

Winter survival of *Culex pipiens* biotype *pipiens* adults in Central Greece

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Summary Winter survival is a key life-history trait of insect vectors in temperate ecosystems, shaping early seasonal population growth and vector-borne disease epidemiology. For West Nile virus infection in Europe, including Greece, *Culex pipiens* is the principal vector. We conducted overwintering experiments in three areas in Central Greece, to assess winter survival of adult *Culex pipiens* biotype *pipiens* under coastal (Nea Anchialos and Volos) and inland (Kalamaki) conditions. A proportion of females survived winter at all sites, whereas males did not overwinter. Female survival reached 23.9% in Kalamaki and 25.3% in Nea Anchialos, while in Volos it ranged from 9.4 to 15.7% across treatments. Surviving females were able to blood-feed and oviposit in spring, producing viable offspring. The proportion of fertile females was 52.3% in Kalamaki and 47.4% in Nea Anchialos, whereas in Volos it ranged from 20.8 to 47.2%. Survival varied among locations and treatments, suggesting an effect of local environmental conditions on overwintering success. The results provide evidence that females of *Cx. pipiens* biotype *pipiens* can survive winter conditions in the study areas and subsequently initiate reproduction in spring. This study contributes to improving the understanding of the overwintering ecology of an important vector and highlights the need for further studies in different habitats.

Additional keywords: longevity, mosquitoes, population dynamics, Thessaly, vector borne diseases

Introduction

In temperate and cold regions, mosquitoes have evolved a variety of overwintering strategies that allow populations to persist through unfavorable environmental conditions. Depending on the species, overwintering may occur at the egg, larval, or adult stage (Bale *et al.*, 2010; Nelms *et al.*, 2013; Denlinger and Armbruster, 2016; Becker *et al.*, 2020). Low temperatures and precipitation are among the most important environmental drivers determining the duration and intensity of overwintering, with effects that vary across latitudinal gradients and lo-

cal climatic regimes (Gill *et al.*, 2017; ECDC, 2020; Rozsypal *et al.*, 2021; Siperstein *et al.*, 2023). In most temperate areas, winter conditions suppress mosquito reproduction and population growth, leading to substantial population declines prior to the onset of the spring generation (Siperstein *et al.*, 2023). From an epidemiological perspective, overwintering survival has been increasingly recognized as a critical determinant of early-season vector abundance and the re-establishment of arbovirus transmission cycles in temperate regions (Brugman *et al.*, 2018; Bellone and Failloux, 2020; Sauer *et al.*, 2022). The proportion of vectors that successfully overwinters can influence the timing and magnitude of population growth in spring, with important implications for the persistence and amplification of mosquito-borne pathogens.

The mosquito species *Culex pipiens* (Diptera: Culicidae) is considered the principal vector of West Nile virus (WNV) in Europe (Kang *et al.*, 2016; Brugman *et al.*, 2018; Rozsypal *et al.*, 2021), including Greece, where repeated outbreaks have been reported

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over the past two decades (Patsoula et al., 2016, 2020; ECDC, 2021; Vakali et al., 2023). The *Cx. pipiens* complex comprises two morphologically indistinguishable but ecologically distinct biotypes, “*pipiens*” (Linnaeus, 1758) and “*molestus*” (Forsk., 1775), as well as their hybrids. These biotypes differ in behavior, physiology, and overwintering biology, traits that may influence their seasonal dynamics and epidemiological importance (Becker et al., 2012; Papadopoulos et al., 2016; Koenraadt et al., 2019; Vinogradova, 2000; Ioannou et al., 2021).

In temperate regions, *Cx. pipiens* biotype *pipiens* overwinters as inseminated adult females that enter facultative reproductive diapause (Mitchell and Briegel, 1989; Denlinger and Armbruster, 2016; Dörge et al., 2020; Rozsypal et al., 2021). Diapause induction is primarily driven by decreasing temperature and photoperiod during late larval and pupal development (Denlinger and Armbruster, 2014; Field et al., 2022). Adults’ diapause triggers a chain reaction of molecular changes, including reduced insulin signaling and juvenile hormone deficiency, leading to ovarian arrest and increased metabolic lipid accumulation (Zhou and Miesfeld, 2009; Sharma et al., 2019; Meuti, 2026). Diapausing females suppress host-seeking and reproductive activity and rely on accumulated lipid reserves to survive the winter months, whereas males do not enter diapause and typically fail to survive winter conditions (Denlinger and Armbruster, 2014; Vinogradova, 2000). With increasing temperatures in spring, females terminate diapause, resume blood feeding, and initiate oviposition, giving rise to the first seasonal generation. Optimal overwintering temperatures for adults’ range between 2 and 6°C. Temperatures below 0°C are generally lethal to overwintering adults if they last for several days. Temperatures above 6°C lead to a gradual increase in metabolic activity, causing depletion of stored lipids and reduced winter survival (Dörge et al., 2020; Bergman et al., 2020; Kampen et al., 2021; Rozsypal et al., 2021).

Overwintering survival is strongly in-

fluenced by microclimatic conditions within hibernation sites, particularly temperature stability and humidity (Koenraadt et al., 2019; Rozsypal et al., 2021; Sauer et al., 2022). Shelters that remain relatively frost-free, such as underground structures, barns, and other protected habitats, are commonly used by overwintering *Cx. pipiens* females. Mild winter conditions or frequent temperature fluctuations may increase metabolic activity and accelerate depletion of lipid reserves, thereby reducing survival, whereas cooler and more stable environments tend to enhance overwintering success (Koenraadt et al., 2019; Rozsypal et al., 2021; Sauer et al., 2022). In southern temperate regions, including the Mediterranean basin, evidence of adult mosquito overwintering has been increasingly documented, highlighting the importance of local microclimatic conditions and human-made shelters (Ravasi et al., 2022; Beleri et al., 2023; Lührs-en et al., 2023).

In the present study, we investigated the overwintering capacity of *Cx. pipiens* biotype *pipiens* adults in three regions of Thessaly, Central Greece, encompassing both coastal and inland environments. Using field-based overwintering experiments, we aimed to (i) assess survival patterns of adult mosquitoes during the winter months, and (ii) determine whether overwintering females are capable of resuming blood feeding and oviposition in spring, thereby contributing to the initiation of the first seasonal generation. By providing field data, this study contributes to a better understanding of the overwintering ecology of *Cx. pipiens* biotype *pipiens* in southern temperate regions and informs future investigations on vector population dynamics and disease risk.

Materials and Methods

Study areas and mosquito colonies

Overwintering experiments with *Cx. pipiens* biotype *pipiens* were conducted in the region of Thessaly, Central Greece, at three locations representing different environ-

mental settings: (a) Kalamaki, an inland village adjacent to Lake Karla, characterized by extensive agricultural activity and wetland-associated vegetation (b) Nea Anchialos, a coastal settlement, and (c) Volos, a coastal urban area (Fig. 1). Nea Anchialos and Volos influenced by maritime climatic conditions, with milder winter temperature fluctuations and urban/peri-urban vegetation. The three sites differ in their microclimatic characteristics, particularly regarding humidity and thermal stability during winter. The experiments were designed as semi-field studies aiming to assess adult overwintering survival under local winter conditions.

Two overwintering experiments were performed. Experiment 1 took place during the winter of 2012–2013 at Kalamaki and Nea Anchialos. Experiment 2 was conducted during the winter of 2013–2014 in Volos and included additional experimental treatments to explore potential effects of organic material present in overwintering shelters.

Adult mosquitoes used in all experiments originated from laboratory colonies of *Cx. pipiens* biotype *pipiens* maintained at the Laboratory of Entomology and Agricultural Zoology, University of Thessaly (Volos, Greece). Colonies were established from egg rafts laid by laboratory-reared adults

and maintained for experimental use.

Rearing of immature stages and adult pre-exposure conditions

Egg rafts (~70 per experiment) were placed in plastic containers containing 12 L of water and reared outdoors at the premises of the Department of Agriculture, Crop Protection and Rural Environment, University of Thessaly, Volos, in protected locations shielded from direct rainfall. Larvae were provided with artificial food (Purina Adult cat food, Friskies) and both food and water were replenished at regular intervals to ensure adequate developmental conditions.

Immature development occurred under natural autumn photoperiod and ambient temperature conditions. Upon pupation, pupae of both sexes were transferred to plastic containers containing 250 mL of water and placed inside Plexiglas cages (20 × 20 × 20 cm, bearing four 15 × 15 cm mesh covered windows to facilitate ventilation) for adults' emergence.

Newly emerged adults were maintained for 10–12 days in a temperature-controlled storage room ($15 \pm 2^\circ\text{C}$) under natural light conditions. During this period, adults were provided with a 10% sucrose solution to allow mating and accumulation of lipid re-



Figure 1. Map showing the locations (Kalamaki, Nea Anchialos, Volos), where the experimental study took place.

serves necessary for overwintering. After this pre-exposure period, sucrose was replaced with plain water prior to transfer to overwintering sites.

Overwintering experimental procedures

For Experiment 1, five cages (~100 adults per cage) were transferred to Kalamaki on December 30, 2012 and to Nea Anchialos on January 4, 2013. Cages were placed in dark, humid, and sheltered storage areas protected from rain and wind, simulating typical overwintering environments. Cages were placed on shelves at 1–1.5 m above the ground and 20–30 cm from the nearest wall.

For Experiment 2 in Volos, ten cages (~100 adults per cage) were transferred to the overwintering site on December 1, 2013. Cages were randomly assigned to one of the following treatments and placed in the overwintering site as above:

- Treatment A: plain water only
- Treatment B: plain water plus organic-rich infusion (a vial containing liquid collected from a composting bin, as a proxy of natural detritus and microbial environment)
- Treatment C: plain water only, with adults originating from a later egg-hatching cohort (October 15, 2013), serving as a temporal control

Each treatment consisted of five cages, with one cage representing the experimental unit. No additional food sources were provided during the overwintering period.

