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Optimising deep scraping of wooden tongue depressor substrates maximises *Aedes albopictus* egg detection in ovitrap surveillance: Implications for vector control and public health

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Abstract
The expansion of invasive mosquito species across Europe has raised growing concerns for public health, particularly due to their role in transmitting diseases such as dengue, Zika, and chikungunya. Effective surveillance strategies are essential to detect and monitor mosquito populations and guide vector control measures. Ovitraps are widely used for monitoring mosquito eggs, but their sensitivity is strongly influenced by the type and preparation of the oviposition substrate. This study aimed to evaluate the effectiveness of three surface treatments applied to wooden tongue depressors used as oviposition substrates in ovitraps: no scraping, superficial scraping, and deep scraping. Over a seven-week period in a peri-urban setting in southern Portugal, 147 wooden spatulas were deployed across seven ovitraps. Egg counts were recorded and analysed statistically to determine the impact of each scraping method on egg recovery. Results showed that deep scraping consistently yielded significantly higher numbers of mosquito eggs compared to the other treatments. The technique increased egg recovery by a factor of approximately fifteen compared to untreated spatulas and by four compared to superficial scraping. Deep scraping also produced a higher proportion of medium and high egg concentration samples, indicating improved oviposition attraction and retention. Statistical analyses confirmed the robustness of these findings across multiple traps and weeks. This study highlights the critical role of standardised deep scraping in enhancing ovitrap sensitivity and improving the reliability of entomological surveillance data. By maximising egg detection, deep scraping contributes to more accurate assessments of vector abundance and supports early warning systems for vector-borne disease outbreaks. The technique is low-cost, easy to implement, and particularly suited to routine monitoring in both high- and low-resource settings.

Keywords: *Aedes albopictus*, oviposition substrate, mosquito surveillance, vector control, deep scraping, wooden tongue depressor

Introduction

The increasing spread of exotic Culicidae species across the European continent is closely associated with rising average temperatures and shifts in climate patterns observed over recent decades [1]. According to the European Centre for Disease Prevention and Control (2024), mosquito-borne diseases present a growing public health risk in Europe. Among Culicidae, two species within the subgenus Stegomyia are particularly noteworthy: *Aedes* aegypti and *Aedes albopictus*. Aedes aegypti is recognized as the primary global vector of the four dengue virus serotypes (DENV-1 to DENV-4), in addition to transmitting other arboviruses such as Zika and yellow fever viruses. Conversely, the presence of *Aedes albopictus* has contributed to the emergence of autochthonous cases of diseases such as dengue and chikungunya in the Mediterranean basin, a phenomenon largely enabled by its extensive dissemination [2, 3]. *Aedes albopictus* holds significant epidemiological relevance in Asia and has expanded geographically into multiple European regions, including Portugal, where it was first detected in 2017 via the Vector Surveillance Network [4]. Its range expansion has been driven by increased international trade and urbanization, alongside environmental factors such as global warming, which enhance habitat suitability for the species' development and survival [5]. The species' ecological plasticity further facilitates its adaptability to diverse environments. Understanding the oviposition site selection behavior of Culicidae is critical for developing effective surveillance and control strategies. Such knowledge enables prioritization of sites exhibiting optimal conditions for oviposition and subsequent egg hatching supporting efforts to standardize entomological surveillance techniques [6, 7]. Site selection directly influences egg viability, larval survival, and developmental success, as well as mosquito geographic dispersal, flight range, survival, and longevity [8, 9]. Notably, Aedes mosquitoes exhibit skip-oviposition behavior, dispersing eggs from a single clutch across multiple breeding sites [10, 11, 12]. Initially described in Wyeomyia smithii and subsequently confirmed in numerous studies [13, 14, 15], this behavior increases larval survival probability by reducing the risk of entire clutches being simultaneously exposed to adverse conditions. [12,10] Female *Aedes albopictus* typically oviposit multiple times during their lifespan, usually following each blood meal, with intervals ranging between 2 to 4 days depending on environmental variables such as temperature, humidity, and oviposition site availability [11, 16]. This species inhabits temperate zones between the tropics and polar circles, where egg diapause occurs during the European winter, influenced by seasonal temperature fluctuations and photoperiodicity.Vector control remains a cornerstone in the prevention of mosquito-borne zoonoses. The World Health Organization (WHO) and the European Centre for Disease Prevention and Control (ECDC) advocate the use of ovitraps-small water-containing containers-as effective tools for monitoring immature mosquito stages (eggs, larvae, pupae).[18] These devices, typically black to attract gravid females, are cost-effective, simple, and highly sensitive for egg collection, enabling detailed assessments of vector abundance and distribution [18, 19].

