



HAL
open science

Can penguins (Spheniscidae) see in the ultraviolet spectrum?

Peter Hadden, Misha Vorobyev, William Hadden, Francesco Bonadonna,
Charles N.J. Mcghee, Jie Zhang

► **To cite this version:**

Peter Hadden, Misha Vorobyev, William Hadden, Francesco Bonadonna, Charles N.J. Mcghee, et al.. Can penguins (Spheniscidae) see in the ultraviolet spectrum?. *Polar Biology*, 2023, 46 (10), pp.1111-1121. 10.1007/s00300-023-03188-8. hal-04230087

HAL Id: hal-04230087

<https://hal.science/hal-04230087>

Submitted on 5 Oct 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Title page**

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

3 Running head: Penguin ocular transmission

4 Peter W. Hadden^a (Orcid ID: 0000-0003-0352-5286) peterh211@gmail.com, Misha Vorobyev^b

5 m.vorobyev@auckland.ac.nz, William H. Hadden^c williamhadden92@gmail.com, Francesco

6 Bonadonna (Orcid ID: 0000-0002-2702-5801)^d francesco.bonadonna@cefe.cnrs.fr, Charles N.J.

7 McGhee^a (Orcid ID: 0000-0001-7048-0706) c.mcghee@auckland.ac.nz, Jie Zhang^a (Orcid ID:0000-

8 0003-1572-8339) jie.zhang@auckland.ac.nz.

9 ^a Department of Ophthalmology, New Zealand National Eye Centre, Faculty of Medical and Health
10 Sciences, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand

11 ^b Department of Optometry, New Zealand National Eye Centre, Faculty of Medical and Health
12 Sciences, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand

13 ^c Department of Computer Science, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand

14 ^d CEFE, Univ Montpellier, CNRS, EPHE, IRD, Montpellier, France

15 Corresponding author: Peter W. Hadden (address and email as above, i.e. peterh211@gmail.com,
16 ph. +6421528252)

17 **Acknowledgements**

18 We would like to acknowledge the staff of SEA LIFE Kelly Tarlton's Aquarium, Eye Institute,
19 University of Auckland and Auckland Zoo, all of Auckland, New Zealand, for their assistance in this
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
65 demonstrated in penguins (Meyer-Rochow et al. 2008), leading to a fierce debate in this journal
66 (Jouventin et al. 2009; Meyer-Rochow et al. 2009; Nolan et al. 2010).

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 (β -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).

89 Clearly, in order for UV reflectance to be important in mate selection such reflectance has to be
90 visible. Given the debate, the primary aim of this study was to determine the ocular transmission of
91 the King Penguin eye and thus determine the potential sensitivity of its visual system to UV light.
92 Little Penguins (*Eudyptula minor*) and Gentoo Penguins were also available to us in Auckland, New
93 Zealand, and represent a different lineage within Spheniscidae (Zusi 1975; Baker et al. 2006; Cole et
94 al. 2022), with recent DNA evidence (Vianna et al. 2020) suggesting that King and Emperor penguins
95 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
111 completed within 6 hours of enucleation post-mortem, and none had any known ocular disease. The
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, had spent time
116 refrigerated, or both. Moreover, their lenticular ocular transmission was not as great as the penguins
117 that we included in this study, probably because of their age and preservation, and we did not wish
118 to combine the results for fear of underestimating potential ocular transmission in the youngest and
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,
132 as were the corneas of G2, noting that these were older animals but being reassured of their validity
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
135 eye was enucleated and then the posterior pole was removed with a Bard-Parker 15 blade (Aspen
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
140 anterior segment, minus cornea, was then inserted into the spectrometer.

141 *Data acquisition using SpectraMax 13x*

142 A plate blank and a well filled with blackened paper tissue were used to verify the calibration. The
143 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_λ). 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 λ_{50} but also the cornea λ_{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
158 purposes of calibration, the spectrum without light on and with just the xenon light on was recorded
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
163 wavelength (T_λ) measured using the USB2000+ was calculated by first subtracting the background
164 light (b_λ) measured at that wavelength within the closed box from that measured with the Xenon
165 source on (Xe_λ) 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_λ).

