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

4

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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/cm^2) and UVB radiation (0.013, 0.025, 0.076 J/cm^2) on the development and behaviour in early life stages (4.5 – 5.5 hours post fertilization, hpf) of the zebrafish (*Danio rerio*). The used doses were all below the median lethal dose (LD_{50}) 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 LD_{50} 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.

Key words: zebrafish; UV; locomotor; heart rate; ROS; lipid peroxidation

41 ## 1 Introduction

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

50 Most studied aquatic organisms, particularly those inhabiting shallow aquatic environments, show
51 susceptibility to the detrimental effects of UV radiation exposure (Häder et al., 2007). In general,
52 it has been reported that fish spawning in shallow waters are most susceptible to the biologically
53 damaging effects of UV radiation due to exposure of the vulnerable early larval stages, at a time
54 when extensive DNA replication and organogenesis is taking place (Béland et al. 1999, Hunter et
55 al., 1979). In sufficient doses (i.e. a longer exposure time), UV radiation can impair embryonic
56 development in fish (Andrade et al., 2016; Fujimoto et al., 2007), and additionally it was found
57 that zebrafish embryos at the gastrulation stage (starting from 5.25 hours post fertilization (hpf)),
58 were more tolerant to UV radiation compared to later developmental stages (Dong et al., 2007).
59 Further, it was shown that even UVC radiation, at a wavelength outside the solar spectrum could
60 inflict severe biological damage, whereby hindering the embryonic development in zebrafish
61 (*Danio rerio*) via impairment of epiboly in the earliest post-zygotic stages (Strähle and Jesuthasan,
62 1993).

63 Moreover, UV radiation in sufficient doses can initiate a series of redox reactions to generate
64 reactive oxygen species (ROS), which cause oxidative stress to cells and tissues during irradiation,
65 but also as a result of disturbed cellular metabolic processes (Stańczyk et al., 2005). Although the
66 exposure effects on oxidative stress parameters in larval fish have been reported after chronic UV
67 irradiation during several days (Lesser et al., 2000; Mekkawy et al., 2010), it is less known whether
68 these effects are persisting at later developmental stages.

69 In addition to the potential of UV radiation to induce oxidative stress, previous studies have shown
70 that UVB exposure also caused differences in physiological and behavioural responses in fish
71 larvae (Icoglu Aksakal and Ciltas, 2018), which are key life fitness traits essential for the growth
72 and survival. Alterations in these responses would have severe consequences for the survival of
73 these vulnerable early life-history stages. For example, an impairment of avoidance behaviour was
74 demonstrated after exposure to environmentally relevant doses of UVB in cod (*Gadus morhua*)
75 larvae (Fukunishi et al. 2012). In an earlier study, Alemanni et al., (2003) investigated the
76 neurobehavioural effects of UVB exposure in juvenile rainbow trout (*Oncorhynchus mykiss*).
77 These authors observed that irradiation with UVB from fluorescent tubes irreversibly increased
78 trout O_2-consumption by individual fish. Further, rapid tail and fin movement as well as rapid and
79 erratic displacements were observed at doses that caused changes in the O_2 consumption. In
80 another study, Häkkinen et al. (2004) reported that exposure of newly fertilized pike (*Esox Lucius*)
81 eggs to UVB-doses similar to one daily erythema weighted ambient dose in Finland in May (0.27
82 J/cm^2, solar radiant exposure weighted by an action spectrum), resulted in neurobehavioural
83 disorders such as inability to swim straight, circular movement and eventual mortality. However,
84 to date insufficient data is available on the potential persistence of deleterious effects of UV
85 irradiation during early life stages prior to hatching in fish.

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

98

99 ## 2 Materials and methods

100 ### 2.1 Fish husbandry

101 The study was performed at The Norwegian Zebrafish Platform of the Norwegian University of
102 Life Sciences, Oslo, Norway. The unit is licensed by the Norwegian Animal Research Authority
103 (NARA) (www.mattilsynet.no) and accredited by the Association for Assessment and
104 Accreditation of Laboratory Animal Care (www.aaalac.org). The study was carried out under the
105 regulations approved by the unit's animal ethics committee (Institutional Animal Care and Use
106 Committee/IACUC) following Norwegian laws and regulations controlling experiments and
107 procedures on live animals in Norway. AB wild-type zebrafish were maintained at 28°C under a
108 14:10 light/dark photoperiod. Adult care and breeding was in accordance with the local protocols

109 previously described in Hurem et al. (2017). To generate embryos, adults were placed in spawning
110 tanks in the afternoon, and the fish were spawned following the cessation of light (08:00) the next
111 day, and the embryos collected (09:00) and maintained in sterile embryo media (60 µg/mL Instant
112 Ocean® sea salts) until the time of exposure.

113 **2.2. Ethical statement**

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

117 **2.3 UV exposure and embryo toxicity**

118 Embryos between the late blastula (4.5 hpf) and early gastrula (5.5 hpf) stage of development were
119 used for the UVA and UVB exposures (Table 1). All exposures were performed in polystyrene 50
120 x 9 mm Petri dishes (VWR, Radnor, PA, U.S) without the lid with 10 embryos in a 1 mL volume.
121 Radiation exposure was performed using a modified exposure unit (Polylux PT, Dreve-Dentamid,
122 Unna, Germany) consisting of three 9 W PL 12 UVB lamps (Philips, Eindhoven, The Netherlands)
123 or three UVA-lamps, Osram GmbH DULUX S BL UVA 9 W/78. In order to remove UV with
124 shorter wavelengths than 280 nm a filter material consisting of 5 mm Poly-Methyl-Methacrylate
125 (Atoglas, Altuglas International) was placed in front of the exposure unit. The transmission of the
126 filter was 100 % for wavelengths above 300 nm. During irradiance measurement of the UVB-
127 lamps, the filter was placed between the lamp and the detector to account for any absorption or
128 light scatter in the material. The samples to be irradiated with UVB were placed 10 cm from the
129 exposure unit. The UVA irradiation was performed with two exposure units placed on top of each
130 other in a "sandwich" configuration with Petri dishes placed on a plate made of Atoglas in the gap
131 between the exposure units. Thereby the dishes transparent to UVA were irradiated from both

132 sides. The irradiance at the level of the dishes was estimated by adding the upward and downward
133 fluxes. The spectrum and irradiance were determined by a scanning spectral radiometer (Bentham,
134 UK, DTM 300 with a fibre optic light guide and cosine adapted diffuser D7). Constancy of the
135 irradiance values was routinely performed with a Solar Light Co, PMA2100 (Philadelphia, USA)
136 radiometer with appropriate detectors. The irradiance levels were 10.4 mW/cm^2 and 0.42 mW/cm^2
137 in UVA and UVB, respectively. The controls for UVB and UVA embryos were kept at room
138 temperature (22°C) during irradiation.

