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eDNA-qPCR outperforms larval monitoring for detecting Aedes albopictus under tropical field conditions: associations with larval density and sun exposure

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Abstract

Mosquito vector surveillance is a major public health priority in tropical regions. Environmental DNA, a promising approach for detecting mosquito larvae in their aquatic breeding sites, remains poorly documented under tropical conditions and must overcome the challenges of DNA degradation and PCR inhibition. This study evaluated a qPCR assay targeting *Aedes albopictus* eDNA in larval breeding sites, compared its performance with conventional larval surveillance, and identified factors influencing detection and signal intensity. We conducted field validation at 28 breeding sites on La Réunion island over five months, from November 2025 to March 2026, totalling 102 site visits. At each visit, we filtered 120 mL water samples at each breeding site (Sterivex, 0.45 µm) and extracted DNA using a Macherey-Nagel NucleoSpin eDNA Water Kit. We amplified DNA by qPCR using Environmental Master Mix in triplicate, alongside larval surveys. A site was considered eDNA-positive if at least two out of three replicates amplified beyond the detection threshold, established based on a calibration curve. We assessed predictors of detection and signal intensity using mixed models. In the field, eDNA-qPCR detected *Ae. albopictus* in 64.7% of site visits, significantly more than larval surveys (39.2%, McNemar's test, $X2(1) = 16.45$, $p < 0.001$). Agreement between the two methods was fair (Cohen's Kappa = 0.30, 95% CI [0.14–0.46]), reflecting their differing temporal sensitivity rather than assay unreliability. Positive and negative percentage agreement were 85% (95% CI: [70.9, 92.9] %) and 48.4% (95% CI: [36.4, 60.6] %), respectively. Larval density was negatively correlated with Ct values (rho=-0.46, $p = 0.007$), confirming a dose-dependent relationship between larval abundance and eDNA signal. Sun exposure affected eDNA signal intensity, but not detection probability. eDNA-qPCR significantly outperformed larval surveys for detecting Ae. albopictus under tropical field conditions, supporting its use for early detection of invasive vectors and monitoring of vector-control interventions.

1. Background

The global expansion of invasive species increasingly challenges mosquito-borne disease control and surveillance. *Aedes albopictus* has expanded from its native range in Southeast Asia to become one of the most successful invasive mosquito species worldwide. Its ecological plasticity allows it to thrive in a wide variety of natural and artificial breeding habitats [1] and, more broadly, under diverse climatic conditions in tropical and temperate regions. On La Réunion, the species has been present for over a century and thrives from the coast to about 1200m elevation, in urban and rural settings, across a wide diversity of breeding habitats [2]. This broad distribution, combined with the risk of introduction of additional invasive species, highlights the importance of sensitive entomological surveillance in vector prevention and control on the island.

One surveillance challenge lies in the early detection of newly introduced mosquito species. Invasive species are expected to occur at low abundance and may initially be spatially restricted, making their early detection difficult using conventional surveillance methods. This is even more relevant at points of entry (ports, airports), where the movements of people and goods create more opportunities for mosquito introductions, especially for island territories. Therefore, species-level entomological surveillance has been implemented at points of entry since late 2021 in La Réunion, with Le Port designated a priority site due to

the risk of *Anopheles stephensi* [3]. This invasive malaria vector has recently spread to the African continent, posing a threat to malaria control efforts [4]. An expert report by ANSES conducted in 2023 on entomological surveillance in France and its tropical territories highlighted that entomological surveillance in Réunion combines larval surveys, oviposition traps, and adult traps [3]. While these methods ensure effective routine entomological surveillance, increasing the probability of detecting newly introduced species remains an important operational target.

