3. Results

3.1. Hemodynamics

Mean arterial blood pressure and heart rate were stable during all imaging procedures at 23 ± 5 mmHg and 39 ± 4 beats per minute, respectively.

3.2. Transthoracic echocardiography

Conventional TTE was attempted but the heart was essentially impossible to visualize due to the impedance offered by various tissues, i.e. gastralia and ribs. This was also the case even after surgical removal of the dermal scutes.

3.3. Left ventricle

With the probe in the esophagus, facing ventrally, slight anteflexion of the tip and multiplane angle set to approximate 90° (range 80–110°) we obtained long-axis views of the left atrium (LA), LV, left ventricular outflow tract (LVOT) and proximal RAo (Fig. 1 and Loop 1). The left ventricle is located caudally and dorsally compared to the RV, which is situated more cranially and ventral (for a detailed anatomical description of the somewhat unusual location of the ventricles, please cf. (Cook et al., 2017)). The atria are relatively large and atrial systole seems to contribute substantially to the ventricular filling, as shown in Loop 1. A pulse-wave Doppler was used to measure flow profile across the mitral valve over several heart cycles (Fig. 2). From the LV the RAo arises at the level of the aortic valve. A flow profile in the RAo could be obtained by pulse-wave Doppler (Fig. 3) immediately distally from the aortic valve and gated to the corresponding EKG.

The probe was then advanced slightly farther into the esophagus, and the multiplace angle returned to approximate 0° to obtain a short axis view of the two ventricles (Loop 2).

The ejection fraction appeared high (i.e. >60%; cf. Loop 1 and 2), but was not quantified due to the highly trabeculated structure of the left ventricular myocardium that makes it difficult to delineate the borders of the LV at both end-diastole and end-systole to for calculation of biplane ejection fraction.

3.4. Right ventricle

Similar to other animals with full separation between the pulmonary and systemic circulations, the RV wraps around the LV such that it was not possible to obtain images to calculate ejection fraction. The right ventricle gives rise to the LAo and the pulmonary trunk; these are separated by a small muscular outlet septum was not possible to visualize on TEE. The two RV outflows were visualized with Doppler color flow (Loop 3) with the multiplane angle at approximate 90°. The aligned orientation of the pulmonary valve and aortic valve can be seen in Loop 4. Directly below the pulmonary valve, in the subpulmonary part of the RVOT, the ‘cog-teeth’ protrudes into the subpulmonary RVOT, giving rise to a classical obstructive flow pattern, with an initial plateau phase, followed by a late-systolic flow acceleration (Fig. 4).

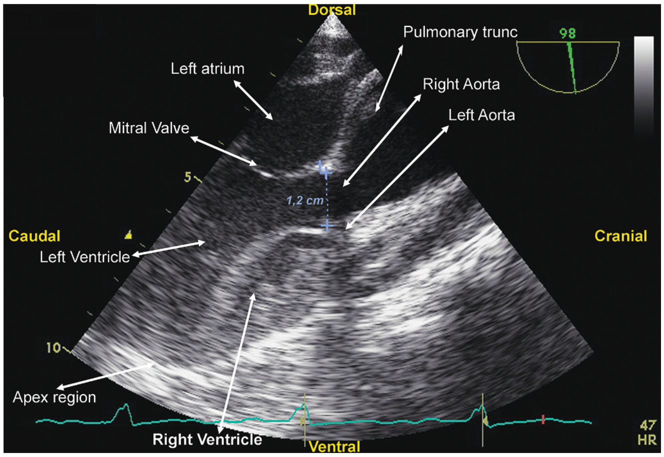

Fig. 1. Transesophageal long axis view of the left side structures. Anatomical structures identified with white arrows. Anatomical directional terms are indicated in yellow. The measurements indicates the size of the aortic annulus at the right aortic arch. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.5. Arterial outflow in the truncus arteriosus

The outflow from the crocodilian heart, from the ventriculo-arterial junction, and distally is initially comprised of RAo (originating from left ventricle), the LAo and pulmonary trunk originating from the right ventricle. The outflow arteries course closely together in the truncus arteriosus, giving off their first branches and coursing farther distally. With the probe in the esophagus, facing ventrally, slight anteflexion of the tip and multiplane angle set at 0° it was possible to obtain high quality images of the truncus (Fig. 5). We recorded EKG gated loops without (Loop 5) and with color Doppler (Loop 6). With the multiplane angle rotated back to approximately 90°, long axis views of the two aortas and pulmonary trunk could be obtained in both early and late systole (Fig. 6 and Loop 7).

3.6. Structures unique to the crocodile heart

Foramen of Panizza: EKG gated Doppler echocardiography showed that shunting between the LAo and RAo occurred exclusively in late systole, as a brief high velocity jet from the right to the left aorta (Loop 8). Due to apical displacement of the heart during heart cycle, and the small size of the FP it was not possible to record a pulse-wave Doppler in the FP.

