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ORIGINAL ARTICLE

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Plasticity contributes to a fine-scale depth gradient in sticklebacks' visual system

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Abstract

The light environment influences an animal's ability to forage, evade predators, and find mates, and consequently is known to drive local adaptation of visual systems. However, the light environment may also vary over fine spatial scales at which genetic adaptation is difficult. For instance, in aquatic systems, the available wavelengths of light change over a few metres depth. Do animals plastically adjust their visual system to such small-scale environmental light variation? Here, we show that in three-spine stickleback (*Gasterosteus aculeatus*), opsin gene expression (an important determinant of colour vision) changes over a 2-m vertical gradient in nest depth. By experimentally altering the light environment using light filters to cover enclosures in a lake, we found that opsin expression can be adjusted on a short time frame (weeks) in response to the local light environment. This is to our knowledge the smallest spatial scale on which visual adjustments through opsin expression have been recorded in a natural setting along a continuously changing light environment.

KEYWORDS
cline, *Gasterosteus aculeatus*, light environment, opsin expression, plasticity, stickleback, three-spine, visual ecology

1 | INTRODUCTION

Sensory systems are important for fitness as they allow an individual to monitor and respond to its local environment (Endler, 1991). Due to the importance of sensory systems, such as vision, for foraging efficiency, predator detection and mate choice, senses are predicted to adapt to spatial differences in the sensory environment, either through changes in genotype frequency or through plasticity. Adjustments of the visual system have been found to take place at different processing stages, from the retina where the initial capture of photons takes place, to the neurological response initiated, and finally to how these stimuli are processed by the brain (Webster, 2015). Despite the awareness of the diversity of ways vision adjusts to the environment, relatively little is known about how the visual system adjusts to differences in light environments at a small spatial scale within an organism's natural environment. This is not surprising as most neurological studies are very hard to conduct under natural conditions. In this study, we focus on one visual adjustment that can be studied under natural conditions, the differential expression of opsin genes (which influences visual sensitivity), to a naturally occurring light gradient experienced by the three-spine stickleback (*Gasterosteus aculeatus*).

The ambient light environment is a key determinant of the performance of the visual system, as it determines photon availability across the wavelength spectrum. This in turn directly affects visual functions such as the ability to see contrast and detect predators, prey and sexual partners. Consequently, populations inhabiting locations with different light conditions often evolve divergent visual characteristics (Boughman, 2001; Cummings, 2007; Fuller, Carleton, Fadool, Spady, & Travis, 2005; Ryan & Cummings, 2013). The resulting visual adaptation leads to correlations between organisms' spectral sensitivity and aspects of their local light environment; this pattern is frequently found in fishes (Carleton, Parry, Bowmaker, Hunt, & Seehausen, 2005; Cummings & Partridge, 2001; Lythgoe,

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Muntz, Partridge, & Shand, 1994; Rennison, Owens, Heckman, Schluter, & Veen, 2016).

Local adaptation of the visual system is generally documented at a fairly broad spatial scale, for example, between allopatric populations exposed to unique light environments (e.g., tannin-stained vs. clear water) (Fuller et al., 2005). However, light environments can vary over quite small spatial scales (e.g., sunspots in a forest) (Endler & Thery, 1996; Mollon, 1989). This is especially true for aquatic environments, where some wavelengths of light are more rapidly attenuated than others as they pass through the water column. The wavelengths most affected depend upon the type and abundance of dissolved organic solutes or suspended particulates within a water body (Kirk, 1994; Lythgoe, 1979; Sabbah et al., 2011). This differential filtering of wavelengths along a depth gradient makes it well suited to the study of fine-scale adjustment to different light environments.

Individuals of many fish species easily travel along light gradients over short timescales (even within seconds), especially in shallower water where light changes markedly across a couple of metres. For animals to adjust their visual system to shifts in the local light environment, individuals must inhabit different light environments (e.g., different water depths) for sufficient time relative to the speed of plasticity. Some visual changes (e.g., pupil dilation) occur on the scale of seconds; such adjustments allow acclimation to fast-changing light conditions. However, changes in opsin gene expression are slower acting and vary diurnally or over a series of days (e.g., Johnson, Stanis, & Fuller, 2013). Thus, for many mobile animals, adjustment of visual gene expression to fine-scale variation in light environment may not be possible. In stickleback, we know that individuals can remain more strictly associated with particular depths and in doing so are exposed to distinct light regimes; male stickleback build and guard nests at depths between 0.5 and 3 m in lakes where the light environment changes markedly across this depth gradient. Although males may move up and down the water column above their nest, shallow- vs. deep-nesting males are exposed to different light environments for extended periods of time while they tend to their nest and raise their young (McPhail, 1994; Vines & Schluter, 2006; Snowberg & Bolnick, 2012; personal observations). We hypothesized that male stickleback inhabiting different depths have adjusted their visual system to their respective light environment. To test this hypothesis, we quantified opsin gene expression and used these measures of expression to estimate the absorbance of light (photons) for males found along a natural depth gradient. We focused our efforts on opsin genes because opsin proteins are found in retinal rod and cone cells and mediate the absorbance of photons and thus are essential for both light detection and image formation. Previous work in stickleback (Rennison et al., 2016) and other fishes (e.g., Fuller et al., 2005) has shown that opsin expression can respond to differences in ambient light. We then asked whether expression and absorbance covary predictably with the light environment.

Changes in opsin expression have previously been found to have a genetic determination in some systems (e.g., Hofmann, O'Quin, Smith, & Carleton, 2010; Rennison et al., 2016) but are a result of phenotypic plasticity in others (e.g., Fuller et al., 2005). Changes in opsin expression along a fine-scale spatial gradient could be genetically determined if individuals choose the depth at which they live based on their spectral phenotype or another correlated trait (habitat matching). Alternatively, nonheritable changes in absorbance could underlie these differences if individuals exploit phenotypic plasticity to rapidly adjust their visual system to a local light environment through differential expression of opsins. To test whether light environment causes plastic changes in opsin expression and absorbance, we conducted an enclosure experiment using light filters to mimic light environments at different depths. Individuals were transplanted to light treatment enclosures that were installed within the lake. We quantified opsin expression and estimated absorbance for each individual after 24 days of exposure. We tested for expression differences between the sexes as the literature is contradictory whether the sexes differ in their visual sensitivity (Boulcott & Braithwaite, 2007; Cronly-Dillon & Sharma, 1968).

