



Shoal choice and ultraviolet reflections in stickleback populations from different photic habitats

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Grouping is a widespread phenomenon in the animal kingdom and the decision to join a group is a function of individual and environmental conditions, meaning that any advantages and disadvantages have to be pondered constantly. Shoaling decisions in fishes are communicated via a variety of factors, such as colour signals, amongst other ultraviolet (UV) signals. The sensitivity for ultraviolet signals is assumed to be costly and a function of the predominant ecological conditions. The island of North Uist, Scotland, comprises bodies of water that possess great variation in their spectral distribution, especially in the UV spectral range. We examined different populations of three-spined stickleback (*Gasterosteus aculeatus* L.), which is known to use UV for visual tasks, consisting of three populations from tea-stained lakes and four from clear-water lakes, concerning their preferences to join a shoal viewed under UV-present and UV-absent conditions. Nonreproductively active sticklebacks from tea-stained lakes significantly preferred the shoal under UV-absent conditions, whereas sticklebacks from clear-water lakes did not show a significant preference. Reflection measurements showed that the UV chroma (intensity) of sticklebacks from tea-stained lakes was higher than that of sticklebacks from clear-water, most likely contrasting maximally against the UV-poor background or compensating for a stronger attenuation of the signal. © 2015 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2015, 00, 000–000.

ADDITIONAL KEYWORDS: fish – *Gasterosteus aculeatus* – grouping – lighting environment – UV signals – UV vision.

INTRODUCTION

Living in groups is a widespread phenomenon in many species throughout the animal kingdom and is very common in fishes (Wright & Krause, 2006) amongst others. Group living provides not only a multitude of advantages for the group member over solitary conspecifics, but also costs (Pitcher & Parrish, 1993; Krause & Ruxton, 2002). Both the advantages and disadvantages of joining or leaving a shoal or to remain solitary on the first hand have to be pondered constantly and are a function of the environmental conditions that an individual is experiencing (Pitcher & Parrish, 1993). The conspicuousness and hence the predation risk of individuals that differ phenotypically from other shoal members ('oddity effect') is, for example, enhanced and has to be

weighed against the disadvantages of being solitary but less conspicuous (Landeau & Terborgh, 1986; Theodorakis, 1989). Thus, joining homogeneous looking shoals should be most advantageous.

The evaluation of shoaling conspecifics can be based on multiple sensory channels (Rosenthal & Lobel, 2005) and is dependent on the species and their repertoire and effectiveness of modalities as a function of the environmental conditions (Candolin, 2003). Visual cues have repeatedly been shown to be important in shoaling decisions, such as shoal size (Tegeer & Krause, 1995; Krause, Longworth & Ruxton, 2000), shoal density (Frommen, Hiermes & Bakker, 2009), and coloration (McRobert & Bradner, 1998; Rosenthal & Ryan, 2005). Besides coloration in the visible part of the spectrum, ultraviolet (UV) light (300–400 nm) has also been shown to be decisive in communicating shoaling decisions (Modarressie, Rick & Bakker, 2006; Modarressie, Günther & Bakker, 2015).

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Generally, the use of visual signals in the ultraviolet spectral range is widespread in many vertebrate species (Bennett *et al.*, 1996; Fleishman *et al.*, 1997; Jacobs, Fenwick & Williams, 2001), especially in many fish species (Losey *et al.*, 1999), despite its damaging effect on ocular tissues (Van Norren & Schellekens, 1990) and the negative photo-oxidative effect (Losey *et al.*, 1999; Sliney, 2002). In detail, UV is used in various contexts, such as foraging (Loew *et al.*, 1993; Viitala *et al.*, 1995; Rubene *et al.*, 2010), navigation (Hawryshyn & McFarland, 1987; Sauman *et al.*, 2005; Cowan & Gries, 2009), and in social contexts such as mate choice (Maier, 1993; Bennett *et al.*, 1996; Smith *et al.*, 2002) and territorial behaviour (Alonso-Alvarez, Doutrelant & Sorci, 2004; Siebeck, 2004).

However, the availability of light and thus the use of certain visual signals varies greatly between habitats (Lythgoe, 1979). This accounts, in particular, for aquatic habitats in which the transmission of light is very complex because any rays entering the liquid medium are scattered and refracted (Johnsen, 2012). Short wavelengths, especially UV wavelengths, are attenuated strongly, making the transmission of UV signals viable only over short distances (Losey *et al.*, 1999). Only those signals that are transmitted through the medium and detected by the receiver most efficiently will be favoured by selection (Endler, 1992, 1993). To optimize the signalling process, animal coloration might be adapted to the local lighting conditions within a specific habitat (Reimchen, 1989; Boughman, 2001; Fuller *et al.*, 2004; Maan *et al.*, 2006; Morrongiello *et al.*, 2010). Consequently, this adaptation in signal design will lead to a differential signal transmission and an adaptation of the sensory system of the signal receiver ('sensory drive hypothesis') (Endler, 1992).