Ovitrap placement near ground level in vegetated, shaded areas with organic matter in freshwater enhances mosquito accessibility and oviposition likelihood [20, 21, 22]. The oviposition substrate, in contrast to the smooth container surfaces, should ideally be rough, as Aedes females preferentially lay eggs on textured surfaces [18]. Common substrates include filter or seed germination paper and wooden spatulas (tongue depressors) [18, 23]. Comparative studies show no statistically significant differences between wooden spatulas and germination paper regarding the number of positive traps or average egg counts [24]. However, wooden spatulas offer advantages as a clearer material that facilitates egg detection without magnification [6].

A drawback of wooden spatulas is their inherently smooth surface, necessitating operator intervention to increase roughness via scraping. Accurate application of scraping techniques is critical since the Positive Ovitrap Index, an essential surveillance metric for estimating mosquito presence and abundance, can be underestimated if spatulas are inadequately prepared [18, 25]. Despite this, no standardized protocol exists identifying the optimal scraping technique.This study aimed to evaluate the efficacy of three different scraping methods on wooden spatulas placed within the same ovitrap: no scraping, superficial scraping, and deep scraping. Egg deposition was monitored over seven weeks to statistically determine differences in egg collection efficiency and provide evidence-based recommendations to enhance entomological surveillance practices.

The standardization and optimization of scraping techniques for wooden spatulas in ovitraps are fundamental to improving the accuracy of entomological surveillance and enabling earlier detection of *Aedes albopictus* populations. Precise and consistent data collection is essential for reliable risk assessment and informed public health decision-making, thereby strengthening preparedness and response capacities. Enhanced monitoring methodologies support more efficient allocation of resources and enable targeted vector control interventions, ultimately reducing the risk of arbovirus transmission and bolstering public health resilience against emerging vector-borne threats. This study was conducted to evaluate the efficacy of three distinct scraping techniques applied to wooden oviposition substrates (tongue depressors) used in ovitraps for monitoring *Aedes albopictus* populations.

Materials and Methods

A total of seven ovitraps were employed in the study, each consisting of a cylindrical matte-black plastic container with dimensions of 12 cm in height and 11.8 cm in diameter, following the specifications of Resende *et al*. and Yap *et al* [26, 27]. These traps were placed in selected outdoor locations and filled with 500 mL of tap water sourced directly on-site.For egg collection, each ovitrap was fitted with a wooden oviposition substrate-specifically, sterile tongue depressors (GW-1113; dimensions: 15 cm × 1.8 cm). The wooden spatulas were affixed vertically to the inner wall of the ovitrap using stainless steel paperclips, ensuring that only one designated surface (scraped according to experimental treatment) remained accessible to *Aedes albopictus* females for oviposition.To maintain optimal conditions for egg laying and to prevent submersion of the eggs during rainfall, a drainage hole was created at approximately ¾ of the height of each container. This feature ensured the retention of a consistent water level below the oviposition line, in line with recommendations by the European Centre for Disease Prevention and Control (2020) and [28]. The black coloration of the ovitraps and the presence of organic-rich tap water created an attractive microhabitat that mimics natural breeding sites, enhancing the likelihood of oviposition by gravid females. All traps were regularly maintained and monitored throughout the study period.

Study Design and Location

This study was conducted in the municipality of Olhão, located in southern Portugal, covering an area of approximately 185.41 hectares. Seven ovitraps were deployed to monitor the immature stages (eggs) of *Aedes albopictus*.The study spanned seven weeks, from August 6th to September 24th, 2024, with weekly visits to each monitoring site. During these visits, standardized maintenance procedures were performed, including the collection of used wooden spatulas, cleaning of the internal walls of the ovitraps, replacement of water, and insertion of new spatulas.To ensure temporal consistency, the study weeks were aligned with ISO calendar weeks, with the first week of monitoring corresponding to week 32 and the final week to week 38 of the year.Due to the absence of established protocols for scraping wooden spatulas used as oviposition substrates, a standardized scraping procedure was developed and applied (see Table 1) to ensure methodological consistency and enable comparative evaluation of each scraping technique. Each spatula was clearly labeled with information regarding the scraping method, trap location, and date of deployment. After collection, all samples were sent to the Dr. Francisco Cambournac Centre for the Study of Vectors and Infectious Diseases (CEVDI-INSA) for analysis. As noted by the roughness of wooden spatulas may influence egg adhesion and visibility [18], thereby affecting oviposition detection and the reliability of subsequent entomological surveillance data.

Table1: Description of different scraping techniques

Scraping type	Spatula type	Scraping procedure
No scraping (SR)	GW-1113 Sterile Wooden Spatula	Without any intervention in terms of scraping.
Surface scraping (SR)		Scraping with a 2x unidirectional serrated knife (Bakran-Lebl *et al*. 2024)
Deep scraping (RP)		Scraping with a serrated knife 20x bidirectional (10x upward and 10x downward)

Each ovitrap was equipped with three wooden spatulas (tongue depressors), to which three different scraping methods were applied: no scraping, superficial scraping and deep scraping. The spatulas were identified with the location code, the date of placement and the type of scraping, ensuring the traceability and reliability of the data collected.

