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PRIMARY RESEARCH PAPER

Adaptive variation in opsin expression of sticklebacks from different photic habitats

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Abstract We studied phenotypic and genetic adaptation of the visual system of three-spined sticklebacks, *Gasterosteus aculeatus*, from North Uist, Scotland. We quantified differences in opsin gene expression of the four cone opsin genes among wild-caught fish from three lakes with clear and from three with tea-stained water and their offspring that were raised in clear water. In addition, visual sensitivity of wild-caught fish was modelled from opsin expression levels. Wild-caught fish from tea-stained waters had a lower SWS1 proportional expression than fish from clear waters, a difference that tended to be maintained in lab-bred fish. Compared to lab-bred fish, wild-caught fish had a higher SWS1 but lower SWS2 proportional expression independent of water clarity. For RH2 and LWS there were significant interactions between generation and water clarity. Reproductively mature fish had a higher LWS but lower proportional expression of RH2 than non-reproductive fish. Sex did not have a significant effect on expression. There was a significant positive association and, depending on chromophore ratio, a distinct match between the centre wavelengths, used as a proxy for spectral distribution, of ambient habitat light and spectral sensitivity indicating that the visual system of sticklebacks is tuned to their local light environment, suggesting adaptation.

Keywords *Gasterosteus aculeatus* · F1 generation · Sex · Water clarity · Phenotypic plasticity · Genetic variation

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Introduction

The aquatic habitat is a variable and dynamic photic environment in which spectral composition and light intensity are influenced by factors like water depth, suspended sediment and dissolved organic matter (Lythgoe, 1979). Many fishes rely on vision, next to other sensory modalities, for foraging, predator avoidance and reproduction. Vision is thus a trait closely connected to fitness in many fishes and thus predicted to be subject to natural and sexual selection (e.g. Horth, 2007; Sabbah et al., 2010; Davies et al., 2012). In vertebrates, cone and rod cells mediate vision, cones under bright light and rods under low light conditions. Visual sensitivity is determined by visual photopigments in the retinal photoreceptors. Visual pigments are composed of opsin proteins that are bound to a chromophore. Vertebrates have one rod opsin and four classes of cone opsins (for a detailed description of vertebrate vision, see, for example, Davies et al., 2012). Differences in opsin gene expression are assumed to reveal differences in colour vision, as opsin expression profiles are good proxies for cone cell abundance (Fuller et al., 2005; Carleton et al., 2008; Shand et al., 2008).

One expects that spectral sensitivities will be adapted to the photic environment. In the present study on three-spined sticklebacks, *Gasterosteus aculeatus* Linnaeus, 1758, we made a comparison of the relative contribution of genetic vs plastic effects during adaptation to divergent spectral conditions to study the nature of adaptation. In fishes, there exist numerous examples of adaptation. Well-known are ontogenetic shifts in opsin gene expression (e.g. Cortesi et al., 2016; Tettamanti et al., 2019), which often coincide with shifts in habitat and diet (Shand, 1993, 1997; Allison et al., 2006; Hoke et al., 2006). Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), reared in captivity under constant light condition underwent the same ontogenetic shift in opsin gene expression as in nature (Spady et al., 2006; Carleton et al., 2008), thereby stressing the genetic basis of the ontogenetic shift.

There are numerous correlational studies in fishes in which natural variation in photic environment relates to variation in opsin expression (for reviews, see Schweikert et al., 2019; Musilova et al., 2021). Some of the population differences in opsin gene expression were based on phenotypic plasticity of the visual system as raising fish during various periods of time under different lighting conditions induced changes in opsin expression. For example, raising bluefin killifish, *Lucania goodei* Jordan, 1880, under clear-water or tea-stained water conditions caused different opsin expressions (Fuller et al., 2005, 2010). Similar results have been obtained in various other fish species (Shand et al., 2008; Hofmann et al., 2009, 2010; Smith et al., 2012; Dalton et al., 2015; Ehlman et al., 2015; Sakai et al., 2016; Härer et al., 2017, 2019; Kranz et al., 2018; Schweikert & Grace, 2018; Wright et al., 2020).

Also keeping adult fish for variable periods of time (ranging from 3 days to 6 months) under changed lighting conditions has been found to induce changes in opsin expression. For example, keeping adult bluefin killifish from different photic habitats for 4 weeks under clear-water or tea-stained water conditions had differential effects on opsin gene expression (Fuller & Claricoates, 2011). Comparable results were obtained with sticklebacks when reproductive fish from different depths were kept in field enclosures for 24 days that were covered with filters mimicking light conditions at different water depths (Veen et al., 2017); however, in contrast, Novales Flamarique et al. (2013) found limited plasticity based on cone distribution and abundance in retinas of adult fish from red-stained waters that were transferred to clear water during 3 weeks–6 months. In several cichlid fishes and in juvenile and adult convict surgeonfish *Acanthurus triostegus* (Linnaeus, 1758), there is evidence for plasticity in cone opsin expression of adult fish after experiencing changes in light conditions (Nandamuri et al., 2017; Härer et al., 2017, 2019, and Fogg et al., 2023, respectively).

Examples of genetic differences of opsin expression profiles are studies on bluefin killifish (Fuller et al., 2005, 2010), three-spined stickleback (Rennison et al., 2016), Atlantic molly, *Poecilia mexicana* Steindachner, 1863 (Tobler et al., 2010) and several cichlid species (Spady et al., 2006; Carleton et al., 2008, 2010; Hoffmann et al., 2009, 2010). For example, the differences in opsin gene expression profiles between wild-caught fish from a lake and a marine population of sticklebacks were largely maintained in laboratory-reared fish in clear water suggesting a substantial genetic component for the differences (Rennison et al., 2016). Novales Flamarique et al. (2013)

also found limited plasticity of stickleback colour vision based on retinal cone composition, abundance and distribution. This was evident in wild-caught fish transferred to clear water in the lab but also of fish introduced into a clear-water pond from a red-stained lake 15–20 years ago (Novales Flamarique et al., 2013).

