Dissolved organic carbon ameliorates the effects of UV radiation on a freshwater fish

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HIGHLIGHTS

• We examined the combined effect of UV radiation and Dissolved Organic Carbon on fish.
• Physiological stress response and epidermal club cell investment were measured.
• Fish exposed to high UVR and DOC had higher ECC investment and reduced cortisol levels.
• DOC plays a role in protecting fish from physiological stress and maintains ECC production.

ARTICLE INFO

Anthropogenic activities over the past several decades have depleted stratospheric ozone, resulting in a global increase in ultraviolet radiation (UVR). Much of the negative effects of UVR in aquatic systems is minimized by dissolved organic carbon (DOC) which is known to attenuate UVR across the water column. The skin of many fishes contains large epidermal club cells (ECCs) that are known to play a role in innate immune responses and also release chemical alarm cues that warn other fishes of danger. This study investigated the effects of in vivo UVR exposure to fathead minnows (Pimephales promelas), under the influence of two sources of DOC: Sigma Aldrich humic acid, a coal based commercial source of DOC and Luther Marsh natural organic matter, a terrigenous source of DOC. Specifically, we examined ECC investment and physiological stress responses and found that fish exposed to high UVR, in the presence of either source of DOC, had higher ECC investment than fish exposed to high UVR only. Similarly, exposure to high UVR under either source of DOC, reduced cortisol levels relative to that in the high UVR only treatment. This indicates that DOC protects fish from physiological stress associated with UVR exposure and helps maintain production of ECC under conditions of UVR exposure. © 2014 Elsevier B.V. All rights reserved.

1. Introduction

Increasing levels of ultraviolet radiation (hereafter UVR) hitting the surface of the earth as a result of reductions in stratospheric ozone have been a topic of concern for several decades (Newman et al., 2006). The implementation of the Montreal Protocol has ameliorated much of the ozone depletion. However, it is difficult to ascertain how persistence of ozone recovery will be due to factors such as changes in cloud cover, air pollutants and aerosols, all of which are influenced by climate change (McKenzie et al., 2011). Extensive studies provide warning about the potentially damaging effects of UVR on freshwater ecosystems ranging from bacteria and phytoplankton to zooplankton and fish (Siebeck et al., 1994; Williamson and Zagarese, 1994; Williamson, 1995). In aquatic ecosystems, the range of solar radiation, including UVR, penetrating water bodies varies due to changes in a variety of abiotic factors like solar zenith angle, ozone depleting chemicals, greenhouse gases, water vapour, density of cloud cover, elevation and absorption and scattering by dissolved and particulate matter (Sullivan et al., 1992). Organisms residing in clear, shallow high elevation lakes, where fluctuations in UVR levels could be more exaggerated, are more vulnerable to harmful effects of UVR due to shallow depth and higher absorption, reducing refuge from damaging levels of radiation (Williamson, 1995; Blaustein et al., 1997). The level of UVR transmission varies across lakes and is greatly influenced by water chemistry. Dissolved organic matter (DOM), a component of natural organic matter (NOM), is an important water chemistry parameter in aquatic ecosystems. DOM is measured as dissolved organic carbon (hereafter DOC measured in mg L⁻¹) (Steinberg et al., 2008). There have been extensive studies on the impacts of NOM on aquatic organisms focussing on its influence on physiological (Campbell et al., 1997; Wood et al., 2003; Matuso et al., 2004; Glover et al., 2005; Galvez et al., 2009) and toxicological effects (Matsu et al., 2006; Meinelt et al., 2007). DOC is generally defined as the fraction of DOM that passes through a 0.45 μm membrane and is

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chemically made of humic and fulvic fractions or acids (Buffie, 1984; Thurman, 1985). The attenuation rate of visible light and UVR in the water column is largely regulated by the concentration and absorptivity of DOC, rightfully terming it a “natural sunscreen” (Kirk et al., 1994; Morris et al., 1995; Porcal et al., 2009). Some studies have suggested that DOC may completely protect aquatic organisms like amphibians from damage caused by UV-B radiation (Adams et al., 2011; Palen et al., 2002). DOC affects other water quality parameters in aquatic systems, namely pH and has the ability to impart color (Porcal et al., 2009). Chromophoric dissolved organic matter (CDOM), is the light dissolving fraction of DOC and it selectively removes the shorter wavelengths of UVR and visible light (Williamson and Rose, 2010). Increased UVR and acidification are known to cause a loss of DOC via photolysis (CDOM absorbance loss) in aquatic ecosystems (Molot and Dillon, 1997; Gennings et al., 2001). On the other hand, a variety of other environmental changes namely increasing atmospheric CO₂ concentration, global warming, nitrogen deposition and decreased sulfate deposition are considered to be causes for an increase in DOC concentrations in many aquatic systems over the last 3 decades (Porcal et al., 2009).

