MALE THREE-SPINED STICKLEBACKS REFLECT IN ULTRAVIOLET LIGHT

by

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Summary

Recent studies evidenced that some fishes use ultraviolet light in social signalling. Using reflection spectrophotometry we showed that reproductively active male sticklebacks also reflect in the UV-light (300-400 nm). Several body regions showed a reflectance peak in the UV-waveband. Silvery appearing regions reflected stronger than red-coloured or darker ones. Males within one population varied in UV-reflection. The UV-contrast of one particular region was positively correlated with physical condition.

Introduction

It is often assumed that ultraviolet reflectance patterns play a role in intraspecific signalling especially in the context of sexual selection. Losey *et al.* (1999) divided UV-reflectance patterns into two functional groups. On the one hand they could enhance the attractiveness of a displaying male by enhancing the contrast between visible and UV-reflectance components. On the other hand they could make a conspicuous colour spot more attractive (Losey *et al.*, 1999). Such UV-patterns are found in a wide variety of taxonomic groups including birds (Andersson & Amundsen, 1997), arthropods (Brunton & Majerus, 1995) and fish (Losey *et al.*, 1999). UV-coloration patterns have been little studied due to the blindness of humans to UV-wavelengths.

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Nevertheless, photoreceptor types sensitive to ultraviolet light (absorption maximum of 360-380 nm) occur numerously in the animal kingdom. For instance, many species of marine and freshwater fish living in shallow-water habitats are able to perceive UV-light (Losey *et al.*, 1999). For example, guppies (*Poecilia reticulata*) possess UV-transparent ocular media (Thorpe *et al.*, 1993) and a visual pigment sensitive in the UV (Archer *et al.*, 1987). In mate choice experiments, females strongly preferred male guppies viewed under UV conditions compared to males viewed under conditions lacking UV (Smith *et al.*, 2002).

Along with the guppy, the three-spined stickleback (*Gasterosteus aculeatus*) has been extensively studied in regard to social signalling in visible light (400-700 nm). Courting male three-spined sticklebacks show nuptial coloration consisting of a red belly and blue eyes (McLennan & McPhail, 1989). The red belly coloration plays a role in female choice as well as male-male competition (Bakker & Milinski, 1993; Bakker, 1994). The redness of the males' belly mostly but not always correlates with greater reproductive activity (Rowland, 1984), lower parasite loads (Milinski & Bakker, 1990), higher aggression levels (Rowland, 1984) and greater social dominance (Bakker & Sevenster, 1983; Bakker, 1986). All these studies deal with signalling in the for humans visible longer wavelengths; the pigment colours of the red belly act as a reliable indicator of male quality. In sticklebacks, it is unknown whether iridescent structural colours reflect in the UV and whether there exist UV-reflectance patterns. Nevertheless a tetrachromatic visual system including a fourth UV-photoreceptor was also described in sticklebacks (C.L. Baube, pers. comm. referenced in Sargent *et al.*, 1998; Rowe *et al.*, 2004). Three-spined sticklebacks reproduce in shallow waters (Wootton, 1976) and consequently are exposed to high UV levels because shortwave light strongly scatters in deeper water (Loew & McFarland, 1990).

We aimed at studying the presence and variation of UV-reflectance patterns in reproductively active male sticklebacks.

Methods

The study was conducted during the breeding seasons of 2002 and 2003. Fish from a shallow pond in Euskirchen (near Bonn, Germany) were collected using minnow traps and transported to the University of Bonn. The fish were released into one-sex outside storage tanks (700 l) which were supplied with running tap-water (15 ± 2°C). Males that became reproductively active were removed from their storage tanks. After their standard length and body mass

