The effect of enriched cyclopoid copepods on the coloration and feeding rate of *Betta splendens*

A Yuslan¹, N Nasir¹, H Suhaimi¹, A Arshad², N W Rasdi¹,3*

¹ Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, 21300, Kuala Terengganu, Malaysia

² International Institute of Aquaculture and Aquatic Sciences, Universiti Putra Malaysia, 71050 Port Dickson, Negeri Sembilan, Malaysia

³ Institute of Tropical Biodiversity and Sustainable Development, Universiti Malaysia Terengganu, 21300, Kuala Terengganu, Malaysia

*Corresponding author: nadiah.rasdi@umt.edu.my

Abstract. Copepods with a wide range of sizes, species, and nutritional compositions are preferred as live food for rearing of *Betta splendens* larvae. This research focuses on evaluating the efficiency of copepod enrichment diets in improving the coloration and feeding rate of *B. splendens*. Copepod were enriched with *Chlorella* sp. (T1), capsicum (T2), mixed vegetable (carrot + spinach), (T3), yeast (T4) and rice bran (T5) in 24 hours prior the feeding tests. As a result, proximate analysis of enriched-copepods showed that T1 (70.88±0.41) has highest protein content and T5 (22.01±0.59) has the highest lipid content. The specific growth rate and survival rate of *B. splendens* was highest in the treatment T1 (2.56±0.07%; 91.11±1.92%) and followed closely by T3 (2.49±0.51%; 85.55±8.39%). Feeding rate, T3 (70.08±3.88%) presented highest rate compared to other treatments. The different enrichment diet used were significantly impact the coloration test on body of L* value (P=0.001, P<0.05), T3 (66.11±3.60) appeared darker in color in contrast to others. As for a* value, the coloration was not impacted with the use of different enrichment on copepods (P=0.158, P>0.05) was detected for T1 (2.84±0.73) that gave a redder shade than other treatments did. T3 (2.40±0.30) exerted a more yellowish shade than the rest for b* value with a significant difference (P=0.015, P<0.05). The current study demonstrates that, rice bran, capsicum and mixed vegetable enrichment (carrot and spinach) have the potential to be an effective means of increasing *B. splendens* coloring and feeding rate. This potential diet can be further used as a substitution to artificial foods in producing sustainable culture of ornamental fish in the aquaculture industry.

1. Introduction

*Betta splendens*, often known as Siamese fighting fish, is a popular fish with aesthetic value that has a distinctive structure and color, so it is kept as an ornamental fish [1, 2]. *B. splendens* can be found in several countries in the world, such as Thailand, Indonesia, Singapore, China, Malaysia, Japan, the United States, and Mexico, and this species is a major contributor to the local economy [3, 4]. However, large-scale production of *B. splendens* is limited by the high mortality rate of fish. In addition, it is also caused by the inability of fish to produce color pigments naturally, but they must obtain it through food [5].
Enhancement of development and body coloration in aquatic animals is determined by the type, quality, and quantity of food consumed [6]. Nutrient intake has a direct effect on egg quality and reproduction [5-7]. Copepods are generally used as nutrient intake at the larval cultivation stage due to their high protein content and lipid profile, which consists of essential fatty acids, and also their ability to assist the development of the digestive system [8-10].

Carotenoids are a group of pigments that express colour in fish. In several studies, ornamental fish feed has been supplemented with this pigment to increase its body coloration, growth performance and market value [2, 11-13]. Carotenoids play a role in the pigmentation of food fish muscles and the colour of ornamental fish and important in eyesight, as transcription regulator precursors, antioxidants, and immunological system [14, 15]. Thus, the application of carotenoid is quite extensive.

Ornamental fishes are primarily pigmented with carotenoid sources that include sections of higher plants, microalgae, seaweed, crustacean waste, and yeast, according to published research by Sathyaruban et al. [12]. Like the fish, live feed that used, as food for ornamental fish such as copepod cannot itself synthesize carotenoid pigments, but instead get it from the enrichment diets such as microalgae [6, 9, 16]. Thus, the purpose of this study was to determine the influence of feeding enriched copepods on color, growth, and feeding rate of *B. splendens*. We hypothesized that the ability of enriched copepod used as food for *B. splendens* might mimic the natural feeding behavior of the fish in wild habitat and is more suitable for scale color development and growth performance of *B. splendens*.

