Predation ability *Toxorhynchites splendens* larvae from Banjarbaru

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Abstract. The population of *Toxorhynchites splendens* in the rural area of the administrative city of Banjarbaru is quite abundant. This can be developed as part of the control management of *Aedes* sp. and *Culex* sp. as disease vectors. The purpose of this study was to test the predation ability of *Tx. splendens* against larvae of *Ae. aegypti* and *Cx. quinquefasciatus* and larval development time of *Tx. splendens* fed with larvae of *Ae. aegypti* and *Cx. quinquefasciatus*. The study used a completely randomized design with 10 replications. The results obtained were the predation ability of *Tx. splendens* larvae from instar 2 to pupae against larvae of *Ae. aegypti* and *Cx. quinquefasciatus* were 5.5 larvae/day and 6 larvae/day, respectively. The average length of the developmental phase of *Tx. splendens* larvae fed with *Ae. aegypti* and *Cx. quinquefasciatus* were 5.5 larvae/day and 6 larvae/day, respectively. The conclusion of this research is *Tx. splendens* originating from the city of Banjarbaru has the power of predation against *Ae. aegypti* and *Cx. quinquefasciatus*.

Keywords: disease vector, mosquito repellent, the controlling agent.

1. Introduction

Housing in densely populated areas is generally very tight. The irregular arrangement of houses inadvertently creates spaces that can become mosquito habitats, both as breeding grounds and as shelters [1]. This supports a relatively stable mosquito population [2]. To overcome mosquito nuisance in the form of mosquito bites, residents use mosquito repellent to repel mosquitoes. The use of mosquito repellents with active ingredients d-allethrin, transfthrin, d-allethrin, and metoflthrin with various forms of application is very familiar in the community. The mosquito repellent formulation can be in the form of spray, coil, electricity, and lotion. The use of these mosquito repellents by the community is considered effective and practical to repel mosquitoes [3-5].

The use of mosquito repellent in the residential environment is not following the standards for using these products. The use of mosquito repellent in the house can be throughout the day. This will add pollutant substances in the home environment especially when used when the room is closed [6]. The consequences of using mosquito repellent in a closed space have been described by many researchers. Exposure to mosquito repellent affects the microscopic appearance of the liver, kidneys and has a teratogenetic effect on mouse fetuses [7]. Lung and liver damage [8]. The finding that the active ingredient of mosquito repellent was also found in stagnant water bodies, although the amount was
small. However, if it accumulates in mosquito larvae, it can make mosquitoes more resistant to these active substances [9].

The use of mosquito repellent for a long time harms human health and the environment [10]. Therefore, there needs to be a serious effort to reduce the use of these mosquito repellents by changing ways of controlling that are safe for both humans and the environment. Biological agents, both parasites, pathogens, and predators are naturally abundant in the environment where mosquitoes are present. The ignorance of the community towards the controlling agent causes community activities to not support conservation [11].

Mosquito predators based on their life phase are divided into two, namely immature phases predators such as fish, odonata larvae, mosquito larvae, and carnivorous plants [12-16] and mature phases such as spiders, lizards [17-19]. These natural enemies generally have a less negative effect on the environment so they can be used as mosquito population control.

The largest mosquito species is Toxorhynchites which in the larval phase is a predator of other mosquito larvae [20]. The distribution of this mosquito is by the distribution of the Aedes mosquito and in Indonesia, there are 12 species [21]. In rural areas and suburbs this mosquito can be found together with Ae albopictus and Ae. aegypti mosquito larvae [22-26]. Therefore these mosquitoes can adapt to residential environments.

One of the many species around rubber plantations is Tx. splendens. This mosquito lives with other mosquito species such as Ae albopictus, Ae butleri, Cx. phangngae, Tripteroides sp. and Amigeres sp. [27] Efforts to make Tx. splendens as part of the integrated management of Aedes control need to be tested for predatory power of Aedes larvae and other mosquitoes, the length of life of the premature and mature phases.

