

1 **Developmental changes of opsin gene expression in ray-finned fishes** 2 **(Actinopterygii)**

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20

21 **Abstract**

22 Fish often change their habitat and trophic preferences during development. Dramatic
23 functional differences between embryos, larvae, juveniles and adults also concern sensory
24 systems, including vision. Here we focus on the photoreceptors (rod and cone cells) in the
25 retina and their gene expression profiles during the development. Using comparative
26 transcriptomics on 63 species, belonging to 23 actinopterygian orders, we report general
27 developmental patterns of opsin expression, mostly suggesting an increased importance of the
28 rod opsin (*RH1*) gene and the long-wavelength sensitive (*LWS*) cone opsin, and a decreasing
29 importance of the shorter wavelength sensitive cone opsin throughout development.
30 Furthermore, we investigate in detail ontogenetic changes in 14 selected species (from
31 Polypteriformes, Acipenseriformes, Cypriniformes, Aulopiformes and Cichliformes), and we
32 report examples of expanded cone opsin repertoires, cone opsin switches (mostly within *RH2*)
33 and increasing rod:cone ratio as evidenced by the opsin and phototransduction cascade genes.

34 Our findings provide molecular support for developmental stage-specific visual palettes of ray-
35 finned fishes and shifts between, which most likely arose in response to ecological, behavioural
36 and physiological factors.

37

38 INTRODUCTION

39 Fish visual systems are very diverse, and they vary in morphology, physiology and spectral
40 sensitivity (Hunt et al. 2014, Carleton et al. 2020, Musilova et al. 2021). Vertebrate vision is
41 enabled by cone and rod photoreceptors in the retina, which carry light-sensitive molecules
42 composed of an opsin protein bound to a light absorbing, vitamin A-derived chromophore
43 (Lamb 2013). In fishes, there are usually four types of cone opsins (*SWS1* and *SWS2*;
44 commonly found in single cones, whereas *RH2* and *LWS* in double cones) used for photopic
45 and colour vision, and one rod opsin (rhodopsin, *RH1* or Rho) for scotopic vision in dim-light
46 conditions (Carleton et al. 2020). Through gene duplications followed by functional
47 diversifications, extant teleost fishes reached a median of seven cone opsin genes within their
48 genomes (Musilova et al. 2019a). Throughout the phylogeny, teleost genomes contain more
49 copies of double-cone genes (middle and longer-wavelength sensitive; *RH2* and *LWS*) than that
50 of single-cones (shorter-wavelength *SWS1* and *SWS2*). While the *SWS1* is often missing from
51 the genome or seen in one, at best two copies (Musilova et al, 2021) and *SWS2* seen in up to
52 three copies (Cortesi et al., 2015), teleost genomes can contain up to eight copies of *RH2*
53 (Musilova & Cortesi, 2021) and up to five copies of *LWS* (Cortesi et al., 2021). Unlike cone
54 opsins, rod opsin duplicates are rarely found, most often in mesopelagic lineages (Pointer et al.
55 2007, Musilova et al. 2019a, Lupše et al. 2021). Higher copy number is considered beneficial
56 by providing more “substrate” for selection, as well as for alternative gene expression of the
57 variants within the opsin type.

58 The formation of the eye, and expression of opsin genes, starts already at the embryonic
59 stage (Hagedorn and Fernald 1992, Carleton et al. 2008). Still, eyes continue to grow, and new
60 photoreceptors are being added throughout life (Fernald 1985). Within the retina, cone
61 photoreceptors are first to develop, followed by temporally and spatially distinct rods
62 (Raymond 1995, Helvik et al. 2001, Shen & Raymond 2004). For example, in zebrafish,
63 photoreceptor progenitor cells start out by first differentiating into cones before rods are added
64 later during development (Sernagor et al. 2006). This cone-to-rod developmental sequence is
65 likely shared across vertebrates (Atlantic cod: Valen et al. 2016; zebrafish: Sernagor et al. 2006;
66 mice: Mears et al. 2001; rhesus monkey: La Vail et al. 1991) and appears to hold even for
67 teleost species with an all-rod retina in the adult stage (Lupše et al. 2021).

68 Photic conditions can change spatially and temporally, resulting in a visually
69 heterogeneous environment in which visual systems of fishes are expected to be under natural
70 selection that favours those that match the local environment (Carleton et al. 2016). For
71 example, longer and shorter wavelengths are scattered and filtered out with increasing water
72 depth and consequently, fishes living in deep-water habitats such as sculpins of Lake Baikal
73 (Hunt 1997), cichlids of lakes Malawi and Tanganyika (Sugawara et al. 2005; Ricci et al. 2022),
74 and African crater lakes (Malinsky et al. 2015; Musilova et al. 2019b), as well as deep-sea
75 fishes (Douglas et al. 2003, Lupše et al. 2021) have visual systems sensitive to the blue-green
76 part of the visible spectrum. Adaptation can be achieved either through functional
77 diversifications of opsin genes when mutations at key-spectral tuning sites shift the peak
78 spectral sensitivity (λ_{\max}) of the photopigment (Yokoyama 2008, 2020), or by regulation of the
79 opsin gene expression. This can be achieved when a subset of opsin genes is expressed and
80 altered among or within species and even within the same individuals during ontogeny
81 (Carleton & Kocher 2001, Manousaki et al. 2013, Carleton 2016, Lupše et al. 2021).

