Philipp Ent 31 (2): 85-105 ISSN 0048-3753 October 2017

BIOLOGY OF A PHILIPPINE POPULATION OF *Toxorhynchites splendens* (Wiedemann) (DIPTERA: CULICIDAE: TOXORHYNCHITINAE) UNDER LABORATORY CONDITIONS WITH *Aedes aegypti* (L.) (DIPTERA: CULICIDAE: CULICINAE) AS PREY[1]

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ABSTRACT

A local population of *Toxorhynchites splendens* (Wiedemann) (Diptera: Culicidae: Toxorhynchitinae) from the College of Forestry and Natural Resources (CFNR), University of the Philippines Los Banos, was observed under laboratory conditions to gather basic data on its biology. Immature stages are briefly described highlighting key characters that differentiate larval instars. Egg incubation and developmental period were shorter in males (1.77 ± 0.35, 36.16 ± 15.57) than in females (1.87 ± 0.26, 38.30 ± 16.23). Males also live shorter (12.9 ±10.44) than females (18.38 ± 14.76). The number of preys, *Aedes aegypti* larvae, offered to *T. splendens* larvae was positively correlated with the duration of the stages of both males and females. Higher juvenile mortality and shorter adult longevity were observed at higher prey densities of 40 and 60. Adults resulting from larvae fed with 10-20 preys daily, lived longest, making it the most appropriate ratio for rearing. The average fecundity was 19.80 eggs (± 16.16), with 96.60% (± 15.60) viability over a reproductive period of 14.80 days, about 15.30 days after mating. Oviposition mostly occurred either during flight above or while resting on the water surface. Hatchability of eggs taken out of the water and cooled or air dried was greatly reduced making the larva more ideal for transport. On the other hand, cannibalism, although present in all larval instars, was reduced when preys are present.

Key words: *Aedes aegypti*, life history, *Toxorhynchites splendens* (Wiedemann)

INTRODUCTION

Toxorhynchites splendens (Wiedemann) is one of the four known species of the genus recorded in the Philippines, alongside *T. gigantulus* (Berlin, 1969), *T. nepenthis* (Dyar & Shannon, 1925), and its resembling counterpart, *T. amboinensis* (Steffan, 1968). The carnivorous *Toxorhynchites* larvae have been successfully utilized as biocontrol agents of mosquito larvae in many neighboring countries including Bangladesh (Begum et al., 1988), Malaysia (Furumizo & Rudnick, 1978), Singapore (Chan, 1968), Indonesia (Annis et al., 1990; 1989), and Thailand (Choochote et al., 2002).

The Philippines, being a major hotspot of mosquito-borne diseases in South East Asia (WHO, 2016), continues to explore all possible prospects for management to complement its recent approval of the Dengue vaccine (DOH, 2016). Although described as early as 1934 (as *Megarhinus,* Barraud, 1934) and many times thereafter (Bohart, 1945; Belkin, 1962; Lee et al., 1988), the potential of this mosquito as a biocontrol agent has not yet been considered locally. This study was, thus, carried from 2013 to 2016 to understand the basic biological characteristics of a local population of *T. splendens* under laboratory conditions, prior to assessing its value in the local mosquito management programs. Also, descriptions of the predatory larval stages are lacking in this species and were, thus, provided for easy identification in prospective studies.

MATERIALS AND METHODS

Insect Collection

Eggs and immature stages of *T. splendens* were collected by inspecting possible water containers around CFNR, UPLB with a torchlight. A modified asepto-syringe was used to siphon larvae and pupae into a small water-filled container. Eggs were transferred using a wooden spatula. Collections were brought to the Institute of Weed Science, Plant Pathology, and Entomology (IWEP), CAFS, UPLB for species confirmation and rearing. In the succeeding texts, *T. splendens* and 'predator', and *A. aegypti* and 'prey' are used interchangeably.

Rearing and Mass Production

***Predator* (*T. splendens*).** A colony was established from the collections adapting the rearing procedure by Furumizo & Rudnick (1978) with slight modifications. Upon emergence, adults were placed in a cylindrical mylar cage (diameter: 22 cm; height: 36 cm) for mating and oviposition. Each cage was provided with 10% honey solution in cotton wicks and water. A wet towel was also hung on the sides to increase moisture. The predators were allowed to

oviposit on dechlorinated tap water in a black plastic cup. Larvae were grown to pupation with a 1:10 predator-prey ratio using *A. aegypti* of the same instar or of proportionate size.

Preys (*A. aegypti*). Egg strips were obtained from the laboratories of Drs. B.L. Caoili and P.A. Javier (Insect Pathology and Postharvest Laboratories, IWEP, UPLB). Rearing protocol by Angeles (2013) was followed.

Biological Studies on *T. splendens*

The species was described based on adults earlier by several authors (Lee et al., 1988; Belkin, 1962; Bohart, 1945; Bonne-Wepster, 1954) while Breland (1949) provided some details on the immature stages of *T. rutilus rutilus*. Here, characters, which help distinguish easily among larval instars, specifically changes in the siphon and saddle of the larva (Figure 1), are provided.

Life History

Egg. Eggs were collected in batches from each female, counted, and placed individually in a 60-cell ice cube mold containing 5 ml dechlorinated tap water. Five batches of 15 eggs were monitored until hatching.

Larva. Hatched larvae were transferred individually to 11.0 cm wide, round plastic containers filled with 200 ml dechlorinated tap water. Each batch was assigned to one of the five treatments based on prey density (A, B, C, D, E). The first set of predator larvae (A) were given five *A. aegypti* of the same instar or a proportionate size, daily, throughout the entire larval stage, the second set (B), 10; third set (C), 20; fourth set (D), 40; and the fifth set (E), 60. After 24 hours, remaining preys were counted and then replaced. Molting was noted by the presence of shed exuviae, which were removed promptly to avoid confusion.

Pupa. Pupae were then transferred to a small plastic cup with dechlorinated tap water and covered with a larger container for emergence.

