Oecologia
DOI 10.1007/s00442-013-2773-x

BEHAVIORAL ECOLOGY - ORIGINAL RESEARCH

The influence of ambient ultraviolet light on sperm quality and sexual ornamentation in three-spined sticklebacks (*Gasterosteus aculeatus*)

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Received: 23 May 2013 / Accepted: 3 September 2013

Abstract Exposure to enhanced levels of ambient ultraviolet (UV) radiation (UVR) can have adverse effects on aquatic organisms including damage at the cellular and molecular level and impairment of development, fecundity and survival. Much research has been conducted on the role of the harmful UVB radiation. However, due to its greater penetration in water the more abundant UVA radiation can also act as an environmental stressor. Little is known about UVR effects on sperm characteristics although sperm cells should be especially prone to UV-induced oxidative stress. Moreover, UV-related changes in oxidative status may affect the phenotypic expression of energetically costly sexual ornaments. We investigated the effects of long-term exposure to ecologically relevant levels of simulated UVA radiation on sperm quality and sexual ornamentation in three-spined sticklebacks (*Gasterosteus aculeatus*). Males were assigned to three spectral exposure treatments differing in the UV spectral part so that they received either enhanced, moderate or no UVA radiation. The results reveal that exposure to enhanced ambient UVA levels had detrimental effects on both male breeding coloration and sperm velocity providing evidence that UVR affects traits targeted by pre- and post-copulatory sexual selection. By highlighting the role of UVA as a factor influencing fitness-relevant traits, our findings may contribute to a better understanding of the consequences of current and future levels of solar UVR for mating systems and life history.

Communicated by David Marcogliese.

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Keywords Breeding coloration · Oxidative stress · Sexual selection · Sperm velocity · Ultraviolet light

Introduction

Solar ultraviolet (UV) radiation (UVR) as an environmental stressor has a considerable impact on organisms in terrestrial and aquatic ecosystems (Häder et al. 1998). Stratospheric ozone reduction over the past few decades has led to a significant increase of radiation reaching the earth's surface (Madronich et al. 1995), especially in the UVB range (280–320 nm) while ozone absorption in the UVA range (320–400 nm) is negligible. Despite the success of the Montreal protocol in reducing the production and use of ozone-depleting chemicals (Solomon 2004) the ozone layer will be damaged for several more decades, thus levels of UVR will continue to increase (e.g., Häder et al. 2011).

The various harmful effects of enhanced levels of UVR, especially of UVB, on organisms include damage to enzymes, DNA and RNA at the cellular and molecular level (e.g., Dahms and Lee 2010). Moreover, UVR can have detrimental effects on development, fecundity and survival rate at the organismal level ultimately leading to ecological consequences (Hansson and Hylander 2009). Some organisms have evolved avoidance or repair mechanisms in terms of behavioral, physiological or molecular responses to increased levels of UVR (e.g., Sinha and Häder 2002; Dahms and Lee 2010).

Most studies investigating the various impacts of UVR on aquatic organisms focus on the more energetic UVB radiation (Häder et al. 2011). However, despite being less harmful than UVB, UVA wavelengths represent the major component of UVR especially in aquatic habitats due to their greater penetration in natural waters (Williamson and

Published online: 28 September 2013

Rose 2010). Moreover, they are of particular importance for visually mediated behaviors in fish and other aquatic organisms (Leech and Johnsen 2009). In fishes, UVA was found to negatively modulate physiological and immunological functions (Winckler and Fidhiany 1996; Salo et al. 2000) but can play a beneficial role in DNA repair as well (Dong et al. 2007). Further investigations of UVA-induced effects on reproductive variables in fish resulted in contradictory results. For instance, although no impact of UVA light on mortality, development and DNA damage in Atlantic cod (*Gadus morhua*) eggs and larvae was found (Béland et al. 1999), the hatching success of medakas (*Oryzias lapites*) was negatively affected by UVA (Bass and Sistrun 1997), and an in situ exposure of yellow perch (*Perca flavescens*) eggs to UVA led to an increased mortality rate (Williamson et al. 1997). While the effects of UVR on eggs, embryos and larvae in aquatic organisms are comparatively well studied (see references above) the impact of UVR on sperm characteristics has been poorly investigated. This is surprising, since sperm should be highly susceptible to UVR as they lack sunscreen compounds (Adams et al. 2001), have a limited antioxidant potential and are prone to lipid peroxidation (LPO) (e.g., Aitken et al. 1998).

In the present study, we thus investigate the effects of ecologically relevant levels of ambient UVA light on selected reproductive variables in males of the three-spined stickleback (*Gasterosteus aculeatus*), a small fish that inhabits shoreline areas of marine, brackish, and freshwater habitats in the Northern Hemisphere (Wootton 1984). During the breeding season between April and August males establish and defend a territory in which they build a nest for their future eggs and court females, all of which takes place in shallow waters with elevated levels of UVR. Furthermore, the stickleback eye is sensitive to UVA radiation and UV signals are used during intraspecific interactions such as female mate choice (Rick and Bakker 2008a) where male UV patterns act in combination with the characteristic red breeding coloration as one of the key determinants of female mate choice (e.g., Bakker and Milinski 1993).

