Jurnal Pijar MIPA
https://jurnalfkip.unram.ac.id/index.php/JPM

ISSN 1907-1744 (Print)
ISSN 2460-1500 (Online)

Effect of Light Intensity on Zebra Fish (*Danio rerio*) Cell Regeneration

Adisty Virakawugi Darniwa*, Tri Cahyanto, Savira Ngesti Rahayu

Biology Department, Faculty of Science and Technology, UIN Sunan Gunung Djati Bandung, Bandung, Indonesia
*E-mail: adistyvd@uinsgd.ac.id

Received: November 17, 2022. Accepted: February 20, 2024. Published: March 20, 2024

Abstract: Zebrafish (*Danio rerio*) is one of the model animals in cell regeneration studies because it has a high ability to regenerate tissue structures, one of which is its caudal fin. This study was conducted to determine the different light interventions on the regeneration of the caudal fin of zebrafish. The research method used is an experimental study with the observed parameters, including specific growth, absolute weight gain, length increase, and survival. A total of 28 male wild-type zebrafish were used in this study at the age of 3 months. The amputated zebrafish on its caudal fin was placed at different light intensities (control, 0 lux, 60 lux, and 90 lux). Parameter measurements were carried out every two days. ANOVA analyzed the data obtained with a significance level of 95%. The results showed that at a light intensity of 90 lux, the zebrafish tail fin regeneration was the most significant based on an increase in total length of 0.10 mm. Using a light intensity of 90 lux is the best condition in the regeneration process of the zebrafish caudal fin. The research findings could be further applied in the healing process of external wounds.

Keywords: Caudal Fin; Cell Regeneration; *Danio rerio*; Light Intensity.

Introduction

Regeneration is a process of formation and growth that strengthens genes, cells, organisms, and ecosystems to face any disturbances or damages [1]. Some vertebrae can carry out the regeneration process in their limbs [2]. Amphibians and Pisces even have a higher ability than mammals to regenerate their organs, especially in the epimorphic process of regenerating severely injured or missing body parts [3].

Zebrafish are often used as animal models for investigating various aspects of biological studies, one of which is related to tissue regeneration [4]. The genetic similarity associated with the mechanism of physiology and genetics (about 70%) with humans makes zebrafish an animal model for determining cell regeneration mechanisms in humans and vertebrates [5]. Also, zebrafish have become animal models for vertebrate tissue regeneration and accompanying genetic models [6]. The high ability of cell regeneration in body parts of zebrafish, especially in the fins, makes this fish effective for learning something related to biological phenomena. The study of biological phenomena and cell regeneration in zebrafish can be done by observing the fin, especially the caudal fin, which has the highest regeneration ability compared to other body parts [7].

Light intensity is one of the abiotic factors that can affect regeneration of living things. If there is a change in light intensity, it can influence the growth of living things [8]. Therefore, it is necessary to manipulate the abiotic environment to improve the cell regeneration process. Light intensity increases the growth of tiger grouper, Siamese sepat fish and zebrafish [9] [10]. Lighting has many characteristics, such as spectrum, intensity, and photoperiod, that can directly or indirectly affect physiological responses, reproduction, and the growth of living things [11]. The LED (Light Emitting Diode) lighting system has just been developed. It can adjust light intensity in a single light source, thus serving as an accurate tool for influencing fish growth and survival. It has been reported that proper light illumination can play a role in zebrafish fin regeneration. If the mechanism of regeneration with light intensity intervention could be understood properly and correctly, it can continue with its application to the regenerative healing process in humans [12]. The latest research on zebrafish regeneration concerns the regeneration of the zebrafish tail fin, determined by the length of the amputee [3]. However, studies on the effect of light intensity on regenerating growth in the caudal fin of zebrafish have not been carried out. Thus, there is a need for a study that discusses the optimal light intensity that will significantly affect the regeneration of the zebrafish caudal fin. Therefore, this study was conducted to determine the different light interventions that can be used to regenerate the caudal fin of zebrafish. Further application from this research could be used in medicine in the form of the healing process of external wounds.