2. Material and Methods

2.1. Copepod stock culture

All feeding trials were conducted in the Faculty of Fisheries and Food Science Hatchery, Universiti Malaysia Terengganu (UMT), Malaysia. Cyclopoid copepod was sampled from Setiu water, Terengganu, Malaysia. The sample was sustained for a few generations for adaptation from wild to the hatchery culture. The copepods culture was maintained and monitored at the optimal water parameter; temperature at (26°C-27°C), salinity (10-15 ppt) and pH (7.5), daily throughout the experimental period by using YSI meter [17]. Every three days, 20%-30% of the water was changed to preserve the experiment's healthy culture.

2.2. Experimental design

Copepod were fed with five diets (Table 1) which is comprises of *Chlorella sp.* (T1), capsicum (T2), mixed vegetable (carrot and spinach) (T3), yeast (T4) and rice bran (T5). Copepod from stocked culture were harvested and placed into five enrichment tanks in triplicate. All of the diet were feed daily on copepod at the concentration of 500 mg L⁻¹ [18] for at least 2 weeks prior to the experiment [19, 20]. Copepods were enriched with respected diet as in Table 1, for 6 hours [21] before feeding experiments were conducted.

| Treatment | Enrichment of Copepod |
|-----------|-----------------------|
| T1        | *Chlorella sp.*       |
| T2        | Capsicum              |
| T3        | Mixed vegetable       |
|           | (carrot and spinach)  |
| T4        | Yeast                 |
| T5        | Rice bran             |

2.3. Proximate analysis

The analyses of protein and lipid compositions in the enriched copepods was carried out at Faculty of Fisheries and Food Science laboratory in UMT. All samples were analysed in triplicates by using the standard protocol from AOAC [22]. Samples of enriched copepods were harvested from enrichment tank, washed and sieve by using 50 µm plankton net. Samples were put in the 50 mL of centrifuge tube
and were centrifuged for 3 minutes at 2500 rpm to remove excess water. All of the samples were dried in an oven for 24 hours at 60°C. Dried samples of enriched copepod were weighed 200 mg for protein and 2 g for lipid analysis. The crude protein was analysed by using the Kjeldahl method with a protein conversion factor (F = 6.25) to transform total nitrogen to crude protein [23]. Meanwhile, crude lipids were extracted from the samples by using a Soxhlet petroleum ether extractor. After successful extraction, the petroleum evaporated, and the residue was dried to a constant weight at 100°C in an oven [24].

Formula used to calculate the percentage of protein and lipid content in the samples are as follows:

**Estimation protein**

\[
\%N \text{ in sample } = \left( \frac{T - B}{W} \times 14.007 \right) \times 100
\]

(1)

\% \text{ Protein in sample } = \%N \times F

(2)

Where,

- T = volume of HCl used in the titration of sample (mL)
- B = volume of HCl used in the titration of blank sample (mL)
- N = Normality of HCl
- W = sample weight (mg)
- F = Protein factor (6.25)

**Estimation lipid**

\[
\% \text{ Lipid } = \left( \frac{W_3 - W_1}{W_2} \right) \times 100
\]

(3)

Where,

- W1 = weight of extracted cup (g)
- W2 = weight original sample (g)
- W3 = weight of extracted cup + essential fat (g)

2.4. Fish and rearing condition.
The experiment was carried out with *B. splendens* juveniles aged 60 days old and had the primary colours red, yellow, and white on their bodies. The fish were separated randomly into four groups based on the enrichment medium with 30 fish each. Four tanks were provided with the specified treatments for enrichment. Each of the tanks was fed in a ratio of 10:1 (10 copepod individuals per fish thrice a day for 30 days of experimental period) [17]. Fishes were observed for growth, survival rate, feeding rate and enhancement of colouration.

