STUDY OF STRESS BEHAVIOUR OF ZEBRA FISH IN REFERENCE TO UV RAYS

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ABSTRACT

Ultraviolet (UV) radiation is one environmental stressor that zebra fish (Danio rerio) are especially susceptible to. Zebra fish stress responses to UV light may provide light on the biochemical and physiological effects of UV stress. A primary motivation for conducting this study was to determine the effect of varying UV radiation doses on zebra fish stress responses. One hundred zebra fish were divided into four groups; one of these groups served as a control and did not expose the fish to UV light. Fish exposed to higher doses of UV radiation exhibited decreased swimming activity, more erratic movements, more time spent at the bottom of the aquarium, and much higher cortisol levels. There was a statistically significant increase in mortality rates among those subjects exposed to moderate to high UV radiation. More proof that ultraviolet radiation is a major stressor for zebra fish, and issues about the broader ecological impacts of UV exposure on aquatic ecosystems are prompted by these findings.

Keywords: Ultraviolet radiation, stress, environment, aquatic, ecosystems

I.INTRODUCTION

An important physiological and behavioral response is stress, which occurs whenever living organisms meet environmental dangers. Sunlight, namely (UV) radiation, is one of the environmental stressors that may be harmful to fish and other aquatic organisms. Aquatic organisms may be positively or negatively impacted by ultraviolet (UV) radiation, which is a part of the sun's natural spectrum. While sun exposure aids vitamin D production in fish, excessive exposure may lead to oxidative stress, DNA damage, immunological suppression, and behavioral issues. Investigating the behavioral changes in stressed zebrafish in response to ultraviolet light may provide light on the physiological and ecological impacts of UV radiation on aquatic environments.

Many studies in the fields of ecology and biology employ zebrafish as a model organism due to its ease of care, rapid reproductive rate, genetic similarity to humans, and transparent embryos. The stress physiology of this species has been extensively investigated because of its behavioral reactions and neurological system. Because zebrafish exhibit obvious behavioral changes in reaction to stress, they may be used to study environmental stressors like UV radiation. Furthermore, genetically modified zebrafish lines now provide a viable model for researchers to investigate the genetic responses to UV-induced stress.

Ultraviolet A and UVB rays may penetrate aquatic ecosystems and cause harm to marine life, even if most ultraviolet C radiation is blocked by Earth's atmosphere. Ultraviolet B radiation is especially dangerous since it may cause oxidative stress, DNA damage, and physiological problems in fish, even in shallow water. Fish behavior, feeding patterns, and survival rates might be impacted by UV radiation in water. Due to their usual shallow habitat and status as freshwater fish, zebrafish are particularly vulnerable to UV-induced stress. Prolonged exposure to ultraviolet light may harm their growth and chances of survival by altering their swimming patterns, social interactions, and stress hormone levels.

Behavioral research is essential for understanding the stress response in zebrafish exposed to ultraviolet light. A zebrafish's social interactions, inquisitive tendencies, anxiety-related behaviors, and locomotion patterns may be utilized to detect stress. Zebrafish exhibit alterations in swimming speed, atypical movement patterns, frozen behavior, and reduced inquisitive tendencies when subjected to UV light, according to researchare often linked to these alterations in behavior. Zebrafish also have an intriguing trait called scototaxis, which makes them avoid bright lights in stressful situations and instead seek out darker areas. Exposure to ultraviolet light causes zebrafish to become more scototactic, which might lead to avoidance behaviors. Understanding these behavioral changes in fish could provide insight on the neurobiology and stress-related circuits impacted by UV radiation.

Zebrafish may undergo a cascade of changes in their biology when exposed to UV rays. Two major ways that ultraviolet radiation (UV) induces stress (ROS). Oxidative stress may damage DNA, proteins, and lipids, among other biological components. Glutathione peroxidase, superoxide dismutase (SOD), and catalase are enzymes that cells may release in a cascade in reaction to oxidative stress. Some DNA damage products are produced by sunlight as well; they include 6-4 photoproducts and cyclobutane pyrimidine dimers (CPDs). If these DNA breaks in zebrafish are not repaired, it might lead to cell death, mutations, and developmental abnormalities. It is obvious that UV radiation has physiological impacts on zebrafish because it activates genes that are associated to stress, such as heat shock proteins (HSPs) and pro-inflammatory cytokines.