2 | METHODS

2.1 | Sample collection

In June and early July 2014, we collected 16 males nesting along a depth gradient (0.32–2.47 m) in Gosling Lake (50°04′03.2″N, 125°30′20.7″W) on Vancouver Island, BC, Canada, to quantify their opsin expression. This location was chosen because earlier work has revealed a consistent gradual change in the light environment across a ~2-m depth gradient within this lake, and a corresponding cline in male nuptial coloration (Brock, Cummings, & Bolnick, 2017). Nesting males were collected by snorkelers using dip nets. We targeted nesting males because during the nesting season they stay in close proximity to their nest (personal observations and Snowberg and Bolnick, 2012) and hence would potentially have the opportunity to plastically adapt their spectral sensitivity to the local light environment. Captured fish were measured (standard length) and weighed and then euthanized in MS-222. Both eyes were immediately removed, placed in RNAlater (Qiagen, The Netherlands) and subsequently frozen.

2.2 | Experimental design

We designed an experiment to test whether opsin expression at different depths was plastic and changed in response to differences in the ambient light environment. To isolate the effect of light from other covariates of depth (e.g., diet [Snowberg & Bolnick, 2012]), we constructed enclosures at a single depth. Forty metal mesh enclosures of approximately 1.5 m by 1.5 m^2 were built in shallow water (~0.5 m deep in the middle of the enclosure) along Gosling Lake's northern shoreline (50°04′04.2″N, 125°30′23.8″W). These enclosures were arranged as 20 adjacent pairs to control for spatial heterogeneity. Within each pair, one cage was assigned a "shallow" light treatment and the other a "deep" treatment. Each cage was wrapped with light filters (LEE Filters www.leefilters.com) that were chosen to mimic the side-welling irradiance at depths of either 0.5 m (#278

Eight Plus Green Filter with 0.15 ND) or 1.8 m (#213 White Flame Green Filter with heat shield, 0.9 trans). From here on, "irradiance" refers to side-welling irradiance unless stated otherwise. The filters covered the top of each cage and the sides of the cages from above the water's surface down to approximately 10 cm underwater. We used the side-welling irradiance from Brock et al. (2017) to choose the most suitable colour filters by minimizing the squared difference of the irradiance at depth 0.5 or 1.8 m and the irradiance of the LEE filters as provided by the manufacturer across the wavelength spectrum. The neutral density (0.15 ND) and heat shield filters were added to equalize the photon flux in both cages. This was done so that any differences in opsin expression found between light treatments would be attributable to the spectral composition, and not depth or photon flux (overall brightness). However, when quantifying the match between irradiance in the two treatment cages with the irradiance measured along the depth gradient, it turned out that our intended shallow treatment best matched the natural light at 1.5 m depth and our deep treatment resembled 2 m (see Appendices S1 and S2). While we did simulate light environments at different depths, they only spanned a 0.5-m range instead of the intended 1.3-m range, and we therefore refer to the two treatments as medium and deep from now on.

At the start of the experiment, we introduced one randomly selected male into each cage and one gravid female later the same day. We only used reproductively active individuals (i.e., nesting males and gravid females) to make sure we stocked each cage with one male and one female. All individuals were captured by dip net, in up to 2.5 m deep water. All cages were checked after 8 days and missing individuals (died or escaped) were replaced. A total of 15 females and seven males were replaced. In half of the cages, extra stickleback had entered the cage (one [eight times], two [once], four [once]). Intruders were successfully identified by comparing the body length of all fish in the cage with the measurements of fish initially introduced into the cage. All cages were thoroughly checked for holes at this stage and adjusted where needed. After 24 days, 27 females and 29 males were retrapped, measured, euthanized and had their eyes extracted and stored for quantification of opsin expression. (Note that not all individuals had been exposed to the light treatment for the full 24 days.) Individuals were trapped in quick succession within each cage and sequentially for each adjacent pair of cages to avoid a potential effect of time of day on opsin expression within a cage pair comparison.

2.3 | Ambient light environment

We collected the side-welling irradiance along the natural depth gradient to validate the previously described irradiance gradient (Brock et al. 2017) and took irradiance measures in the experimental cages to test the effectiveness of our light manipulation. Measures were taken in triplicate just above and below the surface, and at 0.5, 1.0, 1.5, 2.0 and 2.5 m depths along the natural gradient. The light levels were measured at three locations offshore from where the cages were set up, close to where the fish were caught. We measured down- and side-welling (probe facing towards the shore) irradiance at 1-nm intervals using an EPP200C UV-VIS spectrometer coupled to a UV-NIR cosine receptor. The initial irradiance measurements (W/m^2) were translated into $\mu E\ m^{-2}\ s^{-1}$ using a LI-COR Optical Radiation Calibrator (model 1800-02) calibration lamp. The irradiance measures were subsequently normalized (integral equals 1) so that the total available light between measurements and locations was the same, hereby focussing our analyses on differences in the shape of the light spectrum.

2.4 | Opsin expression and absorbance

Stickleback have four cone opsin genes: short-wavelength sensitive 1 (*SWS1*: λ_{max} = 365–382 nm); short-wavelength sensitive 2 (*SWS2*: λ_{max} = 434–441 nm); middle-wavelength sensitive (*RH2*: λ_{max} = 514–546 nm); and long-wavelength sensitive (*LWS*: λ_{max} = 566–638 nm) (Flamarique, Bergstrom, Cheng, & Reimchen, 2013; Rennison, Owens, & Taylor, 2012; Rowe, Baube, Loew, & Phillips, 2004). We measured the relative abundance of mRNAs for each of these four opsin genes. Prior to RNA extraction, the left and right eyes from each fish were pooled and homogenized using a carbide bead in a Retsch mm 400 Mixer Mill (Haan, Germany). Total RNA was extracted from the homogenate using the Aurum™ Total RNA Fatty and Fibrous Tissue kit (Bio-Rad®), which included a DNase I incubation step. The concentration and purity of the extracted RNA were assessed on a NanoDrop® Spectrophotometer (Thermo Scientific). Synthesis of cDNA was accomplished using the iScriptTM cDNA Synthesis Kit (Bio-Rad®); 200 ng of RNA from each sample was used as the input for the cDNA synthesis reaction. The resulting cDNA was diluted 1:100 in ultrapure water for the RT-qPCR analysis.

