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The Potential Use of *Oryzias celebensis* Embryo Heart Rate as a Simple and Non-Invasive Biomarker to Detect the Adverse Effects of UV-C Light

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ABSTRACT

The impact of radiation from UV rays is very important to observe on the environment and aquatic organisms. Research on the use of heartbeat as a biomarker in *Oryzias celebensis* embryos to detect the adverse effects of ultraviolet C light (UV-C) was carried out. This research was conducted using *O. celebensis* embryos, which were exposed to UV-C (250 nm) for 3 days with an exposure time of 15, 10, and 5 minutes. The controls in the study were embryos without UV-C irradiation. The embryo's heart rate was calculated starting from stage 24 until hatching. ANOVA was used to determine differences in embryo heart rate between controls and treatments. The results showed that 15 minutes and 10 minutes of UV-C exposure were significantly different from the control (P<0.05). This proves that the duration of UV-C exposure harms the embryo. The results of the study concluded that the heart rate of *O. celebensis* embryos can be used as a simple and non-invasive biomarker to detect the adverse effects of UV-C.

INTRODUCTION

The medaka fish (*Oryzias* sp.) is a fish that has the advantage of being used as an experimental animal. Some of its advantages are having a fast growth rate, short life, and life cycle, easy to identify and cultivate, and a wide geographical distribution **(Puspitasari, 2016)**. In addition, medaka fish also have a small size. Small fish have become the most popular alternative and choice for vertebrate test organisms because they are considered to be the easiest organisms to handle in laboratory conditions **(Buikema *et al.*, 1982)**. Similar to the broodstock, medaka fish embryos have the advantage of being used as test biota for ecotoxicological studies. The embryo of the medaka Celebes (*Oryzias celebensis*), for example, meets the requirements as a test biota, because its embryo has a high sensitivity to various pollutants **(González-Doncel *et al.*, 2003)**. In addition, medaka Celebes embryos have a chorion and embryonic body that are transparent during their embryogenesis development so that these fish embryos can

provide convenience when used as test biota in the field of aquatic ecotoxicology **(Oxendine *et al.*, 2006)**.

The transparency of the body of the *O. celebensis* embryo makes the activity of internal organs of the embryo easily observable, such as its heart rate. **Yonekura *et al.* (2018)** stated that medaka fish have cardiac ecotoxicological aspects similar to mammalian hearts. The medaka's heartbeat is more similar to that of humans when compared to other animals such as rats. From experiments conducted by **Yonekura *et al.* (2018)**, it was shown that the heart rate of medaka fish embryos had the same response as humans after being given a drug to reduce high blood pressure (verapamil). These results indicate that the medaka can be an appropriate animal model to be used as a sentinel organism for biomonitoring purposes in the field. During the embryogenic development of *O. celebensis* embryos, the heart rate can be observed 40 hours after fertilization or when the embryo has entered stage 23.

Research that has been conducted on *O. celebensis* embryos exposed to pesticides shows a decrease in heartbeat compared to embryos that are not exposed. This decrease in heartbeat was assessed as the impact of pesticide contamination on the embryo **(Istiqomah, 2022)**. In the current study, exposure to ultraviolet C (UV-C) light was used to prove that the heart rate of *O. celebensis* embryos can be used as a simple and non-invasive biomarker in ecotoxicology studies.

MATERIALS AND METHODS

1. Broodstock rearing

Forty pairs of *O. celebensis* broodstock were reared in a 70 × 40 × 40cm aquarium equipped with an aerator in an under gravel circulation system. The broodstock was then fed with Fengli 0 and Artemia pellets. Feeding was carried out at 09:00, 12:00, and 16:00, three times a day so that the fish get an adequate food supply and can produce good egg production **(Kinoshita *et al.*, 2009)**. In addition, the aquarium was cleaned from faeces and leftover feed once a month. Cleaning was performed by removing the water using a siphon cleaner, leaving ¼ of the water in the aquarium, and then filling the aquarium with 80 litres of water.

2. Fertilization of *Oryzias celebensis*

To stimulate fertilization of *O. celebensis* broodstock, they were reared in an aquarium with 12:12 lighting (dark: light) with a male-female ratio of 1:1. After the broodstock were fertilized, the eggs that were clustered in the abdomen of the female broodstock were transferred to a petri dish containing embryo rearing media (ERM) solution. To separate the eggs that were clustered so that we could get a single egg for exposure purposes, the eggs were slowly rotated with the index finger very carefully until they were separated from one another. Separated eggs were ready for selection and experimentation.

