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ORIGINAL PAPER

UV wavelengths make female three-spined sticklebacks (*Gasterosteus aculeatus*) more attractive for males

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Abstract Numerous fishes possess UV vision and show UV patterns, which often play a role in social communication, especially during courtship. UV light is scattered strongly in water and thus might be used for intra-specific close-range communication without being detected by predators. In addition to the red-belly colouration and blue-coloured iris, male three-spined sticklebacks possess UV reflectance on their skin, and females prefer males presented with UV light rather than without. To investigate whether UV also influences male visual preference for females in this species, we used a dichotomous mate choice design in which one female could be viewed by a male in two visual conditions. Courting male sticklebacks preferred females that were presented in full-spectrum conditions including UV to the same females presented in conditions lacking this light component. Furthermore, control trials with neutral-density filters indicated that male preference in the UV treatment was not affected by a difference in achromatic brightness between the UV+ and UV− stimuli. Reflectance measurements of gravid females suggest an enhanced UV contrast between the dark bar pattern, which is characteristic of gravid females, and silvery body parts.

Keywords Male choice · Spectral reflectance · Stickleback · UV vision

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Introduction

UV colour signals (300–400 nm) are of recent interest in studies on social signalling, especially in the context of sexual selection. In several species, there is a UV component to female mate choice (in birds: Bennett et al. 1996, 1997; Andersson and Amundsen 1997; in fish: Smith et al. 2002; Macias Garcia and Burt de Perera 2002; Boulcott et al. 2005; Rick et al. 2006) and male contests (Alonso-Alvarez et al. 2004; Siebeck 2004; Stapley and Whiting 2006). However, only two studies have considered a potential influence of female trait variation in the UV on male mating decisions (Hunt et al. 1999; LeBas and Marshall 2000). Nevertheless, female ornaments can be sexually selected through male mate choice or female–female competition (reviewed by Amundsen 2000), although data are limited to human-visible wavelengths (400–700 nm).

In the three-spined stickleback, females often develop nuptial colouration in the form of a dark, bar-like pattern on their dorsum, upper flanks and tail stem (Rowland et al. 1991). Courting stickleback males preferred dummies of a gravid female with a bar pattern to gravid, uncoloured ones (Rowland et al. 1991). Rowland et al. hypothesized that female nuptial colours might enable males to quickly find a receptive partner and hence increase the females' chances of being courted. Males also preferred dummies of gravid females in head-up courtship posture to horizontal ones (Bakker and Rowland 1995). Furthermore, in female sticklebacks, fecundity correlates with body size (Baker 1994), and when presented with two dummy females, which differed in abdominal distension, males preferentially courted the more distended (more fecund) one (Rowland 1989). Accordingly, Kraak and Bakker (1998) demonstrated that more attractive stickleback males preferred to mate with females that lay more and bigger eggs indicating mutual mate choice in this species. Male sticklebacks might benefit from being choosy, as they have a limited sperm store (Zbinden et al. 2001). Furthermore, they are limited in the number of matings they can obtain during a particular period of time because of a post-mating refractory period (Sevenster-Bol 1962) and exhibit high parental investment in becoming territorial, building nests and caring for the offspring (Wootton 1976).

It was recently shown that stickleback vision is extended into the UV with an additional UV cone type maximally absorbing at 360 nm (Rowe et al. 2004). In choice experiments, females preferred to court males seen under full-spectrum conditions compared to males in conditions lacking UV (Boulcott et al. 2005; Rick et al. 2006). Furthermore, reflectance-spectrophotometrical measurements of reproductively active male three-spined sticklebacks revealed UV reflectance patterns on their body surface (Rick et al. 2004).

Gravid female sticklebacks have conspicuous silvery flanks, which may strongly contrast in UV to the characteristic dark bar pattern, which shows relatively low UV reflectance (see Boulcott et al. 2005). A stronger contrasting appearance of this pattern may give males more reliable information about female receptivity (Rowland et al. 1991).

