JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Temperature influences the development-related phenotypes of Philippine *Aedes albopictus*: implications for vector control

Frances Edillo[1], Rhoniel Ryan Ymbong[1,2], Alyssa Angel Bolneo[1], Ric Jacob Hernandez[1], Mark Windy Suycano[1], Anavaj Sakuntabhai[3,4]

[1] *Mosquito Research Laboratory, Department of Biology, University of San Carlos, Talamban campus, Cebu city, Cebu, 6000, Philippines*
[2] *Cebu Normal University, Main campus, Osmeña Blvd., Cebu city, Cebu, 6000, Philippines*
[3] *Functional Genetics of Infectious Diseases Unit, Insitut Pasteur, Rue de Docteur Roux, 75015, Paris, France*
[4] *Institut Pasteur, Japan Office, Chuo-ku, 104-0061, Tokyo, Japan*

Abstract

Introduction: This study investigated the development-related phenotypes (percent pharate larvae, PPL; hatch rates, HRs; and reproductive outputs, ROs) of *Aedes albopictus* F1 eggs; with parental eggs collected from selected highland and lowland sites of the major islands in the Philippines (Luzon, Visayas, and Mindanao) in 2018–2020.
Methodology: Field-trapped parental *Aedes* eggs were reared and coupled. *Ae. albopictus* F1 eggs were separately placed inside environmental chambers (18 °C, 25 °C, and 38 °C) for 6 weeks. PPL, HRs, and ROs were determined.
Results: Temperature had significant effects on PPL and ROs of F1 eggs of wet season-collected *Ae. albopictus*; and on PPL, HRs, and ROs of F1 eggs of dry season-collected samples. Temperature had significant effects on PPL, HRs, and ROs across all sites. No seasonal differences were found for PPL, HRs, and ROs. The means of PPL (14.01%) and HR (10.18%) were the highest at 25 °C and lowest at 38 °C (0.64% and 0.00%, respectively); mean RO (2.5) was highest at 18 °C. Pharate larvae of *Ae. albopictus* at 38 °C were first reported. PPL, HRs, and ROs were similar between highlands and lowlands in Visayas and Mindanao.
Conclusions: Temperature influences the development-related phenotypes of *Ae. albopictus*. F1 eggs can withstand extreme temperatures, hence are public health threats amidst global warming. Both *Ae. albopictus* and *Ae. aegypti* should be included in all-year round vector control strategies against *Aedes*-borne diseases in the tropics. It is recommended to install water pipelines in the rural mountains to prevent mosquito breeding sites.

Key words: *Aedes albopictus*, pharate larvae, hatching, reproductive output, temperature, Philippines.

J Infect Dev Ctries 2026; 20(2):183-194. doi:10.3855/jidc.20649

(Received 05 August 2024 – Accepted 02 June 2025)

Introduction

Aedes albopictus (Skuse) is an invasive species in the temperate regions, and is the secondary dengue vector in the Philippines that transmits chikungunya (CHIKV), yellow fever, and Zika viruses (ZIKV) [1]. *Ae. albopictus* is an outdoor diurnal feeder with multiple host preferences including humans [2], particularly in rural areas with dense vegetation [3], though limited natural breeding sites allow it to colonize artificial containers in urban areas [4]. *Ae. albopictus* originated in Southeast Asia and has expanded its geographical range to the temperate regions, where it is physiologically challenged [4] owing to human activity and climate change [5]. Moreover, *Ae. albopictus* can survive better in temperate climates than *Ae. aegypti* [6]. Likewise, tropical, subtropical, and temperate strains of *Ae. albopictus* can survive for 2, 10, and 20 days, respectively, when exposed to – 10 °C; however, all these strains survive for 30 days at – 5 °C [7]. Meanwhile, few *Ae. aegypti* responded positively with their development-related phenotypes at 38 °C under laboratory conditions [8]. Inbreeding coefficients of *Ae. aegypti* that vary seasonally and their loci indicated that selection is impacted by relative humidity (RH) in the Philippines [9]. Mosquito survival, dispersion, and transmission rates of dengue viruses (DENVs) are greatly influenced by climate change [10]. Outbreaks in dengue illness escalated in temperate areas such as in Madeira, Portugal in 2012 [11], and in Tokyo, Japan in 2014 [12]. In the Philippines, there were 195,603 suspected dengue cases, 657 deaths, and 0.34% case fatality rate in 2023 [13]. In addition, there were 2,928 chikungunya cases with 0 death between 1 January to 2 December 2023 [14]; and 57 Zika cases with 0 death as of 2 February 2017 [15]. The medical costs of dengue alone were estimated to be US $345 million using an expansion factor model [16] and at US $158.9 million using a dynamic transmission model [17]. *Ae. albopictus'* efficient adaptive behavior to colder regions enables them to further expand their distribution and contribute highly to arboviral transmission [1,18]; hence immediate public health actions are needed.

Certain mosquito species adopt egg dormancy when challenged with unfavorable environmental conditions [19]. Diapause is a genetically programmed and neurohormone-mediated mechanism that allows the survival of *Ae. albopictus* amidst harsh conditions, which cannot be easily terminated by environmental factors unless the process has ended [20]. When a female *Ae. albopictus* in the temperate regions experiences short day lengths, it sends signals to its oocytes to prepare for diapause, and the process is highly dependent on photoperiod. Greater seasonal changes in day lengths are observed within 10° of the Equator, and the physiological reaction of mosquitoes to the length of night or a dark period has been shown in several insect species [21]. The temporal occurrence of diapause depends on the geographic location of mosquito populations. The critical photoperiod (CPP) for *Ae. albopictus* increases with higher latitude, such as in Japan and North America, owing to direct exposure to light [22]; a 0.5 hour increase in CPP per 5° increase in latitude [23]. Quiescence, on the other hand, is an irregular type of dormancy, which slows down metabolic activity of *Ae. aegypti* pharate larvae and heavily relies on environmental cues [24].

Numerous vector and disease control strategies have already been undertaken to address dengue incidence; however, these efforts only partially reduce and hinder the transmission. Edillo *et al.* reported that the temperature and latitude of Luzon, Visayas, and Mindanao, the major islands of the Philippines, play an important role on the development-related phenotypes of *Ae. aegypti* in wet and dry seasons [8]. Thus, extending this study to *Ae. albopictus*, the secondary dengue vector in the country, during a different study period, and different subsites with dense vegetation covers that this species prefers for breeding, is important to understand its biological and ecological survival mechanism. The results of this study may provide valuable insights on the predictions and prevention of chikungunya, dengue, and Zika outbreaks. To understand the underlying molecular mechanism, it is also crucial to understand the functions of genes that regulate its adaptations to the changing

Figure 1. The study sites in Luzon (Baguio and Quezon cities), Visayas (Cebu city mountains and Liloan, Cebu), and Mindanao (Maramag, Bukidnon and Cagayan de Oro (CDO) city).

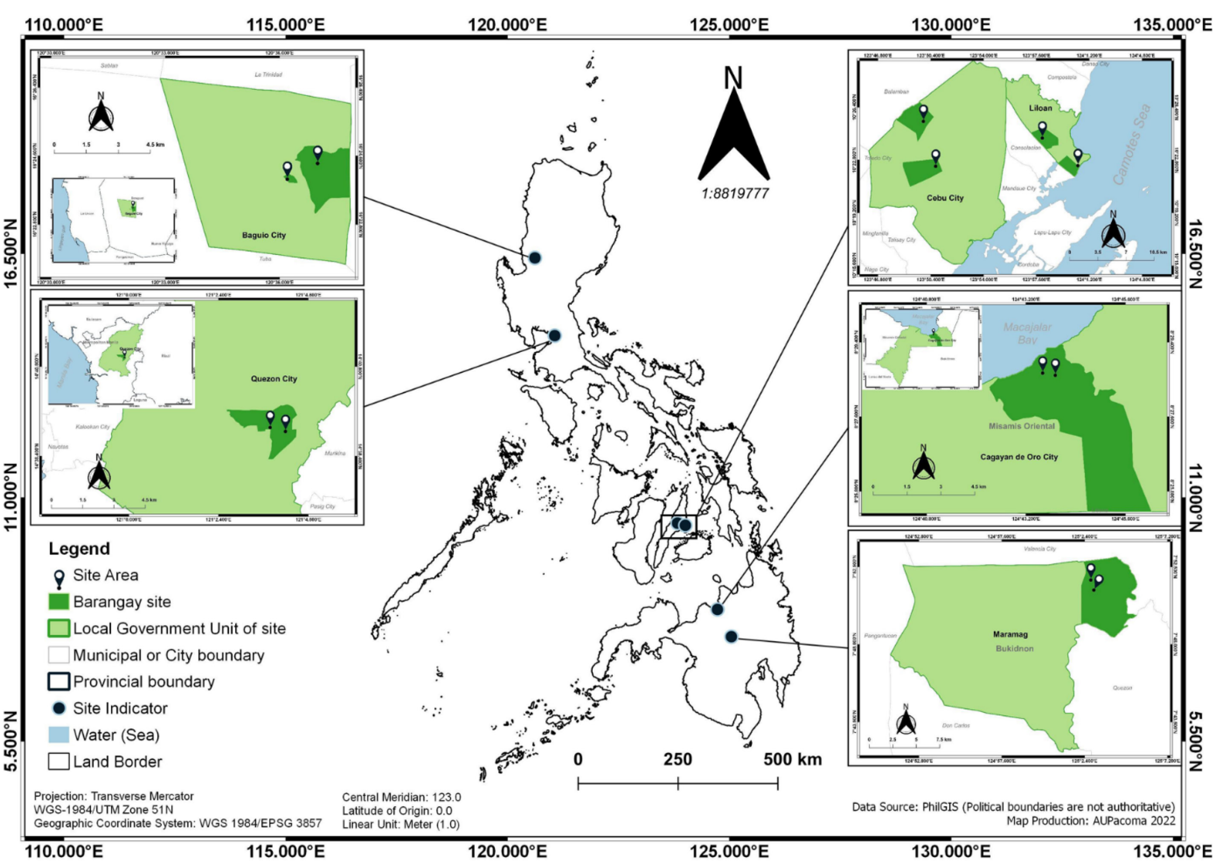


Table 1. The study sites and subsites in the Philippines for parental egg collections of *Ae. Albopictus*, along with the sample size of F1 egg cohorts.

