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Original Study

Ocular Examinations and Investigation of Intraocular Pressure, Tear Production, Central Corneal Thickness, and Corneal Touch Threshold in a Captive Flock of Atlantic Puffins (*Fratercula arctica*)

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Abstract: Ocular examinations were completed on a group of 10 Atlantic puffins (*Fratercula arctica*), 5 males and 5 females that ranged in age from 8 months to older than 30 years. The exams consisted of intraocular pressure/rebound tonometry, tear production/phenol red thread test, central corneal thickness/ultrasound pachymetry, and corneal sensitivity/esthesiometry. On ocular examination, there were no corneal abnormalities observed. Bilateral cataracts were diagnosed in 8 puffins, 6 of which were considered incipient, focal subcapsular opacities. One bird had hypermature cataracts and was removed from the study and excluded from data analysis; the other birds had no evidence of ophthalmic pathology that would interfere with diagnostic results (n = 9). All results for 9 birds were included in the study, with the exception of 1 puffin's tear production, which was too low for accurate assessment and was excluded from data analysis. There were no significant differences between right and left eye measurements for intraocular pressure, corneal thickness, and corneal sensitivity. The median intraocular pressure for both eyes (OU) was 13 mm Hg with an interquartile range [IQR] of 12–15 mm Hg. The median corneal thickness OU was 241 μm, IQR 233–248 μm. The median corneal sensitivity OU was 1.13 cm, IQR 0.81–1.50 cm. There was a significant difference between right and left eye measurements for tear production (right eye median, 7.5 mm/15 s, IQR 6.5–9.3 mm/15 s; and left eye median, 5.0 mm/15 s, IQR 4.0–7.3 mm/15 s) ($P = .03$), with the right eye producing more tears than the left. However, 1 puffin was determined to be an outlier, and when removed, there was no longer a significant difference (OU median, 7.0 mm/15 s, IQR 4.6–8.0 mm/15 s) ($P = .38$). There was no significant difference between sex and intraocular pressure, tear production, and corneal sensitivity. However, there was a significant difference between sex and corneal thickness ($P = .02$), with males (left eye median, 249 μm, IQR 241–249 μm) having thicker corneas than females (left eye median, 236 μm, 234–238 μm). Although sample size precluded statistical testing, there appeared to be an association between opacities and increasing age. There were no associations between age and intraocular pressure, tear production, or corneal thickness. There was a moderate correlation between age and corneal sensitivity, with older birds showing decreased corneal sensitivity ($r = -0.57$). Although the sample size of 9 birds was small, these findings provide preliminary ranges for ocular parameters of Atlantic puffins.

Key words: corneal sensitivity, corneal thickness, intraocular pressure, phenol red thread tear test, ophthalmic, avian, Alcidae, Atlantic puffin, *Fratercula arctica*

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Introduction

The ocular examination is an important part of any avian health assessment. Common components of an ocular examination include direct and indirect ophthalmoscopy. In addition, quantitative measurements may include intraocular pressure (IOP), tear production, corneal thickness, and corneal sensitivity. These measurements are used clinically to evaluate ocular disease and healing. However, results vary by species, so reference intervals cannot be reliably extrapolated among species.[1–3] In addition, factors such as age, sex, morphometrics, and environment can affect results, even within a species.

Intraocular pressure is often measured by applanation or rebound tonometry. Applanation tonometry requires a 3–4-mm corneal diameter and application of topical anesthetic to the cornea.[4] Rebound tonometry can be used with corneal diameters as small as 1.4 mm and without application of topical anesthetics. Consequently, rebound tonometry was used in this study.[1,4] Intraocular pressure can vary by age, head and body position, and species.[5,6] In addition, the tonometry method affects IOP; therefore, ranges determined for IOP of specific avian species should not be extrapolated among methods.