Cog tooth valve: the valves consistent of multiple small connective tissue nodules protruding into the subpulmonary conus of the RVOT. These could be visualized as small hyperechoic noduli protruding into the RVOT (Fig. 7) giving rise to the characteristic outflow pattern described above (Fig. 4).

4. Discussion

In this study, we propose the first standardized echocardiographic evaluation of the crocodilian heart. Using a TEE technique we could visualize the LA, both ventricles, out flow tracts, ‘cog-tooth valve’, truncus arteriosus including separate vessels and FP. Additionally flow profiles were recorded across valves and in central vessels, which allowed for a characterizing flow through the FP with a high temporal resolution and gating to the EKG.

We were not able to do obtain any TTE windows, which does limit the use of ultrasound among researchers for a number of reasons. Firstly, TEE probes are considerably more expensive than their widely used transthoracic counterparts. Secondly, it requires a skilled operator to operate TEE. Thirdly, it probably necessitates anesthesia, although it might be possible in awake animals, but only if adequately physically restrained. Nevertheless, there are some advantages to transesophageal echocardiography that should be emphasized, namely the considerably higher spatial resolution it offers (Lyons and Bhamidipati, 2017). Generally, echocardiographic equipment has been designed for application in humans, and are thus geared towards human heart sizes, i.e. some 0.5% of body mass (Seymour and Blaylock, 2000). In adult crocodiles, heart mass constitutes approximately 0.125% of body mass (Coulson et al., 1989), suggesting that a 400 kg crocodile is needed to image a heart with a similar size of an adult human (80 kg). Crocodiles this size may be challenging experimental subjects. This could to some

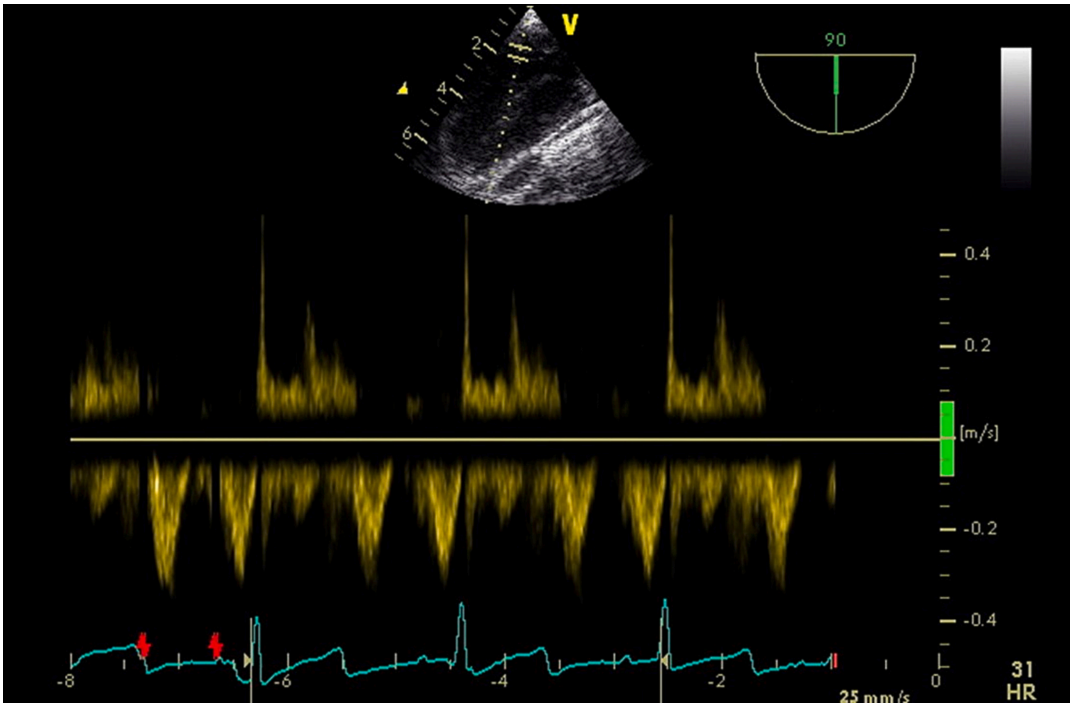

Fig. 2. Pulsed waved Doppler at the left of the mitral valve orifice to measure flow profile across the valve. Flow velocity is measured in m/s and the corresponding EKG is displayed at the bottom of the image.

