Contribution of opsins and chromophores to cone pigment variation across populations of Lake Victoria cichlids

Wilwert, Elodie; Etienne, Rampal S.; van de Zande, Louis; Maan, Martine E.

Published in:
Journal of Fish Biology

DOI:
10.1111/jfb.14969

Received: 2 July 2021 | Accepted: 25 November 2021

DOI: 10.1111/jfb.14969

REGULAR PAPER

JOURNAL OF FISH BIOLOGY fsbi

Contribution of opsins and chromophores to cone pigment variation across populations of Lake Victoria cichlids

Elodie Wilwert | Rampal S. Etienne | Louis van de Zande | Martine E. Maan

Groningen Institute for Evolutionary Life Sciences (GELIFES), Groningen, The Netherlands

Correspondence
Elodie Wilwert, Groningen Institute for Evolutionary Life Sciences (GELIFES), Nijenborgh 7, 9747 AG Groningen, The Netherlands.
Email: e.wilwert@rug.nl

Funding information
This article was funded by an Adaptive Life Programme grant made possible by the Board of the University of Groningen, the Faculty of Science and Engineering and the Groningen Institute for Evolutionary Life Sciences (GELIFES).

Abstract

Adaptation to heterogeneous sensory environments has been implicated as a key parameter in speciation. Cichlid fish are a textbook example of divergent visual adaptation, mediated by variation in the sequences and expression levels of cone opsin genes (encoding the protein component of visual pigments). In some vertebrates including fish, visual sensitivity is also tuned by the ratio of vitamin A_1/A_2–derived chromophores (*i.e.*, the light-sensitive component of the visual pigment bound to the opsin protein), where higher proportions of A_2 cause a more red-shifted wavelength absorbance. This study explores the variation in chromophore ratios across multiple cichlid populations in Lake Victoria, using as a proxy the expression of the gene Cyp27c1, which has been shown to regulate the conversion of vitamin A_1 into vitamin A_2 in several vertebrates. This study focuses on sympatric *Pundamilia* cichlids, where species with blue or red male coloration co-occur at multiple islands but occupy different depths and consequently different visual habitats. In the red species, we found higher *cyp27c1* expression in populations from turbid waters than from clear waters, but there was no such pattern in the blue species. Across populations, differences between the sympatric species in *cyp27c1* expression had a consistent relationship with species differences in opsin expression patterns, but the red/blue identity reversed between clear and turbid waters. To assess the contribution of heritable *vs.* environmental causes of variation, we tested whether light manipulations induce a change in *cyp27c1* expression in the laboratory. We found that *cyp27c1* expression was not influenced by experimental light conditions, suggesting that the observed variation in the wild is due to genetic differences. Nonetheless, compared to other cichlid species, *cyp27c1* is expressed at very low levels in *Pundamilia*, suggesting that it may not be relevant for visual adaptation in this species. Conclusively, establishing the biological importance of this variation requires testing of actual A_1/A_2 ratios in the eye, as well as its consequences for visual performance.

KEYWORDS
Cyp27c1, ecological speciation, haplochromine, phenotypic plasticity, *Pundamilia*, visual adaptation

1 | INTRODUCTION

Local adaptation of sensory traits can initiate or strengthen species divergence. This is because sensory traits are important for both ecological performance and sexual communication (Boughman, 2002; Endler & Basolo, 1998). There are compelling reasons to study the visual system in this context: it is a crucial determinant of fitness in many taxa and highly diverse among species (Endler, 1991; Fernald, 1988; Marshall & Vorobyev, 2003). In particular, aquatic environments induce fine-scale visual adaptation in vision-dependent organisms. Due to the variation in underwater light conditions, divergent selection on visual system properties can be strong (Kullander *et al*., 2014; Loew & McFarland, 1990; Partridge *et al*., 1989), and visual adaptation to local light environments has been documented in numerous fish species (Bowmaker *et al*., 1994; Ehlman *et al*., 2015; Lythgoe *et al*., 1994; Partridge *et al*., 1989; Shand *et al*., 2008). In particular, populations that occur in multiple distinct visual environments provide a good opportunity to explore variation in cone pigments within and between species. This study explores variation in cone pigments across populations of cichlid fish from different visual environments. Cichlids form one of the most species-rich families among vertebrates (Kocher, 2004) and inhabit a broad range of visual environments (Schelly *et al*., 2006). They possess highly diverse visual systems (Carleton & Kocher, 2001; Seehausen *et al*., 2008; Terai *et al*., 2017) and provide some of the best-supported examples of speciation by divergent visual adaptation (Hofmann *et al*., 2009; Seehausen *et al*., 2008; Spady *et al*., 2005).

In fish, as in all vertebrates, visual information is captured by visual pigments in the eye, composed of an opsin protein covalently bound to a photosensitive vitamin-A-derived chromophore. Visual sensitivity depends on the interaction between these components and may be tuned by variation in either component (Carleton *et al*., 2016). Cichlid fish possess several major opsin proteins: one rod opsin (RH1) for dim-light vision and five cone opsins involved in colour vision [UV sensitive (SWS1), blue sensitive (SWS2), green sensitive (Rh2a and Rh2b) and red sensitive (LWS)]. Variation in colour vision among cichlids results from differences in the set of opsin genes that are expressed, from variation in opsin expression levels within that set and from differences in opsin coding sequences (Carleton *et al*., 2005; Carleton *et al*., 2008; Carleton *et al*., 2016; Carleton & Kocher, 2001; Hofmann *et al*., 2009; Larmuseau *et al*., 2009; Terai *et al*., 2006). This variation can be amplified by variation in chromophore composition (Saarinen *et al*., 2012; Sugawara *et al*., 2005; Terai *et al*., 2006; Torres-Dowdall *et al*., 2017). Two types of chromophores occur in fish visual pigments, derived from either vitamin A_1 or vitamin A_2. Higher proportions of vitamin A_2 shift pigment absorption maxima to longer wavelengths, with a stronger effect in longer-wavelength-absorbing opsins (Govardovskii *et al*., 2000; Hárosi, 1994; Parry & Bowmaker, 2000) (Figure 1a). Chromophore composition varies among species: marine fish and some freshwater fish possess solely A_1-derived chromophores, whereas most freshwater fish carry A_2 or A_1/A_2 mixtures (Bridges & Yoshikami, 1970; Morshedian *et al*., 2017; Provencio *et al*., 1992; Reuter *et al*., 1971; Toyama *et al*., 2008; Van der Meer & Bowmaker, 1995). In some species, chromophore ratios are phenotypically plastic, changing with environmental and/or life-history variables, such as migration, development, diet, season or temperature (Munz & McFarland, 1977; Suzuki *et al*., 1984). For example, the migratory Coho salmon (*Oncorhynchus kisutch*) shows annual shifts in A_1/A_2 usage, changing from high proportions of A_1 in the sea to high proportions of A_2 when migrating to freshwater streams for spawning (Temple *et al*., 2006). In the non-migratory rudd, *Scardinius erythrophthalmus*, chromophore ratios covary with age, with older fish expressing higher A_2 proportions (Bridges & Yoshikami, 1970). Switches between the two types of chromophores can occur within a few weeks (Munz & McFarland, 1977) and can, therefore, serve as a way to adjust to short-term changes in light conditions. In cichlids, only a few studies have explored variation in chromophore composition. They suggest that A_1-based chromophores tend to dominate in species inhabiting clear waters (*e.g*., Lake Malawi cichlids) (Carleton *et al*., 2000; Parry *et al*., 2005; Sugawara *et al*., 2005; Torres-Dowdall *et al*., 2017), whereas species occupying turbid waters show higher usage of A_2-based chromophores (Escobar-Camacho *et al*., 2019; Terai *et al*., 2006), indicating that variation in A_2 usage in cichlids may be important for perceiving long-wavelength light. This study explores the potential contribution of differential chromophore usage to visual adaptation in multiple cichlid populations from Lake Victoria.

This study focuses on closely related populations of *Pundamilia* cichlids (red and blue phenotypes) from several locations that differ in water clarity and therefore different light environments. Correlated differences in male coloration, female mate preference, photic environment and visual system properties suggest that visual adaptation to different light regimes contributes to *Pundamilia* divergence. Nonetheless, chromophore usage has not been documented in *Pundamilia*, and therefore, its role in divergent visual adaptation is unknown. Microspectrophotometry (MSP) of pigment absorption suggests that red phenotypes may use higher A_2 proportions than blue phenotypes (Carleton *et al*., 2005).

Previous studies in a variety of vertebrate species have estimated chromophore ratios by MSP or quantified alternative vitamin A derivatives using high-pressure liquid chromatography. Enright *et al*. (2015) showed that in zebrafish, the conversion from vitamin A1 to vitamin A2 is mediated by the enzyme cytochrome p450 family 27 subfamily C polypeptide 1 (CYP27C1). In line with this, studies in bullfrog, zebrafish and lamprey have documented positive correlations between *cyp27c1* expression levels and A2 proportions in retinal pigments (Enright *et al*., 2015; Morshedian *et al*., 2017). This suggests that *cyp27c1* expression levels can be used as a proxy for A2 proportions. This is the approach adopted in the present study. Specifically, we investigate in *Pundamilia* whether (a) *cyp27c1* expression profiles vary between islands and phenotypes, (b) visual conditions with long-wavelength light are associated with higher expression levels of *cyp27c1*, (c) variation in *cyp27c1* expression is correlated with opsin expression patterns and (d) the observed patterns in *cyp27c1* and opsin expression optimize visual performance; finally, given that chromophore usage may be influenced by both genetic and environmental factors, we (e) explored the effect of different light regimes on *cyp27c1* expression levels in laboratory-reared fish.