82 Before reaching the juvenile or sexually mature adult stage, fish larvae undergo major
83 anatomical, physiological, behavioural and quite often, ecological changes (Evans & Browman
84 2004, Carleton et al. 2020). This developmental shift in habitat preference is often suggested
85 to drive ontogenetic changes in opsin expression (e.g. cichlids: Carleton et al. 2016; black
86 bream: Shand et al., 2002; eel: Cottrill et al. 2009; damselfishes: Stieb et al. 2016, bluefin
87 killifish: Chang et al. 2021; gambusia: Chang et al. 2020; rainbow trout: Allison et al. 2006;
88 dottybacks: Cortesi et al. 2016; deep-sea fishes: Lupše et al. 2021). However, changes of photic
89 conditions solely do not always result in different and stage-specific visual system
90 modifications, as seen in the Atlantic cod (Valen et al. 2016) or the spotted unicornfish
91 (Tettamanti et al. 2019). This suggests other factors, such as behaviour, developmental or
92 phylogenetic constraints also play a role in shaping the visual diversity of fishes and potential
93 age-related shifts of it.

94 Here we aim to investigate ontogenetic changes of the opsin and phototransduction
95 cascade gene expression across ray-finned fishes, to estimate presence and relative abundance
96 of opsin gene classes, and to elucidate general and/or taxon-specific patterns. For the purpose
97 of this study we have sequenced and analysed 1) retinal transcriptomes of different
98 developmental stages of 14 species, belonging to five major actinopterygian orders: *Polypterus*
99 *senegalensis* (Polypteriformes), *Acipenser ruthenus* (Acipenseriformes), *Abramis brama* and
100 *Vimba vimba* (both Cypriniformes), *Scopelarchus* spp. and *Coccorella atlantica* (both
101 Aulopiformes), *Coptodon bembini*, *C. imbriferus*, *C. flava*, *C. snyderae*, *C. thysi*, *Sarotherodon*

102 *linnellii*, *S. lohbergeri* and *Stomatepia pindu* (all Cichliformes from the Bermin and Barombi
103 Mbo lakes). 2) We have complemented this data set by publicly available
104 embryonic/larval/juvenile/adult transcriptomes belonging to 49 species and 21 orders, some of
105 which have never been analysed for visual gene expression before. In total, the comprehensive
106 data set of 63 species from 23 ray-finned fish orders allows us to focus on development of the
107 opsin gene expression, and rod and cone cell identity throughout actinopterygian evolution.

108

109 **RESULTS AND DISCUSSION**

110 **General developmental patterns of opsin gene expression across the actinopterygian**
111 **phylogeny – cone-to-rod developmental constraint.** The analysis of the opsin gene
112 expression in 63 ray-finned fishes revealed that generally, the ratio of the rod opsin (*RH1* or
113 Rho, λ_{max} : 447–525 nm) to cone opsin expression increases with age in analysed species (Figs
114 1 and 2, Supp Table). This is in accord with the cone-to-rod development of the retina which
115 starts with cone cells, and rods appearing only later (Sernagor et al. 2006, Valen et al. 2016,
116 Lupše et al. 2021). The increasing rod:cone cell ratio is further confirmed by the expression of
117 the phototransduction cascade gene *GNAT1* (rod specific) vs. *GNAT2* (cone specific), Fig. 3b.
118 Rod opsin and *GNAT1/2* usage increases significantly already during the larval and juvenile
119 stage, before finally transforming retinae of sexually mature adults into predominantly rod-
120 over-cone-expressing tissues (Figs 1 and 2, Supp Table). It thus seems that larval vision is
121 mostly driven by cone vision, while the ability to perform well in low-light conditions appears
122 consequently, at later developmental stages (Evans and Browman 2004, Evans and Fernald
123 1990). Functionally, rods generally allow for an improvement in visual acuity and startle
124 responses in fishes (Fuiman 1993, Pankhurst et al. 1993, Fuiman and Delbos 1998) and are
125 also associated with motion sensitivity and the appearance of novel behaviours, such as
126 schooling (Hunter & Coyne 1982). More specifically, higher rod expression increases
127 individual performance of fishes living in the deep-sea (de Busserolles et al. 2020, Lupše et al.
128 2021). Additionally, laboratory experiments have shown that the ability to follow a rotating
129 stripe pattern (the optomotor drum) might be dependent on rod formation and retinal
130 development, as it is not seen in stages or specimens lacking rods (Blaxter 1986, Carvalho et
131 al. 2002, Magnuson et al. 2020).

