UNIVERSIDADE FEDERAL DO PARANÁ

WANDERLEI DE MORAES

OCULAR MORPHOLOGY, PHISIOLOGY AND SELECTEAL OPHTHALMIC CLINICAL TESTS IN THE HARPY EAGLE (*Harpia harpyja*)

CURITIBA

2018

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OCULAR MORPHOLOGY, PHISIOLOGY AND SELECTEAL OPHTHALMIC CLINICAL TESTS IN THE HARPY EAGLE (*Harpia harpyja*)

Tese apresentada como requisito parcial à obtenção do grau de Doutor em Ciências Veterinárias, no Curso de Pós-Graduação em Ciências Veterinárias, Setor de Ciências Agrárias, da Universidade Federal do Paraná.

Orientador: Prof. Dr. Fabiano Montiani-Ferreira

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CURITIBA, 09 de Março de 2018.

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A minha esposa Inah e aos meus
filhos, Carolina e Luiz Filipe, penso mais
que falo, o quanto amo vocês.

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À oportunidade de estar neste mundo para conviver, aprender, ensinar e não se conformar......

À Deus, de onde viemos e para onde retornamos.

Yo Plantaré mi Árbol

...
Aunque me quieran atajar a veces,
y me arrodille solo y agotado,
y a veces pierda el rumbo a lo sagrado,
yo plantaré mi árbol.
...

JUAN CARLOS CHEBEZ
(1962–2011), 2003.

RESUMO

A presente tese de doutorado compreende uma introdução, onde a espécie estudada é caracterizada e apresentada, abordando a importância da visão para as aves de rapina e a oftalmologia em aves.

O primeiro capítulo descreve as características morfológicas mais relevantes do olho da águia harpia, a órbita óssea e estabelece valores de referência para teste de Schirmer (STT), análise da microbiota conjuntival, tonometria de rebote, tonometria de aplanação, espessura corneana central (CCT), biometria ocular de ultrassom ocular em modo B, comprimento palpebral e medidas biométricas da órbita e crânio, permitindo compreender melhor alguns aspectos importantes do sistema visual desta ave de rapina e ajudando profissionais de medicina aviária e de oftalmologia veterinária a diagnosticar doenças oculares.

O segundo capítulo trata de artigo publicado no periódico Veterinary Ophthalmology, onde são avaliados os parâmetros de velocidade sanguínea da artéria *pectinis oculi* usando ultrassonografia Doppler. O animal modelo utilizado nesta pesquisa foi a águia harpia e os dados encontrados sugerem uma alta atividade metabólica em *pecten oculi* e corroboram a hipótese de uma função nutricional e/ou regulação da pressão intraocular.

Palavras-chave: águia harpia, rapinantes, oftalmologia, veterinária, aves de rapina, animais silvestres.

ABSTRACT

The present doctoral thesis comprises an introduction where the species studied is characterized and presented, addressing the importance of the vision for birds of prey and birds ophthalmology.

The first chapter describes the most relevant morphological characteristics of the harpy eagle's eye, its bony orbit and establishes reference values for Schirmer's tear test (STT), conjunctival microbiota analysis, rebound tonometry, applanation tonometry, central corneal thickness (CCT), B-mode ultrasound ocular biometry, palpebral length and biometric measurements of the orbit and skull, allowing a better understanding of some important aspects of the visual system of this bird of prey and helping veterinary medicine and veterinary ophthalmology professionals in the diagnosis of eye diseases.

The second chapter presents an article published in the journal Veterinary Ophthalmology, where the blood velocity parameters of the pectinis oculi artery are evaluated using Doppler ultrasonography. The animal model used in this research was the harpy eagle and the data found suggest a high metabolic activity in pecten oculi and corroborating with the hypothesis of a nutritional function and/or regulation of intraocular pressure.

Keywords: harpy eagle, prey, ophthalmology, veterinary, birds of prey, wild animals.

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1 INTRODUÇÃO

Os grandes predadores sempre causaram um fascínio na humanidade, quer pela representação do perigo iminente, já que nossa espécie não está no topo da cadeia alimentar, quer pelo símbolo de força e poder que representam por caçarem outras espécies.

Nos ecossistemas terrestres temos predadores alados de diversos tamanhos, mas as águias, sempre foram as que mais se destacaram.

As águias são consideradas símbolos de coragem e independência, frequentemente representadas em brasões de famílias nobres, reinos antigos, cidades e bandeiras de países modernos. O termo "águia" é vulgarmente aplicado às espécies de grande porte da família Accipitridae, de garras bem desenvolvidas e especialistas na captura de vertebrados terrestres ou aquáticos. No planeta temos mais de 70 espécies e no Brasil existem oito espécies de águias, destacando-se a harpia (*Harpia harpyja*), também chamada de gavião-real, considerada a maior e a mais poderosa águia do mundo (MENQ, 2017).

As harpias são inconfundíveis pelo tamanho e robustez, podendo chegar a 105 cm de altura. Cabeça cinzenta com topete formando duas pontas negras. Manto e papo negros. Peito e barriga brancos. Partes inferiores das asas e calções (revestimento das tíbias) brancos com estrias negras e cauda com três faixas cinzentas. Animais imaturos não possuem papo negro e são mais claros que os adultos. É uma espécie florestal que ocorre do México à Bolívia, incluindo o norte da Argentina e todo o Brasil. Em áreas de Mata Atlântica, os registros tornaram-se escassos nas últimas décadas do século passado, possuindo populações significativas somente na Amazônia (ICMBio, 2008).

Nesta espécie as fêmeas pesam pouco mais de 9 kg e possuem uma envergadura de até 2 metros. Suas garras são maiores que as de um urso-pardo (*Ursus arctos*), com unhas do hálux de até 7 cm de comprimento. É uma predadora especializada, por vezes capturando animais com o peso/tamanho da própria ave (MENQ, 2017).

A harpia vive em ambientes florestais, onde constrói seus ninhos em grandes arvores e caça suas presas de preferência no dossel da floresta.

Seus voos são raros e deslocam-se entre as copas das árvores com uma agilidade surpreendente, atingindo suas vítimas em voos rápidos. Permanecem espreitando suas presas por longos períodos de tempo. Sua dieta é composta basicamente por mamíferos arborícolas, como macacos e bicho-preguiça (ROBINSON, 1994).

Segundo Straube (2000) o ornitólogo francês Louis J. P. Vieillot criou para ela um gênero próprio - *Harpia*, uma lógica inspiração nas conhecidas harpias da mitologia grega (PINTO, 1979 *apud* STRAUBE, 2000). As harpias da mitologia grega eram filhas de Taumas e sua esposa Electra, divindades marinhas misteriosas. Eram três: *Coeleno* (a obscuridade), *Aelo* (a tempestade) e *Ocipete* (a rapidez); tinham corpo de abutre e cabeça de velha e desprendiam um cheiro horrível. Contaminavam tudo o que tocassem, fossem objetos ou alimentos, tornando-os pútridos e imprestáveis (SPALDING, 1972; CIVITA ed., 1973; KURY, 1992, *apud* STRAUBE, 2000). Para autores muito antigos (MENESTRIER, 1688 *apud* STRAUBE, 2000), a sua conformação era encimada por faces de mulher e o corpo de águia. Na Teogonia de Hesíodo, as harpias são divindades aladas, e de cabeleira longa e solta, mais velozes que os pássaros e os ventos; no terceiro livro da Eneida de Virgílio, aves com cara de donzela, garras encurvadas e ventre imundo, pálidas de uma fome que não podem saciar. Descem das montanhas e conspurcam as mesas dos festins. São invulneráveis e fétidas; tudo devoram, guinchando e tudo transformam em excremento (J. L. Borges *in* PRIORE, 2000 *apud* STRAUBE, 2000).

Sempre foi troféu cobiçado por índios e caçadores. Eram mantidas em gaiolas pelos caciques que as conservavam para cortar-lhes (e não arrancar-lhes) periodicamente as rêmiges e retrizes de largura ímpar. Eram consideradas a personificação do cacique e quando seu dono morria também eram mortas ou sucumbiam à fome (CARVALHO, 1949 *apud* SICK, 1997). Os caçadores guardam suas garras como amuleto (SICK, 1997).

Esta espécie está ameaçada pela fragmentação do habitat, a caça ilegal, a perseguição e a comercialização, como ameaças imediatas (VARGAS *et al*., 2006).

Também corre risco de extinção pela baixa taxa reprodutiva já que só conseguem criar em média dois filhotes a cada 5 anos. No Brasil as populações mais ameaçadas encontram-se no bioma da Mata Atlântica, sendo que na região sul do Brasil, já é considerada extinta em Santa Catarina e em extinção no Paraná e no Rio Grande do Sul. Atualmente são monitorados ninhos somente no estado do Espirito Santo e no Sul da Bahia, o que demostra a precariedade da população desta espécie neste bioma.

Qualquer animal em seu ambiente natural necessita estar com toda a sua capacidade cognitiva e muscular em um bom estado de funcionamento para conseguir sobreviver. Caso algumas de suas capacidades estejam limitadas não podem estar tão débeis a ponto de impedir que se proteja, se alimente e se reproduza. É quase como ser um atleta em permanente movimento.

A perfeita integração e funcionamento dos sistemas visuais, cognitivos e de locomoção permitem que o animal mantenha sua performance para sobreviver na natureza. A interdependência destes fatores é extremamente relevante e crucial para a sobrevivência de cada indivíduo da espécie e consequentemente da população como um todo.

Os rapinantes além do sistema de apreensão da alimentação, feito preferencialmente pelas garras das patas; do sistema de voo, sustentado principalmente pelas asas e estruturas de redução de peso como os sacos aéreos, também precisam contar com uma perfeita visão para encontrar e capturar suas presas.

A visão nestes animais foi aperfeiçoada por milhares de anos de evolução, em conjunto com os sistemas de locomoção, escolha de habitat e modo de vida, proporcionando em conjunto, as melhores ferramentas para desempenharem seu papel na perpetuação da espécie, cumprindo com sua função ecológica e ajudando na manutenção da homeostase do ambiente.

A posição frontal dos olhos das aves de rapina é resultado de uma adaptação à caça, formando uma visão binocular útil na localização de presas. A proporção do tamanho dos olhos em relação à cabeça, grande quantidade de células da retina (cones e bastonetes) e a presença de duas fóveas em alguns grupos, como os Falconiformes, também auxiliam as aves predadoras na obtenção do alimento (ICMBio, 2008).

