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Robust Sensory Traits Across Light Habitats: Visual Signals but Not Receptors Vary in Centrarchids Inhabiting Distinct Photic Environments

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ABSTRACT

Visual communication in fish is often shaped by their light environment, which influences both sensory (e.g., eye size, opsin gene expression) and signalling traits (e.g., body reflectance). This study explores the phenotypic variation in the visual communication traits of six species of centrarchids (Centrarchidae) inhabiting two contrasting light environments. We measured morphological, molecular and signalling traits to determine their variation across photic conditions. Our findings reveal significant interspecific variation in sensory traits but no consistent phenotypic variation between light environments. Centrarchids showed robust visual systems with green-sensitive *rh2* and red-sensitive *lws* opsin genes representing the main chromatic channels, with their expression remaining largely unaffected between distinct light habitats. We also found significant molecular evolution in the visual opsin genes, although these changes were not associated with environmental conditions. However, body reflectance displayed species-specific responses to environmental conditions, suggesting that signalling traits may be more flexible than sensory traits. Overall, our results challenge the generality of the current paradigm in visual ecology, which portrays visual systems in fish as highly tunable owing to photic conditions. Our study highlights the potential evolutionary or developmental constraints on centrarchid visual systems and their implications for adaptability to various habitats and novel environmental threats.

1 | Introduction

The vast biodiversity in nature prompts questions about why some organisms diversify, while others do not, and which traits are more likely to evolve (Wilson 1992; Schluter 2000). Traits that exhibit substantial heritable phenotypic variation and contribute to reproductive success may evolve via natural selection in response to selective pressures (Fisher 1930; Endler 1986). Habitat conditions create local selection regimes that shape phenotypic distributions between populations (Williams 1966). However, identifying the selective forces behind phenotypic divergence and linking traits to fitness remain a challenge (Whitlock 2015; Wadgymar et al. 2022). Therefore, correlational and comparative studies are often used to make inferences regarding the adaptive value of biological traits (Losos 2011). Similar environmental pressures may result in common phenotypic responses, revealing the traits under selection (Rosenblum et al. 2014). Hence, comparing trait variation across lineages and environments helps determine whether environmental pressures result in patterns of phenotypic variation that are shared among species (i.e., convergent) or that vary in a species-specific manner (i.e., divergent) (Gould 1989). Traits tightly linked to

their habitat are a priori more likely to exhibit strong phenotype–environment interactions. For instance, sensory systems represent organismal traits, the main purpose of which is to perceive signals from the environment, and thus have been widely studied across taxa and habitats to uncover common drivers of sensory diversity and convergence (Baldwin and Ko 2020; Baden 2024).

Organisms inevitably must extract information from their environment to survive; thus, sensory systems tend to be under strong selective pressures (Lythgoe 1979; Oteiza and Baldwin 2021). Not only are sensory systems receivers (e.g., visual sensitivity), but visual signals (e.g., body coloration) are also shaped by environmental conditions and determine the ability of organisms to send reliable cues and communicate in their habitat (Endler 1992; Price 2017). This interplay between signals, receivers and the environment is fundamental for understanding sensory ecology and its implications for the performance of organisms. Particularly in aquatic habitats, visual systems are under strong pressure due to the interactions of sunlight with water and dissolved matter, which generate marked differences in visibility (Kirk 1985; Loew and McFarland 1990). Light in aquatic habitats is attenuated exponentially with depth and is depleted of certain wavelengths, thus narrowing the spectral range available for visual systems and signals (Lythgoe 1975; McFarland and Munz 1975b; Fuller and Endler 2018). Therefore, organisms might need to modulate their visual systems, signalling cues or both along the extensive range of photic conditions they inhabit (Endler 1992; Cummings and Endler 2018). However, measuring all components, that is, signals, receivers and the environment, remains challenging and is usually not accomplished simultaneously (Cummings 2007; Seehausen et al. 2008).

The role of photic conditions as drivers of phenotypic variation in fish visual traits has been extensively studied at morphological, physiological and molecular levels (Bowmaker 1990; Partridge and Cummings 1999; Carleton et al. 2020). Increased eye size has been associated with improved ability to resolve visual details (i.e., spatial acuity), likely as an adaptation to complex habitats (Caves et al. 2017). However, bigger eyes might also be associated with increased sensitivity, that is, larger retinal area for photon catch, enhancing vision in low light conditions (Land 1990; Fritsches et al. 2003). Lens properties might also differ across environments and species, leading to certain wavelengths being filtered out before reaching the retina (Kröger et al. 2001; Hofmann et al. 2010; Torres-Dowdall et al. 2017). The probability of certain wavelengths initiating phototransduction in the retina (i.e., spectral sensitivity) also differs among habitats and species (Partridge and Cummings 1999; Douglas and Djamgoz 2012; Carleton et al. 2016). Spectral sensitivity is primarily mediated by visual pigments, light-sensitive macromolecules composed of an opsin protein and a chromophore (Wald 1939; Dowling 1987). Opsin proteins are commonly found in photoreceptor cells, either cone or rod cells used primarily for colour and dim light vision, respectively (Walls 1942).

Five major visual opsin gene families encode opsin proteins with distinct absorbance properties that confer light sensitivity over spectral regions: rod opsin *rh1* (dim-light) and cone opsins: *sws1* (ultraviolet), *sws2* (blue), *rh2* (green) and *lws* (red) (Yokoyama 2008). Additionally, opsin proteins might be combined with two chromophore types, either vitamin A_1-derived or A_2-derived, that further modulate spectral sensitivity (Corbo 2021). Hence, spectral sensitivity in teleost fish is commonly modulated via the differential expression of opsin genes, structural opsin protein modifications and alternative chromophore usage (Carleton et al. 2020). Opsin gene expression and chromophore switching enable flexible fine-tuning of spectral sensitivity, whereas amino acid substitutions permanently alter the light absorbance of the opsin protein. Complementarily, visual signals in fish also differ in response to ecological factors such as turbidity, depth, or predator presence (Reimchen 1989; Cummings 2007; Seehausen et al. 2008; Schneider et al. 2020). However, most studies assessing the phenotypic variation in fish visual traits have focused on individual traits, for example, measuring only opsin gene expression within a single species. A more comprehensive approach incorporating multiple measurements is needed to extrapolate the sensory ecology from a single species to ecological communities and draw inferences about convergent patterns of visual adaptations.