3. Eggs selection

The eggs previously separated from the broodstock were observed under a microscope with a magnification of 40× to select the eggs to be used in the experiment. If there was still no perivitelline space in the egg, the egg was considered unfertilized and could not be used in the study. If the egg has formed a perivitelline space then the egg was considered fertilized **(González-Doncel *et al.*, 2005)** and the egg can be used in this study. The determination of the fertilized *Oryzias* fish eggs was according to **Iwamatsu (2004)**.

4. Research design

The study was conducted using a completely randomized design with four treatments (the length of exposure) and ten replications (one embryo in the microplate well). Exposure to UV-C light to *O. celebensis* embryos begins at embryonic phase 17. UV exposure was carried out using UV-C light with a wavelength of 250nm. Embryos were divided into four observational treatments, with different exposure times according to **Sayed and Mitani (2016)**. Each treatment consisted of 10 embryos. One treatment was kept under laboratory conditions (not exposed to UV-C), which served as a control. In the other three treatments, exposure to UV-C light was carried out for 3 days with different exposure times, namely for 15, 10, and 5 minutes per day. In this study, the parameters observed were the number of embryo heart rates counted from stage 24 to hatching and the embryo's heart rate counted per minute **(González-Doncel *et al.*, 2005)**.

The maintenance medium used in this study was ERM. ERM is a solution that is commonly used as a laboratory-based fish embryo maintenance medium. The commonly used ERM has components of 10.0g NaCl, 0.3g KCl, 0.4g $CaCl_2$ H_2O, and 1.63g $MgSO_4$ mixed with 1.0ml of $NaHCO_3$ (0.25g/ 20ml H_2O). ERM functions in the protection from bacteria that can damage eggs **(OECD, 2004)**. Afterward, the maintenance medium was put into a 24-well microplate using a dropper pipette. Each well of the microplate was filled with 2ml of maintenance medium. Subsequently, each microplate well was given one fertilized embryo. The total number of embryos observed was 40 embryos obtained from spawning female broodstock in the aquarium.

5. Data analysis

The statistical analysis was performed using GraphPad Prism 5 and SPSS 26 software. A one-way analysis of variance test (one-way ANOVA) was applied if the data were normally distributed and homogenous. Data that were not normally distributed or homogeneous were analyzed using the Kruskal-Wallis test. For data that were normally distributed and homogeneous, post-hoc analysis was conducted using Tukey's multiple comparison test. Conversely, data that were not normally distributed or homogeneous were analyzed using Dunn’s test. Pearson correlation analysis was employed to examine the developmental model of heart rate during embryogenesis.

RESULTS AND DISCUSSION

The heart rate of the embryos in this study was calculated starting from stage 24. The heart rate was counted at each stage for 1.0 minute. The graph of *O. celebensis* embryo heart rate in each treatment can be seen in Fig. (1).

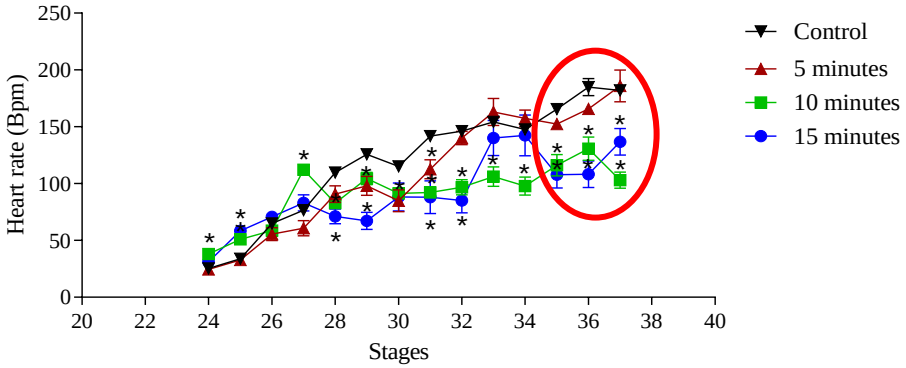

Fig. 1. *Oryzias celebensis* embryo heart rate in each treatment and control. The symbol (*) indicates a significant difference from the control (P<0.05). Bpm: beats per minute. The red circle indicates that the effect of UV-C on heart rate is still present in the final three stages before hatching

