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The dynamics of color signals in male threespine sticklebacks *Gasterosteus aculeatus*

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Abstract

Body coloration and color patterns are ubiquitous throughout the animal kingdom and vary between and within species. Recent studies have dealt with individual dynamics of various aspects of coloration, as it is in many cases a flexible trait and changes in color expression may be context-dependent. During the reproductive phase, temporal changes of coloration in the visible spectral range (400–700 nm) have been shown for many animals but corresponding changes in the ultraviolet (UV) waveband (300–400 nm) have rarely been studied. Threespine stickleback *Gasterosteus aculeatus* males develop conspicuous orange–red breeding coloration combined with UV reflectance in the cheek region. We investigated dynamics of color patterns including UV throughout a male breeding cycle, as well as short-term changes in coloration in response to a computer-animated rival using reflectance spectrophotometry and visual modeling, to estimate how colors would be perceived by conspecifics. We found the orange–red component of coloration to vary during the breeding cycle with respect to hue (*theta*/R50) and intensity (achieved chroma/red chroma). Furthermore, color intensity in the orange–red spectral part (achieved chroma) tended to be increased after the presentation of an artificial rival. Dynamic changes in specific measures of hue and intensity in the UV waveband were not found. In general, the orange–red component of the signal seems to be dynamic with respect to color intensity and hue. This accounts in particular for color changes during the breeding cycle, presumably to signal reproductive status, and with limitations as well in the intrasexual context, most likely to signal dominance or inferiority.

Key words: breeding season, color change, color signals, stickleback, structural coloration, UV coloration

Throughout the animal kingdom, sexual selection often leads to the development of manifold coloration and color patterns (see Andersson 1994). One of the most impressive and exaggerated ornaments is the train of male peacocks (e.g., Petrie et al. 1991). In fishes, the coloration of the skin is derived from two classes of specialized cells: chromatophores and iridophores (Fox and Vevers 1960). Coloration in chromatophores derives from pigments like flavins and red, orange, and yellow colors are mainly produced by carotenoid pigments (Fox 1976). Iridophores often contain layers of guanine crystals that are responsible for the so-called structural coloration, which includes short wavelengths, ultraviolet (UV) wavelengths as well as the silvery, shiny coloration of many fish species (Land 1972). Both, pigment-based and structural coloration often occur in combination (Fox and Vevers 1960).

Coloration is in many cases not a fixed trait of a given individual but a dynamic trait that may vary depending on context, for example camouflage, thermoregulation, and communication, especially during mate-choice (e.g., Kodric-Brown 1998; Stuart-Fox and Moussalli 2009). This accounts in particular for various fish species (e.g., Kodric-Brown 1998). There are basically two types of color change: 1) morphological color change, which is promoted by the modification of chromatophore numbers (Sugimoto 2002) and occurs within days or even months (Stuart-Fox and Moussalli 2009), and 2) physiological color change, which is promoted via

aggregation or dispersal of pigment-containing organelles (e.g., Sköld et al. 2008) or by a changed composition of the reflective capacities of the iridophores and the involved guanine layers (e.g., Kasukawa et al. 1987; Mäthger et al. 2003; Yoshioka et al. 2010) and is usually fast (Thurman 1988). Generally, color changes are observed all over the animal kingdom (crustaceans: Thurman 1988; insects: Filshie et al. 1975; cephalopods: Norman 2000; amphibians: King et al. 1994; reptiles: Cooper and Greenberg 1992; fishes: Kodric-Brown 1998) and are frequent at the beginning of the reproductive season and in the progress of mating (see Kodric-Brown 1998). Seasonal color changes and color changes during mating occur to signal changes in reproductive status, dominance and/or individual quality, for example, the ability to defend a territory or to provide essential resources (see Kodric-Brown 1998 and citations therein).

It is obvious to assume that color changes come along with associated physiological costs and potential fitness consequences (Stuart-Fox and Moussalli 2009). The expression of a pigment-based color change with regard to long-wavelength carotenoid-based coloration has been shown to be condition-dependent (see Hill 1999; Svensson and Wong 2011). In a study on guppies *Poecilia reticulata*, for example, a significantly positive correlation between carotenoid-based orange ornamentation and body condition was found (Nicoletto 1993). In the past years, short-waved UV signals as well have been shown to be condition-dependent in various species (e.g., jumping spider *Cosmophasis umbratica* (Lim and Li 2007) and orange sulfur *Colias eurytheme* (Kemp and Rutowski 2007)). In a study by Jacot and Kempenaers (2007), for example, UV-based plumage coloration in Eurasian blue tits *Parus caerulus* was shown to be positively affected by nestling conditions.