Research Methods

Materials and Tools

This research used twenty-eight wild-type zebrafish (*Danio rerio*) males. Zebrafish which is 3-month-old obtained from the Bojongsari Fish Farm, Depok. Another material used in this research consists of Tetrabits fish pellets (Jakarta, Indonesia) and ice cubes. The tools used include a rearing aquarium, LED lights, 3watt Starlux (Bandung, Indonesia), ruler,

How to Cite:
Darniwa, A. V., Cahyanto, T., & Rahayu, S. N. (2024). Effect of Light Intensity on Zebra Fish (Danio rerio) Cell Regeneration. *Jurnal Pijar Mipa*, *19*(2), 260–264. https://doi.org/10.29303/jpm.v19i2.4364

millimetre block, digital scale, fish filter, pH paper, thermometer, petri dish, scalpel, aquarium aerator, Luxtron LX-102 lux meter, treatment aquarium measuring 12 x 9 x 6.5 (w x l x h).

Zebrafish Preparation

Fish were acclimatized for two weeks by placing them in 4 rearing aquariums. The water temperature in the aquarium is set at 28ºC - 30ºC because too low or too high temperature makes the fish tend to show stress behaviour [13]. During acclimatization, observations were made in the form of fish weight, total length of fish, fish activity, and fish diet. Fish are conditioned with programmed lighting according to their life cycle, which is 14 hours of light and 10 hours of darkness. Zebrafish were fed with Tetrabits twice a day, in the morning and evening, and water was changed every two days. Generally, zebrafish in adult fish are fed twice a day with pellets that are given manually [14].

Different Light Intensity Treatment

Zebrafish were divided into four treatment groups with different lighting intensities (control, 90 lux, 60 lux, and 0 lux) and 24 hours of light duration. Each aquarium consists of 7 fish: P1 as a control group with room lighting; P2 given a light intensity of 0 lux; P3 granted a light intensity of 60 lux; and P3 given a light intensity of 90 lux. Before the caudal fin was amputated, the zebrafish was given anaesthesia using an intervention temperature of 0^0C. The zebrafish's caudal fin is amputated for half of the total fin length. The amputated zebrafish were then reared according to the prescribed lighting treatment. Parameter measurements were carried out every two days for 14 days of treatment.

Test Parameters

Parameters measured include survival, specific growth rate, total length growth, and absolute weight gain. The calculation of these parameters uses the following formula:

Specific Growth Rate

The specific growth rate is the weight every day during the study. The specific growth rate could be calculated using the formula [10]:

SGR = (√(t*Wt)/Wo-1)x100%

SGR = specific growth rate (%)

Wt = average weight of the fish at the end of the study (gram)

Wo = average weight of the fish at the start of the study (gram)

t = observation time (days)

Total Length of caudal fin

Total length growth means the increase in length (difference in final length and initial length) during the study period. The total length growth can be calculated by the formula [10]:

L = Lt-Lo

L = total length (mm)

Lt = average length of the fish at the end of the study (mm)

Lo = average length of the fish at the start of the study (mm)

Absolute Weight Growth

Absolute weight growth is weight gain (difference between final weight and initial weight) during the study period. The absolute weight growth can be calculated by the formula [10]:

Wm = Wt-Wo

Wm = Absolute weight (gram)

Wt = average weight of the fish at the end of the study (gram)

Wo = average weight of the fish at the start of the study (gram)

Survival Rate

Survival was defined as the percentage of the total number of fish alive at the end of the treatment compared to the total number of fish at the start. Survival rates can be calculated by the following formula [10]:

S = Nt/No x100%

S = survival rate (SR) (%)

Nt = total number of fish at the end of the study (individuals)

No = total number of fish at the beginning of the study (individuals)

Data analysis

The increase in the total length of the zebrafish caudal fin obtained from each treatment group was processed by statistical analysis using SPSS 25.0. Parameters were analyzed through specific growth rates and incremental length increases. Statistical analysis was performed using variance analysis (ANOVA) with a significance of 95%.