Sensitive detection at low mosquito occurrence is also important when control interventions suppress mosquito populations. La Réunion has experienced recurrent and increasingly severe arbovirus epidemics. These include a major chikungunya epidemic in 2005–2006 that affected approximately 34% of the island's population [5], sustained dengue transmission since 2017 [6], and a new chikungunya outbreak in 2024–2025, with more than 54,000 confirmed cases and 43 deaths [7]. These recurrent arboviral outbreaks have led to large-scale trials of the boosted Sterile Insect Technique (b-SIT) as a complementary vector control method, involving the release of sterile males treated with a biocide (pyriproxyfen) to suppress wild *Ae. albopictus* populations through non-viable mating and an additional effect on larval breeding sites [8]. Effective mosquito population suppression can make detection and quantification of residual populations more difficult, like early detection of invasive species.

Conventional larval surveillance remains fundamental to mosquito monitoring, but it still relies on field collection by dipping followed by morphological identification in the laboratory by a trained technician. This approach is time-consuming, labour-intensive, and prone to under-detection in cases of low density or hard-to-access sites [9,10]. Another challenge arises from the similarity of species that are morphologically close or difficult to identify in the larval stage, which requires mountings and morphological examinations under a microscope [9,11]. Environmental DNA (eDNA) using qPCR and sequencing represents a promising complementary approach, as organisms release genetic material into their breeding sites that can be detected without the need to collect the organisms themselves. Previous studies have demonstrated the feasibility of detecting mosquitoes from aquatic eDNA and, in some settings, with higher detection probabilities compared with conventional surveys [9,11,12].

However, demonstrating that mosquito genetic material can be detected in breeding sites may not be enough to establish eDNA qPCR as an operationally sensitive surveillance tool. Detection performance depends on the environment in which DNA is shed, persists, and is recovered, and the evidence from tropical systems does not align neatly with general expectations. A meta-analysis of aquatic eDNA studies associated higher temperatures with faster DNA degradation [13]. In an experimental tropical freshwater system, temperatures of 23, 29, and 35°C left degradation rates unchanged, while 35°C increased shedding by the target organism [14]. Field data point to the same result in natural mosquito breeding sites in Ghana; water temperature was not associated with detection probability over 25.8 to 35.8°C [15]. A factor associated with eDNA decay in a broad synthesis does not necessarily translate into a measurable effect on field detection. Turbidity can impair eDNA recovery by reducing filtration efficiency and clogging filters [14]. Dissolved substances present in natural waters can further interfere with amplification. In small, organically loaded mosquito habitats, these three factors all affect the same sample.

Despite the increasing application of eDNA in aquatic biodiversity monitoring, it is important to validate it under real-world tropical conditions, particularly for detecting populations at low abundance. Our study was designed to develop a species-specific qPCR test to detect *Ae. albopictus* eDNA and to evaluate its performance over six months across a network of larval breeding sites. We aimed specifically to: (i) establish the test's sensitivity and compare its performance to that of larval monitoring by dipping and (ii) characterise the factors determining the detection and intensity of the eDNA signal in the field, including its relationship with larval density and local environmental conditions.

2. Methods

2.1. Study sites and sampling design

The study was conducted in the southern part of La Réunion Island, across three zones: Langevin, Saint Joseph, and Vincendo. A network of 28 sentinel breeding sites was established to represent the diversity of larval habitats in the area (anthropogenic domestic sites and natural sites like vacoas trees (*Pandanus sp.)* and ravines. Each breeding site was monitored monthly over five months, from November 2025 to March 2026. This longitudinal monitoring yielded 102 usable samples, with the number of valid visits per site varying due to the occasional drying up of certain sites.

2.2. Field sampling and filtration

The equipment required for each site (sterile 25 mL serological pipettes, sterile 120 mL bottles, gloves, Sterivex cartridges, Luer Lock caps, sterile 60 mL syringes, storage jars, labels, dipper and dip net) was packed by site before travel to the field and stored in a cool box. A pair of workers collected each sample: one contacted the water at the source, and the other operated the filtration device. At each breeding site, larval sampling by dipping was carried out simultaneously with eDNA sampling, from the same pool: all visible larvae were collected.