The threespine stickleback *Gasterosteus aculeatus*, the model organism in the present study, is characterized by elaborate courtship coloration and pronounced sexual dichromatism. Nonreproductive males and females are cryptically colored, but at the beginning of the breeding season males develop conspicuous carotenoid-based orange–red courtship coloration on their throat and belly (Bakker and Mundwiler 1994), which has been studied extensively. In detail, red males have been shown to have a higher condition (Milinski and Bakker, 1990), to be more dominant in intrasexual interactions (Bakker and Sevenster 1983), to court more intensively (Bakker and Milinski 1991) and to be more successful in nest-defense (McKinnon 1996). Furthermore, females prefer intensely red-colored males (e.g., Bakker and Milinski 1993), and may thus indirectly select for higher quality mates. Hence, investing into courtship coloration might be beneficial for males. However, on the other hand more colorful males might inadvertently be confronted with a higher visibility for eavesdropping predators (e.g., Zuk and Kolluru 1998). Besides color signals in the visible part of the spectrum, both sexes reflect UV in several body regions (e.g., Rick et al. 2004; Rowe et al. 2004; Rick and Bakker 2008c) and are able to perceive UV signals as they possess four cone types (UV: λ_{max} 360 nm; S: λ_{max} 435 nm; M: λ_{max} 530 nm; L: λ_{max} 605 nm) (Rowe et al. 2004). UV reflections are used in mate-choice (Rick et al. 2006; Rick and Bakker 2008c), during intrasexual interactions (Rick and Bakker 2008b) and might—next to the carotenoid-based breeding coloration—be an indicator of higher body condition and thus higher quality (Rick et al. 2004). A study by Rick and Bakker (2008a) suggests that the combination of structural UV colors and pigmentary orange–red colors is decisive in visually-mediated social behavior in sticklebacks. Furthermore, it is concluded that both color components of the stickleback male courtship signal may interact and are not to be considered separately (Rick and Bakker 2008a).

The orange–red courtship coloration of stickleback males has been shown to be dynamic with respect to the point of time during the reproductive season (van Iersel 1953; Sevenster 1961; McLennan and McPhail 1989). Moreover, female and male sensitivity for red coloration varies with changing reproductive status (Cronly-Dillon and Sharma 1968; Boulcott and Braithwaite 2007; Shao et al. 2014). There is, however, an evident lack of knowledge about the dynamics of UV signals during the breeding cycle. In general, UV signals are not frequently taken into account when examining dynamics of color patterns, but some studies have found evidence for dynamic color change in the UV spectral range (e.g., Kasukawa et al. 1987; Mäthger et al. 2003; Lim and Li 2007). Lim and Li (2007), for example, found UV reflection patterns of jumping spiders to vary with age, whereas Mäthger et al. (2003) found evidence for very rapid changes in UV-reflective capacities of stripe patterns in the paradise whiptail *Pentapodus pardiseus*. In the present study, we tested for long-term color changes during the breeding cycle of male threespine sticklebacks as well as for short-term changes in coloration in response to an artificial computer-animated male stimulus in the context of intrasexual signaling, especially focusing on the UV spectral range.

Material and Methods

Experimental subjects

Threespine sticklebacks used in this study were the F1 generation of random crosses (May–August 2011) of wild-caught fish (April 2011) from the island of Texel, the Netherlands. Directly after fertilization, clutches ($n = 26$) were kept separated by family in 1-L plastic boxes, which were illuminated by fluorescent tubes mimicking natural daylight, including UV (Truelight, T8/18W, T8/36W, T8/58W). All boxes were located in an air-conditioned room with a constant temperature of 17°C ± 1°C. After hatching fry were fed to excess with *Artemia* nauplii for 20 weeks. Then all fish were transferred into holding tanks (L × W × H, 50 cm × 30 cm × 30 cm) that were equipped with an internal filter and the diet was changed to frozen mosquito larvae (*Chironomus* spec.). All families were raised under standard summer conditions (day/night 16 h/8 h) until October 15th 2011, when the light regime was changed to winter conditions (day/night 8 h/16 h) before it was switched back to summer light regime again on June 15th 2012, four weeks prior to the start of the experiments, to simulate the beginning of the breeding season.

Experimental design

In general, possible changes in courtship coloration of males were recorded during the course of the breeding cycle and as short-term response to an intrasexual stimulus (computer animation of a reproductively active male).

Breeding cycle

Males exhibiting conspicuous orange–red courtship coloration were netted from their holding tanks and reflection measurements (see below for a detailed description) were conducted in the cheek region below the eye (measurement 1: before isolation; $n = 25$, families were never used twice). Directly thereafter males were isolated in individual tanks (30 cm × 20 cm × 20 cm), each equipped with a sand-filled Petri dish and 2 g of green threads as nesting material. During the nest-building phase (mean 1.23 ± standard deviation 0.49 days), males were stimulated with a ripe female twice daily for 15 min to

