

Evolutionary history limits species' ability to match color sensitivity to available habitat light

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1 **Abstract**

2 The spectrum of light that an animal sees – from ultraviolet to far red light – is governed by the
3 number and wavelength sensitivity of a family of retinal proteins called opsins. It has been
4 hypothesized that the spectrum of light available in an environment influences the range of
5 colors that a species has evolved to see. However, invertebrates and vertebrates use
6 phylogenetically distinct opsins in their retinae, and it remains unclear whether these distinct
7 opsins influence what animals see, or how they adapt to their light environments. Systematically
8 utilizing published visual sensitivity data from across animal phyla, we found that terrestrial
9 animals are more sensitive to shorter and longer wavelengths of light than aquatic animals, and
10 that invertebrates are more sensitive to shorter wavelengths of light than vertebrates. Controlling
11 for phylogeny removes the effects of habitat and lineage on visual sensitivity. Closed and open
12 habitat terrestrial species have similar spectral sensitivities when comparing across the Metazoa,
13 and deep water animals are more sensitive to shorter wavelengths of light than shallow water
14 animals. Our results suggest that animals do adapt to their light environment, however the
15 invertebrate-vertebrate evolutionary divergence has limited the degree to which animals can
16 perform visual tuning.

17 **Keywords:** visual tuning, light environment, ciliary opsin, rhabdomeric opsin, λ_{\max}

18

19 **1. Introduction**

20 Animals use vision for many tasks, including finding prey, avoiding toxic animals and plants,
21 identifying predators, assessing mate quality, and navigating their environment [1–5]. In many
22 cases, the objects of interest to the animal need to be distinguished from the background [6,7]. For
23 example, food that does not contrast with the background is harder for foragers to detect than food
24 that does contrast with the background [8–12]. Signals that contrast with background colours and
25 patterns are also used for mating displays [13–18]. Furthermore, many species' body colour
26 patterns have evolved to be simultaneously cryptic to predators while conspicuous to intended
27 receivers [19–21]. Finally, contrasting colours can improve animals' ability to learn the meaning
28 of signals, as when chicks learn more quickly to avoid bitter, aposematically coloured food
29 [2,3,22].

30

31 An animal's ability to detect visual information depends upon the colour and amount of light in its
32 habitat, otherwise known as the *light environment* [6,23,24]. For example, red and blue light are
33 filtered out by chloroplasts, lending forests and estuarine environments a yellow-green cast [24–
34 26]. Likewise, the water column progressively filters red and UV light [23,24]. Animals' signalling
35 behaviours, choice of microhabitat, and visual physiology are thus expected to co-evolve to suit
36 their light environment [6].

37

38 Sighted species' photoreceptors (the light-absorbing neurons which enable vision) are theorized to
39 have undergone adaptation to best absorb the light most often present in their environments [23].

40 This process, called *visual tuning*, is made possible by both filtering pigments [27–35] and
41 differences in the amino acid sequence or 3-dimensional shape of photosensitive proteins called
42 *opsins* [23]. Visual tuning has been found to shift wavelengths of maximum sensitivity in species
43 as diverse as birds, fish, and mammals [23,36,45,46,37–44]. Although the effect of light
44 environment on vision has been extensively studied in fish [47,48], a systematic study of visual
45 tuning in terrestrial animals has not yet occurred; neither have terrestrial animals been
46 systematically compared to aquatic species. Both aquatic and terrestrial animals are found in a
47 variety of light environments, and multiple phyla have independently made the water-to-land
48 habitat transition. Additionally, studies of animals which transition from aquatic larvae to
49 terrestrial adults have found that these species change their visual pigment expression patterns in
50 a manner that matches their changing light environment [49–53]. Understanding whether
51 phylogeny constrains the extent of visual tuning, particularly during these water-to-land
52 transitions, is critical for understanding the evolutionary ecology of animal vision.

53

54 If opsin tuning faces phylogenetic constraints, the evolutionary history of animal vision may have
55 shaped the degree to which different phyla have adapted to their light environments. The types of
56 opsins differ between chordates and other phyla [54]. Chordates use c-opsins in cilia-bearing
57 photoreceptors to transduce photons into vision, while non-chordate animals use r-opsins in
58 rhabdomere-bearing photoreceptors; no animals have been identified that use both c- and r-type
59 photoreceptors for vision [55]. Phylogenetic analyses reveal that c- and r-opsins diverged 400
60 million years ago and were likely both present in the urbilaterian, with r-opsins closely related to
61 the melanopsins used by chordates for non-visual tasks [55]. The r-opsin/ c-opsin divergence may
62 have given rise to different degrees of tuning between chordates and non-chordates.

63

64 The diverse habitats in which animals live, combined with the long evolutionary history of visual
65 pigments, leads to several questions. First, have transitions from aquatic to terrestrial habitats
66 influenced the spectra of light that animals can see? And, are differences in the spectra that animals
67 can see associated with the c-opsin/ r-opsin divergence? Second, do animals that live in visual
68 environments that filter red and blue light, such as closed-canopy forests and estuarine habitats,
69 see colours more similarly to each other than to open terrestrial or freshwater aquatic
70 environments, in which colours are less (if at all) strongly filtered? And, if there is an effect of
71 habitat greenness, is this effect outweighed by phylogeny?