In the present study, we ask whether reproductively active stickleback males respond differently to a gravid female when it is viewed through an optical filter transmitting full-spectrum light including UV-A (300–700 nm) than when it is viewed through an UV-blocking filter (400–700 nm). We hypothesize that if UV wavelengths influence a courting male's visual preference, a male will differentiate between the two female appearances in favour of the stimulus including UV wavelengths. The experiments presented here were conducted using three-spined sticklebacks from the same local freshwater population, which we had used to study the influence of UV on female mate choice (Rick et al. 2006). Additionally, UV reflectance patterns of gravid females were measured.

Materials and methods

Study species

Adult three-spined sticklebacks were collected from a shallow freshwater pond located in a small woodland near Euskirchen, Germany (50°38′N/6°47′E) on 16 March 2005. Details of the study site and treatment of the fish before the experiments are described elsewhere (Rick et al. 2006). In the present study, males were provided with filamentous algae and Java Moss (*Vesicularia dubyana*) for nest building instead of cotton threads.

Experimental procedure

In contrast to the former study, the present choice experiment did not require matching of individuals, as we tested visual preferences by presenting only one female in two conditions (see design in Macias Garcia and Burt de Perera 2002). Hence, pairs of one male and one female were used in the experimental tank (Fig. 1). Our approach should solely test for male preferences towards a female presented in two different visual appearances while controlling for potential female preferences. Consequently, we used an angled arrangement of the optical filters so that the male could locate the female through both filters virtually equal. Thus, the influence of female position on male choice behaviour should be minimized.

Before each trial, we moved a male's nest from the holding aquarium to the male section in the experimental aquarium and placed it centrally in front of the wall, opposite to the optical filters. We then transferred the corresponding male to the male section and a female to the female section. We acclimatized fish to the test aquarium for 30 min. To prevent visual contact between the sexes during this phase, two opaque partitions were placed between them. Thus, the male was given the opportunity to accept its nest in the new environment, which became evident from distinct courtship behaviour during the choice phase.

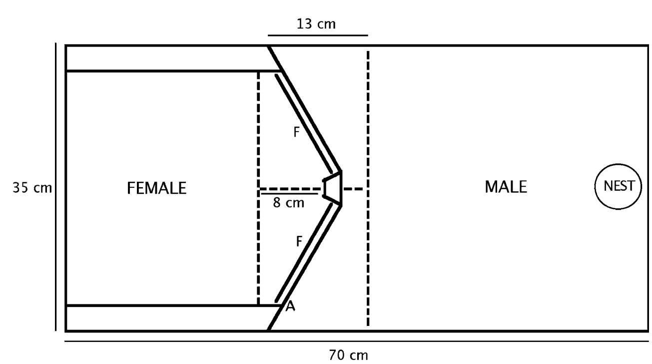

Fig. 1 Overview of the experimental tank. A 70-l aquarium (70×35×35 cm) was divided into a female (*left*) and a male (*right*) section by an opaque plastic frame forming an angle of 115° (*A*). Two rectangular plexiglass windows that transmitted light in the wavelength range between 300 and 800 nm (GS-2458, Röhm, Darmstadt, Germany) were fixed into the frame. For the UV treatment, two changeable UV manipulating filters (*F*) were inserted between the male and female sections. One was UV opaque (UV−: GS-233, Röhm, Darmstadt, Germany), and the other one transmitted UV and visible light (UV+: GS-2458), so that the test male could see the female either under the human visible spectrum or under an extended spectrum including UV-A. The experimental aquarium was filled with tap water to a level of 25 cm. It was placed on a polystyrene tile on which two triangular preference zones in the male section as well as in the female section were marked (*dashed lines*). The tank was surrounded by opaque grey plastic partitions up to a height of 30 cm. A black curtain encased the whole setup

By lifting the opaque partition, we started the observation phase that lasted until the male had entered both preference zones. In case this phase exceeded 15 min, the experimental trial was discarded, otherwise the 10-min test phase began. Thereafter, the opaque partition was replaced for 10 min. To control for side biases, we exchanged the positions of the optical filters. Thus, in the second half of the experiment, the male viewed the female behind exchanged filters. The experimental procedure for the second and first halves was identical, including acclimatization time, observation phase and test phase.