The probe and primer sequences used for RT-qPCR were designed using sequences from the stickleback genome (Jones et al., 2012) and are reported in Table S1. For each gene, one of the primers and/or the RT-qPCR probe spanned an intron, to avoid amplification of genomic DNA. Integrated DNA Technologies (IA, USA) synthesized the primers and probes. We used PrimeTime® qPCR 5′ Nuclease Assays which had a double-quenched probe with 5′ 6-FAM™ dye, internal ZEN™ and 3′ Iowa Black® FQ Quencher.

The RT-qPCR analysis was performed on a Bio-Rad® IQ5 machine (Bio-Rad, CA, USA). The polymerase used was the SsoAdvanced Universal Probes Supermix (Bio-Rad®) in a 25-μl reaction and the reactions were run in 96-well plates (Fisher, MA, USA). The plates were sealed using optical sealing tape (Bio-Rad®). Well factors were collected from each of the experimental plates. Reactions were run in duplicate or triplicate. No-reverse transcription and no-template controls were included for every run. These controls consistently yielded no amplification. RT-qPCR conditions were as follows: 1 cycle at 95°C for 3 min and 40 cycles of 95°C for 10 s and 60°C for 30 s. We used a standardized luminance threshold value of 50 to calculate CT values.

Equation 1 was used to calculate the PCR efficiencies (*E*) for each of the primer pairs.

$$E = e^{-\beta} - 1 \quad (1)$$

where the slope (β) is determined from a linear least-squares regression fit to critical threshold (C_t) data from a cDNA dilution series (1:10, 1:50, 1:100, 1:500, 1:1,000).

When considering colour vision, one informative metric is the expression of each opsin gene relative to the total opsin levels present in the retina (Fuller & Claricoates, 2011). We prefer this measurement as it has been shown to be best for making inferences about colour vision capacity, whereas expression relative to a housekeeping (control) gene is more useful for looking at differential regulation of each opsin gene (Fuller & Claricoates, 2011). The estimates of the initial amount of gene transcript (T_i) were calculated for each individual (i) using Equation 2, where E is the PCR efficiency for a given gene calculated from Equation 1 and C_t is the critical threshold for fluorescence.

$$T_i = \frac{1}{(1+E)^{C_i}} \quad (2)$$

For each individual, we summed the opsin gene expression across the four cone opsin genes and estimated the proportion of total expression for each gene. This provided a measure of relative gene expression.

Opsin expression is one of many steps linking the perception of photons of light to behavioural responses. Opsin expression has been shown convincingly to correlate with colour discriminatory behaviour (Smith, Ma, Soares, & Carleton, 2012) and can provide valuable new insights into visual ecology. However, the molecular basis of variable opsin expression and its ecological function is unknown; it could be due to upregulation of expression in each cell, or more dense opsin packing or differences in optical density. In attempt to further understand the biological implication of changes in opsin expression, we used expression to generate a surrogate phenotypic estimate of spectral absorbance (previously referred to as spectral sensitivity in Rennison et al., 2016). We combined our relative opsin expression estimates with published nonlinear absorbance templates (from Govardovskii, Fyhrquist, Reuter, Kuzmin, & Donner, 2000) and used empirical estimates of the wavelength of maximum absorbance for each opsin gene (Flamarique et al., 2013) to derive the normalized absorbance of each opsin across the visible light spectrum. Combining the absorbance of the four opsins yielded an individual's combined absorbance curve. To calculate absorbance, the ratio of A_1 to A_2 chromophores in visual pigments is needed, but we lack this information for the Gosling population. Earlier work in fish has shown that the ratio can vary between completely A_1 to completely A_2 (Toyama et al., 2008) and that A_2 chromophore domination is common for tannin-stained lakes (e.g., Flamarique et al., 2013). As Gosling has relatively clear water, we chose an equal contribution of both chromophores when calculating the absorbance and validated these results by analysing the only A_1 and only A_2 chromophore scenarios.

Translating opsin expression into a "visual sensitivity phenotype" comes with some severe caveats. Besides the assumption of A_1 to A_2 chromophores ratios, the above approach also assumes that the mRNA and opsin protein concentrations are equivalent and that normalized expression is informative for colour perception (see Smith et al., 2012 for justification of this assumption). It furthermore assumes that the inputs of cone cells expressing the different opsin genes are equivalent in magnitude. Nonetheless, we believe it is useful to calculate the absorbance as it can provide a hint of what the biological effect might be and allows comparison with other studies, of which some have shown a strong and consistent relationships with ambient light suggesting this metric (in stickleback) is biologically informative (Rennison et al., 2016).

2.5 | Relationship between opsin expression and depth along the natural gradient

We quantified the light at a given depth by calculating the cumulative area under the irradiance curve for the green-orange part of the spectrum (501–600 nm), and dividing this by the cumulative area for the UV part of the spectrum (301–400 nm) (sensu Brock et al. 2017). This ratio was regressed against water depth in a linear mixed model, LME4 (Bates, Maechler, Bolker, & Walker, 2015; and LMERTEST packages (Kuznetsova, Brockhoff, & Christensen, 2016) in R (R Development Core Team 2016) with the location of the measurement (three depth gradient replicates) as a random effect.

We tested for a relationship between depth and expression in two steps. First we used a principle component analysis (PCA) to reduce the dimensionality and used the PCs that cumulatively capture >95% of the variance. Subsequently, we conducted a linear regression on each PC to test for an effect of depth and/or time of day. Time of day was included to control for changes of expression throughout the day as found in killifish (Johnson et al., 2013). Model reduction was based on a sequential likelihood ratio test as implemented in the *drop1* function in R. In the second step, a linear regression was performed for each opsin in isolation, with opsin gene expression as the response variable and depth and/or time of day as the explanatory variable. Only the significant explanatory variables from the PCA were included. Because we calculated expression of each opsin as a proportion of total opsin expression, our data are considered "sum-constrained" (i.e., if one opsin is upregulated, the mean of the expression of other three has to go down). To account for this characteristic of the data, we also analysed our data using an *ln*-ratio transformation (Aitchison, 1986; Kucera & Malmgren, 1998) to validate our results. We focus on the nontransformed data as interpretation of the results is much easier, and results are quantitatively similar between the transformed and nontransformed data sets.

We calculated the absorbance across the wavelength spectrum for each individual, but our sample size did not allow us to directly compare the sensitivity of individuals collected at the extremes of the depth gradient. We therefore used the predicted opsin expression at the extremes of the depth range from the linear model described above to calculate the spectral sensitivity of fish at the deep and shallow ends of the gradient and visually compared these two sensitivity curves. This allowed us to interpret the functional consequences of the observed difference in opsin expression across the range of nest depths.