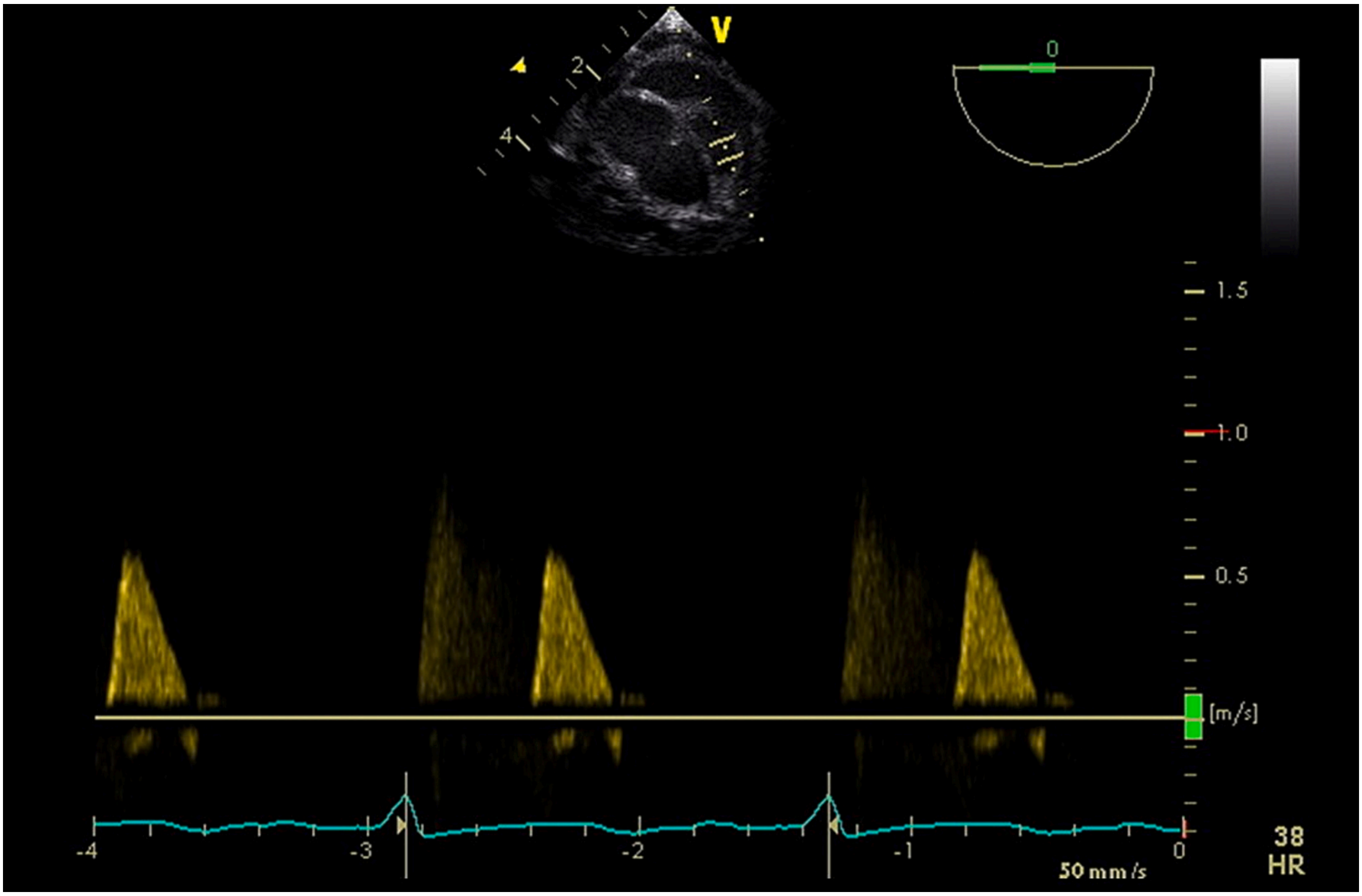

Fig. 3. Pulsed waved Doppler in the proximal right aorta used to measure flow profile during heart cycle. Flow velocity is measured in m/s and the corresponding EKG is displayed at the bottom of the image.