In the present study, the three-spined stickleback (*Gasterosteus aculeatus* L.) was used as a study animal. The stickleback is a small cold water fish and is capable of perceiving, reflecting and using UV in communication (Rick, Modarressie & Bakker, 2004, 2006; Rowe *et al.*, 2004), including during shoal choice (Modarressie *et al.*, 2006). In the latter study, stickleback individuals from a German clear-water population preferred to associate with a shoal viewed under UV-present over a shoal viewed under UV-absent conditions (Modarressie *et al.*, 2006). However, the availability of UV light during ontogeny also has an influence on UV-based shoaling decisions because sticklebacks of the same population raised under UV-absent conditions significantly preferred shoals viewed under UV-absent conditions (Modarressie *et al.*, 2015).

Based on these results, we predicted that, in natural habitats, a varying availability of UV wavelengths

for communication should similarly influence its use in a stickleback's shoaling behaviour. Thus, in the present study, we tested three-spined sticklebacks originating from two habitat types (tea-stained lakes/clear-water lakes) on the island of North Uist, Scotland, with respect to their shoaling preferences for shoals viewed under UV-absent and UV-present conditions, respectively. The two habitat types differ strongly in their transmission of light (Fig. 1). As a result of high amounts of dissolved organic carbon present in tea-stained lakes, short-wave light, especially light in the ultraviolet spectral range, is absorbed more strongly than in clear-water lakes, which results in a poorer transmission and availability of UV wavelengths in these tea-stained water bodies (Fig. 1). In addition, stickleback populations have evolved in the habitat types independently of each other for approximately 15 000 generations subsequent to the retreat of the ice of the last Ice Age (Ballantyne, 2010). As a result of the long time of adaptation, the low transmission of UV wavelengths and thus the more difficult UV-signalling conditions in tea-stained lakes, we predicted that sticklebacks from tea-stained lakes would prefer shoals under UV-absent conditions and show an overall decreased UV signal. On the other hand, we expected sticklebacks originating from clear-water habitats, which provide better circumstances for UV-signalling, to show preferences for a shoal under UV-present conditions and, consequently, to express a well-developed UV signal.

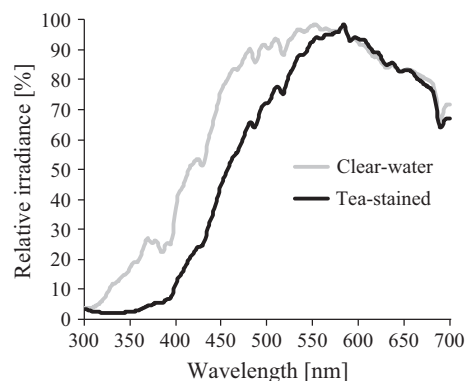


Figure 1. Relative irradiance spectra measured in a water depth of 30 cm [tea-stained lakes: Loch a'Bharpa, Loch Scadavay, Loch Tormasad (black line); clear-water lakes: Loch Eubhal, Loch Grogary, Loch Sandary (grey line)] with a Avantes AvaSpec 2048 fiberoptic spectrophotometer connected to a cosine corrector (Avantes CC-UV/VIS) in the spectral range between 300 and 700 nm. Irradiance calibration was performed vs. an Avantes NIST traceable irradiance application standard.

MATERIAL AND METHODS

EXPERIMENTAL SUBJECTS

Seven populations of three-spined sticklebacks from the island of North Uist, Scotland, were used in UV-based shoal choice experiments and habitat choice experiments (control experiments) in April and May 2010 and in April 2011. The control experiment served to test for the possibility that preferences were not based on shoal characteristics under the differing UV-viewing conditions but on UV-viewing habitat *per se*. Four populations were attributed to the clear-water, alkaline habitat type located in the western part of the island [Loch Eubhal (57°36'46.97"N, 7°29'21.80"W); Loch Grogary (57°36'34.85"N, 7°30'01.31"W); Loch Hosta (57°37'29.57"N, 7°27'59.84"W); Loch Sandary (57°35'10.77"N, 7°27'46.45"W)] and three to the tea-stained, acid habitat type located in the eastern, central part of the island [Loch a'Bharpa (57°34'31.69"N, 7°17'31.74"W); Loch Scadavay (57°35'01.37"N, 7°13'56.05"W); Tormasad (57°33'44.10"N, 7°19'12.93"W)] (Giles, 1983; Hiermes, 2008; De Roij & MacColl, 2012). Lakes of both habitat types vary with respect to their spectral distribution, especially in the UV spectral range (Fig. 1).

All fish were caught using minnow traps (Paladin minnow trap S; Gee Minnow Trap, 1/4", G40M; Gee Exotic Trap, 1/8", G48M). Fish were always caught 1 day prior to the experiments and were transported to our accommodation in Lochmaddy (57°59'73.57"N, 7°15'78.93"W), where fish were kept on the porch and were subsequently used in the experiments that were conducted in a small shed. All fish used for the experiments were nonreproductive and were randomly assigned to serve as shoal fish or test fish. For each population, shoal fish were kept in groups in aerated black 90-litre mortar tubs, whereas test fish were kept isolated in 1-litre plastic boxes. Water required for fish storage and for the experiments was transported in grey, food safe square-end canisters (30 litres; Globetrotter). Test and shoal fish were caught, transported, and kept separately to avoid confounding effects of familiarity. All test and shoal fish were only used once to avoid pseudoreplication.