At the end of winter (early to mid-March), a 10% sucrose solution was reintroduced into all cages, and cages were transferred back to a semi-outdoor area at the University of Thessaly. Transfer dates were March 7, 2013 (Kalamaki), March 14, 2013 (Nea Anchialos), and March 19, 2014 (Volos).

Blood feeding, oviposition and egg hatch

Surviving females from Kalamaki and Nea Anchialos were pooled by location and offered a two-hour blood meal on April 11, 2013, using an artificial feeding device. The mosquitoes were fed certified human blood

maintained at 38°C using two custom-made feeders connected to a circulating warm-water bath (Ioannou et al., 2021).

The mating status of females was supported by published validation assays showing that 1-day-old *Culex pipiens* females with males under laboratory conditions achieve 100% insemination within 5 days, confirmed by spermathecal dissection (Fytrou et al., 2022). Oviposition containers (plastic cups containing 250 mL of water) were placed in the cages from April 20 to April 22, 2013.

In Volos, surviving females were offered a blood meal on April 23, 2014, and oviposition containers were provided on April 28 to April 30, 2014. Percentages of female fertility in all cases were determined by dividing the total number of single egg rafts laid with the total number of surviving females. Egg rafts were collected and examined under a stereomicroscope to assess hatching success. An egg raft was considered as successfully hatched when more than 50% of its total eggs yielded neonate larvae. Since Experiment 1 revealed hatching rates more than 90% (see results) for laid egg rafts, this parameter was not recorded in Experiment 2.

Survival monitoring

Adult survival was monitored at regular intervals throughout the overwintering period. Dead individuals were removed from cages and recorded. Sex was determined based on morphological characteristics. Accidental losses were recorded and excluded from survival analyses where appropriate.

Meteorological data

Ambient temperatures throughout the experimental period, from immature development to oviposition, were recorded with the help of special electronic devices (HOBO, Onset, USA). Temperature data corresponding to each location are presented in Figures 2–4, together with indications of exposure periods, feeding events, and oviposition.

Statistical analysis

Survival analyses were performed using

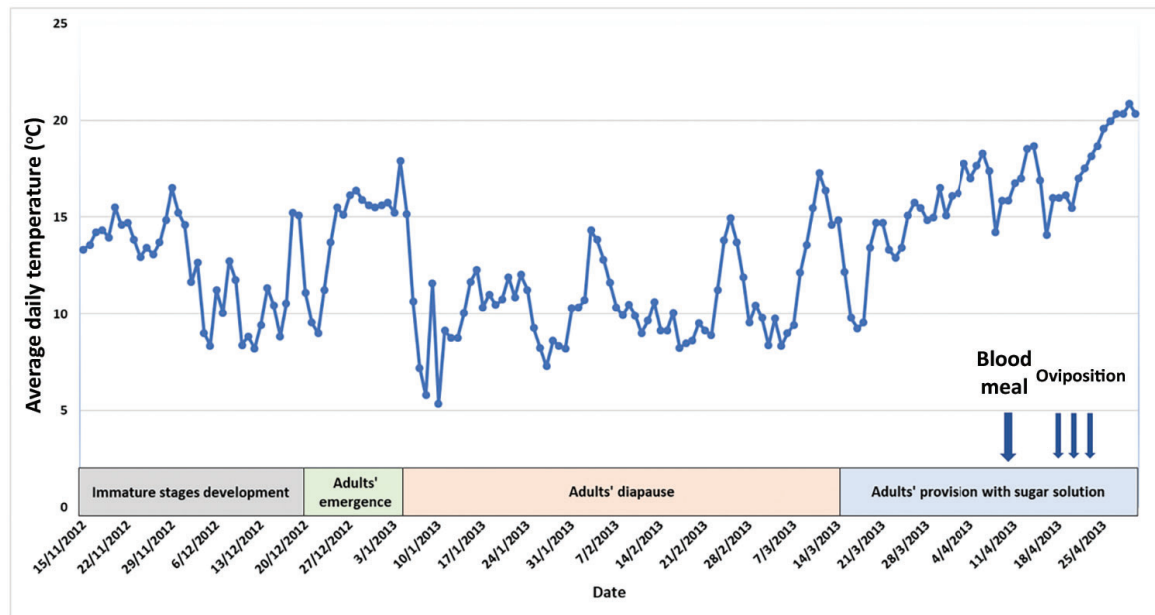


Figure 2. Average daily temperatures that prevailed throughout the experimental study of winter survival of *Culex pipiens* biotype *pipiens* in Nea Anchialos (a coastal area) Thessaly, during the period 2012–2013.

Kaplan–Meier survival curves and log-rank tests to compare survival between sexes and among treatments. Cox regression was employed to examine whether the sex of adults and the overwintering site were sig-

nificant predictors of adult mortality rates. Statistical analyses were conducted using R software (version 4.3.2; R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was set at $\alpha = 0.05$.

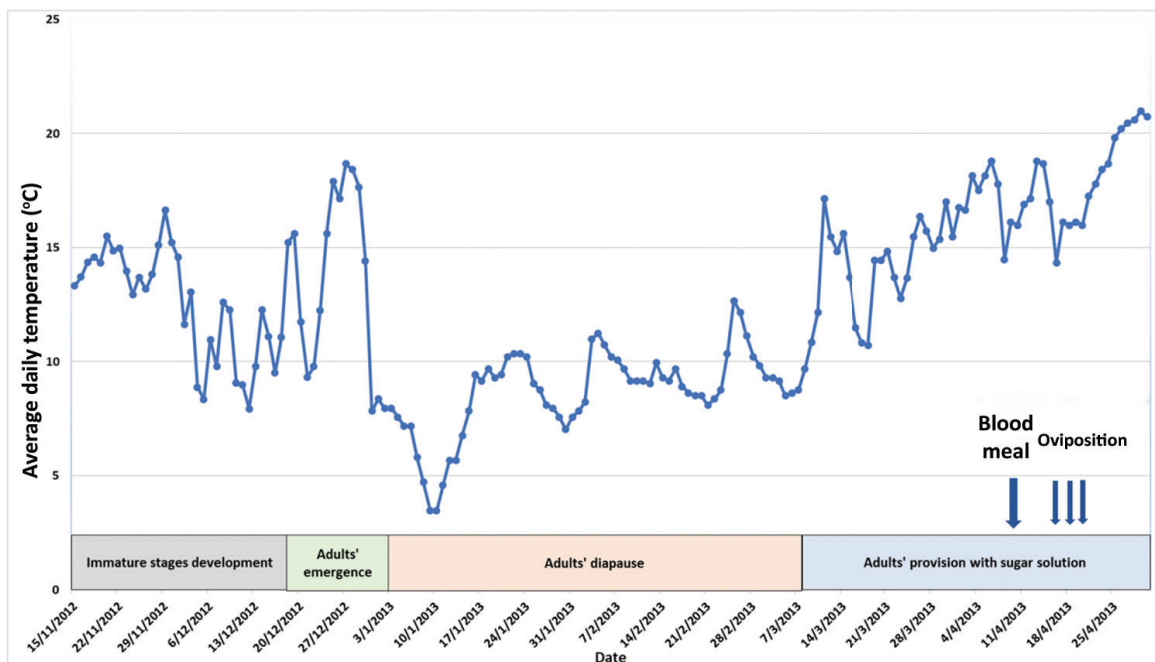


Figure 3. Average daily temperatures that prevailed throughout the experimental study of winter survival of *Culex pipiens* biotype *pipiens* in Kalamaki (a mainland village), Thessaly, during the period 2012–2013.

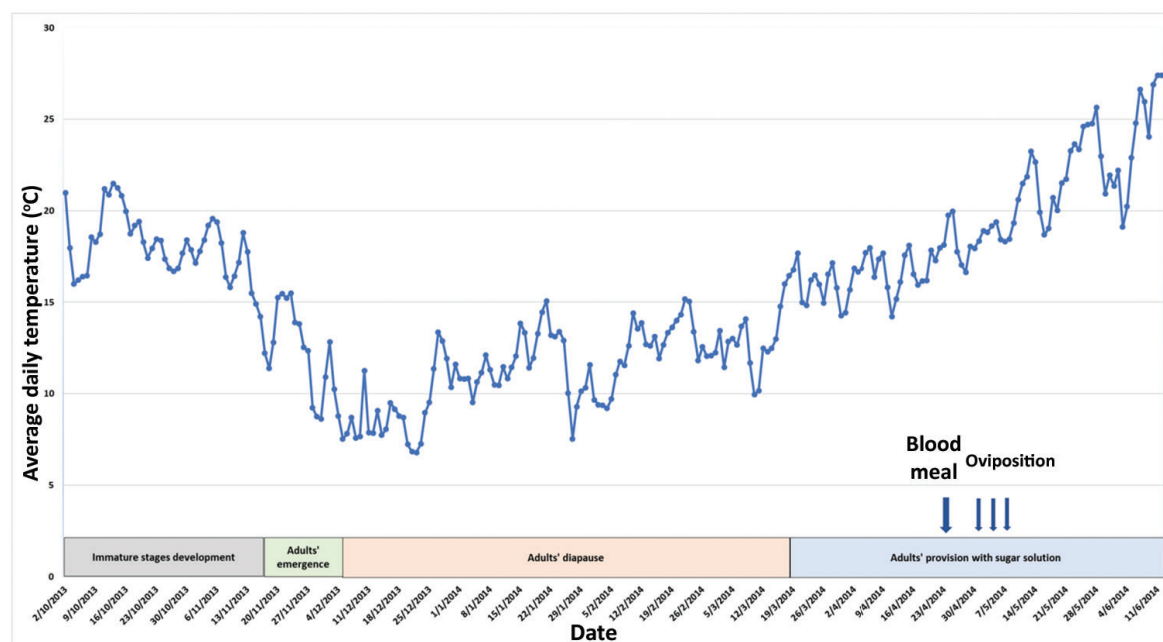


Figure 4. Average daily temperatures that prevailed throughout the experimental study of the winter survival of *Culex pipiens* biotype *pipiens* in Volos, Thessaly, during the period 2013–2014.

