Ultraviolet B radiation alters movement and thermal selection of zebrafish (*Danio rerio*)

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Temperature and ultraviolet B (UV-B) interact in causing cellular damage and impairing locomotor performance. Here, we test the hypothesis that movement and thermal selection of zebrafish (*Danio rerio*) change in the presence of UV-B, and in particular, that fish which were chronically exposed to UV-B avoid high and low temperature extremes to maximize activities of antioxidant enzymes. Fish chronically (two to three weeks) exposed to UV-B had increased reactive oxygen species (ROS)-induced damage to proteins and membranes, and reduced swimming performance at high (more than 26°C) temperatures. In an open field arena with a thermal gradient, chronically exposed fish avoided high and low temperature extremes compared with control fish. Additionally, both control and chronically exposed fish showed slower voluntary swimming speeds in the presence of UV-B. We suggest that in the presence of UV-B fish may reduce muscular activity to minimize intrinsic ROS production. Our data show that the interaction between UV-B and temperature determines movement and microhabitat selection of fish, which is therefore of ecological importance particularly in anthropogenically modified environments.

1. Introduction

Ultraviolet B (UV-B) radiation is a principal environmental driver that can have pronounced negative effects on animal locomotion and ecology [1,2]. Biological damage from UV-B exposure is mediated by excess reactive oxygen species (ROS), which cause damage to proteins and membranes [3]. Animals can defend cells from ROS damage with enzymes such as catalase and superoxide dismutase [3]. Interestingly, the damage caused by UV-B and ROS depends on environmental temperatures, and fish can acclimate locomotor performance to different levels of UV-B and temperature [4]. It is possible that animals can also modulate the effects of UV-B by short-term selection of different thermal microhabitats. Most enzymes have a thermal performance curve in the shape of an inverted ‘U’ where activities are greatest over a narrow optimal temperature range but decline on either side of this range [5]. If shelter from UV-B is not available, animals could select thermal microhabitats that maximize antioxidant enzyme activities and minimize ROS-induced damage. The interactive effect between UV-B and temperature can therefore have pronounced ecological effects by altering movement and microhabitat selection.

Our aim was to determine whether zebrafish (*Danio rerio*) change their spatial behaviour and thermal microhabitat selection in the presence of UV-B. We exposed fish chronically to UV-B to test the hypothesis that chronic exposure to UV-B causes ROS-mediated damage and reductions in locomotor performance. As a result, we predicted that fish chronically exposed to UV-B will avoid high or low temperature microhabitats (relative to their long-term acclimation
2. Material and methods

(a) Study animals and chronic treatments
We chose zebrafish (adult shortfin, mixed sex, from Livefish, Bundaberg, Australia), because the species is an established model for studies on spatial behaviour [6]. We kept fish for one to two weeks at their long-term acclimation temperature of 24–25°C before starting experiments. We then split fish into two chronic treatments (at 25°C) for two to three weeks: UV-B plus visible light (UV-B group) and visible light only (control). Within each treatment group, we dispersed fish across three experimental tanks (280 × 220 × 200 mm; n = 12 fish per tank).

The photoperiod was 12 L : 12 D for all treatments and fish were fed twice daily with fish flakes. We provided UV-B radiation with 120 cm UV-B fluorescent tubes (UVB-313E, Q-lab, Cleveland, USA; 280–390 nm) for 3 h each day from 11.00 to 14.00. UV-B irradiance was measured with a radiometer (IL1500, International Light Inc., Newburyport, MA, USA). Note that UV-B is a natural part of the physical environment of most animals, including zebrafish. Zebrafish naturally occur in relatively shallow, clear water bodies in southern Asia [7], where they are exposed to relatively high average daily UV-B radiation (4000–6000 J m⁻²) year round [8]. The peak absolute irradiance of UV-B generated by our lamps was 3.3 W m⁻² at the water surface, which generated a total daily dose of 1188 J m⁻². We chose this moderate dose of UV-B to reflect natural water conditions where UV-B can be an important factor in the environment for aquatic organisms. We tested temperatures with at least 24 h between measurements.