To investigate the relative contribution of genetic vs plastic effects during adaptation to divergent spectral conditions, we used three-spined sticklebacks from the island of North Uist, Outer Hebrides, Scotland. Lakes in the western part of the island are machair, alkaline, clear-water lakes, while those in the central and eastern part of North Uist are peat-bog, acidic, tea-stained lakes (Giles, 1983). The spectral transmission of water of the lakes differs considerably, especially in the short (UV, blue) wavelength range (Hiermes et al., 2015). Non-reproductive fish from tea-stained water have a higher UV chroma (intensity) than those from clear waters and the photic habitat of origin influences UV-based shoaling decisions (Hiermes et al., 2015). In female mate-choice experiments using wild-caught, reproductive fish from both photic habitats, females from both tea-stained waters and clear waters preferred males seen under UV-present conditions, which was most pronounced under clear-water test conditions (Hiermes et al., 2021a). Males from clear-water lakes had more intense, and red-shifted, breeding coloration than males from tea-stained lakes (Hiermes et al., 2021a). There thus exist adaptations with respect to behaviour and coloration to the photic habitat. The present study aimed at investigating whether this is also the case with respect to opsin expression and visual sensitivity.

Three-spined sticklebacks possess one rod opsin gene (λ_{max} range: 507–531 nm) and four cone opsin genes: short-wavelength-sensitive 1 (SWS1, UV: λ_{max} = 365–382 nm); short-wavelength-sensitive 2 (SWS2, blue: λ_{max} = 434–441 nm); middle-wavelength-sensitive (RH2, green: λ_{max} = 514–546 nm); and long-wavelength-sensitive (LWS, red: λ_{max} = 566–638 nm) (e.g. Rowe et al., 2004; Rennison et al., 2012, 2016; Novales Flamarique et al., 2013). Microspectrophotometry revealed one class of rod receptor and four classes of cone photoreceptors: UV single cones, blue single cones, green/red double cones and red/red double cones (Novales Flamarique et al., 2013).

In sticklebacks, opsin expression patterns are thought to be adaptive, but it is unclear to what extent they are genetically based and phenotypically plastic (Novales Flamarique et al., 2013; Rennison et al., 2016; Veen et al., 2017). The first aim of our study was to assess whether the visual system of wild-caught sticklebacks differs with regard to the photic habitat they inhabit (clear water, tea-stained water) by measuring opsin expression levels in the retina. We predict a difference between habitats based on the frequent association between ambient light and opsin expression found in sticklebacks and other fishes (discussed above). We further estimated spectral sensitivity of wild-caught fish by integrating opsin expression data into a visual modelling approach, to determine the degree of matching between the spectral distribution of sensitivity and ambient light conditions. Next, we aimed at assessing the adaptive nature of differences in the visual system by repeating the opsin analysis with fish from the same populations that had been bred in the lab under clear-water conditions.

Material and methods

Wild-caught generation

In April 2010, three-spined sticklebacks, *G. aculeatus*, were caught using minnow traps from six lakes on the island of North Uist, Scotland (57° 35′ N, 7° 18′ W). The populations are genetically distinct and geographically separated (Rahn et al., 2016b). Three populations lived in clear-water lakes (Grogary, Eubhal, Sandary), the other three in tea-stained lakes (a Bharpa, Scadavay, Tormasad). The spectral distribution of the water from the lakes differed, especially in the UV spectral range between 300 and 400 nm (see Hiermes et al., 2021a, b). Clear-water lakes had an alkaline pH, while that of tea-stained lakes was acidic (e.g. Rahn et al., 2016a, b). Sampling more than one lake of each photic habitat strongly reduces the probability that the effects of photic habitat on the visual system will be due to population characteristics other than the photic environment.

Per lake 6 adult non-reproductive and 4–6 reproductive fish were quickly euthanized by decapitation followed by a cut through the brain, their eyes dissected and each put into 400 µl RNAlater (RNA

Stabilization Reagent, Qiagen) and after return to the Institute in Bonn stored at − 20 °C until RNA extractions were performed. All animals were sacrificed between 9 and 12 h am. Before euthanasia, fish standard body length and body mass were measured. Post euthanasia, the sex of all fishes was determined by inspection of the gonads.

F_1 generation

In April 2010 and April 2011, samples of live fish from each population were transported to the Bonn Institute for Organismic Biology (BIOB), Section II: Animal Biodiversity, University of Bonn, Germany and successfully bred through random mating within each population. Males and females were used once to avoid pseudoreplication. The F_1 generation of the six populations (three from the clear-water lakes Grogary, Eubhal, Sandary, three from the tea-stained lakes a Bharpa, Scadavay, Tormasad) were raised under standardized, clear-water conditions in the lab. Tanks were illuminated by fluorescent full-spectrum tubes (Truelight T8/36W), which provided light similar to natural skylight (for irradiance spectrum see Hiermes et al., 2021a). After shoal-choice and mate-choice experiments at an age of 9–11 and 11–13 months, respectively (Hiermes et al., 2021b), some non-reproductive and reproductive fish (5–7 individuals for each reproductive stage per population, 13 for fish from Tormasad; 1–2 fish per family from each population, between 1 and 4 fish per family from Scadavay and Tormasad) were sacrificed and both eyes stored in each 400 µl RNAlater at − 20 °C until RNA extractions were performed. Before euthanasia, fish standard body length and body mass were determined. After euthanasia, the sex of all fishes was determined by inspection of the gonads (and for the non-reproductive fish confirmed by molecular sexing using dorsal spines (for details, see Bakker et al., 2017)). All animals were sacrificed between 9 and 12 h am.