After male courtship, a female deposits her eggs into the nest, which are then fertilized by the nest owner. Intruding males, so-called sneakers, may attempt to steal fertilizations by creeping through the nest immediately before or after the nest-owning male (Wootton 1984), indicating that sperm competition is of particular relevance in this breeding system. In three-spined sticklebacks spermatogenesis is only active during the short photoperiod, meaning that sperm production completely takes place in late autumn until early winter while being quiescent during the breeding season (Borg 1982). Hence, males are sperm limited over the course of the breeding season so that sperm allocation has a considerable impact on an individual's reproductive success (e.g., Zbinden et al. 2004).

Taken together, the expression of the carotenoid-based red breeding coloration (Bakker and Milinski 1993) and the level of gonadal investment (Cubillos and Guderley 2000) predict a stickleback male's reproductive success and are both related to male oxidative status (Pike et al. 2010). Given that UVR can be a potent agent of oxidative stress in aquatic organisms (Dahms and Lee 2010) we tested whether long-term exposure to different but naturally occurring levels of ambient UVA radiation has an effect on fitness relevant (1) pre-copulatory (sexual ornamentation), and (2) post-copulatory (sperm characteristics) reproductive traits.

Materials and methods

Three-spined sticklebacks from an anadromous population were caught during their spring migration in April 2011 on the island of Texel, the Netherlands, and brought to the Institute for Evolutionary Biology and Ecology in Bonn, Germany. Fish were kept in a large outside tank (750 l), with air ventilation and a constant supply of tap water (3 l min^{-1}) and fed with red mosquito larvae (*Chironomus* spp.) daily in excess. Within one week (16–21 May 2011) 45 males identified by initial signs of nuptial coloration were isolated in single aquaria measuring 30 cm × 20 cm × 20 cm (length × width × height) which were equipped with an airstone and placed in an air-conditioned room under a standardized summer light regime (day length 16:8 h light:dark, temperature 15 ± 1 °C). Standard illumination was provided by daylight fluorescent tubes (Natural Daylight 5500, 36 W, 120 cm; True-Light), which were placed 45 cm above the bottom of the aquaria. These tubes simulate natural skylight conditions including UV light. All aquaria were visually isolated from each other by placing gray opaque plastic sheets between them (40 cm length × 30 cm width) and were covered by UV-transmitting perspex (Röhm GS2458). Males were randomly assigned to one of three spectral exposure treatments differing only in the UV portion of the spectrum: UV depleted [UV(−); $n = 15$] with the UV spectral range being completely removed by a UV-blocking acetate filter (Rosco-3114R; Cinegel), ambient UV [UV(+); $n = 15$] with UVA radiation being only provided by the daylight lamps, and enhanced UV [UV(++); $n = 15$] with additional UVA radiation being emitted by one cold cathode lamp per tank (Conrad no. 581744, 30 cm). These lamps were installed 35 cm above the bottom of the aquaria at 90° to the daylight tubes so that they illuminated one aquarium each from front to back. They were turned on 4 h after sunrise and turned off 4 h before sunset so that fish in the UV(++) treatment received increased levels of UVA radiation for 8 h per day.

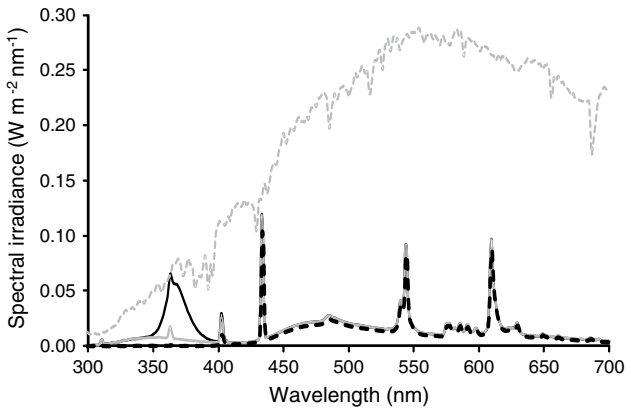

Fig. 1 Spectral irradiance of downwelling light measured in a holding tank under the three exposure treatments UV(−) (*black dashed line*), UV(+) (*gray solid line*) and UV(++) (*black solid line*), and mean solar irradiance collected between 8 a.m. and 4 p.m. on a sunny during the early breeding season on the Island of Texel (*gray dashed line*)

Table 1 Absolute irradiance (W m^{-2}), daily dose (kJ m^{-2}) and total dose (kJ m^{-2}) of ultraviolet-A (*UVA*) radiation used in the three exposure treatments

Measurement	Treatment		
	UV(−)	UV(+)	UV(++)
Absolute UVA irradiance (W m^{-2})	0.027	0.449	1.482
Daily UVA dose (kJ m^{-2})	1.578	25.876	55.622
Total UVA dose (kJ m^{-2})	53.034	869.435	1,868.889