We measured lipid peroxidation (indicated by malondialdehyde [MDA] concentrations) and protein carbonyl concentrations in tail muscle as indicators of ROS-induced damage using commercially available kits (Sigma Aldrich, Castle Hill, Australia; electronic supplementary material).

(b) Swimming and reactive oxygen species damage
Sustained swimming performance was measured as critical sustained swimming speed (Ucrit) according to published protocols [9] (electronic supplementary material). We measured Ucrit in 20 control fish and 12 chronically UV-B treated fish at 18, 28 and 32°C acute test temperatures with at least 24 h between measurements.

We measured lipid peroxidation (indicated by malondialdehyde [MDA] concentrations) and protein carbonyl concentrations in tail muscle as indicators of ROS-induced damage using commercially available kits (Sigma Aldrich, Castle Hill, Australia; electronic supplementary material).

(c) Open field tests and thermal gradients
We recorded movement of fish in open field arenas (600 × 400 mm with a water depth of 30 mm) [6] by filming fish from above at 30 frames s⁻¹ (with 930HD camera, Logitech, China). We chose a relatively shallow water level to facilitate establishment of a thermal gradient. Nonetheless, water depth was several times that of the fish depth, and zebrafish move to the water surface after 3–4 min habituation to a novel environment even if deep water is available [10]. Single fish (n = 16 from chronic UV-B treatments and 20 from controls) were measured in each trial. At the opposite far ends of the arena, we mounted a white metal partition across the width of the arena, taking care that we provided no visual cues for the fish to recognize particular ends of the arena. Behind the partition at one end of the arena, we placed water heaters (2 × 100 W; Heime, Deizisau, Germany), and behind the partition at the other end we installed hollow aluminium cooling elements (2 × 0.3 m length × 0.019 m diameter). Cooling elements were connected with rubber hosing to a pump (150 W, Resun, Longgang, China) submersed in a water/ice slurry (in a 0.6 × 0.45 × 0.28 m plastic container). A series of small holes drilled into the metal partitions allowed water flow between the main arena and the heating or cooling compartments. The heating and cooling areas established a thermal gradient within the main arena that ranged from 18 to 20°C at the cool end to more than 32°C at the warm end (electronic supplementary material, figure S1). We measured water temperatures with a thermal imaging camera (875i, Testo, Lenzkirch, Germany) at the beginning and at the end of each trial to verify the stability of the gradient.

Before each behavioural trial, fish were placed for 10 min into opaque plastic cylinders (150 mm length × 60 mm diameter) standing on their ends at the cool end of the arena. After 10 min, the cylinders were raised remotely without disturbing the fish, and the movement of the fish was filmed for 14 min. To control for potential order effects, half the fish started the trials with visible light only, while the other half started with UV-B plus visible light. After 7 min of the trial the light treatments were reversed. We analysed the movement of fish (in Lolitrack software, Loligo, Tjle, Denmark) for the last 3 min of each light condition [10]. We repeated these trials without the thermal gradient to test whether UV exposure per se affected spatial behaviour.

For each trial in the open field arenas, we subdivided the arena into four temperature zones (in Lolitrack software according to the manufacturer’s instructions) based on the thermal images for each trial (less than 20°C, 20–24.9°C, 25–29.9°C, more than 30°C). In trials without thermal gradients, we approximated these zones along the longitudinal (gradient) axis (0–30 mm from edge, 31–300 mm, 301–569 mm, 570–600 mm).

(d) Statistical analyses
We analysed all data using Bayesian inference in the package MCMCglmm [11] in R [12], and using priors from an inverse Wishart distribution [11]. We used individual fish as random factor to account for non-independence between measures (acute UV exposure, test temperatures, zone). Ucrit was compared between treatments with chronic treatment (UV-B and control) and test temperature as factors, and fish length as covariate. We compared protein carbonyl and lipid peroxidation data with a chronic treatment as factor. We analysed the time that fish spent in each zone with zone, acute and chronic treatments as factors. We analysed the voluntary speed at which fish moved, and activity (i.e. proportion of time for which movement was more than 0.01 m s⁻¹) during each trial with acute and chronic treatments as factors. Monte Carlo Markov chain (MCMC) probabilities are reported for all data, and we used confidence intervals of posterior means to distinguish between levels of significant factors.

