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ORIGINAL ARTICLE

Overwintering survival of adults of *Aedes albopictus* and *Aedes cretinus* (Diptera: Culicidae) in a sheltered microclimate in northern Attica, Greece

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Abstract. The mosquito species *Aedes cretinus*, which is native to Greece, has become increasingly scarce following the invasion of the highly competitive and widespread *Aedes albopictus*. The ability of mosquitoes to survive low winter temperatures plays a pivotal role in their population dynamics of the next season. In this study, we investigated the overwintering capacity of *Ae. albopictus* and *Ae. cretinus* adults under semi-field, sheltered microclimatic conditions in the northern area of Attica, Greece, during the winter of 2023–2024. Our findings revealed that 11% of *Ae. albopictus* females and 21.1% of *Ae. cretinus* females were capable of overwintering, highlighting the importance of sheltered microclimates in enabling overwintering survival under low outdoor temperatures. In contrast, males of both species failed to overwinter. The winter survival ability of adults was significantly greater in *Ae. cretinus* than in *Ae. albopictus*, and this may account for the occurrence of *Ae. cretinus* in the cooler environments of vegetated and wooded locations in northern areas of the Attica region. Nevertheless, the ability of *Ae. albopictus* females to also overwinter under the same sheltered microclimatic environment may affect the potential of interspecific competition in these areas.

INTRODUCTION

Aedes (*Stegomyia*) *albopictus* (Skuse, 1895), commonly known as the Asian tiger mosquito, is a species of significant medical importance due to its role as a competent vector of serious arboviruses, including Dengue, Chikungunya, and Zika viruses (Medlock et al., 2015). It is recognised as the most invasive mosquito species globally, a distinction largely attributed to its remarkable ecological plasticity and strong competitive abilities (Medlock et al., 2015; Bellini et al., 2020). Originally native to tropical and subtropical areas of Southeast Asia, *Ae. albopictus* has demonstrated exceptional adaptability, establishing populations in a wide range of environments, including suburban and urban areas, as well as cooler habitats outside its native range (Kreß et al., 2017; Ibáñez-Justicia et al., 2020). The species' expansion has been further facilitated by climate change and global trade, which have increased the availability of suitable habitats at higher altitudes and latitudes (van Daalen et al., 2022).

The successful establishment of invasive mosquitoes from tropical regions in temperate areas, as in the case of *Ae. albopictus*, largely depends on their ability to overwinter (Medlock et al., 2006; Leisnham & Juliano, 2012; Pluskota et al., 2016). For *Ae. albopictus*, overwintering is primarily associated with its capacity to survive the winter at the egg stage (Hanson & Craig, 1995; Swanson et al., 2000; Medlock et al., 2006; Leisnham & Juliano, 2012; Pluskota et al., 2016). The production of winter diapausing

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eggs is a key adaptation that has facilitated the species' establishment in northern European regions (European Centre for Disease Prevention and Control, 2016). In tropical and subtropical regions, where conditions remain favourable year-round, *Ae. albopictus* adults continue to reproduce without entering diapause at the egg stage. However, in temperate climates, embryonic diapause is employed as a survival strategy during the winter months (Gatt et al., 2009; Lee & Duvall, 2022). Additionally, in temperate regions, *Ae. albopictus* adults may extend their reproductive season by seeking sheltered microclimates when the outdoor temperature declines, allowing them to survive as adults through the winter (Roiz et al., 2010). This behavioural plasticity further enhances their ability to establish and persist in diverse environmental conditions.

Overwintering is a critical factor influencing mosquito population dynamics in the following season and, consequently, the transmission of vector-borne pathogens by competent vectors (ECDC, 2016; Tippelt et al., 2019; Filazzola et al., 2021; Kramer et al., 2021; Vavassori et al., 2022). Temperature affects activity, behaviour, development, fecundity, distribution, population dynamics, and pathogen transmission (Chaves et al., 2014; Liu et al., 2023). It is also a principal factor affecting adult mosquito survival (Yang et al., 2009; Alto & Bettinardi, 2013). For *Ae. albopictus*, winter and mean annual temperatures have been identified as the most significant limiting factors for its expansion to Europe (Roiz et al., 2011). In Northern Italy, winter temperatures significantly impact life cycle parameters of the first generation and influence adult host-seeking behaviour and oviposition activity (Roiz et al., 2010; Carrieri et al., 2023). Interestingly, the activity of *Ae. albopictus* adult females has been documented under both indoor and outdoor conditions throughout the winter season in Italy (Romi et al., 2006; Dutto & Mosca, 2017). Additionally, winter oviposition activity has been reported in specific European areas, further illustrating the adaptability of this species (Collantes et al., 2014; Bueno-Marí & Jiménez-Peydró, 2015; Del Lesto et al., 2022).

Protected microhabitats may serve as sheltered microclimates during winter for *Ae. albopictus* females seeking sugar or host sources (Romi et al., 2006; Dutto & Mosca, 2017). Dutto & Mosca (2017) reported oviposition and blood feeding activity of *Ae. albopictus* on humans under favourable conditions indoors in northwestern Italy. Romi et al. (2006) assumed that the winter oviposition activity of *Ae. albopictus* in Italy could be attributed to the continuous winter trophic activity of long-lived females of the last seasonal generation (emerged in November–December) and to one or more reproductive cycles occurring during winter at particular breeding sites indoors or semi-indoor environments such as greenhouses or nurseries.

