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RESEARCH ARTICLE

UV-B exposure reduces locomotor performance by impairing muscle function but not mitochondrial ATP production

Ensiyeh Ghanizadeh Kazerouni[1], Craig E. Franklin[2] and Frank Seebacher[1,*]

ABSTRACT

Ultraviolet B radiation (UV-B) can reduce swimming performance by increasing reactive oxygen species (ROS) formation. High concentrations of ROS can damage mitochondria, resulting in reduced ATP production. ROS can also damage muscle proteins, thereby leading to impaired muscle contractile function. We have shown previously that UV-B exposure reduces locomotor performance in mosquitofish (*Gambusia holbrooki*) without affecting metabolic scope. Our aim was therefore to test whether UV-B influences swimming performance of mosquitofish by ROS-induced damage to muscle proteins without affecting mitochondrial function. In a fully factorial design, we exposed mosquitofish to UV-B and no-UV-B controls in combination with exposure to *N*-acetylcysteine (NAC) plus no-NAC controls. We used NAC, a precursor of glutathione, as an antioxidant to test whether any effects of UV-B on swimming performance were at least partly due to UV-B-induced ROS. UV-B significantly reduced critical sustained swimming performance and tail beat frequencies, and it increased ROS-induced damage (protein carbonyl concentrations and lipid peroxidation) in muscle. However, UV-B did not affect the activity of sarco-endoplasmic reticulum ATPase (SERCA), an enzyme associated with muscle calcium cycling and muscle relaxation. UV-B did not affect ADP phosphorylation (state 3) rates of mitochondrial respiration, and it did not alter the amount of ATP produced per atom of oxygen consumed (P:O ratio). However, UV-B reduced the mitochondrial respiratory control ratio. Under UV-B exposure, fish treated with NAC showed greater swimming performance and tail beat frequencies, higher glutathione concentrations, and lower protein carbonyl concentrations and lipid peroxidation than untreated fish. Tail beat amplitude was not affected by any treatment. Our results showed, firstly, that the effects of UV-B on locomotor performance were mediated by ROS and, secondly, that reduced swimming performance was not caused by impaired mitochondrial ATP production. Instead, reduced tail beat frequencies indicate that muscle of UV-B exposed fish were slower, which was likely to have been caused by slower contraction rates, because SERCA activities remained unaffected.

KEY WORDS: Reactive oxygen species, Swimming kinematic, Calcium cycling, SERCA, Mitochondrial respiration, Glutathione

INTRODUCTION

Ultraviolet B radiation (UV-B) is an important environmental driver that can damage cells either directly, or indirectly by increasing production of reactive oxygen species (ROS). Increased ROS generation is a significant component of UV-B-induced damage to cells (Gniadecki et al., 2001; Ichihashi et al., 2003; Paz et al., 2008; Birch-Machin and Swalwell, 2010). Excess ROS can have detrimental effects on muscle function and metabolism, thereby impacting performance of animals under natural conditions (Kuwahara et al., 2010; Gram et al., 2015). However, the mechanistic pathways by which UV-B-induced ROS affect animal performance remain unsolved. In a previous study, we demonstrated that UV-B exposure increased lipid peroxidation and protein carbonyl concentrations in muscle of mosquitofish (*Gambusia holbrooki* Girard 1859), and it decreased swimming performance but did not affect metabolic scope (Ghanizadeh Kazerouni et al., 2015). Hence, UV-B can have a negative effect on muscle, but our previous results raise the question of how UV-B affects locomotor performance, particularly because metabolic scope remained unaffected. The aim of this study therefore was to determine whether UV-B-induced ROS impair muscle contractile function without affecting mitochondrial function.

Reduced swimming performance can result from impaired muscle contractile function (Seebacher et al., 2012). Muscle contraction and relaxation are mediated by Ca^{2+} release from the sarcoplasmic reticulum via ryanodine receptors and Ca^{2+} reuptake into the sarcoplasmic reticulum by sarco-endoplasmic reticulum ATPase (SERCA), respectively (Berchtold et al., 2000; Bootman et al., 2001). High concentrations of ROS can oxidise muscle proteins such as ryanodine receptors and SERCA, and therefore affect Ca^{2+} handling dynamics (Videler and Wardle, 1992; Perkins et al., 1997; Jackson, 2008; Bayeva and Ardehali, 2010; Dutka et al., 2012; Lebold et al., 2013). Impaired calcium handling dynamics can reduce the rates of force production and relaxation, which can lead to reduced locomotor performance (Westerblad and Allen, 2011; Bogdanis, 2012; Seebacher et al., 2012).

