

Dynamics of *Toxorhynchites splendens* population in the Larval phase at a rubber plantation in Banjarbaru, South Kalimantan, Indonesia

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Abstract. Muhamat, Hadisusanto S, Umniyati SR, Soesilohadi RCH. 2021. Dynamics of *Toxorhynchites splendens* population in the Larval phase at a rubber plantation in Banjarbaru, South Kalimantan, Indonesia. *Biodiversitas* 22: 4915-4922. This study aims to describe a water-filled rubber sap bowl as a habitat for larval *Toxorhynchites splendens*. The research used a quota drive count method, taking the first 10 rubber sap bowls found in the study area: (i) with larval *Tx. splendens*, (ii) with other mosquito larvae, (iii) with water but without mosquito larvae, and (iv) without water. The number of larval *Tx. splendens* was calculated based on the developmental phase and other mosquito larvae present in each bowl. Environmental factors, such as temperature and humidity, rainfall, wind velocity, and duration of irradiation were the additional data. The results of this study showed that the average frequency of *Tx. splendens* on average, over 20% of bowls contained larval *Tx. splendens*, and the percentage increased in high rainfall. During low rainfall in August and September, *Tx. splendens* used water-filled rubber sap bowls as breeding places. This study concludes that *Tx. splendens* can make use of water-filled rubber sap bowls as places for breeding. Each rubber sap bowl contained one or more individuals of instar larvae 2, and the number decreased as the developmental phase continued because of the limited volume of water in the bowl, cannibalism, and the number of other mosquito larvae as prey.

Keywords: Cannibalism, larval *Tx. splendens*, rubber sap bowl, predator, rainfall

INTRODUCTION

In general, the populations of *Anopheles*, *Aedes* and *Culex* mosquitoes in rural areas can still be reduced by various controlling organisms. This is a sign that the population balance is still maintained between mosquitoes and controlling organisms. These controlling organisms can be predators, parasites and entomopathogens (Saleeza et al. 2014; Benelli et al. 2016; Udayanga et al. 2019). Several insect orders are predators of mosquito larvae, namely Coleoptera, Odonata, and Diptera (Dida et al. 2015; Udayanga et al. 2019; Couret et al. 2020; Eba et al. 2021).

Diptera orders as predators mosquito larvae include species *Toxorhynchites* spp. and *Lutzia* spp. (Moirangthem et al. 2018; Dinithi and Hemantha 2020). Both mosquitoes can be considered as part of integrated vector control. However, to fulfill their energy requirements, *Toxorhynchites* spp. mosquitoes only suck plant fluids, while female *Lutzia* spp. mosquitoes still suck blood (Pramanik et al. 2016). This is a consideration for *Lutzia* spp to be developed as part of integrated mosquito control.

Elephant mosquitoes or *Toxorhynchites* are widely found in tropical and subtropical regions. Twenty-four species of *Toxorhynchites* mosquitoes live in Asia, including 12 species found in Indonesia (Tyagi et al. 2015). These large mosquitos are characterized by a body length

of ± 20 mm, while instar larvae can have a body length of ± 12 mm (Millado et al. 2017).

Toxorhynchites mosquito larvae are predators of other mosquito larvae (Aditya et al. 2007). During the developmental phase, *Toxorhynchites* larvae can hunt up to 300 other mosquito larvae (Collins and Blackwell 2000). *Toxorhynchites* larvae can naturally be found in tree holes flooded with water (Donald 2020). These mosquitoes can also be found in man-made water containers, such as tires, bottles, and drums (Zavortink and Poinar 2008; Mohamad and Zuharah 2014).

Rubber plants are common plantation crops in tropical regions, including Indonesia, data reported that the area of rubber plantations in Indonesia reached 2.8 million hectares in 2018 (Amalia et al. 2020). The expansion of rubber plantations provides new habitats for various species of mosquitoes. Several species of mosquitoes that can adapt to rubber plantations, such as *Aedes albopictus* and *Anopheles* sp., become vectors of diseases for humans (Jomon and Valamparampil 2014; Kaewwaen and Bhumiratana 2015).