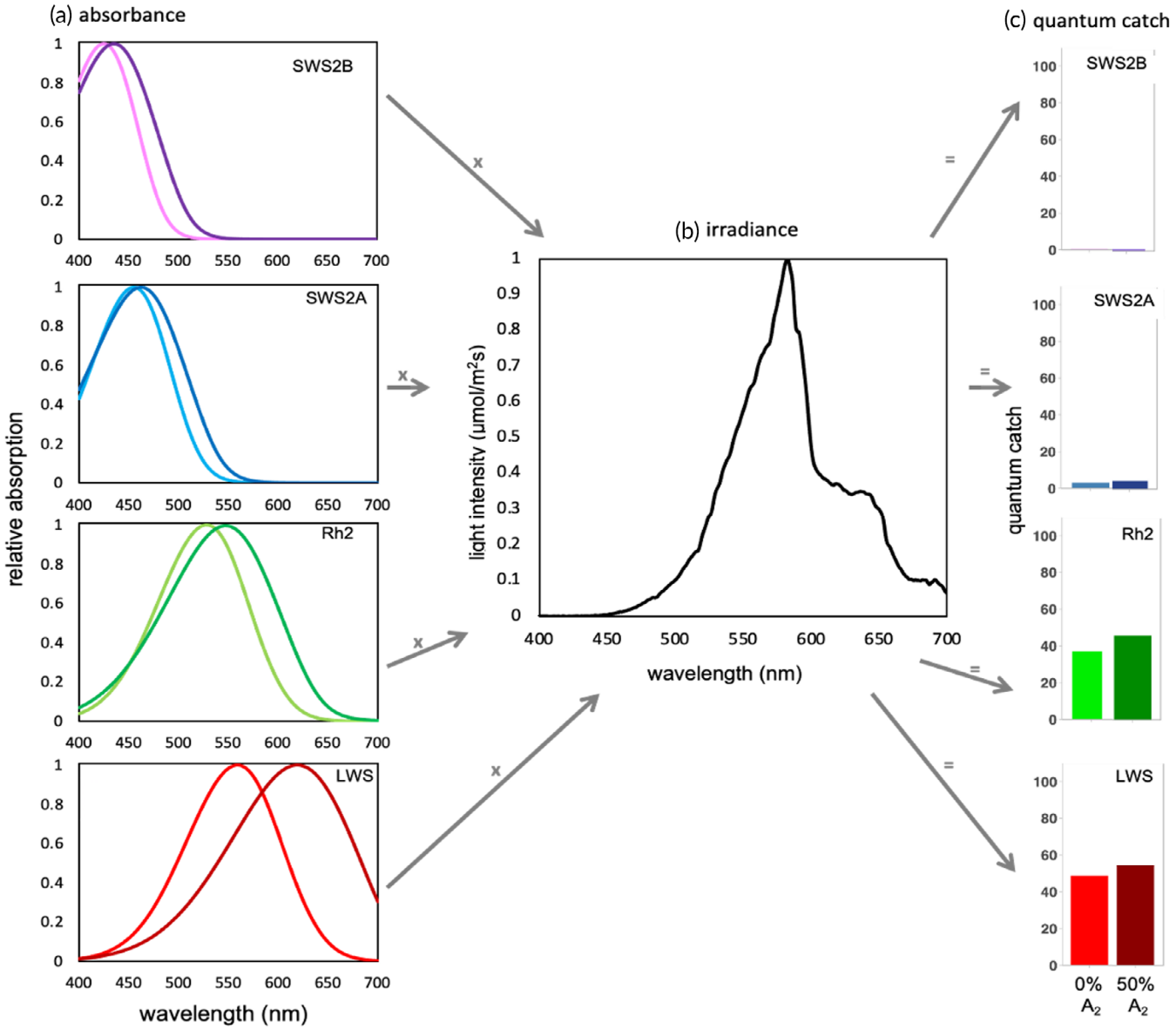

FIGURE 1 Schematic of the quantum catch calculation. (a) Usage of A_2-derived chromophores instead of A_1-derived chromophores generates a shift in pigment peak sensitivity, with larger shifts for opsins with peak sensitivity at longer wavelengths SWS2B (——) A1, (——) A2, SWS2A (——) A1, (——) A2, Rh2 (——) A1, (——) A2, and LWS (——) A1, (——) A2. (b) Pigment absorption curves, for either A_1 or A_2, are multiplied with the ambient light spectrum experienced by the focal population. As an example, depicted is the downwelling irradiance spectrum at Makobe Island at 6 m depth. (c) The resulting quantum catch estimate for different A_1/A_2 ratios for each opsin. Here illustrated for 0% and 50% A_2

2 | MATERIALS AND METHODS

2.1 | Fish

2.1.1 | Wild-caught fish

We included only male fish. We focus on *Pundamilia pundamilia* (Seehausen *et al*., 1998) and *Pundamilia nyererei* (Witte-Maas & Witte, 1985) from the Speke Gulf and north-eastern Mwanza Gulf of Lake Victoria, and similar sympatric *Pundamilia* species pairs from the western and southern Mwanza Gulf of Lake Victoria (*P. sp*. "*pundamilia-like*" *and P. sp*. "*nyererei-like*"; Meier *et al*., 2017, 2018). In particular, *Pundamilia* species were collected in 2014 at five rocky islands in south-eastern Lake Victoria, Mwanza Gulf: Luanso (−2.6889, 32.8842), Kissenda (−2.5494, 32.8276), Python (−2.6238, 35.8566) and Anchor (−2.5552, 32.8848); Speke Gulf: Makobe (−2.3654, 32.9228). Water transparency varies across islands, with more turbid waters at the southern end of the sampled region (*i.e*., Luanso, Kissenda and Python islands) and clearer waters at the northern end (*i.e*., Anchor and Makobe). Males of the species pair differ in nuptial coloration: *P. pundamilia* and *P. sp*. "*pundamilia-like*" display a blue/grey coloration, and *P. nyererei* and *P. sp*. "*nyererei-like*" are yellow on the flanks and orange or red dorsally (Seehausen, 1996). Females are inconspicuously coloured and exert colour-mediated

assortative mate preferences (Haesler & Seehausen, 2005; Seehausen & Van Alphen, 1997; Selz *et al*., 2014; Stelkens *et al*., 2008). For simplicity, "blue phenotype" is for *P. pundamilia/P. sp.* "*pundamilia-like*" and "red phenotype" is for *P. nyererei/P. sp.* "*nyererei-like*." Phenotypes tend to have different depth distributions coinciding with different photic environments: blue phenotypes inhabit shallow waters with broad-spectrum light, whereas red phenotypes occur at greater depths, where long-wavelength light (*i.e*., yellow and red) dominates (Seehausen *et al*., 2008). Until recently, all populations with blue males were classified as *P. pundamilia* and all populations with red males as *P. nyererei*. Nonetheless, population genomic analyses revealed that populations from the southern and western Mwanza Gulf (Kissenda and Python islands) represent a separate speciation event; they are therefore referred to as *P. sp.* "*pundamilia-like*" and *P. sp.* "*nyererei-like*" (Meier *et al*., 2017, 2018). At the most southern island, Luanso, blue and red phenotypes show no genetic differentiation (Meier *et al*., 2018), and fish were categorized into blue or red phenotype by visually scoring coloration (as in Wright *et al*., 2019). Blue and red phenotypes have distinct visual system properties: they differ in the amino acid sequence of the long-wavelength sensitive opsin (LWS) and also show differences in opsin gene expression levels, corresponding to the differences in visual environment between geographic locations and depth ranges (Seehausen *et al*., 2008; Wright *et al*., 2019). In line with these differences, the red phenotype displays greater behavioural sensitivity to long-wavelength light than the blue phenotype (at least in the most northern, clear-water location Makobe; Maan *et al*.,).

Sampling was conducted with permission from the Tanzanian Commission for Science and Technology (COSTECH no. 2013-253-NA-2014-117). We collected 111 adult male fish by gillnetting and angling (Luanso = 10, Kissenda = 32, Python = 29, Anchor = 13 and Makobe = 27). Capture depth was recorded for each individual. Fish were transported to the Tanzanian Fisheries Research Institute (TAFIRI – Mwanza Centre) and sacrificed by using 2-phenoxyethanol (~2.5 ml l^{-1}) and subsequent cutting of the vertebral column. All fish were killed in the early evening on the day of capture (17.00–20.00 hours) to maximize RNA yield and minimize differences due to circadian variation in gene expression (Halstenberg *et al*., 2005; Yourick *et al*., 2019). Eyes were subsequently extracted, preserved in *RNAlater* (Ambion, Austin, TX, USA) and frozen (−20°C).

2.1.2 | Laboratory-reared fish

To explore the effects of light manipulation on *cyp27c1* expression levels, F1 and F2 offspring of wild-caught fish collected in 2010 at Python Island were reared in light conditions mimicking the conditions experienced by each phenotype in their natural habitat at Python Island (Supporting Information Figure S1). Fish were bred opportunistically with 18 dams and 15 sires. We used 75 male offspring resulting from 30 crosses (mother × father: 17 P × P; 21 N × N). *Pundamilia* are female mouthbrooders; eggs were removed from brooding females *c*. 6 days after fertilization and divided evenly between the two light conditions. Fish were housed at 25 ± 1°C on a 12:12 h light–dark cycle and fed with commercial cichlid pellets and frozen Artemia, spirulina and krill. All specimens were sacrificed as adults, by applying an MS-222 (1 g l^{-1}) overdose and subsequent cutting of the vertebral column. Eyes were extracted, preserved in *RNAlater* (Ambion) and frozen (−20°C). All fish were sacrified in the late afternoon (16.00–18.00 hours). This study was conducted with the approval of the Institutional Animal Care and Use Committee of the University of Groningen (DEC6205B; AVD105002016464).

2.2 | Light measurements

In 2010, downwelling light intensity (μmol $m^{-2} \times s^{-1}$) was measured at each island, using a BLK-C-100 spectrometer with an F-600-UV-VIS-SR optical fibre with CR2 cosine receptor (Stellar-Net, Tampa, FL, USA). Measurements were taken between 08.00 and 12.00 hours at depths 13 m at 0.5 m increments. We took two independent series of measurements from Luanso, three from Kissenda and four from Makobe and Python islands. Measurements were collected on different days, and we used the mean across sampling days for each depth measurement. Irradiance measurements were not conducted at Anchor Island.

