Can penguins (Spheniscidae) see in the ultraviolet spectrum?

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Title page

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Running head: Penguin ocular transmission

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Abstract

It has been postulated that ultraviolet reflectance is important in mate choice in King Penguins *Aptenodytes patagonicus*, although not in other penguin species that do not have body parts that reflect UV light. However, this theory has been challenged. Here we aimed to determine the transmission of the ocular media in the large King Penguin as well as the smallest penguin, the Little Penguin *Eudyptula minor*, and a medium-sized penguin, the Gentoo Penguin *Pygoscelis papua*, to determine if the penguin eye is capable of seeing ultraviolet light. In all species the cornea absorbed the most damaging rays at 300 nm or below but it was the lens that predominantly determined the transmission of light between 300 and 400 nm. The lenses of a young King Penguin absorbed almost all light less than 370 nm and had 50% transmission at 406 nm, thus ultraviolet perception in the King Penguin is very limited. In contrast, 50% lenticular transmission was 329 nm in the Little Penguin and 367 nm in the Gentoo. Therefore, we suspect that ultraviolet light may be more important in the behaviour of smaller penguins than in the King Penguin, where it is unlikely to play a significant role.

Keywords

Spheniscidae, lens, cornea, ultraviolet, Gentoo, King, Little

Statements and declarations

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Ethics approval

All procedures performed in the study were in accordance with the ARVO Statement for Use of Animals in Ophthalmic Vision and Research. Ethical approvals were obtained from the New Zealand Department of Conservation (permit numbers 68003-DOA, 28 November 2018 and 89983-DOA, 27 July 2021), Auckland Zoo and SEA LIFE (SL (G) – AR 001

Main document

Introduction

Ultraviolet (UV) reflectance and ultraviolet-sensitive (UVS) vision are thought to play a role in many avian behaviors, including orientation, foraging and sexual selection (Bennett et al. 1996; Johnsen et al. 1998). Color also appears to be important in penguin (Spheniscidae) mate selection (Jouventin 1982; Massaro et al. 2003). The orange beak spots of both King *Aptenodytes patagonicus* and Emperor *A. forsteri* penguins have been found to have UV reflectance (Jouventin et al. 2005) and, in the case of the King Penguin, a multilayer photonic microstructure appears to be the source of this reflectance, which is maximal at 367.5 nm (Dresp et al. 2005). Beak reflectance has also been reported in juvenile Gentoo Penguins *Pygoscelis papua* (Meyer-Rochow and Shimoyama 2008). However, similar beak reflectance has not been found in 10 other species of penguin, nor on the claws, feathers or skin of any penguin (Jouventin et al. 2005).

This finding, together with the observations that UV reflectance from the beak only appeared when the animal was sexually mature and of pairing behavior, led to the suggestion that such UV reflection could be important in King and Emperor penguin breeding (Jouventin et al. 2005). This would appear to be supported by a higher UV beak reflectance amongst early pairing King Penguins (the most successful in breeding) and the observation that experimentally reduced UV reflectance from beak spots is associated with delayed pairing (Jouventin et al. 2009; Nolan et al. 2010). However, this interpretation was challenged on the basis that UVS vision had not been demonstrated in penguins (Meyer-Rochow et al. 2008), leading to a fierce debate in this journal (Jouventin et al. 2009; Meyer-Rochow et al. 2009; Nolan et al. 2010).

Ultraviolet-sensitive visual pigments (<400 nm) are widespread in the animal kingdom (Hunt et al. 2001) and in birds a UVS visual pigment is thought to have evolved from ancestral violet vision on multiple occasions (Wilkie et al. 2000; Hart et al. 2007), although molecular evidence suggests that UVS cones are more widespread in terrestrial birds than in seabirds (Hart and Hunt 2007). A UVS

cone has not been found in penguins; however, a short wavelength 1 (SWS1) cone with maximum sensitivity at 403 – 405 nm has been identified in the Humboldt Penguin *Spheniscus humboldti* (Bowmaker et al. 1985; Wilkie et al. 2000), very close to the UV range. More recently, Gentoo Penguins have been shown to respond behaviourally to UV light maximal at 365 nm, tapering to no output at 390 nm (Cole et al. 2022). Although, this visual pigment does not have maximal absorbance in the UV range, it is close to that range (<400 nm) and the absence of UVS visual pigments is not evidence of blindness to UV, as there is a significant secondary absorption (β-peak) by all visual pigments in the UV range despite maximum absorbance in the visible spectrum (Douglas et al. 2014). This can be demonstrated by the ability of aphakic humans to see in the UV spectrum despite lacking a UVS cone (Stark et al. 1994).