The two filters differ in quantal flux (UV+ to UV−: 18% reduction; Rick et al. 2006). To determine whether male choice behaviour was rather based on the wavelength composition of the two stimulus conditions instead of brightness differences, additional control treatments with neutral density filters (ND1 and ND2) were performed. These filters differ in quantal flux uniformly between 300 and 700 nm without changing wavelength composition. Between 300 and 700 nm, the two filters (ND1 and ND2) show a difference in quantitative transmission of 34% (Rick et al. 2006).

UV treatments and control treatments where conducted in succession, using the same pair of fish, with treatment order being alternated. All trials were recorded from above with a webcam. Filmed trials were analysed without knowledge of the positions of the filters. Male visits were estimated as the number of movements into the preference zones. Additionally, the time was measured that the male (at least half of the body) spent in the two preference zones in front of the female, which was either behind a UV+ and a UV− filter in the UV treatment or behind a ND1 and a ND2 filter in the control treatment. Association time is an approved measure for mating preference in sticklebacks (McLennan and McPhail 1990; Rowland 1995; Milinski et al. 2005).

Because our experimental design cannot rule out a potential female filter preference, which could have influenced male mating decisions, we quantified the amount of time females spent in a specified triangular zone in front of each treatment filter (Fig. 1) to get a crude measure of a potential female filter preference. Fish were used only once. The experimental tank was illuminated by a fluorescent tube (True Light, Natural Daylight 5500, 36 W, 1,200 mm) positioned 35 cm above the water surface. These lights contain a proportion of UV similar to natural skylight. We replaced the water in the tank after each trial. All trials were done between 10 and 17 h. After the study, fish were kept in the laboratory as breeding stock for future experiments.

UV reflectance patterns

Reflectance measurements of six gravid females were taken with a spectrophotometer (Avantes AVS-USB2000, Eerbeek, The Netherlands) connected to a deuterium-halogen light source (Avantes DH-2000) for illumination. A bifurcated 200-μm fibre-optic probe, with unidirectional illumination and recording, was held at a 90° angle to the body surface. For this purpose, females were killed with a blow to the head and immediately placed on a piece of black fabric. Subsequently, scans were collected from three small colour patches on the female's left lateral side, one located centrally on the belly, one on the first distinct dark bar starting from the front end and a further one on the subsequent bright gap (Fig. 2a). The whole procedure took less than 2 min to minimize colour changes caused by, for example, changes in chromatophore pigment dispersion. Reflectance intensity was measured relative to a 98% Spectralon white standard over the range of 300–800 nm at about 0.5-nm resolution in wavelength. Data were recorded with Spectrawin 5.1 (Avantes) and imported into Microsoft Excel. Eight measurements were averaged for each sample region.

Statistical analyses

We tested male behaviour for normality using the Kolmogorov–Smirnov test. All data were normally distributed. We analysed the relative amount of time males spent near the two female appearances behind UV+ and UV− (or ND1 and ND2) with a parametric t test. We also used a parametric *t* test to analyse the relative amount of time females spent behind each filter side and the number of male visits in the preference zone near each filter.

Results

Choice experiment

Of 16 males three did not assess both preference zones and were therefore discarded from statistical analyses. Reproductively active male sticklebacks significantly preferred females seen in UV+ conditions as measured by the number of male visits in the zone near the UV+ filter (mean±SD=29.92±18.31) and UV− filter (18.69±12.24; paired *t* test: t_{12}=2.803, P=0.016; Fig. 3). Accordingly, males spent a significantly greater proportion of time near the UV+ filter (mean SD=58.4%±13.3) compared with the UV− filter (41.6%±13.3; paired *t* test: t_{12}=2.275, P=0.042) (Fig. 4).

Within the 13 valid trials three females failed to enter both preference zones and were therefore excluded from analyses. The relative amount of time females spent near the optical filters did not significantly differ between the UV+ filter (mean±SD=52%±11) and UV− filter (48%±11, paired *t* test: t_9=0.446, P=0.666).