Centrarchids (Centrarchidae) comprise approximately 40 species of freshwater fish native to North America that have been underexplored in terms of visual ecology despite their ecological significance (Cooke and Philipp 2009; Near and Kim 2021). Centrarchid fish encompass key predators and prey in freshwater ecosystems, which are vital in structuring aquatic communities and energy transfer (Carpenter et al. 1985). Additionally, centrarchids hold substantial socioeconomic importance as popular targets in recreational fisheries, contributing significantly to local economies through angling-related activities (Arlinghaus and Cooke 2009). The centrarchid adaptive radiation shows extensive morphological variation linked to diverse ecological roles (Werner 1977; Wainwright and Richard 1995). Their broad distribution and distinct feeding ecologies (e.g., planktivory, piscivory) allow the assessment of how photic environments, foraging and conspecific signalling shape visual ecology. To date, electrophysiological studies have identified only two cone opsin visual pigments, green- and red-sensitive, and one dim-light rhodopsin in centrarchids (Bridges 1965; Hawryshyn et al. 1988). Behavioural data supported their reduced sensitivity to blue hues, and visual modelling has suggested that brightness, rather than colour, is relevant for prey detection (Mitchem et al. 2019; Wale et al. 2021). Given the current pace of human-driven impacts on water clarity (Solomon et al. 2015; Bunnell et al. 2021), it is imperative to better understand the visual ecology of centrarchids. Compounds such as dissolved organic carbon (DOC) and turbidity reduce light in aquatic environments, impairing foraging in species such as bluegill, *Lepomis macrochirus* (Miner and Stein 1993; Weidel et al. 2017). Bluegill in high-DOC lakes also exhibit slower growth and smaller maximum sizes (Craig et al. 2017), suggesting that DOC exerts strong selective pressures. However, the extent and mechanisms of phenotypic variation in visual communication traits of centrarchids remain largely unexplored.

Our study aims to characterise the phenotypic variation of visual communication traits in six centrarchid species inhabiting two contrasting light environments. We use underwater light measurements, retinal transcriptomics, eye morphology and spectral reflectance data to assess the effects of environment

and species identity on eye size, opsin gene expression, chromophore usage and body reflectance. Furthermore, we test for signatures of molecular evolution on the amino acid sequence of visual opsin genes across species and populations. By combining data on the visual ecology of different species from two distinct light environments, we ask: (i) Do the visual traits of centrarchids vary across photic environments? and (ii) Are phenotypic responses to light conditions shared among species or are they species specific? We predict that the dim, long-wavelength-shifted light environment of the high-DOC lake will favour visual phenotypes and body reflectance shifted towards longer wavelengths, compared to fish from the clear water lake (Cronin et al. 2014; Schneider et al. 2020). We found robust visual systems across species (no environmental effect), but variable body reflectance across species and environments (species-by-environment interaction), suggesting that divergent species-specific responses in signalling might help offset the lack of fine-tuning in the visual system of centrarchids.

2 | Materials and Methods

2.1 | Study Design

Fish were collected during June–July 2022 using fyke nets or rod and reel angling from two temperate lakes located in northern Wisconsin, USA (Table S1). All fish were captured at a depth ranging 1–5 m between 8 am and 12 pm. The two lakes are isolated from each other by approximately 45 km and have never been stocked with any of the study species (see Fish Stocking Database, Wisconsin DNR, apps.dnr.wi.gov/fisheriesmanagement/Public/Summary/Index). Additionally, the two study lakes differ in their dissolved organic matter concentrations, leading to DOC-mediated water colour differences that are not erased by winter freezes (Hampton et al. 2017; Solomon et al. 2022). Given the last glaciation period, the lakes were estimated to be less than 12,000 years old (Peterson 1986). A total of 66 specimens from six species of Centrarchidae were collected from either a clear water lake, Big Arbor Vitae (BAV; 45.924001, −89.639012), or a dark water lake, Cranberry Lake (CY; 45.887512, −89.169695). The following species were collected from each site: insectivores such as black crappie, *Pomoxis nigromaculatus* (BAV, $n = 4$; CY, $n = 3$); piscivorous/crayfish predators such as rock bass, *Ambloplites rupestris* (BAV, $n = 7$; CY, $n = 7$), largemouth bass, *Micropterus nigricans* (BAV, $n = 5$; CY, $n = 3$) and smallmouth bass, *Micropterus dolomieu* (BAV, $n = 1$; CY, $n = 1$); zooplanktivores such as bluegill sunfish, *L. macrochirus* (BAV, $n = 9$; CY, $n = 9$); and molluscivores such as pumpkinseed, *Lepomis gibbosus* (BAV, $n = 10$; CY, $n = 7$). All these centrarchid species represent diurnal fish with distinct feeding ecologies and microhabitat preferences (Cooke and Philipp 2009). Fish were then transported under dark conditions to the University of Notre Dame Environmental Research Center (< 2 h). Body reflectance measurements were taken from each fish under dark conditions (see *Signalling Traits: Body Reflectance*). The fish were then anaesthetised by submersion in 0.04% MS-222 solution and euthanised by cervical dislocation. Their eyes were enucleated, and dissected retinas were stored in RNAlater (Sigma-Aldrich) at −80°C until RNA extraction. Then, photographs were taken of each fish on a measuring board. The eye lenses from a subset of individuals were carefully removed and stored in phosphate-buffered saline (PBS) solution at room temperature prior to lens transmission measurements (< 20 min, see *Sensory Traits: Eye Morphology and Lens Transmittance*). Fish were collected under a scientific collector's permit from Wisconsin DNR (SCP-FM-2022-068). All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Notre Dame (IACUC protocol number: 22-08-7369).

2.2 | Underwater Irradiance Measurements

To characterise the ambient photic conditions experienced by centrarchids, we measured the underwater absolute irradiance in their native habitats during the same period when fish were caught under clear skies (late June–July 2002, Table S1). Absolute irradiance measurements were obtained from the main illumination fields available underwater, either light from above, that is, downwelling, or from the side, that is, sidewelling (Cummings 2007; Dalton et al. 2010; Sabbah et al. 2011). All measurements were taken from a boat at the deepest point of each lake between 11 am and 3 pm using a FLAME-S-XR1-ES spectrometer connected to a full-spectrum UV–VIS optical fibre with a cosine corrector (Ocean Insight, USA). Three replicate measurements were taken at 0.5-m intervals from the water surface to the bottom of the lake (BAV = 10 m, CY = 6 m), including one measurement above the water surface and another 0.15 m below. However, the measurement at 5.5 m in the dark lake (CY) was removed due to a lack of reliable signal. The median absolute irradiance (photons/cm^2/s/nm) at each depth was used as the reference spectrum (Bertinetti, Härer et al. 2024). The spectral range between 350 and 700 nm was used to compute photic variables to reduce noise at the extremes of the visible spectrum and in agreement with the relevant wavelengths for fish colour vision in freshwater habitats (Rennison et al. 2016; Carleton et al. 2020). Normalised irradiance, that is, the absolute spectra divided by their respective maximum value, was used to calculate the photon distribution of each spectrum by estimating the spectrum-halving wavelength (λP50) (McFarland and Munz 1975a; Loew and McFarland 1990).