Opsin expression

The expression of each of the four unique cone opsin genes (SWS1 (UV), SWS2 (blue), RH2 (green) and LWS (red)) was measured by quantifying opsin transcripts from whole retinas using real-time PCR with primers from Rennison et al. (2016) (details in the Supplementary Information 1). All qPCR runs were done in duplicate and averages used in the calculations. PCR efficiencies (E) for each of the primer pairs was calculated using Eq. 1:

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

where the slope is determined from a linear least squares regression fit to mean (of two qPCR reactions) critical threshold (C_t) data from a cDNA dilution series (1:1, 1:10, 1:100, 1:1000). The slopes were calculated for three fish separately and then averaged to obtain E (see Supplementary Information 3). The estimates of the initial amount of gene transcript (T_i) were calculated for each individual (i) using Eq. 2:

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

where E is the PCR efficiency for a given gene and C_t is the critical threshold for fluorescence. For each individual, the proportional opsin expression was calculated by dividing the absolute number of transcripts for each gene by the sum of the expression of all four cone opsins. This is a useful measure when one is interested in colour vision capacity instead of calculating opsin expression relative to that of a housekeeping gene (Fuller & Claricoates, 2011).

Some studies apply another way to normalize opsin expression, that is, normalization within single and double cones (e.g. Carleton et al., 2010; Hofmann et al., 2010). For comparison with the most used method, proportional opsin expression was also calculated for the single cones as SWS1/(SWS1 + SWS2) and SWS2/(SWS1 + SWS2) and for the double cones as LWS/(RH2 + LWS) and RH2/(RH2 + LWS). However, given that individual spectral sensitivity was modelled across cone types (see below) data obtained from the latter normalization approach were not considered for subsequent analyses on spectral matching.

Environmental light conditions

Measurements of downwelling irradiance in the six different lakes were taken from a previous study (see Hiermes et al., 2021a for methodological details and irradiance spectra). As a quantitative measure to classify the spectral distribution found in each lake, we

calculated the spectral index λP_{50}-irrad, which describes the centre wavelength at which the total number of photons between 300 and 700 nm is halved (McFarland & Munz, 1975). A higher λP_{50}-irrad is associated with a light spectrum shifted towards longer wavelengths. The tea-stained lakes in our study (a Bharpa: λP_{50}-irrad = 570 nm, Scadavay: λP_{50}-irrad = 576 nm, Tormasad: λP_{50}-irrad = 568 nm) had a higher λP_{50}-irrad compared to the clear-water lakes (Eubhal: λP_{50}-irrad = 557 nm, Grogary: λP_{50}-irrad = 546 nm, Sandary: λP_{50}-irrad = 540 nm).

Spectral sensitivity modelling

In addition to the comparison of opsin expression levels between wild-caught individuals from clear-water and tea-stained habitats, we estimated spectral sensitivity based on proportional opsin expression, which is based on the simplified assumption that opsins contribute additively to spectral sensitivity (see Rennison et al., 2016). Specifically, spectral sensitivity curves between 300 and 700 nm were computed for each individual based by combining estimates of the proportional expression of the four opsin genes with the relative cone absorbance maxima provided by Novales Flamarique et al. (2013) and visual pigment templates from Govardovskii et al. (2000). Given that information on chromophore ratios for fish from our study populations was not available, modelling was based on the assumption that clear-water fish had either pure A1 or A2 retinas (100% A1 or 100% A2) or equal chromophore proportions (50% A1, 50% A2) whereas individuals from tea-stained habitats had either pure A2 or A1 retinas (100% A2 or 100% A1) or equal chromophore proportions (see Novales Flamarique et al., 2013). As we were primarily interested in differences in sensitivity between light habitats, and due to the lack of detailed data on reproductive-state dependent chromophore shifts in three-spined sticklebacks, we used the same chromophore ratios for reproductive and non-reproductive fish in our modelling approach. Data on ocular media transmission are also lacking for the surveyed populations. However, lens transmission of individuals from another stickleback population (IPR unpublished data) suggests that it is unlikely to have a relevant effect. Retina sensitivity curves for each fish were computed for the corresponding chromophore ratios by summing up the integrals of the four cone absorbance curves (see Rennison et al., 2016 for full details).

Matching between spectral sensitivity and environmental light

For a direct comparison between spectral sensitivity and environmental light conditions, we determined the centre wavelength (λP_{50}-sens) of the retina sensitivity curves for the respective chromophore ratios from each individual, as done for the ambient irradiance spectra (λP_{50}-irrad) for each lake (see McFarland & Munz, 1975; Schneider et al., 2020) and tested for an association between the two spectral indices.

Statistical analyses

Data on proportional opsin expression were examined for outliers defined as exceeding 1.5× the interquartile range (IQR) (e.g. Wright et al., 2019, 2020). In total, 9 outliers (4 from the SWS1, 3 from the RH2 and 2 from the LWS opsin) were identified and 7 individuals had to be removed from the data resulting in a final sample size of 87 individuals (Eubhal (clear): $N_{\text{wild-caught}} = 5$, $N_{\text{lab-bred}} = 8$; Grogary (clear): $N_{\text{wild-caught}} = 2$, $N_{\text{lab-bred}} = 6$; Sandary (clear): $N_{\text{wild-caught}} = 8$, $N_{\text{lab-bred}} = 7$; a Bharpa (tea-stained): $N_{\text{wild-caught}} = 7$, $N_{\text{lab-bred}} = 7$; Scadavay (tea-stained): $N_{\text{wild-caught}} = 6$, $N_{\text{lab-bred}} = 4$; Tormasad (tea-stained): $N_{\text{wild-caught}} = 6$, $N_{\text{lab-bred}} = 21$).

