

Ultraviolet reflection enhances the risk of predation in a vertebrate

Ricarda MODARRESSIE, Ingolf P. RICK, Theo C. M. BAKKER*

Institute for Evolutionary Biology and Ecology, University of Bonn, An der Immenburg 1, D-53121 Bonn, Germany

Abstract Many animals are sensitive to ultraviolet light and also possess UV-reflective regions on their body surface. Individuals reflecting UV have been shown to be preferred during social interactions such as mate choice or shoaling decisions. However, whether those body UV-reflections enhance also the conspicuousness to UV-sensitive predators and therefore entail costs for its bearer is less well documented. Two size-matched three-spined sticklebacks *Gasterosteus aculeatus*, one enclosed in a UV-transmitting (UV+) and another in a UV-blocking (UV-) chamber, were simultaneously presented to individual brown trout *Salmo trutta*. “yearlings”. Brown trout of this age are sensitive to the UV part of the electromagnetic spectrum and are natural predators of three-spined sticklebacks. The stickleback that was attacked first as well as the subsequent number of attacks was recorded. Sticklebacks enclosed in the UV-transmitting chamber were attacked first significantly more often compared to sticklebacks enclosed in the UV-blocking chamber. Control experiments using neutral density filters revealed that this was more likely due to UV having an influence on hue perception rather than brightness discrimination. The difference in attack probability corresponded to the difference in chromatic contrasts between sticklebacks and the experimental background calculated for both the UV+ and UV- conditions in a physiological model of trout colour vision. UV reflections seem to be costly by enhancing the risk of predation due to an increased conspicuousness of prey. This is the first study in a vertebrate, to our knowledge, demonstrating direct predation risk due to UV wavelengths [*Current Zoology* 59 (2): 151–159, 2013].

Keywords UV vision, Brown trout, *Salmo trutta*, Ultraviolet reflection, Three-spined stickleback, *Gasterosteus aculeatus*, Predation risk

Sensitivity in the ultraviolet (UV) range of the electromagnetic light spectrum is widespread in the animal kingdom and occurs in all major taxonomic groups (for a review, see Tovée 1995). Furthermore, many animals possess UV-reflective structures on their body surface, which are used in animal communication and play a part in mate attractiveness (Fleishman et al., 1993; Bennett et al., 1996; Kodric-Brown and Johnson, 2002; Smith et al., 2002; Cummings et al., 2003; Rick et al., 2006; Rick and Bakker, 2008a, b; Lim et al., 2008), territorial or aggressive behavior (Siebeck, 2004; Alonso-Alvarez et al., 2004; Whiting et al., 2006; Rick and Bakker, 2008c; Vedder et al., 2010), shoaling decisions (Modarressie et al., 2006) and parent-offspring communication (Jourdie et al., 2004; Tanner and Richner, 2008). It has been suggested that UV signalling affords a private channel for communication that is not accessible to long-lived predators that are often not sensitive to UV wavelengths (Cummings et al., 2003). There is some support for this private communication hypothesis in swordtails *Xiphophorus nigrensis* and damselfish *Pomacentrus amboinensis* (Cummings et al., 2003; Siebeck et al., 2010). UV

vision also facilitates foraging in spiders (Li and Lim, 2005) and birds, where blue tits *Parus caeruleus* detected caterpillars under UV-present condition earlier than when UV light was absent (Church et al., 1998). Furthermore, preferences for UV-reflecting berries could be detected in behavioural experiments on redwings *Turdus iliacus* and black grouse *Tetrao tetrix* (Siitari et al., 1999; Siitari and Viitala, 2002).

Studies on the foraging behavior in fish concerning UV vision are ambiguous. Some fish, e.g. three-spined sticklebacks *Gasterosteus aculeatus* and rainbow trout *Oncorhynchus mykiss*, seemed to use UV vision during foraging on zooplankton (Browman et al., 1994; Rocco et al., 2002; Rick et al., 2012), while in some other studies no significant effect of UV wavelengths on foraging success could be detected, even in cases where the same predator/prey system was used (Rocco et al., 2002; White et al., 2005; Leech and Johnsen, 2006; Modarressie and Bakker, 2007). Most studies examining the function of UV vision during foraging, tested the feeding behavior in presence and/or absence of UV wavelengths, e.g. of fish when preying zooplankters (Brow-

Received April 3, 2012; accepted Aug. 12, 2012.

* Corresponding author. E-mail: t.bakker@uni-bonn.de

© 2013 *Current Zoology*

man et al., 1994; Rocco et al., 2002; Leech and Johnsen, 2006; Modarressie and Bakker, 2007) which are generally assumed to absorb UV wavelengths (Johnsen and Widder, 2001; but see Rick et al., 2012). However, whether animals with UV-reflecting body surfaces themselves face an enhanced predation risk is less well studied.

Enhanced predation risk linked to UV-reflective regions on the body surface was directly demonstrated in butterflies. Lyttinen et al. (2004) manipulated wings of butterflies either to reflect or to absorb UV light and recorded the predation rate in the natural environment. Butterflies without UV-reflections survived better than those with natural UV-reflections. There is also indirect evidence for predation risk in voles due to their UV-reflective scent marks, which attract kestrels *Falco tinnunculus* as well as rough-legged buzzards *Buteo lagopus* (Viitala et al., 1995; Koivula and Viitala, 1999) and also great grey shrikes *Lanius excubitor* (Probst et al., 2002). Guppies *Poecilia reticulata* from a population in which a UV-sensitive prawn is present reflected less in the UV waveband than guppies from a population without UV-sensitive predators (Kemp et al., 2008). A more recent study on visual foraging in three-spined sticklebacks suggests that the UV spectral part is of relative importance compared to other spectral parts when fish prey on live cladoceran *Daphnia magna* (Rick et al., 2012).