Because of this secondary absorption, ocular transmission is more critical to UVS vision than the presence of a UVS cone. Many animals have been shown to have ocular media that allow the transmission of near-ultraviolet (near-UV, <400 nm) light, including many bird species (Emmerton et al. 1980; Siebeck et al. 2001; Mullen et al. 2007; Tsukahara et al. 2013; Lind 2014; Olsson et al. 2021). Although all biological tissues are opaque to light below 300 nm due to absorption by molecules such as nucleic acids and aromatic amino acids, in birds as in mammals the lens is usually more critical than the cornea with regard to UV light transmission, particularly above 345 nm (Douglas et al. 1999; Douglas and Jeffery 2014; Olsson et al. 2021).

Clearly, in order for UV reflectance to be important in mate selection such reflectance has to be visible. Given the debate, the primary aim of this study was to determine the ocular transmission of the King Penguin eye and thus determine the potential sensitivity of its visual system to UV light. Little Penguins (*Eudyptula minor*) and Gentoo Penguins were also available to us in Auckland, New Zealand, and represent a different lineage within Spheniscidae (Zusi 1975; Baker et al. 2006; Cole et al. 2022), with recent DNA evidence (Vianna et al. 2020) suggesting that King and Emporer penguins form a sister clade to all other extant penguins. Little and Gentoo penguins are also of different sizes

(Shirihai 2007), dive to different depths (Montague 1985; Culik et al. 1996) and inhabit vastly different environments (Shirihai 2007) to the Kings, therefore we also wished to determine if these findings were consistent across all penguins.

Methods

Animals and ethics

Permission was obtained for this study from the New Zealand Department of Conservation (permit numbers 68003-DOA, 28 November 2018 and 89983-DOA, 27 July 2021), Auckland Zoo and SEA LIFE (SL (G) – AR 001). A Little Penguin (L1) was recovered from the wild in the Auckland Region, New Zealand, but was unable to be re-released due to a physical disability not involving the eye. Its exact age was unknown, although it was an adult bird and had passed its first moult. Two Gentoo Penguins were examined, G1 aged 7 weeks (lens) and G2 aged 26 years (cornea and vitreous), as well as two King Penguins, K1 aged 23 months and K2 aged 32 years. The Gentoo and King penguins had spent their life in captivity at SEA LIFE Kelly Tarlton's Aquarium in Auckland, New Zealand and were descended from penguins living in South Georgia. With the exception of K2, all examinations were completed within 6 hours of enucleation post-mortem, and none had any known ocular disease. The vitreous of K2 was examined after 24 hours of refrigeration and this animal had moderate cataract; only the vitreous from this eye was examined (Table 1). Several other eyes of all three species were examined to check the accuracy of the two spectrometers that we used against each other. However, we did not use these results as they were from elderly penguins, hadspent time refrigerated, or both. Moreover, their lenticular ocular transmission was not as great as the penguins that we included in this study, probably because of their age and preservation, and we did not wish to combine the results for fear of underestimating potential ocular transmission in the youngest and healthiest eyes of each species, our primary goal being to determine if the penguin optical media

could transmit UV. The results of the right eye of G1 were also discarded, as they were not credible (substantially more than 100% transmission at some wavelengths).