All statistical analyses were conducted in R v. 4.0.3 (R Core Team, 2020) and all reported P values are two-tailed. We used linear mixed effect models (LME, lmer function in the lme4 package: Bates et al., 2015) to study how the fixed effects of water clarity (tea-stained, clear), generation (wild-caught, lab-bred) and the interaction between both, as well as reproductive state (reproductive, non-reproductive) and sex influenced proportional opsin expression. Study population was included as random effect in all models. We used the backward elimination procedure implemented in the "step" function in the lmerTest package (Kuznetsova et al., 2017) to perform a stepwise model reduction where nonsignificant variables are removed in the order of their statistical relevance. Significance values for the fixed effects were calculated using F-tests based on Satterthwaite estimates of degrees of freedom. Opsin expression levels were compared between water clarity conditions within wild-caught and lab-bred fish, and between wild-caught and lab-bred individuals within water clarity conditions, by calculating contrasts from post-hoc

tests with Tukey adjustment for multiple comparisons using the R package "emmeans" based on comparisons between estimated marginal means (EMMs) (Lenth, 2018). For the SWS2, RH2 and LWS opsin, the residuals of the best explaining models did not significantly differ from a normal distribution, confirmed by Shapiro–Wilk tests. Data on the SWS1 opsin had to be Box-Cox transformed to meet the assumption of normality of the residuals of the best explaining model.

Linear mixed effect models (LME) and a stepwise model reduction procedure based on the "step" function were also used for studying the association between spectral sensitivity (for the respective chromophore ratios) and habitat light conditions. In all models, λP_{50}-sens was used as dependent variable, λP_{50}-irrad as fixed effect and study population as random effect. Significance values for the fixed effect were calculated using *F*-tests based on Satterthwaite estimates of degrees of freedom. The residuals of the best explaining models were normally distributed according to Shapiro–Wilk tests.

Results

The expression profile of the SWS1 opsin was influenced by water clarity (Table 1, Supplementary Information 2 Table S1), with wild-caught sticklebacks from clear (UV-blue shifted) habitats showing a significantly higher expression and lab-raised fish tending to have a higher expression than individuals from tea-stained (orange-red shifted) habitats (Fig. 1 and Table 2). Water clarity had no significant effect on the expression profiles of SWS2, RH2 and LWS (Tables 1, 2, S1). In addition, independent of water clarity, generation had a significant effect on the expression of SWS1 and also SWS2 (Tables 1, S1); wild-caught fish had a higher SWS1 expression compared to lab-bred fish, whereas the opposite result was found for the SWS2 opsin (Table 1 and Fig. 1). No significant effect of generation on the expression of RH2 and LWS was found (Tables 1, 2, S1). While the interaction effect between water clarity and generation on opsin expression was not significant for SWS1 and SWS2 (Tables 1, S1), there was, in contrast, a significant interaction term for the RH2 and LWS genes

Table 1 Results of the stepwise model selection performed for the effects of water clarity (clear, tea-stained), generation (wild-caught, lab-bred) and the interaction between both, as well as reproductive state (reproductive, non-reproductive) and sex (male, female) on the proportional expression of the SWS1, SWS2, RH2 and LWS opsin genes. See text for detailed description of variables

Dependent variable	Explanatory variables	*F*	denDF	*P*	Random factor
SWS1	Water clarity	27.805	87.000	**< 0.001**	Population
	Generation	5.699	87.000	**0.019**	
	Water clarity × generation	1.831	87.000	0.179	
	Reproductive state	0.015	87.000	0.902	
	Sex	0.703	87.000	0.404	
SWS2	Water clarity	0.404	5.059	0.553	Population
	Generation	18.560	86.281	**< 0.001**	
	Water clarity × generation	1.295	86.931	0.258	
	Reproductive state	2.270	86.566	0.136	
	Sex	0.356	81.859	0.553	
RH2	Water clarity × generation	4.287	87.000	**0.041**	Population
	Reproductive state	6.834	87.000	**0.011**	
	Sex	0.380	87.000	0.539	
LWS	Water clarity × generation	5.570	87.000	**0.021**	Population
	Reproductive state	7.418	87.000	**< 0.01**	
	Sex	0.072	87.000	0.789	

Study population was included as random effect in all models and never removed from the models. Explanatory variables were removed in order of significance. Degrees of freedom always differed by one during model reduction. Significant results are highlighted in bold ($P < 0.05$). Numerator degrees of freedom were always 1. Denominator degrees of freedom are given as denDF

