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Clinical utility of a complete diagnostic protocol for the ocular evaluation of free-living raptors

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Abstract

Objective: To describe a protocol for the examination of free-living raptors and report the ophthalmic examination findings of seven raptor species native to central Illinois, namely the barred owl, Cooper's hawk, eastern screech owl, great horned owl, American kestrel, red-tailed hawk, and turkey vulture and to determine if the findings relative to visual prognosis affected eligibility for future release.
Animals studied: Seventy-nine free-living raptors.
Procedures: Under manual restraint, complete ophthalmic examination including slit-lamp biomicroscopy and indirect funduscopy, applanation tonometry, rebound tonometry, ocular morphometrics, B-mode ultrasound, and electroretinography (ERG) were performed on each bird. Histopathology of enucleated globes was performed after euthanasia or death in selected cases.
Results: The examination protocol was easily performed using manual restraint alone on all birds. Ocular lesions were detected in 48.1% of birds, with 47.3% affected unilaterally and 52.6% affected bilaterally. Ocular lesions were considered to be vision threatening in 29.0% of the unilaterally affected birds and 29.0% of the bilaterally affected birds. The most common case outcomes were discharge from hospital to rehabilitation facility (45.6%) followed by euthanasia (43.0%). The presence of an ocular lesion or a vision-threatening ocular lesion was not significantly associated with outcome. Reference ranges are reported for B-mode ultrasound, ocular morphometrics, and horizontal corneal diameter in all species.
Conclusion: Complete ophthalmic examination can be supplemented by the use of ocular morphometrics, ultrasound, and ERG in the manually restrained raptor. These advanced diagnostic techniques may be useful in developing more objective criteria for evaluating eligibility for release following rehabilitation of free-living birds of prey.

Key Words: electroretinogram, eye, ophthalmology, raptor, tonometry, ultrasound

INTRODUCTION

The eyes of raptors are very large relative to their body, proportionately greater than that of a human by approximately 45%, and prominently placed in the skull.[1,2] While these features contribute to excellent visual acuity, they predispose the eyes to trauma when birds violently interact with their environment. Ocular lesions in free-living raptor species are commonly seen in rehabilitation settings, with a reported prevalence of 14.5–75% in Strigiformes and Falconiformes.[3–7] Trauma is the major cause of ocular lesions in raptors.[3–8] Congenital and developmental disease is infrequently reported but may be underreported.[9] Ocular lesions may also be associated with systemic infectious disease. In one study from Minnesota, lesions in the posterior portion of the eye were present in 100% of West Nile Virus positive hawks.[10] Sporadic case reports describe acquired ocular disease of free-living raptors.[11–14] Thorough ophthalmic evaluation of raptor patients is generally considered standard of care.[3]

Assessing vision of injured raptors in captivity is challenging.[8,15] Many species, most notably eastern screech owls and

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red-tailed hawks, demonstrate stoic behavior that can be misinterpreted as decreased response to visual stimuli, while other species, such as Cooper's hawks, are highly reactive to any stimulus and may yield false positive results in assessment of visual function.[8] Raptors suffering from systemic disease or head trauma may also be obtunded, reducing their response to visual stimuli.

Little is known about the post-release survivability of raptors with ocular lesions, however strong opinions regarding the prudence of releasing birds of prey with reduced vision are evident in anecdotal reports.[5,8,15] Intuitive to these arguments is knowledge of species differences in prey apprehension, habitat utilization, and reliance on secondary senses such as auditory stimuli. A Cooper's hawk catching prey in flight would have a greater need for binocular vision than a red-tailed hawk that ambushes its prey from a perched position. It is generally accepted that birds of prey with vision deficits in both eyes are deemed nonreleasable and either require euthanasia or permanent placement in a zoological or education facility.[5,8,15] Less clear is the impact of monocular vision in raptors on survivability, as post-release data is lacking. A reliable, systematic protocol for ocular evaluation of birds of prey with a high predictive value for assessing post-release survival is needed in raptor rehabilitation.

The purpose of this study was to establish a complete ophthalmic examination protocol using slit-lamp biomicroscopy, indirect funduscopy, ocular morphometric, B-mode ultrasound, and electroretinography (ERG) in raptor species native to central Illinois. The biological hypotheses were (i) the protocol would be safe and well-tolerated by all raptors, (ii) the presence of ocular lesions would be associated with systemic diagnosis, age, species and outcome, and (iii) the systemic diagnosis would be associated with outcome.

MATERIALS AND METHODS

Free-living raptors presenting to the Wildlife Medical Clinic at the University of Illinois Urbana-Champaign between September 2007 and December 2009 were eligible for inclusion in the study. Only species, in which >4 birds were examined, were included in data analysis. All procedures were performed in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Institutional Animal Care and Use Committee of the University of Illinois. All birds were manually restrained for the entire examination process, including ultrasonography and ERG. The talons of most birds were bandaged to prevent injury to the examiner. A complete ophthalmic examination was performed on every bird including neuroophthalmic examination, slit-lamp biomicroscopy (Kowa-SL2; Kowa, Tokyo, Japan) and indirect funduscopy using a 28D or 60D condensing hand lens (Volk Optical, Inc., Mentor, OH, USA). Horizontal corneal diameter was measured in millimeters using a Jameson caliper. Intraocular pressure (IOP) was estimated using rebound (Tonovet; Icare Finland Oy, Espoo, Finland) and applanation (Tonopen-XL; Reichert Inc., Depew, NY, USA) tonometers. The rebound tonometry was performed using the 'p' mode, which is the appropriate calibration mode for species in which no calibration table has been established. Rebound tonometry was performed first, then a single drop of proparacaine hydrochloride (Proparacaine hydrochloride 0.5% ophthalmic solution USP; Falcon Pharmaceuticals, Ltd, Fort Worth, TX, USA) was administered to each eye and applanation tonometry performed. Fluorescein sodium was instilled into each eye to evaluate the integrity of the corneal epithelium (BioGlo Fluorescein Sodium Ophthalmic Strips USP; Ocularvision Inc., Solvang, CA, USA).

B-mode ocular ultrasonography was performed using a 12-MHz sector probe (LinscanUSB 12 MHz; Ocuscience LLC, Prairie Village, KS, USA). After the administration of one drop of topical proparacaine solution, the probe was applied directly to the cornea using artificial tears ointment as a coupling gel (Artificial Tears Ointment; Rugby Laboratories, Duluth, GA, USA). The ultrasound exam was completed in the sagittal and frontal planes. All images were captured in the sagittal plane. Digital calipers within the computer software associated with the ultrasound probe were used to measure the cornea to anterior lens capsule distance (C-ALC), anterior to posterior lens capsule distance (LENS), posterior lens capsule to retina distance (VIT), axial globe length (A-P), pecten length (PL), and pecten width (PW).