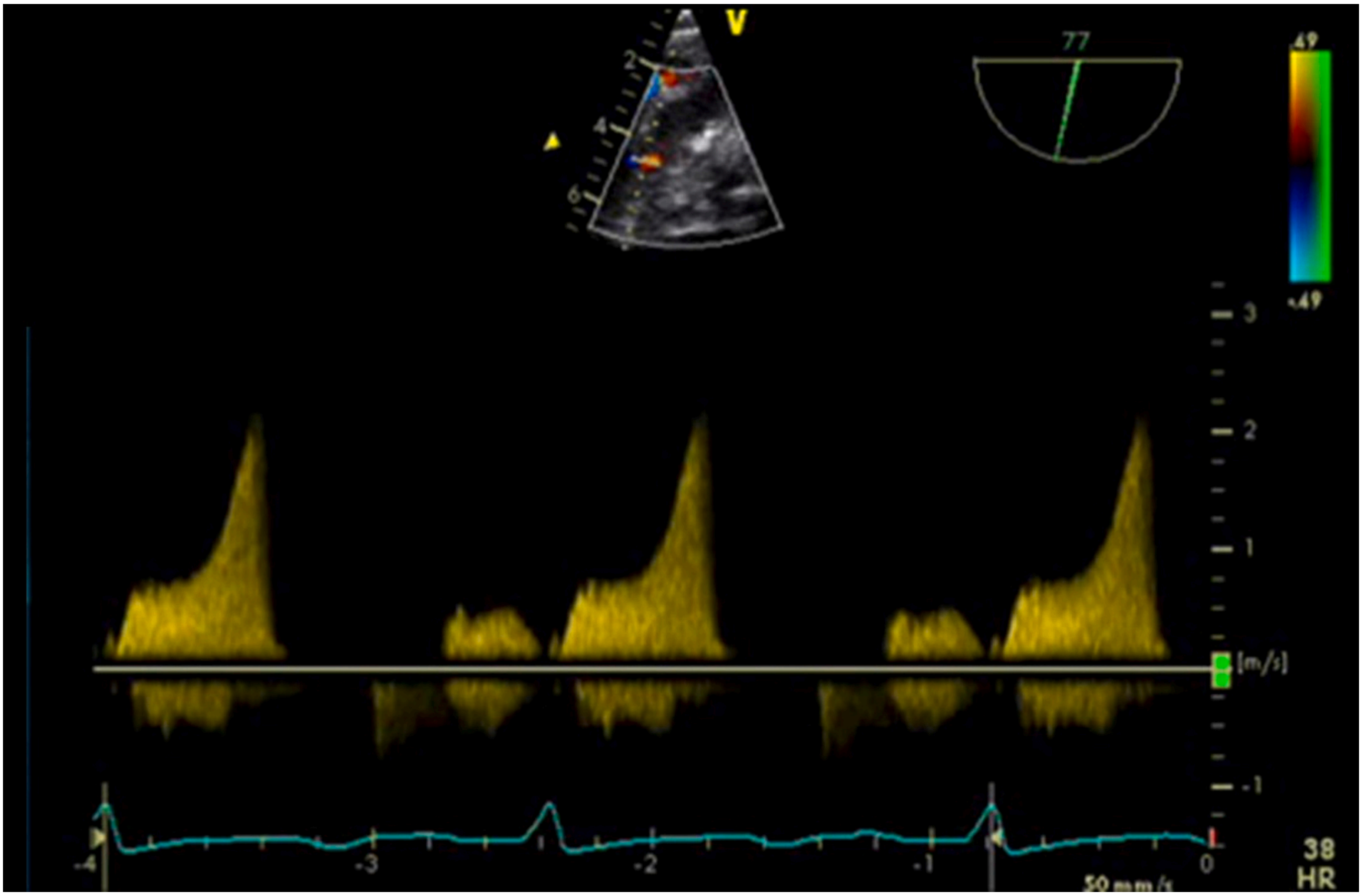

Fig. 4. Pulsed waved Doppler immediately distal from the 'cog-tooth valve' showing late systolic flow acceleration classical for ventricular outflow obstruction. Corresponding EKG is displayed at the bottom of the image.

extent be circumvented by the use of higher frequency neonatal probes, i.e. aimed towards use in a neonate, but these types are less available thus potentially also constraining wider applicability.

Previous studies have relied on angioscopy to visualize cardiac function in crocodiles (Axelsson et al., 1996). However, the invasive nature and limited ability to simultaneous observe other cardiac structures makes it challenging to elucidate overall cardiac function. Similarly ex-vivo perfusion studies cannot be regarded as physiological and conclusions drawn from these must be interpreted with due caution. In crocodiles, one study from 1995 used a transthoracic echocardiography probe (5 MHz, standard probe for imaging of adults in cardiology) to measure flow profiles in central vessels, following surgical exposure of the heart in alligators (mean body weight 4.1 kg) (Malvin et al., 1995). Echocardiographic technology has developed tremendously since this pioneering study from 1995, and in combination with the higher frequency of the TEE probe in the present study it is possible to obtain much higher spatial and temporal resolution to image specific cardiac structures.