139

140 **Table 1.** Doses for zebrafish UVA and UVB exposure experiments, group denotations and
141 comparison to LD_{50}.

UVA exposure, 10.4 mW/cm^2			**UVB exposure, 0.42 mW/cm^2**		
Group	**Exposure time (s)**	**Dose (J/cm^2), (approx. % of LD_{50})**	**Group**	**Exposure time (s)**	**Dose (J/cm^2), (approx. % of LD_{50})**
Control	0	0	Control	0	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 %)

142

143 In order to determine the LD_{50}, 40 embryos distributed in 4 wells (10 embryos/ well) of a 12-well
144 plate (Nunc™, Thermo-Fischer Scientific) were irradiated at approximately 5 hpf over the whole
145 dose range. The number surviving a certain dose was scored at 48 hpf and expressed as surviving
146 fraction relative to an unexposed control. The LD_{50} was found by linear extrapolation of data from
147 4-5 independent experiments (Table 1A). The subsequent lower UVA and UVB doses for the

148 behaviour studies were chosen from the LD_{50} estimation. In order to determine the toxic effects of
149 the used lower doses of UVA and UVB radiation exposure on the survival and development of the
150 embryos and larva, including the LD_{50}, the zebrafish embryo toxicity test (OECD, 2013) was
151 applied. Following exposure, embryos were incubated in 96 well plates (Nunc™, Thermo-Fischer
152 Scientific) until 96 hpf. The survival, occurrence of deformities, and the median hatching time
153 (HT_{50}) were assessed in embryonic and larval zebrafish exposed to doses lower than the LD_{50}
154 presented in Table 1. In addition, body length was assessed at 72 hpf using a stereomicroscope in
155 20-30 replicate larvae without deformities per each exposure dose.

156 **2.4 Heart rate**

157 In order to determine the effects of UVA and UVB radiation exposure on the metabolism, the heart
158 rate was assessed at 50 and 60 hpf using a light microscope and counted as the number of heart
159 beats in a 15 sec period. Eight to ten larvae/group were scored for each biological replicate (n =
160 38-53/group). For UVB, one biological replicate was missing, therefore an additional 8-10 larvae
161 were analyzed within the subsequent biological replicate.

162 **2.5 Oxidative stress**

163 ***2.5.1 ROS formation***

164 Intracellular ROS production was determined in zebrafish after UV irradiation using the
165 fluorescent probe 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA, Invitrogen, Molecular
166 Probes Inc., Eugene, OR, USA) and according to the method described in Hurem et al., (2017).
167 Briefly, embryos were individually collected and incubated in a 96-well black microplate (Corning
168 Costar, Cambridge, MA, USA) for 1.5 hours with H_2DCFDA, with 20-24 replicate embryos per
169 exposure group at 70 hpf. Fluorescence was recorded at approximately 72 and 120 hpf in mean
170 relative fluorescence units (RFU) using the Cytation 3 Cell Imaging Multi-Mode Reader (Biotek,

Winooski Vermont, USA) and analyzed using Gen5 Microplate Reader and Imager Software (Biotek, Winooski Vermont, USA). Natural fluorescence of irradiated egg water in combination with the probes (without presence of embryos) for each dose rate and the resulting fluorescence subtracted, including a positive control (1% H202) were also analysed. The relative fluorescence obtained for each exposure group was expressed as fold induction comparative to the control.

2.5.2 Lipid peroxidation

The lipid peroxidation was assessed by two methods. First, the probe C11-BODIPY$^{581/591}$ was used for measuring LPO in zebrafish larvae in a time-dependent manner. This probe is a fatty acid analogue with specific fluorescence properties, which can easily enter the lipid bilayer and be subject to oxidation by oxyl-radicals together with the endogenous fatty acids, once inside the cellular membrane (Drummen et al., 2002). Similarly to ROS formation, exposed embryos and controls were individually collected and incubated in a 96-well black microplate (Corning Costar, Cambridge, MA, USA) for 2 hours with C11-BODIPY$^{581/591}$ (final concentration 10 μM), with 20-23 replicate embryos per exposure group at 70 hpf. Fluorescence was recorded by use of the same system as for ROS at 72, 96 and prior to 120 hpf and the results expressed as fold induction comparative to the control.

Lipid peroxidation was also determined in 72 hpf larvae by measurement of malondialdehyde (MDA) and 4-hydroxyalkenals (4-HNE) concentrations upon decomposition by polyunsaturated fatty acid peroxides, following the method by Erdelmeier et al. (1998), previously described in zebrafish larvae (Hurem et al., 2017). Here, 15 zebrafish larvae were pooled per sample in triplicate biological samples per dose, with exception of duplicates in UVA2 and UVB3 (where an additional technical replicate was used).

193 **2.6 Behavioural testing**

194 For the behavioural analyses, immediately following exposure, individual embryos were placed in
195 the wells of square 96 well plates (#7701-1651, Whatman, USA) with 500µL of media and placed
196 inside an incubator set to 28°C with a 14:10 day/night cycle. UVA and UVB treated embryos were
197 transferred to separate plates. For the first three biological replicates, the control and all 3 UVA
198 and UVB doses were equally represented across two 96 well plates (n = 24/dose/plate), whereas
199 for the final three biological replicates the control and all 3 UVA and UVB doses were equally
200 represented across only one 96 well plate (n = 24/dose). The locomotor activity (LMR) of the
201 larvae (total 139-143 larvae from 6 experiments) was visualized over a set time interval.
202 Behavioural tests were conducted using a ViewPoint® Zebrabox system and the accompanying
203 video tracking software (ViewPoint Life Sciences, Lyon, France), which is a high-throughput
204 image analysis system that can visualize and quantify the zebrafish behavioural response.
205 Behavioural screening was undertaken at 100 hpf. This corresponds to tests beginning 330 minutes
206 (13:00) and 390 (14:00) minutes after the cessation of light (07:30) in the incubator for UVA and
207 UVB, respectively. Larval behaviour, including the cumulative distance travelled and the time
208 spent active per minute, were simultaneously measured for all larvae on a plate during a 50 minute
209 simulated light-dark-light cycle, consisting of 20 minutes of light, 20 minutes of darkness, and
210 final 10 minutes of light. The average swimming speed was calculated by dividing the cumulated
211 distance travelled with the total time spent active. The light level was set to 100 % on the
212 ViewPoint software. The larval activity was tracked during the dark period. After the behavioural
213 test, the larvae were inspected with a stereo microscope to identify dead or deformed larvae.