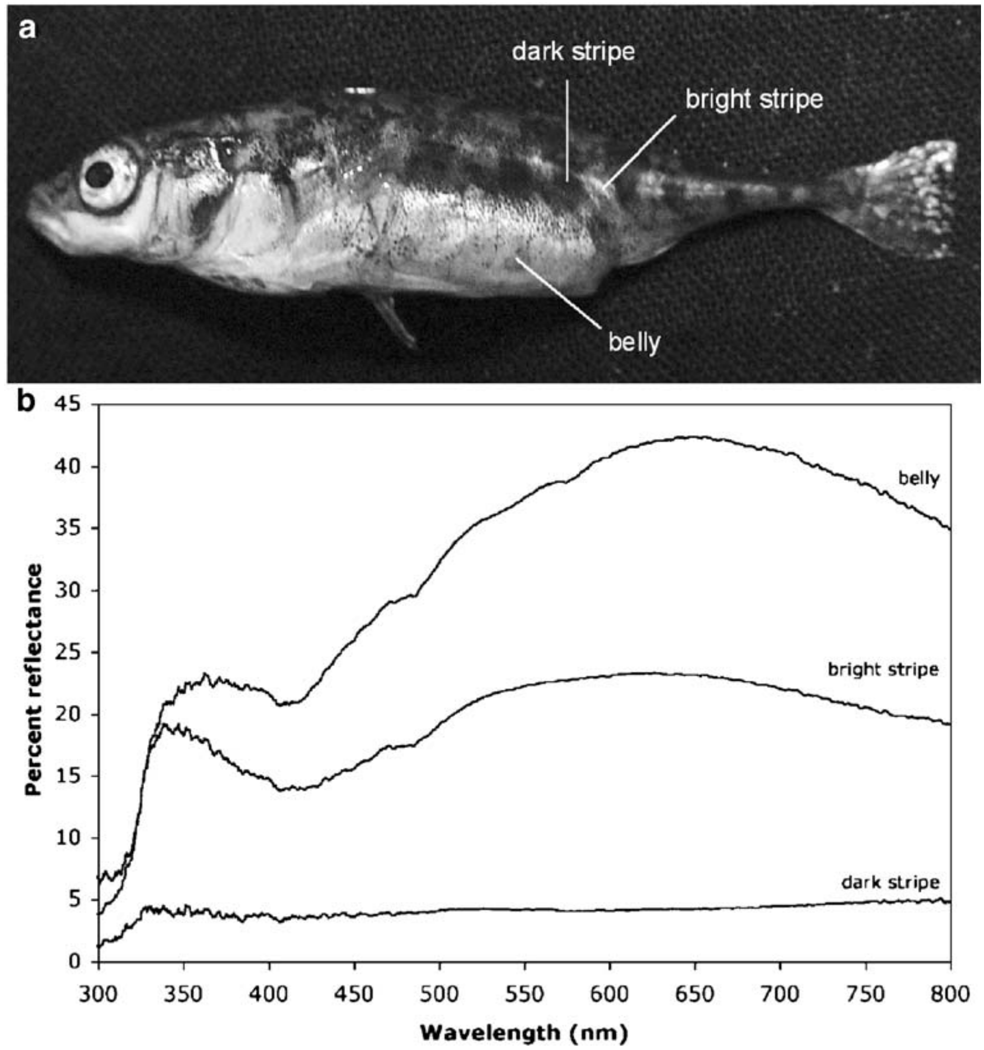

Fig. 2 Female three-spined stickleback in the barring pattern prior to spawning (**a**). Spectral reflectance (proportion of light reflected in relation to a white standard; see the text for explanation) was measured for three body regions of six gravid females. Plotted are the means of the reflectance intensities (%) between 300 and 800 nm (**b**)

In the control treatment, 13 out of 15 males did assess both preference zones and were included in statistical analyses. Males showed a significant preference for females presented in two different brightness levels under full-spectrum conditions as measured by the relative amount of time spent near the ND2 filter (mean±SD=48.2%±19.3) and ND1 filter (51.8%±19.3; paired t test: t_{12}=0.340, P=0.740). Within the control treatment, 12 out of 13 females did enter both preference zones and were thus included in analyses. Females showed, although not significantly, an association preference for the darker ND1 filter (mean±SD=58.3%±13.2) compared with the ND2 filter (mean±SD=41.7%±13.2; paired t test: t_{11}=-2.092, P=0.063).