2.3 | Sensory Traits: Eye Morphology and Lens Transmittance

We measured eye morphology and lens transmittance to estimate differences in phenotypic traits associated with the visual system. Eye size in teleost fish might be associated with higher sensitivity in low-light environments or with higher visual acuity (Land 1990; Fritsches et al. 2003; Caves et al. 2017). To quantify differences in eye size among species and light conditions, we measured eye width (mm) and lens diameter (mm) from 51 photographed fish containing a scale bar using ImageJ (Table S1; Schneider et al. 2012). Eye width and lens diameter were divided by the standard length to compare relative values. Additionally, to assess the spectral range available for centrarchids after lens filtering, we measured

lens transmittance from 1 to 3 individuals from each species at each site ($n = 28$, Table S1). A PX-2 pulsed xenon lamp connected to a FLAME-S-XR1-ES spectrometer equipped with a QR400-7-SR probe (Ocean Insights, USA) was used to measure lens transmittance. Transmission spectra were used to calculate T50, which is the wavelength at which the transmission curve shows the steepest slope between 300 and 700 nm (Torres-Dowdall et al. 2017; Schneider et al. 2020). To test the effects of species, environment, and their interaction on visual traits, we performed ANOVA (Type III) using either (i) lens diameter, (ii) relative eye width or (iii) lens transmittance as a response variable. Because the interaction was not significant, we removed the interaction term and performed a two-way ANOVA (Type II). When significant, differences among groups were assessed using a false discovery rate post hoc test in R (R Core Team 2020).

2.4 | Sensory Traits: Library Preparation and Gene Expression Analysis

Total RNA was extracted from one retina of each fish using standard guanidinium thiocyanate-phenol-chloroform extraction, as described by Bertinetti, Meyer, et al. (2024). The RNA yield and integrity were assessed using a Qubit 4 fluorometer (RNA IQ and RNA HS Assays, Thermo Fischer Scientific, NH, USA). RNA (200 ng) was used for poly(A) selection, and the resulting mRNA was used to generate RNA-Seq libraries using the CORALL RNA-Seq V2 Library Prep Kit (Lexogen, Austria) following the manufacturer's guidelines. Library fragment sizes and concentrations were quantified using the Bioanalyzer 2100 (Agilent, CA, USA). A total of 48 samples were pooled at equimolar ratios and paired-end sequenced on a DNBSEQ 400 platform (PE150; Innomics Inc., CA, USA). Raw data are deposited in the NCBI SRA database under accession number PRJNA1168051. Sequencing adapters, low-quality regions ($Q < 10$), and reads shorter than 20 bp after trimming were filtered from the raw reads using BBDuk (Bushnell et al. 2017). The quality of the remaining reads was assessed using FastQC (Andrews 2010). An average of 11.9 M reads (4.2–57 M reads) with a mean length of 135 bp (125–146 bp) was obtained from each individual. High-quality sequences were mapped to the publicly available genome assembly of bluegill sunfish GCA_024633595.2, using STAR v2.7.3a (Dobin et al. 2012; Ludt et al. 2023). To annotate the genome, we combined ab initio prediction of gene models with Augustus (Stanke et al. 2006), protein evidence from zebrafish (*Danio rerio*) and high-quality reads from bluegill retinal tissue generated in this study following the default pipeline implemented in Funannotate v1.8.16 (Palmer and Stajich 2020). Additionally, the annotation was manually curated using the genomes of largemouth bass (GCF_014851395.1) and smallmouth bass (GCF_021292245.1) to ensure that all visual opsin genes and *cyp27c1*, the main enzyme involved in chromophores usage, were included. Gene counts were normalised using the trimmed mean of M-values (TMM) in *the edgeR* package (Chen et al. 2016). For differential gene expression (DEG) analysis, only genes with a minimum of 100 counts per million in at least one-third of the samples were retained. DEG contrasts and statistics were computed using the constrasts.fit and eBAyes functions in the *limma* package (Ritchie et al. 2015). Proportional opsin gene expression was rank-transformed to achieve normal distribution. Relative gene expression was estimated using the average expression of the two reference genes, *G3PDH* and *EF1-α* (Yourick et al. 2019). To estimate photoreceptor type ratios, rod and cone cells, we used the expression of the cell-specific phototransduction marker genes *gnat1* and *gnat2*, respectively (Ogawa and Corbo 2021). The predicted sensitivity index (PSI), a proxy for visual sensitivity, was computed based on Carleton et al. (2016). The effects of species and environment were tested using ANOVA (Type III) followed by a false discovery rate post hoc test in R (R Core Team 2020).

2.5 | Sensory Traits: Opsin Sequence Analysis

To estimate the role of sequence divergence in the phenotypic variation of visual opsin genes in centrarchids, we extracted coding sequences (CDS) of all visual opsin genes and compared variable sites between species and within populations of the same species. For this, we performed variant calling from RNA-Seq aligned reads following GATK best practices (van der Auwera and O'Connor 2020). Duplicated reads were marked using Picard Tools v3.2 (broadinstitute.github.io/picard) and reformatted as DNA alignments using SplitNCigarReads in GATK v4.6. Haplotype-based variant calling was then performed using standard filters in FreeBayes v1.3.8 (Garrison and Marth 2012). Only variants supported by five or more reads (read depth > 4) were used to generate individual consensus sequences using bcftools (Li 2011). Additionally, to test the signatures of molecular evolution in the visual opsin genes of centrarchids, we used random site models to compare protein divergence (dN/dS) and identify positively selected sites among visual opsin genes using phylogenetic analysis by maximum likelihood (PAML) implemented in EasyCodeML (Yang 2007; Gao et al. 2019). Protein sequence alignments are provided in the data repository (Bertinetti 2024).

2.6 | Signalling Traits: Body Reflectance

The setup used to measure lens transmittance was modified to measure body reflectance (see *Sensory Traits: Eye Morphology and Lens Transmittance*). The reflectance probe was placed at an angle of 45° on the probe holder and held against the surface of the fish body. A WS-1 diffuse standard was used to calibrate reflectance measurements. Reflectance from five points along the body axis was collected from each fish, and covariance-based principal component analysis (PCA) was used for dimensionality reduction ($n = 67$, see Table S2 and Figure S1). Based on PCA unsupervised clustering, we extracted the distances of each individual to the centre of its respective group (centroid distance) and used it as a measure of intra-population variation within the reflectance space represented by PC1–2 (~89% of the variance). Smallmouth bass observations were excluded from this analysis due to sample size constraints for group cluster estimation. To test the effects of species, environment and their interaction on body reflectance, we performed an ANOVA (Type III) using either (i) log-transformed body reflectance (PC1) or (ii) log-transformed centroid distance of each individual as a response variable.

When significant, we measured the differences among groups using a false discovery rate post hoc test (R Core Team 2020).