The sap collection bowl is an important harvesting tool for rubber farmers (Nasruddin and Bahri 2019). The frequently used latex sap bowls are filled with a rubber crust, making them no longer effective to store new latex sap; and therefore, they are replaced with other bowls. However, the old bowls are usually placed around the

rubber plantation area; and thus, in the rainy season, those bowls are filled with rainwater, becoming new breeding places for mosquitoes. An increase in mosquito breeding places is followed by a rise in the population of various mosquito species (Kaewwaen and Bhumiratan 2015; Lestari et al. 2020; Rahma et al. 2020). Otherwise, in the dry season, water in the latex sap bowls evaporates and dries up, reducing many breeding places for various species of mosquitoes, which later causes their population to decline.

Toxorhynchites larvae found in latex sap bowls from the rubber plantation in Banjarbaru are *Tx. splendens* (Muhamat et al. 2020). *Tx. splendens* population in rubber plantation areas are closely related to the number of water-filled latex sap bowls and other mosquito populations. Therefore, this study discusses the fluctuation in the *Tx. splendens* population at the larval phase and other species of mosquito larvae in latex sap bowls in the rubber plantation in Banjarbaru, South Kalimantan.

MATERIALS AND METHODS

This research was conducted in 10 locations of rubber plantations in the villages of Gunung Kupang (GK), Sungai Ulin (SU), and Padang Panjang (PP) in the area around Banjarbaru, South Kalimantan Province, Indonesia. The environmental profile of each location is presented in Table 1 and Figures 1 and 2. This study was conducted from April to October 2020. The research method used was a purposive random sampling method, namely rubber plantations that had not been tapped for a long time. Observations were made on the rubber sap bowl attached to the rubber tree and on the garden floor in the rubber plantation. The sap bowls that were sampled were grouped based on the presence or absence of mosquito larvae and the water content in the bowl into 5 groups, namely: (i) a rubber sap bowl containing larval *Tx. splendens* only; (ii) a rubber sap bowl containing larval *Tx. splendens* and other mosquito larvae; (iii) a rubber bowl containing larval *Tx. splendens* and other mosquito larvae; (iv) a rubber sap bowl containing only water; and (v) a rubber sap bowl that is not filled with water. The method of determining the number of rubber sap bowls as a sample is by using the quota drive account method, that is, from each location, the 100 bowls that are the fastest are found. The bowls containing water were observed and if any larvae were

found, they were taken with a pipette and/or filtered using a 30 mesh filter. The filtered larvae were put into bottles and then brought to the Basic Laboratory of Lambung Mangkurat University, Indonesia for species identification with mosquito identification keys according to Rattanarithikul et al. 2007; Farajollahi and Price 2013. Environmental variables in the form of temperature, humidity, rainfall, duration of irradiation, and wind speed came from the Banjarbaru BMKG class 1 station. Field observations were 2 times a month with a sampling distance of 14 days.

The number of rubber sap bowls observed was classified according to the above classification, the frequency of encounters was calculated and presented in the form of graphic images. Observations on mosquito larvae in the bowl were grouped into 2 groups, namely the *Tx* larval group. *splendens* and other mosquito larvae. Larval *Tx. splendens* were grouped into 4 stages of life, namely (i) to instar 2, (ii) instar 3, (iii) instar 4, and (iv) pupae and counted the number of individuals, while for larval from other mosquito species, only the number of individuals was counted. Number of larval *Tx. splendens* sap bowl as a place of growth and development of larval *Tx. splendens* is presented in the form of monthly frequency graphs. Frequency of encountering bowls containing larval *Tx. splendens* with other mosquito larvae and the frequency of bowl encounters where there are only other mosquito larvae are presented in the form of graphic images. Based on the larval life phase of *Tx. splendens*, larval encounter frequency *Tx. splendens*, either with other mosquito larvae or not with other mosquito larvae and the number of bowl larvae each month are presented in the form of graphic images.

Selection of rubber sap bowls as a place for laying eggs *Tx. splendens* is known from comparing the average number of other mosquitoes larval in the bowl containing larval *Tx. splendens* with other mosquito larvae with the number of other mosquito larvae in the bowl without larval *Tx. splendens*. Both data were subjected to Bartlett test to determine the homogeneity of the data and continued with the T-test. Data on environmental factors and the number of larval *Tx. splendens* obtained every month were tested for normality of Lilliefors followed by Pearson's correlation analysis. The results of this test are to determine the relationship between environmental factors and the monthly population of larval *Tx. splendens*.

