

# Can penguins (Spheniscidae) see in the ultraviolet spectrum?

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#### 1 Title page

## 2 Can penguins (Spheniscidae) see in the ultraviolet spectrum?

#### 3 Running head: Penguin ocular transmission

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- 20 study and for the use of equipment.

#### 21 Abstract

22 It has been postulated that ultraviolet reflectance is important in mate choice in King Penguins 23 Aptenodytes patagonicus, although not in other penguin species that do not have body parts that 24 reflect UV light. However, this theory has been challenged. Here we aimed to determine the 25 transmission of the ocular media in the large King Penguin as well as the smallest penguin, the Little 26 Penguin Eudyptula minor, and a medium-sized penguin, the Gentoo Penguin Pygoscelis papua, to 27 determine if the penguin eye is capable of seeing ultraviolet light. In all species the cornea absorbed 28 the most damaging rays at 300 nm or below but it was the lens that predominantly determined the 29 transmission of light between 300 and 400 nm. The lenses of a young King Penguin absorbed almost 30 all light less than 370 nm and had 50% transmission at 406 nm, thus ultraviolet perception in the 31 King Penguin is very limited. In contrast, 50% lenticular transmission was 329 nm in the Little 32 Penguin and 367 nm in the Gentoo. Therefore, we suspect that ultraviolet light may be more 33 important in the behaviour of smaller penguins than in the King Penguin, where it is unlikely to play 34 a significant role.

#### 35 Keywords

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

#### 37 Statements and declarations

- 38 Funding
- 39 No funding was received for conducting this study. The authors have no relevant financial or non-
- 40 financial interests to disclose.
- 41 Ethics approval
- 42 All procedures performed in the study were in accordance with the ARVO Statement for Use of
- 43 Animals in Ophthalmic Vision and Research. Ethical approvals were obtained from the New Zealand
- 44 Department of Conservation (permit numbers 68003-DOA, 28 November 2018 and 89983-DOA, 27
- 45 July 2021), Auckland Zoo and SEA LIFE (SL (G) AR 001

#### 46 Main document

#### 47 Introduction

48 Ultraviolet (UV) reflectance and ultraviolet-sensitive (UVS) vision are thought to play a role in many 49 avian behaviors, including orientation, foraging and sexual selection (Bennett et al. 1996; Johnsen et 50 al. 1998). Color also appears to be important in penguin (Spheniscidae) mate selection (Jouventin 51 1982; Massaro et al. 2003). The orange beak spots of both King Aptenodytes patagonicus and 52 Emperor A. forsteri penguins have been found to have UV reflectance (Jouventin et al. 2005) and, in 53 the case of the King Penguin, a multilayer photonic microstructure appears to be the source of this 54 reflectance, which is maximal at 367.5 nm (Dresp et al. 2005). Beak reflectance has also been 55 reported in juvenile Gentoo Penguins Pygoscelis papua (Meyer-Rochow and Shimoyama 2008). 56 However, similar beak reflectance has not been found in 10 other species of penguin, nor on the 57 claws, feathers or skin of any penguin (Jouventin et al. 2005). 58 This finding, together with the observations that UV reflectance from the beak only appeared when

59 the animal was sexually mature and of pairing behavior, led to the suggestion that such UV 60 reflection could be important in King and Emperor penguin breeding (Jouventin et al. 2005). This 61 would appear to be supported by a higher UV beak reflectance amongst early pairing King Penguins 62 (the most successful in breeding) and the observation that experimentally reduced UV reflectance 63 from beak spots is associated with delayed pairing (Jouventin et al. 2009; Nolan et al. 2010). 64 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 65 (Jouventin et al. 2009; Meyer-Rochow et al. 2009; Nolan et al. 2010). 66 67 Ultraviolet-sensitive visual pigments (<400 nm) are widespread in the animal kingdom (Hunt et al. 68 2001) and in birds a UVS visual pigment is thought to have evolved from ancestral violet vision on 69 multiple occasions (Wilkie et al. 2000; Hart et al. 2007), although molecular evidence suggests that 70 UVS cones are more widespread in terrestrial birds than in seabirds (Hart and Hunt 2007). A UVS

