The effects of ultraviolet radiation on a freshwater prey fish: physiological stress response, club cell investment, and alarm cue production

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Recent anthropogenic activities have caused deleterious effects to the stratospheric ozone layer, resulting in a global increase in the level of ultraviolet radiation (UVR). Understanding the way that organisms respond to such stressors is key to predicting the effects of anthropogenic activities on aquatic ecosystems and the species that inhabit them. The epidermal layer of the skin of fishes is not keratinized and acts as the primary interface between the fish and its environment. The skin of many species of fishes contains large epidermal club cells (ECCs) that are known to release chemicals (alarm cues) serving to warn other fishes of danger. However, the alarm role of the cells is likely secondary to their role in the immune system. Recent research suggests that ECCs in the epidermis may play a role in protecting the fish from damage caused by UVR. In the present study, we examined the effects of in vivo exposure to UVR on fathead minnows (Pimephales promelas), specifically investigating ECC investment, physiological stress responses, and alarm cue production. We found that fish exposed to UVR showed an increase in cortisol levels and a substantive decrease in ECC investment compared to non-exposed controls. Unexpectedly, our subsequent analysis of the behavioural response of fish to alarm cues revealed no difference in the potency of the cues prepared from the skin of UV-exposed or non-exposed minnows. Our results indicate that, although nonlethal, UVR exposure may lead to secondary mortality by altering the fish immune system, although this same exposure may have little influence on chemically-mediated predator–prey interactions. © 2012 The Linnean Society of London, Biological Journal of the Linnean Society, 2012, 105, 832–841.


INTRODUCTION

Recent anthropogenic activities have caused a considerable reduction in stratospheric ozone, with a corresponding increase in the amount of ultraviolet radiation (UVR) hitting the surface of the earth (Newman et al., 2006). Chromophoric dissolved organic matter (CDOM) functions to protect aquatic organisms from the detrimental effects of UVR by attenuating solar radiation selectively and strongly within the UVR range (Scully & Lean, 1994; Williamson & Zagarise, 1994). However, CDOM concentrations in lakes are declining as a result of anthropogenic disturbances, increasing the levels of potentially damaging UVR reaching aquatic organisms (Schindler et al., 1997; Williamson et al., 2001). An increase in UVR penetration has the potential to cause considerable stress to aquatic organisms. Aquatic species in general, and fishes in particular, are vulnerable to stress-induced changes in their environment primarily through their skin and gills, which are constantly being exposed to the surrounding water. Fish skin lacks the keratinized outer layer, which acts as a protective layer against...
stressors for many vertebrates (Bullock, 1982). Consequently, this multilayered assemblage of cells should serve as an integral part of its defence system and respond rapidly to external stimuli (Zaccone et al., 2001).

The skin of fish comprises two main layers: (1) the epidermis, which is the outer region and includes mucous cells, epidermal club cells (ECCs), and filament cells; and (2) the dermis, which lays under the epidermis and contains scales and various pigment cells. The epidermis is the region that acts as the living interface between the fish and the external environment (Roberts & Bullock, 1981). The selection pressures leading to the evolution of ECCs in fishes have been a topic of great interest to evolutionary ecologists. When damaged during an attack by a predator, these cells release a substance (‘alarm cue’) that elicits a fright response in nearby conspecifics (Chivers & Smith, 1998; Ferrari, Wisenden & Chivers, 2010). Fishes often show graded behavioural responses to alarm cues, exhibiting higher intensity responses to increasing concentrations of alarm cues. Alarm cues have been demonstrated in several groups of fishes including Ostariophysians (minnows, catfishes, sucker), salmonids (trout and salmon), and percids (darters and perch). By their nature, these cues represent a reliable indicator of risk, and individuals responding to these cues with an antipredator response have been shown to increase survival during encounters with predators (Mirza & Chivers, 2001; Brown, 2003). Understanding the evolution of ECCs has been troublesome because it is unclear how these ‘signals’ could benefit the sender. Although many hypotheses have been proposed to explain the evolution of such cells (Smith, 1992), Chivers et al. (2007) provided the first strong support that these cells evolved as part of the immune system, and that the alarm function may have evolved secondarily. As a result of their strategic location, ECCs could provide a first line of defence against agents such as pathogens or parasites that penetrate through the skin, or promote the healing of damaged tissue as a result of agents such as UVR. (Al-Hassen et al., 1985; Smith, 1992; Blazer et al., 1997; Chivers et al., 2007). Chivers et al. (2007) showed that an increase in ECC density was induced in fathead minnows (Pimephales promelas) after exposure to pathogenic water moulds (Saprolegnia ferax and Saprolegnia parasitica) and parasitic larval trematodes (Uvulifer ambloplitis). However, studies by James, Wisenden & Goater (2009) showed that ECC density was not affected by infection from cercariae of the trematode (Ornithodiplostomum sp), Chivers et al. (2007) reported that yellow perch (Perca flavescens) heavily infested with larval trematodes also exhibited a higher ECC density compared to control subjects. Furthermore, Halbegewachs et al. (2009) demonstrated that intraperitoneal injections of cortisol suppressed the innate immune system of minnows and reduced ECC investment. We do not know how alterations in ECC density mediated through immune responses would affect chemically-mediated predator–prey interactions.