72

73 To answer these questions, we performed a phylogenetically weighted systematic analysis of the
74 maximum and minimum wavelength of visual sensitivity, as well as the range of visual sensitivity,
75 across animals.

76

77 **(2) Materials and method**

78 *Paper selection:* We conducted Google Scholar searches in October 2017 and January 2018. Our
79 first search used the search pattern “*visual pigment*” OR *opsin* OR “*absorbance spectrum*” “*λ*
80 *max*” -*human* -*man* -*men* -*woman* -*women* -“*Homo sapiens*” -*disease* -*regeneration*. We
81 conducted a second Google Scholar search using the search pattern *visual pigment*, *opsin*
82 *sensitivity*, *absorbance spectrum*. For both searches, we excluded citations and patents.

83

84 We reviewed candidate articles using a three-step process. First, we screened by title and abstract
85 to identify original research articles and review papers that examined animal visual physiology.
86 We then screened articles to determine if they used microspectrophotometry, electrophysiology,
87 pigment extraction, or *in vitro* mRNA expression followed by spectrophotometry, and that they
88 measured visual sensitivity or visual pigment absorption from at least two animals. Finally, we
89 only kept articles which used animals that were wild-caught or reared in full-spectrum light
90 conditions, to avoid any effects of artificial lighting on visual sensitivity [56,57].

91

92 For review articles, we determined whether the authors had included measurements of the mean
93 wavelength of peak sensitivity (λ_{\max}) of some population in the article's figures or tables. We
94 downloaded the corresponding primary sources and filtered them using the process described
95 above.

96

97 Visual pigment sensitivity data: We recorded the following data for each species of each paper that
98 passed our filters: 1) mean wavelength of peak sensitivity (λ_{\max}) for each visual pigment measured;
99 2) number of animals measured (n); 3) standard deviation of the mean λ_{\max} (SD) (when available);
100 and 4) where animals were caught (when available). We calculated sampling error for visual
101 pigments when possible.

102

103 Habitat data: We used standardized data sources to classify each species by habitat. Sources
104 included field guides [58–60], public databases (BugGuide, <bugguide.net>, Butterflies and Moths
105 of North America, <butterfliesandmoths.org>, FishBase <fishbase.org>, Seabase

106 <sealifebase.org>, IUCN Redlist <iucnredlist.org>) and online encyclopaedias including Animal
107 Diversity Web (<animaldiversity.org>) and Encyclopedia of Life (<eol.org>). After first
108 classifying species as terrestrial or aquatic, we then defined terrestrial sub-habitats: rainforest,
109 forest, woodland, shrubland, grassland, and desert. We recategorized these habitats into three
110 habitat types based on canopy density. Rainforest and temperate forest were designated as “closed”
111 habitats. Woodland was considered to have “intermediate” canopy density [25]. Shrubland,
112 grassland, and desert were classified as "open" habitats.

113

114 Aquatic habitats included river, stream, pond, lake, coastal, estuarine, open-water marine, bottom-
115 dwelling marine, abyssopelagic, abyssodemersal, bathypelagic, and bathydemersal habitats. We
116 recategorized these habitats into two habitat types based on salinity. River, stream, pond, and lake
117 habitats were considered "freshwater" habitats; while coastal, estuarine, open-water marine, and
118 bottom-living marine habitats were "marine" habitats. Animals considered "coastal" were those
119 described as living in water along the coast, near shore, or in estuaries. We also recategorized these
120 habitats into two habitat types based on whether light was abundant or not. Abyssopelagic,
121 abyssodemersal, bathypelagic, and bathydemersal habitats receive little or no sunlight due to their
122 depth in the water column and were considered "lightless" habitats. Species that were considered
123 by our sources as deep-water species were also considered species that lived in "lightless" habitats.
124 All other habitats were considered "lit" habitats. Finally, we used FishBase, SealifeBase or field
125 guides to identify the minimum and maximum depths for each species. We then used these data to
126 calculate average depth per species ($D_{\text{average}} = (D_{\text{max}} + D_{\text{min}}) * 2^{-1}$).

127

128 Phylogenetic control:

129 To control for the effect of evolutionary relatedness on visual sensitivity we built a phylogenetic
130 tree of all animals in our analysis (see the electronic supplementary material: figure S1). We used
131 the function `tnrs_match_names` in the R package *rotl* [61] to acquire data from the Open Tree of
132 Life database (tree.opentreeoflife.org) for each of the species represented in our regression, and
133 to generate a phylogenetic tree using default arguments and excluded species flagged as *incertae*
134 *cedis*; i.e., with uncertain phylogenetic position (25 species) and species which had no sequencing
135 data in the Open Tree of Life database (6 species). We created an induced subtree with the resulting
136 data using the function `tol_induced_subtree` in the package *rotl*.