For DNA analysis, water samples were collected in sterile bottles that were opened without touching the inside of the cap or the neck, then filtered. Filtration was carried out on site immediately after sampling to minimise environmental DNA degradation under tropical conditions and reduce the risk of contamination associated with transporting unfiltered water. Filtration was carried out using the Sterivex syringe system, with Sterivex-HV filters (0.45 µm, PVDF, ref. 2660458, Merck Millipore). After homogenising the sample, the water was filtered by successively refilling the syringe (60 mL) through the same cartridge up to 120 mL. Each cartridge was sealed, labelled, individually packaged, and transported at 4°C from the field to the entomology laboratory, then to the molecular biology laboratory. The samples were stored at 4°C for extraction within 24 hours.

2.3. Operational sampling limitation

Of the 118 breeding sites selected, 16 (14%) could not be sampled because the Sterivex cartridge (0.45 µm) became clogged by excessive turbidity or a high organic load, preventing sufficient water from being

filtered in the field. This exclusion stems from an operational limitation of the filtration system, rather than a predetermined methodological choice.

2.4. Morphological identification of larvae

The larvae collected were taken back to the laboratory for identification. The mosquito larvae were identified using morphological identification keys of Bousses *et al*. (2021) [16] and Robert *et al*. (2022) [17].

2.5. Extraction of environmental DNA

eDNA was extracted directly from each Sterivex cartridge using the NucleoSpin® eDNA Water kit (cat. no. 3613913, Macherey-Nagel) according to the manufacturer's protocol, with minor modifications. After lysis (Dissolve buffer + proteinase K, 30 min) and lysate recovery, the eDNA was purified by removing inhibitors (ACID buffer), binding to NucleoTrap® suspension, and performing successive washes (RESU, WB) before transferring to a column. The purified DNA was eluted in a final volume of 100 µL and stored at -20°C until qPCR analysis. A negative control was included for each series of extractions, where DNA was extracted from an equal volume of nuclease-free water.

2.6. qPCR assay targeting Aedes albopictus

TaqMan™ Environmental Master Mix 2.0 (Thermo Fisher Scientific) was used in each reaction. The final volume was 10 µL and consisted of 5 µL 2X master mix, 0.5 µL each primer (10 µM; final concentration 500 nM each), 0.2 µL probe (5 µM; final concentration 100 nM), 1.8 µL molecular-grade water, and 2 µL template DNA. The amplification programme comprised an initial activation of the Taq polymerase at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 62°C for 30 s. The *Ae. albopictus*-specific primers and probe were used identically in both protocols: ITS1_F440 (5'-GTCAGCAGGGCCGAACC-3', Tm 61.9°C), ITS1_R510 (5'-GACGACCCGCCACTTAGCT-3', Tm 61.8°C) and the AlboITS1P probe (FAM-CAGGGCACATACGTCCGCTTTGGTT-BHQ1, Tm 70.2°C), all from Hill *et al*. (2008) [18].

2.7. Universal COI PCR control of qPCR-negative samples

To verify that DNA was amplifiable at the six sites that were larval-positive but qPCR-negative, extracts were tested by endpoint PCR using universal COI primer mlCOIintF/ jgHCO2198 [19]. Each run included an *Ae. albopictus* DNA extract as a positive control and a negative control. Amplification was assessed on an agarose gel by the presence of a band of the expected size (313 bp).

2.8. Analytical validation: sensitivity, LOD, and LOQ

A standard curve was generated from a 10-fold serial dilution of *Ae. albopictus* DNA extracted in our laboratory (D0 to D6). D0 concentration was verified by Qubit 4 fluorometry (dsDNA Broad Range). Each dilution and sample was analysed in technical triplicate. No-Template Controls (NTC), field and extraction blanks, and a positive control were included on each run to monitor contamination and confirm assay performance. This design meets CSA performance criteria [20] and matches replication levels in comparable mosquito eDNA studies [15,21–23]. A replicate was considered valid when its Ct fell below the assay-specific limit of detection (LOD). A sample was classified as positive when at least two of three replicates were valid. This threshold is stricter than a single detection, yet tolerant of occasional replicate

failure at low target concentrations [10]. The LOD was defined as the lowest dilution showing positive amplification in all three replicates (3/3). The limit of quantification (LOQ) was the lowest dilution within the linear range, at which the coefficient of variation (CV) of the Ct values between replicates remained below 35% [24].