Sites	Codes: subsites	Coordinates	Elevation (m ASL*)	Sample Size (F1)
Luzon				
Baguio city	S1: Sta. Scholastica	N 16°23.990'; E 120°36.207'	1,391	3
	S2: Country Club	N 16°24.405'; E 120°37.004'	1,473	2
	S3: Wright Park	N 16°24.965'; E 120°37.104'	1,503	0
	S4: Leonard's wood	N 16°41.222'; E 120°60.806'	1,437	0
Quezon city	S1: Arboretum, UPD[α]	N 14°39.064'; E 121°04.189'	68	33
	S2: Albert Hall, UPD	N 14°39.170'; E 121°03.777'	74	33
Visayas				
Cebu city mountains	S1: Taptap	N 10°25.439'; E 123°49.904'	540	25
	S2: Babag 2	N 10°22.384'; E 123°50.735'	344	33
Liloan, Cebu	S1: Suso, Catarman	N 10°22.428'; E 124°00.429'	19	18
	S2: Sta. Cruz	N 10°24.302'; E 123°58.002'	85	29
Mindanao				
Maramag, Bukidnon	S1: Market, CMU[β]	N 07°51.151'; E 125°02.931'	324	15
	S2: Forestry, CMU	N 07°51.732'; E 125°02.757'	372	31
Cagayan de Oro city	S1: Tablon	N 08°28.601'; E 124°43.906'	23	31
	S2: Nestle, Tablon	N 08°28.654'; E 124°43.602'	20	33

*Meters above sea level; [α] University of the Philippines – Diliman, Quezon city; [β] Central Mindanao University, Maramag, Bukidnon.

climate [19]. The hypothesis in this study was that *Ae. albopictus* can undergo egg dormancy as its adaptive mechanism to climate change. Hence, this work investigated the hatching rate and viability of *Ae. albopictus* F1 eggs from selected highland and lowland sites in the major islands of the Philippines after separate laboratory exposure to temperature regimes.

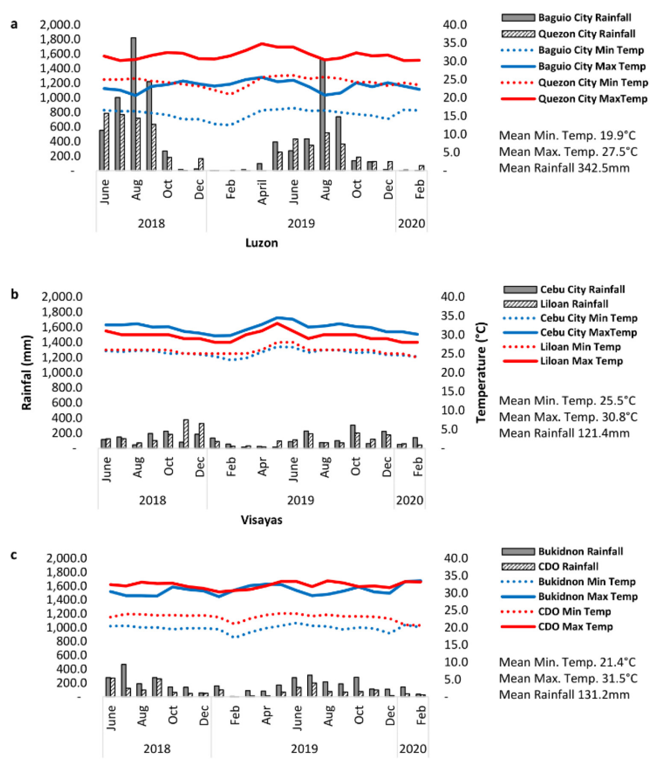
Methodology

Study sites

Sites with dense vegetation cover in each of the major islands (Luzon, Visayas, and Mindanao; Figure 1) were selected according to: 1) elevation (lowlands with < 100 meters above sea level [m ASL]; highlands with > 300 m ASL), 2) varying coordinates of the major islands (Table 1), 3) dengue occurrence, and 4) biotype similarity derived from modified Corona's climate classification [25]. Highland sites in Luzon, Visayas, and Mindanao included Baguio city; mountainous *barangays* (i.e., smallest government units) in Cebu city; and Maramag, Bukidnon; respectively; whereas lowland sites covered Quezon city; Liloan, Cebu; and Cagayan de Oro (CDO) city, respectively (Figure 1). Table 1 shows that the study sites varied in their coordinates, in which Luzon was located at 14.39°N latitude and 121°E longitude, Visayas was located at 10.22°N latitude and 124°E longitude, and Mindanao was located at 7 °N latitude and 125 °E longitude. The longitude coordinates of these major islands represented light intensity although measuring the latter was beyond the scope of this study. Adult *Aedes* can fly as far as 400 m [26]; thus, each site had two subsites that were 500 m or more away from one another. Table 1 shows the different subsites within each site because of the species' preferred breeding in areas with dense vegetation, in reference to previous work [8] conducted for *Ae. aegypti* except for the two subsites in Cebu city mountains.

Meteorological data such as monthly temperatures and mean monthly rainfall (Figure 2) were obtained from the Philippines Atmospheric, Geophysical, and Astronomical Services Administration (PAGASA) through the Philippines Freedom of Information and World Weather online portal [27] for Liloan, Cebu;

Figure 2. Monthly temperatures (minimum and maximum, °C) and monthly rainfall (mm) of Baguio and Quezon cities (A); Cebu city mountains and Liloan, Cebu (B); Bukidnon and Cagayan de Oro (CDO)city (C) from June 2018 until February 2020.



although humidity data was not included in this study. Figure 2 shows that Luzon sites had monthly temperatures between 19.9 °C and 27.5 °C, Visayas sites between 25.5°C and 30.8°C, and Mindanao sites between 21.4°C and 31.5°C. Moreover, Luzon sites had mean monthly rainfall of 342.5 mm, Visayas sites had 121.4 mm, and Mindanao sites had 131.2 mm.

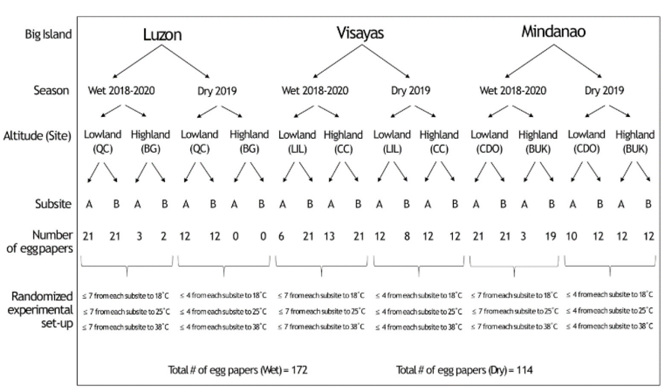
Mosquito egg collections

Aedes eggs were collected twice a month following the method by Edillo *et al.* [8], but in different sampling periods spanning the wet season (June 2018 to February 2020) and dry season (March to May 2019). Parental eggs were sampled using water-filled black plastic ovitraps (Department of Science and Technology, Manila, Philippines) with wooden paddles wrapped with filter paper as an oviposition material for *Aedes* eggs by mother mosquitoes. Ovitraps (30–40 pieces) were put randomly under dense vegetation covers in each sub site. Daily inspection for *Aedes* eggs on the filter paper, hence, called "egg paper", was done. The egg papers were immediately collected after 3–5 days, or until there were enough eggs, to prevent the eggs from hatching, and then air-dried before putting inside a plastic cup, sealed, and stored in a dark place. The egg papers were then shipped with consent from the Philippine Bureau of Quarantine, Cebu city.