To explore the relationship between photic environments and fish visual system properties we calculated the orange ratio of each light spectrum. The orange ratio is the ratio of light transmitted in the 550–700 nm range (yellow, orange and red) over the transmittance in the 400–549 nm range (blue and green). As short wavelengths are more strongly absorbed and scattered with increasing depth in Lake Victoria, orange ratios increase with turbidity and depth (Supporting Information - Figure S2). For each population, we calculated two measures of the orange ratio. First, population-level orange ratios were calculated for each population, based on depth distribution data from larger samples of fish (from Seehausen *et al*., 2008). Second, individual-level orange ratios were based on the capture depth of each individual fish that was sampled in the present study. Because no light measurements were available for Anchor Island and prior work has shown that the water transparency at Anchor Island is intermediate between Python and Makobe islands (Seehausen *et al*., 2008), we estimated the orange ratios at Anchor Island as the medians of the ratios from Python and Makobe islands, following Wright *et al*. (2019).

2.3 | Cyp27c1 gene expression

Cpy27c1 expression was quantified using real-time quantitative PCR (qPCR). We removed the retina from the preserved eyes and extracted total RNA using Trizol (Ambion) followed by a DNase treatment to remove genomic DNA. RNA was reverse transcribed into cDNA using $Oligo(dT)_{18}$ primer (Thermo Fisher Scientific, Carlsbad, CA, USA) and RevertAid H Minus (Thermo Fisher Scientific) at 45°C. cDNA was diluted to a final concentration of 10 ng μl^{-1}. Three housekeeping reference genes (HKGs) were used: *ldh1*, *ß-actin* and *gapdh2* (Jin *et al*., 2013; Torres-Dowdall *et al*., 2017). The stability of the HKG expression was confirmed using RefFinder (Xie *et al*., 2012). qPCRs

were run for 45 cycles (95°C for 3 min, 95°C for 15 s, 60°C for 25 s and 72°C for 30 s) with specifically designed primers using *cyp27c1* sequences from the *P. nyererei* reference genome (Supporting Information Table S1) to amplify short fragments (200 bp). Each 20 μl reaction mixture contained 9 μl gene-specific primer pairs, 1 μl diluted cDNA sample and 10 μl of SYBR Green PCR Master Mix (BioRad, Hercules, CA, USA). Fluorescence was monitored on StepOnePlus Real-Time PCR System (Applied Biosystems StepOnePlusReal-Time PCR System, Foster City, CA, USA). To determine the critical threshold (Ct) and the initial concentration (N_0) of *cyp27c1* and the three HKGs, we used LinRegPCR (Ramakers *et al*., 2003). Expression levels were based on two technical replicates. The following quality criteria were applied: PCR efficiency 1.75–2.25 and Ct standard deviation between duplicates ≤0.5. The following equation was used to calculate *cyp27c1* expression for each sample separately:

$$Re = \frac{No_{,\text{Target}}}{No_{,\text{Reference}}}$$

where $N_{0,\text{Target}}$ is the initial concentration of *cyp27c1* and $N_{0,\text{Reference}}$ is the geometric mean of the starting concentration of the three HKGs.

2.4 | Opsin gene expression

To determine the relationship between opsin gene expression and *cyp27c1* expression in wild-caught fish, we used previously reported opsin gene expression data (*i.e*., SWS2b, SWS2a, Rh2 and LWS) from the same individuals from Wright *et al*. (2019).

2.5 | Quantum catch estimates

To explore whether the observed variation between populations in *cyp27c1* expression (and opsin gene expression) enhances visual performance in the local light environment, we calculated quantum catch estimates (Qc), representing the number of photons captured by visual pigments in a given light environment. Quantum catches were estimated for the red and blue phenotypes at three locations (*i.e*., Kissenda, Python and Makobe islands). We excluded Luanso Island because of the low sample size and Anchor Island because of the lack of light measurements. Quantum catches were calculated considering population-specific LWS genotype, with red phenotypes predominantly carrying LWS alleles conferring a more red-shifted sensitivity (H allele) than the blue phenotypes (P allele) (Seehausen *et al*., 2008), opsin expression profiles and depth ranges (*i.e*., visual environments), for three hypothetical A_1/A_2 proportions (Figure 1). Quantum catches were calculated for each opsin using the following equation:

$$Q = N_i \int_{400}^{700} I(\lambda)\{\alpha R(\lambda, A_2) + (1-\alpha)\, R(\lambda, A_1)\}\, d\lambda$$

where $I(\lambda)$ is the normalized irradiance spectrum at a specific capture depth and island, N_i is the relative opsin expression for each individual reported in Wright *et al*. (2019) and $R(\lambda)$ is the absorption spectrum of the visual pigments calculated for A_1 and A_2 separately (based on Govardovskii *et al*., 2000). We used previously established peak sensitivities for each opsin in association with both A_1 and A_2–based chromophores (Carleton *et al*., 2005) (Table 1). To explore the impact of differential chromophore usage, we estimated quantum catches for three hypothetical proportions of vitamin A_2(designated by α): 10%, 30% and 50%. Quantum catch estimates were obtained using (a) population-level irradiance spectra (based on the depth distributions of each population reported in Seehausen *et al*., 2008) and (b) individual-level irradiance spectra (based on the individual capture depth of each fish).

2.6 | Statistical analysis

All statistical analyses were performed in R (v 4.1.0; R Development Core Team 2021).

2.6.1 | Wild-caught fish

Cyp27c1 expression data were tested for outliers (1.5 × the interquartile range) separately for each population (*i.e*., phenotype–island combination). This resulted in *cyp27c1* expression data for 95 wild-caught fish (six were excluded). After log transformation, we used linear models to explore if *cyp27c1* expression (a) differed between islands and/or phenotypes; (b) covaried with water transparency, using the spectral midpoint from each island as an estimate for water transparency; and (c) covaried with population-level and/or individual-level photic environment (*i.e*., orange ratio) as follows: *cyp27c1* expression ~ island × phenotype + orange ratio. To determine the minimum adequate models, we used stepwise backward selection using likelihood ratio tests (drop1 function, Crawley, 2002). We used ANOVA to estimate parameters and *P*-values (car package, Fox *et al*., 2017). In case of more than two categories per fixed effect (*i.e*., island), we used *post hoc* Tukey's tests (glht – multcomp package, Hothorn *et al*., 2008).

2.6.2 | Laboratory-reared fish

Cyp27c1 expression data were tested for outliers (1.5 × the interquartile range) separately for each phenotype and light treatment. This

TABLE 1 Peak sensitivities for each opsin and A_1 and A_2–based chromophores used for quantum catch calculations (Carleton *et al*., 2005)

Opsin	λ_{max} A_1 (nm)	λ_{max} A_2 (nm)
SWS2b	455	462
SWS2a	425	435
Rh2	528	547
LWS (P-allele)	544	604
LWS (H-allele)	559	61

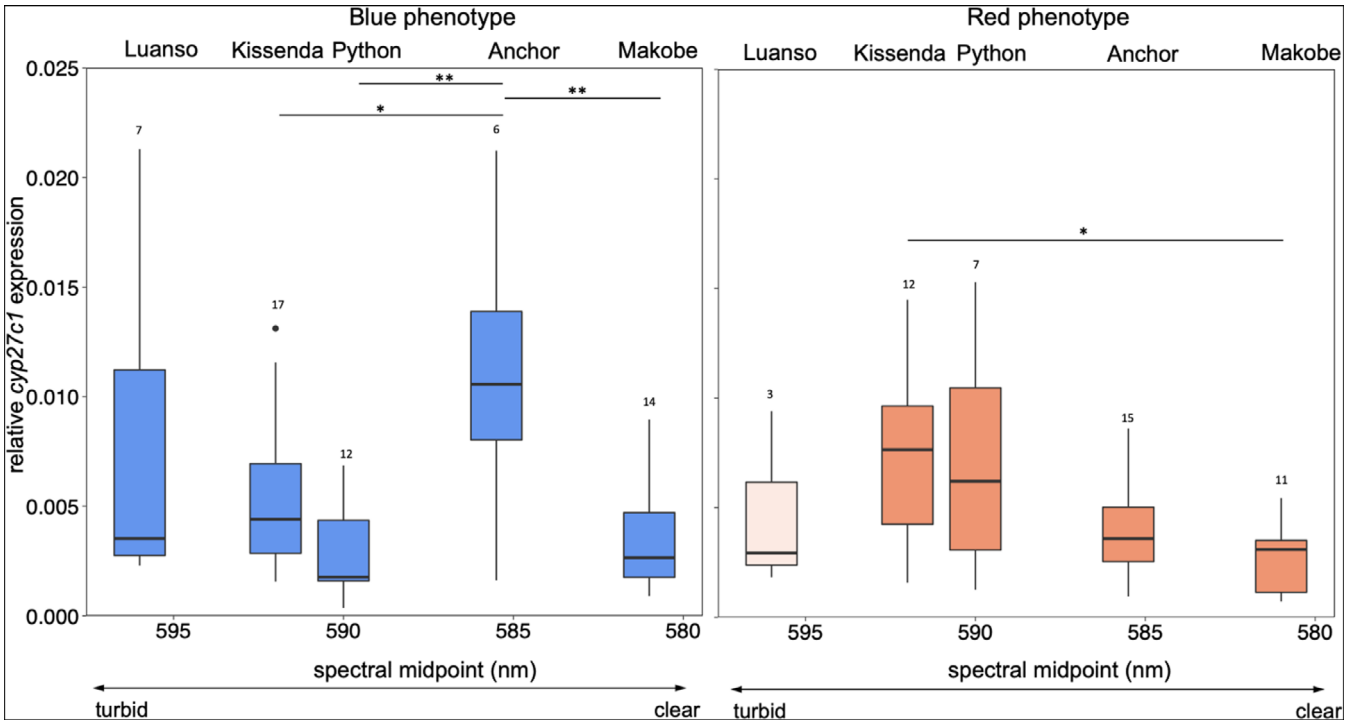

FIGURE 2 Cyp27c1 expression in sympatric *Pundamilia* phenotypes at five locations. For both blue and red phenotypes, *cyp27c1* expression differed between islands. Geographic variation in expression levels was different between the red and blue phenotypes. Colours indicate phenotypes (blue phenotype and red phenotype). Red phenotypes from Luanso were rare ($n = 3$) and represented by a semi-transparent box. Boxes represent 25th–75th percentiles, intercepted by the median. Error bars indicate 95% C.I.; black symbols are outliers. Sample sizes are given above each box. ** indicates $P < 0.01$, * indicates $P < 0.05$