Results

Winter survival of *Culex pipiens* biotype *pipiens* in Kalamaki and Nea Anchialos

According to the average daily temperatures during the experimental period in Nea Anchialos and Kalamaki, from immature development to oviposition of overwintering females, (Figs 2 and 3, respectively), in both locations, temperatures remained relatively high until late December, while the colder winter period began in early January. An increase in temperature was observed in early March, marking the end of the winter period. Overall, winter temperatures were lower in the inland location of Kalamaki compared to the coastal site of Nea Anchialos.

In Kalamaki, a total of 450 adults (90 per cage; 173 females, i.e. 39% of exposed individuals) were exposed to overwintering conditions, while in Nea Anchialos 456 adults were exposed (91.2 per cage; 294 females, i.e. 64.7% of exposed individuals), as determined at the end of the experimental procedure considering also accidental losses. No males survived until the end of the overwintering period at either site (Fig. S1). Male survival was generally longer in Kalamaki

than in Nea Anchialos.

Female mortality in Nea Anchialos increased progressively throughout the winter period (Fig. S1). In contrast, female survival in Kalamaki remained relatively high until late February, followed by a significant decline thereafter (Fig. S1). The reintroduction of sucrose solution in early March coincided with a stabilization of female mortality in both locations. At the onset of oviposition (April 20), the proportion of surviving females reached 23.9% in Kalamaki (44 individuals in total) and 25.3% in Nea Anchialos (76 individuals in total). Of these females, 52.3 and 47.4% oviposited in Kalamaki and Nea Anchialos, respectively, while 95.7 and 91.7% of the laid egg rafts hatched.

Mortality rates of males and females at Kalamaki and Nea Anchialos were compared with the Kaplan Meier curves and the Cox proportional hazards model. Overall, and in both locations, male longevity was shorter than that of females with males failing to overwinter (Fig. 5; $p < 0.01$). The hazard ratio of males compared to females was 12.9 (95%CI: 9.69, 17.2) in Kalamaki and 78.9 (95%CI: 47.4, 131.0) in Nea Anchialos.

Cox regression analysis considering lo-

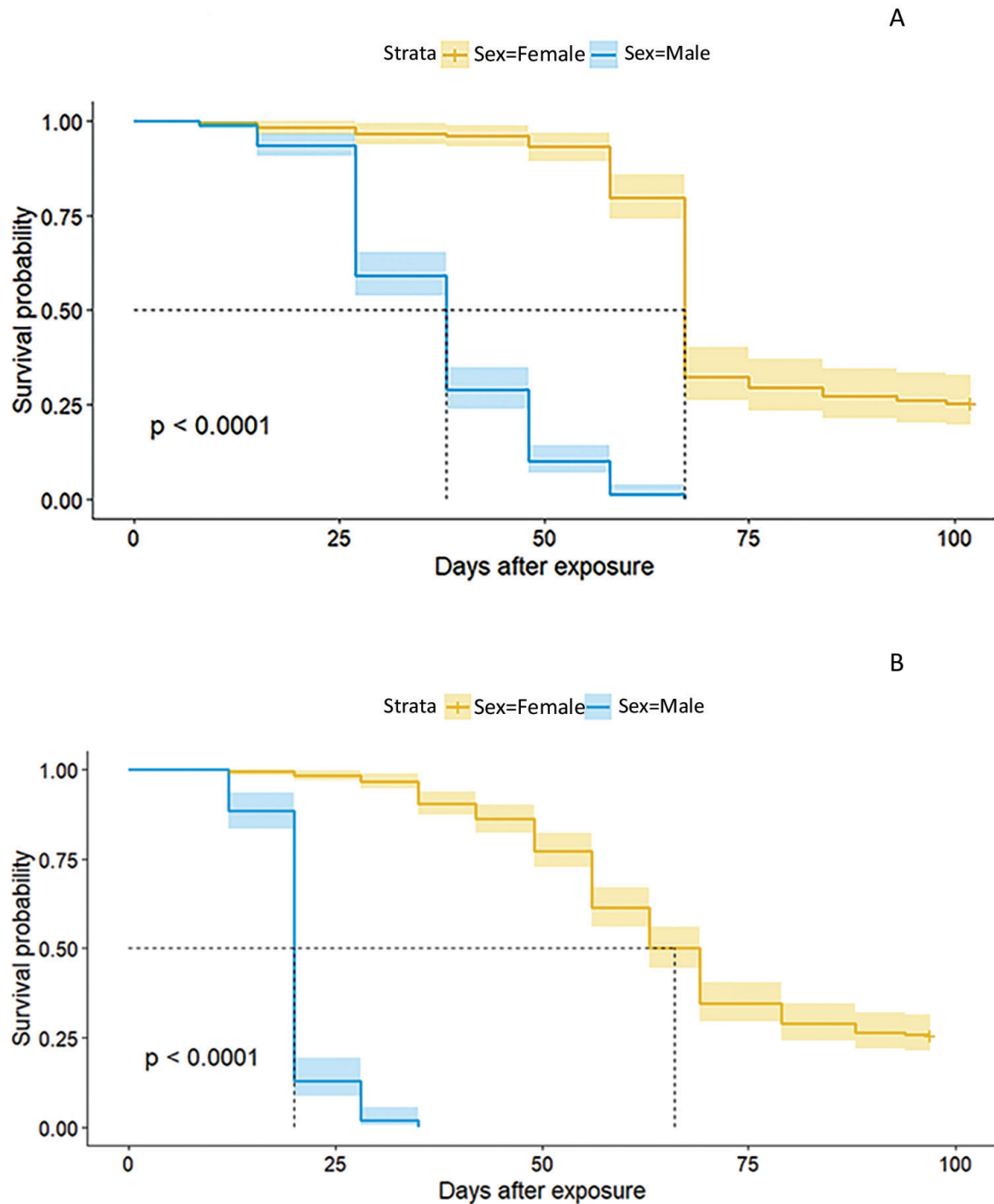


Figure 5. Kaplan Meier curves (with 95%CI) including log-rank test (shown p-value) for the survival of males and females in Kalamaki (A) and Nea Anchialos (B). Dashed lines represent the median survival for males (20 days) and females (66 days).

cation of exposure, sex and their interaction as predictors revealed that males have a significant higher hazard rate compared to females ($p < 0.001$), adjusted for location, and mortality rates were higher in Kalamaki compared to Nea Anchialos ($p < 0.001$) (Fig.

6, Fig. S2). Overall hazard rates considering both males and females were similar between the two locations ($p = 0.425$). The significant interaction between sex and location is associated with higher mortality rates for male in Nea Anchialos compared to Ka-

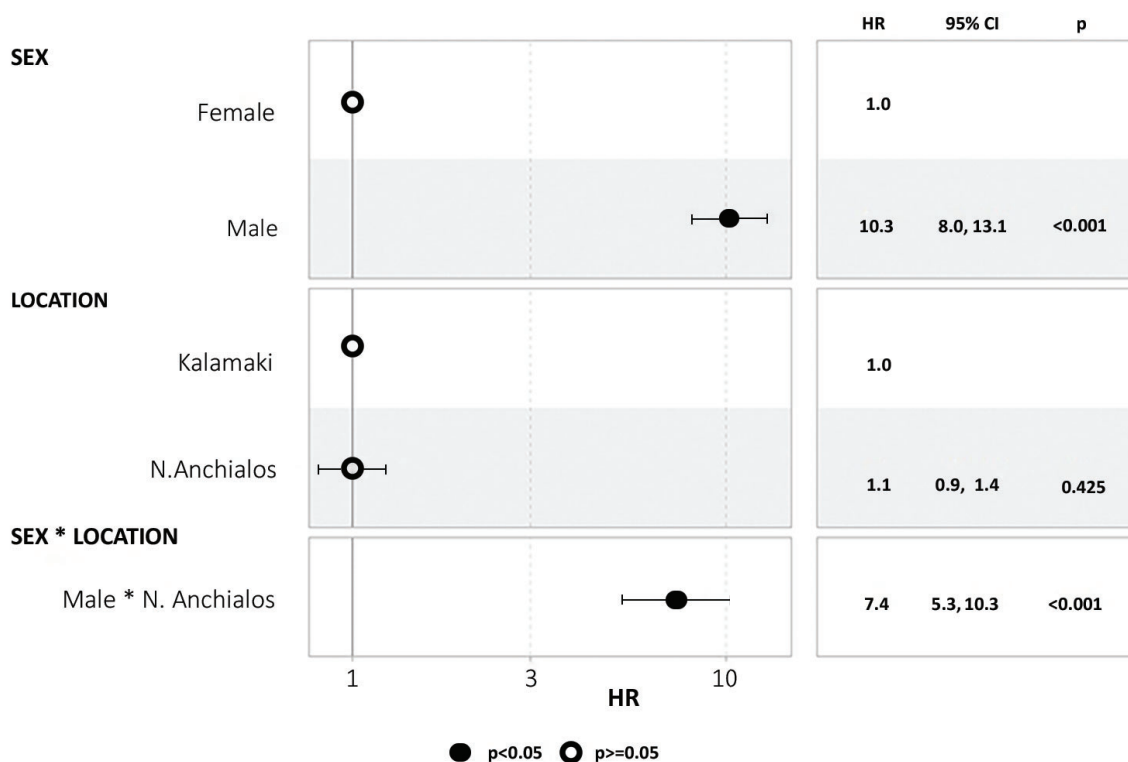


Figure 6. Forest plot depicting the hazard ratio for males and females exposed to Kalamaki and Nea Anchialos.

lamaki. Indeed, comparing survival patterns of females in the two location no significant differences were found (Fig. 6; log-rank test, $p = 0.16$).