Prior to recording the ERG, each bird was dark adapted for a minimum of 20 min. The ERG was performed using a monopolar electrode-contact lens (ERG-jet; Nicolet Instruments, Madison, WI, USA) applied to the cornea with methylcellulose 2.5% gel as a coupling agent (Gonak; Akorn, Inc., Buffalo Grove, IL, USA), male subdermal platinum needle electrodes (FD-E2-24; Astro-Medical, Inc. Warwick, RI, USA) and a hand-held ERG machine, the Handheld Multispecies ERG (HMsERG) (HMsERG Model 1000; RetVetCorp, Columbia, MO, USA). When the corneal diameter was <14 mm, a silver/nylon fiber electrode was utilized to accommodate small corneal size (DTL Plus Electrode; Diagnosys LLC, Lowell, MA, USA). The ground electrode needle was placed subcutaneously at the apex of the occiput. The reference electrode needle was placed approximately 0.5–1 cm lateral to the lateral canthus of the eye being tested. The bandpass was set at 0.3–300 Hz.

Two ERG protocols were used for each patient. A proprietary protocol of the HMsERG machine (QuickRetCheck; RetVetCorp) utilizes only three levels of light stimuli to rapidly obtain an overall evaluation of retinal function. The first recording consisted of the average response to four light flashes (2 s in between flashes) at 10 mcd s/m^2, followed by a single flash at 3000 mcd s/m^2 and, after 20 s, another single flash at 10 000 mcd s/m^2. In dark-adapted conditions, 'pure' rod responses were obtained through the first set of low light intensity flashes, while for the 2nd and 3rd flash stimuli, the responses are derived from a mixture of rod and cone photoreceptors. The second protocol was a pure cone test preceded by 10 min of light adaptation at 3000 mcd s/m^2 followed by 32 flashes at 3000 mcd s/m^2 (0.5 s in between flashes) with a signal-averaged response. The QuickRetCheck protocol was completed first on both eyes, then the eye with the fewest lesions on the ocular examination that might impact the ERG was selected for the cone protocol.

Descriptive data analysis was performed (SPSS 17.0; SPSS Inc., Chicago, IL, USA). The 95% confidence intervals were calculated for binomial proportions. For continuous data, normality was assessed using the Shapiro-Wilk test. Normally distributed data were described using mean and standard deviation (SD) and minimum–maximum (min-max) while non-normally distributed data were reported by median, 10–90% range and min–max. A one-way analysis of variance (ANOVA) was used to evaluate IOP by species. When a significant difference was found, *post-hoc* testing using a Tukey's honestly significant differences test was performed. Significance was set at $P < 0.05$. The chi-squared test was performed to compare types of ocular disease, systemic disease, age and outcome with significance set at $P < 0.05$. A Fisher's exact test was used when expected frequency of any cell values were <5. The Kruskal–Wallis test was used to determine if there was a difference in outcome by systemic diagnosis and vision-threatening ocular disease by systemic diagnosis. Agreement between the applanation and rebound tonometry measurements was determined by use of the Bland-Altman method. Bias was defined as the mean difference between the two methods and limits of agreement were calculated as the bias ±1.96 SD. The alpha was set at $P \leq 0.05$. Statistical analysis was performed by use of a commercially available statistical program (MEDCALC 10.1.9; MedCalc Software, Mariakerke, Belgium).

RESULTS

One hundred sixty-seven raptors were admitted to the University of Illinois Wildlife Medical Clinic during the study period. Approximately half (*n* = 82) of the birds did not receive an ophthalmic examination for one of three reasons; the bird succumbed to its injury or illness prior to the exam being performed (*n* = 44; 26.3%), the bird was humanely euthanized due to the severity of its injury or illness prior to the exam being performed (*n* = 27; 16.2%), or an ophthalmic examination could not be performed due to conflicts in clinic schedules prior to release or transfer of the bird (*n* = 11; 6.6%). Of the 85 birds that received ophthalmic evaluation, a total of 79 birds were included in the study representing seven species: American kestrel (*Falco sparverius*), barred owl (*Strix varia*), Cooper's hawk (*Accipiter cooperii*), eastern screech owl (*Megascops asio*), great horned owl (*Bubo virginianus*), red tailed hawk (*Buteo jamaicensis*), and turkey vulture (*Cathartes aura*). A total of 41 birds (51.9%, 95% CI 40.3–63.3%) were diagnosed as having normal eyes (Figs 1,2) and 38 birds (48.1%, 95% CI 36.7–59.6%) had at least one ocular lesion. Of the birds with ocular lesions, 18 (47.3%, 95% CI 31.0–64.2%) were unilaterally and 20 (52.6%, 95% CI 35.8–69.0%) were bilaterally affected. Of the 38 birds with ocular lesions, 11 birds (29.0%, 95% CI 15.4–45.9%) were affected bilaterally with at least one lesion considered to be vision threatening and 11 birds (29.0%, 95% CI 15.4–45.9%) were affected unilaterally with at least one lesion considered to be vision threatening. Vision-threatening lesions were defined as ulcerative keratitis, uveitis (anterior, posterior, or panuveitis), vitreal hemorrhage, retinal detachment, retinal hemorrhage or chorioretinitis (Fig. 3).

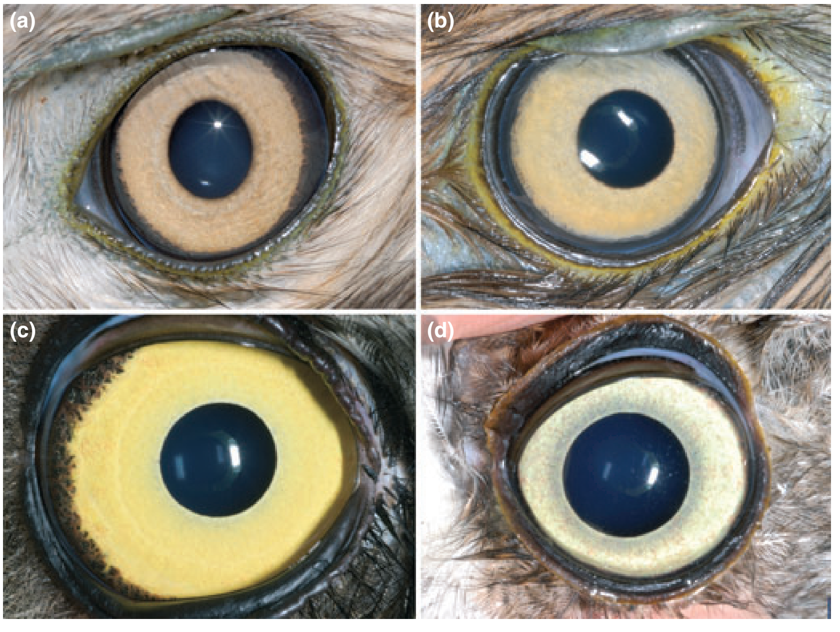

Figure 1. Normal raptor eyes. (a) Red-tailed hawk. (b) Cooper's hawk. (c) Great horned owl. (d) Eastern screech owl.