2.9. Data analysis

Sample positivity was determined according to the analytical validation described in section 2.7). PCR efficiency was calculated from the slope of the standard curve and compared with the 80–110% threshold required by the Canadian standard CSA W219/23 for targeted eDNA qPCR assays [20,25]. Agreement between eDNA detection and larval surveys was assessed using Cohen's Kappa, sensitivity, specificity, and predictive values [26]. In the absence of an absolute reference method, detection performance was expressed as the Positive and Negative Predictive Agreement (PPA and NPA), rather than as sensitivity and specificity. qPCR served as the index method, and larval surveys served as the comparator. The PPA corresponds to the proportion of sites that were positive in both larval surveys and eDNA-qPCR. The NPA corresponds to the proportion of sites that were negative in both larval surveys and eDNA-qPCR. The difference between the paired detection rates for eDNA and larval surveys was tested using a McNemar test with continuity correction [27]. The relationship between larval density and Ct was tested using Spearman's rank correlation.

The relationship between larval density, eDNA detection, and environmental factors (habitat type, water volume category, volume, solar exposure) was initially examined on a factor-by-factor basis, using Mann-Whitney, Kruskal-Wallis, and chi-squared tests. eDNA positivity was compared according to categorical predictors using chi-squared tests.

Generalised linear mixed models (binomial family) were used to assess predictors of eDNA detection (positive/negative). Fixed effects included habitat type, water volume category, volume, turbidity, sun exposure, and sampling period, with breeding site included as a random intercept to account for repeated measurements. Full, reduced, and null models were compared using AIC, BIC, and log-likelihood, and the best-fitting model was retained. Variance inflation factors were checked beforehand to rule out collinearity among fixed effects.

Linear mixed models were subsequently used to assess predictors of eDNA signal intensity (Ct value) among positive samples, using the same fixed and random effect structure. This approach allows the identification of factors associated with strength, independently of detection status alone. For both model types, an adjusted version was additionally fitted by adding log-transformed larval density as a covariate. Adjusted models were fitted on the subset of observations with density data.

All analyses were carried out in R, and the level of statistical significance was set at $\alpha = 0.05$.

3. Results

3.1. *Sampling effort and breeding site characteristics*

A total of 102 breeding sites, including 28 sentinel sites sampled every month from November 2025 to March 2026, were surveyed. The breeding sites sampled varied in water clarity, sun exposure, and water volume: they included clear and turbid waters, shaded, partially shaded, and fully sun-exposed sites, as well as water volumes ranging from less than 1L to more than 5L (Table 1). *Aedes* larvae were detected by morphological examination in 47 of the 102 samples (46.1%). Among the samples containing larvae, *Ae. albopictus* was detected in 40 samples, *Ae. aegypti* in 11, and both species co-occurred in 4 samples (Table 1). In total, 456 *Ae. albopictus* and 20 *Ae. aegypti* larvae were collected over the study period, with larval abundance ranging from 1 to 169 individuals per sample.

Table 1
General description of the study dataset

Variable	Category	Number of samples	Proportion (%)
Habitat	Anthropogenic	23	(22.5)
	Natural	79	(77.5)
Water aspect	Clear	62	(60.8)
	Turbid	40	(39.2)
Sun exposure	Shaded	14	(13.7)
	Partially shaded	54	(52.9)
	Fully sun exposed	34	(33.3)
Water volume	< 1 L	66	(64.7)
	1–5 L	21	(20.6)
	> 5 L	15	(14.7)
Positive by morphology	Any species	47	(46.1%)
	Aedes aegypti	11	(10.8%)
	Aedes albopictus	40	(39.2%)
	Co-occurrence (both species)	4	(3.9%)

3.2. *Analytical performance of the eDNA -qPCR assay*

Amplification remained fully reproducible (3/3 replicates positive) down to dilution D4, corresponding to a limit of detection (LOD) of Ct = 38.13 (0.272000 pg/uL). Quantification precision (CV < 35%) was maintained down to dilution D3, corresponding to a limit of quantification (LOQ) of Ct = 32.31 (2.720000 pg/uL).