In this study we used the predator/prey system of brown trout and three-spined sticklebacks. “Yearling” brown trout and three-spined sticklebacks possess UV vision (Bowmaker and Kunz, 1987; Rowe et al., 2004) and three-spined sticklebacks bear UV-reflecting regions on their body surface (Rick et al., 2004). The conspicuousness of visual signals largely depends on how they contrast with their visual background (Lythgoe, 1968). Hence, the efficiency of visual foraging strongly depends on the prey-to-background-contrast, which could be enhanced by the addition of UV wavelengths in UV-sensitive predatory species. By modelling trout visual perception we thus investigated further if the chromatic contrast generated by stickleback prey against the experimental background is different between the UV+ and UV- conditions and whether this may explain prey preferences of trout used in the choice experiment.

1 Material and Methods

In choice experiments sticklebacks preferred UV-reflecting conspecifics over non-reflecting ones in social

interactions such as female mate choice, male mate choice and shoal choice of non-reproductive fish (Modarressie et al., 2006; Rick et al., 2006; Rick and Bakker, 2008a, b). To assess whether UV-reflections of a vertebrate body surface also enhance the risk of predation, two size-matched sticklebacks were simultaneously presented in two Perspex chambers to trout. One stickleback was enclosed in a UV-transmitting (UV+) chamber and therefore reflected UV light, and the second one in a UV-blocking (UV-) chamber and hence lacked UV-reflections. We then determined whether the difference in spectral content between the two light environments influences the foraging choice of trout.

1.1 Experimental subjects

Sticklebacks (mean $SL \pm SD$, 2.65 cm \pm 0.160) used in the present study were laboratory-bred descendants from a pond population (Euskirchen, Germany 50°38'N/6°47'E'), and held in 10 L aquaria with air ventilation under an 8:16 h light-dark regime. All fish were fed with defrosted chironomid larvae *ad libitum* after the trials. All sticklebacks were used only once.

Brown trout (mean $SL \pm SD$, 12.96 cm \pm 1.14) stemmed from a commercial aquaculture company (Rameil, Lindlar, Germany), and were held in 700 L outdoor tanks provided with continuously fresh water flow-through and air ventilation. Trout were fed daily with commercial fish pellets (BioMar AquaLife) and additionally with defrosted chironomid larvae *ad libitum*. Furthermore, trout were fed with dead sticklebacks and they were starved for one day before the start of the experiment. All trout were used only once.

2–6 days before the start of the experiment, brown trout were transferred into holding aquaria (100 × 40 × 40 cm; l × w × h) in the laboratory, with a permanent water flow-through system, internal filter and ventilation via airstones. Sand covered the bottom of the tanks and java moss *Vesicularia dubyana* provided shelter. Aquaria were filled with tap-water of 13°C up to a height of 24.5 cm. The top of the aquarium was covered by a net to stop trout escaping.

Illumination was provided by fluorescent tubes (Viva-Lite, 36 W, 1200 mm, Light-Office, Germany) hanging 15 cm above the water surface both for trout and stickleback. These lights contain a proportion of UV-A similar to natural skylight.

The experiment was carried out between 16 January 2007 and 5 April 2007 and between 26 January 2008 and 21 February 2008.

1.2 Experimental set-up and procedure

The walls of the test aquarium ($100 \times 45 \times 33$ cm; $l \times w \times h$) were inside fitted out with grey, opaque plastic plates (Fig. 1). The aquarium was filled with tap-water to a height of 8.3 cm. To exclude confounding effects between trials, water was totally replaced after each trial. The aquarium was divided into two halves by a black line drawn on the bottom with a permanent waterproof marker. On one side a bisected opaque grey plastic tube ($14.9 \times 14.7 \times 7.8$ cm; $l \times w \times h$) was placed at the bottom midway against the side wall with its opening facing the middle line. It provided shelter for the trout. On both sides of the shelter one airstone was mounted. Opposite to the shelter-tube, sticklebacks were presented in two Perspex chambers ($4 \times 4.7 \times 26$ cm; $l \times w \times h$, each). Both chambers were made of UV-transmitting Perspex (UV+: GS-2458, Röhm, Darmstadt, Germany), but one was fitted out with a UV-blocking polythene sheet filter (UV-: GS-2548 plus Lee filters # 226, Fig. 2a). The walls of the two chambers (UV+ and UV-) that faced each other were covered with grey opaque plastic slices (30×4 cm; $l \times w$). Thus, the two sticklebacks were visually isolated from each other. Both chambers stuck together 16.8 cm apart by a piece of wood. In total, 37 trials were conducted with UV+ /UV- filter and 37 trials with neutral-density filter. Sticklebacks as well as trout were only used once.

The behavior of the trout was filmed with a web-cam mounted 125 cm above the tank and connected to a laptop. The whole set-up was surrounded by black curtain. Before each trial, the position of the chambers, as well as the position of the sticklebacks, was chosen randomly by tossing a coin. Out of 37 trials in the UV treatment

the UV+ filter was placed 20 times on the right and 17 times on the left side. A test for side bias revealed that trout did not show a significant preference for one side with respect to the first attack (Chi-square test: $\chi^2_1 = 0.027$, $n = 37$, $P = 0.869$). The simultaneously presented sticklebacks were matched for standard length and body mass to the nearest mm and mg, respectively.

Before the test, a trout was gently taken out of the holding tank and introduced into the test aquarium. Then the recording was started. After the trout showed normal swimming behavior (trout does not show fleeing or hiding behavior and swam at an even pace in its tank), or at least after one hour of acclimatisation, and when it was located in the back half of the aquarium, two previously matched sticklebacks were gently introduced into the chambers. Brown trout were measured for standard length and body mass after each trial. The condition factor for both trout and sticklebacks was calculated following (Bolger and Connolly, 1989): $100 \times \text{mass (g)} / \text{length}^3 \text{ (cm)}$.

As a measure of predation risk, we analysed which of the two sticklebacks (UV+ or UV-) was attacked first by trout and subsequently how often each stickleback was attacked during 20 min.