In a resting crocodilian heart, the blood flow pattern is similar to what is observed in avian or mammalian hearts. Venous blood first enters the right atrium and continues through the RV, pulmonary trunk and lung vasculature. From the lungs the blood enters the LA and then LV. The oxygenated blood is then pumped through the RAo suppling the brain, upper extremities and lower body. Under these conditions only a limited amount of blood enters the left aorta through the FP (Axelsson et al., 1996). Some have suggested, that the limited flow is needed to prevent blood clots forming in the LAo (Shelton and Jones, 1991). In the current study no signs of spontaneous echo contrast or thrombus was observed in any cardiac chamber or aortas. Imaging of the FP showed right to left shunting (i.e., LAo to RAo) through FP in late systole, which is in line with Malvin who also reported flow during systole (Malvin et al., 1995), although they reported bidirectional flow. Axelsson et al. reported reverse flow through FP from right to left (i.e. from the LAo to the RAo) in early diastole (Axelsson et al., 1996). However, these measurements were performed on explanted perfused hearts, which might explain the difference between the studies. Whether anesthesia with pentobarbital as used in our study creates alterations in systemic vascular resistance causing a right to left shunt resembling hemodynamic changes observed during diving is not known. But data from anaesthetized crocodilians suggest that mean arterial pressure drops in response to anesthesia, and this could perhaps induce the observed changes in flow profiles (Axelsson et al., 1996; Stegmann et al., 2017). The mean arterial blood pressure of the animals in our study were lower than what have been reported from telemetric studies in conscious animals (Stegmann et al., 2017), suggesting an impact of anesthesia on hemodynamics, and potentially shunt patterns.

In conclusion, we proposed the first echocardiographic standard evaluation of the crocodilian heart. Standard imaging planes are defined as reference for future studies on central hemodynamics and shunting in crocodilians.

Echocardiographic loops

All loops can be accessed and downloaded from Figshare by clicking the hyperlink: https://figshare.com/s/4b5af3656509bc8eb640

DOI: https://doi.org/10.6084/m9.figshare.22561810

Loop 1: Transesophageal long axis view of the left side structures. Please cf. Fig. 1 for identification of anatomical structures.

Loop 2: Transesophageal short axis view of the left and right ventricle at the level of the papillary muscles. Corresponding EKG is

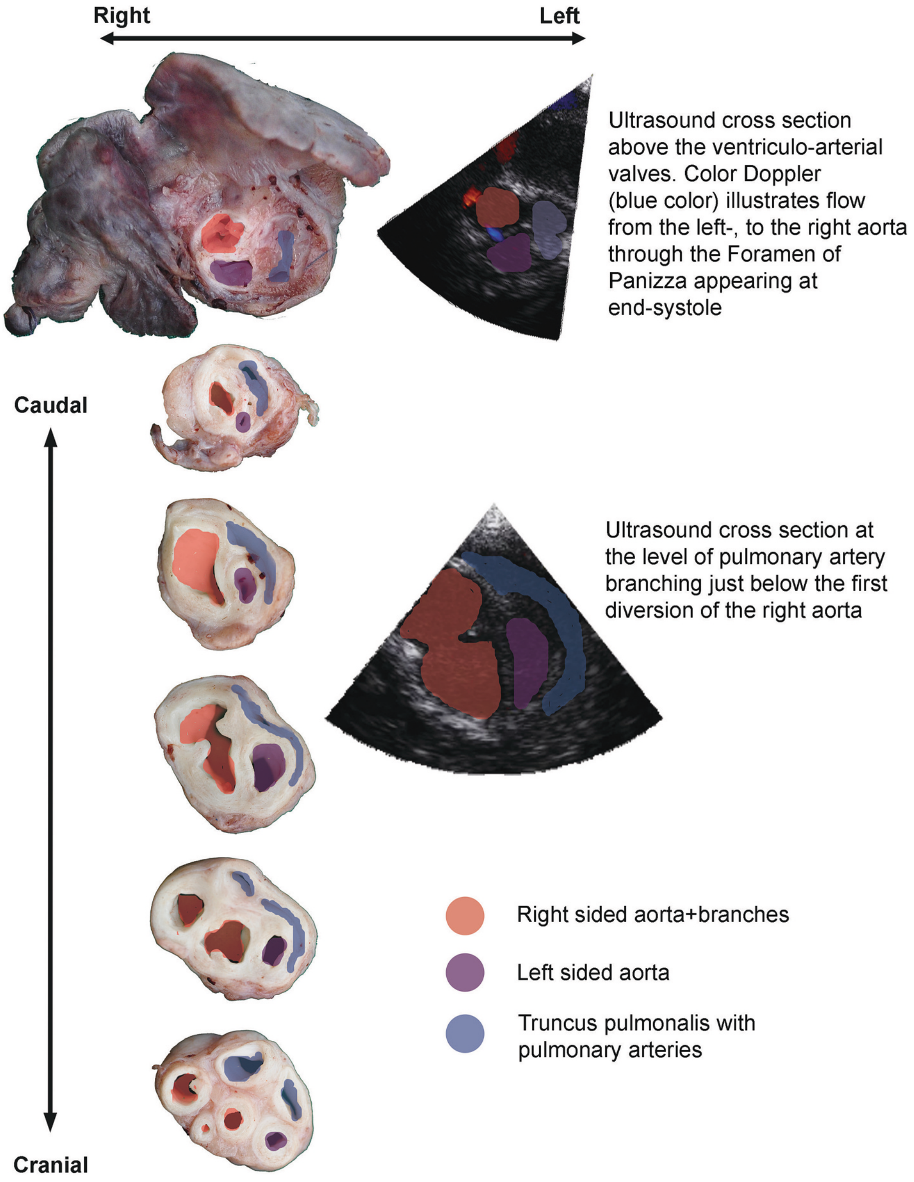

Fig. 5. Consecutive anatomical cross sections from the level of the pulmonary and aortic valves distally through the truncus arteriosus. Relevant vessels in the truncus are identified in on the anatomical sections and corresponsingly on the transesophageal images to allow for identification of the relevant vessels during an echocardiogram.

displayed at the bottom of the image.

