Supplementary Materials

1. Collection and Site Information

Fish were collected from eleven populations from eight different lakes, rivers or lagoons (from now on referred to as *location*): two marine, three solitary and three species-pair locations (see Supplementary Table 1 for details). In order to measure opsin gene expression, six gravid females were collected from each of the populations. All fish from a given population were taken at the same time and the collections were taken between 10 am and 12 pm during the period of May 16th to May 30th 2012. Fish were euthanized at the site using buffered Tricaine methanesulfonate (MS-222). Both eyes were removed and immediately stored in 1 ml RNAlater® (Qiagen, Netherlands) and moved to a -20^{0} C freezer for up to a month until RNA was extracted. Irradiance was measured in July 2012.

Three families of Priest Benthic and three families of Oyster Marine fish were generated by *in vitro* fertilization in May and June 2012 respectively. These fish were hatched and reared in freshwater tanks under fluorescent lights on a 14 and 10 hour light-dark cycle. Animals were treated in accordance with University of British Columbia Animal Care protocols (Animal Care Permit # A11-0402). Gravid females were sacrificed using MS-222 between June 5th and 7th 2013. One fish from each family was surveyed (three fish per population). Both eyes were immediately removed and put directly into RNAlater®. Samples were stored in RNAlater® for one week at -20^{0}C until RNA was extracted.

2. RT-qPCR Protocol

Left and right eyes were pooled for each individual. The pooled eyes were homogenized in a Retsch mm 400 Mixer Mill (Haan, Germany) using a carbide bead. Total RNA was extracted using the Aurum™ Total RNA Fatty and Fibrous Tissue (BioRad®), which included a DNase I incubation step. The concentration and purity of the extracted RNA was assessed on a NanoDrop® Spectrophotometer (Thermo Scientific). Synthesis of cDNA was accomplished using the iScriptTM cDNA Synthesis Kit (Bio-Rad®); 1000 ng of RNA was

used as the input for the cDNA synthesis of each sample. The resulting cDNA was diluted 1:100 in ultra-pure water for RT-qPCR analysis.

RT-qPCR primers and probes were designed using sequences from the stickleback genome (See supplementary Table 2 for primer and probe sequences). Primer sequences were targeted to regions that were divergent between the five opsin gene subfamilies. Despite the fact the stickleback have two *RH2* genes the primers were designed to pick up only one of the duplicates because there is no evidence to suggest they would have different absorption phenotypes and there is some evidence to suggest that the non-targeted duplicate may be a pseudogene (Rennison et al., 2012). For each gene one of the primers and/or the RT-qPCR probe spanned an intron, this was done to avoid amplification of genomic DNA. We used the PrimeTime® qPCR 5' Nuclease Assays from Integrated DNA Technologies® (Iowa, USA) for each of the targeted genes. The assays used had a double-quenched probe with 5' 6-FAM™ dye, internal ZEN™ and 3' Iowa Black® FQ Quencher.

Quantification of gene transcript copy number was done using RT-qPCR analysis on a BioRad®IQ5 machine (BioRad, California USA). The polymerase used was the SsoFast probes supermix (BioRad®) in a 25 µl reaction. Reactions were run in 96-well plates (Fisher, Massachusetts USA), which were sealed using optical sealing tape (BioRad®). 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 and for every run and did not amplify. RT-qPCR conditions consisted of 1 cycle at 95 °C (3 minutes); 40 cycles of 95 °C (10 seconds) followed by 60 °C (30 seconds). 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,

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

where the slope is determined from a linear least squares regression fit to critical threshold (Ct) data from a cDNA dilution series (1:10, 1:50, 1:100, 1:500, 1:1000).

We calculated opsin expression relative to the *beta actin* reference gene, however for the purposes of this study we were more interested in the

expression of each opsin gene relative to the total opsin levels present in the retina, rather than absolute levels of expression, so we used the proportion of total opsin expression for a given gene. The estimate of the initial amount of gene transcript (T_i) was 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.

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

Then for each individual we summed the opsin gene expression across the four opsin genes and calculated the proportion of total expression that each gene exhibited.

Amplicons from the RT-qPCR for each gene (primer pair) were sequenced from one individual and are reported in Supplementary Table 2. Sanger sequencing of the amplicons was done at the NAPS Sequencing Centre at the University of British Columbia.