Winter survival of *Culex pipiens* biotype *pipiens* in Volos

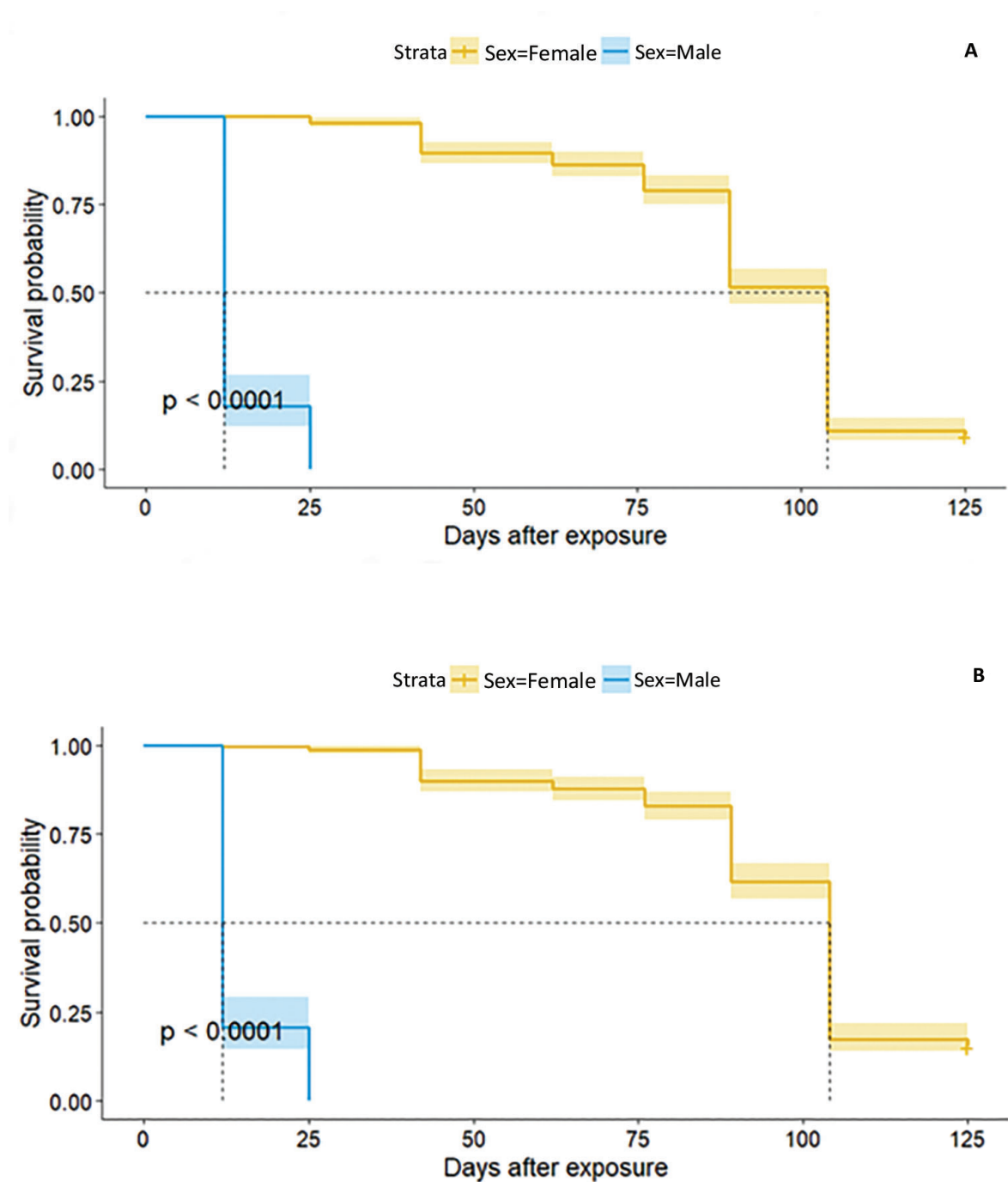
The average daily temperatures recorded during the overwintering experiment in Volos are shown in Figure 4. A decrease in temperature in early December marked the onset of winter conditions, while temperatures increased again in mid-March. The total number of adults exposed to winter conditions was 482 (average per cage 96.4; 371 females, i.e. 76.9%), 475 (average per cage 95; 349 females, i.e. 73.5%) and 509 (average per cage 101.8; 388 females, i.e. 76.2%) for the treatments A, B and C respectively. In all treatments, male survival was markedly lower than female survival, and no males survived beyond late December. Details of female and male survival patterns in each cage are given in Figure S3.

Among cage variation was low in Treatment A and B and quite higher in Treatment C (Figs S3-S5). Overall at the end of the winter period 9.4, 15.7 and 12.4% of the females managed to survive in Treatments A, B and C respectively. Among cage variation in male survival was minimal within the same treatment and overall survival patterns among treatments negligible as well (Fig. S4). Comparing the survival of the two sexes of *Cx. pipiens*, the ability of females (Fig. S5a) to overwinter in all three treatments and the inability of males (Fig. S5b) to survive are evident. In all three treatments provision of sugar solution on March 16, 2014 reduced mortality rates. At the start of oviposition (April 28) in Treatment A, Treatment B and Treatment C, the number of females alive was 35, 53, and 48, of which 13, 25, and 10 oviposited, with the oviposition rate reaching 37.1, 47.2, and 20.8% respectively.

Kaplan–Meier survival analysis followed by the log-rank test revealed the higher mortality of males compared to that of fe-

males within each treatment (Fig. 7; log-rank test, $p < 0.01$). Cox regression analysis including treatment, sex and their interaction as predictors confirmed the overall higher hazard rates of death for males compared to females ($p < 0.001$) and differences among the three treatments (Fig. 8). The hazard ratio of Treatment B and C compared to baseline A was slightly but significantly lower and higher respectively (Fig. 8; $p < 0.05$). The

interaction between sex and treatment was not statistically significant. Kaplan Meier analysis comparing survival patterns of the three female groups followed by log-rank test revealed significant differences among the three treatments (Fig. 7; log-rank test, $p < 0.01$). Nevertheless, the proportion of surviving females at the end of the exposure period was similar among the three treatments (chi-square test, $p > 0.05$).



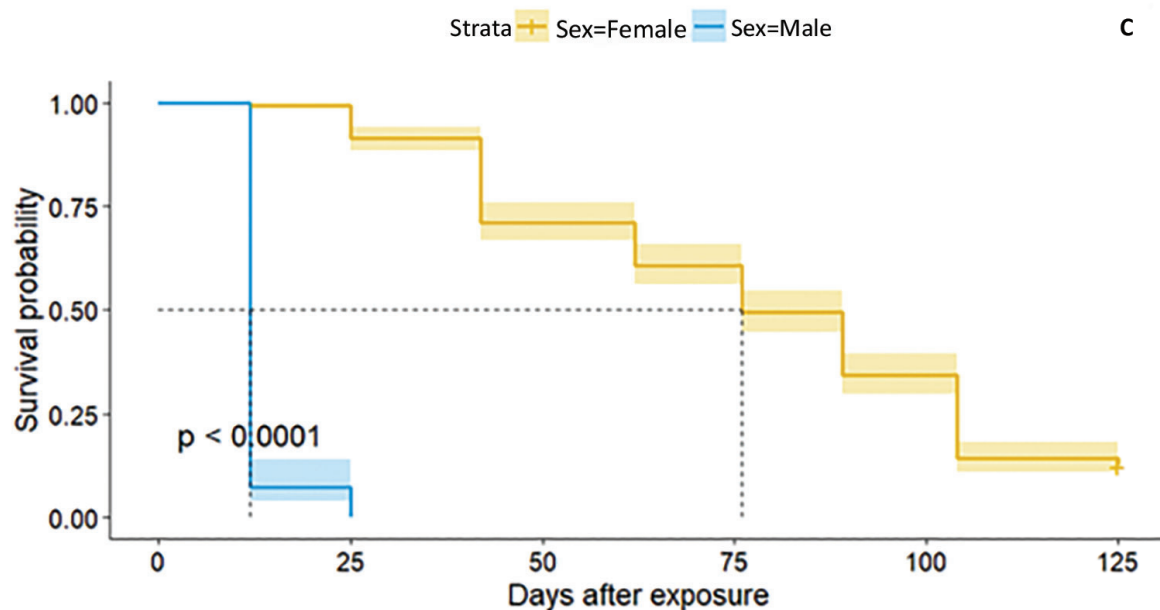


Figure 7. Kaplan Meier curves (with 95%CI) including log-rank test (shown p-value) for the survival of males and females in treatment A (Plain water plus organic-rich infusion exposed to the wild on October 1, 2013), treatment B (Plain water only, exposed to the wild on October 1, 2013), and treatment C (Plain water only exposed to the wild on October 15, 2013). Black dashed lines represent the median survival for males (12 days) and females (76 days).