132 In the selected taxa (Fig. 3), we have specifically focused on the rod vs. cone identity
133 by quantifying the expression of the phototransduction cascade gene *GNAT1* or *GNAT2*,
134 respectively. We found correspondence between the expression of phototransduction cascade
135 gene type and the opsin type (i.e. cone *SWS1*, *SWS2*, *RH2*, *LWS* and rod *RH1*), and detected a

136 clear increase of *GNAT1:GNAT2* ratio with ageing, with the exception of the Aulopiformes
137 deep-sea fishes. In this group, a rare discordance between the dominating opsin type (rod-
138 specific) and phototransduction cascade genes (cone-specific) in adults suggests a presence of
139 possibly transmuted photoreceptors, and an overall intriguing visual system which needs to be
140 investigated further (Fig 3, Lupše et al. 2021).

141

142 **Developmental switch of the short-wavelength sensitive opsin genes.** A trend of age-related
143 shifts in expression also appears within cone opsins. Our data set shows a clear decrease in
144 proportional expression of the ultraviolet or UV-sensitive *SWS1* (λ_{\max} : 347–383 nm) with age.
145 Although *SWS1* expression usually remains in the lower quarter of the total cone opsin
146 expression, it seems to be expressed in early stages throughout the phylogeny (Fig 1, Supp
147 Table). On one hand, UV radiation can result in larval mortality; to mitigate negative effects
148 of exposure, UV avoidance through detection of ultraviolet light and adjustments of vertical
149 position is expected (Ylönen et al. 2004, Guggiana-Nilo and Engert 2016). On the other hand,
150 distinguishing wavelengths belonging to the UV part of the visual spectrum aids younger
151 individuals that feed on zooplankton (Browman et al. 1994, Flamarique et al. 2013, Fattah
152 Ibrahim et al. 2015). With ageing and a shift in diet, UV opsin expression probably becomes
153 irrelevant (Britt et al. 2001), thus potentially explaining why adults never (e.g. *Naso*
154 *brevirostris*, *Oryzias latipes*) or rarely (e.g. *Danio rerio*, *Poecilia reticulata*, cichlids) express
155 *SWS1* cone opsin (Fig 1, Supp Table). Adult expression of *SWS1*, when seen, seems to play a
156 role in species and/or colour discrimination and mate selection (guppies: Smith et al. 2002;
157 damselfishes: Siebeck et al. 2010; cichlids: Carleton et al. 2016), male aggression
158 (sticklebacks: Rick and Bakker 2008) or is associated with migration events (salmonids:
159 Allison et al., 2006). The blue sensitive *SWS2* cone opsin (λ_{\max} : 397–482 nm), shows a more
160 complex pattern (Figs 1 and 2, Supp Table). Interestingly, while some fish (e.g. sturgeons or
161 cyprinids) seem to ontogenetically decrease the proportion of both *SWS1* and *SWS2* opsins, in
162 other fish groups (e.g. cichlids) we observe a replacement of one type by another (Fig 3). This
163 switch in single cone opsin expression between *SWS1* and *SWS2* has been shown before e.g.
164 by Spady et al. (2006) in Nile tilapia or by Cheng and Flamarique (2007) in rainbow trout, and
165 it keeps the total single cone opsin expression similar between different developmental stages
166 (Fig. 2).

167

168 **Middle and long-wavelength sensitive opsins in double cones:** The ontogenetic switch in
169 expression occurs also between the green-sensitive *RH2* (λ_{\max} : 452–537 nm) and the red-

170 sensitive *LWS* (λ_{\max} : 501–573 nm) cone opsin types; plus switching between different *RH2*
171 copies also commonly occurs (Fig 3). Values for these typically double-cone opsins vary
172 considerably across the fish phylogeny, but a general trend of a decrease in relative expression
173 of *RH2*, and an increase of *LWS* with age is detected (Figs 1 and 2, Supp Table), except for
174 groups that completely lost the *LWS* opsin gene. In general, medium-wavelength opsins appear
175 to be of use to all stages (Figs 1 and 2, Supp Table), perhaps due to general presence of
176 corresponding wavelengths in most habitats. Our overview data seem to show the trend of
177 freshwater species exhibiting the dominance of red-sensitive *LWS* opsin gene expression,
178 whereas in marine species, green-sensitive *RH2* gets to be more dominant (with exceptions)
179 (Fig 1). Namely, for species inhabiting the spectrally narrower deep sea at least during certain
180 parts of their lives (Stomiiformes, Aulopiformes, Trachichthyiformes, Anguilliformes,
181 Gadiformes), *RH2* seems to be the most important, if not the only cone opsin expressed (Fig 1,
182 Lupše et al. 2021). On the other hand, expression of *LWS* in adults might be a response to
183 inhabiting freshwater habitats, such as turbid rivers and murky, eutrophic lakes (e.g. Lake
184 Victoria where usually, longer wavelengths penetrate to greater depths and are the most
185 prevalent colour of the ambient light (Hofmann et al. 2009, Carleton et al. 2016). Expression
186 of *LWS* could also be beneficial for foraging in herbivorous reef fishes, providing them with
187 the visual ability to discriminate benthic algae from coral reef backgrounds (Marshall et al.
188 2003, Stieb et al. 2017). In some cases, increased *LWS* expression and expanded *LWS*
189 repertoires might also be explained by sexual selection (e.g. in Poeciliidae), where females
190 evolved mate preferences for red and orange male coloration (Watson et al. 2011).