3. Deriving Spectral Sensitivity

We used the absorbance templates for A1 chromophore (unless otherwise stated) for each of the four cone opsins from Govardovskii *et al.* (2000) and the wavelengths of maximum absorbance (λ_{max}) for each opsin from Flamarique et al. (2013). The spectral sensitivity of an individual (i) at a given wavelength (λ) for a particular opsin (o) is the multiplication of its absorbance ($A_o(\lambda)$) and relative expression ($E_{i,o}$). The overall sensitivity of an individual is the sum over of all opsins and is defined across the visible wavelength range (350 to 700 nm) by

$$S_i(\lambda) = \sum_{0<o<5} A_o\,(\lambda)\; E_{i,o}.$$

4. Plasticity in the Laboratory Environment

To assess the effect of plasticity on opsin gene expression, we looked at the difference between wild and lab-reared fish derived from one marine and one freshwater location (Figure 4). While much of the differentiation in gene expression between marine and freshwater individuals was maintained in the lab, some plasticity was still seen; the level of *SWS1* (UV) expression

was reduced in both types of lab reared fish relative to their wild counterparts (marine difference = 0.16 ± 0.04 SE, p=0.003, $F_{1,7}$=19.9; freshwater difference = 0.06 ±0.02 SE, p=0.007, $F_{1,7}$= 14.3) (Figure 4). There was also a significant increase in *SWS2* (blue) expression for the lab-reared marine fish compared to wild individuals, although the effect size was small (difference =0.013 ±0.004 SE, p=0.009, $F_{1,7}$=12.9) (Figure 4). However, this was not seen for the freshwater population (p=0.2) (Figure 4). There was not a significant difference in the *LWS* or *RH2* expression of wild and lab reared fish (p>0.09) (Figure 4).

These results indicate that there is a small contribution of plasticity in stickleback opsin gene expression. Raising the fish under artificial (fluorescent) lighting that lacked UV wavelengths likely contributed to the reduction in *SWS1* expression that we saw in lab-reared fish. This same pattern has been previously described in cichlids, where lab-reared individuals raised under artificial lighting had reduced *SWS1* expression compared to wild caught fish (Hofmann et al., 2010).

5. Association between differences in Spectral Sensitivity and Ambient Light

We use two functions of wavelength (λ) to characterize ambient light: the irradiance $I_s(\lambda)$ and the transmission $K_s(\lambda)$, with the values of λ between 350 and 700 nm. Recall from the main text that the irradiance is taken to be the irradiance measured at depth 50m. To construct $K_s(\lambda)$ we use transmission coefficients, as defined by the Beer-Lambert law, which gives transmission T_s at depth (d) and wavelength (λ) as

$$T_{s,d}(\lambda) = b(\lambda)e^{-K_s(\lambda)d}.$$

For each site and each value of λ, we estimated the unknown parameters $b(\lambda)$ and $K_s(\lambda)$ using the *nls* function in R. We then smoothed the resulting $K_s(\lambda)$ values using a rolling mean approach (as implemented in the R *zoo* library, Zeileis and Grothendieck, 2005, with window width 10 nm). For each site, these smoothed $K_s(\lambda)$ values were then normalized to sum to 1, as we want to compare the difference in relative absorbance between different locations. Hereafter, $K_s(\lambda)$ refers to the smoothed and normalized values of

the site-specific transmission coefficients. For each location, we then constructed the 'representative' transmission coefficient curve, $\overline{K}_s(\lambda)$, by calculating at each value of λ the median of the $K_s(\lambda)$'s from all sites within that location.

To quantify ambient light differences between freshwater and marine locations, we chose a reference marine location (A), and refer to its curve of transmission coefficients as $\overline{K}_{s,A}(\lambda)$. We then calculated the difference between the transmission coefficients for each freshwater location (B) that we wanted to test and the reference marine location (A) as $\Delta K_s(\lambda) = -1(\overline{K}_{s,B}(\lambda) - \overline{K}_{s,A}(\lambda))$. We multiplied the difference by -1 to facilitate the comparison between $\Delta S_{i,}$, ΔK_s and $\Delta \mathrm{I}_s$ (see Figure 6). Note that, in our definition, ΔK_s is a measure of light propagation (instead of rate of absorbance). A positive value of $\Delta K_s(\lambda)$ indicates more transmission of light (*i.e*. fewer photons are lost as light travels through water) at wavelength λ at the freshwater location *B* than at the reference marine location. For this analysis we used Oyster Lagoon as our marine reference location (A). We repeated this procedure (without multiplying by -1) to calculate the difference between environments in irradiance ($\Delta \mathrm{I}_s$). Again for irradiance a positive value of $\Delta \mathrm{I}_s(\lambda)$ indicates that there are more photons present at wavelength λ at the freshwater location (B) relative to the marine reference environment (A).

