

Article

Influence of lighting environment on social preferences in sticklebacks from two different photic habitats. I. mate preferences of wild-caught females

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Abstract

Ultraviolet (UV) A signals (320–400 nm) are important in mate choice in numerous species. The sensitivity for UV signals is not only assumed to be costly, but also expected to be a function of the prevailing ecological conditions. Generally, those signals are favored by selection that efficiently reach the receiver. A decisive factor for color signaling is the lighting environment, especially in aquatic habitats, as the visibility of signals, and thus costs and benefits, are instantaneously influenced by it. Although ecological aspects of color signal evolution are relatively well-studied, there is little data on specific effects of environmental UV-light conditions on signaling at these shorter wavelengths. We studied wild-caught gravid female 3-spined sticklebacks *Gasterosteus aculeatus* of 2 photic habitat types (tea-stained and clear-water lakes), possessing great variation in their UV transmission. In 2 treatments, tea-stained and clear-water, preferences for males viewed under UV-present (UV+) and UV-absent (UV-) conditions were tested. A preference for males under UV+ conditions was found for females from both habitat types, thus stressing the significance of UV signals in stickleback's mate choice decisions. However, females from both habitat types showed the most pronounced preferences for males under UV+ conditions under clear-water test conditions. Moreover, reflectance measurements revealed that the carotenoid-based orange-red breeding coloration in wild-caught males of both habitat types differed significantly in color intensity (higher in clear-water males) and hue (more red shifted in clear-water males) while no significant differences in UV coloration were found. The differential reflection patterns in longer wavelengths suggest that sticklebacks of both habitat types have adapted to the respective water conditions. Adaptations of UV signals in a sexual context to ambient light conditions in both behavior and coloration seem less evident.

Key words: color signals, female mate preference, *Gasterosteus aculeatus*, lighting environment, sensory drive, UV vision

Many animal species are capable of perceiving ultraviolet A (UVA) wavelengths between 320 and 400 nm (e.g., [Tovée 1995](#)). UVA has been shown to be of influence in various contexts like orientation ([Sauman et al. 2005](#)), foraging ([Viitala et al. 1995](#)), and intraspecific communication, especially during mate choice decisions (e.g., insects: [Kemp 2008](#); reptiles: [Bajer et al. 2010](#); birds: [Bennett et al. 1996](#); [Zampiga et al. 2008](#); fishes: [Smith et al. 2002](#); [Rick et al. 2006](#); [Rick and Bakker 2008a](#)). Besides the advantageous effects, UVA perception is also costly due to the higher energy of UV light compared with visible light which may negatively impact visual tissues ([Losey et al. 1999](#); [Slincy 2002](#)). It can damage the retina (e.g., [Zigman et al. 1996](#)) and will increase the degree of chromatic aberration ([Muntz 1973](#)). In fishes, for example, UVA negatively modulated physiological and immunological functions ([Winckler and Fidhiany 1996](#); [Salo et al. 2000](#)) but may promote DNA repair ([Dong et al. 2007](#)).

The effectiveness of visual signals and visual communication is influenced by (1) the spectral properties of the signal, (2) the visual capacities of the signal receiver, and (3) the ambient photic conditions under which signaling occurs (“sensory drive hypothesis”: [Endler 1992](#)). Sensory drive is defined as the evolution of signals, perceptual systems, and behavior triggered by the physics of signal production and signals transmission as well as the neurobiology of perception ([Endler 1992](#); [Boughman 2002](#)). With respect to mating signals, the hypothesis proposes how certain signals are best designed to attract mates ([Boughman 2002](#)). Light and the proportion of light available for signal transmission vary strongly between habitats ([Lythgoe 1979](#)). This applies particularly to aquatic habitats, in which the transmission of light is quite complex, as sunlight is refracted and scattered when entering the liquid medium and water molecules as well as dissolved particles subsequently scatter and absorb the transmitted light ([Johnsen 2012](#)). Short wavelengths are attenuated relatively strongly, making the transmission of UV signals only viable over short distances ([Losey et al. 1999](#)). However, in oligotrophic waters, ~10% of surface UVA radiation (at 360 nm) can be found at depths between 50 and 70 m ([Lee et al. 2013](#)). Aquatic habitats may vary in water depth, the amount of dissolved organic matter, and/or suspended sediments that affect the transmission of the color signals and the background they are viewed against ([Partridge and Cummings 1999](#)). Those signals that most efficiently reach the receiver are favored by selection ([Partridge and Cummings 1999](#)). Accordingly, the expression of color patterns has been shown to be highly habitat-dependent in a number of species (e.g., [Fuller 2002](#); [Morrongiello et al. 2010](#); [Giery and Layman 2017](#)).

A habitat-dependent expression of color patterns has also been demonstrated in 3-spined sticklebacks *Gasterosteus aculeatus* (e.g., [Reimchen 1989](#); [Scott 2001](#); [Novales Flamarique et al. 2013a](#)), the model organism of this study. Sticklebacks show pronounced sexual dichromatism during the reproductive season. Males usually develop characteristic courtship coloration that is composed of an orange-red cheek, a blue iris, and a blue-green dorsal region ([Bakker and Mundwiler 1994](#)). The typical red courtship coloration has been shown to be of particular relevance in mate choice behavior ([Milinski and Bakker 1990](#); [Bakker and Milinski 1993](#); [Bakker and Mundwiler 1994](#)). However, the evolution of and the preference for the courtship coloration of 3-spined stickleback males—with respect to color variables in the visible part of the spectrum—is dependent on the photic environment ([McDonald et al. 1995](#); [McDonald and Hawryshyn 1995](#); [Boughman 2001](#); [Scott 2004](#)). Besides the typical red courtship coloration, reproductively active sticklebacks reflect in

the UV ([Rick et al. 2004](#); [Rowe et al. 2004](#)) and possess a fourth cone type, which is UV-sensitive ([Rowe et al. 2004](#); [Novales Flamarique et al. 2013b](#)). UV has been shown to be decisive in female mate choice in 3-spined sticklebacks (e.g., [Boulcott et al. 2005](#); [Rick et al. 2006](#)) and [Rick and Bakker \(2008a\)](#) were furthermore able to show that UV and red wavelength are equally important during visual mate choice.