EXPERIMENTAL DESIGN

Choice experiments were conducted in a tank (length 80 cm, width 35 cm, height 40 cm) (Fig. 2). By using UV-transmitting Plexiglas partitions (GS-2458; Röhm), two compartments (15 × 35 × 40 cm) were waterproof separated and served as shoal fish compartments in the shoal choice experiments and as habitat compartments in the control habitat choice experiments. Choice zones measuring 10 cm were

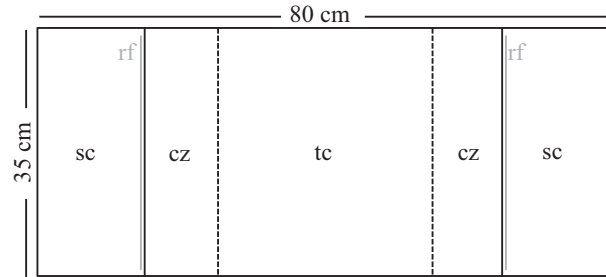


Figure 2. Top view of the experimental tank. The tank was divided into three compartments: two shoal fish compartments (sc), which were empty compartments in control habitat choice experiments, on both ends of the tank and a test fish compartment [tc; dashed lines mark the borders of two choice zones (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, two removable filters (rf) (UV-blocking and UV-transmitting) and a removable opaque partition were installed.

marked by a black line drawn onto the bottom of the tank (Fig. 2).

A UV-blocking filter (LEE 226), a UV-transmitting neutral density filter (Rosco E Color 298 ND), and a grey opaque plastic partition were installed in front of the shoal compartments. The total amount of light transmitted between 300 and 700 nm was determined spectrophotometrically for both filters. Four layers of UV-blocking filter compared to one layer of UV-transmitting neutral density filter were used, resulting in a difference of 0.13% in total light transmission between both filters (Fig. 3) such that any confounding effects as a result of luminance differences were minimized. This represents a suitable approach for balancing total quantal flux over the entire spectral range between 300 and 700 nm because studies have shown that various stickleback populations possess a UV-sensitive cone (Rowe *et al.*, 2004; Novales Flamarique *et al.*, 2013). Furthermore, behavioural experiments on the stickleback populations used in the present study have indicated that all populations are able to perceive light including the UV spectral range (300–400 nm) (M. Hiermes, unpubl. data). The mechanisms involved in luminance discrimination in sticklebacks are still unknown; however, mate choice experiments suggest that UV wavelengths are involved in detecting chromatic cues rather than differences in luminance (Rick *et al.*, 2006; Rick & Bakker, 2008b).

The complete tank was encased by grey plastic partitions that were installed to minimize disturbance from the outside and reflections within the

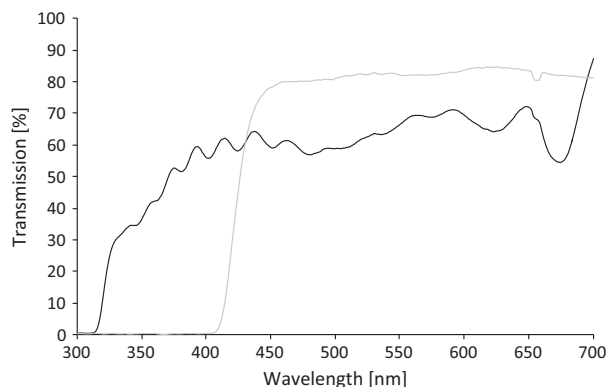


Figure 3. Transmission (%) of the UV-transmitting (black line) and four layers of UV-blocking (grey line) filter. Four layers of UV-blocking filter were used to control for differences in total light intensity between the two filter types, minimizing the differences in total quantal flux between 300 and 700 nm to 0.13%.

tank. Furthermore, the whole set-up was surrounded by a black plastic foil to avoid any disturbances from the outside or by the experimenter. A camera (Logitech Webcam, Pro 9000) and two fluorescent tubes (Truelight, T8/36W; distance to water level 57 cm), which provided light with a proportion of UV similar to natural skylight (Rick & Bakker, 2008a), were installed above the tank.

One hour prior to each experiment, the shoal fish and the stimulus fish were fed to excess with defrosted red mosquito larvae because the hunger status of fish has been shown to influence shoaling decisions (Krause, 1993; Frommen, Luz & Bakker, 2007). Then, the test tank was filled up to a level of 15 cm with water from the lake of the test fish that had been collected the day before. Directly afterwards, the UV-transmitting and UV-blocking filters were adjusted so that the test fish could view one shoal under the spectrum visible for humans (400–700 nm) and the other under a spectrum extended into the UV (300–700 nm). Furthermore, the opaque partitions were lowered on both sides. Filters and opaque partitions could be lifted from outside the set-up via pulleys to avoid any disturbance during the experiments. Sides of filters were exchanged between the experiments to avoid a confounding effect of side preferences. Control experiments (habitat choice) were conducted analogous to the shoal choice trials but without stimulus fish.