3 | Results

3.1 | Centrarchids Inhabit Widely Different Photic Environments

Underwater spectral measurements within a range of depths, that is, 0.15–5.5 m, found in both lakes revealed strong differences in their photic environments (Figure 1). The overall underwater spectra were short-wavelength-shifted in the clear lake, with the spectrum-halving wavelength (λP50) averaging 582 ± 1.28 nm compared to 640 ± 1.28 nm in the dark lake (mean ± SE). Furthermore, λP50 in the clear lake shifted towards shorter wavelengths as depth increased, whereas dissolved particles in the dark lake filtered out short wavelengths, leading to red-shifted conditions with increasing depth. The photic differences in spectral composition and brightness between the lakes were maintained throughout the water column and independent of sensor orientation (Figures S2–S4). Overall, light abundance was consistently lower at any given depth in the dark lake than in the clear lake, with the dark lake containing only 21% of the photons at 1 m depth and less than 10% below 2.5 m depth relative to the clear lake (Figure 1b and Figure S3).

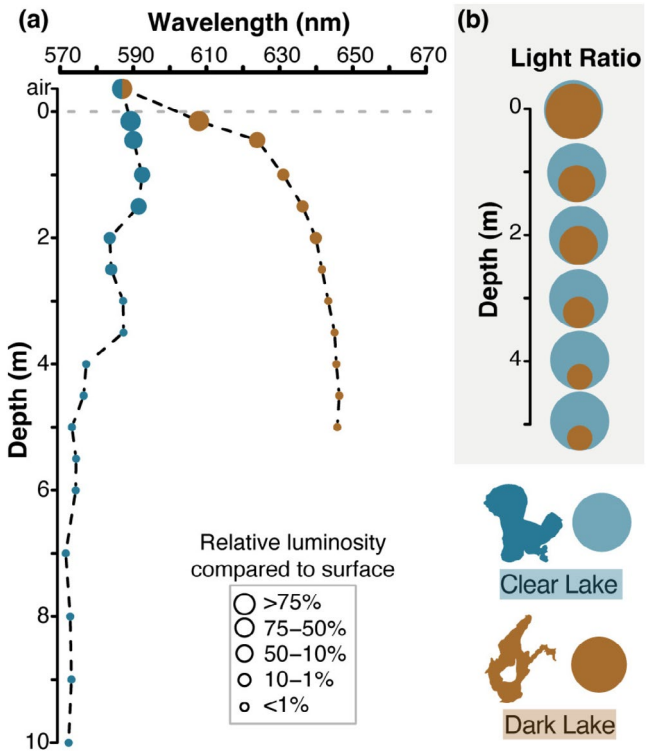

FIGURE 1 | Study lakes inhabited by centrarchids show strong differences in photic conditions. The photic environment in the dark lake shifted towards longer wavelengths and became dimmer and darker with increasing depth compared to the clear lake (dark lake maximum depth = 6 m) (a) Depth profile showing the spectrum-halving wavelength (λP50) from the surface to bottom. (b) Circular area showing the proportion of photons present at each depth.

3.2 | Eye Morphology but Not Lens Transmittance Differs Across Species

We found no differences in eye morphology between lakes; however, there were differences across species. Morphological estimates of visual traits, that is, eye width and lens diameter, were significantly affected by species identity (Figure 2a,b). However, these traits were not significantly affected by the photic environment (Figure 2a,b). There was no significant interaction between species and the photic environment (eye width: $F_{5,37} = 1.56$, $p = 0.19$; lens diameter: $F_{5,37} = 1.5$, $p = 0.21$). The overall results observed in visual trait morphology were maintained when normalising traits by fish standard length (Figure S5). Lens transmittance (T50) did not vary significantly across species or environments, averaging 402 ± 0.33 nm (Figure 2c and Figure S1).

3.3 | Opsin Gene and cyp27c1 Expression Vary Across Species but Not Between Light Habitats

Visual pigment usage, as determined by the expression of opsin genes and *cyp27c1*, did not vary across lakes, although there was variation across species. We compared the expression of cone opsins to rhodopsin and the cell-specific marker genes for photoreceptor types (*gnat1, gnat2*) as a proxy for photoreceptor tissue and cone–rod abundance ratios. Rod opsin gene *rh1* was the most expressed visual opsin gene in the retina, ranging 83%–98% relative to cone opsins, and cell-marker genes revealed rod-predominant retinas ranging 60%–94% of *gnat1* expression relative to *gnat2* (Figure 3a and Figure S6). Both proportional rhodopsin expression and *gnat1/gnat2* ratio did vary significantly among species but not across environments. A similar pattern was observed for proportional cone opsin gene expression, which varied significantly across species but not between light environments (Figure 3b and Figures S7, S8). Estimates of visual sensitivity based on the predicted sensitivity index (PSI) also varied significantly across species but not across environments (Figure 3b). The expression of *cyp27c1*, a gene linked to chromophore switching in fish, was also expressed in a species-specific manner $F_{5,41} = 4.72$, $p = 0.002$, but was not significantly affected by the light environment (Figure 3c). There were no significant interactions between light environment and species identity affecting the expression of vision genes (*gnat1/gnat2*: $F_{5,36} = 1.32$, $p = 0.27$; cone opsin predicted visual sensitivity (PSI): $F_{5,36} = 1.36$, $p = 0.26$; *cyp27c1*: $F_{5,36} = 1.89$, $p = 0.12$). The species-specific gene expression patterns that remained unaffected by the photic environment were also observed for relative gene expression compared to reference genes (Figures S9 and S10). Differential gene expression analysis of the bulk retinal transcriptome revealed that only two species, pumpkinseed and largemouth bass, exhibited differentially expressed genes between photic environments (Figure S11). These genes constituted a small percentage of the total expressed genes, specifically 0.1% for pumpkinseed and 2.6% for largemouth bass. Furthermore, none of the differentially expressed genes were shared between the two species in response to photic environments (Figure S11). Finally, applying the same PCA approach used for body reflectance to analyse cone opsin gene expression did not affect the results, log opsin gene expression PC1: $F_{5,36} = 1.7$, $p = 0.13$; log centroid distance: $F_{4,36} = 0.74$, $p = 0.56$