191

192 **Age-specific cone opsin gene copies in the selected taxa.** We have specifically focused and
193 de-novo sequenced retina transcriptomes of larvae/juveniles and adults of 14 actinopterygian
194 species belonging to five orders spanning the ray-finned fish phylogeny. Apart from the
195 aforementioned rod vs. cone identity assessed by *GNAT* genes, we have additionally focused
196 on switches between copies of the same opsin type in the selected taxa (Fig 3, Supp Table).
197 Namely, we studied the visual opsin gene repertoire in two basal non-teleost fish groups,
198 bichirs (Polypteriformes) and sturgeons (Acipenseriformes), and in teleost riverine cyprinids
199 (Cypriniformes, Ostariophysi), crater-lake cichlids (Cichliformes, Euteleostei) and deep-sea
200 pearleyes and sabretooths (Aulopiformes, Euteleostei). The overall expression patterns are in
201 most cases in accord with the general patterns discussed above (Figure 3, Supp Table), with
202 exceptions seen in the deep-sea fishes (based on our earlier data from Lupše et al. 2021).

203 In all species but the bichir, we found multiple copies within at least one opsin gene
204 type, namely within the rod *RH1* opsin, and cone *SWS2* and *RH2* opsins. In some species
205 (cyprinids, sturgeon, *Scopelarchus* spp.) we found simultaneous expression of two rod *RH1*
206 copies (Fig 1, Supp Table). All three groups possess the two *RH1* genes in their genome
207 resulting from three independent ancestral gene duplication events (Musilova et al. 2021, Lupše
208 et al. 2021). The *RH1* gene duplicates were lost in the later evolution of the euteleost crown
209 group, and hence most teleost species carry only one *RH1* copy, a phenomenon similar to that
210 seen in “non-fish” vertebrates. *RH1* copies do not show any sign of ontogenetic switch in
211 studied species, as is the case for e.g. eels (Hope et al., 1998). On the other hand, we detected
212 several cases of stage-specific copies within cone opsin genes. While *Acipenser ruthenus* and
213 *Abramis brama* + *Vimba vimba* express only one *SWS2* copy, cichlids express two different
214 *SWS2* genes (Fig 3, Supp Table); this corresponds to multiple copies found in their genome
215 due to the neoteleost- and percomorph-specific *SWS2* gene duplications (Cortesi et al. 2015).
216 Most examined species show an expanded *RH2* repertoire (Fig 3, Supp Table) and the existence
217 of clearly larval and adult-specific copies has been observed in cyprinids, cichlids and in the
218 deep-sea aulopiforms (Fig. 3). Expression of multiple copies might enhance colour vision by
219 increased spectral resolution useful in a particular environment, however reasons for these
220 opsin switches are not yet completely understood. The presence of such stage-specific copies
221 means that species adjust their vision to differing light environments not only through a change
222 in opsin class expression, but also through preferential expression of opsin copies within a
223 single class. In cichlids, a group for which the development of visual system is probably best
224 understood, a shift to longer-wavelength copies is generally observed within a single opsin type
225 (*RH2A* copies replacing *RH2B* with age) or among single-cone opsins (*SWS2* replacing *SWS1*)
226 and has been reported before for different groups of cichlids (e.g., Malawi, Carleton et al. 2008;
227 Nile tilapia, Spady et al., 2006).

228 Mesopelagic deep-sea aulopiform species have a limited repertoire of cone opsin
229 classes that reflects living in photon-depleted depths (Musilova et al. 2019, Lupše et al. 2021).
230 *Scopelarchus* spp. and *Coccorella atlantica* express only one cone opsin class, namely *RH2*
231 (Fig 3, Supp Table). However, both expanded their *RH2* repertoires and express larval- and
232 adult-specific copies that are thought to be functionally different and most likely best respond
233 to different wavelengths shallow-water epipelagic larvae and mesopelagic deep-water adults
234 encounter (Fig 3, Supp Table) (Lupše et al. 2021). Genomic analyses by Lupše et al. (2021)
235 reveal a total of three, and seven *RH2* cone opsin copies within the genomes of *Coccorella*
236 *atlantica* and *Scopelarchus michaelsarsi*, respectively. Mesopelagic fish lineages in some cases

237 expand rod opsin repertoires, which are better suited for dim-light conditions (Musilova et al.
238 2019, Lupše et al. 2021). *Coccorella* and *Scopelarchus*, however, seem to inhabit relatively
239 shallower and photon-richer depths than some other deep-sea fishes, such as Stomiiformes, and
240 might thus benefit also from having extra copies of cone opsins (Lupše et al. 2021).