In addition, inadequate ATP supply to support muscle contraction and relaxation can impair locomotor performance. SERCA and myosin ATPase require ATP to facilitate muscle relaxation and contraction, respectively. Hence, insufficient ATP supply may affect muscle force production and fatigue resistance (Allen et al., 2008). ATP is produced by electrons passing through the electron transport chain in mitochondria. Release of the resulting proton gradient across the mitochondrial membrane provides the energy for ADP phosphorylation by complex V (Brand and Nicholls, 2011; Divakaruni and Brand, 2011). Importantly, mitochondria themselves are a source of ROS in skeletal muscle cells, and can be a major target for oxidative damage (Murphy, 2009; Barbieri and Sestili, 2012). ROS damage to mitochondrial membranes can result in decreased membrane potential and impaired ATP production (Ehlers et al., 1999). Additionally, the efficacy of mitochondria, that is the amount of oxygen necessary to produce a molecule of ATP (P:O ratio), may change with the cellular environment and thereby change the ATP supply even if whole-animal oxygen consumption remains the same (Brand, 2005).

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List of symbols and abbreviations

GSH	glutathione
NAC	*N*-acetylcysteine
P:O ratio	amount of ATP produced per atom of oxygen consumed
RCR	respiratory control ratio
ROS	reactive oxygen species
SERCA	sarco-endoplasmic reticulum ATPase
U_{crit}	critical sustained swimming performance
UV-B	ultraviolet B radiation

Cells defend themselves against ROS damage by enzymatic and non-enzymatic antioxidants. Glutathione (GSH) is an important non-enzymatic cellular antioxidant that is present in skeletal muscle fibres and mitochondria at high concentration, and its depletion is correlated with increased ROS damage (Martensson and Meister, 1989; Leeuwenburgh and Ji, 1995; Dam et al., 2012). *N*-acetylcysteine (NAC) is an antioxidant that can protect cells from ROS damage by increasing the concentration of cysteine, a precursor of GSH, and thereby maintaining the biosynthesis of reduced GSH in cells (Kerksick and Willoughby, 2005; Ferreira and Reid, 2008; Westerblad and Allen, 2011; Hernández et al., 2012). In addition, NAC contains a thiol group that can act directly as an effective ROS scavenger (Kerksick and Willoughby, 2005; Oksay et al., 2013).

We tested the hypotheses that: (1) reduced locomotor performance is caused by ROS, in which case treatment with NAC should at least partially alleviate the negative effects of UV-B; (2) UV-B affects muscle function, which manifests itself as reduced SERCA activity and altered swimming kinematics, and can explain reduction in sustained locomotor performance; and (3) UV-B impairs mitochondrial function by reducing ADP phosphorylation (state 3) rate, increasing proton leak (state 4) rate, and reducing the P:O ratio. To test these hypotheses we exposed adult male mosquitofish (*G. holbrooki*) to UV-B and controls (no-UV-B), each with and without NAC in a fully factorial design.

MATERIALS AND METHODS

Experimental animals

Mosquitofish were collected from a wild population in Australia (33°46′33″S, 151°14′52″E), and kept in plastic tanks (645×423×276 mm) at a density of 1–2 fish per litre for 1–2 weeks before starting the experiments. Fish were held, and all experiments were conducted, at 20°C, which was similar to the environmental temperature at the time of capture. We used only male mosquitofish in experiments, and fish were fed twice per day with fish flakes (Wardley Tropical Fish Flakes, Hartz Mountain Corporation, Secaucus, NJ, USA).

NAC treatments

We used NAC (Sigma-Aldrich, Castle Hill, NSW, Australia) as an antioxidant that reduces ROS to test whether the effects of UV-B on locomotor performance are mediated by ROS. Before starting the experiment, a pilot experiment was conducted to determine the NAC concentration necessary to modify GSH concentrations in the tail muscle. Mosquitofish were exposed to four concentrations of NAC (0.0, 0.1, 1.0 and 5 mmol l^{-1}) (Bridgeman et al., 1991; Peña-Llopis et al., 2003; Parng et al., 2006; Underwood et al., 2010) in the presence of 3.3 W m^{-2} UV-B for 1 week. UV-B exposure lasted for 3 h every day from 11:00 to 14:00 h (see below). There were three replicate tanks (40×20×35 mm) for each treatment with 15 fish in each tank. To maintain NAC concentration constant in the tank water, its concentration was measured every day (see below), and the depleted amount was added to the water. GSH concentrations in the tail muscle were measured on days 3 and 6 in eight fish each from 0.0, 0.1 and 1.0 mmol l^{-1} NAC treatments. The 0.1 mmol l^{-1} concentration was chosen for the main experiment because it significantly increased GSH concentration in fish muscle compared with the other concentrations (see Results). Fish treated with 5 mmol l^{-1} NAC died after 3 days, and these results were excluded from statistical analyses.