Suggested location of Table 1

Equipment and tissue preparation

Two spectrometers were used. The first was a USB2000+ (Ocean Optics, Dunedin, Florida, USA, Fig. 1), which could be transported to peripheral locations. This was used for G1 and K1. The second was a SpectraMax 13x (Molecular Devices Inc, San Jose, CA), into which the eyes or components thereof were inserted, and which could only be used when the central laboratory was accessible (Fig. 2). This was used for L1, G2 and K2. It was only possible to measure vitreous transmission using the SpectraMax 13x.

The spectral transmission of the whole eye, cornea and lens were measured in L1 and K1. In G1, only the transmission of the lens was measured. Vitreous samples of L1, G2 and K2 were also measured, as were the corneas of G2, noting that these were older animals but being reassured of their validity as noted above and in the knowledge that, in all birds, it is the lens that determines ocular transmission in any event (Olsson et al. 2021). To measure transmission through the whole eye, the eye was enucleated and then the posterior pole was removed with a Bard-Parker 15 blade (Aspen Surgical Products, Inc., Caledonia, MI). To measure transmission through the cornea, the cornea was then removed and placed in the spectrometer. Then, having removed the cornea, the vitreous was removed as much as possible without touching the lens, leaving the lens attached to the ciliary body (and thus the rigid ossicular ring) for stability so that we could avoid handling the lens. The residual anterior segment, minus cornea, was then inserted into the spectrometer.

Data acquisition using SpectraMax 13x

A plate blank and a well filled with blackened paper tissue were used to verify the calibration. The tissue being measured was then placed into a plastic tray, cushioned with paper for stability (Fig. 1)

and inserted into the machine where it lay immediately above the detector, separated from the latter only by the floor of the clear plastic container. To measure the transmission of the vitreous, a well 14.5 mm high was filled with liquid vitreous. Percentage transmission was directly measured by the machine in steps of 10 nm. This was then calculated as a percentage of the transmission at 700 nm (T_λ). Values obtained for light less than 300 nm were ignored as very little light was transmitted at these wavelengths through the plate blank using the SpectraMax 13x and thus the values obtained for transmission of ocular media as a percentage of transmission by the plate blank were erroneous. An exception was made and the value at 290 nm used if necessary to calculate an intercept, using the method described below (primarily the vitreous λ_{50} but also the cornea λ_{10}).

Suggested location of Figure 1

Data acquisition using Ocean Optics USB2000+

The tissue being measured was placed in a black case that was custom made at the University of Auckland using a 3D plastic printer to keep it steady and to eliminate external sources of light (Fig. 2). Multiple recordings were obtained, and the maximum spectral transmission recorded. For purposes of calibration, the spectrum without light on and with just the xenon light on was recorded using the USB2000+. When the whole eye or the lens (attached to the ciliary body) was being measured, this was placed as close as possible to the detector and cushioned for stability by tissue paper. When the cornea was being measured, it was draped over the detector. The Oceans Optics USB2000+ measured absolute transmission in steps of 0.38 to 0.39 nm. The transmission of each wavelength (T_λ) measured using the USB2000+ was calculated by first subtracting the background light (b_λ) measured at that wavelength within the closed box from that measured with the Xenon source on (Xe_λ) and then comparing that to the light transmitted with both the Xe source on and the optical element under investigation being in the path of the beam (Xt_λ).

Thus $T_\lambda = (Xt_\lambda - b_\lambda) / (Xe_\lambda - b_\lambda)$

This was then calculated as a percentage ($T_{\%}$) of the average transmission between 650 and 750 nm (T_{700}). Due to the small transmission interval of 0.38 to 0.39 nm, there was variability between individual measurements. Therefore, the data was first exponentially smoothed with a damping value of 0.5 using Microsoft 365® Excel. Transmission percentages for light less than 300 nm were ignored on the USB2000+, both for consistency with the SpectraMax and because they appeared to be highly variable, probably a function of how little light was produced by the Xenon source at those wavelengths as compared to background.