241 We have collected a robust data set combining not only our own, but also publicly
242 available genetic data, deposited in databases. This allowed us to detect shared vs. specific
243 expression patterns among different fish groups. We are aware that the collected data set has
244 certain limitations, and many factors could not be controlled in this study. We also do not
245 present any developmental time series but rather snapshots of embryos, larvae, juveniles and
246 adults. As not all stages are available for all species, more subtle or time-restricted expression
247 patterns could not be detected here. Despite this, our combined data provides robust evidence
248 for expression patterns shared across distantly related fish groups, as it highlights general
249 trends, and more detailed conclusions achieved through in-detail analyses of species
250 specifically sequenced within this study.

251

252 **Conclusions**

253 To conclude, this study aims to identify general patterns of the visual opsin gene expression
254 shared among ray-finned fishes, and to detect similarities in the ontogenetic changes between
255 opsin gene types. We found that the rod:cone opsins ratio increased with age in fish species,
256 supporting the conserved cone-to-rod developmental pathway. We also noted the increased
257 importance of the long-wavelength sensitive *LWS* opsin genes, and the decreased importance
258 of the short-wavelength sensitive *SWS1* opsin gene, observed across ray-finned fish phylogeny
259 (e.g. in sturgeons, cyprinids and cichlids). We have further detected the existence of different
260 stage-specific *RH2* copies, which are switched during development. To conclude, fish visual
261 systems are evolutionary and developmentally very dynamic and future studies focused on
262 particular fish groups promise to throw further light on exact mechanisms, patterns and reasons
263 for this extreme sensory system diversity.

264 METHODS AND MATERIALS

265 **Data and sample collection** Transcriptomes belonging to taxa deemed as focal groups, which
266 were inspected for age-specific copies and presented in detail in Figure 3, were obtained from
267 specimens (N=72) caught solely for the purpose of this study. In detail, 16 specimens were
268 classified as larvae, 4 as juveniles, 3 as subadults and 49 as adults (Figure 3, Supp Table).
269 *Polypterus senegalensis* larvae were collected in the rearing facility of the Department of
270 Zoology, Charles University, and the adults were purchased from the aquarium trade.
271 *Acipenser ruthenus* and *Abramis brama* were collected at the rearing facility in Vodňany, and
272 in local water bodies (adults: Velky Tisy pond, Klicava dam, Lipno dam; larvae: Vltava and
273 Elbe rivers), Czech Republic, respectively. Both mesopelagic taxa, *Scopelarchus* spp. and
274 *Coccorella atlantica*, were collected in the Sargasso Sea and originate from Lupše et al. (2021).
275 Crater lake cichlids were collected in lakes Barombi Mbo and Bermin (Cameroon, West
276 Africa) between 2013 and 2018 (research permit numbers:
277 0000047,49/MINRESI/B00/C00/C10/nye, 0000116,117/MINRESI/ B00/C00/C10/C14,
278 000002-3/MINRESI/B00/C00/C10/C11, 0000032,48-50/MINRESI/B00/C00/C10/C12).
279 Larvae were caught by fine-meshed nets and fixed in RNAlater™ immediately. Adults were
280 collected using gill nets and selective capturing by snorkelling in the shallow-water zone. For
281 all species, fin clips were taken from specimens and stored in 96% EtOH for sub-sequent
282 molecular analyses. Larval samples were fixed in RNAlater™ (ThermoFisher) and stored at
283 -80 °C until further use. Adults of all species were euthanised on site with eyes or retinae
284 extracted, fixed in RNAlater™ and stored at -80 °C upon arrival to the laboratory.

285 To obtain publicly available transcriptomes used in this study (Fig 1, Supp Table), we
286 searched the largest publicly available repository of high throughput sequencing data, the
287 Sequence Read Archive (SRA), using the following topic search term: ‘(embryo* OR larva*
288 OR juvenile* OR adult*) AND (retina* OR eye* OR head* OR whole*) AND (taxon name *
289 OR fish*)’. Whenever possible, we have analysed up to three specimens per stage per species
290 (Fig 1, Supp Table). In case of embryos, specimens closest to hatching were analysed (for
291 reasons, see Results and Discussion). The entire dataset analysed, including de-novo
292 transcriptomes described below, includes 215 samples of which, based on morphology, 56 were
293 classified as embryos, 40 as larvae, 25 as juveniles, 3 as subadults and 91 as adults (Figs 1 and
294 3, Supp Table). Sample IDs, number of raw reads, individual accession numbers for BioProject
295 XX and further parameters are listed in the Supplementary Table.