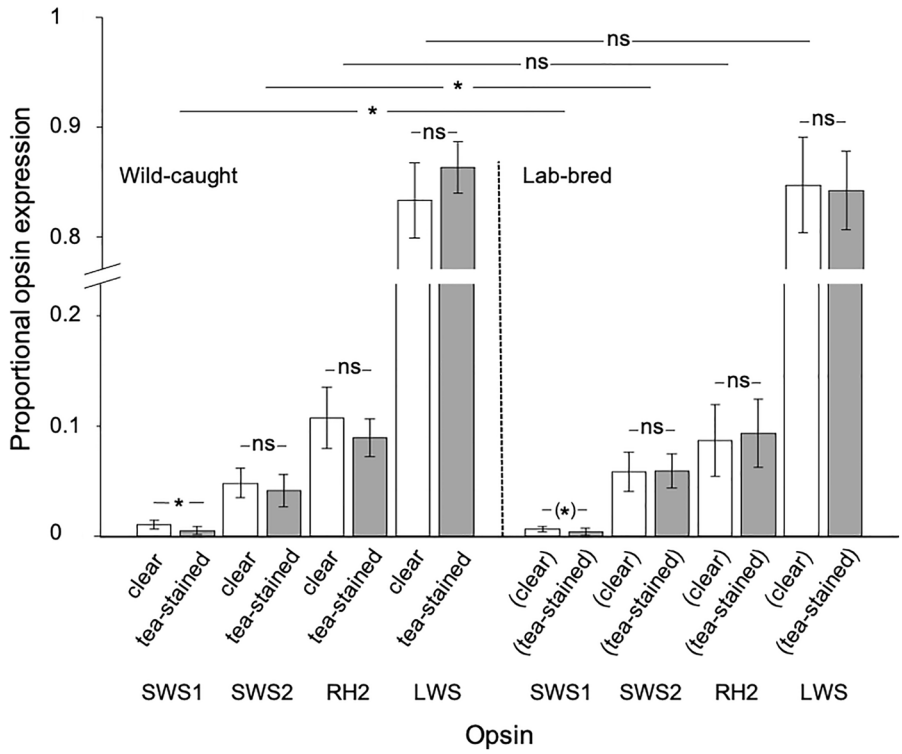

Fig. 1 Proportional opsin expression of the four opsin genes SWS1, SWS2, RH2 and LWS in wild-caught (left half) and lab-bred F1 (right half) fish from clear-water (white bars) and tea-stained lakes (grey bars) on North Uist. Indicated are mean values ± SD. (*) $0.05 < P < 0.1$, * $P < 0.05$, ns $P > 0.05$

Table 2 Results of post-hoc pairwise comparisons of the proportional expression of the SWS1, SWS2, RH2 and LWS opsin genes shown in wild-caught and lab-bred fish from the two water clarity conditions (clear, tea-stained)

Dependent variable	Pairs		*t*	df	*p*
SWS1	*Wild-caught*—clear	*Wild-caught*—turbid	3.765	47.9	**< 0.01**
	Lab-bred—clear	*Lab-bred*—turbid	2.498	28.9	*0.081*
	Wild-caught—clear	*Lab-bred*—clear	2.530	90.7	*0.062*
	Wild-caught—turbid	*Lab-bred*—turbid	0.827	97.0	0.841
SWS2	*Wild-caught*—clear	*Wild-caught*—turbid	1.093	40.5	0.696
	Lab-bred—clear	*Lab-bred*—turbid	− 0.084	23.8	1.000
	Wild-caught—clear	*Lab-bred*—clear	− 1.924	90.3	0.225
	Wild-caught—turbid	*Lab-bred*—turbid	− 3.656	95.7	**< 0.01**
RH2	*Wild-caught*—clear	*Wild-caught*—turbid	1.840	20.92	0.284
	Lab-bred—clear	*Lab-bred*—turbid	− 0.819	8.69	0.844
	Wild-caught—clear	*Lab-bred*—clear	2.298	93.29	0.106
	Wild-caught—turbid	*Lab-bred*—turbid	− 0.370	77.28	0.983
LWS	*Wild-caught*—clear	*Wild-caught*—turbid	− 2.260	37.1	0.126
	Lab-bred—clear	*Lab-bred*—turbid	0.444	20.7	0.970
	Wild-caught—clear	*Lab-bred*—clear	− 1.310	91.2	0.559
	Wild-caught—turbid	*Lab-bred*—turbid	1.849	94.3	0.257

Significant p-values are highlighted in bold ($P < 0.05$) and trends indicated in italics ($0.05 < P < 0.1$)

(Fig. 2a, b and Tables 1, S1). Furthermore, across all treatment groups reproductive state had a significant effect on the proportional expression of RH2 and LWS, but not of SWS1 and SWS2 (Tables 1, S1), i.e. reproductively active sticklebacks had a higher LWS expression and a lower RH2 expression compared to non-reproductive individuals (Fig. 3). Independent of water clarity, generation and reproductive state, there

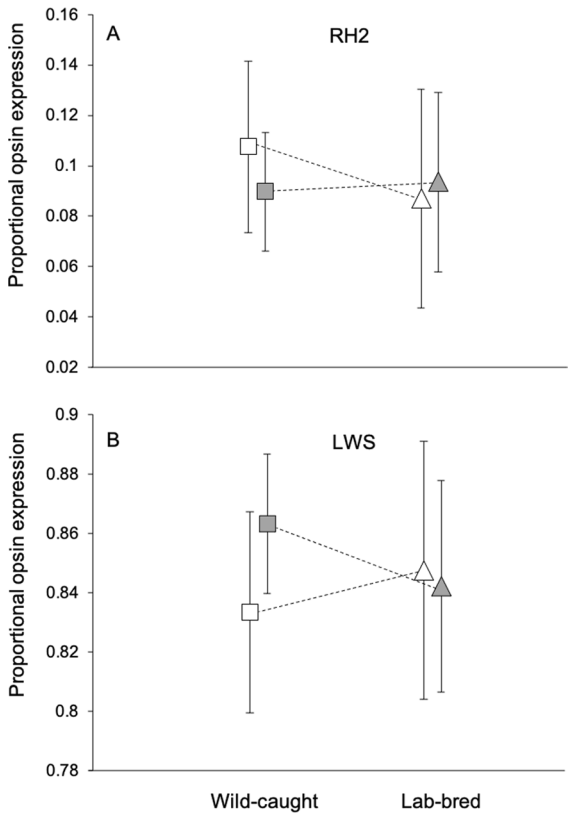

Fig. 2 Visualization of the interaction between water clarity (clear-water: white symbols, tea-stained water: grey symbols) and generation (parental wild-caught and lab-bred F1 generation) on proportional opsin expression of **A** RH2 and **B** LWS. Indicated are mean values ± SD

were no significant differences between the sexes with regard to proportional opsin expression for all four opsin classes (Table 1, S1). The proportional expression of the opsin genes was qualitatively similar among wild-caught fish from the three clear-water and among those from the three tea-stained-water populations (Supplementary Information 2 Fig. S1).

Analyses of proportional gene expression based on within cone classes gave roughly the same results as those based on total opsin expression, except for SWS2. Here the opsin expression in wild-caught fish from clear waters was significantly lower than that in fish from tea-stained waters (Supplementary Information 4).

