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Original Article

Larval mosquito (Diptera: Culicidae) abundance in relation with environmental conditions of pitcher plants *Nepenthes mirabilis* var. *mirabilis* in Songkhla Province, Thailand

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Abstract

This study examined the abundance of various immature mosquito species and ascertained selected environmental conditions in the pitcher. A total of hundred pitchers (*Nepenthes mirabilis* var. *mirabilis*), including 50 upper pitchers and 50 lower pitchers, were collected during rainy and dry seasons from Songkhla Province, Thailand. A total of 546 mosquito larvae belonging to two genera and three species were identified. Among the collections, 526 larvae of *Tripteroides tenax* (96.34%), followed by 11 larvae of *Tripteroides* sp.1 (2.01%), and nine larvae of *Toxorhynchites albipes* (1.65%) were identified. The abundance of mosquito larvae was noted to be higher during rainy season than during dry season. Mosquito larvae abundance positively correlated with pitcher size, amount of detritus present, pH of the medium, abundance of microorganisms, and amount of total fluid present in pitchers. Variation in abundance of mosquito larvae existing in pitchers is influenced by the presence of predators therein and different seasons.

Keywords: abundance, mosquito larvae, *Nepenthes mirabilis* var. *mirabilis*, pitcher characteristics

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1. Introduction

Pitcher plants (family: Nepenthaceae; genus: *Nepenthes*) contains 120 species (Miguel, Hehn, & Bourgaud, 2018). Members of this plant family usually occur in sunny and wet climates that are deficient in nitrogen and phosphorus. These plants possess a variety of pitcher trap patterns, such as size, shape, color, and others, to attract and capture insects or other arthropods for augmenting their nutritional deficiency (Gaume, Bazile, Huguin, & Bonhomme, 2016; Wang, Zhou, Zheng, & Xu, 2009). *Nepenthes* pitcher plants have been considered as natural container habitats that store freshwater and are called pitcher trap (Kitching, 2001). These pitcher plant traps contain fluid to facilitate oviposition and habitation of some arthropods including immature stages of some vectors mosquito species, such as *Aedes aegypti* and *Aedes albopictus* (Chou, Dykes, Wilson, & Clarke, 2016). There are numerous studies concerning *Nepenthes* spp. pitcher plants providing oviposition and larval and pupal development habitats for gravid female mosquitoes. However, at present, there is limited information concerning the environmental factors that prevail both inside and outside of *Nepenthes* species pitcher plants in relation to mosquito populations and communities.

Understanding the environmental factors and their influence on oviposition by gravid female mosquitoes and the survival of mosquito larvae and pupae in *Nepenthes* spp. pitcher traps is fundamental for vector management strategies and understanding the risk of disease transmission in various habitat types (Vanlalruia, Senthikumar, & Gurusubramanian, 2014).

Selecting appropriate oviposition sites of gravid female mosquitoes is important because the survival of mosquito offsprings depends upon sites where eggs are laid (Chou, Dykes, Wilson, & Clarke, 2016; Kershenbaum, Spencer, Blaustein, & Cohen, 2012). Gravid female mosquitoes utilize a variety of complex oviposition cues in order to explore potential oviposition sites. Oviposition cues of female mosquitoes including olfactory, visual, and tactile are associated with abiotic and biotic factors (Grech & Juliano, 2018). These oviposition cues have been well recognized to influence mosquito female decision for oviposition. Mosquito oviposition cues include color and size of container (Chou, Dykes, Wilson, & Clarke, 2016; Torrisi & Hoback,2013) detritus materials and their types (Norman, & Walker, 2018; Yee & Juliano, 2006; Yee, Kneitel, & Juliano, 2010); presence of organic chemicals from conspecific eggs (Ganesan, Mendki, Suryanarayana, Prakash, & Malhotra, 2006); presence of bacteria (Arbaoui & Chua,2014); and presence of conspecific larvae and anuran tadpoles (Mokany & Shine, 2003). Therefore, oviposition behavior of female mosquitoes in responding to a variety of oviposition cues is essential for seeking suitable and secure sites for their offspring. However, after hatching of eggs, larval mosquitoes have to cope with prevailing environmental factors and their survival depends upon availability of food resources including microorganisms and detritus as well as competition, predation and resistance to physical environmental conditions (Duguma, Kaufman, & Simas Domingos, 2017).