Tear production is frequently assessed by the Schirmer tear test or the phenol red thread test.[7] The Schirmer tear test measures residual, basal, and reflexive tearing, and the phenol red thread test measures residual and basal tearing.[8] The phenol red thread test is affordable, faster than the Schirmer tear test, and commonly used in animals with small palpebral fissures and was selected for use in this study.[6,9] Similar to IOP measurements, results from one tear test method cannot be compared with results from a different test method.[9] In mammals, ocular tear production can be affected by age, sex, species, and time of day.[10,11] In avian studies, tear production can be associated with age and species.[6,9,12] In addition, environment may affect tear production; one study in Humboldt penguins (*Spheniscus humboldti*) found that birds housed in saltwater had significantly lower tear production compared with those housed in freshwater.[13]

Corneal thickness is measured by various methods, including ultrasound pachymetry, confocal microscopy, and optical coherence tomography. Ultrasound pachymetry is applicable in animals diagnosed with corneal opacities and has good reproducibility as long as the probe is placed perpendicular to the central surface of the cornea.[4] Ultrasound pachymetry has been used successfully to measure corneal thickness in various avian species, including chickens, raptors, and penguins.[14–16] Some animal species show a positive correlation between corneal thickness and IOP measurements by rebound tonometry.[17,18] Age, region of cornea tested, and weight affect corneal thickness in various species, including dogs, cats, horses, and chickens.[16,19–22] Corneal thickness measurements from different instruments should only be compared if the units and the strength of the filament are the same.[4]

Corneal sensitivity is determined with an esthesiometer, which measures ocular tactile sensitivity. Studies in mammals show differences in corneal sensitivity associated with age, health, iris and coat color, region of cornea tested, and skull type.[23–27] Additionally, the test results can be affected by the patient's temperature and environmental conditions that affect the nylon filament.[23,28] Although there are few studies on corneal sensitivity in birds, a Cochet-Bonnet esthesiometer has been successfully used to evaluate the corneas of multiple species of raptors, and the same technique was used in this study.[23,29]

The Atlantic puffin (*Fratercula arctica*), also known as the common puffin, is a seabird in the auk family (Alcidae). This is the only puffin species to inhabit the rocky coastline of the North Atlantic Ocean.[30] Puffins are represented in many zoos and aquariums, and knowledge of reference interval health parameters is important for the management of this bird species. This study is a preliminary description of ocular parameters, including visual assessment, with quantitative measurements of IOP, tear production, central corneal thickness, and corneal touch threshold in a flock of Atlantic puffins under professional care.

Materials and Methods

Animals

Ten Atlantic puffins housed at the National Aquarium were presented for full ocular examinations in March and April 2012. The group included 5 males and 5 females at ages from 8 months to older than 30 years. Body weight range was 450–536 g at the time of the ocular examinations. Puffins were housed with razorbills (*Alca torda*) and black guillemots (*Cepphus grille*) in a 240 square foot (22.3 m^2) indoor exhibit with concrete rockwork and a 6500-gallon (24 605 L) saltwater pool. Air temperature (7.2–12.8°C [45–55°F]), water temperature (8.9–11.1°C [48–52°F]), and lighting (without ultraviolet B) varied seasonally to mimic natural habitat fluctuations. Puffins were fed daily on a diet of capelin, silversides, smelt, and krill, with a vitamin E and thiamine supplement (Thiamin-E, Stuart Products, Inc, Bedford, TX, USA) administered according to manufacturer instructions. Routine annual examinations (external physical examination, complete blood cell count, and plasma chemistry panel) were completed less than 5 months before the full ophthalmic exams. Diagnostic blood tests from annual examinations showed no significant abnormalities compared with extensive in-house values. One puffin had a history of stifle osteoarthritis, whereas another individual had chronic mild pododermatitis. Animals in the collection were known to have minor lens opacities consistent with incipient cataracts, whereas the 2 oldest birds had more-extensive lens opacities. In 6 birds, the age when lenticular opacities were first noted was 1.3–5.4 years and younger than 2.5 years in 5 of those 6 birds. All birds exhibited behavior and appetite within reference intervals and were considered in good health at the time of full ophthalmic examinations.