To quantify differences in spectral sensitivity between each location B and the reference marine location A, at each wavelength λ we first calculated $\bar{S}_A(\lambda)$ as the median of the spectral sensitivities of all measured individuals from the reference location A. For each individual *i* in location B, we calculated the difference between that individual's spectral sensitivity at each wavelength $S_{i,B}(\lambda)$ and the marine location A spectral sensitivity: $\Delta S_{i,B}(\lambda) = S_{i,B}(\lambda) - \bar{S}_A(\lambda)$.

We are now able to proceed with studying association between differences in spectral sensitivity and ambient light, using each fish's spectral sensitivity and its light environment (transmission and irradiance) at each wavelength, all measured relative to the reference marine location A. A scatterplot of spectral sensitivity difference against difference of light environment at all wavelengths allows us to visually assess the association

between the two variables. To proceed with a statistical analysis, for each fish, we summarized and quantified this strength of association via the correlation coefficient (r). If the correlation coefficient calculated for fish *i* is positive, then, for that fish, wavelengths showing elevated sensitivity (positive $\Delta S_{i,B}\ (\lambda)$'s) are associated with increased light propagation (higher transmission) in our transmission calculations and, in our irradiance calculations, are associated with more photons (higher irradiance). We calculated this association summary for every fish in location B. We tested whether the mean association (that is, the mean of the correlations in the population) in location B was zero using a one-sample t-test. We did this for all locations. Our results are contained in Supplementary Table 3 and reported in the main text. We also tested simultaneously the equality of the means of the individual level measures of association of all freshwater locations by fitting a mixed-effects model to the measure of association, with location as a random effect.

To study differences in environment (pelagic versus littoral) we carried out separate analyses for each of the two species pair lakes with data on light environment (Paxton and Priest). For each lake, we took as reference the limnetic population, and thus the pelagic environment. Our calculations were the same as in the marine/freshwater comparison above, with the littoral environment (with benthic fish) being substituted for 'location B' in our calculations. Thus, in each lake, for each benthic fish, we considered the relationship between differences in its spectral sensitivity (relative to median limnetic sensitivity) and differences in light environment (relative to that lake's median – representative pelagic environment). We summarized that relationship for each benthic fish by the correlation and then tested whether these correlations were expected to equal to 0. The results did not differ greatly if the benthic population was used as the reference. See Supplementary Table 4 for complete results, including those discussed in Supp. Mat. Section 6.

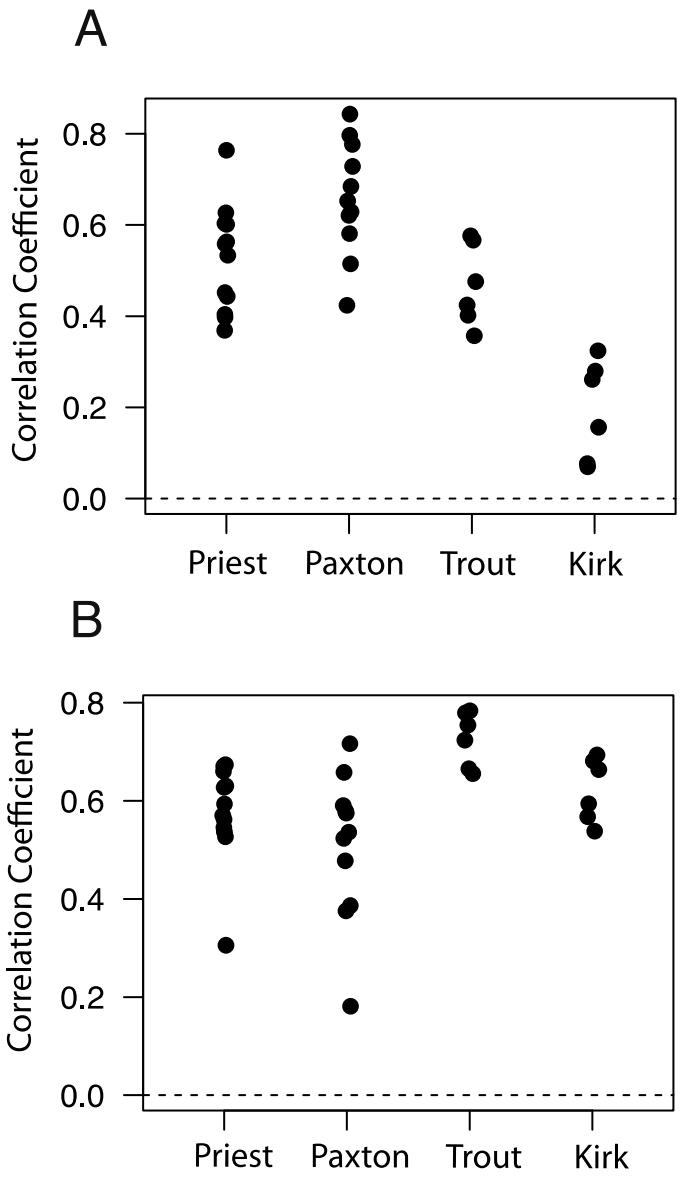
6. Effect of Changing Chromophore or Reference Population in the Analyses of Differences in Sensitivity and Differences in Light Environment