2. Materials and Methods

2.1 Materials, Locations Sample, and Rearing Mosquito
This research was initiated by taking Tx. splendens mosquito larvae from a rubber plantation in Sungai Ulin village (-3.46446,114.906883), Banjarbaru, South Kalimantan. The larvae of Tx. splendens in the sap bowl were taken with a pipette and put into a bottle and brought to the basic laboratory of FMIPA Unlam to be identified and reared as mosquitoes. The larvae were fed with mosquito larvae obtained from the sampling location. The pupae obtained were transferred to a cage measuring 1mx1mx1m and kept until they became mosquitoes. Mosquitoes were identified as male and female using the Rattanarithikul [28] method (the ratio between male and female mosquitoes in the cage was attempted to be 4:1). Mosquitoes will lay eggs after 7 days of being kept in a cage. The eggs obtained are hatched into larvae. These larvae will be used as test samples. The test was carried out in a room with a temperature of 28°C with long irradiation (12:12) with a light intensity of 100 lux.

2.2 Method of Testing
The method of testing is divided into 2, namely (1) the length of life of the immature phase of Tx. splendens and (2) the predation power of Tx. splendens larvae against Ae. aegypti and Cx. quinquefasciatus larvae. Tx. splendens immature phase life length test. as follows: A plastic glass with a volume of 100 ml is washed with well water, dried and as much as 50 ml of well water is put into a plastic cup. Tx. splendens larvae with a length of ± 3mm were put into the plastic cup and fed with 10 Ae. aegypti larvae instar 3 as many as 10 tails. Every day the number of larvae remaining was observed and replaced with new mosquito larvae and the developmental phase (instar). The number of Tx. splendens larvae used in this test was 20 larvae with a completely randomized design.

The method of preparation for testing the predation power of Tx. splendens larvae against Ae. aegypti and Cx. quinquefasciatus larvae are the same as testing the length of life of the immature phase of Tx. splendens as follows: A plastic glass with a volume of 100 ml is washed with well water, dried and 50 ml of well water is inserted into the glass. Plastic. Tx. splendens larvae with a length of ±3mm were included and fed with 5 larvae of Ae. aegypti and Cx. quinquefasciatus (according to the experiment). Feeding in the morning at 07.00-07.30 and in the afternoon at 18.00-18.30. The larvae
before being fed were observed for the number of remaining feed larvae and the stage of instar development. The number of larvae as feed depends on the number of remaining larvae previously, if the larvae of feed run out then the number of feed larvae given is increased by 1. Observations are made every day until they become pupae. This study used a completely randomized design with 10 replications.

2.3 Statistical Analysis
The data was obtained from the testing of the immature phase of the Tx. splendens phase was searched for the average value and analyzed by LSD test analysis. While the test data for larval predation Tx. splendens against larvae of Ae. aegypti and Cx. quinquefasciatus, the average value of the number of prey eaten during the day and night was sought and continued with the t-test, which was previously carried out by the lilieforms normality test.

3. Results and Discussion
Immature mosquitoes consist of larva and pupae, the larvae undergo 4 changes of skin/instar to become pupae. The average length of the larval phase is generally 6-9 days. However, it is different from the larvae of Tx. splendens which has a body size of 2-3 times that of the Aedes or Culex mosquito larvae. From the observations of Tx. splendens larvae fed Ae. aegypti mosquito larvae to become mosquitoes it takes 15 - 21 days with an average of 17.9 days. The development time for each instar is relatively the same. The fastest developmental average is 1st instar, followed by instar 2nd, 3rd, pupae, and instar 4th. The fastest development period for instar 1 takes 2 days, while the other immature development period takes 3 days the fastest. The longest development time range is instar 4 and pupa which is 3-5 days (Fig.1). The LSD test (0.05) showed no difference in developmental time between the developmental phases of immature Tx. splendens.