O sistema visual, integrando desde as estruturas anatômicas, sua fisiologia e funcionalidade com o sistema nervoso, é um dos principais requisitos para a performance plena em rapinantes, pois é graças à visão que estes animais conseguem uma interação plena com o ambiente. A visão, a locomoção através do voo e a captura das presas com o uso da apreensão pelas patas e garras são as três principais peças fundamentais para a sobrevivência destes animais como predadores.

A medicina veterinária de rapinantes tem como princípio básico permitir a manutenção e/ou a recuperação das habilidades necessárias ao vôo e à caça. Por esse princípio, todas as ações executadas com as espécies manejadas tentarão manter a integridade do animal. Dessa forma, o manejo dos animais oriundos de acidentes ou apreensões e os mantidos em cativeiro deverão cumprir com um protocolo básico que contemple esses cuidados especiais. Princípios de medicina veterinária preventiva deverão ser empregados para evitar que as aves adquiram novas enfermidades e que sejam curadas das enfermidades pregressas (ICMBio, 2008).

Os animais mantidos em cativeiro devem ser avaliados periodicamente quanto ao aspecto físico e nutricional, sobretudo os que participam de programas de treinamento e vôo. Anualmente, os animais deverão fazer exames e sempre que possível deverão incluir hematologia, coproparasitologia, exame clínico de pele, exame de cavidades e exames das penas (ICMBio, 2008).

O estudo morfológico, fisiológico e os testes oftálmicos clínicos em águias harpias, além das avaliações clínicas e físicas mais tradicionais, são mais uma ferramenta para auxiliar nos processos de reabilitação de animais oriundos da natureza e que podem voltar ao seu habitat original ou integrar programas de reintrodução, ou ainda incrementar populações nas áreas de ocorrência da espécie. Estes estudos podem reduzir o risco de enviar para o ambiente natural um animal que não está apto fisicamente para desempenhar sua função ecológica ou mesmo manter-se vivo. Recomenda-se que além da análise clínica prévia e do acompanhamento físico e sanitário, os animais liberados também tenham um acompanhamento comportamental pós-soltura, com o auxílio de técnicas de monitoramento, como a radio-telemetria, ou por visualização dos indivíduos.

Bayon *et al*. (2007) relata que a oftalmologia de aves tem se tornado uma parte importante da medicina aviária. Os principais grupos de aves que os oftalmologistas veterinários examinam em suas consultas incluem aves de gaiola, esporte, aves de zoológico e aves silvestres. O conhecimento sobre as peculiaridades anatômicas e fisiológicas dos olhos dessas espécies ajuda na interpretação da investigação ocular e em diagnósticos apropriados. Algumas das diferenças mais importantes que podem ser descritas nos olhos de aves em comparação com os olhos dos mamíferos incluem o pequeno tamanho ocular de algumas espécies, diferenças morfologicas do globo ocular, órbita aberta, contração voluntária da íris, ossículos na esclera, retina avascular e presença do saliente pécten na câmara vítrea (estrutura vascular que nutre a retina). As semiotécnicas e parâmetros oftálmicos para aves podem incluir o exame ocular físico e técnicas complementares, como a tonometria, oftalmoscopia, electrorretinografia e ultrassonografia, entre outros, que além de indicar os parâmetros normais também auxiliam na identificação de lesões oculares e avaliam sua gravidade.

Bayon *et al*. (2007) preconiza como principais exames a serem realizados em aves a inspeção ocular, a análise do segmento anterior e das estruturas perioculares, o exame do reflexo ocular, o teste lacrimal de Schirmer, a coloração tópica com fluoresceína, a coloração com Rosa Bengala, a citologia e cultura, a tonometria, a oftalmoscopia direta e indireta e a ultrassonografia com Doppler a cores que permite a avaliação da vascularização das estruturas oculares, além da radiologia para a avaliação da órbita e crânio.

Rodarte-Almeida *et al*. (2013) descrevem as características morfológicas do bulbo ocular e determinam valores de referência para testes oftálmicos selecionados em corujas-orelhudas (*Asio clamator*), referentes a observações morfológicas do crânio, bulbo ocular e anexos, além de mensuração de testes oftálmicos, incluindo, Teste Lacrimal de Schirmer (TLS), cultura da microbiota normal da conjuntiva, estesiometria, pressão intraocular (PIO), espessura de córnea central (ECC), tamanho da rima palpebral, diâmetro horizontal da córnea e oftalmoscopia indireta. O estudo contribuiu para a caracterização da morfologia ocular e para o estabelecimento de valores de referências de testes diagnósticos oftálmicos em corujas-orelhudas, sendo que os autores indicaram a necessidade de desenvolver estudos complementares sobre histologia ocular nesta espécie.

Montiani-Ferreira (2007) relata que as fontes bibliográficas de valores normais dos parâmetros fisiológicos em oftalmologia de animais exóticos ou silvestres é um ponto limitante para a avaliação oftálmica desses animais.

Para a águia harpia e corroborando com a afirmativa acima, observamos que somente foi relatada a pressão intraocular, usando tonômetro de aplanação de dois exemplares de harpia (15,50 ± 3,40); confirmando que os dados bibliográficos sobre valores normais nestes animais são escassos (MONTIANI-FERREIRA, 2001).

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CAPÍTULO I

THE EYE OF THE HARPY EAGLE (*Harpia harpyja*): MORPHOLOGIC OBSERVATIONS AND REFERENCE VALUES FOR SELECTED OPHTHALMIC TESTS

ABSTRACT

Purpose To carry out a descriptive investigation into the most relevant morphological features of the harpy eagle eye, bony orbit and to perform selected ophthalmic diagnostic tests. This broad investigative approach will enable to better comprehend some important aspects of the visual system of this iconic raptor and to help professionals in both avian medicine and veterinary ophthalmology areas fields. *Methods* Selected morphological ocular observations were made in live animals under physical restraint and skull specimens. The following diagnostic tests were performed: Schirmer tear test (STT), culture of normal conjunctival flora, rebound tonometry, applanation tonometry, central corneal thickness (CCT), B-mode ultrasound ocular biometry, palpebral length and biometric measurements of the orbit and the skull. *Results and Discussion* Normal parameters found for the ocular diagnostic tests were: STT: 17.478 ± 5.476 mm/min; rebound tonometry: 22.250 ± 5.459; applanation tonometry: 22.250 ± 2.652; CCT: 0.563 ± 0.041 mm; anterior chamber depth: 4.866 ± 0.873 mm; axial length of the lens: 7.12 ± 2.296 mm; vitreous chamber depth = 15.267 ± 4.41 mm; globe axial length: 29.239 ± 1.605 mm; palpebral length: 26.417 ± 2.127 mm; nasal canthus distance: 32.176 ± 2.755 mm; lateral canthus distance: 62.367 ± 7.545 mm. The most frequent conjunctival bacterial isolates were *E. coli* (32.76%) followed by *Enterobacter sp.* (15.52%) and *Streptococcus sp*. (10.34%). Reference data and morphologic observations obtained in this investigation might help veterinary ophthalmologists to diagnose ocular diseases in harpy eagles.

Key-words: harpy-eagle, pecten, raptor, ophthalmology, birds of prey

1 INTRODUCTION

The harpy eagle *(Harpia harpyja)* is an endangered avian species belonging to the order Falconiformes, family Accipitridae. It is a top predator bird, considered the largest and powerful raptor.[1-3] Harpy eagle inhabits mainly highly density forests areas, mostly in border of rivers and waterfalls.[4] The harpy eagle occurs from southern Mexico to northeastern Argentina being locally or regionally extinct in large parts of its former range.[5] It is an carnivorous bird that mainly feeds on arboreal mammals such as sloths, monkeys, porcupines [6,15] and less frequently, reptiles and birds.[10, 13,16-18] Its body length ranges from 57 to 90 cm with wingspan reaching 2 meters. Females are heavier, weighing as much as 9.0 kg. As one of the main external characteristics they possess a bipartite dark crest on the top of the head.[4]

It is notorious that avian vision system arises great interest of researches around the globe. It is also well known that eye anatomic and physiologic features vary considerably between avian species. Therefore, we assume that ophthalmology had become an important part of avian medicine. The knowledge of anatomical and physiological ocular features from different raptor species that might need veterinary care someday, will certainly help veterinarians, at the diagnosis moment and prior to establish a therapeutic protocol. The present study aimed to characterize the harpy eagle eye, by recording morphologic features and establishing reference values for selected ophthalmic diagnostic tests: Schirmer tear test, microbiology, rebound tonometry, applanation tonometry, palpebral pachymetry, ocular ultrasonography and ocular biometry, thus generating reference data for future ocular investigations and veterinary care.

2 MATERIAL AND METHODS

All procedures using live harpy eagles were conducted in accordance with UFPR´s Animal Use Committee and with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. A total of 24 adult birds, age ranging from 2 to 88 months of age including 14 males and 10 females, weighing between 3.11 to 8.0 kg were selected and used in this investigation. The animals were part of private collections of the following institutions: Refugio Biológico Bela Vista, located at Foz do Iguaçu, Paraná, Brazil, and Zoológico de Curitiba located at Curitiba, Paraná, Brazil.

Physical examinations and blood collection samples, were performed prior to the ophthalmic examinations to exclude animals with indications of systemic disease. Procedures and tests were split between the investigators. However, to avoid discrepancies related to inter-observer repeatability, the same person always performed the same ocular test on each occasion.

2.1 ANATOMY OF THE BONY ORBIT

The synchranium analyzed in this study was from an adult bird, for that reason, the description of many bones was based on descriptions previously reported on literature about avian osteology. The specimen utilized in this analysis manually prepared such as the lack of the sclera skeleton (ring of scleral ossicles and cartilaginous blade of the sclera) and fractures of bony prominences such as the orbital process of the quadrate bones and the caudal extremity of the palatine bones. However, since these imperfections do not compromise the entire structure of the orbit wall, it was possible to analyze and describe the main constitutional and conformational aspects of the orbit for this study.