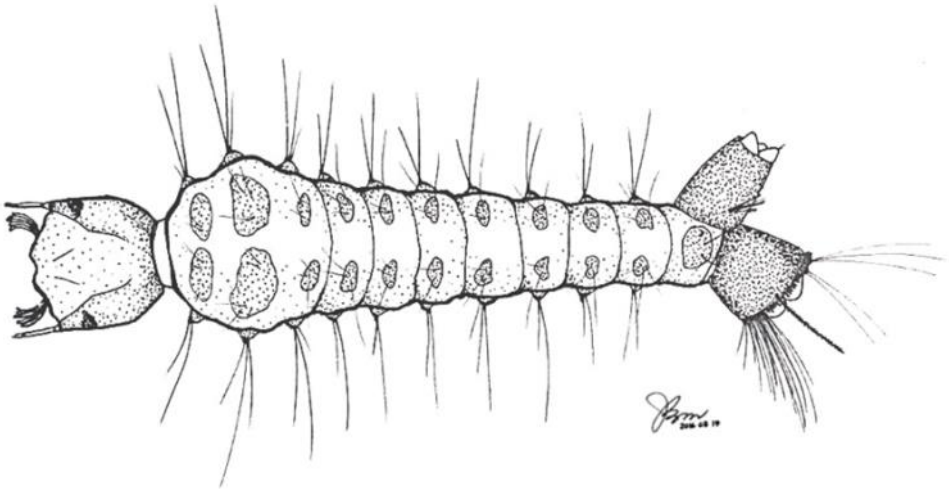

Figure 1. General dorsal habitus of *Toxorhynchites splendens* larva. Abbreviation: siphon (*si*), siphonal hair (*s-1s*), saddle (*sa*), upper dorsal brush (*upd*), lower dorsal brush (*ldb*), and ventral brush (*vb*).

Adult. After eclosion, newly emerged adults were sorted by sex. A pair of newly emerged male and female of the same set was placed inside a cylindrical mylar cage (diameter: 0.30 m; height: 0.46 m) and kept until mating occurs. A black cup with 100 ml water was placed inside the cage as oviposition medium. Adults were maintained in 10% honey solution until death. Mortality was also recorded throughout the study.

Egg Viability Test

Eggs collected 24 hours after laying were subjected to drying or refrigeration for a number of days to determine maximum shelf life. Percent hatchability was determined by placing the eggs in dechlorinated tap water after the drying/refrigeration period was over. Each treatment was composed of 15 individual eggs.

Cannibalism

Two set-ups were used to observe the cannibalistic behavior of *T. splendens* larvae: a) *Toxorhynchites* alone, and b) *Toxorhynchites* with prey.

Without prey. Each set up consisted of four starved, newly hatched or molted *Toxorhynchites* larvae of the same age placed together in 200 ml of dechlorinated tap water. Treatments included a) first instar only, b) second instar only, c) third instar only, d) fourth instar only, and e) combination of all instars. The number of surviving larvae after 24 hours was noted including body remains/parts. Three replications were done per treatment with five set-ups each.

With prey. The same set-up was carried out as the same procedure above, but with each treatment added with forty *A. aegypti* larvae. The same replication scheme was maintained.

Pre-pupal Compulsive Killing Behavior

Experimental set-ups for the life history were observed daily for prey which were partially eaten and/or killed but not eaten until pupation.

RESULTS AND DISCUSSION

Description of Life Stages

T. splendens, like most culicids, after hatching passes through four larval instars, and a pupal stage before reaching the adult stage. Each life stage is shortly described below with emphasis on the larval instars:

Egg. 0.112 ± 0.0038 mm long and 0.083 ± 0.0036 mm wide, floated horizontally on the water surface (Figure 2a). Freshly laid eggs appear white with papilliform ornamentation (Figure 2b), gradually turning cream to ecru toward

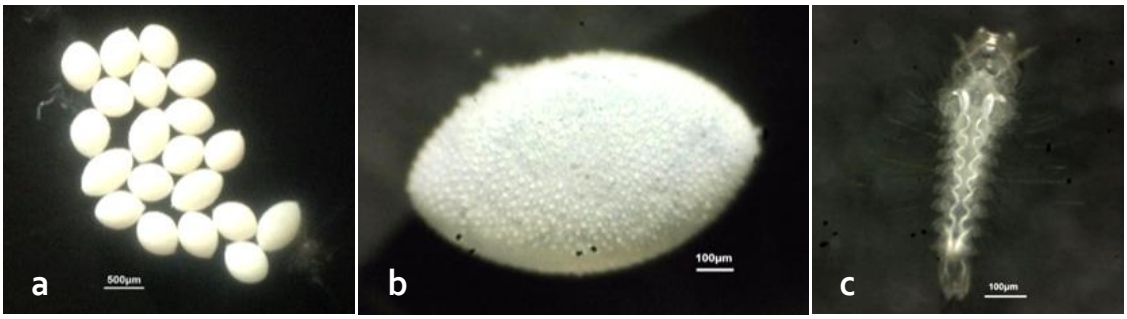

Figure 2. Hydrophobic eggs of *Toxorhynchites splendens* floating on water-filled black oviposition cups and c) a newly hatched first instar larva.

hatching.

Larvae. First instar 2.46 mm long. Newly hatched larvae (Figure 2c) appear translucent white with the dorsal aorta and trachea clearly visible and hefts of long spicules surrounding its body. The first instar's head is round with two black eye spots (Figure 3a). The siphon is short and stout with no apparent siphonal hair. Saddle has no dorsal and ventral brush as well. Soon after hatching, the first instar larva would be capable of movement and feeding.

Second instar 3.08 mm long, cylindrical, body light purplish-red with initial appearance of brown-purple pigmentation of the thorax, and spicules dark brown to black. Head is light brown with dark buccal area (Figure 3b). Siphon with one hair on each side. Saddle with three branches of upper dorsal brush on each side and two branches on the lower dorsal brush. Ventral brush also appeared on this instar.