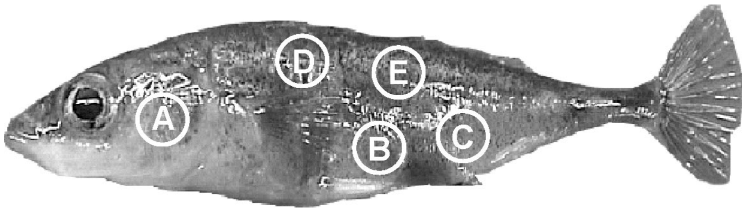

Fig. 1. The five circular standardised body regions with a diameter of 0.4 cm which were measured with regard to their reflection between 310 and 710 nm.

was measured they were individually transferred to aerated and filtrated 12 l glass tanks where they were allowed to build nests under standardised laboratory conditions (17 ± 1°C, daylength 16L:8D). Illumination was provided by fluorescent tubes (True-Light, Natural Daylight 5500, 36W) which produce a spectral emission similar to natural skylight (see Bennett *et al.*, 1996). Fish in the storage tanks and in the territorial aquaria were fed once daily on frozen chironomid larvae.

In order to do reflection measurements each male was killed with a blow to the head and placed on a background of matt black paper. We then measured UV-reflection of the left body surface with an Avantes USB-2000 fibre-optic spectrometer. A bifurcated 200 micron fibre-optic probe, with unidirectional illumination and recording, was held at a 90° angle to the body surface. Illumination was given by a deuterium-halogen-lightsource (Avantes DH-2000, 215-1700 nm). A darkened pipette tip was mounted on the probe end in order to exclude ambient light and to measure reflectance at a fixed distance of 4 mm from the body surface (see Keyser & Hill, 1999). Reflectance intensity over the range of 310-710 nm was recorded relative to a 99% Spectralon white-standard. Scans were collected from three UV-reflective lateral body regions (A, B, C) at about 8 nm resolution in 2002 and from two additional regions (A, B, C, D, E) at about 0.5 nm resolution in 2003 (Fig. 1). In each region the probe was lifted and replaced five times to take a new measurement. These five measurements were averaged for each sample.

Data were recorded with Spectrawin 5.1 (Avantes, Netherlands) and imported into Microsoft Excel. The wavelength of peak reflectance in the UV (hue), reflectance intensity (%-reflectance) at the UV-peak, and the UV-contrast C (difference in intensity between the UV-peak and the lowest value of the reflection curve; Fig. 2) were calculated from the spectral data (see Keyser & Hill, 2000). Analyses were performed with Microsoft Excel and SPSS 11.0 statistical software. Due to a different pipette tip mounted on the probe end resulting in a measurement distance of 3 mm from the body surface we obtained higher reflectance values in 2003 compared to 2002.

Results

Reflectance data of three body regions (A, B, C) with conspicuous UV-reflectance were obtained for 33 reproductively active males in 2002. All three regions showed an intermediate to strong reflection in the ultraviolet

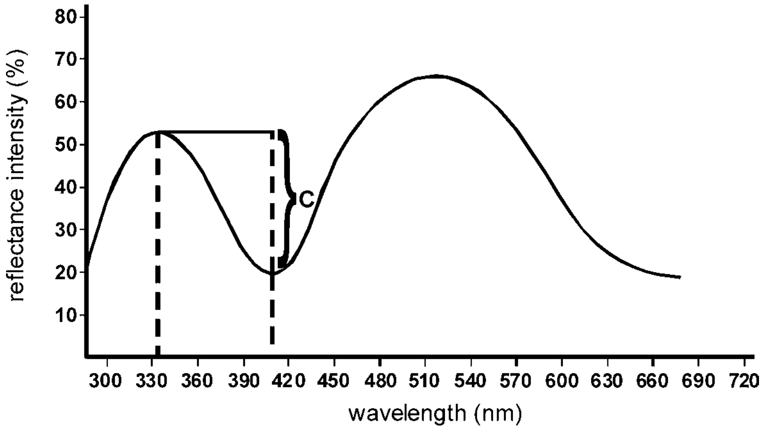

Fig. 2. Representative reflectance spectrum of a measured surface region. The UV-contrast C was defined as the difference between the highest reflectance intensity in the UV (300-400 nm) and the lowest reflectance intensity in the whole waverange (300-700 nm) (see Keyser & Hill, 2000).