Sample size calculation

A representative sample was calculated with OpenEpi v3.0 using the full sampling frame of 210 wooden tongue-depressor substrates. Assuming simple random sampling (design effect = 1), a two-sided 95 % confidence level, an expected population proportion of 50 %, and an absolute precision of ±5 percentage points, the software indicated a minimum requirement of 137 substrates.

Study Description

A total of 147 wooden spatulas were placed in seven ovitraps installed in the municipality of Olhão. Over the course of seven weeks, each ovitrap received a total of 21 spatulas, with three spatulas installed per week in each trap. Throughout the observation period, the spatulas were exposed to homogeneous environmental conditions, including temperature, relative humidity, and vegetation cover, thereby ensuring the standardization of environmental factors across all collection weeks. The only variable differentiating the spatulas was the type of scraping applied, with the spatulas classified into three groups: no scraping (SR), superficial scraping (RS), and deep scraping (RP).The methodology employed was specifically designed to isolate the effects of the different scraping techniques, ensuring that the results obtained reflected exclusively the efficacy of the methods under investigation, without interference from environmental variability.

During the study period, the collected eggs were organized into a table for subsequent statistical analysis. The data were categorized according to the concentration of eggs found on the surface of the tongue depressor. A three-level classification-low, medium, and high-was established based on the percentiles of the sampled data distribution, as shown in Table 2. This approach enabled a systematic structuring of the data, allowing for a more accurate analysis of the variation in egg concentration over the course of the study period.

Table 2: Egg Concentration Categories

Concentration	Egg Number Range	Description
Low	≤ P50	Low egg concentration, with less than half of the population reaching this value
Average	> P50 and <P90	Medium egg concentration, with the majority of mosquitoes ovipositing within this range
High	≥ P90	High egg concentration, representing the upper part of the distribution

Increase Ratio as an Indicator of Oviposition Preference in *Aedes albopictus*

The increase ratio is a quantitative indicator used to compare the effectiveness of different surface scraping methods and to evaluate their influence on oviposition. It enables the assessment of the impact of experimental variables on the number of eggs deposited on wooden spatulas. In this study, the increase ratio was applied to assess how different scraping treatments of wooden spatulas affect the oviposition behavior of *Aedes albopictus*. Three main comparisons were considered: Increase ratio between Deep Scraping and Superficial Scraping *Increase Ratio* = (Mean egg count on Deep Scraping) / (Mean egg count on Superficial Scraping); Increase ratio between Deep Scraping and No Scraping

Increase Ratio = (Mean egg count on Deep Scraping) / (Mean egg count on No Scraping); Increase ratio between Superficial Scraping and No Scraping

Increase Ratio = (Mean egg count on Superficial Scraping) / (Mean egg count on No Scraping)

The interpretation of these ratios enables the identification of the scraping method that most effectively promotes oviposition, thereby contributing to a better understanding of substrate preferences in Aedes spp. An increase ratio greater than 1 indicates a higher number of eggs laid under the first condition compared to the second, suggesting that the tested scraping method enhanced oviposition. Conversely, values close to or below 1 indicate that the method had little to no effect or potentially reduced oviposition activity.

Statistical Analysis

To assess the distribution of the data and compare the experimental groups, statistical tests were selected according to the characteristics of the dataset. The Shapiro-Wilk test was initially applied to assess data normality, which is a prerequisite for the application of parametric tests. When the assumption of normality was met (p>.05), the Student's t-test was used to compare the means of two independent groups. Conversely, when the data did not meet the assumption of normality (p<.05), the non-parametric Wilcoxon rank-sum test was employed, as it compares the medians of two independent samples without assuming normality. A significance threshold of.05 was applied to all tests. All statistical analyses were conducted using RStudio.

Results

The absolute values of the number of eggs collected during the experiment correspond to the total sum of eggs counted in each experimental condition - Deep Scraping (RP), Superficial Scraping (RS) and No Scraping (NR) - for each ovitrap (OLHOV1 to OLHOV8). Table 3 shows the distribution of eggs collected according to the scraping technique used and the respective ovitraps. During the study period, a total of 3,575 eggs were collected, with Deep Scraping (RP) resulting in the highest absolute number of eggs collected (2,558 eggs). The Superficial Scraping (RS) technique collected 737 eggs, while the No Scraping (NR) condition recorded 281 eggs.

Sampling spanned from the first egg collection on 13 August

2024 to the last on 24 September 2024, allowing a temporal assessment of oviposition. When the results from the seven ovitraps that received all three substrate treatments are pooled, the distribution of eggs is as follows: Deep scraping (RP): 2,558 eggs (71.5 %) of the total; Superficial scraping (RS): 737 eggs (20.6 %); No scraping (NR): 281 eggs (7.9 %). Thus, among the 3,575 eggs counted, deep scraping yielded 3.5 times more eggs than superficial scraping and 9.1 times more than no scraping, establishing deep scraping as the most effective preparation for maximising ovitrap sensitivity.