2.2 OPHTHALMIC TESTS

Selected ophthalmic tests were performed while the birds were carefully manually restrained and maintained in the vertical (upright) position during the examination. This technique avoided the generation of pressure on the neck, prevented feather damage and minimized stress for the animal being examined. Not all ophthalmic tests were performed in all animals, which varied depending on the equipment transported to each location. The number of individuals examined for each test is indicated below. Tests included: I. Schirmer tear test type 1 (STT1), II. collection of material for bacterial culture analysis from the ocular surface, III. anterior segment evaluation, IV. central corneal thickness measurement with an ultrasonic pachymeter, V. rebound tonometry, VI. applanation tonometry, VII. B-mode ultra-sonographic biometry, VIII. manual measurements of the palpebral length and distance between both nasal and lateral canthus.

2.3 OCULAR INSPECTION

The anterior segment of 48 eyes from 24 birds was evaluated using a Finoff transiluminator (WELCH ALLYN Inc., Skaneateles Falls, NY, USA) and a slit lamp biomicroscope *(*Hawk Eye, Dioptrix, L'Union, France).

2.4 HISTOLOGICAL STUDY OF THE LOWER EYELID

In order to microscopically analyze the samples of the lower eyelid, they were directly fixed in 10% buffered formaldehyde, embedded in paraffin wax and cut with a sliding microtome into 3–4 µm sections. All of the samples were stained with hematoxylin and eosin (H&E), to visualize the histological structure. Then, the slides were examined using the Zeiss Axio Scope A1 light microscope (Carl Zeiss, Jena, Germany).

2.5 SCHIRMER TEAR TEST TYPE 1 (STT1)

Sterile standardized STT strips (Schering Plough Animal Health, Union, NJ, USA) were used to perform the Schirmer type I test (Figure 1A), which measures the basal portion of the reflex tear secretion in 48 eyes from 24 harpy eagles. These measures were obtained before the instillation of anesthetic medication to avoid influencing the results.

2.6 MICROBIOLOGICAL ANALYSIS

For microbiological analysis, samples from 38 eyes of 19 harpy eagles were obtained by carefully touching the ocular surface with a sterile cotton swab (Fig. 1B). Before sample collection, all the animals were kept in the same environment. The temperature was kept at 23 °C, and humidity level at 47%. No topical anesthetics were used prior to sample collection as this may interfere with growth of microorganisms. Aerobic bacterial culture was performed in BHI broth (brain heart infusion), and on 5% sheep blood agar and MacConkey plates, which were incubated at 37 °C in an aerobic environment for 48 h.

2.7 INTRAOCULAR PRESSURE

As for the IOP measurements, special attention was given to avoid applying any pressure in the neck region during physical restraint in order to prevent iatrogenic IOP alterations. First, IOP was measured bilaterally (left eye was always measured first) on each harpy eagle, using rebound tonometer (Fig. 1C) (Tonovet®, Veterinary Division of S&V Technologies AG, Henningsdorf, Germany) without applying topical anesthetic, followed by use of the applanation tonometer (Fig. 1D) (TonoPen Avia ®, Mentor, Norwell, MA, USA). For use of the applanation tonometer, the topical anesthetic agent (proparacaine hydrochloride 0.5% ophthalmic solution USP; Alcon Laboratories, Forth Worth, TX, USA) was applied on both eyes 5 min after rebound tonometry. IOP measurement was performed 3 min later. The recorded IOP was an average of three successive measurements on the central cornea. Measurements with variance greater than 5% were disregarded. All measurements were conducted by the same investigator (FM).

2.8 CENTRAL CORNEAL THICKNESS

Central corneal thickness (CCT) measurements (Fig.1E) were taken after the instillation of sterile topical anesthetic (proparacaine hydrochloride 0.5% ophthalmic solution USP; Alcon Laboratories, Fort Worth, TX, USA). CCT was measured in 32 eyes from 16 birds using an ultrasonic pachymeter (Model 200P+; Micropach_ Sonomed, Lake Success, NY, USA), with the speed of sound in the cornea preset at 1640 m/s. Three consecutive measurements were made.

2.9 PALPEBRAL FISSURE LENGTH AND CANTHUS DISTANCE

Palpebral fissure length and corneal diameter were measured in 48 eyes from 24 birds using a stainless-steel caliper with an LCD display and an accuracy of ± 0.02 mm (Neiko Tools, Klamath Falls, OR, USA). To measure palpebral fissure length one end of the caliper was positioned over the nasal canthus and the other end over the temporal canthus of the same eye (Fig. 1F). For the measurement of nasal canthus distance one end of the caliper was positioned over the nasal canthus of the right eye (OD) and the other end over the nasal canthus of the left eye (Fig. 1G). For the measurement of lateral canthus distance one end of the caliper was positioned over the lateral canthus of the right eye (OD) and the other end over the lateral canthus of the left eye (Fig. 1H).

Figure 1. Photographs of selected ocular tests being performed in harpy eagle (*Harpia harpyja*). A. Photograph of a STT1 being performed in the right eye of a representative harpy. B. Samples for microbiologic analysis obtained by swabbing the ocular surface. C. Rebound tonometry being performed. D. Applanation tonometry being performed. E. Detail of the CCT being performed. F. Palpebral fissure length being measured. G. and H. Interorbital measurements being made, between both left and right nasal canthus and lateral cantus, respectively.

2.10 B-MODE ULTRA-SONOGRAPHIC BIOMETRY

B-mode scan ultrasonography was performed on 46 eyes from 23 birds using a (MyLab 30 - Esaote, Genova, Italy) equipped with a 14-MHz linear ultrasound transducer. The B-scan 14-MHz probe was gently placed on the corneal surface perpendicular to the center of the cornea using ultrasonic transmission gel (Aquasonic-100; Parker Laboratories Inc., Fairfield, NJ, USA). Care was taken during probe placement to avoid corneal indentation. Reflected ultrasonic waves were captured. Optimal positioning was confirmed when the posterior wall of the globe could be clearly visualized on the B-scan ultrasound image, the image appeared symmetrical and the reflections from the four principal landmarks (cornea, anterior lens surface, posterior lens surface and retinal surface) along the optic axis were perpendicular. The optimal image was frozen on the screen and then all echobiometric measurements were taken.

2.11 STATISTICAL ANALYSES

When applicable, statistical analyses of the measurements including a complete descriptive statistical analysis and *t*-tests (when comparing two groups of data), Fisher's exact test (for comparison of proportions) and Bartlett's correlation test (to compare continuous numeric variables) were performed using the computer software JMP, version 5.0.1 (SAS Institute Inc., Cary, NC, USA). Data were deemed statistically significant when $P < 0.05$ and are expressed as mean ± SD.

3 RESULTS

3.1 GENERAL OBSERVATIONS ON LIVE ANIMALS

All 24 animals were healthy with no systemic or ocular abnormalities. The mean weight of the animals was 5.489 ± 1.152 kg. Females had a mean weight of 6.693 ± 0.129 kg and the mean weight for males was 4.850 ± 0.577 kg. There was significant difference in the mean weight between males and females *(P* = 0.0001).

3.2 BONY ORBIT AND EYE ANATOMY

Harpy eagle bone orbit is delimited primarily by skull bones and has its floor extensively complemented by mandibular muscles. The rostral wall is constituted by the orbital process of the lacrimal bone and the ectetmoid process; the caudal wall mainly composed by the lateroesfenoid bone and the post-orbital process; the medial wall is constituted by the interorbital septum, formed mainly by the mesetmoid bone and, finally, the dorsal wall by a robust supraorbital process of the lacrimal bone. We observed a well-developed super ciliary bone (Fig. 2A, B and Fig. 4) articulated to the supraorbital process of the lacrimal bonend. As the supraorbital process, the superciliary bone protrudes laterally and caudally at a more ventrally than the supraorbital margin. The lachrymal bone eagle articulates to the frontal and nasal bones (Fig. 1A and B), constituting the frontonasolacrimal joint, and represents the rostrolateral limit of the orbit. The rostral orbit wall is completed by the ectetmoid process, which protrudes from the interorbital septum, separating the orbit from the nasal cavity. The ectetmoid process is concave in its orbital face and accompanies the contour of the the eye. A small triangular bone articulated to the ventral extremity of the ectetmoid process was observed bilaterally. On the right side, this bone (Fig. 3B) was about 2 times larger than the left one (Fig. 3A). However, the right ectemoid process was smaller than the left one, making the total proportions of the bone on each side virtually equivalent (Fig. 3). The distance from the bone end to the ventral base of the ectetmoid process was 9.55 mm on the right side and 10.66 mm on the left side. The triangular bone articulated to the ectetmoid process protrudes towards the orbital process of the lacrimal bone, which articulates to the jugal arch (Fig. 3).

The large lateral orbitonasal foramen, represented by an extensive gap located between the orbital part of the frontal bone and the dorsal margin of the ectetmoid process, medially, and the lacrimal bone, laterally, renders the rostral wall of the orbit incomplete (Fig. 4).

The medial wall of the orbit consists majoritarian of the fonticulus allows communication between both orbits (Fig. 4). The olfactory groove for the passage of the olfactory nerve (I) follows the dorsal aspect and extends from the medial orbitonasal foramen to the olfactory foramen, caudally located between the dorsal and caudal walls of the orbit (Fig. 4). An orbitocranial fonticulus is present in the dorsomedial aspect of the caudal wall of the orbit, near the interorbital septum (Fig. 3). When in the fresh state, the font is obliterated by fibrous membranes.

The post-orbital process (Fig. 2 and Fig. 4) is large, robust and has straight angulations, which gives it a square appearance. It protrudes ventral and somewhat rostrally from the caudal end of the supraorbital margin, widens this lateral margin and ventrally, conferring ample bony protection to the orbital cavity. The laterosphenoid bone constitutes the major part of the ventral area of the caudal wall of the orbit and extends from the interorbital septum to the temporal fossa, post-orbital process and tympanic cavity. The optic foramen, which provides passage to the optic nerve (II), is the largest foramen of the orbit and is located medially in the caudal wall of the orbit, near the interorbital septum (Fig. 2B and Fig. 4). In the lateral and ventral view of the optic foramen are four distinct small foramina (Fig. 4).

