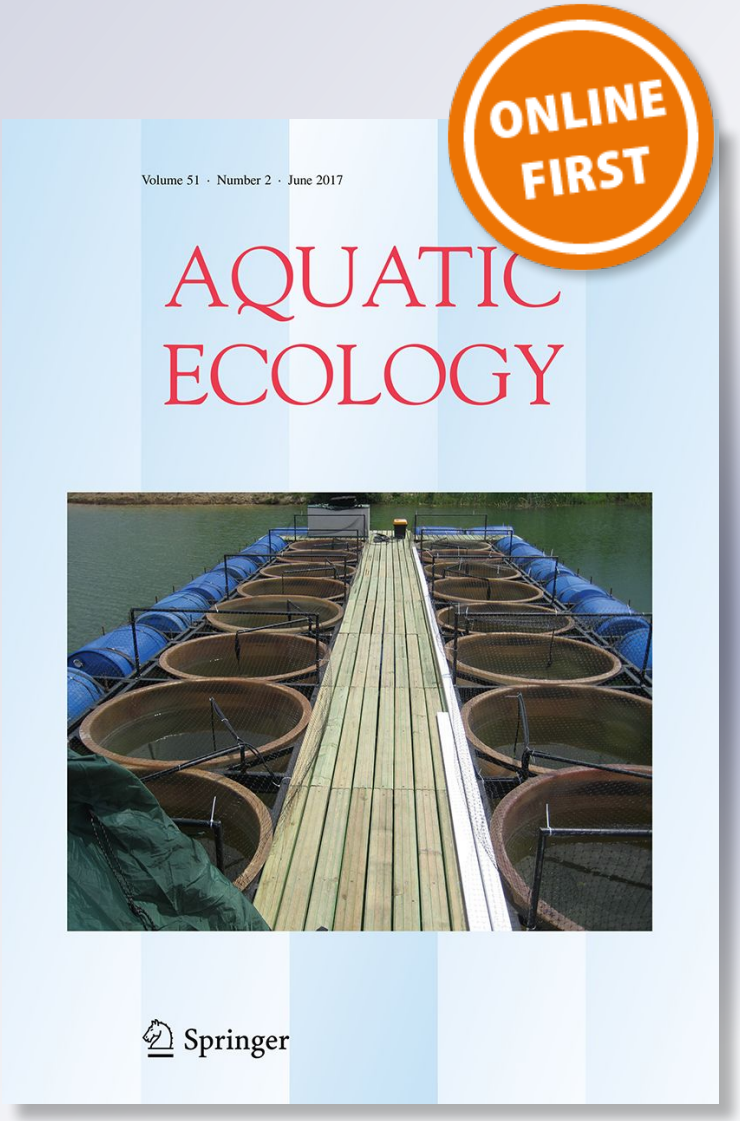
Enhanced ambient UVB light affects growth, body condition and the investment in innate and adaptive immunity in three-spined sticklebacks (Gasterosteus aculeatus)

Simon Vitt, Anna K. Rahn, Lisa Drolshagen, Theo C. M. Bakker, Jörn P. Scharsack & Ingolf P. Rick

Aquatic Ecology
A Multidisciplinary Journal Relating to Processes and Structures at Different Organizational Levels

ISSN 1386-2588

Aquat Ecol
DOI 10.1007/s10452-017-9632-5



Springer

Aquat Ecol
DOI 10.1007/s10452-017-9632-5

Enhanced ambient UVB light affects growth, body condition and the investment in innate and adaptive immunity in three-spined sticklebacks (*Gasterosteus aculeatus*)

Simon Vitt · Anna K. Rahn · Lisa Drolshagen · Theo C. M. Bakker · Jörn P. Scharsack · Ingolf P. Rick

Received: 11 January 2017 / Accepted: 29 May 2017

Abstract With ongoing environmental change, ultraviolet-B radiation (UVB) reaching the Earth's surface has increased over recent decades with consequences for terrestrial and also aquatic ecosystems. Despite evidence for direct physiological and immunological responses of aquatic animals following enhanced UVB exposure, studies investigating indirect impacts of ambient UVB radiation are scarce and mainly used only single doses and/or artificially high amounts of UVB. In the present study, the influence of chronic exposure to elevated UVB levels on growth, body condition and immune function was investigated in three-spined sticklebacks (*Gasterosteus aculeatus*). Fish were kept outdoors for 68 ± 2 days under two different spectral conditions; one group was exposed to natural solar radiation (UVB-normal), while the other group received additional UVB light for four hours daily (UVB-enhanced). Enhanced UVB radiation was within the range of UVB levels measured at the study site. Fish length and weight were determined at the beginning and end of the experiment to compare growth and body condition between the two treatment groups. At the end of the experiment, the splenosomatic index and the granulocyte-to-lymphocyte ratio were determined as immune parameters. Fish from the UVB-enhanced group showed a reduced growth and body condition as well as a lower splenosomatic index compared to the UVB-normal group. Furthermore, UVB-treated fish had a higher granulocyte-to-lymphocyte ratio representing a relatively higher activation of innate compared to adaptive immunity. Consequently, increased but ecologically relevant levels of ambient UVB negatively affect growth and body condition and have a considerable impact on immunity in three-spined sticklebacks.

Keywords Ultraviolet radiation · Environmental change · Immune system · Body size · Teleost fish

Handling Editor: Piet Spaak.

S. Vitt (✉) · A. K. Rahn · L. Drolshagen · T. C. M. Bakker · I. P. Rick
Institute for Evolutionary Biology and Ecology, University of Bonn, An der Immenburg 1, 53121 Bonn, Germany
e-mail: svitt@evolution.uni-bonn.de

J. P. Scharsack
Department of Animal Evolutionary Ecology, Institute for Evolution and Biodiversity, University of Münster, Hüfferstrasse 1, 48149 Münster, Germany

Introduction

Solar ultraviolet radiation, especially in the UVB wavelength range (280–320 nm), has significant effects on terrestrial and aquatic organisms at all levels of biological organisation and plays an important role in the context of environmental change

Published online: 03 June 2017

(Häder et al. 2007; Williamson and Rose 2010; Williamson et al. 2014). Ozone depletion leads to an increase in the amount of solar UVB reaching Earth's surface (Stuart et al. 2004) affecting mainly polar regions, but also the mid-latitudes of the northern and southern hemispheres (Reuder et al. 2001). In aquatic ecosystems, the UVA wavelength range (320–400 nm) represents the main part of solar UV radiation as it penetrates deeper into the water column than UVB, but UVB is more energetic and therefore plays a role as stressor for aquatic organisms especially in shallow habitats (e.g. Bothwell et al. 1994; Bancroft et al. 2007; Sucré et al. 2011).