There are some studies that focus on abundance and survival of larval mosquitoes inhabiting *Nepenthes* spp. pitcher plants and evaluation of the influence of environmental factors inside the pitcher traps of *Nepenthes* spp.; specifically, characteristics of *Nepenthes* spp. impact on behavior of gravid female mosquito decision on oviposition. An investigation of three different *Nepenthes* species, *N. gracilis* (Korth), *N. mirabilis* (Lour), and *N. ampullaria* (Jack) demonstrated that *N. mirabilis* and *N. gracilis* were not suitable for survival of *A. aegypti* and *A. albopictus* mosquito larvae because of the prevailing high acidity levels and microbial activity, concluding that *Nepenthes* spp. pitcher plants were not suitable habitats for survival of *A. albopictus* and *A. aegypti* (Chou, Wilson, Dykes, & Clarke,2015) Further investigation by Chou, Dykes, Wilson, & Clarke (2016) illustrated that both color and size of *N. ampullaria* were not attractive to gravid *A. aegypti* and *A. albopictus* mosquitoes for oviposition purposes. On the other hand, an investigation of mosquitoes in a peat swamp forest in Narathiwat Province of Southern Thailand, revealed larvae of three mosquito species in *N. mirabilis* pitcher plants (Apiwathnasorn, Samung, Prummongkol, Panasoponkul, & Loymek, 2009). Although some observations have been reported concerning mosquito larvae in *Nepenthes* spp. pitcher plants, there remain many *Nepenthes* species that need investigation with reference to larval mosquitoes. Moreover, the relationship of larval mosquito species and prevailing environmental conditions, such as pitcher size, height of pitcher from ground, pitcher color, abundance of microorganisms and detritus in the pitcher, pH, and electrical conductivity prevailing in *Nepenthes* spp. pitcher plants have not been investigation previously.

Therefore, the present study on *Nepenthes mirabilis* var. *mirabilis* in Songkhla Province of Thailand was conducted with specific objectives as follow: (1) identify mosquito species inhabiting *N. mirabilis* var. *mirabilis*; (2) determine the relationship between larval mosquitoes and pitcher characteristics, such as size, color, height of pitcher from ground, and environmental conditions including abundance of microorganisms, detritus, water pH, electrical conductivity, and presence of predatory insects.

2. Materials and Methods

2.1 Study site

The study sites supporting natural populations of pitcher plant, *N. mirabilis* var. *mirabilis,* is located in Bangklam District of Songkhla Province, Thailand (7° 01' 41.4” N and 100° 22' 27.6” E). The climate of Songkhla Province is tropical with, high temperature and humidity levels; dry season remains from middle of January to April and rainy season prevails from May to January (Seephueak, Petcharat, & Phongpaichit, 2010).

2.2 Field sampling of larval mosquitoes and their taxonomic identification

The pitcher plant, *N. mirabilis* var. *mirabilis*, population at the study site was observed to be relatively small. Moreover, at the study site pitcher plants were observed to have insufficient water for blossoming during the dry season and due to this reason blossoming population of this plant were not available all the time. Specifically, growth and reproduction of pitcher plants were supported by their pitcher traps; therefore, sufficient numbers of pitchers were required to fulfill the objectives of the study and the entire population could not be depleted for sampling purpose. Thus, a total of 50 pitchers, containing 25 lower (ca. 10 cm above ground level) and 25 upper pitchers (height ca. 50 cm above ground level) were collected from the field twice, once during rainy season (September-November, 2017) and once during dry season (February-April, 2018). In the laboratory, each pitcher was carefully observed to collect mosquito larvae and the collected larvae were suitably labeled and preserved in 95% alcohol. Thereafter, permanent slides of the larvae were made and the larvae were identified to species level using standard keys based on external morphology of third and fourth instars (Rattanarithikul *et al.*, 2010; Rattanarithikul, Harbach, Harri son, Panthusiri, & Jones, 2005b; Rattanarithikul, Harrison, Panthusiri, & Coleman, 2005a, 2007; Rattanarithikul, Harrison, Panthusiri, Peyton, & Coleman, 2006;).