build a nest. The female was presented in a separate tank, which was positioned in front of the male tank, thus only allowing for visual contact (e.g., Mehlis et al. 2010). The progress of nest-building was checked every day right after males' second stimulation. When a nest was considered completed (indicated by a clearly visible tunnel through the nest (Sevenster 1961)), reflection measurements were conducted immediately (measurement 2: nest-building phase; $n = 25$). A ripe female was then introduced into the male's aquarium in the morning of the following day. Every 15 min it was checked whether the female had spawned. In the case of a successful spawning, females were removed immediately; when a female did not spawn within 2 h ($n = 10$), a second female was introduced with a 2-h delay. In exceptional cases ($n = 2$), the second female did not spawn either and a third female had to be introduced the following day in the morning again. Both times these females successfully spawned. The day following a successful mating, the male was again stimulated with a ripe female twice for 15 min and was remeasured following the second stimulation (measurement 3: egg-collecting phase; $n = 25$). Under natural conditions, the egg-collecting phase of males usually lasts about 2–3 days (Kraak et al. 1999), however, as only one female was introduced per male the brood-caring period began right afterwards. During the subsequent brood-caring phase (measurement 4: brood-caring phase; $n = 24$), measurements were conducted every second day for an 8-day-lasting period. These 4 measurements were later on averaged. Nests were checked daily from the fifth day after spawning for newly-hatched fry. However, in only 9 out of 24 nests fry was detected. These 9 males were measured again 3 times within a 6-day-lasting period of fry-guarding (measurement 5: fry-guarding phase; $n = 9$) and again these measurements were averaged for later analyses. All reflections measurements throughout the different phases of reproduction were conducted between 5 pm and 8 pm during the light phase of the illumination cycle.

Short-term response

In this experiment, males that showed signs of red breeding coloration were also netted out of their holding tank and subsequently isolated in single aquaria (30 cm × 20 cm × 20 cm) each equipped as described above ($n = 25$, families were never used twice). As soon as a male had completed its nest (mean 1.12 ± standard deviation 0.33 days), it was measured spectrophotometrically in the cheek region, put back into the individual aquarium, which also served as experimental aquarium, whereupon it was exposed to a computer animation of a reproductively active male. We used a computer animation to standardize tests on the influence of an intrasexual stimulus on the immediate color change. Computer animations work well in sticklebacks and have been used frequently both in the intersexual context (e.g., Künzler and Bakker 1998; Mehlis et al. 2008) and in the intrasexual context (e.g., Mehlis et al. 2009). The computer-animated male stimulus was adopted from a previous study, where the video colors of the stimulus presentation were modified according to the spectral characteristics of the 'natural' red breeding signal from reproductively active males of the same study population as it is perceived through the stickleback visual system (Richter 2012; see also Fleishman et al. 1998; Gomez et al. 2009). It is important to mention that the computer screen did not emit UV light but only light in the human visible part of the spectrum. The RGB values (R = 238, G = 61, B = 8) assigned to the red courtship coloration of the artificial male correspond to intensely red-colored males from the study population. The basic computer animation used was constructed by Künzler and Bakker (1998) and lasts 150 s in total. The sequence starts with the display of a gray-colored landscape (5 s), followed by the entrance of the computer-animated red-colored male on the left, which shows fanning and zig-zagging movements (28 s). The whole sequence is repeated 4 times after which the male leaves the scene, and an empty background is visible again (see Richter 2012). During the animation time, the aquarium containing the test male was positioned in front of a computer monitor (ViewSonic G90fB, Model VS10794). The screen was covered with black wall paper and a 10 cm x 6 cm window was cut out so that the test male was able to view the computer animation but not the rest of the screen. Furthermore, the set-up was surrounded by a black curtain, to avoid any disturbance of the fish. The lighting conditions (illumination provided by Truelight T8 fluorescent tubes) during testing resembled those during rearing and during the short isolation phase of the males. Males were allowed to acclimate for 10 min, during which the empty landscape was visible on the computer screen. Then the computer animation was started and repeatedly shown for 10 min. After the animation time, males were directly measured spectrophotometrically again. Thus, the first and second measurements were only separated by the acclimation time of 10 min and the animation time of 10 min.

Reflection measurements

All reflection measurements were taken outside the water using an Avantes AvaSpec 2048 fiber-optic spectrophotometer in the cheek region below the eye. The measuring procedure (from catching the male until putting it back into its tank) took less than 1 min, so that short-term color changes related to pigment dispersion or aggregation should be minimal (see also Rick et al. 2014). Light was provided by a deuterium–halogen light source (Avantes AvaLight-DHS Deuterium-Halogen Light Sources, 200–1100 nm), and scans were conducted using a bifurcated 200 micron fiber-optic probe with a fitted black cap (angle: 45°), which was held to the body surface to avoid discrepancies in angle and distance (distance: 3 mm). The device was calibrated with a 98% Spectralon white standard (300–700 nm). To record individual scans (initially 20 per measurement), the software Avantes AvaSoft 7.5 was used. All scans were then exported to Microsoft Excel via an integrated Excel output and were averaged per measurement and later on interpolated and smoothed with the program Avicol_v6 (Gomez 2006) (see Figure 1 for mean reflectance spectra of males throughout the breeding cycle (Figure 1A) and before and after animation with the computer-animated rival (Figure 1B)). To evaluate how sticklebacks might be perceived by conspecifics, we calculated a physiological model using Avicol_v6 (Gomez 2006). First, spectral sensitivity curves for the four stickleback cone receptors were determined from cone absorbance maxima provided in Rowe et al. (2004), and by using parameters for the calculation of visual pigment templates provided in Govardovskii et al. (2000). The determined absolute cone stimulations (UV, S, M, L) were then calculated by multiplying individual reflectance, the ambient light (spectrum of the fluorescent tubes used during rearing and experiments (Truelight T8/36 W)), and the calculated spectral cone sensitivities (see Endler and Mielke 2005; Rick et al. 2011). Absolute cone stimulations were then converted to relative cone stimulations and translated to the Cartesian coordinates x, y, and z, and converted to three spherical coordinates (*theta*, *phi*, chroma *r*), which define a color vector within a tetrahedral color space (see Endler and Mielke 2005; Stoddard and Prum 2008; Rick et al. 2011). Within the tetrahedral color space, the central point is the achromatic point of black, white or gray color (Stoddard and Prum 2008; Drobniak et al. 2014). Hue is defined as the