Aedes albopictus was first recorded in northwestern Greece in 2003–2004 (Samanidou-Voyadjoglou et al., 2005) and subsequently reported in Athens, Attica, in 2008 (Koliopoulos et al., 2008). In the following years, results from mosquito surveillance data in the Attica Region, Greece, revealed that, *Ae. albopictus* has a widespread distribution and was the dominant *Aedes* container breeding mosquito. Its oviposition activity was recorded from May to December, with a peak during the summer months, whereas small numbers of adults were detected in December (Giatropoulos et al., 2012a; Beleri et al., 2021). However, during the winter months beyond December, only diapausing eggs and no adults of *Ae. albopictus* were detected until 2022 (Petrić et al., 2021; Stefopoulou et al., 2022). Interestingly, during the winter of 2022–2023, adult populations of *Ae. albopictus* were captured in BG-Sentinel adult mosquito traps in the Attica Region until the late January 2023, likely due to the remarkably warm winter conditions (Lührsen et al., 2023). Furthermore, a semi-field study conducted in an area of Attica Region demonstrated the overwintering survival capacity of two different strains of *Ae. albopictus* adults in protected cold microclimate conditions, highlighting the potential for this species to persist under certain sheltered environmental settings (Beleri et al., 2023).

Aedes (*Stegomyia*) *cretinus* Edwards, 1921 is a tree-hole mosquito with a limited geographic distribution, reported in Greece, Cyprus, Georgia, Lebanon and Turkey (Becker et al., 2020; Knio et al., 2005). Despite its presence in these regions, little is known about its bioecology, and to date, there is no data on the ability of its adults to survive overwinter. Additionally, the potential of *Ae. cretinus* to act as a vector for pathogens remains unknown (Schaffner & Mathis, 2014). *Aedes cretinus* is described as an aggressive daytime biter, with a preference to rest and bite humans outdoors (Samanidou-Voyadjoglou & Koliopoulos 1998; Koliopoulos, 2011). It is a mosquito native to Greece, where it has primarily been found in vegetated and wooded habitats within urban, peri-urban, and rural residential areas. In Greece, its larvae have been collected from a variety of small, natural or artificial breeding sites, including tree holes, ground pools, flowerpots, dishes, barrels, road drains, and used tyres (Samanidou-Voyadjoglou & Koliopoulos, 1998; Koliopoulos, 2011; Giatropoulos et al., 2012a; Giatropoulos et al., 2024). *Aedes cretinus* has been reported mainly in wooded areas (forests), open fields and road edges in Turkey (Alten et al., 2000; Caglar et al., 2003) and forested habitats in Cyprus (Martinou et al., 2016). The species is closely related to the invasive mosquito species *Ae. albopictus*, as both belong to the subgenus *Stegomyia* and exhibit many behavioural and morphological similarities (Samanidou-Voyadjoglou et al., 2005; Patsoula et al., 2006).

Mosquito survey data from the Region of Attica and other areas in Greece indicate a significant decline in *Ae. cretinus* populations following the invasion and widespread establishment of *Ae. albopictus.* When *Ae. cretinus* was first detected in the Attica Region (1992–1997) prior to the invasion of *Ae. albopictus*, it was primarily found in vegetated and wooded locations of northern Attica (Samanidou, 1998). In Attica Region, over the past decade (2012–2023), *Ae. cretinus* has been reported scarcely in similar habitats, following the widespread establishment of *Ae. albopictus* (Giatropoulos et al., 2024). The observed scarcity of *Ae.*