Table 1. Sampling locations

| Location | Coordinate | | Environmental profile |
|----------|------------|-------------|--|
| | Latitude | Longitude | |
| GK1 | -3.4827524 | 114.8691732 | There are some houses in the area. The area is far from water sources, such as rivers, lakes, and pools. |
| GK2 | -3.4886562 | 114.8892642 | The area is close to chicken coops. |
| GK3 | -3.488188 | 114.883477 | The area is close to houses and rice fields. |
| GK4 | -3.490113 | 114.8767416 | The area is close to water sources, such as lakes. |
| GK5 | -3.490113 | 114.876741 | The area is far from houses but near water sources, such as rivers. |
| MA | -3.464463 | 114.906883 | The area is close to swampy rice fields. |
| PP1 | -3.464330 | 114.914174 | The area is near the chicken coops and water sources. |
| PP2 | -3.46607 | 114.9117217 | The area is near the water sources. |
| PP3 | -3.457512 | 114.913265 | The area is near the chicken coops, houses, and rice fields. |
| PP4 | -3.457512 | 114.913265 | The area is far from water sources. |



Figure 1. Location sample station



Figure 2. Condition and position of the bowl on the rubber tree

RESULTS AND DISCUSSION

Results

The presence of *Tx. splendens* in rubber plantations can be estimated from the frequency of meeting rubber sap bowls containing larval *Tx. splendens*. The results of observations on the frequency of obtaining rubber sap bowls with larval *Tx. splendens* in 10 research locations in Banjarbaru, from April to October 2020, are as the following. From April to July, 48%-83% of rubber sap bowls contained *Tx. splendens*. Between August and September, rubber sap bowls containing water were only found in one location, with 20% and 31%, while on October, 7-25% of rubber sap bowls contained *Tx. splendens* (Figure 3). Observations were also made on rubber sap bowls containing mosquito larvae without larval *Tx. splendens* with the percentage of meeting frequency of 20%-80% in April-September and 100% in October, with the presence of other mosquito larvae (Figure 4).

The weather in the observation site affects the volume of water in the rubber sap bowls. Rainfall in April to June was high, decreased in July-August, and then increased again in September-October. The duration of irradiation increased in August and then decreased in September and October, while the wind velocity increased from July to October. Temperature fluctuation during the study was not high, while the air humidity decreased from July to October (Figure 5). The combination of a decrease in air humidity ($r=0.95$) and rainfall ($r=0.44$), and an increase in wind velocity ($r=-0.81$) led to an increase in evaporation of water in the rubber sap bowls (Table 2), causing the water in the rubber sap bowls to decrease and even run out (Figure 6).

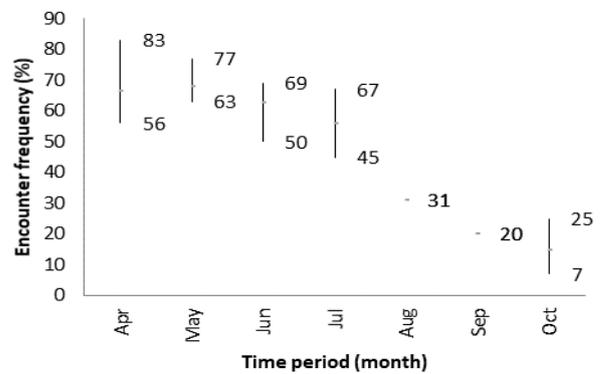


Figure 3. Percentage of rubber sap bowls containing larval *Tx. splendens*

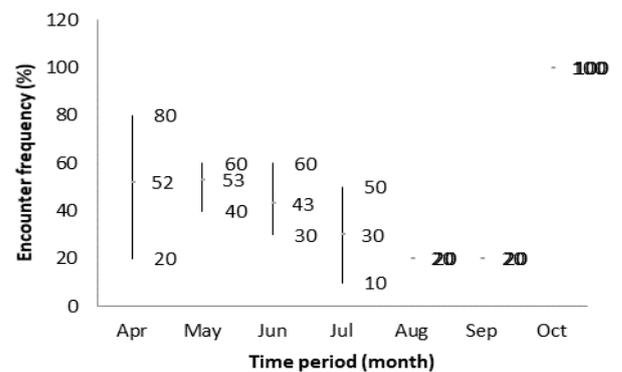


Figure 4. Percentage of rubber sap bowls containing mosquito larvae without larval *Tx. splendens*