UV(−) 16 h of UV-filtered ambient UV, *UV*(+) 16 h of ambient UV, *UV*(++) 8 h of enhanced UV and 8 h of ambient UV

Downwelling light produced by the lamps under the different experimental conditions was measured in 1-nm intervals between 300 and 700 nm using a spectrophotometer (AvaSpec 2048; Avantes, Eerbeek, the Netherlands) and an Avantes CC-UV/VIS cosine corrector. Irradiance was calibrated against an Avantes NIST traceable application standard. For measurements, the irradiance probe was placed in one holding tank at about 10 cm above the bottom and pointed upwards. Irradiance spectra for the three different exposure treatments are shown in Fig. 1 and absolute irradiances (W m^{-2}) in the UVA (320–400 nm) per treatment in Table 1. The daily UVA dose (kJ m^{-2}) for each treatment was calculated by multiplying spectral irradiance by exposure time [UV(++), 8 h of enhanced UV and 8 h of ambient UV; UV(+), 16 h of ambient UV; UV(−), 16 h of UV-filtered ambient UV] and is presented together with the total UVA dose (kJ m^{-2}) that fish received over the experimental period (Table 1).

Solar irradiance measurements in the field were conducted at the beginning of the breeding season at the end of March 2013 with the same setup that was used for measurements in the laboratory. Spectra were recorded on a sunny day at a water depth of 10 cm in a ditch on the island of Texel (53°114′N, 4°898′E) that serves as a common breeding site for sticklebacks from our sample population. The spectrophotometer was programmed to record downwelling irradiance at 1-nm intervals from 300 to 700 nm once per hour from sunrise to sunset for approximately 30 s. Since fish in the UV(++) treatment were exposed daily to enhanced levels of UVA for 8 h we calculated the mean solar irradiance measured in the field for the same period of time around noon, which was 4.291 W m^{-2} compared to 1.482 W m^{-2} for the UV(++) treatment in the lab (Fig. 1; Table 1). While the absolute solar irradiance at 365 nm (0.067 W m^{-2} nm^{-1}) matches well with the UV peak irradiance at 365 nm for the UV(++) conditions (0.063 W m^{-2} nm^{-1}) one has to consider that the light conditions in the holding tanks only provide a rough simulation of natural UVA conditions due to the narrow-banded spectrum compared to the broader solar spectrum between 300 and 400 nm (see Fig. 1). However, although the spectral exposure treatments differ from the field conditions, the comparatively lower amounts of UVA used in the laboratory lie well within the range that sticklebacks experience in their natural habitat so that the chosen illumination conditions were suitable to study long-term radiation effects.

Male standard length (SL) and body mass (M) were determined before isolation and their body condition (BC) was calculated ($BC = 100 \times M \times SL^{-3}$; Bolger and Connolly 1989). During the experimental procedure males received a daily dose of 0.3 g red mosquito larvae that consisted of two types of food equally portioned; red mosquito larvae, and carotenoid-enriched larvae (AHA Frostfutter, Duisburg, Germany). The latter contain an additional amount of astaxanthin, which is one of the main carotenoids in the integument of stickleback males (Wedekind et al. 1998).

Twenty-nine days after isolation, males received 2.5 g of green cotton threads (color 597, length 30 ± 10 mm; Gütermann) and a sand-filled petri dish (diameter 12 cm) for nest building. Males were stimulated daily by presenting a receptive female in a small transparent plastic box in front of the tank for 15 min (see Mehlis et al. 2010). On average males needed 2.18 ± 0.48 days (mean ± SE) to build a nest. Male breeding coloration was first quantified during the peak of reproductive activity, which was on average 2.42 ± 0.14 days (mean ± SE) after nest completion. Therefore, each male was stimulated with a receptive female and as soon as it showed a reaction towards the female, stimulation lasted for 10 min. As female fecundity is size dependent (Wootton 1984) females were chosen in such a way that they were 5–10 % larger than males.

Directly after stimulation the expression of male breeding coloration was quantified using a spectrophotometer

(Avantes AvaSpec-2048) connected to a deuterium-halogen light source (Avantes DH-S) for illumination (Rick et al. 2011). A bifurcated 200-μm fiber-optic probe was held perpendicular to the surface. To exclude ambient light and to ensure that scans were collected at a fixed distance of 0.3 cm the probe end was inserted into a darkened pipette tip. Measurements were taken from three different body regions on the left lateral side (e.g., Rick et al. 2011); first, in the orange-red region directly below the eye (cheek region, breeding coloration); second, at the dorsum below the second dorsal spine (dorsal region); and third, in the gonadal region. Twenty scans were taken in succession without changing probe contact and were averaged for each single region. Reflectance was measured relative to a 98 % Spectralon white standard. Data were recorded with Avasoft 7.5 and imported into Microsoft Excel. The whole procedure took about 1 min so that short-term color changes related to pigment dispersion or aggregation could be ruled out (I. P. R., personal observation). After the reflectance measurements were taken male body condition was determined again (see above).