2.7 Statistical analysis

After evaluating and arranging the data in Excel, the differences in general toxicity, heart rate and LPO between exposure groups were analysed using a one-way ANOVA and Tukey's multiple comparison tests (GraphPad Prism 7 Software Inc., La Jolla, CA, USA). Differences between dose and time were compared for ROS production using a Two-way ANOVA followed by the post-hoc Tukey test (GraphPad Prism 7 Software Inc., La Jolla, CA, USA). For behavioural analyses, data were transferred to R version 2.15.0 (R Development Core Team, http://www.r-project.org). Dead and deformed larvae were excluded from behavioural analyses. Only the cumulative data from the 20 minutes dark period of the test were used, as movement was minimal during the lighted periods. Linear mixed effect (LME) models were used within the "nlme" package of R to assess behaviour. The dependent variable was either the cumulative time spent active, the cumulative distance travelled, or average speed (calculated as the cumulated distance travelled/cumulated time spent active), with dose as a categorical independent variable, and replicate as a random effect. For all models, examination of the residual plots verified that no systematic patterns occurred in the errors (e.g. q-q plots). To assess individual doses to the controls, we used the contrast results provided within R. Significance in all tests was assigned at $p \leq 0.05$.

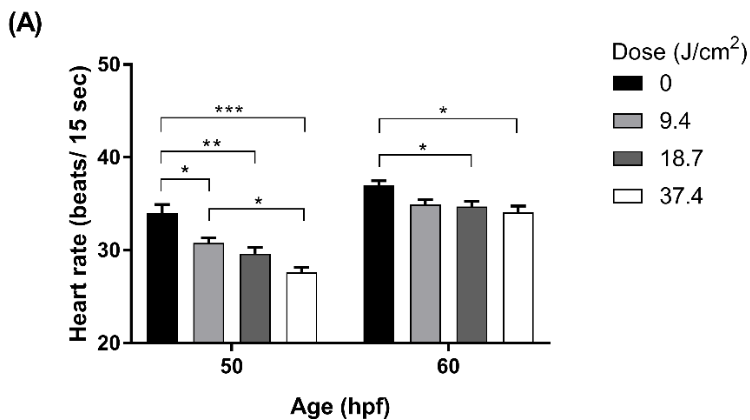
3 Results

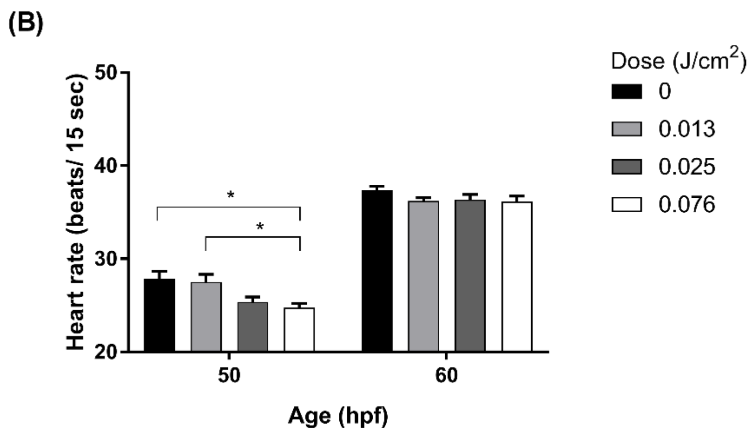
3.1 Developmental effects and heart rate

Analyses of mortality, deformities or hatching at 48, 72 and 96 hpf between controls and the exposed groups using the doses below LD_{50} showed no significant differences compared to controls and were generally below 10% (Table 2A and 3A). There was no difference in mortality

236 between 48 hpf and later time points. Additionally, there was no difference in body length at 72
237 hpf between exposed and control larvae (Table 2A).

238 UVA exposure significantly decreased the heart rate at 50 hpf in all exposed groups compared to
239 controls, while at 60 hpf, the decrease remained significant only in the 18.7 and 37.4 J/cm^2 UVA
240 doses (p = 0.04 and p = 0.003, respectively) (Fig 1A). The results of UVB exposure showed a
241 significant decrease in mean heart rate at the highest UVB dose compared to the controls ($p < 0.01$)
242 at 50 hpf , while no significant differences were observed in the two lower UVB doses compared
243 to controls (Fig 1B). By 60 hpf, no differences in heart rate were found between the UVB exposed
244 and control groups.





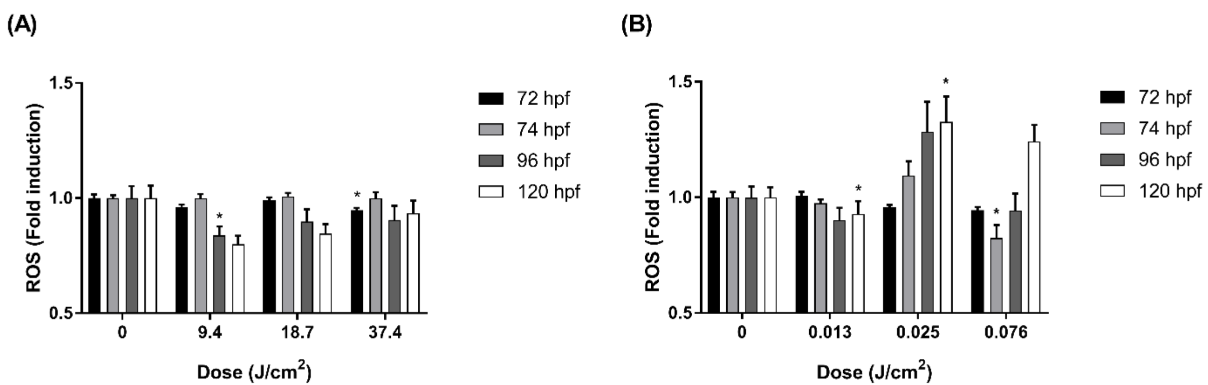
245

246 **Fig 1.** Heart rate measured at 50 and 60 hpf in zebrafish exposed to sub-lethal UV radiation. Data
247 presented as mean ± SEM. (One way ANOVA, $p < 0.006$). Significant difference between groups
248 denoted with asterisks: (*) $p < 0.05$, (**) $p < 0.001$, (***) $p < 0.0001$ according to Tukey's test. **(A)**
249 UVA exposure. **(B)** UVB exposure.