Loop 3: Transesophageal long axis view of the right ventricular outflow vessels, i.e. pulmonary trunk and left aorta. Color Doppler used for visualization of flow during the cardiac cycle. Corresponding EKG is displayed at the bottom of the image.

Loop 4: Transesophageal long axis view of the left ventricular outflow tract and the subaortic portion of the right ventricular outflow tract. Note the parallel orientation of the two aorta and their corresponding bicuspid aortic valves.

Loop 5: Transesophageal short axis view of the truncus arteriosus immediately distal to the pulmonary and aortic valves.

Loop 6: Transesophageal short axis view of the truncus arteriosus immediately distal to the pulmonary and aortic valves with color Doppler.

Loop 7: Transesophageal long axis view of the outflow vessels in the truncus arteriosus with color Doppler.

Loop 8: EKG gated Transesophageal Doppler echocardiography showing right to left aorta shunt over the Foramen of Panizza in late systoli.

Author contributions

CBP: animal instrumentation and echocardiography.

KM: echocardiographic procedures, anatomical photographs and manuscript preparation.

TW: blood pressure recording and anesthesia.

MD: planned the study and drafted paper.

Credit authorship contribution statement

Christian F.B. Poulsen: Conceptualization, Formal analysis, Funding acquisition, Writing – original draft. **Kim Munk:** Data curation, Formal analysis, Methodology. **Tobias Wang:** Conceptualization, Formal analysis, Supervision, Writing – review & editing. **Mads Damkjaer:** Conceptualization, Data curation, Methodology, Supervision, Writing – review & editing.