To quantify male red breeding coloration for the cheek region as viewed by a potential female stickleback we used a physiological model of stickleback color vision. Details on the used parameters and the formulation of the model are provided elsewhere (Rick et al. 2011). In short, absolute excitation values for each of the four stickleback cone receptors [UV, short (S), medium (M), long (L)] were determined between 300 and 700 nm by multiplying spectral reflectance of the cheek region per individual male by cone sensitivity and an ambient irradiance spectrum for D65 standard daylight. Relative excitation values were calculated by dividing absolute excitation of each single cone class by the sum of excitations for all four cone classes. These relative values were converted to Cartesian coordinates in tetrahedral color space (x, y, z), following Endler and Mielke (2005). After conversion to spherical coordinates we obtained the variable chromaticity (r) or spectral purity as the Euclidean distance to the achromatic origin (equal stimulation of all cones). The magnitude of r is an estimate of the intensity of the carotenoid-based red breeding coloration (Rick et al. 2011). For the dorsal and gonadal region we calculated the total brightness (total reflectance between 300 and 700 nm) and for the gonadal region the UV chroma (reflectance between 300 and 400 nm/total brightness, in percentage). We chose these variables to account for potential changes in skin melanin pigmentation in these body regions as a photoprotective response to enhanced levels of UVR ("suntanning"). This was reported for at least two other fish species (Lowe and Goodman-Lowe 1996; Adachi et al. 2005).

On average 89.73 ± 0.23 days (mean ± SE) after isolation males were animated again and reflectance as well as body measurements were repeated as described above. Thereafter, males were quickly euthanized by decapitation. As the testes of three-spined stickleback males are covered with melanophores and there is a striking between-male variation in testicular melanization (Mehlis et al. 2012), the intensity of both testes melanophore pigmentation (L^*_{total}) was quantified using standard digital images (see Mehlis et al. 2012 for details). It is assumed that melanophore pigmentation of the testes might play a role in protection against UV light and/or oxidative stress (Plonka et al. 2009; Galván et al. 2011). Furthermore, the liver was dissected and weighed to the nearest milligram (LM) to determine the hepatosomatic index [HSI = (LM/M) × 100] as an estimate of male energy status (Chellappa et al. 1995).

To obtain sperm each male's right testis was pestled in an Eppendorf tube containing 200 μl artificial ovarian fluid (3.0 g NaCl, 0.1 g KCl, 0.07 g $CaCl_2$ in 1 l aqua destillata; Elofsson et al. 2006), which was set at a constant temperature (16 °C; Thriller, V0410E; PEQLAB). In sticklebacks it is known that the presence of ovarian fluid prolongs the period of sperm motility for up to 24 h (Elofsson et al. 2003) and Bakker et al. (2006) showed that it takes about 10 min to completely fertilize one clutch in this species. Thus, exactly 2 and 30 min (1st and 2nd sperm motility measurement) after pestling the testes sperm motility was videotaped at 320× magnification (Motic microscope B3 Professional Series; VIDO camera CC540X via iMovie) using a Leja counting chamber (12 μ), which was filled with 3 μl of the mixed sperm suspension. Sperm movement was filmed (25 frames per second) for 3 s at ten different positions that were equally distributed over the chamber. For subsequent sperm motility analyses, six randomly chosen sequences lasting 1 s were analyzed with CASA (ImageJ) resulting in the following averaged variables: (1) percentage of motile sperm, (2) velocity curvilinear (VCL; μm s^{-1}), (3) velocity average path (VAP; μm s^{-1}), (4) velocity straight line (VSL; μm s^{-1}), (5) straightness [STR = VSL/VAP × 100 (%)], (6) linearity [LIN = VSL/VCL × 100 (%)] and (7) wobble [WOB = VAP/VCL × 100 (%)]. The threshold values for excluding immotile sperm were specified as 10 μm s^{-1} for VCL, 5 μm s^{-1} for VAP and 2 μm s^{-1} for VSL. Sperm motility measurements were solely performed on mature sperm because the testes of three-spined stickleback males contain almost exclusively spermatozoa during the breeding season (see Borg 1982 for details).

VCL, VAP and VSL represent velocity values that are highly significantly positively correlated with each other in both sperm motility measuerments (Pearson correlations: $n_{1st} = 28$, $n_{2nd} = 24$, all $r_P > 0.728$, all $p < 0.001$). However, it is unknown which of these variables are the most important ones to describe sperm speed in three-spined sticklebacks. Therefore, a principal component