For more than half a century, evolutionary ecologists have been trying to understand the evolutionary role of epidermal club cells (ECCs), which are specialized cells found in the skin of fishes belonging to superorder Ostariophysi, with a few non-ostariophyisan exceptions (e.g. darters and perch) (Ferrari et al., 2010). Initial research indicated that these cells are the primary site of production and maintenance of alarm cues, which are released when the cells are ruptured, as during a predator attack. Anti-predator responses to such alarm cues are found across a wide range of taxa including gastropods, echinoderms, amphibians and fishes (Ferrari et al., 2010). Initial research focused on predation-centered hypothesis (kin selection hypothesis and attraction of secondary predator hypothesis) to explain the evolution of alarm cues. Chivers et al. (2007) deviated from the predation-centered hypothesis and proposed the immune function hypothesis (anti-parasitic/anti-pathogen hypothesis). They indicated through a series of experiments that ECCs have a role to play in innate immune responses. Specifically, they showed that exposure to pathogenic watermolds and larval trematodes causes an increase in ECC investment (upregulation of ECC number/density across the epidermis) highlighting that these cells are part of the innate immune system. Halbgewachs et al. (2009) suggested a link between exposure to an immunosuppressant (intrapertoneal injection of cortisol) and reduced ECC investment. Manek et al., 2012, 2014 showed that exposure to immunosuppressants like UVR or cadmium (Cd) resulted in increased cortisol production and lowered ECC investment, providing further evidence that ECCs have an immune function. Manek et al., 2012, 2013 also showed that despite a reduction in ECC investment due to exposure to UVR and/or Cd, there was no difference in the anti-predator response to alarm cues prepared from the skin of UVR and/or Cd exposed fathead minnows. These results suggest that ECCs have an important role to play in innate immune responses and that the alarm function may have evolved secondarily.

Based on the known effects of DOC on UVR attenuation rates and findings of our previous studies showing that UV radiation decreases ECC investment through altering cortisol production, the objective of this study was to examine the effects of increased DOC levels and UVR exposure on physiological stress responses and ECC investment in fathead minnows (Pimephales promelas). To understand the comparative effect of DOC from different sources on the aforementioned objectives, we studied effects of increased DOC levels using two sources, commercially available Sigma Aldrich humic acid (hereafter SAHA) and field collected Luther Marsh natural organic matter (hereafter LM NOM). In a fully factorial (2 × 3 design), we exposed fish to one of the two DOC treatments along with a water control (3 levels of DOC), and exposed them to UVR in the presence or absence of a UVR blocking filter (2 levels of UVR). We hypothesized that physiological stress and ECC investment will vary in minnows depending on their exposure to UVR under different sources and concentrations of DOC. Specifically, we predicted that exposure to UVR under increased DOC levels would help in maintaining low cortisol levels relative to those exposed to UVR only. We also predicted that under the influence of increased DOC levels and UVR exposure, minnows would be able to maintain a high level of ECC investment.

2. Materials and Methods

2.1. Fish collection and housing for the study

This study was carried out in strict accordance with the approved University of Saskatchewan Animal Care Protocol number 20090091. Adult fathead minnows (standard length ± S.D. = 5.2 ± 0.38 cm, weight ± S.D. = 2.05 ± 0.51 g) were collected between May and June 2011, from the feedlot pond located on the University of Saskatchewan campus using Gee’s improved minnow traps. Male minnows have suppressed ECC numbers due to high testosterone levels in the reproductive phase (Smith, 1973). To ensure that minnows were in the non-reproductive phase, they were acclimated in the laboratory for at least one month prior to the experimental procedure. This could also help reduce any variation in baseline ECC production between individuals (Manek et al., 2013). Fish were housed in 73-L aquaria containing dechlorinated tap water. The water was maintained at around 19 ± 2 °C and the photoperiod was set to a 14:10 h light:dark cycle. The fish were fed commercial flake food ad libitum throughout the acclimation phase and during the experiment. The water used for the experiments originated from the Saskatoon, SK, Canada municipal water supply and was periodically tested every alternate day for water chemistry parameters. Dissolved oxygen, pH, chlorine, ammonia and hardness were measured using Nutrafen water testing kits (Hagen Inc., USA) and pH, dissolved oxygen and temperature were measured using a probe (YSI Professional Plus, YSI Inc., USA). Water temperature in aquaria and breeders during the acclimation and experiment phase was also measured using digital thermometers (Marina Aqua-Minder, Hagen Inc., USA). Results of the water quality parameters are presented in Table 1. Mean hardness (150 ± 0.5 mg L⁻¹) and ammonia levels (0.2 ± 0.1 ppm) were the same for all treatments and hence not presented on the table.