Protocol

Birds were manually restrained in an upright position without pressure on jugular veins or eyelids. The same boarded ophthalmologist (J.A.H.) performed all examinations, and the same boarded ophthalmologist (J.A.H.) or ophthalmology resident (R.M.A.) performed the same tests in the same sequence in all birds. In March 2012, all the puffins included in this study had complete ocular examinations with direct and indirect ophthalmoscopy. Slit-lamp biomicroscopy was performed on all birds. In April 2012, ocular examinations were repeated on the same birds, and quantitative measurements were obtained in each eye. Intraocular pressures were measured with a rebound tonometer (Tonovet rebound tonometer, TiOLAT, Helsinki, Finland) on patient (P) setting, the manufacturer's setting for animals other than dogs or horses. The tonometer was held perpendicular to the eye 4–8 mm from the cornea, and the mean of 6 readings from the central cornea was recorded for each bird. Tear production was measured with the phenol red thread tear test by placing the thread under the lower eyelid for 15 seconds and measuring the length of thread showing a color change because of tear production. A Cochet-Bonnet esthesiometer (Luneau L12 esthesiometer, no. 9147, Luneau Technology Operations, Pont de l'Arche, France) was used to measure central corneal touch threshold. An ultrasound pachymeter (Pachy Meter SP-3000, TOMEY Corporation, Nagoya, Japan) was used to obtain central corneal thickness measurements, 60 seconds after 1 drop of proparacaine (proparacaine hydrochloride ophthalmic solution, Akorn Inc, Lake Forest, IL, USA) was placed on the cornea of each eye. The ultrasound pachymeter probe was placed on the center of the cornea at a 90° angle. The average central corneal thickness was calculated from 10 readings (probe speed 1630 m/s) for each eye.

Statistical analysis

Data were expressed as medians and quartiles because of nonnormal distribution, and nonparametric tests were selected for data analysis. Paired comparison exact-permutation tests were used to compare right and left eye values for IOP, tear production, corneal sensitivity, and corneal thickness. Left eye values were chosen when there were no significant differences between left and right eye values for ocular tests. Random sampling two-sample permutation tests ($R = 1000$) were used to compare male and female left eye values for IOP, tear production, corneal sensitivity, and corneal thickness. The significance level for all tests was set at $\alpha = .05$. Spearman rank correlation coefficients were calculated for age compared with IOP, tear production, corneal sensitivity, and corneal thickness. Values for r were considered moderately to strongly correlated at $\pm 0.5 < r < 1.0$. All statistical analyses were completed with R version 3.2.0 software (R Core Team, Vienna, Austria).[31]

Results

Ocular examination findings by direct and indirect ophthalmoscopy are listed in Table 1. No corneal abnormalities were noted. The 2 youngest puffins (younger than 1 year) had no evidence of lenticular changes. Six birds had bilateral punctate axial anterior subcapsular cataracts (incipient cataracts). One puffin had bilateral flocculent axial anterior subcapsular opacities. One bird had hypermature cataracts and dyscoria with uveitis and was excluded from further evaluation; the remaining 9 birds had no evidence of inflammation or other ocular pathology. Although sample size precluded statistical testing, there appeared to be an association between opacities and increasing age. Posterior segment examinations, including visualization of the atapetal avascular fundus and the pectin, were completed on the 9 birds and were considered within reference intervals.

Table 1. Ocular abnormalities found on direct and indirect ophthalmoscopy in a colony of captive Atlantic puffins, as described in Figure 1, sorted by age.