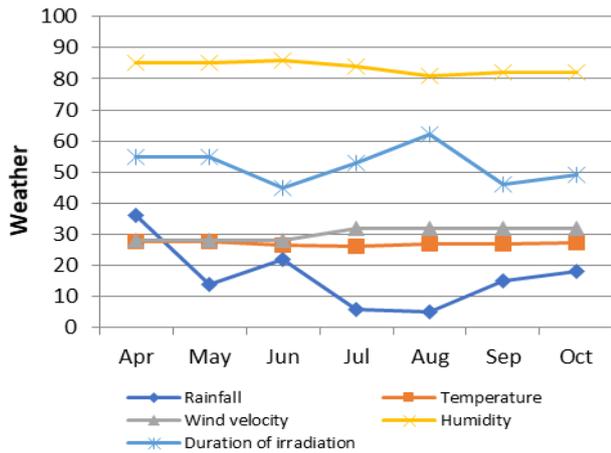


Figure 5. The weather around Banjarbaru (source: analyzed data from Banjarbaru BMKG station)

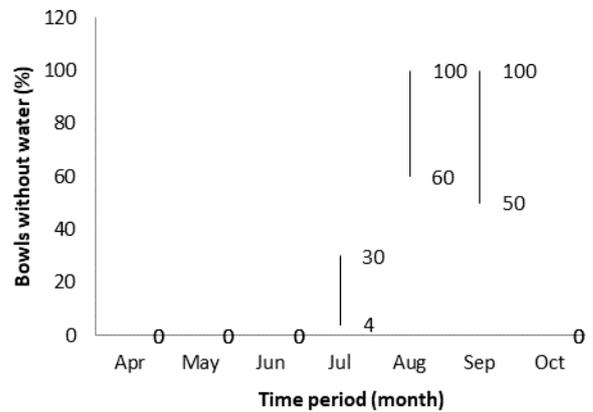


Figure 6. The percentage of meeting rubber sap bowls without water from April to October

Pearson’s correlation test on discovery frequency of rubber sap bowls containing larval *Tx. splendens* and those containing other mosquito larvae without larval *Tx. splendens* in April and May resulted in very strong and strong correlations, respectively. However, the correlation became weak and negative in June. The correlation was moderately negative both in July and October. The correlation in August and September could not be estimated because the rubber sap bowl contained water and larval *Tx. splendens* was only found in one location (Table 3).

The average number of other mosquito larvae in a rubber sap bowl containing larval *Tx. splendens* was between 14 and 50.5 (Figure 7). Meanwhile, a bowl without larval *Tx. splendens* contained 11.5 to 64 larvae (Figure 8). The T-test at the 5% level (p -value = 0.13) of the two conditions did not show any differences in the number of larvae.

The presence of other mosquito larvae in a rubber sap bowl ensures the food availability for *Tx. splendens* larvae that will develop into pupae. The results of observation on the discovery frequency of rubber sap bowl containing 2nd

instar larvae showed that the instar larvae remained with another mosquito larvae. Observation on rubber sap bowl containing 3rd instar larvae and other mosquito larvae conducted from April to July demonstrated a higher frequency of encounter, compared to the bowl containing only *Tx. splendens*. However, from August to July, more bowls contained only 3rd instar larvae. The number of bowls containing 3rd instar larvae and other mosquito larvae reached 100%.

The number of bowls containing *Tx. splendens* 4th instar larvae and pupa together with another mosquito larvae from April to September was fewer than the number of bowls containing only *Tx. splendens* (Figure 9).

The number of rubber sap bowls containing larval *Tx. splendens* with another mosquito larvae for 3rd instar larvae during the observation was more dominating than that of bowls with individuals in other phases of life. The rubber sap bowl containing 4th instar larvae and pupa was smaller in number (Figure 10.A). The number of the rubber sap bowl containing 4th instar larvae and pupa without other mosquito larvae was higher (Figure 10.B).