Eight shoal fish were randomly assigned to one of the two shoals (four fish per shoal). The standard length (SL) of test fish and all shoal fish was determined after the experiments. SL did not differ significantly between the two shoals [Wilcoxon signed rank test; SL: $V = 1250$, $P = 0.460$ (median and first

and third quartile: left, 3.0 cm, 2.8 cm, 3.4 cm; right: 3.0 cm, 2.8 cm, 3.4 cm)]. Shoal fish and test fish were transported to the test tank in 1-litre plastic boxes and were gently released into the respective compartments using a small dip net. The curtain around the set-up was closed and the acclimation time of 15 min began. After the acclimation time had elapsed, the opaque plastic partitions were raised and the 15-min observation period started. After 15 min, the plastic partitions were lowered again, the filters were exchanged on both sides, and an acclimation period of 15 min then followed. The second observation period of 15 min on the same fish was started by lifting the opaque partitions from outside the set-up. Thus, each shoal was viewed once under UV-absent and once under UV-present conditions. We used a paired design to minimize the influence of factors other than UV coloration that might have by chance differed between the two shoals. After the experiment, the test fish was gently netted out of the test tank and the tank was emptied, rinsed, and dried completely.

Reflection measurements in the cheek, gonadal, and back region (spectral range: 300–700 nm) (Fig. 4) were conducted for all test fish. Measurements were performed with a spectrophotometer (Avantes AvaSpec 2048 fibreoptic spectrophotometer) connected to a deuterium-halogen light source [Avantes AvaLight-D(H)-S Deuterium-Halogen Light Sources, 200–1100 nm]. Reflection scans were taken from the fish's left side using a 200- μm fibreoptic probe held at an angle of 90° to the body surface. Reflection measurements were recorded using AVASOFT, version 7.5 (Avantes) for USB2 with an integrated EXCEL (Microsoft Corp.) output. Prior to the measurements, the device was calibrated with a white standard (Spectralon WS-2) and a black standard (shut-off light source). In total, 20 measurements per region and test fish were recorded and exported to EXCEL.

After the reflection measurements, the sex of each test fish was determined because sex has been shown to influence shoaling behaviour in fishes (Arnold, 2000). As the fish were not reproductively active, sexes could not be phenotypically distinguished. Accordingly, test fish had to be killed with a blow to the head for subsequent dissection and gonad inspection.

DATA ANALYSIS

Shoal and control habitat choice experiments

For shoal as well as habitat choice experiments, the time spent in the choice zones in front of the shoals or empty compartments (UV+/UV–) was recorded. A preference index was calculated, which was used in

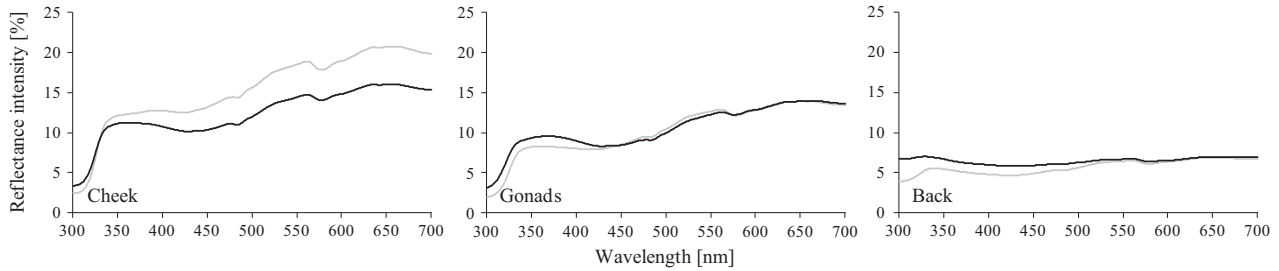


Figure 4. Mean reflectance spectra [proportion of light reflected in relation to a white standard (see text)] of test-fish from clear-water lakes (grey line) and tea-stained lakes (black line) for the cheek, gonadal, and back region.

the statistical analysis: time spent in front of the shoal under UV-present (UV+) minus time spent in front of the shoal under UV-absent conditions (UV-) divided by the total time spent in the choice zones. Only test fish that had entered both choice zones within each of the 15-min lasting observation periods were used in analysis. In the years 2010 and 2011, in total, 108 shoal choice experiments were conducted of which 83 were valid (tea-stained: $N = 40$; clear-water: $N = 43$) and 65 habitat choice experiments were conducted of which 52 were valid (tea-stained: $N = 23$; clear-water: $N = 29$). We calculated the preference index for both 15-min sections and analysed whether preferences differed between the two sections. Because we did not find any significant differences between the 15-min sections (clear-water: $t = 0.119$, d.f. = 42, $P = 0.906$; tea-stained: $t = -1.445$, d.f. = 39, $P = 0.157$), we used the pooled preferences in all analyses.