Recall that, in our initial analyses (reported in the main text), spectral sensitivity was modeled using exclusively the A1 chromophore. Our reference population for the marine-freshwater comparison was Oyster Lagoon and our reference population for the species pair analysis was the limnetic population. We studied the robustness of our results to using different chromophores and different reference populations.

To study the importance of the reference location in the marine/freshwater comparison, we first made a direct comparison of Oyster Lagoon and the other marine location, Little Campbell River, using Oyster Lagoon as reference. That is, Oyster Bay served as “A” and Little Campbell River served as “B” in the analysis in Supp. Mat. Section 5. We found that there was no significant association between differences in sensitivity and differences in transmission (data not shown), but there was a significant association for the irradiance. In other words, changes in sensitivity in Little Campbell relative to the reference Oyster Lagoon population did not significantly covary with changes in transmission but did significantly covary with changes in irradiance. Thus, we might infer that Little Campbell River and Oyster Lagoon are equivalent reference populations for transmission analysis, but perhaps not for irradiance analysis.

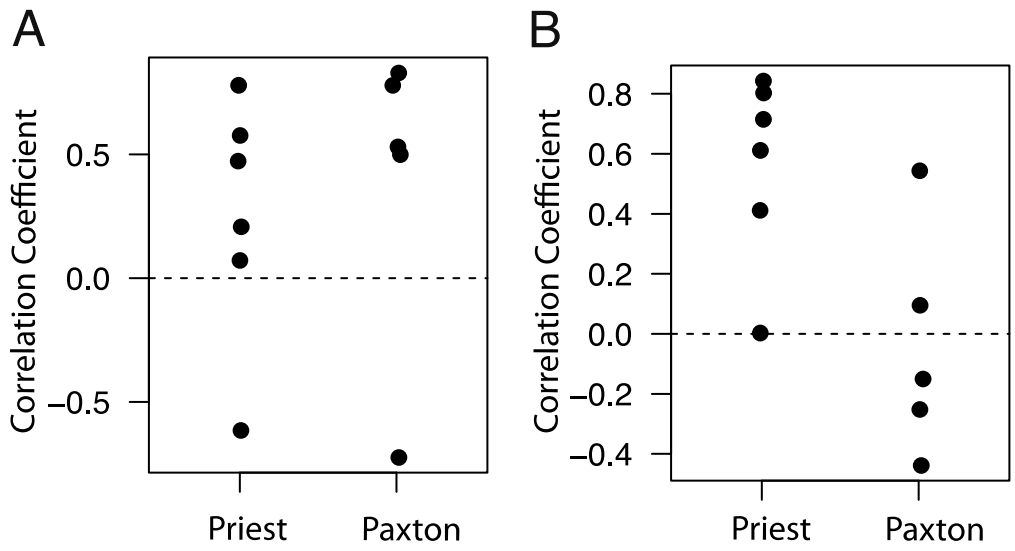
In order to further study whether reference population affected the results we re-did the analysis, described in the main text and in Supp. Mat. Section 5, using Little Campbell River instead of Oyster Lagoon as the marine reference (Supplementary Figure 1A and B). The results are given in Supplementary Table 3. Overall the results with the two base lines agree; the mean correlations and significance levels are very similar for transmission. When the A1 chromophore is used for estimation of sensitivity the correlation for irradiance is also similar, however when other chromophores are used the correlation becomes significantly negative. We believe that Little Campbell River is a much less reliable marine reference population because the

measurements were taken in the tidal (marine) part of the river where turbidity increases significantly when the tide comes in. The light measurements were taken with incoming tide and hence may give a biased view of the light environment the stickleback experience most of the time, and this may explain the odd result for irradiance.