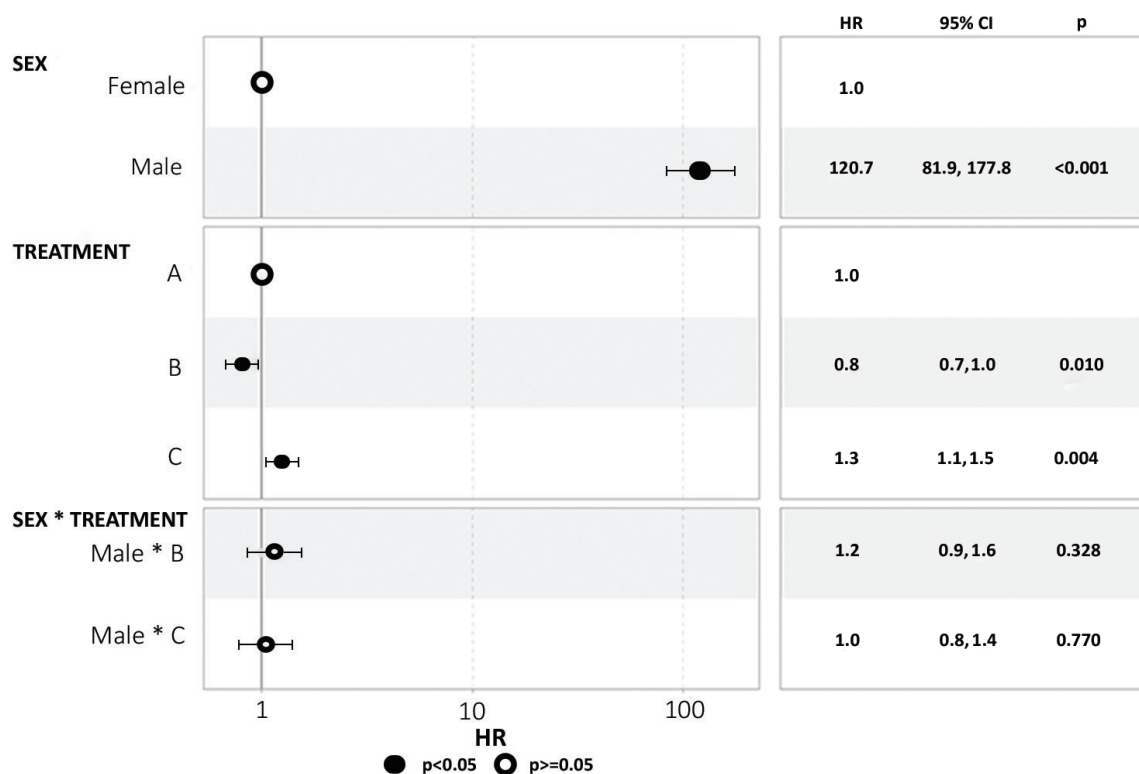


Figure 8. Forest plot depicting the hazard ratios (HR) for different sex, treatment, and interaction between sex and treatment groups exposed to Volos, during the winter 2012-13. Treatment A (Plain water, exposed to the wild on October 1, 2013), treatment B (Plain water plus organic-rich infusion, exposed to the wild on October 1, 2013), and treatment C (Plain water exposed to the wild on October 15, 2013).

Discussion

The overwintering experiments conducted in three regions of Thessaly provide indicative evidence that a proportion of *Cx. pipiens* biotype *pipiens* females are capable of surviving winter conditions in Central Greece and subsequently initiating reproduction in spring. Despite being initiated relatively late in the season, the experiments consistently showed successful overwintering of females at all sites, while males failed to survive until the end of the cold period. These findings are in agreement with the well-established diapause strategy of *Cx. pipiens* biotype *pipiens*, whereby inseminated females overwinter as adults while males do not enter diapause and typically perish during winter.

Female survival patterns differed among locations and treatments, although successful overwintering was observed in both coastal and inland areas. Differences in male longevity between sites may reflect variation in local microclimatic conditions, particularly temperature, with lower temperatures potentially reducing metabolic rates and delaying mortality. In contrast, female survival curves were broadly comparable among locations, suggesting that the overwintering capacity of *Cx. pipiens* biotype *pipiens* females is relatively robust across the range of winter conditions encountered in the study area. Similar patterns have been reported in other temperate regions, where female survival during winter is closely linked to the stability of microclimatic conditions within overwintering shelters (Koenraad et al., 2019; Rozsypal et al., 2021; Sauer et al., 2022).

A significant finding of the present study is that a proportion of overwintering females successfully blood fed, oviposited, and produced viable offspring in spring, demonstrating their potential contribution to the initiation of the first seasonal generation. This observation is epidemiologically relevant, as overwintering females may play a critical role in shaping early-season population dynamics and, consequently, the risk of arbovirus transmission (Brugman et al.,

2018; Bellone and Failloux, 2020). The observed variation in oviposition rates among locations and treatments highlights the importance of pre- and post-winter conditions in determining reproductive success. Although the presence of organic matter in the water did not significantly influence female survival during overwintering, it appeared to have a modest positive effect on survival. In addition, females exposed to this treatment exhibited higher post-overwintering oviposition rates, with the proportion of ovipositing females increasing by approximately 10% relative to Treatment A and 27% relative to Treatment C. The reduction in mortality following the reintroduction of sugar solution in early spring further underscores the importance of energy availability during the post-diapause period, as previously reported for diapausing *Cx. pipiens* females (Mitchell and Briegel, 1989; Denlinger and Armbruster, 2014).

Temperature is widely recognized as a primary driver of overwintering survival in mosquitoes, influencing metabolic rates, activity levels and depletion of lipid reserves. Although the present study was not designed to identify specific thermal thresholds, the observed survival patterns are consistent with previous work indicating that relatively mild and stable winter conditions can support adult overwintering of *Cx. pipiens* biotype *pipiens* (Rozsypal et al., 2021; Sauer et al., 2022). Differences between coastal and inland sites likely reflect microclimatic variation rather than fundamental differences in overwintering strategy, emphasizing the importance of local environmental conditions in shaping overwintering outcomes.

While the ecological and epidemiological roles of *Cx. pipiens* biotypes and their hybrids are well documented, the present study focused exclusively on *Cx. pipiens* biotype *pipiens*. Consequently, no conclusions can be derived from the current data regarding differences among biotypes or the role of hybrids in overwintering and pathogen transmission. Nevertheless, the demonstrated ability of *Cx. pipiens* biotype *pipi-*

ens females to survive winter and reproduce in spring supports the notion that overwintering adults may contribute to the persistence of vector populations and potentially facilitate early-season pathogen circulation, as suggested by studies detecting WNV in overwintering *Culex* females in Europe (Brugman et al., 2018; Kampen et al., 2021).

Several limitations should be considered when interpreting the present findings. The experiments were initiated relatively late in the season, which may have influenced diapause establishment and precluded assessment of mortality earlier in winter. Since the experiments started in December-January, the initial phase of diapause induction and the associated mortality rate are likely to have been incompletely recorded. Subsequently, the reported overwintering survival rates may represent conservative estimates of survival following the initial transition to winter conditions. In addition, the limited number of cages per site and the use of laboratory-reared mosquitoes restrict the extent to which the results can be generalized to natural populations. Interannual climatic variability, which was not captured in the present study, may further influence overwintering success. Despite these limitations, the results provide valuable field-based evidence of overwintering survival of *Cx. pipiens* biotype *pipiens* in a southern temperate region, where experimental data remain comparatively scarce. The use of laboratory-reared mosquitoes is likely to limit the ability to directly generalize the results in natural populations, as laboratory colonies could differ from wild populations regarding the diapause expression, stress tolerance, and energy reserve dynamics. Nevertheless, the use of laboratory populations permitted greater standardization of the experiments and reduced uncontrolled biological variability among treatments (Nelms et al., 2013; Ioannou et al., 2021; Siperstein et al., 2023).

Overall, the findings highlight the importance of overwintering ecology for understanding seasonal population dynamics of *Cx. pipiens* and their implications for vector-borne disease risk. Future studies that

will include multiple winters, additional biotypes, and detailed characterization of natural overwintering shelters will be essential to improve estimates of overwintering survival and to elucidate the role of local environmental conditions in shaping mosquito population dynamics and pathogen transmission.

Supplementary Materials

Figure S1: Winter survival of *Culex pipiens* biotype *pipiens* in Kalamaki (a mainland village) (A) and in Nea Anchialos (a coastal area) (B), Thessaly, 2012-2013.

Figure S2: Kaplan Meier curves (with 95%CI) including log-rank test (shown p-value) for the survival of females in Nea Anchialos and Kalamaki. Black dashed lines represent the median survival for females in Nea Anchialos (66 days) and females in Kalamaki (67 days).

Figure S3: Survival rate of *Culex pipiens* biotype *pipiens* female adults regarding treatment 1 (a), treatment 2 (b), and treatment 3 (c), in each cage, in Volos, Thessaly, 2013-2014.

Figure S4: Survival rate of *Culex pipiens* biotype *pipiens* male adults regarding treatment 1 (a), treatment 2 (b), and treatment 3 (c), in each cage, in Volos, Thessaly, 2013-2014.

Figure S5: Survival rate of *Culex pipiens* biotype *pipiens* adults (a) females, (b) males, regarding the three treatments in Volos, Thessaly, 2013-2014.

Author Contributions

Conceptualization, C.I., C.H. and N.T.P.; Methodology, N.T.P. and C.I.; Software, C.I. and P.T.; Validation, X.X., Y.Y. and Z.Z.; Formal analysis, C.I., S.B. and N.T.P.; Investigation, C.I., P.T.; Resources, C.H., E.P., A.M. and N.T.P.; Data curation, C.I. and P.T.; Writing—original draft preparation, S.B., A.M., E.P. and N.T.P.; Writing—review and editing, S.B., A.M., E.P. and N.T.P.; Visualization, A.M., E.P.; Supervision, N.T.P.; Project administration, N.T.P.; Funding acquisition, C.H. and N.T.P. All authors have read and agreed to the published version of the manuscript.

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Competing interests

The authors declare no competing interests.