137

138 Trees produced using *rotl* are unrooted, without branch lengths, and sometimes with unresolved
139 polytomies. We used the R packages *phytools* [62] and *ape* [63] to resolve these issues. We used
140 the `root` function in *ape* to root the tree using *Saccharomyces cerevisiae* (otid: 5262624) from
141 tree.opentreeoflife.org) as the outgroup. We computed branch lengths using the `compute.brln`
142 function in *ape* with default arguments. Finally, we randomly resolved polytomies using the
143 `multi2di` function in *ape* with default parameters. Subtrees of the primary tree were constructed as
144 needed using the `drop.tips` function in *ape*.

145

146 Statistical Analyses:

147 To determine whether longest λ_{\max} , shortest λ_{\max} , and range of λ_{\max} followed the normal
148 distribution, we used the `shapiro.test` function in R. To determine whether the variances of longest

149 λ_{\max} , shortest λ_{\max} , and range of λ_{\max} differed between broad habitat type (aquatic or terrestrial) or
150 lineage (invertebrates or vertebrates) we used `levene.test` function in R.

151

152 To determine whether there was an effect of broad habitat type or lineage on the longest λ_{\max} ,
153 shortest λ_{\max} , and range of λ_{\max} , we used the `glm` function in R to construct generalized linear
154 models with the formula $\lambda_{\max} \sim \text{broad habitat} * \text{lineage}$.

155

156 To determine whether phylogeny could explain extant differences in longest λ_{\max} , shortest λ_{\max} ,
157 and range of λ_{\max} between broad habitat type or lineage, we constructed phylogenetically
158 controlled linear models using the `phylolm` function in the *phylolm* [64] package for R with the
159 formula $\lambda_{\max} \sim \text{broad habitat} * \text{lineage}$ with a bootstrap of 100. Since we had to exclude 31 species
160 from our phylogenetic tree, we first ran the `glm` described above with the trimmed data set, and
161 then compared those results to the results of our `phylolm` models. For these models, we used the
162 overall phylogenetic tree and our trimmed dataset.

163

164 We then subset our overall dataset for terrestrial animals and aquatic animals, re-tested for normal
165 distributions and variances, and conducted a set of statistical analyses specific to terrestrial or
166 aquatic animals. To determine whether there was an effect of terrestrial habitat type (closed,
167 intermediate, or open) or lineage on longest λ_{\max} , shortest λ_{\max} , and range of λ_{\max} , we used the `glm`
168 function with the formula $\lambda_{\max} \sim \text{terrestrial habitat type} * \text{lineage}$. However, since there were only
169 2 species that were intermediate habitat specialists, and they had similar visual spectra to those in
170 closed canopies, we combined closed and intermediate habitat treatments into a single treatment,

171 closed_intermediate, and re-ran the generalized linear models described above using the new
172 habitat treatment levels (closed_intermediate vs open).

173

174 We examined the effects of depth and habitat on visual sensitivities of aquatic animals. To
175 determine whether there were effects of minimum, maximum, or average depth of habitat on
176 longest λ_{\max} , shortest λ_{\max} , and range of λ_{\max} among aquatic species, we constructed linear models
177 using the `lm` command with the formula $\lambda_{\max} \sim \text{depth}$. To determine whether phylogeny could
178 explain extant differences in minimum, maximum, or average depth of habitat among longest λ_{\max} ,
179 shortest λ_{\max} , and range of λ_{\max} among aquatic species, we first re-ran the linear models without
180 the species excluded from our phylogenetic tree, and then constructed phylogenetically controlled
181 linear models using the `phylolm` function in the *phylolm* package for R with the formula $\lambda_{\max} \sim$
182 depth with a bootstrap of 100. For these models, we used a subtree of our overall phylogenetic tree
183 (see above), which omitted all terrestrial species.

184

185 Finally, we subset our overall dataset for open terrestrial animals and non-deep water aquatic
186 animals (both freshwater and coastal) and conducted the following analyses. To assess whether
187 the visual systems of animals in open terrestrial habitats were more similar to the visual systems
188 of animals in open water habitats (coastal or freshwater) than those of closed terrestrial habitats
189 we compared the longest λ_{\max} , shortest λ_{\max} , and range of λ_{\max} , of species in coastal-aquatic,
190 freshwater-aquatic, terrestrial-closed, and terrestrial-open habitats using the `kruskal.wallis`
191 function in R. Following this, we performed a pairwise (Steel-Dwass) test using the `dscfAllPairs`
192 function in the R package *PCMCRRplus* [65]. To determine whether phylogeny could explain extant

193 differences in longest λ_{\max} , shortest λ_{\max} , and range of λ_{\max} between animals living in these four
194 habitat types we re-ran the above analyses only using species in our phylogenetic tree, and then
195 constructed phylogenetically controlled linear models using the `phylolm` function in the *phylolm*
196 package for R with using the following formula with a bootstrap of 100: $\lambda_{\max} \sim \text{coastal-aquatic} +$
197 $\text{freshwater-aquatic} + \text{terrestrial-closed}$ and $\lambda_{\max} \sim \text{coastal-aquatic} + \text{freshwater-aquatic} + \text{terrestrial-}$
198 open .