2.2. DOC and UVR exposure

Minnows were exposed in vivo to UVR for 8 h a day for 4 days in an Atlas SUNTEST XLS+ solar simulator with a xenon lamp with a Suprax daylight glass filter – 290 nm cut off (Atlas Material Testing Technology LLC, Chicago, USA) in groups of 4 in quartz beakers (diameter of 13.8 cm, height of 16.8 cm, QSI Quartz Scientific, USA). The total level of UVR emitted by the solar simulator in the present study was around 250 W/m² (UVR and photosynthetically active radiation combined). The levels of UVR that fathead minnows are exposed to across their geographical range can vary 2 fold depending on their latitude (Goncalves et al., 2017).

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Temperature (°C)</th>
<th>Dissolved oxygen (mg L⁻¹)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>High UVR + DCW</td>
<td>18.4 ± 2.1</td>
<td>6.5 ± 0.5</td>
<td>7.95 ± 0.23</td>
</tr>
<tr>
<td>Low UVR + DCW</td>
<td>18.5 ± 2.5</td>
<td>7.1 ± 0.4</td>
<td>8.31 ± 0.12</td>
</tr>
<tr>
<td>High UVR + LM NOM</td>
<td>18.2 ± 2.2</td>
<td>6.6 ± 0.4</td>
<td>8.23 ± 0.13</td>
</tr>
<tr>
<td>Low UVR + LM NOM</td>
<td>18.3 ± 2.3</td>
<td>7.2 ± 0.5</td>
<td>8.27 ± 0.22</td>
</tr>
<tr>
<td>High UVR + SAHA</td>
<td>18.6 ± 2.2</td>
<td>6.7 ± 0.5</td>
<td>8.19 ± 0.24</td>
</tr>
<tr>
<td>Low UVR + SAHA</td>
<td>18.2 ± 2.4</td>
<td>7.0 ± 0.4</td>
<td>8.13 ± 0.11</td>
</tr>
</tbody>
</table>
et al., 2010). If we dissect out the actual UVR exposure only, it was around 45 W/m², which is comparable to natural levels of UVR in mid-summer in Saskatchewan (43 W/m²) (Sereda et al., unpublished results). Fish were exposed to UVR in the presence or absence of a UV blocking filter in dechlorinated water (hereafter DCW), SAHA or LM NOM spiked water. The UVR exposure procedure and duration described above was similar to the one described previously by Manek et al. (2012). Some beakers had their top and sides covered with a 2 mm thick Lexan polycarbonate sheet. The polycarbonate sheet removed 76% of the UVB and UVA radiation, hence the level of UVR in the low UVR treatment was less than a quarter of mid-summer UVR levels in Saskatchewan (Sereda et al., unpublished results). Hereafter, we refer to the group exposed to UVR in the presence of a blocking filter as the low UVR group, and the group without the blocking filter as the high UVR group. This gave us a 2 × 3 fully factorial design, where the type of UVR exposure (high UVR vs. low UVR) was crossed with the source of DOC (DCW vs. LM NOM vs. SAHA). A 100% water change was performed per beaker per day. The four minnows in each beaker were not independent, so we considered the ‘beaker’, not the individual minnows, as our replicate unit. We alternated the order of treatments and conducted between 6 and 8 replicates per treatment (6 replicates of the high UVR + LM NOM and the low UVR + LM NOM treatments and 8 replicates of all other treatments).

2.3. LM NOM and SAHA stock solution preparation

Organic matter of an aquatic system can be characterized by source or origin. One such source of origin is terrigenous (NOM produced on land and then transported into the water body). LM NOM is an example of a terrigenous source of DOC. LM NOM was collected from Luther Marsh, Ontario in September 2009, via a portable reverse-osmosis apparatus (Sun et al., 1995), resulting in a stock with a concentration of 502.87 mg of carbon per litre (mg L⁻¹) and a pH of around 2.7. Since the DOC concentration of the stock solution was known, we spiked the stock solution to end up with a final concentration of around 5.5 mg L⁻¹ in the LM NOM water.