The square and pterygoid bones (Fig. 4) constitute the caudal bony part of the floor of the orbit. The square bone is articulated to the skull through the optical process and orbital process, which protrudes rostrodromedially towards the interorbital septum. The pterygoid bone allows the articulation between the square and palatine bones. Rostral the bony floor of the orbit consists of the palatine bone (Fig. 2B, Fig. 3 and Fig. 4). The jugal arch is a thin, rectilinear bone bar that connects the maxilla to the square bone. This arch represents the orbital border of the orbit, articulates with the orbital process of the nasal in its middle third (Fig. 2B) and is formed by the ankylosis of three bone elements: jugal process of the maxillary bone, jugal bone and squarejugal bone.

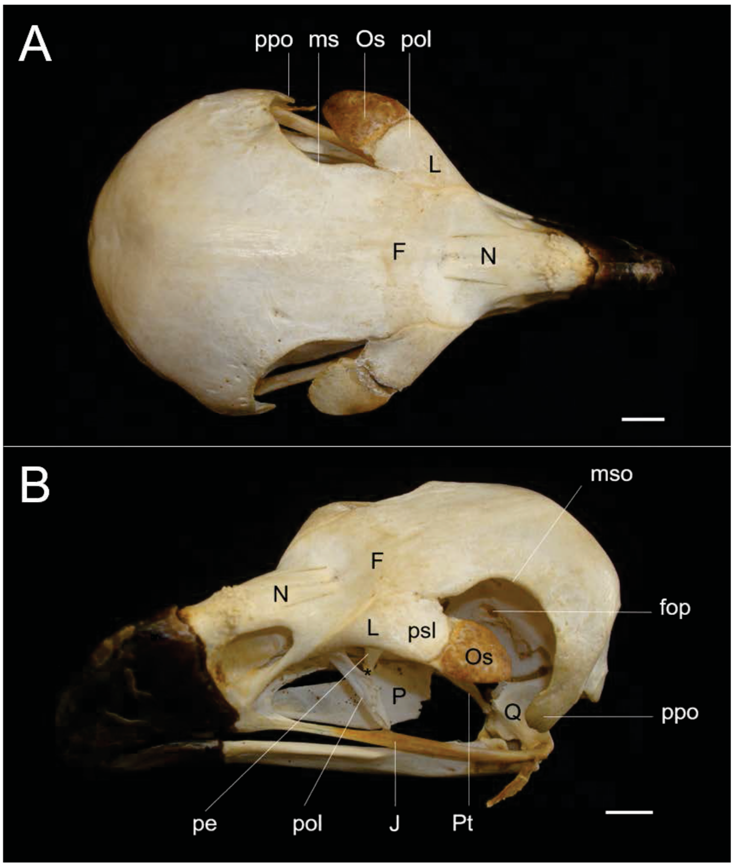

Figure 2. Dorsal (A) and left rostrolateral (B) aspects of an adult harpy (*Harpia harpyja*). Legend: F, frontal bone; fop, optic foramen; J, jugal arch; L, lacrimal bone; mso, supraorbital margin; N, nasal bone; Os, superciliary bone; P, palatine bone; eg, ectetmoid process; pol, orbital process of lacrimal bone; ppo, post-orbital process; Pt, pterygoid bone; psl, supraorbital process of lacrimal bone; Q, square bone. Bars: 1cm.

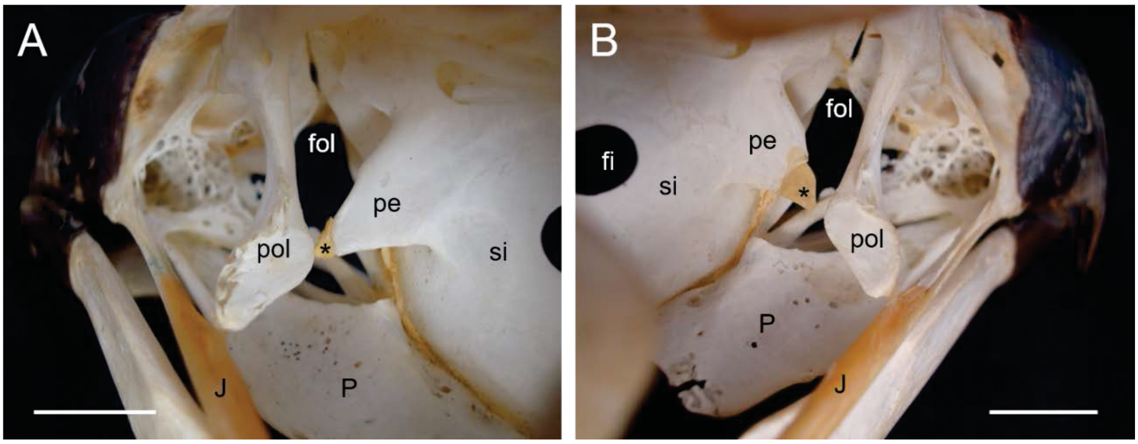

Figure 3. Caudolateral view of the rostral wall of the left (A) and right (B) orbits of an adult harpy (*Harpia harpyja*). Legend: fi, interorbital font; fol, lateral orbitonasal foramen; J, jugal arch; P, palatine bone; eg, ectetmoid process; pol, orbital process of lacrimal bone; Q, square bone; if, interorbital septum. Note the triangular bone (asterisk) articulated to the ectetmoid process and the different proportions of the processes and the triangular bones on each side. Bars: 1cm.

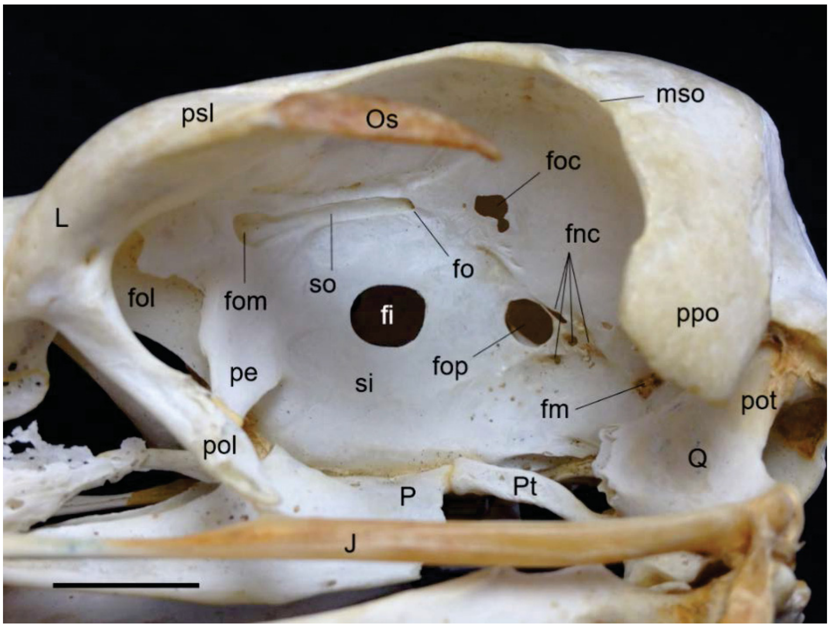

Figure 4. Lateral aspect of the left orbit of an adult harpy eagle (*Harpia harpyja)* synchranium and constituent elements. Legend: fi, interorbital font; fm, maxillomandubular foramen; fnc, set of foramina for cranial nerves; fo, oligo-foramen; foc, orbitocranial font; fol, lateral orbitonasal foramen; fom, medial orbitonasal foramen; fop, optic foramen; J, jugal arch; L, lacrimal bone; mso, supraorbital margin; superciliary bone; P, palatine bone; eg, ectetmoid process; pol, orbital process of lacrimal bone; pot, optical square process; ppo, post-orbital process; psl, supraorbital lacrimal process; Pt, pterygoid bone; Q, square bone; if, interorbital septum; only, olfactory groove. Note the triangular bone (asterisk) articulated to the ectetmoid process and the different proportions of the processes and the triangular bones on each side. Bars: 1cm.

3.3 EXTERNAL OCULAR MORPHOLOGICAL OBSERVATIONS ON LIVE ANIMALS

Externally it was easy to note the rostral located eyes in harpy eagles. The eyelids margin is darkly pigmented. Filoplumes present mainly at the lower eyelid margin. The lower lid is longer than the upper lid, and more mobile. It contains a markedly and circular cartilaginous plate (Fig. 6). Third eyelid is located dorsal medially on the conjunctival fornix between the upper lid and the eye ball. The third lid us thick and have a whitish color covering the entire corneal surface. It is extremely mobile and his movement follows a ventral lateral orientation. Iris anterior surface in harpy eagles can vary individually, ranging from a yellowish predominant surface to light brown and finally to darker brow color, independent of age and sex (Fig. 5).

Figure 5. Different degrees of pigmentation of the anterior surface of the iris of three adult harpy eagles (*Harpia harpyja*).

3.4 HISTOLOGICAL STUDY OF THE LOWER EYELID

The conjunctival surface of the lower eyelid has a distinguishing firm structure that on histologic section revealed to be a cartilaginous plaque, located inside the underlying connective tissue, denominator tarsal plate, a one protective structure peculiar in the eye to the harpy eagle.

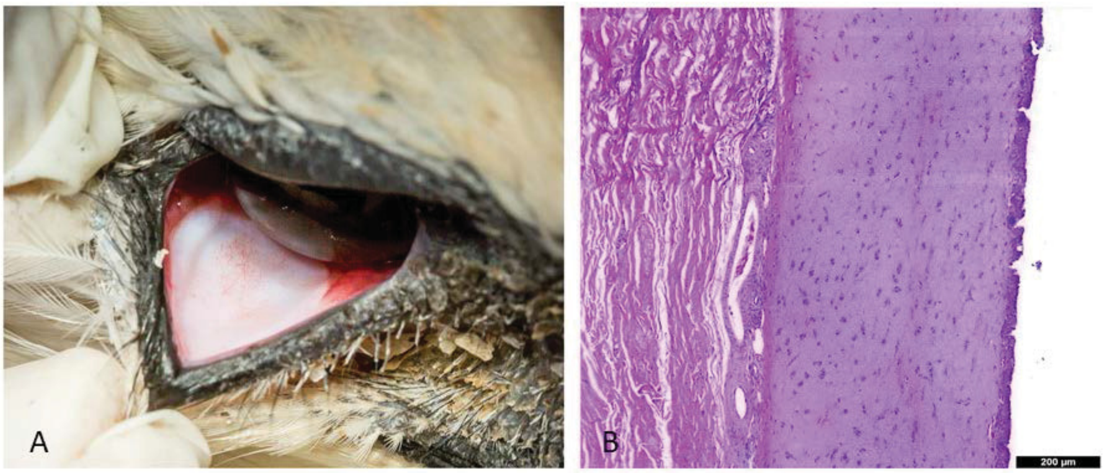

Figure 6. A. Everted lower eyelid showing the conjunctival surface displaying a dense structure, the Tarsal Plate. B. Histologic examination demonstrating the cartilaginous plaque, located inside the underlying connective tissue.