Ecological aspects of color signal evolution are the subject of an increasing number of studies across a variety of taxa (e.g., [Millar et al. 2006](#); [Ng et al. 2013](#)). In contrast, studies on specific effects of environmental UV-light conditions on signaling in the UV spectral range and on preferences for these signals are lacking. Therefore, we investigated the influence of varying lighting conditions (water staining) on the mate preference of female 3-spined sticklebacks *G. aculeatus* with respect to UV-signaling. The island of North Uist (Outer Hebrides), Scotland, comprises clear-water lakes as well as tea-stained bodies of water that strongly differ in their lighting conditions, in which stickleback populations have evolved for ~10,000 generations ([Giles 1981](#)). The tea-stained lakes are characterized by a high amount of dissolved organic carbon and thus a low transmission of short wavelengths, especially UV light ([Losey et al. 1999](#)), and are referred to as red-shifted ([Jerlov 1968](#)). Thus, the lakes of the 2 habitat types (clear-water vs. tea-stained) across the island should possess great variation in their spectral distribution, especially concerning UV wavelengths.

Sensory drive predicts mating signals to be best designed to attract mates. The signaling conditions in both habitat types differ considerably, especially in the UV spectral region. Our predictions were therefore that both mating signals and associated mating preferences will differ between photic habitats with UV and red signals being of less importance in tea-stained habitats compared with clear-water habitats.

Material and Methods

Experimental subjects

Three-spined sticklebacks used in experiments were caught using minnow traps from 6 lakes on the island of North Uist, Scotland (57°35′N, 7°18′W), in April 2010 and 2011 and transported to the Institute for Evolutionary Biology and Ecology, University of Bonn, Germany. The 6 stickleback populations belonged to 2 different habitat types, 3 from clear-water (Lake Grogary, Lake Eubhal, and Lake Sandary), the other 3 from tea-stained, turbid lakes (Lake a Bharpa, Lake Scadavay, and Lake Tormasad), which differed in their spectral distribution, especially in the UV spectral range between 300 and 400 nm ([Figure 1A](#)). The pH of the clear-water lakes was alkaline while that of tea-stained lakes was acidic (e.g., [Rahn et al. 2016](#)). All fish (100 per population) were kept in aerated aquaria (L × W × H, 50 × 50 × 30 cm) in groups of 20 fish separated by population in an air-conditioned room under standardized summer light regime (temperature: 17 ± 1°C, day length: 16L : 8D). All aquaria were illuminated by fluorescent tubes (Truelight, T8/18 W, T8/58 W), which provide light with a proportion of UV similar to natural skylight ([Rick et al. 2006](#)). Fish were fed daily to excess on frozen *Chironomus* spec.

Males showing first signs of courtship coloration were transferred to individual aerated aquaria (30 × 20 × 20 cm) equipped with a petri-dish (diameter: 12 cm) filled with sand as nesting site and 2 g of 3-cm-long green threads as nesting material. Nest-building was initiated by stimulating each male twice daily for 15 min with a receptive female. Only males that had completed their