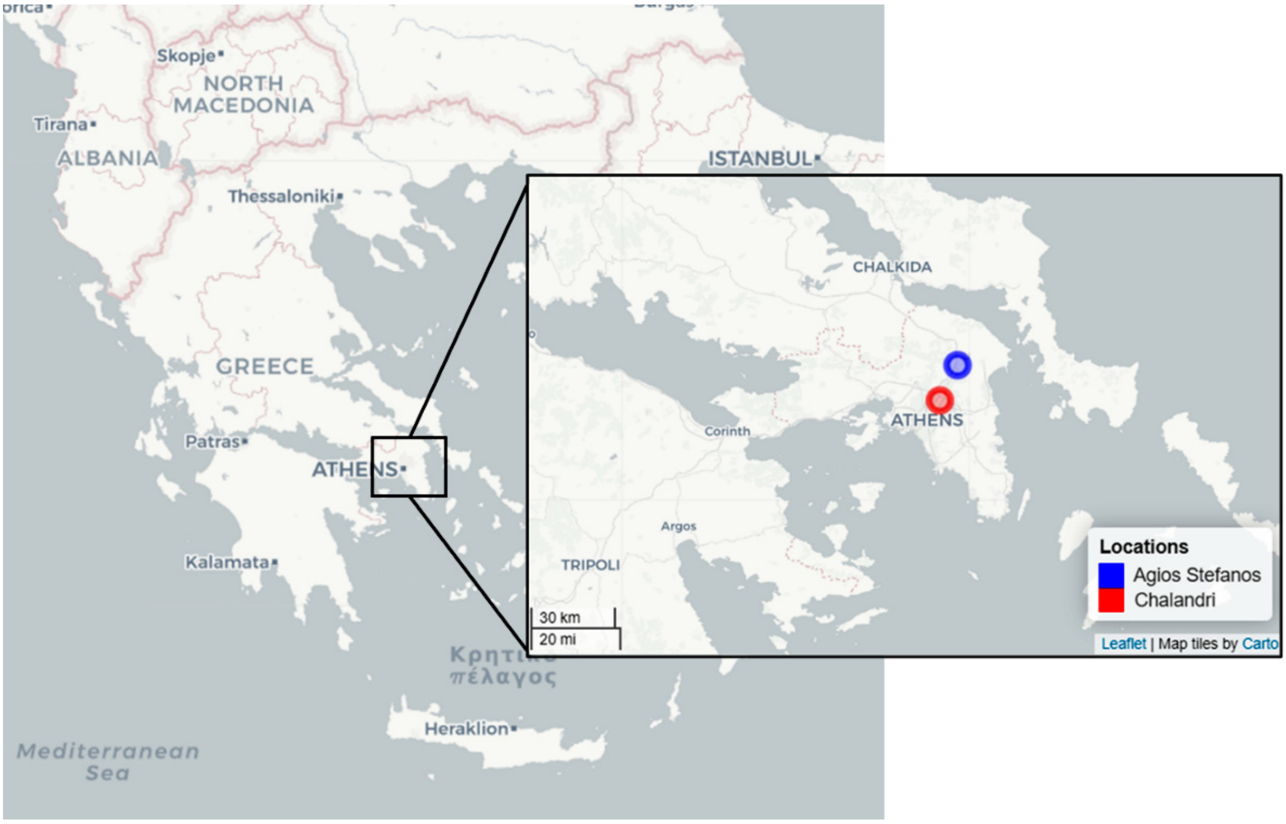

Fig. 1. Locations of egg collections of *Aedes cretinus* (Agios Stefanos) and *Aedes albopictus* (Chalandri), and experimental site (Chalandri).

cretinus is likely linked to the dominance of *Ae. albopictus* in overlapping habitats (Giatropoulos et al., 2024). Laboratory studies on cross-mating and larval competition between these two species have consistently demonstrated a competitive advantage for *Ae. albopictus* (Giatropoulos et al., 2015, 2022). Consequently, the limited presence of *Ae. cretinus* in Attica, particularly in areas where the two species coexist, can be attributed to interspecific competition following the rapid spread of *Ae. albopictus* (Giatropoulos et al., 2024). These findings underscore the ecological impact of *Ae. albopictus* as an invasive species capable of displacing native mosquito populations.

In this study, we aimed to explore how *Ae. albopictus* and *Ae. cretinus* deal with winter conditions within the same microclimatic habitats. Hence, we evaluated the winter survival ability of *Ae. albopictus* and *Ae. cretinus* adult males and females under semi-field, sheltered microclimatic conditions in a northern area of Attica, Greece, to better understand their distribution patterns in this region.

MATERIAL AND METHODS

Origin of mosquito populations

The mosquito populations used in this study were collected using ovitraps from two locations within the northern area of the Attica Region, Greece. The *Aedes cretinus* population originated from Agios Stefanos (38.140846°N, 23.859296°E), situated in the Municipality of Dionysos, while the *Aedes albopictus* population was collected from Chalandri (38.025333°N, 23.802611°E) (Fig. 1). Both locations are found in the northern Attica Region and comprise urban and peri-urban environments with vegetated and wooded areas creating favourable microhabitats for mosquito development and survival.

Mosquito colonies

Mosquito eggs were collected using ovitraps and transferred to the Laboratory of Agricultural Zoology and Entomology at the Agricultural University of Athens. Under controlled laboratory conditions (T = 25 ± 2°C, RH = 75 ± 5%, 14L : 10D), the eggs were hatched in tap water supplemented with powdered fish food (Sera Micron Nature) and the larvae were reared to the adult stage for species identification using appropriate taxonomic keys (Darsie & Samanidou-Voyadjoglou, 1997; Becker et al., 2020). The larvae were fed with baby fish food ad libitum until pupation. Adult mosquitoes of each species were then transferred separately to cages measuring 30 × 30 × 30 cm and provided with a 10% sucrose solution through a cotton wick. Female mosquitoes were fed with chicken blood using a Hemotek membrane feeding system (Hemotek). For oviposition, plastic beakers containing 100 ml of water and strips of moistened filter paper were placed in the cages.