analysis (PCA) was performed using VCL, VAP and VSL resulting in a single significant factor for sperm velocity for both the 1st (eigenvalue, 1.63; proportion of variance, 88.1 %) and the 2nd sperm motility measurement (eigenvalue, 1.65; proportion of variance, 90.8 %). The trajectory of sperm movement is described by the three ratios of the velocities (LIN, STR and WOB) and the corresponding PCA yielded a single significant factor for sperm linearity for the 1st (eigenvalue, 1.48; proportion of variance, 73.2 %) and the 2nd sperm motility measurement (eigenvalue, 1.66; proportion of variance, 91.7 %). Both for the 1st and the 2nd sperm motility measurement there was a significant positive relationship between PC1 of sperm velocity and VCL, VAP and VSL (Pearson correlations: $n_{1st} = 28$, $n_{2nd} = 24$, all $r_P > 0.883$, all $p < 0.001$) as well as PC1 of sperm linearity and LIN, STR (exponentially transformed to achieve normal distribution) and WOB (Pearson correlations: $n_{1st} = 28$, $n_{2nd} = 24$, all $r_P > 0.667$, all $p < 0.001$). As a measure of sperm longevity the difference in the percentage of motile sperm between the 1st and the 2nd sperm motility measurement was calculated.

Sperm number was determined using a Neubauer improved counting chamber (0.0025 mm^2, depth 0.1 mm; Labor Optik). The average sperm count in 64 cells yielded the total number of sperm (see Mehlis et al. 2012 for details). Moreover, sperm morphology variables were determined (head length, head width, mid-piece length, mid-piece width and tail length) using 30 sperm per male analyzed in ImageJ; 5 µl of the sperm suspension was fixed on a glass slide with a cover slip using nail polish. Sperm were photographed at 1,000× magnification via cell^D 5.1 (Olympus) with a camera (Olympus ColorView IIIu) mounted on a microscope (Olympus BX51).

Statistical analyses

Six males failed to build a nest [$n_{UV(-)} = 2$, $n_{UV(+)} = 1$, $n_{UV(++)} = 3$] within 10 days and nine males died during the experiments and were thus excluded [$n_{UV(-)} = 2$, $n_{UV(+)} = 4$, $n_{UV(++)} = 3$], resulting in a final sample size of $n_{UV(-)} = 11$, $n_{UV(+)} = 10$, $n_{UV(++)} = 9$ for the analyses of breeding coloration, testis traits and sperm morphology. Furthermore, sperm of two further males from the UV(−) treatment did not show any swimming activity in both sperm motility measurements. In addition, in four males [$n_{UV(+)} = 2$, $n_{UV(++)} = 2$] sperm stopped moving between the 1st and the 2nd sperm motility measurement. Thus, we had a final sample size of $n_{UV(-)} = 9$, $n_{UV(+)} = 10$, $n_{UV(++)} = 9$ for the 1st and $n_{UV(-)} = 9$, $n_{UV(+)} = 8$, $n_{UV(++)} = 7$ for the 2nd sperm motility measurement.

Analyses were conducted in R 2.9.1 statistical package and *p*-values are based on two-tailed tests. For each male three values for body measurements and two values for total brightness (dorsal region and gonadal region), UV chroma (gonadal region) and chromaticity (cheek region) exist because we determined these variables repeatable during the experimental procedure (see above). We constructed different linear models (lme) using the measured variables as dependent variables. The treatment (daily dose, kJ m^{-2}), the days of exposure and the interaction between these two variables were included as explanatory variable. To account for repeated measures male identity was included as random factor. For variables that were determined at the end of the experiments (e.g., testis and sperm traits) we used the treatment (daily dose) as an explanatory variable and days of exposure as random factor. For an overview, all models used are listed in Table 2. Tests of significance were based on likelihood-ratio tests and in all models explanatory variables were removed stepwise in the order of statistical relevance. SL and total brightness (gonadal region) were logarithmically transformed and testis melanization (L^*_{total}) was exponentially transformed to achieve normal distributions of the residuals of the best explanatory models according to Kolmogorov–Smirnov tests with Lilliefors correction.

Results

There was a significant treatment × days of exposure interaction for chromaticity of the cheek region (breeding coloration) (lme, $\chi^2 = 4.197$, $p = 0.041$; Table 2). In addition, chromaticity was significantly influenced by days of exposure (lme, $\chi^2 = 8.044$, $p = 0.018$) and the three UV exposure treatments (lme, $\chi^2 = 7.535$, $p = 0.023$) with males from the UV(−) treatment being more intensely colored (Fig. 2a; Table 2). Over the course of the experiments there was a significant decrease in total brightness for the dorsal as well as the gonadal region (lme, both $\chi^2 \geq 10.241$, both $p \leq 0.001$; Table 2). However, total brightness for both regions was not significantly influenced by treatment or treatment × days of exposure interaction (lme, all $\chi^2 \leq 1.885$, all $p \geq 0.170$; Table 2). Male SL increased over the course of the experiments (lme, $\chi^2 = 45.235$, $p < 0.001$) but these changes were not significantly influenced by the three UV exposure treatments and treatment × days of exposure interaction (lme, both $\chi^2 \leq 1.866$, both $p \geq 0.172$; Table 2). Male body condition and UV chroma (gonadal region) were neither influenced by treatment, days of exposure nor treatment × days of exposure interaction (lme, all $\chi^2 \leq 2.752$, all $p \geq 0.097$; Table 2).