Third instar 8.12 mm long and a generally larger version of the previous instar. However, unlike the latter, the former has a larger head-prothorax ratio and the color is deeper brown-red. At this stage, the larva appear to have a larger body but with a smaller and more quadrate head dorsally. Head is more round compared to the second instar (Figure 3c) but siphon has 3-4 lightly barbed branches. Upper portion of the dorsal brush of saddle with 4-5 branches each and lower portion with three branches each. Ventral brush same with previous instar but hairs are coarser.

Fourth instar, also the last and largest among the four, about 13.63 mm long (Figure 3d). Body is robust with dense, but short spicules at the lateral portion of each tergite. Head-pronotum ratio is less than the third instar, body deep purple-red, and the head is more quadrate. Siphon hair has 4-7 barbed branches each while saddle hairs appear fused with 10-12 branches on the upper dorsal brush and 8-10 branches on the lower dorsal brush. From the purple-red color, older larvae appear darker and brownish-red with a patched-like exterior around the dorsal part of the thorax. A comparison of the four larval instars can be seen in Figure 4. After several days, the lateral portion of the thoracic area appears whitish as the cuticle loosens in preparation for pupation (Figure 5a) as with *T. rutilus rutilus* (Breland, 1949).

Just like in *A. aegypti* (Timmerman & Briegel, 1999), measurements of the larva's thoracic width could also effectively express its growth within the stadium. This is due to the increasing deposition of protein reserves as the

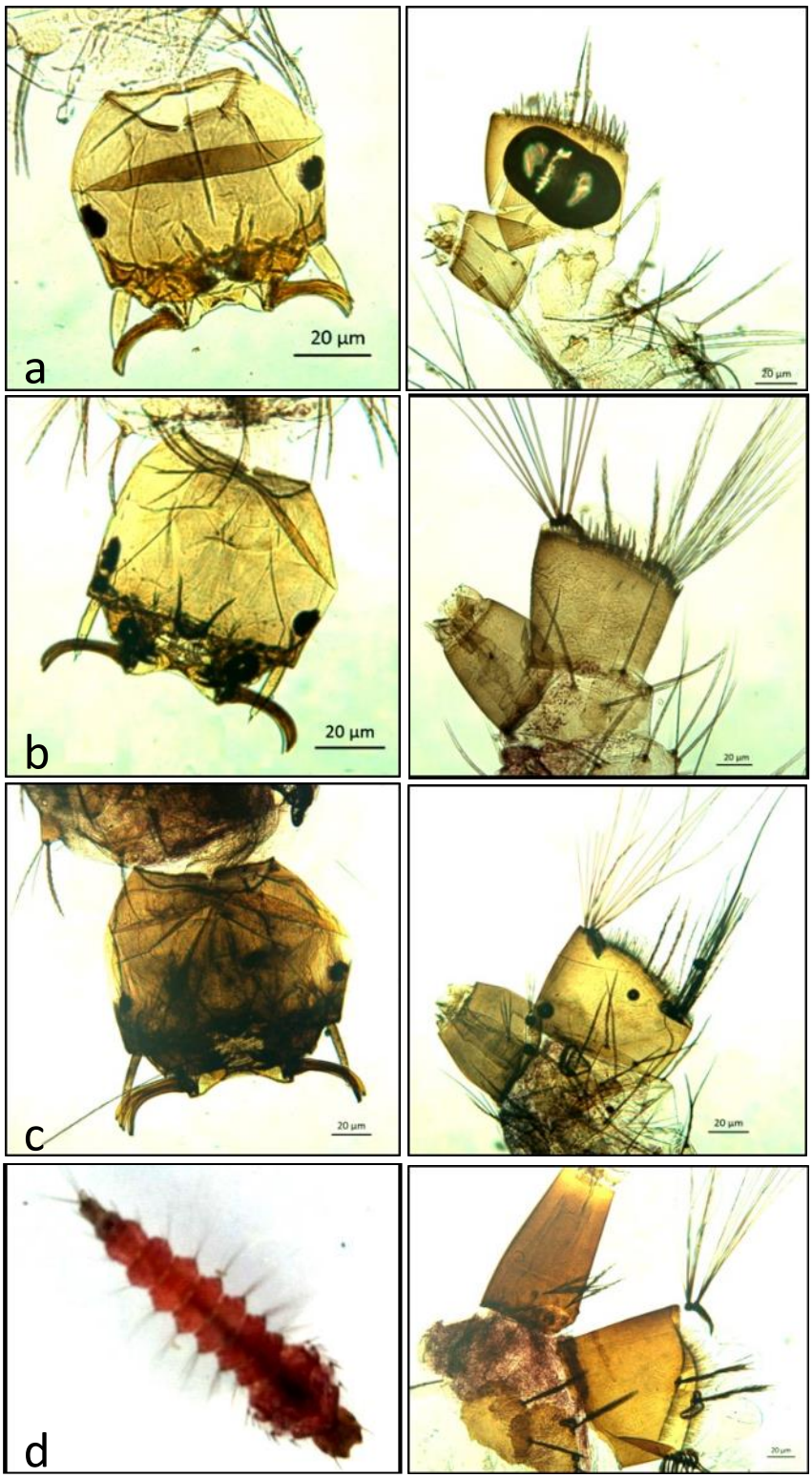

Figure 3. Dorsal habitus and siphon and saddle of *Toxorhynchites splendens* larval instars. **a**. first, **b**. second, **c**. third, and **d**. fourth instar.