UVR forms a part of the electromagnetic spectrum, which is divided into three groups: UV-A (320–400 nm), UV-B (280–320 nm), and UV-C (100–280 nm). UV-A, despite being the main component of the solar UVR and having greater penetration power in the ecosystems, is far less harmful than UV-B. UV-C is absorbed by molecular oxygen (O3) in the atmosphere and most of the UV-B is absorbed by the ozone layer (O3) (Madronich et al., 1998). Ambient levels of UV-B have been shown to cause mortality in embryonic and larval amphibians (Blaustein et al., 1997, 1998). A comparative study of the effects of UV-A and UV-B on roach (Rutilus rutilus) has shown that exposure to both UV-A and UV-B causes suppression in transiently mitogenic proliferation of blood lymphocytes. UVA radiation decreases haematocrit, plasma protein, and plasma immunoglobulin levels, and increases the proportion of blood cells (Salo et al., 2006b). UV-B affects the functioning of the head kidney and blood phagocytes, induces granulocytosis and lymphocytopenia in the blood, and increases plasma cortisol concentrations (Salo et al., 2000a). UV-B also causes skin burns in brown trout (Noceda, Sierra & Martinez, 1997). These results indicate that ambient levels of UVR can act as a potential stressor and an immunosuppressant in fish.

The present study aimed to investigate the responses of fathead minnow to UVR exposure. Previous studies have demonstrated a correlation between UVR exposure and ECC investment in two different groups of fishes (percids and ostariophysians). For example, johnny darters (Etheostoma nigrum) and yellow perch (Perca flavescens) both had significantly more ECCs on their dorsal surface than their ventral, and more ECCs on their flank than on their ventral surface (Chivers et al., 2007). Similarly, fathead minnows have more ECCs on their dorsal surface than their ventral surface (Hugie, 1990). Consequently, as a result of this indirect evidence, we propose that UVR exposure could increase ECC investment in fathead minnows. However, if UVR exposure acts as an immunosuppressant via increasing cortisol, the epidermal immune response might be inhibited. We also examined whether the number of mucous cells was reduced by exposure to UVR, as previously documented by Blazer et al. (1997) and Kaweewat & Hofer (1997). Finally, we tested whether minnows respond differentially to skin extracts produced by UV-exposed and non-exposed minnows. Changes in ECC numbers associated with the UVR
exposure could lead to a change in the behavioural response of the minnows to skin extracts. Ferrari, Kapitania-Kwok & Chivers (2006) demonstrated that minnows displayed a greater intensity antipredator response when the amount of skin extract introduced into their tank was increased. In the present study, we control the volume of skin added to each tank and test for differential responses that arise from the UVR exposure treatments.

**MATERIAL AND METHODS**

**FISH COLLECTION AND MAINTENANCE**

Adult fathead minnows (mean ± SD, standard length = 4.97 ± 0.08 cm; weight = 2.9 ± 0.7 g) were collected between April and May 2009, from the Feedlot pond located on the University of Saskatchewan campus using Gee’s improved minnow traps. Fish were housed in a 1600-L flow-through pool containing dechlorinated tap water. The water was maintained at approximately 19 ± 2 °C under a 14 : 10 h light/dark cycle. Fish were fed commercial flake food *ad libitum* throughout the experiment and were acclimated for several months before the experimental procedure. The water used for the experiments originated from the Saskatoon, SK, Canada municipal water supply and was run through an active carbon filter. Water chemistry parameters (temperature, dissolved oxygen, pH, total alkalinity, and total hardness; Table 1) were monitored every alternate day during the acclimation and experiment phase.