waveband (Fig. 3). Two distinct peaks of UV-reflectance intensity could be recognised: a pronounced peak at about 340 nm and a lower and less obvious one at about 390 nm. In the visible waveband (400-700 nm), regions B and C had a consistently high reflectance intensity over a broad range (520-700 nm) indicating the grey to white iridescent colours at these body regions. Region A, on the other hand, showed a narrower range of high reflectance intensity in visible light, pointing to the red nuptial colour which often extended into this region. In comparison with region A, regions B and C showed higher reflectance intensities in both the UV and the visible spectrum with the highest UV-reflectance occurring in region C. In addition, regions B and C exhibited low reflectance intensities in blue-violet wavelengths around 420 nm whereas region A showed an extended range of low reflectance intensities in blue-green wavelengths between 420 and 500 nm (Fig. 3).

There was a conspicuous variation in the location of the UV-reflectance peak (hue) (Fig. 4a-c). For region A the waveband of the UV-reflectance peak for the 33 males was normally distributed [Kolmogorov-Smirnov test, $D = 0.193$, p (Lilliefors) $= 0.200$]; most individuals had an UV-reflectance peak between 330 nm and 360 nm. For regions B and C the distributions also did not significantly deviate from normality [Kolmogorov-Smirnov test, $D = 0.284$, p (Lilliefors) $= 0.141$, and $D = 0.269$, p (Lilliefors) $= 0.200$, for region B and C, respectively] although there occurred two distinct wave-

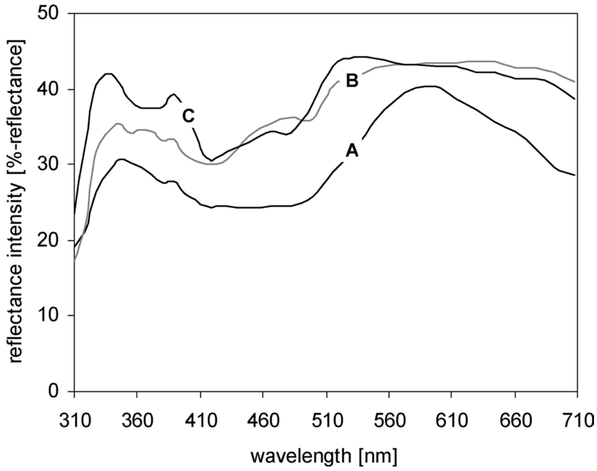

Fig. 3. Average reflectance spectra (proportion of light reflected in relation to a white standard; see text) of 33 males for three UV-reflective body regions (A, B, C). Plotted are the medians of the reflectance intensities (%) between 310 and 710 nm.

bands as to the location of the UV-reflectance peak (Fig. 4b,c). For example, in region C 22 out of 33 males showed a maximum UV-reflectance between 330 nm and 350 nm and eight males peaked between 360 nm and 380 nm (Fig. 4c).

Spectral data revealed variation in the reflectance contrast in the UV between individual males in all three regions: medians (quartiles) were 8.38 (6.28; 14.39), 7.92 (5.16; 14.68), 11.64 (5.23; 16.63) for region A, B and C, respectively. There was a significant positive correlation between the body condition factor [BCF $= 100 \times$ (mass in g)/(length in cm)^3 where the exponent is the slope of the regression of log(mass) on log(length); Bolger & Conolly, 1989] and the UV-peak contrast for the region near the anal fin (C) (Fig. 5c) whereas for the regions A and B no significant correlation was found (Fig. 5a, b).