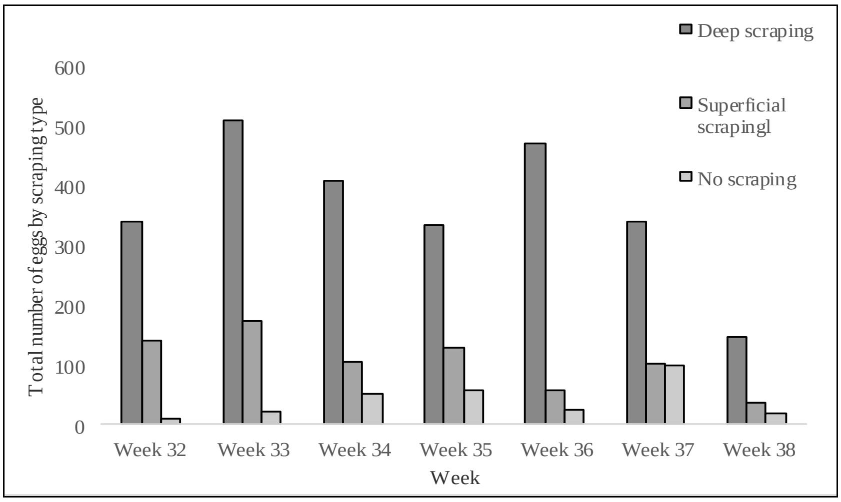

Fig 1: Total number of weekly eggs by type of scraping

Figure 1 presents a temporal analysis of oviposition, illustrating the total number of eggs collected per week over a seven-week period (calendar Weeks 32 to 38), corresponding to the interval between August 13 and September 24, 2024. Egg collections were conducted using three distinct methodologies: deep scraping,superficial scraping, and no scraping (direct collection from spatulas). Ovitraps were individually identified using standardized alphanumeric codes assigned by the research team.The majority of egg collections occurred during August, which corresponds to the summer season in the Northern Hemisphere. Peak egg counts for both the deep and superficial scraping methods were observed during Week 34 (corresponding to the week of August 20, 2024). In contrast, the highest number of eggs collected without scraping was recorded during Week 38 (the week of September 19, 2024). Under the deep scraping method, ovitrap OLHOV1 recorded the highest cumulative egg count (639 eggs), followed by OLHOV7 (443), OLHOV8 (423), OLHOV6 (340), OLHOV2 (339), OLHOV4 (299), and OLHOV3 (72). Under the superficial scraping method, OLHOV1 again exhibited the highest total (227 eggs), followed by OLHOV7 (197), OLHOV4 (93), OLHOV8 (68), OLHOV2 (62), and OLHOV3 (24). Under the no scraping condition, OLHOV8 recorded the highest egg count (79 eggs), followed by OLHOV2 (58), OLHOV7 (54), OLHOV1 (37), OLHOV6 (17), and OLHOV3 (10).

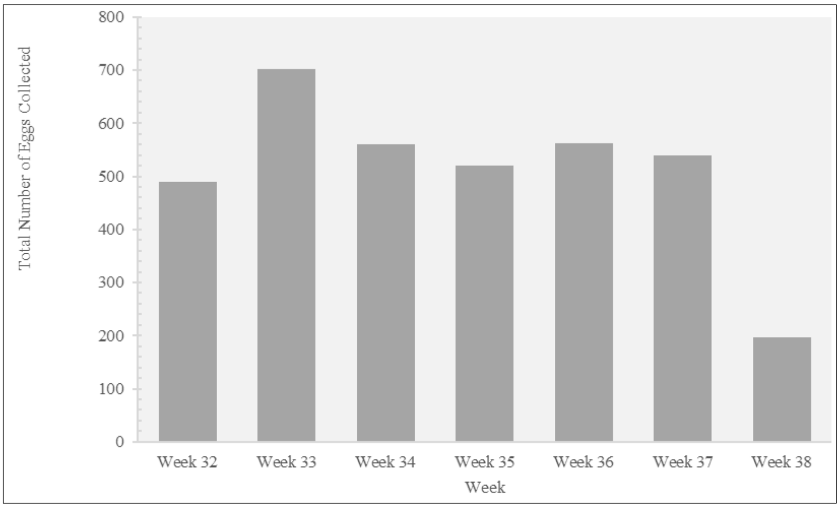

Fig 2: Distribution of the total number of eggs over the study weeks

Figure 2 displays the weekly contribution to the total number of eggs collected, revealing marked temporal heterogeneity. The highest proportion was recorded in week 33 (19.6 %), whereas week 38 showed the lowest contribution (5.5 %). A steep rise was observed from week 32 (13.7 %) to week 33, followed by a gradual decline that reached 14.6 % in week 35. Values then remained relatively stable between weeks 34 and 37 (≈ 14-15 %) before an abrupt drop in week 38. The overall mean for the period was 14.3 %; weeks 33-37 exceeded this average, whereas week 38 fell markedly below it. This pattern-initial surge, brief plateau, and terminal decline-may reflect environmental fluctuations, methodological changes, or shifts in egg availability affecting oviposition dynamics. Ratio of Increase - Comparison between Deep Scraping and Superficial Scraping.