Sperm swimming speed was significantly different between the three UV exposure treatments; this was true for the 1st (lme, $\chi^2 = 4.645$, $p = 0.031$) and the 2nd sperm motility measurement (lme, $\chi^2 = 5.529$, $p = 0.019$) with sperm velocity being decreased in UV-exposed males

Table 2 All linear mixed effects models used

Dependent variable	Explanatory variable									Random factor
	Treatment (daily dose; kJ m^{-2})			Days of exposure			Treatment (daily dose; kJ m^{-2}) × days of exposure			
	χ^2	Δdf	*p*-value	χ^2	Δdf	*p*-value	χ^2	Δdf	*p*-value	
Standard length (cm)	0.044	1	0.835	45.235	1	*<0.001*	1.866	1	0.172	Male identity
Body condition	0.502	1	0.479	2.752	1	0.097	0.373	1	0.542	Male identity
Total brightness (dorsal region)	0.202	1	0.653	13.741	1	*<0.001*	0.017	1	0.896	Male identity
Total brightness (gonadal region)	0.012	1	0.912	10.241	1	*0.001*	1.885	1	0.170	Male identity
UV chroma (gonadal region)	1.210	1	0.271	0.013	1	0.908	<0.001	1	0.993	Male identity
Chromaticity (cheek region)	7.535	2	*0.023*	8.044	2	*0.018*	4.197	1	*0.041*	Male identity
Sperm velocity (1st sperm motility measurement)	4.645	1	*0.031*		–			–		Days of exposure
Sperm velocity (2nd sperm motility measurement)	5.529	1	*0.019*		–			–		Days of exposure
Sperm linearity (1st sperm motility measurement)	0.085	1	0.770		–			–		Days of exposure
Sperm linearity (2nd sperm motility measurement)	0.876	1	0.349		–			–		Days of exposure
Percentage of motile sperm (1st sperm motility measurement)	2.204	1	0.137		–			–		Days of exposure
Percentage of motile sperm (2nd sperm motility measurement)	0.506	1	0.477		–			–		Days of exposure
Sperm longevity	0.152	1	0.697		–			–		Days of exposure
Tail length (μm)	1.419	1	0.234		–			–		Days of exposure
Mid–piece volume (μm^3)	0.082	1	0.775		–			–		Days of exposure
Head to tail length ratio	1.102	1	0.294		–			–		Days of exposure
Sperm quantity	0.752	1	0.386		–			–		Days of exposure
Testis melanization	0.388	1	0.533		–			–		Days of exposure
Hepatosomatic index	0.035	1	0.851		–			–		Days of exposure

To control for possible influences in all models the random factor was never removed. Significant results are in *italics*

(Fig. 2b; Table 2). In comparison, there was no significant difference in terms of sperm linearity and the percentage of motile sperm, both for the 1st (lme, both $\chi^2 \leq 2.204$, both $p > 0.137$) and the 2nd sperm motility measurement (lme, both $\chi^2 \leq 0.876$, both $p > 0.349$; Table 2). Males from the three treatments did not differ significantly with regard to sperm longevity (lme, $\chi^2 = 0.152$, $p = 0.697$), sperm morphology (tail length, mid-piece volume and head to tail length ratio), sperm quantity, testis melanization and HSI (lme, all $\chi^2 \leq 1.419$, all $p \geq 0.234$; Table 2).

Discussion

Exposure to different levels of environmental UVA radiation induced changes in sperm swimming ability in stickleback males with increasing amounts of UVA leading to a significant decline in sperm velocity. To the best of our knowledge, this is the first study providing experimental evidence for a detrimental effect of ecologically relevant levels of UVA light on sperm swimming ability before sperm release, i.e., with sperm cells being not directly exposed to environmental UVR.

Most studies dealing with UVR effects on reproduction-related variables in fish focus on UVR-induced damage of eggs, embryos and larvae (Béland et al. 1999). However, sperm cells should generally be even more prone to the effects of UV irradiation due to the lack of UV-absorbing sunscreen compounds (Adams et al. 2001), low DNA repair abilities (Donnelly et al. 2000; Tremellen 2008), limited antioxidant properties because of the small cytoplasmic volume (Aitken et al. 1998) and the susceptibility to LPO based on high concentrations of cellular polyunsaturated fatty acids in their membrane (Aitken et al. 1998; Tremellen 2008).