199

200 **(3) Results**

201 Our dataset included 1,114 opsins from 446 species, extracted from a total of 156 articles (See the
202 electronic supplementary materials: table S1). Of these, 868 opsins were recorded from 355 aquatic
203 species, and 246 opsins were recorded from 91 terrestrial species. Our data were not normally
204 distributed (Shapiro-Wilk test: longest λ_{\max} : $p < 0.05$, $W = 0.94$; shortest λ_{\max} : $p < 0.05$, $W = 0.83$;
205 range of λ_{\max} : $p < 0.05$, $W = 0.70$). Shortest, but neither longest nor range of λ_{\max} were found to
206 have equal variances when compared across broad habitat and lineage (Levene's test: longest λ_{\max} :
207 $p = 0.16$, $t = 1.74$; shortest λ_{\max} : $p < 0.05$, $t = 25.28$; range of λ_{\max} : $p < 0.05$, $t = 21.62$).

208

209 (a) *Terrestrial species were maximally sensitive to longer wavelengths of light than aquatic*
210 *species*

211 Terrestrial species were maximally sensitive to longer wavelengths of light than aquatic species,
212 independent of opsin type (GLM, $n=433$: habitat $p = 3.83 \times 10^{-8}$, $t = 5.600$, lineage $p = 0.309$, $t = -$
213 1.019 ; interaction: $p = 0.595$, $t = -0.532$; λ_{\max} longest long-wavelength terrestrial species: 535 ± 41.6

214 nm, aquatic species: 506 ± 30.6 nm, invertebrate species: 513 ± 38.9 nm, vertebrate species:
215 512 ± 33.0 nm) (figure 1a).

216

217 (b) Terrestrial species saw shorter wavelengths of light than aquatic species

218 Terrestrial species were maximally sensitive to shorter wavelengths of light than aquatic species,
219 but there was a significant interaction between habitat and lineage: aquatic vertebrate species were
220 more sensitive to short wavelengths than aquatic invertebrate species, but terrestrial invertebrate
221 species were more sensitive to short wavelengths than terrestrial vertebrate species. Additionally,
222 invertebrates trended towards seeing short wavelengths of light (GLM, $n = 433$: habitat $p = 0.045$,
223 $t = -2.012$, lineage $p = 0.051$, $t = 1.960$, interaction: $p = 2.34 \times 10^{-3}$; $t = -3.061$; λ_{\max} shortest short-
224 wavelength terrestrial species: 442 ± 79.2 nm, aquatic species: 476 ± 39.3 nm; invertebrate species:
225 466 ± 70.2 nm, vertebrate species: 471 ± 41.3 nm) (figure 1b).

226

227 (c) Terrestrial species and invertebrates saw a larger range of colours than aquatic species
228 and vertebrates

229 Terrestrial species saw a larger range of colours than aquatic species. In addition, there was a
230 significant interaction between habitat and lineage: aquatic invertebrates saw a narrower range of
231 colours than aquatic vertebrates, but terrestrial invertebrates saw a broader range of colours than
232 terrestrial vertebrates (GLM, $n = 443$: habitat $p = 2.51 \times 10^{-6}$, $t = 4.772$, lineage $p = 0.03$, $t = -2.184$,
233 interaction: $p = 0.00261$; $t = 2.232$; λ_{\max} range terrestrial species: 92 ± 85.6 nm, aquatic species:
234 30.9 ± 51.6 nm; invertebrate species: 47.2 ± 80.4 nm, vertebrate species: 40.7 ± 56.7 nm) (figure 1c).

235 (d) Accounting for phylogeny removes the effect of habitat and lineage on visual pigment
 236 sensitivity

237 When we ran our analyses with only the subset of species included in our phylogenetic tree, we
 238 found that we lost the effect of lineage on the range of visual pigment sensitivity but did not lose
 239 the effect of lineage on the longest or shortest wavelengths of maximum sensitivity (see the
 240 electronic supplementary materials: tables S2 and S3). With this in mind, we controlled for
 241 phylogeny. Controlling for phylogeny removed the effect of habitat and lineage on longest λ_{\max} ,
 242 shortest λ_{\max} and range of λ_{\max} (table 1).

Table 1. No effect of habitat or lineage on visual sensitivity following phylogenetic control.

Variable	<i>Longest λ_{\max}</i>			<i>Shortest λ_{\max}</i>			<i>λ_{\max} Range</i>		
	<i>p</i>	<i>t</i>	<i>SE</i>	<i>p</i>	<i>t</i>	<i>SE</i>	<i>p</i>	<i>t</i>	<i>SE</i>
<i>Habitat</i>	0.257	-1.13	11.4	0.439	0.774	17.0	0.226	-1.211	21.7
<i>Lineage</i>	0.953	0.058	12.5	0.940	0.075	18.5	0.978	-0.028	23.7
<i>Habitat * Lineage</i>	0.474	-0.72	20.3	0.214	-1.25	30.1	0.552	0.596	38.6

243

244 (e) Forest-woodland and open habitat species have similar spectral sensitivities

245 There was no effect of tree canopy openness on λ_{\max} , shortest λ_{\max} , and range of λ_{\max} (table 2; see
 246 the electronic supplementary materials: figure S2, table S4 and table S5).