In addition to control measurements of regions A, B, C, two regions (D, E) showing low or none UV-reflectance were measured on 10 males in 2003 (Fig. 6). The three silvery body regions (A, B, C) exhibited high UV-reflectance whereas the two other regions (D, E) offered little or none UV-reflectance (Fig. 6). As in the former measurements in 2002 region C showed the highest reflectance intensity. The darker regions D and E had a comparatively low reflection over the whole spectrum. There was no or only

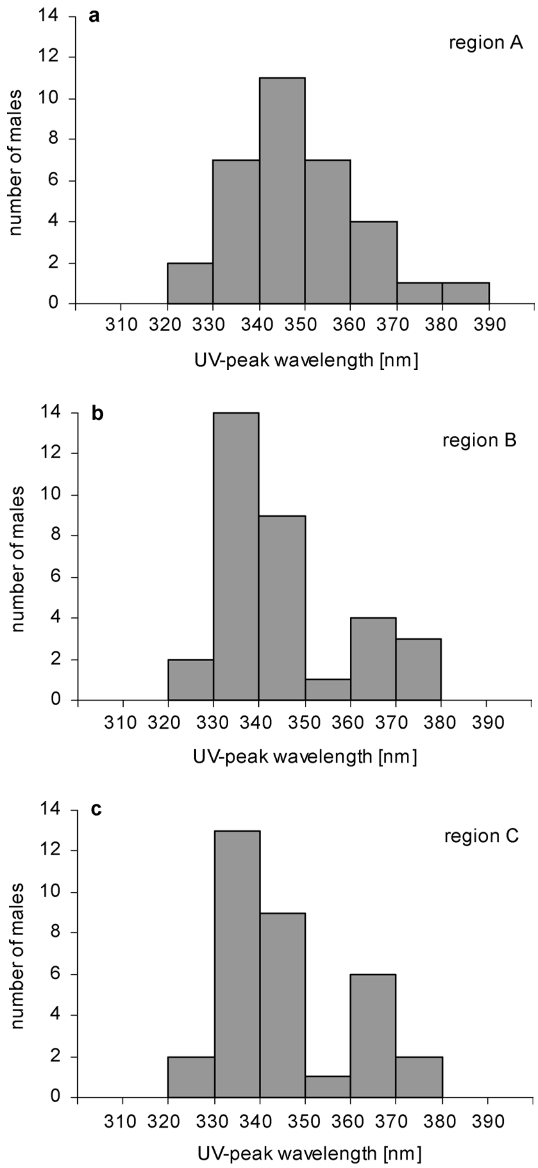

Fig. 4. Distribution of wavelengths with a maximum reflectance intensity in the UV (UV-peak) for region A (a), region B (b) and region C (c) from 33 males.