2.3 Biometric data and measurement of environmental parameters

Air temperature, relative humidity, and soil temperature at the study site were recorded during field sampling of pitcher plants. To collect a pitcher, it was cut from the plant at the petiole of the leaf by using knife. Height of a pitcher in meters from soil surface up to the tip of lip of pitcher plant was measured by using a suitable measure stick. Width of pitcher opening of each sampled pitcher was measured at the broadest point of the opening while the length of a pitcher was measured from the pitcher's spur to the pitcher bottom by using a caliper (Bauer, Clemente, Renner, & Federle, 2012). The color of a pitcher was estimated according to Nastase, De La Rosa, and Newell (1995) whereas, fluid in the pitcher was estimated according to Nastase, De La Rosa, and Newell (1995). The pH of pitchers fluid was measured by using a digital pH meter (Selvan, Jebanesan, Divya, & Ramesh, 2015) and electrical conductivity of pitcher fluid was measured by using electrical conductivity meter as used by Yee, Kneitel, and Juliano (2010). The pitcher fluid was transferred to a labeled vial in order to measure the fluid volume. The volume of active zone of pitcher was measured by filling the empty pitcher's active zone with water and then transferring water into the vial for measurement purpose. All pitcher fluid samples were processed according to (Hoekman, 2011) to separate detritus and microorganisms. The detritus was dried at 50 °C for 48 hours to measure its weight by using digital electric balance (Yee, Kneitel, & Juliano, 2010). Food sources such as Protozoa, Rotifera, etc., were estimated all pitcher fluids. Pitcher fluids were centrifuged for 10 minutes and each pitcher fluid sample was examined by using an auto-pipette to pick up fluid 100 µl of the fluid on a hemocytometer slide to count microorganisms.

2.4 Data analysis

Measured characteristics of *N. mirabilis* var. *mirabilis* were analyzed for correlation analysis. Spearman rank correlation coefficients were used to determine influence of pitcher characteristics on abundance of each larval mosquito species. Chi-square test was applied to determine the significance of the association mosquitoes between rainy and dry seasons. All data analysis was carried out using "R" program version 3.5.1 (R Core Team, 2018).

3. Results

3.1 Larval mosquito composition in field collected *Nepenthes mirabilis* var. *mirabilis*

A total of 546 mosquito larvae belonging to two genera and three species were collected during rainy and dry seasons. Among these collections, larval of *Tripteroides tenax* were the highest in number (526) forming 96.34% of the total larval collection, while the total number of *Tripteroides* sp.1 amounted to 11 (2.01%), and *Toxorhychites albipes* to 9 (1.65%). Thus, the density of larval mosquitoes per pitcher amounted to: *Tripteroides tenax*, 5.26; *Tripteroides* sp.1, 0.11; and *Toxorhychites albipes*, 0.09. The abundance of larval mosquitoes differed between rainy and dry seasons; their abundance was significantly higher during rainy season than during dry season ($x^2 = 43.93$, $P = 0.01$) (Figure 2).

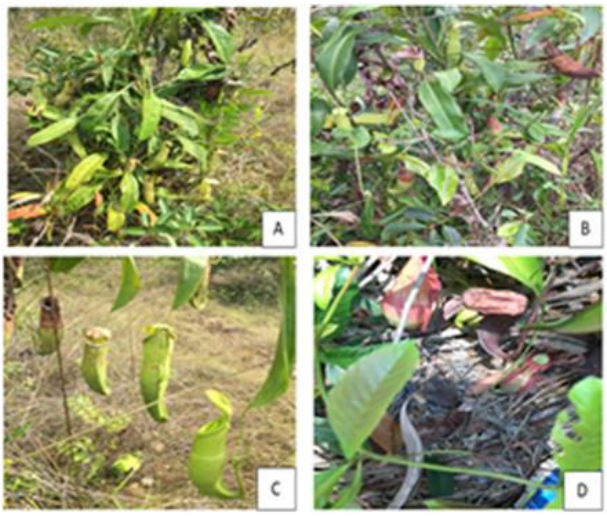

Figure 1. A. *N. mirabilis* var. *mirabilis* population. B. A pitcher plant *N. mirabilis* var. *mirabilis*. C. Upper pitchers *N. mirabilis* var. *mirabilis*. D. Lower pitchers of *N. mirabilis* var. *mirabilis*.