250

251 **3.2 Oxidative stress**

252 To assess the potential of UV radiation to generate ROS in zebrafish, the time dependent formation
253 of ROS using a fluorescent probe was measured in all exposure groups. The results showed that
254 for UVA both time and dose were significant for the differences seen in exposed larvae ($p < 0.0001$
255 and $p = 0.0025$, respectively). No clear pattern of increasing ROS formation was observed in the
256 larvae after UVA exposure (Fig 2A), while a trend of increasing ROS formation at the two highest
257 UVB-doses was observed (Fig 2B), although significantly increased only in the highest dose at
258 120 hpf. Two-way ANOVA also showed that both time and dose affected ROS formation
259 significantly for the UVB exposed groups ($p = 0.0006$ and $p < 0.0001$, respectively), and that their
260 interaction was also significant ($p < 0.0001$) (Table 4A).



261

Fig 2. ROS fold induction in zebrafish larvae from 72 hpf to 120 hpf exposed to UV radiation. Results presented as mean ± SEM. Significance in comparison to control denoted with (*) (Two-way ANOVA, $p < 0.05$; Tukey's test, $p < 0.05$). (A) UVA exposure. (B) UVB exposure.

The formation of oxyl-radicals ($HO^{\cdot}$, $ROO^{\cdot}$, $RO^{\cdot}$ and peroxynitrite) responsible for lipid oxidation was assessed by the fluorescent dye C11-BODIPY$^{581/591}$ in zebrafish larvae exposed to UVA and UVB. Results showed a small decrease in LPO after 72 hpf in the two highest UVA-doses when compared to the control (Fig 3A), while no LPO was detected after 96 hpf. However, no formation of oxyl-radicals was detected in larvae exposed to UVB (Fig 3B).

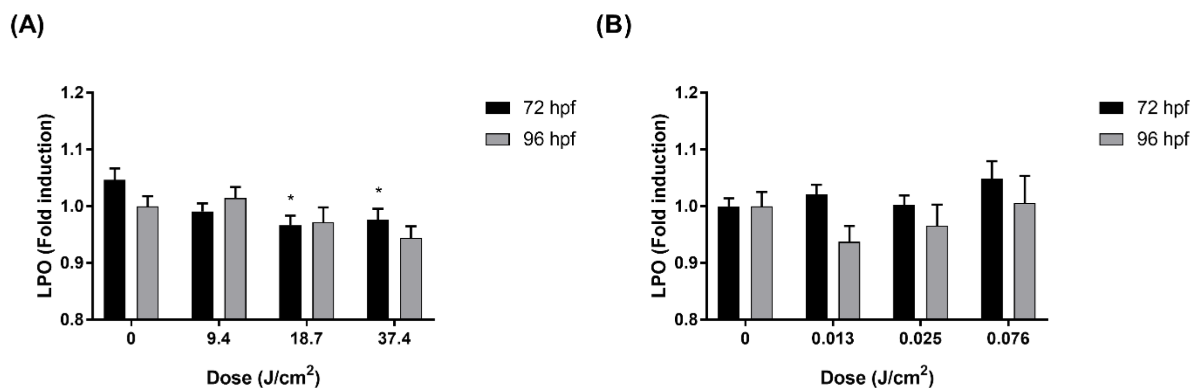
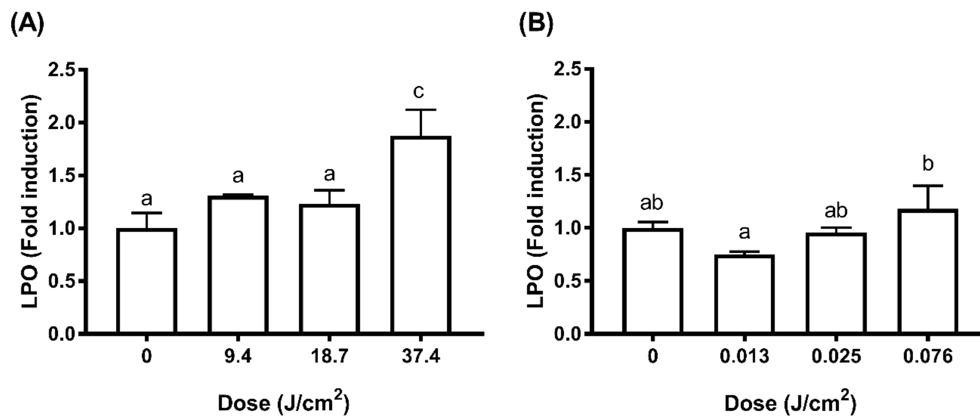


Fig 3. Formation of oxyl-radicals in 72 hpf to 96 hpf zebrafish larvae exposed to UV radiation. Results presented as mean ± SEM. Significance in comparison to control denoted with (*). (One way ANOVA, $p < 0.05$; Tukey's test, $p < 0.05$). (A) UVA exposure. (B) UVB exposure.

On the other hand, the end-products of LPO, MDA and 4-HNE were determined at 72 hpf, where an increase (1.9-fold) in the highest dose was detected after the UVA exposure (Fig 4A). In the

278 UVB exposure doses, the highest UVB dose demonstrated a non-significant increase in lipid
279 peroxidation compared to control, while the lowest UVB dose caused a significantly decreased
280 lipid peroxidation compared to control (Fig 4B). Therefore, in both wavelength regions, a dose
281 dependent increasing trend in LPO was observed.