2.6 | Opsin expression in the experiment

In the first step, we analysed whether opsin expression differed between the two treatments for each opsin using a mixed-effects model with enclosure (cage) pair as a random effect to control for potential heterogeneity along the shoreline and effect of time of day (fish from paired cages being collected in quick succession). We included sex and a sex–treatment interaction to the full model because previous work suggested that males were slightly more sensitive to shorter wavelengths (Cronly-Dillon & Sharma, 1968; but see Boulcott & Braithwaite, 2007). We employed analysis of deviance for model reduction and only included a term in the final model if it contributed significantly to the variance explained for the dependent variable (using the ANOVA function in R). The order of terms tested during model reduction was based on *p*-values (high values first).

To help interpret the results of our experiment in terms of the natural light gradient, we identified the depths along the gradient for which the irradiance best matched the irradiance from each of the filter treatments. To increase our precision, we interpolated irradiance measures for 0.1-m intervals using locally weighted polynomial regressions as implemented in the LOEWESS function in R, applied to each wavelength. This provided an estimate of the spectral composition at 0.1 m depth increments. We then compared the irradiance measured in each cage to each natural depth. Specifically, we calculated the squared difference between the irradiance in the cage (the effect of the filter plus the water) and the irradiance at different depths along the natural light gradient (only effect of water). The depth with the lowest squared difference represents the best match within a given treatment.

We then used a resampling routine to test whether the irradiance differed significantly between the two cage light treatments. We first performed a wavelength-by-wavelength linear model analysis to obtain a *F*-value for the differences between the irradiance measured in each treatment. We used the sum of *F*-values across the spectrum as our test statistic. To obtain a null distribution, we used a permutation test (10,000 iterations), which redistributed the cage irradiance measurements randomly to a treatment and allowed us to obtain a *p*-value for our sensitivity comparison (North, Curtis, & Sham, 2002). Next, we calculated the normalized absorbance for each individual using its opsin expression data and tested whether absorbance differed between the two treatments, using a resampling routine as described above but replacing irradiance with the absorbance of individuals.

If relative levels of opsin expression are plastic, we predicted that fish that were moved from an initially shallow depth to a deep-like light environment would show a greater change in opsin expression (compared to other shallow nesting males), than fish moved from a deep nest into a deep-like light environment. To quantify the magnitude of the change in opsin gene expression for individuals, we compared their predicted absorbance at the beginning of the experiment to their estimated absorbance (using their opsin expression data) at the end of the experiment. We predicted the expression of these individuals at the beginning of the experiment using the depth at which they were collected at and the linear model from the natural depth gradient. This gave us an estimate of the extent to which individuals' opsin expression may have changed, assuming their pre-experiment expression followed the estimated regression trend for wild-caught fish. This assumption is necessary because opsin expression requires destructive sampling and so cannot be obtained both pre- and postexperiment using the same fish. We then regressed the inferred change in expression (predicted expression upon capture–expression at the end of the experiment) against the change in depth (depth of capture–depth of treatment light environment). If plasticity of opsin expression is strong, we expect a positive correlation between the change in depth and the change in opsin expression or sensitivity. To test this, we used a linear model with change in expression as the response variable and change in depth as the explanatory variable focusing on the males of the experiment only (as only males were collected along the natural depth gradient).

3 | RESULTS

3.1 | Natural depth gradient

3.1.1 | Changes in irradiance

The spectral composition of irradiance changed with depth (slope = 0.830 (0.146 *SE*), $df = 52$, $t = 5.691$, $p < .001$). The trend indicates that longer wavelengths are more heavily represented as depth increases (i.e., short wavelengths were filtered out). This depth gradient is quantitatively comparable to depth gradients found in three separate years by Brock et al. (2017).

3.1.2 | Opsin expression differences

The first and second principle components (PCs) combined explained more than 99.9% of the variance in opsin expression (Table 1). Based on the likelihood ratio test, neither depth ($p = .488$) nor time ($p = .186$) contributed substantially to explaining PC1, but depth ($p = .030$) was maintained in the final model for PC2 (time: $p = .962$). SWS1 has the strongest loading on PC2, followed by LWS, RH2 and SWS2 (Table 1).

In analysing each opsin separately, we only tested the effect of depth because time had no significant contribution to either PC1 or PC2. The expression of *SWS1* had a significant negative covariance with depth for *SWS1* (Figure 1 and Table 2), suggesting that males become less sensitive to shorter wavelengths with increasing depth.

TABLE 1 A principle component analysis of the expression of four opsins. The first row provides the percentage of the variance explained for each principle component (PC) and the subsequent rows the loadings for each opsin

	PC 1	PC 2	PC 3	PC 4
Variance explained (%)	86.0	13.9	<0.001	<0.001
SWS1	0.1978	0.799	−0.269	0.500
SWS2	0.002	−0.022	0.866	0.500
RH2	−0.786	−0.217	−0.293	0.500
LWS	0.586	−0.560	−0.304	0.500

The other three opsins did not covary significantly with depth (Figure 1 and Table 2). The analyses with the *ln*-transformed data show similar results, but *SWS1* turned nonsignificant (see Appendix S2).

To estimate absorbance, we used the linear models to first predict opsin expression at extreme ends of the natural gradient, 0.32 m and 2.47 m, and subsequently calculated the absorbance of predicted expression phenotypes at these depths (Figure 2a). As we lack proper sample sizes on the extreme ends of the depth gradient to conduct a formal statistical test, we visually evaluated the data. We see this approach as an exploratory analysis to help inform future work. Deep fish showed a small decrease in absorbance in the shorter part of the wavelength range and an increase of absorbance in the mid-range relative to the shallow fish (Figure 2b).

3.2 | Differences in opsin expression in the experiment

We next assessed the effects of the light treatment (estimates are relative to the deep treatment), sex (estimates are relative to females) and their interaction using linear mixed-effects models. We find that individuals in the medium depth treatment had significantly higher *RH2* expression and lower *LWS* expression relative to deep treatment (Figure 3 and Table 3). The expression of *SWS1* and *SWS2* was not significantly affected by the treatment. In summary, the light treatment changed the expression of opsins that affect the mid- to long-wavelength range mostly. Significant differences in *SWS1* were found between the sexes with lower expression for males (Figure 3 and Table 3). All other opsins showed no significant differences between the sexes. The interaction between treatment and sex was only significant for *SWS2* with males having lower expression in medium depth treatment and higher in the deep treatment compared to females (Figure 3 and Table 3). The results of the *ln*-transformation were qualitatively similar but nonsignificant, except for the interaction between treatment and sex for *SWS2* (see Table S4).