Table 2. Effect of habitat greenness on shortest λ_{\max} . P-values are above diagonal; q-values are below diagonal. Numbers in bold are statistically significant.

q \ p		<i>Forest + Intermediate</i>	<i>Freshwater</i>	<i>Open Terrestrial</i>	<i>Mean (nm)</i>	<i>SD (nm)</i>
	<i>Coastal</i>	--	5.75*10⁻⁷	0.903	3.92*10⁻⁶	254.4
<i>Forest + Intermediate</i>	-7.54	--	2.62*10⁻⁵	0.24	470.4	68
<i>Freshwater</i>	-0.970	-6.49	--	1.92*10⁻⁵	261.1	56.5
<i>Open Terrestrial</i>	-8.26	-2.64	-7.034	--	423.5	82.1

247

248 (f) Average and maximum depth, but not minimum depth, influenced sensitivity to blue but
249 not red light

250 Species living at deeper average depth had longer shortest λ_{\max} than shallow-living species ($p =$
251 0.046 , $t = 2.03$; see the electronic supplementary material: figure S3a). Additionally, species
252 living at a deeper maximum depth had shortest λ_{\max} that were longer than shallow-living species
253 shortest λ_{\max} ($p = 0.033$, $t = 2.18$; electronic supplementary material: figure S4). Average depth
254 and minimum depth did not affect species' longest λ_{\max} or range of λ_{\max} , and there was no effect
255 of maximum depth on spectral sensitivity (electronic supplementary material: figures S3b-c and
256 S5). Controlling for phylogeny removes the effect of average depth on shortest λ_{\max} (phylolm: $t =$
257 $-4.688 \cdot 10^{-1}$, $p = 0.641$).

258

259 (g) Animals in coastal and freshwater habitats saw shorter wavelengths while animals in
260 forest+intermediate or open-canopy habitats saw longer wavelengths

261 We found that coastal animals' and freshwater animals' shortest λ_{\max} were shorter than both
262 forest+intermediate animals' shortest λ_{\max} and open terrestrial animals' shortest λ_{\max} (omnibus test:
263 $p = 1.359 \cdot 10^{-12}$, $\chi^2 = 53.296$, $df = 3$, pair-wise comparisons: table 2; figure 2). We also found that
264 forest+intermediate animals' longest λ_{\max} and open terrestrial animals' longest λ_{\max} were longer
265 than freshwater animals' and coastal animals' longest λ_{\max} (omnibus test: $p = 1.19 \cdot 10^{-14}$, $\chi^2 = 67.92$,
266 $df = 3$; pairwise comparisons: table 3 and figure 2).

267

268 (h) Open terrestrial animals had a broader visual range than coastal animals

269 We found that open terrestrial animals had a larger range (longest λ_{\max} – shortest λ_{\max}) than coastal
 270 animals, but all other habitat groups were statistically similar (omnibus test: $p = 0.03366$, $\chi^2 =$
 271 8.6931 , $df = 3$; pairwise comparisons: table 4).

Table 3. Effect of habitat greenness on longest λ_{\max} . P-values are above diagonal; q-values are below diagonal. Numbers in bold are statistically significant.

q \ p	Coastal	Forest + Intermediate	Freshwater	Open Terrestrial	Mean (nm)	SD (nm)
Coastal	--	1.72×10^{-7}	0.77	6.23×10^{-10}	297.3	72.4
Forest + Intermediate	-7.82	--	7.54×10^{-6}	0.22	547.1	17.7
Freshwater	-2.40	-6.85	--	2.77×10^{-5}	314.3	73.8
Open Terrestrial	-9.14	-2.71	-7.73	--	534.1	49.1

272

273 (i) Accounting for lineage removed the effects of water and dissolved particles on visual
 274 pigment sensitivity

275 Controlling for phylogeny removed the effect of habitat greenness on shortest λ_{\max} , longest λ_{\max} ,
 276 and λ_{\max} range (table 5), even when we accounted for the species absent from our phylogenetic
 277 tree (see the electronic supplementary material: tables S6 – S8).

Table 4. Effect of habitat greenness on λ_{\max} range. P-values are above diagonal; q-values are below diagonal. Numbers in bold are statistically significant.

q \ p	Coastal	Forest + Intermediate	Freshwater	Open Terrestrial	Mean (nm)	SD (nm)
Coastal	--	0.239	0.411	0.0497	42.88	70.2
Forest + Intermediate	-2.65	--	0.827	0.756	76.63	72.6
Freshwater	-2.18	-1.21	--	0.561	53.26	69.3
Open Terrestrial	-3.64	-1.40	-1.84	--	110.6	98.9

278

279

Comparison	Dependent variable	t	p	SE
Coastal vs forest + intermediate	Shortest λ_{\max}	-0.576	0.566	253.78
Coastal vs open terrestrial	Shortest λ_{\max}	-0.490	0.625	251.87
Freshwater vs open terrestrial	Shortest λ_{\max}	-0.445	0.658	254.04
Coastal vs open terrestrial	Longest λ_{\max}	-1.37	0.174	164.14
Forest + intermediate vs freshwater	Longest λ_{\max}	-1.74	0.0847	166.79
Freshwater vs open terrestrial	Longest λ_{\max}	-1.69	0.0941	165.55
Open terrestrial vs coastal	λ_{\max} Range	-0.397	0.692	255.87