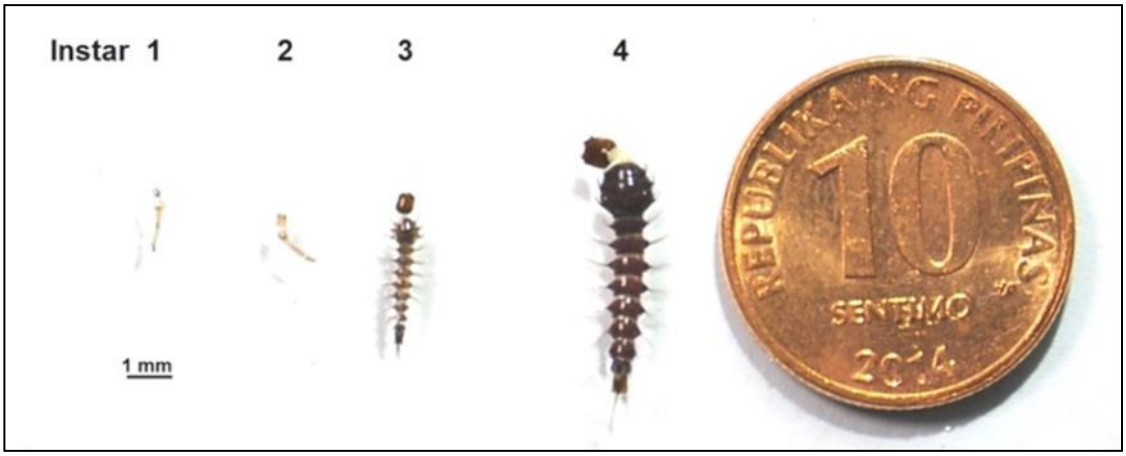

Figure 4. Larval instars of *Toxorhynchites splendens*: **a**. first, **b**. second, **c**. third, and **d**. fourth instar.

larva's body enlarges. Measurements of the head capsule (even possibly from exuvia, Figure 5b), on the other hand, are more helpful when discriminating between the four larval instars.

Bodily proportions (head, thorax, abdomen) of males and females were also found to be similar and the smaller size of males was the only general indication of sexual dimorphism. In case of starved larvae and those reared in crowding, the amount of protein followed the same trend. Although, fourth instar larvae stopped growing earlier, resulting to smaller pupae and adults. Aside from the general size, starved larvae can also be distinguished from their well-fed counterparts based on the more translucent appearance of the abdomen due to lower accumulation of lipids throughout the body. On the other hand, well-fed larvae do not shrink in size but instead lose the opacity of the abdomen as seen on starved individuals (Timmerman & Briegel, 1999).

Pupa comma-shaped like that of other culicine mosquitoes but larger (Figure 5c). Freshly pupated individuals appear light brown while those nearing emergence are a darker brown to black.

Biological Studies on *T. splendens*

Life History

The life history data of *T. splendens* is summarized in Table 1. Eggs hatch in 1-2 days for both males and females. The entire larval stage or Total Larval Period (TLP) takes about a month with the males pupating about two days earlier than females. Males also emerge a day shorter, taking a Total Development Period (TDP) of 36.16 ± 15.57, compared to females with 38.30 ± 16.23 days. While adult males complete development faster, they also lived shorter than their female counterparts. Between sexes, the medians were found to be significant at 95% for the TLP (Mann-Whitney, P=0.0316), pupal period (P=0.0174), TDP (P=0.0145), and adult longevity (P=0.0164).

Mating was observed only twice under laboratory conditions. This occurred

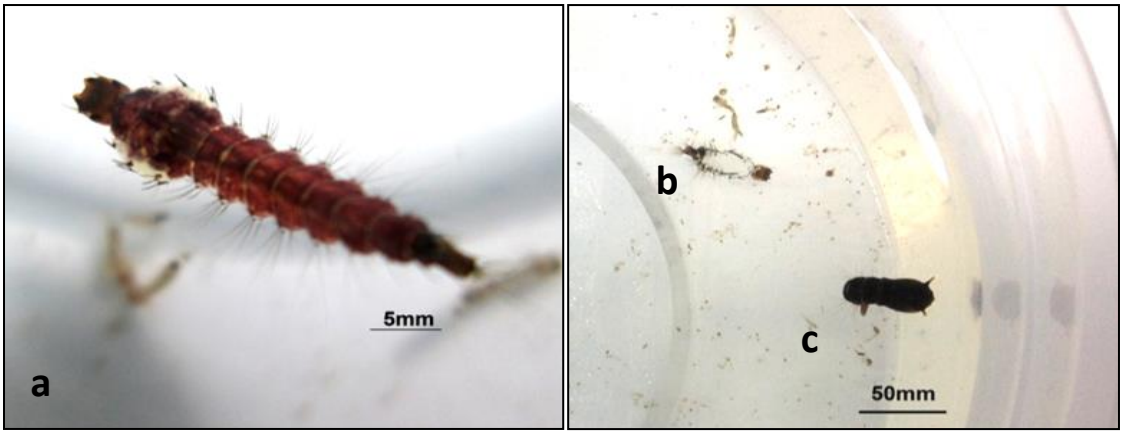

Figure 5. a. Late fourth instar *Toxorhynchites splendens* showing changes in appearance before casting its exuvia (**b**) and turn into a pupa (**c**).

Table 1. Life history of *Toxorhynchites splendens* (Wiedemann) using composite data from set-ups fed with different *Aedes aegypti* densities (in days).

DEVELOPMENTAL STAGE	MALE		FEMALE	
	Range	Mean	Range	Mean
Egg				
Incubation Period	1.0 – 2.0	1.77 ± 0.35	1.0 – 2.0	1.87 ± 0.26
Larva				
1st Instar	1.0 – 5.0	2.32 ± 0.9	1.0 – 6.0	2.35 ± 0.95
2nd Instar	1.0 – 15.0	2.90 ± 1.98	1.0 – 8.0	2.96 ± 1.25
3rd Instar	2.0 – 15.0	6.15 ± 2.94	2.0 – 16.0	6.98 ± 3.24
4th Instar	8.0 – 59.0	17.5 ± 12.6	8.0 – 65.0	19.21 ± 13.59
Total Larval Period*	12.0 – 79.0	28.94 ± 15.45	15.0 – 83.0	31.94 ± 16.03
Pupa*	3.0 – 7.0	4.76 ± 0.72	3.0 – 6.0	4.96 ± 0.64
Total Developmental Period*	15.0 – 86.0	36.16 ± 15.57	22.0 – 90.0	38.30 ± 16.23
Adult Longevity*	2.0 – 55.0	12.9 ± 10.44	1.0 – 73.0	18.38 ± 14.76
Pre-Oviposition Period			10.0 – 22.0	15.3 ± 3.99
Reproductive Period			1.0 – 47.0	14.8 ± 11.23
Fecundity			1.0 – 76.0	19.8 ± 16.16
Egg Viability (%)			16.0 – 100.0	96.6 ± 15.6

n=174
*significant at 95% confidence interval

when the male, while in flight, swiftly approached the resting female from the back and made efforts to link the ends of their abdomens. The pair then falls to the cage floor.

