

**Mosquito aquatic predator diversity and its abundance
at Phetchaburi Province, Thailand**

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Abstract

This research investigates mosquito control strategies at Kaeng Krachan Dam, Thailand, emphasizing the negative environmental impacts of traditional chemical insecticides. The study explores the role of predatory aquatic insects in regulating mosquito larvae populations and assesses the impact of distance from the dam on mosquito and predator diversity. Findings reveal a higher concentration of mosquito larvae in closer proximity to the dam, with earthen containers (jars, cisterns, etc.) showing the highest larval abundance. The study advocates for environmentally friendly alternative mosquito control measures and proposes community-based interventions for disease prevention. Collaborative efforts with STEM professionals and advanced data analysis techniques enhance the study's interdisciplinary nature.

Keywords: GLOBE Observer: MHM App, Mosquito Predator, Thailand

1.Introduction

The natural regulation of mosquito larvae relies on a diverse cast of predators. Though predatory aquatic insects have received notable attention in research (Knight et al., 2003; Tuno et al., 2005; Mogi, 2007; Quiroz-Martinez & Rodriguez-Castro, 2007; Shaalan et al., 2007; Forio et al., 2020), further investigation reveals a broader range of potential predators. Besides insects, other organisms contributing to mosquito larvae control in diverse habitats include arachnids, crustaceans, amphibians, fish, birds, and even mammals.

Dengue is a complicated illness with several presentations, including dengue fever, dengue hemorrhagic fever, and dengue shock syndrome/expanded dengue syndrome (WHO, 1997; 2011). This disease is of significant concern in tropical and subtropical areas, especially Southeast Asia (WHO, 2009; 2012). Dengue has been endemic in Thailand for >60 years, with an unpredictable outbreak pattern (Diseases Control Department, 2015). A retrospective study of dengue outbreaks in 2014, 2015, 2016, 2017, and 2018 reported morbidity rates of 63.25, 222.58, 96.76, 79.55, and 128.41 cases/100,000 inhabitants and mortality rates of 0.10%, 0.10%, 0.10%, 0.12%, and 0.13%, respectively (Vector Borne Diseases Division, 2019).

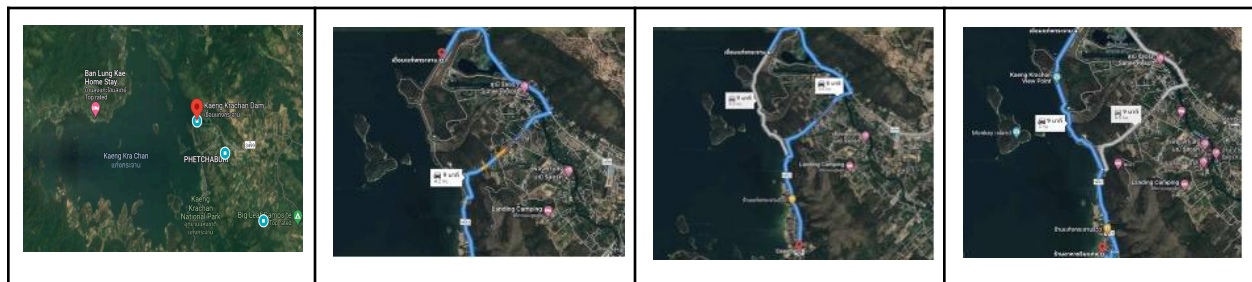
Thailand is one of many countries with have a dengue fever problem. The outbreak has been going on for more than 50 years with a higher morbidity rate than the average for the past 13 years (1997 to 2009). The central region has a higher dengue fever rate than other regions of Thailand. 2020, the central region had the highest sickness rate, 18.06 per 100,000 people. In 2020, the Department of Disease Control reported that the central region had 4,052 dengue cases with a case fatality rate of 0.10 percent (DDC, 2020). From epidemiological disease surveillance data in 2023, there were 79,475 cumulative cases of dengue fever, calculated as an illness rate of 120.25 per 100,000 population, with 73 deaths as a death rate of 0.09 per 100,000 population.

In this study, we investigate whether the distance from the dam affects the diversity of mosquito larvae and predators at Kaeng Krachan Dam, Phetchaburi Province, Central Thailand. To test this, we conducted our mosquito larvae and predator survey at three sites: (1) within 1 km from Kaeng Krachan Dam, (2) 3 km from Kaeng Krachan Dam, and (3) 5 km from Kaeng Krachan Dam. We randomly selected 30 houses at each sampling site to collect mosquito larvae and mosquito predators in all outdoor containers within 15 m of a house. We also consider the following factors: (1) natural/artificial containers, (2) metal/ plastic / earthen containers, (3) containers with lid / without lid, (4) water levels (0-25%, 26-50%, 51-75%, 76-100%), (5) water temperature, (6) water pH, (7) water salinity, (8) positive/negative containers, (9) mosquito species: *Aedes* (*Ae. albopictus*, *Ae. aegypti*), *Culex spp.*, *Armigeres*, *Toxorhynchites* (10) predator species: *Arachnida*, *Crustacea*, *Odonata*, Heteroptera, Coleoptera, Diptera, fish, and Amphibia.

2. Materials and methods

2.1 Study site

Survey of mosquito larvae and aquatic insect predators was conducted at Kaeng Krachan Dam Phetchaburi Province, the Central Thailand (12.9170° N, 99.6300° E) November-December 2023. In Phetchaburi Province, there are three seasons: summer season (mid-February to mid-May), rainy season (mid-May to mid-October), and winter season (Mid-October to mid-February).



(a)map Phetchaburi	(1b) 1 km from Kaeng Krachan Dam	(2b) 3 km from Kaeng Krachan Dam	(3b) 5 km from Kaeng Krachan Dam
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Figure 1. (a) Map of Phetchaburi and (b) Map of study sites at Kaeng Krachan Dam Phetchaburi Province **[the maps were unclear to brad when proofreading]**

2.2 Sampling of mosquito larval predators and mosquito larvae.

In this study, we surveyed three areas with 30 houses per area and then collected samples of mosquito larvae and larval predators for classification. We collected all samples from every water container, both with and without water, and we measured pH, temperature, and salinity in containers with water.

We recorded the water level of the container and whether or not it had a lid. We scooped up mosquito larvae and predators into plastic bags. We used the GLOBE Observer: MHM app to locate water container latitude and longitude coordinates, the number of mosquito larvae, and the mosquito species in the GLOBE database. The mosquito larvae and larval predators were classified up to the species level.