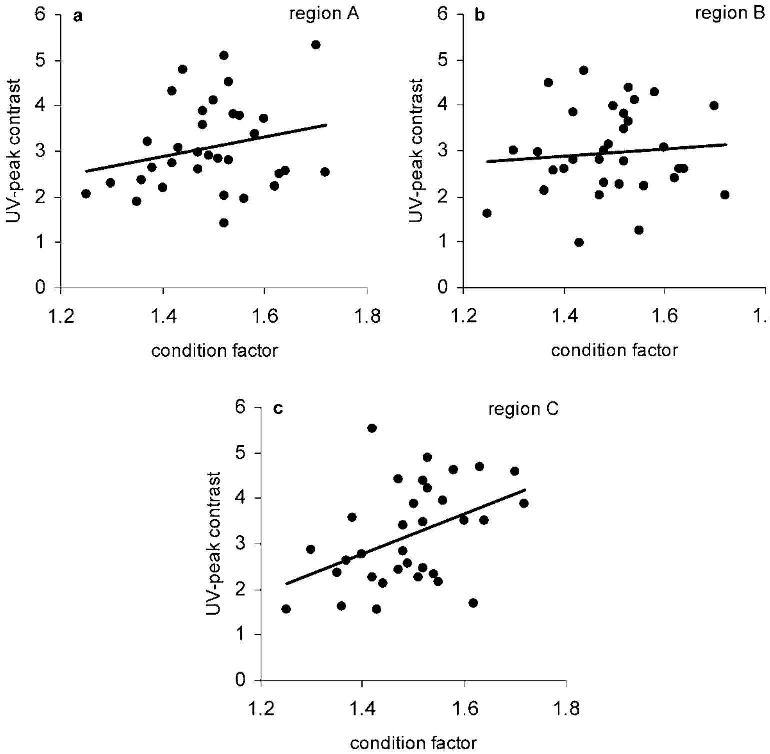

Fig. 5. Correlations between the males' condition factor and their UV-peak contrast (C) (square root transformed) of region A (a), region B (b) and region C (c). Sample size is 33 males. The lines are least-square regressions. Region A: y = 2.2176x − 0.225; $r_p = 0.238$; $F = 1.862$; $p = 0.182$. Region B: y = 0.7736x + 1.8072; $r_p = 0.089$; $F = 0.248$; $p = 0.622$. Region C: y = 4.3538x − 3.3231; $r_p = 0.433$; $F = 7.140$; $p = 0.012$.

marginal reflection in the ultraviolet which is particularly well demonstrated for region D.

Discussion

Male courtship signals are generally formed by sexual selection to increase male conspicuousness (Andersson, 1994). Increased conspicuousness normally comes along with increased conspicuousness to predators (Andersson, 1994). Private sexual signals can be developed without being restricted by predation (Endler, 1982). UV-reflectance patterns could support private signalling when predators are insensitive to ultraviolet light or when the dis-

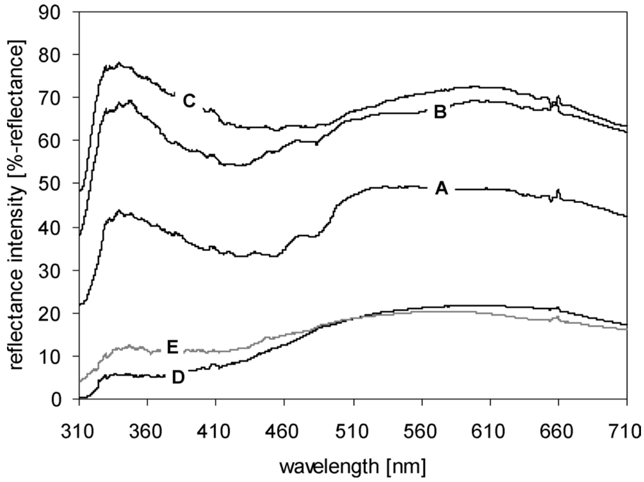

Fig. 6. Average reflectance spectra (proportion of light reflected in relation to a white standard; see text) of ten males for three body regions which reflected in the UV (A, B, C) and two regions which did not (D, E). Plotted are the medians of the reflectance intensities (%) between 310 and 710 nm.

tance between prey and predator is usually too great for detection of UV-signals (Losey *et al.*, 1999).

In this study, we demonstrated for the first time the existence of UV-reflective regions on the lateral body surface of reproductively active male sticklebacks. Due to the fact that transmission of ultraviolet light through clear water is rather weak, intraspecific UV-signals are only effective over short distances and in shallow waters (Siebeck & Marshall, 2001). The lateral side in fish is categorized as typical for the development of UV-coloration patterns in terms of spots, stripes or bigger areas which have a potential function in sexual signalling (Losey *et al.*, 1999). Zahavi & Zahavi (1997) consider many colour patterns to play an amplifying role in courtship behaviour. The measured UV-reflective regions on the stickleback male's lateral side (regions A, B, C) could support courtship by offering females a more precise view and evaluation of the male's zigzag dance. The more dorsal regions of the stickleback's body (regions D, E) showed relatively low UV reflectance. This near absence of UV reflection could be associated with a reduced detection by UV-sensitive predators from above (see Cummings *et al.*, 2003). The obtained reflectance spectra suggest that the UV reflection is a combination of specific visible colours with a pronounced peak in the ultraviolet spectrum (*e.g.* green-red/UV for region A). Such reflectance spectra have to be separated from a UV reflection as part of a colour which is extended from the human visible wavelengths into the UV (see Losey *et al.*, 1999).