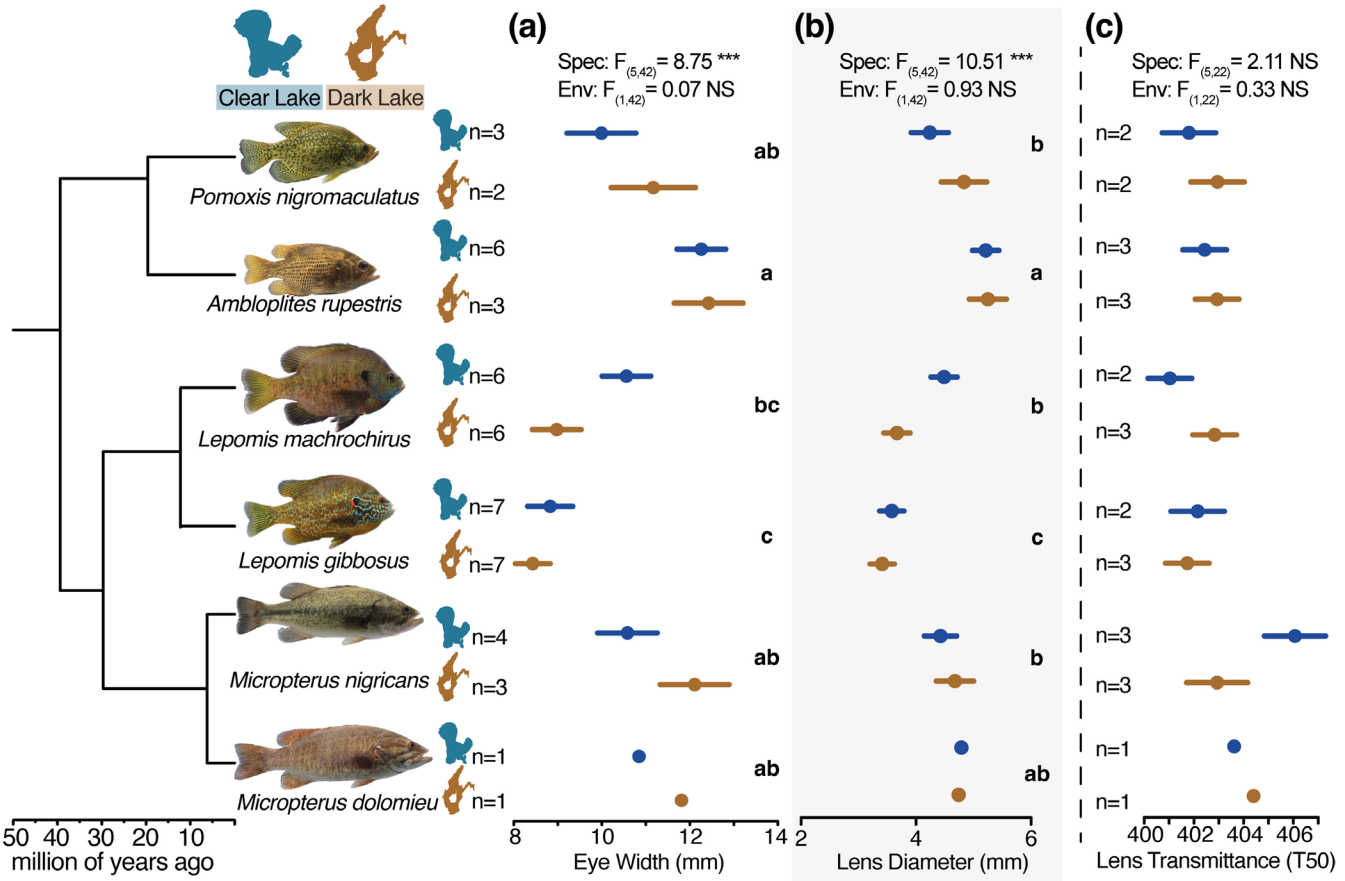

FIGURE 2 | Morphological variation of visual traits: (a) eye width; (b) lens diameter; and (c) lens transmittance, in six species of centrarchids inhabiting distinct photic environments. Dots and horizontal bars represent mean ± SE. The numbers displayed next to each species correspond to the sample size for each lake. Model estimates from ANOVA (Type II) are shown above each trait. Letters display groups based on a post hoc least-significance difference test using the false discovery rate. Phylogenetic tree based on Near and Kim (2021). *** and NS denote $p < 0.001$ and $p > 0.05$, respectively.

(Figures S12, S13 and S14). However, intra-specific variation in opsin gene expression represented by PC1–2 (90% of the variance) was increased in the clear water, broad-spectrum photic environment (Figure S14).

3.4 | Signatures of Molecular Evolution in Visual Opsin Genes

A comparison of the amino acid residues of the four cone opsin genes and rhodopsin (*rh1*) revealed that 29% of the amino acid residues were variable in at least one species (Tables S3–S8). No sites diverged between the environments, although some populations exhibited polymorphic sites. Furthermore, tests of molecular evolution using random site models showed evidence of positively selected sites in *rh1*, *rh2_1* and *lws* (Table S4). Residues 39 and 304 in *rh1*, 27, 214 and 309 in *rh2_1*, and 115 and 166 in *lws* (numbering based on bovine rhodopsin) were identified as having a high posterior probability (≥90% Bayes Empirical Bayes, BEB) of evolving under positive selection. All sites were found in the transmembrane domains of the opsin protein, except for site 27, which was located at the N-terminus. Additionally, site 163 in *lws*, which had an 89.3% posterior probability of evolving under positive selection, was the only site found to be directed into the binding pocket of the chromophore (Carleton et al. 2005). However, no functional implications could be found for the candidate sites (Frazer et al. 2024). None of the sites were polymorphic within species but were divergent across species (Tables S3–S8).

3.5 | Centrarchids Exhibit Divergent Body Reflectance Patterns Between Photic Habitats

A significant interaction between species and the photic environment was observed for log-transformed body reflectance (PC1 = 79% of the variance; Figures S1, S15 and S16), with largemouth bass and bluegill showing significantly divergent responses to ambient light conditions (Figure 4a; $F_{4,55} = 3.8$, $p = 0.008$). For instance, dark lake bluegill reflect more long-wavelength light, that is, brownish appearance, in their lower body part compared to clear lake fish, likely increasing background matching (body points 1 and 3; Figures S1, S15 and S16). In contrast, largemouth bass in dark lakes show increased short-wavelength reflectance for those body points, potentially increasing background contrast. The sex of the individuals did not significantly affect body reflectance and was, therefore, excluded from the analysis (log body reflectance: $F_{3,42} = 1.18$, $p = 0.32$; log centroid distance: $F_{3,42} = 0.03$, $p = 0.99$). Further, intra-population variation in body reflectance estimated as