Reflection measurements

In the analysis, the mean of 20 measurements was used for the three body regions. We calculated the colorimetric variable 'UV chroma' for the cheek and the gonadal region. To determine the chroma, the amount of light reflected in the UV spectral range (300–400 nm) was calculated relative to the total amount of light reflected in the spectral range between 300 and 700 nm (Rick *et al.*, 2004; Shawkey *et al.*, 2006). Furthermore, brightness, the total amount of light in the spectral range between 300 and 700 nm, was calculated for the three body regions (Rick *et al.*, 2014). Brightness in the dorsal region (back) was calculated to account for possible differences in melanin pigmentation as a potential photoprotective response to different levels of UV in the habitats (Rick *et al.*, 2014).

Statistical analysis

All data were checked for normal distribution using Shapiro–Wilk tests. All normally distributed data were analysed using parametric tests, whereas

colorimetric variables, which were all not normally distributed, were tested using nonparametric statistics. R, version 2.9.1 (R-Development-Core-Team, 2009) was used for all analysis. Linear mixed effect models were conducted using the 'lme' function of the 'nlme' library for shoal and habitat choice experiments. The preference index was used as a dependent variable in all conducted models [shoal choice for test fish from clear-water and test fish from tea-stained lakes; habitat choice (control experiment) for test fish from clear-water and test fish from tea-stained lakes]. 'Population' and 'year' were included as random factors and never removed from the model to control for possible population-specific and year-specific influences, respectively. UV chroma (cheek), sex of test fish, and sequence (sequence of the 15-min periods) were used as explanatory variables. To test for differences in choice behaviour between test fish of both habitat types, two further models (shoal choice and habitat choice preferences) were conducted adding habitat type (tea-stained/clear-water) as an explanatory variable. Furthermore, for the model concerning shoal choice, the interaction between habitat type and sequence was added. The preferences shown in the main experiment (shoal choice) were tested against the preferences shown in the control experiment (habitat choice) both for test fish from clear-water and tea-stained lakes using two additional models.

Nonsignificant explanatory variables were stepwise removed from the models in descending order of significance. All tests of significance were based on likelihood-ratio-tests. Furthermore, we used Akaike's information criterion (AIC) for model comparisons; in the present study, the model with the lowest AIC values represents the best-explaining model (Symonds & Moussalli, 2011).

Wilcoxon rank sum tests were used to compare variables between test fish from the two habitat types used in the shoal and habitat choice experiments. All test probabilities are two-tailed.

STATEMENT OF ANIMAL RIGHTS

The study conforms to the Animal Behaviour Society guidelines for the use of animals in research, as well as to the legal requirements of Germany and was carried out in accordance with German laws for animal experiments. All wild-caught fish were held under near optimal conditions (a detailed description of rearing conditions is provided above).

RESULTS

SHOAL CHOICE EXPERIMENTS

Test fish from tea-stained lakes significantly preferred the shoal that was visible through the UV-blocking filter over the shoal viewed through the UV-transmitting filter ('lme', $N = 40$, $t = -2.110$, d.f. = 37, $P = 0.042$) (Fig. 5), whereas test fish from clear-water lakes showed no significant preference ('lme', $N = 43$, $t = -1.062$, d.f. = 39, $P = 0.295$)

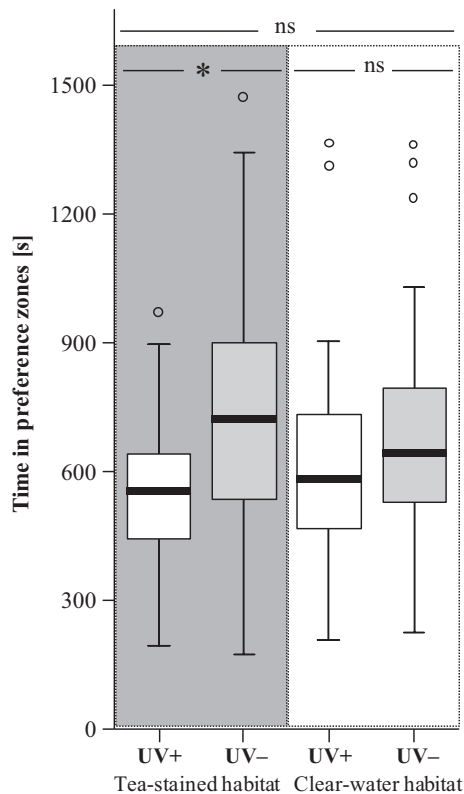


Figure 5. Time in front of shoal viewed under UV-present (white bars) or UV-absent (grey bars) lighting conditions for sticklebacks from tea-stained habitats (grey background) and for fish from clear-water lakes (white background). Plotted are medians, quartiles and percentiles. * $P < 0.05$; ns, $P > 0.10$.

(Fig. 5). UV chroma and sex of the test fish did not have a significant influence on the observed shoaling preferences (Table 1). However, for sticklebacks from tea-stained lakes, the sequence of the 15-min blocks had a significant influence (Table 1); in the second 15-min block, they preferred the shoal that they had preferred in the first 15-min block, irrespective of filter type. UV-based shoaling preferences of sticklebacks from tea-stained and clear-water lakes did not differ significantly ('lme', $N_{\text{tea-stained}} = 40$, $N_{\text{clear-water}} = 43$, $\chi^2 = 0.569$, $P = 0.451$) (Fig. 5). However, the interaction between habitat type and sequence of the 15-min blocks tended to be significant (Table 1); although test fish from tea-stained lakes preferred the same shoal that they had preferred in the first 15-min block, in the second 15-min block, test fish from clear-water lakes did not show a preference based on sequence (Table 1).