Nevertheless, the strong UV reflectance in the silvery and primarily unpigmented regions might be considered as a simple byproduct of the lighter surfaces on the stickleback's body and thus would not imply a function in signalling. However, in region A most males exhibited some red pigmentation combined with a distinct amount of UV reflectance. Furthermore, additional measurements on lighter ventral parts of the body did not reveal remarkable reflectance in the ultraviolet wave range compared with the measured highly UV-reflective lateral regions (I.P. Rick, pers. obs.). In the end, mate choice experiments will decide whether the UV-reflective surfaces play a role in sexual signalling in *Gasterosteus aculeatus*. Smith *et al.* (2002) demonstrated a strong female preference for guppy males seen under conditions that allowed UV-light. Nevertheless, White *et al.* (2003) could not relate natural variation in UV-reflectance to information about male quality or female preference for one examined guppy population. We quantified variation in the UV-coloration between males as seen in both the location of peak wavelength (hue) and the UV-peak contrast. UV-signals normally represent structural colours which are produced by differentiated surface structures. Differences in the expression of structurally based colours can reveal different qualities of their bearers affected by nutritional status (see McGraw *et al.*, 2002). Keyser & Hill (1999) demonstrated that UV-reflectance intensity of male plumage regions in a bird, the blue grosbeak (*Guiraca caerulea*) correlated positively with its nutritional status and therefore seemed to be condition dependent. In our study, we tested for a possible relationship between UV-colour pattern and physical condition by comparing the UV-peak contrast with the males' body condition factor. Region C showed a significant positive relationship whereas regions A and B did not. Further knowledge about the costs of developing a particular UV-reflectance pattern are necessary in order to examine a potential condition dependency.

A major task will be to study the visual apparatus of the three-spined stickleback with respect to capacities in the ultraviolet. For the stickleback, Cronly-Dillon & Sharma (1968) presumed that the female visual system was tuned to male courtship signals in the visible light spectrum. However, a spectral tuning between signaller and receiver could also have coevolved in the ultraviolet spectrum in the stickleback.

References

Andersson, M. (1994). Sexual selection. — Princeton University Press, New Jersey.

Andersson, S. & Amundsen, T. (1997). Ultraviolet colour vision and ornamentation in bluethroats. — Proc. R. Soc. Lond. B 264, p. 1587-1591.

Archer, S.N., Endler, J.A., Lythgoe, J.N. & Partridge, J.C. (1987). Visual pigment polymorphism in the guppy *Poecilia reticulata*. — Vision Res. 27, p. 1243-1252.

Bakker, T.C.M. (1986). Aggressiveness in sticklebacks (*Gasterosteus aculeatus* L.): a behaviour-genetic study. — Behaviour 98, p. 1-144.

— — (1994). Evolution of aggressive behaviour in the threespine stickleback. — In: The evolutionary biology of the threespine stickleback (M.A. Bell & S.A. Foster, eds). Oxford University Press, Oxford, p. 345-380.

— — & Milinski, M. (1993). The advantages of being red: sexual selection in the stickleback. — Mar. Behav. Physiol. 33, p. 287-300.

— — & Sevenster, P. (1983). Determinants of dominance in male sticklebacks (*Gasterosteus aculeatus* L.). — Behaviour 86, p. 55-71.

Bennett, A.T.D., Cuthill, I.C., Partridge, J.C. & Maier, E.J. (1996). Ultraviolet vision and mate choice in zebra finches. — Nature 380, p. 433-435.

Bolger, T. & Conolly, P.L. (1989). The selection of suitable measurements and analysis of fish condition. — J. Fish Biol. 34, p. 171-182.

Brunton, C.F.A. & Majerus, M.E.N. (1995). Ultraviolet colors in butterflies — intra- or inter-specific communication. — Proc. R. Soc. Lond. B 260, p. 199-204.