280

281 **(4) Discussion**

282

283 *(a) The transition from aquatic to terrestrial habitats has influenced animal vision*

284 We found that terrestrial species see longer long-wavelength light and a larger range of colours
285 overall compared to aquatic species. Few other studies have broadly investigated the effect of
286 animals' evolutionary transitions between aquatic and terrestrial habitats on colour vision.
287 However, transitions from aquatic to terrestrial life stages that lead to the development of different
288 visual abilities can indicate whether differences between terrestrial and aquatic lifestyles
289 themselves necessitate different strategies for perceiving the world [49]. Such studies have been
290 conducted within single species: for example, in several species of dragonflies, adults have short
291 wavelength-shifted vision, express more visual pigments than larvae, and have dorsal eye regions
292 specialized to detect shorter wavelengths of light refracted from the sky [49,50]. Similar types of
293 visual shifts have been observed in southern leopard frogs [66]. Just as animal development
294 favours the expression of environmentally matched opsins over an intra-generational timescale,
295 our results suggest that evolutionary adaptation favours the use of environmentally matched opsins
296 over an inter-generational timescale.

297

298 The results of our terrestrial vs. aquatic models are congruent with the visual tuning hypothesis,
299 that animal visual systems undergo adaptation to best detect the light most often present in their
300 environments [23]. Terrestrial animals are exposed to a dynamic range of colours that changes
301 throughout the day, including both short and long-wavelength light, as well as ultraviolet light in
302 large forest gaps and open environments [25,26]. By contrast, aquatic animals, which we found to
303 be less sensitive to long-wavelength and ultraviolet light, live in environments that are exposed to
304 relatively less long-wavelength and ultraviolet light [24]. Absent phylogenetic controls, our
305 regressions suggest that animals are likely to be maximally sensitive to colours most often present
306 in their environment, and insensitive to colours likely to be absent.

307

308 *(b) Canopy coverage does not influence visual tuning*

309 We found that animals which live in densely forested environments do not differ in their visual
310 sensitivities from animals that live in open, prairie-like habitats. Although the forest light
311 environment directly beneath the canopy is dominated by middle wavelengths (i.e., greens and
312 yellows) under most conditions [25,26], spatial and temporal variations in forest light's spectral
313 qualities may require forest animals to possess visual sensitivities similar to those of animals living
314 in open habitats.

315

316 Additionally, animals may choose to use light microhabitats which are suitable to their current
317 visual physiology. Endler and Théry observed that forest birds use areas in which they are most
318 conspicuous to advertise to potential mates [67]. Some species also modify their habitats to

319 improve the visibility of their visual displays. For example, male golden-collared manakins clean
320 the arenas they use to court females; the background of a cleaned arena contrasts better with male
321 manakins' plumage than the background of the forest surrounding the arena [68]. Arena cleaning
322 also seems to improve white-bearded manakins' ability to detect predators [69]. In such cases,
323 evolution may be driving site preferences which match vision rather than driving vision to match
324 site preferences, a complete reversal of the mechanism being investigated in our study.

325

326 *(c) The ciliary/rhabdomeric opsin divergence may impact the colours that animals can see*

327 We found that animals that use rhabdomeric opsins for vision see a broader range of wavelengths
328 of light than animals that use ciliary opsins for vision. Many animals that use rhabdomeric
329 photoreceptors for vision, especially arthropods, have opsins that are maximally sensitive to
330 ultraviolet light [31,70–75]. By contrast, comparatively few animals that use ciliary photoreceptors
331 for vision have opsins that are maximally sensitive to UV light, although several species of birds
332 and fish are sensitive to ultraviolet light [40,76–78]. Additionally, many mammals that utilize high
333 acuity colour vision and whose short wavelength sensitive photoreceptors are sensitive to UV light,
334 have corneas that selectively filter UV, inhibiting their ability to see those wavelengths [79,80].
335 Both ciliary and rhabdomeric opsins are thought to have been present in the urbilaterian, the
336 common ancestor of all modern animals save sponges, cnidarians, placozoans, and ctenophores
337 [55]. The emergence in chordates of ciliary opsins for vision rather than photoentrainment
338 represents a singular event, one that may have also heralded differences in visual perception
339 associated with reduced sensitivity to short wavelengths of light.

340

341 *(d) Phylogeny outweighs the effect of habitat*

342 We found that the effects of habitat upon the spectrum of light animals can see were reduced once
343 we controlled for phylogenetic history. These findings differ from those of studies looking at
344 individual animal clades. For example, a 2018 survey of ray-finned fish found that species living
345 at depth have reduced chromacy even after controlling for phylogeny [47]. Similarly, a historic
346 study of cottoid fish in Lake Baikal found that there was a correlation between λ_{\max} and habitat
347 depth [81]. While studies of marine mammals found that species that forage near the surface have
348 visual pigments that resemble those of terrestrial mammals while those that foraged at depth had
349 visual pigments with amino acid substitutions that shifted the λ_{\max} towards shorter wavelengths
350 [82]. We found that, when expanding to include multiple clades – both chordates and non-
351 chordates – a similar pattern emerged: terrestrial species had broader sensitivity to light and more
352 sensitivity to long wavelengths of light compared to aquatic animals. However, these effects are
353 lost once we account for phylogeny. This loss of an effect might be because the historical
354 divergence between the visual pigments used by vertebrates and invertebrates is an important
355 limiting factor on the degree to which visual pigments can accommodate for light environment,
356 something that would not be detected in analyses limited to vertebrates.