Table 2. The results of Pearson’s correlation test on the relationship between the weather and the number of rubber sap bowls containing *Tx. splendens* in Banjarbaru

| | | Air humidity | Rainfall | Air temp. | Wind velocity | Irradiation duration | Multiple correlations |
|--------------------------------------|---------------------------------------|--------------|----------|-----------|---------------|----------------------|-----------------------|
| The number of bowls containing water | Pearson’s Correlation (p-value <0.05) | 0.95 | 0.44 | 0.15 | -0.81 | -0.04 | 0.42 |

Table 3. The results of Pearson’s correlation test on the frequency of discovering the rubber sap bowls containing larval *Tx. splendens* and the rubber sap bowls containing other mosquito larvae

| Month | Pearson’s correlation (r) (p-value <0.05) | Month | Pearson’s correlation (r) | Description of r score | Interpretation |
|-------|---|-----------|---------------------------|------------------------|----------------|
| April | 0.85 | August | - | 0-1.99 | Very weak |
| May | 0.72 | September | - | 2.00-3.99 | Weak |
| June | -0.02 | October | -0.54 | 4.00-5.99 | Fair |
| July | -0.48 | | | 6.00-7.99 | Strong |
| | | | | 8.00-10.00 | Very strong |

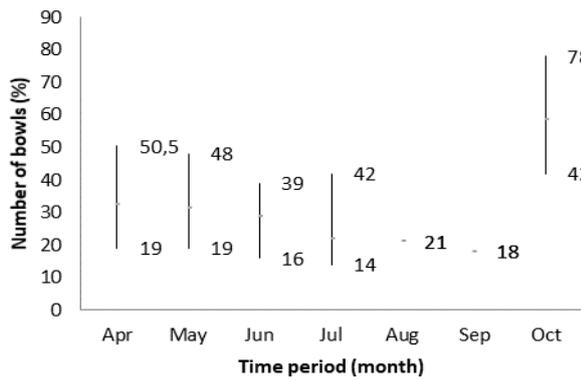


Figure 7. The average number of other mosquito larvae in rubber sap bowls containing larval *Tx. splendens*

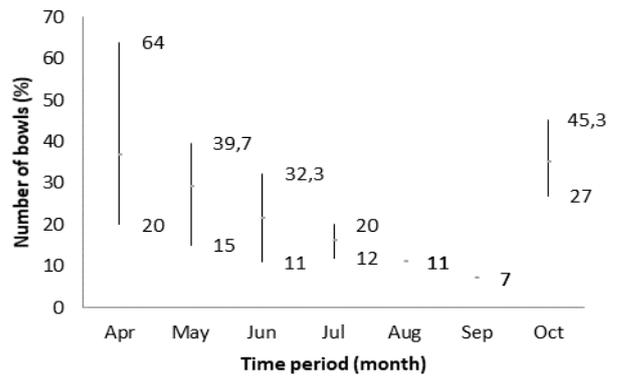


Figure 8. The average number of other mosquito larvae in rubber sap bowls without larval *Tx. splendens*