The retina sensitivity of wild-caught fish was associated with habitat light conditions found in the corresponding lake of origin as revealed by a significantly positive relationship between the spectral indices λP_{50}-irrad and λP_{50}-sens (Table 3 and Fig. 4). While this relationship was qualitatively similar for the different chromophore ratio combinations (Table 3 and Fig. 4), it was weaker when similar retina chromophore compositions (either 50:50 mix of A1 and A2 or pure A1 or pure A2) were modelled for fish from both water clarity conditions (Table 3 and Fig. 4B, E, F). Despite the strong positive relationship between λP_{50}-irrad and λP_{50}-sens there was a noticeable wavelength difference between both indices when separately considering the six different lakes at least for five of the six hypothetical chromophore ratio combinations (Fig. 4A–C, E, F). In contrast, assuming a pure A1 chromophore for clear-water fish and a 50:50 mix of A1 and A2 for fish from tea-stained habitats resulted in a comparably strong match between centre wavelengths λP_{50}-irrad and λP_{50}-sens (Fig. 4D).

Discussion

Living in tea-stained waters instead of clear waters had a clear effect on the expression of SWS1, but not on the expression of the SWS2, RH2 and LWS genes (Fig. 1). Fish in tea-stained waters were less sensitive for UV, which coincides with a shift in breeding coloration to shorter wavelengths (Hiermes et al., 2021a) and a greater UV intensity of non-reproductive fish (Hiermes et al., 2015) from the same tea-stained populations (cf Brock et al., 2018 for sticklebacks and

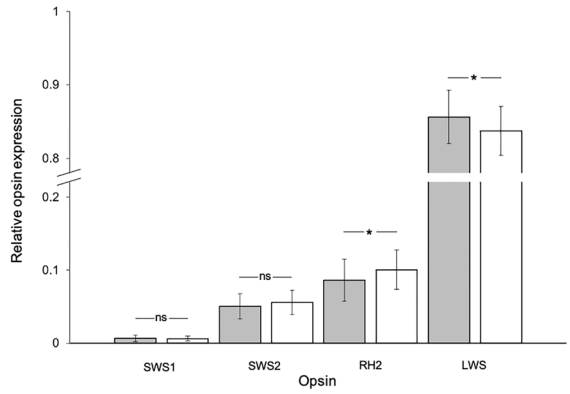

Fig. 3 Proportional opsin expression of the four opsin genes SWS1, SWS2, RH2 and LWS in reproductively active (grey bars) and reproductively inactive (white bars) fish (wild-caught and F1 fish pooled). Indicated are mean values ± SD. *$P < 0.05$, ns $P > 0.05$

Table 3 Results of the stepwise model selection performed for the effects of the spectral index of ambient light (λP_{50}-irrad) in the six different lakes of two habitats (clear, tea-stained) on the spectral index of retina sensitivity (λP_{50}-sens) of wild-caught individuals for different hypothetical chromophore ratio combinations. See text for detailed description of variables

Dependent variable	Explanatory variable	Chromophore ratio		*F*	*R*2	denDF	*P*	Random factor
		Clear	Tea-stained					
λP50-sens	λP50-irrad	A1/A2 (50%)	A2	25.953	0.801	6.045	**0.002**	Population
		A1/A2 (50%)	A1/A2 (50%)	6.956	0.174	34.000	**0.013**	Population
		A1	A2	26.523	0.827	6.011	**0.002**	Population
		A1	A1/A2 (50%)	25.892	0.817	6.023	**0.002**	Population
		A1	A1	5.835	0.150	34.000	**0.021**	Population
		A2	A2	8.254	0.200	34.000	**0.007**	Population

Study population was included as random effect in all models and never removed from the models. Degrees of freedom always differed by one during model reduction. Significant results are highlighted in bold ($P < 0.05$). Numerator degrees of freedom were always 1. Denominator degrees of freedom are given as denDF

water depth and Owens et al., 2022 for *P. mexicana* in sulphidic waters). Although opsin expression between tea-stained and clear-water fish was only significantly different for the SWS1 opsin (and SWS2 when using another normalization procedure, see Supplementary Information 4), there was an overall positive correlation between the spectral distribution of ambient light and of retina sensitivity revealing that visual sensitivity is, at least to a certain extent, spectrally tuned to the prevailing light conditions (Fig. 4). As expected, this association was more pronounced when the chosen chromophore ratios were in line with the ones previously described for dystrophic (A2-dominated) and clear-water systems (A1-dominated) (see Novales Flamarique et al., 2013).

As the stickleback populations on North Uist are largely genetically independent (e.g. Rahn et al., 2016b), the consistent differences in proportional opsin expression between wild-caught fish from the three replicate clear-water lakes and fish from the three replicate tea-stained lakes (Figs. 4, S1) is another example of parallel evolution in sticklebacks. This finding is in line with parallel evolution of the visual system to environmental light in sticklebacks (Rennison et al., 2016) and other fishes (e.g. Owens et al., 2022; Ricci et al., 2023). Overall, these stickleback populations seem to have evolved in response to natural and sexual selection to match environmental light conditions. This adaptive shift appears to be a result of a combination of changes in visual signals and visual sensitivities, especially in the UV wavelength range. UV in sticklebacks plays a role in several contexts like shoaling (Modarressie et al., 2006; Hiermes et al., 2015, 2021a, b), foraging (Rick et al., 2012), male–male competition (Rick & Bakker, 2008b) and mate choice (Boulcott et al., 2005; Rick et al., 2006; Rick & Bakker, 2008a; Hiermes et al., 2021a, b). In tea-stained waters, UV is attenuated strongly and fish have reduced sensitivity in the UV (this study). This interacts with changed coloration in non-reproductive (Hiermes et al., 2015) and reproductive fish (Hiermes et al., 2021a). Further research will be needed to evaluate the net benefit of UV vision under tea-stained compared to clear-water conditions.