357

358 The effect of the c-opsin/ r-opsin divergence is lost once we account for phylogeny in our analyses,
359 but since this transition happened once and maps onto the metazoan phylogenetic tree, this loss of
360 effect might be expected. Outside of this transition, opsin evolutionary history such as mutation
361 biases may account for the effect of phylogeny on visual ability in our analysis. Retinal is
362 covalently bonded to opsin *via* a Schiff base and the charge of the amino acid residues near the
363 Schiff base influence the ability of retinal to change conformation and λ_{\max} of the associated opsin

364 [83,84], which has been experimentally confirmed using directed mutagenesis [37,85,86]. Future
365 research should consider whether there are inherent differences in the electronic charge of the
366 binding pocket between ciliary and rhabdomeric type opsins. Additionally, studies examining
367 whether non-opsin means of visual tuning, including the differential absorption of light by
368 screening pigments, differ between animals which use ciliary and rhabdomeric opsins and which
369 live in the same light environment may prove particularly illuminating.

370

371 *(e) Conclusions*

372 Here we used visual sensitivity data from nearly 450 animal species and 3 phyla to conduct a
373 systematic survey of the effects of habitat light on the colours animals can see. We found that
374 terrestrial animals and aquatic animals possess different ranges of spectral sensitivity from each
375 other, but that evolutionary processes such as the c-opsin/r-opsin divergence may have limited
376 chordates' ability to tune their opsins to short-wavelength light. Additionally, the eyes of animals
377 living in terrestrial habitats are not specifically tuned to forest canopy cover. Future research
378 should consider whether inherent differences between chordate and non-chordate opsin amino acid
379 sequences, or downstream neural signalling, are responsible for the evolutionary limitations to
380 visual tuning.

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384 valuable feedback on early versions of this project and manuscript.

385

386 *Data Accessibility:*

387 Data pertaining to this study are available in the supplementary materials or available at Dryad
388 repository <https://doi.org/10.5061/dryad.47d7wm3fc>. Code pertaining to this study is available at
389 GitHub repository: <https://github.com/mjosmurphy/opsin-evolutionary-ecology>.

390

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394

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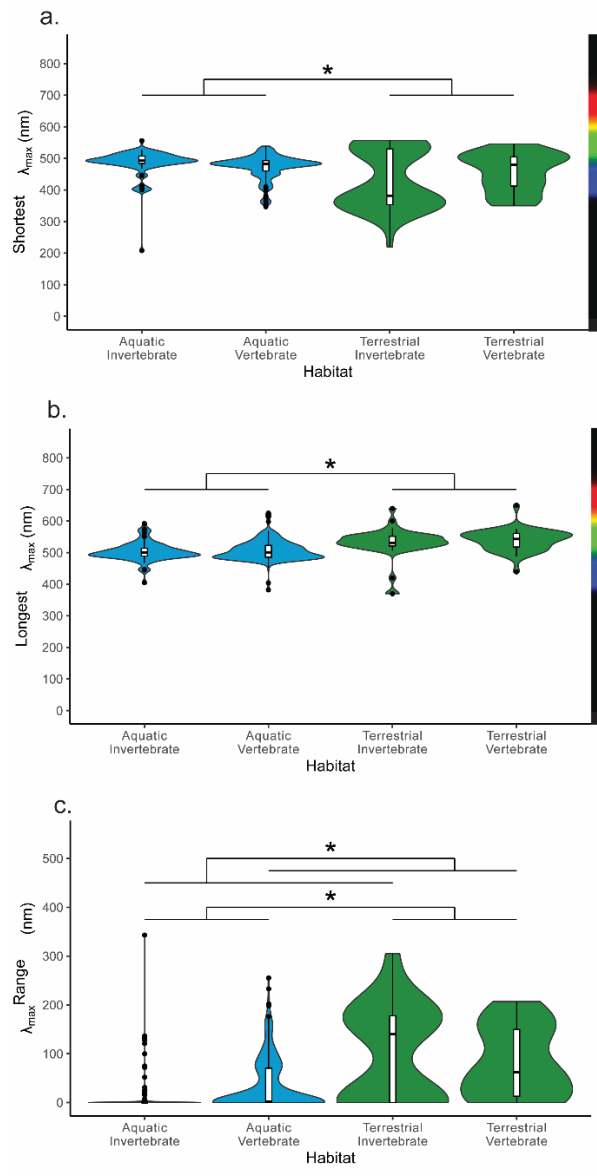