CONTROL HABITAT CHOICE EXPERIMENTS

Test fish from tea-stained lakes ('lme', $N = 23$, $t = -0.809$, d.f. = 20, $P = 0.428$; UV+: 583.26 ± 225.12 s (mean \pm SD); UV-: 654.43 ± 288.06 s), nor test fish from clear-water lakes ('lme', $N = 29$, $t = -1.008$, d.f. = 25, $P = 0.323$; UV+: 588.21 ± 311.76 s; UV-: 691.17 ± 309.78 s) significantly preferred either of the habitats viewed under UV-absent or UV-present conditions. UV chroma and sex did not have a significant influence on habitat choice (Table 1). Habitat choice of sticklebacks from tea-stained and clear-water lakes did not differ significantly from each other ('lme', $N_{\text{tea-stained}} = 23$, $N_{\text{clear-water}} = 29$, $\chi^2 = 0.059$, $P = 0.809$) (Table 1).

SHOAL CHOICE VS. CONTROL HABITAT CHOICE EXPERIMENTS

The preference indices of test fish from tea-stained lakes did not differ significantly between shoal choice and habitat choice (control) ('lme', $N_{\text{shoal choice}} = 40$, $N_{\text{habitat choice}} = 23$, $\chi^2 = 0.321$, $P = 0.571$; shoal choice: 162.63 ± 400.02 s; habitat choice: -71.17 ± 410.54 s) (Table 2). Furthermore, the shoal and habitat choice preferences of test fish from clear-water lakes did not differ significantly as well ('lme', $N_{\text{shoal choice}} = 43$, $N_{\text{habitat choice}} = 29$, $\chi^2 = 0.126$, $P = 0.723$; shoal choice: -69.49 ± 442.37 s; habitat choice: -102.96 ± 577.58 s) (Table 2).

REFLECTANCE MEASUREMENTS

Test fish from both habitat types differed significantly with respect to the UV chroma and brightness in the cheek region (Table 3). UV chroma was

Table 1. Summary of the conducted lmes for shoal and control habitat choice experiments investigating UV preferences of test fish from tea-stained and clear-water lakes, as well as differences between habitat types

Dependent variable	Explanatory variable	AIC	Explanatory variable	χ^2	δ d.f.	<i>P</i>
Preference index (shoal choice) for fish from tea-stained habitats	Sex + chroma + sequence	18.989	Sex	1.291	1	0.256
	Chroma + sequence	18.279	Chroma	2.503	1	0.114
	Sequence	17.782	Sequence	6.296	1	0.012
Preference index (shoal choice) for fish from clear-water habitats	1	23.078				
	Sex + chroma + sequence	32.412	Sequence	0.014	1	0.905
	Chroma + sequence	30.426	Sex	0.015	1	0.901
	Chroma	28.441	Chroma	0.472	1	0.492
Preference index (habitat choice) for fish from tea-stained habitats	1	26.913				
	Sex + chroma	24.145	Sex	0.548	1	0.760
	Sex	20.693	Chroma	1.288	1	0.257
Preference index (habitat choice) for fish from clear-water habitats	1	19.981				
	Sex + chroma	38.485	Chroma	< 0.001	1	0.984
	Sex	36.485	Sex	1.306	1	0.521
Preference index (shoal choice)	1	33.791				
	Habitat type \times sequence	42.192	Habitat type \times sequence	3.320	1	<i>0.068</i>
	Habitat type + sequence	43.512	Habitat type	0.768	1	0.381
	Habitat type	42.280	Sequence	2.520	1	0.122
Preference index (habitat choice)	1	44.032				
	Habitat type	48.972	Habitat type	0.059	1	0.809
	1	47.031				

The dependent variable was the preference index ((time (s) in front of UV-present compartment - time (s) in front of UV-absent compartment)/total time in preference zones). Explanatory variables included were 'sex' (sex of test fish), 'UV chroma' [UV chroma (in the cheek region)], sequence (sequence of the 15-min periods), 'habitat type' (tea-stained or clear-water), respectively, and the interaction between 'habitat type' and 'sequence'. To control for possible influences of population and year, both variables were included as random factors and were never removed from models. Also shown are Akaike's information criterion (AIC) values, with the model with the lowest AIC representing the best approximating model, as well as the results of stepwise removal of explanatory variables and respective statistics. Significant results ($P < 0.05$) are shown in bold; trends ($0.05 < P < 0.10$) are shown in italics. 1 (explanatory variable) represents the model with no explanatory variable included.