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

168 This was then calculated as a percentage ($T_{\%}$) of the average transmission between 650 and 750 nm
169 (T_{700}). Due to the small transmission interval of 0.38 to 0.39 nm, there was variability between
170 individual measurements. Therefore, the data was first exponentially smoothed with a damping
171 value of 0.5 using Microsoft 365[®] Excel. Transmission percentages for light less than 300 nm were
172 ignored on the USB2000+, both for consistency with the SpectraMax and because they appeared to
173 be highly variable, probably a function of how little light was produced by the Xenon source at those
174 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_{λ} 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% (λ_{10}),
180 50% (λ_{50}) and 90% (λ_{90}) of that recorded at 700 nm for species was determined by using the forecast
181 function of Microsoft 365[®] 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
187 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}
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*

191 The dimensions of eyes of these penguins have been the subject of a previous report (Hadden et al.
192 2022). The average corneal thickness (measured with ultrasound pachymetry) was 0.303, 0.472 and
193 0.605 mm in the Little, Gentoo and King penguins respectively, the lens thickness (measured with
194 callipers) 4.0, 7.14 and 7.75 mm, excluding the lens of K2 which was cataractous and 10 mm thick,
195 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),
214 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 λ_{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 λ_{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 β -peak in the
262 UV range possessed by all visual pigments, allowing the transmission of UV light would make the
263 task of color differentiation much more difficult (Pye 2011). There would appear to be a correlation
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 Furthermore, 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*

284 We suspect there is an artifactual drop-off in the transmission of the left lens of G1, particularly
285 above 50% transmission, given that the rise in shortwave transmission is less rapid above this point
286 than in the King Penguin despite a much lower λ_{10} and a substantially lower λ_{50} . This could be due
287 to a greater separation of the tissue from detector in the USB2000+ by mistake, increasing chromatic
288 aberration which is more significant at shorter wavelengths. This could also have been the reason
289 why the reading from the right lens was not credible. There was also a dip in transmission at 410 to
290 420 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 λ_{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**

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

335 **Author contributions**

336 PH, MV, JZ and CM conceived and designed the research. PH, WH and JZ conducted the experiments.
337 PH, WH and JZ analysed the data. PH, MV and FB interpreted the data. CM and JZ provided resources
338 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, <https://doi.org/10.17608/k6.auckland.c.6427706.v1>

342

343 **References**

- 344 Ambach WM, Blumthaler T, Schöpf E, Ambach F, Katzgraber F, Daxecker F, Daxer A (1994) Spectral
345 transmission of the optical media of the human eye with respect to keratitis and cataract formation.
346 *Doc Ophthalmol* 88:165-173 <https://doi.org/10.1007/BF01204614>
- 347 Baker AJ, Pereira SL, Haddrath OP, Edge K-A (2006) Multiple gene evidence for expansion of extant
348 penguins out of Antarctica due to global cooling. *P R Soc B* 273:11-17
349 <https://doi.org/10.1098/rspb.2005.3260>
- 350 Bennett ATD, Cuthill IC, Partridge JC, Maier EJ (1996) Ultraviolet vision and mate choice in Zebra
351 Finches. *Nature* 380:433-435 <https://doi.org/10.1038/380433a0>
- 352 Bowmaker K, Martin G (1985) Visual pigments and oil droplets in the penguin, *Spheniscus humboldti*.
353 *J Comp Physiol A* 156:71-77 <https://doi.org/10.1007/BF00610668>
- 354 Chiaradia AY, Ropert-Coudert Y, Kato T, Mattern T, Yorke J (2007) Diving behaviour of Little Penguins
355 from four colonies across their whole distribution range: Bathymetry affecting diving effort and
356 fledging success. *Mar Biol* 151:1535-1542 <https://doi.org/10.1007/s00227-006-0593-9>
- 357 Coimbra JP, Nolan PM, Collin SP, Hart NS (2012) Retinal ganglion cell topography and spatial
358 resolving power in penguins. *Brain Behav Evolut* 80:254-268 <https://doi.org/10.1159/000341901>
- 359 Cole TL, Zhou C, Fang M, Pan H, Ksepka DT, Fiddaman SR, Emerling CA, Thomas DB, Bi X, Fang Q et al
360 (2022) Genomic insights into the secondary aquatic transition of penguins. *Nat Commun* 13:1-13
361 <https://doi.org/10.1038/s41467-022-31508-9>
- 362 Croxall JP, Davis RW, Connell MJO (1988) Diving patterns in relation to diet of gentoo and macaroni
363 penguins at South Georgia. *Condor* 90:157-167 <https://doi.org/10.2307/1368444>
- 364 Culik BM, Pütz K, Wilson R, Allers D, Lage J, Bost C, Le Maho Y (1996) Diving energetics in King
365 Penguins (*Aptenodytes patagonicus*). *J Exp Biol* 199:973-983 <https://doi.org/10.2307/2937173>
- 366 Delcourt C, Cougnard-Grégoire A, Boniol M, Carrière I, Doré J-F, Delyfer M -N, Rougier M -B, Le Goff
367 M, Dartigues J -F, Barberger-Gateau P, Korobelnik J -F (2014) Lifetime exposure to ambient
368 ultraviolet radiation and the risk for cataract extraction and age-related macular degeneration: The
369 alienor study. *Invest Ophth Vis Sci* 55:7619-7627 <https://doi.org/10.1167/iovs.14-14471>
- 370 Douglas RH, Jeffery G (2014) The spectral transmission of ocular media suggests ultraviolet
371 sensitivity is widespread among mammals. *P R Soc B* 281:20132995
372 <https://doi.org/10.1098/rspb.2013.2995>
- 373 Douglas RH, Marshall NJ (1999) A review of vertebrate and invertebrate ocular filters. In: Archer et al
374 (eds) *Adaptive mechanisms in the ecology of vision*. Springer, Dordrecht, pp 95-162
375 https://doi.org/10.1007/978-94-017-0619-3_5
- 376 Dresch B, Jouventin P, Langley K (2005) Ultraviolet reflecting photonic microstructures in the King
377 Penguin beak. *Biology Lett* 1:310-313 <https://doi.org/10.1098/rsbl.2005.0322>
- 378 Emmerton J, Schwemer J, Muth I, Schlecht P (1980) Spectral transmission of the ocular media of the
379 Pigeon (*Columba livia*). *Invest Ophth Vis Sci* 19:1382-1387