The adaptive character of vision under changed environmental light agrees with findings in other fishes both within and between species (reviewed in Musilova et al., 2021), e.g. bluefin killifish from clear springs and from tea-stained swamps showed different opsin expression profiles (Fuller & Travis, 2004) or opsin expression changed in cave fish compared to surface fish of related species (Tobler et al., 2010; Meng et al., 2013). Comparable changes in opsin expression with photic environment have been found in various other fish species (Terai et al., 2006; Hofmann et al., 2009; Smith et al., 2011; Stieb et al., 2016; Härer et al., 2018; Owens et al., 2022; Ricci et al., 2023). Three-spined stickleback, *G. aculeatus*, from freshwater populations differed in opsin gene expression from those from marine populations and within freshwater populations benthic and limnetic fish had different opsin expression (Rennison et al., 2016). Opsin gene expression of *G. aculeatus* varied also with water depth (Veen et al., 2017), and between marine habitat and lakes with different photic habitats

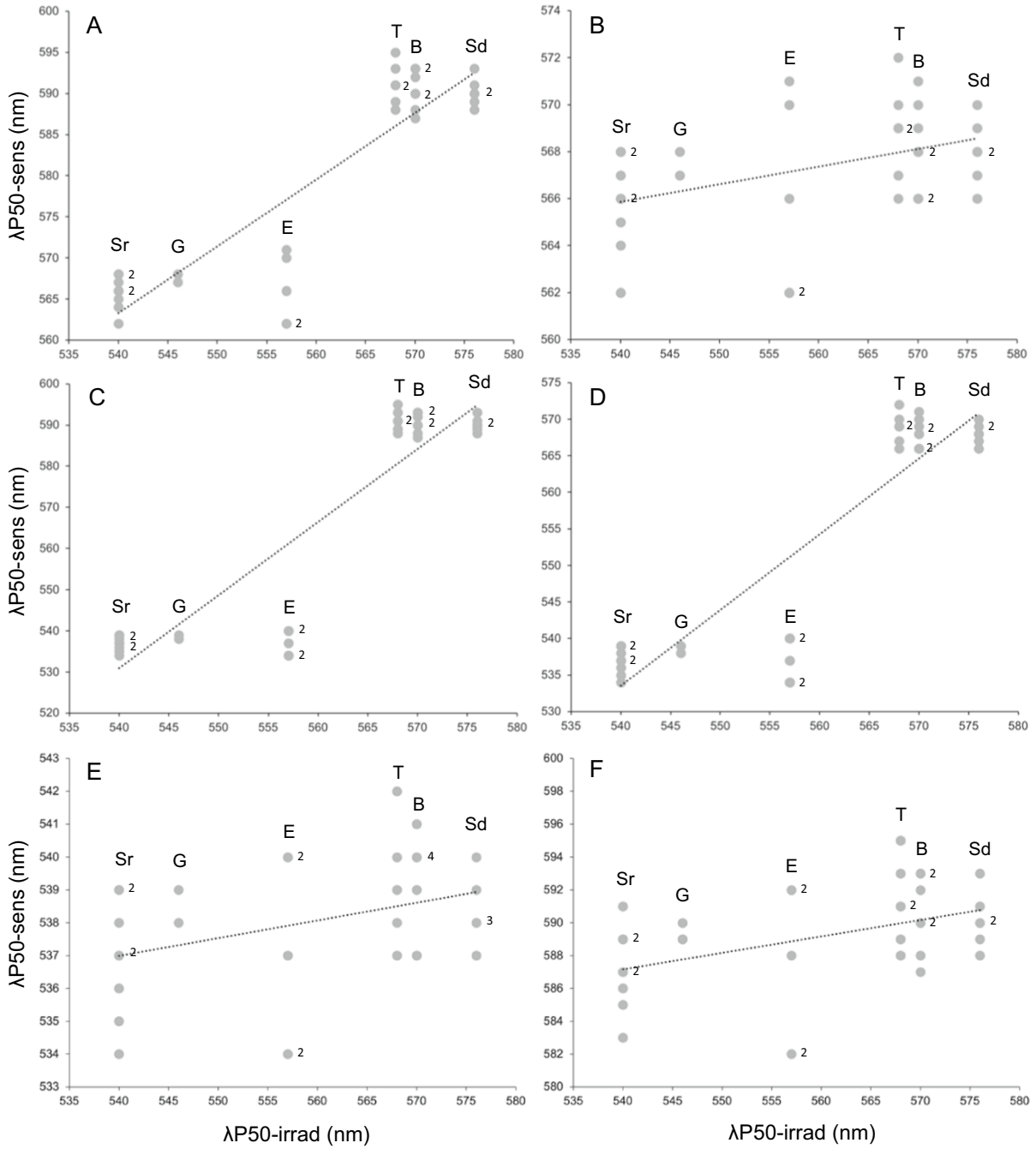

Fig. 4 Relationship between the spectral indices λP50-irrad (centre wavelength of ambient light) for the six lakes of two habitats (clear: Sandary (Sr), Grogary (G), Eubhal (E), tea-stained: Tormasad (T), a Bharpa (B), Scadavay (Sd)) and λP50-sens (centre wavelength of retina sensitivity) of individual fish. Shown are data for the different hypothetical chromophore ratio combinations **A** clear—A1/A2 (50%), tea-stained—A2, **B** clear—A1/A2 (50%), tea-stained—A1/A2 (50%), **C** clear—A1, tea-stained—A2, **D** clear—A1, tea-stained—A1/A2 (50%), **E** clear—A1, tea-stained—A1 and **F** clear—A2, tea-stained—A2. Note that some data points are overlapping

in British Columbia ranging from clear waters to red-stained waters (Novales Flamarique et al., 2013).