In an experimental study on sea urchins (*Anthocidaris crassispina*) direct irradiation of sperm with natural levels of UVA and UVB had a negative effect on sperm motility and fertilization success based on an increase in reactive oxygen species (ROS) production (Lu and Wu 2005). In fish, adverse effects of a direct exposure to UVA and UVB on the motility and fertility of sperm due to elevated ROS fluxes were found in tilapia (*Oreochromis niloticus*) (Zan-Bar et al. 2005). High ROS levels may reduce sperm quality either through a reduction

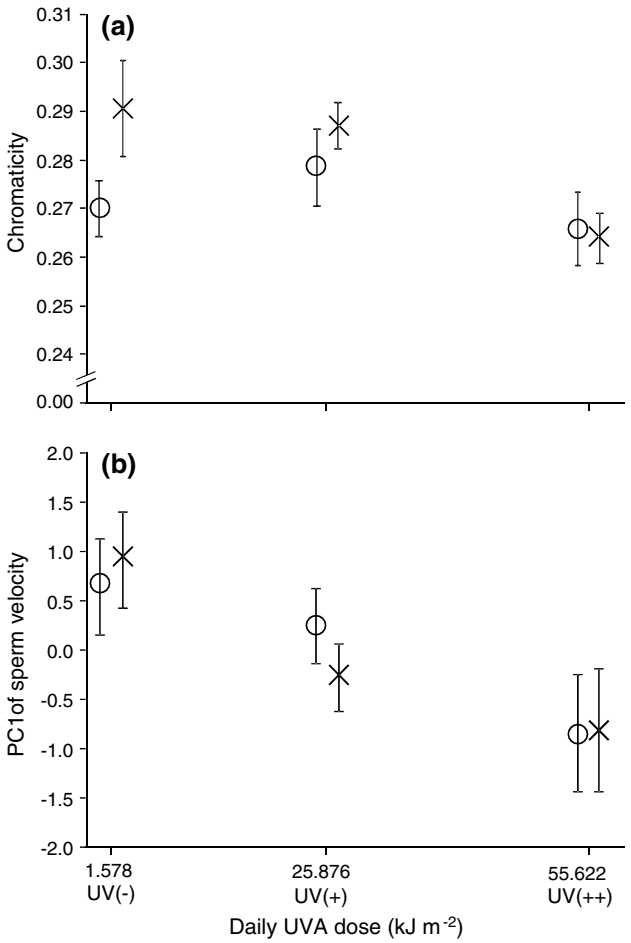

Fig. 2 Effects of daily UVA dose on **a** chromaticity of the red cheek region and **b** sperm velocity (principal component 1; *PC1*) of reproductively active males for the three exposure treatments [UV(−), UV(+) and UV(++)]. **a** *Open circles* represent male chromaticity at the breeding peak and *crosses* show chromaticity at the end of the experiment. **b** *Open circles* represent the early measurement of sperm velocity and *crosses* show the late measurement. Mean values ± SEs are shown. For other abbreviations, see Fig. 1

in mitochondrial membrane potential (Wang et al. 2003) or through effects on the plasma membrane in addition to LPO, leading to reduced egg penetration (DeBaulny et al. 1997).

In the present study, the decrease in sperm velocity in UV-irradiated stickleback males suggests that prolonged exposure of fish to UVA light during the reproductive period incurs costs in generating enhanced levels of oxidative stress. It appears likely that the reduction in sperm swimming velocity is based on negative effects of ROS on mitochondrial function (Barja 2004). Nonetheless, specific identification of the underlying mechanisms requires direct measures of oxidative damage in terms of LPO (malondialdehyde levels), in situ production of ROS inside the mitochondria as well as levels of sperm DNA fragmentation, which should be addressed in future investigations.

The chromaticity of male breeding coloration, which reflects the concentration of carotenoid antioxidants deposited in the integument (Rick et al. 2011), was found to be lower in UVR-exposed males at the end of the experiment. This further indicates that males may have suffered from accumulated oxidative damage during the course of the experiment so that carotenoids were increasingly mobilized from the skin for self-maintenance functions in other parts of the body (von Schantz et al. 1999). Recent experimental studies have confirmed a link between ornament expression and sperm quality through oxidative stress. For instance, high levels of oxidative stress in great tits (*Parus major*) led to a stronger reduction in sperm motility and swimming ability and enhanced levels of sperm LPO in less colorful males compared to more colorful ones (Helfenstein et al. 2010). Moreover, carotenoids and other dietary antioxidants have been shown to increase sperm quality in birds, fish and mammals (Catoni et al. 2008; Helfenstein et al. 2010). Almbro et al. (2011) provided male crickets (*Teleogryllus oceanicus*) with diets differing in antioxidant content and demonstrated that males reared on large amounts of vitamin E in combination with beta-carotene had a higher reproductive success under sperm competition conditions. In sticklebacks it has been shown that under non-competitive mating conditions males supplemented with increased levels of carotenoids had a higher functional fertility (proportion of fertilized eggs) than carotenoid-deprived males (Pike et al. 2010). In the case of the present study, it is likely that the observed effects on reproductive traits did arise through the interplay between the antioxidant system and sexual ornamentation (von Schantz et al. 1999) with stickleback males facing an UVR-induced oxidative challenge. Nonetheless, there is controversy about the antioxidant role of carotenoids (e.g., Blount et al. 2003; Pérez Rodríguez 2009) and the mechanisms involved need to be verified physiologically before one can draw more definite conclusions (Garratt and Brooks 2012).