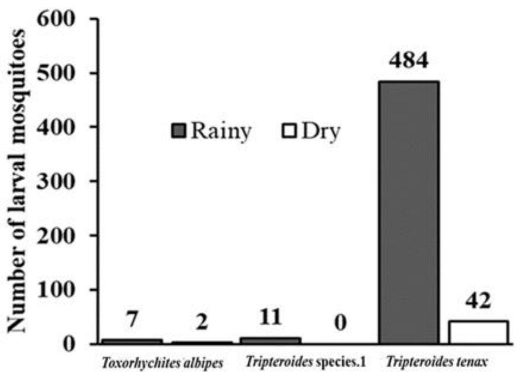

Figure 2. Abundance of larval mosquito species in the study area during rainy and dry seasons.

3.2 Correlation of pitcher plant *Nepenthes mirabilis* var. *mirabilis* characteristics and abundance of mosquito larvae

The length and width measurement data of *Nepenthes mirabilis* var. *mirabilis* revealed the mean length value of 12.07±2.68 cm and mean width (opening of peristome) 2.84±0.68cm. Mean value of actual fluid per pitcher was 9.67±7.00 ml with active zone volume amounting to 19.91±10.43 ml. The dry detritus weight per pitcher was 0.47±0.87g. The mean pH of fluids was 5.51±1.15 and electrical conductivity was 72.91±10.43µs/cm. The mean number of Protozoa was 8000.28±16301.22 and Rotifera 2719.56±7581.1. Correlation analysis of pitcher charac teristics revealed significant positive correlation with pitcher variables, such as pitcher length, dry detritus, active zone, and actual fluids. Abundance of Protozoa was also positively correlated with actual fluids, active zone, and dry detritus. Similarly, abundance of Rotifera was positively correlated with volume of actual fluids. However, mean pH value of pitcher fluids was negatively correlated with pitcher size, dry detritus, actual fluids, and active zone (Table 1).

Abundance of larval mosquitoes in pitchers was correlated with pitcher characteristics. Positive relationship between larval mosquito abundance and active zone and actual fluids was noted. On the other hand, larval mosquito abundance was negatively correlated with prevailing pH, and electrical conductivity. Abundance of *Tripteroides tenax* was significantly associated with actual fluids ($P<0.01$) and active zone ($P<0.05$) but was negatively correlated pH ($P<0.05$). The abundance of *Tripteroides* sp.1 was also positively correlated with actual fluids ($P<$ 0.05). Abundance of all species were negatively associated with electrical conductivity ($P<0.05$). No relationship between abundance of larval mosquito and pitcher size, dry detritus, abundance of Protozoa and Rotifera was observed (Table 2).

Table 1. Spearman's correlation coefficient values among pitcher characteristics of *N. mirabilis* var. *mirabilis*

	Width opening	pH	Dry detritus	Active zone	Actual fluid	Electrical conductivity	Protozoa	Rotifera
Length	0.79**	-0.37**	0.36*	0.60*	0.48*	0.16*	0.12*	0.06
Width opening		-0.39*	0.36**	0.52**	0.41*	0.21*	0.14	0.14
pH			-0.17	-0.35**	-0.31**	0.02	-0.06	-0.15
Dry detritus				0.35**	0.53**	0.17	0.27**	0.08
Active zone					0.62**	-0.11	0.29**	0.15
Actual fluid						-0.13	0.53**	0.23*
Electrical conductivity							-0.005	-0.08
Protozoa								0.35*

Note: *= P < 0.05, ** = P < 0.01

Table 2. Spearman's correlation coefficients among the pitcher characteristics relating abundance of larval mosquitoes to individual pitcher parameters in Songkhla Province, Thailand