3.5 SCHIRMER TEAR TEST (STT1)

The mean value obtained in 48 eyes of 24 animals was 17,478 ± 5.476 mm/min. There were no significant differences between males and females (*P* = 0.8304) and no significant differences between right and left eyes (*P* = 0.6149).

3.6 ANALYSIS OF THE CONJUNCTIVE MICROBIOTA

Bacterial organisms were identified in microbiological samples from 35 of the 38 samples (92.10 %). Table 1 contains the condensed results of the microbiological analysis. A single bacterial colony was isolated in 12 eyes (34.28%). In some eyes, more than one type of bacterial colony was isolated: 20 (57.14%) showed growth of two different bacterial colonies and two (5.71%) showed growth of three bacterial colony types isolated.

We observed 58 isolates. 17 (29.31%) were gram-positive and 41 (70.69 %) were gram-negative. The most frequent isolate was *Escherichia coli* (32.76%) followed by *Enterocbacter sp.* (15.52%) and *Streptococcus sp.* (10.34%). In small numbers we found: Coagulase negative *Staphylococcus* (6.89%), *Staphylococcus sp.* (5.17%), *Enterococcus sp. (*3.45%*), Citrobacter sp. (*3.45%*), Proteus sp. (*3.45%*), Serratia liquefaciens (*3.45%*), Enterobacter aerogenes (*1.72%*), Enterobacter aglomerans (*1.72%*), Enterobacter cloacae (*1.72%*), Klebsiela sp. (*1.72%*), Morganella morganii (*1.72%*), Shigella sp. (*1.72%*), Staphylococcus epidermidis (*1.72%*)* and *Staphylococcus saprophyticus (*1.72%*).*

Table 1. Results obtained from the microbiological bacterial analyses in 42 eyes of 21 harpy eagle (*Harpia harpyja)*.

Bacterial type	**N (%)**
Gram positive	
Streptococcus sp.	6 (10.34%)
Coagulase – negative *Staphylococcus*	4 (6.89%)
Staphylococcus sp.	3 (5.17%)
Enterococcus sp.	2 (3.45%)
Staphylococcus epidermidis	1 (1.72%)
Staphylococcus saprophyticus	1 (1.72%)
Gram negative	
Escherichia coli	19 (32.76%)
Enterobacter sp.	9 (15.52%)
Citrobacter sp.	2 (3.45%)
Proteus sp.	2 (3.45%)
Serratia liquefaciens	2 (3.45%)
Serratia sp.	1 (1.72%)
Enterobacter aerogenes	1 (1.72%)
Enterobacter aglomerans	1 (1.72%)
Enterobacter cloacae	1 (1.72%)
Klebsiela sp.	1 (1.72%)
Morganella morganii	1 (1.72%)
Shigella sp.	1 (1.72%)
Samples without growth	3 (-)
Total samples with growth	**58**

3.7 INTRAOCULAR PRESSURE (IOP)

The mean IOP of 42 eyes from 21 animals taken using applanation tonometry (TonoPen Avia ®, Mentor, Norwell, MA, USA) was 14,119 ± 2.652 mm/Hg. The mean IOP using rebound tonometry (Tonovet® Veterinary Division of S&V Technologies AG, Henningsdorf, Germany) of 16 eyes from 8 animals was 22.250 ± 5.459. There were no significant differences between males and females *(P* = 0.8951) and no significant differences between right and left eyes (*P* = 0.7853*)* in both methods*.* However, significant difference between the mean values of each Tonometer was found *P* = 0.0001).

3.8 CENTRAL CORNEAL THICKNESS

The mean central corneal thickness was 0,563 ± 0,041 mm. CCT was significant (*P* = 0.0005*)*. Higher in females (0,603 ± 0.024 mm) when comparing to males (0.553 ± 0.031 mm). No significant differences between right and left eyes (*P* = 0.8951*).*

3.9 PALPEBRAL FISSURE LENGTH

The mean palpebral fissure length recorded for 44 eyes from 22 birds were 26.417± 2.127 mm. There were no significant differences between males and females (P = 0.6615) and no significant differences between right and left eyes (*P* = 05644).

3.10 NASAL CANTHUS DISTANCE

The mean value for the distance between both nasal canthus was 32.176 ± 2.755 mm. There was significant difference in nasal canthus distance between males and females *(P = 0.0001)* with females having higher values (34,514 ± 2.003) then males (31,146 ± 2.342). No significant differences between right and left eyes was found (*P* = 0.8581).

3.11 LATERAL CANTHUS DISTANCE

The mean value for the distance between both nasal canthus was 62.367 ± 7.545 mm. There was no significant difference in lateral canthus distance between males and females (*P* = 0.392) and no significant differences between right and left eyes *(P* = 0.4353*).*

Table 2 contains the results of the descriptive statistics of the ophthalmic tests in harpy eagle (*Harpia harpyja).*

Table 2. Results of the descriptive statistics of the ophthalmic tests in Harpy eagle (*Harpia harpyja).*

Ophthalmic test/unit	Mean	SD	Minimum and maximum values	CV
Schirmer tear test I (mm/min)	17.478	5.476	3 – 28	0.313
Central corneal thickness (mm)	0.563	0.041	0.476 – 0.65	0.072
Rebound tonometry (mm/Hg)	22.250	5.459	13 – 33	0.092
Applanation tonometry (mm/Hg)	14.119	2.652	10 – 20	0.188
Palpebral fissure length (mm)	26.417	2.127	28.8 – 30.1	0.081
Distance between nasal canthus (mm)	32.176	2.755	28.3 – 37.2	0.086
Distance between lateral canthus (mm)	62.367	7.545	21.9 – 69.3	0.121

3.12 B-MODE ULTRA-SONOGRAPHIC BIOMETRY

The mean axial globe length was 29.239 ± 1.605 mm. Mean anterior chamber depth (axial chamber length) was 4.866 ± 0.873 mm. Mean lens thickness (axial lens length) was 7.12± 2.296. Mean vitreous chamber depth (axial chamber length) was 15.267 ± 4.41. Mean pecten length was 8.174 ± 2.206 mm. Mean globe height - Posterior segment (dorsonal) was 33.195 ± 1.138 mm. Globe width - Posterior segment (temporonasal) was 35.285 ± 0.375 mm. There were significant difference (*P* = 0.0042) between males (7.081 ± 2.893) and females (9.012 ± 1.154) in the pecten length.

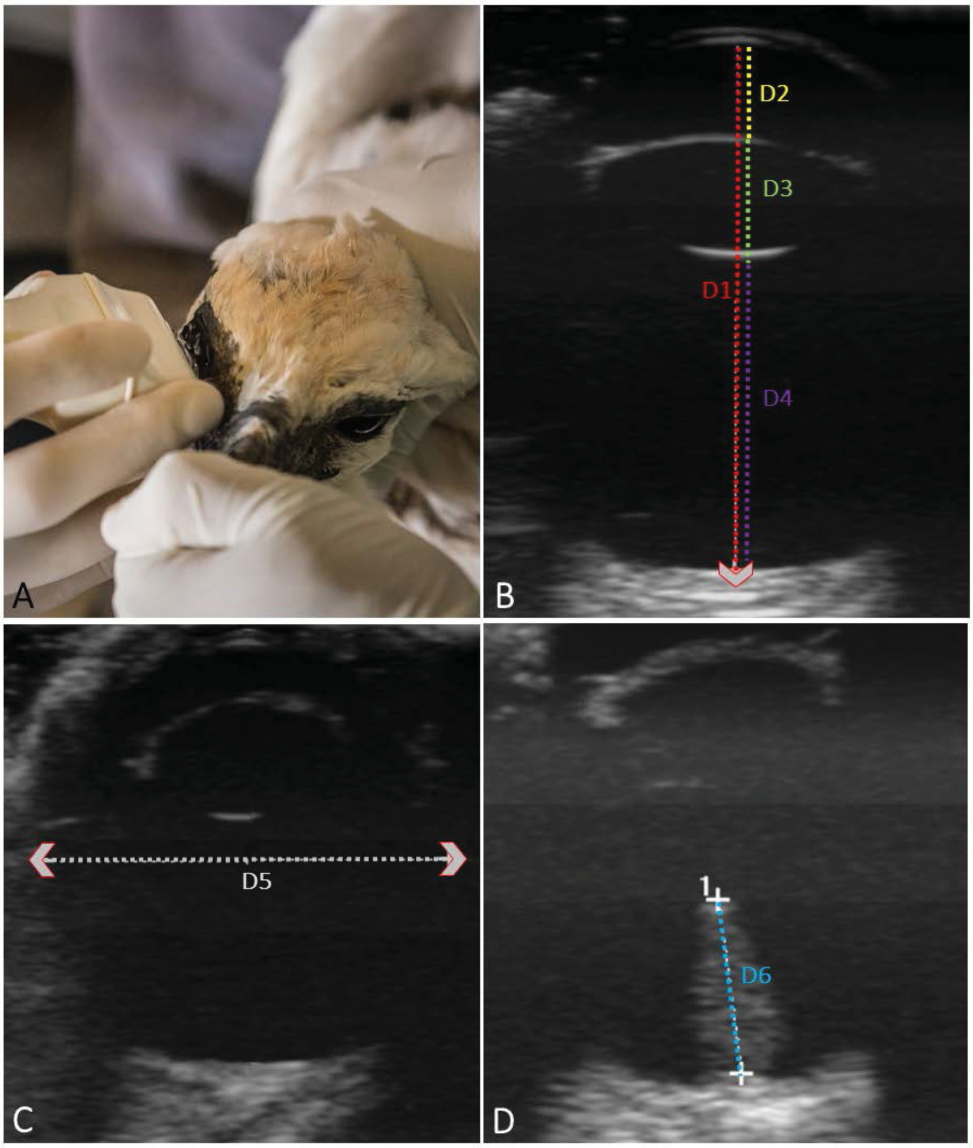

Figure 7. (a) Ultrasound gel was applied and the transducer was gently positioned with minimal pressure on the eye surface (b) Representative B-scan ultrasonogram after optimal positioning. The four principal landmarks (cornea, anterior lens surface, posterior lens surface and retinal surface) along the globe axis are all perpendicular. Four measurements were performed on the B-scan image: D1: Axial globe length (anterior cornea to the chorioretinal surface) D2: Anterior chamber depth (posterior cornea to anterior lens capsule); D3: Lens thickness (anterior lens capsule to the posterior lens capsule); D4: Vitreous chamber depth (posterior lens capsule to the chorioretinal surface). (c) Transversal globe length (medial globe wall to the lateral globe wall). (d) D6: Pecten length.