Breeding of Ae. albopictus for F1 egg cohort collections

The method described by Edillo *et al.* for F1 egg cohort collections was followed but with some modifications [8]. Briefly, *Aedes* eggs sampled from the study sites were brought inside the insectary of the Mosquito Research Laboratory – University of San Carlos Talamban campus (MRL-USCTC), Cebu city under controlled environment conditions of 23–25 °C

Figure 3. Summary of the randomized experimental setup for wet (2018-2020) and dry (2019) season-collected Ae. albopictus F1 egg papers. Details of the study sub-sites are listed in Table 1.



temperature, 75–80% RH, and 12 hour light and 12 hour dark (12L:12D) photoperiod. These eggs were hatched using 10% ascorbic acid solution to hasten egg hatching. The larvae were transferred into fine-mesh cloth-covered plastic cups containing distilled water (DW) after 3 days. Egg papers with unhatched eggs were air dried and submerged again in ascorbic acid hatching solution, with several dry-wet cycles done until no more viable eggs were observed. The larvae were fed with refined fish food (Sakura; All Aquariums Co., Ltd. Bangkok, Thailand) every day until adult emergence. Adult *Aedes* were classified based on sex and species [28] and were coupled by subsite. Only *Ae. albopictus* adults were transferred to individual plastic cups. Each *Ae. albopictus* couple was placed in fine-mesh cloth covered plastic cup (base radius = 4.5 cm, height = 10 cm, mouth radius = 5 cm) with a filter paper at its base. Adult mosquitoes were flushed twice daily with 10% sucrose solution wet in cotton balls that were put on top of the mesh cloth and were given a 5-day mating window. Females that were unfed for 6–8 hours were later flushed with chicken blood anti-coagulated with ethylendiaminetetraacetic acid (EDTA). The blood meal was placed on the outside bottom depression of a plastic bottle covered with parafilm, filled with lukewarm water to simulate the human body temperature, and was placed in an upright position, on top of the mesh cloth-covered plastic cup for blood-feeding of females. Then, the wet filter paper at the base of the plastic cup served as the egg laying material. After enough eggs were laid, each egg paper with ~30 morphologically viable F1 eggs was air-dried and kept inside a parafilm-sealed plastic cup inside a dark cabinet for 1.75–2 months.

Experimental design for the collected Ae. albopictus

F1 egg papers of *Ae. albopictus* from different subsites (Table 1) were placed individually inside falcon tubes and exposed separately in environmental chambers (Bio-Integrated Philippines Inc., Manila, Philippines) set at 18 °C, 25 °C (control), and 38 °C under 12L:12D photoperiod (225–745 lx) and 65–75% RH for 6 weeks [29]. Temperature regimes and photoperiod were based on the readings of PAGASA in the sampling months of 2018–2019. The environmental chambers were monitored twice daily to maintain the regulated conditions throughout the experimental period. Randomly selected egg papers were subjected to experimental treatment under separate temperature regimes following complete randomized design (Figure 3).

Table 2. Descriptive statistics of percent pharate larvae (PPL), hatch rates (HRs), and reproductive outputs (ROs) of F1 *Ae. albopictus* for wet season (2018–2020) by the major islands of the Philippines and temperature.

Major islands	Temperature (°C)	PPL (%)		HRs (%)		ROs	
		Mean	SD	Mean	SD	Mean	SD
Luzon	18	23.71	26.69	0.93	1.85	0.25	0.50
	25	19.21	22.21	0.33	0.65	0.25	0.50
	38	1.31	2.61	0.00	0.00	0.00	0.00
Visayas	18	23.46	19.54	6.30	5.61	2.00	1.83
	25	26.54	10.65	4.23	4.88	0.75	0.96
	38	0.00	0.00	0.00	0.00	0.00	0.00
Mindanao	18	16.76	15.35	5.24	8.13	1.00	1.15
	25	32.82	35.86	24.70	39.62	1.00	0.82
	38	0.42	0.85	0.00	0.00	0.00	0.00

Based on the G*Power sample size software [30], *Ae. albopictus* F1 egg cohorts per major island included 84 (i.e., 21 egg papers per site with ~7 egg cohorts per sub-site per temperature) for wet season and 48 (i.e., 12 egg papers per site with ~4 egg cohorts per sub-site per temperature) for dry season. The sample size provided a statistical power of at least 80% to detect significant differences among the development-related phenotypes with 2 types of elevations (lowland and highland), 3 major islands, 2 sub-sites from each type of elevation per major island (Table 1), and 3 temperature regimes.

After 6 weeks, F1 egg cohorts of *Ae. albopictus* in their respective chambers were submerged with 10% ascorbic acid solution for 3 days to remove oxygen from water and spare egg diapause. These F1 egg cohorts were then subjected with 50% sodium hypochlorite (NaOCl) solution at room temperature to remove the egg chorion's coloration [31]. Pharate larvae or unhatched embryonated eggs and unhatched unembryonated eggs were counted and examined [32–33] using a light microscope. Important morphological characteristics of pharate larvae that completed embryogenesis include ocelli, egg burster, and abdominal segments [29]. Phenotypic adaptations were measured by percent pharate larvae (PPL), reproductive output (RO) (i.e., the number of egg papers with hatched larvae), and hatching rate (HR) calculated using the equation below [33]:

$$HR = \frac{HE}{(eHE + HE)}\ x\ 100 \quad (1)$$

where: HE = hatched eggs; eHE = embryonated unhatched eggs.

Statistical analyses

Normality distributions for all data sets (wet season of 2018–2020, dry season of 2019, and across seasons) were determined using Kolmogorov-Smirnov and Shapiro-Wilk tests (SSPS v 21; IBM Corporation, NY, USA). Data sets of the wet season 2018–2020 (Supplementary Table 1) and dry season 2019 (Supplementary Table 2) showed normal distributions and were subjected to multivariate analysis of variance (MANOVA) to determine whether independent variables (i.e., temperature and major islands) had main or interaction effects on PPL, HR, and RO. Combined seasonal data set (Supplementary Table 3) showed a non-normal distribution, and hence, was subjected to nonparametric tests such as Mann-Whitney (i.e., to compare 2 independent groups) and Kruskal-Wallis (i.e., to compare 3 or more independent groups) to determine significant differences among the PPL, HRs, and ROs within and across the major islands of the Philippines (Luzon, Visayas, and Mindanao) as affected by elevation (highland and lowland), temperature (18 °C, 25 °C, and 38 °C), and season (wet and dry).

Results

Wet season (2018–2020)-collected Ae. albopictus

Descriptive statistical results of the wet season data set showed that PPL, HRs, and ROs of *Ae. albopictus*

Figure 4. Morphology of pharate larvae of Aedes albopictus (dorsal view) obtained from this study showing body regions, namely, the head (H), thorax (T), abdominal segments (AS), respiratory siphon (RS), ocelli (Oc), and egg burster (EB).

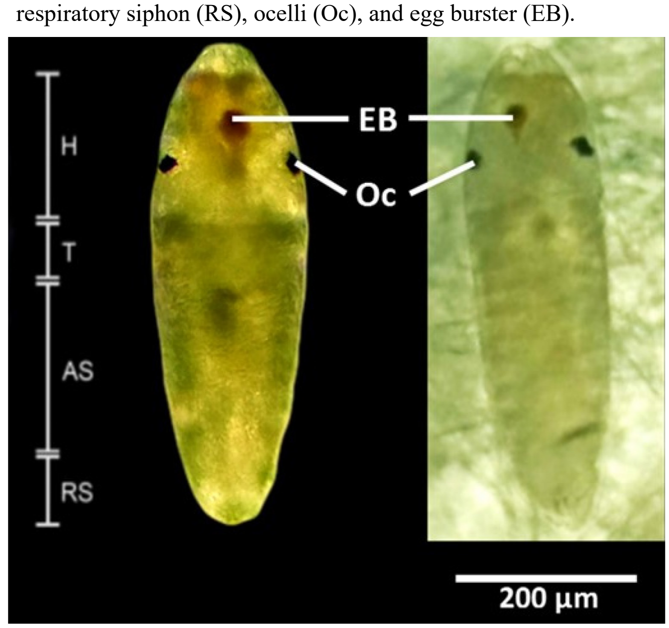


Table 3. Results of MANOVA on the main and interaction effects of the major islands of the Philippines and temperature on pharate larvae (PPL), hatch rates (HRs), and reproductive outputs (ROs) of F1 *Ae. albopictus* for wet season (2018—2020).

Source	***df***	**PPL**		**HRs**		**ROs**	
		F	***p* value**	**F**	***p* value**	**F**	***p* value**
Model	9	4.39	0.001	1.64	0.153	3.96	0.003
Major island	2	0.04	0.959	1.52	0.237	2.33	0.116
Temperature	2	6.18	0.016	1.53	0.236	4.78	0.017
Major island * temperature	4	0.33	0.857	1.15	0.356	1.28	0.303
Error	27						
Total	36						

were lowest at 38 °C across all the major islands of the Philippines (Table 2). Pharate larvae (Figure 4) exposed at 38 °C were observed only in Luzon and Mindanao (Table 2). MANOVA results (Table 3) using Pillai's trace (observed power of 0.964) revealed significant ($p < 0.01$) main effect of temperature on PPL and ROs during the wet season. PPL and ROs differed significantly ($p < 0.05$) across the three temperature regimes but not with HRs. Tukey's test revealed that PPL and ROs were similar between 18 °C and 25 °C but were lowest ($p < 0.05$) at 38 °C, suggesting high egg mortality at higher temperature. However, both the main effect of the major islands and interaction effects between major islands and temperature on PPL, HRs, and ROs were insignificant ($p > 0.05$).