II.REVIEW OF LITERATURE

Alves, Ricardo & Agustí, Susana. (2020) UVR is endangering several fish species. UV radiation's effects on fish have been studied since the start of the 20th century, and progress has been achieved. We cover all reported examples of UVB and UVA radiation harming fish from embryo to adult. Early mortality and developmental defects, especially in larvae's skin and gills, are the most visible effects. UVB radiation slows development, degrades physical condition, and alters behavior, physiology, and metabolism in children and adults. Adults and adolescents' skin undergoes significant morphological and functional changes after acute UVR exposure. DNA damage, apoptosis, and tissue antioxidant status changes characterized molecular and cellular process disruption during development. Updates were made to photo-protective techniques that cope with UVR light. Since climate change and stratospheric ozone dynamics are interconnected, fish may be exposed to greater UV radiation underwater. New and intricate interactions between ultraviolet radiation (UVR) and other pressures may affect fish development and survival due to these environmental changes. Understanding how fish may adapt to and survive these habitat changes is essential for assessing fisheries impacts and addressing environmental challenges.

Hurem, Selma et al., (2018) Climate change may increase UV radiation. This radiation may affect organisms at different life stages differently. This research examined the developmental and behavioral effects of sub-lethal and ecologically relevant concentrations of ultraviolet A (9.4, 18.7, 37.7 J/cm2) and ultraviolet B (0.013, 0.025, 0.076 J/cm2) radiation in zebrafish (Danio rerio) early life stages (4.5-5.5 hpf). Since the doses were all below the median lethal dose (LD50), neither the exposed nor the control groups showed any variation in survival, deformities, or hatching. Compared to the control group, UVA and UVB radiation alone decreased heart rate dose-dependently at 50 and 60 hpf, respectively. UVB light enhanced ROS production, but only at 120 hpf for the second-highest dose. Both UVA and UVB showed that LPO increased at 72 hpf at the maximal levels. At 100 hpf, the control group and the two highest UVA dosage groups decreased larval movement, including time spent active and total distance traveled, but not swimming speed. Even modest UVA doses did not influence behavior. The maximal UVB dose did not significantly influence active time or average swimming speed ($p = 0.07$). At UV doses below LD50, zebrafish larvae may undergo oxidative stress, behavioral and physiological changes, ROS generation, and LPO. More study is required to discover how this radiation may affect fish population dynamics.

Kysil, Elana et al., (2017) Adult zebrafish (Danio rerio) models of anxiety and stress are increasingly used in neuroscience and central nervous system medication development. The new tank test (NTT) and light-dark test are popular zebrafish anxiety models. The first uses zebrafish's natural dive in response to fear, while the latter uses fish scototaxis. We compare and contrast in-vivo behavioral and whole-body endocrine (cortisol) tests to evaluate their utility. Meta-analyses suggest that the NTT and LDT are sensitive to anxiety-like states in zebrafish in-vivo, although they have a substantial cross-test association. Since it generates more cortisol than LDT, NTT may be more stressful. Overall, this study reevaluates NTT and LDT for zebrafish anxiety-like states and shows their rising application in neurobehavioral investigations. These data may help us identify better animals to test anxiolytic or anxiogenic drugs, improving drug screening.

Andrade, Thayres et al., (2016) Over the last century, rising global temperatures have changed freshwater physicochemical qualities including pH, dissolved oxygen, and UV light abundance. These traits may impact pollutant toxicity. To assess the embryonic tolerance of zebrafish (Danio rerio) to variations in pH (3-12), dissolved oxygen (3.9-237 µmol/L), and UV intensity (55-467 mW/m2), we used endpoints to assess survival and sublethal outcomes. The sublethal endpoint examination measured hatching success, developmental delay, body length reduction, edema frequency, and morphological abnormalities. The 96 h-LC50 was 3.68 for acidic pH and 10.21 for alkaline pH. The 96 h-LC50 of 0.42 mg/L indicated moderate oxygen deprivation resistance in embryos. Edema and developmental retardation occurred at dosages below 6 mg/L. Continuous ultraviolet radiation (UVR) caused pericardial edema, abnormalities, and a reduced hatching rate and survival rate in zebrafish. Through intensity-response modeling, a 72-h-LC50 of 227 mW/m2 was obtained. Concentration-response parameters from our data enable further studies of chemical and environmental stress features.