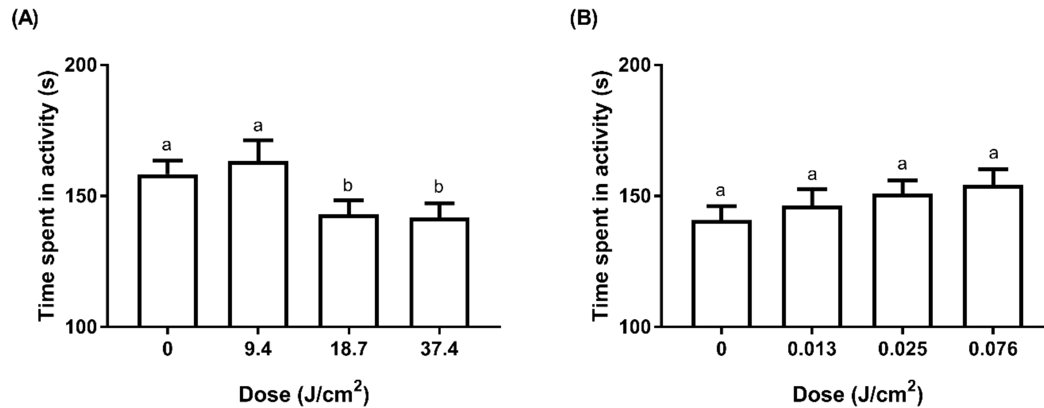
282

283 **Fig 4.** Lipid peroxidation in 72 hpf zebrafish larvae after exposure to UV radiation. Results
284 presented as mean ± SD. Significant difference between groups denoted with different letters,
285 whereby letters shared by groups represent no significant difference. (One way ANOVA, $p < 0.05$;
286 Tukey's test, $p < 0.05$). **(A)** UVA exposure. **(B)** UVB exposure.

287 **3.3 Behaviour**

288 Analysis of the locomotor activity (LMR) assay data showed that exposure to the highest and
289 second highest dose of UVA significantly reduced the time spent active (p = 0.02 and 0.04,
290 respectively) (Fig 5A), while the highest dose also decreased the total distance moved compared
291 to controls (p = 0.03), but had no effect on swimming speed. The lowest dose of UVA had no
292 effect on behaviour. Exposure to the highest dose of UVB led to an increase in the time spent
293 active (Fig 5B), but a slower average swimming speed although these effects were not significant
294 (p = 0.07). Neither of these tendencies were observed at lower UVB doses.

295



296

297 **Fig 5.** Time spent in activity during the dark cycles of the locomotor assay measured in 100 hpf
298 larval zebrafish after exposure to UV radiation. Data presented as means ± SEM. Significance
299 between groups denoted with different letters, whereby letters shared by groups represent no
300 significant difference (linear mixed effect models $p \leq 0.05$, n = 139-143 larvae). **(A)** UVA
301 exposure. (**B**) UVB exposure.

302

303 ## 4 Discussion

304 This study examined the biological effects in zebrafish larvae following a short and low dose
305 exposure to UVA and UVB radiation during a very sensitive life stage. The results demonstrated
306 that the heart rate, oxidative stress parameters and the behaviour in fish aged 72 to 120 hpf may be
307 persistently altered even at very low doses and that these alterations are wavelength and dose-
308 dependent.

309 **4.1 General toxicity and heart rate**

310 UV radiation in high levels is able to induce acute toxicity in fish embryos and larvae. The LD_{50}
311 determined following exposure to the mentioned UVA and UVB regimes confirmed that the doses
312 used in this study are below the acute toxic levels. Comparable to the present results, Icoglu
313 Aksakal and Ciltas (2018) reported effects on different parameters in zebrafish, whereby a
314 mortality of 20 % was observed at 24 hpf in embryos exposed to 0.1 J/cm^2 during a 3h period in
315 the blastula stage of development. Dong et al. (2007) used doses and dose rates higher than in the
316 present study, but with different spectra, with LD_{50} about 20x higher in UVB and about 10x higher
317 in UVA, whereby the segmentation stage (12-24 hpf) was more sensitive than the mid-blastula
318 stage. Banerjee and Leptin (2014) exposed zebrafish embryos to lamps with wavelength around
319 320 nm, i.e., between UVA and UVB. The dose inducing close to 50 % embryo mortality at 24
320 hpf was 0.024 J/cm^2, which is lower than here, but in accordance with the data of Dong et al.
321 (2007), who found that mortality after UVB was higher at 24 hpf than at 3 hpf.

322 In order to study the behavioural effects and other effects not induced by acute toxicity, the used
323 doses for late blastula to early gastrula (4.5-5.5 hpf) embryo exposure to UVA and UVB radiation
324 corresponded to maximum 68 and 76% of the LD_{50}, respectively. Correspondingly, no differences
325 were observed in development, hatching and body length at later stages of development from these
326 exposures. Deformities included spinal aberrations, yolk sac or cardiac oedema, aberrations in
327 pigmentation, and loss of equilibrium, but they were not statistically significant (Table 2A).
328 However, the heart rate was significantly decreased compared to control after exposure to both
329 UVA and UVB radiation at 50 hpf. This difference persisted until 60 hpf in the two highest doses
330 of UVA compared to control groups, while in UVB no differences were observed at this stage.
331 Together these results indicate that heart rate is a very sensitive endpoint susceptible to change

332 after exposure to sub-lethal UV radiation in fish larvae, but that it also might be a temporary effect
333 in the lower doses. In addition, the lowered heart rate may be connected to changes in metabolism
334 and/or other physiological parameters, such as oxidative stress, as reported by Icoglu Aksakal and
335 Ciltas (2018). Embryos of Atlantic cod (*Gadus Moruha*) exposed to UVB from the sun seemed to
336 be less sensitive by a factor >10 than zebrafish (Béland et al., 1999). It should be emphasized that
337 the exposure conditions and the shape of the spectra varied widely in the studies cited above and
338 direct comparisons of lethal doses are therefore impractical.