Suggested location of Figure 2

Data analysis and plotting

Since, except for G1, both eyes were examined, the results from each eye were first averaged and then the average T_{λ} for each species, at 10 nm intervals, was plotted against the respective wavelengths. The wavelength at which the cornea, lens, vitreous and whole eye reached 10% (λ_{10}), 50% (λ_{50}) and 90% (λ_{90}) of that recorded at 700 nm for species was determined by using the forecast function of Microsoft 365® Excel to predict where the 10%, 50% and 90% intercepts would be based on the two recordings 10 nm and 20 nm before 10%, 50% or 90% transmission was reached and the two recordings 10 and 20 nm after.

Results

The averaged transmission curves, combining both eyes of each penguin (except in the case of the lens of G1, which was only the left eye), are presented in figures 3, 4, 5 and 6. λ_{10}, λ_{50} and λ_{90} intercepts are listed in table 2.

Suggested location of Figure 3, Figure 4, Figure 5 and Figure 6

Suggested location of Table 2

The dimensions of eyes of these penguins have been the subject of a previous report (Hadden et al. 2022). The average corneal thickness (measured with ultrasound pachymetry) was 0.303, 0.472 and 0.605 mm in the Little, Gentoo and King penguins respectively, the lens thickness (measured with callipers) 4.0, 7.14 and 7.75 mm, excluding the lens of K2 which was cataractous and 10 mm thick, and the axial length (measured with ultrasound) 17.4, 21.7 and 26.5 mm respectively.

Discussion

Importance of UV reflectance in King Penguin mate selection

Because of reduced lenticular UV transmission, the eye of the King Penguin transmitted very little UV light, with almost all light at wavelengths less than 370 nm being blocked and 50% transmission only being reached at 406 nm. In King Penguins, the only part of the body that reflects UV are the orange bands of the beak (Jouventin et al. 2005). The UV peak in these bands, according to the literature, is at 401 nm in museum specimens and 380 nm in live penguins (Jouventin et al. 2005). In a King Penguin taxidermy available at CEFE (Univ Montpellier, CNRS, EPHE, IRD, Montpellier, France), the UV peak of the bands of the beak, as measured with an Avantes spectrophotometer, is at 360 nm (FB personal data). Our results, showing that no light below 370 nm and only half of the light even at 400 nm is transmitted to the retina in this species, suggests that the use of UV in mate choice behaviour (Jouventin et al. 2005; Nolan et al. 2010) should be reconsidered, or at least confirmed with further experimental protocols. We also note that, in the later paper (Nolan et al. 2010), the range of the spectrum examined in the experiment was from 320 nm to 450 nm, thus including some visible light (> 400 nm), which could have been easily perceived by King Penguins.

Variation between species

The cornea of all specimens examined absorbed almost all light at or below 300 nm (Fig. 3), consistent with what is known about the absorption of all biological tissues generally (Douglas et al.

1999). However, in contrast to King Penguin, the lens of the Little Penguin did not further restrict the ability of UV light to be perceived at the retina and thus the animal will be more sensitive to light of these wavelengths. The Gentoo Penguin eye would appear to lie somewhere between these two, although clearly the optical transmission would enable it to see the 365 nm torch previously reported (Cole et al. 2022). The behavioural relevance of this finding in these species is unclear. In regard to mate selection, adult Gentoo Penguins have no body parts that reflect UV, although beak reflectance has been reported in juveniles (Jouventin et al. 2005; Meyer-Rochow and Shimoyama 2008).

Why are shorter wavelengths transmitted more readily through the lenses of Little and Gentoo penguins but absorbed in the King? There is a weak association between lens thickness and UV transmission in birds (Olsson et al. 2021), presumably because of the increased tissue thickness across which light can be absorbed. However, the difference in UV transmission between the lens of the Little Penguin and the King Penguin is much more marked than that between the corneas of the two species, despite both anatomical structures being proportionately thicker in the King Penguin (Hadden et al. 2022). Furthermore, despite the aforementioned weak association, many birds have a higher λ_{50} than would be expected from eye size; for instance, the Long-eared Owl *Asio otus* has a 7.0 mm thick lens but a lenticular λ_{50} of 323.6 nm (Olsson et al. 2021).