![Figure 1. duration of development of immature Tx. splendens fed Ae. aegypti larvae.](image)

Testing the predation ability of Tx. splendens larvae against 2 prey species, namely Ae. aegypti and Cx. quinquefasciatus originating from Sungai Besar Village (-3.450460,114.852038) in the city of Banjarbaru (Table 1 and 2). 2nd instar Tx. splendens larvae fed Ae. aegypti larvae to pupa took an average of 14.55 days, the number of larvae eaten was 158.9 larvae or 10.6 larvae/day. Meanwhile, the second instar Tx. splendens larvae fed with Cx. quinquefasciatus larvae required an average of 12 days to pupae with the number of larvae being eaten as many as 146 or 12 larvae/day. The larvae of Tx. splendens prey during the day and night. The t-test (0.05) of the number of larvae preyed on by the larvae of Tx. splendens between the larvae of Ae. aegypti and Cx. quinquefasciatus during the day and night, the results were obtained successively as follows: F hit (1.0314) < Ftab (2.2622) and F hit (0.7646) < Ftab (2.2622). There was no difference in the predation ability of Tx. splendens larvae against Ae. aegypti and Cx. quinquefasciatus larvae during the day and night (Table 3). The results of this study showed that the predation ability of Tx. splendens larvae were not affected by environmental light intensity.
Table 1. Number of *Ae. aegypti* larvae preyed on by *Tx. splendens* instar 2 until it becomes a pupa.

| No. | Condition | Replication | Average |   |
|-----|-----------|-------------|---------|---|
| 1.  | light     | 3 2 2 2 2 2 3 3 3 2 | 2.4  |
|     | dark      | 4 3 3 3 3 3 3 4 4 4 | 3.4  |
| 2.  | light     | 3 3 3 3 4 4 4 3 3 3 | 3.3  |
|     | dark      | 5 4 4 4 3 3 3 3 3 3 | 3.5  |
| 3.  | light     | 5 4 4 4 3 4 4 4 4 4 | 4.4  |
|     | dark      | 5 4 4 4 5 4 5 3 3 3 | 4.3  |
| 4.  | light     | 4 4 4 5 3 5 3 5 7 6 | 4.6  |
|     | dark      | 5 5 5 6 5 4 5 5 5 5 | 4.9  |
| 5.  | light     | 5 4 5 3 6 6 5 5 5 5 | 4.9  |
|     | dark      | 6 6 5 6 6 5 4 4 4 4 | 5.5  |
| 6.  | light     | 6 6 5 5 6 6 5 7 4 4 | 5.4  |
|     | dark      | 5 5 6 5 4 6 6 6 5 5 | 5.3  |
| 7.  | light     | 5 5 5 5 6 6 5 5 5 5 | 5.3  |
|     | dark      | 6 5 5 5 6 5 6 5 5 5 | 5.3  |
| 8.  | light     | 4 6 5 6 5 4 6 4 5 5 | 5.5  |
|     | dark      | 6 6 5 5 5 5 5 6 4 5 | 5.3  |
| 9.  | light     | 7 6 6 4 4 6 5 4 6 5 | 5.3  |
|     | dark      | 4 5 4 6 6 4 4 6 5 7 | 5.1  |
| 10. | light     | 7 7 5 5 5 6 6 5 5 5 | 5.4  |
|     | dark      | 7 7 4 7 7 4 5 5 4 3 | 5.3  |
| 11. | light     | 4 4 7 4 3 6 5 5 6 8 | 5.2  |
|     | dark      | 7 7 7.6 6 8 6 9 8 7 6 | 7.1  |
| 12. | light     | 7 7 7 6 8 6 8 7 6 6 | 6.8  |
|     | dark      | 8 5 7 4 2 5 5 4 4 6 | 5.6  |
| 13. | light     | 5 6 5.7 11 8 7 7 9 7 7 | 7.2  |
|     | dark      | 5 4 6 7 5 5 6 7 6 10 | 6.1  |
| 14. | light     | 6 3 5.10 10 10 10 10 10 10 8.4 |
|     | dark      | 14 10 15 10 10 10 10 10 10 10.9 |
| 15. | light     | 0 0 3 0 6 10 10 10 10 5.9 |
|     | dark      | 0 0 0 0 0 10 0 10 9 3.9 |
| total|          | 158 143 151 147 155 168 161 169 168 158.9 |
| average |        | 11 10 10.1 11 10.3 11 10.7 11 11 11 10.6 |
| average (light) | 5.1 4.8 4.7 4.9 5.4 5.9 5.73 5.5 6.1 5.5 5.35 |
| average (dark)   | 5.4 5.7 5.6 5.3 5.33 5.4 5.8 5.2 5.7 53 5.56 |
| length (day)     | 14 14 14.5 14 14.5 15 14.5 15 15 15 14.55 |
### Table 2. Number of *Cx. quinquefasciatus* larvae preyed on by *Tx. splendens* instar 2 until it becomes a pupa