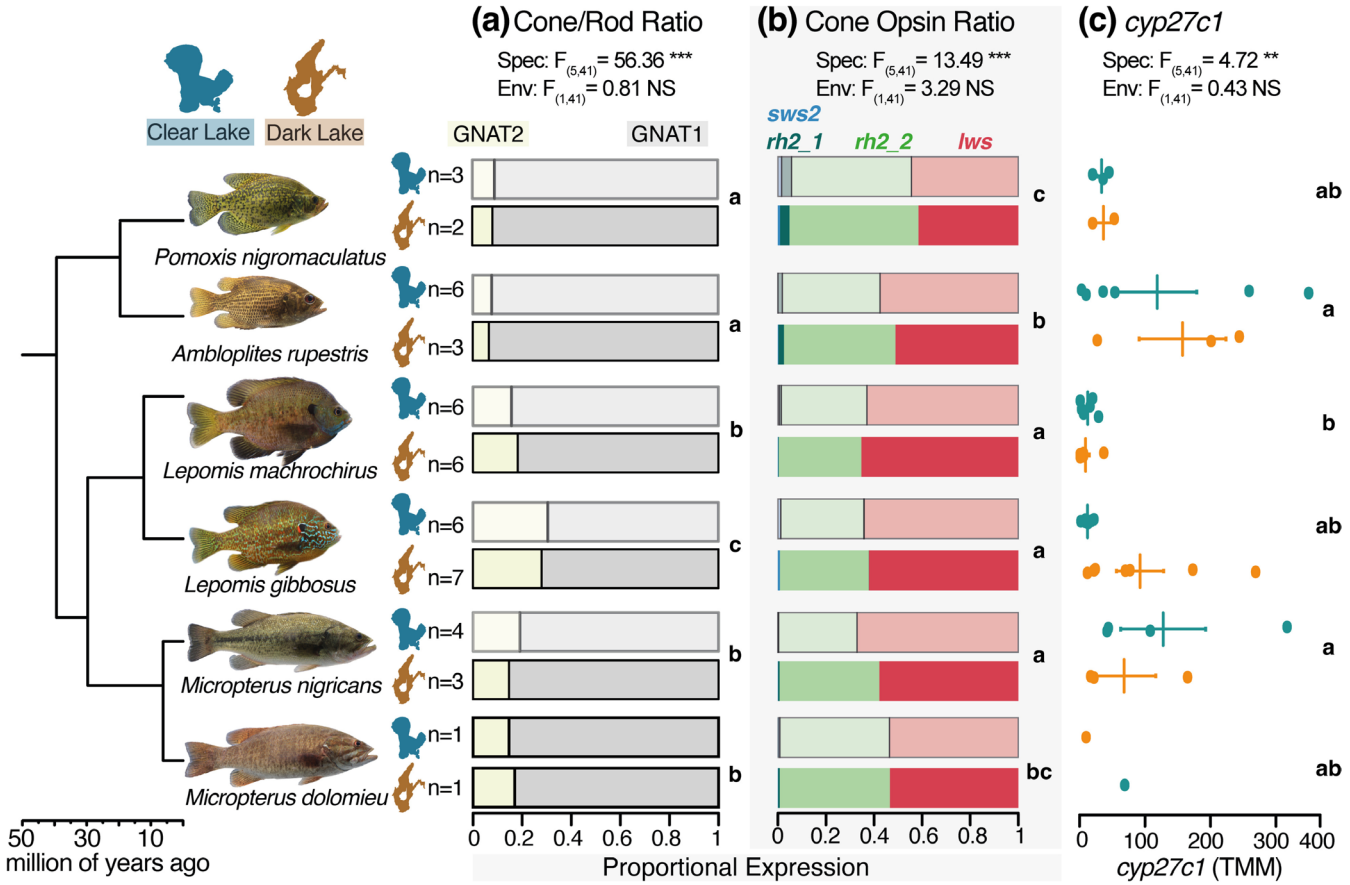

FIGURE 3 | Expression of visual opsin genes and *cyp27c1* in six centrarchid species from two different photic environments. (a) Ratio of rod-specific cell marker gene gnat1 to cone-specific cell marker gene gnat2. (b) Proportional expression of cone opsin genes. (c) Normalised expression of *cyp27c1*. Model estimates from ANOVA (Type II) are shown above each trait. Letters display groups based on a post hoc least-significance difference test using the false discovery rate. For a detailed visualisation of individual gene expression values see supplemental material (Figures S6, S7, S9 and S10). Phylogenetic tree based on Near and Kim (2021). ***, ** and NS denote $p < 0.001$, $p < 0.01$ and $p > 0.05$, respectively.

centroid distances in reflectance space (PC1-2, 89% variation; Figures S15 and S16) also showed a significant species-by-environment interaction (Figure 4b; $F_{4,55} = 6.18$, $p < 0.001$).

4 | Discussion

Most studies on visual ecology have found strong associations between photic environments and phenotypic variation in fish visual communication traits (Lythgoe 1979; Partridge and Cummings 1999; Cummings and Endler 2018). Specifically, studies focusing on sensory traits (e.g., cone opsin gene expression) have consistently found significant correlations with photic conditions (Cummings and Partridge 2001; Fuller et al. 2004; Hofmann et al. 2009; Rennison et al. 2016; Stieb et al. 2016; Torres-Dowdall et al. 2017; Veen et al. 2017; Härer et al. 2018; Bertinetti, Härer et al. 2024). A similar pattern has been observed in studies focusing on signalling traits, including body coloration (Reimchen 1989; Dalton et al. 2010; Maan et al. 2010; Morrongiello et al. 2010). While some of the strongest evidence favouring environmentally driven diversification of sensory and signalling traits comes from aquatic habitats, evidence incorporating multiple species, environmental data and variation in both signals and receivers simultaneously is restricted to a few examples (Cummings 2007; Seehausen et al. 2008; Brock et al. 2018). Here, we used an integrated approach to characterise molecular and morphological variations in the visual communication traits of six centrarchid species inhabiting two contrasting light environments. We found interspecific differences for most traits measured, including eye morphology, body reflectance, and the sequence and expression of cone opsin genes. However, sensory traits and signalling traits exhibited different patterns of phenotypic variation. Phenotypic variation in sensory traits was minimal in the six species across photic environments (i.e., robust phenotypes), whereas visual signals exhibited species-specific divergence across environments.

The most striking pattern observed in our study was the limited phenotypic variation exhibited by the visual systems of centrarchids between different photic conditions. Photic environments impose selective pressures on centrarchids, for example, high DOC concentrations leading to dim and long wavelength shifted environments that decrease foraging and reproductive success (Craig et al. 2017; Weidel et al. 2017). This sets up the expectation that sensory systems in centrarchids should be shaped by photic conditions, as seen in other fish taxa with overlapping feeding ecologies inhabiting similar ecosystems and latitudes (Munz and McFarland 1977; Lythgoe 1980; Loew 1995). For instance, based on convergent eco-morphological features, cichlid fish (Cichlidae) occupy similar ecological niches yet exhibit