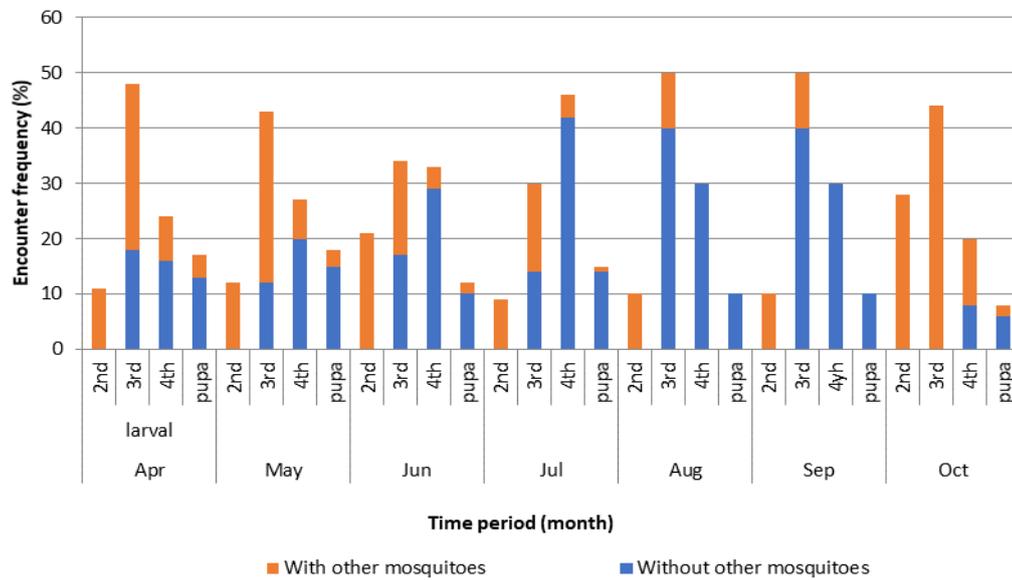


Figure 9. The comparison of the number of bowls containing *Tx. splendens* along with other mosquito larvae and the number of bowls containing only larval *Tx. splendens*

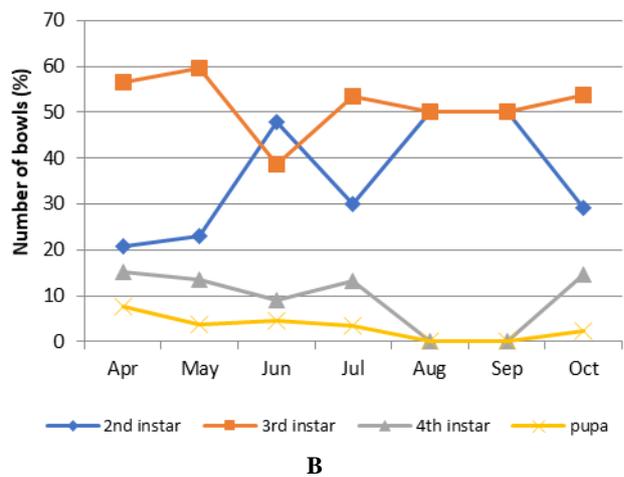
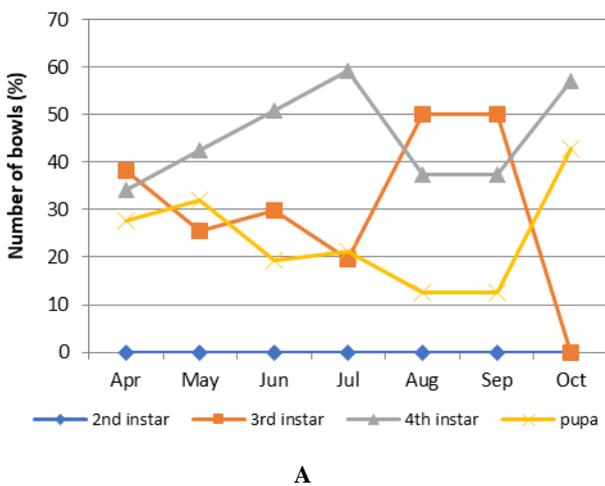


Figure 10. The number of (A) bowls containing *Tx. splendens* along with other mosquito larvae, and (B) bowls containing only larval *Tx. splendens*

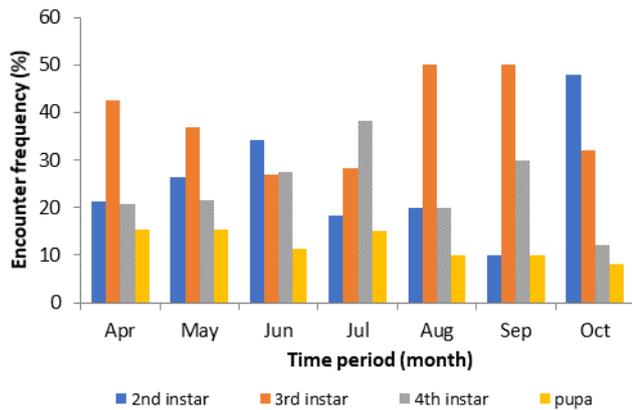


Figure 11. The percentage of the number of larval *Tx. splendens* in rubber sap bowl