The results of calculating the heart rate of *O. celebensis* embryos per minute at each stage of embryo development can be seen in Fig. (1). It showed that the heart rate of the embryos in all treatments and controls, as well as between treatments and other treatments was significantly different (P<0.05) based on the results of the one way ANOVA statistical test. Given the current findings, it was monitored that UV-C light affected the embryo's heart rate and the length of time of exposure showed a different effect. Heart rate in all treatments and controls was slower and varied compared to the heart rate of *O. latipes* embryos **(González-Doncel *et al.*, 2005)**.

The graph of the results of the correlation analysis of the control, exposure of 5, 10, and 15-minute heart rates of *O. celebensis* embryos can be seen in Figs. (2, 3, 4, and 5, respectively).

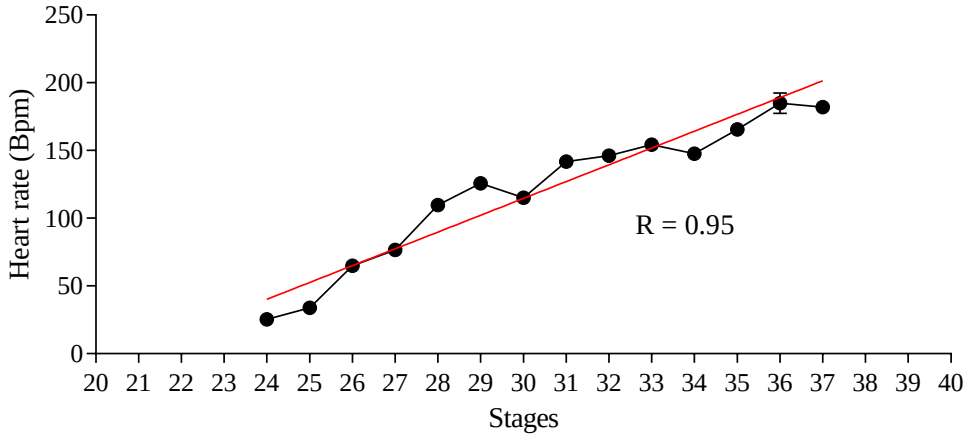

Fig. 2. Heart rate of control *O. celebensis* embryos based on the results of correlation analysis. Bpm: beats per minute

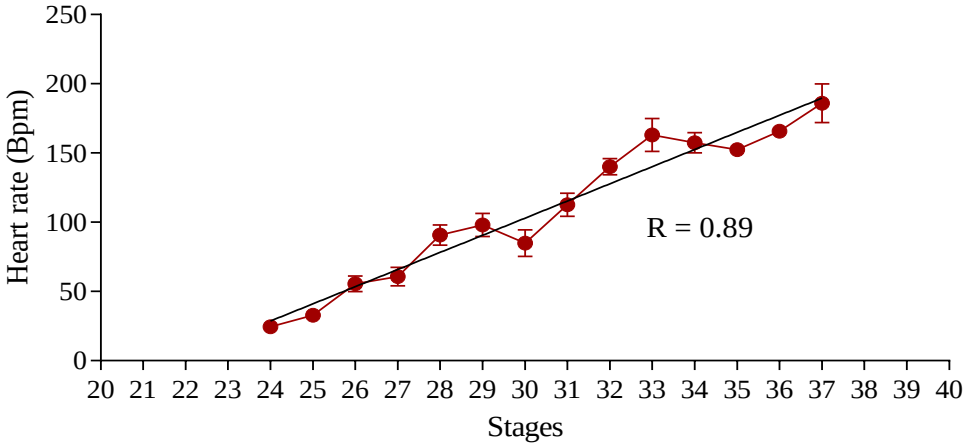

Fig. 3. Heart rate of *O. celebensis* embryos treated with UV-C light exposure for 5 minutes based on the results of correlation analysis. Bpm: beats per minute