**UV SYSTEM**

Fathead minnows were exposed to artificial UVR (250 W m⁻²) *in vivo* in an Atlas SUNTEST XLS + Solar Simulator with Xeon lamp with a Suprax Daylight Glass Filter – 290 nm cut-off (Atlas Material Testing Technology LLC, Chicago, USA). Minnows were maintained in quartz beakers (diameter 13.8 cm, height 16.8 cm; QSI Quartz Scientific, USA) placed in a constant flow water bath to maintain ambient water temperature. Water temperature throughout the experiment was maintained at 19 ± 2 °C, which is representative of ambient surface water temperatures in Saskatchewan lakes throughout July and August. The water quality parameters (Table 1) were checked daily during the exposure period. We set the periodicity to a 8 : 16 h light/dark cycle. The 8-h exposure included UV-A (320–400 nm), UV-B (280–320 nm), and photosynthetically active radiation (PAR; 400–700 nm). The spectroscopic readings of UVR were measured every 0.5 nm through the UVR (280–400 nm) and PAR (400–700 nm) spectrum, using an Ocean Optics USB 2000 spectroscopic radiometer. The spectral characteristics of UVR were analyzed using SPECTRA SUITE software (Ocean Optics). The spectral output from the Suntest Solar Simulator was comparable to mean noon time solar irradiance measured in Saskatoon, SK (J. M. Sereda & J. J. Hudson, unpubl. data). Total UVR emitted by the solar simulator was 45 W m⁻²; mean natural solar irradiance was 43 W m⁻². Although there was little difference in the total UVR emitted from the solar simulator and the natural noon time solar irradiance in Saskatoon, the 8-h periodicity that we choose would result in a cumulative dose somewhat higher than normal for minnows at our latitude. However, the cumulative dose that we choose would be lower for minnows living at different latitudes. Indeed, the amount of UV exposure that the minnows receive across their geographical range will vary by as much as two-fold depending on latitude (Goncalves et al., 2010).

**EXPERIMENTAL DESIGN**

The objective of this experiment was three-fold: (1) to observe the effects of UVR on ECC and mucous cell investment; (2) to observe the effects of UVR on physiological stress response; and (3) to observe the effect of UVR on potency of alarm cues prepared from the skin of minnows exposed to UVR.

Male minnows have suppressed ECC numbers due to high testosterone levels; consequently, the experiment was performed from September 2009 to March 2010, outside the breeding season of minnows. In the nonreproductive phase, male and female minnows are difficult to morphologically differentiate. Four randomly chosen minnows were introduced in each of the two quartz beakers in the solar simulator. We used a design, whereby the fish in two beakers were exposed

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**Table 1.** Mean ± SE water quality parameters for ultraviolet UV-exposed and UV-filtered groups of fish

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Temperature (°C)</th>
<th>Dissolved oxygen (mg L⁻¹)</th>
<th>pH</th>
<th>Alkalinity (mg L⁻¹, CaCO₃)</th>
<th>Hardness (mg L⁻¹, CaCO₃)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV filtered</td>
<td>19 ± 2</td>
<td>7.0 ± 0.3</td>
<td>7.42 ± 0.03</td>
<td>94 ± 0.2</td>
<td>120 ± 1.3</td>
</tr>
<tr>
<td>UV exposed</td>
<td>19 ± 2</td>
<td>6.3 ± 0.1</td>
<td>7.45 ± 0.02</td>
<td>94 ± 0.3</td>
<td>120 ± 2.5</td>
</tr>
</tbody>
</table>