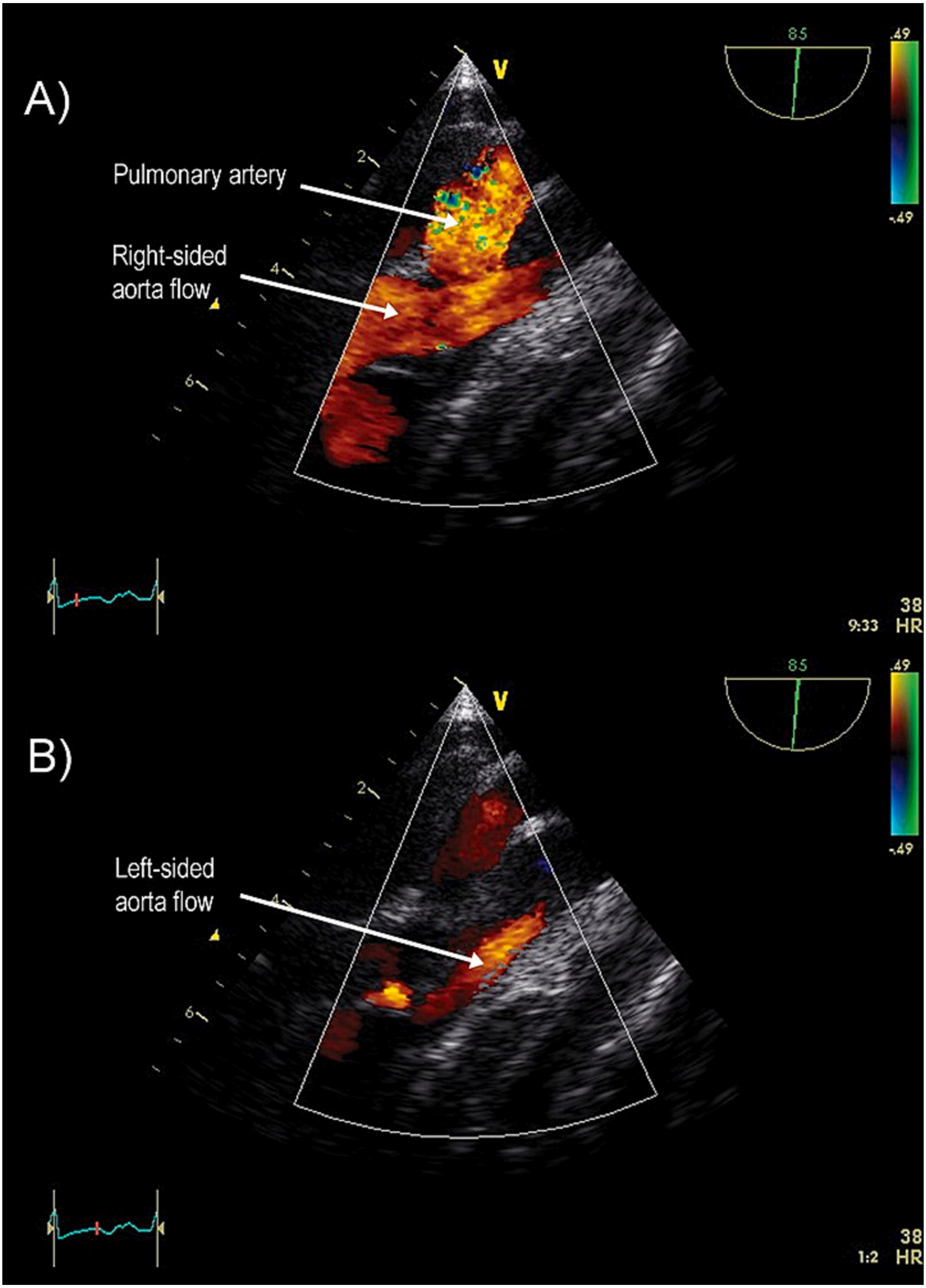

Fig. 6. Transesophageal long axis view in A) early systole demonstrating flow in the right-sided aorta and the main pulmonary artery, and in B) late systole (ECG traces in lower left corner) demonstrating flow in the left sided aorta.

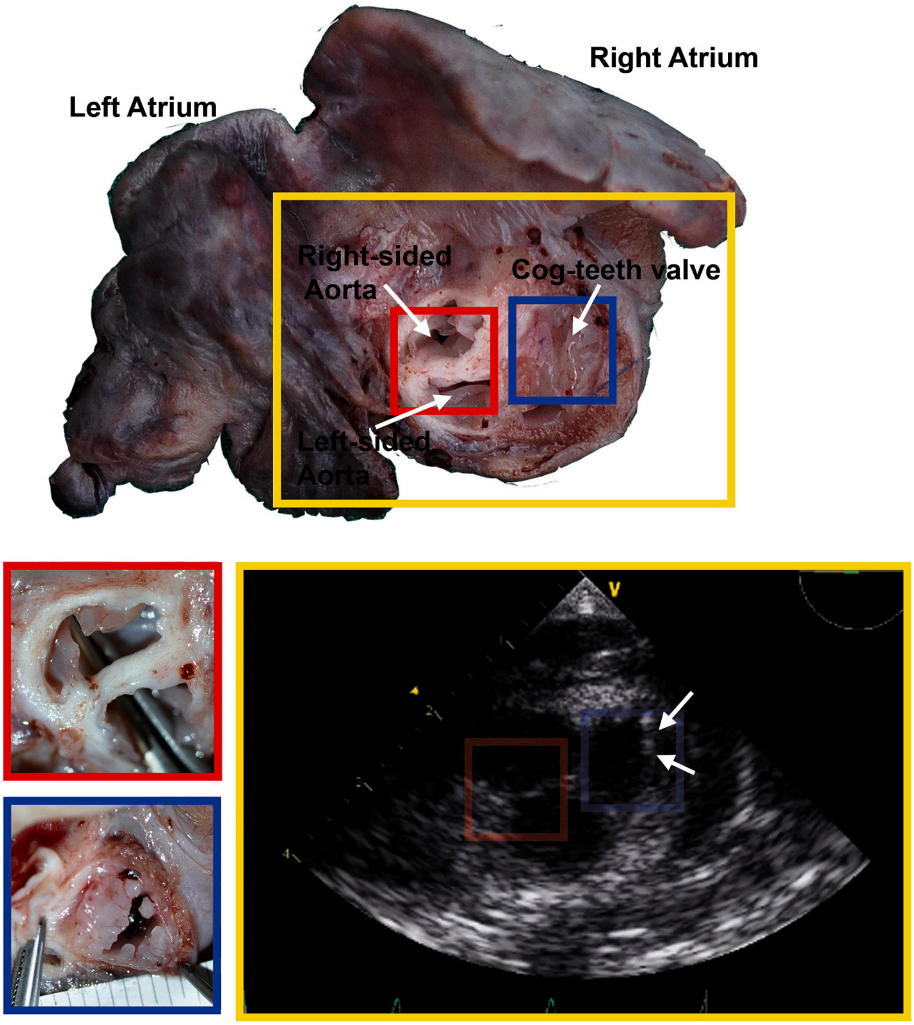

Fig. 7. Anatomic and transesophageal ultrasound pictures of the crocodilian heart cross sectioned at the level of the ventricular arterial valves. The red box demonstrates the Foramen of Panizza connecting the right-sided Aorta (arising from the left ventricle) and left-sided Aorta (arising from the right ventricle). The blue box shows the cog-teeth Valve in the subpulmonary conus. The yellow box demonstrates the ultrasound picture, with small structures (white arrows) of a higher echogenicity representing connective tissue in the cog-teeth Valve. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Declaration of Competing Interest

None.

