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Animal behaviour

Long-term UVB exposure promotes predator-inspection behaviour in a fish

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Ultraviolet-B radiation (UVB) reaching the earth's surface has increased due to human-caused stratospheric ozone depletion. Whereas the harmful effects of UVB on aquatic organisms are well studied at the molecular and cellular level, recent studies have also begun to address behavioural changes caused by sublethal amounts of UVB. However, the behavioural consequences of long-term exposure to ecologically relevant UVB levels over several life stages are virtually unknown, particularly with regard to predator–prey behaviour. We found increased predator-inspection behaviour together with a smaller body length in three-spined sticklebacks (*Gasterosteus aculeatus*) after fish were exposed for about seven months to natural sunlight conditions with enhanced UVB, compared with full siblings exposed to natural sunlight only. The observed change in antipredator behaviour may reflect a direct behavioural response mediated through UVB-induced oxidative stress during development. Alternatively, the smaller body size in UVB-exposed fish may result in an increased inspection effort allowing them to spend more time foraging. Our findings suggest that, within the scope of environmental change, UVB radiation constitutes an important stress factor by eliciting behavioural responses that influence crucial ecological processes, such as predator–prey interactions.

1. Introduction

Ultraviolet radiation, and especially short-wavelength ultraviolet-B radiation (UVB, 280–320 nm), constitutes an important environmental factor that negatively affects organisms (e.g. [1]). UVB exposure of organisms living in aquatic habitats is expected to be influenced by climate change, particularly through recent and ongoing stratospheric ozone depletion, alterations in cloud cover and acidification [1]. UVB radiation causes direct damage to DNA and has indirect effects at molecular and cellular levels by inducing the formation of reactive oxygen species (ROS) [2]. Furthermore, UVB affects development, fecundity and physiology at the whole-organism level with potentially far-reaching ecological consequences (e.g. [1,3]).

In fishes, in addition to physiological responses to increased UVB levels [4], recent studies confirmed that UV can influence behaviours involved in predator–prey interactions in terms of a reduced escape performance in prey fish [5], a decrease in prey detection [6], as well as alterations in locomotion [7] and habitat selection [8]. However, these laboratory studies have focused mainly on consequences either of an acute direct UV exposure (e.g. [4,8]) or on effects following medium-term UVB exposure at larval or adult stages [5–7]. In comparison, it is unknown how long-term exposure to ecologically relevant UVB levels shapes behaviours that play a role in a predation context, although this is necessary for understanding the ecological consequences of chronic environmental stress for predator–prey interactions.

Table 1. Mean irradiance at midday (W m^{-2}) and daily dose (kJ m^{-2}) of ultraviolet-A (UVA) and ultraviolet-B (UVB) radiation used in the exposure treatments.

measurement	UVB-winter conditions				UVB-summer conditions			
	UV$_{enhanced}$		UV$_{normal}$		UV$_{enhanced}$		UV$_{normal}$	
spectral range	UVA	UVB	UVA	UVB	UVA	UVB	UVA	UVB
mean irradiance – midday (W m^{-2})	2.14	0.36	2.04	0.11	3.68	0.47	3.31	0.27
daily dose (kJ m^{-2})	45.58	2.36	45.20	1.46	85.23	5.85	82.42	4.27

When threatened by predators, behavioural responses of prey individuals may include direct predator avoidance or antipredator behaviour [9]. A widespread, but seemingly counterintuitive form of antipredator behaviour is predator inspection, where one or more potential prey individuals approach a predator while benefiting from gaining information about the predatory threat (e.g. [10]).

In our study species, the three-spined stickleback (*Gasterosteus aculeatus*), predator-inspection behaviour is a well-studied phenomenon in the context of behavioural predator–prey interactions (e.g. [11]). Furthermore, sticklebacks typically inhabit shallow waters where UVB radiation constitutes a significant environmental stressor [3]. Taken together, the aim of the present study was to examine whether long-term exposure to increased, but naturally occurring UVB levels, for several months during the period of major growth, affects predator-inspection behaviour in sticklebacks.

2. Material and methods

(a) Study animals and treatments

Sticklebacks used in this study were laboratory-bred offspring of fish caught from a large anadromous population in Texel, The Netherlands. On 2 September 2015, at an age of three months, 40 fish from each of 28 full-sib families were split into two equally sized groups and transferred to 56 enclosures (20 l) installed in four large (2500 l) outdoor tanks. All fish were fed on six days a week ad libitum with defrosted chironomid larvae.