*t*-tests indicate no significant differences between treatments at *P* = 0.05.
to UVR (i.e. UV-exposed group) for 4 days. Subsequently, two beakers had their top and sides covered with a 2-mm thick Lexan polycarbonate sheet with the fish exposed to the treatment for the same 4-day period. The polycarbonate sheeted removed 76% of the UVB and UVA radiation; hence, this treatment is referred to as the UV-filtered group. We alternated having UV-exposed and UV-filtered fish in the solar simulator to control for order effects. Filtering efficiency of the polycarbonate sheets was monitored throughout the study to ensure that there was no change in radiation treatment. The fish were left to acclimate for 24 h before the start of the exposure. After this acclimation period, fish were exposed to artificial solar radiation of 250 W m\(^{-2}\) for 8 h every day over a 4-day period. A preliminary trial indicated no effect of UVR on ECC numbers after only 2 or 3 days. We ran 12 blocks containing two UV-exposed beakers, and 13 blocks containing two UV-filtered beakers. The four minnows in each beaker were not independent, so that we used the ‘beaker’, and not the individual minnows, as our replicate unit.

Eight of 96 minnows in the UV-exposed group died, whereas no fish died in the UV-filtered group. Minnows that did not survive until the end of the exposure were excluded from further analysis. After 96 h of exposure, the fish were sacrificed with a blow to the head (University of Saskatchewan Animal Care Protocol Number 2009091) and blood was immediately extracted from eight blocks of each group for cortisol analysis. Two UV-exposed minnows and two UV-filtered minnows were randomly selected for histological analysis and were preserved in 10% neutral buffered formalin until tissue processing could be performed. Epidermal samples were taken from the dorso/lateral surface just behind the operculum to the dorsal fin. An automatic tissue processor (MUP1, Modular Vacuum Processor) was used to dehydrate the fixed skin tissue in a series of ethanol grades and perfused with paraffin wax. Tissues were then manually embedded in paraffin wax and sectioned using a rotary microtome (HM330; Heidelberg) at 5-μm thickness. After sectioning, three to five sections were placed on a pre-cleaned supra-frost slide (VWR micro slides). After the slides were dried on a slide warmer, they were deparaffinized, rehydrated, and then stained with periodic acid Schiff's reagent with Harris' haematoxylin (PAS-H) to darken the mucous cells and the basement membrane (PAS) and the nucleus (haematoxylin), rendering ECCs colourless and easily recognizable (Fig. 1). Images of each epidermal cross section were captured with a Zeiss Axioplan Fluorescence Microscope in conjunction with an AxioCamICc1 (Colour, 1.4 MP) digital camera at ×10 magnification. For each slide, the parameters recorded were: epidermal thickness, number of mucous cells, number of ECCs, and ECC area, which were all quantified using the image processing and analysis software IMAGE J, version 1.32, (http://rsb.info.nih.gov/ij/). The observer was blind with respect to the treatment. The size of minnows used for the histological measurements had a mean ± SD fork length of 5.5 ± 0.5 cm and a mass of 2.1 ± 0.6 g. We tested a total of 12, 13, and eight blocks in the UV-exposed, UV-filtered, and control groups, respectively.

**Experimental protocol for blood extraction**

Blood extraction for cortisol analysis was performed *sensu* Halbegewachs *et al.* (2009). Blood samples (25–50 μL) were extracted from the caudal vein near the anal fin region of euthanized minnows. To obtain sufficient blood for the analysis, we pooled blood from four fish from the same beaker (UV-exposed, UV-filtered, and control). This blood was placed on ice and allowed to clot for at least 1 h. Serum was extracted from the blood after centrifugation and then frozen at −20 °C until it was used for analysis. The cortisol level in the extracted serum was measured by the Endocrine Laboratory at Prairie Diagnostic Service (University of Saskatchewan) in a Coat-A-Count radioimmunoassay, which is designed for the quantitative measurement of cortisol in serum.