2.5. Growth and survival rate
The growth and survival aspects of fishes were observed once in every six days throughout the 30 days experimental period. Their specific growth rate and survivability were calculated by the formula given by [17]:

\[
\text{Specific growth rate(%) } = \left( \frac{\text{In final weight} - \text{In initial weight}}{\text{Days of culture}} \right) \times 100
\]

(4)

\[
\text{Survival(%) } = \left( \frac{\text{Final number of fish}}{\text{Initial number of fish}} \right) \times 100
\]

(5)

2.6. Feeding rate
The feeding rate of *B. splendens* was measured by identifying how many copepods that they consume during each mealtime. Each of the tanks in triplicates was fed in a ratio of 10:1 (10 copepod individuals per fish thrice a day for 30 days of experimental period) [17]. Excess copepods were collected from each tank after each mealtime to calculate the feeding rate of *Betta splendens*.
2.7. Coloration
Each fish's colour was determined by examining the bodies of five randomly selected fish in each replicate. The fishes were anaesthetised for 10 minutes at cool temperature before the skin colour evaluation begins. Skin colour was measured in the centre of the body area of all fish. The Konica Minolta Chroma meter was used on the final day of the feeding test (day 30) and measurements were taken based on the colour system mode shown in the International Commission on Illumination (CIE), where L* = lightness, dark = 0, white = 100, a* = red (positive values = red and negative values = green), and b* = yellow (positive values = yellow and negative values = blue) [25, 44].

2.8. Data analysis
All data were recorded and presented as mean ± standard deviation. The collected data were tested for normality, homogeneity, and independence to meet the ANOVA hypothesis (IBM SPSS Version 25.0, 2017). All the data were analyses by using one-way analysis of variance (ANOVA) to see the effect of enriched copepods on the growth, survival, feeding rate and coloration of the B. splendens for 30 days of feeding trial. Differences are considered significant at the P<0.05 levels. The variances were significant at P<0.05 levels. The post-hoc Tukey test was conducted when the main treatment impact was significant.

3. Result and Discussion

3.1. Proximate analysis of cyclopoid copepod
Protein and lipid content analysis of cyclopoid copepod was significantly elevated with the types of feed used as enrichment diet for cyclopoid copepod (P=0.001, P < 0.05, Table 2). The highest protein content was recorded in T1 on copepods enriched with Chlorella sp. (70.88±0.41 %) compared with other dietary treatments. While, the highest lipid content was in T5, when copepod enriched with rice bran (22.01±0.59 %).

Table 2. Protein and lipid analysis of enriched cyclopoid copepod.

| Proximate analysis (%) | T 1       | T 2       | T 3       | T 4       | T 5       |
|------------------------|-----------|-----------|-----------|-----------|-----------|
| Protein                | 70.88±0.41<sup>a</sup> | 61.63±0.6<sup>b,c</sup> | 62.08±0.74<sup>c</sup> | 59.78±0.68<sup>d</sup> | 68.25±0.70<sup>e</sup> |
| Lipid                  | 19.42±0.24<sup>a</sup> | 17.97±0.7<sup>a</sup> | 18.97±0.79<sup>a</sup> | 13.87±0.67<sup>b</sup> | 22.01±0.59<sup>c</sup> |

All values are mean ± standard deviation (n = 3). Different letters on the same row indicate a significant difference (P < 0.05).

The nutrient importance, metabolism and function in the food chain can be explained by the biochemical investigations in zooplankton [26]. The biochemical components of three mostly marine pond copepods, Acartia grani, Centropages hamatus, and Eurytemora affinis, were analyzed by average protein (38.3 % -56.5 %) and identified by intermediate lipid levels (6.9 % - 22.5 %) [27][28]. A diet high in protein and lipids is required to enhance B. splenden growth performance and survivability [29].

3.2. Growth performance of B. splendens fed with enriched cyclopoid copepod
After 30 days of culture, B. splendens fry's specific growth rate and survival rate were calculated. The results were tabulated in Table 3 and Figure 1. Specific growth rate and survival rate of B. splendens fry fed with enriched cyclopoid copepod were impacted with the different enrichment used on (P=0.03; P=0.002, P<0.05). The highest specific growth rate was obtained in T1 (2.56±0.07 %) compared with other diets. While, the highest survival percentage was obtained in T1 (91.11±1.92 %), followed by T3 (85.56 ± 8.39 %), T2 (81.11 ± 5.09 %), T5 (72.77 ± 3.87 %) and T4 (70.00 ± 3.33 %) respectively.

A recent study by [7], found that feeding juvenile B. splendens on freshwater live feeds (Moina micrura) supplemented with Chlorella sp. resulted in the highest growth and survival rate. This is
consistent with the findings of [30], who discovered that feeding larvae of the loach fish *Misgurnus anguillicaudatus* on live food such as *Moina* sp. and *Daphnia* sp. enriched with *Chlorella* sp. as a continuous diet resulted in significant increases in both growth and survival rates since microalgae was always considered as a good resource for high-value substances [31, 32, 45].