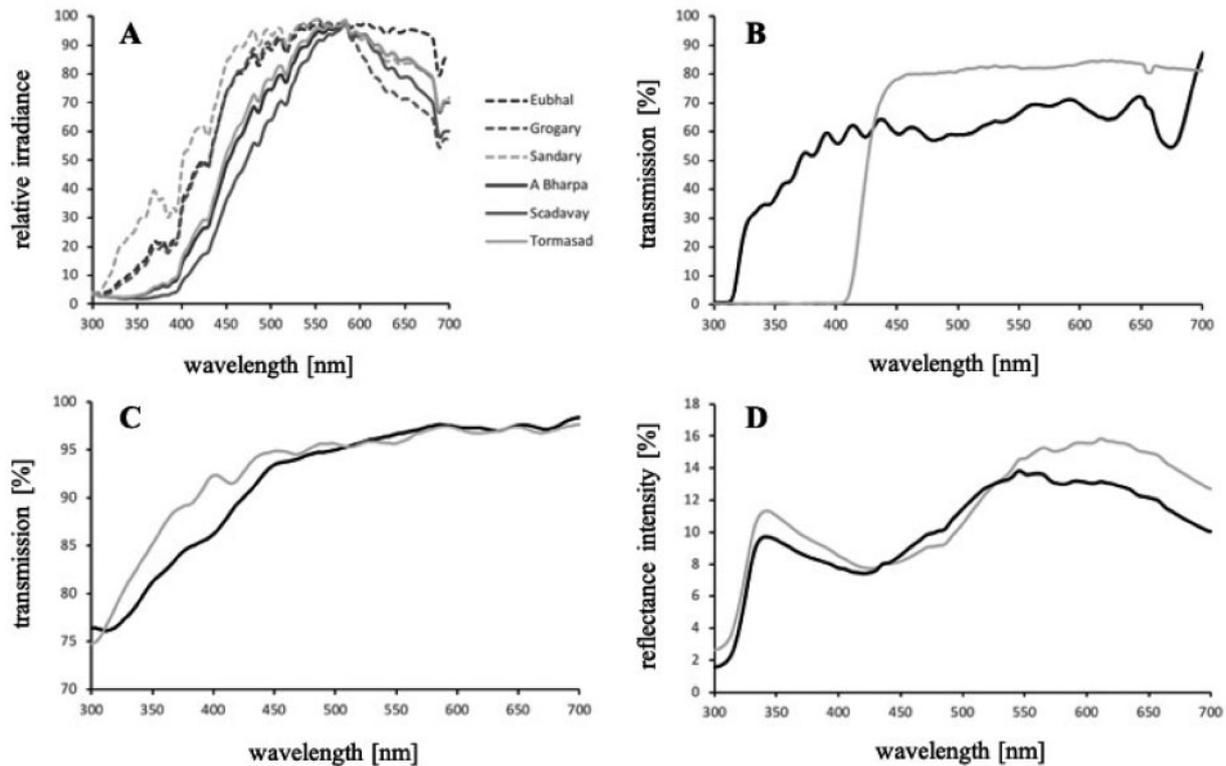


Figure 1. (A) Relative downwelling solar irradiance spectra measured in a water depth of 30 cm (tea-stained lakes (lochs): Loch a Bharpa, Loch Scadavay, Loch Tormasad [solid lines]; clear-water: Loch Eubhal, Loch Grogary, Loch Sandary [dashed lines]) with an Avantes AvaSpec 2048 fiber-optic spectrometer connected to a cosine corrector that collects from an 180° angle (Avantes CC-UV/VIS) in the spectral range between 300 and 700 nm. (B) Transmission [%] of the UV-transmitting (black line) and the UV-blocking (grey line) filter. To control for differences in total quantal flux between the 2 filter types, 4 layers of UV-blocking filter were used, minimizing the differences in total quantal flux between 300 and 700 nm to 0.13%. (C) Mean transmission [%] of the water samples taken from the 3 tea-stained lakes (Scadavay, A Bharpa, and Tormasad) (black line) and the experimental water stained with rooibos tea (grey line), measured using a transmission dip probe. (D) Mean reflectance spectra (proportion of light reflected in relation to a white standard (see text) of males from clear-water lakes (grey line) and tea-stained lakes (black line).

nest (indicated by a clearly visible tunnel through the nest) were used in experiments. Receptive females of the respective populations served as test fish. Males and females were weighed and measured ahead of experiments and the condition factor was calculated following Bolger and Connolly (1989).

Experimental design

Experiments were conducted between June and July 2010 and June and August 2011. To test female mate preferences, test females were offered a single male under 2 different viewing conditions [UV-present (UV+)/UV-absent (UV-)]. The advantage of such a paired mate preference design is that a preference for other male traits—besides the different viewing conditions—can be ruled out (Macías Garcia and Burt de Perera 2002; Rick and Bakker 2008c). To exclude that preferences shown during experiments were based on a preference for the viewing environment per se and not on the manipulated male coloration, experiments on habitat preference were conducted using fish from the same populations like those in this study in the course of another study. The results showed that juvenile test fish (~6 months of age) of both habitat types did not show significant preferences for either viewing condition ($P > 0.09$; M.H. et al., unpublished data). Similar results had been obtained in another study on 3-spined sticklebacks: both non-reproductive and gravid females did not show a preference for the UV+ or UV- compartment ($P > 0.15$; Boulcott et al. 2005). The experimental aquarium

(70 × 35 × 35 cm; water level: 15 cm; Figure 2A) was illuminated by 2 fluorescent tubes (Truelight, T8/36 W) installed 72 cm above the bottom of the tank (Figure 2B). It was divided into 2 compartments, a smaller male (MC) and a larger female compartment (FC), separated by 2 grey opaque plastic partitions placed at an angle of 115° to each other. A Plexiglas window (GS-2458, Röhm, Darmstadt, Germany), transparent to light between 300 and 800 nm, was glued into an opening (24 × 12 cm) cut into each opaque partition and allowed visual but no olfactory contact between the male and the test female. In front of each Plexiglas window 2 removable optical filters as well as a removable opaque partition were installed. One of the filters was UV-blocking (LEE 226), the other UV-transmitting (Neutral-Density (ND) Filter, Rosco E Color 298 ND). After raising the opaque partition and one of the optical filters the test female could either view the male under the spectrum visible for humans (400–700 nm) or under a spectrum extended into the UV spectral range (300–700 nm). Four layers of UV-blocking filter and 1 layer of UV-transmitting filter were used to equalize for transmitted quantal flux (0.13% difference between 300 and 700 nm) (Figure 1B) as determined spectrophotometrically for both filters (see Hiermes et al. 2015b). Controlling for quantal flux was only reasonable after assuring that sticklebacks of all populations were able to perceive light in the complete spectral range between 300 and 700 nm, including UV (learning experiments: M.H. et al., unpublished data; opsin data: T.C.M.B. et al. unpublished data), otherwise an