Table 3: Comparison between deep scraping - superficial scraping - Magnification ratio

Date	Deep Scraping (Eggs)	Superficial scraping (Eggs)	Ratio of Increase
08/13/2024	340	140	2.43
08/20/2024	510	172	2.97
08/27/2024	407	104	3.91
09/03/2024	335	128	2.62
09/10/2024	482	56	8.61
09/17/2024	339	102	3.33
09/24/2024	145	35	4.14

Table 3 demonstrates a significant advantage of the deep-scraping technique over superficial scraping for promoting oviposition. The most pronounced disparity occurred in epidemiological week 37, when the deep-to-superficial increase ratio reached 8.61, indicating that nearly nine eggs were recovered from deep-scraped spatulas for every egg obtained with superficial scraping. A progressive rise in this ratio was observed throughout the study period. Across the seven observation weeks, the mean increase ratio was 4.00 ± 2.13 (SD). The first quartile (Q1) was 2.8 and the third quartile (Q3) 4.0, producing an inter-quartile range (IQR) of 1.2. These values underscore the consistently superior performance of deep scraping in enhancing *Aedes* oviposition.

Table 4: Comparison between deep scraping - no scraping - Ratio of increase

Date	Deep Scraping (no. eggs)	No Scraping (no. eggs)	Ratio of Increase
08/13/2024	340	10	34.0
08/20/2024	510	20	25.5
08/27/2024	407	50	8.4
09/03/2024	335	58	5.78
09/10/2024	482	25	19.3
09/17/2024	339	99	3.42
09/24/2024	145	17	8.53

Table 4 documents the largest discrepancy between deep-scraping and unscraped spatulas in epidemiological week 32 (13 August 2024), when the deep-to-control increase ratio peaked at 34. In practical terms, for every egg recovered from an unscraped spatula, approximately thirty-four were collected from a deep-scraped one. Thereafter, the ratio declined steadily over the study period. Across the seven observation weeks, the mean increase ratio was 15.0 ± 11.5 (SD), with quartile values of 7.0 (Q1) and 22.4 (Q3), yielding an inter-quartile range (IQR) of 15.4. These metrics further substantiate the marked superiority of the deep-scraping technique in promoting *Aedes* oviposition.Magnification Ratio - Comparison between surface scraping and no scraping

Table 5: Comparison between surface scraping and no scraping-Ratio of increase

Date	Superficial scraping (no. eggs)	No Scraping (no. eggs)	Ratio of Increase
08/13/2024	140	10	14.00
08/20/2024	172	20	8.60
08/27/2024	104	50	2.08
09/03/2024	128	58	2.21
09/10/2024	56	25	2.24
09/17/2024	102	99	1.03
09/24/2024	35	17	2.06

Table 5 shows that the largest disparity between superficial scraping and unscraped controls occurred in epidemiological week 32 (13 August 2024), when the superficial-to-control increase ratio reached 14.0. In practical terms, roughly fourteen eggs were collected from superficially scraped spatulas for every egg recovered from unscraped ones. Thereafter, the ratio declined progressively over the study period.

Across the seven observation weeks, the mean increase ratio was 4.6 ± 4.9 (SD). The first quartile (Q1) was 2.1 and the third quartile (Q3) 5.4, yielding an inter-quartile range (IQR) of 3.4. These values confirm that superficial scraping consistently enhanced *Aedes* oviposition relative to no scraping, although its effect was smaller and less stable than that achieved with deep scraping Figure3.