Dry season (2019)-collected Ae. albopictus

Table 4 summarizes the descriptive statistical results from the data set of the dry season (2019) samples of *Ae. albopictus*, which were comparable with the wet season (2018–2020). PPL, HRs, and ROs of dry season-collected samples were lowest at 38 °C, and higher at 18 °C and 25 °C (Table 4). Pharate larvae exposed to 38 °C were only observed in Luzon and Mindanao samples. MANOVA results (Table 5) using Roy's largest root (observed power of 0.891) revealed that temperature had main effects ($p < 0.001$) on PPL ($p < 0.001$), HRs ($p < 0.05$), and ROs ($p < 0.01$) of dry season-collected *Ae. albopictus* but insignificant ($p > 0.05$) in both the main effect of the major islands, and interaction effect between temperature and major island. PPL and ROs were similar between 18 °C and 25 °C but were significantly lowest at 38 °C ($p < 0.01$) by Tukey's test. HRs were comparable between 18 °C and 25 °C, and between 18 °C and 38 °C, but differed ($p < 0.05$) between 25 °C and 38 °C. Based on Tukey's test, HRs of F1 eggs of *Ae. albopictus* from Luzon and Visayas were similar between 18 °C and 38 °C, and were much lower than those exposed at 25 °C. HRs differed ($p < 0.05$) between 25 °C and 38 °C because no eggs across major islands hatched when exposed at 38 °C, while HRs of F1 eggs of *Ae. albopictus* from Luzon and Visayas were highest at 25 °C.

Table 4. Descriptive statistics of pharate larvae (PPL), hatch rates (HRs), and reproductive outputs (ROs) of F1 *Ae. albopictus* for dry season (2019) by the major islands of the Philippines and temperatures.

Major Islands	**Temperature (°C)**	**PPL (%)**		**HRs (%)**		**ROs**	
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Luzon	18	13.55	15.72	4.33	8.66	0.25	0.50
	25	11.35	13.16	9.98	18.08	1.00	1.15
	38	0.11	0.22	0.00	0.00	0.00	0.00
Visayas	18	16.58	3.37	8.77	6.66	1.50	1.29
	25	33.42	10.13	12.94	14.12	1.25	0.96
	38	0.00	0.00	0.00	0.00	0.00	0.00
Mindanao	18	17.31	5.69	18.40	17.27	2.50	1.29
	25	30.02	15.49	13.73	17.21	1.75	1.71
	38	2.68	5.35	0.00	0.00	0.00	0.00

Table 5. Results of MANOVA on the main and interaction effects of the major islands of the Philippines and temperatures on pharate larvae (PPL), hatch rates (HRs), and reproductive outputs (ROs) of F1 *Ae. albopictus* for dry season (2019).

Source	***df***	**PPL**		**HRs**		**ROs**	
		F	***p* value**	**F**	***p* value**	**F**	***p* value**
Model	9	13.95	0.000	2.87	0.016	6.49	0.000
Temperature	2	18.98	0.000	3.81	0.035	7.80	0.002
Major Island	2	2.99	0.067	0.78	0.470	3.09	0.062
Temperature * Major island	4	1.68	0.183	0.42	0.793	1.37	0.270
Error	27						
Total	36						

Table 6. Results of Mann-Whitney statistical test showing no significant seasonal differences in pharate larvae (PPL), hatch rates (HR), and reproductive outputs (ROs) of F1 *Ae. albopictus* across the major islands of the Philippines.

Statistics	PPL	HR	RO
Mann-Whitney U	641.000	548.000	557.000
Wilcoxon W	1307.000	1214.000	1223.000
Z	– 0.082	– 1.282	– 1.172
Asymptotic Significance (2-tailed)	0.935	0.200	0.241

Seasonal differences in phenotypic adaptation of Ae. albopictus

Mann-Whitney statistical test results (Table 6) showed insignificant differences ($p > 0.05$) among the PPL, HRs, and ROs of *Ae. albopictus* between the two seasons, although temperature still played an important role in both seasons, as shown in the individual statistical analysis for wet and dry season data sets.

Combined data of wet- and dry season-collected Ae. albopictus: descriptive statistics for both wet (2018–2020) and dry seasons (2019)

Table 7 shows that mean PPL (14.01 ± 9.39) and mean HR (10.18 ± 15.12) of *Ae. albopictus* were greatest at 25 °C and least at 38 °C (0.64 ± 1.30 and 0.00, respectively) across the major islands. Mean RO (2.50 ± 2.32) peaked at 18 °C and was zero at 38 °C. Within the Philippines major islands, mean HR was greatest at 25 °C for Luzon (5.54 ± 6.68) and Mindanao (17.34 ± 24.79), but highest at 18 °C for Visayas (7.75 ± 5.20). Mean RO was highest at 18 °C for Visayas (3.50 ± 2.52) and Mindanao (3.50 ± 2.08), but highest at 25 °C for Luzon (1.25 ± 1.50) (Table 7). PPL were highest at 25 °C for samples from Luzon (13.57 ± 15.81), Visayas (14.35 ± 5.38), and Mindanao (14.12 ± 6.62). Fewer pharate larvae were observed in lowland sites in Luzon and Mindanao. Pharate larvae were observed at 38 °C; incomplete embryonated eggs were observed prevalently at 38 °C.

Within Visayas and Mindanao data analyses

Apparently, *Ae. albopictus* might not be successfully adapted in Baguio city with the highest elevation of 1,391–1,503 m ASL (Table 1), as evidenced by a very few or no samples obtained during the wet and dry seasons of 2018–2020, respectively, even after increasing the number of subsites and sampling efforts. Hence, further statistical analyses excluded Luzon. Table 8 shows that the Mann-Whitney statistical analysis revealed that insignificant differences ($p > 0.05$) were observed among combined PPL, HRs, and ROs between highland and lowland sites in Visayas and Mindanao. However, mean PPL (8.39 ± 8.59) and mean HR (9.26 ± 15.79) in highland sites were slightly higher than those from lowland sites (mean PPL = 8.27 ± 6.72; mean HR = 6.14 ± 8.36) in Visayas and Mindanao, whereas mean ROs (2.00 ± 2.37) in lowland sites were slightly higher than in

Table 7. Descriptive statistics of pharate larvae (PPL), hatch rates (HRs), and reproductive outputs (ROs) of F1 *Ae. albopictus* for combined data sets (wet and dry seasons) by the major islands of the Philippines and temperatures.

Major Islands	Temperature (°C)	PPL (%)		HRs (%)		ROs	
		Mean	SD	Mean	SD	Mean	SD
Luzon	18	10.49	12.60	2.16	4.33	0.50	1.00
	25	13.57	15.81	5.54	6.68	1.25	1.50
	38	0.95	1.69	0.00	0.00	0.00	0.00
Visayas	18	10.65	6.33	7.75	5.20	3.50	2.52
	25	14.35	5.38	7.65	8.55	2.00	1.83
	38	0.00	0.00	0.00	0.00	0.00	0.00
Mindanao	18	9.92	7.26	13.47	12.19	3.50	2.08
	25	14.12	6.62	17.34	24.79	2.75	2.50
	38	0.97	1.58	0.00	0.00	0.00	0.00
Philippines (Combined)	18	10.35	8.29	7.79	8.73	2.50	2.32
	25	14.01	9.39	10.18	15.12	2.00	1.91
	38	0.64	1.30	0.00	0.00	0.00	0.00

Table 8. Results of Mann-Whitney statistical test showing no significant difference in pharate larvae (PPL), hatch rates (HR), and reproductive outputs (ROs) of F1 *Ae. albopictus* between highland and lowland sites of Visayas and Mindanao.

Statistics	PPL	HR	RO
Mann-Whitney U	69.00	67.00	69.50
Wilcoxon W	147.00	145.00	147.50
Z	– 0.18	– 0.30	– 0.15
Asymptotic Significance (2-tailed)	0.86	0.76	0.88

Table 9. Results of the Mann-Whitney test showing no significant differences in pharate larvae (PPL), hatch rates (HRs), and reproductive outputs (ROs) of F1 *Ae. albopictus* between highland and lowland sites in Visayas and Mindanao at different temperature regimes.