71 cone has not been found in penguins; however, a short wavelength 1 (SWS1) cone with maximum 72 sensitivity at 403 – 405 nm has been identified in the Humboldt Penguin Spheniscus humboldti 73 (Bowmaker et al. 1985; Wilkie et al. 2000), very close to the UV range. More recently, Gentoo 74 Penguins have been shown to respond behaviourally to UV light maximal at 365 nm, tapering to no 75 output at 390 nm (Cole et al. 2022). Although, this visual pigment does not have maximal 76 absorbance in the UV range, it is close to that range (<400 nm) and the absence of UVS visual 77 pigments is not evidence of blindness to UV, as there is a significant secondary absorption ( $\beta$ -peak) 78 by all visual pigments in the UV range despite maximum absorbance in the visible spectrum (Douglas 79 et al. 2014). This can be demonstrated by the ability of aphakic humans to see in the UV spectrum 80 despite lacking a UVS cone (Stark et al. 1994).

81 Because of this secondary absorption, ocular transmission is more critical to UVS vision than the 82 presence of a UVS cone. Many animals have been shown to have ocular media that allow the 83 transmission of near-ultraviolet (near-UV, <400 nm) light, including many bird species (Emmerton et 84 al. 1980; Siebeck et al. 2001; Mullen et al. 2007; Tsukahara et al. 2013; Lind 2014; Olsson et al. 85 2021). Although all biological tissues are opaque to light below 300 nm due to absorption by 86 molecules such as nucleic acids and aromatic amino acids, in birds as in mammals the lens is usually 87 more critical than the cornea with regard to UV light transmission, particularly above 345 nm 88 (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

96 (Shirihai 2007), dive to different depths (Montague 1985; Culik et al. 1996) and inhabit vastly

97 different environments (Shirihai 2007) to the Kings, therefore we also wished to determine if these

98 findings were consistent across all penguins.

99

#### 100 Methods

#### 101 Animals and ethics

102 Permission was obtained for this study from the New Zealand Department of Conservation (permit 103 numbers 68003-DOA, 28 November 2018 and 89983-DOA, 27 July 2021), Auckland Zoo and SEA LIFE 104 (SL (G) – AR 001). A Little Penguin (L1) was recovered from the wild in the Auckland Region, New 105 Zealand, but was unable to be re-released due to a physical disability not involving the eye. Its exact 106 age was unknown, although it was an adult bird and had passed its first moult. Two Gentoo Penguins 107 were examined, G1 aged 7 weeks (lens) and G2 aged 26 years (cornea and vitreous), as well as two 108 King Penguins, K1 aged 23 months and K2 aged 32 years. The Gentoo and King penguins had spent 109 their life in captivity at SEA LIFE Kelly Tarlton's Aquarium in Auckland, New Zealand and were 110 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 111 112 vitreous of K2 was examined after 24 hours of refrigeration and this animal had moderate cataract; 113 only the vitreous from this eye was examined (Table 1). Several other eyes of all three species were 114 examined to check the accuracy of the two spectrometers that we used against each other. 115 However, we did not use these results as they were from elderly penguins, hadspent time 116 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 117 to combine the results for fear of underestimating potential ocular transmission in the youngest and 118 119 healthiest eyes of each species, our primary goal being to determine if the penguin optical media

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

122 Suggested location of Table 1

#### 123 Equipment and tissue preparation

124 Two spectrometers were used. The first was a USB2000+ (Ocean Optics, Dunedin, Florida, USA, Fig. 125 1), which could be transported to peripheral locations. This was used for G1 and K1. The second was 126 a SpectraMax 13x (Molecular Devices Inc, San Jose, CA), into which the eyes or components thereof 127 were inserted, and which could only be used when the central laboratory was accessible (Fig. 2). This 128 was used for L1, G2 and K2. It was only possible to measure vitreous transmission using the 129 SpectraMax 13x. 130 The spectral transmission of the whole eye, cornea and lens were measured in L1 and K1. In G1, only 131 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 132 133 as noted above and in the knowledge that, in all birds, it is the lens that determines ocular 134 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 135 136 Surgical Products, Inc., Caledonia, MI). To measure transmission through the cornea, the cornea was 137 then removed and placed in the spectrometer. Then, having removed the cornea, the vitreous was 138 removed as much as possible without touching the lens, leaving the lens attached to the ciliary body

- 139 (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.