One group was exposed to enhanced ambient UVB radiation (UVB$_{enhanced}$) while the other group received natural sunlight (UVB$_{normal}$). UVB radiation was applied for 2 h daily for UVB-summer conditions (7 September 2015–18 November 2015) and reduced to 1 h daily for UVB-winter conditions (19 November 2015–14 April 2016). Mean irradiance and daily doses of UVA and UVB are given in table 1. The maximum UVB intensity measured under UVB$_{enhanced}$ conditions reached 0.51 W m^{-2} and was within levels measured in March 2013 during midday under sunny weather conditions at 10 cm water depth in the natural habitat of our study population on the island of Texel (53°114′ N, 4°898′ E), peaking at 0.63 W m^{-2}. See the online electronic supplementary material for methodological details.

For the behavioural experiments, one randomly chosen stickleback out of each enclosure was used ($N_{UVB\text{-}normal} = 28$, $N_{UVB\text{-}enhanced} = 28$). Sixteen rainbow trout *Oncorhynchus mykiss* (standard length (SL) 15.3 ± 1.4 cm) served as potential predators and each trout was used for the maximum of one experimental trial per day.

(b) Predator-inspection experiments

From 30 March 2016 to 14 April 2016, at the age of 10.5 ± 0.5 months, fish were individually tested in an experimental tank (100 × 45 × 35 cm, water level 16.5 cm) consisting of a predator compartment (25 × 45 cm) separated by a fixed sheet of perforated clear plexiglas, an experimental compartment (60 × 45 cm) and a start zone (15 × 45 cm). The inspection zone was located within the experimental compartment, next to the predator compartment (figure 1; see electronic supplementary material for details).

Before each experiment, a transparent partition between the start zone and the experimental compartment and an opaque partition between the inspection zone and the predator compartment were lowered. For the experiments, a trout was placed in the predator compartment and a test fish was introduced in the start zone. After 10 min of acclimation time both partitions were lifted for 30 min of trial time so that the stickleback could enter the experimental compartment (figure 1). Trials were filmed from above using a webcam connected to a laptop. After each experiment, the SL of the test fish was measured and the first dorsal spine was clipped off and used for molecular genetic sexing (for methods, see [12]).

(c) Data analyses

The latency of the test fish to initially leave the start zone and the absolute inspection time (IT$_{abs}$ = absolute time in inspection zone) (following [13]) were quantified using the video recordings. Furthermore, we calculated the relative inspection time (IT$_{rel}$ = IT$_{abs}$/time after leaving start zone) to control for inter-individual differences in the latency to leave the start zone. Moreover, the numbers of visits to the start zone and to the inspection zone were used to calculate absolute activity (activity$_{abs}$ = visits to start zone + visits to inspection zone) and relative activity (activity$_{rel}$ = activity$_{abs}$/time after leaving start zone). Data were analysed using R v. 3.3.0 statistical package [14]. Shapiro–Wilk's tests were performed to test for normality. Except for SL, all variables were Box–Cox transformed to meet the assumptions of normality. Linear mixed-effects models (lme) were applied using the 'lmer' function in the 'lme4' library. Hierarchical random effects were used by nesting family in outdoor tank. A backward stepwise model reduction was conducted by removing the explanatory variables in the order of statistical relevance (e.g. [15]). Tests of significance were based on likelihood-ratio tests.

3. Results

Sticklebacks from the UVB$_{normal}$-group were, independent of sex, significantly larger compared with fish from the UVB$_{enhanced}$-group (lme, $\chi^2 = 22.162$, $p < 0.001$) and, independent of treatment, females ($N = 25$: $N_{UVB\text{-}enhanced} = 11$, $N_{UVB\text{-}normal} = 14$) were significantly larger than males ($N = 31$: $N_{UVB\text{-}enhanced} = 17$, $N_{UVB\text{-}normal} = 14$) (lme, $\chi^2 = 18.092$, $p < 0.001$).