380 Gales R, Williams C, Ritz D (1990) Foraging behaviour of the Little Penguin, *Eudyptula minor*: Initial
381 results and assessment of instrument effect. J Zool 220:61-85 [https://doi.org/10.1111/j.1469-](https://doi.org/10.1111/j.1469-7998.1990.tb04294.x)
382 [7998.1990.tb04294.x](https://doi.org/10.1111/j.1469-7998.1990.tb04294.x)

383 Gondo M, Ando H (1995) Comparative histophysiological study of oil droplets in the avian retina. Jap
384 J Ornithol 44:81-91 <https://doi.org/10.3838/jjo.44.81>

385 Grosser S, Scofield RP, Waters JM (2017) Multivariate skeletal analyses support a taxonomic
386 distinction between New Zealand and Australian *Eudyptula* penguins (Sphenisciformes:
387 Spheniscidae). Emu 117:276-283 <https://doi.org/10.1080/01584197.2017.1315310>

388 Hadden PW, Vorobyev M, Cassidy SB, Gokul A, Simkin SK, Tran H, McGhee CNJ, Zhang J (2022)
389 Selected ocular dimensions of three penguin species. Vision Res 201:108122
390 <https://doi.org/10.1016/j.visres.2022.108122>

391 Hart NS, Hunt DM (2007) Avian visual pigments: Characteristics, spectral tuning, and evolution. the
392 Am Nat 169(S1):S7-S26 <https://doi.org/10.1086/510141>

393 Hunt DM, Wilkie SE, Bowmaker JK, Poopalasundaram S (2001) Vision in the ultraviolet. Cell Mol Life
394 Sci 58:1583-1598 <https://doi.org/10.1007/PL00000798>

395 Iida T, Odate T (2014) Seasonal variability of phytoplankton biomass and composition in the major
396 water masses of the Indian Ocean sector of the Southern Ocean. Polar Sci 8:283-297
397 <https://doi.org/10.1016/j.polar.2014.03.003>

398 Johnsen A, Andersson S, Ornborg J, Lifjeld JT (1998) Ultraviolet plumage ornamentation affects social
399 mate choice and sperm competition in Bluethroats (Aves: Luscinia s. svecica): A field experiment. P R
400 Soc B 265:1313-1318 <https://doi.org/10.1098/rspb.1998.0435>

401 Jouventin P (1982) Visual and vocal signals in penguins, their evolution and adaptive characters.
402 Fortschritte der Verhaltensforschung 24:148 <https://doi.org/10.1002/iroh.3510680523>

403 Jouventin P, Couchoux C, Dobson FS (2009) UV signals in penguins. Polar Biol 32:513-514
404 <https://doi.org/10.1007/s00300-008-0564-3>