The same experimental procedure was conducted with neutral-density chambers to test whether brightness differences influence attack behavior instead of differences in wavelength composition. This control experiment was necessary as the UV-transmitting and UV-blocking filters not only differed in wavelength transmission but also in overall quantal flux in the 300–700 nm range, which potentially could have affected the predator's choice. Neutral-density filters were produced through printing various shades of grey with a laser printer on overhead transparencies (Xerox Type A P/N 003R96019) (see Fig. 2a and Siebeck, 2004). Both neutral-density filters transmitted UV wavelengths and the visible spectrum, but differed in the amount of achromatic transmission. The two UV-transmitting chambers were fitted out with the neutral-density filters such that the brightness difference (24%) between them came close to but exceeded the brightness difference between the UV filters (18% difference: Rick et al., 2006). The spectral transmission of all treatment filters was quantified with a spectrophotometer (Avantes AvaSpec 2048, Eerbeek, The Netherlands).

We also checked for differences in behavior of sticklebacks enclosed in the UV-transmitting or UV-blocking chamber. The same experimental set-up as

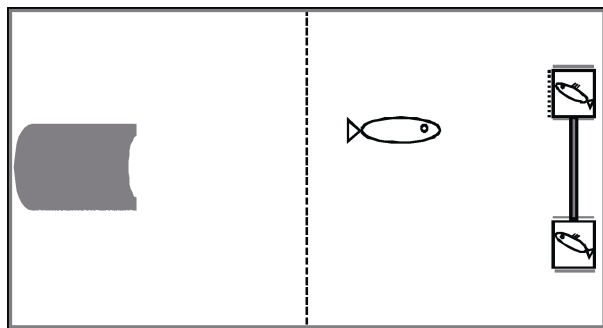


Fig. 1 Experimental aquarium containing one shelter-tube for the trout predator on the left side and two chambers for the stickleback prey on the opposite side

Black lines indicate UV-transmitting partitions, the small dotted black line indicates the position of the UV-blocking filter in front of one prey chamber and grey lines indicate grey, opaque plastic slices.

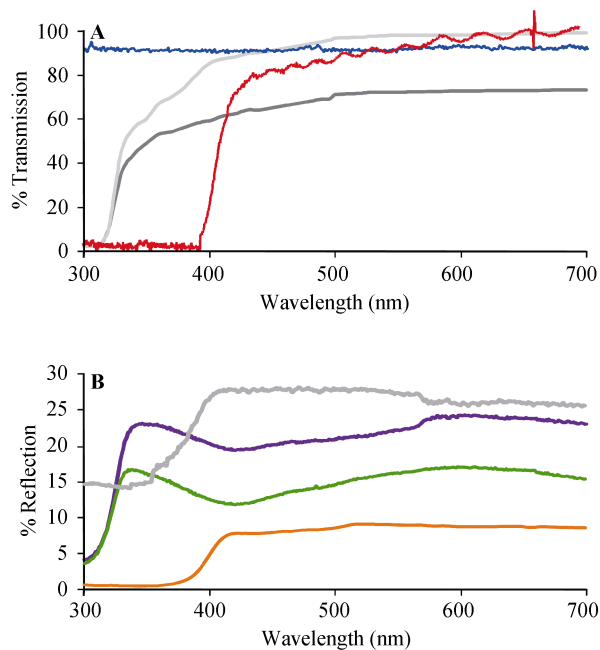


Fig. 2 (A) Transmission (%) spectra of the used optical filters (UV+: blue, UV-: red, ND bright: bright grey, ND dark: dark grey). (b) Mean reflection spectra (%) of the operculum (violet) and abdominal region (green) of ten subadult three-spined sticklebacks as well as reflection of the grey experimental background (grey) and of natural background substrates found in a common freshwater habitat (orange)

before was used. Now stickleback behavior was filmed from the front side of the aquarium. As a measure of activity, the time spent in the upper half of the chamber and number of crossings between the upper and lower halves was recorded for the sticklebacks in each chamber. We run 11 trials with new sticklebacks and trout for this control experiment.

1.3 Spectral reflection of prey and the experimental background

Subadult sticklebacks (mean $SL \pm SD$, $2.56 \text{ cm} \pm 0.217$) from the same population and were similar age and size as the fish used in the choice tests were collected in February 2011. Reflection measurements were taken from ten individuals using a bifurcated 200- μm fibre-optic probe connected to a spectrophotometer (Avantes AvaSpec 2048) in combination with a deuterium-halogen light source (Avantes AvaLight D/H-S) for illumination. Scans were collected from two conspicuous silvery-coloured patches on the ventro-lateral surface of the left side, one located in the opercular area and one in the abdominal region. Both regions showed reflection in the UV spectral range, which is mainly based on structural coloration and characteristic for the

silvery skin parts found in non-reproductive three-spined sticklebacks as well as mature females (Rick and Bakker, 2008c; Fig. 2b). Reflection was measured relative to a 98% Spectralon white standard over the range of 300–700 nm at about 0.5 nm resolution in wavelength. Data were recorded with AvaSoft 7.5 (Avantes) and imported into Microsoft Excel. Fifteen measurements were averaged for each sample region. The measurement procedure took less than 2 min per individual so that colour changes caused by pigment aggregation or dispersion could be ruled out (IPR, personal observation).

Spectral reflection of the visual background in the stimulus compartments, which consisted of grey plastic partitions, was measured analogous to the stickleback skin. The spectrum reveals lower reflection intensities at UV wavelengths followed by an increase in reflection between 350 and 400 nm and an extended plateau region between 400 and 600 nm as well as a slight decrease in reflection intensity towards 700 nm (Fig. 2b). This was found to resemble the spectral composition of natural background substrates consisting of mud with overlaying organic matter found in common German freshwater habitats of our study species (Fig. 2b).

1.4 Experimental irradiance levels

Downwelling irradiance produced by the fluorescent tubes under the experimental conditions was measured with an Avantes CC-UV/VIS cosine corrector. Therefore, the probe end was placed in the prey fish position at about 10 cm above the bottom of each, the UV+ and UV- as well as the ND bright and ND dark chamber, and was pointing upwards. Spectral irradiance was measured between 300 and 700 nm and was calibrated against an Avantes NIST traceable application standard.