The NAC concentration in water was measured according to a published protocol (Kukoc-Modun and Radić, 2011). The method is based on a coupled two-step redox reaction where, initially, NAC reduces Fe (III) to Fe (II), and subsequently Fe (II) is complexed with 2,4,6-tripyridyl-s-triazine (TPTZ), which can be measured spectrophotometrically at 593 nm. In a cuvette, we added 1 ml of reaction solution consisting of: 800 µl of 0.5 mmol l^{-1} acetate buffer (pH 3.6), 50 µl of 10 mmol l^{-1} Fe (III), 50 µl of 10 mmol l^{-1} TPTZ, 40 µl water samples containing NAC, and 60 µl water, which we kept at room temperature (25°C) for 60 min before measuring the absorbance. A standard curve was determined using a prepared NAC solution (range 0–10 mmol l^{-1}; Sigma-Aldrich) to determine the unknown concentration of NAC in the tank water.

Experimental design

For the main experiment, male mosquito fish were transferred to a controlled temperature room, and were divided into UV-B and no-UV-B (control) treatments. Within each UV treatment, we conducted NAC treatments with no NAC (–NAC) or 0.1 mmol l^{-1} NAC (+NAC). There were three replicate tanks (40×20×35 mm) per treatment with 20 fish in each tank. Fish were chosen randomly from across all tanks within treatments for experimental measures to minimise potential tank effects. The photoperiod was 12 h:12 h light:dark for all treatments.

UV-B radiation was provided by 120-cm UV-B fluorescent tubes (UVB-313 EL, Q-lab, Cleveland, OH, USA; 280–390 nm) for 3 h each day from 11:00 to 14:00 h, which coincided with the time of greatest natural UV-B irradiation. The control groups received only visible light, and experimental groups received visible plus UV light. The peak natural dose of solar UV-B in summer at the capture site is 7.4 W m^{-2} at midday, and the total daily natural dose is 5100 J m^{-2} (Beckmann et al., 2014). We arranged our lamps so that the peak irradiance was 3.3 W m^{-2} UV-B at the water surface, which led to a total daily dose of 1188 J m^{-2}. This lower dose of UV-B was chosen to better reflect the average UV-B dose across seasons (Beckmann et al., 2014). We measured sustained swimming performance of 12–15 fish from each treatment after 1 week in treatments, which was sufficiently long to observe effects of UV-B and NAC where they existed (see pilot experiment; Ghanizadeh Kazerouni et al., 2015).

Swimming performance and kinematics

Critical sustained swimming speed (U_{crit}) (Brett, 1964) was measured according to published protocols (Hammill et al., 2004; Seebacher et al., 2015). U_{crit} was measured in a Blazka-style flume consisting of a cylindrical clear Perspex tube (150 mm length and 38 mm diameter). The flume was fitted tightly over the intake end of a submersible pump (12V DC, iL500, Rule, Hertfordshire, UK). The pump drew water through the flume and a bundle of hollow straws at the inlet end of flume helped maintain laminar flow. A plastic grid prevented fish from entering the pump. The flume and pump were submerged in a plastic tank (645×423×276 mm). A variable DC power source (NP9615; Manson Engineering Industrial, Hong Kong, China) was used to adjust flow speed by changing the voltage input. The water flow in each flume was measured in real-time by a flow meter (DigiFlow 6710 M, Savant Electronics, Taichung, Taiwan) connected to the outlet of each pump. To measure U_{crit}, fish swam at an initial flow rate of 0.06 m s^{-1} for 20 min, before flow speed was increased by 0.02 m s^{-1} every 5 min until fish were exhausted and unable to hold their position in the water column. The first time fish fell back onto the grid in the flume, the flow was stopped for several seconds before returning it to the previous speed, and the trial was terminated when the fish fell back the second time. U_{crit} is reported as body lengths per second (BL s^{-1}).

We measured tail beat amplitude and frequency of nine fish from each treatment by filming fish from above at 60 frames s^{-1} (with an Exilim EX ZR200 camera, Casio, Tokyo, Japan) according to Seebacher et al. (2012). The fish were filmed while holding their position in the swimming flume at 12, 15 and 18 cm s^{-1}, which correlated to 60, 75 and 90% of the average U_{crit} velocities of all groups, respectively. The films were analysed using

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Tracker Video Analysis and Modeling tool (Open Source Physics, www.opensourcephysics.org). The amplitude was measured as the maximal displacement of the tail at the peduncle during one tail beat cycle. Frequency was calculated as 1/period, where period is the time taken for the tail to complete one cycle. In the analyses, we used average tail beat frequencies and amplitudes from five independent tail beat events.

Lipid peroxidation and protein carbonyl determination

Fish were euthanised by immersion in an MS-222 solution (0.4 g l^{-1} buffered to pH 7), and the tail muscle was immediately frozen in liquid nitrogen and kept at −80°C for biochemical analyses. Lipid peroxidation was measured in tail muscle of nine to 12 fish from each treatment using a commercial kit (MAK085, Sigma-Aldrich) following the manufacturer's instructions. The assay is based on measuring malondialdehyde (MDA), which is the end product of the lipid peroxidation chain. To determine the amount of MDA in tissue samples, a standard curve was determined using a prepared MDA standard solution. Lipid peroxidation in each sample was expressed as nmol MDA per mg tissue.

