




## Article

# Influence of lighting environment on social preferences in sticklebacks from two different photic habitats. II. Shoaling and mate preferences of lab-bred fishes

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## Abstract

Different environmental conditions may lead to diverse morphological, behavioral, and physiological adaptations of different populations of the same species. Lighting conditions, for example, vary vastly especially between aquatic habitats, and have been shown to elicit adaptations. The availability of short-wave ultraviolet (UV) light is especially fluctuating, as UV wavelengths are attenuated strongly depending on water properties. The island of North Uist, Scotland, comprises 2 differential habitat types, tea-stained and clear-water lakes, varying considerably in UV transmission. In previous studies, wild-caught 3-spined stickleback *Gasterosteus aculeatus* populations (3 populations of each habitat type) were tested with respect to their shoaling and mate preferences for fish viewed under UV-present and UV-absent conditions. The results revealed a habitat-dependent preference of UV cues during shoal choice (tea-stained populations: preference for UV-absent condition in tea-stained water; clear-water populations: no preference in clear-water) but an overall preference for UV-present conditions during mate choice. To assess genetic influences on these behavioral patterns, similar experiments were conducted with lab-bred F1-generations of the same stickleback populations that were raised in a common environment (i.e. standardized clear-water conditions). Offspring of sticklebacks from tea-stained lakes tended to prefer shoals viewed under UV-absent conditions (only in tea-stained water), while sticklebacks from clear-water lakes showed a significant preference for the shoal viewed under UV-present conditions in clear-water but not in tea-stained water. Mate-preference experiments demonstrated that females from the tea-stained lakes significantly preferred and females from the clear-water lakes preferred by trend the male viewed under UV-present conditions in the clear-water treatment. The results for both shoaling- and mate-preference tests were largely similar for wild-caught and lab-bred sticklebacks, thus hinting at a genetic basis for the preference patterns.

**Key words:** *Gasterosteus aculeatus*, genetic adaptation, mate preference, shoaling preference, UV signals, water staining

There are 2 possible avenues for organisms to adapt to environmental conditions. They can adapt within 1 generation by changing their phenotype by a process called phenotypic plasticity (West-Eberhard 2003). Alternatively, the mean phenotype of a population can change over generations by a process called selection (Darwin 1859). One process does not exclude the other and often both and their interaction are in force. Visual signals and visual communication systems are strongly influenced by the ambient lighting conditions (e.g., Endler 1992, 1993). Variation in environmental light is especially pronounced in aquatic habitats, where spectral transmission is very complex as water molecules and dissolved particles selectively scatter and absorb wavelengths, thus creating unique lighting conditions within each specific aquatic habitat (e.g., Partridge and Cummings 1999; Johnsen 2012). The availability of short-wave ultraviolet (UV) light between 300 and 400 nm is particularly fluctuating between aquatic habitats, as UV wavelengths are attenuated strongly depending on water properties (e.g., Losey et al. 1999). Based on differences in ambient lighting conditions between habitats, signals that are highly efficient in one habitat type may be less effective in other habitats due to among others signal detectability (Leal and Fleishman 2004).

To optimize the efficiency of signaling, fishes have been shown to adapt their coloration in one way or the other to local lighting conditions (e.g., Reimchen 1989; Seehausen et al. 1997; Boughman 2001; Fuller 2002; Maan et al. 2006; Lewandowski and Boughman 2008; Morrongiello et al. 2010; Giery and Layman 2017; Côte et al. 2019), or choose light environments that enhance courtship patterns (Cole and Endler 2016), and the signal receiver's sensory system will in turn be tuned to the different signal and signal transmission ("sensory drive hypothesis": Endler 1992).

In most of the above-mentioned studies on coloration, the nature of the adaptation is unclear as they concern correlation studies. Exceptions are experiments in which either the environment and/or the genotype is controlled for. In the gudgeon *Gobio occitaniae*, for example, reciprocal transplant experiments between habitats of different turbidity, showed that the melanin coloration was highly plastic (Côte et al. 2019). Experiments, for example, with 3-spined stickleback *Gasterosteus aculeatus* from different photic environments that were bred and reared in clear and red-shifted water, showed that the correlation between male color and photic environment is due to both genetic evolution and phenotypic plasticity (Lewandowski and Boughman 2008).

Immediate responses of visual systems to changing local environmental conditions (phenotypic plasticity), for example, are shifts in habitat preferences of fish during ontogeny (e.g., Bowmaker and Kunz 1987; Shand 1993, 1997; Allison et al. 2006; Hoke et al. 2006; Savelli et al. 2018) or responses to short-term changes in environmental light quality (e.g., Schweikert and Grace 2018; Escobar-Camacho et al. 2019). A longtime exposure to different lighting habitats and resulting selection may instead eventually lead to genetic differences in sensory systems (e.g., Fuller et al. 2004; Tobler et al. 2010; Novales Flamarique et al. 2013). More recent studies revealed that observed adaptations to different lighting conditions are a combination of genetic adaptation and developmental plasticity acting on visual systems, e.g. in fishes (e.g., Endler et al. 2001; Fuller and Noa 2010; Fuller et al. 2010; Härer et al. 2017). Fuller and Noa (2010), for example, found in bluefin killifish *Lucania goodei* 3-way interactions between immediate testing environment, developmental plasticity (rearing environment), and genetics in a mate-choice context. Here, preferences were highest when

fish of a certain genetic background were raised and tested under lighting conditions of habitat of origin (Fuller and Noa 2010).

The 3-spined stickleback is a small cold-water fish, which is widespread in various lighting habitats throughout the Holarctic (Bell and Foster 1994). Marine and anadromous sticklebacks have frequently colonized new freshwater habitats and a huge number of these freshwater populations have evolved independently under different environmental conditions (Bell and Foster 1994), which have resulted in evolutionary changes in morphology, physiology, and behavior (Barrett et al. 2010; Greenwood et al. 2011; Wark et al. 2011). Due to its wide geographical distribution and its migratory lifestyle (Bell and Foster 1994), the stickleback has potential for the evolution of plastic responses (e.g., Day et al. 1994; Wund et al. 2008; Frommen et al. 2011).