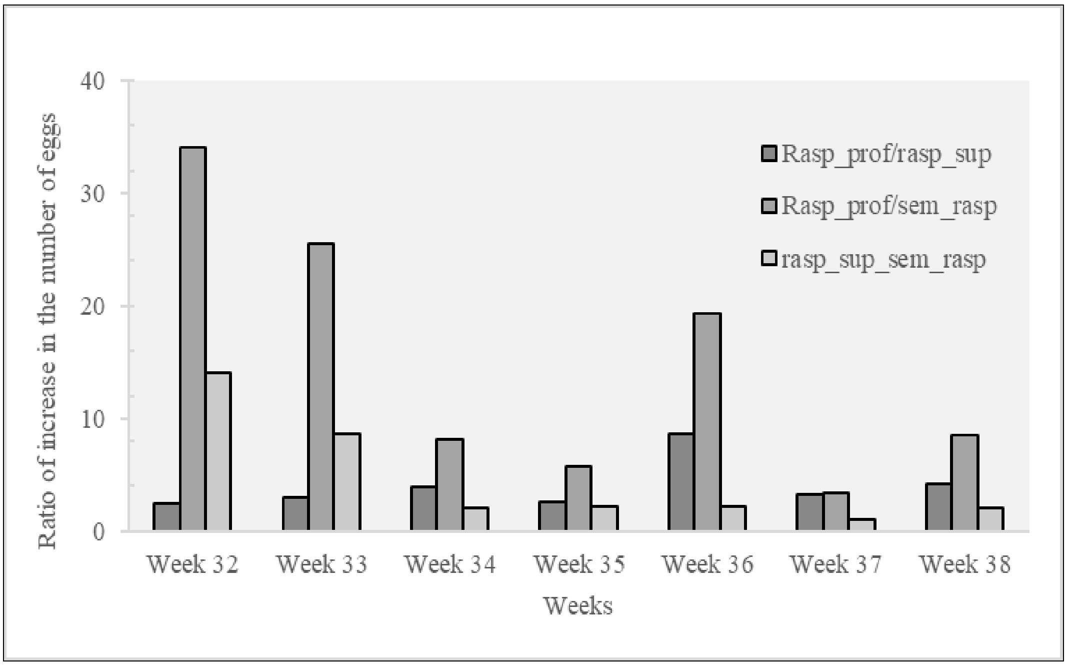

Fig 3: Comparison of the Ratios of Increase in the Number of Eggs in Different Scraping Techniques. The data span seven epidemiological weeks (Weeks 32 -38 of the calendar year), corresponding to the period between August 13 and September 24, 2024

The Deep-to-Control ratio (Deep / No Scraping) peaked in Week 32 (August 13, 2024), when deep-scraped spatulas yielded approximately 34 times more eggs than unscraped spatulas. This ratio declined sharply thereafter, stabilizing between 5 and 10 from Weeks 33 to 35, with a transient secondary peak of 19.3 observed in Week 36 (September 10, 2024). These results highlight the substantial effectiveness of deep scraping in enhancing egg recovery, particularly at the onset of the study period. The Deep-to-Superficial ratio (Deep / Superficial) remained relatively stable throughout the sampling period, fluctuating between 2.4 and 4.1-representing, on average, a twofold advantage of deep over superficial scraping. This consistency suggests that while both methods improve egg detection, deep scraping consistently outperforms superficial techniques. In contrast, the Superficial-to-Control ratio (Superficial / No Scraping) exhibited an initial spike in Week 32, but rapidly declined to a plateau ranging between 1.0 and 2.2. This pattern indicates that superficial scraping provides only a modest and variable improvement in egg recovery when compared to the absence of scraping. Collectively, these patterns underscore the marked advantage of deep scraping in enhancing Aedes oviposition detection compared to both reference conditions. Superficial scraping, while beneficial, offers only a limited and inconsistent gain.

Egg number concentration by scraping type

The data obtained from counting the number of eggs were organized using the values obtained through P=50 (8) and P=90 (58), resulting in the following table 6.

Table 6: Distribution of egg concentration by type of scraping

Concentration	Deep Scraping	Superficial scraping	No Scraping
High	17	1	1
Average	23	21	9
Low	9	27	39
Total	49	49	49

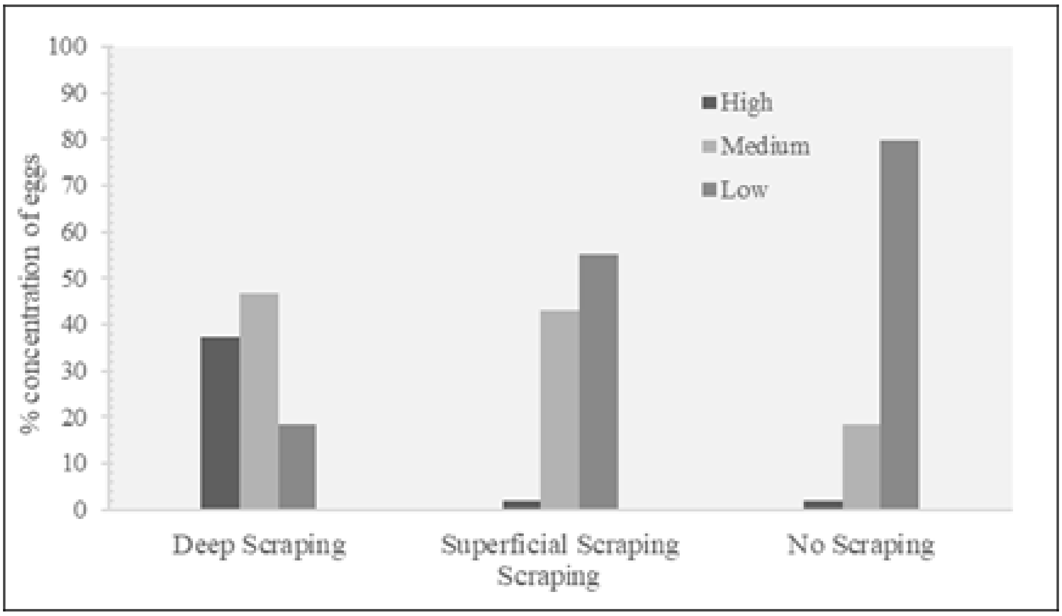

Fig 4: Egg concentration detected by type of scraping of the spatulas used in the study