1.5 Visual modelling

Since visual systems encode colour signals in terms of a contrast relative to its visual background we used a photoreceptor noise-limited colour discrimination model (Vorobyev and Osorio, 1998) to determine how trout predators perceive background contrast of stickleback prey presented behind a UV transmitting compared to a UV blocking filter. Therefore, we included reflection data of stickleback prey and the experimental background, data on filter transmission and the illumination spectrum as well as data on trout spectral sensitivity in our calculations. The model establishes a chromatic distance ΔS which describes the colour contrast between prey and the experimental background with small ΔS values corresponding to different colour signals that

appear similar to a receiver whereas large ΔS values account for highly contrasting signals. The chromatic

$$\Delta S^2 = \frac{\left[(e_1 e_2)^2 (\Delta f_4 - \Delta f_3)^2 + (e_1 e_3)^2 (\Delta f_4 - \Delta f_2)^2 + (e_1 e_4)^2 (\Delta f_2 - \Delta f_3)^2 + (e_2 e_3)^2 (\Delta f_4 - \Delta f_1)^2 + (e_2 e_4)^2 (\Delta f_3 - \Delta f_1)^2 + (e_3 e_4)^2 (\Delta f_2 - \Delta f_1)^2 \right]}{\left[(e_1 e_2 e_3)^2 + (e_1 e_2 e_4)^2 + (e_1 e_3 e_4)^2 + (e_2 e_3 e_4)^2 \right]}$$

where Δf_i is the natural log ratio of the quantum catches for cone receptor i , for each of the two stickleback color patches A and the experimental background B and e_i is the signalling noise for each cone type i .

$$\Delta f_i = \ln \left(\frac{Q_{iA}}{Q_{iB}} \right) = \ln \left(\frac{\int_{300}^{700} R_A(\lambda) I(\lambda) S_i(\lambda) d\lambda}{\int_{300}^{700} R_B(\lambda) I(\lambda) S_i(\lambda) d\lambda} \right)$$

where $R_A(\lambda)$ is the reflection of the colour patch A, $R_B(\lambda)$ is the reflection of the background B, $I(\lambda)$ is the irradiance spectrum of the illuminant for either the UV+ or UV- conditions and $S_i(\lambda)$ is the spectral sensitivity of the cone type i , summed across wavelengths between 300 and 700 nm.

Irradiance curves for both conditions as well as spectral sensitivity curves were normalised to one. Absorption curves were obtained from published cone absorbance maxima for “yearling” trout with sensitivity peaks at 355 nm, 441 nm, 535 nm and 600 nm (Bowmaker and Kunz, 1987) by using pigment template parameters provided in Govardovskii et al. (2000). The spectral sensitivity S_i of each cone type was calculated as

$$S_i = P_i(\lambda) C_i(\lambda) L_i(\lambda) T_i(\lambda)$$

where $P_i(\lambda)$ denotes the normalised absorbance of cone type i , $C_i(\lambda)$ the transmission of the cornea, $L_i(\lambda)$ the transmission of the lens, and $T_i(\lambda)$ the transmission of the vertically mounted optical filters, either UV+ or UV-, located in the light path between the trout predator and the stimulus fish. Data on the transmission of cornea and lens in “yearling” brown trout were taken from Douglas (1989). Since prey perception of trout in our experimental setup took place over short distances and at low water depths, absorption and scatter of water was not considered in our computations. For the noise calculations we used a cone ratio of 1:1:2 for the brown trout (for UV-sensitive, short-wavelength sensitive, long- and middle-wavelength sensitive, respectively) (Bowmaker and Kunz, 1989), and assumed that the signalling noise for each cone receptor was dependent on the light intensity

$$e_i = \sqrt{\omega^2 / n_i + 2 / (Q_{iA} + Q_{iB})}$$

where ω is the Weber fraction (taken as 0.05), n_i is the relative density of the cone type i on the retina, Q_{iA} is

distance was calculated within the perceptual space of the trout predator as:

the quantum catch for cone type i and the color patch A and Q_{iB} is the quantum catch for cone type i and the background colour B (Osorio et al., 2004).

1.6 Statistical analysis

All analyses were performed using SPSS v.11.0 for Windows. When data were not normally distributed according to the Kolmogorov-Smirnov test with Lilliefors correction, and could not be transformed, non-parametric statistics were used. Given P -values are two-tailed throughout.

2 Results

2.1 Prey choice experiment

When given the choice between two simultaneously presented sticklebacks, brown trout attacked first sticklebacks seen behind a UV-transmitting filter ($n = 25$) significantly more often than sticklebacks seen behind a UV-blocking filter ($n = 12$) (Chi-square test: $\chi^2_1 = 4.568$, $n = 37$, $P = 0.033$; Fig. 3). No such preference was found in the neutral-density filter treatment. Sticklebacks presented in the brighter cylinder were attacked first at the same rate as sticklebacks presented in the darker cylinder (Chi-square test: $\chi^2_1 = 0.027$, $n_1 = 18$, $n_2 = 19$, $P = 0.869$; Fig. 3).

The distribution of first attacks of brown trout in the UV treatment tended to differ from the distribution in the ND treatment with regard to brightness differences, although this was statistically not significant (Chi-square test: $\chi^2_1 = 2.72$, $P = 0.099$).

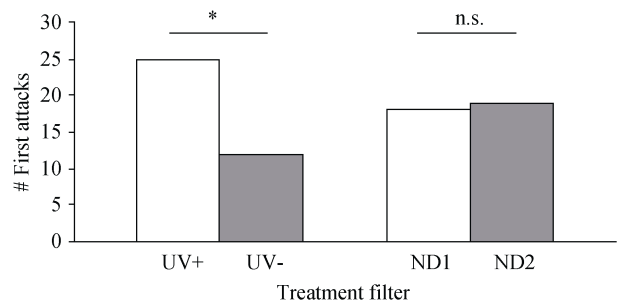


Fig. 3 Number (#) of first attacks by brown trout toward the stickleback in the UV-transmitting (UV+) and UV-blocking (UV-) chamber

Trout attacked first the UV+ stickleback significantly more often. No significant preference was found in the ND treatment. ND1 = brighter chamber; ND2 = darker chamber. * $P < 0.05$; n.s. $P > 0.05$.