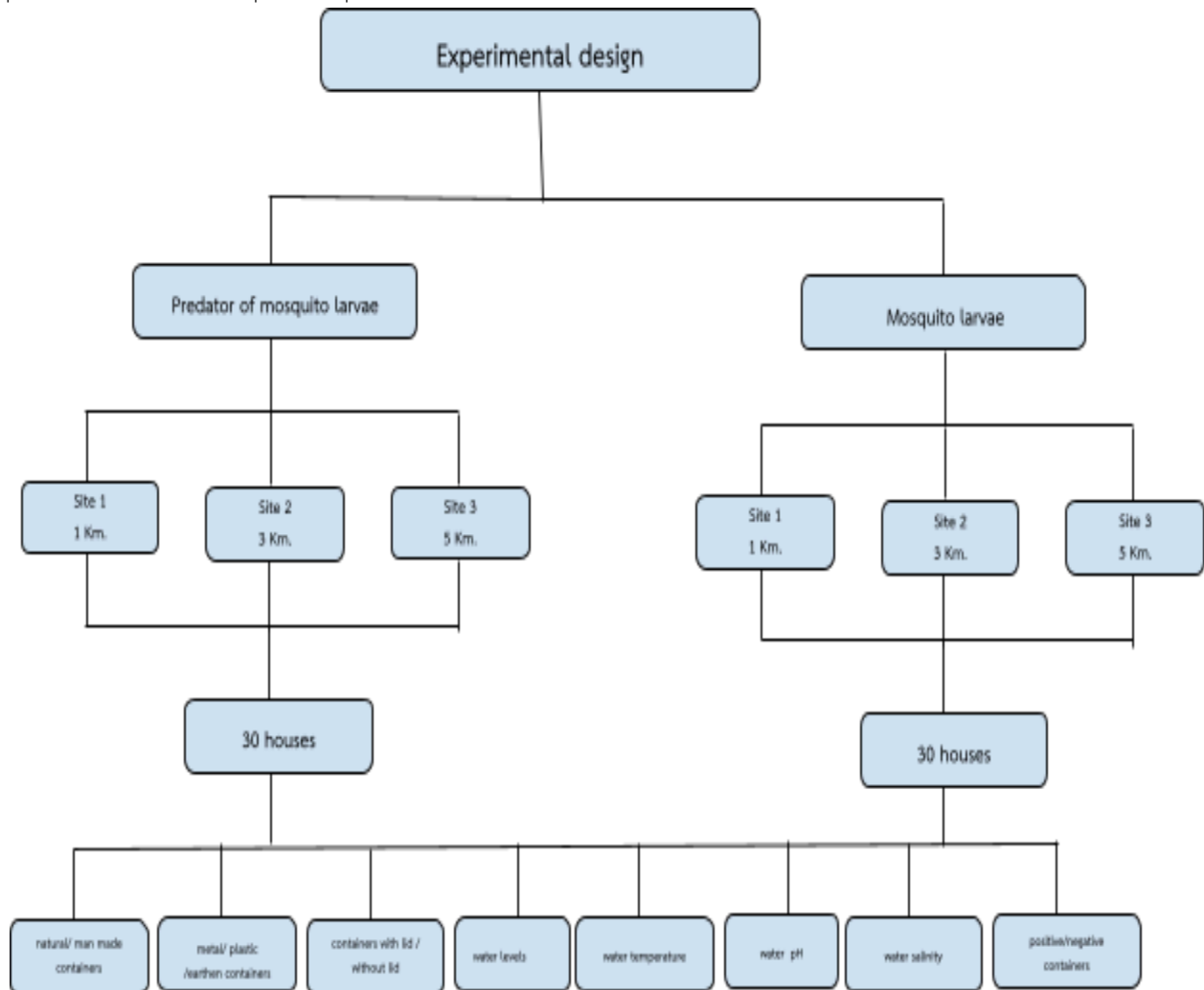


Figure 2. Experimental design in the study

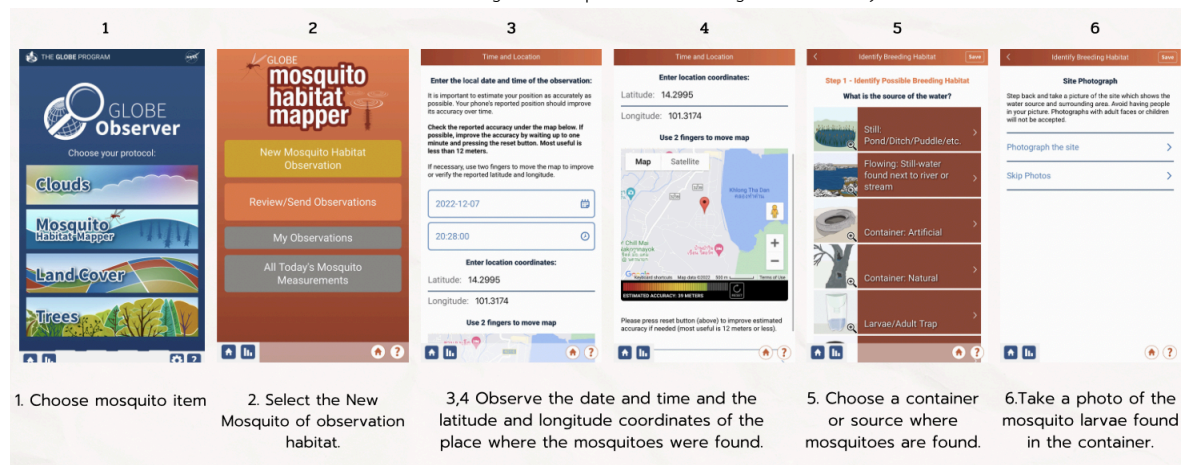


Figure 3. GLOBE Observer: MHM app

2.3 Data collection

In this study, there were three sampling sites: (1) within 1 km from Kaeng Krachan Dam, (2) 3 km from Kaeng Krachan Dam and (3) 5 km from Kaeng Krachan Dam. We randomly selected 30 houses at each sampling site to collect mosquito larvae and mosquito predators in all outdoor containers within 15 m of a house.

All probable habitat types found at every site were recorded, classified, and inspected for the presence or absence of mosquito larvae and their predators. Each habitat was dipped 20 times using a standard mosquito dipper. A D-frame dip net of 0.3 m width attached to a long pole and with a cone-shaped bag for capturing the mosquito larvae predators was used. The captured mosquito larvae and predators were immediately preserved in 90% ethanol for further identification. Water physico-chemical parameters (pH, temperature, conductivity, total dissolved solids (TDS), and salinity) were measured in situ using a hand-held multi-parameter- meter.

2.4 Entomological studies

All water containers were sampled for mosquito larvae and predators in a rural community living at Kaeng Krachan Dam, 1-5 km from the dam. Tiny water containers were emptied through the containers. Larger water containers were sampled by dipping the net in the water, starting at the top of the container and continuing to the bottom in a swirling motion that sampled all edges of the container. All live mosquito larvae were collected in plastic bags, taken to the laboratory, preserved, and identified up to species level using Rattanarithkul and Panthusiri's keys. This study discarded the first-second instars and pupae because immature mosquitoes could not be identified at these stages. There were a total of 129 container categories in this study. Plastic water containers were divided into two categories: large plastic containers used for water storage (>100 L) and plastic bottles (i.e., 0.5–2.0 L water bottles). Earthen jars were classified into two categories: small earthen jars with a volume of ≤100 L and large earthen jars with a volume of >100 L.

Water containers that were found at each house were classified as (1) natural/artificial containers, (2) metal/ plastic / earthen containers, (3) containers with lid / without lid, (4) water levels (0-25%, 26-50%, 51-75%, 76-100%), (5) water temperature, (6) water pH, (7) water salinity, (8) positive/negative containers. The mosquito larvae were identified as follows:

Aedes (*Ae. albopictus*, *Ae. aegypti*), *Culex* spp., *Armigeres*. Predator species were identified as Arachnida, crustacea, Odonata, heteroptera, Coleoptera, Diptera, fish, and amphibia.

2.5 Statistical analysis

All variables were tested for normality using the Komogorov-Smirnov test. The equality of variances was evaluated using Levene's test. Descriptive statistics of the data were analyzed. The number of mosquito larvae in different types of water containers, the number of positive containers, the number of households with positive containers, and the number of mosquito Larvae were analyzed using a one-way ANOVA test. The number of mosquitoes was analyzed using the Chi-square test. The relationship between water parameters, the number of mosquitoes, and the number of predators was analyzed using simple linear regression. All significant tests were two-tailed.

Three larval indices (i.e. house index (HI), container index (CI) and Breteau index (BI)) were worked out as per standard WHO guidelines. There were three larval indices calculated as per standard WHO guidelines: House Index (HI), Container Index (CI), and Breteau Index (BI). House index (HI) was calculated by the number of cheerful houses divided by the total number of houses inspected timed 100. The container index (CI) was calculated by the number of positive containers divided by the total number of containers inspected timed 100. Breteau index (BI) was calculated by the number of positive containers divided by the total number of houses inspected timed 100.

3. Results

Ae. aegypti and Ae. albopictus larvae

In other containers, we found 5 car tires containers, 3 puddles containers, 2 drain containers, 1 paper containers. In Kaeng Krachan Dam area, we found 41 plastic containers, 56 earthen containers. From all four types of water containers, we found the highest number of mosquito larvae in earthen containers (17 larvae), followed by other containers (13 larvae) and lowest in metal containers (0 larvae) (Table 1).

