Study of patterns in the relationship of ecdysis with the age of freshwater crayfish *Cherax quadricarinatus* aged 76 Days

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Abstract. The purpose of this research study was to examine the growth pattern of ecdysis freshwater crayfish, especially crayfish type *Cherax quadricarinatus* aged 76 days to 116 days. The descriptive method was used. The data was collected through a direct observation. The data that was obtained consisted of an analysis done using simple linear regression. Because the weight of the crayfish was divergent, the data material was divided into 3 groups as a way to obtain data that was more accurate. For group 1, we obtained a linear regression with an equation of $Y = 6,00 + 2,600X$. For group 2, we obtained a linear regression with an equation of $Y = 8,500 + 2,300X$. Group 3 obtained a linear regression with equation of $Y = 9,100 + 2,500X$. The result explained a positive correlation between ecdysis with number of days. During the experiment, the water quality was still in the range of optimum, the DO ranged from 6,5 – 7 mg/l, the temperature was between 24° C – 27° C, the pH was about 7,5 – 8 and the ammonia was still at the range of normal <0,5.

1. Introduction

Crustacean growth has a relationship that parallels with skin changing. In crustaceans, shape and size changing can only occur if the hard calcareous exoskeleton is removed before the new cuticle is in the process of hardened. Periodically changing skin, or ecdysis, continuously happens by separating the old cuticle from the new instar. Additionally, absorbing water through the digestive tract are the common characteristics of the Crustacean class, as well as other aquatic insects. The water is then absorbed gradually, replaced by a protein produced by biosynthesis. This growth process occurs continuously, and the molting cycle can be divided into four stages: i.e proecdysis, ecdysis, metecdysis and intermoult [1].

Ecdysis is controlled by two types of hormones, namely the molting hormones (Ecdysone) and moult-inhibiting hormone (MIH). The negative regulation of ecdysteroidogenesis (by MIH) seems to have the strongest influence on the molting hormones, among several of the other factors that control molting. Ecdysone, which is synthesized by the Y-organs, is gained from the hemolymph and converted / circulated into 20-hydroxyecdysone (20-E). In *Cherax quadricarinatus*, it was found that 3-dehydroecdysone (3-dhE) is the main biosynthetic product of the Y-organ in-vitro physiologically, which is used as a molting hormone. MIH is released from the X-organs located on the eyestalk. The function of MIH is to inhibit the beginning of molting and to extend the molt-cycle [2].
In the inhibition strategy of the negative regulatory system of MIH, the small molecule that causes MIH activity is 3-hydroxy-L-kynurenine (3-OHK). In the X-organs, 3-OHK is enzymatically transaminated into xanthurenic acid (XA). The process of ecdysteroidogenesis in the Y-organs, which is mediated by cytochrome P450, is inhibited by XA [3]. The endocrine control of the carbohydrate metabolism of LAT (Figure 1) during the inter-molt period is dominated by the Pentose Monophosphate Shunt (PMS) cycle. At the beginning of proecdysis, the direction of the reaction changes to the glycolysis pathway. In the same way as the PMS cycle in the inter-molt phase, the proecdysis phase of MIH activity is pressed by the molting hormone, and then the activity of glucose-6-phosphate-dehydrogenase is directed to the glycolysis pathway [4].

At the stage of ecdysis, the death of LAT often occurs. Death occurs mainly due to cannibalism during the ecdysis process. The age of each individual is diverse, therefore the timing of ecdysis does not occur simultaneously. Thus, this condition provides an opportunity for birth that does not involve ecdysis or cannibalism, to be analyzed, thus impacting the reduced survival rate (SR). In order to be simultaneous, it must be induced by the MH (Moulting hormone) hormone externally either through feed or immersion. This MH is given during proecdysis. To find out if the proecdysis happens early, the pattern of ecdysis must be known first. The main problem is how and when the pro-analysis period occurs. The study of the ecdysis pattern of the Freshwater Lobster Cherax quadricarinatus is necessary as a starting point for the study of hormonal manipulation in subsequent studies.

2. Material and methods
2.1 Research preparation
The preparation of the research tools and materials was as followed. The bottles to be used as cages were prepared. The aquarium was cleaned and sterilized and the researcher set up the tools and places required. The supporting tools were prepared (the tools which were used to measure parameters). The test lobster was prepared and the lobster used in the test was 76 days old. In the lobster test, they were transported by being put into a plastic bag with oxygen. The location of the lobster test was sampled, taking up to as many as 20 tails that were adapted for about 2 hours before being stocked in each bottle in the aquarium.