The values obtained for *T. splendens* in the Philippines fit well with the figures obtained by Chan (1968) and Furumizo & Rudnick (1978). However, the current data has a wider range mainly due to the amount of prey offered which ranged from 5-60 per day. Other factors such as prey capturing and searching ability, light, and temperature may also vary to some extent.

The average number of deposited eggs was lower in this study than those recorded by for *T. amboinensis* (Steffan et al., 1980) which averaged to 14.3 eggs

per day with gradual decline on the hatchability as the female ages. The differences in individual fecundity of females could be due to the rearing method used (Focks et al., 1977; 1979). Confinement of females in small cages during rearing could affect the dispersal behavior of the female which were intended to oviposit over a wide area (Trimble, 1979). Females may exhibit high fecundity during initial release. However, subsequent dispersal of multiple females may reduce deposition over the same containers. This behavior prevents crowding and ensures that other potential sites could also be covered.

Oviposition on the water was done either in flight or by resting on top of the water surface. For in-flight oviposition, the female first performs 6-54 backward flight loops, with a second or shorter per loop, above the water and ejects an egg into the water surface at the end of the loop. Normally, the female performed shorter loops in between eggs while loops during oviposition were generally longer. Consequently, other females were observed to rest on the water surface and deposit an egg by pushing it outward its genitalia (Figure 6). This novel behavior was not mentioned in previous studies. Newly laid eggs bounce as they hit the water surface.

Female *Toxorhynchites* usually deposit 1-3 eggs and over a wide number of sites to reduce crowding and cannibalism among siblings with depleting prey. Egg production in *Toxorhynchites* is achieved through a continuous production of egg follicles. This autogenous process allows females to produce batches of eggs without a bloodmeal and even with water as food source. Unlike other culicines, gravid females are also able to interrupt deposition which allows them to store eggs until an appropriate habitat becomes available (Steffan & Evenhuis, 1981). Additionally, the unique looping behavior exhibited by the gravid females allows them to access contained habitats which would be impossible with

Figure 6. Gravid female *Toxorhynchites splendens* depositing eggs while resting on the water surface.

conventional surface deposition (Furumizo & Rudnick, 1978). This also reduces

the risk of predation from spiders and other predators commonly found around mosquito habitats (Trimble, 1979).

Development at different prey densities

Male larvae of *T. splendens* fed with five *A. aegypti* larvae daily have shorter durations (50.38 ± 22.09) than females (56.06 ± 22.63) (Figure 7). At 10 preys per day, larval development shortens abruptly to almost half in both sexes. A gradual decrease was then recorded for those fed with 20, 40, and 60 preys.

Predators given five, 40, and 60 larvae per day did not entirely consume the food given compared to those given 10 or 20. This was observed even as the larva aged and pupated. Also, predators needed to accumulate a certain amount of proteins and lipids to complete development. Higher prey density results to higher consumption, eventually allowing the larva to attain the needed protein and lipid requirement faster. This explains why those given fewer preys took longer time to develop than those fed with more preys.

The faster development and early pupation by males in light of competition and survival in the field was discussed by Crans & Slaff (1977) as a reason for compulsive killing. As males develop faster than females, they are also expected to exhibit surplus killing earlier than their female counterparts to protect themselves from their siblings that develop days later.

Development at higher prey densities also produced larger larvae that resulted to larger adults (Figure 8). Conversely, the rate of larval mortality was also higher in immature stages and adults fed with more preys (Figure 9).

Larger adult mosquitoes resulting from larvae fed with more preys eventually have more lipid, carbohydrate, and protein reserves and are expected to live longer and produce more eggs within a shorter period (Briegel 1990).

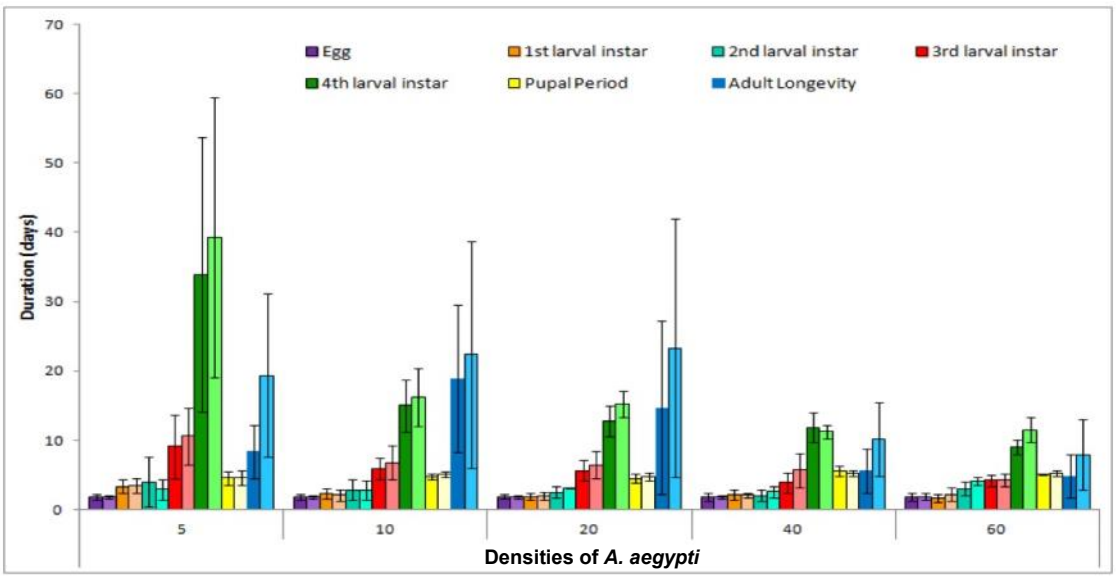

Figure 7. Duration of life stages and adult longevity of *Toxorhynchites splendens* at different prey *Aedes aegypti* larvae densities. Legend: Males, darker shade; Females, lighter shade.