Variables	*Tripteroides tenax*		*Tripteroides* species.1		*Toxorhychites albipes*	
	r_s	*P*	r_s	*P*	r_s	*P*
Length (cm)	0.14	0.15	-0.008	0.93	0.02	0.79
Width opening (cm)	0.043	0.66	0.05	0.56	-0.10	0.28
pH	-0.21*	0.03	-0.027	0.78	0.11	0.25
Dry detritus weight (g)	0.11	0.24	0.10	0.30	-0.01	0.80
Active zone (ml)	0.22*	0.02	0.10	0.30	-0.02	0.83
Actual fluid (ml)	0.47**	0.00	0.23*	0.01	0.05	0.58
Electrical conductivity (µs/cm)	-1.42**	0.00	-0.24*	0.01	-0.40	0.00
Protozoa abundance	0.16	0.09	-0.03	0.75	0.10	0.32
Rotifera abundance	0.04	0.66	-0.02	0.80	-0.02	0.79

Note: * = $P < 0.05$, ** = $P < 0.01$

4. Discussion

4.1 Correlation of pitcher characteristics

The pH of fluid in *N. mirabilis* var. *mirabilis* pitchers was negatively correlated with amount of detritus, pitcher size, active zone, and actual fluids. Bauer, Willmes, and Federle (2009) revealed that pH of pitcher fluid decreased after opening independent of the amount of prey. After pitcher opening, pitchers were realized to secrete protons consistent with proteolytic enzymes secretion in order to provide similar pH optimum of the enzymes to respond to digestion of the present prey items (Morrissey, 1955). Recent investigation supports that a number of prey items in pitcher were digested into nutrients including ammonium, which support pitcher plant nutrient deficiency. All ammonium elements from prey digestion were known to induce decline pH of pitcher fluid (Higashi, Nakashima, Ozaki, Abe, & Uchiumi, 1993; An, Fukusaki, & Kobayashi, 2001). Therefore, increase in amount of prey items in pitcher fluids probably influence decline in pH of fluid.

Active zone or digestive zone of pitchers covered half of the inside of pitcher length and had glandular surface that developed from single epidermal cells. All glands located in digestive zone at bottom of the pitcher most likely had two functions: to produce acidic digestive fluid which contained digestive enzymes and antimicrobial compounds, and acidic polysaccharides (Buch *et al.*, 2013, Gaume & Forterre, 2007). The changing shape and size of glands depended on the level of the digestive zone (Wang, Zhou, Zheng, & Xu, 2009). Large size pitchers probably have many glands to produce sufficient amount of enzymes to respond to the amount of prey items in pitcher fluids. Neprosin and aspartic proteases enzyme were isolated from digestive fluids of *Nepenthes* and had been realized to be active only during decrease in pH of pitcher fluids (Athauda *et al.*, 2004; Schrader *et al.*, 2017). This suggests that most enzymes were secreted from pitcher's glands due to decline in pH level.

The low pH of pitcher fluids under low water level conditions was essential for digestion efficiency of prey for gaining nutrients in relatively short time. Moreover, pitcher fluids were secreted from inside the pitcher while pitchers were not opened but pitcher fluids were mixed with water rain during pitcher opened their lips. This point of view, pitcher fluids pH may be probably positively correlated with increasing water in pitcher. However, most pitcher fluids of *Nepenthes* contained viscoelasticity that plays important role to kill and retain preys. Rost and Schauer (1997) found that viscosity was strongly based on pH levels. Alternatively, viscoelasticity was also dependent on a polysaccharide and decreased gradually by microbial and infauna digested polysaccharide (Gaume & Forterre, 2007). Consequently, pitcher fluids pH decreased as polysaccharide was digested even though rainy water added in pitcher fluids.

The positive correlation among measurements of pitcher size, dry detritus, active zone, actual fluids, and electrical conductivity were significant. Characteristics of pitcher size, which include width of pitcher hood and pitcher length, were significant related positively. Similarly, Heard (1998); Nastase, De La Rosa, and Newell (1995) found that width of hood of pitcher was positively related with length and other pitcher measurements. The amount of dry detritus was indicated to have positively correlation with pitcher size and actual fluids (Nastase, De La Rosa, & Newell, 1995).