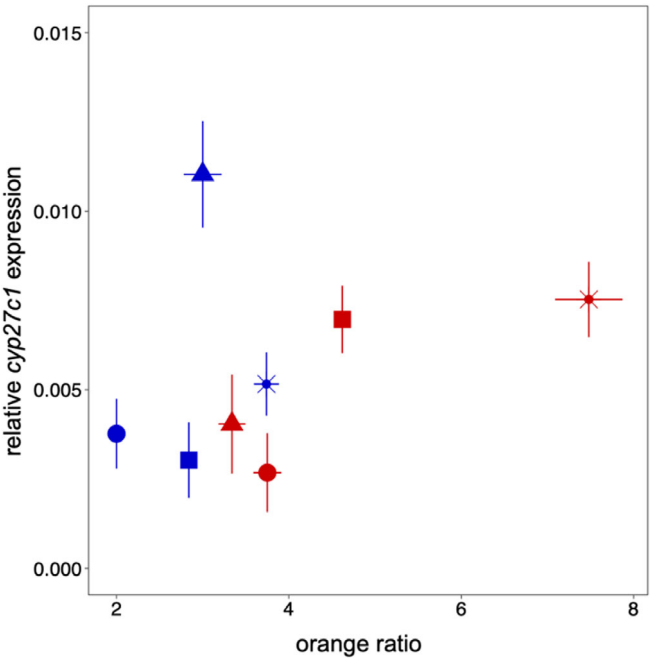

FIGURE 3 No covariation between *cyp27c1* expression and population-level orange ratio for *Pundamilia* populations at four locations. Symbols: Kissenda (∗), Python (■), Anchor (▲) and Makobe (•). Population-level orange ratios were derived from population-specific depth distributions presented in Seehausen *et al.* (2008). Colours indicate phenotypes (blue phenotype and red phenotype). Error bars represent ±S.E

resulted in 66 laboratory samples for *cyp27c1* expression (9 removed). We tested for (a) differences between wild-caught and laboratory-reared fish, using general linear models: *cyp27c1* expression ~ phenotype × origin × light treatment, where "origin" denotes wild-caught or laboratory-reared fish (*i.e.*, including laboratory-reared fish from both light treatments) and light treatment denotes broad-spectrum light or red-shifted light; and (b) differences between light treatments, including only the laboratory-reared fish and using linear mixed effects modelling (lmer, package lme4) after log transforming the data: relative gene expression ~ phenotype × treatment + (1|mother ID) + (1|father ID). To estimate the parameter effects, *P*-values and degrees of freedom, we performed "KRmodcomp" (pbkrtest package; Halekoh & Højsgaard, 2014).

3 | RESULTS

We found that *Pundamilia* from all five locations expressed *cyp27c1*, but the expression levels differed between islands [$\chi^2(4) = 3.23$, $P = 0.015$]. There was no overall difference between blue and red phenotypes [$\chi^2(1) = 0.75$, $P = 0.387$]. Indeed, differences between islands were inconsistent between phenotypes, indicated by a significant island-by-phenotype interaction [$\chi^2(4) = 4.12$, $P = 0.004$] (Figure 2): *cyp27c1* expression decreased with water transparency in the red phenotypes [$\chi^2(1) = 8.93$, $P = 0.003$)] but not in the blue phenotypes [$\chi^2(1) = 0.62$, $P = 0.429$].

We then evaluated whether *cyp27c1* expression could be predicted by the local light environment. We found no evidence that *cyp27c1* expression covaried with population-level orange ratio [$\chi^2(1) = 0.13$, $P = 0.716$; Figure 3] nor with individual-level orange ratio [$\chi^2(1) = 0.02$, $P = 0.898$; Supporting Information Figure S3], indicating that the spectral composition of the local light environment did not explain variation in *cyp27c1* expression levels.

Regarding the within-island, between-phenotype differences in *cyp27c1* expression, we found that sympatric blue and red phenotypes showed significant differences in *cyp27c1* expression at several islands, but the direction of the difference was inconsistent between islands (Figure 4a). At locations with higher turbidity (*i.e.*, Kissenda and Python), *cyp27c1* expression tended to be higher in the red phenotypes, whereas at clear-water locations (*i.e.*, Anchor and Makobe), *cyp27c1* expression tended to be higher in the blue phenotypes. Previous work in the same populations (Wright *et al.*, 2019) reported that at the clear-water islands (Makobe and Anchor) the red phenotypes expressed higher LWS (and lower Rh2) than the blue phenotypes, whereas at the turbid-water locations (Python and Kissenda), the pattern was reversed (Figure 4b). The present study observed that this reversal in Rh2/LWS expression is matched by a reversal in *cyp27c1* expression, suggesting that there is a consistent relationship between blue–red differences in *cyp27c1* expression and blue–red differences in opsin gene expression. In other words, at all locations, the phenotypes with the lower level of Rh2 expression (and higher level of LWS) tended to express lower levels of *cyp27c1*, but the identity of the phenotypes reversed between clear- and turbid-water locations (Figures 4 and 5). In contrast to this general consistency in the differences between sympatric blue and red phenotypes, we observed substantial individual variation (Figure 5) and no consistent relationships between *cyp27c1* and opsin gene expression at the individual level (Supporting Information Figure S4).

We used visual modelling to evaluate whether the observed patterns of *cyp27c1* expression maximize visual performance. Based on previous studies (Enright *et al.*, 2015; Morshedian *et al.*, 2017; Torres-Dowdall *et al.*, 2017), models incorporated the assumption that higher levels of *cyp27c1* expression would cause higher vitamin A_2 levels in the visual pigments and thereby a

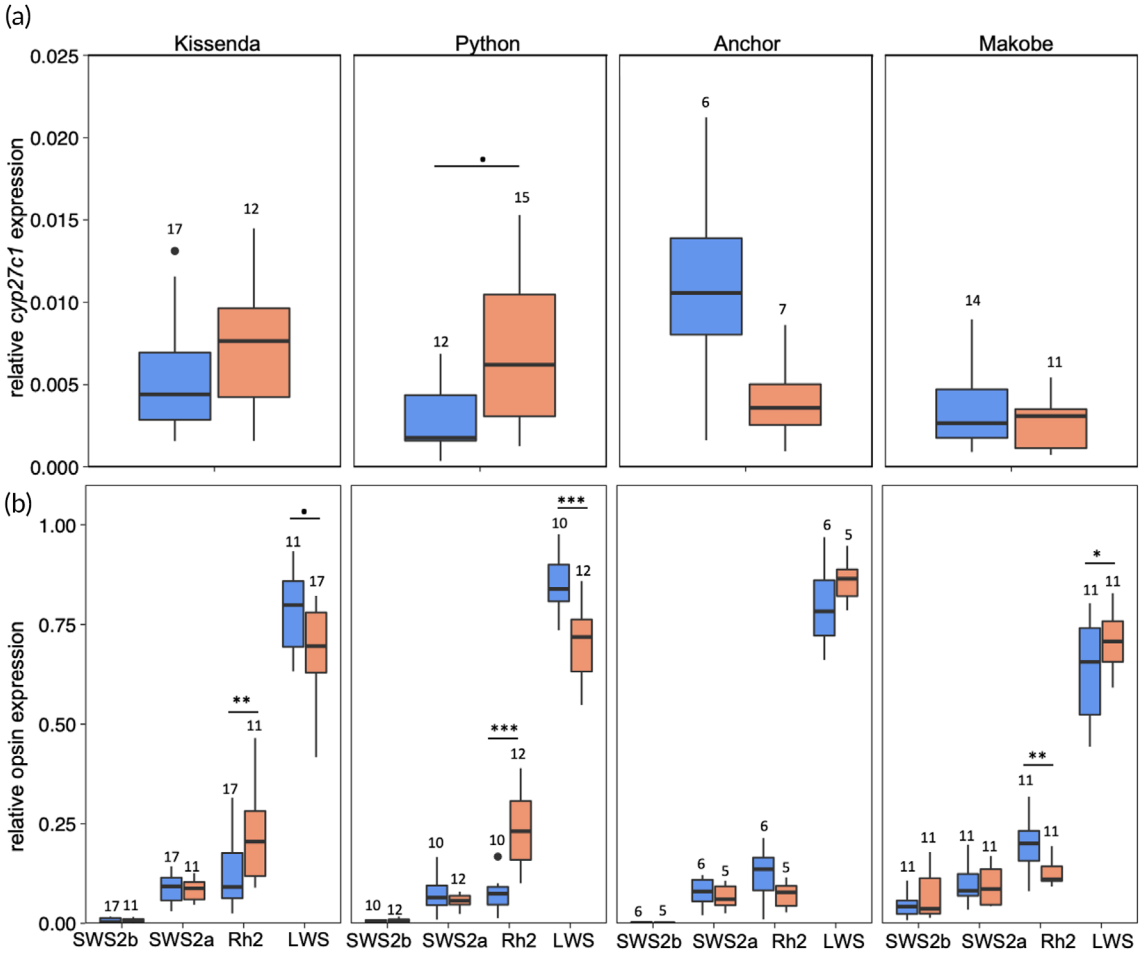

FIGURE 4 Cyp27c1 and opsin gene expression patterns in sympatric blue and red *Pundamilia* phenotypes from four locations. (a) *cyp27c1* expression levels. (b) Opsin gene expression levels (from Wright *et al.*, 2019). Boxes represent 25th–75th percentiles, intercepted by the median. Error bars indicate 95% C.I.; black symbols are outliers. Sample sizes are indicated above each boxplot. ***indicates $P < 0.001$, **indicates $P < 0.01$, *indicates $P < 0.05$ and •indicates $P < 0.1$