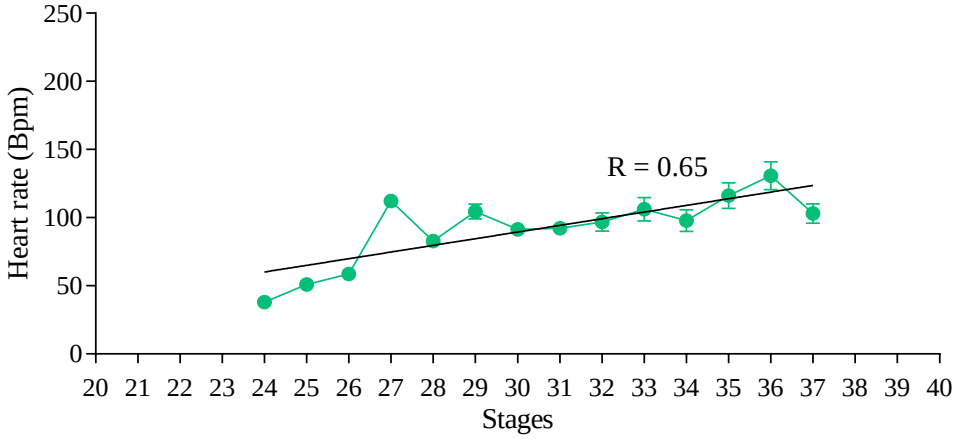

Fig. 4. Heart rate of *O. celebensis* embryos treated with UV light exposure for 10 minutes based on the results of correlation analysis. Bpm: beats per minute

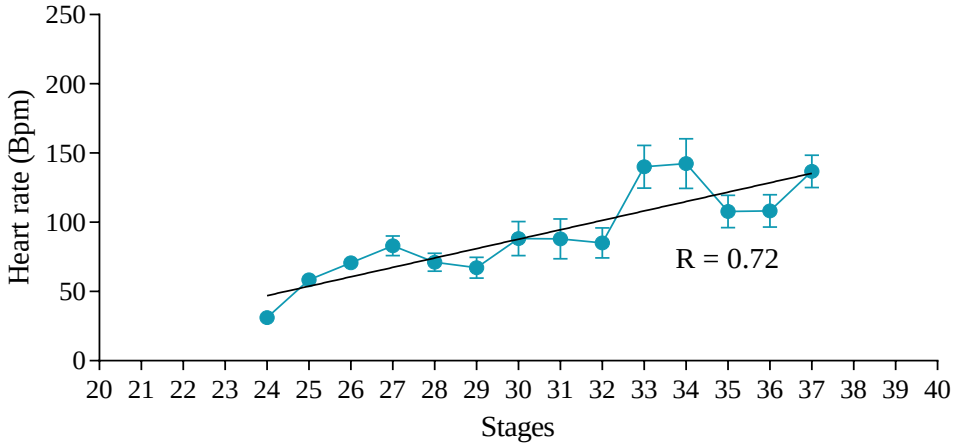

Fig. 5. Heart rate of *O. celebensis* embryos treated with UV light exposure for 15 minutes based on the results of correlation analysis. Bpm: beats per minute

Based on the results of the correlation analysis, embryos treated with UV-C light exposure had lower R-values compared to control embryos. However, the embryos treated for 10 minutes had a higher R-value than the other two treatments. The R-value of embryos treated for 5 minutes had an R-value close to the R-value of control embryos.

Heart rate can be used as a biomarker in ecotoxicology studies. In *O. celebensis* embryos the heartbeat begins to form at stage 23, although it still looks unclear or faint because the heart rate at this stage still beats weakly **(González-Doncel *et al.*, 2005; Yaqin, 2021)**. In this study, embryos without treatment or control had a heart rate that tended to increase with increasing stages of embryogenesis. This can happen because the more developed the embryo, the faster the heart rate will be produced **(Agatha *et al.*, 2021; Yaqin *et al.*, 2024)**.

Embryo's heart rate with UV-C light exposure in the early stages of the appearance of a heartbeat, has a heart rate that continues to increase, but at later stages, it experiences an inconsistent heart rate. Increased heart rate can be categorized as a form of embryo stress due to the treatment that exposed them. According to **Jain-Schlaepfer *et al.* (2018)**, heart rate provides an indicator that can be categorized as a stress response, indicating increased energy mobilization and use in fish embryos. Heart rate (mediated by β-adrenoceptor) increases directly in response to stressors induced by neural stimulation of the hypothalamic-sympathetic-chromaffin cell axis and catecholamine production **(Barton, 2002; Bagatto, 2005)**. The decrease in heart rate experienced by the embryo can also occur due to the toxic response that occurs in the embryo as a result of the treatment given **(Aksakal and Ciltas, 2018; Zhang *et al.*, 2021)**.