4.2 Abundance of larval mosquitoes in relation to pitcher characteristics

Three species of mosquito larvae were collected from pitchers of *N. mirabilis* var. *mirabilis* in this study with *Tripteroides tenax* being the predominant species. This observation is consistent with the previous finding of Mogi and Chan (1997) who had reported that *Tripteroides tenax* was dominant filter feeder in *Nepenthes* species. The larvae of *Toxorhynchites albipes* have large body size and are known as predators. The previous observation of Apiwathnasorn, Samung, Prummongkol, Panasoponkul, and Loymek (2009) also reported three larval mosquito species surviving in pitchers of *N. mirabilis*; however, the mosquito species in their study were different compared to the present study. Variation of mosquito species occurring in pitcher plants depends upon their distribution.

The present study results show that abundance of larval mosquitoes was relates to pitcher detritus, actual fluid, and pitcher size, pH, and abundance of some microorganisms. The presence of detritus in habitat of mosquitoes, particularly in pitcher plants, plays an important role as main food resources to support microorganisms such as, bacteria, Protozoa, and Rotifera (Duguma, Kaufman, and Simas Domingos, 2017). Hoekman (2007) found that density of larval mosquitoes in pitcher was based on detritus amount that was digested by microorganisms in order to obtain nutrients for supporting their population. Alternatively, it has been reported that abundance of microorganisms, such as Protozoa and Rotifera in pitchers declined dramatically when larval mosquitoes were present (Hoekman, 2011; Trzcinski, Walde, & Taylor, 2005). Thus, amount of detritus in pitchers is an essential food resource and correlated positively with abundance of larval mosquitoes.

Gravid female mosquitoes were affected by morphological characteristics of pitchers. Gravid female mosquitoes preferred to deposit their eggs in large size pitchers, perhaps to ensure that the larvae would have better survival possibility (Heard, 1994). The results of present study indicate that actual mean volume of fluids (9.67±7.00 ml) in *N. mirabilis* var. *mirabilis*, was rather small and may not be attractive for gravid female mosquitoes to deposit their eggs (Chou, Dykes, Wilson, & Clarke, 2016). Chou, Dykes, Wilson, and Clarke (2016) had reported their observations concerning mosquito oviposition in *N. ampullaria* pitchers and suggested that the smaller pitcher size was unattractive to gravid female mosquitoes for oviposition. However, mosquito larval presence and survival in pitchers having very low fluid levels indicates that such low levels cannot deter gravid mosquitoes to oviposit their eggs in pitchers. Giusto, Grosbois, Fargeas, Marshall, and Gaume (2008) suggested that *Nepenthes* pitchers have ability to emit sweet scent for insect attraction particularly flying insects. Furthermore, in fluid of open as well as unopen pitchers of the genus *Nepenthes*, diverse bacteria was discovered (Takeuchi *et al.*, 2015). The process of changing abundance of bacteria and digestion of detritus with other organics in containers may produced metabolites that attracted gravid mosquitoes for oviposition (Ponnusamy, Wesson, Arellano, Schal, & Apperson, 2010).

The color of containers has been realized to serve as visual cue to attract gravid female mosquitoes. Green color of *N. mirabilis* var. *mirabilis* was not attractive to gravid female mosquitoes particularly *Aedes* spp. (Chou, Dykes, Wilson, & Clarke, 2016; Torrisi & Hoback, 2013). Results of the present study are consistent with previous observations that low densities of larval mosquitoes per pitcher prevailed in *N. mirabilis* var. *mirabilis*.