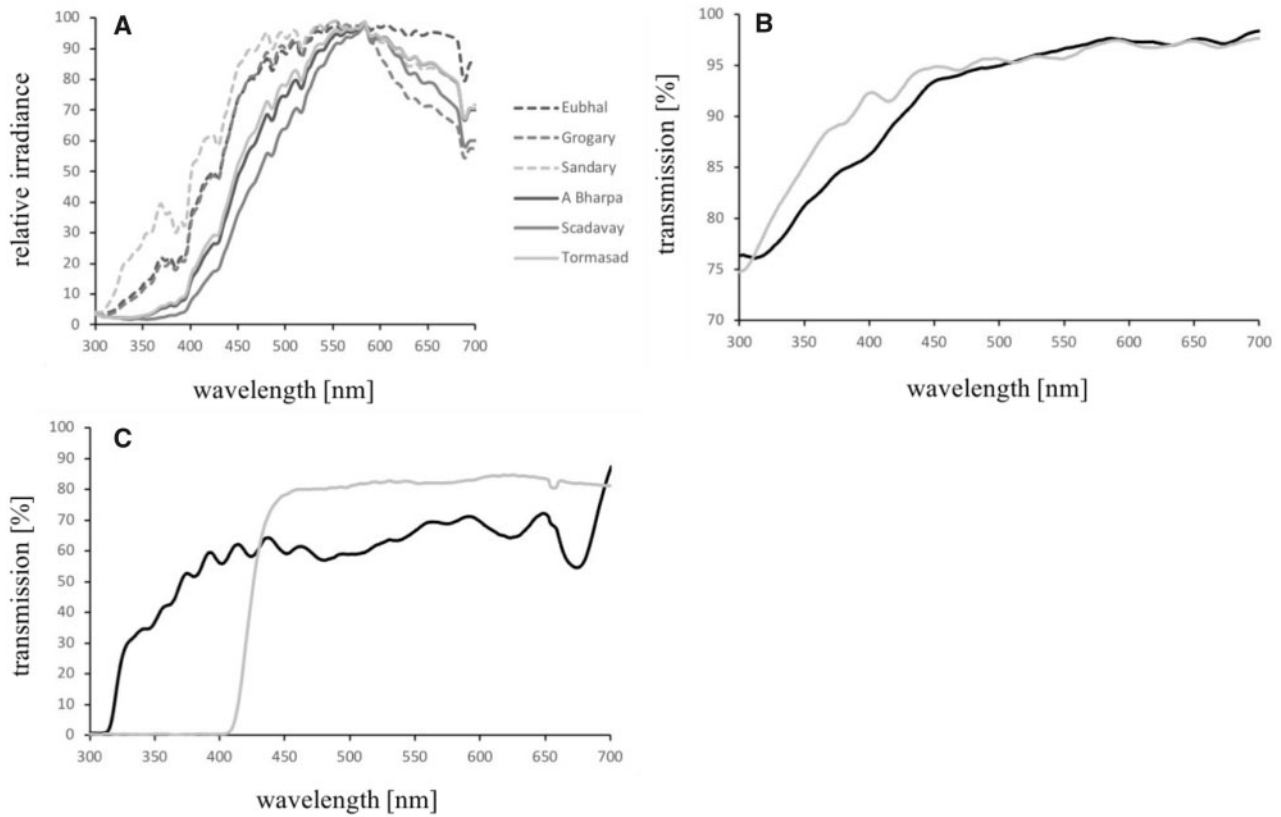
The aim of the present study was to determine genetic influences on UV-mediated social behavior (shoal preference and mate preference). This was done by using sticklebacks originating from 2 photic habitat types (clear-water and tea-stained lakes) on the island of North Uist, Scotland, which strongly differ in the spectral transmission of light, especially at UV wavelengths (see below). For UV signals in particular, ecological aspects of signaling evolution have been largely neglected. Three-spined sticklebacks live in large shoals outside the reproductive season (Keenleyside 1955). During the reproductive season, males leave the shoal and occupy territories in the littoral zone (Wootton 1976). Shoaling behavior (e.g., Van Havre and FitzGerald 1988; Ward et al. 2002; Frommen and Bakker 2004) and mate-choice behavior (e.g., Rowland 1982; Milinski and Bakker 1990; Bakker 1993; Milinski et al. 2005) of sticklebacks have been studied intensively over the past decades but the influence of UV in both contexts has only in recent years received interest (e.g., shoal choice: Modarressie et al. 2006, 2015; mate choice: Rick et al. 2006; Rick and Bakker 2008a, 2008b). The sticklebacks have adapted to these habitats on North Uist for at most 10,000 years since the retreat of glaciers after the last Ice Age (e.g., Giles 1981; Ballantyne 2010).

To investigate genetic influences resulting from selection under different lighting conditions on UV preferences, a F1-generation of wild-caught parents from populations of different photic habitats was raised under common environmental conditions (i.e., standardized laboratory clear-water conditions) and shoal- and mate-preference experiments were conducted. In detail, test fish had the choice between a shoal or a reproductively active male, respectively, viewed under UV-present and UV-absent conditions, and were tested in 2 water-color treatments (tea-stained and clear-water), which matched lighting conditions in the habitats of origin. The results are compared with data from similar experiments conducted with wild-caught fish from the same study populations (Hiermes et al. 2015, 2021). We would expect comparable UV-preference patterns of wild-caught and lab-bred fish when there exist genetic influences on UV preferences.

## Materials and Methods

### Experimental subjects

All fish used in the experiments were the F1-generation of 6 populations of 3-spined stickleback that were caught on the island of North Uist (Scotland) in April/May 2010 and in April 2011. Wild-caught fish originated from 2 habitat types: 3 tea-stained, acidic lakes (a Bharpa, Scadavay, Tormasad) and 3 clear-water, alkaline lakes (Eubhal, Grogary, Sandary), that differ substantially in their spectral distribution, especially in the UV spectral range between



**Figure 1.** (A) Relative irradiance spectra measured in a water depth of 30 cm (tea-stained: 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 spectrophotometer connected to a cosine corrector (Avantes CC-UV/VIS) in the spectral range between 300 and 700 nm. Irradiance calibration was performed versus an Avantes NIST traceable irradiance application standard. (B) 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 (gray line), measured using a transmission dip probe. (C) Transmission (%) of the UV-transmitting (black line) and the UV-blocking (gray line) filter. To control for differences in total light intensity between the 2 filter types, 4 layers of UV-blocking filter were used, minimizing the differences in total quantum flux between 300 and 700 nm to 0.13%.