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Χειμερινή επιβίωση των ενηλίκων του *Culex pipiens* biotype *pipiens* στην Κεντρική Ελλάδα

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Περίληψη Η επιβίωση κατά τη χειμερινή περίοδο αποτελεί κρίσιμο στοιχείο της βιολογίας των εντόμων-διαβιβαστών σε εύκρατα οικοσυστήματα, καθώς συνδέεται άμεσα με την έναρξη και τη δυναμική αύξηση των πληθυσμών κατά την επόμενη αναπαραγωγική περίοδο, με σημαντικές επιπτώσεις στην επιδημιολογία των μεταδιδόμενων νοσημάτων. Το είδος *Culex pipiens* αποτελεί τον κύριο διαβιβαστή του ιού του Δυτικού Νείλου στην Ευρώπη, συμπεριλαμβανομένης της Ελλάδας. Στην παρούσα μελέτη παρουσιάζονται πειράματα διαχείμασης που πραγματοποιήθηκαν σε τρεις περιοχές της Κεντρικής Ελλάδας, με στόχο την αξιολόγηση της ικανότητας διαχείμασης των ενηλίκων του *Cx. pipiens* biotype *pipiens* υπό φυσικές, τοπικές χειμερινές συνθήκες. Διεξήχθησαν δύο πειραματικές προσεγγίσεις σε παράκτιες περιοχές (Νέα Αγχίαλος και Βόλος) και σε ηπειρωτική περιοχή (Καλαμάκι). Τα αποτελέσματα έδειξαν ότι ένα ποσοστό των θηλυκών του *Cx. pipiens* biotype *pipiens* επέζησε επιτυχώς της χειμερι-

νης περιόδου σε όλες τις μελετώμενες περιοχές μελέτης, ενώ τα αρσενικά δεν παρουσίασαν ικανότητα διαχείμασης. Τα ποσοστά επιβίωσης των θηλυκών στο τέλος της χειμερινής περιόδου έφτασαν το 23,9% στο Καλαμάκι, το 25,3% στη Νέα Αγχίαλο, ενώ στο Βόλο κυμάνθηκε από 9,4% έως 15,7%. Τα θηλυκά που επέζησαν μετά τη χειμερινή περίοδο ήταν ικανά να τραφούν με αίμα και να ωοτοκήσουν κατά την άνοιξη, παράγοντας βιώσιμους απογόνους. Σημαντικός αριθμός θηλυκών που επιβίωσαν έδωσαν αυγά (σχεδίες αυγών) τόσο στο πείραμα στο Καλαμάκι όσο και στη Νέα Αγχίαλο (52,3% και 47,4% αντίστοιχα). Το ποσοστό των θηλυκών που ωοτόκησαν στο πείραμα στον Βόλο κυμάνθηκε από 20,8% έως 47,2%. Η επιβίωση των θηλυκών παρουσίασε διαφοροποιήσεις μεταξύ των περιοχών και των πειραματικών μεταχειρίσεων, υποδηλώνοντας πιθανή επίδραση των τοπικών περιβαλλοντικών συνθηκών στη δυναμική της διαχείμασης. Τα ευρήματα παρέχουν ενδεικτικά στοιχεία ότι τα θηλυκά του *Cx. pipiens* biotype *pipiens* μπορούν να διαχειμάζουν επιτυχώς στις περιοχές μελέτης και στη συνέχεια να ξεκινήσουν την αναπαραγωγική τους διαδικασία την άνοιξη. Τα αποτελέσματα της παρούσας μελέτης συμβάλλουν στη βελτίωση της κατανόησης της οικολογίας της διαχείμασης ενός σημαντικού διαβιβαστή παθογόνων και αναδεικνύουν την ανάγκη για περαιτέρω μελέτες σε διαφορετικά ενδιαίτηματα.

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Supplementary material

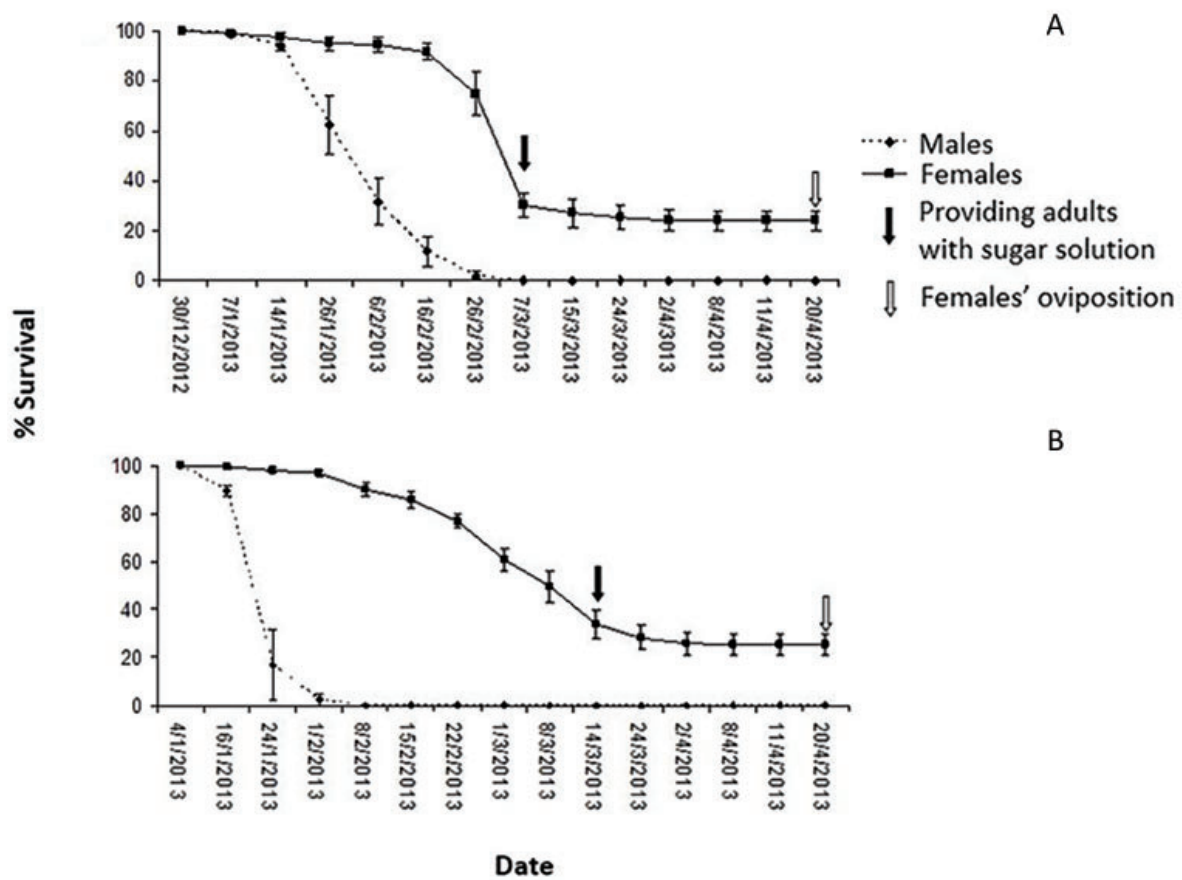


Figure S1: Winter survival of *Culex pipiens* biotype *pipiens* in Kalamaki (a mainland village) (A) and in Nea Anchialos (a coastal area) (B), Thessaly, 2012-2013.