| No. | Condition | Replication | Average |
|-----|-----------|-------------|---------|
| 1.  | light     | 2 2 2 4 3 4 0 1 | 2       |
|     | dark      | 2 4 6 0 2 1 1 2 | 2.4     |
| 2.  | light     | 4 5 5 1 1 0 0 2 | 2.5     |
|     | dark      | 5 4 4 6 3 1 3 2 | 3.7     |
| 3.  | light     | 4 3 4 6 2 1 0 2 | 2.6     |
|     | dark      | 7 7 6 5 7 6 5 3 | 5.4     |
| 4.  | light     | 5 4 5 8 8 6 5 6 | 5.6     |
|     | dark      | 7 9 2 2 3 4 2 5 | 4.2     |
| 5.  | light     | 7 7 8 8 6 5 8 6 | 6.8     |
|     | dark      | 5 7 7 7 7 6 8 6 | 6.6     |
| 6.  | light     | 8 7 7 4 4 6 8 2 | 6.4     |
|     | dark      | 8 6 8 9 6 7 9 6 | 7.5     |
| 7.  | light     | 8 9 7 8 5 7 8 9 | 8       |
|     | dark      | 7 8 8 9 5 5 10 9 | 8       |
| 8.  | light     | 9 10 6 6 7 8 7 8 | 7.6     |
|     | dark      | 7 6 7 10 8 10 10 8 | 8.6     |
| 9.  | light     | 8 7 8 9 8 7 10 10 | 8.6     |
|     | dark      | 9 9 9 9 9 10 10 8 | 8       |
| 10. | light     | 8 8 8 9 7 8 10 7 | 8.3     |
|     | dark      | 5 9 9 9 7 8 6 7 | 7.6     |
| 11. | light     | 8 4 9 1 8 7 4 10 | 8.8     |
|     | dark      | 8 8 7 10 3 4 9 8 | 8.8     |
| 12. | light     | 7 0 5 0 0 3 0 6 | 3.6     |
|     | dark      | 2 0 6 0 9 0 0 3 | 2.8     |
| total|          | 151 146 156 144 141 137 135 155 145 150 150 146 | 146     |
| average|        | 15 12 13 11 13 13 13 13 13 13 13 12 | 12      |
| average (light)|    | 6.5 6.0 6.2 5.64 5.8 5.25 5.7 6.2 5.5 5.8 5.9 | 5.9     |
| average (dark) |    | 5.7 6.7 6.7 6.2 6.5 6.7 6 6.2 5.9 5.4 5.5 6 | 6       |
| length (day)   |    | 12 12.2 12 13.1 11 10.5 12 12 11 11 12 | 12      |

### Table 3. T test (0.05) of the predation ability of *Tx. splendens* on larvae (a) *Ae. aegypti* and (b) *Cx. quinquefasciatus* at light and dark condition.