The differences in opsin expression were subsequently used to estimate the light absorbances of each individual. The absorbances of the two treatment groups were not statistically different based on a permutation test ($p = .079$, Figure 4a; for chromophore ratios fixed for A1, $p = .089$, and fixed for A2, $p = .119$). Figure 4b shows that the absorbance differences were most pronounced in the mid- and long-wavelength regions, as predicted from the opsin expression results.

3.3 | Small differences in magnitude of plasticity among treatments

The opsin expression differences between the two treatments indicate that expression can respond on short timescales (weeks) to the local light environment. We tested whether we could detect this as a positive correlation between change in depth (depth of

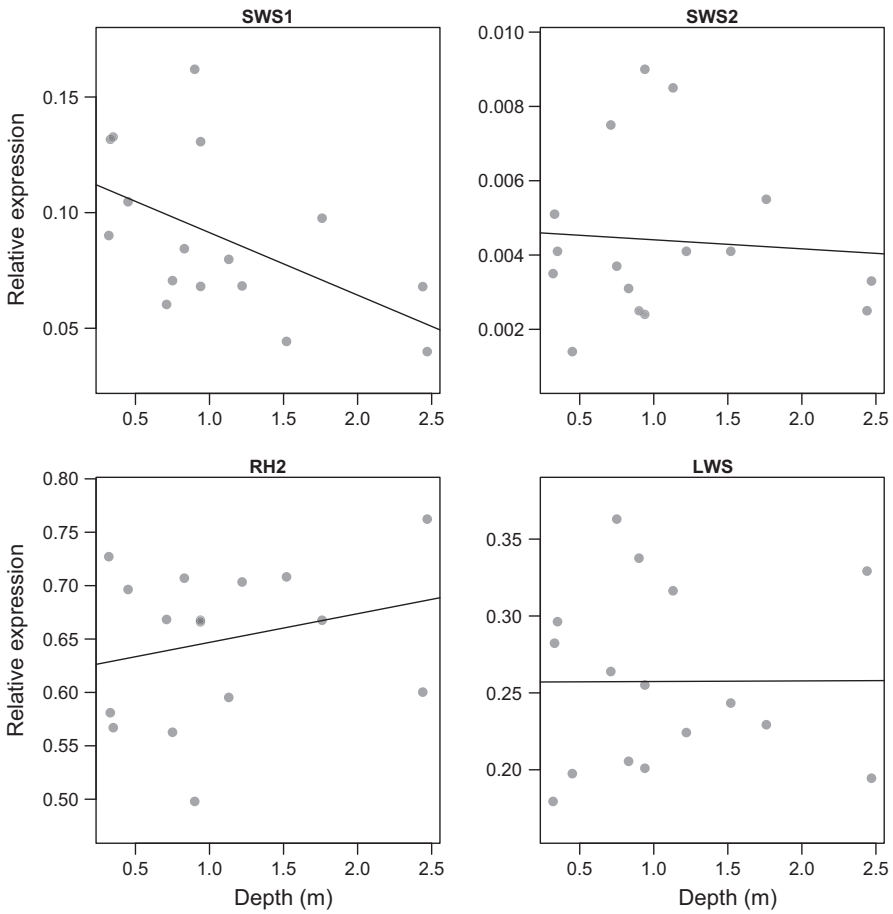

FIGURE 1 Relative expression of four opsin genes (SWS1, SWS2, RH2 and LWS) against the nesting depth of the collected males. The solid line is estimated using a linear model (see Section 2 for details)

TABLE 2 Regression analysis of relationship between depth and the expression of each of the four opsin genes

	Estimate (*SE*)	$t_{1,14}$	*p*	Adjusted R^2
SWS1	−0.027 (0.012)	−2.326	.036*	.227
SWS2	−0.001 (<0.001)	−0.279	.784	−.066
RH2	0.027 (0.028)	0.969	.349	−.004
LWS	<0.001 (0.023)	0.017	.987	−.071

*$p < .05$.

capture–depth of light treatment) and change in opsin expression (predicted opsin expression at depth of capture–measured opsin expression after experiment). We found suggestive evidence for this trend in males in *SWS2* (females do not have a clearly defined depth of capture, so we could not impute their expected pre-experiment expression). The change of *SWS2* showed a positive (but not statistically significant) relationship with change in depth (Figure 5 and Table 4). In other words, fish originating in shallow water but transplanted into a light treatment mimicking the deeper habitat (negative depth change) had a weak decrease in *SWS2* expression and thus reduced sensitivity to the mid- to low-wavelength range. There was no significant relationship for the other genes (Figure 5 and Table 4).

4 | DISCUSSION

Sensory systems can be tuned to different types and intensities of stimuli. We provide evidence that in nature, the visual system adjusts to heterogeneity in the light environment at remarkably small spatial scales, on the order of metres. As far as we are aware, this is among the smallest scales on which visual adjustment has been found in nature, although the magnitude of the effect is small.

4.1 | Natural light gradient

The side-welling light environment in Gosling Lake becomes enriched for longer wavelengths (greens, yellows and oranges) with increasing depth along a 2-m depth gradient. We find a corresponding change in expression of *SWS1* opsins along this gradient in the resident population of three-spine stickleback. Individuals at the deep end of the gradient have lower absorbance across the shorter wavelengths and elevated absorbance across mid-wavelengths relative to individuals inhabiting the shallow end of the depth gradient. Male stickleback nesting at deeper sites had elevated absorbance broadly matching the available light. These differences in absorbance were found across a very fine spatial scale.

Previous work has documented spatial covariance between ambient light and visual system properties, but at much larger spatial or taxonomic scales. Most examples entail visual differences between allopatric populations or even different species (e.g., Cummings & Partridge, 2001; Fuller et al., 2005). Differences in absorbance have been described between Lake Victoria cichlid species occupying habitats differing by 4–8 m in depth (Seehausen et al., 2008) or larger depth differences (Smith et al., 2011). However, this is still a much greater spatial difference than what we describe here. In cichlids, the *LWS*-driven adaptation (affecting absorbance of longer wavelengths) contrasts with our results, in which changes mostly involved *SWS1* (absorbing shorter wavelengths). These contrasting results could be attributed to differences in the local light environments of the respective study systems, as these water bodies likely differ in dissolved solutes.