Seebacher, Frank et al., (2016) Interactions between temperature and ultraviolet B (UV-B) harm cells and reduce their ability to move. We tested the theory that zebrafish (Danio rerio) adapt their thermal selection and movement patterns to ultraviolet B light. We test the hypothesis that chronically UV-B-exposed fish avoid extreme cold or heat to maximize antioxidant enzyme activity. At temperatures exceeding 26°C, fish exposed to UV-B for two to three weeks had decreased swimming capacity and increased ROS-induced protein and membrane damage. Unlike controls, chronically exposed fish avoided hot and cold places in a thermal gradient field arena. Both control and chronically exposed fish had lower voluntary swimming rates after UV-B exposure. We propose that UV-B-exposed fish may reduce muscle activity to reduce intrinsic ROS. Our results have ecological implications, particularly in disturbed ecosystems, since they reveal that fish movement and microhabitat selection are driven by the combination of UV-B and temperature.

Bai, Yiming et al., (2016) The clear and simple vertebrate brain of a larval zebrafish, which has around 100 thousand neurons, makes it an ideal model for understanding the whole neural circuitry that drives behavior. Additionally, larval zebrafish are ideal for high-content screening due to their small size (~4-5 mm) that fits in 96-well plates. Despite these appealing traits, larval zebrafish have few behavioral characterizations compared to other model species and adults. Final Product We described the behavioral and physiological responses of developing zebrafish to temperature, ultraviolet light, mechanical disruption, and social isolation. These stimuli are selected because larval zebrafish dislike them. In a light/dark choice paradigm, larval zebrafish have an inherent fear of the dark (scotophobia). Exposure to heat, cold, and UV stimuli greatly increases their scotophobia, with heat having the greatest effect. MD and SI have little effect. The cortisol test, a physiological stress indicator, shows that all stimuli except heat and SI significantly increase whole body cortisol levels. According to these studies, zebrafish larvae may be exposed to stressors. Understanding the mechanics of inputs that drive physiological and behavioral responses requires circuit-level study. Anxiolytics reduce dark avoidance and stressors increase it in larval zebrafish, supporting this fear/anxiety-related light/dark preference behavior.

Blaser, Rachel & Peñalosa, Y.M.. (2011) Ethanol may reduce zebrafish anxiety by inhibiting the new tank diving reflex and certain social activities. This study examined fish's anxiety-like behavior in the light/dark preference test to address the question, "How does acute ethanol exposure affect behavior in the light/dark task?" The first experiment examined numerous cue manipulation approaches to induce light/dark preference in zebrafish. When light was altered, participants displayed phototaxis, or light preference. When wall and substrate colors were altered, they demonstrated scototaxis, or dark preference. Color preference affected locomotion, with animals choosing darker locations to freeze. In Experiment 2, the black-and-white test was used instead of the open/covered one owing to behavior interpretation ambiguity. Zebrafish were submerged in water for 30 minutes or exposed to 0.25%, 0.5%, or 1.0% ethanol in the second trial. For another 30 minutes, individuals were evaluated in a black/white preference tank with the same ethanol or water concentrations. Ethanol increased freezing and locomotion. Locomotor activity and ethanol administration also interacted considerably on side preference. Low ethanol concentrations increased white avoidance in normally moving fish, while high doses did not.

III.RESEARCH METHODOLOGY

Sample

One hundred zebrafish were chosen for the research and split into four groups according to the amount of ultraviolet light they would be exposed to during the trial. The following were the categories:

- **Group 1**: No UV exposure (Control group)
- **Group 2**: Low UV exposure
- **Group 3**: Moderate UV exposure
- **Group 4**: High UV exposure.