All sticklebacks left the start zone within the experimental time (median: 20.50 s; interquartile range: 5.00–56.25 s) and showed characteristic predator-inspection behaviour in

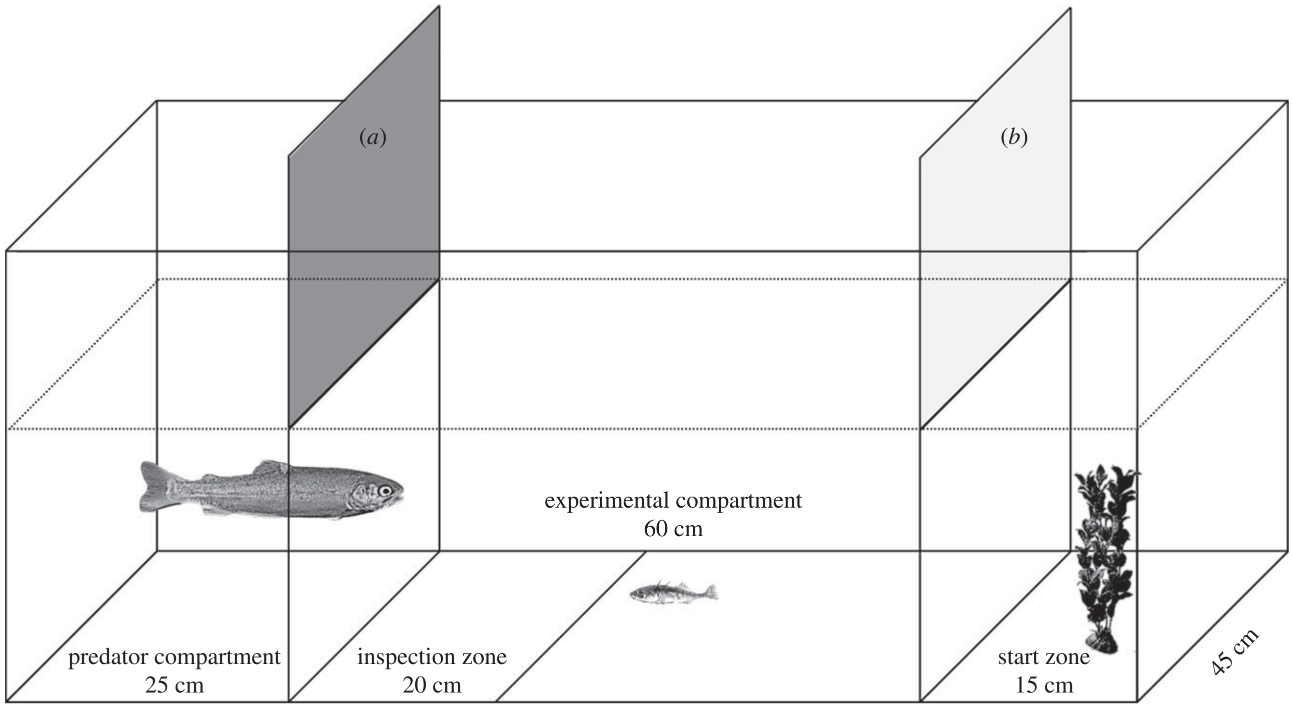

Figure 1. The experimental tank with the predator compartment on the left, the experimental compartment in the middle and the start zone with an artificial plant on the right. The inspection zone is located next to the predator compartment, within the experimental compartment. An opaque partition (*a*) and a transparent partition (*b*) were lowered during the acclimation phase and lifted for the experimental phase.

terms of repeated sequences of approaching the predator, pausing, turning and moving back to the opposite side during all trials. Trout were active and orientated towards the test fish most of the time. Sticklebacks from the $UVB_{enhanced}$-group showed on average a significantly higher absolute (IT_{abs}: lme, $\chi^2 = 5.538$, $p = 0.019$; figure 2) as well as relative inspection time (IT_{rel}: lme, $\chi^2 = 5.213$, $p = 0.022$) and thereby their predator-inspection behaviour was improved compared with UVB_{normal}-fish. Absolute and relative activity were not significantly different between treatments ($activity_{abs}$: lme, $\chi^2 = 0.025$, $p = 0.875$; $activity_{rel}$: lme, $\chi^2 = 0.223$, $p = 0.637$). Moreover, there was no significant difference between treatments with regard to the latency to leave the start zone (lme, $\chi^2 = 2.495$, $p = 0.114$). See electronic supplementary material (table S1) for detailed results.