Experimental design

Adult mosquitoes of both species were reared under laboratory conditions to the sixth generation from field-collected populations. *Aedes cretinus* and *Ae. albopictus* males and females < 24 h old were transferred from the colonies to transparent plastic (polypropylene) cages sized 28 × 16 × 16 cm (L × W × H). The top and the sides of the cages had openings covered with fine netting to allow adequate airflow and prevent mosquitoes from escaping. For *Ae. albopictus*, two cages housed 25 males along with 30 females, while one cage contained 30 males and 30 females (80 males and 90 females, in total). For *Ae. cretinus*, all three cages housed 30 males along with 30 females (90 males and 90 females, in total). The mosquitoes were installed into the cages and placed under semi-field conditions in a sheltered storage room located in Chalandri, northern Attica Region, on December 13, 2023 (Fig. S2). The storage room was unheated and exposed to natural light through a wide opening (window), allowing seasonal photoperiod and temperature fluctuations. Mosquito adults into the cages were supplied with a 10% sucrose solution through a cotton wick, which was replaced every 10 days (no-blood meals were provided). Every day, alive mosquito adults were counted, and dead individuals were removed using fine-tipped tweezers. Mosquitoes were considered dead in the absence of any movement. Mosquito survival was monitored throughout winter, with observations continuing until April 12, 2024.

Meteorological data

The external environmental weather data (open-air) for the Municipality of Chalandri were obtained from penteli.meteo.gr/stations-Chalandri (Meteo, 2023). For the experimental semi-field conditions in the sheltered storage room, a table thermometer (TP-LINK Tapo T315) was used. The thermometer continuously recorded the min-max temperature and min-max Relative Humidity (RH) daily. These data were transferred to a mobile phone database via the TP-LINK Tapo H100 Smart Hub (extra device to connect the thermometer sensor with the Wi-Fi network).

Statistical analysis

The mean survival times and standard errors of the mean (SEM) were calculated using Microsoft Excel. Survival probabilities and percentiles (25th, median [50th], and 75th) were analysed using the Kaplan-Meier estimator implemented through the survival package in RStudio, and the standard error (SE) derived from the confidence intervals (CIs) of each percentile. To compare survival curves between the two species, separately for males and females, the log-rank test in RStudio was applied. Kaplan-Meier survival curves, where log-rank tests were performed, were visualised using the survival, survminer, lubridate and gridExtra packages (Auguie & Antonov, 2017; Grolemund & Wickham, 2011; Kassambara et al., 2021; Therneau, 2024). Cox regression analysis in RStudio was conducted to examine whether sex was a significant factor in determining Hazard Ratios (HR) for event occurrence (death) between sexes within the same species. Overwintering rates were assessed by calculating the percentage of females surviving beyond March 1, 2024 (end of the winter period). A chi-square test was conducted to compare overwintering rates of females between the two species. For data analysis, the significance level of $\alpha = 0.05$ was set. Statistical analysis was conducted using R (version 4.3.2) within the RStudio environment (R Core Team, 2023).

RESULTS

Throughout the experimental period, the outdoor weather was generally mild, with no recorded temperature dropping below 2°C. January was the coldest month in winter, with an average minimum temperature of 7.2°C, followed by the second half of December and February with minimum temperatures of 9.3°C and 9.4°C, respectively (Fig. 2). The experimental semi-field conditions maintained higher minimum temperatures compared to the outdoor environment (approx. 4°C higher), reaching a minimum monthly temperature of 12.4°C during the coldest month of January. Throughout winter, the average monthly temperature in the semi-field environment remained 2–3°C higher than that outdoors (Fig. 2). As shown in Fig. 3, several rapid declines in temperature were recorded in the experimental space: from December 16 to 18, reaching a minimum of 11.5°C on December 17; from January 9 to 15, reaching a minimum of 9.8°C on January 14; and from January 29 to February 2, reaching the overall lowest daily minimum of 8°C on February 2.

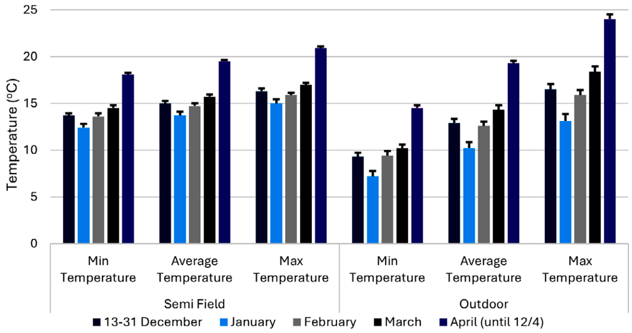

Fig. 2. Mean (± SEM) temperatures (°C) from semi-field and outdoor conditions in Chalandri, Attica, Greece, from 13-12-2023 to 12-4-2024.

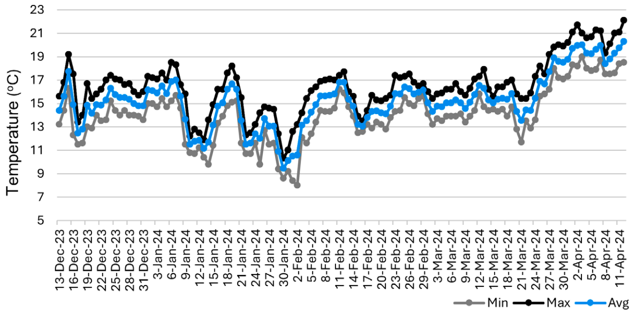

Fig. 3. Winter daily mean temperature (°C) in semi-field conditions in the experimental storage-room in Chalandri, Attica, Greece, from 13-12-2023 to 12-4-2024.