Impacts of UVB radiation include detrimental effects on various levels of animal organisation. On the molecular level, photo-induced damage to macromolecules can be related to UVB in terms of DNA and RNA mutations as well as damage to enzymes and membranes (Häder et al. 1998; Dahms and Lee 2010). Furthermore, direct effects of UV radiation (UVR) on fatty acids in the skin, ocular tissue and on dorsal muscle as well as growth were found in Atlantic salmon (*Salmo salar*) (Arts et al. 2010). UV-induced genetic alterations may lead to negative impacts on the ontogenetic development (Sinha and Häder 2002). Exposure to UVB can further lead to modifications of osmoregulatory functions as was found for the larval stage of the European seabass (*Dicentrarchus labrax*) in terms of lower numbers of integumentary ionocytes and mucous cells. Moreover, UVB is known to negatively affect growth and body condition (Jokinen et al. 2008, 2011) and can lead to an increased mortality as shown for eggs of the Atlantic cod (*Gadus morhua*) (Kouwenberg et al. 1999) and embryos of other fishes (Llabrés et al. 2013). UVB can also interact with other environmental factors such as thermal conditions (Carreja et al. 2016; Seebacher et al. 2016), and it can, for example, increase the susceptibility to parasitic infections in fish (Cramp et al. 2014). Increases in UVR can have substantial effects on trophic-level interactions as well, even though the detailed relationships are far from being understood (but see Bothwell et al. 1994; Häder et al. 2007).

A number of studies have also demonstrated immunomodulatory effects of UVB radiation. The immune system of teleost fishes generally consists of non-specific innate and highly specific adaptive or acquired immunity components (Magnadóttir 2006; Uribe et al. 2011). On the cellular level, immediate but rather unspecific responses are carried out by cells of the innate immune system, such as granulocytes, whereas cells of the adaptive immune system, such as B and T lymphocytes, mediate highly specific immune responses and long-lasting immune memory (Flajnik and Kasahara 2010).

UVR-induced immunosuppression has been shown in fishes (e.g. Subramani et al. 2015), insects (e.g. Debecker et al. 2015), amphibians (e.g. Ceccato et al. 2016), birds (e.g. Blount and Pike 2012), mammals (e.g. Uberoi et al. 2016) and humans (reviewed in Ullrich 2016). In fish, Salo et al. (1998) studied the effects of a single dose of UVB on the non-specific immune system of the roach (*Rutilus rutilus*) with regard to random and directed migration of granulocytes. Spontaneous cytotoxicity of granulocytes towards target cells was suppressed one day after irradiation and even at 14 days post-irradiation (Salo et al. 1998). Further photo-immunological studies showed an increased proportion of granulocytes and decreased proportion of lymphocytes in the peripheral blood of roach, carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*) in response to a single dose of UVB (Salo et al. 2000a, b). Additionally, decreased lymphoproliferative responses were observed in roach after short-term exposure to UVB (Jokinen et al. 2001). In a long-term experiment using juvenile Atlantic salmon (*Salmo salar*), enhanced UVB reduced haematocrit value and plasma protein concentrations and affected plasma immunoglobulin concentrations (Jokinen et al. 2008). Most studies on fish have explored the effects on embryos and larval stages using a single dose of UVB or short exposure times of only a few days (but see Markkula et al. 2005; Fukunishi et al. 2013). Furthermore, artificial conditions were used such as restriction of motion during exposure concomitant with additional stress through handling of the experimental fish (e.g. Markkula et al. 2005, 2006). Moreover, studies on long-term effects exposed fish to UVB radiation several times a week but did not use a chronic UVB exposure by daily application (Markkula et al. 2009).

In the present study, three-spined sticklebacks (*Gasterosteus aculeatus*) were chronically exposed to either elevated, but naturally occurring levels of UVB radiation (UVB-enhanced) or natural daylight conditions as control treatment (UVB-normal) on a daily basis for several weeks. Three-spined

sticklebacks inhabit shoreline areas of marine, brackish and freshwater habitats in the northern hemisphere (Wootton 1984), with shallow waters being characterised by elevated levels of UVR. Sticklebacks are capable of perceiving light in the UVA range (Rowe et al. 2004) and UV wavelengths are used in this species for intraspecific communication (e.g. Modarressie et al. 2006; Rick et al. 2006; Rick and Bakker 2008; Hiermes et al. 2015b), visual foraging (Rick et al. 2012) and habitat selection (Rick and Bakker 2010). Moreover, increased levels of ambient UVA light during the nesting period had negative effects on sperm quality and sexual ornamentation in stickleback males (Rick et al. 2014).

Here, the effects of a long-term UVB exposure on growth, body condition, the relative spleen mass and immune functioning were quantified and compared between the two treatment groups (UVB-enhanced, UVB-normal). Our expectation was that chronic exposure to enhanced UVB radiation leads to a shifted immune activation, resulting in a higher ratio of granulocytes to lymphocytes due to UVB-induced non-specific inflammatory processes.

Materials and methods

Study animals

About 320 juvenile three-spined sticklebacks were collected in November and December 2012 from a shallow freshwater pond near Euskirchen, Germany (50°38′N, 6°47′E) with the permission of the local forestry department. At the Institute for Evolutionary Biology and Ecology, University of Bonn, fish were maintained in one outside stock tank (volume 700 l; temperature 8 ± 1 °C with a tap-water flow rate of 3 l min^{-1} and air ventilation) for three months prior to the start of the experiments. Fish were fed ad libitum three times a week with defrosted chironomid larvae. To rule out a possible influence of UVB on ectoparasites (e.g. *Gyrodactylus* sp.), which may have an indirect effect on fish immunity, all sticklebacks were treated with an anthelmintic agent (Gyrodol 2, JBL, Neuhofen, Germany). The absence of ectoparasites was confirmed by microscopy before the start of the experiments.

The study conforms to the Association for the Study of Animal Behaviour Guidelines for the use of animals in research as well as to the legal requirements of Germany. No further licences were needed.