Protein carbonyl concentrations in tail muscle of 10–11 fish from each treatment were determined using a commercial kit (MAK094, Sigma-Aldrich) and following the manufacturer's instructions. ROS oxidise proteins and form a stable carbonyl group that can be measured as a marker of oxidative damage. The amount of protein carbonyl in each sample was reported as nmol protein carbonyl per mg total protein. A bicinchoninic acid protein assay kit (BCA1 and B9643, Sigma-Aldrich) was used to determine the concentration of the total protein per sample.

GSH concentration and SERCA activity

GSH concentrations were determined in tail muscle of eight fish from each treatment using a commercial kit (CS0260, Sigma-Aldrich) and following the manufacturer's instructions. Reduced GSH, the main form of GSH present inside cells, is a tripeptide and the major free thiol in most living cells. Reduced GSH stabilises ROS by donating an electron. A standard curve was determined using a prepared reduced GSH solution (range 3.12–50 µm) to determine the amount of GSH in muscle samples. GSH in each sample was expressed as nmol GSH per mg tissue.

SERCA activity was measured in the tail muscle of 10–12 fish from each experimental group according to a published protocol (James et al., 2011). We measure SERCA activity to determine whether ROS impaired Ca^{2+} handling dynamics and muscle relaxation.

Mitochondrial respiration

Mitochondrial respiration was measured in the tail muscle of seven to nine fish from each experimental group according to published protocols (Walter and Seebacher, 2009; Dos Santos et al., 2013). The fish were killed by immersion in MS-222 solution (0.4 g l^{-1} buffered to pH 7) and the tail muscle was dissected and chopped on an ice-cold surface. The tissue was then homogenised in nine volumes of ice-cold isolation buffer (in mmol l^{-1}): KCl, 140; EDTA, 2; $MgCl_2$, 5; HEPES, 20; ATP, 1; 1% bovine serum albumin (BSA), pH 7 in a Teflon glass homogeniser and centrifuged at 500 ***g*** for 5 min. The supernatant was extracted and centrifuged at 1400 ***g*** for 5 min at 4°C, and then at 9000 ***g*** for 10 min. The pellet was resuspended in assay medium at 1:2000 (g tissue μl^{-1} assay medium) dilution. The assay medium contained (in mmol l^{-1}): Tris-HCl, 60; mannitol, 60; KCl, 5; KH_2PO_4, 5; $MgCl_2$, 0.5; EDTA, 0.2; 0.2 mg ml^{-1} BSA fatty acid free, pH 7.4. Mitochondrial oxygen consumption was measured in duplicate in a respiration chamber (Mitocell MT200; Strathkelvin Instruments, North Lanarkshire, UK) with a microelectrode (model 1302; Strathkelvin Instruments) connected to an oxygen meter (model 782; Strathkelvin Instruments). State 2 respiration was measured after adding a final concentration of 5 mmol l^{-1} malate to spark the Krebs cycle and then 2.5 mmol l^{-1} pyruvate acid as substrate. State 3 respiration was induced by adding 5 µl of 50 mmol l^{-1} ADP (final concentration, 1 mmol l^{-1}). State 4 respiration was obtained after adding oligomycin dissolved in ethanol at a final concentration of 4 µg ml^{-1} to inhibit the F_o/F_1 ATPase. Protein concentrations of the mitochondrial solutions were measured by the bicinchoninic acid assay kit (BCA, Sigma-Aldrich) following the manufacturer's instructions.

Statistical analysis

We conducted a two-way ANOVA with day and NAC concentrations as fixed factors to test for the effect of different concentrations of NAC on GSH concentrations in muscle in the pilot experiment. We conducted one-way ANOVA to compare GSH concentrations within days for different NAC treatments. We excluded the data for 5 mmol l^{-1} NAC treatment from the analysis because all fish died after 3 days.

We performed a two-way ANOVA with UV-B (UV-B and control) and NAC (+NAC and −NAC) as fixed factors to analyse swimming performance, GSH concentration, protein carbonyl concentration, lipid peroxidation, mitochondrial respiration and SERCA activity data. To test for the effect of UV-B and NAC on tail beat frequency and amplitude, a repeated-measures ANOVA was conducted (using Pillai's trace as the test statistic) with water flow velocity as the repeated measure, and UV-B and NAC as fixed factors. All data fulfilled the assumption of homogeneity of variance (Levene's test) and were not transformed. A significance level of 0.05 was used for all analyses, and the data are presented as means±s.e.m.