Linear regression restricted to dilutions within the confirmed range from D0 to D3 (Fig. 1) showed strong linearity (R^2 = 0.9997), with a slope of − 3.61 corresponding to a PCR efficiency of 89.2%, which falls within the 80–110% range recommended by CSA performance criteria (Abbott et al., 2023). No amplification was

detected in the no-template controls or extraction blanks, indicating no reagent or extraction contamination.

3.3. *Field detection of Aedes albopictus by eDNA-qPCR and larval surveillance*

Overall, eDNA-qPCR detected *Ae. albopictus* in a higher proportion of positive samples than larval surveys (McNemar's test, X^2 =16.447, df = 1, $p < 0.001$). *Aedes albopictus* eDNA was detected in 64.7% (66/102) of samples, compared with 39.2% (40/102) in which larvae were detected by conventional surveillance. Over the entire five-month sampling period, eDNA-qPCR detected more positive samples than conventional larval surveys, and both methods showed a similar temporal pattern (Fig. 2). The two approaches showed concordant positive results in 33.3% (34/102) of the samples and concordant negative results in 29.4% (30/102) of the samples. However, 31.4% (32/102) of the samples tested positive by eDNA-qPCR but negative by larval surveillance, whilst 5.9% (6/102) tested negative by eDNA-qPCR but positive by larval surveillance.

All six extracts showed positive amplification in conventional PCR. Of these, three samples showed a partial qPCR signal. For two of these, two out of three replicates amplified, but the mean Ct value exceeded the assay's LOD (38.39 and 39.65). For the third, only one of the three replicates amplified, with a valid Ct value below the LOD (37.63).

3.4. Diagnostic performance of eDNA-qPCR

eDNA detection showed a Positive Percent Agreement of 85% (95% CI: [70.9, 92.9] %) and Negative Percent Agreement of 48.4% (95% CI: [36.4, 60.6] %) relative to larval surveys. Cohen's Kappa coefficient was 0.30 (95% CI: [0.14, 0.46]), indicating fair agreement between the two methods. The Ct values for *Ae. albopictus* ranged from 21.4 to 37.0 (median: 32.8; IQR: [30.6, 34.3]). Among the 176 replicate pairs from 66 positive samples, the mean Ct difference was 1.72 ± 1.59 cycles. The Ct difference was below 2 cycles in 116 pairs (65.9%) and below 3 cycles in 145 pairs (82.4%). The interclass correlation coefficient (ICC2, two-way absolute agreement) was 0.735 (95% CI: [0.635–0.818]).

3.5. Effects of breeding sites and sun exposure on larval density

Larval density did not differ significantly by breeding site type (Mann-Whitney, W = 977.5, p = 0.53), water aspect (Kruskal-Wallis, H = 0.079, df = 1, p = 0.78), or water volume (Kruskal-Wallis, H = 4.037, df = 2, p = 0.1328). Conversely, larval density differed significantly across sun exposure categories (Kruskal-Wallis, H = 7.150, df = 2, p = 0.03), with the highest median density in shaded breeding sites.

3.6. Relationship between larval density and eDNA signal

Figure 3 shows a significant negative correlation between the number of larvae counted by morphological analysis and eDNA Ct values across all sites, with a significant negative correlation between eDNA and

morphological detection (Spearman ρ= -0.455, p = 0.007, n = 34). This indicated a tendency towards lower Ct values (strong signal) at higher larval abundance. Ct values remained relatively stable at low to moderate larval densities, with a more pronounced decline observed above approximately 19 larvae. Considerable dispersion was observed around this overall trend.