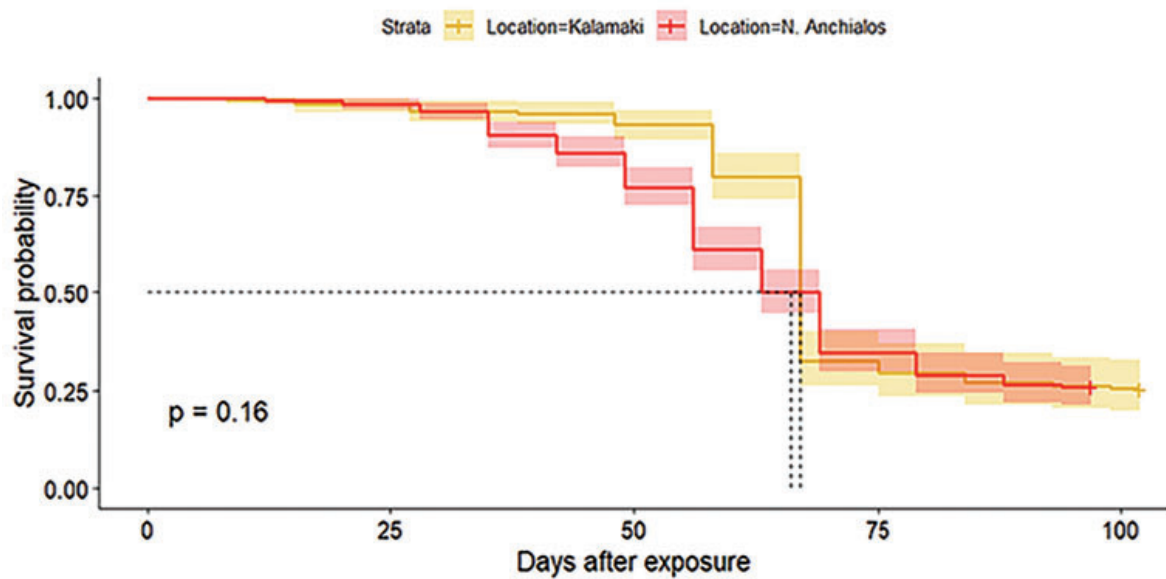
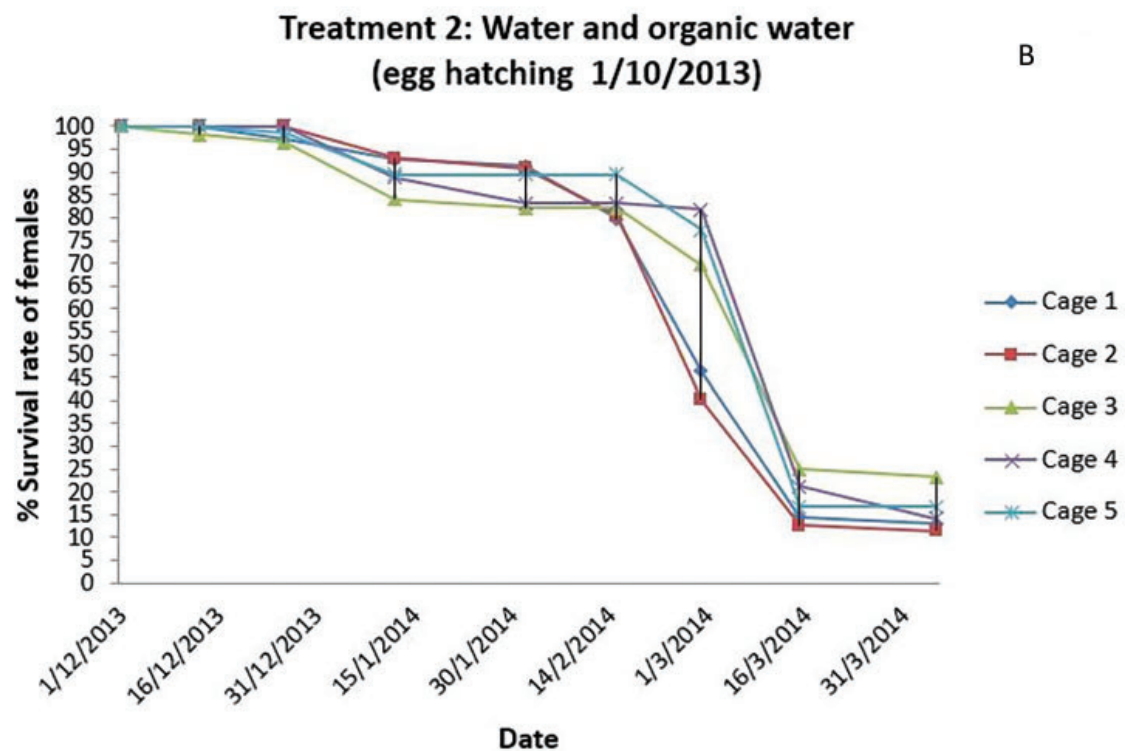
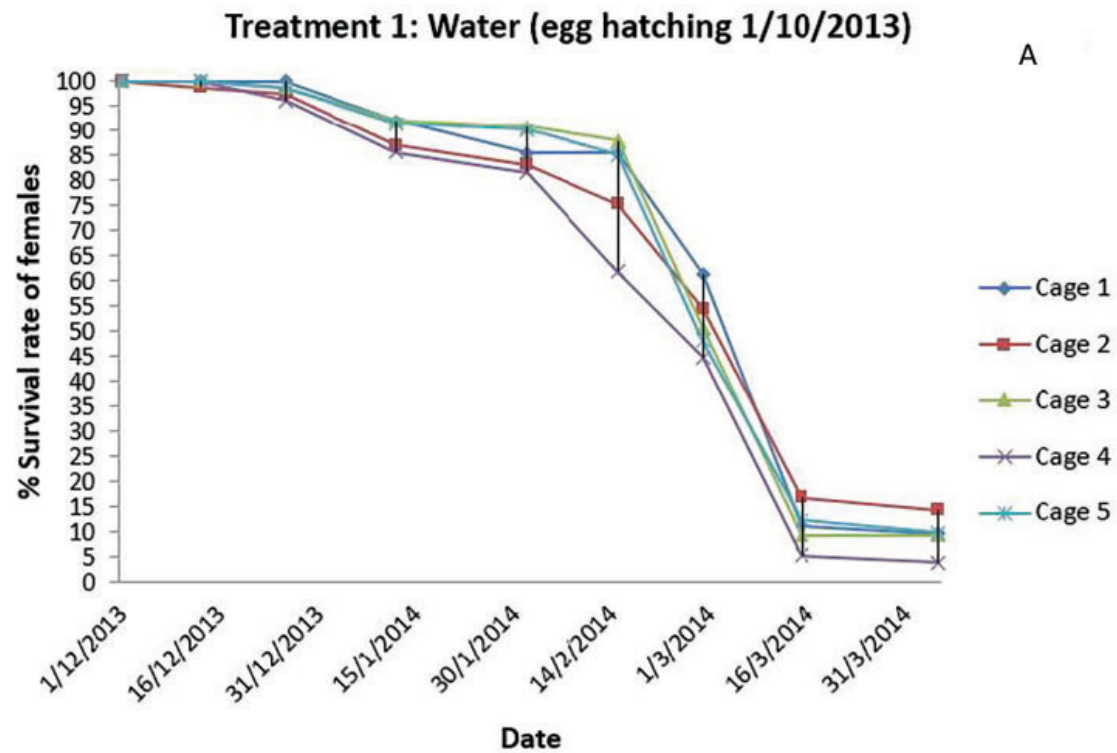


Figure S2: Kaplan Meier curves (with 95%CI) including log-rank test (shown p-value) for the survival of females in Nea Anchialos and Kalamaki. Black dashed lines represent the median survival for females in Nea Anchialos (66 days) and females in Kalamaki (67 days).



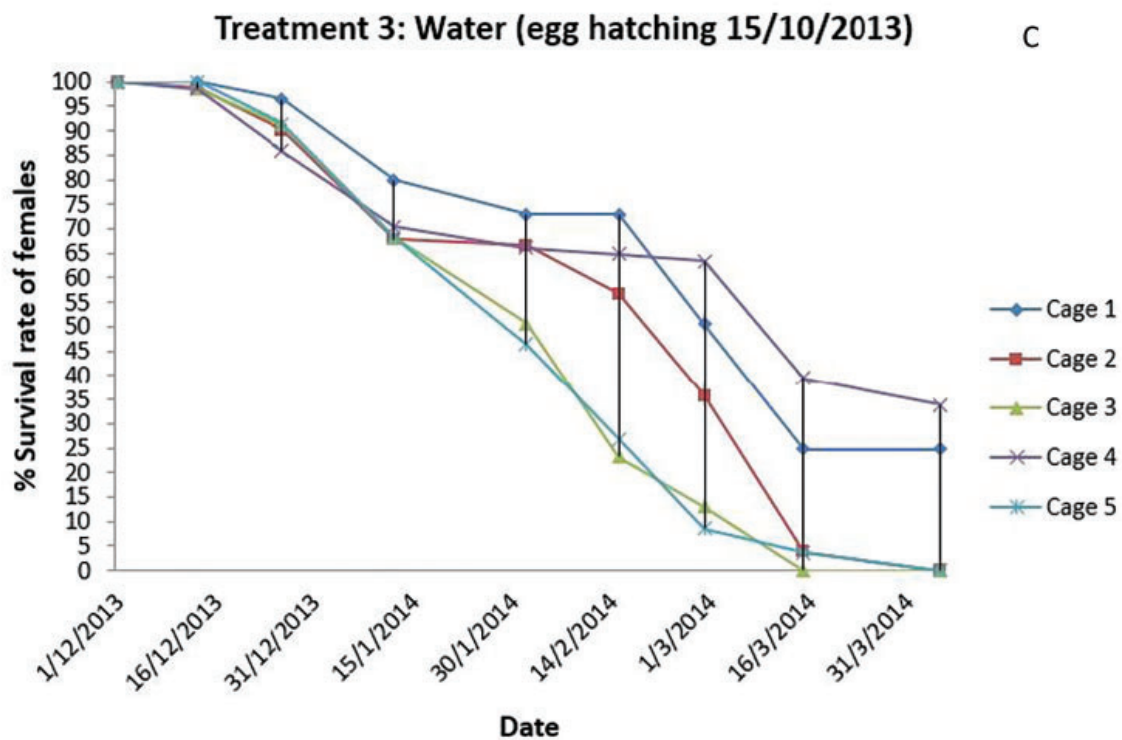
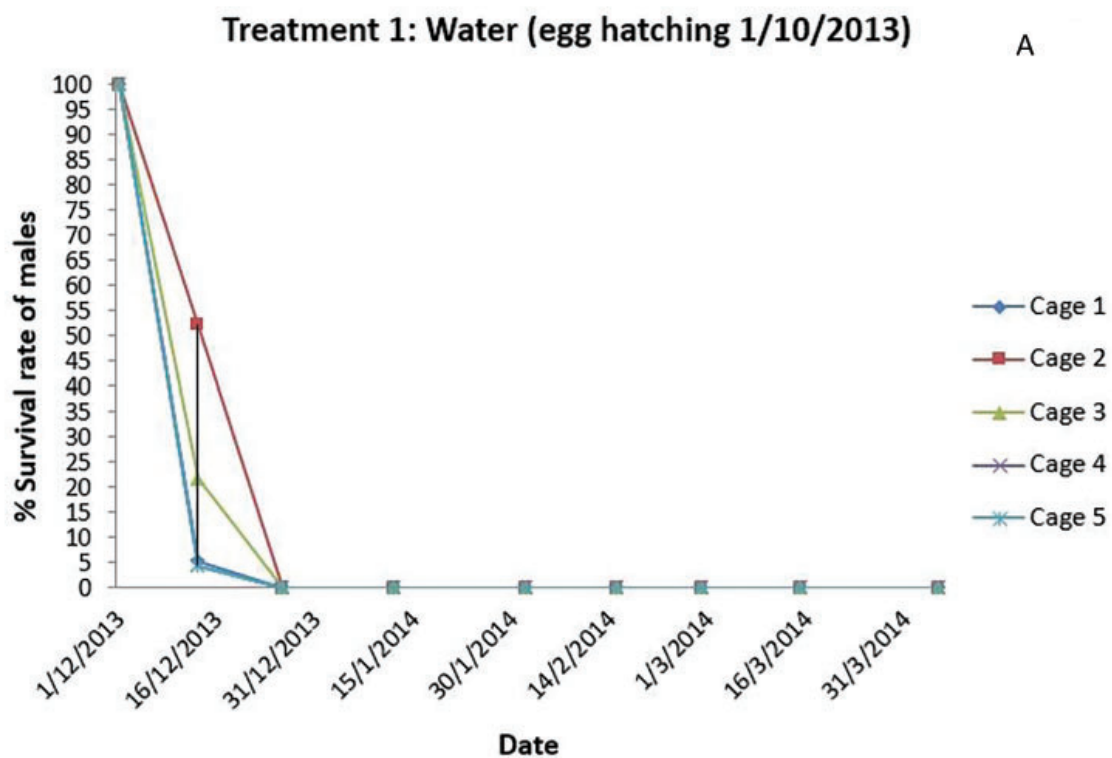


Figure S3: Survival rate of *Culex pipiens* biotype *pipiens* female adults regarding treatment 1 (A), treatment 2 (B), and treatment 3 (C), in each cage, in Volos, Thessaly, 2013-2014.