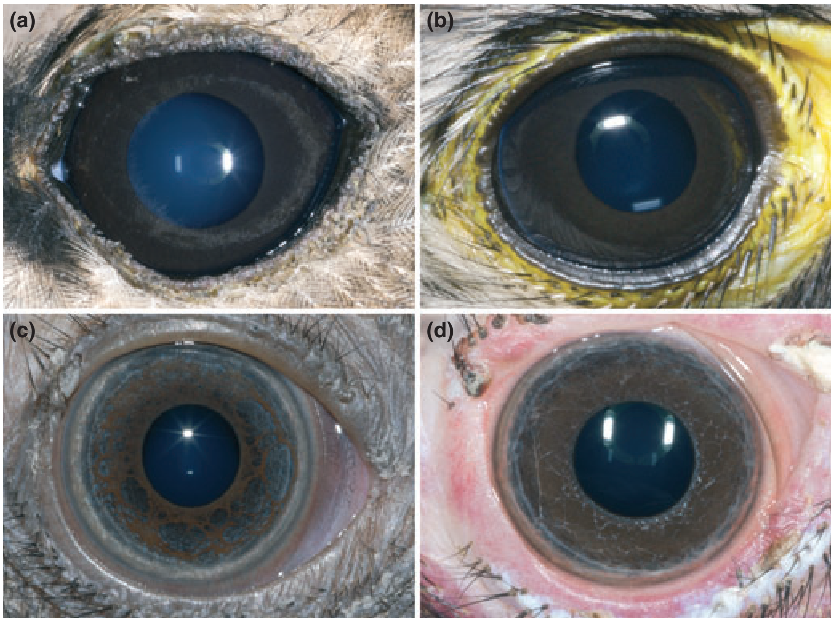

Figure 2. Normal raptor eyes. (a) Barred owl. (b) American kestrel. (c) Turkey vulture. (d) Turkey vulture.

Figure 3. Focal hemorrhagic fibrin clot in the anterior chamber with focal iris disinsertion in a Red-tailed hawk.

Figure 4 demonstrates the frequency of unilateral vs. bilateral ocular lesions as well as vision-threatening ocular lesions by species. American kestrels and turkey vultures are not included in this table as no ocular abnormalities were noted in these species. Table 1 lists the ocular lesions noted in each species by anatomic localization. The most common ophthalmic diagnoses were anterior uveitis (including hyphema) (n = 15; 19.0%, 95% CI 11.0–29.4%), vitreal hemorrhage (n = 12; 15.2%, 95% CI 8.1–25.0%), and retinal detachment (n = 8; 10.1%, 95% CI 4.5%–19.0%). Species was not significantly associated with the presence of ocular lesions (P = 0.174) or vision-threatening ocular lesions (P = 0.169).

All birds were divided into categories based on their primary systemic diagnosis: congenital (n = 1), healthy (n = 10), systemic inflammatory disease/systemic infectious disease (n = 10), head trauma (n = 15) or body trauma

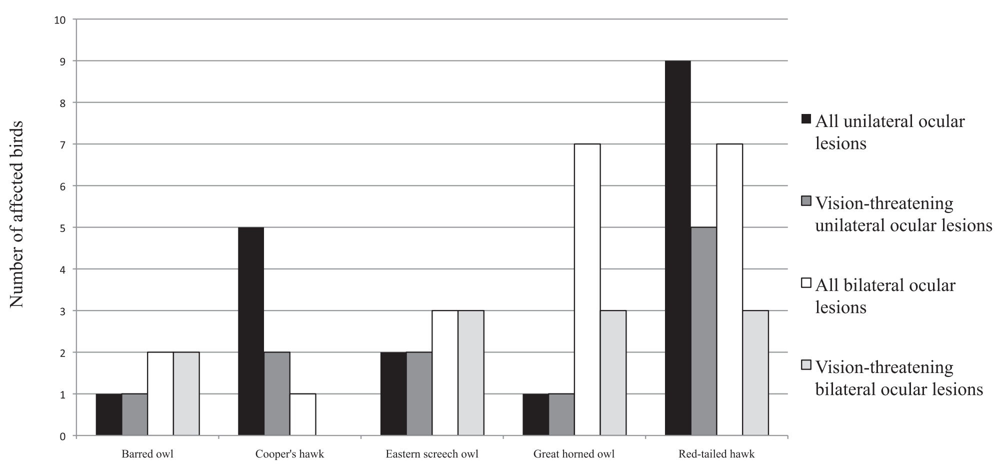

Figure 4. Number of unilateral vs. bilateral ocular lesions as well as vision-threatening ocular lesions by species.

Table 1. Ocular lesions by anatomical region and species

	Adnexa	Conjunctiva/ Cornea	Anterior uvea/ anterior chamber	Lens	Vitreous	Retina/ Choroid	Globe	Neuroophthalmic
Barred owl	1	2	3	1	1	3	0	0
Cooper's hawk	3	2	0	1	1	1	0	1
Eastern screech owl	1	1	3	0	1	3	0	0
Great horned owl	5	3	3	4	2	5	0	0
American kestrel	0	0	0	0	0	0	0	0
Red-tailed hawk	4	5	3	4	6	3	1	0
Turkey vulture	0	0	0	0	0	0	0	0

Numbers within the table represent number of birds in each category.

Table 2. Proportion of ocular lesions by primary systemic disease category

Systemic disease category	Total number of birds	% of total with no ocular lesions	% of total with unilateral ocular lesions	% of total with bilateral ocular lesions	% of total with vision-threatening unilateral ocular lesions	% of total with vision-threatening bilateral ocular lesions
Congenital	1	100.0	0.0	0.0	0.0	0.0
Healthy	10	80.0	10.0	10.0	0.0	0.0
Systemic	10	50.0	20.0	30.0	20.0	10.0
Body trauma	43	58.1	25.6	16.3	11.6	2.3
Head trauma	15	13.3	26.7	60.0	26.7	60.0

Table 3. Proportion of ocular lesions by outcome category

	Total number of birds	% of total with no ocular lesions	% of total with unilateral ocular lesions	% of total with bilateral ocular lesions	% of total with vision-threatening unilateral ocular lesions	% of total with vision-threatening bilateral ocular lesions
REHAB	36	58.3	13.9	27.8	11.1	11.1
EUTH	34	47.1	29.4	23.5	14.7	14.7
DIED	3	33.3	33.3	33.3	0.0	33.3
RELEASE	6	50.0	33.3	16.7	33.3	16.7

REHAB = discharged from hospital to licensed raptor rehabilitator; EUTH = humanely euthanized while in hospital; DIED = raptor died while in hospital; RELEASE = raptor released in local wildlife habitat.