In 23 out of 30 analysed UV as well as ND treatment experiments brown trout showed subsequent attacks after their first attack. Brown trout attacked sticklebacks in the UV- transmitting chamber more often than sticklebacks in the UV-blocking chamber, although this result was not statistically significant [median (interquartile range), UV+: 4 (1– 8.5), UV-: 3 (0–5), Wilcoxon matched-pairs signed-ranks test: $z = -1.791$, $n = 23$, $P = 0.073$]. In the neutral-density experiment there was no significant difference between the brighter and in the darker chamber in attack frequency [median (interquartile range), brighter: 3 (0–6.5), darker: 4.5 (0–8.5), Wilcoxon matched-pairs signed-ranks test: $z = -1.318$, $n = 23$, $P = 0.188$].

In the UV treatment, the simultaneously presented sticklebacks did not differ significantly with respect to standard length (mean $SL \pm SD$: 2.67 ± 0.16 and 2.65 ± 0.17 , paired t test, $t_{37} = 0.728$, $P = 0.471$), body mass [median mass (interquartile range): 0.21 (0.187–0.236) and 0.21 (0.178–0.242), Wilcoxon-matched-pairs signed-ranks test, $n = 37$, $z = -0.181$, $P = 0.857$], or condition factor [median CF (interquartile range): 1.08 (1.01–1.97) and 1.13 (1.05–1.23), Wilcoxon-matched-pairs signed-ranks test, $n = 37$, $z = -0.038$, $P = 0.97$]. Furthermore, there were also no significant differences regarding any body measurement between sticklebacks that were presented in the UV-transmitting chamber and those that were presented in the UV-blocking chamber [mean $SL \pm SD$, UV+: 2.72 ± 0.18], UV-: 2.71 ± 0.2), median mass (interquartile range): UV+: 0.226 (0.189–0.292), UV-: 0.225 (0.193–0.279), median CF (interquartile range), UV+: 1.163 (1.064–1.280), UV-: 1.164 (1.082–1.292), all $P > 0.5$]. The same was true for the simultaneously presented sticklebacks in the ND treatment [mean $SL \pm SD$: 2.8 ± 0.21 and 2.81 ± 0.17 , median mass (interquartile range): 0.27 (0.21–0.3) and 0.25 (0.21–0.31), median CF (interquartile range): 1.19 (1.1–1.27) and 1.19 (1.03–1.3), all $P > 0.28$].

However, despite matching sticklebacks for standard length and body mass to the nearest mm and mg, in some trials the simultaneously presented individuals differed although not significantly so with respect to absolute length and mass in the range of 1–2 mm and 0.1–0.2 mg. Therefore, we tested whether those differences had any effect on brown trout behavior. In the neutral-density treatment, trout attacked first the stickleback with the higher condition factor significantly more often (Chi-square test: $\chi^2_1 = 7.811$, $n_1 = 27$, $n_2 = 10$, $P = 0.005$; Fig. 4a) and the heavier one was attacked

more often too, although this result was statistically not significant (Chi-square test: $\chi^2_1 = 3.27$, $n_1 = 24$, $n_2 = 13$, $P = 0.071$, Fig. 4a). In 34 trials of the UV treatment, sticklebacks differed although not significantly so with respect to body mass, in 31 trials with respect to standard length and in 36 trials with respect to condition factor. No significant preference of trout for heavier or better-conditioned sticklebacks was found in the UV treatment (Chi-square test: mass: $\chi^2_1 = 0.471$, $n_1 = 19$, $n_2 = 15$, $P = 0.493$; condition factor: $\chi^2_1 = 1$, $n_1 = 15$, $n_2 = 21$, $P = 0.317$; Fig. 4b).

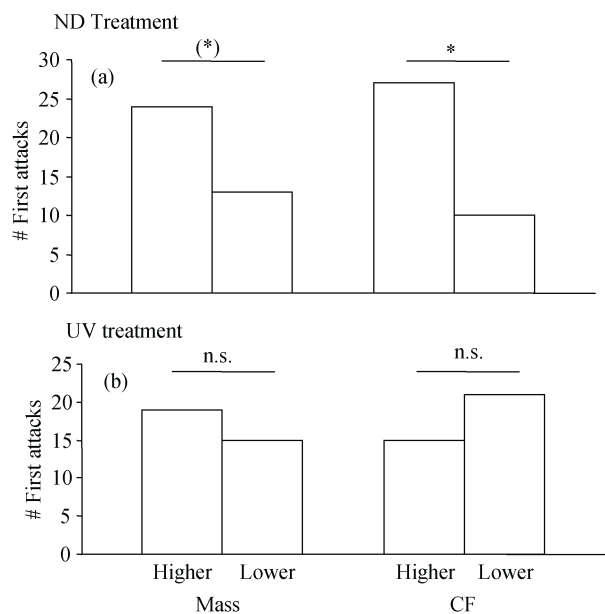


Fig. 4 Number (#) of first attacks by brown trout toward simultaneously presented sticklebacks differing in body mass and condition factor (CF) in the (a) ND treatment and (b) UV treatment

In the ND treatment brown trout attacked first the heavier and the one with a higher CF significantly more often. No significant differences were found in the UV treatment. * $P < 0.05$; (*) $P < 0.1$; n.s. $P > 0.1$.

Sticklebacks' standard length was not significantly associated with the first attack rate neither in the UV nor in the ND treatment (Chi-square test: $\chi^2_1 = 0.29$, $n_1 = 17$, $n_2 = 14$, $P = 0.178$ and $\chi^2_1 = 0.59$, $n_1 = 10$, $n_2 = 12$, $P = 0.67$, respectively).

Trout which attacked the UV+ stickleback first did not differ significantly from trout which attacked the UV- stickleback first with respect to standard length, body mass or condition factor (Mann-Whitney U test: $n_1 = 25$, $n_2 = 12$, $U = 146.5$, $P = 0.908$ and $n_1 = 25$, $n_2 = 12$, $U = 135$, $P = 0.626$ and $n_1 = 25$, $n_2 = 12$, $U = 116$, $P = 0.27$, respectively).