Table 1. The number of households ($X \pm SD$) that had indoor/outdoor water containers in three topographical areas: 1 km from Kaeng Krachan Dam, 3 km from Kaeng Krachan Dam and 5 km from Kaeng Krachan Dam at Phetchaburi Province, Thailand

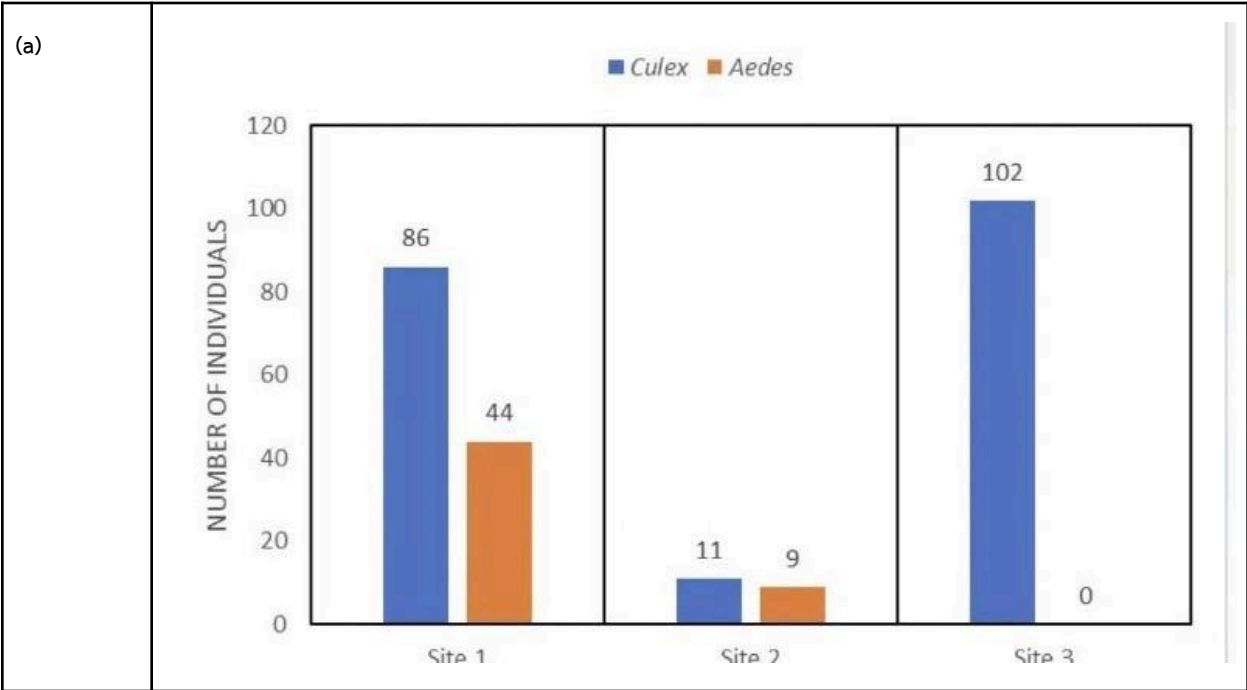
(*P<0.05, **P<0.01, ***P<0.001)

Container types	The number of water container			
	1 km from Kaeng Krachan Dam	3 km from Kaeng Krachan Dam	5 km from Kaeng Krachan Dam	Number of mosquito larvae
Metal	0	0	0	0
Plastic	3	3	5	11
Earthen	9	3	5	17
Other container	5	6	2	13

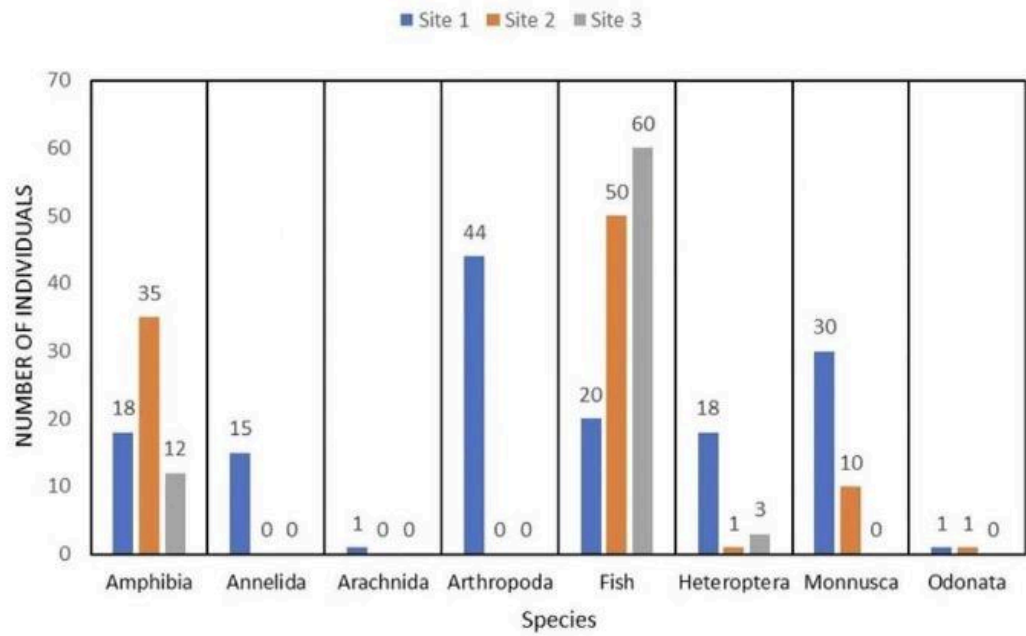
	The number of water container
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Container types

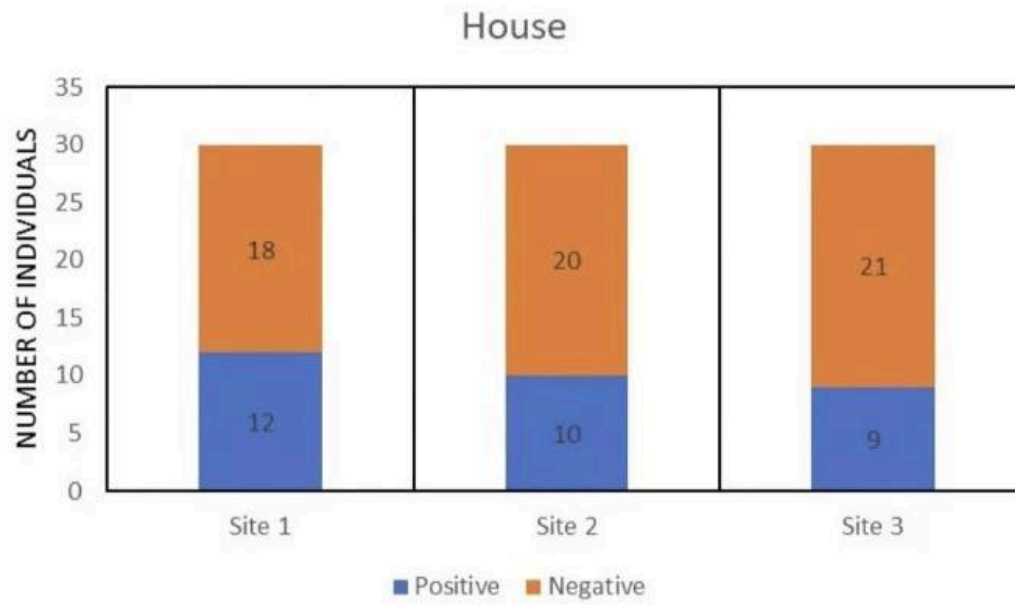
	1 km from Kaeng Krachan Dam	3 km from Kaeng Krachan Dam	5 km from Kaeng Krachan Dam	Number of predators
Metal	0	0	0	0
Plastic	1	4	2	7
Earthen	4	1	2	7
Other container	3	9	4	16