4 DISCUSSION

Regardless of avian represents an extremely diverse class, and notoriously (with few exceptions) being animals that depends incredibly of the eyes and visual system to daily basis activities, and the fact that harpy eagles have been extensively studied since from the late 1960's[6] in various aspects, such as nesting behavior[3,6,7] foraging,[10] interactions,[19] breeding[13,16,20] little is still known about his eyes. Important information, like the functioning ways of visual system and also reference values for ophthalmic tests, remains unknown. Therefore, the purpose of this study was to contribute to partially fill this gap of information, by developing knowledge of important aspects of the visual system in one iconic top predator raptor. The data complained here, might be useful for both veterinarian practitioners and people involved in projects to reestablish a solid population of free living harpy eagles in the wild.

In the present study, females are markedly heavier than males *(P* = 0.0001). This was previously seen in some other birds[21] particularly in raptors.[22]

In adult birds a huge fusion between certain bones of the skeleton often makes difficult or even impossible to distinguish adjacent bones, however many of them can be easily identified in young individuals. Classical descriptions[23] report that the orbit of the birds is delimited by 4 bone walls: rostral, caudal, medial and dorsal orbital wall. In addition, each of the orbital walls consists of bony elements that may exhibit variations among different species. The well-developed superciliary bone (Fig. 1A, B and Fig. 3) articulated to the supraorbital process of the lacrimal bone, seen here was previously reported in harpy eagles[24] and in other acciptriformes.[25] Just as the supraorbital process, the superciliary bone protrudes laterally and caudally at a more ventrally than the supraorbital margin, which confers the characteristic "threatening" outline of the orbit in birds of prey, as observed in birds of this order.[24] The concave aspect of the ectetmoid process in its orbital face, accompanying the contour of the eye may play an important role in the rostral support of the eye, as it was reported to occurs in other species of predators.[26] Considering a vast review of the lacrimal-ectetmoid complex in different orders of birds, no mention of the triangular bone was reported but it is mentioned that the ectetmoid process could be very or poorly developed and may or may not reach the orbital process lacrimal bone, depending on the genus and species.[25] Migotto (2013), when analyzing syncranius of harpy eagles did not mention such bone and reported that the contact between the ectetmoid and orbital processes of the lacrimal in Accipitridae occurs through a joint, with no bone bridge between the two parts. Moreover, the same author suggests that the establishment of contact between such bone parts could be contested in some Accipitridae, since the formation of a lacrimal-ectetmoid complex could be considered absent, given the existence of a considerable distance between the orbital process of the and the lateral extremity of the ethmoid process, observed in some specimens of its study. However, the author suggests that the observation of the lack of contact between the bones may be a result of a preparation artifact, which would make it impossible to determine the actual characteristic in dry material. In our study, the observation of such bone, bilaterally, can corroborate the hypothesis of the preparation artifact, since the possibility of loss of bone elements during the preparation of dry pieces, especially smaller bones, is known and reported in the literature.[25,26] The large lateral orbitonasal foramen may provide passage to the lateral branch of the ophthalmic nerve and to the ducts of the nasal gland as it was commonly, in several species.[23] In several species the medial orbitonasal foramen commonly provides passage to the medial branch of the ophthalmic nerve.[1] The small se of foramens in the lateral and ventral aspect of the optic foramen, are suggested to provide passage to the oculomotor (III), trochlear (IV), ophthalmic of V) and abducent (VI) (Fig. 3).[23,26-28] Lateral and ventrally to this set of foramens is the maxillomandibular foramen (Fig. 4), which provides passage to the maxillomandibular branch of the trigeminal nerve (V).[23]

The eye position within the skull varies through the enormous amount of avian species on the globe. Birds that have narrow skulls usually have laterally located eyes while birds with wider skulls generally have anteriorly located eyes.[29] Harpy eagles have a large skull and rostral located eyes, similar to what was previously reported in several birds of prey.[30,31] External ocular adnexa in harpy eagles reminds the aspect well described in avians,[26,32-34] with the presence of pigmented margins and filoplumes that are supposed to act like cilia in mammals, providing protection to the ocular surface. The third eyelid is thick with a heavy whitish color, which cannot let the observation of the eye, like it is possible in several birds, due to some degree of transparence.[33-36]

Palpebra inferioris support a dense connective tissue structure called tarsal plate. Tarsal plate in the harpy eagle has a strong structure and suitable form to protect eye and is similar to estructure in Buzzard's.[37] Histological examination demonstrated that the tarsal plaque is a cartilaginous tissue, located within the underlying connective tissue.

The iris of harpy eagles can range from a yellowish predominant surface to light brown and finally to darker brow color, independent of age and sex. It corroborates with other authors that reports that iris color can vary dramatically between individuals and species depending on the amount of pigments, pigment types and degree of vascularity,[38] which can all vary with age, sex and diet.[39] It is known that melanophores are responsible for brown iris colors, and that other colors may be produced by structures such as lipids, carotenoids, collagen fibers and hemoglobin.[39] Combinations of purines, pteridines and carotenoids are also common. It is unknown what properties, pigments or vasculature, leads to the coloration of the harpy eagle iris.

Lacrimal secretion evaluation, is commonly studied in ophthalmic research with birds.[26,40-42] The Schirmer tear test (STT) is an important component of the evaluation of lacrimal gland functionality (GUM, 1999), being the most common method used to measure lacrimal secretion on veterinary ophthalmology routine.[43] STT can be performed in harpy eagles with only physical restraint, but careful was need to make sure that the strip was correctly inserted on the eyelid margin, because the movement of the third eyelid is extremely strong in this bird, and, consequently can easily remove the strip from the eye. This situation was reported to occur in psittacines,[41,42] in some cases, is necessary to put the strip in the superior conjunctival fornix.[42] Another study with falconiformes, suggest to keep eyelids closure during the STT, to avoid the strip to be dislodged. In harpy eagles, the entire test was performed with birds with eyelids open, without problem. STT results here (17,478 ± 5.476 mm/min), were markedly higher to those previously reported in psittaciformes, falconiformes and other Acciptriformes.[41,42,44]

The ocular surface (corneoconjunctival) microbiota have been commonly included in studies with avian subjects.[26,34,45-48] The knowledge of the ocular surface symbiotic microbes it is essential, since these organisms are important to inhibit the overgrowth of ocular pathogens.[46,49-51] This information also, can help to choose a better antibiotic therapeutic approach when is necessary. Curiously, different to what was previously described in studies about the ocular microbiota in owls,[26,34] birds of prey,[46] psittacines,[52] and penguins,[53] that report a high prevalence of gram-positive isolates, in harpy eagles we found a great predominance of gram negative isolates. This fact can be partially explained by the high prevalence of *E. coli* (34.28 % of total isolates). *E. coli* can play an important role in pathogenic ocular conditions, being the most common isolate from newborns in a retrospective study with neonatal conjunctivitis patients. and also, is incriminated to cause severe blefaroconjunctivitis in an ostrich.[54] As in the case of *E. coli*, other members of the family enterobacteriacae are very present on isolates in harpy eagles and do not appear to be residents of the eye. This isolation in this study may indicate possible fecal contamination, being in this case only a transient bacterium of the conjunctiva. Further investigation of the ocular surface microbiome in harpy eagles from different locations, including free living individuals, as well as the microbiome of other areas of the body such as the oral mucosa and cloacae, and their role in the pathophysiology of diseases is a significant, emerging field of research, and someday might enable the development of an consensus about the real ocular surface commensal microbiome in these birds, and to establish novel therapeutic approaches and prevention of ophthalmic diseases.

Intraocular pressure had been evaluated in several avian species, including pigeon,[55] Anatidae,[56] penguins,[53,57] owls,[26,34,55,58,59] Psittaciformes,[41] and falconiformes.[55,59] There are few reports in the literature, testing accuracy and comparing different IOP evaluation methods in birds. Rebound tonometry was found to overestimate or underestimate IOP in comparison with manometry or to overestimate IOP in comparison with applanation tonometry in avian species.[58,60,61,62] With rebound tonometry incriminated to increasingly overestimate IOP in accipitriformes,[60] being exactly what we found in harpy eagle (P = 0.0001), when comparing to our applanation results, with a mean difference of -8.131 mm/Hg.

The mean IOP (14,119 ± 2.652 mm/Hg) value recorded in our research using applanation tonometry (TonoPen Avia®, Mentor, Norwell, MA, USA) were slightly lower than in Bald Eagles (*Haliaetus leucocephalus)* 21.5 ± 1.7 mm/Hg.[63] This may be due species-specific differences, but the influence of each tonometer cannot rule out, since, despite comparison between different applanation tonometers had been described,[64,65] differences related to these two-specific tonometer's (Tonopen Vet® and Tonopen Avia®) have not been investigated yet. Another study, comparing rebound tonometry and applanation tonometry in owls, also suggests overestimated values when using rebound tonometry, similar to what we are reporting here.[58] Considering the high number of investigations of IOP in birds of prey, showing vast diversity in IOP evaluation methods and consequently an equal diverse world of results, all different methodologies should be considered to influencing values. Here, despite restraint methods and tonometer technologies used being distinct when comparing to another studies, harpy eagle can now be included in the list of important endangered species who have IOP diagnostic parameters established using two different IOP evaluation devices. Despite several corneal studies focused both in normal parameters and pathologic corneal changes nowadays being made using more recent technologies like high-resolution ultrasound biomicroscopy (UBM)[66-68] spectral domain coherence tomography (OCT)[69-72] and other anterior segment analysis devices,[73,74] ultrasonic pachymetry have been proved to be a useful method when considering to establish normal CCT values for domestic[69,70,75,76] and wild[26,77,78] animals. The corneal thickness had been incriminated as one important related factor, which may influence the results of tonometry in humans[62,79,80] dogs[64,81] and birds such as owls[77] but have been rare included in studies about birds of prey.[26,77] The harpy eagle central corneal thickness measured using ultrasonic pachymetry (0,563 ± 0,041 mm) is higher to values obtained in owls (0,29 ± 0,03 mm), chicks (0.242 ± 0.0002 mm),[82] and penguins (0.348 ± 0.003)[78] fact which could be easily explained by the great difference of size between harpy eagles and these another avian species. The significant difference found in CCT values found in females, also can be explained by the markedly difference of size found, where females are markedly bigger than males. CCT have been commonly included in mammal's studies,[83-86] and curiously, the values obtained in our study being similar to those reported in dogs (0.5192 ± 0.0056 mm)[87] and quite close to values reported in human beings (0.46 ± 0.04 mm). Although harpy eagles do not fit as animals models, since it is a very endangered species, those close values with dogs and humans can provide useful information when facing pathologic conditions that can affect this parameter, since these two species are the most studied.