Experimental set-up

At the beginning of the experimental phase, on 16 January 2013, 144 subadult fish with an average standard length of 3.362 (±SEM 0.02 cm) and a weight of 0.461 (±SEM 0.01 g) were transferred from the outside stock tank to four circular outdoor tanks with a volume of 2500 l each and a diameter of 2 m (AquaTech, Kitzbühel, Austria), arranged in a square of 36 m^2 with uniform sun exposure. The tanks were equipped with a filter (PonDuett 3000, Pontec, Hörstel, Germany) and six water-permeable enclosures (39 × 28 × 28 cm), which were dipped 20 cm deep into the water column. UVB lamps (G8T5E, 8 W, Sankyo Denki, Kanagawa, Japan) were installed 10 cm above the water surface of every second enclosure to create lighting conditions consisting of natural daylight and artificially enhanced UVB radiation (UVB-enhanced). The remaining enclosures were illuminated by natural daylight (UVB-normal). In this case, a dummy (grey PVC, 2 × 40 cm) was installed above the enclosures, providing the same shading as the UVB lamps used in the UVB-enhanced treatment. Twelve days after transferring the test fish into the outdoor tanks, UVB lamps were switched on for four hours daily around noon (11:00 a.m.–03:00 p.m.). Fish were kept in equal-sized, mixed-sex groups of six individuals per enclosure for nine weeks resulting in 144 individuals (24 groups); half of them were assigned to UVB-enhanced and half to UVB-normal conditions. Sticklebacks in each enclosure were marked individually by clipping the tip of the dorsal spines in various combinations to enable the determination of change in growth and body condition for each individual. During the whole experimental phase, fish were fed three times a week ad libitum with defrosted chironomid larvae, equally distributed in the whole enclosure. Leftover food was removed using a tube after 5–10 min.

Photic conditions

For the two different experimental conditions, downwelling irradiance between 280 and 700 nm was measured in one enclosure which was specifically used for collecting irradiance data and placed at randomly

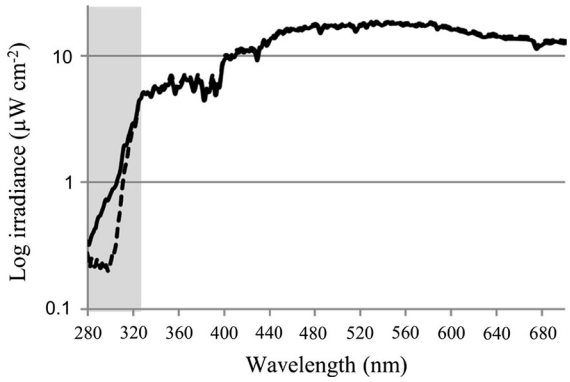


Fig. 1 Average spectral irradiance of downwelling light in a holding tank measured on 16 days under various weather conditions between 11:00 a.m. and 03:00 p.m. in February and March 2013. Displayed are the UVB range (*shaded area*), the two exposure treatments UVB-enhanced (*black solid line*) and UVB-normal (*black dashed line*)

chosen locations within all four outdoor tanks. Measurements were taken on 16 days under various weather conditions between 11:00 a.m. and 03:00 p.m. in February and March 2013 by using a spectrophotometer (AvaSpec 2048, Avantes) equipped with a cosine corrector (CC-UV/VIS, Avantes, Netherlands). Irradiance was calibrated against an Avantes NIST traceable application standard. For measurements, the irradiance probe was kept in a fixed position at 18 cm water depth. Fifteen single spectra were recorded within one measuring series using the software AvaSoft (Version 7.5, Avantes) and transferred to Microsoft Excel (Microsoft Office 2007) to calculate a mean spectrum. Average irradiance spectra for the two exposure treatments are shown in Fig. 1, and absolute irradiances (W m^{-2}) in the UVB (280–320 nm), UVA (320–400 nm) and PAR (400–700 nm) wavelength range are given in Table 1.

The maximum amount of UVB was 0.76 W m^{-2} (±SEM 0.30 W m^{-2}) for UVB-enhanced and 0.48 W m^{-2} (±SEM 0.29 W m^{-2}) for UVB-normal which is below values measured in August 2012 during sunny conditions in the same experimental set-up (0.79 W m^{-2}). Mean values of absolute irradiance for the experimental phase were 0.33 W m^{-2} (±SEM 0.12 W m^{-2}) for UVB-enhanced and 0.21 W m^{-2} (±SEM 0.08 W m^{-2}) for UVB-normal conditions. UVB-enhanced fish were exposed to a daily dose of 6.48 kJ m^{-2} (±SEM 0.73 W m^{-2}) and an absolute dose of 440.39 kJ m^{-2} (±SEM 49.32 W m^{-2}), whereas UVB-normal fish received 3.89 kJ m^{-2} (±SEM 0.47 W m^{-2}) as daily and 264.80 kJ m^{-2} (±SEM 32.19 W m^{-2}) as the absolute dose (Table 1).

Sampling

Immune tests were conducted 66 days (04 April 2013) and 70 days (08 April 2013), respectively, after UVB lamps were switched on for the first time. One day before the tests, fish were transferred to the laboratory in their holding groups and kept in plastic tanks similar in size to the outdoor enclosures (L 39 cm × W 22 cm × H 25 cm) at a light–dark cycle of 11L:13D and a temperature of 15 ± 1 °C. At the time when fish were transferred from the outdoor tanks to the laboratory, the difference in water temperature between outdoor and laboratory conditions was between 1 and 3 °C. Illumination was provided by fluorescent tubes (Natural Daylight 5500, 36 W, 120 cm; TrueLight). Prior to dissection, the standard length (SL) (to the nearest mm) and body mass (to the nearest mg) of each individual were measured to calculate the body condition index (BCI) after Bolger and Connolly (1989). Changes in SL and BCI from the beginning to the end of

Table 1 Maximum and mean irradiance (W m^{-2}), daily dose (kJ m^{-2}) and absolute dose (kJ m^{-2}) of ultraviolet-A (UVA) and ultraviolet-B (UVB) radiation used in the exposure treatments

Measurement	Treatment			
	UV-enhanced		UV-normal	
Spectral range	UVB	UVA	UVB	UVA
Maximum irradiance (W m^{-2})	0.76 (±0.30)	8.27 (±1.14)	0.48 (±0.29)	8.67 (±1.32)
Mean irradiance (W m^{-2})	0.33 (±0.12)	3.47 (±0.61)	0.21 (±0.08)	3.69 (±0.70)
Daily dose (kJ m^{-2})	6.48 (±0.73)	64.12 (±7.08)	3.89 (±0.47)	67.96 (±8.12)
Absolute dose (kJ m^{-2})	440.39 (±49.32)	4360.39 (±481.51)	264.80 (±32.19)	4621.32 (±551.99)