141 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)

144 and inserted into the machine where it lay immediately above the detector, separated from the 145 latter only by the floor of the clear plastic container. To measure the transmission of the vitreous, a 146 well 14.5 mm high was filled with liquid vitreous. Percentage transmission was directly measured by 147 the machine in steps of 10 nm. This was then calculated as a percentage of the transmission at 700 148 nm ( $T_{\lambda}$ ). Values obtained for light less than 300 nm were ignored as very little light was transmitted 149 at these wavelengths through the plate blank using the SpectraMax 13x and thus the values 150 obtained for transmission of ocular media as a percentage of transmission by the plate blank were 151 erroneous. An exception was made and the value at 290 nm used if necessary to calculate an 152 intercept, using the method described below (primarily the vitreous  $\lambda_{50}$  but also the cornea  $\lambda_{10}$ ).

#### 153 Suggested location of Figure 1

#### 154 Data acquisition using Ocean Optics USB2000+

155 The tissue being measured was placed in a black case that was custom made at the University of 156 Auckland using a 3D plastic printer to keep it steady and to eliminate external sources of light (Fig. 157 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 158 159 using the USB2000+. When the whole eye or the lens (attached to the ciliary body) was being 160 measured, this was placed as close as possible to the detector and cushioned for stability by tissue 161 paper. When the cornea was being measured, it was draped over the detector. The Oceans Optics 162 USB2000+ measured absolute transmission in steps of 0.38 to 0.39 nm. The transmission of each wavelength ( $T_{\lambda}$ ) measured using the USB2000+ was calculated by first subtracting the background 163 164 light ( $b_{\lambda}$ ) measured at that wavelength within the closed box from that measured with the Xenon 165 source on  $(Xe_{\lambda})$  and then comparing that to the light transmitted with both the Xe source on and the 166 optical element under investigation being in the path of the beam (Xt<sub> $\lambda$ </sub>).

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

This was then calculated as a percentage (T<sub>%</sub>) of the average transmission between 650 and 750 nm (T<sub>700</sub>). 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<sup>®</sup> 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.

#### 175 Suggested location of Figure 2

#### 176 Data analysis and plotting

- 177 Since, except for G1, both eyes were examined, the results from each eye were first averaged and
- 178 then the average  $T_{\lambda}$  for each species, at 10 nm intervals, was plotted against the respective
- 179 wavelengths. The wavelength at which the cornea, lens, vitreous and whole eye reached 10% ( $\lambda_{10}$ ),
- 180 50% ( $\lambda_{50}$ ) and 90% ( $\lambda_{90}$ ) of that recorded at 700 nm for species was determined by using the forecast
- 181 function of Microsoft 365<sup>®</sup> Excel to predict where the 10%, 50% and 90% intercepts would be based
- 182 on the two recordings 10 nm and 20 nm before 10%, 50% or 90% transmission was reached and the
- 183 two recordings 10 and 20 nm after.

184

#### 185 Results

- 186 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.  $\lambda_{10}$ ,  $\lambda_{50}$  and  $\lambda_{90}$
- 188 intercepts are listed in table 2.
- 189 Suggested location of Figure 3, Figure 4, Figure 5 and Figure 6
- 190 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.

196

#### 197 Discussion

#### 198 Importance of UV reflectance in King Penguin mate selection

199 Because of reduced lenticular UV transmission, the eye of the King Penguin transmitted very little UV 200 light, with almost all light at wavelengths less than 370 nm being blocked and 50% transmission only 201 being reached at 406 nm. In King Penguins, the only part of the body that reflects UV are the orange 202 bands of the beak (Jouventin et al. 2005). The UV peak in these bands, according to the literature, is 203 at 401 nm in museum specimens and 380 nm in live penguins (Jouventin et al. 2005). In a King 204 Penguin taxidermy available at CEFE (Univ Montpellier, CNRS, EPHE, IRD, Montpellier, France), the 205 UV peak of the bands of the beak, as measured with an Avantes spectrophotometer, is at 360 nm 206 (FB personal data). Our results, showing that no light below 370 nm and only half of the light even at 207 400 nm is transmitted to the retina in this species, suggests that the use of UV in mate choice 208 behaviour (Jouventin et al. 2005; Nolan et al. 2010) should be reconsidered, or at least confirmed 209 with further experimental protocols. We also note that, in the later paper (Nolan et al. 2010), the 210 range of the spectrum examined in the experiment was from 320 nm to 450 nm, thus including 211 some visible light (> 400 nm), which could have been easily perceived by King Penguins.