Results and Discussion

After the amputation, the zebrafish's caudal fin showed growth in the form of a thin transparent layer (Day 0, Figure 1.). This condition is thought to indicate the occurrence of the initial wound-healing process. Poss *et al*. described that epidermal cells moved to cover the wound area during the first tweleve hours after injury or amputee [15]. After that, the epithelium in the wound area forms several apical epidermal layers. During development, the apical epidermis plays an essential role in the apical ectodermal ridge [16]. The signal transduction process of wound healing, according to Paredes *et al.*, involves the gene expression of BMP-7 (Bone Morphogenetic Protein), PI3k (Phosphoinositide 3-kinase), and PS6K protein [13]. Genes from the Wnt-Signing pathway (β-catenin, lef1, and Wnt5) were also expressed in the epidermal area around the wound during wound healing [16]. It is hypothesized that signals from the distal blastema indicate that the Wnt/β-catenin and aldhla2 pathways are expressed in the initial wound.

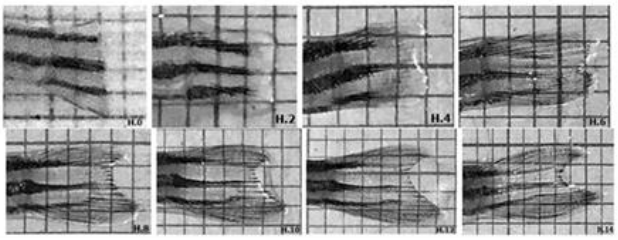
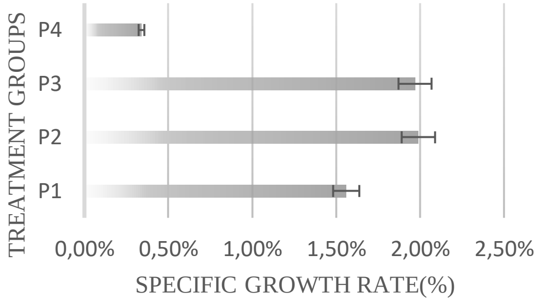


Figure 1. Zebrafish caudal fin regeneration process (Day 0 – Day 14)

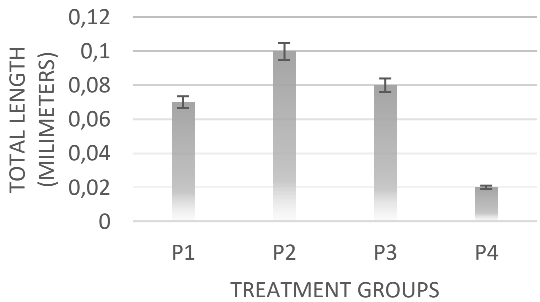
Figure 1. on part H.2 shows the condition of the caudal fin on the second day after the amputee. The visible layer started to look and began thickening after the fin amputation. The thickening process occurs on the entire surface of the former amputation. These results are in accordance with research by Poss *et al*. [15], where blastema formation occurs 12-24 hours after amputation (second phase of regeneration). Blastema is a group of cells derived from differentiated mesenchymal cells in the proximal area of the amputation area. According to Paredes *et al*. [4], injured epidermal areas display msxA and msxD expression and initiate initiation of the Wnt/β-catenin pathway. Then, Sehring & Weidinger [16] explained that bone morphogenic protein 4 (bmp4), which is a transcription factor for the homeobox hoxA11B and hoxA13B, as well as the retinic acid receptor resinate-γ (rar-γ) were also expressed in the blastema stage.

On day three, after the amputee, the fish's caudal fin developed around the periphery of the caudal fin. The structure of the caudal fin has been formed from the beginning of regenerative development. It will continue to grow until the 8^{th} day after amputee (Figure 1. Part H.4 - H.8). Then on the 9th day after amputee, finger joints on the caudal fin began to form until the 10th day after amputee (Figure 1. Part H.10). Entering the 11^{th} to 14^{th} after amputee day (Figure 1. Part H.12 - H.14), overall regenerating radius has formed resembling a segment in the proximal area. From the above stages, observations of the regeneration process of the caudal fin are supported by the research of Poss *et al*. [17], which described the last phase of fin regeneration called regeneration growth, which is 48 hours after amputation until regeneration is complete. According to Paredes *et al.* [4], during the regenerative growth of the epidermis in the wound area, the fgf gene and signalling her4 and msxB were expressed and followed by notch1b, lfng, her6, and her15 expressions.