Mean values ± SEM are given

the experimental phase were calculated for each individual separately ($\Delta SL = (SL_{end} - SL_{start}) / SL_{start}$; $\Delta BCI = (BCI_{end} - BCI_{start}) / BCI_{start}$). Fish were then killed by incision of the brain after being anaesthetized by a blow on the head. Because sexes in sticklebacks are monomorphic outside the breeding season sexes were identified histologically using gonad tissue. Head kidneys were removed and transferred into 1.5-ml tubes containing 800 µl R-90 (RPMI 1640 with 10% Millipore H_20). Samples were stored on ice for 6 ± 1 h before being used for further analyses. The mass of the spleen, a lymphoid organ involved in adaptive immunity (Zapata et al. 2006), was measured (to the nearest 0.1 mg) to calculate the splenosomatic index [SSI = 100 × spleen mass/fish mass (Kurtz et al. 2007)]. In total, 47 fish were randomly chosen out of all outdoor tanks and used for *G/L* ratio analyses, 24 (8 males and 16 females) of the UVB-enhanced and 23 (8 males and 15 females) of the UVB-normal treatment. From each enclosure, two sticklebacks were used, except for one enclosure of the UVB-enhanced treatment, where only one fish could be analysed due to damage of the head kidney during dissection. Forty-six fish (8 males and 38 females) from the UVB-enhanced treatment and 56 fish (9 males and 47 females) from the UVB-normal treatment were used for calculating BCI and SSI, respectively. Due to harsh winter conditions during the experimental phase, 42 fish died, 26 from the UVB-enhanced and 16 from UVB-normal treatment. Mortality was not significantly different between treatments (Chi square test, $N_{deathsUVB\text{-}enhanced} = 26$, $N_{deathsUVB\text{-}normal} = 16$, $\chi^2 = 2.381$, $df\,1$, $P = 0.123$). During dissection, all fish were visually checked for internal macroparasites, including an examination of the lens to check for eye flukes.

Flow cytometric analyses and granulocyte-to-lymphocyte ratio

For flow cytometric analyses, samples were transferred to the Institute for Evolution and Biodiversity at the University of Münster, Germany. Cell suspensions from head kidney leucocytes (HKL) were made by forcing tissues through 40 µm nylon screen cell strainers (BD-Falcon, USA) and transferred into 96-deep-well plates. Samples were washed twice at 4 °C with R-90 and resuspended to a volume of 1 ml (Scharsack et al. 2004). Differential cell numbers of HKL were determined by a flow cytometer (BD FACS Canto II; Becton–Dickinson, USA) according to their forward and side scatter values (FSC/SSC characteristics). Analyses were performed using the Software FACS DIVA version 6.1.2 software (Becton–Dickinson, USA). Cellular debris and aggregated cells were identified by their scatter characteristics and excluded from further evaluation. Ten microlitre of each cell suspension were supplemented with 45 µl Sheath Fluid (BD Flow), 20 µl propidium iodide solution (10 mg l^{-1}, Sigma Aldrich) and 25 µl Latex-Beads (Beads, 30,000/25 µl Flow). Dead cells (propidium iodide positive) were not included in further analyses. To obtain information regarding the relative activity of the innate versus the adaptive immune system, the granulocyte-to-lymphocyte ratio (*G/L* ratio) was estimated by using forward and side scatter values (FSC/SSC characteristics) to identify proportions of granulocytes and lymphocytes (for detailed methods see Scharsack et al. 2004).

Statistical analyses

Analyses were conducted in R 3.3.0 statistical package (R Core Team 2016). Data were tested for normality using Shapiro–Wilk tests. *G/L* ratio, and SSI data were Box–Cox-transformed (Box and Cox 1964) to meet the assumptions of normality. Linear mixed-effect models were fitted using the 'lme' function in the 'nlme' library (Pinheiro et al. 2009). *G/L* ratio, ΔBCI and SSI were used as dependent variables, respectively. Treatment (UVB-enhanced, UVB-normal) and sex were included as fixed factors in each model. Additionally, BCI_{end} was included in the models regarding *G/L* ratio and SSI. The initial models included interactions between treatment and sex as well as treatment and BCI_{end}. Hierarchical random effects were used by nesting enclosure within outdoor tank as random factors. Non-significant interactions and explanatory variables were removed by using a backward stepwise model reduction (e.g. Engqvist 2005). Therefore, the statistical significance of the interaction terms was determined by comparing the full model, including the interaction terms, to a model without the corresponding interaction term. The statistical significance of each explanatory variable was tested by removing variables in the order of their statistical relevance and comparing models with and without the variable of interest (e.g. Engqvist 2005; Mehlis and Bakker 2014; Hiermes et al. 2015a). Tests of significance were based on likelihood-ratio tests. To test

for ΔBCI deviations from zero, indicating no change, intercept models were conducted for each group separately with enclosure nested in outdoor tank as random factors. Initial standard length (SL) and ΔSL were not normally distributed and failed to respond to transformation; therefore, nonparametric Wilcoxon rank-sum tests were used to compare fish from both treatments.

Results

Body variables

Standard length (SL), body mass and body condition (BCI) did not significantly differ between the two exposure groups at the beginning of the experimental phase (SL: Wilcoxon signed-rank tests, $N_{\text{UVB-enhanced}} = 46$, $N_{\text{UVB-normal}} = 55$, $W = 1606.5$, $P = 0.884$; body mass: LME, $N_{\text{UVB-enhanced}} = 46$, $N_{\text{UVB-normal}} = 55$, $\chi^2 = 2.289$, $P = 0.130$; BCI: LME, $N_{\text{UVB-enhanced}} = 46$, $N_{\text{UVB-normal}} = 55$, $\chi^2 = 1.179$, $P = 0.278$). The change in standard length (ΔSL) was significantly lower for fish from the UVB-enhanced group compared to fish from the UVB-normal group (Wilcoxon signed-rank test, $N_{\text{UVB-enhanced}} = 46$, $N_{\text{UVB-normal}} = 55$, $W = 725.5$, $P < 0.001$). Fish exposed to additional UVB showed a significantly smaller change in body condition index (lower ΔBCI) compared to fish without additional UVB (LME, $N_{\text{UVB-enhanced}} = 46$, $N_{\text{UVB-normal}} = 55$, $\chi^2 = 10.237$, $P = 0.001$; Table 2; Fig. 2a). Within the UVB-enhanced treatment group body condition decreased significantly (LME, $N_{\text{UVB-enhanced}} = 46$, intercept estimate $= -0.029$, $df = 31$, $t = -2.609$, $P = 0.014$; Fig. 2a), whereas no significant change in ΔBCI was observed within the UVB-normal treatment group (LME, $N_{\text{UVB-normal}} = 55$, intercept estimate $= 0.016$, $df = 43$, $t = 1.559$, $P = 0.126$; Fig. 2a). The interaction between treatment and sex as well as sex as explanatory variable did not have a significant influence on ΔBCI, and therefore, both were not included in the best-explaining model (LME, $N_{\text{UVB-enhanced}} = 46$, $N_{\text{UVB-normal}} = 55$, all $P > 0.682$; Table 2).