RESULTS

Effect of NAC on GSH production

In the pilot experiment, there was a two-way interaction between the effects of day of exposure and NAC concentration on GSH production in tail muscle (F=3.302, P=0.047). In fish treated with 0.1 mmol l^{-1} NAC, the concentration of GSH in tail muscle was significantly increased on days 3 and 6 (Fig. 1).

GSH and ROS-induced damage

There was a two-way interaction between UV-B exposure and NAC treatment in their effect on GSH concentrations in tail muscle (Table 1). UV-B-exposed fish treated with NAC had higher GSH concentrations than fish not treated with NAC (Fig. 2A). However, NAC did not increase the concentration of GSH in tail muscle of fish that were not exposed to UV-B (Fig. 2A).

A two-way interaction between UV-B exposure and NAC treatment determined protein carbonyl concentrations and lipid

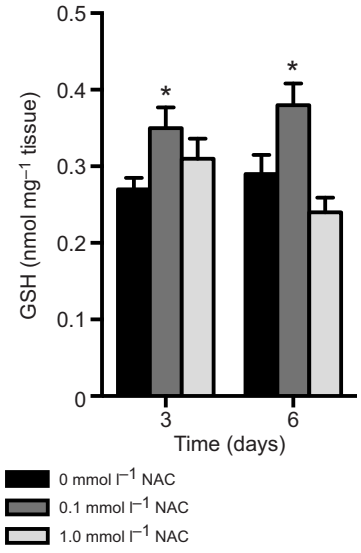

Fig. 1. The effect of *N*-acetylcysteine (NAC) administration on glutathione (GSH) concentration over 6 days of exposure to ultraviolet B radiation (UV-B). Treatment with 0.1 mmol l^{-1} NAC significantly increased GSH concentration at days 3 and 6 of exposure to UV-B (NAC×day interaction). Asterisks above bars indicate significant differences (*P<0.05) between the concentrations of GSH on different days. N=8 fish per time point and treatment; means±s.e.m. are shown.

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Table 1. Results of two-way ANOVA testing for the effect of ultraviolet B radiation (UV-B) and *N*-acetylcysteine (NAC) on critical sustained swimming speed (U_{crit}), glutathione concentration, protein carbonyl concentration (PC), lipid peroxidation (LP), sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) activity, state 3 and state 4 rates of mitochondrial respiration, respiratory control ratio (RCR), and amount of ATP produced per atom of oxygen consumed (P:O ratio)

	UV-B			NAC			UV-B×NAC		
	d.f.	*F*	*P*	d.f.	*F*	*P*	d.f.	*F*	*P*
U_{crit}	1	15.40	0.000	1	2.15	0.149	1	4.78	**0.034**
Glutathione	1	10.46	0.003	1	0.09	0.767	1	7.22	**0.012**
PC	1	11.03	0.002	1	1.57	0.218	1	4.45	**0.041**
LP	1	19.88	0.000	1	1.42	0.240	1	4.49	**0.040**
SERCA	1	0.041	0.841	1	0.076	0.785	1	0.27	0.606
State 3	1	1.66	0.208	1	1.21	0.281	1	0.99	0.326
State 4	1	0.18	0.676	1	0.291	0.594	1	0.77	0.385
RCR	1	4.94	**0.034**	1	1.26	0.270	1	0.115	0.737
P:O ratio	1	0.521	0.476	1	0.605	0.443	1	0.163	0.689

The relevant (highest interaction) significant results are highlighted in bold.

peroxidation (Table 1, Fig. 2). NAC significantly reduced the concentrations of protein carbonyl (Fig. 2B) and lipid peroxidation (Fig. 2C) in the UV-B-exposed fish, while there was no effect of NAC on fish not exposed to UV-B.

SERCA activity and locomotor performance

SERCA activity was not affected by NAC or UV-B (Table 1, Fig. 3A). Fish exposed to UV-B without NAC had significantly lower U_{crit}, but swimming performance increased significantly when UV-B-treated fish were exposed to NAC (Table 1, Fig. 3B). There was no effect of NAC on U_{crit} in control fish not exposed to UV-B (interaction between UV-B and NAC; Table 1, Fig. 3B).

Control fish had significantly higher tail beat frequencies than UV-B exposed fish at 15 and 18 cm s^{-1} water flow speeds (flow speed and UV-B interaction; Table 2, Fig. 3C). NAC alleviated the effects of UV-B at 15 and 18 cm s^{-1} water flow speeds, and tail beat frequencies of fish exposed to both UV-B and NAC were significantly higher than in the UV-B group with no NAC. There was no effect of NAC on tail beat frequencies of control fish that were not exposed to UV-B fish (NAC and UV-B interaction; Table 2, Fig. 3C). Tail beat amplitudes increased significantly with increasing water flow speeds in all treatments (main effect of flow speed), but there was no effect of NAC or UV-B on tail beat amplitudes (Table 2, Fig. 3D).