300 and 400 nm (Figure 1A). The pH of the clear-water lakes was basic while that of tea-stained lakes was acidic (e.g., Rahn et al. 2016). In 2010, 21 families (a Bharpa:  $N=4$ ; Tormasad:  $N=6$ ; Grogary:  $N=6$ ; Sandary:  $N=5$ ) and in 2011, 40 families (a Bharpa:  $N=4$ ; Scadavay:  $N=6$ ; Tormasad:  $N=13$ ; Eubhal:  $N=6$ ; Grogary:  $N=6$ ; Sandary:  $N=5$ ) were successfully bred through random matings within a population. All parental individuals were only used once to avoid pseudoreplication. Fertilized egg clutches were removed from the nest and were kept in aerated 1-L plastic boxes, illuminated by fluorescent full-spectrum tubes (Truelight T8/36W), which provided light similar to natural skylight (Rick et al. 2006), and were fed with *Artemia* spp. from the fourth day after hatching. They were kept in an air-conditioned room (air temperature  $17 \pm 1^\circ\text{C}$ ; water temperature  $15 \pm 1^\circ\text{C}$ ) under standard summer conditions (day/night 16 h/8 h). At an age of 4 weeks after hatching, fish were transferred to plastic tanks (length  $\times$  width  $\times$  height: 40 cm  $\times$  20 cm  $\times$  25 cm), which were equipped with an internal filter (Dohse Aquaristik). Eight weeks after hatching the number of fish per aquarium was reduced to a maximum of 20 individuals. From now on, fish were daily fed with defrosted mosquito larvae (*Chironomus* spp.). At an age of 4–5 months, light conditions were changed to standard winter conditions (day/night 8 h/16 h). After the shoal-preference experiments (conducted between March and May 2011 and 2012 at an age of 9–11 months), conditions were changed back to summer conditions to mimic the beginning of the

breeding season. The mate-preference experiments were conducted between June and August 2011 and 2012 at an age of 11–13 months.

### Experimental design

To investigate the influence of UV in stickleback visual communication, combined shoal- and habitat-preference experiments as well as female mate-preference experiments were conducted. In the shoal-preference experiments, a non-reproductive test fish was given the choice between a shoal viewed under UV-present and another shoal viewed under UV-absent conditions. In the habitat-preference experiment, the choice was between the 2 photic habitats without shoals. We used a paired design, thus each test fish was tested for its shoal and habitat preferences. The habitat choice served as a control experiment to exclude the possibility that a potentially significant preference might be based on the light habitat but not on the shoal viewed under the particular light condition. In mate-preference experiments, a gravid female had the choice of viewing a single reproductively active male under UV-present or UV-absent conditions. In both the shoal- and mate-preference experiments, the preference behavior was tested under 2 light-transmission conditions: 1) under clear-water conditions and 2) under tea-stained conditions. For the clear-water treatment, 1-day-old tap-water was used, while tea-stained conditions were created by dyeing 1-day-old tap-water with

**Table 1** Overview of the 8 different trial sequences used during shoal- and habitat-preference experiments

Trial sequence	Choice experiment type and water-color treatment
1	1 2 3 4
2	1 2 4 3
3	2 1 3 4
4	2 1 4 3
5	3 4 1 2
6	3 4 2 1
7	4 3 1 2
8	4 3 2 1

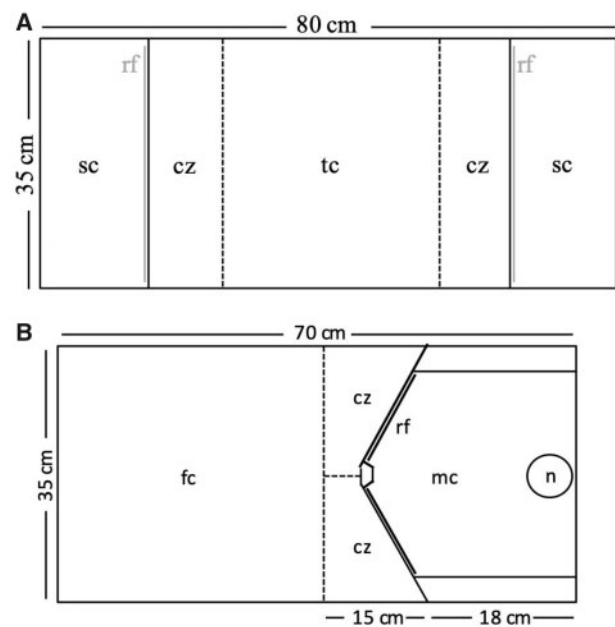
1 = Shoal choice/clear-water, 2 = habitat choice/clear-water, 3 = shoal choice/tea-stained, and 4 = habitat choice/tea-stained.

caffeine-free rooibos tea (Westcliff), so that the transmission spectrum closely resembled the original habitat-water (Figure 1B). In the experiments, the testing of fish from different habitat types and populations and position of the UV filters were randomized. All fish were fed to excess 1 h prior to experiments and only used once.

### Shoal and habitat preference

Shoal- and habitat-preference experiments were conducted in 8 different sequences, which were randomly assigned to the test fish (Table 1). Experiments were conducted in an aquarium (80 cm × 35 cm × 40 cm; water level: 15 cm), which was divided into 3 compartments, 2 shoal fish compartments (shoal choice) or empty compartments (habitat choice), respectively, and 1 test-fish compartment (see Figure 2A). The test aquarium was lit by 2 fluorescent tubes (Truelight T8/36 W) installed 72 cm above the aquarium ground, mimicking natural daylight (see Rick and Bakker 2008a). The whole set-up was surrounded by a black plastic curtain to prevent external disturbances. Furthermore, the inner aquarium walls were covered with gray plastic sheets. The compartments were divided by UV-transmitting Plexiglas (GS-2458, Röhm), allowing visual but no olfactory communication. Behind the Plexiglas dividers a gray, opaque plastic partition as well as 2 optical filters (UV-blocking [LEE 226] and UV-transmitting [Rosco E Color 298 ND]) were attached, which were liftable independently of each other and controlled from outside the plastic curtain. To control for differences in the transmittance of the 2 optical filters, 4 layers of UV-blocking filter were used thereby reducing the difference in transmitted quantal flux in the spectral range between 300 and 700 nm to 0.13% (Figure 1C). The light intensity control was valid, as behavioral experiments on the stickleback populations used in this study have indicated that individuals from all populations are UV sensitive (learning experiments: Hiermes M et al., unpublished data; opsin data: Bakker TCM et al., unpublished data). Behavioral experiments in sticklebacks from a German freshwater population suggest that UV wavelengths might be involved in the detection of chromatic cues in a mate choice context (Rick et al. 2006; Rick and Bakker 2008a). However, conclusive data on the mechanisms involved in luminance and color processing across the whole wavelength range are still lacking for sticklebacks. All experiments were filmed from above with a webcam (Logitech Webcam, Pro 9000) and recorded via Windows Media Encoder.