B-mode ultrasonographic evaluation demonstrated that the axial globe length (29.239 ± 1.605 mm) observed in our investigation (4.866 ± 0.873 mm) were higher than that observed in amazon parrots (12.50 ± 0.5 mm),[88] flamingos (13.8 ± 0.16 mm)[88] and owls (23.76 ± 0.92 mm),[90] fact which can be easily explained considering the higher size of the harpy eagles in comparison to those birds. The anterior chamber depth (axial chamber length) observed in our investigation (4.866 ± 0.873 mm) is also higher when compared to the three prementioned studies (1.7 0.17 ± 0.3 mm for amazon parrots,[88] 1.75 ± 0.05 mm for flamingos[89] and owls (4.27 ± 0.47 mm),[90] following the logical explanation of the size disparity between those birds in comparison with harpy eagles. Mean lens thickness (axial lens length) is relatively similar when compared to owls (7.79 ± 0.27 mm)[91] relatively smaller in harpy eagles (7.12 ± 2.296).

Ophthalmology in raptors, had been an important subject of interest of veterinarians, with specific studies being published.[91] However, until nowadays, a lot of information remains undiscovered, even considering iconic predators, such is the harpy eagle. We believe the morphological features and reference data for ocular tests obtained in this investigation will help veterinary ophthalmologists to recognize ocular abnormalities in this important species and thus improve diagnostic of eye disease and consequently help to provide the optimal eye care for these animals.

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CAPÍTULO II

DOPPLER ULTRASONOGRAPHY OF THE *PECTINIS OCULI* ARTERY IN HARPY EAGLES (*Harpia harpyja*)

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Doppler ultrasonography of the *pectinis oculi* artery in harpy eagles (*Harpia harpyja*)

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Abstract
Twenty harpy eagles (*Harpia harpyja*) without systemic or ocular diseases were examined to measure blood velocity parameters of the *pectinis oculi* artery using Doppler ultrasonography. Pectinate artery resistive index (RI) and pulsatility index (PI) were investigated using ocular Doppler ultrasonography. The mean RI and PI values across all eyes were 0.44±0.10 and 0.62±0.20 respectively. Low RI and PI values found in the harpy eagle´s *pectinis oculi* artery compared with the American pekin ducks one and other tissue suggest indeed a high metabolic activity in *pecten oculi* and corroborates the hypothesis of a nutritional function and/or intraocular pressure regulation.
Keywords: Avian posterior segment, Pulsatility index, Raptors, Resistive index.

Introduction

The harpy eagle (*Harpia harpyja*) is the largest and most powerful neotropical raptor. They primarily inhabit the Amazon rainforest, but can be found extending from Mexico to South of Brazil (Banhos *et al*., 2016). They are considered a vulnerable species according to the Brazilian list of endangered fauna (Brazil, 2014) due to deforestation, given their dependency on rainforest habitat, where they builds nests within the canopy and hunts tree-dwelling mammals like sloths (Banhos *et al*., 2016). As are all eagles, they are primarily visual hunters, with eyes that contain many adaptations for increased visual acuity, including large globes (Güntürkün, 2000; Jones *et al*., 2007), accommodation by corneal and lenticular measures (Samuelson, 2007), bifoveate retinae (Tucker, 2000), and an anangiotic (avascular) retina (Ruggeri *et al*., 2010).

As with all avian species, an anangiotic retina leaves the inner retinal layers without a direct blood supply, therefore limiting delivery of nutrients and rapid removal of wastes. However, a vascular structure called the *pecten oculi* is found projecting anteriorly from the optic disc into the vitreous body of all birds (class Aves or clade Avialae) (Brach, 1977; Kern, 2006; Kiama *et al*., 2006; Rahman *et al*., 2010; Micali *et al*., 2012; Mustafa and Ozaydjn, 2013). *Pecten oculi* are pigmented, vascularized, and are traditionally classified into one of three morphologies: 1) conical (e.g. as found in kiwis); 2) vaned (e.g. as found in ostriches, rheas, and tinamous), or 3) pleated (e.g. as found in all the other avian species) (Brach, 1977; Baumel, 1993; Montiani-Ferreira, 2001; Kern, 2006; Kiama *et al*., 2006; Rahman *et al*., 2010; Micali *et al*., 2012; Mustafa and Ozaydjn, 2013).

Other than a structure providing nutritional support for the anangiotic retina (Rodriguez-Peralta, 1975), the *pecten oculi* has been hypothesized to function in intraocular pH and pressure regulation (Brach, 1975), stabilization of the vitreous body (Tucker, 1975), reduction of intraocular glare and maintenance of intraocular pressure (Seaman and Himelfarb, 1963), and in maintenance of the blood ocular barrier for the retina and vitreous body (Barlow and Ostwald, 1972).

Color and pulsed Doppler ultrasonography have been used to further investigate the function of *pecten oculi* by measuring blood velocity parameters (BVPs) such as peak systolic velocity (Vmax), end diastolic velocity (Vmin), pulsatility index (PI), resistive index (RI) and time-averaged maximum frequency (TAmax) (Ferreira *et al*., 2015). These parameters may assist to evaluate vascular integrity in many organs and tissues (Carvalho and Chammas, 2011; Ferreira *et al*., 2015). Moreover, it is known that RI and PI values are directly related to the level of metabolism within a particular tissue or organ. When diastolic velocity increases relative to systolic velocity, RI and PI values decrease (Carvalho and Chammas, 2011). High diastolic velocity suggests

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a high metabolic rate due to a demand for continuous blood flow (Carvalho and Chammas, 2011). Consequently, knowledge of BVPs provides information that is suggestive of the metabolic activity of the *pecten oculi* and may offer insight to its function within the eye (Greenfield *et al*., 1995; Gellat-Nicholson *et al*., 1999; Carvalho and Chammas, 2011; Ferreira *et al*., 2015). To the authors´ knowledge, only one study applying this concept using Doppler imaging ultrasonography of the *pecten oculi* was previously performed on American pekin ducks (*Anas platyrhynchos domestica*) (Ferreira *et al*., 2015).

The objective of this investigation was to evaluate the BVPs in the *pectinis oculi* artery of harpy eagles in order to make conjectures about the metabolic activity of the *pecten oculi* and its function. A second objective was to verify these earlier findings and help establish repeatability and validity of using Doppler imaging ultrasonography as a reliable method to assess metabolic activity of ocular tissues.

Materials and Methods

This study was approved by the Federal University of Paraná´s Animal Welfare Committee and was conducted according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Animals

Twenty harpy eagles, born and raised in captivity, consisting of 13 males and seven females (confirmed by cytogenetics) where evaluated. All of the birds were born and belonged to the ITAIPU BINACIONAL´s Biological Sanctuary, located on the border between Brazil and Paraguay, in the State of Paraná, Brazil. The birds had a mean weight of 5.85± 1.2kg, and a mean age of 41.94 months (ranging from 12 to 88 months). Only individuals with no evidence of ocular abnormalities following slit lamp biomicroscope (Hawk Eye; Dioptrix, L'Union, France) and indirect ophthalmoscope (EyeTech; São Paulo, Brazil) evaluation were included in the study. All ophthalmic examinations were performed by the same veterinary ophthalmologist (TF).

Ocular Doppler Ultrasonography

All animals in this study are handled and manually restrained regularly as part of their husbandry and management program. This practice conditions them to handling, thus facilitating veterinary examinations and treatments, and reduces the overall stress of the birds. For all procedures described herein, all animals were physically restrained by experienced and well-trained personnel wearing adequate protective equipment (leather gloves and aprons). All birds were hooded just prior to having measurements taken, with claws closed with adhesive tape and beak closed manually by the examiner.

Blood velocity parameters in the *pecten oculi* were evaluated using an ultrasound system (Logiq 5 GE; Chicago, United States) coupled with a high-frequency 12 MHz ultrasound transducer in both eyes. After instillation of one drop of a topical analgesic solution (proximetacaine 0.5% - Anestalcon - Alcon do Brasil, São Paulo, Brazil), ultrasonic gel (Carbogel, São Paulo, Brazil) was applied over the corneal surface. The ultrasound transducer was perpendicularly positioned in a horizontal plane, from 4 to 10 o'clock. The long axis of the transducer was placed on top of the corneal surface (transcorneal technique), with the marker pointing nasally and inclined 45°. First the base of the pecten was observed by B-mode ultrasound, near the optic nerve. Doppler settings used were: frequency (5.0 MHz), gain (24.5 dB), pulse repetition frequency (1.4 KHz) and wall filter (89 Hz). The *pectinis oculi* artery was located on the more nasal portion using the power Doppler mode to inspect the blood flow. The formulas to determine the resistive index and pulsatility index for the *pectinis oculi* artery were RI=(Vmax-Vmin)/Vmax and PI=(Vmax-Vmin)/TMAX, respectively (Greenfield *et al*., 1995; Gellat-Nicholson *et al*., 1999; Carvalho and Chammas, 2011; Ferreira *et al*., 2015), where RI= resistance index; Vmax= the maximum, peak systolic velocity of the *pectinis oculi* artery; Vmin= the minimum, end diastolic velocity of the *pectinis oculi* artery; PI= pulsatility index; and; TAMAX= is the time-averaged mean of the maximum velocity. RI values can vary from 0 to 1 (Nelson and Pretorius, 1988; Martinoli *et al*., 1998; Gellat-Nicholson *et al*., 1999; Brooks *et al*., 2007).