Table 4. Proportion of species in each outcome category

	Total number of birds	% of birds sent to rehabilitator	% of birds euthanized	% of birds died	% of birds released
Barred owl	6	66.7	33.3	0.0	0.0
Cooper's hawk	11	27.3	63.6	9.1	0.0
Eastern screech owl	8	37.5	12.5	12.5	37.5
Great horned owl	13	46.2	46.2	7.7	0.0
American kestrel	4	75.0	25.0	0.0	0.0
Red-tailed hawk	33	51.5	39.4	0.0	9.1
Turkey vulture	4	0.0	100.0	0.0	0.0

(n = 43). Head trauma and body trauma were combined into one group for portions of the statistical analysis. Table 2 details the proportion of ocular lesions and vision-threatening ocular lesions in birds based on primary systemic diagnosis. Primary systemic diagnosis was significantly associated with the presence of ocular disease (P = 0.005) and the presence of vision-threatening ocular disease ($P < 0.005$). Birds that presented with head trauma were significantly more likely to have unilateral (26.7%) and bilateral (60%) vision-threatening ocular disease than all other groups (unilateral <20%; bilateral <10%).

All birds experienced one of four possible outcomes: discharge from hospital to a rehabilitation facility (n = 36; 45.6%), euthanasia (n = 34; 43.0%), death during hospitalization (n = 3; 3.8%) or release to a local wildlife habitat (n = 6; 7.6%). Table 3 details the proportion of birds and their outcomes for all ocular lesions and vision-threatening ocular lesions. Table 4 details the proportion of outcomes by species. There was no significant difference in outcome by systemic disease diagnosis ($P = 0.057$); however, the probability did approach significance (0.05). Because the power analysis done for this comparison was 0.3, it does suggest that there is the potential for a Type II error. While not significant, raptors presenting with head trauma or body trauma were more likely to be euthanized (30/57; 52.6%) than healthy raptors (1/10; 10%) or raptors with infectious disease (1/10; 10%). As a result, birds presenting with trauma were also less likely to be transferred to a rehabilitation facility (21/57; 36%) than healthy birds (8/10; 80%) or birds with infectious disease (7/10; 70%). Birds presenting with systemic disease were more likely to die in captivity (2/10; 20%) than healthy birds (1/10; 10%) or birds presenting for trauma (1/57; 1.7%). Animals with body trauma were significantly more likely to euthanized (61%) than any other group.

Although determining exact age in this free-living raptor population was difficult, all birds were classified into three age groups based on morphology and plumage: nestling/fledgling ($n = 20$), juvenile ($n = 12$), and adult ($n = 47$). Age was not statistically significantly associated with the presence of ocular lesions ($P = 0.059$) or the presence of vision-threatening ocular lesions ($P = 0.105$) but was associated with outcome ($P = 0.022$). Birds that were classified as adults (55%) were significantly more likely to be euthanized than nestlings (15%) or juveniles (41%).

Reference values for ocular measurements by B-mode ultrasound are presented in Table 5. Ocular ultrasound was successful in all birds and was not affected in any bird by restraint or technical limitations. Abnormalities were noted on ocular ultrasound in 9/79 birds (11.3%; 95% CI 5.3–20.5%). The most frequent abnormality noted on ultrasound was increased vitreal echogenicity in 7/9 birds (77.8%; 95% CI 40.0–97.2%) and retinal detachment in 5/9 birds (55.6%; 95% CI 21.2–86.3%). Figure 5 demonstrates selected abnormalities observed on ultrasonographic examination. Horizontal corneal diameter by species and age is presented in Table 6. Although ocular ultrasound and corneal diameter measurements were performed in all birds, all species were not represented in all three age categories (nestling/fledgling, juvenile, adult), thus data are only reported for species/age categories with >2 birds.

Electroretinography recording was successful in all birds. In no bird was the ERG QuickRetCheck protocol unable to be harvested due to restraint or technical limitations; however, some patients became unduly stressed with the light-adaptation time required for the cone test. Harvesting of further ERG tracings after the recording of the QuickRetCheck protocol was aborted in any bird that showed signs of physical stress, including agitation, tachypnea, or weakness. Inferential statistics comparing the ERG results of birds of different species could not be performed due to the limited number of normal birds in each species and age category. Table 7 details the ERG results in all species. Three normal ERGs in species common to central Illinois (red-tailed hawk, eastern screech owl, great horned owl) are presented in Fig. 6.

Table 5. Morphometric B-mode ultrasound measurements by species in mm

Species	Age	C-ALC	LENS	VIT	A-P	PL	PW
Barred owl	Adult (n = 8 eyes)						
	Mean	4.8	9.8	12.5	27.6	5.9	2.2
	SD	1.6	1.6	0.9	3.4	0.4	1.0
Cooper's hawk	Nestling (n = 10 eyes)						
	Mean	2.9	4.0	8.4	15.0	5.0	2.9
	SD	0.6	0.8	0.6	1.3	0.9	0.5
	Juvenile (n = 4 eyes)						
	Mean	2.6	4.4	9.4	16.0	5.0	2.7
	SD	1.0	0.3	0.1	0.7	0.3	1.0
	Adult (n = 8 eyes)						
	Mean	3.0	4.2	9.4	16.6	5.1	2.9
	SD	0.5	0.5	0.2	0.5	1.0	1.7
Eastern screech owl	Nestling (n = 8 eyes)						
	Mean	4.0	5.7	7.5	16.1	4.0	2.3
	SD	0.5	1.0	1.1	2.1	0.6	0.5
	Adult (n = 8 eyes)						
	Mean	3.9	6.2	9.5	19.4	4.2	1.9
	SD	0.4	0.8	0.3	0.8	0.4	0.3
Great horned owl	Nestling (n = 4 eyes)						
	Mean	5.6	8.2	13.7	27.5	8.0	3.7
	SD	1.1	1.3	0.6	0.8	0.4	1.7
	Adult (n = 22 eyes)						
	Mean	5.6	9.3	17.3	31.9	7.3	3.4
	SD	1.3	0.9	0.5	1.7	0.9	1.5
American kestrel	Nestling (n = 4 eyes)						
	Mean	1.8	3.4	7.3	13.1	5.7	3.0
	SD	0.2	0.4	0.1	1.7	0.6	0.6
	Adult (n = 4 eyes)						
	Mean	3.0	3.3	7.3	12.0	4.0	1.8
	SD	2.0	0.1	0.2	0.4	0.3	0.5
Red-tailed hawk	Nestling (n = 10 eyes)						
	Mean	3.7	5.7	14.6	23.8	7.0	3.6
	SD	0.2	0.5	0.3	0.9	0.4	0.4
	Juvenile (n = 18 eyes)						
	Mean	4.2	6.3	14.5	24.1	7.5	3.7
	SD	1.0	1.7	0.7	1.0	0.8	0.9
	Adult (n = 38 eyes)						
	Mean	4.1	6.3	14.9	24.3	7.6	3.4
	SD	1.6	1.9	1.9	0.9	1.1	0.6
Turkey vulture	Adult (n = 6 eyes)						
	Mean	3.1	3.5	10.4	16.8	7.4	3.3
	SD	0.5	0.4	0.5	1.1	0.6	0.6

C-ALC = cornea to anterior lens capsule; LENS = lens axial length; VIT = vitreous axial length; A-P = axial globe length; PW = pecten width; PL = pecten length.