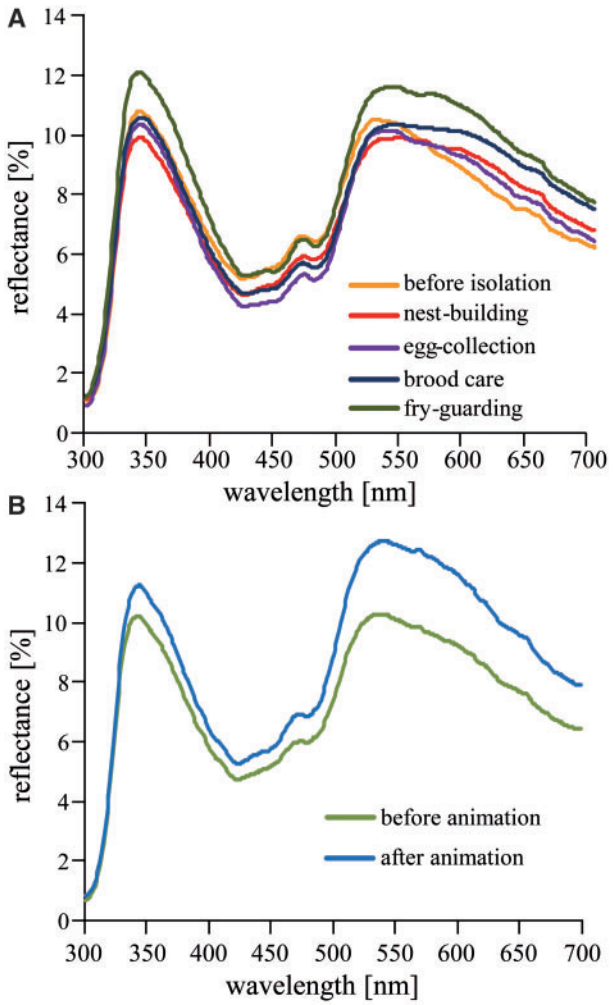

Figure 1. Mean reflectance spectra (proportion of light reflected in relation to a white standard, see text). (A) Taken during the 5 stages of the breeding cycle. (B) Before and after the animation with a reproductively active computer-animated male.

direction of a color vector and is given by two angles: *theta* and *phi* (see Stoddard and Prum 2008). Hue describes a specific color, whereas chroma describes the intensity of the color. *Theta* (longitudinal hue) describes the human-visible part of the spectrum and is used as a measure of "hue" of the carotenoid-based component of stickleback male breeding coloration (see Pike et al. 2011). Higher values indicate orange-shifted and lower values more red-shifted hues (see Pike et al. 2011). *Phi* is the vertical angle (range from +90° to −90°), and represents the short-wave (UV) contribution to the perceived color with more positive values indicating more UV perceived (see Pike et al. 2011). The color intensity (chroma) is defined as the distance of the achromatic point from a given color point (defined by the angles *phi* and *theta*). The larger the magnitude of the chroma, the larger is the distance from the achromatic point and thus the higher is the color intensity. We used achieved chroma r_A as a measure of color intensity, which is the value for chroma r in comparison to the maximum possible value of for a specific hue (r/r_{max}) (Stoddard and Prum 2008; Drobniak et al. 2014).

To allow for better comparison with other studies, we furthermore calculated colorimetric variables (intensity, hue, and brightness), which correspond to the variables obtained by the physiological model. For the cheek region two measures of intensity ("UV chroma", "red chroma") and the "R50 value", as a measure of hue in the human-visible spectral region, were calculated. "UV chroma", a measure of the relative intensity in the UV spectral range between 300 nm and 400 nm, was calculated relative to the total amount of light in the spectral range between 300 nm and 700 nm (Rick et al. 2004; Shawkey et al. 2006). The "red chroma" was calculated the same way, including the relative amount of orange–red reflections between 575 nm and 700 nm (Rick et al. 2011). For carotenoid-based color, the "R50 value" is defined as the wavelength that corresponds to the point of the spectrum that is centered between the minimum reflection between 400 nm and 500 nm and the maximum reflection between 500 nm and 700 nm, and is an indicator of hue (Rick and Bakker 2008b; Pike et al. 2011). Furthermore, the total brightness was determined.

Statistics

The R statistical package (R 2.9.1) was used for statistical analyses (R-Development-Core-Team 2009). Shapiro–Wilk tests were used to check for normal distribution. As data differed significantly from normal distribution, nonparametric statistics were used. For all colorimetric variables, the Friedman rank-sum test was used to be able to control for repeated measures. Differences in coloration were assessed for the course of the breeding cycle (isolation, nest, egg, brood care, fry) and as short-term response (before animation, after animation). All *P*-values are two-tailed.