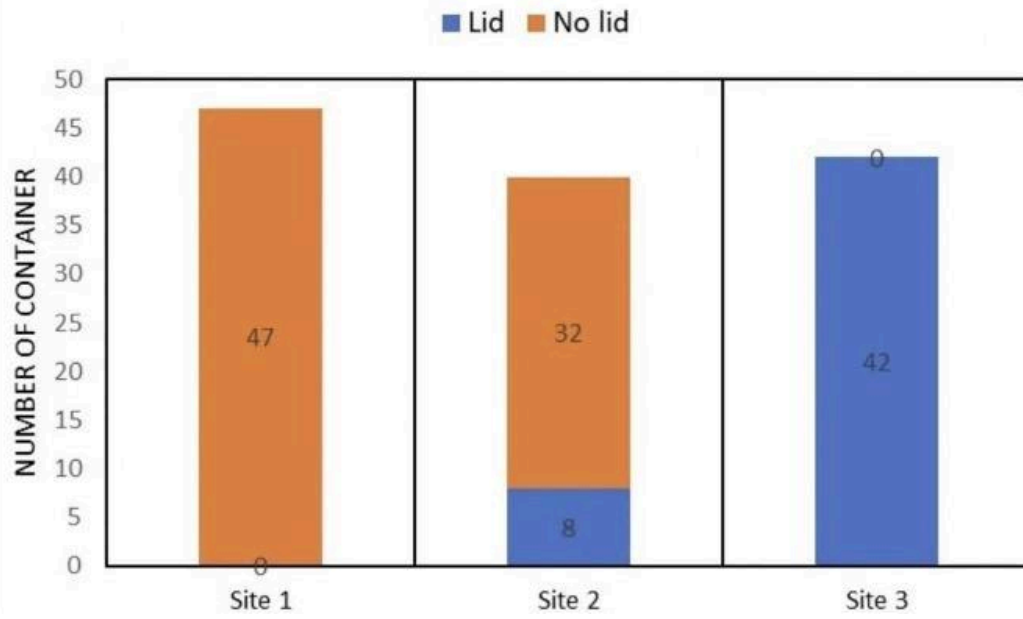
(b)



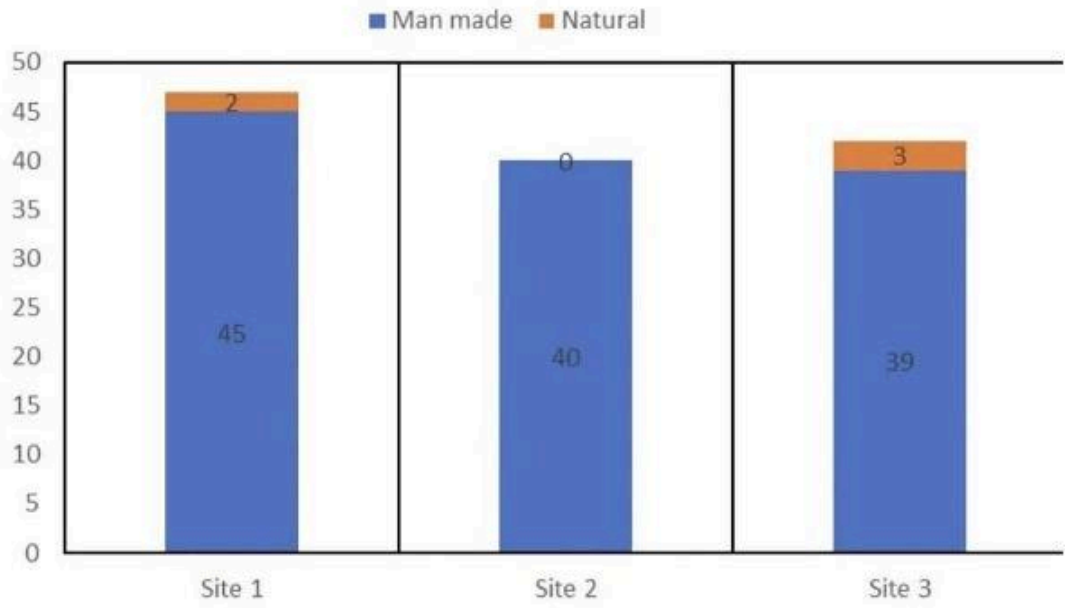
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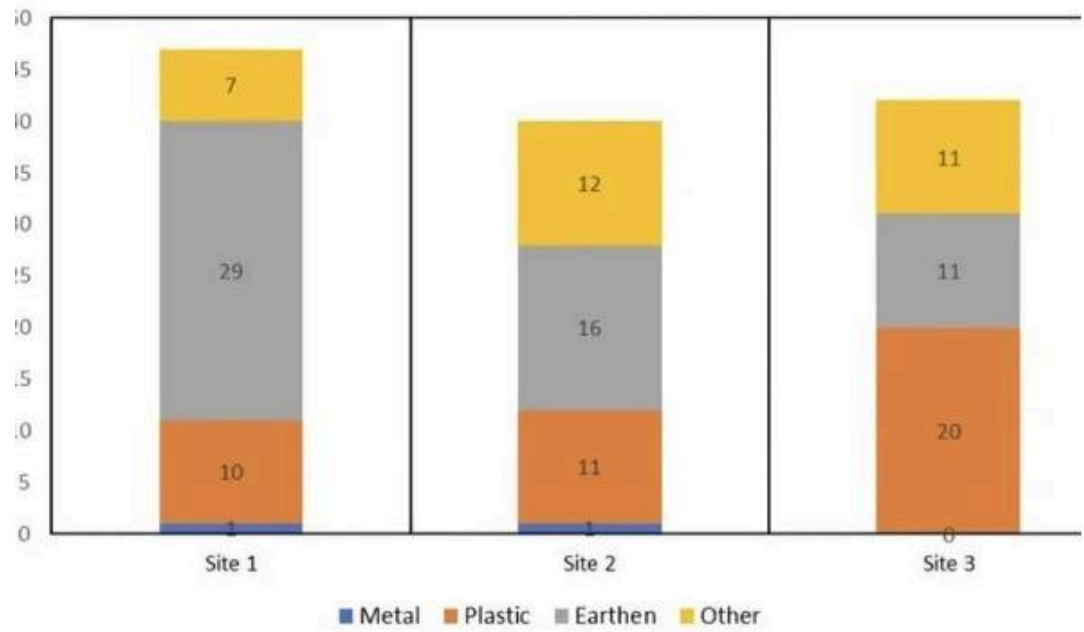
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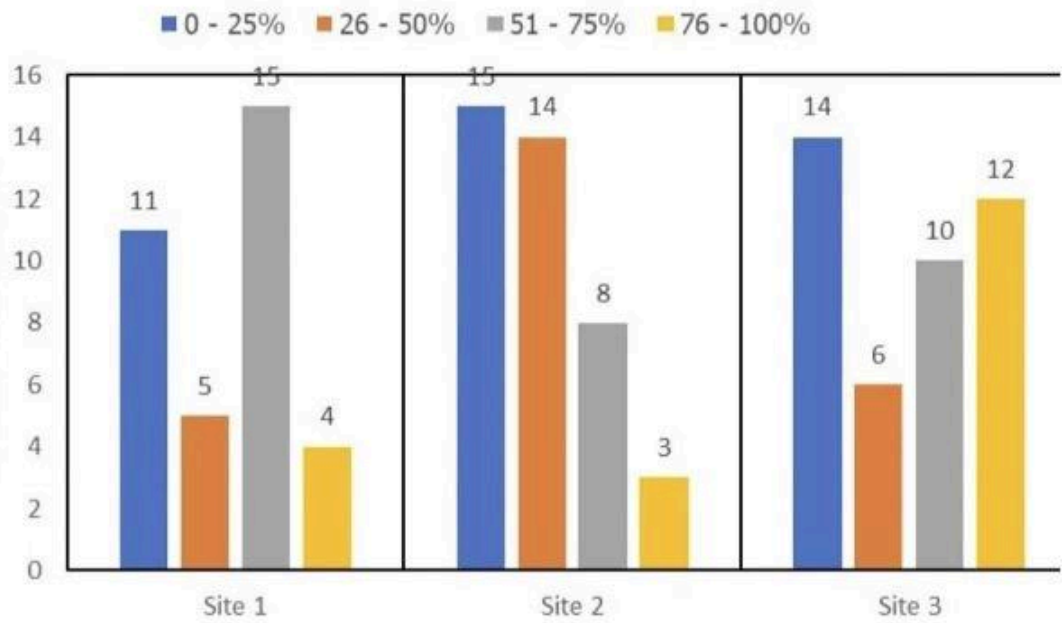
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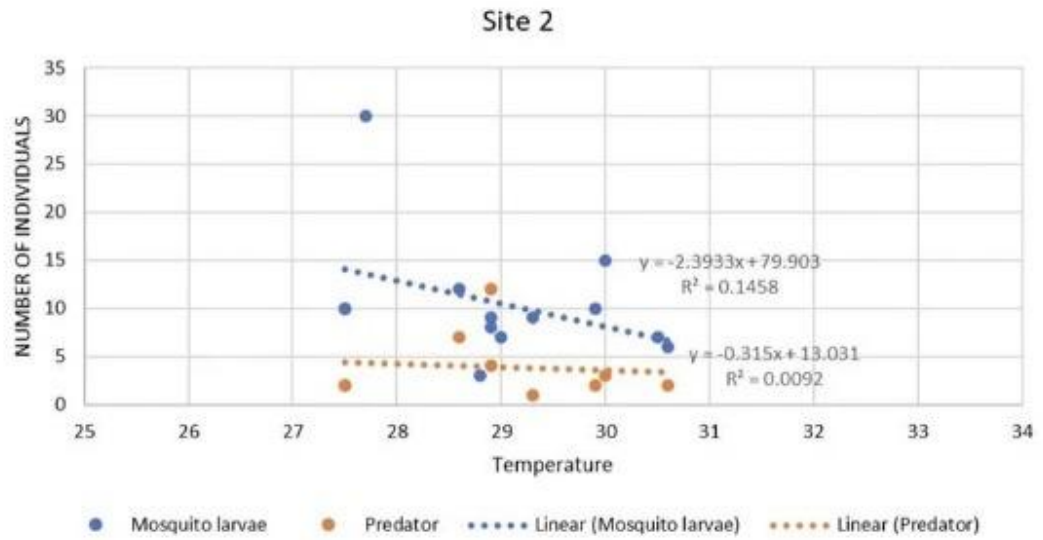
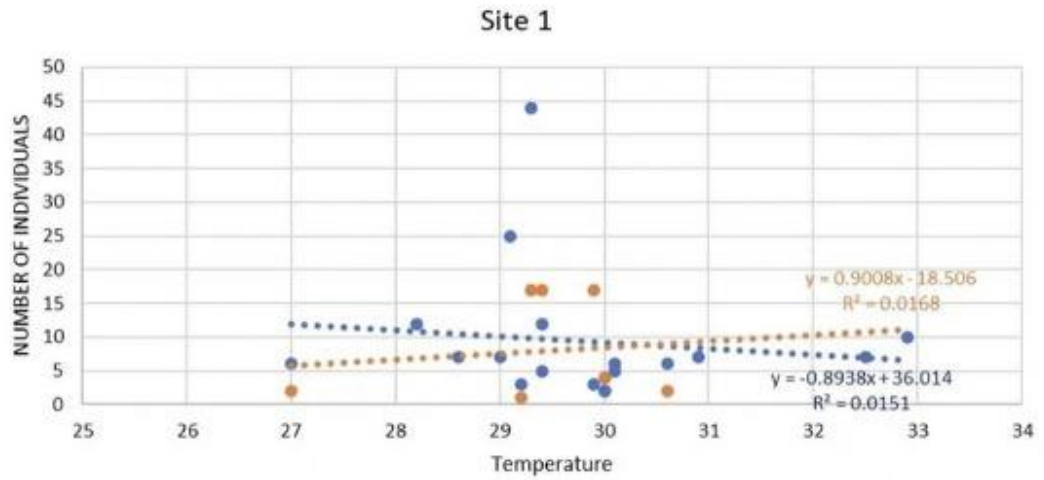
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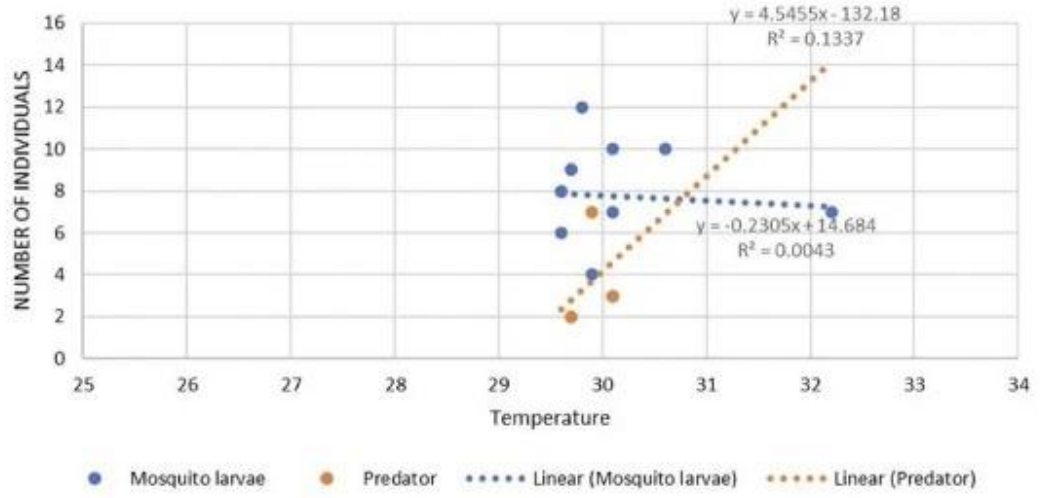
(g)