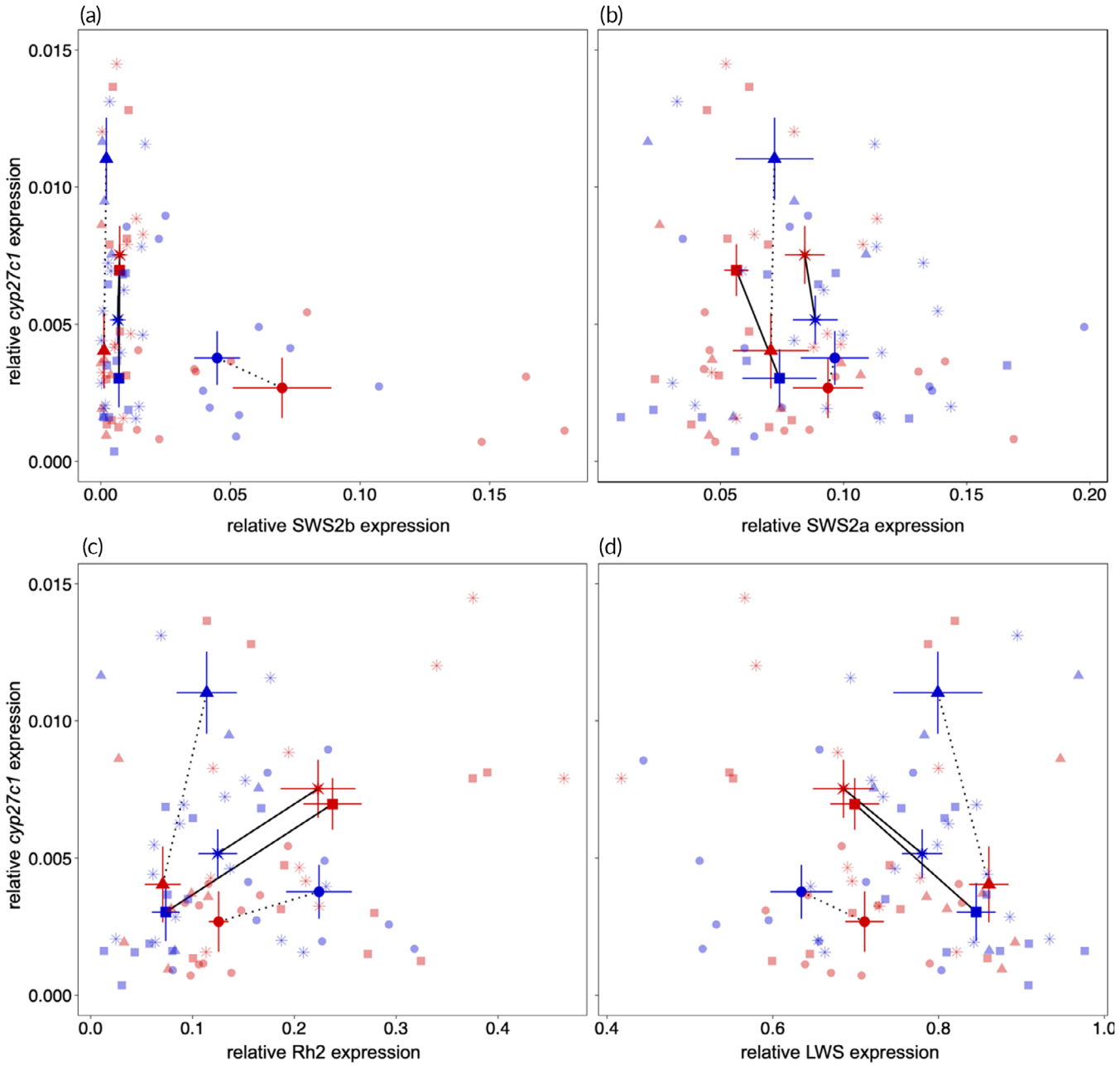

FIGURE 5 Relationships between *cyp27c1* and opsin gene expression [(a) SWS2b, (b) SWS2a, (c) Rh2 and (d) LWS] in sympatric blue and red *Pundamilia* phenotypes from four locations. Colours indicate phenotypes (blue phenotype and red phenotype). Symbols indicate population means [Kissenda (*), Python (■), Anchor (▲) and Makobe (•)]. The lines do not indicate statistically established relationships, but connect sympatric populations. Solid black lines indicate populations from turbid water locations, whereas black dashed lines indicate populations from clear-water locations. Shaded symbols indicate individual data points. Error bars represent ±S.E

red-shifted visual sensitivity. Thus, visual models predict that populations with higher *cyp27c1* expression levels, and therefore presumably higher A_2 levels, obtain higher quantum catches (Qc) in red-shifted light conditions. The analysis did not support this hypothesis: a hypothetical increase in A_2 proportions generates higher quantum catch estimates for every phenotype at each location (Figure 6). Quantum catch estimates based on individual depth ranges yielded similar results (Supporting Information Figure S5). Therefore, these results do not provide evidence that the observed differences between populations in *cyp27c1* expression contribute to locally adapted visual performance.

To evaluate whether the observed variation in *cyp27c1* expression in the wild is due to genetic differences and/or phenotypic plasticity, we reared both *Pundamilia* phenotypes from one population (*i.e.*, Python Island) under two different light conditions, mimicking the light spectra in the natural shallow-water and deep-water environments of Python Island (*i.e.*, broad-spectrum light *vs.* red-shifted light). We found that overall, laboratory-bred individuals showed similar

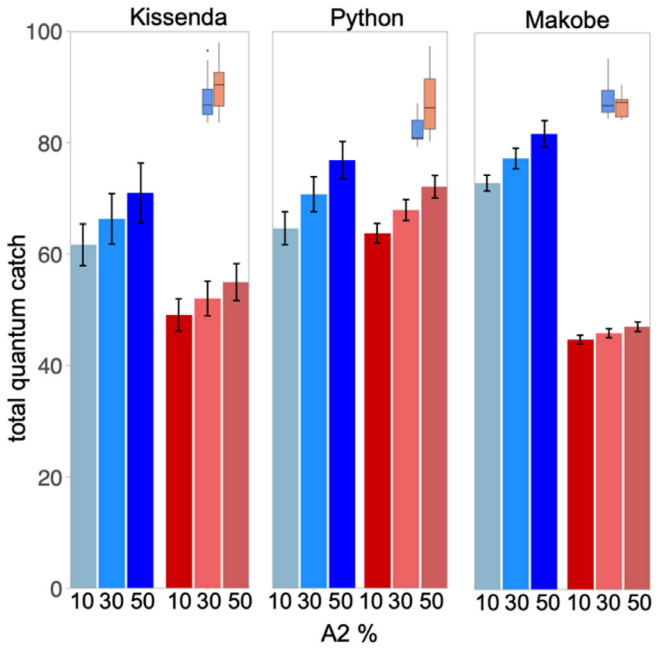

FIGURE 6 Variation in A_2 proportion does not maximize visual performance for *Pundamilia* phenotypes at three locations. Bars represent different quantum catches for three hypothetical proportions of vitamin A_2 (*i.e.*, 10%, 30% and 50%). Colours indicate phenotypes (blue phenotype and red phenotype) and error bars represent ±S.E. The top-right panels show reported phenotype differences in *cyp27c1* expression (from Figure 4a)

cyp27c1 expression levels as their wild-caught counterparts (Figure 7a). Nonetheless, there was a statistical trend indicating that the red–blue phenotype difference in the field (see earlier) was not maintained in the laboratory [phenotype by origin interaction: $\chi^2(1) = 3.63$, $P = 0.05$]. Consequently, analysis including only laboratory-reared fish showed no difference between red and blue phenotypes [$\chi^2(1) = 0.180$, $P = 0.675$; Figure 7a]. This was not explained by the different light conditions employed in the laboratory: they found no influence of the two rearing light conditions on *cyp27c1* expression (Figure 7b; *P. sp.* "*pundamilia*-like": $z = 1.33$, $P = 0.56$; *P. sp.* "*nyererei*-like": $z = 0.03$, $P = 1.00$). The difference between field and lab data was mostly due to the fact that laboratory-bred individuals of the red phenotype tended to have lower *cyp27c1* expression levels than their wild-caught counterparts, irrespective of the light conditions ($t = -2.25$; $P = 0.05$). In the blue phenotype, levels did not differ between laboratory-bred and wild-caught individuals ($t = 2.18$; $P = 0.97$).