Mitochondrial respiration

State 3 (Fig. 4A) and 4 (Fig. 4B) respiration and P:O ratios (Fig. 4C) were not affected by NAC or UV-B treatments (Table 1). However, UV-B significantly decreased respiratory control ratios (main effect of UV-B, Table 1, Fig. 4D).

DISCUSSION

Swimming performance plays an important role by enabling essential behaviors such as migration, foraging, finding mates and avoiding predation (Husak et al., 2006; Mowles et al., 2010; Sinclair et al., 2014). Hence, it is important to understand the environmental drivers and physiological mechanisms that constrain locomotor performance. Here we found that UV-B-induced ROS reduced locomotor performance by impairing muscle contractile function but not the efficacy of mitochondrial ATP production. We accept our hypothesis that UV-B damage is mediated by ROS, because NAC significantly increased sustained swimming performance in fish exposed to UV-B and these increases were paralleled by decreased ROS-induced damage to membranes and proteins.

Swimming performance depends on muscle function, which is modified by factors such as excitation–contraction coupling, muscle protein function and metabolic capacity (Berchtold et al., 2000; Johnston and Temple, 2002; Seebacher et al., 2012; Seebacher and Walter, 2012). In isolated muscle fibres, ROS can damage SERCA, which contributes to reduce Ca^{2+} reuptake into the sarcoplasmic reticulum and therefore slows muscle relaxation (Dutka et al., 2012). Contrary to the results for isolated muscle fibres (Dutka et al., 2012), SERCA activity was not affected by UV-B radiation or NAC treatment in our experiment. Nonetheless, the lower U_{crit} of fish exposed to UV-B in the absence of NAC was paralleled by lower tail beat frequencies. Tail beat frequency reflects the speed of muscle contraction and relaxation, which is mediated by the sequence of events that start with Ca^{2+} release from the sarcoplasmic reticulum, followed by actin–myosin interaction and muscle contraction. Muscle relaxation comprises actin–myosin cross-bridge detachment, and Ca^{2+} re-uptake into the sarcoplasmic

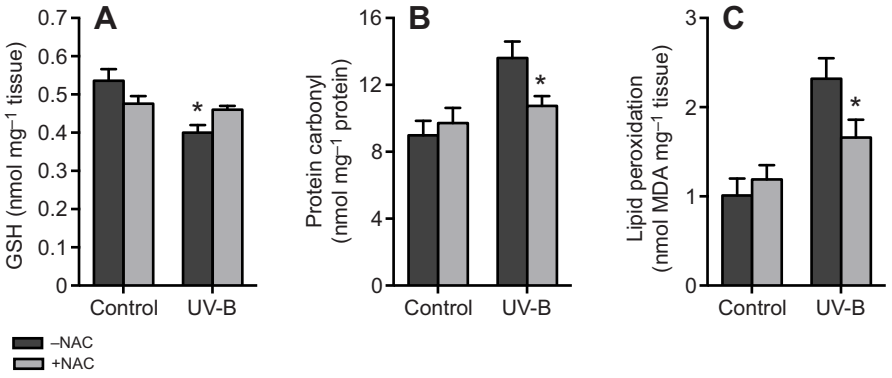

Fig. 2. GSH concentration and ROS-induced damage in response to NAC and UV-B. The effect of UV-B on concentrations of (A) GSH (*N*=8 fish per treatment), (B) protein carbonyl (*N*=10–11 fish per treatment) and (C) lipid peroxidation (*N*=9–12 fish per treatment) are shown with (grey bars) and without (black bars) NAC. NAC significantly increased GSH concentrations and decreased lipid peroxidation and protein carbonyls in UV-B-exposed fish, but it had no effect on fish not exposed to UV-B (control; UV-B×NAC interaction). Asterisks above bars indicate significant differences (*P<0.05) between treatments. Means±s.e.m. are shown.

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reticulum (Berchtold et al., 2000). Considering that SERCA was not affected by UV-B, it appears that the lower tail beat frequency was not caused by slowing of muscle relaxation. It is therefore likely that tail beat frequency was affected by the rate of muscle contraction. For example, ROS can oxidise ryanodine receptors and myofibrillar proteins (Kourie, 1998), which can lead to decreased force production and rates of contraction. Oxidised myofibrillar proteins have decreased Ca^{2+} sensitivity, and disrupted actin–myosin interactions (Allen et al., 2008). In addition, UV radiation and ROS can affect the nervous system and therefore influence excitation–contraction coupling, which may also impair muscle contractile function (Scholzen et al., 1999; Oka et al., 2015).