An environmental difference seems unlikely, despite the habitat of King and Little penguins being markedly dissimilar, because of the relatively high UV transmission of the Gentoo Penguin lens despite a habitat much more similar to the King Penguin than to the Little Penguin. Both King and Gentoo penguins predominantly breed on subantarctic islands, although the gentoo can also be found on the Antarctic Peninsula and both can be found as rare vagrants on the New Zealand mainland (Shirihai 2007). The Gentoo and King penguins in our study were also both of South Georgian descent and therefore share the same pelagic environment, although King Penguins hunt in open pelagic waters down to 300 m, which seems to correlate with their main prey, the midwater

lanternfishes Myctophidae (Kooyman et al. 1992), while gentoos, which at South Georgia have been recorded diving to over 100 m (Croxall et al. 1988), are both nearshore benthic and pelagic foragers, consuming crustaceans, particularly Antarctic krill *Euphasia superba* and *Themisto gaudichaudii,* as well as fish (Xavier et al. 2017; McClintock et al. 2020). The latitudes in which they live means that the hours of night and day are more a function of seasonality than time of day and they forage at all times of the year. By contrast, Little Penguins (grouping both *Eudyptula minor* and *E. novaehollandiae* together) inhabit mainland New Zealand, southern Australia and Tasmania (Shirihai 2007; Grosser et al. 2017). They feed on small shoaling fish (particularly Clupeiformes), cephalopods and crustaceans such as krill and forage at shallower depths, around 10-50 m (Montague 1985; Gales et al. 1990; Chiaradia et al. 2007; Iida et al. 2014). Little Penguins also breed in burrows, under rocks and in other hollows, in dunes or amongst vegetation, rather than on the harsher shores of South Georgia; they can be active at night but are predominantly diurnal foragers (Shirihai 2007). The lower latitudes in which Little Penguin live, with illumination coming from more directly overhead, means that the Little Penguin lives in a generally brighter environment; the atmospheric transmission of UV also increases with increasing solar elevation in a similar fashion to light in the visible spectrum (Spitschan et al. 2016). However, if this greater luminance is the explanation for increased UV transmission in the Little Penguin, it is one that is contrary to the tendency of diurnal mammals to block UV (Douglas et al. 2014).

It might be that the larger eyes of the larger King Penguin block UV light to maximise the increased visual acuity their size affords, since the elimination of UV reduces chromatic aberration, to which their large pupils renders them more sensitive; elimination of UV will also reduce Rayleigh scatter (Walls 1931; Pye 2011; Douglas and Jeffery 2014; Olsson et al. 2021). Also, given the β-peak in the UV range possessed by all visual pigments, allowing the transmission of UV light would make the task of color differentiation much more difficult (Pye 2011). There would appear to be a correlation between larger eyes, which generally possess higher spatial sensitivity given that more pixels (photoreceptors) can be made available per visual angle, and lack of transparency of the lens to UV

(Walls 1931; Ambach et al. 1994;Tsukahara et al. 2013; Douglas and Jeffery 2014). Furthermore, the ocular media of raptors, which are notable for their excellent spatial sensitivity, up to 140 cycles / degree in the Golden Eagle *Aquila audax*, are relatively opaque to UV, with 50% transmissions ranging from 369 nm to 394 nm in those that have been studied, although this does not include the Golden Eagle (Reymond 1985; Lind et al. 2013; Potier et al. 2016). In humans, a drop in potential visual acuity has been documented in pseudophakic individuals who have intraocular lenses that do not absorb UV, although intraocular lenses may introduce retinal images that do not occur in the phakic eye (Rog et al. 1986). On the other hand, the potential spatial resolution of the King Penguin eye (20.40 cycles / degree in water) is only marginally greater than that of the Little Penguin (17.07-17.46 cycles / degree in water), despite its greater size, because of a lesser ganglion cell density (Coimbra et al. 2012). Of course, this resolution was based on morphological ganglion cell counts rather than actual measurements of acuity so may not reflect the true spatial sensitivity.

urthermore, UV transmission is of less relevance underwater, the environment in which penguins forage, because it is more rapidly attenuated than are wavelengths in the visible spectrum (Williams 1973). Attenuation also increases with depth, which suggests it may be even less relevant in the deeper diving King Penguin than in the Little Penguin, although the latter forage in more estuarine waters where UV attenuation is higher (Zielinski 2013).