Immune variables

Splenosomatic index was influenced by sex with males having a significantly smaller relative spleen size

Table 2 All linear mixed-effect models calculated

Dependent variable	Explanatory variable														Random factors
	Treatment			Sex			BCI_{end}			Treatment × Sex			Treatment × BCI_{end}			
	χ^2	Δdf	P	χ^2	Δdf	P	χ^2	Δdf	P	χ^2	Δdf	P	χ^2	Δdf	P	
ΔBCI	10.237	1	**0.001**	0.168	1	0.682	–	–	–	2.494	1	0.114	–	–	–	Outdoor tank/enclosure
SSI	6.672	1	**0.010**	8.043	1	**0.005**	0.163	1	0.687	0.334	1	0.563	0.195	1	0.659	Outdoor tank/enclosure
G/L ratio	27.880	1	**<0.001**	0.119	1	0.730	3.614	1	0.057	0.068	1	0.794	1.229	1	0.268	Outdoor tank/enclosure

Significant results are printed in bold ($P < 0.05$)

Change in body condition (ΔBCI), splenosomatic index (SSI), and the granulocyte-to-lymphocyte ratio (*G/L* ratio) were used as dependent variable

Treatment, sex, body condition (BCI) and the interactions between treatment and sex (Treatment × Sex) as well as between treatment and body condition (Treatment × BCI_{end}) were used as explanatory variables. Hierarchical random effects were used in each model by nesting enclosure within outdoor tank as random factors

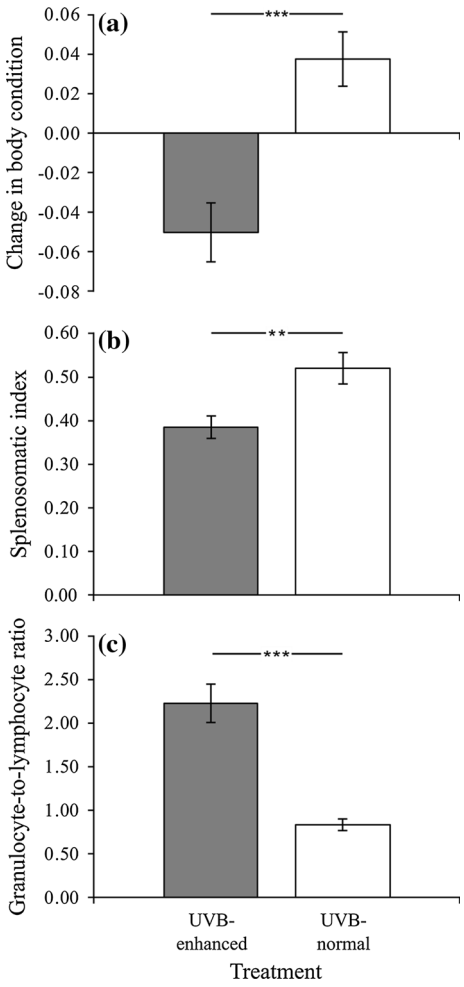


Fig. 2 Effects of the exposure treatments UVB-enhanced (*grey*) and UVB-normal (*white*) on **a** changes in body condition (ΔBCI), **b** splenosomatic index (SSI) and **c** the granulocyte-to-lymphocyte ratio (*G*/*L* ratio). Non-transformed data are presented for visual purposes only. Mean values ± SEM are shown. $^{**}P < 0.01$; $^{***}P < 0.001$

(LME, $N_{\text{UVB-enhanced}} = 46$, $N_{\text{UVB-normal}} = 55$, $\chi^2 = 8.043$, $P = 0.005$; Table 2). Independent of sex, UVB-normal fish had a significantly higher SSI compared to fish from the UVB-enhanced treatment (LME, $N_{\text{UVB-enhanced}} = 46$, $N_{\text{UVB-normal}} = 55$, $\chi^2 = 6.672$, $P = 0.010$; Table 2; Fig. 2b), but the interaction between sex and treatment was not significant and therefore not included in the best-explaining model (LME, $N_{\text{UVB-enhanced}} = 46$, $N_{\text{UVB-normal}} = 55$, $\chi^2 = 0.334$, $P = 0.563$; Table 2).

Furthermore, there was no significant interaction between BCI_{end} and treatment as well as no significant influence of BCI_{end} on SSI (LME, $N_{\text{UVB-enhanced}} = 46$, $N_{\text{UVB-normal}} = 55$, all $P > 0.659$; Table 2).

There was no significant interaction between treatment and sex as well as treatment and BCI_{end} regarding the *G*/*L* ratio (treatment × sex: LME, $N_{\text{UVB-enhanced}} = 24$, $N_{\text{UVB-normal}} = 23$, $\chi^2 = 0.068$, $P = 0.794$; treatment × BCI_{end}: LME, $N_{\text{UVB-enhanced}} = 24$, $N_{\text{UVB-normal}} = 23$, $\chi^2 = 1.229$, $P = 0.268$; Table 2). Sex and BCI_{end} were not included in the best-explaining model as they did not have a significant influence on *G*/*L* ratio (LME, $N_{\text{UVB-enhanced}} = 24$, $N_{\text{UVB-normal}} = 23$, all $P > 0.057$).

Sticklebacks from the UVB-enhanced treatment had a significantly higher *G*/*L* ratio (LME, $N_{\text{UVB-enhanced}} = 24$, $N_{\text{UVB-normal}} = 23$, $\chi^2 = 27.880$, $P < 0.001$; Table 2; Fig. 2c), representing a relatively reduced activation of the adaptive immune system.

Parasite load

All sticklebacks were free from external and internal macroparasites, and no eye flukes were observed in the lenses.

Discussion

Persistent exposure of sticklebacks to increased, but ecologically relevant, levels of UVB radiation had strong effects on body condition variables and immune function. In contrast to previous studies on long-term UVB exposure, seminatural holding and exposure conditions were chosen. Experimental animals received chronic UVB doses in the form of daily exposure while remaining in their holding tanks. First, after being regularly exposed to enhanced UVB levels for two months, subadult sticklebacks showed reduced growth and loss in body condition compared to individuals that were exposed to natural solar conditions. Fish from both treatment groups were kept in equal-sized groups under identical temperature and food regimes. Consequently, the reduced growth as well as the decrease in body condition in UVB-exposed fish suggests that these individuals had to invest a higher amount of available resources in processes other than growth and somatic maintenance. Trade-offs regarding the investment in different life-history components are presumed to be the result of differential allocation of limited resources between

competing physiological needs (Monaghan et al. 2009) and become more pronounced under stressful conditions (e.g. Alonso-Alvarez et al. 2006). Direct effects of increased UVB exposure include damage to lipids, nucleic acids and proteins (Bancroft et al. 2007), whereas indirect effects are related to the formation of reactive oxygen species (ROS) (e.g. Seebacher et al. 2016), leading to oxidative damage to proteins and membranes (Lesser et al. 2001). Thus, exposure to UVB radiation can cause cell damage, resulting in energetically costly cell repair mechanisms or apoptosis (Groff et al. 2010), which might explain the lower body condition of sticklebacks exposed to enhanced UVB levels.