Here we show that differences in absorbance that correspond to the environment can occur within a population. Our experimental work using enclosures (discussed below) provided further support for this idea that that light environment is an important factor which influences small-scale shifts in phenotype. However, as temperature has been shown to effect opsin expression in butterflies (Macias-Muñoz, Smith, Monteiro, & Briscoe, 2015), we cannot exclude a role of this factor in our study, as it likely covaries to some degree with water depth. Although typically we find negligible shifts in water temperature over the vertical depth range examined in this study (D. I. Bolnick, unpublished data), the thermocline in Gosling Lake occurs much deeper than the range of nest depths surveyed here. Regardless of the

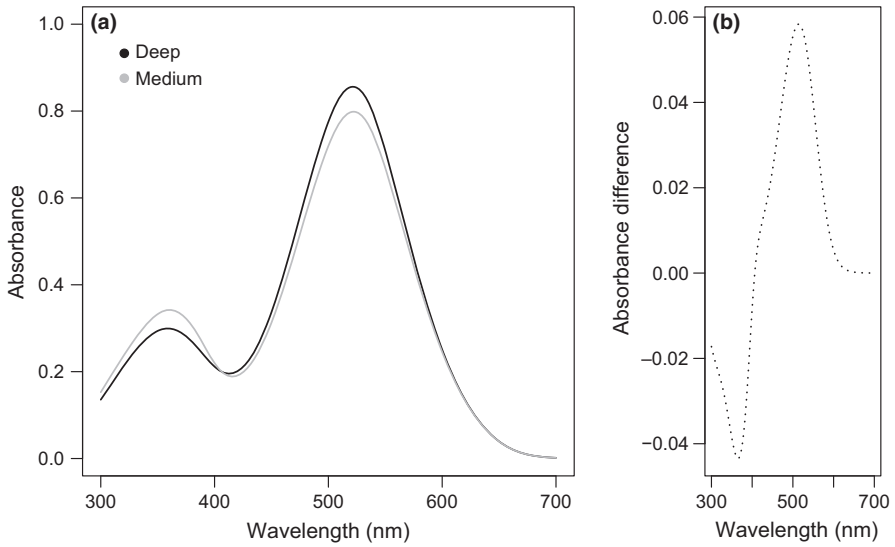

FIGURE 2 (a) The predicted mean normalized absorbance of individuals in the shallow (0.32 m: grey) and deep (2.47 m: black) end of the natural depth gradient. (b) The difference between the shallow and deep individuals on the gradient. Absorbance based on an equal A_1/A_2 chromophore ratio

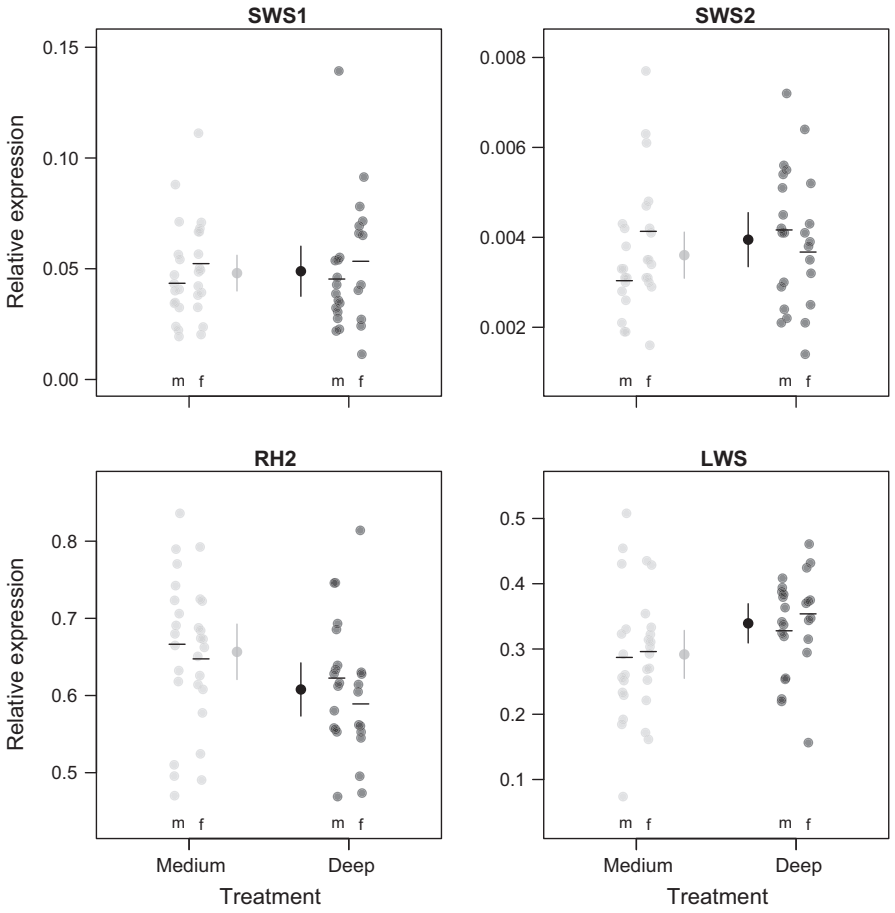

FIGURE 3 Relative expression of each of the four cone opsins in the medium depth (grey) and deep (black) light treatment for both males and females. The mean for the males (m) and females (f) is given by a horizontal line and the grand mean of each treatment with 95% confidence intervals is depicted next to each treatment

TABLE 3 Effects of light treatment, sex and their interaction on expression of the four opsins. In the case of a significant interaction, no further model reduction was performed, and hence, no χ^2 and *p*-value are available for the two fixed effects. Estimates are relative to the deep treatment and to females for sex

Opsin	Fixed effect	Estimate (*SE*)	χ^2_1	*p*
SWS1	Treatment	<−0.001 (<0.005)	0.010	.919
	Sex	−0.010 (0.005)	4.279	.039*
	Treatment × sex	<−0.003 (<0.010)	0.071	.790
SWS2	Treatment	<0.001 (<0.001)		
	Sex	<0.001 (<0.001)		
	Treatment × sex	−0.002 (<0.001)	5.280	.022*
RH2	Treatment	0.0488 (0.024)	3.991	.046*
	Sex	0.026 (0.024)	1.152	.282
	Treatment × sex	−0.015 (0.049)	0.093	.760
LWS	Treatment	−0.047 (0.023)	4.074	.044*
	Sex	−0.017 (0.024)	0.525	.469
	Treatment × sex	0.017 (0.048)	0.134	.714

*$p < .05$.

causal mechanism, phenotypic variation along small geographical scales may be more common than previously appreciated and may play an important role in maintaining genetic and phenotypic diversity (Anderson, Perera, Chowdhury, & Mitchell-Olds, 2015; Langin et al., 2015; Richardson, Urban, Bolnick, & Skelly, 2014).