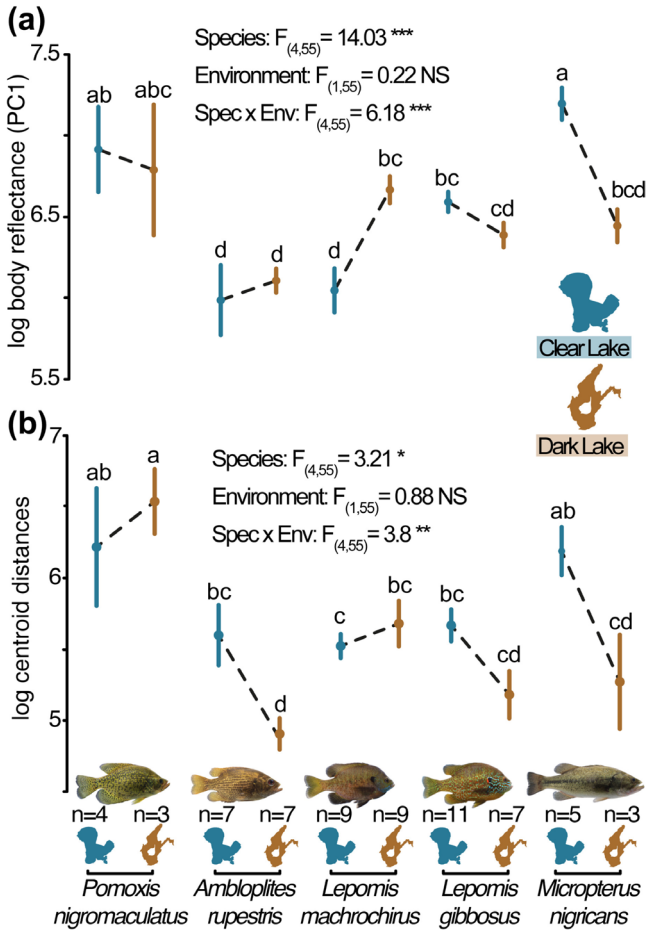

FIGURE 4 | Variation in body reflectance in centrarchids across the two contrasting light habitats. (a) Logarithmic body reflectance (PC1, 79% of variance; Figures S15 and S16). (b) Logarithmic centroid distance as an estimate of the intra-population reflectance space (PC1-2; Figure S15). Estimates from ANOVA (Type III) are shown above each trait. Letters display groups based on a post hoc least-significance difference test using the false discovery rate. ***, **, * and NS denote $p < 0.001$, $p < 0.01$, $p < 0.05$ and $p > 0.05$, respectively.

broad and highly tunable visual sensitivities that diverge with changes in photic conditions, even lower than the ones found in this study ($\Delta\lambda P50 \sim 60$ nm) (Maan et al. 2010; Montaña and Winemiller 2013; Torres-Dowdall et al. 2017, 2021; Härer et al. 2018; Bertinetti, Härer et al. 2024). This includes top predatory fish such as *Parachromis managuensis* (Härer et al. 2018) and *Cichla monoculus* (Escobar-Camacho et al. 2019). The native geographic range of cichlids might partially explain their enhanced spectral sensitivity in UV-blue range compared to centrarchids, given that tropical waters are abundant in short wavelengths compared to higher latitudes (McFarland and Munz 1975a; McFarland et al. 1999). However, fish inhabiting similar native latitudinal ranges to centrarchids, such as three-spine sticklebacks (*Gasterosteus aculeatus*) or red shiners (*Cyprinella lutrensis*) do exhibit phenotypic variation in their visual system across light habitats (Novales Flamarique et al. 2013; Rennison et al. 2016; Marques et al. 2017; Foster et al. 2024). Further, taxa inhabiting similar light habitats dominated by low versus high DOC, such as the bluefin killifish (*Lucania goodei*) or the sailfin tetra (*Crenuchus spilurus*) also show environmentally driven visual trait diversity (Fuller et al. 2004; De Almeida Borghezan et al. 2024). In contrast, sensory traits in centrarchids did not vary across photic conditions at either the morphological or the molecular level.

In contrast to most studies reporting widespread phenotypic variation across photic habitats, the flat responses of centrarchids resulted in robust phenotypic traits across environments. However, it seems that most studies have focused on complex chromatic organisms, such as tri-, tetra- and pentachromatic organisms (Carleton et al. 2020; Torres-Dowdall et al. 2021). The centrarchids analysed in this study possess four cone opsin genes, *sws2, rh2_1, rh2_2 and lws*, but the expression of *rh2_2* and *lws* accounted for over 98% and the blue-sensitive opsin gene *sws2* for 0.05% of the total cone opsin expression on average. While unlikely, the contribution of *sws2* cones to colour vision cannot be excluded and could instead be linked to larval stages, as seen in other fish where short-wavelength sensitivity decreases through ontogeny (Cheng and Novales Flamarique 2004; Carleton et al. 2008; Härer et al. 2017; Lupše et al. 2022). Therefore, the expression of certain cone opsins in centrarchids may vary with ontogenetic diet changes (Werner and Hall 1988). In this study, we focused on adult fish and found no variation in opsin gene expression between photic habitats. One potential explanation is that the retinal structure limits visual pigment variation. Cone opsins are usually expressed in a cone-specific manner in the photoreceptor cells (Palacios et al. 1998; Ogawa and Corbo 2021). Adult centrarchids show only two types of cone photoreceptors: single cones expressing only *rh2* (green) and twin cones expressing only *lws* (red). Hence, cone opsin gene expression in centrarchids possessing only single and twin cones may be constrained by both retinal structure and ontogeny. Understanding the ontogeny of visual systems in centrarchids and their relationship to their ecology might explain the biological role of lowly expressed cone opsin genes and why adult fish seem restricted to a predominant red-green chromatic system.

While the predominantly red-green chromatic system of centrarchids might represent a rather simple, constrained phenotype lacking responses to environmental pressures, it could also represent a robust, generalist trait that performs sufficiently well under different photic conditions (Marshall et al. 2015). Studies in dichromatic freshwater darters (*Etheostoma* spp.) found differences between populations using microspectrophotometry (MSP), but did not establish any link between spectral variation and light environments (Gumm et al. 2012; Zhou et al. 2015). By combining MSP, reflectance and underwater irradiance data, Cummings (2007) found evidence of signal and sensory divergence among marine dichromatic surfperch species of Embiotocidae occupying different light habitats. Hence, a systematic MSP study of variation across centrarchid populations might reveal sensory divergence in pigment peak sensitivities that is not evident from the patterns of opsin gene evolution. To date, only one such comparison is available for *M. nigricans*, where no differences were found between Florida and Illinois populations (Mitchem et al. 2019). Dichromatic systems, as presumed for centrarchids, inherently have a trade-off between luminance and chromatic sensitivity (Lythgoe and Partridge 1991; Chiao et al. 2000). If red and green pigments overlap in their spectral absorbance, enhanced sensitivity to photons reaching

the overlapping spectral region is achieved (i.e., luminance contrast). Alternatively, little overlap between red and green pigments in their absorbance enables better colour discrimination of photons in distinct spectral regions (i.e., chromatic contrast). The difference in peak pigment sensitivities of 83 nm found in centrarchids aligns better with visual systems that prioritise chromatic contrast (green pigment: 535 nm, red pigment: 618 nm; Dearry and Barlow 1987; Hawryshyn et al. 1988; Mitchem et al. 2019). Counterintuitively, visual modelling studies of bluegill predation suggest that photic conditions do not affect the detection of zooplankton prey (Wale et al. 2021). However, a different explanation than visual limitation would need to be evoked for the decreased foraging success of centrarchids in high-DOC environments (Weidel et al. 2017). Hence, the centrarchid visual system may provide sufficient chromatic detection under different photic conditions. A detailed study modelling the visual performance of centrarchids across multiple photic environments could determine whether this chromatic system represents a constrained or robust trait.