Mean ± SD IOP for all normal adult birds as measured with applanation and rebound tonometry is presented as Table 8. Mean ± SD IOP for normal nestling/fledgling birds is reported separately as Table 9. Only birds with normal intraocular examinations or examinations that showed them to be free of intraocular disease that could reasonably be expected to alter IOP are included in this data analysis. One-way ANOVA revealed a significant difference in IOP between adult species ($P < 0.001$). The IOP of barred owls were significantly lower than the IOP of red-tailed hawks ($P < 0.001$). The IOP of Cooper's hawks were significantly

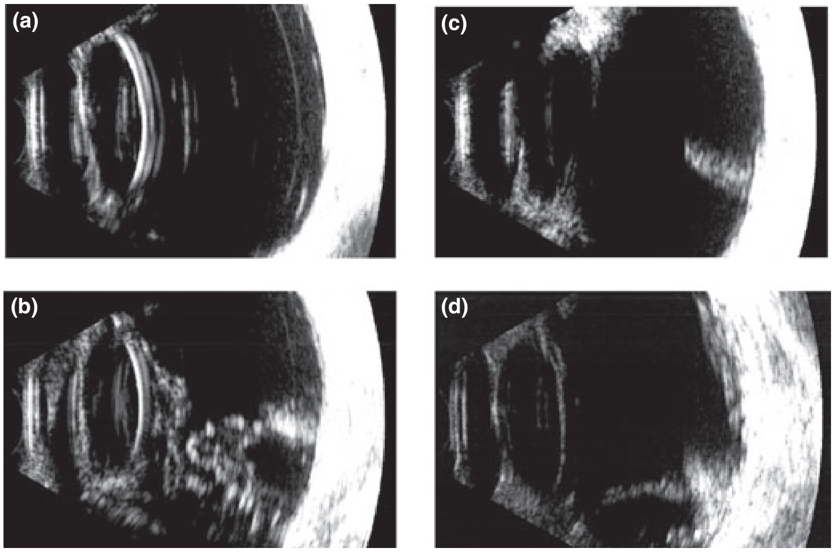

Figure 5. B-mode ultrasound sagittal ultrasound images. (a) Normal adult red-tailed hawk. Note reverberation artifact present within the vitreous and anterior to the retina. (b) Marked echogenic debris in the vitreous associated with the pecten and extending to the posterior lens capsule with increased echogenicity of the pecten consistent with vitreal hemorrhage and intra-pecten hemorrhage in a red-tailed hawk. (c) Normal adult red-tailed hawk, pecten visible. (d) Mild echogenic debris in the vitreous with markedly abnormal pecten profile consistent with vitreal hemorrhage, and partial pecten avulsion in a red-tailed hawk.

higher than the IOP of eastern screech owls ($P < 0.001$), great horned owls ($P < 0.001$), and kestrels ($P < 0.001$). The IOP of eastern screech owls were significantly lower than the IOP of Cooper's hawks ($P < 0.001$) and red-tailed hawks ($P < 0.001$). The IOP of Great horned owls were significantly lower than the IOP of Cooper's hawks ($P < 0.001$), red-tailed hawks ($P < 0.001$), and turkey vultures ($P = 0.001$). The IOP of kestrels were significantly lower than Cooper's hawks ($P < 0.001$), red-tailed hawks ($P < 0.001$), and turkey vultures ($P = 0.005$). The IOP of red-tailed hawks were significantly higher than the IOP of barred owls ($P < 0.001$), eastern screech owls ($P < 0.001$), great horned owls ($P < 0.001$), kestrels ($P < 0.001$), and turkey vultures ($P = 0.001$). The IOP of turkey vultures were significantly higher than the IOP of eastern screech owls ($P = 0.02$), great horned owls ($P = 0.001$), kestrels ($P = 0.005$) and significantly lower than the IOP of red-tailed hawks ($P = 0.001$).

One-way ANOVA was repeated with the barred owls and eastern screech owls combined into a Strigidae group (owl), the Cooper's hawks and red-tailed hawks combined into a Accipitridae group (hawk) and with American kestrels and turkey vultures each left as separate groups. A significant difference in IOP was detected between groups for both rebound and applanation tonometry ($P < 0.001$) (Figs 7,8). The IOP of the owl group was significantly lower than the IOP of the hawk group ($P < 0.001$) and turkey vulture group ($P < 0.001$) but not significantly different than the kestrel group ($P = 0.77$). The IOP of the hawk group was significantly higher than the IOP of the owl group ($P < 0.001$), kestrel group ($P < 0.001$), and turkey vulture group ($P = 0.002$). The IOP of the kestrel group was significantly lower than the IOP of the hawk group ($P < 0.001$) and turkey vulture group ($P = 0.003$) but not significantly different than the owl group ($P = 0.77$). The IOP of the turkey vulture group was significantly higher than the IOP of the owl group ($P = 0.001$), and kestrel group ($P = 0.003$) but lower than the hawk group ($P = 0.002$).

The Bland Altman plots for the left eye (bias 2.3, limits of agreement −5.7 to 10.2) and right eye (bias 2.1, limits of agreement −5.1 to 9.2) were found to have moderate agreement based on the bias; however, the large limits of agreement suggest that there is the potential for a discrepancy between these two measuring devices (Figs 9,10).

Ocular histopathology was performed in 23/37 (62.2%) of the birds that either died in the hospital or were euthanized. Histopathology confirmed the clinical exam findings in all cases. 12/23 birds evaluated using histopathology had normal ophthalmic clinical exams and lacked significant histologic lesions. The most common histopathologic diagnoses were vitreal hemorrhage (5/23 cases) and retinal detachment (3/23 cases), consistent with the clinical findings in these patients.

DISCUSSION

This study successfully established an advanced ophthalmic examination protocol including slit-lamp biomicroscopy, indirect funduscopy, ocular morphometrics, B-mode ultrasound, and ERG for use in evaluating raptors.

Red-tailed hawks were the most frequently examined raptor species, which is consistent with the University of Illinois Urbana-Champaign Wildlife Medical Clinic patient population. Trauma to the body followed by trauma to the head were the most common systemic diagnoses. Previous reports also suggest trauma as the most common reason for raptors needing medical care.[4–7] Our findings of anterior uveitis, vitreal hemorrhage, and retinal detachment as the most frequent ophthalmic abnormalities are also in agreement with previous studies.[5,7]

The clinical and statistical findings between primary systemic diagnosis and the presence of both ocular disease and vision-threatening ocular disease is not surprising, given that the probably etiology for the three most frequent abnormalities in this population is trauma. More interesting is the lack