Limitations and further work

We suspect there is an artifactual drop-off in the transmission of the left lens of G1, particularly above 50% transmission, given that the rise in shortwave transmission is less rapid above this point than in the King Penguin despite a much lower λ_{10} and a substantially lower λ_{50}. This could be due to a greater separation of the tissue from detector in the USB2000+ by mistake, increasing chromatic aberration which is more significant at shorter wavelengths. This could also have been the reason why the reading from the right lens was not credible. There was also a dip in transmission at 410 to 420 nm in the cornea of G2 and in the vitreous of all animals. 420 nm is an absorption peak of

oxygenated hemoglobin, and we speculate that some tissues may have been contaminated by blood when they were dissected out and placed in the spectrometer. Such contamination may mean that the λ_{90} measurements are less reliable than the others, particularly in the case of the cornea of G2 and the lens of G1.

In the future, more individuals per species across different ages could be tested to confirm these results and, for those species that can see UV, experimental behavioural protocols should be designed to confirm the use of potential UV cues. In addition, more species of penguin could be studied to determine if the apparent relationship between size and UV transmission in these three species of penguin is consistent across the family. For instance, it would be useful to know whether the lens of the larger Emperor Penguin blocks UV light, given that it shares the same lineage as the King and UV reflection off the beak has been demonstrated (Jouventin et al. 2005). It would also be interesting to understand if Galápagos (*Spheniscus mendiculus*) or African (Jackass, Black-footed, *Spheniscus demersus*) penguins, which inhabit yet lower latitudes than the Little Penguin, transmit UV to a similar extent to that observed in the similarly sized but subantarctic Gentoo Penguin. It would also be useful to correlate behaviour and vision between species with different UV transmissions, for instance in mate choice. Previous authors have speculated that, since the UVS form of the SWS opsin is ancestral, a lens that transmits UV may also be ancestral (Olsson et al. 2021). Investigation of the spectral transmission of oil droplets in the retina would also be instructive, as light passes through them before being detected by cone photoreceptor outer segments. The King Penguin retina has been found to contain only pale green droplets, similar to nocturnal birds. This may reflect a habitat of little colour and their need to see well at depth in a dark sea, necessitating maximisation of retinal sensitivity. Maximal absorption of these droplets was between 500 and 600 nm; the absorption in UV wavelengths was not measured but it was approximately 0.35 at 400 nm (Gondo and Ando 1995). Humboldt *Spheniscus humboldti* and Rockhopper *Eudyptes chrysocome* penguins were found to have droplets of four different colours, similar to diurnal birds and potentially because they live in areas with vegetation (Gondo and Ando

1995). The closer phylogenetic relationship (Cole 2022) and, for the Little Penguin, habitat similarity might suggest that Gentoo and Little penguin oil droplets may be more akin those in Humboldt and Rockhopper penguins than to those in King Penguins. However, they have not been examined in this regard and the similarly related Magellanic Penguin *Spheniscus magellanicus* was found to possess yet different droplets (Suburo and Scolaro 1999). Finally, cataract is known to be associated with UV exposure (Delcourt et al. 2014). Although UV radiation has been shown to cause cataract in Sprague-Dawley rats (Michael et al. 1998), where it is unlikely that the lens constitutes an additional barrier to UV transmission over and above the cornea (Douglas and Jeffery 2014), it would be useful to know if absorbing this energy in the lens rather than transmitting it increases the risk of cataract; if this was the case, there may be a relationship between lack of UV transmission and increased incidence of cataract. On the other hand, absorbing UV in the lens may reduce the risk of UV-induced retinal damage (Meyer-Rochow 2000).

Conclusions

Because of the UV absorbing characteristics of the King Penguin lens, its ability to perceive UV will be poor and well-nigh impossible below 370 nm. Gentoo and Little penguins, on the other hand, can perceive near UV light almost down to 300 nm, near the transmission cut off of the cornea and biological tissues in general. Therefore, UV reflectance should be of much lesser importance in the behaviour of the King Penguin than of its smaller relatives.