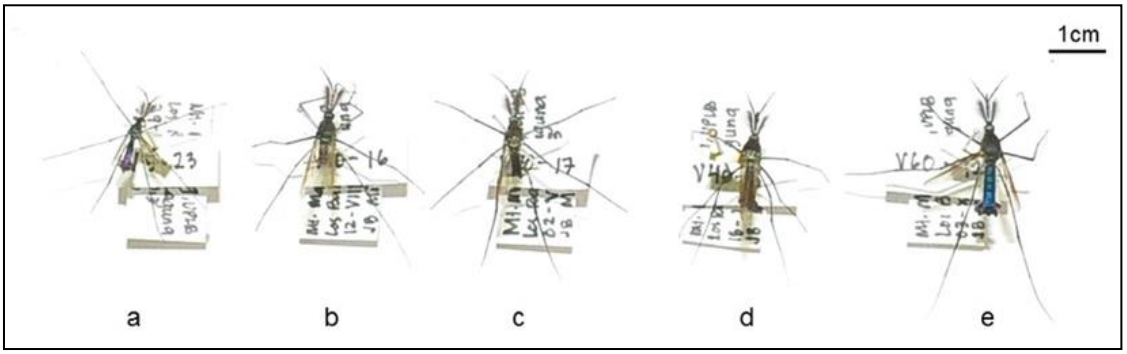

Figure 8. Size differences of adult male *Toxorhynchites splendens* when larvae were fed with a) 5, b) 10, c) 20, d) 40, and e) 60 *Aedes aegypti* larvae per day.

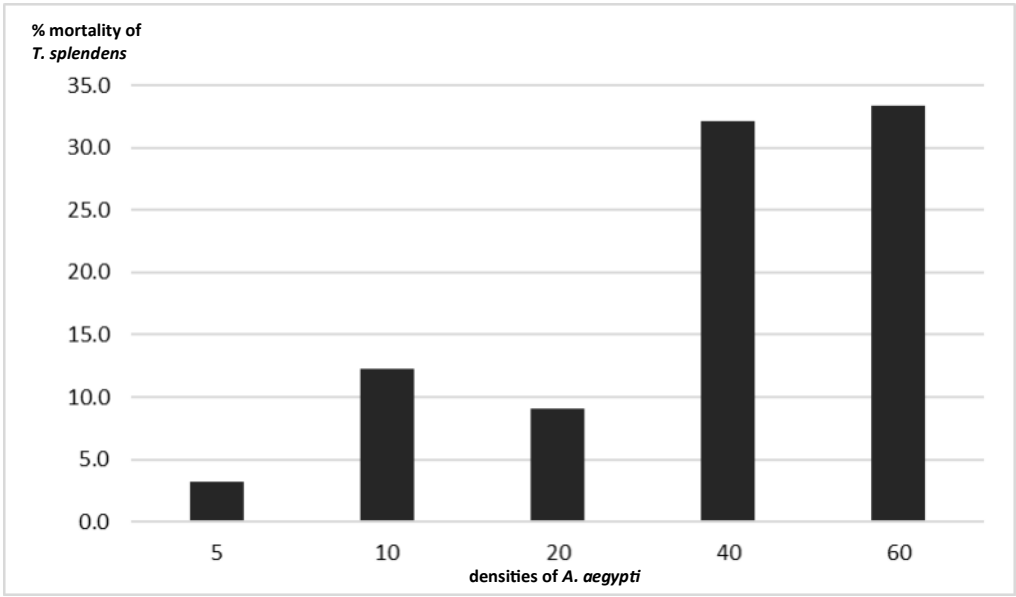

Figure 9. Percent mortality of immature *Toxorhynchites splendens* given different densities of *Aedes aegypti* larvae as prey.

However, in this study, mortality was high for both immature stages and adults fed with more preys than their smaller counterparts. As observed under laboratory conditions, mortality of larger adults was mainly because they were unable to detect the honey-soaked cotton balls inside rearing cages without manual intervention. Smaller adults, however, had less difficulty finding their artificial food source. Timmermann & Briegel (1999) explained that when well-fed mosquito larvae and adults are subjected to continuous starvation, their lipid and protein reserves decline tremendously resulting to 73% mortality compared to larvae with restricted food sources. This, however, may not be reflective of field conditions.

Adult longevity for both males and females in this study was optimal at

prey densities of 10-20 per day since densities lower or higher than this range resulted to more short-lived individuals. This could be regarded as the optimum values for mass production for future laboratory and field studies.

Between sexes, the duration of the fourth instar, TLP, and TDP were significantly shorter in males and females fed with 20 and 60 larvae per day. Compared to other densities, the longer development of females compared to males was more pronounced in densities 40 and 60.

The pupal period was also found to be considerably distinct in individuals given 10 preys per day, where males emerge one day earlier than females. Consequently, adult longevity was longer in larvae fed with five preys per day. Compared to other densities where the difference is minimal, at five preys per day, females live twice as long as their male counterparts.

Egg Viability Test

The viability of eggs of *T. splendens* was tested after drying or refrigeration at 4°C for a number of days. In both set-ups, only those eggs taken out of the water or cooled for 24 hours were able to produce viable eggs. Sixty-seven percent of eggs refrigerated for one day was able to hatch compared to 20.0% in eggs that were dried (Figure 10). Eggs exposed to both circumstances longer than one day failed to hatch. Here, refrigerated eggs were kept moist by placing them in a slightly dampened paper towel. Without the damp towel, both dried and refrigerated eggs lost shape and failed to hatch after the two-day expected incubation period.