SAHA is a commercial source of DOC derived from coal, and has been extensively used as a DOM analogue in various physiological and toxicological studies (Glover et al., 2005; Glover and Wood, 2005). A stock solution of SAHA was prepared by mixing 1 g of SAHA sodium salt (Sigma Aldrich, USA) in one litre of de-ionized water. The water was thoroughly stirred using a magnetic stirrer and then stored in a flask which was tin fined and kept at 4 °C in the refrigerator until used for spiking in the exposure water. We spiked the stock solution to obtain a final concentration of around 4.5 mg L⁻¹ in the SAHA water. We originally aimed for similar (but not exact) final concentrations of DOC in SAHA and LM NOM. We decided to use a slightly lower amount of SAHA than LM NOM due to the greater chromomorphic properties of SAHA and its lower solubility (Al-Reasi et al., 2012). Our pilot studies showed significant treatment effects even with a marginal increase in DOC concentration.

2.4. DOC level analysis

Water samples were collected from beakers (DCW, LM NOM spiked water and SAHA spiked water) pre- and post-exposure to UVR. DOC levels were analyzed at the Saskatchewan Research Council (SRC) using UV persulfate digestion with IR detection on a Shimadzu TOC-VCPN analyzer equipped with an ASI-V autosampler (detection limit: 0.2 mg L⁻¹). Saskatchewan Research Council Analytical Laboratories is accredited by the Standards Council of Canada, in cooperation with the Canadian Association for Environmental Analytical Laboratories.

2.5. Euthanization

Dušan et al. (2006) and Manek et al. (2014) showed that minnows euthanized with an overdose of Aquacalm (methomylate hydrochloride) had a lower cortisol elevation from the baseline than those euthanized with MS222. Thus, at the end of the 96 h UVR exposure, minnows were euthanized with an overdose of Aquacalm for blood extraction and cortisol analysis. After extracting blood from the caudal vein region, all minnows were preserved in 10% neutral buffered formalin until further processing to obtain skin sections for histological analysis.

2.6. Experimental protocol for blood extraction

Blood extraction for cortisol analysis followed the method described by Halbgewachs et al. (2009). Blood samples (25–50 µL) were extracted from the caudal vein near the anal fin region of euthanized minnows. In order to obtain enough blood for the analysis, we pooled blood from four fish in the same beaker. This blood was placed on ice and allowed to clot for at least one hour. 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 (Immumite-1000 Cortisol, Diagnostic Products Corporation, USA), which is designed for quantitative measurement of cortisol in serum.

2.7. Histological analysis of the skin

Tissue preparation for the analysis of the minnow epidermis followed the method described by Manek et al. (2012). Skin sections were stained with periodic acid Schiff’s reagent and with Harris’ hematoxylin (PAS-H) to darken the mucous cells, the basement membrane and the nucleus, rendering ECCs colourless and easily recognizable. Images of each epidermal cross-section were captured with a Zeiss Axioplan fluorescence microscope with an AxioCamICc1 (color, 1.4 MP) digital camera at 10× magnification. For each slide, we recorded the following parameters: epidermal thickness, number of ECCs, ECC density and ECC area, which were all quantified using ImageJ 1.32, an image processing and analysis program (available on the National Institute of Health’s web page http://rsb.info.nih.gov/ij/). The observer was blind with respect to the treatment.

2.8. Statistical analysis

Levene’s tests were performed to check for homoscedasticity and Kolmogorov–Smirnov tests were performed to check for normality distribution. All statistical analyses were performed using SPSS (ver. 19, SPSS Inc., USA). We performed a series of 2 × 3 ANOVAs to assess the effect of DOC (DCW vs. SAHA vs. LM NOM) and UVR exposure (low UVR vs. high UVR) on DOC levels, cortisol levels and histological data (which included ECC density, epidermal width and ECC area). In all cases we used α = 0.05 as or default significance threshold, however, we did observe instances where we observed a statistically significant difference in cortisol levels when the biological difference was, in our opinion, somewhat ambiguous.

3. Results

3.1. Evaluation of mortality

In the experiment, 10% of the minnows in the high UVR + DCW group died and 5% of the minnows in the high UVR + SAHA group died. There was no mortality recorded in the low UVR + DCW group, high UVR + LM NOM group, low UVR + LM NOM group and low UVR + SAHA group. In the event that more than one of four minnows...
died in the test beaker the replicate was removed because there was not enough blood available for the cortisol analysis.