#### 212 Variation between species

213 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.

215 1999). However, in contrast to King Penguin, the lens of the Little Penguin did not further restrict the 216 ability of UV light to be perceived at the retina and thus the animal will be more sensitive to light of 217 these wavelengths. The Gentoo Penguin eye would appear to lie somewhere between these two, 218 although clearly the optical transmission would enable it to see the 365 nm torch previously 219 reported (Cole et al. 2022). The behavioural relevance of this finding in these species is unclear. In 220 regard to mate selection, adult Gentoo Penguins have no body parts that reflect UV, although beak 221 reflectance has been reported in juveniles (Jouventin et al. 2005; Meyer-Rochow and Shimoyama 222 2008).

223 Why are shorter wavelengths transmitted more readily through the lenses of Little and Gentoo 224 penguins but absorbed in the King? There is a weak association between lens thickness and UV 225 transmission in birds (Olsson et al. 2021), presumably because of the increased tissue thickness 226 across which light can be absorbed. However, the difference in UV transmission between the lens of 227 the Little Penguin and the King Penguin is much more marked than that between the corneas of the 228 two species, despite both anatomical structures being proportionately thicker in the King Penguin 229 (Hadden et al. 2022). Furthermore, despite the aforementioned weak association, many birds have a 230 higher  $\lambda_{50}$  than would be expected from eye size; for instance, the Long-eared Owl Asio otus has a 231 7.0 mm thick lens but a lenticular  $\lambda_{50}$  of 323.6 nm (Olsson et al. 2021).

232 An environmental difference seems unlikely, despite the habitat of King and Little penguins being 233 markedly dissimilar, because of the relatively high UV transmission of the Gentoo Penguin lens 234 despite a habitat much more similar to the King Penguin than to the Little Penguin. Both King and 235 Gentoo penguins predominantly breed on subantarctic islands, although the gentoo can also be 236 found on the Antarctic Peninsula and both can be found as rare vagrants on the New Zealand 237 mainland (Shirihai 2007). The Gentoo and King penguins in our study were also both of South 238 Georgian descent and therefore share the same pelagic environment, although King Penguins hunt 239 in open pelagic waters down to 300 m, which seems to correlate with their main prey, the midwater 240 lanternfishes Myctophidae (Kooyman et al. 1992), while gentoos, which at South Georgia have been 241 recorded diving to over 100 m (Croxall et al. 1988), are both nearshore benthic and pelagic foragers, 242 consuming crustaceans, particularly Antarctic krill Euphasia superba and Themisto gaudichaudii, as 243 well as fish (Xavier et al. 2017; McClintock et al. 2020). The latitudes in which they live means that 244 the hours of night and day are more a function of seasonality than time of day and they forage at all 245 times of the year. By contrast, Little Penguins (grouping both Eudyptula minor and E. 246 novaehollandiae together) inhabit mainland New Zealand, southern Australia and Tasmania (Shirihai 247 2007; Grosser et al. 2017). They feed on small shoaling fish (particularly Clupeiformes), cephalopods 248 and crustaceans such as krill and forage at shallower depths, around 10-50 m (Montague 1985; 249 Gales et al. 1990; Chiaradia et al. 2007; Iida et al. 2014). Little Penguins also breed in burrows, under 250 rocks and in other hollows, in dunes or amongst vegetation, rather than on the harsher shores of 251 South Georgia; they can be active at night but are predominantly diurnal foragers (Shirihai 2007). 252 The lower latitudes in which Little Penguin live, with illumination coming from more directly 253 overhead, means that the Little Penguin lives in a generally brighter environment; the atmospheric 254 transmission of UV also increases with increasing solar elevation in a similar fashion to light in the 255 visible spectrum (Spitschan et al. 2016). However, if this greater luminance is the explanation for 256 increased UV transmission in the Little Penguin, it is one that is contrary to the tendency of diurnal 257 mammals to block UV (Douglas et al. 2014).