Author contributions

PH, MV, JZ and CM conceived and designed the research. PH, WH and JZ conducted the experiments. PH, WH and JZ analysed the data. PH, MV and FB interpreted the data. CM and JZ provided resources and supervision. PH and FB wrote the manuscript. All authors read and approved the manuscript.

Data availability

The raw data that support the findings of this study are available in the Digital Science (London, UK) online open access repository, https://doi.org/10.17608/k6.auckland.c.6427706.v1

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Table legends

Table 1 Demographic data of penguins with, in brackets, the names and / or identification numbers, where known, for each penguin. Fresh means the ocular examination was completed within 6 hours of death, without being stored in any medium

Table 2 The wavelength at which transmission reached 10% (λ_{10}), 50% (λ_{50}) and 90% (λ_{90}) of the transmission at 700 nm for each of the cornea, lens and whole eye. Footnotes: a. The λ_{10} values were unable to be calculated as the eyes never recorded less than 10% transmission even at the lowest wavelength tested, 230 nm. b. The whole eye of the Gentoo Penguin was not examined

Figure legends

Fig. 1 The 3D printed box in which the eye or components thereof were placed, to measure their spectral transmission using the Oceans Optics USB2000+ instrument. The fiber optic cables delivering the xenon light source (left) and the receiver (right) were screwed in at opposite ends. A lid was placed on top to eliminate room light while the experiment was undertaken

Fig. 2 The tray in which the eyes were placed to be examined by the SpectraMax 13x, in this case the left and right eyes of Little Penguin *Eudyptula minor* L1. Note the tissue paper holding the eye stable and central

Fig. 3 Averaged transmission of light through the cornea of Little (*Eudyptula minor*), Gentoo (*Pygoscelis papua*) and King (*Aptenodytes patagonicus*) penguins L1, G2 and K1. Note a dip in transmission maximum around 400–420 nm in G2. Created with Microsoft 365® Excel

Fig. 4 Averaged transmission of light through the lens of Little (*Eudyptula minor*), Gentoo (*Pygoscelis papua*) and King (*Aptenodytes patagonicus*) penguins L1, G1 (left eye only) and K1. Note that L1 transmits much more of the shorter wavelengths than does K1, while G1 is intermediate between the two. The rise in shortwave transmission is less rapid in G2, especially above 50%, which may be the result of methodological error (see discussion). Created with Microsoft 365® Excel

Fig. 5 Averaged transmission of light through the vitreous of Little (*Eudyptula minor*), Gentoo (*Pygoscelis papua*) and King (*Aptenodytes patagonicus*) penguins L1, G2 and K2. Created with Microsoft 365® Excel

Fig. 6 Averaged transmission of light through all ocular structures anterior to the retina of Little (*Eudyptula minor*) and King (*Aptenodytes patagonicus*) penguins L1 and K1. There is greater transmission of short wavelengths through L1 than K1. The shape of each curve most closely approximates that seen with transmission through the lens alone. Created with Microsoft 365® Excel

Table 1

Penguin identification	Age at examination (years)	Sex	Preservation prior to examination	Lens transparency	Equipment used	Tissue examined
Little Penguins (*Eudyptula minor*)						
L1 (LP1, B80231)	Adult	Unknown	Fresh	Clear	SpectraMax 13x	2 eyes: cornea, lens, vitreous and whole eye
Gentoo Penguins (*Pygoscelis papua*)						
G1 (Goose, G120)	7 weeks	Unknown	Fresh	Clear	USB2000+	1 eye: lens
G2 (Twinkles, G194)	26 years	Female	Fresh	Mild nuclear cataract	SpectraMax 13x	2 eyes: cornea and vitreous
King Penguins (*Aptenodytes patagonicus*)						
K1 (Berta, K191)	23 months	Unknown	Fresh	Clear	USB2000+	2 eyes: cornea, lens and whole eye
K2 (Maggie, K155)	32 years	Female	24 hours refrigeration	Moderate cataract	SpectraMax 13x	2 eyes: vitreous