The presence of larvae in pitchers was not only dependent upon the ability of gravid female mosquitoes to seek a suitable oviposition site but also included the tolerance of mosquito larvae to cope with the prevailing environmental conditions. The results of present study indicate that abundance of microorganisms was negatively correlated with larval mosquito abundance (Table 2). Trzcinski, Walde, and Taylor (2005) had reported that abundance of microorganisms play an important role in survival of mosquito larvae in pitchers. Additionally, positive relationship between detritus and mosquitoes indicated that detritus was necessary food resource for microorganisms and larval mosquitoes consume microorganisms. Therefore, abundance of microorganisms had significant negative correlation with abundance of larval mosquitoes. Nevertheless, pitcher habitats can provide food resources for survival of mosquito larvae but larval mosquito density was still low in pitchers of *N. mirabilis* var. *mirabilis*. The lower density of larval mosquitoes in pitchers may be caused by the pH levels. The present study results revealed mean pH levels of 5.51±1.15 of pitcher fluid *Nepenthes mirabilis* var. *mirabilis*. This pH level was suggested to have slightly lethal condition, which impact on oxygen transportation and osmoregulation of larval mosquito species (Selvan, Jebanesan, Divya, & Ramesh, 2015). Moreover, the results of present study showed that during samples collection red crab spiders, *Misumenops nepenthicola*, existed and constructed their nests on surface wall inside pitchers. The observation of Chua and Lim (2012) supported that *Misumenops nepenthicola* were predators of *Tripteroides* spp. and density mosquito larvae decreased dramatically in pitchers where the red crab spiders were present. Thus, the lower density larval mosquitoes in pitchers in the present study might be due to the presence of red spiders in pitchers.

The present study results show that there was no or little correlations between the predators mosquito, *Toxorhynchites* and *Tripteroides tenax* (r_s = 0.19, P = 0.05), *Toxorhynchites* and *Tripteroides* sp.1 (r_s = 0.040, P = 0.69). Larvae of all mosquito species were not interacting with each other but were precisely responding in a similar fashion to pitcher environmental factors (Table 2). Also, *T. tenax* and *Tripteroides* sp.1 may have developed behavioral trait of avoiding predators, such as species of *Toxorhynchites*. Larval mosquitoes are consumed by *Toxorhynchites* because of their behavioral movement; mosquito larvae that are active for extended time period move to the surface of the fluid to breathe and become exposed to predations, such as *Toxorhynchites*. This potential body movement of larvae, e.g., of *A. albopictus* creates vibration in the water column that predators detect to chase them and consume them (Chou, Wilson, Dykes, & Clarke, 2015). In the present study, detailed behavioral observations of *Toxorhynchites albipes* were not made; also, no *Aedes* mosquito larvae in *N. mirabilis* var. *mirabilis* pitchers were observed to occur. *Nepenthes mirabilis* var. *mirabilis* may provide suitable oviposition site for gravid female *Aedes* mosquitoes as *Aedes albopictus* larvae occurred in pitchers of *Nepenthes ampullaria*. Also, *Aedes dybasi* Bohart and *Aedes maehleri* Bohart were found in *N. mirabilis* pitchers on the islands of Pala and Yap (Sota & Mogi, 2006). Pitcher plants, *N. mirabilis* var. *mirabilis*, populations in the present study were located in a rural area and due to this reason the encounter frequency of gravid female *Aedes* mosquitoes for egg laying purpose in pitchers was much reduced. Nevertheless, due to rapid urban expansion and destruction of forest habitats, the possibility of *Aedes* mosquito habitation in pitchers of *N. mirabilis* var. *mirabilis* may increase. Reference (Yee, Kneitel, & Juliano, 2010) suggested that detritus was the best predictor of presence *Aedes* spp. larvae, whereas microorganisms were strongly predictors of occurrence of larvae *Culex* spp. mosquitoes. Further studies are needed on the interaction of environmental conditions of *N. mirabilis* var. *mirabilis* and *Aedes* and *Culex* larval mosquitoes where *Nepenthes* spp. occur near human populations and support *Aedes* and *Culex* mosquitoes.

The presence or absence of mosquito larvae in pitchers may be influenced by microclimate at the observation site. The present study revealed abundance of larval mosquitoes during rainy season compared to dry season. This finding is agreement with that of Nastase, De La Rosa, and Newell (1995) who explained that the pitchers were likely to dry out during summer and anaerobic water full of decomposing materials does not support mosquito larvae and eggs deposited therein.

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