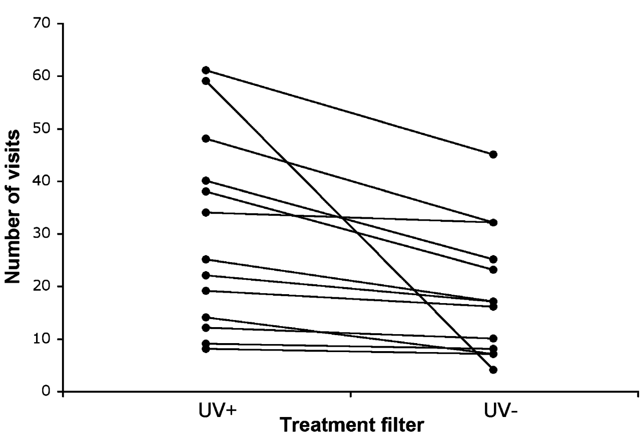

Fig. 3 Number of visits by 13 males in the preference zone in front of UV+ and UV− females during the mate assessment phase

Reflectance patterns

The mean proportion reflectance of six gravid females revealed a double-peaked spectrum with a separated UV peak for the belly and the gap between the first and second dark bar (Fig. 2b). UV peak reflectance is located at 339 nm for the gap and 363 nm for the belly. In contrast, the dark bars show only low reflectance intensities without any prominent peaks over the whole UV-Vis wave range.

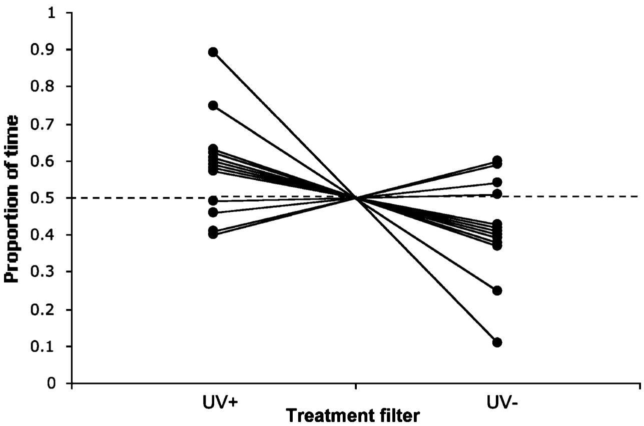

Fig. 4 Proportion of time spent by 13 males within the preference zone in front of UV+ and UV− females during the mate assessment phase. The *dashed line* corresponds to the null expectation of no preference

Discussion

Courting male three-spined sticklebacks assessed a female under UV-present conditions more frequently and spent significantly more time with it compared to the same female presented under UV-absent conditions. We thus demonstrated for the first time an effect of UV wavelengths on male choice decisions in a fish. To our knowledge, there is only one previous study, on a bird, in which a direct effect of UV on male mate choice behaviour was demonstrated (Hunt et al. 1999). Although our results might be interpreted as mate preferences based on female UV patterns as specific quality signals, one cannot rule out that other factors have influenced the male's preference for females viewed under full-spectrum conditions. For example, the particular visual environment, in which the female stimulus is presented, may have an effect. We recently tested the role of visual background cues in sticklebacks and found that differences in UV background reflectance had no significant impact on at least female viewing preferences (Rick and Bakker, unpublished data). Nevertheless, the contrast between a visual signal and its background is affected by various factors namely, the spectral properties of the signal and the background, the sensory properties of the receiver and the lighting conditions under which signalling occurs (Endler 1992, 1993a). Hence, psychophysical approaches in terms of receptor modelling and visual contrast calculations are needed to get more insights into how stickleback males and females may perceive and evaluate each other.