2.2 Research implementation
The lobster was weighed first. The tested lobsters were stocked in bottles that were prepared in the aquarium. The tested lobster was fed commercial pellets. The feed was given once a day, every afternoon at 5:00 p.m. The observations were carried out every day to determine the analysis phase and the weight gain in the lobster. The quality of the water measurements included temperature, pH, DO, and ammonia, carried out once a week. The aquariums were dissipated every day and the water was changed every two days.

2.3 Main variables
The main variable was the period of the molting cycle, especially the ecdysis phase. This occurred in the Freshwater Lobster, Cherax quadricarinatus, aged 76 to 116 days old.

2.4 Supporting variables
The supporting variable in this study was the quality of the water in the media that the freshwater lobster seeds were placed into, including DO (dissolved oxygen), temperature, pH, and ammonia. The quality of the water as a supporting variable was measured every two days, while the ammonia was measured every week.

2.5 Data analysis
The data obtained from the research was analyzed using simple linear regression, which was obtained from the classical assumption test including the normality and autocorrelation tests.

3. Results and discussion

This study used 20 *Ceraxquadricarinatus* lobsters, as many as 20 of 76 days old with a diverse weight between 1.67 gr - 4.49 gr. Because the weight was random, the data was divided into 3 groups in order to conduct the data analysis more accurately.

Based on the previous research, there was a division of the sample into three groups based on the initial weight at the start of the study (age 30 days), which was between 0.35 gr - 1.32 gr. For more detail, the division of the groups can be seen in Table 1.

| Table 1. Group division based on weight |
|----------------------------------------|
| Group | Initial Wight       |
|-------|---------------------|
| 1.    | 0,1 gr – 0,60 gr    |
| 2.    | 0,61 gr – 1,00 gr   |
| 3.    | 1,01 gr – 1,50 gr   |

3.1. Classic assumption test

To find out if the regression model met the basic assumptions of the observational data, it is necessary to test the classic assumption in order to obtain the best unbiased model or BLUE (Best Linear Un-based Estimated). This test included the normality test and autocorrelation test. From this test, the assumption is expected to get a good regression model that will allow for more precise decision making or a conclusion bias, as well as describing the actual conditions.

Normality Test. The use of the regression model must qualify that the data is normally distributed. The qualified requirements of the normality conditions will ensure that the analysis model used can be accounted for, so then the conclusions taken can also be accounted. The normality test of the data from group 1 using the normality graph presented in Figure 5a shows that the data was spread around the diagonal line and that the distribution follows the direction of the diagonal line; the data is therefore normally distributed.

Autocorrelation Test. To find out whether there are symptoms of autocorrelation in the regression analysis model used, we used the serial correlation model test with the Durbin-Watson (DW) method. Conventionally, it can be said that a regression equation is said to have qualified the assumption of no autocorrelation if the value of the Durbin Watson number is between -2 to +2 [6]. The calculation result for group 1 was 0.735, for group 2 was 0.754, and for group 3 was 1.950, which means that there was no autocorrelation in the regression model. Therefore, from the normality and autocorrelation test, it can be concluded that the data analysis that qualifies to meet the requirements of Simple Linear Regression Analysis.

3.2 Simple linear regression analysis

Group 1

From the observation of the pattern of *ecdysis*’s relationship with age during the study, it was found that each time the LAT reached the stage of *ecdysis* and molting was experienced, it was followed by the increasing weight of the LAT. The number of days needed by the LAT to get longer in order to reach the next stage of *ecdysis* also increased. For group 1, the following data was obtained:
Table 2. Ecdysis correlation with the average number of days in group 1

| Ecdysis (X) | Average number of days (Y) |
|-------------|---------------------------|
| 1           | 9                         |
| 2           | 11                        |
| 3           | 13                        |
| 4           | 17                        |
| 5           | 19                        |

To determine the relationship pattern of *ecdysis* by age, linear regression analysis was performed. The results of the analysis can be seen as follows: by entering the values obtained from the calculation results, a simple linear regression equation was obtained as follows:

\[ Y = 6 + 2.6X \]

Based on the simple linear regression equation, it can be seen that a constant of 6.00 means that the number of days needed by the LAT to reach the stage of *ecdysis* before the study began ± 6 days. The regression coefficient of 2.6 means that the analysis had a positive and direct effect on the number of days, which means that each LAT reached the next stage of *ecdysis*, causing an increase in the number of days taken by 1 - 2 days.