524 **Table 2**

	Cornea			Lens			Vitreous			Whole eye		
	λ_{10}	λ_{50}	λ_{90}	λ_{10}	λ_{50}	λ_{90}	λ_{10}	λ_{50}	λ_{90}	λ_{10}	λ_{50}	λ_{90}
Little Penguin *Eudyptula minor*	293	335	393	301	322	440	N/A[a]	300	362	316	340	392
Gentoo Penguin *Pygoscelis papua*	295	325	481	347	415	611	N/A[a]	301	343	-[b]	-[b]	-[b]
King Penguin *Aptenodytes patagonicus*	299	341	409	376	400	614	N/A[a]	301	330	371	404	492

525

Figure 1

Figure 2

Figure 3

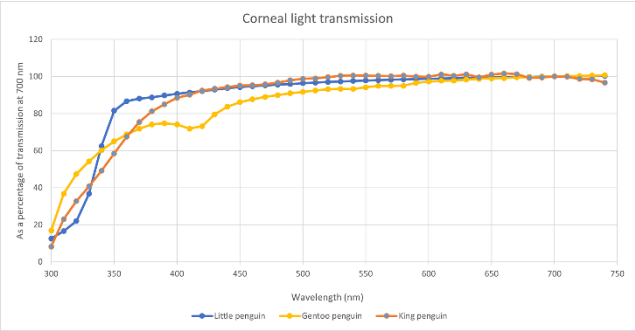
Corneal light transmission
As a percentage of transmission at 700 nm
120
100
80
60
40
20
0
300
350
400
450
500
550
600
650
700
750
Wavelength (nm)
Little penguin
Gentoo penguin
King penguin

Figure 4

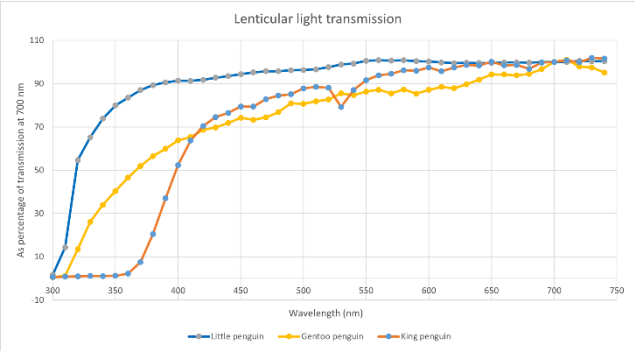
Lenticular light transmission
As percentage of transmission at 700 nm
110
90
70
50
30
10
-10
300
350
400
450
500
550
600
650
700
750
Wavelength (nm)
Little penguin
Gentoo penguin
King penguin

Figure 5

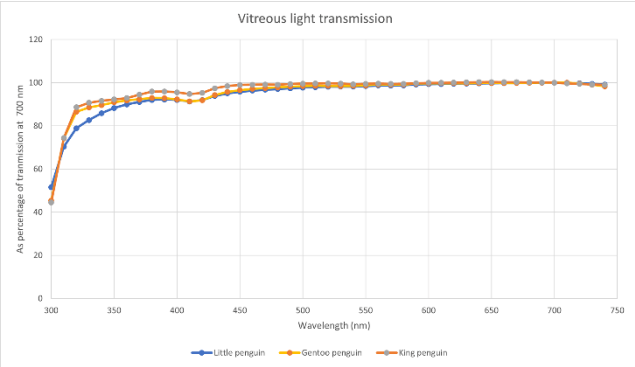
Vitreous light transmission
As percentage of tranmission at 700 nm
120
100
80
60
40
20
0
300
350
400
450
500
550
600
650
700
750
Wavelength (nm)
Little penguin
Gentoo penguin
King penguin

Figure 6


Whole eye transmission
As percentage of transmission at 700 nm
120
100
80
60
40
20
0
300
350
400
450
500
550
600
650
700
750
Wavelength (nm)
Little penguin
King penguin