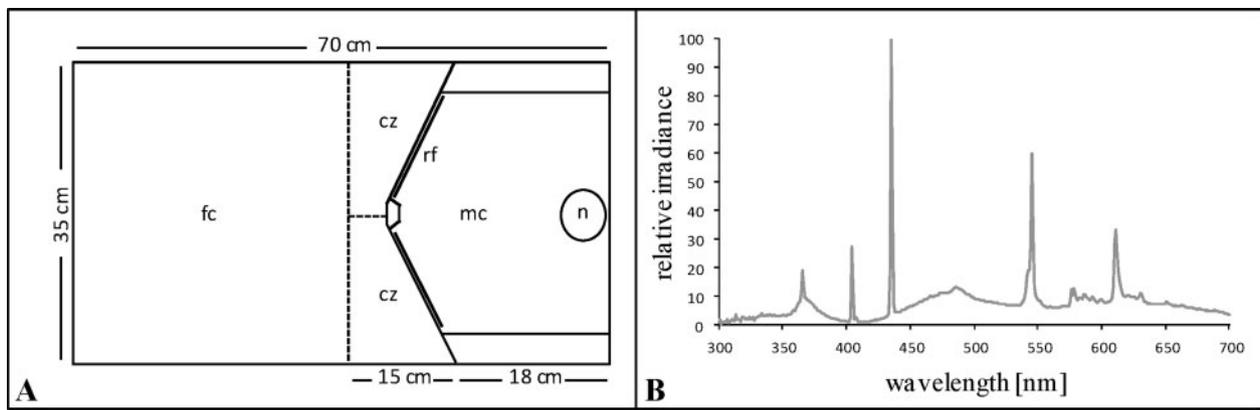


Figure 2. (A) Top view of the experimental tank. The aquarium was divided into 2 compartments: female compartment (FC) and male compartment (MC) with the male's nest (N) positioned centrally on the back wall. The 2 compartments were separated by opaque plastic partitions with an embedded UV-transmitting Plexiglas window on each side. In front of each of the windows, 2 removable filters (UV-blocking and UV-transmitting) and a removable opaque partition were installed. Dashed lines mark the borders of 2 choice zones (CZ). (B) Relative irradiance spectrum of the 2 fluorescent tubes (Truelight T8/36 W) used for illumination of the experimental set-up in the spectral range between 300 and 700 nm.

unintentional difference in light intensity would have been created. The mechanisms involved in luminance discrimination in sticklebacks are still unknown, however, mate choice experiments in sticklebacks from a German freshwater population suggest that UV wavelengths are involved in detecting chromatic cues rather than differences in luminance (Rick et al. 2006; Rick and Bakker 2008c). Both filters and opaque partitions could be lifted from outside the set-up via a pulley with minimal disturbance of the male and test female. Two choice zones (CZs) were marked in the FC in front of the filters (Figure 2A). To prevent possible disturbances, the test tank was surrounded by opaque, grey plastic partitions and the whole set-up was shielded by a black plastic curtain, which was fixed to a wooden frame.

Each experiment consisted of 2 water-color treatments, a tea-stained and a clear-water treatment. To mimic the water color of the original habitats, water samples were taken from the lakes, transported in opaque bottles to the lab in Germany and the transmission was later analyzed using a transmission dip probe with an integrated white standard (Avantes, Apeldoorn, Netherlands). To produce tea-stained water that matched the average water color of the tea-stained lakes, 2 tea bags of theine-free (caffeine-free) rooibos tea (Westcliff) were steeped for 6 min in 400 mL of freshly boiled water. The brown-colored tea was diluted in 52 L of 1-day-old tap water. The transmission of the tea-stained water was measured using the same method and was compared with the light transmission of the original lake water and closely resembled it (Figure 1C). For the clear-water treatment plain 1-day-old tap water was used.

Before the experiments started, the test tank was filled with water of the respective treatment up to a level of 15 cm. Then the filter positions (left/right) were randomly assigned and adjusted accordingly. To exclude possible side effects we used a paired design for each test fish, switching filter positions within both treatments. The whole experiment was filmed from above with a webcam connected to a laptop positioned outside the set-up to exclude disturbances by the experimenter. The nest was moved from the holding tank to the test tank and placed centrally in front of the back wall (Figure 2A). The orientations of the nest entrance in the holding tank and in the test tank were kept identical. The nest owner and a receptive female of the same population, which had been originally kept in different stock tanks to exclude confounding effects of familiarity, were then gently released into the MC and FC, respectively.

Fish were acclimated for 15 min, during which visual contact was prevented as the opaque partitions in front of the optical filters were lowered. After acclimation, the opaque partitions in front of the filters were gently lifted from outside the set-up and a 15 min lasting observation period started. Then the opaque partitions were lowered again, the positions of the UV+ and UV- filters were exchanged and fish were allowed to acclimate again for 15 min. This period was again followed by an observation period of 15 min. Immediately thereafter, males and females were netted out of the aquarium and the water was replaced according to treatment. Meanwhile, males and females were kept in 2 1-L-plastic boxes, without visual contact. Subsequent to water replacement, the same experimental procedure of acclimation and observation was performed again twice under the different water treatment using the same fish. During the tests males and females usually showed courtship behavior, that is zigzagging and nest-directed behavior in the males and head-up posture in the females.

After the experiments, females had to spawn with the respective test male in its holding tank within 24 h to ensure that they had been willing to spawn during the choice tests and that the measured social preferences were thus based on mate choice behavior (Bakker et al. 1999; Mehlis et al. 2008). Females that failed to spawn within that time interval were excluded from analysis. In 2010, 4 out of 15 tested females failed to spawn (2 tea-stained/2 clear-water) and in 2011, 9 out of 60 tested females did not spawn (5 tea-stained/4 clear-water). All test females and test males were only used once.

Data analysis

All videos were analyzed blind with respect to the position of the UV filters. We measured the absolute time females spent in each of the CZs (UV+ or UV-) for both water treatments. A preference index (time in front of male under UV+ viewing condition divided by total time spent in both CZs) was calculated for further analyses. Females that failed to enter both CZs within each of the 4 observation periods were not taken into account (2011: 6 females [2 tea-stained/4 clear-water]). Sample sizes for 2010 were thus $N_{(\text{tea-stained})} = 2$ and $N_{(\text{clear-water})} = 9$ and for 2011, $N_{(\text{tea-stained})} = 16$ and $N_{(\text{clear-water})} = 29$. As preferences did not significantly differ between the 2 years (Welch 2-sample *t*-test, $t = -0.100$, $df = 14.832$, $P = 0.922$), data were pooled resulting in a total sample size of $N_{(\text{tea-stained})} = 18$ (Lake a Bharpa: $N = 5$, Lake Scadavay: $N = 5$,

Lake Tormasad: $N = 8$) and $N_{(\text{clear-water})} = 38$ (Lake Grogary = 14, Lake Eubhal = 10, Lake Sandary = 14).