405 Jouventin P, Nolan PM, Örnberg J, Dobson FS (2005) Ultraviolet beak spots in King and Emperor
406 penguins. Condor 107:144-150 <https://doi.org/10.1093/condor/107.1.144>

407 Kooyman G, Cherel Y, Maho YL, Croxall J, Thorson P, Ridoux V, Kooyman C (1992) Diving behavior
408 and energetics during foraging cycles in King Penguins. Ecol Monogr 62:143-163
409 <https://doi.org/10.2307/2937173>

410 Lind O, Mitkus M, Olsson P, Kelber A (2013) Ultraviolet sensitivity and colour vision in raptor
411 foraging. J Exp Biol 216:1819-1826 <https://doi.org/10.1242/jeb.082834>

412 Lind OM, Mitkus M, Olsson P, Kelber A (2014) Ultraviolet vision in birds: The importance of
413 transparent eye media. P R Soc B 281:1-9 <https://doi.org/10.1098/rspb.2013.2209>

414 Massaro M, Davis LS, Darby JT (2003) Carotenoid-derived ornaments reflect parental quality in male
415 and female Yellow-eyed Penguins (*Megadyptes antipodes*). Behav Ecol Sociobiol 55:169-175
416 <https://doi.org/10.1007/s00265-003-0683-3>

417 McClintock JB, Amsler CD, Amsler MO, Fraser WR (2020) Intertidal foraging by Gentoo Penguins in a
418 macroalgal raft. Antarct Sci 32:43-44 <https://doi.org/10.1017/S095410201900052X>

419 Meyer-Rochow VB (2000) Risks, especially for the eye, emanating from the rise of solar UV-radiation
420 in the Arctic and Antarctic regions. *Int J Circumpolar Health*. 59:38-51

421 Meyer-Rochow VB, Shimoyama A (2008) UV-reflecting and absorbing body regions in gentoo and
422 King Penguin: Can they really be used by the penguins as signals for conspecific recognition? *Polar*
423 *Biol* 31:557-560 <https://doi.org/10.1007/s00300-007-0387-7>

424 Meyer-Rochow VB, Shimoyama A (2009) Is there any proof of UV-sensitivity and its role in King
425 Penguins (*Aptenodytes patagonicus*)? *Polar Biol* 32:515-516 [https://doi.org/10.1007/s00300-008-](https://doi.org/10.1007/s00300-008-0565-2)
426 [0565-2](https://doi.org/10.1007/s00300-008-0565-2)

427 Michael R, Söderberg P, Chen E (1998) Dose-response function for lens forward light scattering after
428 in vivo exposure to ultraviolet radiation. *Graefes Arch Clin Exp Ophthalmol* 236:625–629 [https://doi-](https://doi.org/10.1007/s004170050132)
429 [org/10.1007/s004170050132](https://doi.org/10.1007/s004170050132)

430 Montague T (1985) A maximum dive recorder for Little Penguins. *Emu* 85:264-267
431 <https://doi.org/10.1071/MU9850264>

432 Mullen P, Pohland G (2007) Studies on UV reflection in feathers of some 1000 bird species: Are UV
433 peaks in feathers correlated with violet-sensitive and ultraviolet-sensitive cones? *Ibis* 150:59-68
434 <https://doi.org/10.1111/j.1474-919X.2007.00736.x>

435 Nolan PM, Dobson FS, Nicolaus M, Karels TJ, McGraw KJ, Jouventin P (2010) Mutual mate choice for
436 colorful traits in King Penguins. *Ethology* 116:635-644 [https://doi.org/10.1111/j.1439-](https://doi.org/10.1111/j.1439-0310.2010.01775.x)
437 [0310.2010.01775.x](https://doi.org/10.1111/j.1439-0310.2010.01775.x)

438 Olsson P, Lind O, Mitkus M, Delhey K, Kelber A (2021) Lens and cornea limit UV vision of birds - a
439 phylogenetic perspective. *J Exp Biol* 224: jeb243129 <https://doi:10.1242/jeb.243129>

440 Potier S, Bonadonna F, Kelber A, Duriez O (2016) Visual acuity in an opportunistic raptor, the
441 chimango caracara (*Milvago chimango*). *Physiol Behav* 157:125-128
442 <https://doi.org/10.1016/j.physbeh.2016.01.032>

443 Pye D (2011) To add another hue unto the rainbow—near ultraviolet in nature. *Opt Laser Technol*
444 43:310-316 <https://doi.org/10.1016/j.optlastec.2009.01.007>