339 **4.2 Oxidative stress**

340 The use of fast and direct assays using fluorescent dyes has proven effective for the detection of
341 oxidative stress caused by radiation at a whole-organism level, as previously seen in zebrafish
342 larvae exposed to gamma radiation (Hurem et al., 2017). Results obtained using the H_2DCFDA
343 probe showed that no significant formation of ROS was generated in zebrafish larvae exposed to
344 UVA, contrary to what was expected. On the other hand, a more clear time and dose dependent
345 increase was seen at the two highest UVB-doses, even though only significant at 120 hpf for the
346 second highest dose. The reason for not observing an increase in ROS might be related to the time
347 point of the ROS assessment. Zebrafish larvae exposed to both UV wavelengths could have
348 undergone different chain reaction processes involved in the oxidative stress mechanism during
349 exposure that could have accounted for a continuous formation and recycling of reactive species,
350 which after 72 hpf were not present or not detected by H_2DCFDA. Another possibility for this lack
351 of ROS formation in exposed larvae is the combined action of the antioxidant defence system that
352 might have mediated the ROS formed during and after exposure. A study of chronic exposure of
353 Atlantic cod (*Gadus morhua*) larvae to UVA and UVB radiation (0.001 and 0.006 J/cm^2 weighted

354 dose), reported a significant increase in antioxidant enzyme activity after constant irradiation for
355 12 days, which is consistent with the previous notion.

356 LPO is a marker of oxidative damage and can potentially lead to cell death (Ayala et al., 2014;
357 Livingstone, 2001; Halliwell and Gutteridge, 2007). In this study, no significant increase in LPO
358 levels were detected using the C11-BODIPY$^{581/591}$ probe in zebrafish larvae exposed to both UVA
359 and UVB (Fig A1), even at the UVB doses where an increase in ROS levels was seen. This non-
360 linearity between the formation of ROS and LPO levels detected in zebrafish larvae can be
361 explained by the specificity of the two fluorescent probes towards different reactive species. The
362 fluorescent probe H_2DCFDA has been shown to be reactive to a variety of ROS, particularly H_2O_2,
363 $HO^{\cdot}$, NO, $ROO^{\cdot}$, O2− and peroxide-derived oxidants, while the C11-BODIPY$^{581/591}$ probe is
364 triggered only in the presence of oxyl-radicals such as $HO^{\cdot}$, $ROO^{\cdot}$, $RO^{\cdot}$ and $ONOO^{-}$ (Drummen et
365 al., 2002). The absence of oxyl-radicals formation in exposed zebrafish can also be related to the
366 formation of other end products of LPO, which are not detectable by the this probe. In fact, the
367 results obtained for LPO when measured as MDA and 4-HNE were more consistent to those
368 obtained for ROS formation and showed significant damage at the highest UVA and UVB doses
369 at 72 hpf. Although the ROS and LPO levels determined were not significantly different between
370 the highest UVB dose and controls, the lowest UVB dose (0.013 J/cm^2) demonstrated a significant
371 decrease in both parameters at 72 and 120 hpf, respectively. Together, these findings indicate that
372 UVA at doses $\geq$ 37.4 J/cm^2 is potent enough to cause lipid peroxidation and consequently oxidative
373 damage. It could be speculated that adaptive responses to the highest level of UVB exposure could
374 lead to decreased LPO. This study has shown that oxidative stress parameters such as time
375 dependent ROS formation and LPO can demonstrate changes persisting a longer time after early
376 life UV exposure.

377 **4.3 Behaviour**

378 UV radiation levels in aquatic environments are strongly influenced by UV absorption in the water
379 and sediments, and current levels have the potency to affect aquatic organisms and induce
380 behavioural changes (Bais et al., 2017). Changes in behaviour may represent either compensatory
381 and reversible adaptive responses in order to mitigate potential overt effects after perception of
382 stress), such as reported in Atlantic cod after sea temperature changes (Alemanni et al., 2003;
383 Freitas et al., 2015). They also may be irreversible effects of a toxicant on a behavioural
384 mechanism or expression after toxicokinetic and toxicodynamic processes have started (Nellore,
385 2015) and are found to be an indicator of overall welfare (Martins et al., 2012). Some claim that
386 behavioural changes might be pointing to neurodevelopmental toxicity of studied agents (Levin
387 and Cerutti, 2009; Rihel and Schier, 2012).

388 Zebrafish larval behaviour was previously shown to be affected after exposure to various toxicants
389 at the early embryonic stages (Nellore, 2015; Fraser et al., 2017). Here, behavioural changes
390 resulting from a short duration early life exposure to UV were assessed 5 days post fertilization,
391 and results showed that exposure to the two highest UVA doses resulted in a significant decrease
392 in larval activity compared to the controls. As an example, a decrease in total movement can be an
393 indicator of differences in anti-predator behaviour, concurring with earlier reports showing
394 impaired escape behaviour in fish larvae after UV exposure (Fukunishi et al., 2012). The same
395 exposure groups demonstrated a decrease in heart rate, which together with the decreased
396 locomotor activity may be indicative of an overall lower metabolic activity as a consequence of
397 UVA exposure. UVB exposure had no significant effect on larval activity. This result contradicts
398 results obtained in studies of behaviour after exposure to environmentally relevant doses of UVB,

399 whereby decreases in total movement in cod (*Gadus morhua*) larvae (Fukunishi et al. 2012) as
400 well as behavioural differences in juvenile rainbow trout (*Oncorhynchus mykiss)* (Alemanni et al.,
401 2003), were observed. Additionally, in pike eggs (*Esox Lucius*), mortality occurred after exposure
402 to UVB doses similar to one daily erythema weighted ambient dose in Finland in May, in addition
403 to swimming disorders (about 0.27 J/cm^2) (Häkkinen et al., 2004), indicating that influence of
404 UVB irradiation effects on the behaviour could have been a factor contributing to increased
405 mortality.

406 Even though studies demonstrating an interaction of ROS production and behaviour in zebrafish
407 larvae are lacking in the literature, at later developmental stages in zebrafish, an interaction of ROS
408 production and movement was observed after chronic 3-hour daily exposure to UVB radiation
409 (Seebacher et al., 2016). In this study, the ROS formation was significantly decreased in the 37.4
410 J/cm^2 UVA dose at 72 hpf. The LPO in this group on the other hand, was increased at the same
411 time point. In addition to the increased LPO in the highest UVA dose, the displayed decreased
412 locomotor activity in these larvae might indicate that oxidative damage is affecting the behaviour.

413

414 5 Conclusion

415 Taken in account that climate change may increase exposure of aquatic organisms to increased
416 UV radiation levels, it is important to assess how subtle changes in the UV regime might affect
417 the physiological parameters and the behavior as a key life fitness trait in aquatic populations.
418 From the present findings, it can be concluded that an early life stage exposure to UVA and UVB
419 radiation to sub-lethal and non-detrimental doses to zebrafish development can lower the
420 metabolic activity in later stage embryos and fish larvae. However, depending on the exposure
421 duration and wavelength, this effect persists only temporarily in the shortly exposed UVB groups.