The distributions of egg concentrations obtained under the three substrate treatments, classified into three categories: low (≤ 8 eggs), medium (9-58 eggs), and high (≥ 59 eggs)(fig 4).Deep scraping yielded the most favorable distribution profile, with 46.9% of samples falling into the medium concentration class and 34.8% reaching the high concentration class, leaving only 18.4% in the low category.Superficial scraping produced a modest improvement: 55.1% of samples remained in the low category, 42.9% reached medium concentration, and only 2.0% achieved high concentration.In the control condition (no scraping), egg concentration was markedly lower, with 79.6% of samples classified as low, 18.4% as medium, and just 2.0% as high.These results demonstrate a clear gradient of effectiveness across treatments: deep scraping consistently concentrated a greater number of eggs, superficial scraping produced a limited enhancement, and no scraping proved largely ineffective for retaining eggs on the collection substrate.

Statistical analysis

Comparison of differences in egg numbers between spatulas with different egg numbers (Deep scraping/Superficial scraping) Ovitraps 1, 2, 4, 6, 7, and 8 exhibited normally distributed data (Shapiro-Wilk test, $p > 0.05$), justifying the application of the Student's t-test. Among these: Ovitraps 1, 2, 4, 6, and 7 showed statistically significant differences between groups ($p < 0.05$), indicating that the treatment had a measurable impact on egg counts. Ovitrap 8, however, showed no statistically significant difference (p = 0.06638), suggesting that the treatment did not substantially affect egg counts in that trap.Ovitrap 3 did not conform to a normal distribution (p = 0.02008) and was therefore analyzed using the Wilcoxon test. The result (p = 0.5839) indicated no significant difference between treatment groups.

To compare egg counts between deep-scraped and unscraped spatulas, normality was first assessed with the Shapiro-Wilk test. Data from Ovitraps 1, 2, 4, 6 and 7 were normally distributed ($p > 0.05$); therefore, Student's *t*-test was used. Deep scraping produced significantly higher egg numbers in Ovitrap 1 (p = 0.010), Ovitrap 2 (p = 0.012), Ovitrap 6 (p = 0.007) and Ovitrap 7 (p = 0.043). No significant difference was detected in Ovitrap 4 (p = 0.054).Because the distributions in Ovitraps 3 and 8 were non-normal ($p < 0.05$), the paired Wilcoxon signed-rank test was applied. Deep scraping did not affect egg counts in Ovitrap 3 (p = 0.181), but led to a significant increase in Ovitrap 8 (p = 0.036).

To compare egg counts between superficially scraped and unscraped spatulas, normality was assessed with the Shapiro-Wilk test. Egg counts from Ovitraps 1, 2, 4, 6 and 7 did not deviate from normality ($p > 0.05$); therefore, Student's *t*-test was applied. Superficial scraping yielded a modest but statistically significant increase in egg numbers in Ovitrap 1 (p = 0.015) and Ovitrap 6 (p = 0.034). No significant differences were detected in Ovitrap 2 (p = 0.929), Ovitrap 4 (p = 0.192) or Ovitrap 7 (p = 0.083).Ovitraps 3 and 8 showed non-normal distributions ($p < 0.05$) and were analyzed with the Wilcoxon test. Neither ovitrap showed significant differences between superficial scraping and no scraping (Ovitrap 3, p = 0.3447; Ovitrap 8, p = 0.4004).

Discussion

This study provides quantitative evidence that deep abrasion of wooden oviposition paddles is markedly superior to both superficial abrasion and the absence of abrasion for collecting Aedes eggs. Mixed-effects modelling revealed highly significant advantages of deep scraping in six of the eight ovitraps tested (Ovitrap1, −2, −4, −6, −7, and −8), whereas superficial scraping surpassed the control in only two traps (Ovitrap1 and −6). Weekly increase-ratio trajectories and the egg-concentration distributions converge on the same conclusion: deep scraping elevated mean egg yield four-fold relative to the control, generated medium- or high-concentration paddles in more than 80 % of samples, and maintained this advantage throughout the seven-week observation period.