Table 2. Summary of the conducted two models testing UV-based preferences of shoal choice experiments (main experiments) vs. preferences of control habitat choice experiments for fish from tea-stained and clear-water lakes

Dependent variable	Explanatory variable	AIC	Explanatory variable	χ^2	δ d.f.	<i>P</i>
Preference index of fish from tea-stained habitats	Experimental type	37.212	Experimental type	0.321	1	0.571
	1	35.533				
Preference index of fish from clear-water habitats	Experimental type	57.084	Experimental type	0.126	1	0.723
	1	55.210				

The dependent variable was the preference index ((time (s) in front of UV-present compartment - time (s) in front of UV-absent compartment)/total time in preference zones). The explanatory included was 'experimental type' (shoal choice or habitat choice). To control for possible influences of population and year, both variables were included as random factors and were never removed from models. Also shown are Akaike's information criterion (AIC) values, with the model with the lowest AIC representing the best approximating model, as well as the results of stepwise removal of explanatory variables and respective statistics. 1 (explanatory variable) represents the model with no explanatory variable included.

significantly higher for test fish from tea-stained lakes and brightness was significantly higher for test fish from clear-water lakes. No significant differences

were measured in UV chroma and brightness for the gonadal region, nor for brightness in the back region (Table 3).

Table 3. Tests for differences in reflectance variables taken at sticklebacks' cheek region, gonadal region, and back region for test fish from tea-stained and clear-water lakes

Variable	<i>W</i>	<i>P</i>	Tea-stained Median (first quartile, third quartile)	Clear-water Median (first quartile, third quartile)
UV chroma (cheek)	1142	< 0.001	0.195 (0.181, 0.215)	0.165 (0.148, 0.190)
Brightness (cheek)	2804	0.018	3250.2 (2436.2, 4983.2)	4387.9 (2899.9, 7175.8)
UV chroma (gonads)	1971	0.191	0.233 (0.189, 0.257)	0.199 (0.158, 0.255)
Brightness (gonads)	2012	0.260	2022.6 (1098.3, 3686.7)	1608.4 (859.9, 3808.0)
Brightness (back)	2195	0.749	2545.5 (1641.2, 4117.3)	2388.4 (1205.7, 4705.5)

Wilcoxon rank sums tests were used throughout. $N_{\text{tea-stained}} = 72$, $N_{\text{clear-water}} = 63$. Significant results are shown in bold. UV, ultraviolet.

DISCUSSION

In accordance with our predictions concerning lighting habitat-dependent UV-based shoaling behaviour, sticklebacks from tea-stained lakes, which provide only low amounts of UV light for communication, significantly preferred the shoal under UV-absent conditions. Thus, the limited availability of UV wavelengths within the tea-stained habitat appears to have caused a decreased reliance on ultraviolet signals, at least during shoal choice. In contrast to our predictions that clear-water sticklebacks would prefer shoals viewed under UV-present conditions, no significant preference was found during shoaling experiments. These results are contradictory to a study on a German clear-water population of the three-spined stickleback, which described a preference for UV-reflecting shoals (Modarressie *et al.*, 2006). Often, the use of UV coloration in social contexts and potential benefits of using UV are discussed in the context of 'private communication' because this may allow communication with conspecifics but may disrupt communication and conspicuousness towards UV-blind heterospecifics and predators (Cummings, Rosenthal & Ryan, 2003; Hastad, Victorsson & Odeen, 2005; Siebeck *et al.*, 2010). However, all examined lakes are known to be inhabited by brown trout (*Salmo trutta lacustris* L.) (Giles, 1983; MacColl, El Nagar & de Roij, 2013), which is UV-sensitive in its juvenile stage up to an age of 2 years (Bowmaker & Kunz, 1987). Thus, the advantages of using UV may be outweighed by an increased risk of predation, at least in the context of shoal choice, and at short distance from predators.

The results of sticklebacks from tea-stained lakes are somewhat comparable to the findings of a study by Modarressie *et al.* (2015). In that study, an F_1 generation of the German freshwater population used by Modarressie *et al.* (2006) was raised under UV-absent conditions in the laboratory. In subsequent shoal choice experiments, these sticklebacks showed a

significant preference for the shoal viewed under UV-absent conditions. During the nonreproductive season, sticklebacks form shoals in limnetic zones, stay away from the shores, and inhabit deeper water layers (Keenleyside, 1955; Wootton, 1976). The lighting conditions within the tea-stained lakes may thus be comparable to the UV-absent lighting conditions in the laboratory because the availability of UV in the lakes is very limited and, in addition, further attenuates with increasing depth (Losey *et al.*, 1999). UV light penetrates to considerably deeper waters in clear-water than in tea-stained lakes. Thus, sticklebacks from clear-water lakes should be confronted with UV-poor (at greater depth) and UV-rich (at the surface) lighting conditions during their nonreproductive season and their lack of a decision between the shoals might be simply based on a preference of familiar conditions; in this case, both lighting conditions.

The results also showed that the sequence of the 15-min blocks had a significant influence on the choice behaviour of tea-stained test fish and, furthermore, the interaction between habitat type and sequence showed that test fish from clear-water and tea-stained lakes differed in their behaviour. The choice of test fish from tea-stained lakes during the second 15 min was influenced by a familiarity with or habituation to the shoal that they had preferred during the first 15 min, indicating that preferences based on colour signals might be influenced by other variables over time. Nevertheless, independent of this familiarity effect, preferences for shoals under UV-absent conditions were still significant over the total testing time. No sequence effect was found for test fish from clear-water lakes, indicating that their lack of significant choice was independent of any familiarity or shoal characteristics and that a lack of choice for either UV condition was unaffected by that potentially confounding effect.