3.2. DOC levels

The $2 \times 3$ ANOVA revealed that mean DOC levels differed among DOC treatments ($F_{2,12} = 80.15, P < 0.001$), but not among UVR treatments ($F_{1,12} = 1.22, P = 0.291$) and that there was no interaction between the two ($F_{2,12} = 0.13, P = 0.714$) on mean DOC levels (Fig. 1).

Tukey post-hoc comparisons revealed that the 3 groups differed in DOC levels (all $P_s < 0.001$), with the water control having the lowest level and the LM NOM having the highest level of DOC.

3.3. Cortisol levels

The $2 \times 3$ ANOVA revealed a significant interaction between UVR and DOC on mean cortisol levels ($F_{2,37} = 26.91, P < 0.001$). In the control group, the presence of elevated UVR causes a 10-fold increase (from 3 to 30 ng mL$^{-1}$) in cortisol level ($P < 0.001$). In contrast, the presence of either source of DOC (SAHA vs. LM NOM) led to a reduction in cortisol level under high UVR, as compared to the low UVR conditions ($P = 0.009$ and 0.035 respectively). Even though we found a significant reduction in cortisol (~5–8 ng mL$^{-1}$) this reduction may not be biologically meaningful (Fig. 2).

3.4. Histological parameters

3.4.1. ECC density

The $2 \times 3$ ANOVA revealed a synergistic interaction between DOC source and UVR ($F_{2,38} = 10.19, P < 0.001$) on mean ECC density of minnows (Fig. 3). In the control group there was a much greater density of cells in the low UVR treatment than the high UVR treatment ($P < 0.001$), while in both of the DOC groups (SAHA vs. LM NOM) there was no difference in cell density between the high and low UVR treatments ($P = 0.558$ and 0.655 respectively).
stress response and ECC investment to a real-world aquatic humic substance LM NOM. Both sources of DOC have different specific UV absorption coefficients (index of aromaticity, measured using the absorbance of DOC at 340 nm, and expressed as cm$^2$ mg$^{-1}$; Curtis and Schindler, 1997). The specific absorption coefficient of SAHA is 79.98 cm$^2$ mg$^{-1}$ (Al-Reasi et al., 2012). LM NOM is primarily humic acid-like material (74%) and its specific absorption coefficient is 37.8–39.3 cm$^2$ mg$^{-1}$ (Al-Reasi et al., 2012; Gheorghiu et al., 2010). A higher specific UV absorption coefficient of SAHA compared to LM NOM indicated that the former has greater chromomorph attributes (potentially greater UVR absorption capacity) than the latter (Al-Reasi et al., 2011).

DOC by itself has some beneficial effects on fish physiology such as facilitating ion uptake, and regulation and amelioration of low pH associated stress (Wood et al., 2011). The concentration of DOC in DCW that minnows were housed in prior to and during UVR exposure was around 2.7 mg L$^{-1}$. We initially aimed at nearly doubling the concentration of DOC by spiking LM NOM or SAHA to obtain a final concentration of around 5–5.4 mg L$^{-1}$. These are levels that typically occur in aquatic ecosystems across the Canadian Prairies; however, there is extreme variation in DOC levels, with some lakes and wetlands in the Great Plains exceeding 150 mg L$^{-1}$ (Arts et al., 2000). Indeed, we found that a marginal increase (67–104%) in DOC levels (in mg L$^{-1}$) lowered cortisol levels in some groups and correspondingly increased ECC density. It is particularly important to note here that LM NOM, which is a natural aquatic DOC with lesser chromomorph properties, appeared to be more effective than SAHA in ameliorating stress response and ECC investment in fathead minnows under high UVR exposure in the present study. Indeed, LM NOM, actually had a stronger positive response than SAHA for epidermal width and ECC area. The potential cause(s) for the difference in response between the two sources of DOC is not clear at present, and deserves further attention. Previous studies have shown that increased exposure to UVR and altered pH (acidification) are known to have a negative effect on DOC by resulting in loss of DOC via photolysis (CDOM absorbance loss) in aquatic systems (Molot and Dillon, 1997; Gennings et al., 2001). We found no significant difference in pH levels across groups exposed to UVR with DCW, SAHA or LM NOM (F$_{5,48}$ = 0.87, $P = 0.50$) (Table 1). In fact, the pH of the feedlot pond was around 8.4 at the time the minnows were collected. This information underscores that fathead minnows used for this study were pre-exposed to alkaline conditions and that we estimated very similar pH levels in the experimental waters. Preliminary analysis of DOC concentration also revealed no significant difference in DOC levels in DCW, LM NOM or SAHA treatment water pre- and post-UVR exposure, in the presence or absence of a UVR blocking filter. Since our study was laboratory based and under controlled conditions, the duration of UVR exposure and concentration of DOC that we selected for this study possibly was not enough to result in loss of DOC or drastically alter the pH.