Reflectance measurements

As the fertilized eggs of the test females were used in another study, we waited for 2 h after spawning before doing the reflection measurements. That gave the eggs enough time to harden (Kraak and Bakker 1998), while differences in male coloration are relatively stable. Two hours after females had spawned, males were removed from the tank, and reflection measurements in the spectral range between 300 and 700 nm were carried out at 3 defined body regions: the cheek region below the eye (Figure 1D), the abdominal testis region, and the dorsal region below the second spine. These body regions—with the exception of the back—are known to reflect in the UV in other populations of the 3-spined stickleback (Rick et al. 2004; Rowe et al. 2004; Pike et al. 2011). The reflection of the left body surface was measured at an angle of 90° with an Avantes AvaSpec 2048 fiber-optic spectrometer and a bifurcated 200-micron fiber-optic probe. Unidirectional illumination was provided by a deuterium-halogen lightsource (Avantes AvaLight-D(H)-S Deuterium-Halogen Light Source, 200–1100 nm). Measurements were done quickly (<2 min per individual) to minimize color changes based on, for example, pigment dispersion. Reflection measurements were recorded relative to a Spectralon WS-2 white-standard with Avantes AvaSoft version 7.5 for USB2 and afterward exported to Microsoft Excel. The average of 20 measurements per body region was used in subsequent analyses. A physiological model, simulating how stickleback males might be perceived by female conspecifics, was calculated with Avicol_v6 (Gomez 2006). The spectral sensitivity curves were determined for the 4 stickleback cone receptors from absorbance maxima provided in Rowe et al. (2004) by using parameters for the calculation of visual pigment templates provided in Govardovskii et al. (2000). Absolute cone stimulations (UV, S, M, and L) for the spectral range between 300 and 700 nm were then calculated by multiplying individual reflectance, the ambient light (spectrum of the fluorescent tubes used during experiments (Truelight T8/36 W; Figure 2B)) and the calculated spectral cone sensitivity (see Endler and Mielke 2005). Absolute stimulations were then converted to relative cone stimulations and translated to the Cartesian coordinates x , y , and z , which were mapped within a tetrahedral color space (see Endler and Mielke 2005; Stoddard and Prum 2008; Rick et al. 2011). Within the tetrahedral color space, the central point is the achromatic point, in which all 4 cone classes are stimulated equally and thus represents the absence of all colors (Drobnjak et al. 2014). The direction of a vector of the achromatic point describes hue, and this vector in turn is represented by 2 angles (θ and ϕ) (see Stoddard and Prum 2008; Hiermes et al. 2015a). θ (longitudinal hue) describes a measure of hue of the carotenoid-based coloration in the human-visible spectral range and is associated with the composition of carotenoids in the red breeding coloration of stickleback males (see Pike et al. 2011; Vitt et al. 2019, 2020). Higher values indicate orange-shifted and lower values more red-shifted hues (see Pike et al. 2011). ϕ (latitudinal hue) represents a measure of the stimulation of the UV cone; low values of ϕ describe a reduced stimulation of the UV cone whereas high values describe an increased stimulation of the UV cone (see Pike et al. 2011). The color intensity (chroma) is defined as the distance of the achromatic point from a given color point. The larger the magnitude of the chroma, the larger is the distance from the achromatic point and thus the higher is the color intensity. We used achieved chroma r_A as measure of color intensity, which is the value for chroma r in comparison to the maximum possible value of for a

specific hue (r/r_{max}) (Stoddard and Prum 2008; Mehliş et al. 2015; Hiermes et al. 2016; Vitt et al. 2019, 2020).

To allow for better comparison with other studies, further colorimetric variables were calculated that correspond to the variables calculated with the help of the physiological model. For the cheek region “UV chroma” (roughly corresponding to the stimulation of the UV cone ϕ), “red chroma” (corresponding to achieved chroma r_A) and the “R50 value” (corresponding to θ) were calculated. To determine the “red chroma,” a measure of the relative orange-red intensity, the amount of light in the spectral range between 575 and 700 nm was calculated relative to the total amount of light in the spectral range between 300 and 700 nm (Rick et al. 2011). The “UV chroma” was calculated the same way, including the relative amount of UV reflection between 300 and 400 nm (Rick et al. 2004; Shawkey et al. 2006). For carotenoid-based color the “R50 value” is defined as the wavelength that corresponds to the point of the spectrum that is centered between the minimum reflection between 400 and 500 nm and the maximum reflection between 500 and 700 nm, and is an indicator of hue (Rick and Bakker 2008b; Pike et al. 2011).

For the testis region, which lacks the orange-red courtship coloration, only “UV chroma” and ϕ were determined and for the dorsal region the total brightness was used in analyses and compared between the males of both habitat types.

Statistical analyses

Some data had to be logarithmically transformed (UV chroma [testis] & brightness [back]) or put to the power of 6 (R50-value [cheek]) to reach normal distribution.

Parametric statistics were used throughout as data (raw or after transformation) and the residuals of the best-fitting models did not significantly deviate from normal distribution according to Shapiro-Wilk tests.

R version 4.0.3 (R Development Core Team 2020) statistical package was used for all analyses. Linear mixed effect models were conducted using the “lme” and VarCorr functions of the “nlme” library (Pinheiro et al. 2020). The preference indices of females from tea-stained habitats and clear-water habitats (for both treatments pooled, for the tea-stained, and for the clear-water treatment) served as dependent variables in separate models. “Population” was included as random factor in all models and never removed to control for population-linked preferences, but had no significant effect on UV preference (all $\chi^2 < 0.001$, all $P > 0.99$). “Condition factor (male)” and “condition factor (test female)” were included as explanatory variables and non-significant variables were stepwise removed from the models in the order of statistical relevance. Furthermore, to test whether the UV preference index of fish differs between the 2 water-color treatments depending on the habitat of origin an additional model was fitted including an interaction term between treatment and habitat as explanatory variables and “test fish individual” nested within “population” as random factor. The models were compared with likelihood-ratio-tests that follow a χ^2 -distribution, so that degrees of freedom always differed by 1.