Adaptation may not only occur at the opsin expression level (Musilova et al., 2021). For instance, males from sympatric *Pundamilia* species pairs (*Pundamilia pundamilia* Seehausen & Bouton 1998 or *Pundamilia* sp. "*pundamilia-like*" and *Pundamilia nyererei* (Witte-Maas & Witte, 1985) or *Pundamilia* sp. "*nyererei-like*") that occur at different depths (shallow and deeper, respectively) in Lake Victoria differ in breeding coloration (blue and red, respectively). The sister species differ in LWS and RH2A opsin expression; LWS expression was higher and RH2A expression lower in the red species but only in clear-water habitats (Wright et al., 2019). However, at other more turbid locations, the relationships may be reversed (Wright et al., 2019, 2020). Interestingly, opsin alleles and female mate preferences (Kanazawa et al., 2020) and variation in opsin expression and female mate preference (Wright et al., 2020) may be linked and provide a plausible mechanism for the reported genetic correlation between male red coloration and female preference for and visual sensitivity to red in sticklebacks (Bakker, 1993; Rick et al., 2011).

Only for SWS1 did we have indications for a genetic influence on opsin proportional expression as differences in SWS1 expression between fish from clear-water and tea-stained lakes tended to be maintained in the F_1 generation (Fig. 1). For RH2 and LWS there were significant interactions between generation and water clarity (Fig. 2). For SWS2 there was no significant difference associated with water clarity in both generations. Thus, phenotypic plasticity seems to be the major cause for adaptation to different photic habitats in the present study. These results contrast with those of Novales Flamarique et al. (2013), who found genetic variation in opsin expression between stickleback populations only in RH2 and LWS. Rennison et al. (2016) found genetic effects for the expression of SWS1 and RH2 in sticklebacks. So, with respect to genetic influences on opsin expression in sticklebacks the evidence is mixed and may be population specific. Further evidence for a mix of plastic and genetic involvement in opsin expression comes from studies on bluefin killifish (Fuller et al., 2005, 2010), Atlantic molly (Tobler et al., 2010) and several cichlid species (Spady et al., 2006; Carleton et al., 2008, 2010; Hofmann et al., 2009, 2010).

SWS1 proportional expression was higher in wild-caught fish than in lab-reared fish independent of water clarity (Fig. 1). For SWS2 the reverse was true. SWS1 opsin expression level was also reduced after lab-rearing in sticklebacks (Rennison et al., 2016) and cichlids (Hofmann et al., 2010). Rennison et al. (2016) attributed this to a lack of UV wavelengths during rearing, which cannot be the cause in our case, as in the fish in our study were raised under fluorescent full-spectrum tubes. There may, however, be a difference between the natural and artificial light environments.

Reproductive state was found to have an effect on LWS (higher in reproductive fish) and RH2 (lower in reproductive fish) proportional expression but not on that of SWS1 and SWS2 (Fig. 3). This finding confirms that of a previous study in sticklebacks (Shao et al., 2014), who found that when sticklebacks become reproductively active, they became more sensitive for long wavelengths as measured as proportional LWS expression (Shao et al., 2014). This pattern has also been shown using electroretinogram data (Shao et al., 2014) and optomotor response (Cronly-Dillon & Sharma, 1968; Boulcott & Braithwaite, 2007). Red coloration in males plays a crucial role in female mate choice (e.g. Milinski & Bakker, 1990) and male–male competition (e.g. Bakker & Sevenster, 1983). Through the process of sexual selection, red coloration and female preference become genetically correlated (Bakker, 1993; Rick et al., 2011) and this may be mediated by LWS gene expression (Brock et al., 2018). Reproductive state also has an effect on opsin expression in other fishes like the guppy *Poecilia reticulata* Peters, 1859 (Laver & Taylor, 2011) and the cichlid *Astatotilapia burtoni* (Günther, 1894) (Butler & Maruska, 2021).

Proportional opsin expression levels were similar for all four opsin genes in both sexes, which agrees with a previous study of Shao et al. (2014) in sticklebacks. Boulcott and Braithwaite (2007) also found similar optomotor responses under red illumination for both sexes of sticklebacks. However, sex-specific effects in sticklebacks have been found for optomotor response when conducted using reproductive females (Cronly-Dillon & Sharma, 1968), and for proportional SWS1 expression in reproductive adults from one wild population (Veen et al., 2017). Also, in some other fishes like *P. reticulata* (Laver & Taylor, 2011; but see Sandkam et al., 2018),

Melanochromis auratus (Boulenger, 1897) (Sabbah et al., 2010), *A. burtoni* (Butler & Maruska, 2021) and two *Pundamilia* chichlid species (Wright et al., 2020), some opsins showed sex-specific changes in expression levels with reproduction. However, in a study using 10 African and Neotropical cichlids, Schneider et al. (2020) did not find sex-specific expression of opsins, neither did Sabbah et al. (2010) in *Protomelas taeniolatus* (Trewavas, 1935). So, evidence for the sex-specific expression of opsins is rather mixed and seems to be highly variable among populations and species.

In conclusion, we found adaptive variation in opsin expression profiles associated with water clarity in sticklebacks. Phenotypic plasticity seems to be the major cause. Only in the case of SWS1, variation in expression may be attributed to genetic effects.

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Data availability All data needed to generate the results are provided in the Supplementary Information 3.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national and institutional guidelines for the use of animals were followed. Animal care and experimental procedures were in accordance with the legal requirements of Germany. Holding and rearing conditions were approved by the City of Bonn, Amt für Umwelt, Verbraucherschutz und Lokale Agenda after § 11 Abs. 1 TierSchG (licence 56-2 for keeping and breeding fishes). No additional license was required for this study.

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