The longevity of both adult males and females *Ae. cretinus* was higher than that of *Ae. albopictus* (Table 1). The superior survival ability of *Ae. cretinus* was even more profound in males, where an almost two-fold increase in average survival time was recorded. The median (50%)

Table 1. Life span (days ± SEM) of *Aedes albopictus* and *Aedes cretinus* adult males and females exposed to semi-field conditions in Chalandri, Attica, Greece, during the winter 2023–2024.

Aedes adults (sex)	n	Average	Percentiles*			min	max
			25%	50%	75%		
Aedes cretinus (♂)	90	28.25 ± 1.3	34 ± 2.8	30 ± 0.8	21 ± 2.8	1	51
Aedes albopictus (♂)	80	15.78 ± 1.3	30 ± 1.3	8.5 ± 3.6	6 ± 0.25	1	37
Aedes cretinus (♀)	90	54.25 ± 3	53 ± 9.95	50 ± 2.3	33 ± 2.6	18	121
Aedes albopictus (♀)	90	32.48 ± 3	35 ± 4.6	31 ± 0.25	7 ± 5.9	4	116

* Percentiles represent the age by which 25%, 50%, and 75% of the population are expected to have survived, respectively. The min and max values refer to the shortest and longest recorded adult survival time (in days).

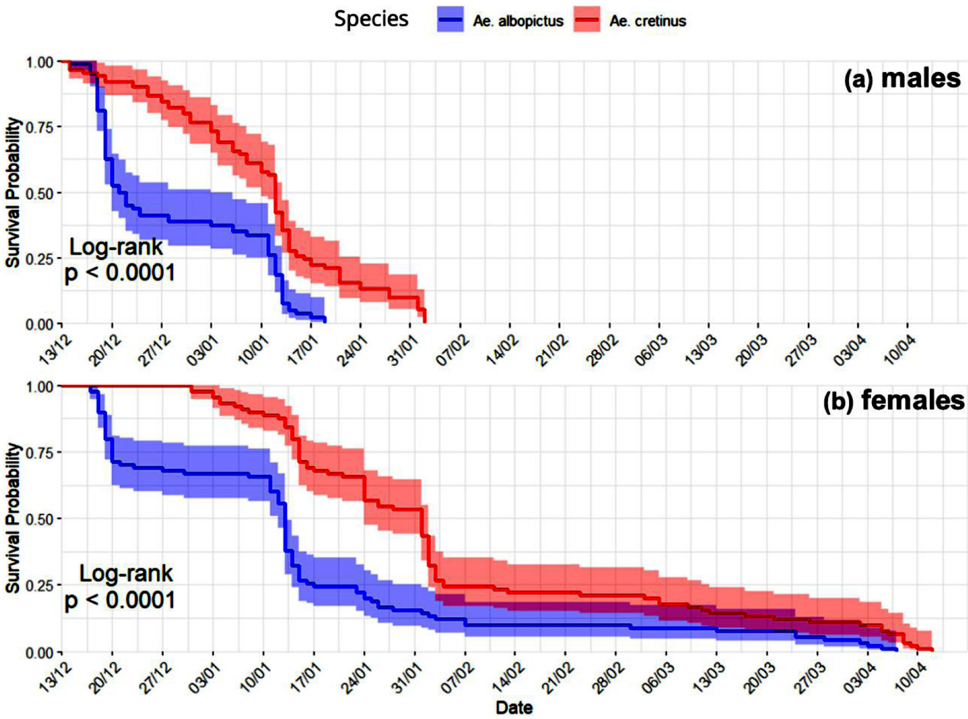

Fig. 4. Comparative survival of *Aedes albopictus* and *Aedes cretinus* adult males (a) and females (b) exposed to semi-field conditions in Chalandri, Attica, Greece, in winter 2023–2024 (Kaplan Meier survival curves with log-rank test).

survival time for *Ae. cretinus* males was 30 days from the start of the experimental period, whereas 50% of *Ae. albopictus* males reached the 50% survival point shortly after the first week, with a median survival time of 8.5 days. Approximately 25% of males from both species remained alive for 30 days, with approximately 75% of *Ae. albopictus* males remaining alive at 6 days, compared to 21 days for *Ae. cretinus* males.

For females, *Ae. cretinus* exhibited a markedly longer average survival time, living approximately 22 days longer than *Ae. albopictus* females (54.25 ± 3 days for *Ae. cretinus* females vs 32.48 ± 3 days for *Ae. albopictus* females). Fifty percent (50%) of *Ae. cretinus* females remained alive at 50 days, whereas 50% of *Ae. albopictus* females remained alive at approximately 31 days. A few females from both species survived for 116–121 days.