Temperature (°C)	Statistics	PPL	HR	RO
18	Mann-Whitney U	7.000	7.000	5.000
	Wilcoxon W	17.000	17.000	15.000
	Z	– 0.289	– 0.289	– 0.893
	Asymptotic Significance (2-tailed)	0.773	0.773	0.372
25	Mann-Whitney U	6.000	7.500	6.500
	Wilcoxon W	16.000	17.500	16.500
	Z	– 0.577	– 0.145	– 0.438
	Asymptotic Significance (2-tailed)	0.564	0.885	0.661
38	Mann-Whitney U	4.000	8.000	8.000
	Wilcoxon W	14.000	18.000	18.000
	Z	– 1.512	0.000	0.000
	Asymptotic Significance (2-tailed)	0.131	1.000	1.000

highland sites (1.92 ± 2.15). Furthermore, Table 9 shows the Mann-Whitney test results, in which PPL, HRs, and ROs were similar between highland and lowland sites in Visayas and Mindanao at each temperature regime. In addition, residents in the rural mountains did not have water pipelines installed in their houses. They stored water in drums and containers for household usage that may have served as breeding sites for dengue mosquitoes.

Across the major islands of the Philippines

Kruskal-Wallis test results (Table 10) revealed that temperature impacted ($p < 0.01$) the PPL, HRs, and ROs of *Ae. albopictus* across all sites in the Philippines, implying that survival and proliferation of this mosquito species were highly influenced by temperature.

Discussion

This study extended our previous work on *Ae. aegypti* [8], but during a different study period and mostly different sub sites with dense vegetation covers (Table 1), to investigate the impacts of temperature, elevation, major island, and season on the viability and hatching rate of *Ae. albopictus* in the Philippines. The findings of this work are very relevant because of the arboviral vectorial capacity of *Ae. albopictus*. Edillo *et al*. [1] reported the silent co-circulation of CHIKV, DENVs, and ZIKV in *Ae. albopictus* from selected sites in Cebu city, Philippines, even if the country has not experienced Zika outbreak thus far, unlike in neighboring Asian countries [34] such as India, Thailand, and Vietnam.

There are three major findings of this study. First, temperature played significant main effects on two development-related phenotypes (PPL and ROs), but not HRs of *Ae. albopictus* during the wet season (2018–2020), and on all the 3 phenotypes during the dry season (2019). These results were consistent with a previous study on *Ae. aegypti* in the Philippines, where temperature had significant main effects on these development-related phenotypes in both seasons [8]. Moreover, PPL and ROs of *Ae. albopictus* in the current study were similar between 18 °C and 25 °C, but were significantly lower at 38 °C in both seasons; whereas HRs differed significantly between 25 °C and 38 °C for dry season-collected samples. These results partly coincided with that of Delatte *et al.* who reported that *Ae. albopictus* fully develops at a temperature range from 15 °C to 35 °C, both sexes survive longer at lower temperature, and lower developmental zero temperature is at 10.4 °C with 29.7 °C as optimum [35]. However, the current study showed that a few F1 pharate larvae of *Ae. albopictus* were observed at 38 °C; the first report, thus far, implying that the species might have gradually adapted to survive in the higher temperatures that are a consequence of global warming. Most F1 eggs exposed at 38 °C did not complete embryogenesis and did not hatch. Consistently, Dickerson (2007) reported that 32 °C and 35 °C have a negative effect on the HRs of *Ae. albopictus* [29]. Monteiro *et al*. (2007) showed that at 35 °C, *Ae. albopictus* has very low HR, no adult emergence, lower larval development, and that 35 °C may be its limiting temperature for larval development [36]. This species develops and survives at a wider temperature range than *Ae. aegypti*, and can grow completely between 15 °C and 35 °C [6], with a minimum threshold for development at 10.4 °C [35].

Table 10. Kruskal-Wallis test results showing significant differences in the pharate larvae (PPL), hatch rates (HRs), and reproductive outputs (ROs) of F1 *Ae. albopictus* across the major islands of the Philippine as grouped by temperature. Significant *p*-values are underlined.

Statistics	PPL	HR	RO
Chi square	16.398	12.567	12.743
df	2	2	2
Asymptotic Significance	0.000	0.002	0.002

Ae. albopictus is more favorable at 30 °C in both the percentage of individuals that fully develops and development time (i.e., up to adult emergence) [37]. Its tolerance to cold depends on its region of origin; the strains adapted to North American and colder Asian conditions survive more than the tropical strains [38]. The latter can hatch at a very low temperature of – 10 °C [39]; the temperature threshold for larval development is ~ 9 °C [40]. Furthermore, summer air does not affect that much on *Ae. albopictus* owing to their exophagic nature [41], hence, current results had similar significant main effects of temperature on both PPL and ROs for samples collected in both dry and wet seasons. Little perturbations within the normal thermal range of *Ae. albopictus* has a stronger effect on individuals but a meager effect under stressful conditions (i.e., during dry season) [42].

Second, PPL, HRs, and ROs of *Ae. albopictus* did not significantly differ between wet (2018–2020) and dry (2019) seasons, although temperature still played an important role in both seasons. Seasonality of *Ae. albopictus* has been known to be governed by climatic factors such as temperature fluctuations [43], with a one-month lag in its seasonality compared to mean temperature fluctuations [44]. Although, Ryan *et al*. (2019) predicted that the climate suitability of *Ae. albopictus* under severe climate change is reduced for southeast Asia [45], a global shift towards seasonal risk is expected for *Ae. albopictus* across regions. Rainfall has a larger effect on *Ae. albopictus* [44], whereas the combined effects of temperature and rainfall greatly influence vector indices of both *Ae. aegypti* and *Ae. albopictus*, more so than RH [45]. The combined results of the wet and dry seasons of the current study were consistent with previous studies [46–47], in which thermal stress causes protein denaturation, disruption of cellular processes, dehydration, and affects oxygen supply that leads to a halt in development and death of pharate larvae. Dickerson (2007) showed a significant decrease in HRs of *Ae. albopictus* collected from Texas, USA at 15 °C from 21 °C [29]. Subtropical *Ae. albopictus* can also oviposit eggs that do not enter diapause [48]. Length of the gonotrophic cycles of mosquitoes, in general, is shorter in higher temperature that increases the reproductive capacity [49] and biting frequency [50] of female mosquitoes. *Ae. albopictus* shows the shortest gonotrophic cycle at 30 °C for 3.5 days with 3.9 cycles as the highest number [51]; however, it lays apparently similar number of eggs per gonotrophic cycle at 20 °C, 25 °C, 30 °C, and 35 °C [35].

Third, the current results suggest that elevation and temperature did not have differential effects on development-related phenotypes of *Ae. albopictus* within Visayas and Mindanao highland and lowland sites. Also, there was the lack of significant main effect of the Philippines major islands with latitudinal differences on HRs and ROs of *Ae. albopictus*; contrary to that of Tsunoda *et al.*, who reported that day length affects egg hatching under milder tropical conditions [52]. The results within Visayas and Mindanao sites suggest that *Ae. albopictus* might have gradually adapted in finding the balance between existing and thriving as they colonized areas of higher elevation (324–540 m ASL) (Table 1), although not yet in Baguio city with the highest elevation (1,391–1,503 m ASL) among the study sites. *Ae. albopictus* in the tropical islands of La Réunion and Mauritius are abundant up to 800 m ASL and are present only up to 1,200 m ASL [53], and in Thailand forested areas between 450 and 1,800 m ASL [54]. Moreover, this species has a narrower distributional potential in the tropics and subtropics than *Ae. aegypti* [55]; it has an inverse relationship between its presence and elevation [56]. In areas where both mosquito species co-occur, temperature, precipitation [57], breeding site heterogeneity [48], and interspecific competition within a breeding site [3] influence their spatial and temporal abundance patterns. Interestingly, the elevation (Table 1) and temperature (Figure 2B-C) in Visayas and Mindanao sites were similar and that might also explain the similar effects of elevation and temperature within these sites. Temperature decreases as elevation increases, with temperature theoretically decreasing by 9.8 °C for every 1,000 m ASL; however, due to the presence of water vapor, observed drop in the environment only occurs by 6.5 °C for every 1,000 m ASL in most cases [58]. Increased elevation lowers environmental temperature, but warming climate has been shown to amplify in the mountains around the world [59]. Mosquito invasion in higher elevation can be exacerbated by warming global temperature. Neteler *et al*. predicted that *Ae. albopictus* will continue to spread to the highlands in an increasing temperature scenario in the coming years [60]. This implies that with increasing atmospheric temperature caused by global warming, both Philippines highlands and lowlands are at risk of arboviral-mosquito borne outbreaks.

Studies [9,45] supported the global and local geographic expansion of both dengue mosquito vectors owing to climate change and related human activities, evident from mosquito movement from regions of lower latitudes and elevations to higher latitudes and elevations. In this study, temperature undeniably showed a great deal of influence on the phenotypic adaptation of *Ae. albopictus* eggs in both wet and dry seasons. This current study showed that the viability, survivability, and expansion of *Ae. albopictus* in higher elevations were temperature-dependent. The difference in mean temperature between the northernmost and southernmost regions of the Philippines is negligible (mean temperature at ~26.60 °C), suggesting that there is a similar annual mean temperature that falls within the optimal temperature ranges for viral transmissions of *Aedes* (21.3 °C – 34.0 °C for *Ae. aegypti*; 19.9 °C – 29.4 °C for *Ae. albopictus*) [45]. This indicates that the Philippines is highly affected by the health and economic burden brought about by these mosquito species. The country might face new challenges in the future with *Aedes*-associated outbreaks such as Zika outbreak since RH and temperature influence minimum infection rate (MIR) of ZIKV in *Ae. albopictus* in the Philippines [1] since places with higher temperature are more likely to produce more mosquito adults [37].