In addition to the negative effects on body variables, sticklebacks from the UVB-enhanced group had a lower splenosomatic index compared to control fish. The spleen, as a lymphoid organ, plays a major role in adaptive immunity (Zapata et al. 2006; Kurtz et al. 2007), and spleen size has frequently been used as an intraspecific measurement of immunocompetence in studies using fish (Skarstein et al. 2001; Kortet et al. 2003; Ottová et al. 2005). The SSI is reported to be positively related to the state of immune activation when being infected with a chronic parasite (Seppänen et al. 2009), thereby referring to an increased adaptive immunity. In the present study, the SSI was found to be lower in UVB-exposed sticklebacks and consequently may indicate a reduced investment in adaptive immunity, but this requires further investigation.

Sex differences in spleen sizes, with males having smaller spleens, have been shown before in various species of birds (e.g. Møller et al. 1998; Roberts et al. 2004) and also in mammals (Fernández-Llario et al. 2004). In birds, males are predicted to be subject to androgen-induced immunosuppression, resulting in a reduced spleen size (Møller et al. 1998). However, these differences were found to be age-dependent, occurring only in sexually mature birds (Møller et al. 1998). In the present study, subadult sticklebacks in a monomorphic non-reproductive stage were used. Nevertheless, given that data collection took place in early April shortly before the start of the breeding season, differences in androgen levels cannot be completely ruled out. Thus, a potentially enhanced level of testosterone in males may be responsible for the sex difference in SSI. Testosterone levels are associated with aggressiveness in fish (Li et al. 2014), and the so-called pre-breeding aggression described for sticklebacks (Bakker 1994) may be beneficial in the impending phase of territory occupation. However, the *G*/*L* ratio was not significantly influenced by sex which stays in contrast to previous studies showing that testosterone suppresses innate immunity (e.g. Kurtz et al. 2007). This may suggest that males in the present study were not reproductively active.

Permanent stimulation of the innate immune system in fish from the UVB-enhanced group could have led to a shift towards innate immune responses resulting in a reduced splenosomatic index. The assumed trade-off between innate and adaptive immunity (Norris and Evans 2000) is also supported by the higher *G*/*L* ratio in sticklebacks from the UVB-enhanced group compared to fish from the UVB-normal group. The higher granulocyte-to-lymphocyte ratio in UVB-enhanced fish indicates a relatively increased activity of the innate compared to the adaptive immune system in response to a long-term exposure to enhanced UVB radiation. Although no visible signs of skin alterations such as sunburn were observed in the present study, UVB could have induced inflammatory responses (i.e. erythema). Similar effects have been found in mammals, in which the granulocyte–macrophage colony-stimulating factor (GM-CSF) was shown to be introduced in keratinocytes after exposition to ultraviolet radiation (Imokawa et al. 2015).

In fish, it has been demonstrated that inflammatory processes like those caused by enteric helminths in the digestive tract mainly involve the innate immunity (Dezfuli et al. 2016). In the present study, long-term application of UVB radiation may have promoted inflammatory processes, which are accompanied by a permanent activation of the innate immune system and therefore may have led to its intensification. It is important to note that due to the fact that fish were reared in a closed tank system, the adaptive immune system was not particularly stimulated as there was no contact to parasites and contagious diseases, and even though sticklebacks were wild-caught, no internal macroparasites were observed during dissection. Reinforcing the relative activation of the innate immune system (higher *G*/*L* ratio) may be beneficial for an organism when dealing with an environmental stress factor, such as enhanced UVB. Altered immune activation in fish caused by long-term UVB radiation has been shown for juvenile Atlantic salmon where it affected the plasma immunoglobulin concentration,

which is affiliated with the adaptive immune system (Jokinen et al. 2008). Considering the well-studied connection between the innate and adaptive immunity (reviewed in Tort et al. 2003; Magnadóttir 2006), effects of the interactions between these two components of the immune system deserve attention and have consequences for the whole organism. Particularly with regard to photic conditions in the context of environmental change, the present study shows how enhanced UVB radiation under seminatural conditions affects the investment in innate and adaptive immunity.

In summary, the present findings reveal a UVB-induced shifted balance of the immune system towards the innate immunity. It can thus be assumed that UVB radiation as an environmental stressor has a strong impact on the defence mechanisms against viral infections and parasites by reducing the adaptive immune response. Further studies using multiple stressors are required to examine potential interactive effects of different abiotic and biotic factors on the immune system. For instance, it has been shown for juvenile Atlantic salmon that UVB radiation combined with increased temperature revealed additive effects on the plasma immunoglobulin concentration (Jokinen et al. 2011). Additional demands on the innate immunity, as those observed in the present study, are very costly for the whole organism and could eventually lead to a reduced fitness as shown for mosquitoes (Ahmed et al. 2002). Although resource availability was sufficient and additional stressors such as predation risk were excluded in the present study, individuals could not compensate for the negative effects of an enhanced UVB radiation. How the described effects will occur under natural conditions needs further investigation.

Acknowledgements We are grateful to Frederik Franke for laboratory assistance and the Bakker research group for discussion.

References

Ahmed A, Baggott S, Maingon R, Hurd H (2002) The costs of mounting an immune response are reflected in the reproductive fitness of the mosquito *Anopheles gambiae*. Oikos 97:371–377

Alonso-Alvarez C et al (2006) An experimental manipulation of life-history trajectories and resistance to oxidative stress. Evolution 60:1913–1924

Arts MT, Browman HI, Jokinen IE, Skiftesvik AB (2010) Effects of UV radiation and diet on polyunsaturated fatty acids in the skin, ocular tissue and dorsal muscle of atlantic salmon (*Salmo salar*) held in outdoor rearing tanks. Photochem Photobiol 86:909–919

Bakker TCM (1994) Evolution of aggressive behaviour in the threespine stickleback. In: Bell MA, Foster SA (eds) The evolutionary biology of the threespine stickleback. Oxford University Press, Oxford, pp 345–380

Bancroft BA, Baker NJ, Blaustein AR (2007) Effects of UVB radiation on marine and freshwater organisms: a synthesis through meta-analysis. Ecol Lett 10:332–345