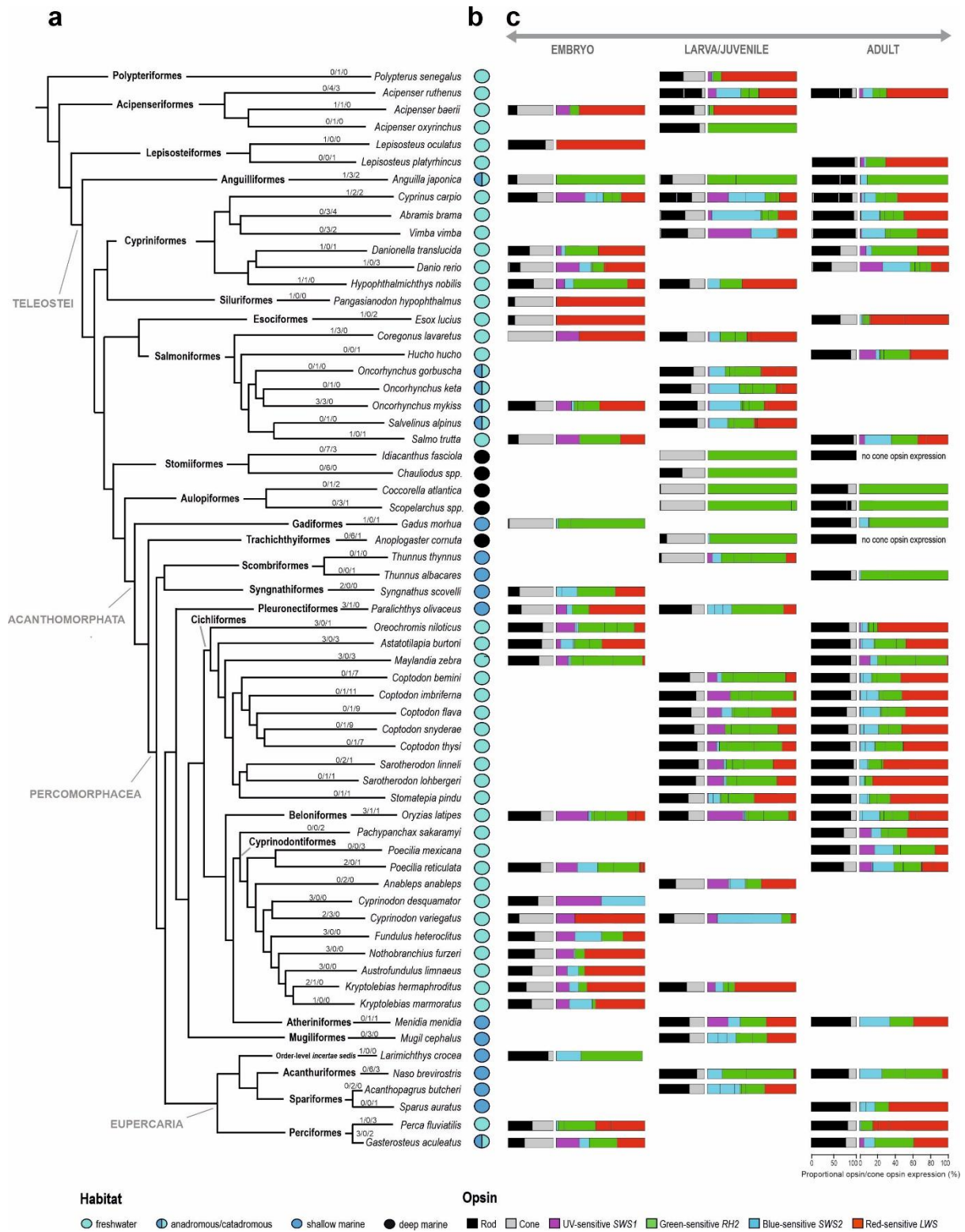
296 **Transcriptome sequencing and analyses** Total RNA was extracted from the whole eyes or
297 retinal tissue using either the RNeasy micro or mini kit (Qiagen). The extracted RNA
298 concentration and integrity were verified on a 2100 Bioanalyzer (Agilent) and Qubit
299 Fluorometer (ThermoFisher Scientific). RNAseq libraries were constructed in-house from
300 unfragmented total RNA using Illumina's NEBNext Ultra II Directional RNA library
301 preparation kit, NEBNext Multiplex Oligos and the NEBNext Poly(A) mRNA Magnetic
302 Isolation Module (New England Biolabs). Multiplexed libraries were sequenced on the
303 Illumina HiSeq 2500 platform as 150 bp paired-end (PE) reads. The sequence data was quality-
304 checked using FastQC (Andrews 2017). Opsin gene expression was then quantified using
305 Geneious software version 11.0.3 (Kearse et al. 2012). In case of each sample, we mapped the
306 reads against a genomic reference dataset for all opsin genes, obtained from the NCBI,
307 belonging to the exact or a closely related species, using the Medium-sensitivity settings in
308 Geneious. This enabled us to identify cone and rod opsin specific reads. If needed, paralogous
309 genes were subsequently disentangled following the methods in Musilova et al. 2019a and de
310 Busserolles et al. 2017. Created species-specific opsin references were re-mapped to the
311 transcriptome reads with Medium-Low sensitivity to obtain copy-specific expression levels.
312 We report opsin gene proportional expression in relation to the total opsin gene expression, and
313 to the total cone opsin gene expression (Supp Table). The abovementioned quantification of
314 opsin gene expression was also used on transcriptomes obtained from SRA. Identical pipeline
315 was used for quantification of *GNATI/2* genes in selected taxa (Fig 3).