Conclusions

Temperature, and not season, had an undeniable significant main effect on the phenotypic adaptation of F1 *Ae. albopictus* eggs across the major islands of the Philippines, with a broad temperature range for hatching and egg viability. The species' phenotypic traits were not significantly influenced by elevation in Visayas and Mindanao sites, and were not yet successfully adapted in Baguio city, the site with the highest elevation. *Ae. albopictus* is the secondary dengue vector in the Philippines; but owing to global shifts in climatic conditions as a result of global warming, nationwide campaigns for vector control against chikungunya, dengue, and Zika illnesses should also include *Ae. albopictus* for a more holistic and effective all-year round approach. This study recommends the installation of water pipelines in mountainous countrysides to avoid creating ideal conditions for *Ae. albopictus* hatchery due to the need of water storage for household usage. The results are definitely relevant in the tropics and subtropics.

Acknowledgements

We extend our gratitude to our coordinators and collectors for *Aedes* collections (Alan Dargantes of Central Mindanao University, Bukidnon; Cesar Carloman of CDO city; Chiara dela Peña of Liloan, Cebu; Benny Sunga of Baguio city; Jose Enrico Lazaro of the University of the Philippines – Diliman [UP-D], Quezon city (QC) for mediating the Baguio coordinator; and Liza Marie Erabon, Maintenance Unit of UP-D, QC. We also thank Enriqueta Reston of the University of San Carlos (USC), Cebu city who worked as consultant for the data analysis using SPSS, and to Arvin Pacoma for making the map of all study sites.

Funding

This study was supported by funds from the Philippines Council for Health Research and Development—Department of Science and Technology, Manila; and from the University of San Carlos, Cebu city, Philippines to FE. The funding agencies had no role in the study design, collection, data analysis and interpretation, manuscript writing, and in the decision for publication.

Authors' contributions

FE, AS, conceptualization; FE, RRY, supervision; RRY, AAB, RJH, MWS, research; RRY, AAB, RJH, MWS, data curation; FE, RRY, AAB, data analysis; RRY, MWS, figures; RRY, AAB, RJH, writing—original manuscript; FE, RRY, AS, manuscript review and editing; FE, project proposal and funding acquisition.

Corresponding author

Frances E. Edillo, PhD.
Department of Biology, University of San Carlos-Talamban campus,
Cebu city, Cebu, 6000, Philippines.
Tel: +63 (32) 230-0100 x 156
Email: feedillo@usc.edu.ph

Conflict of interest

No conflict of interest is declared.

References

1. Edillo F, Ymbong RR, Navarro AO, Cabahug MM, Saavedra K (2022) Detecting the impacts of humidity, rainfall, temperature, and season on chikungunya, dengue and Zika viruses in *Aedes albopictus* mosquitoes from selected sites in Cebu city, Philippines. Virol J 21: 1–15. doi: 10.1186/s12985-024-02310-4.
2. Kek R, Hapuarachchi HC, Chung CY, Humaidi MB, Razak MA, Chiang S, Lee C, Tan CH, Yap G, Chong CS, Lee KS, Ng LC (2014) Feeding host range of *Aedes albopictus* (Diptera: Culicidae) demonstrates its opportunistic host-seeking behavior in rural Singapore. J Med Entomol 51: 880–884. doi: 10.1603/ME13213.
3. Edillo FE, Roble ND, Otero ND 2nd (2012) The key breeding sites by pupal survey for dengue mosquito vectors, *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse), in Guba, Cebu City, Philippines. Southeast Asian J Trop Med Public Health 43: 1365–1374.
4. Armstrong PM, Andreadis TG, Shepard JJ, Thomas MC (2017) Northern range expansion of the Asian tiger mosquito (*Aedes albopictus*): analysis of mosquito data from Connecticut, USA. PLoS Negl Trop Dis 11: e0005623. doi: 10.1371/journal.pntd.0005623.
5. Xia D, Guo X, Hu T, Li L, Teng PY, Yin QQ, Luo L, Xie T, Wei YH, Yang Q, Li SK, Wang YJ, Xie Y, Li YJ, Wang CM, Yang ZC, Chen XG, Zhou XH (2018) Photoperiodic diapause in a subtropical population of *Aedes albopictus* in Guangzhou, China: optimized field-laboratory-based study and statistical

models for comprehensive characterization. Infect Dis Poverty 7: 89. doi: 10.1186/s40249-018-0466-8.
6. Reinhold JM, Lazzari CR, Lahondère C (2018) Effects of the environmental temperature on *Aedes aegypti* and *Aedes albopictus* mosquitoes: a review. Insects 9: 158. doi: 10.3390/insects9040158.
7. Tippelt L, Werner D, Kampen H (2020) Low temperature tolerance of three *Aedes albopictus* strains (Diptera: Culicidae) under constant and fluctuating temperature scenarios. Parasit Vectors 13: 587. doi: 10.1186/s13071-020-04386-7.
8. Edillo F, Ymbong RR, Bolneo AA, Hernandez RJ, Fuentes BL, Cortes G, Cabrera J, Lazaro JE, Sakuntabhai A (2022) Temperature, season, and latitude influence development-related phenotypes of Philippines *Aedes aegypti* (Linnaeus): implications for dengue control amidst global warming. Parasites Vectors 15: 74. doi: 10.1186/s13071-022-05186-x.
9. Edillo F, Ymbong RR, Cabahug MM, Labiros D, Suycano MW, Lambrechts L, Sakuntabhai A (2022) Yearly variations of the genetic structure of *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) in the Philippines (2017-2019). Infect Genet Evol 102: 105296. doi: 10.1016/j.meegid.2022.105296.
10. Tran BL, Tseng WC, Chen CC, Liao SY (2020) Estimating the threshold effects of climate on dengue: a case study of Taiwan. Int J Environ Res Public Health 17: 1392. doi: 10.3390/ijerph17041392.
11. Lourenço J, Recker M (2014). The 2012 Madeira dengue outbreak: epidemiological determinants and future epidemic potential. PLoS Negl Trop Dis 8: e3083. doi: 10.1371/journal.pntd.0003083.
12. Kutsuna S, Kato Y, Moi ML, Kotaki A, Ota M, Shinohara K, Kobayashi T, Yamamoto K, Fujiya Y, Mawatari M, Sato T, Kunimatsu J, Takeshita N, Hayakawa K, Kanagawa S, Takasaki T, Ohmagari N (2015) Autochthonous dengue fever, Tokyo, Japan, 2014. Emerg Infect Dis 21: 517–520. doi: 10.3201/eid2103/141662.
13. World Health Organization-Regional Office for the Western Pacific (2024) Dengue situation update — Number 700: update on dengue situation in the Western Pacific Region. Available: https://cdn.who.int/media/docs/default-source/wpro---documents/emergency/surveillance/dengue/dengue-20240613.pdf?sfvrsn=fc80101d_141. Accessed: 21 July 2024.
14. Montemayor MT (2023) Chikungunya cases in the Philippines on downtrend — DOH. Available: https://www.pna.gov.ph/articles/1216031. Accessed: 23 June 2024.
15. Bureau of Quarantine (2017) Philippines now has 57 Zika cases, 3 more confirmed pregnant cases included in latest report. Available: https://quarantine.doh.gov.ph/ph-now-has-57-zika-cases-3-more-confirmed-pregnant-cases-included-in-latest-report/. Accessed: 21 March 2024.
16. Edillo FE, Halasa YA, Largo FM, Erasmo JNV, Amoin NB, Alera MTP, Yoon I-K, Alcantara AC, Shepard DS (2015) Economic cost and burden of dengue in the Philippines. Am J Trop Med Hyg 92: 360–366. doi: 10.4269/ajtmh.14-0139.
17. Cheng KJG, Lam HY, Rivera AS, Tumanan-Mendoza BA, Alejandria MM, Wu DB (2018) Estimating the burden of dengue in the Philippines using a dynamic transmission model. Acta Med Philipp 52: 153–159. doi: 10.47895/amp.v52i2.427.
18. Rezza G (2012) *Aedes albopictus* and the reemergence of dengue. BMC Public Health 12: 72. doi: 10.1186/1471-2458-12-72.
19. Poelchau MF, Reynolds JA, Elsik CG, Denlinger DL, Armbruster PA (2013) Deep sequencing reveals complex mechanisms of diapause preparation in the invasive mosquito, *Aedes albopictus*. Proc Biol Sci 280: 20130143. doi: 10.1098/rspb.2013.0143.
20. Armbruster PA (2016) Photoperiodic diapause and the establishment of *Aedes albopictus* (Diptera: Culicidae) in North America. J Med Entomol 53: 1013–1023. doi: 10.1093/jme/tjw037.
21. Denlinger DL (1986) Dormancy in tropical insects. Annu Rev Entomol31:239–264.doi: 10.1146/annurev.en.31.010186.001323.
22. Tauber MJ, Tauber CA, and Masaki S (1986) Seasonal adaptation of insects. New York: Oxford Univ. Press.
23. Urbanski JM, Benoit JB, Michaud MR, Denlinger DL, Armbruster P (2010) The molecular physiology of increased egg desiccation resistance during diapause in the invasive mosquito, *Aedes albopictus*. Proc Biol Sci 277: 2683–2692. doi: 10.1098/rspb.2010.0362.
24. Vinogradova EB (2007) Diapause in aquatic insects, with emphasis on mosquitoes. Diapause in aquatic invertebrates: theory and human use. Netherlands: Springer Science and Business Media. 84: 83–113. doi: 10.1007/978-1-4020-5680-2_5.
25. Corporal-Lodangco IL, Leslie LM (2007) Defining Philippines climate zones using surface and high-resolution satellite data. Procedia Comput Sci 114: 324–332. doi: 10.1016/j.procs.2017.09.068.
26. World Health Organization 2019 Dengue and severe dengue: Who spread dengue and severe dengue? Available: https://www.who.int/news-room/q-a-detail/dengue-and-severe-dengue. Accessed: 14 February 2021.
27. Worldweatheronline (2021) Liloan monthly climate averages, Cebu,Philippines.Available: https://www.worldweatheronline.com/liloan-weather-averages/cebu/ph.aspx. Accessed: 15 February 2021.
28. Belkin JN (1962) The mosquitoes of the South Pacific (Diptera: Culicidae). California: University of California Press. 1: 620 p.
29. Dickerson CZ (2007) The effects of temperature and humidity on the eggs of *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) in Texas. [Doctoral dissertation]. Texas A&M University, USA. Available: http://hdl.handle.net/1969.1/ETD-TAMU-2508. Accessed: 10 January 2024.
30. Statistics Solution (2025) How to determine sample size from G*Power.Available: https://www.statisticssolutions.com/how-to-determine-sample-size-from-gpower/. Accessed: 15 February 2021.
31. Vargas HC, Farnesi LC, Martins AJ, Valle D, Rezende GL (2014) Serosal cuticle formation and distinct degrees of desiccation resistance in embryos of the mosquito vectors *Aedes aegypti*, *Anopheles aquasalis* and *Culex quinquefasciatus*. J Insect Physiol 62: 54–60. doi: 10.1016/j.jinsphys.2014.02.001.
32. Farnesi LC, Martins AJ, Valle D, Rezende GL (2009) Embryonic development of *Aedes aegypti* (Diptera: Culicidae): influence of different constant temperatures. Mem Inst Oswaldo Cruz 104: 124–126. doi: 10.1590/S0074-02762009000100020.
33. Lacour G, Vernichon F, Cadilhac N, Boyer S, Lagneau C, Hance T (2014) When mothers anticipate: effects of the prediapause stage on embryo development time and of maternal photoperiod on eggs of a temperate and a tropical strain of *Aedes albopictus* (Diptera: Culicidae). J Insect Physiol 71: 87–96. doi: 10.1016/j.jinsphys.2014.10.008.