To check for differences between the preference of females from the 2 habitat types, Welch 2-sample t -tests were conducted. Paired t -tests were used to test for differences in female preference between the 2 water-color treatments within the 2 habitat types. Welch 2-sample t -tests were also used to compare the colorimetric variables of males from the 2 habitat types. All test probabilities are 2-tailed throughout.

Results

Female mate preference

Fish from both tea-stained lakes and clear-water lakes significantly preferred the male viewed under UV+ conditions when water-color treatments were pooled (tea-stained lakes: “lme,” $t = 3.136$, $df = 15$, $P = 0.007$; clear-water lakes: “lme,” $t = 3.787$, $df = 35$, $P < 0.001$; Figure 3). There was no significant difference in preference between females of different habitat types in the pooled dataset (Welch 2-sample t -test, $t = -0.294$, $df = 35.426$, $P = 0.771$; Figure 3). In the non-pooled datasets, females from tea-stained lakes tended to prefer UV+ males when tested under the visual conditions of origin (tea-stained treatment: “lme,” $t = 1.911$, $df = 15$, $P = 0.075$). Moreover, females from tea-stained lakes significantly preferred UV+ males under non-native visual conditions (clear-water treatment: “lme,” $t = 2.645$, $df = 15$, $P = 0.019$; Figure 4), albeit male body condition had a confounding effect on the result, that is, females with lower

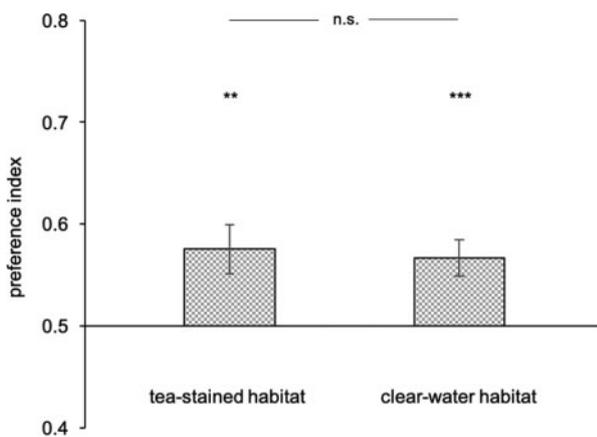


Figure 3. Preference index (time in front of male under UV+ viewing condition divided by total time spent in both CZs) for stickleback females from tea-stained and clear-water lakes irrespective of water-color treatment. Plotted are mean differences and standard errors. Values >0.5 indicate a UV+ preference. *** $P < 0.001$; ** $P < 0.01$; n.s.: $P > 0.1$.

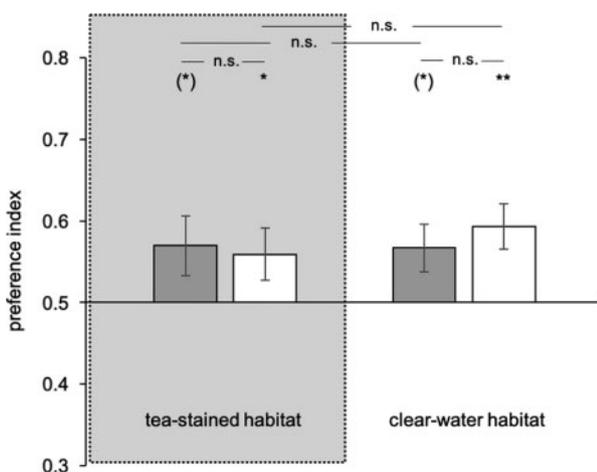


Figure 4. Preference index (time in front of male under UV+ viewing condition divided by total time spent in both CZs) for stickleback females from tea-stained (grey background) and clear-water lakes (white background) in the tea-stained treatment (grey bars) and in the clear-water treatment (white bars). Plotted are mean differences and standard errors. Values >0.5 indicate a UV+ preference. ** $P < 0.01$; * $P < 0.05$; (*) $0.05 < P < 0.1$, n.s.: $P > 0.1$.

body condition showed a greater preference for UV+ males (“lme,” $\chi^2 = 4.871$, $df = 1$, $P = 0.027$). While showing a tendency to prefer UV+ males under tea-stained conditions (tea-stained treatment: “lme,” $t = 2.024$, $df = 35$, $P = 0.051$; Figure 4), females from clear-water habitats significantly preferred males under UV-presence when tested under the visual conditions of origin (clear-water treatment: “lme,” $t = 3.301$, $df = 35$, $P = 0.002$; Figure 4). Other than for fish from tea-stained lakes under clear-water conditions, as mentioned above, body condition of males and females had no significant influence on preference (all $\chi^2 < 2.371$, all $P > 0.124$). There was no effect of experimental treatment on female preferences for UV+ males in the face of habitat of origin, indicated by a non-significant interaction (“lme,” $\chi^2 = 0.450$, $df = 1$, $P = 0.503$). Accordingly, a significant difference in preference between females of different habitat types was neither found in the tea-stained treatment (Welch 2-sample t -test, $t = -0.231$, $df = 38.403$, $P = 0.819$; Figure 4) nor in the clear-water treatment (Welch 2-sample t -test, $t = 0.798$, $df = 42.347$, $P = 0.429$; Figure 4). There was no significant difference in preference between water-color treatments either for fish from clear-water lakes (paired t -test, $t = 0.715$, $df = 37$, $P = 0.479$, Figure 4) or for fish from tea-stained lakes (paired t -test, $t = -0.230$, $df = 17$, $P = 0.821$, Figure 4).