**Histological analysis of the skin**

Tissue preparation for the analysis of the minnow epidermis was performed *sensu* Hugie (1990) with specific modifications. The entire fish was initially fixed in 10% neutral buffered formalin until tissue processing could be performed. Epidermal samples were taken from the dorso/lateral surface until tissue processing could be performed. Epidermal samples were taken from the dorso/lateral surface just behind the operculum to the dorsal fin. An automatic tissue processor (MUP1, Modular Vacuum Processor) was used to dehydrate the fixed skin tissue in a series of ethanol grades and perfused with paraffin wax. Tissues were then manually embedded in paraffin wax and sectioned using a rotary microtome (HM330; Heidelberg) at 5-μm thickness. After sectioning, three to five sections were placed on a pre-cleaned supra-frost slide (VWR micro slides). After the slides were dried on a slide warmer, they were deparaffinized, rehydrated, and then stained with periodic acid Schiff's reagent with Harris' haematoxylin (PAS-H) to darken the mucous cells and the basement membrane (PAS) and the nucleus (haematoxylin), rendering ECCs colourless and easily recognizable (Fig. 1). Images of each epidermal cross section were captured with a Zeiss Axioplan Fluorescence Microscope in conjunction with an AxioCamICc1 (Colour, 1.4 MP) digital camera at ×10 magnification. For each slide, the parameters recorded were: epidermal thickness, number of mucous cells, number of ECCs, and ECC area, which were all quantified using the image processing and analysis software IMAGE J, version 1.32, (http://rsb.info.nih.gov/ij/). The observer was blind with respect to the treatment. The size of minnows used for the histological measurements had a mean ± SD fork length of 5.5 ± 0.5 cm and a mass of 2.1 ± 0.6 g. We tested a total of 12, 13, and eight blocks in the UV-exposed, UV-filtered, and control groups, respectively.

**Behavioural assay on potency of skin extract**

The skin extract for the behavioural assay was produced from UV-exposed (*N* = 20, mean ± SE standard length: 4.97 ± 0.08 cm) and UV-filtered fathead minnows (*N* = 20, mean ± SE standard length: 5.35 ± 0.14 cm). Skin from the lateral epidermal layer on either side of the body was removed and placed in 40 mL of chilled distilled water. We collected a total of
50.1 cm² of skin from the UV-exposed group and 54.9 cm² from the UV-filtered group. The skin fillets were homogenized with a Polytron homogenizer and filtered through filter floss to remove large particles. Serial dilutions were used to obtain a final concentration of 1 cm² of skin per 40 litres, a concentration known to elicit overt antipredator response in fathead minnows (Ferrari et al., 2005, 2006). The alarm substance was frozen at -20 °C in 20-mL aliquots until used.

The behavioural bioassay was carried out to evaluate the difference in the potency of alarm cues prepared from the skin of UV-exposed and UV-filtered minnows on the antipredator response of control minnows. The assay was performed in 74-L aquaria (60 × 30 × 40 cm), which were wrapped in black plastic on three sides so that fish in adjacent aquaria were not visible to each other. Each aquarium was filled with dechlorinated water and equipped with a single air stone. Three randomly selected minnows were acclimated in each aquarium for at least 24 h before the assay. Each day, one-third of the aquaria (randomly chosen) were exposed to alarm cues from the UV-exposed group, a third were exposed alarm cues from the UV-filtered group, and the remaining third were exposed to water (control). We tested a total of 20 groups of fish in each of the three treatment groups. The experiment was divided into three phases: an 8-min pre-stimulus phase, a 1-min stimulus injection phase, and an 8-min post-stimulus phase (Pollock & Chivers, 2004). We used a well established protocol for measuring the antipredator responses of minnows (Ferrari et al., 2005). This included recording an index of shoaling and an estimate of activity level, as measured by line crossing. The shoaling index of three fish was measured every 15 s. Shoaling index was analyzed by evaluating the distance between the three fish per aquarium every 15 s during the pre- and post-stimulus time (1 = no fish within a body length of another; 2 = two fish within a body length of each other; 3 = all the fish within a body length of each other). As a measure of line crossing, the number of line crosses was also recorded for one of the three minnows during the first 10 s of the 15-s period. The same fish was randomly selected and observed until the end of the conditioning period. An increase in shoaling index and a decrease in activity level are two typical antipredator responses in minnows (Chivers & Smith, 1998).

**Statistical analysis**

For parameters relating to histology, blood cortisol, and behavioural responses, Levene’s tests were performed to check for homoscedasticity and Kolmogorov–Smirnov tests were performed to check for normality. All statistical analyses were performed using SPSS 17, version 17 (SPSS Inc.).

We used one-way analysis of variance (ANOVA) followed by Tukey’s post-hoc test to compare the physiological stress response [cortisol levels (in ng mL⁻¹), and skin parameters (epidermal thickness, number of ECCs per mm of skin, area of ECCs (in µm²) and number of mucous cells)] among UV-exposed minnows, UV-filtered minnows, and control minnows. For the behavioural responses, we used the differences in shoaling index and line crossing from the pre-stimulus baseline as our raw data. The effect of cues (skin extract from UV-exposed and UV-filtered minnows and water) on the behavioural response of control minnows was tested using a one-way ANOVA followed by a post-hoc Tukey test.