The Kaplan-Meier survival curves for *Ae. albopictus* and *Ae. cretinus* adults are presented separately for each sex in Fig. 4. Both male and female *Ae. cretinus* exhibited superior ability to survive compared to *Ae. albopictus*, as the log-rank tests revealed, that survival curves differed significantly between the two species for both sexes ($p < 0.0001$). The steep declines in the survival curves indicate mass number of deaths on the following dates: December 18–20 and January 11–15 for both males and females of *Ae. albopictus*, and January 11–15 for both males and females *Ae. cretinus*, with an additional drop for *Ae. cretinus* females from February 1–3.

The survival curves show that 50% of the *Ae. cretinus* males and females survived approximately 20 days longer than the corresponding *Ae. albopictus* populations in both sexes (Table 1 and Fig. 4). The median (50%) survival time for *Ae. cretinus* males was observed in mid-January while for females occurred early February. In contrast, for *Ae. albopictus*, the 50% survival point for males was reached approximately 8 days after their introduction into the cages on December 13, whereas for females it occurred in mid-January. Moreover, the percentage of females surviving beyond 1st March, thus after the end of winter, was 11% for *Ae. albopictus* and 21.1% for *Ae. cretinus*, though this difference was not statistically significant (chi-squared test $\chi^2 = 3.32$, df = 1, p = 0.07).

The Cox analysis demonstrated the superior survival prospects of females compared to same species males, as shown by the hazard ratio (HR) calculations. For *Ae. albopictus*, males had an HR = 2.65, thus 2.65 times significantly higher risk of mortality compared to females ($p < 0.001$). Similarly, the HR of *Ae. cretinus* males is significantly higher than that of females (HR = 4.1, $p < 0.001$).

DISCUSSION

In the current study, adult females of *Ae. albopictus* and *Ae. cretinus* demonstrated the ability to survive winter under semi-field conditions in a sheltered microclimatic environment in northern Attica, Greece. The sheltered storage room offered a microclimate with more stable and favourable conditions with higher minimum temperatures compared to the outdoor environment, enabling the mosquitoes to survive. This is particularly important during winter, a period characterised by limited availability of resources and challenging environmental conditions (Becker et al., 2020). The ability of both species to overwinter was influenced by the conditions of the experimental space, which mimicked natural refuges such as urban shelters where these species are often found. Notably, under semi-field conditions, some females of both species survived winter temperatures as low as 8°C, recorded on February 2, 2024.

Our findings are consistent with those of Beleri et al. (2023), who reported the successful overwintering of *Ae. albopictus* females in a protected microclimatic shelter in central Attica. That study revealed that females of *Ae. albopictus* could survive winter conditions in human-made shelters where the temperature was moderated compared to the outdoor environment. Similarly, our study highlights the importance of sheltered microclimates in enabling overwinter survival, even under relatively low outdoor temperatures. The ability of *Ae. albopictus* females to survive winter temperatures in sheltered environments in Attica likely contributed to the high adult mosquito populations observed in Attica during warmer winters, such as the winter of 2022–2023, when Lührsen et al. (2023) recorded considerable *Ae. albopictus* activity in BG-Sentinel traps. The minimum winter temperature plays a pivotal role for the survival of *Ae. albopictus* (Hawley, 1988). The cold tolerance observed in females of the Greek strain of *Ae. albopictus* in the present study aligns with findings from Italian strains which demonstrated survival for less than a week at 10°C under stable laboratory conditions (Marini et al., 2020). Furthermore, field data from Northern Italy support these observations, indicating that 9°C represents the threshold temperature for adult female activity in *Ae. albopictus* (Roiz et al., 2010).

In contrast to females, adult males of both species failed to overwinter temperatures under the semi-field conditions of this study. *Aedes albopictus* males exhibited particularly high vulnerability, with the highest mortality observed during cold waves in mid-December and mid-January. By January 19, 2024, all males of this species had succumbed to the unfavourable winter conditions. Similarly, *Ae. cretinus* males experienced significant losses, particularly in mid-January, with complete mortality observed by February 2, 2024. These findings differ from those reported by Beleri et al. (2023), who observed that some *Ae. albopictus* males were able to withstand winter conditions and survive beyond March 1st, in a sheltered microclimatic environment in the Attica Region. The higher survival in that study was likely facilitated by relatively warmer conditions within the protected shelter, where temperatures ranged from approximately 20 to 12°C, highlighting the critical role of microclimatic variability in overwintering success.

While specific data on the effects of temperature on the adult longevity of *Ae. cretinus* are lacking, the cold tolerance exhibited by its females under semi-field conditions may explain the species' preference for cooler microclimatic environments as it has been primarily reported in vegetated and wooded areas in northern urban and suburban areas of the Attica Region (Giatropoulos et al., 2024). Our results indicate a greater longevity of *Ae. cretinus* adults compared to *Ae. albopictus* in the protected microclimate during winter (Fig. 4, Table 1). It is notable that prior to the invasion of *Ae. albopictus* into the Attica Region in 2008, *Ae. cretinus* was widely distributed. However, following the establishment of *Ae. albopictus*, *Ae. cretinus* has become scarce and is mostly detected in vegetated and wooded locations (Giatropoulos et al., 2024). This may be attributed to the displacement of *Ae. cretinus* by *Ae. albopictus,* driven by interspecific competition, as evidenced by laboratory studies on cross-mating and larval competition (Giatropoulos et al., 2015, 2022).