Table 3. Specific growth rate of *B. splendens* fed with enriched cyclopoid copepod.

|       | T 1    | T 2    | T 3    | T 4    | T 5    |
|-------|--------|--------|--------|--------|--------|
| Rate  | 2.56±0.07<sup>a</sup> | 2.16±0.27<sup>a,b</sup> | 2.49±0.51<sup>a,b</sup> | 1.71±0.17<sup>b</sup> | 2.44±0.27<sup>a,b</sup> |

All values are mean ± standard deviation (*n* = 3). Different letters on the same row indicate significant difference (*P* < 0.05).

Figure 1. Survival rate (%) of *B. splendens* fed with enriched cyclopoid copepod.

3.3. Feeding rate

Many studies have focused on the fact that small live food is required for the development of fry production [33]. The optimal feeding rate is directly proportional to the frequency of feeding. Thus, it is critical to determine the number of meals that the specified amount of food will serve. When freshwater fish larvae are fed marine live feed, such as *Artemia nauplii*, optimal feeding frequency is critical to increase the potential for freshwater fish larvae to consume nauplii, enhancing the nutrient uptake and avoiding potential water quality issues [34][35]. According to Abe et.al [36, 44], angelfish fed with a high frequency of live feeds also had a higher feeding rate and were more successful in terms of growth. Thus, a sufficient feed frequency can lead to higher feed rate, which makes it easier for ornamental fish to be managed and marketed [34][37].
Figure 2. Feeding rate (%) of *B. splendens* fed with enriched cyclopoid copepod.

3.4. Coloration

Table 4 shows the L* values of *B. splendens* that indicated lightness. The varied enrichment copepod displayed significant differences on the L* values of the *B. splendens* body, *P*=0.001, *P*<0.05. *B. splendens* fed with copepod enriched in T3 (66.11±3.60) revealed a more whitish shade than those fed with copepod enriched in T4 (25.63±6.00). All treatments had positive values to signify the presence of red pigment on the *B. splendens* body. The a* value for all treatments had positive values to signify the presence of red pigment on the fish body. A significant variance (*P*=0.158, *P*>0.05) was noted as *Chlorella* sp. had the reddest shades (2.84±0.73) compared with other dietary treatments. The positive b* values of all *B. splendens* in the treatments indicate the presence of yellowish colour on the *B. splendens* body. Significant difference was recorded (*P*=0.015, *P*<0.05). *B. splendens* fed with copepod enriched in T3 resulted in the most yellowish colour (2.40±0.30), whereas the T5 gave the least yellowish shade (0.52±0.41).

Table 4. Coloration of *B. splendens* fed with enriched cyclopoid copepod.

|       | T1        | T2        | T3        | T4        | T5        |
|-------|-----------|-----------|-----------|-----------|-----------|
| L*value| 48.41±5.32a | 55.90±1.01a,b | 66.11±3.60b | 25.63±6.00c | 33.28±1.99c |
| a* value| 2.84±0.73a | 1.87±1.01a | 2.30±1.19a | 1.53±0.18a | 1.20±0.13a |
| b* value| 0.80±0.20a | 1.43±0.90ab | 2.40±0.30b | 0.86±0.69a | 0.52±0.41a |

All values are mean ± standard deviation (*n*= 3). Different letters on the same row indicate significant difference (*P* < 0.05).