Figure 3: Correlation between larval abundance and eDNA qPCR cycle threshold (Ct) across positive breeding sites. Points show individual eDNA-positive samples, and larval density is expressed as log(n + 1). The solid line shows a LOESS smoother, and the grey shaded area represents the 95% confidence interval. Horizontal dashed lines mark the limit of quantification (LOQ; orange) and the limit of detection (LOD; red). Spearman correlation: rho=-0.455, p = 0.007.

3.7. Factors influencing eDNA detection and signal intensity using multivariate models

Detection probability and signal intensity were modelled using the same complete-case dataset comprising 102 samples collected across 28 breeding sites, of which 66 were eDNA-positive, using a binomial GLMM and an LMM, respectively, each including a random breeding site effect (Fig. 4).

Factors associated with eDNA detection

In the unadjusted detection model, breeding sites with a volume of 1–5 L had higher odds of eDNA detection than those with a volume of less than 1L (OR = 6.22, 95% CI [1.08, 35.95], p = 0.04), whereas sites with values above 5L did not differ from the reference (OR = 1.06, 95% CI [0.22, 5.03]). Detection was lower under partial shade than under full shade (OR = 0.09, 95% CI [0.01, 0.84], p = 0.035), with a similar estimate under full sun (OR = 0.12, 95% CI [0.01, 1.29], p = 0.08). It was also lower in January (OR = 0.20, 95% CI [0.05, 0.92], p = 0.038) and in March 2026 (OR = 0.12, 95% CI [0.03, 0.60], p = 0.009) than in November 2025, with no monotonic seasonal trend. Confidence intervals for December, February, habitat type (OR = 1.65, 95% CI [0.33, 8.13]), and water turbidity (OR = 0.44, 95% CI [0.17, 1.15]) included 1 (Fig. 4a).

After adjustment, larval density was associated with detection probability (OR = 2.45, 95% CI [1.05, 5.70], p = 0.037). The associations with volume and exposure were attenuated relative to the unadjusted model, with all estimates moving towards the null and their confidence intervals including 1 (1-5L: OR = 5.54, 95% CI [0.94, 32.50], p = 0.0578; partial shade: OR = 0.12, 95% CI [0.01, 1.25], p = 0.076). Detection remained lower in January (OR = 0.19, 95% CI [0.04, 0.91], p = 0.0376) and in March (OR = 0.14, 95% CI [0.03, 0.72], p = 0.0179) than in November. Confidence intervals for December, February, habitat type, and turbidity also included 1 (Fig. 4c).

Factors associated with eDNA signal intensity

A linear mixed model was subsequently fitted to the Ct values among eDNA-positive samples, using the same fixed and random effect's structure, to assess predictors of eDNA signal intensity independently of detection status (Fig. 4b). Sun exposure was significantly associated with Ct values (p = 0.011), with Ct increasing by 2.4 cycles under partial shade (β = 2.41, 95% CI [0.34, 4.48], p = 0.0223) and by 3.1 cycles under full sun exposure (β = 3.11, 95% CI [1.07, 5.16], p = 0.0029) relative to shaded sites. Ct values were

lower in February than in November (β = -2.51, 95% CI [-4.54, -0.48], p = 0.015), corresponding to higher eDNA concentrations.

After adjustment (Fig. 4d), larval density was associated with signal intensity (β = -0.77, 95% CI [-1.38, -0.16], p = 0.0137). Full sun exposure remained significant, with a slightly reduced beta (β = 2.44, 95% CI [0.41, 4.47], p = 0.0184). Partial shade (β = 1.57, 95% CI [-0.52, 3.66], p = 0.1410) was attenuated relative to the unadjusted model and was no longer significant. The difference in signal intensity between November and February persisted after adjustment for larval density (β = -2.43, 95% CI [-4.37, -0.48], p = 0.0144).