Table 6. Horizontal corneal diameter by species and age in mm

Species	Age	Horizontal corneal diameter
Barred owl	Nestling (n = 4 eyes)	
	Mean	12.5
	SD	0.6
	Adult (n = 8 eyes)	
	Mean	20.9
	SD	5.1
Cooper's hawk	Nestling (n = 10 eyes)	
	Mean	8.8
	SD	
	Juvenile (n = 4 eyes)	
	Mean	10.5
	SD	0.6
	Adult (n = 8 eyes)	
	Mean	10.0
	SD	0.8
Eastern screech owl	Nestling (n = 8 eyes)	
	Mean	11.3
	SD	1.2
	Adult (n = 8 eyes)	
	Mean	14.8
	SD	
Great horned owl	Nestling (n = 4 eyes)	
	Mean	18.3
	SD	2.1
	Adult (n = 22 eyes)	
	Mean	23.5
	SD	1.1
American kestrel	Nestling (n = 4 eyes)	
	Mean	7.3
	SD	0.3
	Adult (n = 4 eyes)	
	Mean	7.5
	SD	0.6
Red-tailed hawk	Nestling (n = 10 eyes)	
	Mean	14.6
	SD	0.8
	Juvenile (n = 18 eyes)	
	Mean	14.8
	SD	1.2
	Adult (n = 38 eyes)	
	Mean	14.5
	SD	1.3
Turkey vulture	Adult (n = 6 eyes)	
	Mean	9.7
	SD	0.5

of statistical association between outcome and ocular lesions or vision-threatening ocular lesions. Outcome was significantly associated with primary systemic diagnosis and age, suggesting that the ocular lesions were less of a determinant of outcome than the patient's major systemic abnormality. Although vision is necessary for raptor survival, mobility is equally important as evidenced by the high percentage of poor outcomes (euthanasia or death) in the body trauma group.

For raptors with systemic disease from which recovery is possible, thorough assessment of ocular abnormalities and rendering an accurate prognosis is essential for optimal case outcome. Histopathology, although useful for confirming clinical diagnoses in this study, does not hold any predictive value in forming a prognosis when performed on enucleated globes. Ocular ultrasonography has previously been demonstrated to be an effective, rapid and economical technique for evaluating the avian globe.[16–18] Vitreal hemorrhage, the second most common ocular abnormality in this study, precluded direct examination of the posterior segment in a number of birds, making ultrasound a useful diagnostic modality. The overall frequency of lesions seen on ocular ultrasound was approximately 10%. In the raptor medicine setting where financial limitations may dictate selection of diagnostic procedures, ultrasound is considered to be most useful in evaluating cases where anterior segment opacity limits posterior segment examination. Establishing anatomical measurement reference ranges for frequently observed species, as was performed in this study, allows for more accurate assessment of pathology and potentially improves the clinician's ability to render an accurate prognosis. For example, when evaluating a raptor with head trauma and vitreal hemorrhage, having normal pecten size values for that species is essential for determining the integrity of the pecten. B-mode ultrasound, which is more widely available than A-mode, has been established as an accurate method for evaluating avian ocular anatomy.[16]

Tonometry is the estimation of IOP. Both applanation and rebound tonometry have previously been reported as accurate methods of estimating IOP in raptors.[7,17,19–21] Significant differences can be found between species, which makes establishing reference ranges in normal birds essential for accurate interpretation of results.[20] The results of this study agree with previous studies that suggest that the IOP of owls is lower than that of hawks. Additionally, the results of this study suggest that the IOP of American kestrels and turkey vultures is also lower than the IOP of hawks. The cause of variability in IOP between raptor species warrants further investigation, and differences in corneal curvature and corneal thickness may play a role in this variation.[22–24]

Although rebound tonometry may significantly overestimate IOP, the clinical difference in the two instruments appears minimal.[19] Results of another study stated that mean IOP values obtained by rebound tonometry were lower than that obtained with applanation tonometry.[17] A third study suggested that rebound tonometry may underestimate IOP in owl species while overestimating IOP in hawk species.[21] All studies agreed that rebound tonometry using a Tonovet® tonometer is rapid and may be better tolerated in raptors, particularly those with small corneas, because of the smaller footprint of the device contacting the cornea.[17,19,21] Although the prevalence of glaucoma in free-living raptors appears low, tonometry is still indicated as part of a complete ophthalmic examination as it is easy to perform, minimally invasive and economical.[4–7]

While the overall prevalence of congenital lesions in this study was low, and no cases of abnormal globe size were noted, reference ranges for horizontal corneal diameter in

Table 7. Electroretinogram values in normal adult and juvenile raptors

	10 mcd/m^2		3000 mcd/m^2				10 000 mcd/m^2			
	b amplitude	b implicit	a amplitude	a implicit	b amplitude	b implicit	a amplitude	a implicit	b amplitude	b implicit
Barred owl (*n* = 3 eyes)										
Mean	*	*	86.9	17.4	332.8	60.2	138.1	17.9	236.5	48.4
SD	*	*	17.0	3.4	70.5	24.3	54.8	3.1	49.7	3.4
Minimum	*	*	74.8	15.0	282.9	43.0	76.7	14.6	182.9	44.5
Maximum	*	*	98.9	19.8	382.6	77.3	181.9	20.7	280.9	50.7
Cooper's hawk (*n* = 8 eyes)										
Mean	87.1	48.5	81.8	11.6	199.6	29.6	147.9	13.3	183.4	27.7
SD	30.0	4.1	54.3	4.4	88.8	6.8	176.9	5.1	91.0	5.0
Minimum	39.0	44.1	23.7	8.4	74.2	18.9	22.8	8.8	75.3	21.9
Maximum	117.9	55.1	176.0	22.1	350.2	38.7	604.0	22.1	336.4	38.9
Eastern screech owl (*n* = 3 eyes)										
Mean	86.8	80.9	51.5	23.4	481.0	57.2	74.6	24.1	279.7	60.7
SD	71.6	30.4	47.0	0.4	344.1	0.6	46.8	2.2	12.1	7.0
Minimum	36.2	59.4	18.2	23.1	237.7	56.7	41.5	21.3	271.1	54.9
Maximum	137.4	102.4	84.7	23.7	724.3	57.6	107.7	25.6	672.5	65.6
Great horned owl (*n* = 14 eyes)										
Mean	49.8	73.6	64.8	19.5	148.7	47.2	78.3	19.7	167.2	44.5
SD	19.2	11.7	40.1	6.0	76.0	9.4	31.0	3.7	71.3	8.9
Minimum	13.7	55.3	6.7	7.6	64.8	34.2	38.7	14.6	62.2	33.7
Maximum	75.7	92.6	140.7	31.4	341.1	62.9	136.8	29.3	254.6	61.9
American kestrel (*n* = 4 eyes)										
Mean	53.9	56.0	166.0	10.5	473.7	38.8	198.5	11.0	468.2	39.0
SD	26.8	8.4	95.3	0.8	224.6	7.2	81.1	1.5	240.1	7.8
Minimum	21.8	47.6	62.9	9.4	275.6	32.9	142.7	9.2	243.5	31.6
Maximum	84.7	67.3	293.4	10.9	759.9	47.6	318.8	12.5	744.4	46.8
Red-tailed hawk (*n* = 39 eyes)										
Mean	93.9	56.2	148.2	11.9	372.7	33.3	179.2	11.8	422.8	33.2
SD	46.1	16.6	67.6	2.0	103.4	4.0	66.7	1.4	131.7	5.1
Minimum	31.2	10.1	11.4	4.9	140.1	25.6	25.4	9.0	127.6	23.3
Maximum	251.2	82.0	328.5	16.6	522.1	42.0	381.1	14.4	695.3	47.2
Turkey vulture (*n* = 8 eyes)										
Mean	129.8	57.1	161.1	11.2	307.9	43.0	196.6	12.3	368.9	35.6
SD	63.4	17.4	84.5	1.0	129.1	19.0	76.3	1.4	73.6	5.4
Minimum	48.3	42.4	13.0	9.6	48.0	25.8	104.3	9.4	247.6	24.6
Maximum	188.7	80.4	298.7	12.1	421.8	83.0	336.3	13.8	488.1	41.6