258 It might be that the larger eyes of the larger King Penguin block UV light to maximise the increased 259 visual acuity their size affords, since the elimination of UV reduces chromatic aberration, to which 260 their large pupils renders them more sensitive; elimination of UV will also reduce Rayleigh scatter 261 (Walls 1931; Pye 2011; Douglas and Jeffery 2014; Olsson et al. 2021). Also, given the  $\beta$ -peak in the 262 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 263 264 between larger eyes, which generally possess higher spatial sensitivity given that more pixels 265 (photoreceptors) can be made available per visual angle, and lack of transparency of the lens to UV 266 (Walls 1931; Ambach et al. 1994; Tsukahara et al. 2013; Douglas and Jeffery 2014). Furthermore, the 267 ocular media of raptors, which are notable for their excellent spatial sensitivity, up to 140 cycles / 268 degree in the Golden Eagle Aquila audax, are relatively opaque to UV, with 50% transmissions 269 ranging from 369 nm to 394 nm in those that have been studied, although this does not include the 270 Golden Eagle (Reymond 1985; Lind et al. 2013; Potier et al. 2016). In humans, a drop in potential 271 visual acuity has been documented in pseudophakic individuals who have intraocular lenses that do 272 not absorb UV, although intraocular lenses may introduce retinal images that do not occur in the 273 phakic eye (Rog et al. 1986). On the other hand, the potential spatial resolution of the King Penguin 274 eye (20.40 cycles / degree in water) is only marginally greater than that of the Little Penguin (17.07-275 17.46 cycles / degree in water), despite its greater size, because of a lesser ganglion cell density 276 (Coimbra et al. 2012). Of course, this resolution was based on morphological ganglion cell counts 277 rather than actual measurements of acuity so may not reflect the true spatial sensitivity. 278 urthermore, UV transmission is of less relevance underwater, the environment in which penguins 279 forage, because it is more rapidly attenuated than are wavelengths in the visible spectrum (Williams 280 1973). Attenuation also increases with depth, which suggests it may be even less relevant in the 281 deeper diving King Penguin than in the Little Penguin, although the latter forage in more estuarine 282 waters where UV attenuation is higher (Zielinski 2013).

#### 283 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  $\lambda_{10}$  and a substantially lower  $\lambda_{50}$ . 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 291 oxygenated hemoglobin, and we speculate that some tissues may have been contaminated by blood 292 when they were dissected out and placed in the spectrometer. Such contamination may mean that 293 the  $\lambda_{90}$  measurements are less reliable than the others, particularly in the case of the cornea of G2 294 and the lens of G1.

295 In the future, more individuals per species across different ages could be tested to confirm these 296 results and, for those species that can see UV, experimental behavioural protocols should be 297 designed to confirm the use of potential UV cues. In addition, more species of penguin could be 298 studied to determine if the apparent relationship between size and UV transmission in these three 299 species of penguin is consistent across the family. For instance, it would be useful to know whether 300 the lens of the larger Emperor Penguin blocks UV light, given that it shares the same lineage as the 301 King and UV reflection off the beak has been demonstrated (Jouventin et al. 2005). It would also be 302 interesting to understand if Galápagos (Spheniscus mendiculus) or African (Jackass, Black-footed, 303 Spheniscus demersus) penguins, which inhabit yet lower latitudes than the Little Penguin, transmit 304 UV to a similar extent to that observed in the similarly sized but subantarctic Gentoo Penguin. It 305 would also be useful to correlate behaviour and vision between species with different UV 306 transmissions, for instance in mate choice. Previous authors have speculated that, since the UVS 307 form of the SWS opsin is ancestral, a lens that transmits UV may also be ancestral (Olsson et al. 308 2021). Investigation of the spectral transmission of oil droplets in the retina would also be 309 instructive, as light passes through them before being detected by cone photoreceptor outer 310 segments. The King Penguin retina has been found to contain only pale green droplets, similar to 311 nocturnal birds. This may reflect a habitat of little colour and their need to see well at depth in a 312 dark sea, necessitating maximisation of retinal sensitivity. Maximal absorption of these droplets was 313 between 500 and 600 nm; the absorption in UV wavelengths was not measured but it was 314 approximately 0.35 at 400 nm (Gondo and Ando 1995). Humboldt Spheniscus humboldti and 315 Rockhopper Eudyptes chrysocome penguins were found to have droplets of four different colours, 316 similar to diurnal birds and potentially because they live in areas with vegetation (Gondo and Ando