In order to simplify the description of the experimental procedure, trial sequence 1 will be explained in detail. All other sequences were conducted following the same procedure (Table 1). In general, each trial sequence comprised 4 trials and each trial comprised 2 sub-trials, which were similar except for the changed position of the



**Figure 2.** (A) Top view of the experimental shoal-preference tank. The tank was divided into 3 compartments: 2 shoal fish compartments (sc), which contained no fish in habitat-preference experiments, on both ends of the tank and a test fish compartment (tc; dashed lines mark the borders of 2 choice zones [cz] within the test fish compartment). The shoal fish compartments were separated by UV-transmitting, water impermeable Plexiglas windows on each side. In front of each of the windows, 2 removable filters (rf) (UV-blocking and UV-transmitting) and a removable opaque partition were installed. (B) Top view of the experimental mate-preference 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 (rf) (UV-blocking and UV-transmitting) and a removable opaque partition were installed. Dashed lines mark the borders of 2 choice zones (cz).

UV-filters. Between trials, water in the test aquarium was exchanged. Before the experiment started, the opaque plastic partitions were lowered and the optical filters were adjusted so that, without the opaque partition, one shoal compartment was viewed under UV-absent and the other shoal compartment under UV-present conditions. Then 1-day-old tap-water (clear-water treatment) was filled into the test aquarium. The test fish and the 8 shoal fish, which always originated from the same population but never from the same family as the test fish, were dip-netted from their holding tanks and transferred to 3 separate 1-L plastic boxes. Due to winter conditions, all fish used were reproductively inactive. The test fish and the 2 shoals, consisting of 4 fish each, were then transferred to the designated compartments and had 15 min to acclimatize. After 15 min the opaque partitions were lifted from outside the set-up and the test fish had 15 min to associate with either of the 2 shoals. After these 15 min the opaque partitions were lowered again, the shoal and test fish were gently netted out of the compartments, put back into their 1-L boxes, and the position of the filters was exchanged, so that the compartment that had been viewed under UV-absent conditions was afterward visible under UV-present conditions and vice versa. All fish were put back in their compartments. Again, the test fish had 15 min to acclimatize and afterward a 15-min observation period followed. Subsequent to the second observation period, the fish were gently netted out of the aquarium and put back into

their 1-L plastic boxes. For the next trial, the test aquarium was emptied, rinsed, and dried and again clear-water was filled in. Now only the test fish was put back in the middle compartment for a habitat-preference experiment in clear-water. Again a 15-min acclimatization with lowered opaque partitions and a 15-min observation period with lifted opaque partitions followed. After 15 min observation, the opaque partitions were lowered again, the test fish gently netted out of the middle compartment and put back into its 1-L box. Then the position of the filters was exchanged and the test fish tested again as above, netted out, and put back into its box. For the shoal- and habitat-preference tests in tea-stained water, the test aquarium was emptied, rinsed, and dried and tea-stained water was filled in. Then the shoal and test fish were put back into their compartments and the whole experimental procedure (shoal choice and habitat choice) was re-done with tea-stained water.

Shoal fish were size matched by visual judgment to avoid differences in body size between the shoals. Test fish were also similar in size to shoal fish. After the experiment, all fish were weighed, measured for length, and the condition factor (CF) following [Bolger and Connolly \(1989\)](#) was determined (mean  $\pm$  SD;  $CF_{\text{left shoal}}$ :  $1.13 \pm 0.10$ ;  $CF_{\text{right shoal}}$ :  $1.12 \pm 0.09$ ).

### Female mate-preference

During summer conditions, reproductively active males were separated in single aquaria (30 cm  $\times$  30 cm  $\times$  20 cm), which were equipped with a Petri-dish filled with sand as nesting site and 2 g of dark-green 3-cm-long threads (Toldi Lock, COL 8065) as nesting material. Before introducing a male to the single aquarium, it was weighed and measured and its CF was determined ([Bolger and Connolly 1989](#)). To initiate nest-building, all males were enticed with a ripe female twice daily at least for 15 min. Nest-building was considered to be finished as soon as a clearly visible tunnel through the nest was observed ([Wootton 1976](#)). Two days later males were used in mate-preference experiments.

The basic experimental procedure was equivalent to the shoal-preference experiments. However, the test aquarium used measured 70 cm  $\times$  35 cm  $\times$  35 cm (water level: 15 cm) and was divided into 2 compartments: a female-choice compartment and a male compartment that also incorporated the nest (see [Figure 2B](#)). Again, a gray opaque partition and the 2 optical filters (UV-blocking/UV-transmitting) were installed in front of each of the 2 UV-transmitting Plexiglas partitions that separated the male and female compartment ([Figure 2B](#)). The Plexiglas partitions were arranged in a V-shaped design so that the same male could be viewed under both UV conditions. The advantage of such a set-up is that a preference for other male traits, such as the conspicuous red breeding coloration or body size, can be excluded as confounding factors ([Macías Garcia and Burt de Perera 2002](#); [Rick and Bakker 2008a](#)).

The test aquarium was prepared ahead of experiments as described above (see shoal and habitat preference). The test male was netted out of its single aquarium and a gravid female of the same population, but never from the same family, out of a holding aquarium and transferred to 1-L plastic boxes. After both male and female were located in separated plastic boxes, the Petri-dish with the nest was gently taken from the male's aquarium and placed at the back wall of the male compartment (see [Figure 2B](#)). Afterward the female was introduced into the tank and the experimental procedure described above (see shoal and habitat preference) followed (details in [Hiermes et al. 2021](#)). Thus, test fish were tested in clear- and tea-stained water, acclimatization and observation periods were 15 min, and fish were tested twice in each water-color treatment

with filters exchange after the first acclimatization and observation period. Afterward, males with their nests were returned to their holding tanks, and females were weighed, measured, and introduced in the males' holding tanks. To ensure that mate preference had been observed in choice experiments, test females had to spawn within 24 h after experiments in the males' holding tanks (see [Mehlis et al. 2008](#)). Females that did not spawn ( $N=8$ ) were excluded from all further analyses.