Supplementary Figure 1. Quantification of correlation between differences in spectral sensitivity and differences in local light transmission (A) and irradiance (B) for marine and freshwater populations. Circles indicate individuals' correlations. All populations are presented relative to the marine reference location, Little Campbell River.

To determine the importance of the reference population in the analyses for the two species pair locations (Priest and Paxton), we repeated the analysis using the benthic ecotype as reference instead of the limnetic. For all chromophore combinations the results are very similar to those obtained using the limnetics as a reference for both transmission (Supplementary Figures 2 and Supplementary Table 4).



Supplementary Figure 2. Quantification of correlation between differences in spectral sensitivity and differences in local light transmission (A) and Irradiance (B) for benthic and limnetic populations. Circles indicate individuals' correlations, using the benthics as reference population.

7. Effect of Changing Chromophore in the Analysis of the Correlation Between Spectral Sensitivity and Ambient Light (Spectral Matching).

To study the effect of chromophore on our spectral matching results, we repeated the analysis reported in the main text with various different chromophore combinations in the freshwater population. The combinations and the results are presented in Supplementary Table 5. Switching the ratio of

chromophore used did little to affect the magnitude of the correlation between spectral sensitivity and Transmission. However the magnitude of the correlation strengthened for irradiance when there was a 50:50 mix of A_1 and A_2 used.

8. Supplementary Figures and Tables

Supplementary Table 1. Overview of the different stickleback populations used, locations, and sample sizes (# of fish) for opsin expression and environmental light conditions (irradiance) for shallow (≤ 6m) and deep (> 6m) sampling sites.

Name	Latitude	Longitude	Type		Sample Size	Irradiance (# of sites)	
						≤6m	>6m
Oyster Bay	49.61210	-124.03186	Marine		6	10	0
Little Campbell River	49.01543	-122.77662	Marine		6	10	0
Trout	49.50820	-123.87641	Solitary		6	10	5
Cranby	49.69537	-124.50812	Solitary		6	*failed*	
Kirk	49.73897	-124.58680	Solitary		6	7	5
Paxton	49.70789	-124.52492	Species -pair	Benthic	6	10	5
				Limnetic	6		
Priest	49.74517	-124.56519	Species -pair	Benthic	6	10	5
				Limnetic	5		
Little Quarry	49.66266	-124.10888	Species -pair	Benthic	6	*failed*	
				Limnetic	6		

Lake						

Supplementary Table 2.

Primer, probe and amplicon sequences from RT-qPCR assays.

Gene	Probe Sequence 5'-3'	Primer Sequences 5'-3'	Amplicon Size (bp)	RT-qPCR Amplicon Sequence
SWS1	CCGTAGCAGGACTGGTGACAGC	Forward: ACATCACCTTGGCAGGATTC Reverse: GTGGGCTGGAACAACAGATT	279	GGTGTTTGTCGCATCTGCGAGGGGTTACTACTTCCTGGGTTACACCTTGTGCGCGCTGGAGGCTGCGATGGGATCCGTAGCAGGACTGGTGACAGCCTGGTCTTTGGCTGTTTTGTCTTTCGAGAGATATCTGATCATCTGTAAACCTTTTGGAGCCTTTAAGTTTACCAGTAACCACGCTCTCGGTGCTGTCGCCTTCACCTGGTTTATGGGAATCTGTTGTTCCAGCCCA
SWS2A	GAAAATGGCGGCAAAGGCC	Forward: TCTGCACAATTTGCTTCTGC Reverse: GGTTGTAAACTGCGGAGGAC	261	GGCGGCAAGGCCCAAGCAGAATCCGCCTCGACCCAGAAGGCGGAGCGGGAGGTGACCAGGATGGTGGTTCTCATGGTGATGGGCTTCCTGGTGTGCTGGATGCCGTACGCCTCATTCGCTCTTTGGGTGGTCAACAACCGCGGGCAGACTTTTGACCTGAGGTTTGCTTCTATTCCGTCCGTCTTTTCCAAGTCCTCCGCAGTTTACAAC
RH2	TTGGCTGGTCCAGGTACCTTCC	Forward: GGGATTCATGGCCACATTAG Reverse: TAGTCAGGTCCACACGAGCA	174	CTGGATCCTTTTCCCTGGACCATGGCTATGGCATGTGCTGCTCCCCCTCTTTTTGGTGGCCAGGTACCTTCCTGAGGGCATGCAGTGCTCGTGTGGACCTGA