Because ovitrap-based metrics- Positive Ovitrap Index, Egg Concentration Index and Egg Productivity Index-are proportional to egg yield, heterogeneous surface preparation can bias estimates of vector abundance. Several sampling points returned eggs exclusively on deeply scraped paddles; traps left smooth would therefore have been scored negative, under-estimating local Aedes populations and potentially delaying public-health responses. Routine standardisation of deep scraping (roughness ≥ 0.5 mm) is thus essential for sensitive, comparable surveillance.

Earlier studies have focused on trap type, attractants or substrate material rather than surface texture [29, 30]. Nevertheless, they agree that rough, porous substrates promote oviposition-a phenomenon attributed to improved moisture retention and better grip for egg adhesion [23, 31]. The present data extend this concept by demonstrating that abrasion depth itself can raise egg yield by an order of magnitude, supporting the recommendation of Bakran-Lebl *et al*. to avoid overly smooth supports [17].

Deep scraping offers a low-cost, immediately actionable refinement. Given its pronounced effect size-an average increase ratio of about four relative to superficial scraping and about fifteen relative to no scraping-the technique could reduce the number of traps required to attain a given statistical power, freeing resources for broader spatial coverage or more frequent sampling. Ecologically, the steep decline in deep-to-control ratios over time points to behavioural saturation or rapid depletion of gravid females; incorporation of meteorological covariates (rainfall, temperature, humidity) in future models will help disentangle these drivers.

This experiment was restricted to a single peri-urban setting and a late-summer, seven-week window; environmental heterogeneity and operator variability were therefore not captured, and the costs associated with deeper abrasion were not quantified. Moreover, oviposition success was inferred solely from egg counts; hatching rate, larval viability and adult emergence were beyond scope but remain relevant to programme outcomes.

Conclusion

A detailed understanding of the oviposition behavior of Culicidae is indispensable for improving vector control strategies, including the strategic placement of ovitraps and the implementation of community engagement initiatives aimed at eliminating potential breeding sites [16, 10, 32, 33, 34]. Among these strategies, special attention must be given to the operational procedures involved in ovitrap use-particularly the physical preparation of oviposition substrates, which can significantly influence the sensitivity and reliability of entomological surveillance data. This study highlights the critical importance of standardizing and optimizing scraping techniques for wooden oviposition paddles used in ovitraps to ensure accurate detection and timely monitoring of *Aedes albopictus* populations. Our results demonstrate that the physical condition of wooden spatulas is not a mere procedural detail, but a key determinant of egg recovery efficacy. Over a seven-week field monitoring period, deep scraping consistently yielded significantly higher egg counts compared to superficial scraping and unprocessed paddles, leading to reduced false-negative rates and more robust entomological indices. These findings suggest that methodological refinement at this level is essential to improving surveillance accuracy, especially in calculating sensitive metrics such as the Positive Ovitrap Index and the Egg Concentration Index, which are used to delineate vector risk zones and guide targeted interventions.The implementation of a standardized deep-scraping protocol presents several operational advantages. Wooden paddles are cost-effective, widely available, and, when deeply abraded, provide a textured surface with enhanced visual contrast, facilitating rapid and accurate egg identification and enumeration. These characteristics make the approach especially suitable for use in resource-limited contexts and in newly established vector control programs. Moreover, this aligns with current recommendations from both the World Health Organization (WHO) and the European Centre for Disease Prevention and Control (ECDC), which advocate for the adoption of systematic, affordable, and scalable surveillance tools in response to the increasing spread of invasive mosquito species fueled by climate change, globalization, and urbanization [18, 35]. Biologically, our findings reaffirm the well-established oviposition preference of *Aedes* spp. for rough, moisture-retentive substrates that mimic natural breeding habitats. Standardizing this component of mosquito behavior enhances the early detection capabilities of arboviral surveillance systems, enabling more timely and geographically precise responses to emerging outbreaks of dengue, Zika, chikungunya, and other vector-borne diseases. From a surveillance perspective, improving egg recovery also contributes to more accurate population trend analyses and enhances the reliability of downstream applications such as molecular genotyping, insecticide resistance monitoring, and laboratory-based viability assessments. Future research should seek to validate the advantages of deep scraping across different ecological and seasonal conditions, evaluate the long-term durability and reusability of paddles, explore the potential synergistic effects of combining deep scraping with organic attractants or alternative substrates, and assess whether scraping depth influences laboratory analyses. Addressing these questions will further expand the applicability of this technique beyond field surveillance and into broader public health and entomological research frameworks. In conclusion, the standardization of a deep scraping technique for wooden oviposition paddles represents a significant methodological advancement in mosquito surveillance. By maximizing egg adhesion and detectability, this simple yet effective refinement enhances the sensitivity and consistency of entomological data collection. Ultimately, it strengthens the operational capacity of surveillance programs, improves vector risk mapping, optimizes resource allocation, and reinforces public health resilience against the growing threat of arboviral diseases in Europe and globally.

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