### Data processing

All videos were analyzed by an observer who was blind with respect to the tested population and the filter position. The absolute time test fish or test females, respectively, spent within each choice zone was recorded and a preference index (time [s] in front of UV-transmitting filter)/total time [s] in both preferences zones) was calculated and used in all statistical analyses. To exclude the possibility that a test fish had not been aware of both shoals or of the male under both UV conditions, respectively, only experiments were taken into account in which test fish entered both choice zones during the 15 min-lasting observation periods. Otherwise, experiments had to be discarded (shoal- and habitat choice [ $N=10$ ]; female mate choice [ $N=8$ ]). Sample sizes for shoal- and habitat-preference experiments were  $N_{\text{clear-water}} = 35$ ,  $N_{\text{tea-stained}} = 42$  and for mate-preference experiments  $N_{\text{clear-water}} = 38$ ,  $N_{\text{tea-stained}} = 41$ . The position and behavior of the stimulus shoal-fish or males were not recorded.

### Statistical analysis

The R 4.0.3 statistical package was used for all analyses ([R Development Core Team 2020](#)). Linear mixed-effect models ("lmer" function of the "lme4" library) were used throughout as data did not deviate significantly from normal distribution according to Shapiro–Wilk tests. Initial models for the shoal-preference, habitat-preference, and mate-preference experiments were fitted, including "habitat type," "water-color treatment," and the interaction between the 2 as explanatory variables and "preference index" as dependent variable. The data for "preference index" were transformed by subtracting 0.5 from the observed proportions to test whether the intercept of the best-explaining model deviated significantly from 0 (no preference) in the mixed-effect models. The interaction term was used to test whether the preference index of test fish differs between the 2 water-color treatments as a function of habitat type. "CF of test fish" and "year" served as additional explanatory variables in models for shoal preference and habitat preference and "CF (male)," "CF (female)," and "year" were included in the model for mate preference. "Individual" nested within "family" nested within "population" served as random factors and were never removed from the analyses to control for population-specific and family-specific influences, respectively, as well as for repeated measures. Post-hoc tests with Tukey adjustment for multiple comparisons were conducted to calculate contrasts among preferences of each water-color treatment between habitat types and between water-color treatments within habitat types using the R package "emmeans" based on comparisons between estimated marginal means (EMMs) ([Lenth 2018](#)).

To test whether preferences deviated from chance level (no preference), models were constructed for each combination of habitat type and water-color treatment, including "CF of test fish" and "year" as explanatory variables in models for shoal preference and habitat preference. The variables "CF (male)," "CF (female)," and "year" were used as explanatory variables in models for mate



preference. “Population” and “family” served as random factors in intercept models for the different combinations of habitat type and water-color treatment. For comparison, in the [Supplementary Materials](#), we give one-sample *t*-tests with Bonferroni correction that tested whether preferences deviated from random (0.5) but do not control for confounding variables.

Shoal- and control habitat-preferences were tested against each other to exclude that test fish showed a preference for lighting habitat but not for the shoal viewed under the respective lighting conditions. The respective model included the preference index as dependent variable, “experiment type” (shoal preference, habitat preference), “water-color treatment,” and “habitat type” as well as “CF of test fish” as explanatory variables and “individual” nested within “family” nested within “population” as random factors. For pairwise comparisons of shoal- and habitat-preferences, post-hoc tests with Tukey adjustment for multiple testing (R package “emmeans”: [Lenth 2018](#)) were used for the following groups: clear-water and tea-stained habitat type fish in (1) clear-water and (2) tea-stained treatment, clear-water habitat type fish in (3) clear-water and (4) tea-stained treatment, tea-stained habitat type fish in (5) clear-water and (6) tea-stained treatment.

In all models, non-significant explanatory variables were stepwise removed from the models in the order of descending significance using the backward elimination procedure of the “step” function in the lmerTest package ([Kuznetsova et al. 2017](#)). Significance values for the fixed effects were determined using *F*-tests with Satterthwaite’s approximation. Tests of random effects were based on likelihood ratio tests that follow a  $\chi^2$  distribution. Random factors “population,” “family,” and “individual” had no significant effect in all models for mate-preference and shoal-preference experiments (all  $\chi^2 < 1.696$ ,  $df = 1$ , all  $P > 0.193$ ; [Supplementary Table S1](#)). However, in the habitat-preference experiment, “individual” significantly influenced behavior ( $\chi^2 = 7.524$ ,  $df = 1$ ,  $P = 0.006$ ; [Supplementary Table S1](#)) indicating that individual fish showed consistent habitat preference.