The enrichment of cyclopoid copepods had a substantial effect on the colouring of *B. splendens* distribution, as measured by L* value. According to Pereira and Campos [11], carotenoid is the primary source of pigmentation in ornamental tropical fish that functioning to build the colour such as yellow, orange, red and other related colours. Based on the L* value result, the lowest value of lightness was at T4 and T5 enrichment of copepods with yeast and rice bran. The darker the colour, the lower the brightness reading. This shows that the fish's skin pigmentation was improved with a dark pigment called eumelanin produced from melanin, which has the ability to block ultraviolet (UV) rays. *B. splendens* fed with yeast and rice bran enriched copepods, showed the darkest reading value compared to others. Kim et.al [38] demonstrated that even low doses of rice bran improved the pigmentation of fish.
Carotenoids, melanin and astaxanthin are one of the main ingredients needed to produce pigmentation of skin colour in ornamental fish [39]. The enhancement of the red colour on the *B. splendens* body can be measured through the *a* value reading. T1 and T3 recorded the highest reading compared to the other treatments. The results revealed that the fish fed with mixed vegetable (carrot and spinach) that has been proved as an astaxanthin source by [40], have developed more of red colour. It is supported by Wagde et al. [41], the colour intensity and prominence of red-orange colour increased in the fish with diets enriched with natural sources of carotenoids such as carrot and spinach. From these observations, it can be inferred that in order to maintain appropriate colour in ornamental fish, suitable pigment enhancing agent should always be a part of fish diet on a prolonged basis. This proves that copepod enriched in T3 rise to high value of astaxanthin that helps in red colour enhancement. As for the *b* value reading, the value resulting different kind of colours which blue (-) value meanwhile yellow (+). *B. splendens* fed with copepods in T2 and T3 the highest value (+) that depicts that the skin colour pigmentation was more likely to be yellowish. Capsicum said to be one of the naturally available carotenoid rich ingredients [42]. Capsicum proves that the present of xanthophyll pigments in it helps in enhancing the yellow colour on the fish body. As for the negative value of *b* presented in the results of T5 *B. splendens* fed with copepod enriched with rice bran and T4 *B. splendens* fed with copepod enriched with yeast show it serve blue colour in the coloration.

4. Conclusion
Copepods enriched with rice bran produced a high result of protein, lipid and it is comparable with the results of copepods enriched with *Chlorella* sp. This is showed that, the use of agro-industrial residue such as rice bran also can be used to substitute microalgae culture or supplemented together with microalgae, to boost the production of live feed and fish larvae. Enrichment of mixed diet (carrot and spinach) has produced high results of specific growth rate, survival rate and better enhancement of coloration on *B. splendens*. The findings reported in this study may serve as guidelines for further investigate the development of an enrichment medium for copepods to improve the growth performance, survival and coloration of *B. splendens*, which important in Betta trade industry.

5. Acknowledgment
The Industrial Matching Grant funded the writing of this article issued under grant Vote No. 58904 by the Malaysian Ministry of Higher Education (MOHE) in order to generate new ideas and methodology for the development of live food nutrition in Malaysian aquaculture industry.