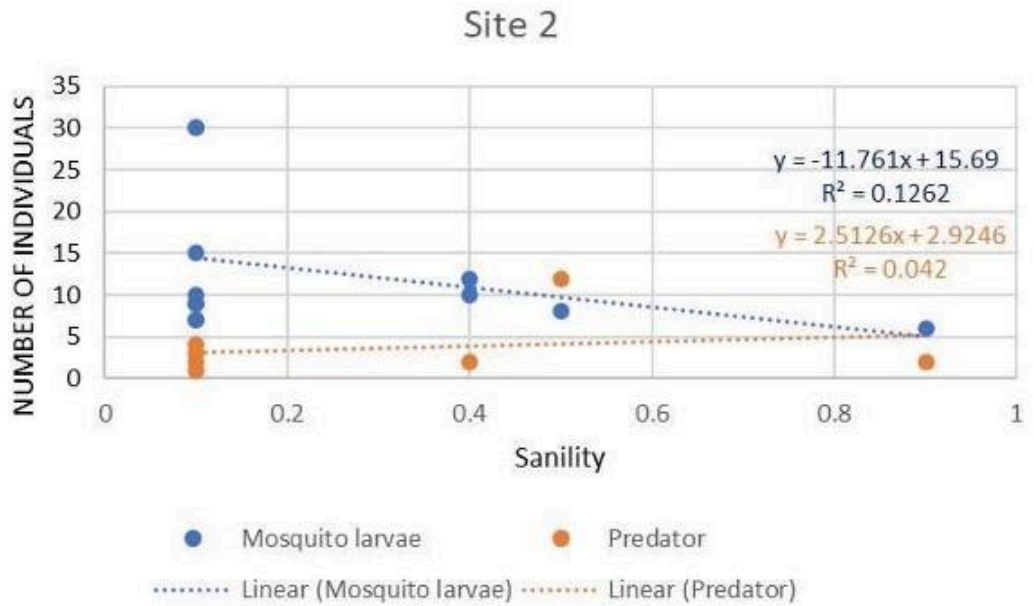
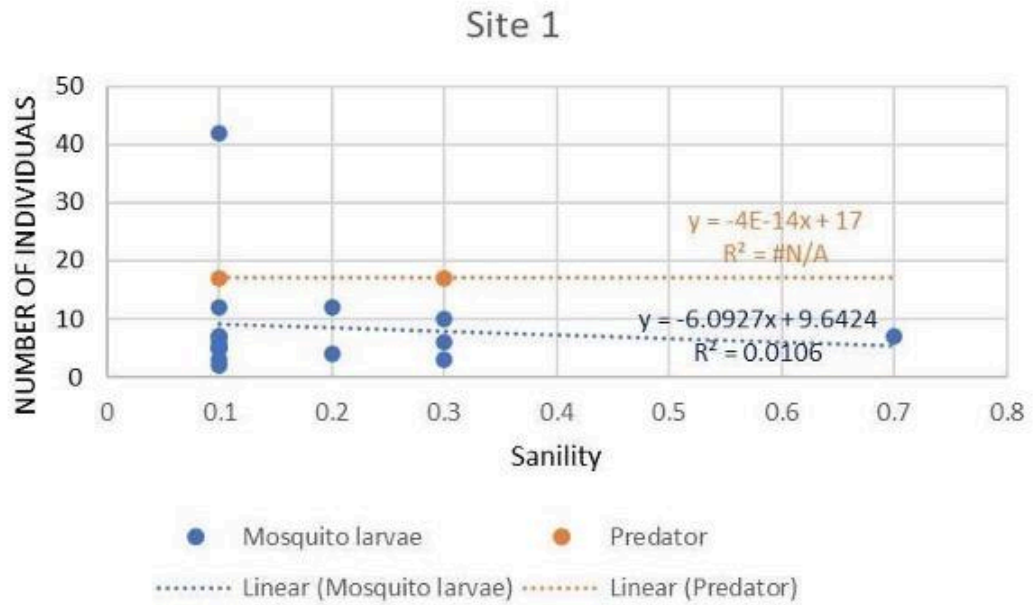
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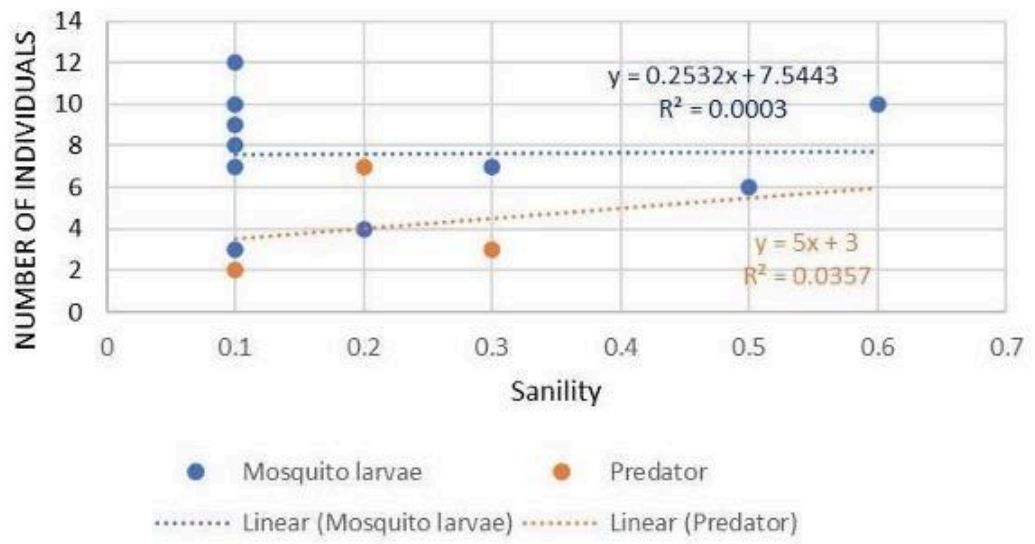
Site 3