Cronly-Dillon, J.R. & Sharma, S.C. (1968). Effect of season and sex on the photopic spectral sensitivity of the three-spined stickleback. — J. Exp. Biol. 49, p. 679-687.

Cummings, M.E., Rosenthal, G.G. & Ryan, M.J. (2003). A private ultraviolet channel in visual communication. — Proc. R. Soc. Lond. B 270, p. 897-904.

Endler, J.A. (1982). Convergent and divergent effects of natural selection on color patterns in two fish faunas. — Evolution 36, p. 178-188.

Keyser, A.J. & Hill, G.E. (1999). Condition-dependent variation in the blue-ultraviolet coloration of a structurally based plumage ornament. — Proc. R. Soc. Lond. B 266, p. 771-777.

— — & — — (2000). Structurally based plumage coloration is an honest signal of quality in male blue grosbeaks. — Behav. Ecol. 11, p. 202-209.

Loew, E.R. & McFarland, W.N. (1990). The underwater visual environment. — In: The visual system of fish (R.H. Douglas & M. Djamgoz, eds). Chapman & Hall, New York, p. 1-43.

Losey, G.S., Cronin, T.W., Goldsmith, T.H., Hyde, D., Marshall, N.J. & McFarland, W.N. (1999). The UV visual world of fishes: a review. — J. Fish Biol. 54, p. 921-943.

McGraw, K.J., Mackillop, E.A., Dale, J. & Hauber, M.E. (2002). Different colours reveal different information: how nutritional stress affects the expression of melanin- and structurally based ornamental plumage. — J. Exp. Biol. 205, p. 3747-3755.

McLennan, D.A. & McPhail, J.D. (1989). Experimental investigations of the evolutionary significance of sexually dimorphic nuptial colouration in *Gasterosteus aculeatus* (L.): the relationship between male colour and female behaviour. — Can. J. Zool. 68, p. 482-492.

Milinski, M. & Bakker, T.C.M. (1990). Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. — Nature 344, p. 330-333.

Rowe, M.P., Baube, C.L., Loew, E.R. & Phillips, J.B. (2004). Optimal mechanisms for finding and selecting mates: how threespine stickleback (*Gasterosteus aculeatus*) should encode male throat colors. — J. Comp. Physiol. A 190, p. 241-256.
Rowland, W.J. (1984). The relationship among nuptial coloration, aggression and courtship of male three-spined sticklebacks, *Gasterosteus aculeatus*. — Can. J. Zool. 62, p. 999-1004.
Sargent, R.C., Rush, V.N., Wisenden, B.D. & Yan, H.Y. (1998). Courtship and mate choice in fishes: Integrating behavioral and sensory ecology. — Am. Zool. 38, p. 82-96.
Siebeck, U.E. & Marshall, N.J. (2001). Ocular media transmission of coral reef fish — can coral reef fish see ultraviolet light? — Vision Res. 41, p. 133-149.
Smith, E.J., Partridge, J.C., Parsons, K.N., White, E.M., Cuthill, I.C., Bennett, A.T.D. & Church, S.C. (2002). Ultraviolet vision and mate choice in the guppy (*Poecilia reticulata*). — Behav. Ecol. 13, p. 11-19.
Thorpe, J.W., Douglas, R.H. & Truscott, R.J.W. (1993). Spectral transmission and short-wave absorbing pigments in the fish lens — 1. Phylogenetic distribution and identity. — Vision Res. 33, p. 289-300.
White, E.M., Partridge, U.C. & Church, S.C. (2003). Ultraviolet dermal reflection and mate choice in the guppy, *Poecilia reticulata*. — Anim. Behav. 65, p. 693-700.
Wootton, R.J. (1976). The biology of the sticklebacks. — Academic Press, London.
Zahavi, A. & Zahavi, A. (1997). The handicap principle. — Oxford University Press, Oxford.