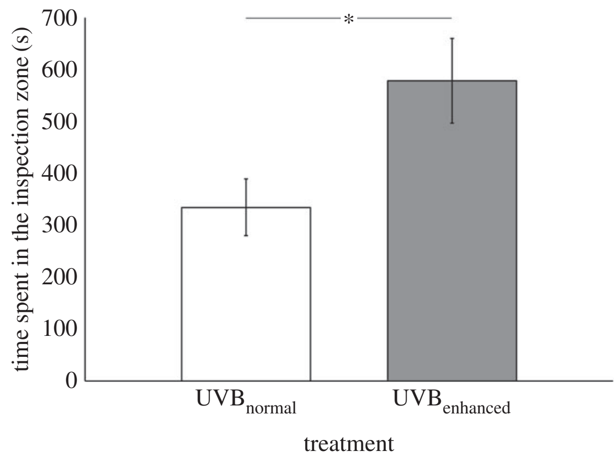

Figure 2. Absolute times (mean $\pm$ s.e.m.) sticklebacks from the UVB_{normal} (white) and $UVB_{enhanced}$ (grey) treatments spent in the inspection zone. Non-transformed data are presented for visual purposes only. $^*p < 0.05$.

4. Discussion

Three-spined sticklebacks showed an increased predator inspection as a consequence of prolonged UVB exposure. In addition, $UVB_{enhanced}$-fish were smaller in size (SL), as has also been shown in a recent study dealing with UVB stress in sticklebacks [3]. This is possibly a result of resource allocation to repair mechanisms of UVB-induced ROS-mediated photodamage at the expense of growth [16].

Although predator-inspection behaviour is risky [17] and smaller fish are subject to a higher predation risk [18], smaller $UVB_{enhanced}$-fish showed an increased inspection compared with larger UVB_{normal}-fish. It can be assumed that UVB-exposed sticklebacks face higher physiological costs, resulting in increased nutritional requirements. Consequently, these individuals may gain a foraging benefit through an increased predator inspection [19]. Moreover, it is important for physically constrained individuals to gather detailed information about a predator, as had been shown for gravid female sticklebacks [10]. Furthermore, the increased inspection could be based on a demonstration of alertness or flight abilities in accordance to the ‘attack deterrence hypothesis’ [20] with smaller sticklebacks being potentially more manoeuvrable. As long as prey individuals benefit from an increased inspection behaviour by gaining more information about a potential predator, they should be able to compensate for their UVB-induced physical disadvantage. However, the net benefit should be outweighed by the costs of an increased mortality risk when the amount of predator-inspection behaviour is very high. Further investigations are needed to elucidate whether UVB-stressed prey individuals ultimately suffer from an increased mortality or not.

Independent of the aforementioned effects of UVB exposition on body size and possibly related behavioural responses, an enhanced formation of ROS may be responsible for the behavioural changes in $UVB_{enhanced}$-fish. Seebacher *et al*. [7] found an increase in ROS-induced damage in UVB-exposed zebrafish (*Danio rerio*) together with a reduced swimming performance at high temperatures compared with fish from a control group. By contrast, activity levels were not different between treatment groups in the present study so that comparable ROS-mediated effects on swimming performance can largely be ruled out. Nevertheless, further research is required to identify the mechanisms and pathways underlying UVB-caused alterations in physiology and behaviour (e.g. [21]).

In summary, long-term exposure to increased but natural levels of UVB radiation is capable of affecting predator–prey interactions through alterations in predator-inspection behaviour in sticklebacks. Short-term UVB exposure was found to affect predator–prey interactions, e.g. through impaired escape performance, as demonstrated in cod larvae (*Gadus morhua*) threatened by two-spotted gobies (*Gobiusculus flavescens*) [5]. In comparison, our findings demonstrate for the first time to our knowledge that long-term exposure to even lower, ecologically relevant UVB levels influences antipredator behaviour in a fish. Given that UVB radiation is abundant in shallow water environments, UVB-mediated changes in ecological processes such as predator–prey interactions are likely to have important consequences for population and community dynamics in these ecosystems.

Ethics. Experiments complied with the current laws of Germany and were approved by the regional office for nature, environment and consumer protection North-Rhine Westphalia (LANUV NRW, reference no. 84-02.04.2015.A580).

Data accessibility. The datasets supporting this article have been uploaded as part of the electronic supplementary material.

Authors' contributions. S.V., J.E.Z., T.C.M.B. and I.P.R. designed the study. S.V. and J.E.Z. collected the data. S.V., J.E.Z. and I.P.R. did the statistical analyses. S.V. wrote the manuscript supported by T.C.M.B., J.E.Z. and I.P.R. All authors agree to be held accountable for the content of this paper and approved the final version of the manuscript.

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