Statistical Analysis

Descriptive statistics of blood flow parameters is presented. The normality of the distribution of residuals was tested graphically and confirmed by a *p* value > 0.05 on the Shapiro-Wilk test. *T*-tests were performed to compare values obtained on right and left eyes and on males and females. Differences were considered significant when $P<0.05$. Statistical analysis was performed using StatView (StatView, Mountain View, CA).

Results

Doppler ultrasonography was successfully performed in 39/40 eyes (one eye had a severe corneal lesion at the time of the Doppler investigation and was excluded from the study). During ultrasonography the pecten was easily located in the posterior segment of each eye as a hyperechoic structure emerging directly anteriorly from the optic nerve. The *pectinins oculi* artery was identified directly underneath the most nasal portion of the *pecten oculi* and Doppler blood velocity parameters were determined. Ocular Doppler ultrasonography examination allowed images to be obtained of the *pectinis oculi* artery in spectral Doppler mode (Fig. 1A). The artery was located directly underneath the nasal portion of the *pecten* and was clearly identified for all BVP measurements (Fig. 1B).

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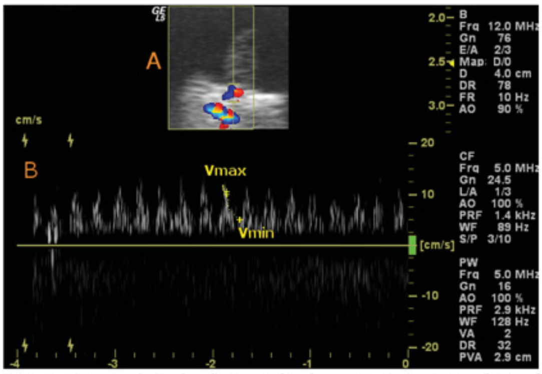

Fig. 1. Spectral (Pulsed) Doppler images. (A): Detection of *pectinis oculi* artery in the most nasal portion of *pecten oculi*; (B): Doppler waves indicated as Vmax (peak systolic velocity) and Vmin (end diastolic velocity).

All blood velocity parameters are described in Table 1. No significant differences between right and left eyes were found, and no significant differences were found between sex and age of the harpy eagles (P>0.05).

Table 1. *Pectinis oculi* artery blood velocity parameters of twenty harpy eagles (*Harpia harpyja*).

Both eyes (n=39)	Mean	Variance	Std. Dev.	Std. Err
Vmax	15.34	30.457	5.519	0.884
Vmin	2.912	17.87	4.227	0.677
RI	0.44	0.011	0.103	0.017
PI	0.618	0.042	0.204	0.033
TAmax	11.174	23.129	4.809	0.77

Vmax - peak systolic velocity; Vmin - end diastolic velocity; RI - pectinate artery resistive index; PI - pulsatilityindex;TAmax- time-averaged maximum frequency.

Discussion

Investigations of BVPs have been made in many organs and tissues in different species such as cat (Carvalho, 2009; Reis *et al*., 2014), chicken (Barua *et al*., 2007), dog (Lamb *et al*., 1999; Lee *et al*. 2014; Souza *et al*., 2014), rabbit (Abdallah *et al*., 2010), crab-eating foxes (Silva *et al*., 2014) and more recently, in ducks (Ferreira *et al*., 2015). Assumptions about a tissue's metabolism can be made from its blood supply's Vmax, Vmin, RI and PI parameters (Carvalho and Chammas, 2011). Higher metabolism is signified by lower RI and PI values, whereas low or slower metabolism is signified by higher RI and PI values (Carvalho and Chammas, 2011).

For the purpose of comparison, the anterior ciliary artery and short posterior ciliary artery in dogs have been shown to have RI values of 0.53 and 0.44 respectively, the first supplies blood to the ciliary body whereas the second to the choroid and retina that have higher metabolism than ciliary body (Gellat-Nicholson *et al*., 1999).

RI values of the long posterior ciliary artery (LPCA) have been demonstrated in the rabbit and the dog as a means to evaluate the importance of the LPCA as a vascular supply to the retina, which is a very highly metabolic tissue (Gellat-Nicholson *et al*., 1999; Abdallah *et al*., 2010; Ferreira *et al*., 2015). The rabbit has been shown to have a very low RI (0.09±0.05) compared to the dog (0.51±0.006), suggesting that the LPCA is of greater importance as a vascular supply to the non-central region of the merangiotic rabbit retina than in the holangiotic retina of dogs (Tokoro, 1972; Bill, 1985; Gellat-Nicholson *et al*., 1999; Samuelson, 2007; Abdallah *et al*., 2010; Yang *et al*., 2011; Ferreira *et al*., 2015).

A previous study of the RI and PI values of the *pectinis oculi* artery in American pekin ducks showed similar RI and PI values of the short posterior ciliary artery that supplies the ciliary body in the dog (Gellat-Nicholson *et al*., 1999; Abdallah *et al*., 2010; Ferreira *et al*., 2015). The RI and PI values of the *pectinis oculi* artery in harpy eagles found in the present study were also similar. Considering these findings, perhaps there are similarities between the function of the *pecten oculi* and ciliary body, as suggested by the previous theory that the *pecten oculi* could be used for maintenance of IOP through fluid production/excretion into the eye (Seaman and Himelfarb, 1963).

However, another investigation contradicts this theory, as they found several structures that would suggest otherwise: choroidal lacunas within the endothelium, absence of well-delimited basal lamina muscular tunica, innervation and acellular material filling their lumens, and the same characteristic of lymphatic vessels (De Stefano and Mugnaini, 1997). Furthermore, the choroidal lacunae become smaller and less numerous near the optic nerve, *pecten oculi,* and iridotrabecular angle, and therefore are not a part of a Schlemm's canal (De Stefano and Mugnaini, 1997).

Several other observations made on the *pecten oculi* also give support to the hypothesis of a secretory function, including 1) extrusion of dye from the pecten accompanying each heart beat during fluorescein angiography (Bellhorn and Bellhorn, 1975), 2) the pecten´s high content of carbonic anhydrase and alkaline phosphatase (Bawa and YashRoy, 1972; Amemiya, 1982), 3) evidence that the pecten acts as an agitator to propel perfusate towards the central retina (Pettigrew *et al*., 1990), and 4) evidence of the pecten having a crucial role in maintaining retinal health (appearance of retinal damage after *pectin oculi* ablation (Wingstrand and Munk, 1965).

In this study, we showed that Doppler imaging is capable of successfully enabling characterization of the main hemodynamic features of the *pectinis oculi* artery in harpy eagles.

Based on the evidence provided here, i.e. high BVP results, as similarly reported in American pekin ducks, we provide indirect evidence for a potential secretory function of the *pecten oculi*. Future studies should also specifically evaluate the *pecten oculi* using other methods of investigation and in other species of birds.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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ANEXO A – APROVAÇÃO DO COMITÊ DE ÉTICA

UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS AGRÁRIAS
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o protocolo número 075/2016, referente ao projeto **"OFTALMOLOGIA CLÍNICA E INVESTIGATIVA EM GAVIÃO-REAL (*Harpia harpija* Linnaeus, 1758)"**, sob a responsabilidade de **Fabiano Montiani Ferreira** – que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de Outubro, de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DO SETOR DE CIÊNCIAS AGRÁRIAS DA UNIVERSIDADE FEDERAL DO PARANÁ - BRASIL, com grau 2 de invasividade, em reunião de 14/09/2016.

Vigência do projeto	Outubro/2016 até Março/2017
Espécie/Linhagem	*Harpia harpyja* (ave) / Harpia
Número de animais	21
Peso/Idade	4 a 9 kg / 0 a 18 anos
Sexo	Ambos (13 machos e 8 fêmeas)
Origem	Zoológico Roberto Ribas Lange do Refúgio Biológico Bela Vista ITAIPU Binacional em Foz do Iguaçu – PR

CERTIFICATE

We certify that the protocol number 075/2016, regarding the project **"CLINICAL AND INVESTIGATIVE OPHTHALMOLOGY IN HARPY EAGLES (*Harpia harpija* Linnaeus, 1758)"** under **Fabiano Montiani Ferreira** supervision – which includes the production, maintenance and/or utilization of animals from Chordata phylum, Vertebrata subphylum (except Humans), for scientific or teaching purposes – is in accordance with the precepts of Law nº 11.794, of 8 October, 2008, of Decree nº 6.899, of 15 July, 2009, and with the edited rules from Conselho Nacional de Controle da Experimentação Animal (CONCEA), and it was approved by the ANIMAL USE ETHICS COMMITTEE OF THE AGRICULTURAL SCIENCES CAMPUS OF THE UNIVERSIDADE FEDERAL DO PARANÁ (Federal University of the State of Paraná, Brazil), with degree 2 of invasiveness, in session of 14/09/2016.

Duration of the project	October/2016 until March/2017
Specie/Line	*Harpia harpyja* (bird) / Harpia
Number of animals	21
Wheight/Age	4 to 9 kg / 0 to 18 years
Sex	Both (13 males and 8 females)
Origin	Roberto Ribas Lange Zoo of the Bela Vista ITAIPU Binacional Biological Refuge at Foz do Iguaçu – PR

Curitiba, 14 de setembro de 2016.

Simone Tostes de Oliveira Stedile
Coordenadora CEUA-SCA

Comissão de Ética no Uso de Animais do Setor de Ciências Agrárias - UFPR

VITA

Médico Veterinário formado pela Universidade Federal Fluminense (UFF), em 1985.

Especialista em Primatologia pela Universidade de Brasília (UNB), em 1986.

Professor da disciplina de Medicina Veterinária de Animais Silvestres na Universidade Federal Fluminense (UFF), em 1987/1988.

Responsável técnico pelo Zoológico “Roberto Ribas Lange”, pertencente à ITAIPU Binacional onde trabalha desde 1989.

Aperfeiçoamento em Meio Ambiente e Gestão Ambiental pela Universidade Paranaense – UNIPAR em 1999.

Mestre em Ciências Veterinárias pela Universidade Federal do Paraná (UFPR), em 2011.