4 | DISCUSSION

Cichlids are a major model system for visually mediated ecological speciation, based on evidence for divergent adaptation in opsin genes and opsin gene expression levels. In many vertebrates, particularly in fish, variation in chromophore usage also contributes to visual adaptation. This study provides the first investigation of differential

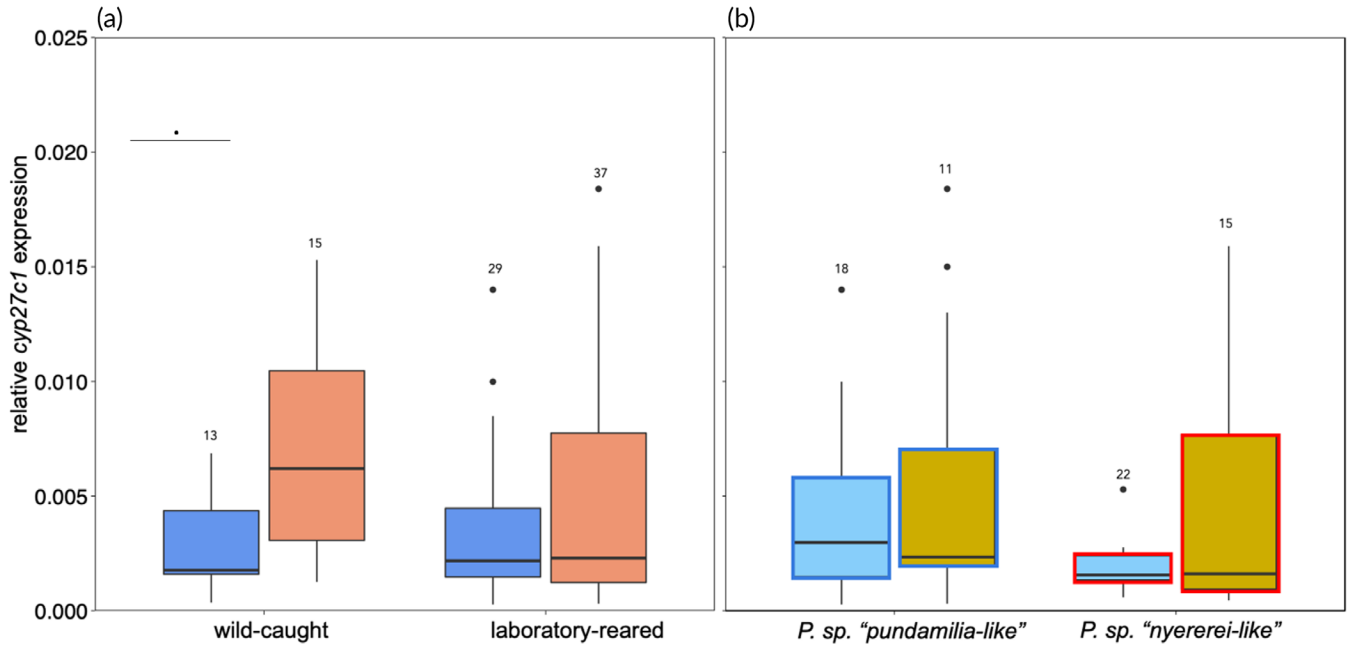

FIGURE 7 Cyp27c1 expression in wild-caught and laboratory-reared fish. (a) Cyp27c1 levels in laboratory fish were similar to the levels found in wild-caught fish. (b) Laboratory-reared phenotypes were not influenced by the rearing light conditions ((■) broad-spectrum light *vs.* (■) red-shifted light (■)). Blue borders indicate *P. sp* "*pundamilia-like*" and (■) red borders indicate *P. sp* "*nyererei-like*". Boxes represent 25th–75th percentiles, intercepted by the median. Error bars indicate 95% C.I.; black symbols are outliers. Sample sizes are indicated above each bar. •indicates $P < 0.1$

chromophore usage across multiple cichlid populations from Lake Victoria, using as a proxy *cyp27c1*, an enzyme responsible for converting A_1 to A_2-derived chromophores (Enright *et al*., 2015). We found that *Pundamilia* cichlids from five different islands express *cyp27c1* in the eye and that expression levels differ between populations. Nonetheless, we also found substantial individual variation and no clear association between the visual environment and *cyp27c1* expression levels. We also find that overall expression levels are low, possibly indicating that *cyp27c1* has no significant role in *Pundamilia* visual adaptation.

Typically, fish that inhabit red-shifted visual conditions (as in many turbid waters) use higher vitamin A_2 proportions (Bowmaker, 1995). Yet evidence for this pattern in cichlid species is mixed. Based on MSP, it has been shown that cichlids from clear waters of Lake Malawi use only A_1 (Carleton *et al*., 2000), whereas cichlids from turbid waters of Lake Victoria incorporate A_2 (Carleton *et al*., 2005; Terai *et al*., 2006; Van der Meer & Bowmaker, 1995). Nonetheless, indirect measurements of chromophore usage through *cyp27c1* expression are inconsistent. For example, Härer *et al*. (2018) observed that several closely related Neotropical cichlid species expressed higher levels of *cyp27c1* in turbid waters (Lake Managua and Lake Nicaragua) than in clear waters (Lake Xiloa). Nonetheless, in the Amazonian cichlid *Cichla monoculus* from Lake Gatun, fish from turbid waters expressed lower *cyp27c1* than fish from clear waters (Escobar-Camacho *et al*., 2019). The present study observed that both *Pundamilia* phenotypes (*i.e*., red and blue) express *cyp27c1*, but it found no clear relationship with visual conditions (*i.e*., differences in water transparency across locations and variation in orange ratio across depths). In particular, it found that the red *Pundamilia* phenotype displayed higher *cyp27c1* expression in turbid waters than in clear waters, but the blue phenotype showed no such pattern. A possible explanation for this species difference is the difference in depth distribution between the red and the blue phenotypes: the consistently shallow depth range of the blue phenotype entails a relatively consistent light environment across locations, whereas the red phenotypes occur in deeper waters where variation in water transparency has a much greater impact, generating larger variation in light conditions (Figure 3; Supporting Information Figure S6).

In various aquatic taxa including cichlids, species and population differences in opsin expression have been shown to correlate with variation in visual conditions (Carleton 2009; Carleton *et al*., 2016; Hofmann *et al*., 2009). Recent work in *Pundamilia* (Wright *et al*., 2019) demonstrated that at locations with higher turbidity (*i.e*., Kissenda and Python), the red phenotypes express more Rh2 and less LWS than the blue phenotypes, whereas this difference reverses at clear-water locations (*i.e*., Anchor and Makobe). Visual modelling suggested that these patterns do not maximize quantum catch at each location, implying that the observed patterns could not be explained by local adaptation. Here, we explored whether *cyp27c1* expression could act as a compensatory mechanism and thereby explain these findings. Strikingly, we observed a similar reversal for *cyp27c1* expression levels across locations: at locations with higher turbidity (*i.e*., Kissenda and Python), *cyp27c1* expression tended to be higher in the red phenotypes than in the blue phenotypes, whereas at clear-water locations (*i.e*., Anchor and Makobe), we found the opposite. Therefore, there is a consistent relationship between phenotype differences in *cyp27c1* expression levels and phenotype differences in opsin expression levels, where the phenotype expressing more Rh2 consistently also expresses more *cyp27c1* than its sympatric relative, but the red/blue identity reverses between clear-water and turbid-water locations. We propose three possible explanations for these findings. First, the observation that phenotypes with low LWS levels expressed high *cyp27c1* levels could reflect a compensatory mechanism, where reduced long-wavelength sensitivity (*i.e*., lower LWS expression) might be compensated with higher vitamin A_2 usage (*i.e*., higher *cyp27c1* expression) and *vice versa*. Nonetheless, the present quantum catch estimates suggest that the observed variation does not maximize local visual performance, as any hypothetical increase in A_2 proportion generates higher quantum catch estimates for every phenotype at each location. It should be noted that quantum catch estimates are a fairly crude measure of visual performance, considering only the ability to capture ambient light and ignoring more complex perceptual abilities such as colour discrimination and object recognition. Either way, a second explanation could be non-adaptive: the parallel reversal in opsin expression and *cyp27c1* expression between blue and red phenotypes may simply be a by-product of the evolutionary history of the study populations. Meier *et al*. (2017) found that the speciation event resulting in *P. pundamilia* and *P. nyererei* occurred outside the Mwanza Gulf, after which the species pair settled at Makobe Island. Many generations later, *P. pundamilia* colonized the Western Mwanza Gulf (including Python Island). After 1000 generations, *P. nyererei* from outside the Mwanza Gulf immigrated into the Western Mwanza Gulf and hybridized with the local *P. pundamilia* population, which resulted in a new speciation event in which *P. sp*. "*pundamilia-like*" and *P. sp*. "*nyererei-like*" emerged. Given this recent history, the characteristic expression patterns observed at Python and Kissenda, but not at Makobe and Anchor, may be a non-adaptive by-product of this event rather than an adaptation to the visual environment at these locations. Third, in the past decades, intensive agriculture, deforestation and urban runoff have significantly increased nutrient loading causing the eutrophication of Lake Victoria (Scheren *et al*., 2000). This resulted in an increase in algal biomass, which in turn, together with silt carried by the rivers, decreased water transparency (Nyamweya *et al*., 2020). Therefore, *Pundamilia* populations have recently been exposed to environmental changes, allowing very little time to adapt and possibly explaining the lack of a relationship between opsin expression, *cyp27c1* expression and current visual conditions.

Rapid adjustments in visual system properties may improve individual visual performance in changing light conditions. Such plasticity may enhance population persistence and thereby provide a starting point for evolutionary change (Fusco & Minelli, 2010; Price *et al*., 2003; West-Eberhard, 2003). Several cichlid species have been shown to change opsin gene expression levels over development and in response to different light regimes (Härer *et al*., 2019; Nandamuri *et al*., 2017; Wright *et al*., 2020), suggesting a potential role of phenotypic plasticity in optimizing visual performance. Also in *Pundamilia*, expression levels of long-wavelength-sensitive (LWS) and short-wavelength-sensitive (SWS2a) opsins can be influenced by light

manipulations (Wright *et al*., 2020). The present study is the first to explore plasticity in chromophore usage in *Pundamilia*. We observed that the phenotype difference in *cyp27c1* expression observed in the wild was reduced in laboratory-reared fish, which indicates a potential but modest contribution of plasticity to the variation observed in the wild. Nonetheless, when fish were reared under different light conditions in the laboratory, we did not detect an effect on *cyp27c1* expression levels. Together, these findings indicate that *cyp27c1* expression in *Pundamilia* is less plastic than opsin gene expression and that the observed variation in *cyp27c1* expression among natural populations largely reflects genetic differences. Nonetheless, compared to other cichlid species, *cyp27c1* expression in *Pundamilia* is very low (Torres-Dowdall *et al*., 2017), questioning the biological relevance of the observed variation in *cyp27c1* expression between phenotypes and locations. Therefore, the present study suggests that *cyp27c1* expression may not be relevant for visual adaptation in *Pundamilia*, and this may also explain the absence of a strong plastic response in expression level to different light environments.