The superior ability of *Ae. cretinus* females to survive at low winter temperatures in protected microclimatic environments in northern Attica may provide *Ae. cretinus* with an advantage, enabling it to develop higher populations outdoors early in the spring and better withstand competition from *Ae. albopictus* in these areas. However, the ability of *Ae. albopictus* females to also overwinter under the same semi-field conditions may result in an increased population size at the beginning of the following growing season and therefore affect the potential of interspecific competition with *Ae. cretinus* at the same outdoor habitats. Following the first recording of *Ae. albopictus* in the Attica Region (in the Municipality of Athens) in 2008, both *Ae. cretinus* and *Ae. albopictus* were documented to coexist in the study area of Chalandri for two years (2010–2011) after the invasion *Ae. albopictus* into Chalandri in 2010 (Giatropoulos et al., 2012b; Giatropoulos, 2014). However, *Ae. cretinus* has not been reported since then in Chalandri (Giatropoulos et al., 2024). Although *Ae. cretinus* survived longer than *Ae. albopictus* in this study, the ability of *Ae. albopictus* to survive winter temperatures, combined with its competitive advantage over *Ae. cretinus* (Giatropoulos et al., 2015; Giatropoulos et al., 2022), may explain the disappearance of *Ae. cretinus* from the study area following the establishment of *Ae. albopictus*.

The overwintering survival ability of females observed under the protected microclimatic conditions in the current study indicates the potential of these species to build up large populations early in spring. In winter, the majority of the *Aedes (Stegomyia)* populations in temperate areas go into diapause at the egg stage (Hawley, 1988, Kramer et al., 2021). Nevertheless, the winter survival of females may contribute to population dynamics the following growing season considering that *Ae. albopictus* winter activity has already been evidenced in temperate southern European countries, namely oviposition activity outdoors in Italy (Romi et al., 2006) and Spain (Collantes et al., 2014; Del Lesto et al., 2022), collection of adult females using traps outdoors in Greece (Lührsen et al., 2023), detection of different life stages (eggs, larvae, males and females) and blood feeding activity outdoors in southeast Spain (Bueno-Marí & Jiménez-Peydró, 2015), and oviposition and human-biting (blood-feeding) activity under favourable indoor conditions in northwestern Italy (Dutto & Mosca, 2017).

Based on our findings, it is strongly recommended that mosquito surveillance be conducted year-round, with appropriate control measures implemented at the beginning of spring, or even during winter on specific occasions, to suppress the first mosquito generation and prevent the establishment of large populations early in the season. Understanding the characteristics of mosquito population dynamics is essential for optimizing surveillance and control strategies (Petrić et al., 2021). In Mediterranean countries such as Greece, spring egg hatching typically occurs between late February and early March (Petrić et al., 2021). Knowledge of the timing and magnitude of peak mosquito activity, as well as the factors affecting it, is essential for guiding effective surveillance methods, implementing timely control activities, and estimating the risk of pathogen transmission (Caputo & Manica, 2020).

Although the current study provides evidence of the overwintering abilities of *Ae. albopictus* and *Ae. cretinus* in a protected microclimatic environment, some limitations must be acknowledged. The semi-field study design does not fully capture the variability of natural settings, including those found outdoors, which may influence mosquito survival and behaviour. Exposure of mosquitoes to various storage rooms and environmental conditions would provide a deeper understanding about the winter survival under different microclimatic conditions. Therefore, future research incorporating more diverse experimental conditions would help validate and expand upon these findings. In our test design, we provided mosquitoes with *ad libitum* food (sucrose) in order to simulate sheltered anthropogenic microhabitats for mosquitoes through winter such as plant nurseries, greenhouses and food-storage premises where *Aedes* mosquitoes may have access to sucrose-based food sources. Throughout the trial, we frequently observed females of both species feeding from the sucrose solution, and this behaviour may account for their survival. Hence, it seems that the females of the tested *Aedes* mosquito species would require a sheltered microclimate and sugar sources to survive during the winter. The importance of sugar availability for the survival of mosquito females in winter has also been observed in obligately overwintering adult mosquitoes such as *Cx. pipiens pipiens*, which show lower mortality rates when provided with fructose in hibernation sites (Sauer et al., 2022). Nevertheless, further experimentation is needed to explore the survival of female adults of the tested *Aedes* species during winter in protected microhabitats with limited food sources. Moreover, the present study focused on winter survival of adults in a protected microhabitat and did not evaluate the ability of females to reproduce during winter, which should be further investigated to assess the potential implications to mosquito population the following growing season.

To conclude, our findings highlight the importance of year-round monitoring of *Ae. albopictus* and *Ae. cretinus* populations, particularly during winter in sheltered environments within the Attica Region and other areas where these species coexist. Such monitoring is essential for understanding their distribution patterns and the dynamics of interspecific competition, which are key elements in shaping mosquito populations and their potential public health impact. By documenting the survival and activity of these species during the winter months, surveillance efforts can better guide targeted control strategies to mitigate population build-up early in the spring. The observed ability of both species to survive winter in protected microclimates provides valuable insights into their population dynamics in urban and peri-urban areas, where sheltered environments such as storage rooms, vegetated areas, and urban refuges may serve as critical overwintering sites. Understanding these dynamics is pivotal for designing effective management programs aimed at reducing mosquito populations and the associated risks of pathogen transmission in temperate regions like the Mediterranean.