Table 2. Results from repeated-measures ANOVA testing for the effects of UV-B and NAC on tail beat frequency and amplitude of fish at three water flow speeds

	Frequency			Amplitude		
	d.f.	*F*	*P*	d.f.	*F*	*P*
UV-B	1	51.92	0.000	1	0.002	0.969
NAC	1	1.87	0.181	1	0.24	0.629
Speed	2	119.2	0.000	2	22.54	**0**
Speed×UV-B	2	9.02	**0.001**	2	0.64	0.535
Speed×NAC	2	0.15	0.855	2	0.49	0.619
UV-B×NAC	1	4.79	**0.036**	1	0.29	0.592
Speed×NAC×UV-B	2	0.66	0.525	2	1.45	0.242

The relevant (highest interaction) significant results are highlighted in bold.

The supply of ATP required for SERCA and myosin ATPase activities is another factor that can restrict muscle performance, and the availability of sufficient ATP is necessary to maintain normal muscle function and locomotion. We reject our hypothesis that UV-B decreases state 3 rates and P:O ratios. However, UV-B significantly decreased mitochondrial respiratory control ratio (RCR), which is an indicator of the relationship between basal proton leak and ATP supply. ROS damage to mitochondrial membranes can increase proton leak, which results in decreased membrane potential and ATP production (Skulachev, 1998; Brand et al., 2005). In addition, ROS can impair ATP production by disrupting substrate oxidation (Gniadecki et al., 2001; Paz et al., 2008). However, ADP phosphorylation (state 3 respiration) and

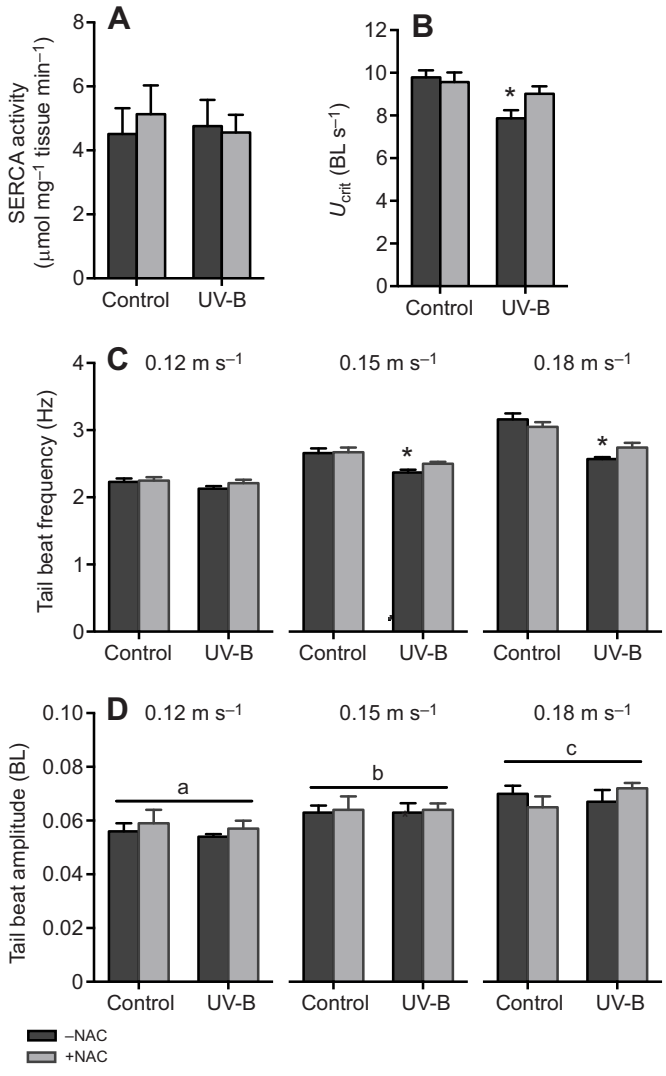

Fig. 3. The effects of UV-B and NAC exposure on sarco-endoplasmic reticulum ATPase (SERCA) activity and swimming performance. (A) There was no effect of NAC or UV-B on SERCA activity (*N*=10–12 fish per treatment). (B) NAC (grey bars) significantly increased critical sustained swimming performance (U_{crit}; *N*=12–15 fish per treatment) in fish exposed to UV-B, but had no effect on the control group (UV-B×NAC interaction). (C) Fish from the control treatment had significant higher tail beat frequency (*N*=9 fish per treatment) than fish exposed to UV-B at 15 and 18 cm s^{-1} water flow speed (UV-B×speed interaction). In the presence of UV-B, NAC significantly increased tail beat frequency of fish at 15 and 18 cm s^{-1} water flow speed (UV-B×NAC interaction). (D) Tail beat amplitude (*N*=9 fish per treatment) increased with increasing water flow speed in all groups (main effect of flow speed). Asterisks above histograms indicate significant differences (*P<0.05) between NAC treatments in the UV-B group. Horizontal lines above bars indicate significant differences between flow rates. Means±s.e.m. are shown.

