



Sub-lethal UV radiation during early life stages alters the behaviour, heart rate and oxidative stress parameters in zebrafish (*Danio rerio*)

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ABSTRACT

Environmental UV radiation in sufficient doses, as a possible consequence of climate change, is potent enough to affect living organisms with different outcomes, depending on the exposure life stage. The aim of this project was to evaluate the potentially toxic effects of exposure to sub-lethal and environmentally relevant doses of UVA (9.4, 18, 7, 37.7 J/cm²) and UVB radiation (0.013, 0.025, 0.076 J/cm²) on the development and behaviour in early life stages (4.5–5.5 h post fertilization, hpf) of the zebrafish (*Danio rerio*). The used doses were all below the median lethal dose (LD₅₀) and caused no significant difference in survival, deformities, or hatching between exposed and control groups. Compared to controls, there were transient UVA and UVB exposure effects on heart rate, with dose dependent reductions at 50 hpf, and at 60 hpf for UVA only. The UVB exposure caused an increasing trend in reactive oxygen species (ROS) formation at the two highest doses, even though only significant at 120 hpf for the second highest dose. Both UVA and UVB caused an increasing trend in lipid peroxidation (LPO) at the highest doses tested at 72 hpf. Furthermore, UVA exposure led to significant reductions in larval movement following exposure to the two highest doses of UVA, i.e., reduction in the time spent active and the total distance moved compared to control at 100 hpf, while no effect on the swimming speed was observed. The lowest dose of UVA had no effect on behaviour. In contrast, the highest dose of UVB led to a possible increase in the time spent active and a slower average swimming speed although these effects were not significant ($p = 0.07$). The obtained results show that UV doses below LD₅₀ levels are able to cause changes in the behaviour and physiological parameters of zebrafish larvae, as well as oxidative stress in the form of ROS formation and LPO. Further testing is necessary to assess how this type of radiation and the effects observed could affect fish population dynamics.

1. Introduction

Ultraviolet light is ubiquitously present in the environment and classified into three categories: UVA (400–315 nm), UVB (315–280 nm), and UVC (280–100 nm), which is absorbed by the ozone layer and does not occur as part of the solar spectrum reaching the troposphere. The depletion of the ozone layer and climate change together are increasing the exposure of aquatic organisms to increasing levels of UVB and UVA radiation (Bais et al., 2018). It has been proposed that exposure to an altered UV regime can potentially cause differences in behavioural responses and possibly influence the level of biodiversity and food web dynamics in aquatic ecosystems (Bais et al., 2018).

Most studied aquatic organisms, particularly those inhabiting

shallow aquatic environments, show susceptibility to the detrimental effects of UV radiation exposure (Häder et al., 2007). In general, it has been reported that fish spawning in shallow waters are most susceptible to the biologically damaging effects of UV radiation due to exposure of the vulnerable early larval stages, at a time when extensive DNA replication and organogenesis is taking place (Béland et al., 1999; Hunter et al., 1979). In sufficient doses (i.e. a longer exposure time), UV radiation can impair embryonic development in fish (Andrade et al., 2017; Fujimoto et al., 2007), and additionally it was found that zebrafish embryos at the gastrulation stage (starting from 5.25 h post fertilization (hpf)), were more tolerant to UV radiation compared to later developmental stages (Dong et al., 2007). Further, it was shown that even UVC radiation, at a wavelength outside the solar spectrum could inflict severe biological damage, whereby hindering the embryonic

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development in zebrafish (*Danio rerio*) via impairment of epiboly in the earliest post-zygotic stages (Strähle and Jesuthasan, 1993).

Moreover, UV radiation in sufficient doses can initiate a series of redox reactions to generate reactive oxygen species (ROS), which cause oxidative stress to cells and tissues during irradiation, but also as a result of disturbed cellular metabolic processes (Stańczyk et al., 2005). Although the exposure effects on oxidative stress parameters in larval fish have been reported after chronic UV irradiation during several days (Lesser et al., 2001; Mekki et al., 2010), it is less known whether these effects are persisting at later developmental stages.

In addition to the potential of UV radiation to induce oxidative stress, previous studies have shown that UVB exposure also caused differences in physiological and behavioural responses in fish larvae (Icoglu Aksakal and Ciltas, 2018), which are key life fitness traits essential for the growth and survival. Alterations in these responses would have severe consequences for the survival of these vulnerable early life-history stages. For example, an impairment of avoidance behaviour was demonstrated after exposure to environmentally relevant doses of UVB in cod (*Gadus morhua*) larvae (Fukunishi et al., 2012). In an earlier study, Alemanni et al. (2003) investigated the neurobehavioural effects of UVB exposure in juvenile rainbow trout (*Oncorhynchus mykiss*). These authors observed that irradiation with UVB from fluorescent tubes irreversibly increased trout O₂ consumption by individual fish. Further, rapid tail and fin movement as well as rapid and erratic displacements were observed at doses that caused changes in the O₂ consumption. In another study, Häkkinen et al. (2004) reported that exposure of newly fertilized pike (*Esox lucius*) eggs to UVB-doses similar to one daily erythema weighted ambient dose in Finland in May (0.27 J/cm², solar radiant exposure weighted by an action spectrum), resulted in neurobehavioural disorders such as inability to swim straight, circular movement and eventual mortality. However, to date insufficient data is available on the potential persistence of deleterious effects of UV irradiation during early life stages prior to hatching in fish.