LWS	TGGATGGAGC AGGTACTGGC C	Forward: GATATGGTCTG CCGTCTGGT Reverse: GCCACAATCAT GACAACGAC	297	TGGAAGTGAAGACC CTGGAGTCCAGTCC TACATGATTGTTCTC ATGATCACATGCTGT CTCATTCCTCTGGC CATCATCATATTGTG CTACCTTGCAGTCT GGTTGGCTATCCGT GCTGTGGCCATGCA GCAGAAGGAATCAG AGTCAACTCAAAAA GCTGAAAGAGACGT ATCCAGAATGGTCG TTGTCATGATTGTGG C
Beta Actin	CTGTGCTACG TCGCCCTGGA	Forward: GGCTACTCCTT CACCACCAC Reverse: CAGGACTCCAT ACCGAGGAA	329	CACAGCTGAGAGGG AAATCGTGCGTGAC ATCAAGGAGAAGCT GTGCTACGTCGCCC TGGACTTCGAGCAG GAGATGGGTACCGC TGCCTCCTCCTCCT CCCTGGAGAAGAGC TACGAGCTGCCCGA CGGACAGGTCATCA CCATCGGCAATGAG AGGTTCCGTTGCCC AGAGGCCCTCTTCC AGCCTTCCTTCCTC GGTACGTTTCCCTA CTCGAGCCTAACAG T CTCATAATGTAAATA TGTTGCTCCCTTGG TTACTCTGCACCGC CACATGCTTACAAGT GTCATCTCCCCTCA G

Supplementary Table 3. Marine-fresh water comparison of correlation between differences in spectral sensitivity and differences in ambient light. O.L. is the location Oyster Lagoon, L.C.R. is the location Little Campbell River. Correlations that are significantly different from 0 are in bold.

Reference Population	Ambient Light Measure	Chromophore		Mean Corr.	SE	T-value	Raw p-value	Adjusted p-value
		Marine	Fresh					
O.L.	Transmission	A1	A1	**0.39**	0.12	3.30	0.002	0.004
L.C.R.	Transmission	A1	A1	**0.46**	0.1	4.76	< 0.0001	< 0.0001
O.L.	Transmission	A1	A2	0.14	0.09	1.53	0.136	0.148
L.C.R.	Transmission	A1	A2	**0.26**	0.07	3.91	0.001	0.001
O.L.	Transmission	A1	50:50 A1/A2	0.22	0.12	1.85	0.074	0.088
L.C.R	Transmission	A1	50:50 A1/A2	**0.55**	0.09	6.19	< 0.0001	< 0.0001
O.L.	Irradiance	A1	A1	**0.32**	0.07	4.94	< 0.0001	< 0.0001
L.C.R.	Irradiance	A1	A1	**0.6**	0.05	13.3	< 0.0001	< 0.0001
O.L.	Irradiance	A1	A2	**-0.32**	0.12	-2.74	0.010	0.015
L.C.R.	Irradiance	A1	A2	**-0.48**	0.05	-9.29	< 0.0001	< 0.0001
O.L.	Irradiance	A1	50:50 A1/A2	**-0.23**	0.10	-2.15	0.039	0.052
L.C.R	Irradiance	A1	50:50 A1/A2	-0.13	0.09	-1.41	0.168	0.168