316

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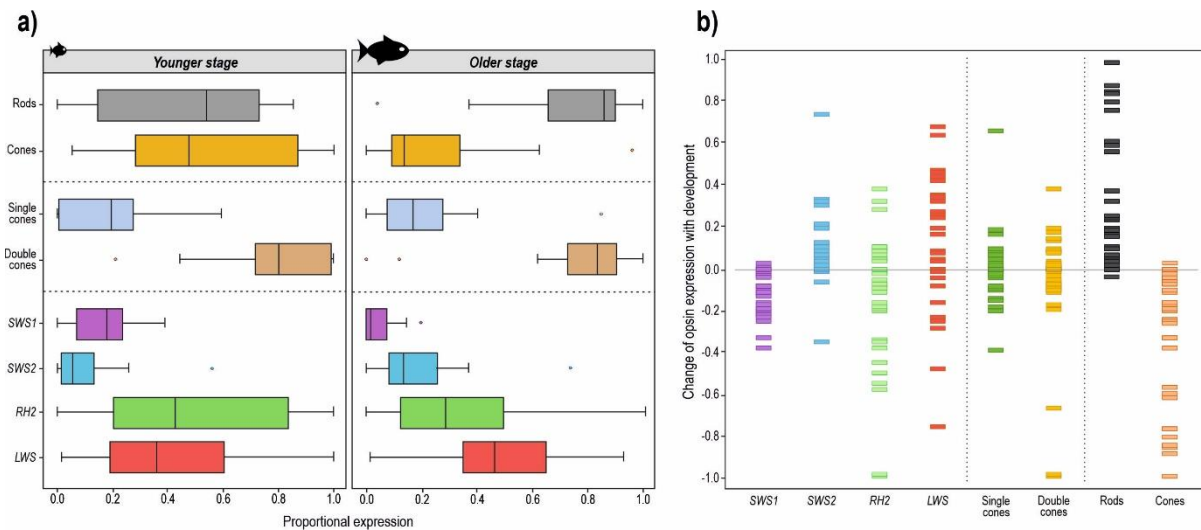
328