Results

Breeding cycle

Table 1 comprises pairwise comparisons of the variables obtained from the physiological model and the colorimetric variables between the different parts of the breeding cycle (before isolation, nest-building, egg-collection, brood care, fry-guarding) as well as medians and quartiles. We examined two corresponding variables for hue in the human-visible part of the spectrum: angle *theta* and the R50 value. Values for angle *theta* ranged from high values at the beginning of the breeding cycle before males were isolated (rather orange-shifted hues) to low values during brood care and fry-guarding (rather red-shifted hues) (Table 1A; Figure 2A). The values of *theta* differed significantly between the first measurement point (before isolation) and "egg-collection" as well as "brood care" (Table 1A; Figure 2A). For R50, the values were low at the start and increased over the course of the breeding season (Table 1B); here, the first measurement point (before isolation) differed significantly from "egg-collection" and "brood care" (Table 1B). Furthermore, there was a significant difference between "nest-building" and "egg-collection" as well as "brood care" (Table 1B). The orange–red part of the courtship coloration increased in color intensity (achieved chroma r_A and red chroma) over the course of the breeding cycle (Table 1C and Table 1D; Figure 2B). For the achieved chroma r_A, the variable for color intensity according to the physiological model, "before isolation" differed significantly from "egg-collection" and "brood care" (Table 1C; Figure 2B). Moreover, "nest-building" differed significantly from "brood care" (Table 1C). For red chroma, the first measurement (before isolation) differed significantly from "nest-building", "egg-collection", and "brood care" (Table 1D). Furthermore, the red chroma measured during "nest-building" differed significantly from "brood care" (Table 1D). UV chroma and

Table 1. Results of the pairwise analysis of male courtship color variables (A: *theta*, B: R50 value, C: achieved chroma r_A, D: red chroma, E: *phi*, F: UV chroma) taken in the cheek region at 5 points of time during the male breeding cycle: 1) before isolation (isolation), 2) throughout the nest-building process (nest), 3) during the egg-collecting phase (egg), 4) during brood care (brood care), and 5) while guarding the hatched fry (fry)