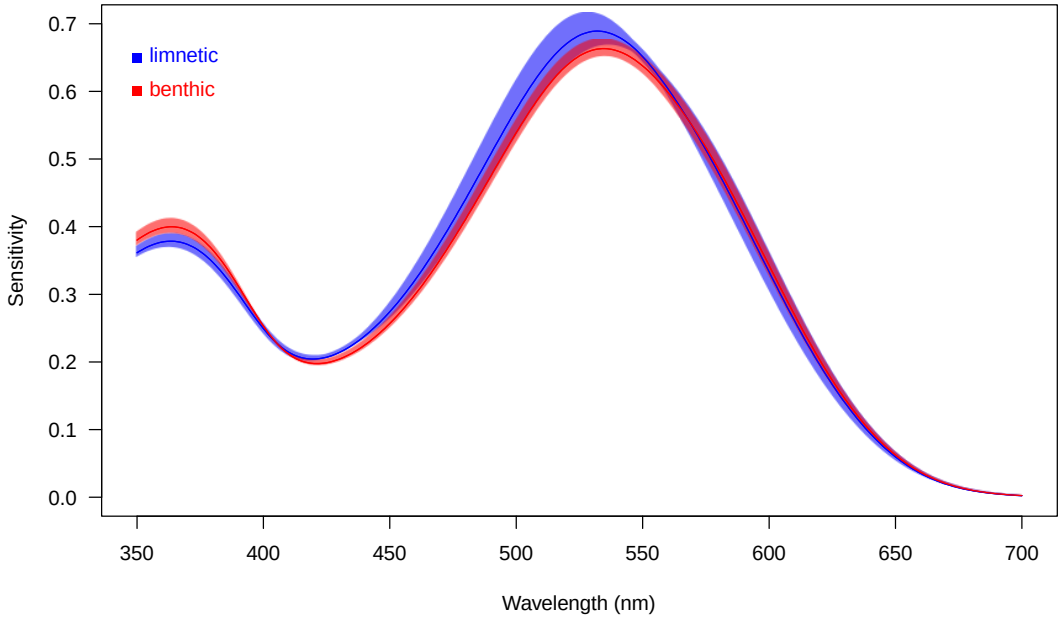
Supplementary Table 4. Benthic - limnetic comparison of correlation between differences in spectral sensitivity and differences in ambient light.

Reference Group	Ambient Light Measure	Chromophore	Mean Corr.	SE	t-value	Raw p-value	Adjusted p-value
Limnetic	Transmission	A1	0.27	0.13	1.97	0.077	0.185
Benthic	Transmission	A1	0.31	0.16	1.91	0.088	0.185
Limnetic	Transmission	A2	0.30	0.15	1.99	0.075	0.185
Benthic	Transmission	A2	0.34	0.18	1.88	0.093	0.185
Limnetic	Transmission	50:50 A1/A2	0.28	0.15	1.92	0.084	0.185
Benthic	Transmission	50:50 A1/A2	0.33	0.17	1.88	0.094	0.185
Limnetic	Irradiance	A1	0.18	0.18	1.00	0.339	0.581
Benthic	Irradiance	A1	0.27	0.30	0.87	0.402	0.603
Limnetic	Irradiance	A2	0.04	0.14	0.29	0.775	0.775
Benthic	Irradiance	A2	0.08	0.24	0.35	0.735	0.775
Limnetic	Irradiance	50:50 A1/A2	0.11	0.17	0.63	0.539	0.696
Benthic	Irradiance	50:50 A1/A2	0.18	0.31	0.57	0.580	0.696

Supplementary Table 5. Marine-Fresh comparison of correlation between ambient light and spectral sensitivity. Correlations that are significantly different from 0 are in bold.

Population Type	Water Type	Ambient Light Measure	Chromophore	Mean Corr.	SE	p-value	Adjusted p-value
Fresh	Fresh	Transmission	A1	**0.07**	0.03	0.0184	0.029
Fresh	Fresh	Transmission	50:50 A1/A2	0.04	0.04	0.2983	0.341
Fresh	Fresh	Transmission	A2	-0.04	0.04	0.3859	0.386
Fresh	Fresh	Irradiance	A1	**0.12**	0.02	< 0.0001	0.0002
Fresh	Fresh	Irradiance	50:50 A1/A2	**0.15**	0.02	< 0.0001	0.0002
Fresh	Fresh	Irradiance	A2	**0.09**	0.03	< 0.0001	0.0002

Marine	Marine	Transmission	A1	**-0.66**	0.11	0.0017	0.003
Marine	Marine	Irradiance	A1	**-0.16**	0.05	0.0230	0.031



Supplementary Figure 3. Estimated mean spectral sensitivity of benthic and limnetic populations. Benthic populations are indicated in red, limnetics in blue. The thin lines are the fitted values of spectral sensitivity from the mixed effects model. The shaded regions are one standard error above and below the fitted values, with standard errors also derived from the mixed-effects model.

Supplementary Materials References:

1.

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