Puffin	Age, y/mo/d	Left eye	Right eye
1	0/8/2	No abnormalities	No abnormalities
2	0/8/9	No abnormalities	No abnormalities
3	1/8/13	PAASO	PAASO
4	5/8/13	PAASO	PAASO
5	8/9/2	PAASO	PAASO
6	8/9/5	PAASO	PAASO
7	9/9/6	PAASO, focal nuclear opacity	PAASO, focal nuclear opacity
8	14/6/7	PAASO, insipient bladder cells or PPT on posterior capsule at 7 o'clock	PAASO, insipient bladder cells or PPT on posterior capsule at 4 o'clock
9	18/8/19	Flocculant axial anterior subcapsular opacity	Flocculant axial anterior subcapsular opacity, more severe
10	30/7/27	Hypermature cataract, dyscoria, lens-induced uveitis	Hypermature cataract, dyscoria, lens-induced uveitis, less severe

Abbreviations: PAASO indicates punctate axial anterior subcapsular opacity; PPT, precipitate.

Bilateral measurements of IOP, corneal thickness, and corneal sensitivity were successfully collected on all 9 birds. Tear production measurements were collected from 8 of 9 puffins; 1 bird's results were excluded from data analysis because the tear production was too low for accurate assessment. There were no significant differences found between right and left eye measurements of IOP, corneal thickness, or corneal sensitivity (Table 2). There was a significant difference between right and left eye measurements for tear production ($P = .03$), with the right eye producing more tears than the left eye (Table 2). However, 1 puffin was determined to be an outlier, and when removed, there was no longer a significant difference ($P = .38$) (Table 2). Because of the lack of significant findings between right and left eyes, results from the left eyes were chosen for comparisons between males and females for all ocular parameters. There was no significant difference between sex and IOP, tear production, and corneal sensitivity. However, there was a significant difference between sex and corneal thickness ($P = .02$): males (left eye median, 249 μm, interquartile range [IQR], 241–249 μm) had thicker corneas than females (left eye median, 236 μm, IQR 234–238 μm). Male puffins were generally larger than females (males, 454–536 g; females, 450–476 g), although no significant correlation between weight and corneal thickness was detected. There were no associations between age and IOP, tear production, or corneal thickness. There was moderate correlation between age and corneal sensitivity, with older birds showing decreased corneal sensitivity (left eye, $r = -0.57$; right eye, $r = -0.28$) (Fig 1).

Table 2. Median and interquartile range values (IQR) for right (OD), left (OS), and both (OU) eye ocular measurements (intraocular pressure [IOP], central corneal thickness, corneal sensitivity, and tear production) in a colony of Atlantic puffins (*Fratercula arctica*).

Parameter	Eye	n	Median	IQR
IOP, mm Hg	OD	9	13	12–15
	OS	9	13	13–15
	OU	9	13	12–15
Central corneal thickness, μm	OD	9	236	230–248
	OS	9	241	236–249
	OU	9	241	233–248
Corneal sensitivity, cm	OD	9	1.25	0.75–1.50
	OS	9	1.00	1.00–1.50
	OU	9	1.13	0.81–1.50
Tear production, mm/15 s	OD	8	7.5	6.5–9.3
	OS	8	5.0	4.0–7.3
	OU	7	7.0	4.6–8.0

Discussion

The results of this study show that IOP, tear production, corneal thickness, and corneal sensitivity can be successfully measured in Atlantic puffins. Ocular measurements are important when evaluating avian health, particularly when evaluating ocular pathology, and baseline values need to be available for each species because of the interspecies variability. Though the sample size was small in this study, these findings provide preliminary ranges for ocular parameters in Atlantic puffins in similar environmental conditions.