Blount JD, Pike TW (2012) Deleterious effects of light exposure on immunity and sexual coloration in birds. Funct Ecol 26:37–45

Bolger T, Connolly PL (1989) The selection of suitable indices for the measurement and analysis of fish condition. J Fish Biol 34:171–182

Bothwell ML, Sherbot DMJ, Pollock CM (1994) Ecosystem response to solar ultraviolet-b radiation: influence of trophic-level interactions. Science 265:97–100

Box GE, Cox DR (1964) An analysis of transformations. J R Stat Soc Ser B (Stat Method) 26:211–252

Carreja B, Fernández M, Agustí S (2016) Joint additive effects of temperature and UVB radiation on zoeae of the crab *Taliepus dentatus*. Mar Ecol Prog Ser 550:135–145

Ceccato E, Cramp RL, Seebacher F, Franklin CE (2016) Early exposure to ultraviolet-B radiation decreases immune function later in life. Conserv Physiol 4:cow037

Cramp RL, Reid S, Seebacher F, Franklin CE (2014) Synergistic interaction between UVB radiation and temperature increases susceptibility to parasitic infection in a fish. Biol Lett 10:20140449

Dahms H-U, Lee J-S (2010) UV radiation in marine ectotherms: molecular effects and responses. Aquat Toxicol 97:3–14

Debecker S, Sommaruga R, Maes T, Stoks R (2015) Larval UV exposure impairs adult immune function through a trade-off with larval investment in cuticular melanin. Funct Ecol 29:1292–1299

Dezfuli B, Bosi G, DePasquale J, Manera M, Giari L (2016) Fish innate immunity against intestinal helminths. Fish Shellfish Immunol 50:274–287

Engqvist L (2005) The mistreatment of covariate interaction terms in linear model analyses of behavioural and evolutionary ecology studies. Anim Behav 70:967–971

Fernández-Llario P, Parra A, Cerrato R, De Mendoza JH (2004) Spleen size variations and reproduction in a Mediterranean population of wild boar (*Sus scrofa*). Eur J Wildl Res 50:13–17

Flajnik MF, Kasahara M (2010) Origin and evolution of the adaptive immune system: genetic events and selective pressures. Nat Rev Genet 11:47–59

Fukunishi Y, Masuda R, Robert D, Yamashita Y (2013) Comparison of UV-B tolerance between wild and hatchery-reared juveniles of red sea bream (*Pagrus major*) and black sea bream (*Acanthopagrus schlegeli*). Environ Biol Fishes 96:13–20

Groff AA et al (2010) UVA/UVB-induced genotoxicity and lesion repair in *Colossoma macropomum* and *Arapaima gigas* Amazonian fish. J Photochem Photobiol B Biol 99:93–99

Häder DP, Kumar HD, Smith RC, Worrest RC (1998) Effects on aquatic ecosystems. J Photochem Photobiol B Biol 46:53–68

Häder D-P, Kumar H, Smith R, Worrest R (2007) Effects of solar UV radiation on aquatic ecosystems and interactions with climate change. Photochem Photobiol Sci 6:267–285

Hiermes M, Mehlis M, Rick IP, Bakker TCM (2015a) Habitat-dependent olfactory discrimination in three-spined sticklebacks (*Gasterosteus aculeatus*). Anim Cogn 18:839–846

Hiermes M, Vitt S, Rick IP, Bakker TCM (2015b) Shoal choice and ultraviolet reflections in stickleback populations from different photic habitats. Biol J Linn Soc 116:761–772

Imokawa G, Nakajima H, Ishida K (2015) Biological mechanisms underlying the ultraviolet radiation-induced formation of skin wrinkling and sagging II: over-expression of neprilysin plays an essential role. Int J Mol Sci 16:7776–7795

Jokinen EI, Salo HM, Markkula SE, Immonen AK, Aaltonen TM (2001) Ultraviolet B irradiation modulates the immune system of fish (*Rutilus rutilus*, Cyprinidae). Part III: lymphocytes. Photochem Photobiol 73:505–512

Jokinen IE, Markkula ES, Salo HM, Kuhn P, Nikoskelainen S, Arts MT, Browman HI (2008) Exposure to increased ambient ultraviolet B radiation has negative effects on growth, condition and immune function of juvenile Atlantic salmon (*Salmo salar*). Photochem Photobiol 84:1265–1271

Jokinen IE, Salo HM, Markkula E, Rikalainen K, Arts MT, Browman HI (2011) Additive effects of enhanced ambient ultraviolet B radiation and increased temperature on immune function, growth and physiological condition of juvenile (parr) Atlantic salmon, *Salmo salar*. Fish Shellfish Immunol 30:102–108

Kortet R, Taskinen J, Sinisalo T, Jokinen I (2003) Breeding-related seasonal changes in immunocompetence, health state and condition of the cyprinid fish, *Rutilus rutilus*, L. Biol J Linn Soc 78:117–127

Kouwenberg J, Browman H, Cullen J, Davis R, St-Pierre J-F, Runge J (1999) Biological weighting of ultraviolet (280–400 nm) induced mortality in marine zooplankton and fish. I. Atlantic cod (*Gadus morhua*) eggs. Mar Biol 134:269–284

Kurtz J, Kalbe M, Langefors S, Mayer I, Milinski M, Hasselquist D (2007) An experimental test of the immunocompetence handicap hypothesis in a teleost fish: 11-ketotestosterone suppresses innate immunity in three-spined sticklebacks. Am Nat 170:509–519

Lesser MP, Farrell JH, Walker CW (2001) Oxidative stress, DNA damage and p53 expression in the larvae of Atlantic cod (*Gadus morhua*) exposed to ultraviolet (290–400 nm) radiation. J Exp Biol 204:157–164

Li C-Y, Earley RL, Huang S-P, Hsu Y (2014) Fighting experience alters brain androgen receptor expression dependent on testosterone status. Proc R Soc B 281:20141532

Llabrés M, Agustí S, Fernández M, Canepa A, Maurin F, Vidal F, Duarte CM (2013) Impact of elevated UVB radiation on marine biota: a meta-analysis. Glob Ecol Biogeogr 22:131–144

Magnadóttir B (2006) Innate immunity of fish (overview). Fish Shellfish Immunol 20:137–151

Markkula SE, Salo HM, Immonen AK, Jokinen EM (2005) Effects of short- and long-term ultraviolet B irradiation on the immune system of the common carp (*Cyprinus carpio*). Photochem Photobiol 81:595–602

Markkula SE, Salo HM, Rikalainen A-K, Jokinen EI (2006) Different sensitivity of carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*) to the immunomodulatory effects of UVB irradiation. Fish Shellfish Immunol 21:70–79