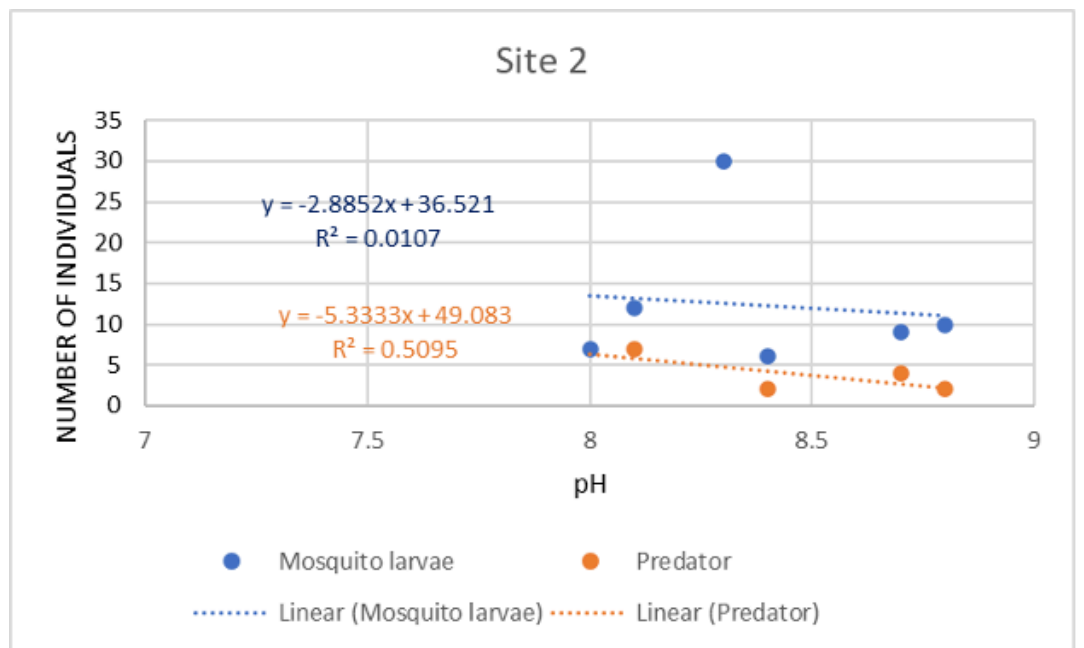
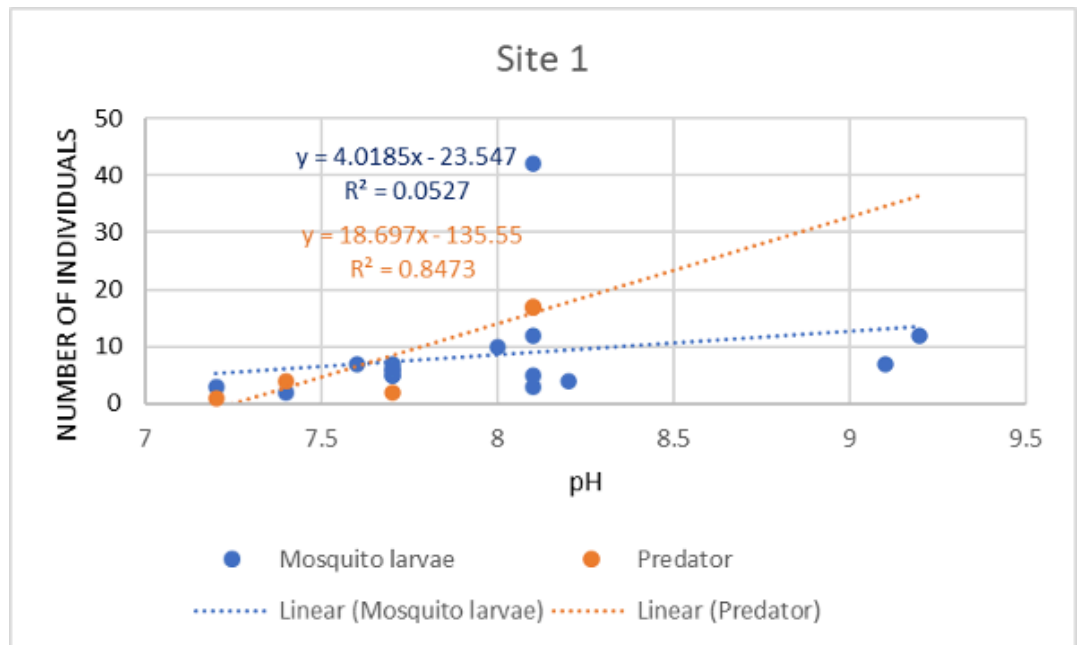
(i)



Site 3



(j)