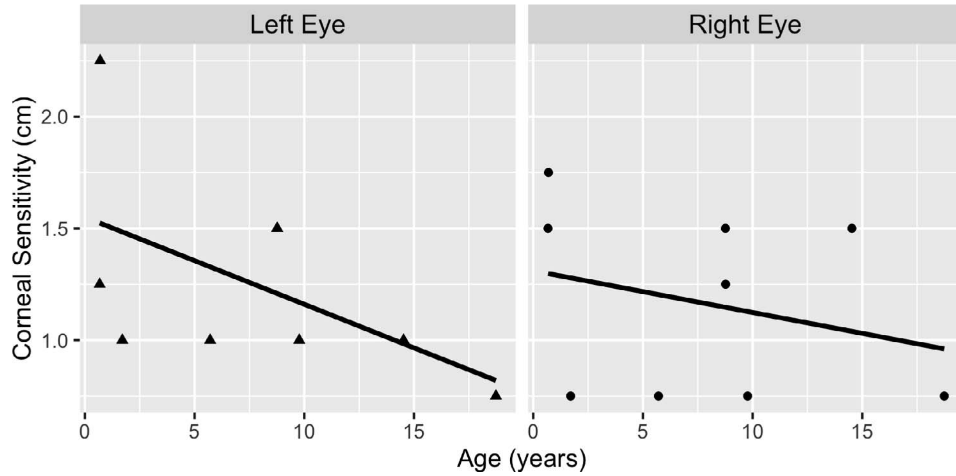


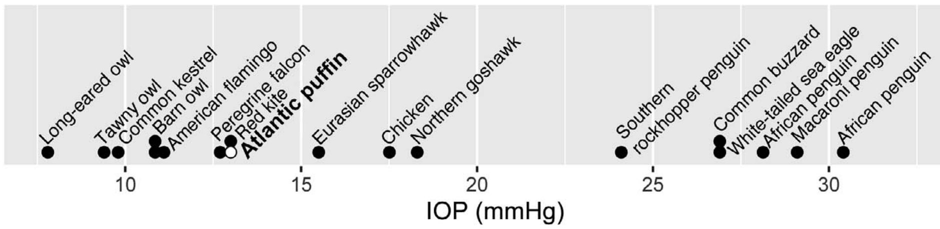
Figure 1. Scatterplot with line of best fit (linear regression line) of corneal sensitivity in 9 Atlantic puffins (5 males, 4 females, ages 8 months to 19 years) maintained in an indoor, mixed species, climate-controlled environment with access to a salt water pool. Results showed a moderate negative correlation (left eye, $r = -0.57$; right eye, $r = -0.28$) between increased age and decreased corneal sensitivity.

Slit lamp biomicroscopy, direct, and indirect ophthalmoscopy showed no abnormalities of the cornea or posterior segment in any of the subject birds. Cataracts were common but generally small, focal, and subcapsular and, apart from 1 older bird, showed no association with intraocular inflammation. The older bird with hypermature cataracts was removed from the study and excluded from data analysis. Cataracts appear to be relatively common in captive Arctic and Antarctic sea birds, including the southern rockhopper (*Eudyptes chrysocome*), macaroni (*Eudyptes chrysolophus*), African (*Spheniscus demersus*) and Humboldt penguins, tufted puffins (*Fratercula cirrhata*), and Inca terns (*Larosterna inca*).[3,6,13,32] The etiology of cataracts in these birds is unknown, but cataracts can be associated with trauma, inflammation, infection, nutritional deficiencies, toxins, excessive ultraviolet light, genetics, and increased age.[6,33–39] Where cataracts are associated with lens-induced uveitis, IOP is often found to be significantly decreased.[40] The IOPs from the 2 puffins in this study without cataracts were similar to the IOPs for puffins with cataracts and no visual evidence of uveitis. Because of the lack of any evidence for conditions that would significantly affect ocular diagnostic readings, ocular parameters were reported in these 9 puffins.

In this research study, puffin IOP was measured by rebound tonometry. It is important to account for the method of IOP measurement (applanation versus rebound tonometry) when making comparisons.[6,41] Avian IOPs measured by rebound tonometry vary significantly across species, with the median value in this puffin colony (13 mm Hg, IQR 12 – 15 mm Hg) being most similar to peregrine falcons (*Falco peregrinus*), and red kites (*Milvus milvu*) (Fig 2). This variability highlights the need to establish IOP reference intervals for each avian species.