Table 3. Linear regression analysis group 1

| Variabel       | Koefisien Regresi | T<sub>arithmetic</sub> | Sig. |
|----------------|--------------------|------------------------|------|
| Ecdysis        | 2.6                | 13                     | 0.001|
| Konstanta      | 6                  |                        |      |
| R square       | 0.991              |                        |      |

Dependent variable: Number of days

From the results of the linear regression analysis shown above, it can be seen that the coefficient of determination (R square) was 0.983. This figure shows that the *ecdysis* variable can explain variations or that it is able to influence 98.3% of the dependent variable (number of days).

Group 2
For group 2, the following data obtained is as follows:

Table 4. *Ecdysis* correlation with the average number of days in group 2.

| Ecdysis (X) | Average number of days (Y) |
|-------------|---------------------------|
| 1           | 11                        |
| 2           | 13                        |
| 3           | 15                        |
| 4           | 18                        |
| 5           | 20                        |

To determine the relationship pattern of *ecdysis* by age, linear regression analysis was performed. The results of the analysis can be determined by entering the values obtained from the calculation results, a simple linear regression equation was created as follows:
Y = 8.500 + 2.300X

Based on the simple linear regression equation, it can be seen that a constant of 8,500 means that the number of days needed by the LAT to reach the stage of *ecdysis* before the study began was ± 9 days. A regression coefficient of 2.300 means that *ecdysis* has a positive effect on the number of days, which means that it has a direct effect on the number of days. This means that each LAT reaches the next *ecdysis* stage, causing an increase in the number of days taken for 2 - 3 days.

**Table 5. Linear regression analysis group 2**

| Variabel    | Koefisien Regresi | Tarithmatic | Sig. |
|-------------|--------------------|-------------|------|
| Ecdysis     | 2,300              | 2,300       | 0,000|
| Konstanta   | 8                  |             |      |
| R square    |                     |             |      |
| Dependent variable: Number of days |

From the results of the linear regression analysis above, it can be seen that the coefficient of determination (R square) was 0.994. This number indicates that the *ecdysis* variable can explain the variations or that it is able to influence 99.4% of the dependent variable (number of days).

Group 3
To determine the relationship pattern of *ecdysis* by age, linear regression analysis was performed. The results of the analysis can be seen in the table below.

**Table 6. Ecdysis correlation with the average number of days in group 3.**

| Ecdysis (X) | Average number of days (Y) |
|-------------|---------------------------|
| 1           | 12                        |
| 2           | 14                        |
| 3           | 16                        |
| 4           | 19                        |
| 5           | 23                        |

By entering the values obtained from the calculation results, a simple linear regression equation was created as follows:

Y = 9,100 + 2,500X

Based on the simple linear regression equation, it is known that the constant of 9.100 means that the number of days needed for the LAT to reach the stage of *ecdysis* before the study began was ± 9 days. The regression coefficient of 2.500 means that *ecdysis* has a positive and direct influence on the number of days, which means that when each LAT reaches the next stage of *ecdysis*, it causes an increase in the number of days by 3 - 4 days.

From the results of the linear regression analysis above, it can be seen that the coefficient of determination (R square) was 0.989. This figure shows that the *ecdysis* variable can explain variation or that it is able to influence 98.9% of the dependent variable (number of days).

3.3. *Ecdysis* pattern
Ecdysis has a positive effect on the number of days, which means that it has a direct effect on the number of days as well. This means that an increase of 1 unit of ecdysis will cause an increase in the number of days and conversely, a decrease in 1 unit of ecdysis will cause a decrease in the number of days. This increase can be seen in the following picture.

**Figure 1.** Ecdysis pattern

From the graph, it can be seen that there is an increase in the number of days when the LAT reaches ecdysis. This can mean that when the LAT reaches the next ecdysis, the number of days will be longer compared to the number of previous ecdysis days.

The ecdysis process is a growth stage for LAT; a new exoskeleton will appear. In the beginning, the skin is still very soft and will begin to harden, along the increase in the body size of the LAT. When forming a new skin, the LAT requires calcium carbonate so then the chitin can be fully formed. When molting, the LAT experiences severe stress, their appetite drops dramatically and its energy is being depleted [7].

4. Conclusion
From the results of the study, we were able to determine the pattern of LAT analysis, especially for Freshwater Lobster Cheraxquadricarinatus in aged from 76 days up to 116 days old. It can be concluded that there is a positive (+) correlation between Ecdysis and the number of days. In group 1, the linear regression equation was \( Y = 6.00 + 2.600X \); for group 2 it was \( Y = 8.500 + 2.300X \) and for group 3, it was \( Y = 9.100 + 2.500X \).

5. References
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