In control habitat choice experiments, neither sticklebacks from tea-stained lakes, nor fish from

clear-water lakes showed significant preferences for either lighting habitat. The habitat choice experiments served as control experiments to eliminate the possibility that test fish based their choice on the lighting habitat and not on the shoal under the respective lighting conditions. Rick & Bakker (2010), for example, conducted habitat choice experiments under predatory threat and showed that sticklebacks raised under full-spectrum conditions escape to a habitat that lacks UV, probably because they will be less conspicuous for predators. By contrast, the results of our habitat choice experiments indicate that shoal choice based on lighting habitat appears to be an implausible explanation for the observed preferences. Nevertheless, when shoaling preferences were tested against habitat preferences, there was no significant difference between the choice behaviours, possibly as a result of limited sample sizes. It can thus not be ultimately excluded that habitat choice had no influence and this requires further study.

We had predicted that UV reflections should be reduced in tea-stained lakes with limited UV light available for communication, whereas sticklebacks from clear-water lakes were expected to reflect stronger in the ultraviolet spectral range. However, completely opposite to our expectations, the results revealed that the UV reflections of sticklebacks from tea-stained lakes were higher than for sticklebacks from clear-water lakes, at least in the cheek region. Test fish from clear-water lakes on the other hand were significantly brighter in that body region. The development of an enhanced UV reflection in a habitat with a very restricted amount of UV available for communication appears to be counterintuitive at first sight. However, tea-stained lakes are strongly red-shifted (Scott, 2001) and thus short-wave UV reflections will contrast very well against the long-wave background irradiance in these lakes. Visual contrast against background light is required for the perception of visual signals (Endler, 1993). In his 'sensory drive' hypothesis, Endler (1992) postulated a habitat specific transmission of signals, which would in turn cause adaptations of signaller and signal receiver. Accordingly, McDonald, Reimchen & Hawryshyn (1995) and Boughman (2001) described stickleback males that developed a dark, blackish courtship coloration, instead of a typical red coloration, in red-shifted waters. Fuller (2002) showed that bluefin killifish [*Lucania goodei* (Jordan)] with blue anal fins are more common in bodies of water with predominant long-wave lighting conditions, thus enhancing the contrast against background light. Furthermore, the opsin expression in several stickleback populations has been found to maximize sensitivity to the background light (Novales Flamarique *et al.*, 2013).

However, because no data on opsin expression and the ratio and retinal distribution of cones are available for sticklebacks from the two habitat types, it cannot be definitively concluded that the overall conspicuousness of the UV colour patterns within the tea-stained habitat is enhanced, diminished or differs at all compared to the clear-water habitat. An alternative explanation is that stickleback compensate for a stronger attenuation of the signal in tea-stained lakes by enhancing the signal in the UV spectral range in habitats with low transmission of UV wavelengths. Further studies incorporating detailed information on spectral sensitivity and habitat-specific variation in background radiance are required to quantify how sticklebacks contrast against the water background when viewed by conspecifics.

An enhanced reflection in the UV region might also be explained in the context of an adaptation of the body surface to the acidic nature of the tea-stained lakes because UV reflections are based on structural properties of the reflecting body regions (Losey *et al.*, 1999). To adapt to acidic environments, modifications of mucous membranes might occur. Zuchelkowski, Lantz & Hinton (1981) described an adaptation of epidermal mucous cells to increased acidity in brown bullhead catfish (*Ictalurus nebulosus* Lesueur). Modifications of the mucous membrane can alter UV reflections. In the saddle wrasse [*Thalassoma duperrey* (Quoy & Gaimard)], for example, it was shown that a change in mucous cells led to an enhanced UV absorption (Zamzow, 2004).

Taken the preferences found in the shoal choice experiment and the differences in UV reflections into account, the results appear to be contradictory: sticklebacks from tea-stained lakes have a higher UV chroma but prefer shoals lacking UV reflections. The existing and well-elaborated UV reflections, however, may be indicative of an important function of UV signals in other contexts, such as mate choice (Rick *et al.*, 2006; Rick & Bakker, 2008a). During the reproductive season, three-spined sticklebacks remain in shallow waters close to the shore where males establish territories and build nests (Wootton, 1976). More UV light is available in the littoral zone, allowing a better detection of UV signals (Jerlov, 1968; Conde, Aubriot & Sommaruga, 2000; Kjeldstad *et al.*, 2003; Fuller & Travis, 2004).

In summary, sticklebacks from tea-stained lakes showed a significant preference for shoals lacking UV reflections, whereas those from clear-water lakes did not show a preference for a shoal under particular lighting conditions, although sticklebacks from tea-stained lakes reflected stronger in the UV. Thus, the habitat of origin has an influence on UV-based shoaling decisions and UV signals. However, the overall importance of UV reflections appears to be

limited in the context of shoaling in these stickleback populations, probably because UV-sensitive predators are present and therefore the advantages of private UV communication might be outweighed by the increased risk of predation.

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