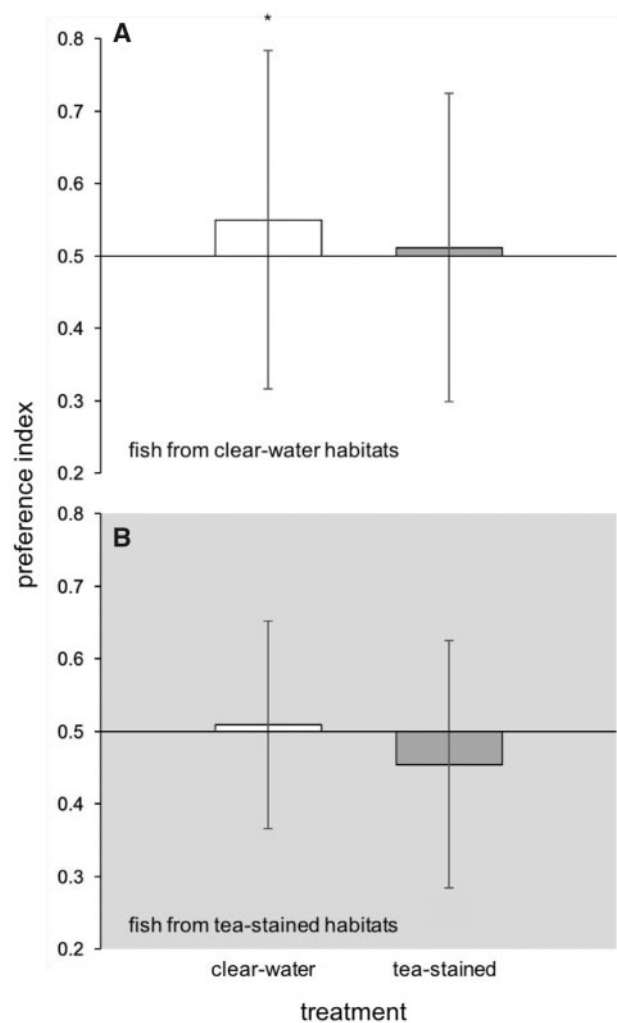
## Results

### Shoal preference

In the pooled dataset, there was no significant interaction effect between “habitat type” and “water-color treatment” on stickleback’s shoal-preference behavior (lme:  $F = 0.079$ ,  $df = 1, 77$ ,  $P = 0.780$ ; [Supplementary Table S1](#)). Furthermore, “habitat type,” “CF,” and “year” did not have a significant influence on the preference index of test fish (all  $F \leq 2.476$ , all  $P \geq 0.120$ ; [Supplementary Table S1](#)), while “water-color treatment,” however, tended to influence shoal-preference behavior ( $F = 2.781$ ,  $df = 1, 77$ ,  $P = 0.099$ ; [Supplementary Table S1](#)). Sticklebacks from clear-water habitats significantly preferred to shoal with fish viewed under UV-present conditions in the clear-water treatment ([Figure 3A](#) and [Table 2](#), but see [Supplementary Table S2](#)) while fish from tea-stained habitats tended to prefer the shoal viewed under UV-absent conditions in the tea-stained treatment ([Figure 3B](#), [Table 2](#), and [Supplementary Table S2](#)). CF of the testfish had a significant positive effect on shoaling preference in fish from clear-water habitats in the clear-water treatment in the intercept tests (lme:  $t = 2.289$ ,  $df = 34.96$ ,  $P = 0.028$ ). Differences between preferences were in all cases not significant (EMM: all  $P > 0.381$ ).

### Habitat preference

In the pooled dataset, the interaction between “habitat type” and “water-color treatment” (lme:  $F = 0.692$ ,  $df = 1, 77$ ,  $P = 0.408$ ) as



**Figure 3.** Preference index (time [s] in front of shoal viewed under UV+ conditions/total time [s] in preferences zones) for sticklebacks from (A) clear-water habitats (white background) and (B) tea-stained habitats (gray background) in the clear-water treatment (white bars) and in the tea-stained treatment (gray bars). Plotted are mean differences and standard deviations. Values above 0.5 indicate a UV+ preference. Non-transformed data are presented for visual purposes only. \* $P < 0.05$ .

well as “habitat type,” “water-color treatment,” and “year” did not have a significant influence on the preference index of test fish (all  $F \leq 1.546$ , all  $P \geq 0.217$ ) ([Supplementary Table S1](#)). However, “CF” of test fish had a significant negative effect on habitat-preference behavior ( $F = 4.046$ ,  $df = 1, 77$ ,  $P = 0.048$ ; [Supplementary Table S1](#)). Fish from clear-water habitats did not show a preference for either habitat in the clear-water and the tea-stained treatment ([Table 2](#) and [Supplementary Table S2](#)). Sticklebacks from tea-stained habitats tended to prefer UV-present conditions in the clear-water treatment but did not show significant preferences in the tea-stained treatment ([Table 2](#) and [Supplementary Table S2](#)). Differences between preferences were in all cases not significant (EMM: all  $P > 0.464$ ).

### Shoal versus habitat preference

Testing preference of shoal and habitat against each other, no significant differences were found for fish from both habitat types under clear-water (EMM:  $t = 0.102$ ,  $df = 237$ ,  $P = 1$ ) or tea-stained treatment (EMM:  $t = 0.716$ ,  $df = 237$ ,  $P = 0.891$ ). Moreover, there

**Table 2.** Summary of the conducted lme testing whether the preference index (time [s] in front of UV-transmitting filter/total time [s] in both preference zones) deviates from chance level (no preference) for shoal-, habitat- (control), and mate-preference experiments investigating UV preferences of lab-bred test fish from clear-water and tea-stained habitats in the clear-water treatment and in the tea-stained treatment

Experiment	Habitat type	Treatment	<i>t</i>	Estimate	SE	<i>P</i>	UV+ (mean ± SD)	UV- (mean ± SD)
Shoal choice	Clear-water	Clear-water	-2.141	-0.679	0.317	<b>0.039</b>	398.29 s ± 196.77	319.20 s ± 181.35
	Clear-water	Tea-stained	0.674	0.027	0.04	0.506	359.69 s ± 172.55	338.97 s ± 160.60
	Tea-stained	Clear-water	0.354	0.009	0.025	0.725	318.76 s ± 131.15	308.33 s ± 127.38
	Tea-stained	Tea-stained	-1.834	-0.045	0.025	<i>0.074</i>	306.95 s ± 114.26	375.24 s ± 137.32
Habitat choice	Clear-water	Clear-water	0.396	0.013	0.033	0.695	349.91 s ± 162.54	338.66 s ± 169.29
	Clear-water	Tea-stained	0.270	0.010	0.038	0.805	348.14 s ± 167.63	330.31 s ± 152.43
	Tea-stained	Clear-water	1.758	0.052	0.029	<i>0.086</i>	397.29 s ± 173.83	312.17 s ± 139.04
	Tea-stained	Tea-stained	0.193	0.006	0.029	0.849	354.07 s ± 140.71	354.74 s ± 152.74
Mate choice	Clear-water	Clear-water	1.686	0.041	0.024	0.100	396.11 s ± 213.55	353.82 s ± 244.38
	Clear-water	Tea-stained	-0.681	-0.019	0.029	0.500	376.87 s ± 240.67	408.79 s ± 256.73
	Tea-stained	Clear-water	3.095	0.071	0.023	<b>0.004</b>	459.05 s ± 247.98	316.29 s ± 164.58
	Tea-stained	Tea-stained	-0.665	-0.018	0.026	0.510	413.88 s ± 250.41	431.46 s ± 234.66

Significant preferences ( $P < 0.05$ ) are printed in bold, trends ( $0.05 < P < 0.10$ ) are printed in italics. SD, standard deviation. Means ± SD of UV-present and UV-absent preferences are also given.  $N_{\text{clear-water}} = 35$ ,  $N_{\text{tea-stained}} = 42$  for shoaling and habitat preferences, and  $N_{\text{clear-water}} = 38$ ,  $N_{\text{tea-stained}} = 41$  for mate preferences.

were no significant differences in preference between shoal- and habitat-preference experiments when separated for habitat type and water color treatment (EMM: all  $P > 0.926$ ).