Growth values on zebrafish caudal fin showed varying results. The results of the specific growth rate in each treatment are shown in **Figure 2.** The highest specific growth rate in P2 is 1.99%, and P3 is 1.97%, while the lowest specific growth rate is found in P3, just 0.34% ($\alpha \leq 0.05$). In the P2 and P3 treatments, the specific growth rate can produce high values, presumably because zebrafish can adapt to the tolerance range of light intensity exposed to be optimal in foraging. Optimal foraging provides nutrients to support the amputated zebrafish's caudal fin growth. According to Mahardhika *et al.* [18], fish growth can also be influenced by other factors, namely the ability of each fish to convert feed into growth energy. The zebrafish at P4 showed a shallow growth rate. According to the research of Carrillo & McHenry [19], the ability to forage in the dark at all stages of zebrafish age is very lacking. The life cycle of zebrafish is 14 hours of light and 10 hours of darkness. In light time, the fish are used for foraging activities using light-catching receptors on their organs of vision, while in the dark, the fish are used to mate [14] [20].



Specific Growth Rate (%) on Zebra Fish Caudal Fin



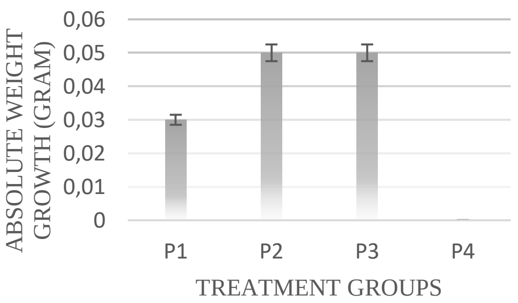
Total Length (mm) on Zebra Fish Caudal Fin

Figure 2. Specific Growth Rate on zebrafish caudal fin and Total Length on zebrafish caudal fin.

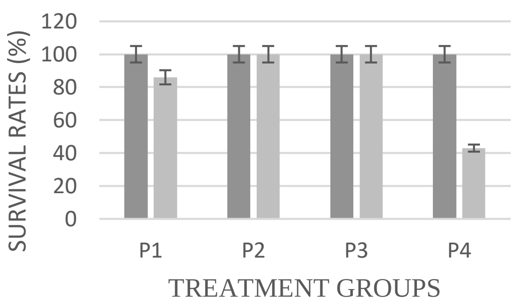
Absolute Weight was measured to determine the weight gain of zebrafish in each treatment group. Figure 2. shows that the highest weight growth was in the P2 and P3 treatments, which was 0.05 grams, while in P4, there was no absolute weight gain. There was no significant difference between P2 and P3 ($\alpha \leq 0.05\%$). In the P4 treatment, the absolute weight gain was 0.00. This is presumably because exposure to a light intensity of 0 lux causes very low or no light exposure and is below the zebrafish tolerance threshold. Very low light intensity is one of the factors that causes zebrafish to be unable to find food and eat it usually. Light intensity below the required tolerance causes fish to have difficulty finding and devouring the feed provided so that the fish will die of starvation [9]. The intensity of light that can still be received by zebrafish is in the range of 54-324 lux [21].

Measurement of Total Length was carried out every two days. Figure 2. illustrates the increase in total length with the highest yield at 0.1 mm P2. At the same time, the lowest result is seen in P4, which is 0.02 mm. It is known that the function of the caudal fin of fish is to support the fish in moving by directing the caudal fin to the side and as a driving force for its forward movement. When the caudal fin is amputated, the fish will find it difficult to move and even move from one position to another, especially in finding food to support the growth of the caudal fin. Changes to the fish's living environment and unfulfilled nutritional needs are thought to affect the increase in total fish length.

In light conditions, the fish's tail fin regeneration will be more visible than in constant dark conditions. Significantly seen on the P4, with a total length increase of only 0.02 mm. Fish need light to survive and grow (Figure 2.). Light intensity, spectrum, and irradiation significantly affect fish at all life stages [22]. In addition to external factors, in this case, light intervention, the zebrafish's growth or regeneration process is thought to be influenced by internal factors. Internal factors, including light-suppressed genes, are considered to affect mRNA expression [23]. Also, during caudal fin regeneration, the wee1 mRNA expression could fluctuate rhythmically during a 24-hour period [4].