**Results**

**Physiological stress response**

The one-way ANOVA revealed a significant effect of treatment on the serum cortisol levels measured in minnows (N = 8 per treatment, F_{2,21} = 25.1, P < 0.001; Fig. 2). Post-hoc Tukey tests revealed no significant difference in the serum cortisol levels of UV-filtered and control groups (P = 0.709). However, the tests
revealed a much higher level of cortisol in the UV-exposed group compared to that in the UV-filtered group ($P < 0.001$) and control group ($P < 0.001$). Serum cortisol levels of UV-exposed minnows were almost five-fold higher than those found in the blood of the UV-filtered minnows.

**Histological Parameters**

One-way ANOVA revealed no significant effect of treatment on epidermal thickness ($N = 12, 13$, and $8$ for the UV-exposed, UV-filtered and control groups respectively, $F_{2,30} = 0.300, P = 0.743$). Similarly, there was no effect of treatment on mean ECC area, although there was a trend for ECCs to be smaller in the UV-exposed group ($F_{2,30} = 3.1; P = 0.059$). By contrast, there was a significant effect of treatment on the mean number of ECCs between the UV-filtered and control group ($P = 0.988$). However, there was a reduction in the number of ECCs to almost half in the UV exposed group compared to the UV filtered group ($P = 0.008$) and control group ($P = 0.015$). Similarly, one-way ANOVA revealed a significant effect of treatment on the mean number of mucus cells ($F_{2,30} = 11.8, P < 0.001$; Fig. 4). Post-hoc Tukey tests revealed no difference in mean number of mucus cells between the UV-filtered group and control group ($P = 0.934$). However, there was a three-fold reduction in the number of mucous cells in the UV-exposed group compared to the UV-filtered group ($P < 0.001$) and control group ($P = 0.003$).

**Behavioural Assay**

One-way ANOVA revealed a significant effect of cue on the behavioural responses of minnows for both shoaling index ($N = 20$ per treatment, $F_{2,57} = 19.2, P < 0.001$; Fig. 5) and line crosses ($N = 20$ per treatment, $F_{2,57} = 17.1, P < 0.001$; Fig. 6). For both behavioural measures, post-hoc Tukey tests revealed a stronger antipredator response displayed by minnows exposed to alarm cues than minnows exposed to water (all $P < 0.001$). However, no difference were found between the responses of minnows to alarm cues from UV-exposed and UV-filtered minnows (shoaling index: $P = 0.99$; line crosses: $P = 0.273$).

**Discussion**

Previous studies have suggested a link between exposure to UVR and ECC investment in both cyprinid (minnows; Hugie, 1990) and percid fishes (Johnny darters, yellow perch; Chivers et al., 2007). Wild captured fish have more ECCs on their dorsal surface than their lateral surface and even fewer ECCs on...
their ventral surface. Consequently, we may expect that fish should increase ECC investment upon exposure to UVR. By contrast, minnows did not show an increase but rather a decrease in ECC investment when exposed to UVR. Interestingly, we did observe a five-fold increase in cortisol levels in the UV-exposed group, whereas UV-filtered minnows and control minnows did not differ in their levels of serum cortisol. Detection of increased cortisol levels has been used as an indicator of stress in fish (Barton, 2002). This indicates that UVR exposure per se, and not holding conditions, elicited this five-fold increase in cortisol in the UV-exposed group. Other studies have documented similar UVR induced increases in plasma cortisol levels (Salo et al., 2000a, b). The observed cortisol levels in minnows from the UV-filtered group (mean ± SE, 68.7 ± 13.5 ng mL$^{-1}$) and control group (34.9 ± 23.1 ng mL$^{-1}$) is comparable to the levels of cortisol (Palić et al., 2006) reported in minnows exposed to handling stress (53 ng mL$^{-1}$). These levels, even though slightly higher than levels of unstressed minnows are five-fold lower than levels of cortisol from the blood of UV-exposed minnows (311.1 ± 44.7 ng mL$^{-1}$). The elevation in cortisol levels probably prevented the adaptive epidermal responses that we predicted. Halbegewachs et al. (2009) showed that minnows exposed to cortisol had a suppressed immune system, as measured by a respiratory burst assay, and also a corresponding reduction in investment in ECCs.
The present study dictates that future research should focus on investigating long-term effects of UVR on the stress responses of fishes. Wild captured fishes often showed marked variation in ECC density (A. K. Manek, R. J. Pollock, M. C. O. Ferrari, D. Vicente and D. P. Chivers, unpubl. data). Consequently, in the present study, we held the minnows in the laboratory under standard conditions for several months in an attempt to reduce the variation in ECC number that we had at the beginning of the experiment. This means that, when we began our experiment, the minnows had been held for a long period in the absence of UVR. This is equivalent to minnows being held under the ice for several months with limited exposure to UVR. The stress response that we observed to UVR exposure may be a short-term response that reflects their limited UVR exposure over the past several months. If fish that are exposed to UVR for an extended period of time lose their stress response, then it is possible that UVR could indeed increase ECC investment, as suggested by the distribution of ECCs on the dorsal and ventral surface of darters, perch, and minnows. If UVR is not responsible for the difference in ECC distribution over the body of the fish, then we should consider other possibilities for these patterns, including the possibility that pathogenic agents differentially prefer to penetrate the dorsal surface than the ventral surface of the fish.