34. CDC (2018) Zika travel information.Available: https://wwwnc.cdc.gov/travel/page/zika-travel-information. Accessed: 24 July 2024.
35. Delatte H, Gimonneau G, Triboire A, Fontenille D (2009) Influence of temperature on immature development, survival, longevity, fecundity, and gonotrophic cycles of *Aedes albopictus*, vector of chikungunya and dengue in the Indian Ocean. J Med Entomol 46: 33–41. doi: 10.1603/033.046.0105.
36. Monteiro LC, de Souza JR, de Albuquerque CM (2007) Eclosion rate, development and survivorship of *Aedes albopictus* (Skuse) (Diptera: Culicidae) under different water temperatures. Neotrop Entomol 36: 966–971. doi: 10.1590/S1519-566X2007000600021.
37. Alto BW, Juliano SA (2001) Precipitation and temperature effects on populations of *Aedes albopictus* (Diptera: Culicidae): implications for range expansion. J Med Entomol 38: 646–656. doi: 10.1603/0022-2585-38.5.646.
38. Hawley WA, Pumpuni CB, Brady RH, Craig GB Jr (1989) Overwintering survival of *Aedes albopictus* (Diptera: Culicidae) eggs in Indiana. J Med Entomol 26: 122–129. doi: 10.1093/jmedent/26.2.122.
39. Tippelt L, Werner D, Kampen H (2019) Tolerance of three *Aedes albopictus* strains (Diptera: Culicidae) from different geographical origins towards winter temperatures under field conditions in northern Germany. PLoS One 14: e0219553. doi: 10.1371/journal.pone.0219553.
40. Teng HJ. Apperson CS (2000) Development and survival of immature *Aedes albopictus* and *Aedes triseriatus* (Diptera: Culicidae) in the laboratory: effects of density, food, and competition on response to temperature. J Med Entomol 37: 40–52. doi: 10.1603/0022-2585-37.1.40.
41. Weaver SC, Reisen WK (2010) Present and future arboviral threats. Antiviral Res 85: 328–345. doi: 10.1016/j.antiviral.2009.10.008.
42. Chaves LF (2016) Climate change and biology of insect vectors of human pathogens. In Johnson SN, Jones TH, editors. Global Climate Change and Terrestrial Invertebrates. Hoboken: Wiley-Blackwell. 126–147. doi: 10.1002/9781119070894.ch8.
43. Zheng X, Zhong D, He Y, Zhou G (2019) Seasonality modeling of the distribution of *Aedes albopictus* in China based on climatic and environmental suitability. Infect Dis Poverty 8: 98. doi: 10.1186/s40249-019-0612-y.
44. Rajarethinam J, Ong J, Neo ZW, Ng LC, Aik J (2020) Distribution and seasonal fluctuations of *Ae. aegypti* and *Ae. albopictus* larval and pupae in residential areas in an urban landscape. PLoS Negl Trop Dis 14: e0008209. doi: 10.1371/journal.pntd.0008209.
45. Ryan SJ, Carlson CJ, Mordecai EA, Johnson LR (2019) Global expansion and redistribution of *Aedes*-borne virus transmission risk with climate change. PLoS Negl Trop Dis 13: e0007213. doi: 10.1371/journal.pntd.0007213.
46. Tuladhar R, Singh A, Banjara MR, Gautam I, Dhimal M, Varma A, Choudhary DK (2019) Effect of meteorological factors on the seasonal prevalence of dengue vectors in upland hilly and lowland Terai regions of Nepal. Parasit Vectors 12: 42. doi: 10.1186/s13071-019-3304-3.
47. Denlinger DL, Hallman GJ (1998) Temperature sensitivity in insects and application in integrated pest management. Boulder: Westview Press.
48. Higa Y, Yen NT, Kawada H, Son TH, Hoa NT, Takagi M (2010) Geographic distribution of *Aedes aegypti* and *Aedes albopictus* collected from used tires in Vietnam. J Am Mosq Control Assoc 26: 1–9. doi: 10.2987/09-5945.1.
49. Jetten TH, Focks DA (1997) Potential changes in the distribution of dengue transmission under climate warming. Am J Trop Med Hyg 57: 285–297. doi: 10.4269/ajtmh.1997.57.285.
50. Chang AY, Fuller DO, Carrasquillo O, Beier JC (2014) Social justice, climate change, and dengue. Health Hum Rights 16: 93–104.
51. Christophers SR (1960) *Aedes aegypti* (L.) the yellow fever mosquito. London: Cambridge University Press.
52. Tsunoda T, Chaves LF, Nguyen GT, Nguyen YT, Takagi M (2015) Winter activity and diapause of *Aedes albopictus* (Diptera: Culicidae) in Hanoi, Northern Vietnam. J Med Entomol 52: 1203–1212. doi: 10.1093/jme/tjv122.
53. Delatte H, Dehecq JS, Thiria J, Domerg C, Paupy C, Fontenille D (2008) Geographic distribution and developmental sites of *Aedes albopictus* (Diptera: Culicidae) during a chikungunya epidemic event. Vector Borne Zoonotic Dis 8: 25–34. doi: 10.1089/vbz.2007.0649.
54. Preechaporn W, Jaroensutasinee M, Jaroensutasinee K (2006) The larval ecology of *Aedes aegypti* and *Aedes albopictus* in three topological areas of southern Thailand. WHO Regional Office for South-East Asia 30: 204–213. Available: https://apps.who.int/iris/handle/10665/170210.
55. Kamal M, Kenawy MA, Rady MH, Khaled AS, Samy AM (2018) Mapping the global potential distributions of two arboviral vectors *Aedes aegypti* and *Ae. albopictus* under changing climate. PLoS One 13: e0210122. doi: 10.1371/journal.pone.0210122.
56. Dhimal M, Gautam I, Joshi HD, O'Hara RB, Ahrens B, Kuch U (2015) Risk factors for the presence of chikungunya and dengue factors (*Aedes aegypti* and *Aedes albopictus*), their altitudinal distribution and climatic determinants of their abundance in Central Nepal. PLoS Negl Trop Dis 9: e0003545. doi: 10.1371/journal.pntd.0003545.
57. Chaves LF (2016) Globally invasive, withdrawing at home: *Aedes albopictus* and *Aedes japonicus* facing the rise of *Aedes flavopictus*. Int J Biometeorol 60: 1727-1738. doi: 10.1007/s00484-016-1162-7.
58. Tabony RC (1985) Relations between minimum temperature and topography in Great Britain. J Climatol 5: 503–520. doi: 10.1002/joc.3370050504.
59. Barry RG (2008) Mountain weather and climate, 3rd ed. New York: Cambridge University Press. doi: 10.1017/CBO9780511754753.
60. Neteler M, Roiz D, Rocchini D, Castellani C, Rizzoli A (2011) Terra and Aqua satellites track tiger mosquito invasion: modelling the potential distribution of *Aedes albopictus* in north-eastern Italy. Int J Health Geogr 10: 49. doi: 10.1186/1476-072X-10-49.