UV Exposure

An adjustable UV lamp was used to deliver the radiation, allowing for exact control over the degrees of UV exposure. To ensure uniform exposure, the UV light was set at a constant distance from the water tank. In order to replicate long-term exposure to environmental factors, the exposure time was set at 2 hours daily for 30 days.

Parameters Measured

We investigated behavioural and physiological markers to assess the effects of UV radiation on zebra fish:

- Behavioral Characteristics: Propensity to swim, irregular gait, and bottom living frequency.
- Measurements of Physiology: pulse rate, cortisol levels, and fatality rates.

Data Collection

In order to monitor the changes in stress behavior and physiological reactions, data was gathered at regular intervals, namely every 5 days.

Statistical Techniques

Analyzed were means and standard deviations.

IV.DATA ANALYSIS AND INTERPRETATION

Table 1: Behavioral observations of Zebra Fish under different UV exposure levels

Group	Swimming Activity (in laps/min)	Erratic Movement (incidents/min)	Bottom Dwelling (minutes)
Group 1	8 ± 1	0.5 ± 0.1	2 ± 0.5
Group 2	6 ± 1.5	1.2 ± 0.2	5 ± 1
Group 3	4 ± 1	2 ± 0.3	8 ± 1.5
Group 4	2 ± 0.5	4 ± 0.5	15 ± 2

According to what is shown in Table 1, The fish in Group 1, the control group that did not get any UV light, swam the fastest (8 laps/min), exhibited the fewest erratic movements (0.5 incidents/min), and spent the least amount of time bottom dwelling (2 minutes). The fish's behavior began to deteriorate as the amount of UV radiation grew. Swimming activity dropped to 6 laps per minute, erratic movements spiked to 1.2 occurrences per minute, and bottom dwelling duration increased to 5 minutes in the low UV exposure group (Group 2). Group 3, which was exposed to moderate UV radiation, had even more stress symptoms, such as a decrease in swimming activity to 4 laps per minute, an increase in erratic movements to 2 incidents per minute, and bottom dwelling duration to 8 minutes. Group 4, which was exposed to high levels of UV radiation, showed the most severe signs of stress, including a dramatic decrease in swimming activity (two laps per minute), an increase in erratic movements (four occurrences per minute), and an extension of bottom residing duration (15 minutes).

Table 2: Physiological Measurements of Zebra Fish under Different UV Exposure Levels

Group	Heart Rate (beats/min)	Cortisol Levels (ng/ml)	Mortality Rate (%)
Group 1	120 ± 5	10 ± 1	5%
Group 2	115 ± 4	15 ± 2	8%
Group 3	100 ± 6	25 ± 3	15%
Group 4	85 ± 7	40 ± 5	30%

Relatively low mortality rate of 5%, cortisol levels of 10 ng/ml, and a heart rate of 120 beats per minute were all indicators of normal physiological responses in the control group (Group 1) of Zebra Fish, according to facts shown in Table 2. Heart rate and cortisol levels, which indicate stress, clearly rose with increasing UV exposure. Group 2's low UV exposure resulted in a little drop in heart rate to 115 bpm, a rise in cortisol levels to 15 ng/ml, and an 8% increase in mortality. Group 3, which was exposed to moderate UV radiation, had more severe physiological stress, including a mortality rate of 15%, cortisol levels of 25 ng/ml, and a heart rate of 100 beats per minute. The fourth group, which was exposed to the largest amount of UV radiation, showed the most severe physiological distress. Their heart rates were 85 beats per minute, cortisol levels were 40 ng/ml, and their death rate was 30%.

V.CONCLUSION

The results showed that the fish showed obvious symptoms of stress, including less swimming activity, more erratic movements, and longer bottom living, as the UV exposure rose. Also, when UV exposure escalated, physiological measures including heart rate and cortisol levels were negatively impacted, with a considerable rise in both. The greater UV exposure groups also had an elevated death rate, providing further evidence that UV radiation has harmful consequences. These findings highlight the significance of ultraviolet radiation as a stressor in aquatic environments, which may have far-reaching ecological effects. The study emphasizes the importance of further research into how ultraviolet radiation affects aquatic organisms and how to lessen that impact, especially in light of the fact that our planet is experiencing rapid environmental change.

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