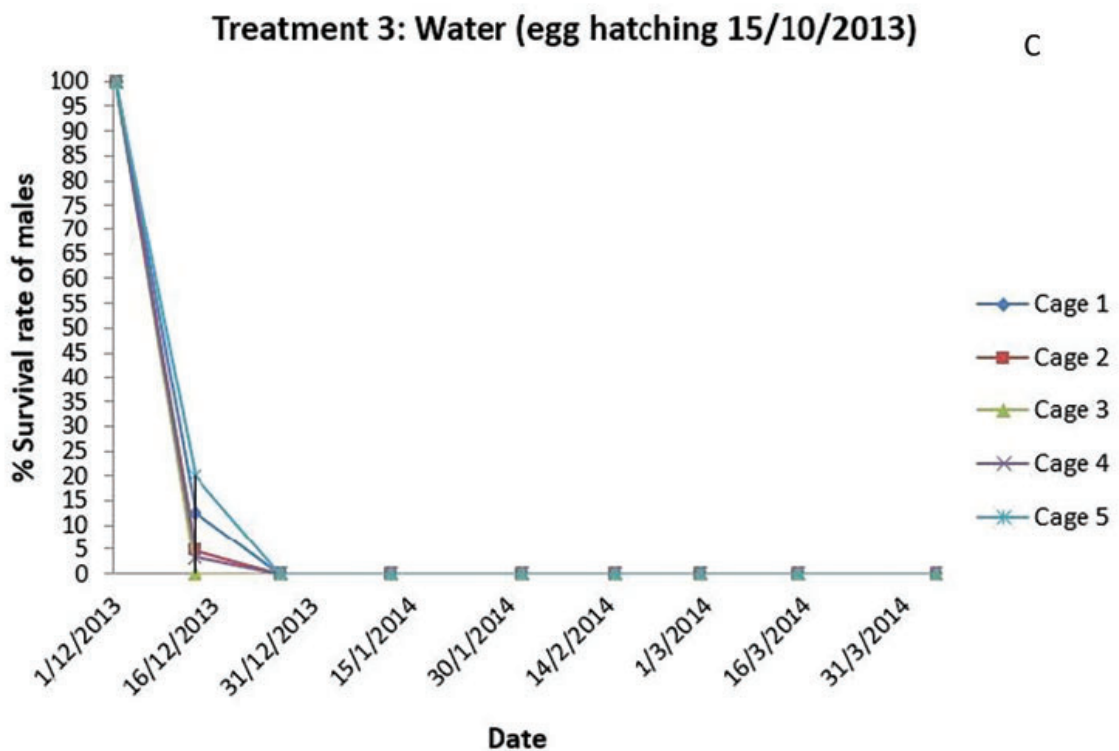
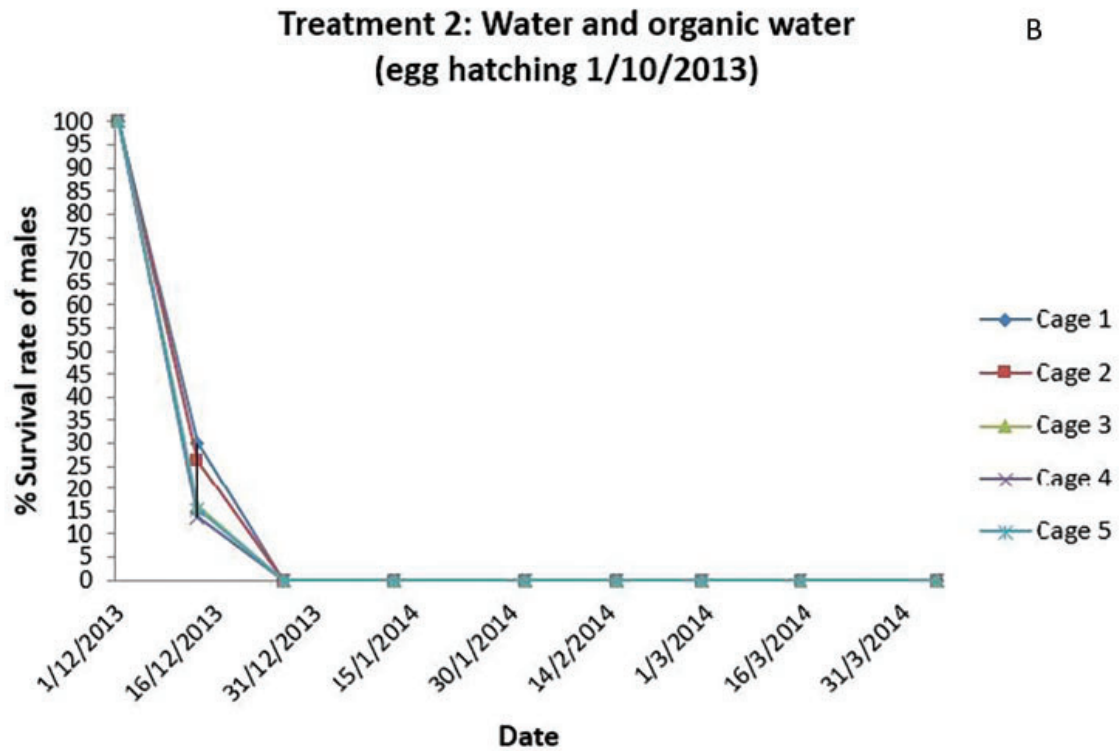


Figure S4. Survival rate of *Culex pipiens f. pipiens* male adults regarding treatment 1 (A), treatment 2 (B), and treatment 3 (C), in each cage, in Volos, Thessaly, 2013-2014.

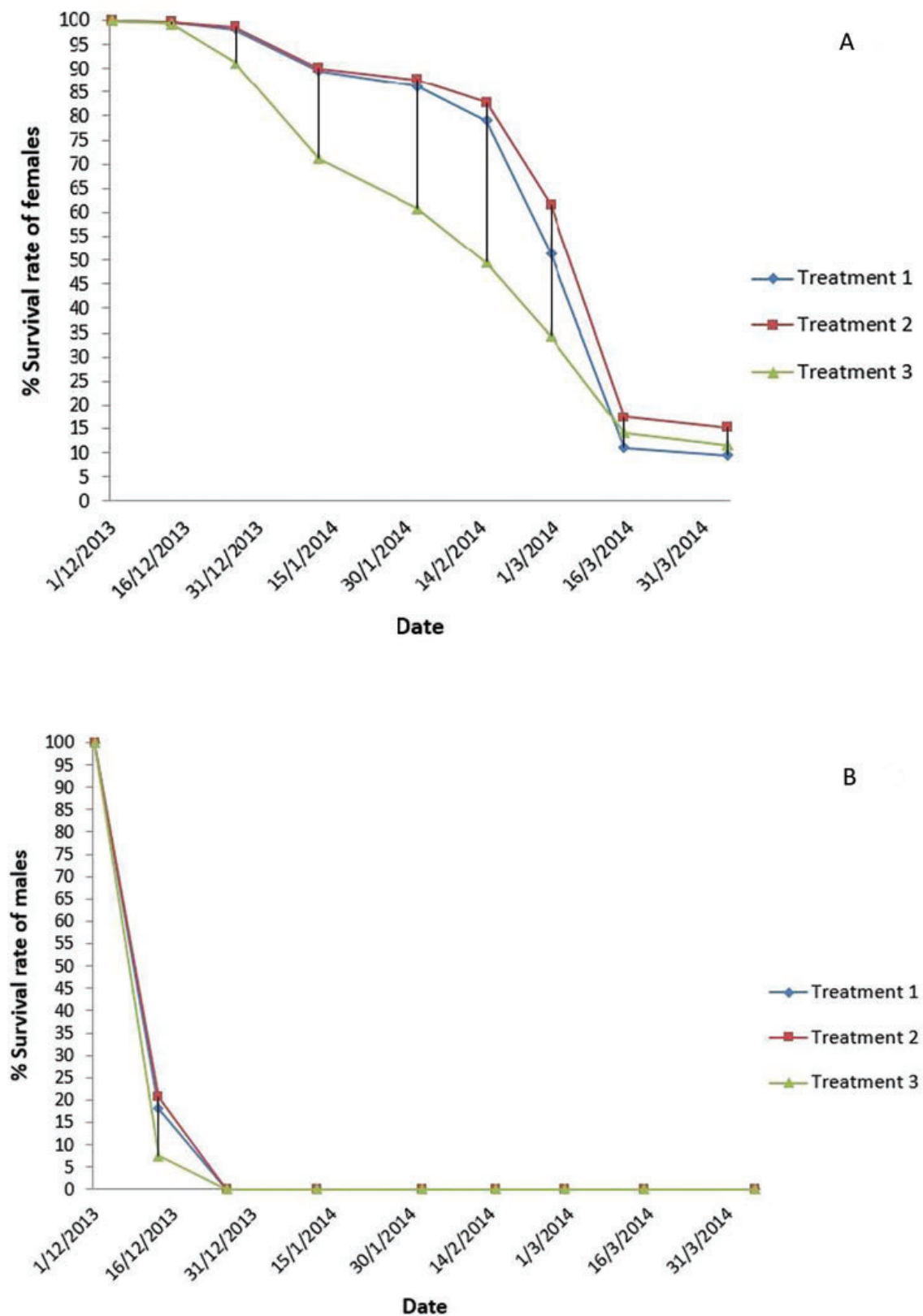


Figure S5. Survival rate of *Culex pipiens f. pipiens* adults (A) females, (B) males, regarding the three treatments in Volos, Thessaly, 2013-2014.