A key assumption in this study was that *cyp27c1* expression and A_2 proportion are positively correlated. Nonetheless, only a few datapoints in Neotropical cichlids are available to substantiate this assumption, with inconsistent results (Escobar-Camacho *et al*., 2019; Torres-Dowdall *et al*., 2017). Consequently, Escobar-Camacho *et al*. (2019) hypothesized that in cichlid fish, conversions between A_1 and A_2 might be regulated differently. Thus, further studies are needed to explore whether *cyp27c1* expression does actually reflect A_1/A_2 ratios in cichlid fish and how this gene functions and interacts with other genes in visual system functioning. Also, there is a need for data from additional cichlid species, from different visual habitats, to evaluate the contribution of chromophore usage to cichlid visual adaptation.

ACKNOWLEDGEMENTS

We thank the Tanzanian Commission for Science and Technology for research permission and the Tanzanian Fisheries Research Institute for hospitality and facilities. We thank Daniel Shane Wright for collecting the wild-caught samples and performing RNA isolation; Roel van Eijk and Jolien Gay for helping design the qPCR protocol; and Sjoerd Veenstra, Brendan Verbeek, Lucia Irazábal-González and Willem Diderich for taking care of the fish in the laboratory.

AUTHOR CONTRIBUTIONS

M.E.M. designed the study, together with R.S.E., L.Z. and E.W. E.W. designed the qPCR protocol for *cyp27c1* and completed the laboratory work. E.W. performed the analysis, with assistance from M.E.M., R.S.E. and L.Z. E.W. wrote the manuscript with contributions from M.E.M., R.S.E. and L.Z. All authors approved the contents of this manuscript.

ORCID

Elodie Wilwert https://orcid.org/0000-0003-4135-1811

REFERENCES

Boughman, J. W. (2002). How sensory drive can promote speciation. *Trends in Ecology and Evolution*, *17*(12), 571–577.

Bowmaker, J. K. (1995). Visual pigments of fishes. *Progress in Retinal and Eye Research*, *15*, 1–31.

Bowmaker, J. K., Govardovskii, V. I., Shukolyukov, S. A., Zueva, J. L. V., Hunt, D. M., Sideleva, V. G., & Smirnova, O. G. (1994). Visual pigments and the photic environment: The Cottoid fish of Lake Baikal. *Vision Research*, *34*(5), 591–605.

Bridges, C. D. B., & Yoshikami, S. (1970). The rhodopsin-porphyropsin system in freshwater fishes −1. Effects of age and photic environment. *Vision Research*, *10*(12), 1315-IN4.

Carleton, K. L., Dalton, B. E., Escobar-Camacho, D., & Nandamuri, S. P. (2016). Proximate and ultimate causes of variable visual sensitivities: Insights from cichlid fish radiations. *Genesis*, *54*(6), 299–325.

Carleton, K. L., Hárosi, F. I., & Kocher, T. D. (2000). Visual pigments of African cichlid fishes: Evidence for ultraviolet vision from microspectrophotometry and DNA sequences. *Vision Research*, *40*(8), 879–890.

Carleton, K. L., & Kocher, T. D. (2001). Cone opsin genes of African cichlid fishes: Tuning spectral sensitivity by differential gene expression. *Molecular Biology and Evolution*, *18*(8), 1540–1550.

Carleton, K. L., Parry, J. W., Bowmaker, J. K., Hunt, D. M., & Seehausen, O. (2005). Colour vision and speciation in Lake Victoria cichlids of the genus *Pundamilia*. *Molecular Ecology*, *14*(14), 4341–4353.

Carleton, K. L., Spady, T. C., Streelman, J. T., Kidd, M. R., McFarland, W. N., & Loew, E. R. (2008). Visual sensitivities tuned by heterochronic shifts in opsin gene expression. *BMC Biology*, *6*, 1–14.

Crawley, M. J. (2002). *Statistical computing: An introduction to data analysis using S-PLUS*. John Wiley & Son.

Ehlman, S. M., Sandkam, B. A., Breden, F., & Sih, A. (2015). Developmental plasticity in vision and behavior may help guppies overcome increased turbidity. *Journal of Comparative Physiology A*, *201*(12), 1125–1135.

Endler, J. A. (1991). Variation in the appearance of guppy color patterns to guppies and their predators under different visual conditions. *Vision Research*, *31*(3), 587–608.

Endler, J. A., & Basolo, A. L. (1998). Sensory ecology, receiver biases and sexual selection. *Trends in Ecology and Evolution*, *13*(10), 415–420.

Enright, J. M., Toomey, M. B., Sato, S. Y., Temple, S. E., Allen, J. R., Fujiwara, R., … Corbo, J. C. (2015). Cyp27c1 red-shifts the spectral sensitivity of photoreceptors by converting Vitamin A1 into A2. *Current Biology*, *25*(23), 3048–3057.

Escobar-Camacho, D., Pierotti, M. E., Ferenc, V., Sharpe, D. M., Ramos, E., Martins, C., & Carleton, K. L. (2019). Variable vision in variable environments: The visual system of an invasive cichlid (*Cichla monoculus*) in Lake Gatun, Panama. *Journal of Experimental Biology*, *222*(6), jeb188300.

Fernald, R. D. (1988). Aquatic adaptations in fish eyes. In *Sensory biology of aquatic animals* (pp. 435–466). New York, NY: Springer.

Fox, J., Adler, D., Bates, D., Baud-Bovy, G., Ellison, S., Firth, D., Friendly, M., Gorjanc, G. & Graves, S. (2017). Package 'car'.

Fusco, G., & Minelli, A. (2010). Phenotypic plasticity in development and evolution: Facts and concepts. *Philosophical Transactions: Biological Sciences*, *365*(1540), 547–556.

Govardovskii, V. I., Fyhrquist, N., Reuter, T., Kuzmin, D. G., & Donner, K. (2000). In search of the visual pigment template. *Visual Neuroscience*, *17*(4), 509–528.

Haesler, M. P., & Seehausen, O. (2005). Inheritance of female mating preference in a sympatric sibling species pair of Lake Victoria cichlids: Implications for speciation. *Proceedings of the Royal Society B: Biological Sciences*, *272*(1560), 237–245.

Halekoh, U., & Højsgaard, S. (2014). A kenward-roger approximation and parametric bootstrap methods for tests in linear mixed models–the R package pbkrtest. *Journal of Statistical Software*, *59*(9), 1–30.

Halstenberg, S., Lindgren, K. M., Samagh, S. P., Nadal-Vicens, M., Balt, S., & Fernald, R. D. (2005). Diurnal rhythm of cone opsin expression in the teleost fish *Haplochromis burtoni*. *Visual Neuroscience*, *22*(2), 135–141.

Härer, A., Karagic, N., Meyer, A., & Torres-Dowdall, J. (2019). Reverting ontogeny: Rapid phenotypic plasticity of colour vision in cichlid fish. *Royal Society Open Science*, *6*(7), 190841.

Härer, A., Meyer, A., & Torres-Dowdall, J. (2018). Convergent phenotypic evolution of the visual system via different molecular routes: How Neotropical cichlid fishes adapt to novel light environments. *Evolution Letters*, *2*(4), 341–354.

Hárosi, F. I. (1994). An analysis of two spectral properties of vertebrate visual pigments. *Vision Research*, *34*(11), 1359–1367.

Hofmann, C. M., O'Quin, K. E., Marshall, N. J., Cronin, T. W., Seehausen, O., & Carleton, K. L. (2009). The eyes have it: Regulatory and structural changes both underlie cichlid visual pigment diversity. *PLoS Biology*, *7*(12), e1000266.

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, *50*(3), 346–363.

Jin, Y. H., Park, J. W., Kim, J. H., & Kwon, J. Y. (2013). The expression pattern of melatonin receptor 1a gene during early life stages in the Nile tilapia (*Oreochromis niloticus*). *Development and Reproduction*, *17*(1), 45–53.

Kocher, T. D. (2004). Adaptive evolution and explosive speciation: The cichlid fish model. *Nature Reviews Genetics*, *5*(4), 288–298.

Kullander, S. O., Karlsson, M., Karlsson, M., & Noren, M. (2014). *Chalinochromis cyanophleps*, a new species of cichlid fish (Teleostei: Cichlidae) from Lake Tanganyika. *Zootaxa*, *3790*(3), 425–438.

Larmuseau, M. H., Raeymaekers, J. A., Ruddick, K. G., Van Houdt, J. K., & Volckaert, F. A. (2009). To see in different seas: Spatial variation in the rhodopsin gene of the sand goby (*Pomatoschistus minutus*). *Molecular Ecology*, *18*(20), 4227–4239.

Loew, E. R., & McFarland, W. N. (1990). The underwater visual environment. In *The visual system of fish* (pp. 1–43). Netherlands: Springer.

Lythgoe, J. N., Muntz, W. R. A., Partridge, J. C., Shand, J., & Williams, D. M. B. (1994). The ecology of the visual pigments of snappers (*Lutjanidae*) on the great barrier reef. *Journal of Comparative Physiology A*, *174*(4), 461–467.

Maan, M. E., Seehausen, O., & Groothuis, T. G. (2017). Differential survival between visual environments supports a role of divergent sensory drive in cichlid fish speciation. *The American Naturalist*, *189*(1), 78–85.

Marshall, N. J., & Vorobyev, M. (2003). The design of color signals and color vision in fishes. In *Sensory processing in aquatic environments* (pp. 194–222). New York, NY: Springer.

Meier, J. I., Marques, D. A., Wagner, C. E., Excoffier, L., & Seehausen, O. (2018). Genomics of parallel ecological speciation in Lake Victoria cichlids. *Molecular Biology and Evolution*, *35*(6), 1489–1506.

Meier, J. I., Sousa, V. C., Marques, D. A., Selz, O. M., Wagner, C. E., Excoffier, L., & Seehausen, O. (2017). Demographic modelling with whole-genome data reveals parallel origin of similar *Pundamilia* cichlid species after hybridization. *Molecular Ecology*, *26*(1), 123–141.