3. Results

(a) Swimming performance
There was a significant interaction between chronic UV treatment and test temperature in their effect on Ucrit (p < 0.006; figure 1). At 18°C test temperature, Ucrit was similar in fish from both treatments; however, Ucrit of chronically UV-B-exposed fish was lower than controls at 26 and 32°C.

(b) Reactive oxygen species damage
Both MDA (p < 0.05; UV-B: 2.25 ± 0.28 (s.e.m.), control: 1.67 ± 0.17 (s.e.m.) nmol g⁻¹ tissue) and protein carbonyl (p < 0.04; UV-B: 9.74 ± 0.91 (s.e.m.), control 7.77 ± 0.37 (s.e.m.) nmol mg per protein) concentrations were significantly higher in fish chronically exposed to UV-B compared with controls.

(c) Open-field tests
Fish chronically exposed to UV-B spent less time at the cold and warm extremes of the arena and more time at the
centre compared with control fish (chronic treatment × zone interaction, p < 0.02; figure 2a). There was no effect of the acute treatment on the time spent in different thermal zones (main effect and interactions p > 0.16). Chronic or acute UV treatments did not affect movement in different zones in the absence of a thermal gradient (all p > 0.15; electronic supplementary material, figure S2a).

Chronically UV-B-exposed fish were less active when acutely exposed to UV-B than control fish, but were more active than controls in visible light only (with and without thermal gradient: acute × chronic treatment interaction p < 0.002; figure 2b; electronic supplementary material, figure S2b). In the presence of a thermal gradient, voluntary speed was less under acute UV-B exposure compared with visible light only (acute treatment main effect p < 0.02; figure 2c), regardless of the chronic treatment (chronic treatment main effect and interaction p > 0.40). There was no effect of UV-B treatment in the absence of a thermal gradient (all p > 0.17).

4. Discussion
Chronically UV-B-exposed fish avoided acute temperature extremes, and we accept our hypothesis that UV-B alters thermal microhabitat selection. Preferences for microhabitats at the long-term acclimation temperatures may maximize activities of antioxidant enzymes and thereby performance [4]. The reduction in swimming performance at high temperatures may result from increased mitochondrial ROS production, which increases during activity and at high temperatures [3,13]. Hence, intrinsic ROS production may exacerbate UV-B-induced ROS damage. The decline in voluntary swimming speed and the lower activity of chronically UV-B-exposed fish in the presence of UV-B are consistent with this suggestion. ROS may also exacerbate inflammatory responses [13], which may act as a trigger for behavioural changes. UV-B-induced ROS damage is not the only avenue by which UV-B affects movement of fish. Fish use UV wavelengths for intraspecific signalling, and UV can modify responses to predators [14]. It may be that the presence of UV alters movement patterns independently from damage even if there are no predators present. Additionally, exposure to novel environments may increase anxiety [8], and may lead to avoidance of novel (extreme) temperatures. However, these effects would have been similar in all treatment groups and therefore cannot explain differences between treatments.

The interactions between environmental drivers in determining thermal selection implies signalling pathways in addition to thermal sensors. ROS are important signalling molecules at low concentrations, and increased ROS generation can signal physiological responses ranging from transcription factor expression to protein–protein interactions and histone modifications [13], which potentially may affect movement and behaviour. Regardless of the mechanisms, microhabitat requirements change with UV-B exposure. The interaction between UV-B and temperature may be particularly important in areas where human impact leads to clearing of vegetation and increases in water temperature.

Ethics. All procedures were approved by the University of Sydney Animal Ethics Committee (approval no. 3907).

Data accessibility. Data are deposited in Dryad http://dx.doi.org/10.5061/dryad.8f901 [15].

Authors’ contributions. F.S. and C.E.F. conceived and designed the project, F.S., C.E.F. and E.G.K. acquired data, F.S. analysed data, F.S. drafted the article and C.E.F. and E.G.K. revised it critically for important intellectual content. All authors gave final approval of the version to be published and agreed to be accountable for all aspects of the work.

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References