The results obtained in the present study also show that UVR exposure resulted in a reduction in number of mucous cells. This finding supports an earlier experiment on salmonids and cyprinids strengthening the hypothesis that UVR down-regulates mucous producing cells (Blazer et al., 1997; Kaweewat & Hofer, 1997). Despite the reduction in both ECCs and mucous cells, there was no reduction in the thickness of the epidermis between treatments. Changes in epidermal thickness are often associated with a reduction in body condition. We do not know whether there was a change in body condition for fish held in the different treatments because we did not weigh and measure the length of the fish before the experiment. Measuring the fish before the experiment would risk injury to the epidermis. It is possible that fish experiencing a reduction in body condition, perhaps as a stress response to UVR, could be responsible for the reduction in ECC and mucous cells, although this possibility remains to be investigated.

Past evidence suggests that alarm cues are located in ECCs (Smith, 1973). Indeed, laboratory and field experiments have shown that skin extract prepared from breeding male minnows, which lack ECCs, fails to elicit antipredator responses in conspecifics (Mathis & Smith, 1992; Pollock et al., 2005). However, Carreau-Green et al. (2008) recently reported that adult minnows displayed an antipredator response to skin extracts from larval minnows that have not yet developed ECCs, suggesting that alarm cues may not just be produced in the ECCs but elsewhere in the skin of the minnows. In the present study, we did not observe any difference in the potency of the alarm cues prepared from the skin of UV-exposed or UV-filtered minnows. There was an approximately 40% reduction in the number of ECCs in the UV-exposed group compared to the UV-filtered group. We know that minnows are known to exhibit a graded response to different concentrations of skin extracts (Ferrari et al., 2005); consequently, we would predict that there should have been a reduction in behavioural response of minnows exposed to skin extract from minnows in the UV-exposed group compared to the UV-filtered group. Our results appear consistent with the findings of Carreau-Green et al. (2008) suggesting that alarm cues may be produced outside of ECCs. However, it is also possible that minnows could up-regulate alarm cue production independent of ECC number. This explanation is not satisfying given that the primary role of ECCs appears to be to act as immune cells and not as producers of alarm cues. A final alternative for the lack of behavioural difference between skin extract from UV-exposed and UV-filtered group fish is that the behavioural sensitivity to different concentrations is not sufficiently fine-tuned to detect a 40% reduction in ECC concentration. Ferrari et al. (2005) showed graded responses to alarm cues, although the magnitude of the differences in concentrations that they used were greater than 40%.

The level of detrimental UVR reaching aquatic organisms is expected to increase as a result of anthropogenic disturbances, including decreases in stratospheric ozone and decreases in chromophoric dissolved organic carbon, which attenuates UVR (Schindler et al., 1997). The findings of the present study show that short-term exposure to UVR increases the physiological stress response in fishes, with the consequence that there is a reduction in ECC investment. Such a reduction in ECCs may lead to an increase in the vulnerability of the fish to secondary infections through suppression of the immune function.

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