Reflectance measurements

Males from different habitat types differed significantly with respect to some aspects of their courtship coloration (Table 1). With respect to color intensity, males from clear-water lakes had a significantly higher achieved chroma r_A (clear-water males: mean \pm standard deviation (SD) = 0.256 ± 0.118 ; tea-stained males: 0.181 ± 0.122), and corresponding to the color intensity measure of the physiological model, also a significantly higher red chroma at the cheek (clear-water males: mean \pm SD = 0.411 ± 0.069 ; tea-stained males: 0.345 ± 0.049) (Table 1). In addition, θ , the measure for hue in the human-visible part of the spectrum, of males from clear-water lakes was significantly shifted further to the red part of the spectrum in comparison to their tea-stained counterparts (clear-water males: mean \pm SD = 14.620 ± 25.664 ; tea-stained males: 38.068 ± 26.412). Correspondingly, the R50 value was significantly higher in clear-water males (512.579 ± 36.499) compared with tea-stained males (486.833 ± 19.479), which also indicates a more red-shifted courtship coloration of males from clear-water lakes (Table 1). There were no significant differences in UV chroma or ϕ , neither in the cheek nor in the testis region (Table 1). The brightness in the back region did not differ significantly between males from different habitat types (Table 1).

Discussion

Some specific predictions of sensory drive regarding transmission properties, which emphasize the importance of the environmental conditions under which signaling occurs, would have proposed a limited role of UV communication in tea-stained bodies of water, in which the transmission of UV and the availability for communication purposes is expected to be low. However, we found an overall female preference for males viewed under UV+ conditions, irrespective of habitat of origin (Figure 3). Given $\sim 10,000$ generations of adaptation to the environmental conditions (Giles 1981) and the marine (clear-water) origin of the freshwater stickleback populations on North Uist, one could have expected a reduced UV signal in males and also a reduced female preference of UV in tea-stained lakes compared with those from clear-water lakes. However, in the

Table 1. Differences in courtship coloration variables taken at stickleback male's cheek, abdomen (testis region), and back for both males from tea-stained and clear-water lakes

Variable	<i>t</i>	<i>df</i>	<i>P</i> -value	Mean ± SD (tea-stained)	Mean ± SD (clear-water)
UV chroma (cheek)	-1.360	34.470	0.183	0.211 ± 0.052	0.191 ± 0.054
<i>pbi</i> (cheek)	-0.829	37.602	0.412	-16.968 ± 58.248	-31.405 ± 66.043
achieved chroma r_A (cheek)	2.178	32.370	0.037	0.181 ± 0.122	0.256 ± 0.118
red chroma (cheek)	4.396	42.699	<0.001	0.348 ± 0.049	0.417 ± 0.065
R50 value	3.436	53.093	0.001	486.83 ± 19.48	512.58 ± 36.50
<i>theta</i> (cheek)	-3.131	32.609	0.004	38.068 ± 26.412	14.620 ± 25.664
UV chroma (testis)	-1.249	41.405	0.219	0.191 ± 0.041	0.175 ± 0.052
<i>pbi</i> (testis)	-0.640	30.921	0.527	-43.960 ± 48.139	-52.539 ± 44.044
brightness (back)	0.651	48.703	0.518	1991.01 ± 1836.57	2402.69 ± 2845.18

Welch 2-sample *t*-tests were used throughout. Significant results are printed in bold.

reproductive season between April and August reproductively active males in most populations establish territories in shallow shore regions, build a tunnel-shaped nest from plant material, and court females once the nest is finished (Wootton 1976). It thus cannot be ruled out that courtship in both tea-stained and clear-water lakes, takes place in shallow areas that experience a substantial amount of UV radiation, independent of general water-staining conditions in the 2 habitat types. Further field research, including the collection of behavioral data on water depth preferences of courting fish from both habitat types, would help reveal whether sensory adaptation to UV-rich courtship environments can be observed in fish from our study populations.

Irrespective of habitat, possible positive effects linked to the presence and expression of UV coloration, for example, an enhanced physical condition of the mating partner (see Rick et al. 2004) seem to outweigh possible negative effects of communicating in the UV spectral range. Possibly, UV mate-preferences have evolved through sensory bias (Ryan and Rand 1990; Endler and Basalo 1998; see also Smith et al. 2004). Studies on other stickleback populations have demonstrated the importance of UV signals, also relative to red signals (Rick and Bakker 2008a), on female mating decisions (Boulcott et al. 2005; Rick et al. 2006), although the significance of UV has been doubted in a study by Pike et al. (2011). The results of our study on 6 additional populations, however, corroborate the relevance of UV in visual mate preference and emphasize the need of including the UV spectral range in experiments of UV-sensitive test animals.

Females from both habitats showed the most pronounced UV preferences when tested under clear-water conditions (Figure 4), which for females of the clear-water habitat might be explained by a fine-scale tuning between the female visual system and male courtship signals triggered by the signal transmitting medium. Males might have evolved ornaments that are most attractive under certain visual conditions and that match the female sensory systems. This assumption is corroborated by the fact that we found substantial differences in color variables between males of different habitat types. In bluefin killifish, Fuller and Noa (2010) demonstrated that lighting environment had an effect on mate-choice decisions, probably by altering the immediate perception of male color patterns, which corresponds to our results of more pronounced, albeit non-significantly, mate preferences under clear-water conditions. In bluefin killifish, rearing light conditions also affected mating preference (Fuller and Noa 2010), which contrasts with our finding of similar mate preferences of fish from different habitat types (Figure 3). Rearing light conditions also affected inter-specific female preferences in 2 closely related African cichlid species (Wright et al. 2017).