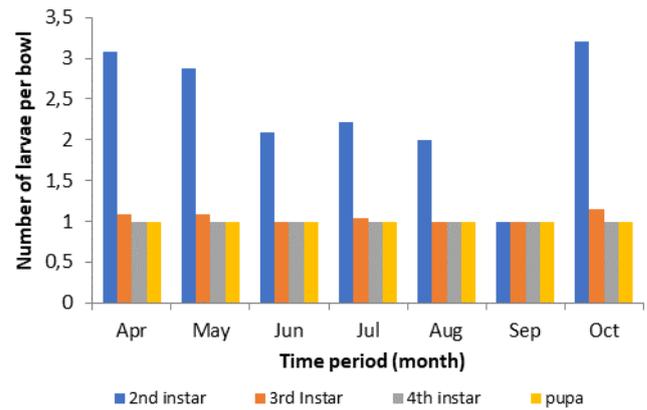


Figure 12. The average number of larval *Tx. splendens* in each rubber sap bowl

The ease of discovering *Tx. splendens* larvae and pupae in rubber plantations depicted the development phase of the larvae and pupae in various rubber sap bowls. The most frequently met bowls contained larval *Tx. splendens* 3rd instar larvae, followed by 2nd and 4th instar larvae (Figure 11). The highest average number of larvae or pupae in each bowl during the observation was 2nd instar larvae, followed by 3rd instar larvae, 4th instar larvae, and pupa, respectively (Figure 12).

Discussion

The presence of larval *Tx. splendens* as a predator of mosquito larvae in an environment is strongly influenced by external factors that work in the environment. The larval phase that lives in water is very sensitive to changes in the volume of water in the container. In this study, population of larval *Tx. splendens* was observed in the sap bowl of rubber plantations during the rainy and dry seasons.

The results of observations of the frequency of encounters different the rubber sap bowls containing the larval stages of *Tx. splendens* and other mosquito larvae can be seen in Figures 3 and 4. Encounters of rubber bowls containing the larval stages of *Tx. splendens* and other mosquito larvae in April to July and October more often than August and September. April to July and October are rainy with enough rainfall to keep the sap bowls filled with water. In this month all the sap bowls filled with water are there so that it will maintain the population of both *Tx. splendens* and other mosquitoes. The decrease in rainfall in August and September caused many bowls of rubber sap to dry up, resulting in fewer breeding places for mosquitoes (Figure 6). This causes a decrease in the encounter of the rubber sap bowl containing the larval stages of *Tx. splendens*. Population fluctuations were also experienced by *Aedes albopictus* and *Anopheles maculatus* as the main prey larvae of *Tx. splendens* (Tangena et al. 2017; Zahouli et al. 2017; Nair and Gayathri 2019;) The addition and reduction of water-filled containers in rubber plantations will result in increase and decrease in population of various mosquito species (Boonklong and Bhumiratana 2016).

In general, the dynamics of the mosquito population in an area is strongly influenced by the water condition. The water condition is significantly attributed to the water area, rainfall, evaporation, humidity, air temperature, and wind velocity (Majumder 2014). Air humidity, rainfall, and wind velocity are the environmental factors that play a role in the water condition in rubber plantations (Table 1).

Tx. splendens mosquitoes choose a water container for breeding based on the presence of prey in the container. The selection of the number of prey in the rubber sap bowl can be considered before choosing the bowl as a breeding site. The results of the *F*-test on the rubber sap bowl containing larval *Tx. splendens* along with other mosquito larvae and the rubber sap bowl containing only other mosquito larvae do not show any differences (Table 2). This indicates that all sap bowls have an equal opportunity as a breeding place for *Tx. splendens*. *Tx. splendens* in choosing a water container as a breeding place is based on the presence of prey first in the container. The selection of the number of prey in the bowl can be considered before choosing a rubber sap bowl as a breeding place (Klecka 2012; Odo et al. 2015).