Future work is required to further examine the patterns that our study has revealed. For example, the differences found in this study are relatively small and their functional implications need to be tested directly. It is currently unclear what aspect of colour vision (e.g., photon capture, wavelength discrimination) is important for driving the observed shift in absorbance. The independent evolutionary origin of many stickleback populations on Vancouver Island allows for replication of this study in the future to test whether the visual adaptation has evolved in parallel and thus may be adaptive (sensu Rennison et al., 2016). In future studies, the inclusion of "black-water" lakes, where the light gradient is reversed compared to the clear-water lakes like Gosling, could help to uniquely verify the effect of the light environment; we predict we will find reversed opsin gradients in these lakes.

4.2 | Plasticity in opsin expression

Fish in the simulated medium depth and deep light environments exhibited weakly differentiated (but not statistically significant, $p = .061$) opsin expression. Oddly, this plastic change entailed different opsins (*RH2* and *LWS*) than those underlying the natural gradient, *SWS1*. This disconnect is likely because our light filters did not achieve the intended goal of mimicking shallow and deep light environments. Rather, the light filters generated light conditions that most resembled medium–deep vs. deep natural light environments.

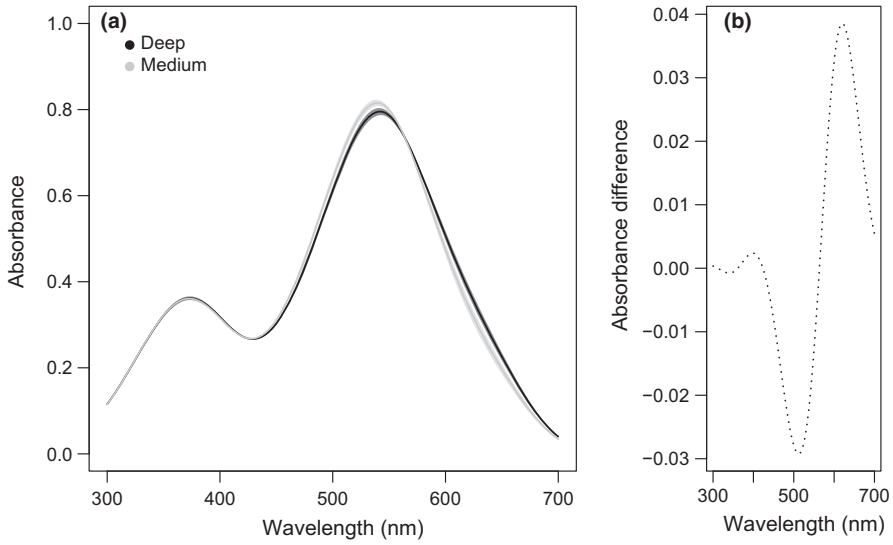

FIGURE 4 (a) The mean normalized absorbance of individuals in the medium (grey) and deep (black) depth treatments (solid line). The shaded areas represent the standard error around the means. (b) The difference between the mean of the deep and medium depth treatments

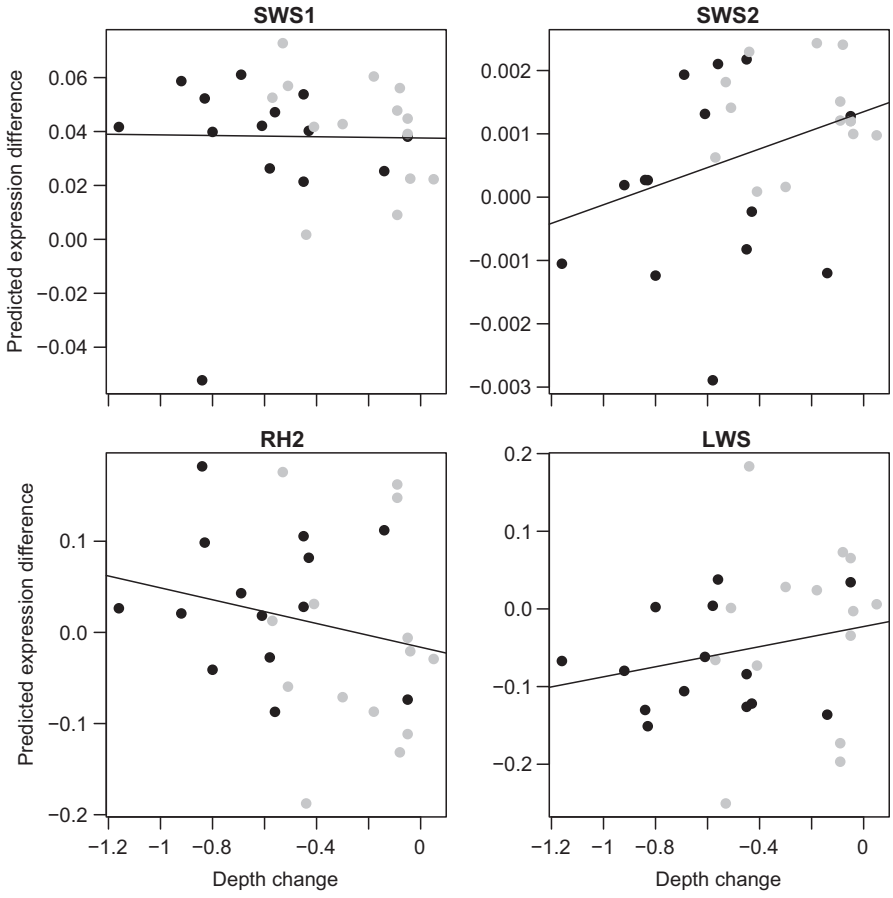

FIGURE 5 The difference in predicted opsin expression of males at the start of the experiment and the measured expression at the end (expression change) against the difference in depth at which the male was caught and the depth of the deep (black) and medium depth (grey) experimental light treatments (depth change). Negative values thus indicate a reduction of expression or depth between the location the males were caught and the experimental treatment

Accordingly, we had to adjust our predictions such that fish from both treatments would generally shift towards a better match to the middle and deeper end of the gradient. *SWS1* largely mediates differences along the natural cline (with lower expression at greater depths); correspondingly, we see that individuals in both treatments reduced their *SWS1* expression. The differences between our two treatments in *RH2* and *LWS* indicate that opsin expression may be "fine-tuned" to the local light environment, which may be a response to unanticipated effects of the filters.