### Female mate-preference

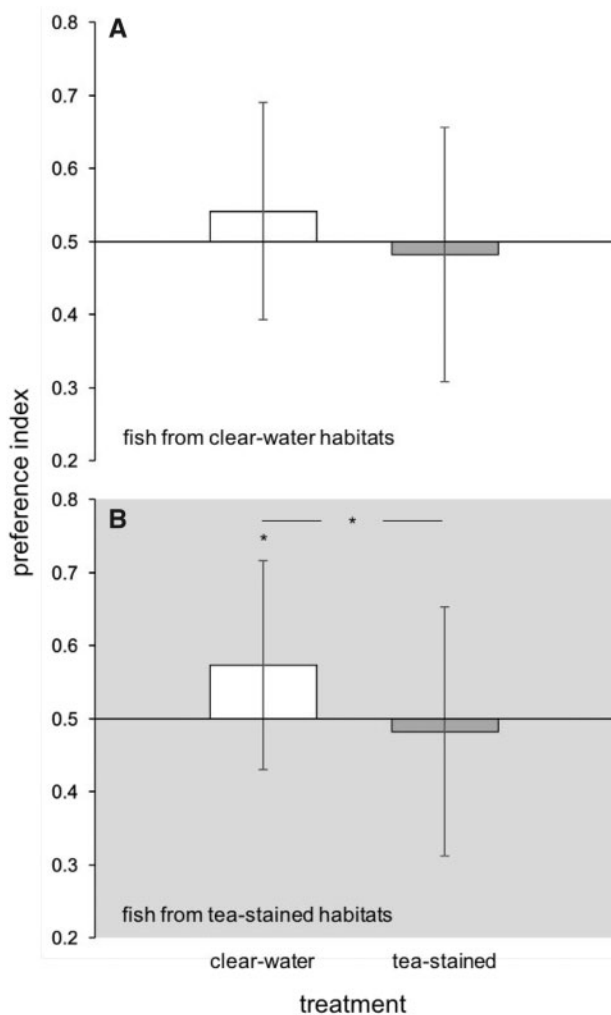
In the pooled dataset, mate-preference behavior was not significantly influenced by the interaction between “habitat type” and “water-color treatment” (lme:  $F=0.447$ ,  $df = 1$ ,  $81.29$ ,  $P=0.506$ ; Supplementary Table S1). “Habitat type,” “CF” of males and females, and “year” did not have a significant influence on the preference index of test fish (all  $F \leq 0.299$ , all  $P \geq 0.586$ ), while “water-color treatment” had a significant effect on mate-preference behavior ( $F=10.442$ ,  $df = 1$ ,  $81.75$ ,  $P=0.002$ ) (Supplementary Table S1). Females from the clear-water habitat tended to prefer the male viewed under UV-present conditions when tested in the clear-water treatment (Figure 4A, Table 2, and Supplementary Table S2) but showed no significant preference in the tea-stained treatment. Females from tea-stained habitats significantly preferred the male viewed under UV-present conditions in the clear-water treatment (Figure 4B, Table 2, and Supplementary Table S2) but did not show a significant preference for a male under either UV-condition in the tea-stained treatment (Figure 4B, Table 2, and Supplementary Table S2). Family nested in population had a significant effect on mate preference in fish from clear-water habitats both in the clear-water and the tea-stained treatment in the intercept tests (lme:  $\chi^2 = 16.128$ ,  $df = 1$ ,  $P < 0.001$ ;  $\chi^2 = 15.027$ ,  $df = 1$ ,  $P < 0.001$ , respectively). The preferences from tea-stained females differed significantly between water-color treatments (EMM:  $t=2.764$ ,  $P=0.035$ ; Figure 4B). All other contrasts between preferences were not significant (EMM: all  $P > 0.206$ ).

### Discussion

The preferences of lab-bred F1 sticklebacks from both clear-water and tea-stained habitats during UV-based female-mate preference, which we tested in the present study, were largely similar to those of wild-caught females (Hiermes et al. 2021): an overall preference for

males under UV-present conditions in the clear-water treatment (Figure 4A, B, Table 2, and Supplementary Table S2). This result points to a genetic influence on UV mate preferences. The UV mate preferences in the tea-stained treatment were non-significant (Figure 4A, B, Table 2, and Supplementary Table S2) resulting in a significant water-color treatment effect in females from tea-stained lakes (Supplementary Table S1). Thus, water color during the testing of preferences affected UV-based female preference of fish from tea-stained lakes even after being raised under full light spectrum conditions in clear water. In the wild-caught females (Hiermes et al. 2021), there were no significant water-color treatment effects as the females from both habitat types tended to prefer males under UV-present conditions in both water-color treatments. There thus may be differences in UV-preferences in the tea-stained treatment between wild-caught and F1 females that may point to an effect of developmental plasticity. Fuller and Noa (2010) demonstrated in bluefin killifish that the environment experienced during development, the immediate conditions during mate choice, as well as the genetics (i.e., population of origin) account for observed mate preferences. In sticklebacks, reproduction-related plasticity of the visual system with respect to long wavelengths have been documented by Cronly-Dillon and Sharma (1968) and Boulcott and Braithwaite (2007). To exclude or confirm effects of developmental plasticity on UV mate preferences in sticklebacks, further common-garden experiments, thereby raising offspring of both clear-water lakes and tea-stained lakes under both clear-water and tea-stained conditions, must be performed.