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Table S1. Monthly temperature (°C) and relative humidity (%) in semi-field and outdoor conditions.

Environment	Period (Months)	Average temperature (°C) (Average ± SE)	Average min temperature (°C) (Average ± SE)	Relative humidity (%) (Average ± SE)
Semi-field conditions	December (from 13/12)	15 ± 0.25	13. 7±0.25	67.2 ± 1.8
	January	13.7 ± 0.4	12.4 ± 0.4	60.8 ± 1.4
	February	14.7 ± 0.3	13.6 ± 0.3	64.1 ± 1.3
	March	15.7 ± 0.25	14.5 ± 0.3	62.4 ± 1.2
	April (until 12/4)	19.5 ± 0.15	18.1 ± 0.15	47.2 ± 1.2
Outdoor conditions	December (from 13/12)	12.9 ± 0.4	9.3 ± 0.4	74.2 ± 1.8
	January	10.2 ± 0.6	7.2 ± 0.6	72.3 ± 1.3
	February	12.6 ± 0.4	9.4 ± 0.5	70 ± 1.5
	March	14.3 ± 0.5	10.2 ± 0.4	65.6 ± 1.3
	April (until 12/4)	19.3 ± 0.3	14.5 ± 0.25	49.5 ± 1.75

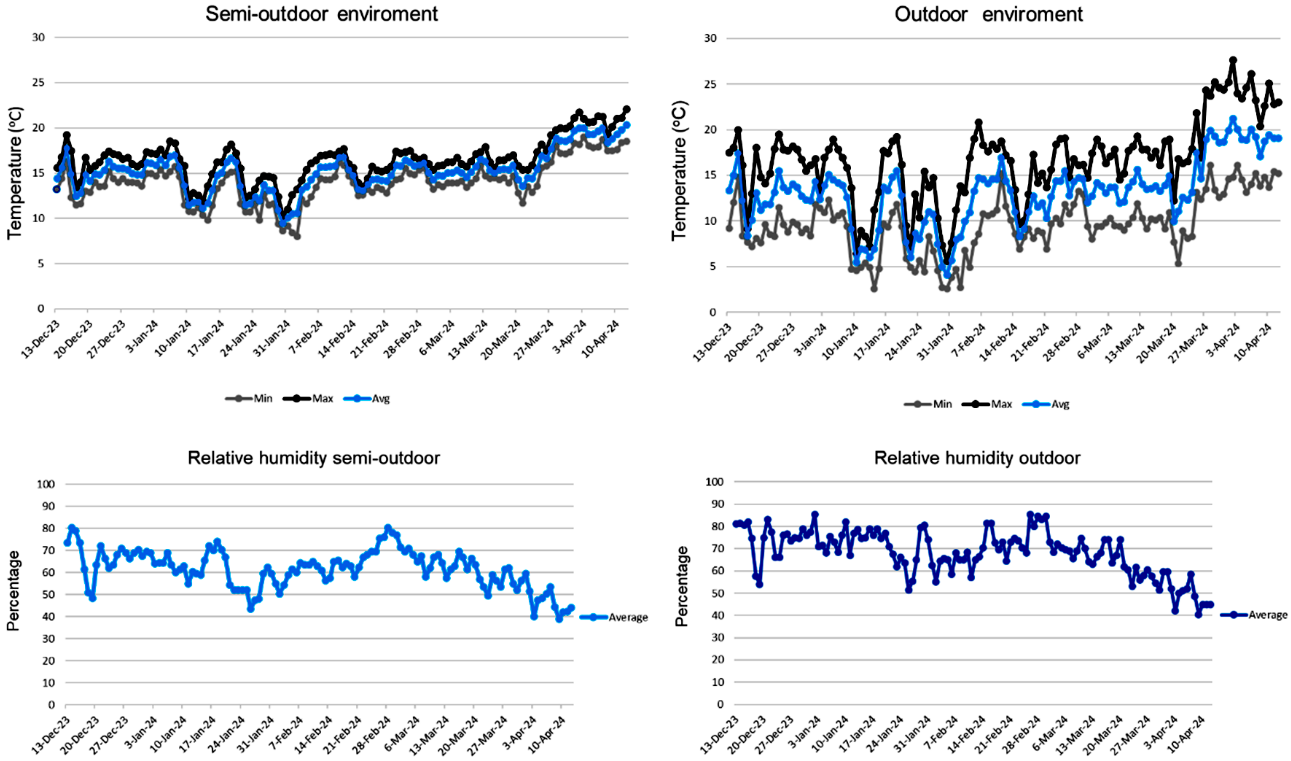

Fig. S1. Daily temperature (°C) and relative humidity (%) in semi-field and outdoor conditions.

Fig. S2. Experimental sheltered storage room (left) and cages (center and right).