References

[1] Dong H, Senapin S, Phiwsaiya K, Techatanakitarnan C, Dokladda K, Ruenwongsa P and Panijpan B 2018 *Microbial pathogenesis* 122 46-52
[2] Mejia-Mejia M, Arce E, García-Rodríguez J and Burciaga L 2021 *International Aquatic Research* 13 71-9
[3] Lim L C, Dhert P and Sorgeloos P 2003 *Aquaculture* 227 319-31
[4] Thongprajukaew K, Kovitvadhi U, Kovitvadhi S, Somsueb P and Rungruangsk-Torrissen K 2011 *Aquaculture* 322 1-9
[5] Chua K, SH T, Liew J and Hasnita C 2017 *Journal of Tropical Resources and Sustainable Science* 5 51-4
[6] Uribe E A, Archundia M P F and Luna-Figueroa J 2018 *Agricultural Sciences* 9 171
[7] Rasdi N, Ramlee A, Abol-Munafi A, Ikhanuddin M, Azani N, Yuslan A, Suhaime H and Arshad A 2020 *Journal of Environmental Biology* 41 1257-63
[8] Naman N, Kassim Z and Rasdi N 2021. In: *Secondary*: IOP Publishing 674 p 012081
[9] Rasdi N W, Suhaime H, Yuslan A, Sung Y Y, Ikhanuddin M, Omar S S, Qin J G, Kassim Z and Yusoff F M 2018 *Aquaculture, Aquarium, Conservation & Legislation* 11 1658-71
[10] Rasdi N W and Qin J G 2016 *Aquaculture Research* 47 1-20
[11] Pereira da C D and Campos M-F K 2020 Reviews in Aquaculture 12 1567-78
[12] Sathyaruban S, Uluswaduge D J, Yohi S and Kugananathan S 2021 Aquaculture International 29 1-22
[13] Yigit N O, Ozdal A M, Bahadir Koca S and Ozmen O 2021 Aquaculture Research 00 1-6
[14] Hill G E and Johnson J D 2012 The American Naturalist 180 E127-E50
[15] Sefé K M, Brown A C and Clotfelter E D 2014 Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 173 42-51
[16] Luna-Figueroa J, Arce E, Figueroa J and Archundia M 2019 Int. J. of Aquatic Science 10 55-9
[17] Islam S N 2007 Research Journal of Fisheries and Hydrobiology 2 21-5
[18] Rasdi N W and Qin J G 2018 Aquaculture International 26 1281-95
[19] Rasdi N W, Qin J G and Li Y 2016 Aquaculture Research 47 3254-64
[20] Rasdi N W and Qin J G 2018 Aquaculture Research 49 3606-13
[21] Estévez A and Giménez G 2017 Aquaculture Nutrition 23 1264-73
[22] AOAC 2000 Official Methods of Analysis of the Association of Official Agricultural Chemist HeIritz K. (Ed.). 2 (USA: AOAC International)
[23] Pearson D 1999 Pearson’s Composition and Analysis of Foods (UK: University of Reading, Reading)
[24] Bligh E G and Dyer W J 1959 Canadian journal of biochemistry and physiology 37 911-7
[25] Yilmaz S and Ergün S 2011 The Israeli Journal of Aquaculture-Bamidgeh 63 633-8
[26] Müller-Navaur D C 2008 International Review of Hydrobiology 93 489-505
[27] van der Meeren T, Olsen R E, Hamre K and Fyhn H J 2008 Aquaculture 274 375-97
[28] Rasdi N, Ikhwanuddin M, Syafika C, Azani N and Ramli A 2021 Iranian Journal of Fisheries Sciences 20 986-1003
[29] Lemos M V, Arantes T Q, Souto C N, Martins G P, Araujo J G and Guimarães I G 2014 Ciência e Agrotecnologia 38 76-84
[30] Wang Y, Hu M, Cao L, Yang Y and Wang W 2008 Aquaculture International 16 361-8
[31] Jusoh M, Kasan N A, Hashim F S, Haris N, Zakaria M F, Mohamed N N, Rasdi N W, Abd Wahid M E, Katayama T and Takahashi K 2020 International Aquatic Research 12 74-83
[32] Yuslan A, Najuwa S, Hagiwara A, Ghaffar M A, Suhaimi H and Rasdi N W 2021 Diversity 13 105
[33] Mona M H, Rizk E-S T, El-feky M and Elawany M E 2019 The Egyptian Journal of Experimental Biology 15 135-42
[34] Campelo D A V, Marques M H C, Marim O P, de Moura L B, Eiras B J, Fernandes e C, Brabo M F and Veras G C 2019 International Journal of Fisheries and Aquaculture 11 23-8
[35] Samat N A, Yusoff F M, Rasdi N W and Karim M 2020 Animals 10 2457
[36] Abe H A, Dias J A R, Reis R G A, Sousa N d C, Ramos F M and Fujimoto R Y 2016 Embrapa Tabuleiros Costeiros-Artigo em periódico indexado (ALICE)
[37] Gonçalves Júnior L, Pereira S, Matielo M and Mendonça P 2013 Arquivo Brasileiro de Medicina Veterinária e Zootecnia 65 1176-82
[38] Kim Y-M, Lee E-C, Lim H-M and Seo Y-K 2019 International journal of molecular sciences 20 2172
[39] Kaur R and Shah T K 2017 International Journal of Fisheries and Aquatic Studies 5 684-6
[40] Maoka T, Kawase N, Ueda T and Nishida R 2020 Biochemical Systematics and Ecology 89 104001
[41] Wagde M S, Sharma S K and Sharma B K 2018 Journal of Entomology and Zoology Studies 6 2112-5
[42] Tamhane V A, Chougule N P, Giri A P, Dixit A R, Sainani M N and Gupta V S 2005 Biochimica et Biophysica Acta (BBA)-General Subjects 1722 156-67
[43] Rasdi N W, Qin J G, Naseer N M, Ikhwanuddin M, Yik Sung Y, Hagiwara 2021 Songklanakarin Journal of Science & Technology 43 3
[44] Azani N, Rasdi N W 2020 *UMT Journal of Undergraduate Research* **3** 2
[45] Rasdi N W, Arshad A, Ikhwanuddin M, Hagiwara A, Yusoff F M, Azani N 2020 *Journal of Environmental Biology* **41** 1239-1248