Dependent variable	Explanatory variable	χ^2	*df*	*P*	First point of time			Second point of time		
					Median	*1st quartile*	*3rd quartile*	*Median*	*1st quartile*	*3rd quartile*
A *theta*	isolation vs nest	2.462	1	0.117	30.290	22.717	47.157	24.526	17.219	35.067
	isolation vs egg	12.462	1	**<0.001**	30.290	22.717	47.157	23.016	16.504	28.189
	isolation vs brood care	14.440	1	**<0.001**	30.290	22.717	47.157	18.145	13.792	26.197
	isolation vs fry	2.778	1	*0.096*	30.290	22.717	47.157	19.155	13.848	25.120
	nest vs egg	1.385	1	0.239	24.526	17.219	35.067	23.016	16.504	28.189
	nest vs brood care	1.960	1	0.162	24.526	17.219	35.067	18.145	13.792	26.197
	nest vs fry	0.111	1	0.739	24.526	17.219	35.067	19.155	13.848	25.120
	egg vs brood care	4.840	1	0.028	23.016	16.504	28.189	18.145	13.792	26.197
	egg vs fry	2.778	1	*0.096*	23.016	16.504	28.189	19.155	13.848	25.120
	brood care vs fry	0.111	1	0.739	18.145	13.792	26.197	19.155	13.848	25.120
B R50 value	isolation vs nest	0.000	1	1.000	496	483	502	498	456	502
	isolation vs egg	10.667	1	**0.001**	496	483	502	502	499	504
	isolation vs brood care	6.000	1	**0.014**	496	483	502	501	498	504
	isolation vs fry	1.000	1	0.317	496	483	502	502	496	502
	nest vs egg	5.539	1	**0.019**	498	456	502	502	499	504
	nest vs brood care	4.167	1	**0.041**	498	456	502	501	498	504
	nest vs fry	1.000	1	0.317	498	456	502	502	496	502
	egg vs brood care	0.040	1	0.842	502	499	504	501	498	504
	egg vs fry	0.111	1	0.739	502	499	504	502	496	502
	brood care vs fry	0.111	1	0.739	501	498	504	502	496	502
C achieved chroma r_A	isolation vs nest	0.154	1	0.695	0.174	0.125	0.238	0.206	0.154	0.243
	isolation vs egg	7.539	1	**0.006**	0.174	0.125	0.238	0.280	0.183	0.296
	isolation vs brood care	6.760	1	**0.009**	0.174	0.125	0.238	0.246	0.189	0.288
	isolation vs fry	2.778	1	*0.096*	0.174	0.125	0.238	0.229	0.169	0.283
	nest vs egg	5.539	1	**0.019**	0.206	0.154	0.243	0.280	0.183	0.296
	nest vs brood care	1.960	1	0.162	0.206	0.154	0.243	0.246	0.189	0.288
	nest vs fry	0.111	1	0.739	0.206	0.154	0.243	0.229	0.169	0.283
	egg vs brood care	1.960	1	0.162	0.280	0.183	0.296	0.246	0.189	0.288
	egg vs fry	1.000	1	0.317	0.280	0.183	0.296	0.229	0.169	0.283
	brood care vs fry	1.000	1	0.317	0.246	0.189	0.288	0.229	0.169	0.283
D red chroma	isolation vs nest	7.539	1	**0.006**	0.328	0.308	0.341	0.340	0.325	0.366
	isolation vs egg	5.539	1	**0.019**	0.328	0.308	0.341	0.354	0.330	0.369
	isolation vs brood care	14.440	1	**<0.001**	0.328	0.308	0.341	0.367	0.349	0.376
	isolation vs fry	2.778	1	*0.096*	0.328	0.308	0.341	0.358	0.353	0.385
	nest vs egg	0.000	1	1.000	0.340	0.325	0.366	0.354	0.330	0.369
	nest vs brood care	4.840	1	**0.028**	0.340	0.325	0.366	0.367	0.349	0.376
	nest vs fry	0.111	1	0.739	0.340	0.325	0.366	0.358	0.353	0.385
	egg vs brood care	1.960	1	0.162	0.354	0.330	0.369	0.367	0.349	0.376
	egg vs fry	2.778	1	*0.096*	0.354	0.330	0.369	0.358	0.353	0.385
	brood care vs fry	2.778	1	*0.096*	0.367	0.349	0.376	0.358	0.353	0.385
E *phi*	isolation vs nest	0.154	1	0.695	41.918	−26.281	71.198	30.937	2.177	72.899
	isolation vs egg	0.154	1	0.695	41.918	−26.281	71.198	37.452	12.257	71.380
	isolation vs brood care	0.040	1	0.842	41.918	−26.281	71.198	19.945	−12.767	70.764
	isolation vs fry	1.000	1	0.317	41.918	−26.281	71.198	35.491	17.501	68.772
	nest vs egg	0.154	1	0.695	30.937	2.177	72.899	37.452	12.257	71.380
	nest vs brood care	0.360	1	0.549	30.937	2.177	72.899	19.945	−12.767	70.764
	nest vs fry	0.111	1	0.739	30.937	2.177	72.899	35.491	17.501	68.772
	egg vs brood care	1.960	1	0.162	37.452	12.257	71.380	19.945	−12.767	70.764
	egg vs fry	1.000	1	0.317	37.452	12.257	71.380	35.491	17.501	68.772
	brood care vs fry	0.111	1	0.739	19.945	−12.767	70.764	35.491	17.501	68.772
F UV chroma	isolation vs nest	0.615	1	0.433	0.252	0.222	0.276	0.250	0.226	0.282
	isolation vs egg	0.154	1	0.695	0.252	0.222	0.276	0.251	0.242	0.266
	isolation vs brood care	1.000	1	0.317	0.252	0.222	0.276	0.246	0.238	0.261
	isolation vs fry	1.000	1	0.317	0.252	0.222	0.276	0.245	0.238	0.269
	nest vs egg	0.154	1	0.695	0.250	0.226	0.282	0.251	0.242	0.266
	nest vs brood care	0.360	1	0.549	0.250	0.226	0.282	0.246	0.238	0.261
	nest vs fry	0.111	1	0.739	0.250	0.226	0.282	0.245	0.238	0.269
	egg vs brood care	0.040	1	0.842	0.251	0.242	0.266	0.246	0.238	0.261
	egg vs fry	0.111	1	0.739	0.251	0.242	0.266	0.245	0.238	0.269
	brood care vs fry	0.111	1	0.739	0.246	0.238	0.261	0.245	0.238	0.269

Friedman rank-sum tests were used throughout. Significant results ($P < 0.05$) are printed in bold, tendencies ($0.05 < P < 0.10$) are printed in italics.

angle *phi*, describing the stimulation of the UV cone, did not differ significantly between any of the stages of the reproductive cycle (Table 1E and Table 1F). In addition, brightness did not differ significantly between any of the different stages of the reproductive cycle (all $\chi^2 < 2.778$, all $P > 0.096$).

Short-term response

The achieved chroma r_A tended to be lower before (less saturated coloration) than after the animation (more saturated coloration) (Table 2; Figure 3). All other variables obtained by the physiological model and the corresponding colorimetric variables did not differ significantly before and after the animation (Table 2). Furthermore, brightness did not differ significantly before and after the animation ($\chi^2 = 1.960$, $P = 0.162$).