To test whether sticklebacks' behavior itself was affected by being either in a UV+ or UV- chamber, the

mean time sticklebacks spent in the upper half of the chamber and the number of changes between the upper and lower halves were recorded. The mean time sticklebacks spent in the upper half of the chamber did not differ significantly between fish enclosed in the UV-transmitting or UV-blocking chamber (mean \pm SD, UV+: 971.63 \pm 204.55 s; UV-: 992.91 \pm 179.53 s; paired *t* test: *t* = -0.275, *n* = 11, *P* = 0.789). Similarly, no significant difference was found in the number of changes between chamber halves of fish presented in the UV-transmitting or UV-blocking chamber (Wilcoxon matched-pairs signed-ranks test: *n* = 11, *z* = -0.356, *P* = 0.722).

2.2 Chromatic background contrast

The UV+ preference of trout predators found in the UV treatment was also mirrored in the magnitude of the chromatic contrast values calculated in the visual model. Chromatic background contrast ΔS of stickleback prey as perceived by the brown trout visual system is significantly higher when being viewed through the UV+ filter compared to the UV- filter for both, the opercular region (paired *t* test: *t* = 3.65, *df* = 9, *P* < 0.01, Fig. 5a) as well as the abdominal region (paired *t* test: *t* = 3.46, *df* = 9, *P* < 0.01, Fig. 5b).

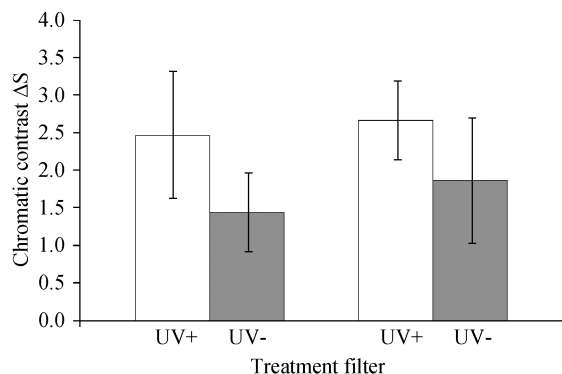


Fig. 5 Mean chromatic contrast ΔS of two body areas (opercular region, abdominal region) from ten subadult sticklebacks when viewed by a yearling brown trout predator against the visual background under experimental conditions for both the UV+ (white bar) and UV- chamber (grey bar)

The error bars show the standard deviation of the mean. Chromatic contrast values of both skin regions were significantly higher for UV+ sticklebacks compared to UV- sticklebacks. *****P* < 0.01.**

3 Discussion

The presence of UV wavelengths did affect the prey choice behavior of brown trout, sticklebacks inside the UV-transmitting chamber were significantly more often attacked first than those enclosed in the UV-blocking

chamber. This result shows that, beside the benefits of UV-reflections e.g. through mate attraction (e.g. Fleishman et al., 1993; Bennett et al., 1996; Rick et al., 2006), UV-reflections are costly by enhancing an individual's visual conspicuousness and thus the risk of predation. However, in the present study only non-reproductive sticklebacks were used. In order to directly compare the context-dependent benefits and costs of UV-reflections further experiments using sexually mature individuals are required. Modarressie et al. (2006) showed that non-reproductive sticklebacks prefer to shoal with UV-reflecting conspecifics compared to non-reflecting ones. These findings, together with the present results, give support for the oddity theory in that way, that UV-reflecting sticklebacks benefit from shoaling with groups that possess UV-reflections due to a lower risk of being eaten by UV-sensitive predators.

An increase in conspicuousness of stickleback prey presented under UV-rich conditions is also predicted by the results of the visual model of trout perception. Prominent skin areas of prey fish generated a higher chromatic contrast against the background when being observed under UV+ conditions compared to UV- conditions. A substantial increase in chromatic contrast based on UV cues and its functional importance has been shown for visual interactions between thomisid spiders and their hymenopteran prey (Heiling et al., 2003, 2005) as well as bird-fruit interactions (Schaefer et al., 2007). Furthermore, our results are in accordance to a study on fish foraging behavior in which sticklebacks as predatory fish preferred zooplankton prey under UV-rich lighting conditions that provided a larger prey-to-background contrast compared to UV-deficient conditions (Rick et al., 2012).

In the present study, the UV-reflecting sticklebacks were attacked first significantly more often. Subsequent attacks of trout only tended to be more frequently targeted at the UV reflecting one. Assuming a similar success of hunting by trout for both the UV-reflecting and non-reflecting stickleback once attacked, the UV-reflecting one will suffer a higher risk of being detected and preyed upon. The matter of fact that trout were fed with dead sticklebacks under UV+ holding conditions prior to the experimental trials might have accounted for the preference for UV+ prey because it looked similar to the food previously experienced. Nevertheless, trout were fed with dead stickleback only on very few occasions so that some sort of learning effect can be virtually excluded.

Cummings et al. (2003) demonstrated that UV-reflecting swordtails were not more conspicuous com-

pared to non-reflecting ones to their major, UV blind, predator, the Mexican tetra *Astyanax mexicanus*. The evolution of signalling in the UV waveband, which is invisible for specific predators but attracts potential mates, is discussed as part of a private communication channel (Cummings et al., 2003). A study on colour vision in passerines and their predators (corvids and raptors) also showed the option of private communication in the UV range (Håstad et al., 2005). Although the brown trout used as predators in the present study are capable of UV vision, it is conceivable that signalling in the ultraviolet could be beneficial and functions as a private communication channel in three-spined sticklebacks as well since there are other predators like eel *Anguilla anguilla* and herring *Clupea harengus* that are less sensitive to UV. In order to test the hypothesis whether natural selection drives the use of the UV part of the electromagnetic spectrum during communication, sticklebacks from habitats with different predators and predation risks should be compared for their UV-reflecting properties. Among juvenile sticklebacks, there exists considerable variation in the intensity of UV reflection (RM pers. observ.). Whether individuals with stronger UV reflection are more exposed to predation needs to be tested, too.

In the neutral density experiment, the appearance of the presented sticklebacks only differed in brightness, while both were reflecting UV wavelengths. Trout showed no significant preference for the simultaneously presented brighter or darker one. Instead, brown trout showed a significant preference for the stickleback with the higher condition factor and tended to prefer the heavier one. This result is in concordance with optimal foraging theory, which predicts predators to consume the most energy-efficient prey item first (MacArthur and Pianka, 1966; Charnov, 1976; Pyke et al., 1977). This result further suggests UV wavelengths to be more important in the context of predation risk than small differences in body measurements, which had no significant influence on predation risk in the UV treatment.