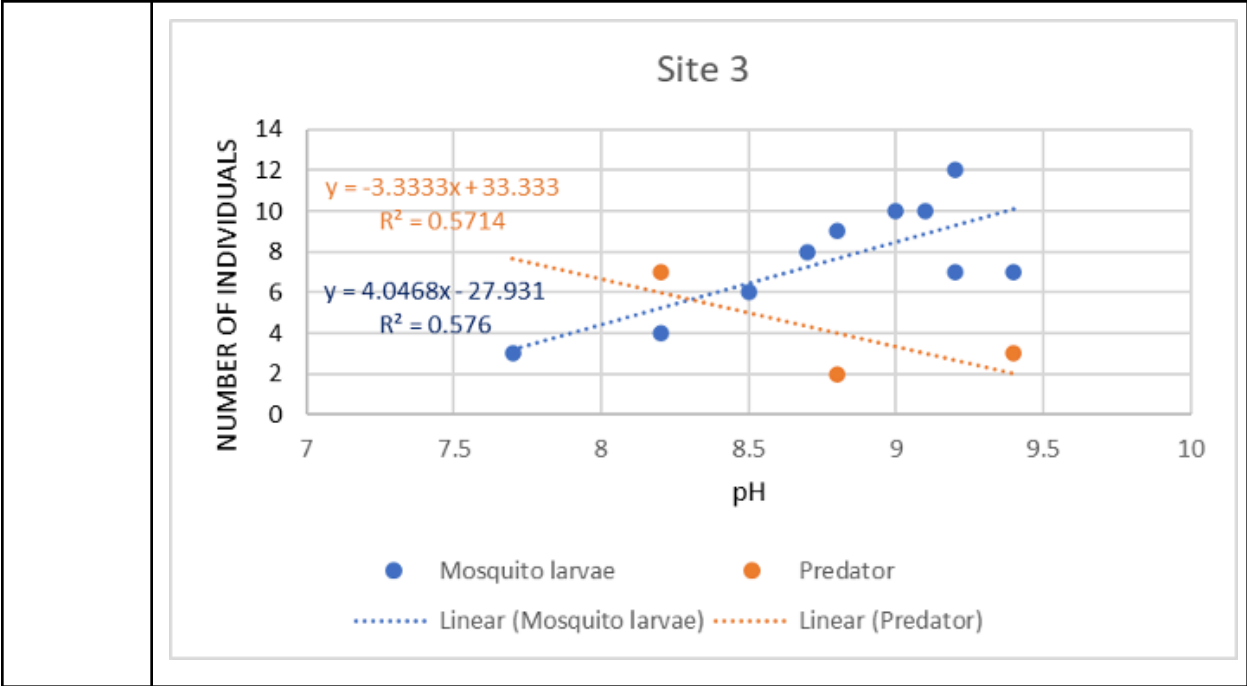


Figure 2. Water containers predators and mosquito larvae. (a) *Ae. aegypti*, *Ae. albopictus*, *Culex* spp., *Armigers* spp. and *Toxorhynchites* spp., (b) Predator species: Amphibia, Annelida, Arachnida, Arthropoda, Fish, Heteroptera, Monnusca, Odonata., (c) containers: breeding sites, (d) lid / without lid, (e) natural/man made container, (f) metal/ plastic /earthen /other containers, (g) water levels (0-25%, 26-50%, 51-75%, 76-100%), (h) water temperature, (i) water salinity and (j) water pH

pH

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.115 ^a	.013	-.014	.61670

a. Predictors: (Constant), Mosquito larvae

ANOVA^a

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.182	1	.182	.480	.493 ^b
	Residual	13.691	36	.380		
	Total	13.874	37			

a. Dependent Variable: Water pH

b. Predictors: (Constant), Mosquito larvae

Coefficients^a

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	8.171	.158		51.772	.000
	Mosquito larvae	.009	.013	.115	.693	.493

a. Dependent Variable: Water pH

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.247 ^a	.061	.035	1.18457

a. Predictors: (Constant), Mosq

ANOVA^a

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	3.288	1	3.288	2.343	.135 ^b
	Residual	50.515	36	1.403		
	Total	53.803	37			

a. Dependent Variable: Temperature

b. Predictors: (Constant), Mosq

Coefficients^a

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	30.046	.303		99.113	.000
	Mosq	-.037	.024	-.247	-1.531	.135

a. Dependent Variable: Temperature

Ae. aegypti, Ae. albopictus, Culex spp. , Armigeres spp. and Toxorhynchites spp.

From the survey of all 3 areas, it was found that in area 1 Found 86 Culex spp. mosquito larvae and 44 Aedes mosquito larvae in area 2. Found Culex spp. mosquito larvae and 9 Aedes mosquito larvae in area 3. Found 102 Culex spp. mosquito larvae and no Aedes mosquito larvae found. (Figure 2a).

Predator species: Amphibia, Annelida, Arachnida, Arthropoda, Fish, Heteroptera, Monnusca, Odonata.

Based on the survey conducted in all three areas, the findings indicate the distribution of various animal species. Amphibia was discovered in all three areas, with area 2 showing the highest occurrence. Annelida was exclusively found in area 1, while Arachnida and Arthropoda were also limited to area 1. Fish, on the other hand, was observed in all three areas, with area 3 having the highest prevalence. Heteroptera were present in all three areas, with area 1 having the highest numbers. Monnusca was identified in both area 1 and area 2, and Odonata was also observed in area 1 and area 2. (Figure 2b).

Containers / Breeding Sites

According to the survey, the highest number of water containers and the highest prevalence of containers being used as breeding grounds for mosquito larvae were found in Area 3, which is located approximately 5 kilometers away from the Kaeng Krachan Dam. Conversely, the lowest occurrence of such containers was observed in area 1, which is only 1 kilometer away from the dam. (Figure 2c).

Lid / Without Lid Containers

The survey revealed that in Area 1, only 47 containers were discovered without lids. In Area 3, only 42 containers with lids were found. In Area 2, both containers with lids and containers without lids were identified, with 8 containers having lids and 32 containers lacking lids. (Figure 2d).

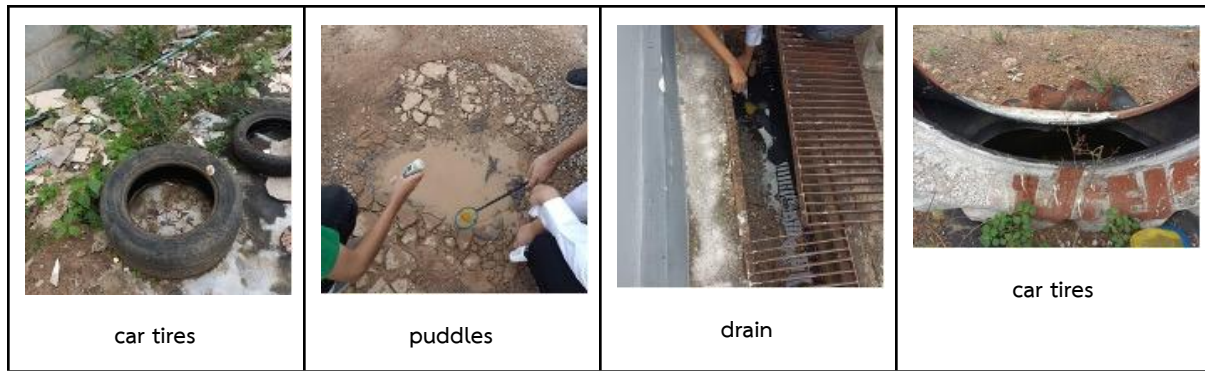
Natural /Man made Containers

The survey results indicate that in Area 1, 45 man-made containers and 2 natural containers were discovered. In Area 2, only 40 man-made containers were found. In Area 3, 39 man-made containers and 3 natural containers were identified. (Figure 2e).

Metal/ Plastic /Earthen /Other Containers

After examining the container types across all three areas, it was discovered that there are four categories: metal, plastic, earthen, and other containers. In Area 1, the highest number of earthen containers was observed, totaling 29, while the lowest number of metal containers was just one. Similarly, in Area 2, the maximum number of earthen containers was 16, with only one metal container. In Area 3, the predominant containers were plastic, totaling 20, and no metal containers were encountered. (Figure 2f).