| t-Test: Paired Two Sample for Means | a | b |
|------------------------------------|---|---|
| Means                              | light | dark | light | dark |
| Mean                               | 5.3519 | 10.2824 | 5.8515 | 5.9810 |
| Variance                           | 0.2123 | 229.5648 | 0.1333 | 0.1738 |
| Observations                       | 10 | 10 | 10 | 10 |
| Pearson Correlation                | 0.0892 | 0.0666 |
| df                                 | 9 | 9 |
| t Stat                             | -1.0314 | -0.7646 |
| P(T<=t) one-tail                    | 0.1646 | 0.2321 |
| t Critical one-tail                 | 1.8331 | 1.8331 |
| P(T<=t) two-tail                   | 0.3293 | 0.4641 |
| t Critical two-tail                | 2.2622 | 2.2622 |
All mosquito larvae of the genus *Toxorhynchites* have a very strong mouthpart designed to prey on other mosquito larvae [29]. According to Tyagi [21], there are 12 species of *Toxorhynchites* in Indonesia, however, the 12 species have not been explored much in terms of their biology, ecology, and predation capabilities.

One of the species that are still commonly found in rural areas in Kalimantan and which has only recently been explored for bioecological exploration is *Tx. splendens*. The habitat of this mosquito is in plantation areas where there are small water pools. In rubber plantations, these mosquitoes can use sap bowls filled with water as immature habitats with other mosquito larvae as prey [27].

The *Tx. splendens* mosquito is relatively large, therefore its life cycle takes a long time. This is very different from other mosquito life cycles. The duration of the larval phase of *Aedes* sp and *Culex* sp to become mosquitoes takes 10-14 days, this is also not much different from other mosquito larvae [30,31]. While the larvae of *Tx. splendens* need a long time of 15-21 days.

The duration of development of insects undergoing a metamorphosis in each instar is different [32]. The fastest duration of larval development of *Tx. splendens* is 1st instar. This short duration is common in other mosquito larvae such as larvae of *Cx. pipiens* and *Cx. Quinquefasciatus* [33,34]. First instar larvae prey on smaller prey in the water. The larger 2nd instar starts hunting for mosquito larvae that are the same size or larger than it. This hunt requires time and energy so that the 2nd instar is longer than 1st instar. 3rd instar with sufficient hunting ability so that it can prey on more prey. However, the duration of this phase becomes longer because of the larger body size compared to 2nd instar.

The duration of 4th instar is longer than the duration of the other instars. Several reasons cause the 4th instar duration too long, namely reduced appetite and preparing to change phases from larva to pupa. Pupae duration is also relatively long. This is very different from the pupa duration of other mosquitoes which generally have a shorter pupa duration than the instar duration.

The larval phase is the feeding phase. The availability of food in the waters will ensure that the larval phase becomes a pupa. As a predator, the larvae of *Tx. splendens* prey on other mosquito larvae [35,36]. Similarly, the larvae of *Tx. splendens* from Banjarbaru can prey on the larvae of *Ae. aegypti* and *Cx. quinquefasciatus*. *Tx. splendens* larvae tend to prey more on *Cx. quinquefasciatus* larvae compared to larvae of *Ae. aegypti* with an average of 12 individuals and 10.6 individuals. The results of this study were also similar to that of *Tx. splendens* larvae from India which preferred *Cx. Quinquefasciatus* [37]. Preference for prey generally occurs in predators [38,39].

Preference for prey will also affect the length of the larval development phase. *Tx. splendens* larvae prefer *Cx. quinquefasciatus* larvae than *Ae. aegypti* larvae. This affects the duration of the larval phase to pupa, where larvae that prey on *Cx. quinquefasciatus* are faster than those that prey on *Ae. aegypti* larvae.

4. Conclusion
The preying activity of *Tx. splendens* larvae based on light and dark conditions was no different. This shows that *Tx. splendens* larvae hunt and prey are not influenced by environmental conditions, especially light. *Tx. splendens* originating from the city of Banjarbaru has the power of predation against *Ae. aegypti* and *Cx. quinquefasciatus*

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