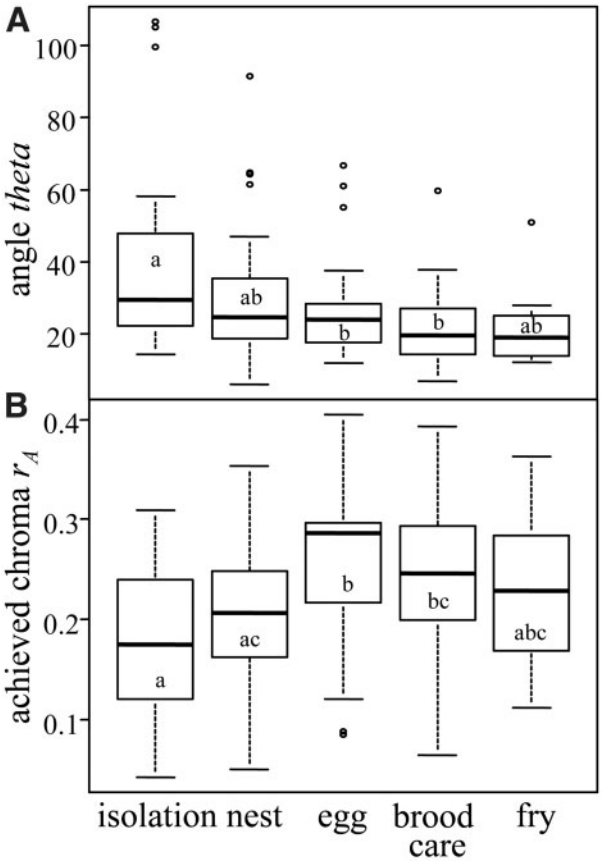

Figure 2. (A) Angle *theta* and (B) achieved chroma r_A taken in the cheek region at 5 points of time during the male breeding cycle: 1) before isolation (isolation), 2) throughout the nest-building process (nest), 3) during the egg-collecting phase (egg), 4) during brood care (brood care), and 5) while guarding the hatched fry (fry). Friedman rank-sum tests were used throughout. Plotted are medians and quartiles, whiskers (defined as 1.5 × inter-quartile range) and outliers. Significant differences ($P < 0.05$) between groups are indicated by different letters.

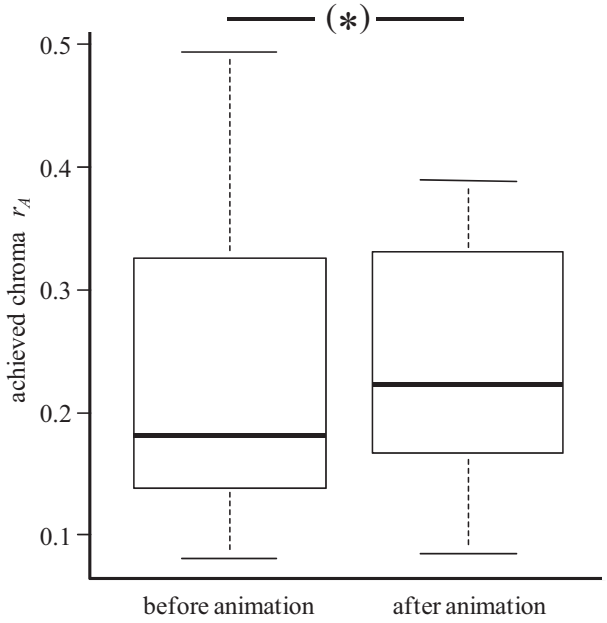

Figure 3. Achieved chroma r_A of males before and after confrontation with the animation of a reproductively active computer-animated male. Plotted are medians, quartiles and whiskers (defined as 1.5 × inter-quartile range). (*): $0.05 < P < 0.1$.

Table 2. Results of the analysis of male courtship color variables taken in the cheek region before and after being animated by a reproductively active computer-animated male

Dependent variable	χ^2	*df*	*P*	Before animation			After animation		
				Median	*1st quartile*	*3rd quartile*	*Median*	*1st quartile*	*3rd quartile*
theta	1.960	1	0.162	22.978	17.661	33.939	18.813	16.017	24.057
R50 value	0.391	1	0.532	502	494	506	503	501	506
achieved chroma r_A	3.240	1	*0.072*	0.166	0.119	0.262	0.249	0.188	0.339
red chroma	1.960	1	0.162	0.340	0.310	0.371	0.359	0.335	0.386
phi	0.040	1	0.842	27.463	−30.073	62.672	38.887	−1.499	65.675
UV chroma	0.360	1	0.549	0.237	0.213	0.276	0.251	0.231	0.277

Friedman rank-sum tests were used throughout. Tendencies ($0.05 < P < 0.10$) are printed in italics.

Discussion

The results of the present study show that the reflection component in the human-visible part of the spectrum (*theta* and R50) was shifted toward longer wavelengths over the course of the breeding season (indicated by lower values of *theta* and higher values of R50). The intensity of orange–red coloration increased over the course of the breeding cycle, represented by the two according measures (achieved chroma r_A and red chroma). Coloration in the UV spectral range did not change significantly, neither in hue nor in intensity.