	Cone Function			
	a amplitude	a implicit	b amplitude	b implicit
Barred owl (*n* = 3 eyes)				
Mean	17.9	18.6	133.0	53.6
SD				
Minimum				
Maximum				
Cooper's hawk (*n* = 8 eyes)				
Mean	64.4	10.3	201.2	23.4
SD	37.1	0.6	80.2	2.6
Minimum	36.0	9.6	116.6	21.9
Maximum	117.2	10.9	303.1	27.3
Eastern screech owl (*n* = 3 eyes)				
Mean	8.6	24.9	51.1	52.2
SD	5.6	0.8	45.3	4.4
Minimum	4.6	24.3	19.0	49.1
Maximum	12.5	25.4	83.1	55.3
Great horned owl (*n* = 14 eyes)				
Mean	27.9	23.2	122.1	45.6
SD	13.0	2.3	76.7	4.7
Minimum	11.9	20.4	47.2	39.5
Maximum	42.4	26.0	251.3	53.4
American kestrel (*n* = 4 eyes)				
Mean	97.3	11.3	521.2	36.3

Table 7. (Continued)

	Cone Function			
	a amplitude	a implicit	b amplitude	b implicit
SD	64.6	0.6	344.2	1.9
Minimum	51.6	10.9	277.8	34.9
Maximum	142.9	11.7	764.6	37.6
Red-tailed hawk (*n* = 39 eyes)				
Mean	75.0	11.8	339.2	31.8
SD	21.5	0.9	97.4	2.1
Minimum	43.2	10.1	166.4	28.3
Maximum	105.5	13.4	512.2	37.9
Turkey vulture (*n* = 8 eyes)				
Mean	56.7	12.5	232.1	30.2
SD	15.9	0.5	48.7	1.3
Minimum	44.2	11.7	163.4	28.3
Maximum	80.1	12.8	267.7	31.0

All amplitude values are given in μV and all implicit times are in ms.

common raptor species are useful for ruling out abnormalities such as microphthalmos, exophthalmos and buphthalmos. Ocular ultrasonography can also be utilized in conjunction with measurements of the corneal diameter to further evaluate globe size.[16]

Electroretinography has only been previously reported in the research setting on two little owls (*Athene noctua*), two tawny owls (*Strix aluco*) and in a clinical case on one great horned owl.[11,25,26] The ERG is the summation of the electrical potentials and currents generated within the cells of the retina and is widely used in veterinary ophthalmology for assessing retinal function.[27] Traditional ERG units are bulky and nonportable; however, a new hand-held ERG system makes electrodiagnostic assessment of nontraditional

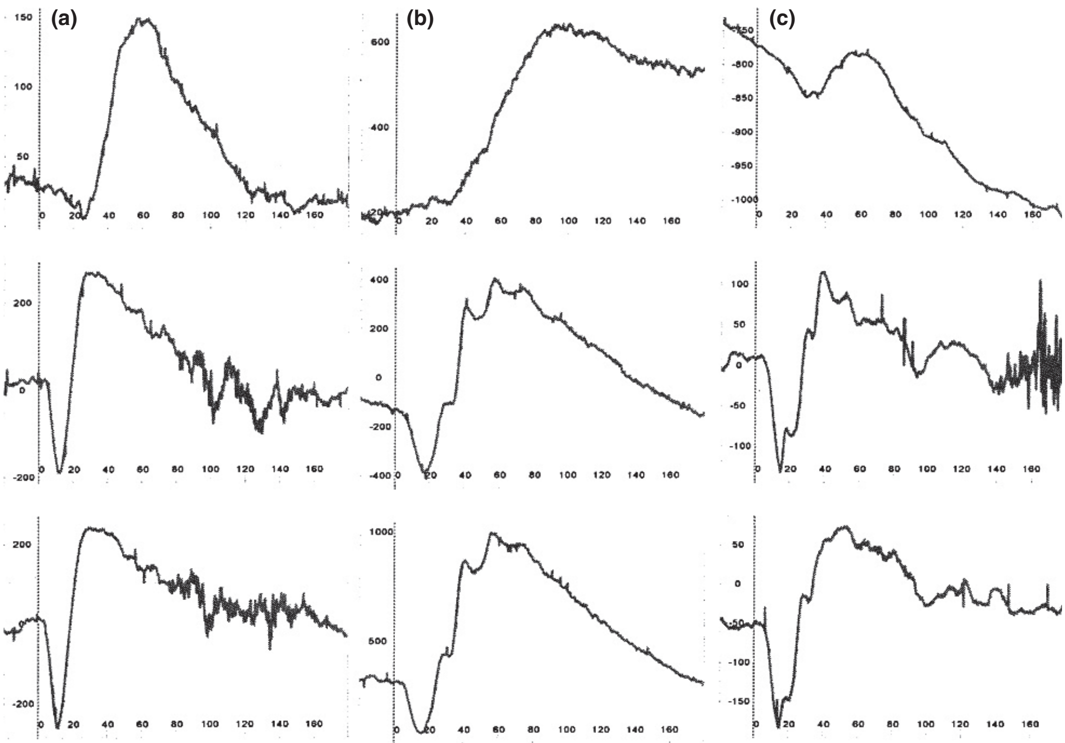

Figure 6. Normal electroretinograms in selected raptor species. The first row of tracings represents the retinal electrical response to a single white flash at 10 mcd s/m^2 (primarily a response of the rod photoreceptors), the second row at 3000 mcd s/m^2 (mixed rod and cone photoreceptor response) and the third row at 10 000 mcd s/m^2 (primarily a response of the cone photoreceptors). Amplitude is listed in μV on the *Y*-axis and implicit time is listed in ms on the *X*-axis. Note that the scale of the *Y*-axis changes in each tracing. (a) Normal adult red-tailed hawk. (b) Normal eastern screech owl. (c) Normal great horned owl.