The relative frequency of first attacks of brown trout with respect to brightness tended to be different between the UV and ND treatment suggesting that the preference for UV-reflecting prey was based on differences in wavelength composition instead of achromatic brightness differences between simultaneously presented prey. However, a direct comparison between both experiments should be treated with caution since brightness cues in the ND treatment were tested in isolation of the UV treatment. Moreover, the physiological mechanism for brightness discrimination in brown trout is unknown.

Nonetheless, our findings indicate that prey preferences of trout were more likely influenced by UV-based hue discrimination and not simply by brightness differences.

In conclusion, our study provides experimental evidence that UV body-reflections enhance the risk of predation in a vertebrate, which suggests that in addition to their beneficial functions during social, and especially intraspecific interactions, they can also impose significant costs on their bearer. Finally, future investigations incorporating visual predator-prey interactions also need to especially consider the effects of spectral variation of ambient light and diverse visual backgrounds under natural conditions.

Acknowledgements We are grateful to JG Frommen and T Thünken for discussion. We thank A Hömberg and K Kunz for indispensable help with the experiments and L Engqvist and T Schmoll for statistical discussion. We gratefully acknowledge the permission of J Wittler and T Schaper for catching sticklebacks at the field site. The study was supported by the Deutsche Forschungsgemeinschaft (BA 2885/1-3).

References

- Alonso-Alvarez C, Doutrelant C, Sorci G, 2004. Ultraviolet reflectance affects male-male interactions in the blue tit *Parus caeruleus ultramarinus*. *Behav. Ecol.* 15: 805–809.
- Bennett ATD, Cuthill IC, Partridge JC, Maier EJ, 1996. Ultraviolet vision and mate choice in zebra finches. *Nature* 380: 433–435.
- Bennett ATD, Cuthill IC, Partridge JC, Lunau K, 1997. Ultraviolet plumage colors predict mate preferences in starlings. *Proc. Natl. Acad. Sci. USA* 94: 8618–8621.
- Bolger T, Connolly PL, 1989. The selection of suitable indices for the measurement and analysis of fish condition. *J. Fish Biol.* 34: 171–182.
- Bowmaker JK, Kunz YW, 1987. Ultraviolet receptors, tetrachromatic color-vision and retinal mosaics in the brown trout *Salmo trutta*: Age-dependent changes. *Vision Res.* 27: 2101–2108.
- Browman HI, Novales Flamarique I, Hawryshyn CW, 1994. Ultraviolet photoreception contributes to prey search behavior in 2 species of zooplanktivorous fishes. *J. Exp. Biol.* 186: 187–198.
- Charnov EL, 1976. Optimal foraging, marginal value theorem. *Theor. Popul. Biol.* 9: 129–136.
- Church SC, Bennett ATD, Cuthill IC, Partridge JC, 1998. Ultraviolet cues affect the foraging behavior of blue tits. *Proc. R. Soc. Lond. B* 265: 1509–1514.
- Cummings ME, Rosenthal GG, Ryan MJ, 2003. A private ultraviolet channel in visual communication. *Proc. R. Soc. Lond. B* 270: 897–904.
- Douglas RH, 1989. The spectral transmission of the lens and cornea of the brown trout *Salmo trutta* and goldfish *Carassius auratus*: Effect of age and implications for ultraviolet vision. *Vision Res.* 29: 861–869.
- Fleishman LJ, Loew ER, Leal M, 1993. Ultraviolet vision in lizards. *Nature* 365: 397.
- Govardovskii VI, Fyhrquist N, Reuter T, Kuzmin DG, Donner K, 2000. In search of the visual pigment template. *Vis. Neurosci.*