445 Reymond L (1985) Spatial visual acuity of the eagle *Aquila audax*: A behavioural, optical and
446 anatomical investigation. *Vision Res* 25:1477-1491 [https://doi.org/10.1016/0042-6989\(85\)90226-3](https://doi.org/10.1016/0042-6989(85)90226-3)

447 Rog SJ, White CW, Williams TT (1986) Ultraviolet effects on visual acuity in pseudophakia. *Am J*
448 *Optom Phys Opt* 63:867-872 <https://doi.org/10.1097/00006324-198611000-00002>

449 Shirihai H (2007) A complete guide to Antarctic wildlife: The birds and marine mammals of the
450 Antarctic continent and the Southern Ocean. A&C Black, London
451 <https://doi:10.1017/S003224740800764X>

452 Siebeck UE, Marshall NJ (2001) Ocular media transmission of coral reef fish—can coral reef fish see
453 ultraviolet light? *Vision Res* 41:133-149 [https://doi.org/10.1016/S0042-6989\(00\)00240-6](https://doi.org/10.1016/S0042-6989(00)00240-6)

454 Spitschan MG, Aguirre GK, Brainard DH, Sweeney AM (2016) Variation of outdoor illumination as a
455 function of solar elevation and light pollution. *Sci Rep* 6:26756 <https://doi.org/10.1038/srep26756>

456 Stark WS, Wagner RH, Gillespie CM (1994) Ultraviolet sensitivity of three cone types in the aphakic
457 observer determined by chromatic adaptation. *Vision Res* 34:1457-1459
458 [https://doi.org/10.1016/0042-6989\(94\)90147-3](https://doi.org/10.1016/0042-6989(94)90147-3)

459 Suburo AM, Scolaro JA (1999). Environmental adaptations in the retina of the Magellanic Penguin:
460 photoreceptors and outer plexiform layer. *Waterbirds* 22:111-119 <https://doi.org/10.2307/1522000>

461 Tsukahara N, Tani Y, Kikuchi H, Sugita S (2013) Light transmission of the ocular media in birds and
462 mammals. *J Vet Sci* 76:93-95 <https://doi.org/10.1292/jvms.13-0293>

463 Vianna JA, Fernandes FA, Frugone MJ, Figueiró HV, Pertierra LR, Noll D, Bi K, Wang-Claypool CY,
464 Lowther A, Parker P (2020) Genome-wide analyses reveal drivers of penguin diversification. *Proc*
465 *Natl Acad Sci USA* 117:22303-22310 <https://doi.org/10.1073/pnas.2006659117>

466 Walls GL (1931) The occurrence of colored lenses in the eyes of snakes and squirrels, and their
467 probable significance. *Copeia* 1931:125-127 <https://doi.org/10.2307/1437335>

468 Wilkie SE, Robinson PR, Cronin TW, Poopalasundaram S, Bowmaker JK, Hunt DM (2000) Spectral
469 tuning of avian violet-and ultraviolet-sensitive visual pigments. *Biochemistry-US* 39:7895-7901
470 <https://doi.org/10.1021/bi992776m>

471 Williams J (1973) Optical properties of the ocean. *Rep Prog Phys* 36:1567-1608
472 <https://doi.org/10.1088/0034-4885/36/12/002>

473 Xavier JC, Trathan PN, Ceia FR, Tarling GA, Adlard S, Fox D, Edwards EWJ, Vieira RP, Medeiros R, De
474 Broyer C, Cherel Y (2017) Sexual and individual foraging segregation in Gentoo Penguins *Pygoscelis*
475 *papua* from the Southern Ocean during an abnormal winter. *Plos One* 12:e0174850-e0174850
476 <https://doi.org/10.1371/journal.pone.0174850>

477 Zielinski O (2013) Subsea optics: an introduction. In: Watson J, Zielinski O (eds) *Subsea optics and*
478 *imaging*. Woodhead Publishing, Oxford, pp 3-16 <https://doi.org/10.1533/9780857093523.1.3>

479 Zusi R (1975) An interpretation of skull structure in penguins. In: Stonehouse B (ed) *The biology of*
480 *penguins*. MacMillan Press, London and Basingstoke, pp 59-84

481 **Table legends**

482

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

486

487 **Table 2** The wavelength at which transmission reached 10% (λ_{10}), 50% (λ_{50}) and 90% (λ_{90}) of the
488 transmission at 700 nm for each of the cornea, lens and whole eye. Footnotes: a. The λ_{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