In addition to species and measurement method, IOP is affected by other factors, such as sex, age, and body position. Other avian IOP studies have found no significant difference between right and left eyes or sexes, but some have found a correlation with age.[1,42] In a study of clinically normal African penguins, a correlation was found between increasing age and decreasing IOP.[15] Another study found significantly lower IOP in juvenile goshawks (*Accipiter gentilis*), common kestrels (*Falco tinnunculus*), and common buzzards (*Buteo buteo*) compared with adults.[1] The puffin IOPs in this study had no correlation with the sex or age of birds. The lack of correlation between age and IOP may be due to the small sample size of this study, although ages were generally evenly distributed. Intraocular pressure can also be affected by the position of the bird.[1,5] To avoid unnecessary variation among IOP measurements in this study, birds were all held upright, and measurements were made by the same investigator.

Puffin tear production was measured by the minimally invasive phenol red thread test. The test was easy to use on the small puffin eyes. Compared with other avian species (mean ± SD), tear production in puffins (intraocular pressure for both eyes [OU] median, 7.0 mm/15 s, IQR 4.6–8.0 mm/15 s) was lower than Amazon parrots



Family	Genus species	Common name	IOP
Accipitridae	*Accipiter gentilis*	Northern goshawk	18.3 +/- 3.8
	Accipiter nisus	Eurasian sparrowhawk	15.5 +/- 2.5
	Buteo buteo	Common buzzard	26.9 +/- 7.0
	Haliaeetus albicilla	White-tailed sea eagle	26.9 +/- 5.8
	Milvus milvu	Red kite	13.0 +/- 5.5
Falconidae	*Falco perequinus*	Peregrine falcon	12.7 +/- 5.8
	Falco tinnunculus	Common kestrel	9.8 +/- 2.5
Phasianidae	*Gallus gallus domesticus*	Chicken	17.51 +/- 0.13
Phoenicopteridae	*Phoenicopterus ruber*	Flamingo	11.1 +/- 2.3 (OS) 10.9 +/- 1.8 (OD)
Spheniscidae	*Eudyptes chrysocome*	Southern rockhopper penguin	24.1 +/- 5.09
	Eudyptes chrysolophus	Macaroni penguin	29.1 +/- 7.16
	Spheniscus demersus	African penguin	28.13 +/- 6.84 (OS) 30.41 +/- 4.27 (OD)
Strigidae	*Asio otus*	Long-eared owl	7.8 +/- 3.2
	Strix aluco	Tawny owl	9.4 +/- 4.1
Tytonidae	*Tyto alba*	Barn owl	10.8 +/- 3.8

Abbreviations: OD = right eye, OS = left eye

Figure 2. Dot plot and list of mean intraocular pressure (IOP) values (mean mm Hg ± SD) by rebound tonometry reported from the literature compared with the median IOP by rebound tonometry in Atlantic puffins from this study, as described in Figure 1 (13 mm Hg).[1,3,5,6,15,49]

(*Amazona ventralis*; 12.5 ± 5 mm/15 s), screech owls (*Megascops asio*; 15 ± 4.3 mm/15 s), various large birds of the order Psittaciformes (19.1 ± 3.3–20.1 ± 3.9 mm/15 s), macaroni penguins (24.7 ± 6.37 mm/15 s), southern rockhopper penguins (25.1 ± 7.07 mm/15 s), and birds of the order Falconiformes (30.6 ± 4.2 mm/15 s).[6,8,9,42–44] Puffin tear production was similar to that of the long-eared owl (*Asio otus*; 8.0 ± 2.8 mm/15 s) in one study.[9] This emphasizes the need to establish reference interval tear production ranges for each species. The difference may be due to the different ocular anatomy and physiology across avian species or may be specific to the environmental conditions of the colony.[43] One study that used the Schirmer tear test in Humboldt penguins found that individuals housed in freshwater showed nearly twice the tear production of those housed in saltwater.[13] The puffins in this study were housed in saltwater, and this should be considered when comparing tear production values. In addition to the environment, other factors, such as sex and age, may affect tear production. In mammals, tear production can decrease with increasing age.[10] One study found that black vulture (*Aegypius monachus*) hatchlings have higher tear production than adults; therefore, other avian species may show this trend.[12] In this study, there were no significant correlations between age or sex and tear production.