In experiments with guppies, males spent more time in the light environment in which their color signal had the highest contrast but only in the presence of receptive females (Cole and Endler 2016). Novales Flamarique et al. (2013b) examined several stickleback populations from different photic regimes with respect to heritable variability and phenotypic plasticity in opsin expression. The influence of phenotypic plasticity on opsin expression was found to be minor, when lighting conditions were altered (Novales Flamarique et al. 2013b). This finding agrees with the similar UV preferences of females from different habitats in this study (Figures 3 and 4), although the correlation between preference and opsin expression may be weak (Fuller and Noa 2010).

In a non-sexual context, a study by Fuller et al. (2010) on foraging preference of lab-reared offspring of bluefin killifish, which were raised in 2 rearing environments (tea-stained/clear-water) and later on tested in 2 lighting environments (tea-stained/clear-water) showed that lighting environment had an immediate effect on foraging preferences. The observed foraging preference was also shown to be dependent on the light conditions fish experienced during growth (Fuller et al. 2010). Rearing light conditions also affected preferences in sticklebacks. Three-spined sticklebacks raised under UV deprivation had a different UV shoaling preference (Modarressie et al. 2015) as well as a different UV-based habitat choice under predatory threat (Rick and Bakker 2010) than those raised under full-spectrum conditions. Recently, several studies on fishes showed developmental plasticity of the visual system, that is, opsin gene expression (e.g., Shand et al. 2008; Dalton et al. 2015; Ehlman et al. 2015; Härer et al. 2017; Schweikert and Grace 2018). It would be very interesting to study developmental plasticity of the visual system of female sticklebacks by testing preferences in females from different photic populations that had been reared under different lighting conditions.

Many studies have shown that lighting habitats correlate with the respective color patterns (e.g., Reimchen 1989; Scott 2001; Fuller 2002; Novales Flamarique et al. 2013a; Giery and Layman 2017; Côte et al. 2019). We also found substantial differences between the color patterns at longer but not at shorter wavelengths of males of the tea-stained and clear-water habitat type (Table 1) that correlate with the lighting habitat. The achieved chroma r_A and red chroma, both measures of the intensity of the orange-red courtship coloration, were significantly higher in males from clear-water lakes. The same accounted for the corresponding measures *theta* and the R50 value, which means that the hue of the courtship coloration was shifted further to the red part of the spectrum in comparison to males from tea-stained lakes. In clear freshwater habitats, short and long wavelengths are filtered out of the spectrum with greater

depths, resulting in a high proportion of green wavelength (Lythgoe 1979). Males with a high proportion of red are particularly visible under these conditions (Scott 2001). Thus, males from clear-water lakes should also be highly visible within their habitat. Bodies of water rich in organic material, on the contrary, filter mostly short wavelengths, shifting the light spectrum toward the red proportion of light (Lythgoe 1979; Reimchen 1989). Red-colored males would appear rather inconspicuous viewed against a red-shifted background (Reimchen 1989; Scott 2001). Males from our tea-stained lakes were less intensively red-colored and had a higher, but not significantly so, UV chroma and a higher value of *p_{hi}*, indicating a generally reduced carotenoid concentration (Shawkey et al. 2006) and an overall reflection pattern shifted to shorter wavelengths. Again, as for males from clear-water lakes, males from tea-stained lakes should thus be particularly visible for conspecifics. Similar results have been found in the bluefin killifish, which exhibits blue anal fins in tea-stained waters (Fuller 2002). Novales Flamarique et al. (2013a) compared courtship coloration in 3-spined sticklebacks from clear and red-shifted habitats and suggested that male redness is expressed to enhance the contrast of the blue eye. Females preferred males with the highest contrast between the eye and throat color (Novales Flamarique et al. 2013a). A correlation between water color and area and intensity of red coloration in stickleback was found by Boughman (2001), who in addition found a correlated female preference. Taken with our results, contrast between color patterns and the visual background seems to be maximized under the local lighting conditions, enhancing male conspicuousness in both habitat types. It is thus important to take ambient light, color patterns, and the visual background the latter are viewed against into account (Endler 1990). Further research effort including precise measurements of ambient light and the visual background as well as population-specific spectral sensitivity is thus required to be able to estimate visual contrast.

In conclusion, stickleback mate preference is influenced by UV wavelengths, irrespective of habitat type, which contradicts other studies that have doubted an important role of UV in female mate choice for carotenoid-based red coloration in males (Pike et al. 2011) despite earlier studies that have clearly shown that UV is a decisive factor in stickleback mate choice (Boulcott et al. 2005; Rick et al. 2006). Maybe variation in UV reflectance rather than presence or absence plays a role in mate choice in some populations. The overall preference for UV is certainly based on the fact that mate choice in sticklebacks takes place in shallow shore regions, so that the differences in transmission properties between habitat types are less pronounced in a sexual context. In other social contexts, like UV-based shoaling behavior, instead, differences in lighting conditions have been shown to influence stickleback behavior (Modarressie et al. 2006, 2015). Data on the distribution of opsins in the retinal cone photoreceptors of the 6 stickleback populations will give further insight into visual communication under the prevailing conditions (T.C.M.B. et al., unpublished data). As we found differences in male color patterns in long wavelengths between habitat types, it can be speculated that male color signals have adapted to the photic conditions under which signaling occurs. UV preferences from females of both habitat types were similar and most pronounced under clear-water test conditions, and male color signals in the short wavelengths were also similar between habitat types. There thus is little evidence that in both the female visual system and male coloration there are adaptations of UV signals in a sexual context to differences in ambient light conditions due to water staining.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

T.C.M.B. and I.P.R. conceived the study; T.C.M.B., M.H., and I.P.R. designed the study; M.H. and S.R. collected the data; M.H., S.R., and I.P.R. analyzed the data; T.C.M.B., M.H., and I.P.R. wrote the manuscript.

Data Availability

The datasets generated and/or analyzed during this study are available in the supplementary Excel file.

Supplementary Material

Supplementary material can be found at <https://academic.oup.com/cz>.

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