Similar findings were reported by Newkirk (1947) on *T. (Megarhinus) splendens.* Eggs that were taken out of the water failed to hatch, while the other half left on the water surface hatched in two days. In addition, it was noted that temperatures lower than 14°C and higher than 37°C are considered lethal for developing eggs (Trpis, 1972). Under these situations, the hydrophilic inner chorion of the egg becomes rigid and hatching larvae are unable to eclose (Dodge, 1964). Since eggs are fragile and transport of floating eggs in water is difficult, unlike other aedine mosquitoes, the best stage for shipping is still the larva.

Cannibalism

Table 2 presents the number of cannibalized *T. splendens* (no. of cannibalized larvae/total no. of larvae per container) in the presence or absence of larval prey. In both occasions, cannibalism decreased in relation to the age or instar of the predator. In the absence of prey, a greater risk of cannibalism was observed for all instars compared to set-ups with available prey. In a mix of different larval instars, the fourth instar always predominated over younger larvae.

The same observations were noted by Steffan & Evenhuis (1981) where *T. splendens* tend to cannibalize when large numbers of the predator were placed in a small container even in the presence of copious prey. Fox (1975) explains that cannibalism serves as a regulatory mechanism to limit competition for available resources. This trait was a response to the selective pressure of limited food

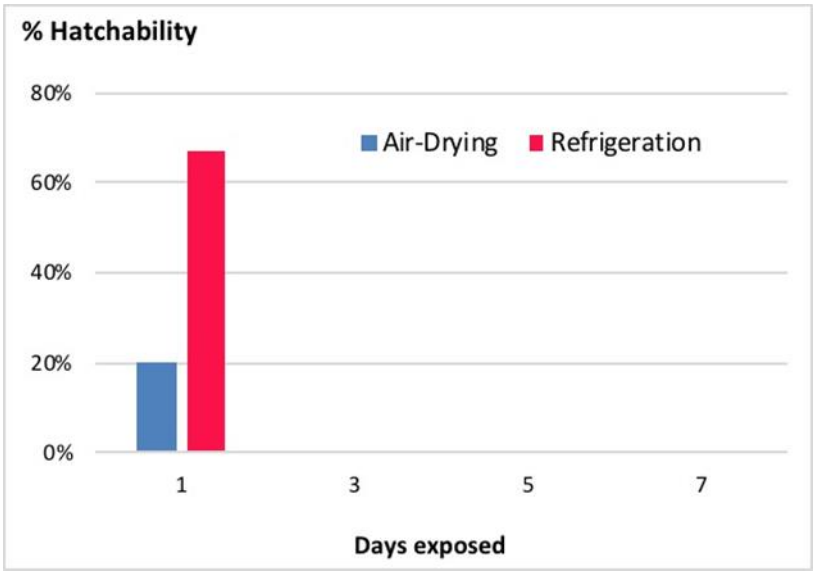

Figure 10. Percent viability of *Toxorhynchites splendens* eggs exposed to refrigeration and drying.

Table 2. Average number of cannibalized *Toxorhynchites splendens* larvae in the presence or absence of 40 *Aedes aegypti* larvae as prey

	TX. SPLENDENS* INSTAR				
	I	II	III	IV	Mixed
w/o prey	0.8	1.4	2.4	2.8	2.8
with prey	0.6	1.0	2.0	2.8	3.0
Prey eaten	18.0	30.6	33.6	40.0	40.0

*15 set-ups/ instar

supply in the field and competition with conspecifics, often, siblings (Russo, 1986).

Furthermore, this behavior is determined by a combination of the following factors: prey density, container size, water volume, predator's age and density, size difference, and hunger level. Based on prey density alone, cannibalism was reduced if there were at least 20 preys available for each predator. However, high number of *Toxorhynchites* larvae present in the same container will still result to higher instances of cannibalism even in the presence of 20 preys per predator (Annis et al., 1990). On the contrary, less cannibalism was observed in containers with a larger surface area compared to narrow containers with the same water volume wherein likelihood of predator contact is increased. This was especially observed in habitats such as bamboo stumps where the presence of two or more *Toxorhynchites* larvae is highly unlikely compared to used car tires. They have also noted that cannibalism is more likely to occur between dissimilar -sized individuals with at least 5 mg weight difference, although belonging to the same instar. This was even higher among different instars where younger instars are highly at risk (Russo, 1986). Cannibalism and pre-pupal killing ensure the pupating *Toxorhynchites* larva that other potential predators, most probably from

another batch of eggs, does not reach the stage where it is able to pose a threat to it and its siblings' highly vulnerable stage.

The collection of more than one larva in the field, even from small containers, could be mainly attributed to the presence of detritus which serve as additional food source and debris where the larvae can hide (Focks, 1982).

Pre-pupal Compulsive Killing Behavior

Successfully reared *T. splendens* exhibited pre-pupal killing of prey larvae especially those nearing pupation starting at prey density of 20 *A. aegypti* per day. Here, Figure 11 presents a newly pupated predator with the prey cadavers intact at the bottom of the container. Some preys were half-eaten while some were still intact suggesting that the behavior is not solely for feeding. In some cases, preys for surplus killing were released within five seconds of successful strike whether it is alive or dead. Live prey released after this period eventually died within a few minutes (Russo, 1986). After release, *Toxorhynchites* larvae exhibit an interesting act of wiping the mandibles across the marginal spicules of its saddle or aciculae to clear remaining debris as humans would with a toothpick.

The same conditions were observed by Chan (1968) where all instars around the molting period exhibit the behavior and only become more pronounced as pupation approaches. This behavior of killing other organisms in the habitat ensures that the predator will be safe during a period where it is most vulnerable, especially from other *Toxorhynchites* larvae (Corbet & Griffiths, 1963). Aside from this, killing of available prey would also deprive younger *Toxorhynchites* present in the same container the needed food requirement to reach the instar where it would pose a threat (Russo, 1986).