Further research effort should be directed to the whole range of potential UVA-related effects on immunological and physiological functions. For example, Salo et al. (2000) exposed roach (*Rutilus rutilus*) to either UVA or UVB light and showed that both types of irradiation have significant immune-modulatory effects. In this study, UVA was especially found to reduce hematocrit, plasma protein and immunoglobulin levels and increase the amount of leukocytes. UVA-induced effects on metabolism were described in the convict cichlid (*Cichlasoma nigrofasciatum*) where long-term exposure to UVA light caused a decrease in metabolic performance and survival (Winckler and Fidhiany 1996). For stickleback males in particular, reproductive activities such as territorial defense, nest building and courtship behavior are energy consuming (e.g., Smith and Wootton 1995). However, males in the present study were

individually kept under laboratory conditions. Consequently, energetic costs for them should have been lower than under natural conditions due to the absence of territorial behavior and parental care. But significant UV-dependent effects on metabolic performance cannot be ruled out since males had to build nests, invest in sexual ornamentation and show courtship behavior when confronted with females, all of which contribute to total energy expenditure.

Unlike the effects of enhanced UV-exposure on sperm swimming abilities and breeding coloration we did not find a significant influence on male body condition and hepatosomatic index, both of which are often used to estimate the energy status in sticklebacks (but see Chellappa et al. 1995). This suggests that although UV-induced effects on these variables were not immediately recognized, deleterious effects mediated through oxidative stress may still become visible over the longer term, which requires further study.

The adverse effects of UVA radiation on reproductive variables in males may have important fitness consequences. In terms of ejaculate quality, sperm with a higher swimming velocity were found to have a higher fertilization success in a non-competitive context (Donnelly et al. 1998; Malo et al. 2005) and a fertilizing advantage in a sperm competition context (Gage et al. 2004; Denk et al. 2005). Stickleback males face an increased risk of sperm competition (Goldschmidt et al. 1992; Jamieson and Colgan 1992; Largiadèr et al. 2001) and there is clear evidence that sperm velocity predicts fertilization success under competitive conditions (M. M., in preparation) as was already shown for other external fertilizers (see Pizzari and Parker 2009). Hence, our findings reveal that environmental UVR might be capable of limiting male reproductive success at the post-mating level, through reduced sperm competitive ability and consequently a lower percentage of paternity.

Exposure of stickleback males to UVR had no significant effect on sperm morphology as was shown for tilapia sperm when being directly irradiated with ecologically irrelevant doses of highly energetic UVB and UVC radiation (Don and Avtalion 1993). In the present study fish were exposed to low energy UVA light, thus changes in the cellular structure of sperm were unlikely. Moreover, spermatogenesis in sticklebacks takes place during the short photoperiod (Borg 1982) and was therefore completed before the start of the experiment so that UVR-related effects on sperm morphology can be largely discounted. The same holds true for the total number of sperm, which also did not differ depending on the amount of environmental UVR. The amount of motile sperm was negatively affected by oxidative stress in some studies (e.g., Helfenstein et al. 2010) but not in others (Losdat et al. 2011). In the present study no effect of UVR on the percentage of motile sperm was found, indicating that UVA-induced changes in the functional characteristics of sperm were not profound enough to inhibit sperm motility completely. Moreover, no UV-induced response was detected with regard to testis melanization, thus it is unlikely that melanophore pigmentation plays a role in protecting against UV light and/or oxidative stress, at least for the light conditions used in this study.

As previously mentioned, the red breeding coloration of stickleback males is of importance in female mate choice (Milinski and Bakker 1990). The finding that UV-exposed males were less colorful thus indicates that UVR may negatively affect pre-mating reproductive traits as well. By avoiding less colorful males with a higher susceptibility to UVR-induced oxidative challenges females may thus decrease the risk of reduced fertility. This is intriguing since stickleback courtship takes place in shallow waters so that males are frequently exposed to increased levels of UVA radiation and UV wavelengths play an important role in visual communication in this species (Rick and Bakker 2008b). Consequently, reproductively active stickleback males may face a selective trade-off between beneficial and adverse effects of ambient UVA radiation in their natural habitat. Moreover, it is possible that females may prefer higher quality males which court in areas of intense UV exposure as these males may be more resistant to oxidative stress and thus can invest more in their breeding coloration associated with increased sperm performance.

In conclusion, we found experimental evidence that exposure to ecologically relevant levels of UVA light affect different components of reproductive effort in this species. Global change can affect UVR levels in aquatic habitats in the UVB but also in the UVA range through, for example, variations in cloud cover and the concentration of dissolved organic matter (Häder et al. 2003). Hence, further research is required in order to better understand potential consequences of not only current but also future levels of solar UVR for animal mating systems and life history.

Acknowledgments We are grateful to the Bakker research group for discussion, Jan Hottentot for catching the sticklebacks and Lukas K. Hilke for analyzing sperm motility. Furthermore, we would like to thank two anonymous reviewers for their constructive comments on an earlier version of this manuscript and Thomas Bartolomaeus for use of the microscope equipment. This research was funded by the Deutsche Forschungsgemeinschaft (BA 2885/4-1).

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