Although photic conditions did not significantly influence within-species variation in centrarchid visual systems, substantial differences were observed between species. Hence, even though intraspecific variation is limited, evolutionary history seems responsible for phenotypic diversity among species. The study species encompass a broad range of trophic ecologies (e.g., planktivores, molluscivores, piscivores, crayfish predators), suggesting that visual adaptations might reflect species-specific ecological demands. Further, our molecular evolution tests suggest that visual systems among species of centrarchids have evolved (Tables S3–S8). Although none of the positively selected sites in opsin proteins were found in known spectral tuning sites, some were adjacent (Hagen et al. 2023). Further analysis is required to characterise the functional implications of these point mutations on spectral sensitivity. In addition, eye morphology differed among species and seemed robust to environmental conditions, likely due to genetic divergence among species. Indeed, increased eye size might relate to foraging demands, for example, demersal foraging rock bass requiring high image resolution to locate prey in complex substrates (Williamson and Keast 1988; Caves et al. 2017). Interestingly, we found significant differences across species in *cyp27c1* expression, a key enzyme involved in chromophore-type usage (Bridges 1964; Enright et al. 2015). In theory, higher *cyp27c1* expression is associated with higher vitamin A2-derived chromophores and, thus, with long-wavelength-shifted sensitivities (Corbo 2021). So far, only A2-derived visual pigments have been identified in centrarchids, despite the significant differences in *cyp27c1* expression in our study (Dearry and Barlow 1987; Hawryshyn et al. 1988; Mitchem et al. 2019; Corbo 2021). The expression of *cyp27c1* in centrarchids resembles values found in other freshwater fish which predominantly use A2-derived chromophore types such as cichlid fish from turbid water bodies (Torres-Dowdall et al. 2017; Härer et al. 2018; Escobar-Camacho et al. 2019; Bertinetti, Meyer, et al. 2024). While our results further support the widespread link between *cyp27c1* expression and chromophore-type abundance in freshwater fish, the significant species-specific differences suggest that the association between *cyp27c1* expression and chromophore usage might be affected by factors that remain unknown.

Despite sensory traits showing no variation across photic environments, variation in body reflectance was significantly affected by species-by-environment interactions, showing divergent patterns of phenotypic variation across species (Figure 4). This pattern of variation in body coloration suggests that centrarchids modulate signalling traits based on photic conditions. While the transmission of visual signals is confined within the boundaries of the light environment, other ecological factors, such as predator–prey interactions or conspecific recognition, might act as selective agents (Endler 1978; Seehausen et al. 1997; Schneider et al. 2020). For instance, reducing conspicuousness to match the ambient light conditions might reduce predator risk at the expense of decreasing the chances of attracting potential mates. Therefore, signalling cues that are conspicuous for mates but cryptic for predators should be favoured and adjusted to the spectral regions available within the environment (Endler 1992). The different reflectance patterns observed in centrarchids support the idea that signalling is modulated by species-specific ecological demands in a context-dependent manner. Our study suggests that all sensory traits in centrarchids vary across species, yet modulating only the signal component might be sufficient to offset environmental pressures set by photic conditions. Additionally, closely related and hybridising species, such as bluegill and pumpkinseed, showed a wide overlap in their signalling space, which might impact mate recognition (Figure S15). The effect of photic conditions on mate-signalling traits, such as body coloration, may have implications for interspecific hybridization in the wild (Seehausen et al. 1997). Turbidity has been mentioned as a factor facilitating hybridization in centrarchids, although this has not been empirically tested (Cooke and Philipp 2009). A closer look at the sensory biology of centrarchids, with particular emphasis on the link between mate signalling and the environmental context, could help elucidate the mechanisms facilitating centrarchid hybridization in nature.

Overall, our study provides an integrated overview of the role of photic conditions in the visual communication traits of six centrarchid species. Phenotypic variation of centrarchids across light conditions, particularly in terms of molecular characterisation, has remained relatively understudied despite their widespread range and ecological relevance (Cooke and Philipp 2009). Centrarchid species vary significantly in phenotypic traits of their visual system but show a lack of variation across underwater photic conditions. These robust visual systems challenge the current paradigm of highly tunable sensory traits in teleosts, in response to distinct photic environments (Lythgoe 1979; Partridge and Cummings 1999; Carleton et al. 2020). Despite the selective pressures imposed by light climates on centrarchids (Craig et al. 2017; Weidel et al. 2017), their lack of phenotypic variation to a common environmental stressor suggests that evolutionary/developmental constraints operate, or that a single phenotype provides an adequate fit in multiple habitats. The detrimental effect of decreased water clarity on foraging success (Miner and Stein 1993; Weidel et al. 2017) or fish productivity (Craig et al. 2017) seems to favour the presence of constraints given that fine-tuning sensory traits would be beneficial. Regarding tasks related to conspecific recognition, the presence of species-by-environment interactions in body reflectance suggests that some species can modulate body coloration and thus signalling across photic conditions. Hence, it remains important to examine the performance consequences of environmental conditions on the robust sensory traits and

variable signalling traits of centrarchids. Furthermore, major threats to aquatic ecosystems, such as water quality degradation and invasive species, may have strong implications for the sensory biology of these species (Dudgeon et al. 2006; Thomsen et al. 2014; Solomon et al. 2015). In particular, competition with introduced species possessing tunable sensory traits may lead to centrarchid underperformance under certain photic conditions. Therefore, examining the visual ecology of centrarchids to understand their adaptability to novel habitats and the ecological consequences of species interactions is relevant for improving the management of aquatic ecosystems.

Author Contributions

J.T.-D. and C.B. developed the project, S.J. and C.M. helped to coordinate and plan fieldwork, J.T.-D. supervised the project, S.J. and C.M. provided access to equipment and advised on logistics, C.B. collected and analysed the data, and C.B. led the writing of the manuscript. All the authors contributed critically to the draft and approved the final manuscript for publication.

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Disclosure

Benefit-Sharing Action: Benefits Generated: Benefits from this research accrue from the sharing of our data and results in public databases, as described above.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Sequence reads and metadata are deposited in the NCBI SRA database under accession number PRJNA1168051. Additionally, the data and code used for analysis are publicly available at the Zenodo Digital Repository (Bertinetti 2024).

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.