Markkula E, Salo HM, Rikalainen K, Jokinen IE (2009) Long-term UVB Irradiation Affects the Immune Functions of Carp (*Cyprinus carpio*) and Rainbow Trout (*Oncorhynchus mykiss*). Photochem Photobiol 85:347–352

Mehlis M, Bakker TCM (2014) The influence of ambient water temperature on sperm performance and fertilization success in three-spined sticklebacks (*Gasterosteus aculeatus*). Evol Ecol 28:655–667

Modarressie R, Rick IP, Bakker TCM (2006) UV matters in shoaling decisions. Proc R Soc Lond B 273:849–854

Møller AP, Sorci G, Jø Erritz (1998) Sexual dimorphism in immune defense. Am Nat 152:605–619

Monaghan P, Metcalfe NB, Torres R (2009) Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. Ecol Lett 12:75–92

Norris K, Evans MR (2000) Ecological immunology: life history trade-offs and immune defense in birds. Behav Ecol 11:19–26

Ottová E, Šimková A, Jurajda P, Dávidová M, Ondračková M, Pečínková M, Gelnar M (2005) Sexual ornamentation and parasite infection in males of common bream (*Abramis brama*): a reflection of immunocompetence status or simple cost of reproduction? Evol Ecol Res 7:581–593

Pinheiro J, Bates D, DebRoy S, Sarkar D (2009) The R Core team (2009) nlme: linear and nonlinear mixed effects models. R package version 3.1-96. R Foundation for Statistical Computing, Vienna

R Core Team (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Reuder J, Dameris M, Koepke P (2001) Future UV radiation in Central Europe modelled from ozone scenarios. J Photochem Photobiol B Biol 61:94–105

Rick IP, Bakker TCM (2008) Males do not see only red: UV wavelengths and male territorial aggression in the three-spined stickleback (*Gasterosteus aculeatus*). Naturwissenschaften 95:631–638

Rick IP, Bakker TCM (2010) Ultraviolet light influences habitat preferences in a fish under predation risk. Evol Ecol 24:25–37

Rick IP, Modarressie R, Bakker TCM (2006) UV wavelengths affect female mate choice in three-spined sticklebacks. Anim Behav 71:307–313

Rick IP, Bloemker D, Bakker TCM (2012) Spectral composition and visual foraging in the three-spined stickleback (Gasterosteidae: *Gasterosteus aculeatus* L.): elucidating the role of ultraviolet wavelengths. Biol J Linn Soc 105:359–368

Rick IP, Mehlis M, Eßer E, Bakker TCM (2014) The influence of ambient ultraviolet light on sperm quality and sexual ornamentation in three-spined sticklebacks (*Gasterosteus aculeatus*). Oecologia 174:393–402

Roberts ML, Buchanan KL, Evans MR (2004) Testing the immunocompetence handicap hypothesis: a review of the evidence. Anim Behav 68:227–239

Rowe MP, Baube CL, Loew ER, Phillips JB (2004) Optimal mechanisms for finding and selecting mates: how three-spine stickleback (*Gasterosteus aculeatus*) should encode male throat colors. J Comp Physiol A 190:241–256

Salo HM, Aaltonen TM, Markkula SE, Jokinen EI (1998) Ultraviolet B irradiation modulates the immune system of fish (*Rutilus rutilus*, Cyprinidae). I. Phagocytes. Photochem Photobiol 67:433–437

Salo HM, Jokinen EI, Markkula SE, Aaltonen TM (2000a) Ultraviolet B irradiation modulates the immune system of fish (*Rutilus rutilus*, Cyprinidae) II: blood. Photochem Photobiol 71:65–70

Salo HM, Jokinen EI, Markkula SE, Aaltonen TM, Penttilä HT (2000b) Comparative effects of UVA and UVB irradiation on the immune system of fish. J Photochem Photobiol B Biol 56:154–162

Scharsack JP, Kalbe M, Derner R, Kurtz J, Milinski M (2004) Modulation of granulocyte responses in three-spined sticklebacks *Gasterosteus aculeatus* infected with the tapeworm *Schistocephalus solidus*. Dis Aquat Org 59:141–150

Seebacher F, Kazerouni EG, Franklin CE (2016) Ultraviolet B radiation alters movement and thermal selection of zebrafish (*Danio rerio*). Biol Lett 12:20160258

Seppänen E, Kuukka H, Voutilainen A, Huuskonen H, Peuhkuri N (2009) Metabolic depression and spleen and liver enlargement in juvenile Arctic charr *Salvelinus alpinus* exposed to chronic parasite infection. J Fish Biol 74:553–561

Sinha RP, Häder D-P (2002) UV-induced DNA damage and repair: a review. Photochem Photobiol Sci 1:225–236

Skarstein F, Folstad I, Liljedal S (2001) Whether to reproduce or not: immune suppression and costs of parasites during reproduction in the Arctic charr. Can J Zool 79:271–278

Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues ASL, Fischman DL, Waller RW (2004) Status and trends of amphibian declines and extinctions worldwide. Science 306:1783–1786

Subramani PA, Hameed B, Michael RD (2015) Effect of UV-B radiation on the antibody response of fish: implication on high altitude fish culture. J Photochem Photobiol B Biol 143:1–4

Sucré E, Vidussi F, Mostajir B, Charmantier G, Lorin-Nebel C (2011) Impact of ultraviolet-B radiation on planktonic fish larvae: alteration of the osmoregulatory function. Aquat Toxicol 109:194–201

Tort L, Balasch J, Mackenzie S (2003) Fish immune system. A crossroads between innate and adaptive responses. Inmunologia 22:277–286

Uribe C, Folch H, Enriquez R, Moran G (2011) Innate and adaptive immunity in teleost fish: a review. Vet Med 56:486–503

Uberoi A, Yoshida S, Frazer IH, Pitot HC, Lambert PF (2016) Role of ultraviolet radiation in papillomavirus-induced disease. PLoS Pathog 12:e1005664

Ullrich SE (2016) Mechanisms by which UV radiation, a natural component of sunlight, suppresses the immune response. In: Esser C (ed) Environmental influences on the immune system. Springer, Vienna, pp 155–179

Williamson CE, Rose KC (2010) When UV meets fresh water. Science 329:637–639

Williamson CE et al (2014) Solar ultraviolet radiation in a changing climate. Nat Clim Change 4:434–441

Wootton RJ (1984) A functional biology of sticklebacks. Croom Helm, London

Zapata A, Diez B, Cejalvo T, Gutierrez-de Frias C, Cortes A (2006) Ontogeny of the immune system of fish. Fish Shellfish Immunol 20:126–136