Annex – Supplementary Items

Supplementary Table 1. Development-related phenotypes of *Ae. albopictus* from wet season 2018–2022.

Case #	Island group *	Altitude **	Temperature	Subsite	Sample size ***	% pharate larvae	Weighted average. % hatching rate	Reproductive output
1	1	1	18	1	3	60.00	0.00	0.00
2	1	1	25	1	0	0.00	0.00	0.00
3	1	1	38	1	0	0.00	0.00	0.00
4	1	1	18	2	0	0.00	0.00	0.00
5	1	1	25	2	2	40.00	0.00	0.00
6	1	1	38	2	0	0.00	0.00	0.00
7	1	2	18	3	7	7.96	0.00	0.00
8	1	2	25	3	7	0.00	0.00	0.00
9	1	2	38	3	7	0.00	0.00	0.00
10	1	2	18	4	7	26.87	3.70	1.00
11	1	2	25	4	7	36.82	1.30	1.00
12	1	2	38	4	7	5.22	0.00	0.00
13	2	1	18	5	4	15.33	3.13	1.00
14	2	1	25	5	7	12.41	0.00	0.00
15	2	1	38	5	2	0.00	0.00	0.00
16	2	1	18	6	7	35.40	11.24	3.00
17	2	1	25	6	7	36.86	8.58	2.00
18	2	1	38	6	7	0.00	0.00	0.00
19	2	2	18	7	0	0.00	0.00	0.00
20	2	2	25	7	6	32.11	8.33	1.00
21	2	2	38	7	0	0.00	0.00	0.00
22	2	2	18	8	7	43.12	10.81	4.00
23	2	2	25	8	7	24.77	0.00	0.00
24	2	2	38	8	7	0.00	0.00	0.00
25	3	1	18	9	0	0.00	0.00	0.00
26	3	1	25	9	2	1.92	83.33	2.00
27	3	1	38	9	1	0.00	0.00	0.00
28	3	1	18	10	7	13.46	0.00	0.00
29	3	1	25	10	7	84.62	0.00	0.00
30	3	1	38	10	5	0.00	0.00	0.00
31	3	2	18	11	7	16.46	17.14	2.00
32	3	2	25	11	7	22.78	14.29	1.00
33	3	2	38	11	7	0.00	0.00	0.00
34	3	2	18	12	7	37.13	3.83	2.00
35	3	2	25	12	7	21.94	1.19	1.00
36	3	2	38	12	7	1.69	0.00	0.00

* 1 = Luzon, 2 = Visayas, 3 = Mindanao; ** 1= high, 2= low; *** Baguio subsites: 1,2; Quezon city subsites: 3, 4; Cebu city subsites: 5, 6; Liloan subsites: 7, 8; Bukidnon subsites: 9, 10; CDO subsites: 11, 12.

Supplementary Table 2. Development-related phenotypes of *Ae. albopictus* from dry season 2019.

Case #	Island group*	Altitude **	Temperature	Subsite ***	Sample size	% pharate larvae	Weighted average. % hatching rate	Reproductive output
1	1	1	18	1	0	0.00	0.00	0.00
2	1	1	25	1	0	0.00	0.00	0.00
3	1	1	38	1	0	0.00	0.00	0.00
4	1	1	18	2	0	0.00	0.00	0.00
5	1	1	25	2	0	0.00	0.00	0.00
6	1	1	38	2	0	0.00	0.00	0.00
7	1	2	18	3	4	29.07	0.00	0.00
8	1	2	25	3	4	24.23	37.02	2.00
9	1	2	38	3	4	0.00	0.00	0.00
10	1	2	18	4	4	25.11	17.31	1.00
11	1	2	25	4	4	21.15	2.88	2.00
12	1	2	38	4	4	0.44	0.00	0.00
13	2	1	18	5	4	20.14	16.15	3.00
14	2	1	25	5	4	38.85	1.47	1.00
15	2	1	38	5	4	0.00	0.00	0.00
16	2	1	18	6	4	14.39	10.00	1.00
17	2	1	25	6	4	26.62	24.28	2.00
18	2	1	38	6	4	0.00	0.00	0.00
19	2	2	18	7	4	18.69	0.00	0.00
20	2	2	25	7	4	23.36	26.00	2.00
21	2	2	38	7	4	0.00	0.00	0.00
22	2	2	18	8	3	13.08	8.93	2.00
23	2	2	25	8	4	44.86	0.00	0.00
24	2	2	38	8	1	0.00	0.00	0.00
25	3	1	18	9	4	9.38	21.63	3.00
26	3	1	25	9	4	17.71	38.87	4.00
27	3	1	38	9	4	0.00	0.00	0.00
28	3	1	18	10	4	20.31	0.76	1.00
29	3	1	25	10	4	52.60	0.00	0.00
30	3	1	38	10	4	0.00	0.00	0.00
31	3	2	18	11	4	17.21	40.93	4.00
32	3	2	25	11	4	26.51	9.09	2.00
33	3	2	38	11	2	10.70	0.00	0.00
34	3	2	18	12	4	22.33	10.29	2.00
35	3	2	25	12	4	23.26	6.94	1.00
36	3	2	38	12	4	0.00	0.00	0.00

* 1 = Luzon, 2 = Visayas, 3 = Mindanao; ** 1= high, 2= low; *** Baguio subsites: 1,2; Quezon city subsites: 3, 4; Cebu city subsites: 5, 6; Liloan subsites: 7, 8; Bukidnon subsites: 9, 10; CDO subsites: 11, 12.

Supplementary Table 3. Combined wet and dry season development-related phenotypes of *Ae. albopictus*.

Case #	Island group *	Altitude **	Temperature	Subsite ***	Sample size	% pharate larvae	Weighted average. % hatching rate	Reproductive output
1	1	1	18	1	3	0.47	0.00	0.00
2	1	1	25	1	0	0.00	0.00	0.00
3	1	1	38	1	0	0.00	0.00	0.00
4	1	1	18	2	0	0.00	0.00	0.00
5	1	1	25	2	2	0.32	0.00	0.00
6	1	1	38	2	0	0.00	0.00	0.00
7	1	2	18	3	11	15.46	0.00	0.00
8	1	2	25	3	11	23.03	13.46	2.00
9	1	2	38	3	11	0.32	0.00	0.00
10	1	2	18	4	11	26.03	8.65	2.00
11	1	2	25	4	11	30.91	8.69	3.00
12	1	2	38	4	11	3.47	0.00	0.00
13	2	1	18	5	8	11.13	9.64	4.00
14	2	1	25	5	11	13.99	0.53	1.00
15	2	1	38	5	6	0.00	0.00	0.00
16	2	1	18	6	11	18.60	11.11	4.00
17	2	1	25	6	11	21.94	14.29	4.00
18	2	1	38	6	11	0.00	0.00	0.00
19	2	2	18	7	4	3.18	0.00	0.00
20	2	2	25	7	10	9.54	15.77	3.00
21	2	2	38	7	4	0.00	0.00	0.00
22	2	2	18	8	10	9.70	10.24	6.00
23	2	2	25	8	11	11.92	0.00	0.00
24	2	2	38	8	8	0.00	0.00	0.00
25	3	1	18	9	4	2.59	21.63	3.00
26	3	1	25	9	6	5.03	53.69	6.00
27	3	1	38	9	5	0.00	0.00	0.00
28	3	1	18	10	11	6.61	0.28	1.00
29	3	1	25	10	11	20.83	0.00	0.00
30	3	1	38	10	9	0.00	0.00	0.00
31	3	2	18	11	11	10.92	25.79	6.00
32	3	2	25	11	11	15.95	12.40	3.00
33	3	2	38	11	9	3.30	0.00	0.00
34	3	2	18	12	11	19.54	6.18	4.00
35	3	2	25	12	11	14.66	3.28	2.00
36	3	2	38	12	11	0.57	0.00	0.00

* 1 = Luzon, 2 = Visayas, 3 = Mindanao; ** 1= high, 2= low; *** Baguio subsites: 1,2; Quezon city subsites: 3, 4; Cebu city subsites: 5, 6; Liloan subsites: 7, 8; Bukidnon subsites: 9, 10; CDO subsites: 11, 12.