491

492 **Figure legends**

493

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

498

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

502

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

506

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

512

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

516

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

521 **Table 1**

Penguin identification	Age at examination (years)	Sex	Preservation prior to examination	Lens transparency	Equipment used	Tissue examined
Little Penguins (<i>Eudyptula minor</i>)						
L1 (LP1, B80231)	Adult	Unknown	Fresh	Clear	SpectraMax 13x	2 eyes: cornea, lens, vitreous and whole eye
Gentoo Penguins (<i>Pygoscelis papua</i>)						
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 (<i>Aptenodytes patagonicus</i>)						
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

522

523

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 <i>Eudyptula minor</i>	293	335	393	301	322	440	N/A ^a	300	362	316	340	392
Gentoo Penguin <i>Pygoscelis papua</i>	295	325	481	347	415	611	N/A ^a	301	343	_b	_b	_b
King Penguin <i>Aptenodytes patagonicus</i>	299	341	409	376	400	614	N/A ^a	301	330	371	404	492

526 **Figure 1**



527

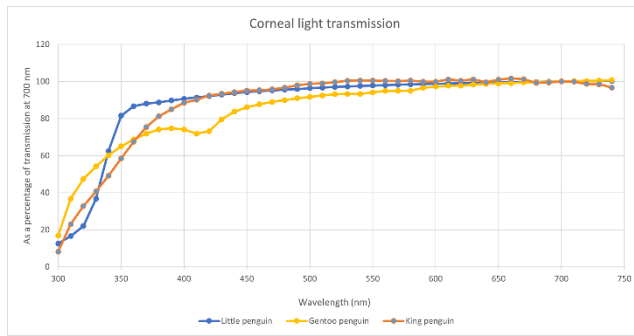
528 **Figure 2**



529

530

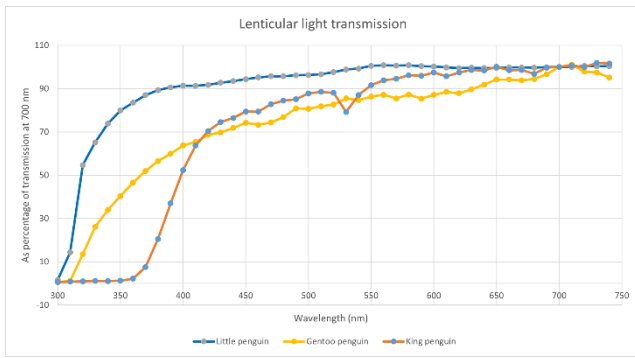
531 **Figure 3**



532

533

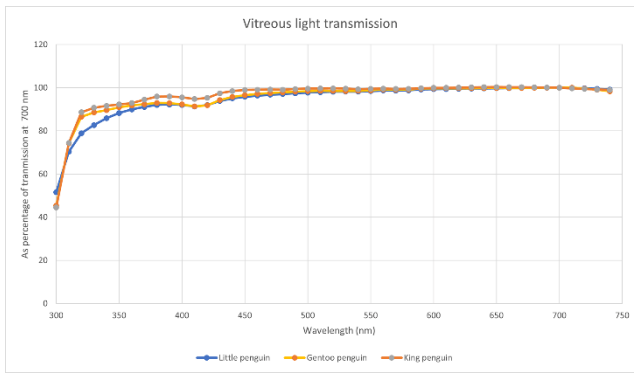
534 **Figure 4**



535

536

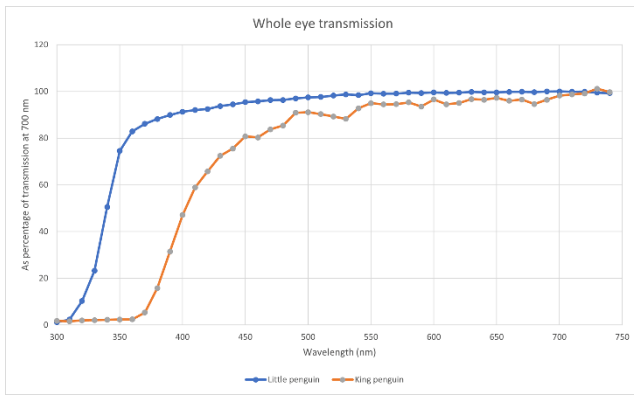
537 **Figure 5**



538

539

540 **Figure 6**



541