Table 8. IOP in normal adult raptors by species. All values are listed in mmHg

	Barred owl (n = 3 eyes)	American kestrel (n = 8 eyes)	Cooper's hawk (n = 6 eyes)	Red-tailed hawk (n = 44 eyes)	Eastern screech owl (n = 4 eyes)	Turkey vulture (n = 6 eyes)	Great horned owl (n = 15 eyes)
Applanation							
Mean	11.7	8.5	16.0	20.3	9.3	15.0	9.9
SD	3.8	4.4	1.8	2.8	2.6	2.1	2.4
Minimum	9.0	3.0	13.0	15.0	7.0	11.0	6.0
Maximum	16.0	12.0	18.0	26.0	12.0	17.0	14.0
Rebound							
Mean	8.3	6.8	10.7	19.8	6.3	11.7	9.9
SD	3.2	1.7	1.4	4.9	1.3	1.0	2.2
Minimum	6.0	5.0	9.0	14.0	5.0	10.0	6.0
Maximum	12.0	9.0	12.0	34.0	8.0	12.0	14.0

Table 9. IOP in normal nestling and fledgling raptors by species. All values are listed in mmHg

	Barred owl (n = 4 eyes)	Great horned owl (n = 4 eyes)	Cooper's hawk (n = 8 eyes)	American kestrel (n = 4 eyes)	Eastern screech owl (n = 4 eyes)	Red-tailed hawk (n = 8 eyes)
Applanation						
Mean	12.0	11.3	12.5	7.3	9.8	16.0
SD	2.9	0.5	2.7	4.9	4.3	1.7
Minimum	9.0	11.0	9.0	3.0	6.0	14.0
Maximum	16.0	12.0	17.0	12.0	14.0	19.0
Rebound						
Mean	5.3	11.8	8.4	6.3	4.8	12.1
SD	1.7	3.1	1.3	1.0	4.1	1.0
Minimum	3.0	9.0	7.0	5.0	1.0	11.0
Maximum	7.0	16.0	10.0	7.0	10.0	14.0

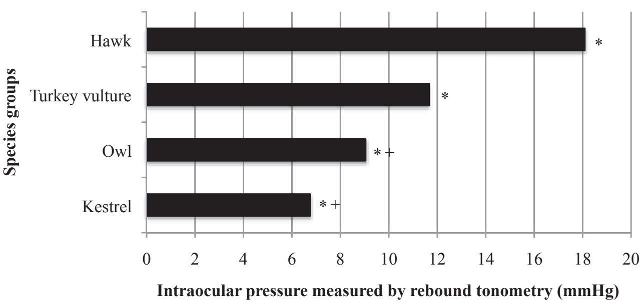

Figure 7. Bar graph of the mean intraocular pressure of the four combined species groups as measured with rebound tonometry. '*' indicates that the group is statistically significantly different from the other groups. The two groups indicated by '+' are not statistically significant from one another.

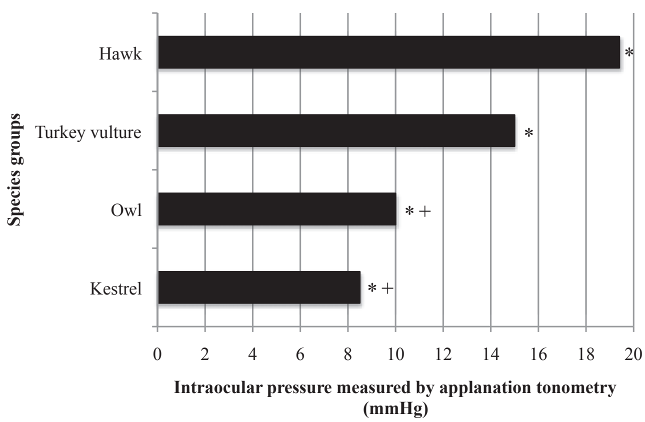

Figure 8. Bar graph of the mean intraocular pressure of the four combined species groups as measured with applanation tonometry. '*' indicates that the group is statistically significantly different from the other groups. The two groups indicated by '+' are not statistically significant from one another.

patients more practical and feasible.[28–30] The present study demonstrates that harvesting ERGs in awake, manually restrained raptors is simple and easy to perform, eliminating the need for general anesthesia which can be both expensive and high risk in the raptor patient.[31] Anesthetic drugs can impact the ERG, so performing ERG with manual restraint alone also eliminates pharmacological confounders.[32] Lack of objective criteria on which to determine eligibility for release plagues raptor rehabilitation.[5,8,15] No bird in this study was declared a poor candidate for release or rehabilitation based on ERG results alone, however an ERG in conjunction with ophthalmic examination provides an objective quantification of retinal function that may prove to be useful in either assessing retinal function after ocular disease is diagnosed or monitoring a raptor's recovery from ophthalmic disease. It is important to note that the ERG is a panreti-

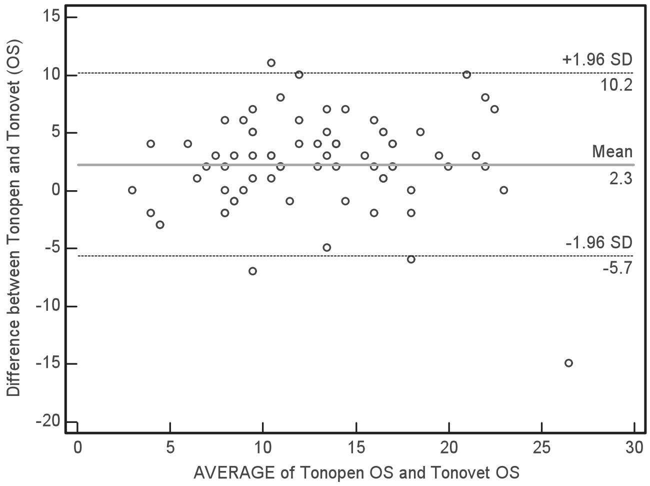

Figure 9. Bland-Altman plot of IOP OS as measured using rebound (Tonovet) and applanation (Tonopen) tonometry.

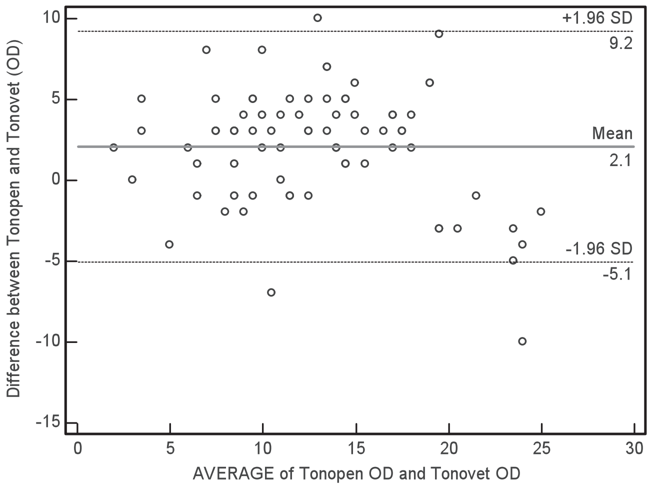

Figure 10. Bland-Altman plot of IOP OD as measured using rebound (Tonovet) and applanation (Tonopen) tonometry.

nal response, and a focal lesion in the area of the fovea could have devastating effects on vision and thus predation but not be detectable via ERG. Electroretinography is not a replacement for complete ophthalmic examination and behavioral evaluation in determining an individual bird's capacity for vision.

This study successfully demonstrates a protocol for the thorough ocular evaluation of free-living raptors presented for veterinary evaluation. This protocol may prove useful in determining objective criteria on which to base decisions regarding release and rehabilitation of injured free-living raptors.

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