Data availability

Data will be made available on request.

References

Axelsson, M., 2001. The crocodilian heart; more controlled than we thought? Exp. Physiol. 86, 785–789.

Axelsson, M., Franklin, C.E., 2001. The calibre of the Foramen of Panizza in Crocodylus porosus is variable and under adrenergic control. J. Comp. Physiol. B. 171, 341–346.

Axelsson, M., Franklin, C., Ouml, Fman C., Nilsson, S., Grigg, G., 1996. Dynamic anatomical study of cardiac shunting in crocodiles using high-resolution angioscopy. J. Exp. Biol. 199, 359–365.

Burggren, W.W., 2015. Form and function in reptilian Circulations1. Am. Zool. 27, 5–19.

Cook, A.C., Tran, V.H., Spicer, D.E., Rob, J.M.H., Sridharan, S., Taylor, A., Anderson, R. H., Jensen, B., 2017. Sequential segmental analysis of the crocodilian heart. J. Anat. 231, 484–499.

Coulson, R.A., Jack, D.H., Thomas, D.C., 1989. Biochemistry and physiology of alligator metabolism in vivo. Am. Zool. 29, 921–934.

Eme, J., Gwalthney, J., Blank, J.M., Owerkowicz, T., Barron, G., Hicks, J.W., 2009. Surgical removal of right-to-left cardiac shunt in the American alligator (Alligator mississippiensis) causes ventricular enlargement but does not alter apnoea or metabolism during diving. J. Exp. Biol. 212, 3553–3563.

Eme, J., Gwalthney, J., Owerkowicz, T., Blank, J.M., Hicks, J.W., 2010. Turning crocodilian hearts into bird hearts: growth rates are similar for alligators with and without right-to-left cardiac shunt. J. Exp. Biol. 213, 2673–2680.

Franklin, C.E., Axelsson, M., 2000. An actively controlled heart valve. Nature 406, 847–848.

Friedberg, M.K., 2016. Hemodynamic measurements. In: Echocardiography in Pediatric and Congenital Heart Disease, pp. 73–95.

Frommelt, P.C., 2016. Diastolic ventricular function assessment. In: In: Echocardiography in Pediatric and Congenital Heart Disease, pp. 132–153.

Grigg, G., 1991. Central cardiovascular anatomy and function in Crocodilia. In: Wood, S. C., W, R.E., Hargens, A.R., Millard, R.W. (Eds.), Physiological Adaptations in Vertebrates: Respiration, Circulation and Metabolism, pp. 339–354. New York.

Jones, D.R., Shelton, G., 1993. The physiology of the alligator heart: left aortic flow patterns and right-to-left shunts. J. Exp. Biol. 176, 247–270.

Lyons, K., Bhamidipati, K., 2017. 4 comparison of transthoracic and transoesophageal echocardiography in the diagnosis of infective endocarditis – a tertiary Centre experience. Heart 103, A3.

Malvin, G.M., Hicks, J.W., Greene, E.R., 1995. Central vascular flow patterns in the alligator Alligator mississipiensis. Am. J. Phys. 269, R1133–R1139.

Mertens, L., Friedberg, M.K., 2016. Systolic ventricular function. In: Echocardiography in Pediatric and Congenital Heart Disease, pp. 96–131.

Seymour, R.S., Blaylock, A.J., 2000. The principle of Laplace and scaling of ventricular wall stress and blood pressure in mammals and birds. Physiol. Biochem. Zool. 73, 389–405.

Seymour, R.S., Bennett-Stamper, C.L., Johnston, S.D., Carrier, D.R., Grigg, G.C., 2004. Evidence for endothermic ancestors of crocodiles at the stem of archosaur evolution. Physiol. Biochem. Zool. 77, 1051–1067.

Shelton, G., Jones, D.R., 1991. The physiology of the alligator heart: the cardiac cycle. J. Exp. Biol. 158, 539–564.

Stegmann, G.F., Williams, C.J., Franklin, C., Wang, T., Axelsson, M., 2017. Long-term surgical anaesthesia with isoflurane in human habituated nile crocodiles. J. S. Afr. Vet. Assoc. 88, e1–e6.

Syme, D.A., Gamperl, K., Jones, D.R., 2002. Delayed depolarization of the cog-wheel valve and pulmonary-to-systemic shunting in alligators. J. Exp. Biol. 205, 1843–1851.

Webb, G.J.W., 1979. Comparative cardiac anatomy of the reptilia. III. The heart of crocodilians and an hypothesis on the completion of the interventricular septum of crocodilians and birds. J. Morphol. 161, 221–240.