Luonibos (1979) explained that this killing behavior is related to larval weight. Studies using *T. brevipalpis* revealed that compulsive killing only occurred when a certain weight threshold was attained. Larvae which weighed less than 30 mg were rarely observed to exhibit the behavior. As the amount of prey given increased, the more likely did the larva reach its threshold and

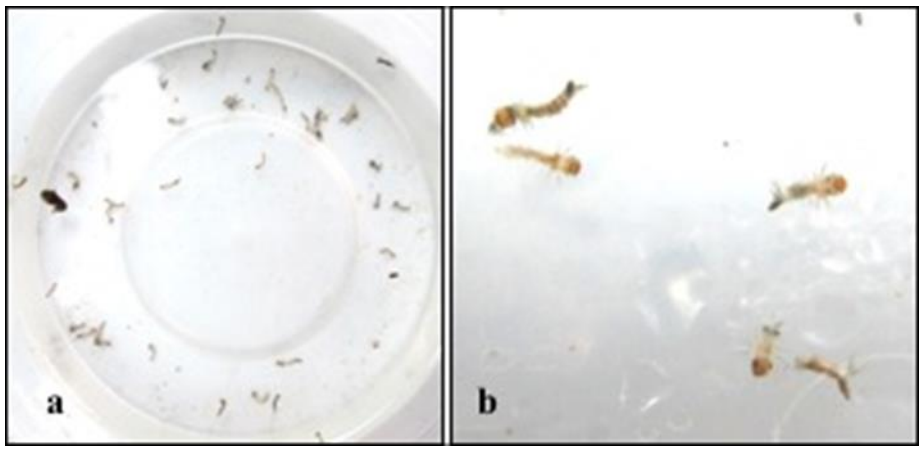

Figure 11. a. Newly pupated *Toxorhynchites splendens* with prey cadavers. **b**. close -up of cadavers of half-eaten and completely uneaten preys.

displayed more intensive killing-without-eating until it reached a plateau at weights more than 40 mg. In *T. splendens*, the threshold was 31 mg. This behavior often intensifies as the larva ages and nears pupation. Compared to other species, the onset of this act in *T. splendens* is delayed and only starts on day 12 after it has ensured itself that it has reached the minimum requirements for pupation (Russo, 1986).

ACKNOWLEDGEMENTS

This study was partly funded by the Accelerated Science and Technology Human Resource Development Program of the Department of Science and Technology (DOST-ASTHRDP). The authors would also like to thank Dr. Gautam Aditya of the University of Calcutta for the species confirmation, Ms. Rachelyn S. Araña for assisting in the statistical analysis, and all reviewers for the useful comments.

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Supplemental Table 1. Different developmental stages of male and female *Toxorhynchites splendens* fed with varying densities of *Aedes aegypti* per day.

PREY DENSITY	DEVELOPMENT STAGE	MALE		FEMALE	
		Range	Mean	Range	Mean
5	Egg	1-2	1.85 ± 0.38	1-2	1.94 ± 0.25
	Larva I	2-5	3.31 ± 0.95	2-6	3.44 ± 1.03
	II	1-15	4.00 ± 3.61	2-7	2.88 ±1.41
	III	2-15	9.15 ± 4.58	2-16	10.56±4.07
	IV	12-59	33.92 ± 19.79	12-65	39.19±20.21
	TLP	20-79	50.38 ± 22.09	23-83	56.06±22.63
	Pupa	3-6	4.54 ± 0.97	3-6	4.56±1.03
	TDP	27-86	56.77 ± 22.58	29-90	62.56±23.26
	AL	2-15	8.38 ± 3.88	3-53	19.38±11.85
10	Egg	1-2	1.79±0.42	1-2	1.89±0.31
	Larva I	1-3	2.30±0.80	1-3	2.10±0.84
	II	1-7	2.80±1.47	1-8	2.73±1.41
	III	3-9	5.95±1.54	3-14	6.77±2.43
	IV	10-24	15.00±3.73	9-26	16.17±4.20
	TLP	23-34	27.10±2.88	20-38	29.07±4.36
	Pupa	4-5	4.80±0.41	4-6	5.10±0.40
	TDP	28-40	32.65±3.18	25-46	34.77±5.01
	AL	3-53	18.90±10.60	3-73	22.33±16.33
20	Egg	1-2	1.88±0.33	1-2	1.93±0.27
	Larva I	1-3	1.88±0.47	1-3	2.00±0.55
	II	2-5	2.53±0.80	1-5	3.07±0.10
	III	4-10	5.59±1.46	3-11	6.43±1.95
	IV	10-16	12.71±2.23	12-24	15.21±1.89
	TLP	20-26	22.71±2.23	23-34	26.71±3.29
	Pupa	4-6	4.47±0.62	4-6	4.79±0.58
	TDP	25-33	29.06±2.19	30-40	33.43±3.08
	AL	2-55	14.71±12.47	2-71	23.29±18.63
40	Egg	1-2	1.67±0.52	1-2	1.92±0.28
	Larva I	1-3	2.14±0.69	2-3	2.08±0.29
	II	1-3	2.0±0.82	2-4	2.58±0.79
	III	3-7	3.86±1.46	3-12	5.67±2.46
	IV	9-16	11.86±2.19	10-13	11.25±0.97
	TDP	5-23	17.43±5.86	15-28	21.33±3.17
	Pupa	5-7	5.57±0.79	5-6	5.27±0.39
	TDP	12-29	24.71±5.99	22-35	28.33±3.14
	AL	2-11	5.57±3.26	3-19	10.17±5.37
60	Egg	1-2	1.67±0.52	1-2	1.67±0.50
	Larva I	1-2	1.60±0.55	1-3	2.20±0.92
	II	2-4	3.0±1.0	3-5	4.10±0.57
	III	3-5	4.20±0.84	2-5	4.20±0.92
	IV	8-10	9.0±1.0	8-14	11.5±1.84
	TLP	17-18	17.8±0.45	18-24	22.0±2.36
	Pupa	5	5.0	5-6	5.20±0.42
	TDP	23-25	24.40±0.89	25-32	28.9±2.42
	AL	2-9	4.8±3.11	1-18	7.9±5.09