422 On the other hand, in the longer exposed UVA group (68% of the LD_{50}); lipid peroxidation persists
423 for a longer time after the exposure, including the change in resting heart rate, while the total
424 activity of fish larvae is reduced. The findings in this study show that even a very small change in
425 the UV regime during a sensitive developmental stage can induce behavioral changes. Considering
426 that these changes persist long after exposure to low doses of UV radiation during early life, they
427 might have further implications for the fish population dynamics and warrant further studies.

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434

435 ## 7 References

436 Alemanni, M.E., Lozada, M., Zagarese, H.E., 2003. Assessing sub-lethal effects of
437 ultraviolet radiation in juvenile rainbow trout (*Oncorhynchus mykiss*). Photochem. Photobiol. Sci.
438 2, 867–70. doi:10.1039/b301564e

439 Andrade, T.S., Henriques, J.F., Almeida, A.R., Soares, A.M.V.M., Scholz, S., Domingues,
440 I., 2017. Zebrafish embryo tolerance to environmental stress factors-Concentration-dose response
441 analysis of oxygen limitation, pH, and UV-light irradiation. Environ. Toxicol. Chem. 36, 682–690.
442 doi:10.1002/etc.3579

Ayala, A., Muñoz, M.F., Argüelles, S., Ayala, A., Muñoz, M.F., Argüelles, S., Muñ, oz, M.F., Argü, elles, S., 2014. Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. Oxid. Med. Cell. Longev. 2014, 1–31. doi:10.1155/2014/360438.

Bais, A.F., Lucas, R.M., Bornman, J.F., Williamson, C.E., Sulzberger, B., Austin, A.T., Wilson, S.R., Andrady, A.L., Bernhard, G., McKenzie, R.L., Aucamp, P.J., Madronich, S., Neale, R.E., Yazar, S., Young, A.R., de Gruijl, F.R., Norval, M., Takizawa, Y., Barnes, P.W., Robson, T.M., Robinson, S.A., Ballaré, C.L., Flint, S.D., Neale, P.J., Hylander, S., Rose, K.C., Wängberg, S.-Å., Häder, D.-P., Worrest, R.C., Zepp, R.G., Paul, N.D., Cory, R.M., Solomon, K.R., Longstreth, J., Pandey, K.K., Redhwi, H.H., Torikai, A., Heikkilä, A.M., 2018. Environmental effects of ozone depletion, UV radiation and interactions with climate change: UNEP Environmental Effects Assessment Panel, update 2017. Photochem. Photobiol. Sci. 17, 127–179. doi:10.1039/c7pp90043k.

Banerjee, S., Leptin, M., 2014. Systemic Response to Ultraviolet Radiation Involves Induction of Leukocytic IL-1β and Inflammation in Zebrafish. J. Immunol. doi:10.4049/jimmunol.1400232.

Béland, F., Browman H.I., Rodriguez, C.A., St-Pierre, J.F. (1999). Effect of solar ultraviolet radiation (280–400 nm) on the eggs and larvae of Atlantic cod (*Gadus morhua*). Canadian Journal of Fisheries and Aquatic Sciences 56: 1058–1067.

Colwill, R.M., Creton, R., 2011. Locomotor behaviors in zebrafish (*Danio rerio*) larvae. Behav. Processes 86, 222–9. doi:10.1016/j.beproc.2010.12.003.

464 Dong, Q., Svoboda, K., Tiersch, T. R., & Todd Monroe, W. (2007). Photobiological effects
465 of UVA and UVB light in zebrafish embryos: Evidence for a competent photorepair system.
466 Journal of Photochemistry and Photobiology B: Biology, 88(2), 137–146.
467 doi:10.1016/j.jphotobiol.2007.07.002

468 Drummen, G.P.C., van Liebergen, L.C.M., Op den Kamp, J.A.F., Post, J.A., 2002. C11-
469 BODIPY(581/591), an oxidation-sensitive fluorescent lipid peroxidation probe:
470 (micro)spectroscopic characterization and validation of methodology. Free Radic. Biol. Med. 33,
471 473–90. doi:10.1016/S0891-5849(02)00848-1

472 Erdelmeier, I., Gerard-Monnier, D., Yadan, J.-C., Chaudi?re, J., 1998. Reactions of N -
473 Methyl-2-phenylindole with Malondialdehyde and 4-Hydroxyalkenals. Mechanistic Aspects of
474 the Colorimetric Assay of Lipid Peroxidation. Chem. Res. Toxicol. 11, 1184–1194.
475 doi:10.1021/tx970180z.

476 Fraser, T.W.K., Khezri, A., Lewandowska-Sabat, A.M., Henry, T., Ropstad, E., 2017.
477 Endocrine disruptors affect larval zebrafish behavior: Testing potential mechanisms and
478 comparisons of behavioral sensitivity to alternative biomarkers. Aquat. Toxicol. 193, 128–135.
479 doi:10.1016/j.aquatox.2017.10.002.

480 Freitas, C., Olsen, E.M., Moland, E., Ciannelli, L., Knutsen, H., 2015. Behavioral
481 responses of Atlantic cod to sea temperature changes. Ecol. Evol. 5, 2070–83.
482 doi:10.1002/ece3.1496.

483 Fujimoto, T., Sakao, S., Yamaha, E., Arai, K., 2007. Evaluation of different doses of UV
484 irradiation to loach eggs for genetic inactivation of the maternal genome. J. Exp. Zool. A. Ecol.
485 Genet. Physiol. 307, 449–62. doi:10.1002/jez.398Fukunishi, Y., Browman, H.I., Durif, C.M.F.,

486 Bjelland, R.M., Skiftesvik, A.B., 2012. Effect of sub-lethal exposure to ultraviolet radiation on the
487 escape performance of Atlantic cod larvae (Gadus morhua). PLoS One 7, e35554.
488 doi:10.1371/journal.pone.0035554.

489 Gutteridge, J.M.C., Halliwell, B., 2010. Antioxidants: Molecules, medicines, and myths.
490 Biochem. Biophys. Res. Commun. 393, 561–564. doi:10.1016/j.bbrc.2010.02.071.