Morshedian, A., Toomey, M. B., Pollock, G. E., Frederiksen, R., Enright, J. M., McCormick, S. D., … Corbo, J. C. (2017). Cambrian origin of the CYP27C1-mediated vitamin A1-to-A2 switch, a key mechanism of vertebrate sensory plasticity. *Royal Society Open Science*, *4*(7), 170362.

Munz, F. W., & McFarland, W. N. (1977). Evolutionary adaptations of fishes to the photic environment. In *The visual system in vertebrates* (pp. 193–274). Berlin: Springer.

Nandamuri, S. P., Yourick, M. R., & Carleton, K. L. (2017). Adult plasticity in African cichlids: Rapid changes in opsin expression in response to environmental light differences. *Molecular Ecology*, *26*(21), 6036–6052.

Nyamweya, C. S., Natugonza, V., Taabu-Munyaho, A., Aura, C. M., Njiru, J. M., Ongore, C., … Kayanda, R. (2020). A century of drastic change: Human-induced changes of Lake Victoria fisheries and ecology. *Fisheries Research*, *230*, 105564.

Parry, J. W., Carleton, K. L., Spady, T., Carboo, A., Hunt, D. M., & Bowmaker, J. K. (2005). Mix and match color vision: Tuning spectral sensitivity by differential opsin gene expression in Lake Malawi cichlids. *Current Biology*, *15*(19), 1734–1739.

Parry, J. W. L., & Bowmaker, J. K. (2000). Visual pigment reconstitution in intact goldfish retina using synthetic retinaldehyde isomers. *Vision Research*, *40*(17), 2241–2247.

Partridge, J. C., Shand, J., Archer, S. N., Lythgoe, J. N., & van Groningen-Luyben, W. A. H. M. (1989). Interspecific variation in the visual pigments of deep-sea fishes. *Journal of Comparative Physiology A*, *164*(4), 513–529.

Price, T. D., Qvarnström, A., & Irwin, D. E. (2003). The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1523), 1433–1440.

Provencio, I., Loew, E. R., & Foster, R. G. (1992). Vitamin A2-based visual pigments in fully terrestrial vertebrates. *Vision Research*, *32*(12), 2201–2208.

Ramakers, C., Ruijter, J. M., Deprez, R. H. L., & Moorman, A. F. (2003). Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. *Neuroscience Letters*, *339*(1), 62–66.

Reuter, T. E., White, R. H., & Wald, G. (1971). Rhodopsin and porphyropsin fields in the adult bullfrog retina. *Journal of General Physiology*, *58*(4), 351–371.

Saarinen, P., Pahlberg, J., Herczeg, G., Viljanen, M., Karjalainen, M., Shikano, T., … Donner, K. (2012). Spectral tuning by selective chromophore uptake in rods and cones of eight populations of nine-spined stickleback (*Pungitius pungitius*). *Journal of Experimental Biology*, *215*(16), 2760–2773.

Schelly, R., Salzburger, W., Koblmüller, S., Duftner, N., & Sturmbauer, C. (2006). Phylogenetic relationships of the lamprologine cichlid genus *Lepidiolamprologus* (Teleostei: Perciformes) based on mitochondrial and nuclear sequences, suggesting introgressive hybridization. *Molecular Phylogenetics and Evolution*, *38*(2), 426–438.

Scheren, P. A. G. M., Zanting, H. A., & Lemmens, A. M. C. (2000). Estimation of water pollution sources in Lake Victoria, East Africa: Application and elaboration of the rapid assessment methodology. *Journal of Environmental Management*, *58*(4), 235–248.

Seehausen, O. (1996). *Lake Victoria rock cichlids: Taxonomy, ecology, and distribution*. Lauenau, Germany: Verduyn Cichlids.

Seehausen, O., Lippitsch, E., Bouton, N., & Heleen, Z. (1998). Mbipi, the rock-dwelling cichlids of Lake Victoria: Description of three new genera and fifteen new species (Teleostei). *Ichthyological Exploration of Freshwaters*, *9*, 129–228.

Seehausen, O., Terai, Y., Magalhaes, I. S., Carleton, K. L., Mrosso, H. D., Miyagi, R., … Imai, H. (2008). Speciation through sensory drive in cichlid fish. *Nature*, *455*(7213), 620–626.

Seehausen, O., & Van Alphen, J. J. M. (1997). The effect of male coloration on female mate choice in closely related Lake Victoria cichlids (*Haplochromis Nyererei* complex). *Behavioral Ecology and Sociobiology*, *42*(1), 1–8.

Selz, O. M., Pierotti, M. E., Maan, M. E., Schmid, C., & Seehausen, O. (2014). Female preference for male color is necessary and sufficient for assortative mating in 2 cichlid sister species. *Behavioral Ecology*, *25*(3), 612–626.

Shand, J., Davies, W. L., Thomas, N., Balmer, L., Cowing, J. A., Pointer, M., … Hunt, D. M. (2008). The influence of ontogeny and light environment on the expression of visual pigment opsins in the retina of the black bream, *Acanthopagrus butcheri*. *Journal of Experimental Biology*, *211*(9), 1495–1503.

Spady, T. C., Seehausen, O., Loew, E. R., Jordan, R. C., Kocher, T. D., & Carleton, K. L. (2005). Adaptive molecular evolution in the opsin genes of rapidly speciating cichlid species. *Molecular Biology and Evolution*, *22*(6), 1412–1422.

Stelkens, R. B., Pierotti, M. E., Joyce, D. A., Smith, A. M., van der Sluijs, I., & Seehausen, O. (2008). Disruptive sexual selection on male nuptial coloration in an experimental hybrid population of cichlid fish. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1505), 2861–2870.

Sugawara, T., Terai, Y., Imai, H., Turner, G. F., Koblmüller, S., Sturmbauer, C., … Okada, N. (2005). Parallelism of amino acid changes at the RH1 affecting spectral sensitivity among deep-water cichlids from Lakes Tanganyika and Malawi. *Proceedings of the National Academy of Sciences*, *102*(15), 5448–5453.

Suzuki, T., Makino-Tasaka, M., & Eguchi, E. (1984). 3-Dehydroretinal (vitamin A2 aldehyde) in crayfish eye. *Vision Research*, *24*(8), 783–787.

Temple, S. E., Plate, E. M., Ramsden, S., Haimberger, T. J., Roth, W. M., & Hawryshyn, C. W. (2006). Seasonal cycle in vitamin A 1/A 2-based visual pigment composition during the life history of coho salmon (*Oncorhynchus kisutch*). *Journal of Comparative Physiology A*, *192*(3), 301–313.

Terai, Y., Miyagi, R., Aibara, M., Mizoiri, S., Imai, H., Okitsu, T., … Mrosso, H. D. (2017). Visual adaptation in Lake Victoria cichlid fishes: Depth-related variation of color and scotopic opsins in species from sand/mud bottoms. *BMC Evolutionary Biology*, *17*(1), 200.

Terai, Y., Seehausen, O., Sasaki, T., Takahashi, K., & Mizoiri, S. (2006). Divergent selection on opsins drives incipient speciation in Lake Victoria cichlids. *PLoS Biology*, *4*(12), 433.

Torres-Dowdall, J., Pierotti, M. E., Härer, A., Karagic, N., Woltering, J. M., Henning, F., … Meyer, A. (2017). Rapid and parallel adaptive evolution of the visual system of Neotropical Midas cichlid fishes. *Molecular Biology and Evolution*, *34*(10), 2469–2485.

Toyama, M., Hironaka, M., Yamahama, Y., Horiguchi, H., Tsukada, O., Uto, N., … Hariyama, T. (2008). Presence of rhodopsin and porphyropsin in the eyes of 164 fishes, representing marine, diadromous, coastal and freshwater species: A qualitative and comparative study. *Photochemistry and Photobiology*, *84*(4), 996–1002.

Van der Meer, H. J., & Bowmaker, J. K. (1995). Interspecific variation of photoreceptors in four co-existing haplochromine cichlid fishes. *Brain, Behavior and Evolution*, *45*(4), 232–240.

West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*. New York, NY: Oxford University Press.

Witte-Maas, E. L. M., & Witte, F. (1985). *Haplochromis nyererei, a new cichlid fish from Lake Victoria named in honour of Mwalimu Nyerere President of Tanzania: 1-13—Private publication* (p. 47). Witte & Van Ojen: Lake Victoria Haplochromine Trophic Groups.

Wright, D. S., Meijer, R., van Eijk, R., Vos, W., Seehausen, O., & Maan, M. E. (2019). Geographic variation in opsin expression does not align with opsin genotype in Lake Victoria cichlid populations. *Ecology and Evolution*, *9*(15), 8676–8689.

Wright, D. S., van Eijk, R., Schuart, L., Seehausen, O., Groothuis, T. G., & Maan, M. E. (2020). Testing sensory drive speciation in cichlid fish: Linking light conditions to opsin expression, opsin genotype and female mate preference. *Journal of Evolutionary Biology*, *33*(4), 422–434.

Xie, F., Xiao, P., Chen, D., Xu, L., & Zhang, B. (2012). miRDeepFinder: A miRNA analysis tool for deep sequencing of plant small RNAs. *Plant Molecular Biology*, *80*, 75–84.

Yourick, M. R., Sandkam, B. A., Gammerdinger, W. J., Escobar-Camacho, D., Nandamuri, S. P., Clark, F. E., … Carleton, K. L. (2019). Diurnal variation in opsin expression and common housekeeping genes necessitates comprehensive normalization methods for quantitative real-time PCR analyses. *Molecular Ecology Resources*, *19*(6), 447–1460.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Wilwert, E., Etienne, R. S., van de Zande, L., & Maan, M. E. (2022). Contribution of opsins and chromophores to cone pigment variation across populations of Lake Victoria cichlids. *Journal of Fish Biology*, *101*(2), 365–377. https://doi.org/10.1111/jfb.14969