UV-based shoaling preferences of the lab-bred F1-generation both from clear-water and tea-stained habitats, which we tested in the present study, resembled those of their wild-caught ancestors tested in a previous study (Hiermes et al. 2015). In the present study, which additionally tested preferences of all test fish in 2 water-color treatments (clear-water and tea-stained water), we found that “water-color treatment” tended to have an influence on UV preferences (Supplementary Table S1). Test fish from tea-stained habitats tended to prefer the shoal viewed under UV-absent conditions, but only in tea-stained water (Figure 3B, Table 2, and Supplementary Table S2). The study on the wild-caught parental generation showed



**Figure 4.** Preference index (time [s] in front of male viewed under UV+ conditions/total time [s] in preference zones) for stickleback females from (A) clear-water habitats (white background) and (B) tea-stained habitats (gray background) in the clear-water treatment (white bars) and in the tea-stained treatment (gray bars). Plotted are mean differences and standard deviations. Values above 0.5 indicate a UV+ preference. Non-transformed data are presented for visual purposes only. \* $P < 0.05$ .

that sticklebacks from tea-stained lakes preferred the shoal viewed under UV-absent conditions in the tea-stained water of lake of origin (Hiermes et al. 2015). Wild-caught sticklebacks from clear-water lakes did not show any significant preferences for one of the 2 shoals when tested in clear-water (Hiermes et al. 2015). In the present study, we assessed a preference of fish from clear-water lakes for the UV+ shoal in clear-water but not in tea-stained water (Figure 3A and Table 2, but see Supplementary Table S2). Thus, the combination of lighting habitat of origin and water-color treatment might be important for sticklebacks' UV-based shoaling preference, and may hint at a genetic basis of shoal preferences. There was a positive effect of body condition on UV-based shoaling preferences in F1 fish from clear-water lakes. In sticklebacks, condition-dependent preferences are known in a mating context (Bakker et al. 1999). The impact of condition in a shoaling context needs further study and may be related to predation risk and/or competitive feeding ability.

We did not study the F2-generation for logistical reasons and can therefore not exclude maternal effects on shoal preference. As

all F1-fish, including those from tea-stained habitats, had been reared under full-spectrum, clear-water conditions in the lab, it is important to note that changed rearing conditions did not change preferences of tea-stained populations. Whether different lighting conditions during development (e.g., tea-stained water) would have an influence on UV shoaling preferences and whether transgenerational effects are present deserve further investigation.

Developmental plasticity of UV shoaling-preferences was, however, observed in F1-sticklebacks from another clear-water population (Euskirchen, Germany) that had been deprived of UV light during development, thus contrasting to the photic rearing-environment in the present study. In a similar set-up to the current study, wild-caught sticklebacks from this population preferred the shoal viewed under UV-present conditions (Modarressie et al. 2006). Interestingly, when F1-descendants of this population were raised in the lab under UV-absent conditions, they showed a significant preference for shoals under UV-absent conditions (Modarressie et al. 2015). F1-descendants that were raised under UV-present conditions, in contrast, did not show any significant preference (Modarressie et al. 2015), thereby deviating from the UV preference shown by their wild-caught parents (Modarressie et al. 2006). Although the results of the Modarressie et al. (2015) study suggest developmental plasticity of UV shoaling-preferences, experiential effects cannot be ruled out as generally tank mates will have looked differently under the different rearing water-color treatments. Developmental plasticity of the visual apparatus has frequently been observed in fishes (e.g., Kröger et al. 1999, 2003; Cronin et al. 2001; Shand et al. 2008; Schartau et al. 2009; Dalton et al. 2015; Ehlman et al. 2015; Härer et al. 2017; Schweikert and Grace 2018). Fuller et al. (2005), for instance, found environmental factors (clear water or tea-stained water) and genetics (habitat of origin) to have an effect on the visual system of bluefin killifish (*Lucania goodie*). However, Novales Flamarique et al. (2013) found limited plasticity of cone number and opsin expression in sticklebacks and thus the question arises, whether visual plasticity in sticklebacks will be ecologically meaningful. Nevertheless, the present study as well as the studies by Modarressie et al. (2015) and Hiermes et al. (2015), hint at a limited importance of UV reflections in shoaling behavior in sticklebacks (but see Modarressie et al. 2006) when compared with the role of UV in mate-choice behavior (Rick et al. 2006; Rick and Bakker 2008a, 2008b). Probably the fitness costs of UV exposure are higher in a reproductive context. Rick et al. (2014) showed that in sticklebacks, long-term exposure to enhanced but ecologically relevant UVA levels had negative effects on both male red breeding coloration and sperm velocity. In sticklebacks, sperm velocity predicted fertilization success under competitive conditions (Mehlis et al. 2015).

It is hard to disentangle whether preferences during shoal choice are truly based on characteristics of the shoal or on characteristics of the habitat, in this case the lighting (UV) habitat. To be able to exclude confounding effects, control habitat-preference experiments were conducted and no significant preferences for either lighting habitat were shown by the test fish; however, test fish from tea-stained lakes in the clear-water treatment tended to prefer the UV-present habitat (Table 2 and Supplementary Table S2). To be able to exclude the influence of habitat preference on shoal preference, the UV preferences (preference index) of the main shoal experiment were tested against the control habitat experiment and the differences were not significantly different. Thus, an influence can still not be ultimately excluded, which is most likely a problem of sample size. However, the different, although non-significant, choice



decisions of fish from the tea-stained habitat in the tea-stained treatment (shoal choice: preference by trend for UV-lacking shoal, habitat choice: no preference) and in the clear-water treatment (shoal choice: no preference, habitat choice: preference by trend for UV-present habitat) suggest a negligible effect of habitat preference on shoal preference. The UV-based habitat preference in itself may be worth further study as we assessed a negative relationship between body condition and UV-based habitat preferences in the pooled dataset (Supplementary Table S1). Effects of body condition on habitat preferences may be related to predation risk and/or prey visibility. A study by Rick and Bakker (2010) demonstrated an influence of rearing conditions with respect to habitat preferences of sticklebacks under predation risk. In that study, sticklebacks raised under UV-present conditions escaped to a habitat lacking UV background reflections, presumably to reduce conspicuousness toward the predator, while sticklebacks raised under UV-deprived conditions did not show significant habitat preferences.

In summary, the results of the present UV shoaling- and UV mate-preference experiments and previous experiments with wild-caught fish (Hiermes et al. 2015, 2021) suggest genetic variation in UV preferences that depended on the immediate lighting conditions and the habitat of origin but were less affected by the developmental lighting conditions. An influence of developmental plasticity on UV mate preferences could not be ruled out without further experiments with different rearing conditions.

### 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 and wrote the manuscript. M.H., M.B.M., S.R., and S.V. collected the data. I.P.R., M.H., M.B.M., S.R., and S.V. analyzed the data.

### Data availability

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

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### Conflict of Interest

The authors declare that they have no conflict of interest.

### Supplementary Material

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

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