Absolute Weight (gram) on Zebra Fish



Survival Rate (%)

Figure 3. Absolute Weight on Zebrafish and Zebrafish Survival Rate.

Survival was observed every two days for 14 days of treatment and was carried out by recording dead fish [24]. The highest survival value was found in treatments P2 and P3 (Figure 3), while the lowest results were found in treatment P4 at an intensity of 0 lux. It is suspected that the light intensity at P2 and P3 is the light intensity that can be tolerated by zebrafish because it can make it easier for fish to find and eat their food. At P4, zebrafish were given post-amputation treatment with 0 lux light, which was thought to cause a very low survival rate compared to other treatments. According to Bianingrum [25], intensive light intensity treatment at a specific size can stress and even kill fish. This is because the treatment does not include the tolerance range of light intensity in zebrafish. Almost all environmental factors, including light intensity, can affect fish responses to stressors that can threaten fish health and welfare.

The amount of feed consumed will affect the growth of fish, where the fish's immune system becomes strong and causes a high rate of zebrafish survival. Light exposure, either acute (24 hours) or chronic (14-20 days), results in increased activity throughout the night, but this behaviour change is not accompanied by an increase in cortisol concentrations throughout the body, indicating that the condition lacks sleep does not cause stress in zebrafish [26]. The critical regulatory genes of zebrafish circadian rhythms Clock1a and Bmal1a may have decreased their expression, so melatonin production will reduce or even stop. Therefore, zebrafish should be allowed to ignore the need for regular rest periods.

Conclusion

The light intensity of 90 lux on P3 is the optimal light intensity for the regeneration of the caudal fin of zebrafish (*Danio rerio*), which is indicated by a specific growth rate of 1.99%, an absolute weight gain of 0.05 grams, an increase in total length of 0.10 mm, and survival rate reach the highest of 100%.

References

[1] Olsen, A. S., Sarras, M. P., & Intine, R. V. (2010). Limb regeneration is impaired in an adult zebrafish model of diabetes mellitus. *Wound Repair and Regeneration. 18(85)*, 532–542.

[2] Mahardhika, K. N., Rejeki, S., & Elfitasari, T. (2017). Performa Pertumbuhan Dan Kelulushidupan Benih Ikan Patin (Pangasius hypophthalamus) Dengan Intensitas Cahaya yang Berbeda. *Journal of Aquaculture Management and Technology. 6(4)*, 130–138.

[3] Ye, Q., Chen, D., Xi, Y., Li, J., Shao, J., & Li, L. (2012). Effects of Light Illumination and The Expression Of Wee1 on Tissue Regeneration in Adult Zebrafish. *Biochemical and Biophysical Research Communications, 428(1)*, 132–136.

[4] Paredes, L. C., Camara, N. O. S., & Braga, T. T. (2019). Understanding The Metabolic Profile of Macrophages During The Regenerative Process in Zebrafish. *Frontiers in Physiology. 10(1)*, 1–19.

[5] Darniwa, A., Cahyanto, T., Eka Putri, Z., Kusumorini, A., Ulfa, R., Adawiyah, A., & Hizqiyah, I. (2021). Uji Perilaku dan Preferensi Area pada Ikan Zebrafish (Danio rerio) yang Diinduksi Stres. Biosfer: *Jurnal Biologi Dan Pendidikan Biologi. 5(2)*, 32-38.

[6] Lisdalia, S. (2013). *Pengaruh Intensitas Cahaya Terhadap Kecepatan Regenerasi non-alami Ikan Planaria*. Skripsi. Universitas Negeri Semarang.

[7] Uemoto, T., Abe, G., & Tamura, K. (2020). Regrowth Of Zebrafish Caudal Fin Regeneration Is Determined by The Amputated Length. *Scientific Reports, 10(1)*, 1–11.

[8] Reed, B., & Jennings, M. (2011). Guidance On The Housing and Care Of Zebrafish. In Research Animal Department, Science Group, RSPCA. Retrieved from papers2://publication/uuid/4F68076E-5B1F-4412-9366-E2017C6ADCD0

[9] Rahmawati, R., Cindelaras, S., & Kusrini, E. (2016). Keragaman Pertumbuhan dan Warna Ikan WILD BETTA (Betta sp.) Dengan Rekayasa Intensitas Cahaya dan Warna Latar. *Jurnal Riset Akuakultur, 11(2)*, 155–162.