Fig. (1). shows that at stage 24 and stage 25, embryos that were treated with exposure to 10 and 15 minutes had statistically different heart rates to the control, namely heart rates were higher compared to the control embryos. This showed that the embryo had tachycardia, which was where the heart rate was higher than it should be **(Chen *et***

***al.,* 2020)**. These results are different from studies of UV-A exposure on the zebrafish embryos, which showed that the embryos experience bradycardia, namely a lower heart rate than they should **(Mosselhy *et al.,* 2016)**. Meanwhile, embryos exposed to 5 minutes showed no significant difference from control embryos. At stage 26 onward, the heart rate of the control embryos continued to increase, and the embryos given the 10 and 15-minute exposure had lower heart rates than the controls. Embryos treated for 5 minutes exhibited no significant difference from control embryos.

The decrease in heart rate that occurred in embryos treated for 10 and 15 minutes is in line with the study of **Hurem *et al.* (2018)**, which stated that the heart rate of *Danio rerio* embryos exposed to UV-A and UV-B light decreased significantly compared to embryos without treatment or control. These results indicate that the heart rate is susceptible to changes after exposure to UV radiation. Decreased heart rate is related to changes in metabolism and/or other physiological parameters **(Aksakal & Ciltas, 2018)**.

However, the results are different from a study conducted by **Cha *et al.* (2012)**, which showed an increase in heart rate occurred in *D. rerio* embryos exposed to UV-B light. Embryos exposed to UV-B light had an average heart rate higher than embryos without treatment. The average heart rate of embryos treated with UV exposure was 245bpm, while the average heart rate of embryos without treatment or control was only 177bpm.

Correlation coefficient values can be categorized into several categories ranging from weak to very strong categories **(Fowler *et al.,* 1998)**. Based on the correlation coefficient value obtained in this study, it was revealed that control embryos had a correlation value of 0.95, which means a very strong and positive correlation, and embryos exposed to UV-C for 15 minutes displayed a correlation value of 0.72, which means a strong and positive correlation. Whereas, embryos exposed to UV-C for 10 minutes had a correlation value of 0.65, which means a moderate and positive correlation. The embryos exposed to UV-C for 5 minutes had a correlation value of 0.89 which meant a strong and positive correlation. A correlation value that is close to 1.0 indicates a correlation or close relationship **(Mustika *et al.,* 2014)**. In this study, control embryos were embryos that had a correlation value close to 1.0, meaning that there was an increase in heart rate along with the development of embryogenesis. An embryo heart rate that had a correlation value that was close to that of the control embryo was the embryo with 5 minutes of UV-C exposure, while the embryo with 10 minutes of UV-C exposure had a correlation value that did not approach the correlation value of the control embryo.

By using this correlation approach, we can determine that the longer UV-C exposure given to *O. celebensis* embryos, the greater the heart rate damage. This can be seen from the fluctuation or inconsistency of the embryo's heartbeat during its development. If we look at Fig. (1) on the red circle, which are stages 35, 36, and 37, it

appears that the longer the exposure, the greater the damage to the rhythm of the heartbeat of the *O. celebensis* embryo.

In ecotoxicological studies, the heart rate phenomenon, as indicated by the heart rate of *O. celebensis* embryos, can be used as a simple and non-invasive biomarker **(Yaqin, 2019)**. This is because its use does not require expensive and sophisticated equipment available in various laboratories. Furthermore, using the biomarker does not require the sacrifice of the sentinel organisms, which is why it is referred to as a non-invasive biomarker. In the future, the heart rate biomarker, because the measurement involves an optical one, is very likely to be integrated into the internet of things (IOT) concept so that its use can be easier and more widespread, for example, used for monitoring in the field.

CONCLUSION

Exposure to UV-C light showed a negative impact on the heart rate of *Oryzias celebensis* embryos. This is indicated by the difference between the treatment exposed to UV-C light and the control. Heart rate showed a difference between each duration of UV-C light exposure. Therefore, the heart rate is a parameter that is strongly influenced by exposure to UV-C light, as well as proving that heart rate can be used as a simple and non-invasive biomarker in ecotoxicology studies.

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