Despite not capturing as large of a range of the light gradient as we anticipated, our experiment showed a strong plastic response of *SWS1* expression in the predicted direction and evidence of fine-tuning of expression to relatively small differences in light environment. This result suggests that plasticity contributes strongly to variation in the stickleback sensory system across the small-scale natural light gradient described above. Furthermore, our study shows that experimentally manipulating light environments in the wild is possible. However, we advise future researchers to choose light filters after

TABLE 4 Correlation between change in depth (depth of capture–depth of light treatment) and change in opsin expression (predicted opsin expression at depth of capture–measured opsin expression after experiment) for male stickleback

	Estimate (*SE*)	$t_{1,26}$	*p*	Adjusted R^2
SWS1	−0.001 (0.015)	−0.078	.939	−.038
SWS2	<0.001 (<0.001)	1.961	.061	.095
RH2	−0.065 (0.058)	−1.122	.272	<.01
LWS	0.065 (0.057)	1.141	.264	.011

testing their effect in the intended environment, rather than on the basis of the light transmission of the filters alone.

We also tried to examine the plasticity of opsin expression by comparing the predicted expression at individuals' original capture depth (using the natural gradient) with the expression at the end of the experiment. We would expect that fish experiencing a larger change in light environment (the difference between depth of capture and the "depth" of the light treatment) would exhibit larger changes in opsin expression. Again, we would expect this to be most pronounced for *SWS1*. This expectation was not supported by our analyses, as no substantial correlation was found. One plausible reason why this failed is that our proxy for opsin expression at the depth of capture when estimated from the linear model is too crude of a measure, and with the relatively low sample sizes we have, we are unable to detect a signal, particularly if the effect size was small. Furthermore, most fish used in the experiment were caught in quite shallow water which, when combined with having only relatively deep light treatment environments, only gave us one part of the opsin change spectrum, namely from shallow to deep, which reduced the power of our approach. Future studies should increase sample sizes and ideally have light treatments spanning a larger part of the depth range, as males do nest deeper than our deepest male.

4.3 | Sex differences

In stickleback, the male defends the nest and hence remains most consistently at a certain (nest) depth (personal observations, Snowberg and Bolnick, 2012). Female stickleback move around different depths which could affect the strength of selection for adjustment to the local light environment. The literature contains conflicting reports of sex-specific spectral sensitivity in stickleback. Cronly-Dillon and Sharma (1968) found that females were more sensitive to longer wavelengths compared to males in summer, but not different in winter. Boulcott and Braithwaite (2007), however, found that both sexes become more responsive to longer wavelengths during the breeding season. Although we cannot contrast different seasons, we did find a significant lower expression in males for one opsin (*SWS1*). This is predicted to lead to reduced absorbance, by males, of the short end of the wavelength spectrum. Although our result suggests a sex difference during the breeding season, the biological relevance and strength of the difference should be validated ideally by sampling both sexes across the same depth gradient at the same period of time or from schools consisting of both sexes just before the breeding season starts.

4.4 | Challenges of studying visual adaptation

Understanding visual adaptation is challenging and requires important assumptions about how opsin gene expression translates into photon absorption, nerve activation, brain perception and behaviour (e.g., mate choice). However, there is good evidence that the visual system adjusts to the local light environment and that shifts in opsin usage are biologically relevant. In cichlids, protein coding sequences vary with different light environments at different depths (Seehausen et al., 2008). In birds, the distribution and relative abundance of photoreceptor pigments within the avian retinal mosaic are strongly correlated with habitat type, diet and feeding behaviour, strongly suggesting that changes in photoreceptors have significant functional effects (Hart, 2001). In stickleback, it has also been shown that there are consistent and strong associations between estimates of spectral sensitivity and light environment (Rennison et al., 2016). All of these findings suggest that changes in opsins are biologically relevant. However, it remains unclear what functional effect these changes have on visual perception.

Translating opsin gene expression to visual sensitivity in a meaningful way is difficult. The current approaches, such as those used to calculate absorbance in this study, rely on strong assumptions that need much more empirical support. We hope that future empirical and theoretical studies will work towards refining the models that predict the visual capacities of organisms, to aid in linking molecular changes in the visual system to the ecological and evolutionary consequences. We also believe that controlled experiments under laboratory conditions will provide valuable insights and further our ability to distinguish the relative importance of genetic determination of opsin expression vs. plastic response. We believe that a combination of correlational studies from the field (described here) and experiments in the field and (in the future) in the laboratory combined with neurological studies will be important to formulate a predictive theory of visual ecology which allows for more powerful empirical testing.

5 | CONCLUSION

Our results indicate modest adjustments of the visual system of wild fish to environmental differences on a very small spatial scale, which is likely due to plasticity in opsin expression. Both the mechanisms and implications of this rapid adjustment remain uncertain. The most immediately obvious implication is that small-scale light environment variation may promote phenotypic variance in the visual system within populations. This microgeographic variation may be confused for nonadaptive "noise" in studies that focus on visual differences among geographically defined populations (including our own work [Rennison et al., 2016]). In reality, such phenotypic noise may be a form of fine-tuned visual adaptation. The impact that these

differences have on other processes such as foraging, predator evasion and mate choice remains to be evaluated. Is environmentally induced variation in vision responsible for some of the dramatic variation in individual foraging behaviour? Or, is the simultaneous change of male nuptial colour signals and receiver vision responsible for some of the assortative mating observed within stickleback populations (Ingram, Jiang, Rangel, & Bolnick, 2015; Snowberg & Bolnick, 2008, 2012)? Our findings open a new window on the potential for heterogeneity in light environments to drive phenotypic variation with potentially wide-ranging consequences in behaviour, ecology and evolution.

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AUTHOR CONTRIBUTION

T.V., C.D.B. and D.I.B. designed the study; T.V., C.D.B. and D.J.R. conducted the fieldwork; D.J.R. and T.V. conducted the opsin expression analysis; T.V., C.D.B., D.J.R. and D.I.B. contributed to data analysis and writing the paper.

DATA ACCESSIBILITY

Data and R code available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.45k67.

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