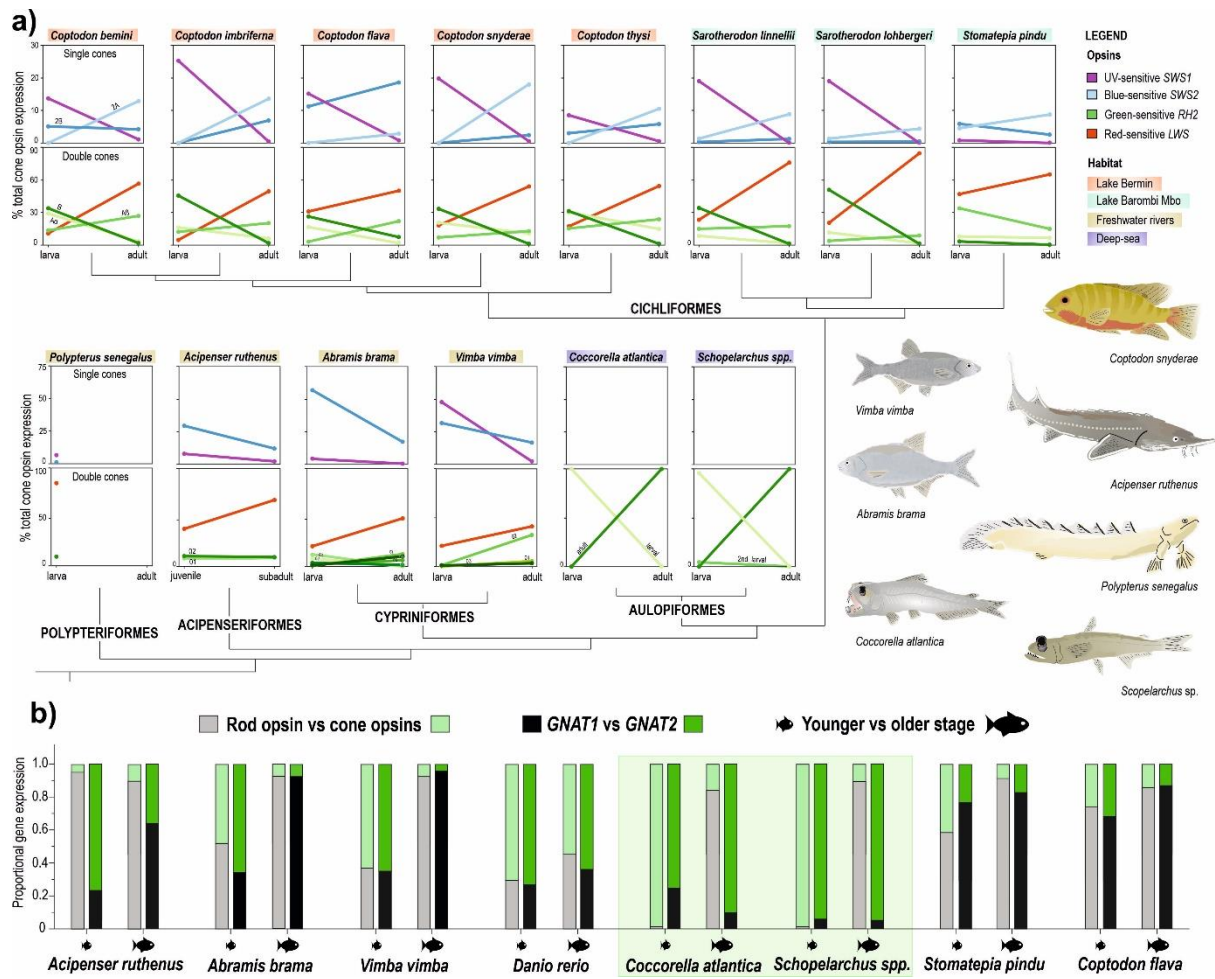
329

330 **Fig. 1: Opsin gene expression in different developmental stages of ray-finned fishes (Actinopterygii).** (A)
 331 Simplified phylogeny of the 63 species, belonging to twenty-three orders, for which the transcriptomes were
 332 analysed (topology after Betancur et al. 2017). Numbers above branches represent number of individuals per
 333 developmental stage analysed (embryo/larva+juvenile/adult). (B) Information on habitat preference, obtained
 334 from <https://www.fishbase.de>. Separation between the shallow and deep marine species is 200m. Information on

335 depth obtained from <https://obis.org/>. (C) Proportional opsin gene expression (horizontal bars) at different
 336 developmental stages. First (shorter) bar represents mean proportional expression of rod and cone opsins. Cone
 337 opsin expression (grey) is depicted as the sum of the expression of all four classes of cone opsin genes (SWS1,
 338 SWS2, RH2, and LWS). If several rod opsin genes (black) were expressed, the different proportions of their
 339 expression are distinguished with white vertical bars. Second (longer) bar represents mean proportional
 340 expression of different cone opsins. Black vertical bars within gene classes separate different copies, if co-
 341 expressed. For details, see Supplementary Table.
 342
 343



344
 345 **Fig. 2: General patterns of age-related opsin expression changes.** (A) Interquartile ranges (25th and 75th
 346 percentiles) and whiskers show data dispersion (relative expression) across different opsins for the youngest and
 347 oldest analysed stage. Data medians are presented as solid vertical lines. To avoid over-representation of certain
 348 taxa (e.g. five *Coptodon* species), data points represent mean genus values, comprised only of species that had at
 349 least two developmental stages analysed (N=31). (B) Change of opsin expression (positive/negative) with
 350 development, calculated as a difference between the mean opsin expression in the oldest and the youngest stage
 351 of a certain genus. Resulting values are represented by rectangles (N=31), centred at the mean. Lower half of the
 352 plot (values below 0.0) shows a decrease, and the upper half (values above 0.0) an increase in relative expression
 353 with age.



354

355 **Fig. 3: Cone opsin switches, age-specific copies and phototransduction cascade gene expression of**
 356 **representative taxa specifically sequenced for this study. (A) Detailed presentation of ontogenetic changes of**
 357 **opsin expression in selected polypteriform, acipenseriform, cypriniform, aulopiform and cichliform species-**
 358 **Interconnected dots are coloured according to specific single and double cone opsins and present mean**
 359 **expression values for specific developmental stages. In cases of gene duplications, copies are named and coloured**
 360 **with different shades. For details on number of individuals and exact values, see Supplementary Table. (B)**
 361 **Ontogenetic changes of rod/cone opsin gene expression, and to it related shifts in expression of phototransduction**
 362 **cascade genes GNAT1 (rod-specific) and GNAT2 (cone-specific) for selected teleost taxa. Highlighted in green**
 363 **are special cases of the two aulopiform species that exhibit a discordance between the dominating opsin type**
 364 **(rod-specific) and phototransduction cascade genes (cone-specific) in adults (Lupše et al. 2021).**

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