- 17: 509–528.
- Håstad O, Victorsson J, Odeen A, 2005. Differences in color vision make passerines less conspicuous in the eyes of their predators. *Proc. Natl. Acad. Sci. USA* 102: 6391–6394.
- Heiling AM, Herberstein ME, Chittka L, 2003. Pollinator attraction: Crab-spiders manipulate flower signals. *Nature* 421: 334.
- Heiling AM, Cheng K, Chittka L, Goeth A, Herberstein ME, 2005. The role of UV in crab spider signals: Effects on perception by prey and predators. *J. Exp. Biol.* 208: 3925–3931.
- Johnsen S, Widder EA, 2001. Ultraviolet absorption in transparent zooplankton and its implications for depth distribution and visual predation. *Mar. Biol.* 138: 717–730.
- Jourdie V, Moureau B, Bennett ATD, Heeb P, 2004. Ultraviolet reflectance by the skin of nestlings. *Nature* 431: 262.
- Kemp DJ, Reznick DN, Grether GF, 2008. Ornamental evolution in Trinidadian guppies *Poecilia reticulata*: Insights from sensory processing-based analyses of entire colour patterns. *Biol. J. Linn. Soc.* 95: 734–747.
- Kodric-Brown A, Johnson SC, 2002. Ultraviolet reflectance patterns of male guppies enhance their attractiveness to females. *Anim. Behav.* 63: 391–396.
- Koivula M, Viitala J, 1999. Rough-legged buzzards use vole scent marks to assess hunting areas. *J. Avian Biol.* 1999: 329–332.
- Leech DM, Johnsen S, 2006. Ultraviolet vision and foraging in juvenile bluegill *Lepomis macrochirus*. *Can. J. Fish. Aquat. Sci.* 63: 2183–2190.
- Lim MLM, Li J, Li D, 2008. Effect of UV-reflecting markings on female mate-choice decisions in *Cosmophasis umbratica*, a jumping spider from Singapore. *Behav. Ecol.* 19: 61–66.
- Li DQ, Lim MLM, 2005. Ultraviolet cues affect the foraging behavior of jumping spiders. *Anim. Behav.* 70: 771–776.
- Lyytinen A, Lindstrom L, Mappes J, 2004. Ultraviolet reflection and predation risk in diurnal and nocturnal Lepidoptera. *Behav. Ecol.* 15: 982–987.
- MacArthur RH, Pianka ER, 1966. On optimal use of a patchy environment. *Am. Nat.* 100: 603.
- Modarressie R, Rick IP, Bakker TCM, 2006. UV matters in shoaling decisions. *Proc. R. Soc. Lond. B* 273: 849–854.
- Modarressie R, Bakker TCM, 2007. A limited role for ultraviolet radiation when threespine sticklebacks *Gasterosteus aculeatus* prey upon *Daphnia*. *Can. J. Fish. Aquat. Sci.* 64: 1573–1580.
- Novalis Flamarique I, 2000. The ontogeny of ultraviolet sensitivity, cone disappearance and regeneration in the sockeye salmon *Oncorhynchus nerka*. *J. Exp. Biol.* 203: 1161–1172.
- Obara Y, Koshitaka H, Arikawa K, 2008. Better mate in the shade: Enhancement of male mating behavior in the cabbage butterfly *Pieris rapae crucivora* in a UV-rich environment. *J. Exp. Biol.* 211: 3698–3702.
- Osorio D, Smith AC, Vorobyev M, Buchanan-Smith HM, 2004. Detection of fruit and the selection of primate visual pigments for color vision. *Am. Nat.* 164: 696–708.
- Probst R, Pavlicev M, Viitala J, 2002. UV reflecting vole scent marks attract a passerine, the great grey shrike *Lanius excubitor*. *J. Avian Biol.* 33: 437–440.
- Pyke GH, Pulliam HR, Charnov EL, 1977. Optimal foraging: Selective review of theory and tests. *Q. Rev. Biol.* 52: 137–154.
- Rick IP, Modarressie R, Bakker TCM, 2004. Male three-spined sticklebacks reflect in ultraviolet light. *behavior* 141: 1531–1541.
- Rick IP, Modarressie R, Bakker TCM, 2006. UV wavelengths affect female mate choice in three-spined sticklebacks. *Anim. Behav.* 71: 307–313.
- Rick IP, Bakker TCM, 2008a. UV wavelengths make female three-spined sticklebacks *Gasterosteus aculeatus* more attractive for males. *Behav. Ecol. Sociobiol.* 62: 439–445.
- Rick IP, Bakker TCM, 2008b. Males do not see only red: UV wavelengths and male territorial aggression in the three-spined stickleback *Gasterosteus aculeatus*. *Naturwissenschaften* 95: 631–638.
- Rick IP, Bakker TCM, 2008c. Color signalling in conspicuous red sticklebacks: Do ultraviolet signals surpass others? *BMC Evol. Biol.* 8: 189.
- Rick IP, Bloemker D, Bakker TCM, 2012. Spectral composition and visual foraging in the threespine stickleback (*Gasterosteidae*: *Gasterosteus aculeatus* L.): Elucidating the role of ultraviolet wavelengths. *Biol. J. Linn. Soc.* 105: 359–368.
- Rocco V, Barriga JP, Zagarese H, Lozada M, 2002. How much does ultraviolet radiation contribute to the feeding performance of rainbow trout *Oncorhynchus mykiss* juveniles under natural illumination? *Environ. Biol. Fish.* 63: 223–228.
- Rowe MP, Baube CL, Loew ER, Phillips JB, 2004. Optimal mechanisms for finding and selecting mates: How threespine stickleback *Gasterosteus aculeatus* should encode male throat colors. *J. Comp. Physiol. A* 190: 241–256.
- Schaefer HM, Schaefer V, Vorobyev M, 2007. Are fruit colors adapted to consumer vision and birds equally efficient in detecting colourful signals? *Am. Nat.* 169: 159–169.
- Siebeck UE, 2004. Communication in coral reef fish: The role of ultraviolet colour patterns in damselfish territorial behavior. *Anim. Behav.* 68: 273–282.
- Siebeck UE, Parker AN, Sprenger D, Mähnger LM, Wallis G, 2010. A species of reef fish that uses ultraviolet patterns for covert face recognition. *Cur. Biol.* 20: 407–410.
- Siitari H, Honkavaara J, Viitala J, 1999. Ultraviolet reflection of berries attracts foraging birds: A laboratory study with redwings *Turdus iliacus* and bilberries *Vaccinium myrtillus*. *Proc. R. Soc. Lond. B* 266: 2125–2129.
- Siitari H, Viitala J, 2002. Behavioural evidence for ultraviolet vision in a tetraonid species foraging experiment with black grouse *Tetrao tetrix*. *J. Avian Biol.* 33: 199–202.
- Smith EJ, Partridge JC, Parsons KN, White EM, Cuthill IC et al., 2002. Ultraviolet vision and mate choice in the guppy *Poecilia reticulata*. *Behav. Ecol.* 13: 11–19.
- Tanner M, Richner H, 2008. Ultraviolet reflectance of plumage for parent-offspring communication in the great tit *Parus major*. *Behav. Ecol.* 19: 369–373.
- Tovée MJ, 1995. Ultra-violet photoreceptors in the animal kingdom: Their distribution and function. *Trends Ecol. Evol.* 10: 455–460.
- Vedder O, Schut E, Magrath MJL, Komdeur J, 2010. Ultraviolet crown coloration affects contest outcomes among male blue tits, but only in the absence of prior encounters. *Funct. Ecol.* 24: 417–425.
- Viitala J, Korpimäki E, Palokangas P, Koivula M, 1995. Attraction of kestrels to vole scent marks visible in ultraviolet-light. *Nature* 373: 425–427.
- Vorobyev M, Osorio D, 1998. Receptor noise as a determinant of colour thresholds. *Proc. R. Soc. Lond. B* 265: 351–358.
- White EM, Church SC, Willoughby LJ, Hudson SJ, Partridge JC, 2005. Spectral irradiance and foraging efficiency in the guppy *Poecilia reticulata*. *Anim. Behav.* 69: 519–527.
- Whiting MJ, Stuart-Fox DM, O'Connor D, Firth D, Bennett NC et al., 2006. Ultraviolet signals ultra-aggression in a lizard. *Anim. Behav.* 72: 353–363.