All sap bowls contained 2nd instar larvae *Tx. splendens* that were still with other mosquito larvae (Figure 9). The early developmental period of predators is generally still with prey, the predator's body size affects the ability to prey. The body length of the 2nd instar larvae is almost the same as the 4th instar larvae of other mosquitoes (Millado et al. 2017). With a small body size, 2nd instar larvae only prey on prey whose body size is smaller than themselves. This is common in preying on predatory larvae (Gvoždík and Smolinský 2015; Younes et al. 2015; Venkatesh and Tyagi 2015; Priyadarshana 2021).

Figure 10). Observations of predators in their habitat are strongly influenced by body size (Shultz and Finlayson 2010). The body length of the 3rd instar larvae ranging from 4-8 mm will be very easy to distinguish from other mosquito larvae (Millado et al. 2017). Other mosquito larvae are still in large numbers so that the cannibalism of 3rd instar larvae has not yet emerged (Figure

9). These two reasons make 3rd instar larvae more common in every observation.

Rubber sap bowl containing 3rd instar larvae is more frequently found, either containing 3rd instar larvae with the other mosquito larvae or without other mosquito larvae, compared to rubber sap bowl containing 4th instar larvae and pupa. Rubber sap bowls containing 4th instar larvae and pupa at each observation time and location were more often found without other mosquito larvae (Figure 8.B). The preying ability of predatory larvae in the early development period is still low and will continue to grow due to the need for more nutrition (Kundu 2014). As the growth and development of predators increases, the prey is eaten also increases (Altwegg 2003). The limited number of prey causes prey to run out quickly. This will affect the process of further instar development.

The decrease in the number of larvae in August and September is due to the reduced rainfall, resulting in many dry bowls and weather condition that is unsuitable for mosquito reproduction. This is common in larval predators, which are closely related to water volume, such as the predatory larvae of the *Platynectes* sp. which has decreased population in the dry season (Gowhar 2018).

The percentage of rubber bowl containing *Tx. splendens* pupa is low, only 1 pupa was found in each bowl. This is because some of the older instar larvae cannot develop into pupae. The need for increased nutritional sources in older instars causes predatory activity to also increase. This also occurs in *Harmonia axyridis* larvae that prey on *Mizus persicae* (Endarto and Wuryantini 2019). However, larval *Tx. splendens* that live in narrow ponds and limited prey causes many old instar larvae to starve to death.

The average number of larval *Tx. splendens* in the bowl at each stage are not uniform (Figure 11). The average number of 2nd instar larvae in each rubber sap bowl is more than 1. The number of *Tx. splendens* 3rd instar larvae in each bowl are decreasing until reaching 1 individual. In each bowl containing 4th instar larvae and pupae, only 1 individual remains. A small-sized bowl has a smaller carrying capacity as a habitat for larval *Tx. splendens* and can only support one larva to develop into a pupa. This is also common in predators that live in confined spaces such as larval populations of *Ceriagrion coromandelianum* and *Platynectes* sp. decreased when environmental conditions were unfavorable for prey and predators (Kumar et al. 2014; Payra 2020).

Tx. splendens produce 14 eggs per day on average with 96% viability (Millado et al. 2017). In the early development phase, the larvae have adequate food. In further development, the larvae of *Tx. splendens* in the limited area and amount of feed will prey on other mosquito larvae until they run out, and when starving, cannibalism will happen, where the larvae will eat each other (Millado et al. 2017; Soda et al. 2018). This condition happens in a rubber sap bowl containing 4th instar larvae and pupae, which finally only 1 larva left.

The sample locations are rubber plantations that are close to cattle pens, rice fields, and roads that are closely related to human activities. Observations from all locations found larval *Tx. splendens*. This shows that the *Tx.*

splendens mosquito can adapt to human activities. The success of *Tx. splendens* originating from rubber plantations in the Banjarbaru area in adapting to the human environment, can be used as a biological control for *Aedes* sp mosquitoes in an integrated vector control program in residential areas (Thomas 2017; Leung et al. 2020).

In conclusion, *Tx. splendens* can use a rubber sap bowl filled with water as a breeding site. The number of larval *Tx. splendens* in each bowl in the early development of instar is greater than 1 and decreases as the developmental phase progresses due to the limited volume of water in the bowl, cannibalism, and the number of other mosquito larvae as prey.

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