Male mate preferences in the present study might be based on a general preference for conspecifics that look more familiar under full-spectrum conditions than under conditions lacking UV. However, during the experimental trials, males showed characteristic courtship behaviour including zigzagging and bumping towards both female appearances (Rick, personal observation) so that species and mate recognition was ensured.

From the results of the control treatment (with ND filters), we extrapolate that the male preference for females under full-spectrum conditions in the UV treatment was rather not based on the higher achromatic brightness caused by the UV+ filter. This is in accordance to experiments conducted on UV and female visual preferences in sticklebacks (Rick et al. 2006). Jacobs (1981) refers to the 'brightness problem,' which describes that if an individual has to discriminate between two signals of different chromatic and achromatic content, it rather may use the difference in brightness for discrimination. However, until yet, it is neither known if an achromatic coding mechanism even exists in sticklebacks nor in which way a presumed achromatic channel may be used. Thus, the results of our control treatment cannot be interpreted adequately without having more knowledge about visual processing in the three-spined stickleback.

Females in the control treatment preferred, although not significantly, the zone in front of the darker ND filter. An analogous preference has been found in a study on shoaling behaviour in sticklebacks (Modarressie et al. 2006) suggesting a potential tradeoff between UV reflectance and brightness. However, ND filters did not affect female viewing preferences for males in another study (Rick et al. 2006). Thus, it is possible, that these contrasting results emerged from discrepancies in the experimental design.

In two previous choice studies, female sticklebacks, when presented with males under UV+ and UV− conditions, preferred the UV+-viewing conditions (Boulcott et al. 2005; Rick et al. 2006). Because in the present study, females could also observe the choosing male under UV+ and UV− conditions, the question arises whether male UV+ filter preferences were the result of female preferences for the UV+ view. In spite of the angled filter arrangement in our experimental setup and the lack of a distinct female filter preference, we cannot completely rule out that male filter preference was affected by female response behaviour.

If female UV patterns play a role in male mating decisions, which extra information may males acquire from these patterns? In fish, female fecundity is often correlated with body size (Reznick and Endler 1982; Baker 1994; Herdman et al. 2004; Ojanguren and Magurran 2004). Males may benefit from mating with large and thus more fecund females. Male mating preferences for large females have been shown for numerous species (Pelabon et al. 2003; Dosen and Montgomerie 2004; Plath et al. 2006). In three-spined sticklebacks, males possibly use UV wavelengths to get more reliable information about female size. Nevertheless, stickleback males are capable of discriminating differently sized females without UV wavelengths (Kraak and Bakker 1998).

The female's distended, silvery shimmering abdomen is reflective in visible as well as UV wavelengths. When UV is present, a courting male may perceive this belly in an increased visual contrast to other body parts, which exhibit a lower overall reflectance. Thus, UV may help stickleback males in reliably assessing female breeding status. Thereby, time costs of courting non-receptive females as well as the risk of courting cannibalistic females, which may consume already collected eggs (FitzGerald and Whoriskey 1992), will be reduced. On the other hand, females may also benefit from conspicuously signalling their receptivity in terms of increased chances of being courted (see Rowland 1989). The characteristic barred spawning pattern of females shows a distinct difference in reflectance intensities between the dark bars and the bright gaps in the UV as well as human-visible wave range. Hence, UV might enable males to better detect gravid females and assess their breeding status more precisely. Nevertheless, to gain more insight into a potential signalling value of structural UV colour patterns in fish, and if they are subject to sexual selection, the effects of environmental or physiological constraints on the development of such colour patterns need to be considered.

To conclude, this study shows that stickleback males are more attracted to females presented in conditions including UV. However, because of the experimental design used in the present and several other studies on UV communication, one cannot strictly assume a mate preference. In fact, it would be necessary to incorporate data on spectral properties of stimulus signals as well as on colour-processing mechanisms of potential receivers to make safe assumptions about visual signals in a mate choice context. Nonetheless, our survey contributes to the fact that in our study population, UV radiation is of importance in different visual interactions between stickleback conspecifics.

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