Figure 1. Effect of coarse habitat and lineage on mean visual pigment sensitivity, prior to phylogenetic control. A. Shortest opsin: Aquatic invertebrates: $n = 78$, $\mu = 485.3$ nm; Aquatic vertebrates: $n = 273$, $\mu = 473.0$ nm; Terrestrial invertebrates: $n = 43$, $\mu = 430.1$ nm; Terrestrial vertebrates: $n = 39$, $\mu = 456.1$ nm. B. Longest opsin: Aquatic invertebrates: $n = 78$, $\mu = 503.3$ nm; Aquatic vertebrates: $n = 273$, $\mu = 507.6$ nm; Terrestrial invertebrates: $n = 43$, $\mu = 530.3$ nm; Terrestrial vertebrates: $n = 39$, $\mu = 539.1$ nm. C. Opsin range: Aquatic invertebrates: $n = 78$, $\mu = 18.0$ nm; Aquatic vertebrates: $n = 273$, $\mu = 34.6$ nm; Terrestrial invertebrates: $n = 43$, $\mu = 100.2$ nm; Terrestrial vertebrates: $n = 39$, $\mu = 83.0$ nm. *: $p < 0.05$.

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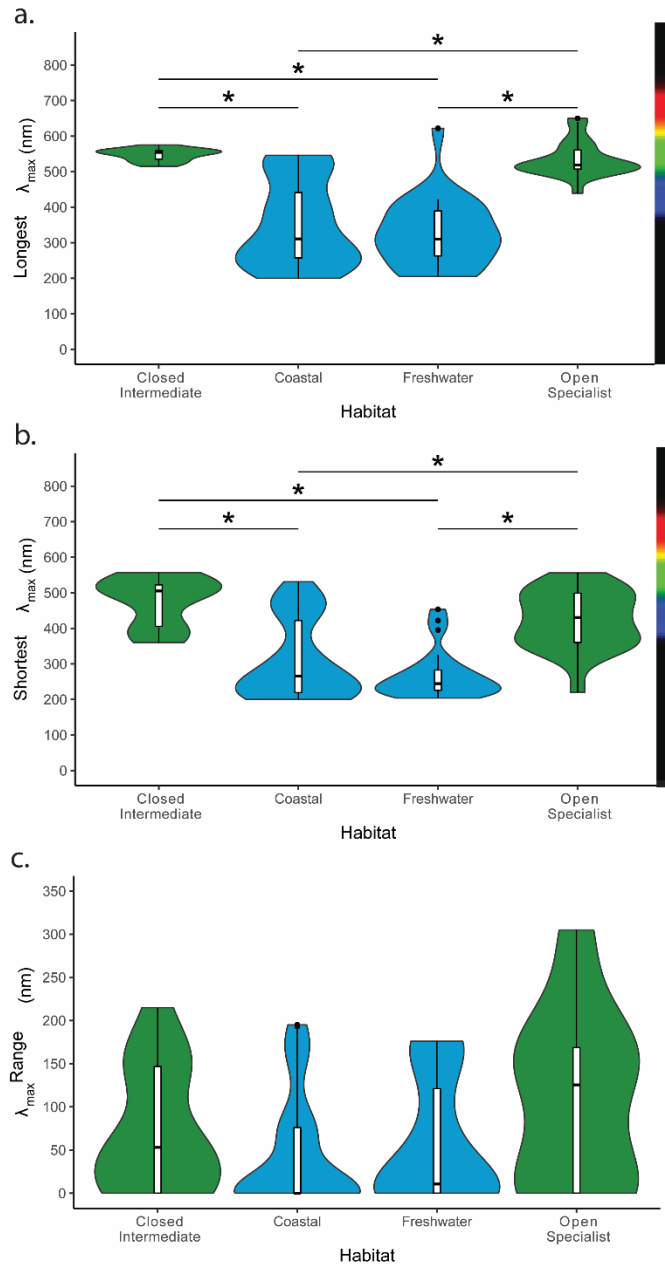


Figure 2. Effect of habitat greenness on visual sensitivities. A. Longest opsin: forest + intermediate: $n = 14$, $\mu = 547.1$ nm, $sd = 17.71$ nm; coastal: $n = 40$, $\mu = 297.3$ nm, $sd = 72.42$ nm; freshwater: $n = 23$, $\mu = 314.3$ nm, $sd = 73.77$ nm; open terrestrial: $n = 22$, $\mu = 534.1$ nm, $sd = 49.09$ nm; B. Shortest opsin: forest + intermediate: $n = 14$, $\mu = 470.4$ nm, $sd = 60.00$ nm; coastal: $n = 40$, $\mu = 254.4$ nm, $sd = 55.16$ nm; freshwater: $n = 23$, $\mu = 261.1$ nm, $sd = 56.52$ nm; open terrestrial: $n = 22$, $\mu = 423.4$ nm, $sd = 82.06$ nm C. Opsin range: forest + intermediate: $n = 14$, $\mu = 76.63$ nm, $sd = 72.61$ nm; coastal: $n = 40$, $\mu = 742.88$ nm, $sd = 70.15$ nm; freshwater: $n = 23$, $\mu = 53.26$ nm, $sd = 69.30$ nm; open: $n = 22$, $\mu = 110.6$ nm, $sd = 98.82$ nm. *: $p < 0.05$.

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