317 1995). The closer phylogenetic relationship (Cole 2022) and, for the Little Penguin, habitat similarity 318 might suggest that Gentoo and Little penguin oil droplets may be more akin those in Humboldt and 319 Rockhopper penguins than to those in King Penguins. However, they have not been examined in this 320 regard and the similarly related Magellanic Penguin Spheniscus magellanicus was found to possess 321 yet different droplets (Suburo and Scolaro 1999). Finally, cataract is known to be associated with UV 322 exposure (Delcourt et al. 2014). Although UV radiation has been shown to cause cataract in Sprague-323 Dawley rats (Michael et al. 1998), where it is unlikely that the lens constitutes an additional barrier 324 to UV transmission over and above the cornea (Douglas and Jeffery 2014), it would be useful to 325 know if absorbing this energy in the lens rather than transmitting it increases the risk of cataract; if 326 this was the case, there may be a relationship between lack of UV transmission and increased 327 incidence of cataract. On the other hand, absorbing UV in the lens may reduce the risk of UV-328 induced retinal damage (Meyer-Rochow 2000).

#### 329 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.

#### 335 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.

#### 339 Data availability

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

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#### 481 Table legends

482

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

486

487 **Table 2** The wavelength at which transmission reached 10% ( $\lambda_{10}$ ), 50% ( $\lambda_{50}$ ) and 90% ( $\lambda_{90}$ ) of the

488 transmission at 700 nm for each of the cornea, lens and whole eye. Footnotes: a. The  $\lambda_{10}$  values

489 were unable to be calculated as the eyes never recorded less than 10% transmission even at the

490 lowest wavelength tested, 230 nm. b. The whole eye of the Gentoo Penguin was not examined

492	Figure legends
493	
494 495 496 497	<b>Fig. 1</b> 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
498	
499 500 501	<b>Fig. 2</b> 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 <i>Eudyptula minor</i> L1. Note the tissue paper holding the eye stable and central
502	
503 504 505	<b>Fig. 3</b> Averaged transmission of light through the cornea of Little ( <i>Eudyptula minor</i> ), Gentoo ( <i>Pygoscelis papua</i> ) and King ( <i>Aptenodytes patagonicus</i> ) penguins L1, G2 and K1. Note a dip in transmission maximum around 400–420 nm in G2. Created with Microsoft 365® Excel
506	
507 508 509 510 511	<b>Fig. 4</b> Averaged transmission of light through the lens of Little ( <i>Eudyptula minor</i> ), Gentoo ( <i>Pygoscelis papua</i> ) and King ( <i>Aptenodytes patagonicus</i> ) 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 <sup>®</sup> Excel
512	
513 514 515	<b>Fig. 5</b> Averaged transmission of light through the vitreous of Little ( <i>Eudyptula minor</i> ), Gentoo ( <i>Pygoscelis papua</i> ) and King ( <i>Aptenodytes patagonicus</i> ) penguins L1, G2 and K2. Created with Microsoft 365 <sup>®</sup> Excel
516	
517 518 519	<b>Fig. 6</b> Averaged transmission of light through all ocular structures anterior to the retina of Little ( <i>Eudyptula minor</i> ) and King ( <i>Aptenodytes patagonicus</i> ) 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

## 521 Table 1

Penguin identification	Age at examination	Sex	Preservation prior to	Lens transparency	Equipment used	Tissue examined
	(years)		examination			
Little Penguins (Eudyptula minor)						
L1 (LP1, B80231)	Adult	Unknown	Fresh	Clear	SpectraMax 13x	2 eyes: cornea, lens, vitreous and whole eye
Gentoo Penguins ( <i>Pygoscelis</i> 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 W			Whole eye		
	λ <sub>10</sub>	$\lambda_{50}$	λ90	λ <sub>10</sub>	$\lambda_{50}$	λ90	λ <sub>10</sub>	$\lambda_{50}$	$\lambda_{90}$	λ <sub>10</sub>	$\lambda_{50}$	λ <sub>90</sub>
Little Penguin	293	335	393	301	322	440	N/A <sup>a</sup>	300	362	316	340	392
Eudyptula minor												
Gentoo Penguin	295	325	481	347	415	611	N/A <sup>a</sup>	301	343	_b	_b	_b
Pygoscelis papua												
King Penguin	299	341	409	376	400	614	N/A <sup>a</sup>	301	330	371	404	492
Aptenodytes												
patagonicus												
525												

