[10] Boykin, J. C. (2016). The Behavioral, Biochemical and Genetic Effects of Sleep Deprivation in Zebrafish (Danio Rerio). Georgia Southern University.

[11] Aras, A. K., Nirmala, K., Soelistyowati, D. T., & Sudarto. (2016). Manipulasi Spektrum Cahaya Terhadap Pertumbuhan dan Kualitas Warna Yuwana Ikan Botia Chromobotia macracanthus. *Jurnal Iktiologi Indonesia. 16(1)*, 45–55.

[12] Schebesta, M., Lien, C. L., Engel, F. B., & Keating, M. T. (2006). Transcriptional profiling of caudal fin regeneration in zebrafish. *The Scientific World Journal, 6(S1)*, 38–54.

[13] Delabbio, J. (2015). Advantages of LED Lighting Systems in Larval Fish Culture. *World Aquaculture. 1(1)*, 25–29.

[14] Sari, Kartika, N., Listyorini, Dwi, Gofur, & Abdul. (2016). Proses Regenerasi Sirip Ekor Pada Ikan Zebra. *Edubiotik, 1(1)*, 25–29.

[15] Putri Anica Rahmawati, A., Hudaidah, S., & Wijayanti, H. (2016). Pengaruh Intensitas Cahaya Selama Pemeliharaan Benih Ikan Kerapu Macan (Epinephelus fuscoguttatus). *E-Jurnal Rekayasa Dan Teknologi Budidaya Perairan, 5(1)*, 547–558.

[16] Sehring, I. M., & Weidinger, G. (2019). Recent Advancements In Understanding Fin Regeneration In Zebrafish. *Wiley Interdisciplinary Reviews: Developmental Biology, 9(1)*, 1–16.

[17] Poss, K. D., Keating, M. T., & Nechiporuk, A. (2003). Tales Of Regeneration In Zebrafish. *Developmental Dynamics, 226(2)*, 202–210.

[18] Matthews, M., Trevarrow, B., & Matthews, J. (2002). A Virtual Tour Of The Guide For Zebrafish Users. *Lab Animal. 31(3)*, 34–40.

[19] Cavalcante, A. K., Rogero, S. O., & Rogero, J. R. (2017). Evaluation Of Resveratrol Toxicity In The Embryolarval Stage Of Danio rerio fish. *Ecotoxicol. 12(1)*, 133–139.

[20] Carrillo, A., & McHenry, M. J. (2016). Zebrafish learn To Forage In The Dark. *Journal of Experimental Biology. 219(4)*, 582–589.

[21] Novianti, T. (2018). *Ekspresi Gen Sitoglobin Pada Fase Wound Healing Proses Regenerasi Jaringan*. Skripsi. Universitas Essa Unggul.

[22] Fernando, R. (2019). *Pengaruh Penambahan Tepung Wortel (Daucus Carota) Pada Pakan Buatan Terhadap Peningkatan Kecerahan Warna Ikan Cupang (Betta splendens Regan)*. Skripsi. Universitas Muhammadiyah Pontianak.

[23] Adamskaya, N., Dungel, P., Mittermayr, R., Hartinger, J., Feichtinger, G., Wassermann, K., Van Griensven, M. 2011. Light Therapy by Blue LED Improves Wound Healing in an Excision Model in Rats. *Injury. 42(9)*, 917–921.

[24] Gemberling, M., Bailey, T. J., Hyde, D. R., & Poss, K. D. (2013). The Zebrafish As A Model For Complex Tissue Regeneration. *Trends in Genetics. 1(1)*, 1–10.

[25] Bianingrum. (2015). Perbedaan Intensitas Cahaya Terhadap Performa Pertumbuhan dan Sintasan Benih Ikan Sepat Siam Trichopodus pectoralis. Skripsi. Institut Pertanian Bogor.

[26] Fleisch, V. C., & Neuhauss, S. C. (2006). Visual behavior in zebrafish. *Zebrafish*, *3*(2), 191-201.