Other Containers



Water Levels (0%, 25%, 50%, 75%, 100%)

Based on the survey findings, in Area 1, the prevailing water level falls within the range of 51-75%, while in both Area 2 and Area 3, the most frequent water level is in the 0-25% range. (Figure 2g).

Water Temperature

In the vicinity of Kaeng Krachan Dam, within a 1 km radius, container water temperatures averaged between 29-30 °C. At a distance of 3 km, the containers maintained an average temperature of 28-29 °C. In the farthest area, 5 km from Kaeng Krachan Dam, container water temperatures averaged around 32.2 °C. (Figure 2h).

Water salinity

In the areas within a 1 km radius of Kaeng Krachan Dam, the average water pH in containers measured 0.1. Similarly, at a distance of 3 km and 5 km from Kaeng Krachan Dam, the water pH in containers maintained an average of 0.1.(Figure 2i).

Water pH

Within the regions situated 1 km from Kaeng Krachan Dam, the average water pH in containers was around 7.7 pH. In the 3 km vicinity from Kaeng Krachan Dam areas, the containers exhibited an average water pH of 8.8. Lastly, in the areas 5 km away from Kaeng Krachan Dam, the containers showed an average water pH ranging between 9 and 9.5. (Figure 2j).

Larval indices

Within the areas located 1 km from Kaeng Krachan Dam, both the total number of containers and the number of positive containers surpassed those in the regions situated 3 km from Kaeng Krachan Dam areas, as indicated in Table 2. Moreover, the House Index (HI), Container Index (CI), and Breteau Index (BI) in the 1 km radius from Kaeng Krachan Dam areas were notably higher compared to the corresponding indices in the regions 3 km and 5 km away from Kaeng Krachan Dam. (Table 2).

Table 2: This table indicates the number of households and containers, and larvae indices in 1 km from Kaeng Krachan Dam areas, 3 km from Kaeng Krachan Dam areas and 5 km from Kaeng Krachan Dam areas.

	1 km from Kaeng Krachan Damareas	3 km from Kaeng Krachan Damareas	5 km from Kaeng Krachan Damareas
No. of households	30	30	30
No. of positive households	12	10	8
No. of containers	47	40	42
No. of positive containers	16	12	9
Larval index			
HI (%)	40	30	26.7
CI (%)	34.04	30	21.4
BI	53.3	40	30

Households that had water containers

4. Discussion

Expanding upon the results, the study underscores the importance of container preferences in shaping mosquito breeding dynamics. This observation aligns with the prior investigations conducted by Knight et al. (2003) and Tuno et al. (2005), accentuating the prevalence of earthen containers as primary breeding sites, particularly in the immediate vicinity of the dam. This underscores the imperative for targeted interventions that account for specific container materials and characteristics to successfully mitigate mosquito proliferation.

An insightful addition to the discussion involves the examination of environmental factors. The study unravels variations in water temperature, pH, and salinity across different areas. Integrating these factors into the analysis provides a nuanced understanding of their potential impact on mosquito breeding and the presence of predators. This aligns with the research conducted by Quiroz-Martinez & Rodriguez-Castro (2007), who emphasize the importance of considering environmental variables in mosquito control strategies.

The calculated larval indices present a noteworthy revelation, indicating a higher risk of dengue transmission in the 1 km zone. This aligns with the findings of Shaalan et al. (2007), underlining the critical role of spatial analysis in assessing disease risk. The study provides crucial information for public health planning and prioritizing mosquito control efforts, contributing to the growing body of research on the epidemiology of mosquito-borne diseases.

The practical implications are seamlessly integrated, deriving insights from the study's findings. It echoes the sentiments expressed by Mogi (2007) and Quiroz-Martinez & Rodriguez-Castro (2007), endorsing the implementation of community-based interventions. The proposed recommendations, including the promotion of specific container types, encouragement of lid usage, and the integration of environmental factors into control strategies, harmonize with the broader discourse surrounding sustainable and community-centric approaches to mosquito control measures.

The survey reveals a notable discrepancy in mosquito species abundance across the three areas. In Area 1, a higher number of *Culex* spp. larvae (86) is observed compared to *Aedes* mosquito larvae (44). This distribution is consistent with the ecological preferences of *Culex* spp., which are known to exploit a broader range of breeding habitats compared to *Aedes* species (Rochlin et al., 2013). In contrast, Area 3 displays a higher concentration of *Culex* spp. larvae (102) with no *Aedes* mosquito larvae found. This unexpected absence of *Aedes* larvae in Area 3 prompts further investigation into the ecological factors influencing the distribution of these mosquito species.

The observed pattern of mosquito species distribution, with higher *Culex* spp. prevalence closer to the dam, aligns with the findings of a study by Liu et al. (2017) conducted in wetland ecosystems. Liu et al. proposed that the preference of *Culex* spp. for habitats in proximity to water bodies is linked to their adaptation to humid environments. The current study's alignment with such findings underscores the ecological consistency of mosquito species preferences in diverse geographical settings.

The distribution of predator species across the surveyed areas introduces an additional layer of complexity to the ecosystem. Amphibia, noted for their larval consumption, exhibit a higher occurrence in Area 2, suggesting a potential correlation between amphibian abundance and mosquito larvae control (Perrin et al., 2023). The exclusive presence of Annelida in Area 1 and the limited occurrence of Arachnida and Arthropoda in the same area resonate with the findings of Silva et al. (2020), who identified these organisms as key predators in freshwater habitats.

The variations in predator species distribution may be influenced by diverse environmental factors, including water temperature, pH, and salinity. Research by Lumpkin and Will Patrick. (2020) highlights the sensitivity of aquatic predators,

such as Heteroptera and Odonata, to water quality parameters. The highest occurrence of Heteroptera in Area 1, coupled with their sensitivity to environmental changes, suggests a potential correlation between their abundance and water quality parameters.

5. Result

The study elucidates the relationship between container types and mosquito larvae abundance, underscoring the role of materials and container characteristics in influencing breeding. The spatial distribution of containers across surveyed areas revealed varying preferences, with earthen containers dominating the 1 km radius and plastic containers gaining significance in the 3 km and 5 km zones. The consistent prevalence of containers without lids in all areas raises concerns about increased mosquito breeding, emphasizing the importance of lid promotion or alternative strategies. Examining environmental factors, the study found variations in water temperature, pH, and salinity across different areas, potentially impacting mosquito breeding and predator presence. Calculated larval indices indicated a higher risk of dengue transmission in the 1 km zone, providing crucial information for public health planning and mosquito control prioritization. The discussion references previous studies, highlighting the significance of predator-prey relationships in mosquito population control and reinforcing the current findings. Practical implications are emphasized, suggesting community-based interventions for mosquito control based on the study's insights. Recommendations include promoting specific container types, advocating lid usage, and integrating environmental factors into mosquito control strategies. Acknowledging study limitations, such as the regional focus and exclusion of certain larval stages, the discussion calls for future research to expand the study to different regions and include additional ecological variables. This comprehensive discussion contributes valuable insights to the understanding and mitigation of mosquito-borne diseases.

I would like to claim IVSS badges

1. I make an impact

The report clearly describes how a local issue led to the research questions or makes connections between local and global impacts. The students need to clearly describe or show how the research contributed to a positive impact on their community through making recommendations or taking action based on findings. The study of mosquito larvae ecology can give the information to preserve and reduce the infection of the disease via the animal vector by reducing or changing the materials of the container.

2. I am a STEM professional.

The report clearly describes collaboration with a STEM professional that enhanced the research methods, contributed to improved precision, and supported more sophisticated analyses and interpretations of results. Data were analyzed by using independent-sampled t-tests to compare the numbers of mosquito larvae in different types of container.

3. I am a data scientist

The report includes in-depth analysis of students' own data as well as other data sources. Students discuss limitations of these data, make inferences about past, present, or future events, or use data to answer questions or solve problems in the represented system. Consider data from other schools or data available from other databases. Latitude and longitude of the area where mosquito larvae are available were recorded by using GLOBE observer, MHM application.

4. I am a problem solver

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