Results of our current study support previous findings related to the immune-function of ECCs (Chivers et al., 2007; Halbgewachs et al., 2009; Manek et al., 2012; Manek et al., 2014). As previously mentioned, ECCs were originally linked with production and maintenance of “alarm cues” which are chemical cues that elicit anti-predator response in conspecifics (Ferrari et al., 2010). In our previous work, we evaluated if changes in ECC numbers as a result of immunosuppression (UVR and/or Cd exposure) would have any effect on the level of anti-predator response to cues prepared from the skin of UVR and/or Cd exposed fathead minnows. We found no significant difference in anti-predator response to alarm cues prepared from the skin of UVR and/or Cd exposed or filtered fathead minnow, as a result of which we concluded that focussing on the immune function of ECCs was of prime importance (Manek et al., 2012, 2014). Since we have established that ECC investment can be modulated by exposure to an environmental stressor causing immunosuppression (UVR and/or Cd exposure), the main focus of this study was to indirectly ascertain if DOC played a role in affecting the immune-function of ECCs (cortisol production and ECC investment).

4. Discussion

The attenuation rate of UVR in water bodies is highly regulated by DOC, the concentration of which is known to be highly variable across aquatic ecosystems (Arts et al., 2000; Steinberg et al., 2008). Since it is well established that DOC acts as a natural sunscreen, it seemed logical to evaluate if altered DOC concentrations played a role in ameliorating physiological stress responses by lowering characteristic elevation of cortisol, and upregulating ECC investment in fathead minnows. Our work provides clear evidence that this is the case.

Previous studies focused on understanding how DOC influences toxicity of various metals have shown that different sources of NOM can have substantially different protective effects (Al-Reasi et al., 2011). This work cautions us against considering only the quantity of DOC, and suggests that we should also consider the sources of the DOC. To emphasize this aspect, we used two different sources of DOC: (i) a natural terrigenous source of DOC in the form of LM NOM, and (ii) a coal based readily available commercial source of DOC in the form of SAHA. Given that SAHA is a commercial humic substance, we wanted to compare the protective effects of SAHA on physiological

Fig. 5. Mean ± S.E. ECC area from the skin of minnows exposed to DCW, LM NOM or SAHA in the presence and absence of a UV blocking filter (white bar graph denotes the high UVR group and grey bar graph denotes low UVR group; n = 6–8/treatment). Different letters denote significant difference.
Most studies examining the effects of stressors on ECC investment have found effects on ECC numbers. There are a few cases where differences in epidermal thickness and in area of ECC were reported (Wisenden and Smith, 1997; Iger et al., 1994). Here we found that minnows specifically in LM NOM treatment had a thicker epidermis and a larger mean cell size (measured as cross-sectional area of the cells) compared to those in SAHA spiked water or DCW group. In our previous study (Manek et al., 2014), we found that exposure to cadmium and UVR resulted in a 25% thicker epidermis as compared to other groups. It is unclear as to why LM NOM exposure resulted in a thicker epidermis and larger ecc area. Additional manipulative experiments are needed to explain these patterns. From a biological significance point of view, to ascertain how much of a difference in ecc density would represent a difference outside the range of ecc density normally associated with healthy or non-stressed fish in the absence of predators, would totally depend on the pre-existing conditions to which fathead minnows are exposed, since baseline ecc densities are highly variable across sites (Manek et al., 2013).

Our current study reveals interesting ameliorative effects of increased DOC concentration on stress response and ECC investment in fish during a short term exposure to UVR under controlled laboratory conditions. Future research should evaluate the long term exposure effects and determine if the chemical properties of DOC that we selected for this study are altered over a prolonged period of exposure to UVR, and what effect this could have on physiological stress response and ECC investment in fathead minnows. It would also be interesting to examine the effects of lower DOC levels as a result of photolysis observed in natural systems due to various anthropogenic activities and climate change. With recent changes to aquatic systems resulting from anthropogenic disturbances, it is becoming critical to understand how changing water quality is affecting fish health. Since fathead minnows represent a widely distributed freshwater fish belonging to superorder Ostariophysi, our current results could be extrapolated to similar aquatic freshwater systems.

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