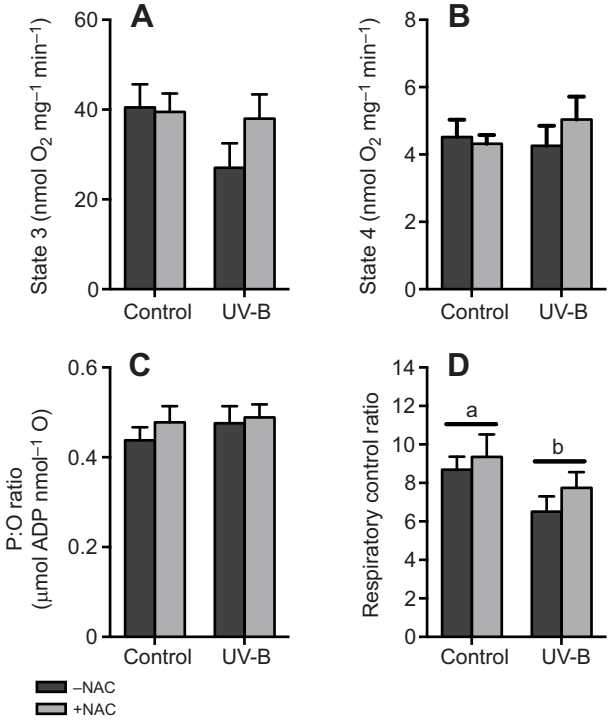

Fig. 4. Mitochondrial respiration in response to NAC and UV-B exposure. State 3 (A) and state 4 (B) respiration rates, and amount of ATP produced per atom of oxygen consumed (P:O ratio) (C) were not affected by UV-B or NAC exposure. However, the respiratory control ratio (D) decreased in the presence of UV-B (main effect of UV-B). Horizontal lines above histograms indicate significant differences between UV-B and control fish. *N*=7–9 fish per treatment; means±s.e.m. are shown.

basal proton leak (state 4) were not affected by UV-B or NAC, so the reduced RCR may be the result of slight decreases in state 3 and slight increases in state 4 that are not significant by themselves, but when combined in a ratio become significant. Increased proton leak can be a defensive mechanism that protects mitochondria by reducing membrane potential and therefore ROS generation (Brand, 2000; Jiang et al., 2009; Mailloux and Harper, 2011). Hence, to avoid extra ROS generation, basal uncoupled proton leak and therefore state 4 oxygen consumption may increase (Brand, 2005). However, UV-B or NAC did not alter the amount of ATP produced per atom oxygen consumed (P:O ratio). This implies that although the ratio of ADP phosphorylation to basal proton leak is affected by UV-B, proton leak at high membrane potential during state 3 of respiration is similar for all treatments. UV-B therefore did not affect the efficacy of ATP production. Hence, mitochondrial ATP production was not a limiting factor for swimming performance.

Mitochondria play an important role in energy metabolism, and mitochondrial changes can influence the ability of animals to live in changing environments. In cell culture, UV-B-induced ROS affected mitochondrial membranes and thereby led to impaired ATP production (Shimmura and Tsubota, 1997; Gniadecki et al., 2001; Wallace, 2005; Paz et al., 2008). However, contrary to the results from cell culture studies, mitochondrial function was not affected by UV-B-induced ROS in our study. Mitochondrial resistance to UV-B-induced ROS in mosquitofish indicates that UV-B might not necessarily be an environmental factor constraining energy metabolism. Nonetheless, UV-B impaired swimming performance by reducing muscle contractile function, which suggests that muscle proteins have a higher sensitivity to UV-B-induced ROS than mitochondria in mosquitofish, thereby contradicting the paradigm of high mitochondrial vulnerability to ROS (Richter et al., 1988; Yakes and Van Houten, 1997; Cadenas and Davies, 2000; Kujoth et al., 2005; Prakash et al., 2015).

Impaired swimming performance of fish exposed to UV-B shows that UV-B is an important environmental driver that influences locomotion. Our experimental UV-B exposure was well below maximal levels experienced by animals under natural conditions, and higher irradiation is likely to lead to more severe effects. However, our experimental levels are probably representative of natural conditions because maximal UV-B levels occur only for relatively brief periods during the day and year, and animals can avoid these behaviourally. However, even our relatively low levels of UV-B affect animal movement and potentially spatial ecology by modifying the dynamics of dispersal, migration and interactions. For example, exploration of a novel environment is dependent on swimming performance (Sinclair et al., 2014), thus in the presence of UV-B animals may explore less and perhaps show lower dispersal (Patterson et al., 2008).

Competing interests

The authors declare no competing or financial interests.

Author contributions

E.G.K., F.S. and C.E.F. conceived the ideas, E.G.K. and F.S. designed the experiments and wrote the manuscript, E.G.K. conducted the experiments, and C.E.F. edited the manuscript.

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