There was a significant difference in tear production between right and left eyes, with the right eye producing more tears than the left. The small sample size likely contributed to this finding because removal of one outlier resulted in a nonsignificant difference in tear production values between right and left eyes. Other studies have not typically found significant differences between right and left eye tear production.[6,9,42,43] However, in one study that used the Schirmer tear test in vultures, black vultures ($n = 54$) had significantly higher tear production in the left than the right eye.[12]

Central corneal thickness measurements with ultrasound pachymetry were easily obtained from the puffins evaluated in this study. Puffin central corneal thickness (OU median, 241 μm; IQR 233–248 μm) is similar to domestic chickens (mean ± SD, 242 ± 0.2 μm) and less than that of African penguins (mean ± SD, 384.08 ± 30.9 μm).[15,21] Similar to the puffins in this study, domestic chickens and striped owls (*Rhinoptynx clamator*) do not have significant differences between right and left corneal thickness.[16,45] Other factors that may affect central corneal thickness include sex, age, and location of corneal measurement. Studies comparing relationship between sex and body weight and corneal thickness in birds have shown variable results.[16,45] Male puffins had significantly thicker corneas than females did in this investigation, similar to findings in striped owls.[45] In striped owls, females are larger than males, which is in contrast to puffins.[45] Despite seasonal fluctuations in puffin body weight, male puffins are typically heavier than females, which was corroborated in this study.[30] However, individual puffin weight did not correlate with corneal thickness measurements. In a study of domestic chickens, no sex differences were found with respect to corneal thickness.[16]

There was no correlation between puffin age and central corneal thickness, which is similar to findings in African penguins and striped owls.[15,45] A study in domestic chickens showed that central corneal thickness initially decreased as chicks matured, but in adulthood, there was no correlation between corneal thickness and age.[16] At the time of this study, there were no chicks to assess whether corneal thickness changes as puffins mature. Only central corneal measurements were obtained from the puffins; however, future investigations looking at the effect of measurement location may also be informative.

Puffin corneal sensitivity was also successfully measured in this study, with no significant differences between right and left eyes or sex of bird. Compared with other avian species, puffins (OU, 1.13 cm; IQR 0.81–1.50 cm) appear to have less-sensitive corneas than some bird of prey species (mean ± SD): little owls (*Athene noctua*) (3.5 ± 2.1 cm), common kestrels (4.9 ± 1.7 cm), Eurasian scops owl (*Otus scops*) (5.5 ± 0.9 cm), and tawny owls (*Strix aluco*) (5.7 ± 0.8 cm).[29] Puffins have more sensitive corneas than Amazon parrots (mean, 0.133 cm).[8] Previous studies in mammals show that the region of cornea tested influences corneal sensitivity results, with the central cornea being the most sensitive.[25,46,47] However, no location differences in corneal sensitivity have been found in birds of prey.[29] Only the central cornea was tested in the puffins. Environmental temperature and humidity were consistent during testing and should have had no effect on the results of corneal sensitivity measurements.

Puffin eyes showed a negative correlation between age and corneal sensitivity. In a bird of prey study, adult birds had less-sensitive corneas than fledglings (mean, 4.1 ± 2 cm compared with 5.4 ± 1.2 cm).[29] This trend of increased age associated with decreased corneal sensitivity is also reported in horses, alpacas, and humans.[23,24,48]

Although most puffins in this study had incipient cataracts, the individuals that were analyzed had no evidence of pathology that would interfere with the assessed diagnostic results. This study of ocular reference interval parameters in a colony of Atlantic puffins showed that IOP/rebound tonometry, tear production/phenol red thread test, corneal thickness/ultrasound pachymetry, and corneal sensitivity/esthesiometry can be successfully measured. Puffin ocular parameter values were different from other avian species, highlighting the need for species-specific values. The values reported here should serve as an initial reference for Atlantic puffins under similar environmental conditions.

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