Studies focusing on coloration in the visible part of the spectrum have shown that the red courtship coloration of male sticklebacks varies between the different stages of reproduction (e.g., Craig-Bennett 1931; van Iersel 1953; McLennan and McPhail 1989). Most studies (e.g., Craig-Bennett 1931; McLennan and McPhail 1989) found the red coloration to be relatively low during the nest-building phase, to be maximal during courtship, and then to slightly decrease during brood care and fry-guarding, presumably to reduce visibility for potential predators. An increase in red coloration (red-shifted hue) and an additional influence of color intensity (increasing orange–red intensity) was also found in our study. A reduction during brood care was not found in the present study; instead, hue was even more red-shifted during this period compared with previous ones. However, the saturation of the red coloration (achieved chroma r_A) followed the pattern described above and slightly decreased during brood care and fry-guarding. McLennan and McPhail (1989) explained the reduction in red coloration during brood care as a masking of red by melanic coloration that would allow males to rapidly return to courtship in a next breeding cycle. It appears, that this masking or reduction of red coloration/red hues did not occur in males of the population used in this study. However, the results agree with reports of an increase in redness in some stickleback populations during brood care and especially fry-guarding (Moodie 1972a), potentially serving as defense signal for intruders in a phase in which the fry are especially endangered (Moodie 1972b). Von Hippel (1999) found threespine stickleback males to have a high red color score during courtship, which decreased during brood care but was actually maximal when the fry hatched and swam free. McLennan (2007) also reported that the red intensity is usually gradually declining during brood care, but peaks again during the fry-guarding phase. Candolin (2000), in addition to changes over the course of one breeding cycle, found body size-dependent changes in orange–red coloration over the course of the whole breeding season. In general, it is important to mention that the other studies on stickleback breeding coloration were not based on spectrophotometric measurements but on evaluations based on color scores or on photographs. *Theta* and achieved chroma represent objective measures of hue and chroma in the visible spectral range of the respective study species (see Stoddard and Prum 2008). However, our results concerning the human-visible component of courtship coloration are in accordance with these results, thus underlining that the orange–red color component in general seems to be highly dynamic.

As mentioned above, coloration in the UV spectral range did not change significantly, neither in hue nor in intensity. However, UV reflections have been shown to be decisive in female mate-choice in sticklebacks (Boulcott et al. 2005; Rick et al. 2006), and to be equally important as the orange–red proportions of light (Rick and Bakker 2008a). It was assumed that structural and pigmentary color components interact, for example, that an altered deposition of carotenoids would lead to differences in UV reflectance as well. The results of the present study, however, indicate that the UV reflective component of the color signal is not as dynamic or as important in signaling changes, for example, in the reproductive status. A possible reason for that is that the cost-benefit ratio might be driven toward an increased investment in pigment-based orange–red coloration, even though both components of stickleback male coloration have already been shown to be condition-dependent in sticklebacks (Milinski and Bakker 1990; Rick et al. 2004). An alternative explanation could be that stickleback males, contrary to pigment-based colors, have limited control over the alteration of structural-based colors.

In the intrasexual context, the achieved chroma r_A tended to be enhanced after the exposure to the reproductively active computer-animated male stimulus. Our finding of a slightly enhanced chroma corresponds to other studies on intrasexual encounters, which have as well found sticklebacks' red courtship coloration to be dynamic in response to potential rivals (e.g., Candolin 1999; Kim and Velando 2014). Kim and Velando (2014) demonstrated that stickleback males enhance the area of red courtship coloration after encountering a potential rival. The study by Candolin (1999) showed that male–male competition influences signal expression by increasing the difference between males in signaling, for example, a reduction in coloration in response to a superior rival and *vice versa*, likely to reduce socially imposed costs of signaling. Thus, the increase in red coloration in the present study might as well be explained by the superiority of males in comparison with image of the computer-animated male. In contexts of competition, red coloration reflects male dominance (Bakker and Sevenster 1983) and dominance in turn correlates with male quality, which is preferred in an intersexual context (Milinski and Bakker 1990). However, the increase in chroma, implying higher conspicuousness, might also result in enhanced or decreased contrast with other body parts (e.g., Rush et al. 2003) or the background (e.g., Reimchen 1989; Scott 2001) and consequently enhanced or reduced conspicuousness of color patterns. An explanation for lack of dynamics in the UV spectral range might be that the computer animation did not emit UV light and thus the color change reaction of the male in that part of the spectrum was minor. However, we decided to use the computer animation to standardize tests on the influence of the intrasexual stimulus. Still, it would be interesting to conduct similar experiments with natural rivals, which also reflect UV, to examine whether these stimuli would generate a more pronounced color change, also including the UV spectral range. An alternative explanation could be that a change in the structural coloration in sticklebacks is only exhibited over longer time frames in an intrasexual context. However, in female sticklebacks the UV component of coloration has been shown to be highly dynamic and context-dependent changes in structural coloration were shown within minutes (Hiermes et al. 2015). Furthermore, other studies on structural coloration have also shown that color changes are executed quite fast in fishes (e.g., Kasukawa et al. 1987; Mäthger et al. 2003). Thus, it is more likely that a change in UV coloration might be more costly and, as for the dynamics over the breeding cycle, the cost-benefit ratio might be driven toward changes in the pigmentary orange–red part of the color signal. Nevertheless, it is important to note that the color signal, including both structural and pigmentary colors, is probably decisive in visually-mediated signaling and communication and is to be considered as unity, and not by its separate components.

In summary, reflections in the human-visible part of the spectrum, contrary to the UV parts of the spectrum, seem in general quite dynamic in male threespine sticklebacks. This accounts, in

particular, for measurements during the course of the breeding cycle and with limitations also for short-term responses to a computer-animated courting rival. The function of dynamic color changes is likely to signal changes in reproductive status during the breeding cycle and to signal changes in dominance or inferiority, respectively. Future research should assess the influence of changes in color intensity and hue on potential mating partners and/or potential rivals, and its dependence on an individual's cost-benefit ratio. Moreover, it would be interesting to examine whether other colorimetric variables, like the area of orange–red and/or UV breeding coloration, are dynamic as well over time.

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