491 Häder, D.-P., Kumar, H.D., Smith, R.C., Worrest, R.C., 2007. Effects of solar UV radiation
492 on aquatic ecosystems and interactions with climate change. Photochem. Photobiol. Sci. 6, 267–
493 85. doi:10.1039/b700020kHäkkinen, J., Vehniäinen, E., Oikari, A., 2004. High sensitivity of
494 northern pike larvae to UV-B but no UV-photoinduced toxicity of retene. Aquat. Toxicol. 66, 393–
495 404. doi:10.1016/j.aquatox.2003.11.001.

496 Hunter, J.R., Taylor J.H., Moser, H.G. (1979). Effect of ultraviolet irradiation on eggs and
497 larvae of the northern anchovy, *Engraulis mordax*, and the pacific mackerel, *Scomber japonicus*,
498 during the embryonic stage. Photochemistry and Photobiology 29: 325–338.

499 Hurem, S., Gomes, T., Brede, D.A., Lindbo-Hansen, E., Mutoloki, S., Fernandez, C.,
500 Mothersill, C., Salbu, B., Kassaye, Y.A., Olsen, A.K., Oughton, D.H., Aleström, P., Lyche, J.L.,
501 2017. Parental gamma irradiation induces reprotoxic effects accompanied by genomic instability
502 in zebrafish (*Danio rerio*) embryos Env Res 159, 564-578 doi:10.1016/j.envres.2017.07.053.

503 Icoglu Aksakal, F., Ciltas, A., 2018. The impact of ultraviolet B (UV-B) radiation in
504 combination with different temperatures in the early life stage of zebrafish (*Danio rerio*).
505 Photochem. Photobiol. Sci. 17, 35–41. doi:10.1039/c7pp00236j.

506 Kimmel, C.B., Ballard, W.W., Kimmel, S.R., Ullmann, B., Schilling, T.F., 1995. Stages of
507 embryonic development of the zebrafish. Dev. Dyn. 203, 253–310. doi:10.1002/aja.1002030302.

508 Lesser, M.P., Farrell, J.H., Walker, C.W., 2001. Effects of ultraviolet radiation on cod
509 larvae. J. Exp. Biol. 204, 157–164.

510 Levin, E.D., Cerutti, D.T., 2009. Behavioral Neuroscience of Zebrafish. In: Buccafusco JJ,
511 Methods of Behavior Analysis in Neuroscience. 2nd edition. Boca Raton (FL): CRC Press/Taylor
512 & Francis; 2009. Chapter 15. Frontiers in Neuroscience.

513 Livingstone, D.R., 2001. Contaminant-stimulated Reactive Oxygen Species Production
514 and Oxidative Damage in Aquatic Organisms. Mar. Pollut. Bull. 42, 656–666. doi:10.1016/S0025-
515 326X(01)00060-1.

516 Martins, C.I.M., Galhardo, L., Noble, C., Damsgård, B., Spedicato, M.T., Zupa, W.,
517 Beauchaud, M., Kulczykowska, E., Massabuau, J.-C., Carter, T., Planellas, S.R., Kristiansen, T.,
518 2012. Behavioural indicators of welfare in farmed fish. Fish Physiol. Biochem. 38, 17–41.
519 doi:10.1007/s10695-011-9518-8.

520 Mekkawy, I.A.A., Mahmoud, U.M., Osman, A.G., Sayed, A.E.-D.H., 2010. Effects of
521 ultraviolet A on the activity of two metabolic enzymes, DNA damage and lipid peroxidation during
522 early developmental stages of the African catfish, Clarias gariepinus (Burchell, 1822). Fish
523 Physiol. Biochem. 36, 605–626. doi:10.1007/s10695-009-9334-6

524 Montero, J.-A., Carvalho, L., Wilsch-Bräuninger, M., Kilian, B., Mustafa, C., Heisenberg,
525 C.-P., 2005. Shield formation at the onset of zebrafish gastrulation. Development 132, 1187–98.
526 doi:10.1242/dev.01667

527 Nellore, J., P., N., 2015. Paraquat exposure induces behavioral deficits in larval zebrafish
528 during the window of dopamine neurogenesis. Toxicol. Reports 2, 950–956.
529 doi:10.1016/J.TOXREP.2015.06.007Ton, C., Lin, Y., & Willett, C. (2006). Zebrafish as a model

530 for developmental neurotoxicity testing. Birth Defects Research. Part A, Clinical and Molecular
531 Teratology, 76(7), 553–67. doi:10.1002/bdra.20281.

532 Parng, C., Roy, N. M., Ton, C., Lin, Y., & McGrath, P. (2007). Neurotoxicity assessment
533 using zebrafish. Journal of Pharmacological and Toxicological Methods, 55(1), 103–12.
534 doi:10.1016/j.vascn.2006.04.004.

535 OECD/OCDE, 2013. OECD GUIDELINES FOR THE TESTING OF CHEMICALS Fish
536 Embryo Acute Toxicity (FET) Test. doi:10.1787/20745761.

537 Rihel, J., Schier, A.F., 2012. Behavioral screening for neuroactive drugs in zebrafish. Dev.
538 Neurobiol. 72, 373–85. doi:10.1002/dneu.20910.

539 Selderslaghs, I. W. T., Hooyberghs, J., De Coen, W., & Witters, H. E. (2010). Locomotor
540 activity in zebrafish embryos: a new method to assess developmental neurotoxicity.
541 Neurotoxicology and Teratology, 32(4), 460–71. doi:10.1016/j.ntt.2010.03.002

542 Seebacher, F., Kazerouni, E.G., Franklin, C.E., 2016. Ultraviolet B radiation alters
543 movement and thermal selection of zebrafish (*Danio rerio*). Biol. Lett. 12, 20160258.
544 doi:10.1098/rsbl.2016.0258.

545 Stańczyk, M., Gromadzińska, J., Wasowicz, W., 2005. Roles of reactive oxygen species
546 and selected antioxidants in regulation of cellular metabolism. Int. J. Occup. Med. Environ. Health
547 18, 15–26.

548 Strähle, U., Jesuthasan, S., 1993. Ultraviolet irradiation impairs epiboly in zebrafish
549 embryos: evidence for a microtubule-dependent mechanism of epiboly. Development 119, 909–
550 19.

551 Tierney, K. B. (2011). Behavioural assessments of neurotoxic effects and
552 neurodegeneration in zebrafish. Biochimica et Biophysica Acta, 1812(3), 381–9.
553 doi:10.1016/j.bbadis.2010.10.011.