The objective of this study was to investigate whether zebrafish sub-lethal UVA and UVB exposures during a vulnerable early life stage can cause persisting changes in physiological, oxidative stress parameters and lead to locomotor behavioural changes later in life. For this purpose, the zebrafish was selected as a model organism as it is a well-known model for developmental and behavioural toxicity assessment following environmental toxicant exposures (Ton et al., 2006; Parnig et al., 2007; Selderslaghs et al., 2010; Colwill and Creton, 2011; Tierney, 2011). The doses used in this study correspond to a typical mid-summer, midday and clear sky average outdoor exposure in Oslo (60°N) of 10 and 150 min of UVB and UVA, respectively. Zebrafish from the late blastula to early gastrula stages (4.5–5.5 hpf), when the cell fate specification onset takes place (Kimmel et al., 1995; Montero et al., 2005) were used for the exposure studies. In addition to changes in larval behaviour, changes in heart rate as well as changes in oxidative stress were assessed.

2. Materials and methods

2.1. Fish husbandry

The study was performed at The Norwegian Zebrafish Platform of the Norwegian University of Life Sciences, Oslo, Norway. The unit is licensed by the Norwegian Animal Research Authority (NARA) (www.mattilsynet.no) and accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (www.aalac.org). The study was carried out under the regulations approved by the unit's animal ethics committee (Institutional Animal Care and Use Committee/IACUC) following Norwegian laws and regulations controlling experiments and procedures on live animals in Norway. AB wild-type zebrafish were maintained at 28 °C under a 14:10 light/dark photoperiod. Adult care and breeding was in accordance with the local protocols previously described in Hurem et al. (2017). To generate embryos, adults were placed in spawning tanks in the afternoon, and the fish

were spawned following the cessation of light (08:00) the next day, and the embryos collected (09:00) and maintained in sterile embryo media (60 µg/mL Instant Ocean® sea salts) until the time of exposure.

2.2. Ethical statement

All animal experiments in this study were performed in accordance with the Norwegian Animal Protection Act (implemented EU Directive 2010/63/EU) and larvae were euthanized at 120 hpf using an overdose of Tricaine (MS-222, Sigma Aldrich), followed by rapid freezing at (-70 °C).

2.3. UV exposure and embryo toxicity

Embryos between the late blastula (4.5 hpf) and early gastrula (5.5 hpf) stage of development were used for the UVA and UVB exposures (Table 1). All exposures were performed in polystyrene 50 × 9 mm Petri dishes (VWR, Radnor, PA, U.S) without the lid with 10 embryos in a 1 mL volume. Radiation exposure was performed using a modified exposure unit (PolyLux PT, Dreve-Dentamid, Unna, Germany) consisting of three 9 W PL 12 UVB lamps (Philips, Eindhoven, The Netherlands) or three UVA-lamps, Osram GmbH DULUX S BL UVA 9 W/78. In order to remove UV with shorter wavelengths than 280 nm a filter material consisting of 5 mm Poly-Methyl-Methacrylate (Atoglas, Altuglas International) was placed in front of the exposure unit. The transmission of the filter was 100% for wavelengths above 300 nm. During irradiance measurement of the UVB-lamps, the filter was placed between the lamp and the detector to account for any absorption or light scatter in the material. The samples to be irradiated with UVB were placed 10 cm from the exposure unit. The UVA irradiation was performed with two exposure units placed on top of each other in a “sandwich” configuration with Petri dishes placed on a plate made of Atoglas in the gap between the exposure units. Thereby the dishes transparent to UVA were irradiated from both sides. The irradiance at the level of the dishes was estimated by adding the upward and downward fluxes. The spectrum and irradiance were determined by a scanning spectral radiometer (Bentham, UK, DTM 300 with a fibre optic light guide and cosine adapted diffuser D7). Constancy of the irradiance values was routinely performed with a Solar Light Co, PMA2100 (Philadelphia, USA) radiometer with appropriate detectors. The irradiance levels were 10.4 mW/cm² and 0.42 mW/cm² in UVA and UVB, respectively. The controls for UVB and UVA embryos were kept at room temperature (22 °C) during irradiation.

In order to determine the LD₅₀, 40 embryos distributed in 4 wells (10 embryos/ well) of a 12-well plate (Nunc™, Thermo-Fischer Scientific) were irradiated at approximately 5 hpf over the whole dose range. The number surviving a certain dose was scored at 48 hpf and expressed as surviving fraction relative to an unexposed control. The LD₅₀ was found by linear extrapolation of data from 4 to 5 independent experiments (Table 1A). The subsequent lower UVA and UVB doses for the behaviour studies were chosen from the LD₅₀ estimation. In order to determine the toxic effects of the used lower doses of UVA and UVB radiation exposure on the survival and development of the embryos and

Table 1

Doses for zebrafish UVA and UVB exposure experiments, group denotations and comparison to LD₅₀.

UVA exposure, 10.4 mW/cm ²			UVB exposure, 0.42 mW/cm ²		
Group	Exposure time (s)	Dose (J/cm ²), (% of LD ₅₀)	Group	Exposure time (s)	Dose (J/cm ²), (% of LD ₅₀)
Control	0	0	Control	0	0
UVA 1	900	9.4, (17%)	UVB 1	30	0.013, (13%)
UVA 2	1800	18.7, (34%)	UVB 2	60	0.025, (25%)
UVA 3	3600	37.4, (68%)	UVB 3	180	0.076, (76%)

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