4. Discussion

This study assessed the field performance of a qPCR test specific to *Ae. albopictus* e-DNA in larval habitats on La Réunion Island. Overall, eDNA-qPCR detected a higher proportion of breeding sites than conventional larval monitoring (64.7% vs 39.2%). The study also identified biological and environmental factors thought to be associated with detection and signal intensity. After accounting for larval density, associations between habitat characteristics and detection probability were attenuated. Full-sun exposure reduced eDNA signal intensity.

eDNA-qPCR offers a complementary approach to traditional entomological methods. However, agreement between the two methods remained fair (Kappa = 0.30). The McNemar test ($p < 0.001$) confirms this asymmetry, with 32 samples tested positive by eDNA-qPCR but negative in larval surveys, compared with only 6 showing the opposite pattern. eDNA-qPCR therefore detects signals more frequently than larval surveys, as expected, since the two approaches do not measure the same thing. Larval surveys confirm the presence of immature stages at the time of sampling, whereas eDNA enables the detection of a species' recent presence through persistent DNA traces, even when larvae are hidden or missed during dipping [28].

This increased sensitivity has also been reported in several field comparisons, notably in *Ae. albopictus* [11], in *Culex quinquefasciatus*, where eDNA enabled the species to be detected up to ten times more frequently than by larval dipping [28], and in *Culex pipiens pallens* in urban environments, particularly in breeding sites with low density [29]. The persistence of eDNA in the water after larvae have disappeared could partly explain this finding, which is well documented in vector mosquitoes. According to these authors, eDNA from *Ae. albopictus* remained detectable for up to 25 days [11], for approximately two weeks in *An. stephensi* [21], and for at least one week in *Culex pipiens pallens* [22].

Conversely, six samples exhibited the opposite pattern, i.e., they tested negative by targeted qPCR but positive by conventional PCR. This discrepancy is attributable less to a lack of sensitivity in eDNA-qPCR than to stochastic factors inherent in molecular detection at low copy numbers. One explanation lies in the stochastic loss of signals near the detection threshold [24]. Among these six sites, two exhibited a signal above the limit of detection, and a third was positive in only one of the three qPCR replicates. Furthermore, the environmental matrix partially inhibits qPCR, whereas conventional PCR is less susceptible to this effect [30]. This is why the present study reports diagnostic performance in terms of PPA and NPA, rather than in terms of strict sensitivity and specificity. Neither of these two methods constitutes an absolute

gold standard. Larval surveying itself is prone to false negatives, due to the imperfect detectability of larvae.

Across the three complementary analyses, larval density consistently proved to be the strongest predictor of eDNA signal, both in terms of detection and intensity. The adjusted models corroborated the negative correlation between larval density and the Ct value reported above. This relationship suggests that higher larval abundance is associated with stronger and more reliably detected signals, indicating that eDNA may provide information not only on species presence but also on their relative abundance. A similar pattern has been reported in previous eDNA validation studies in vector mosquitoes. Odero *et al*. (2018) also reported a positive correlation between larval density and eDNA detection in *An. gambiae* s.l. [10], while Kristan *et al*. (2023) demonstrated an explicit dose-response relationship in *An. stepehensi* and *Ae. aegypti*, with Ct values decreasing across species and container volume as larval numbers increased from 1 to 32 individuals [21]. Taken together, these findings suggest that the density-eDNA relationship is a general feature of eDNA detection in mosquitoes, and not specific to *Ae. albopictus*.

The correlation observed in our study, although significant, was moderate in strength (rho=-0.455), indicating that factors other than larval abundance alone contribute to the variation in eDNA signal intensity. This result is consistent with the detection stochasticity reported by Odero *et al*. (2018) at low larval abundances, when the quantity of DNA present in the sampled water volume approaches the amplification threshold. Larval development stage could also modulate this relationship, with eDNA shedding peaking around the time of pupation and emergence rather than increasing uniformly with larval number [22].

Our study demonstrated that the probability of detection was not affected by exposure to sunlight. This finding is consistent with Kristan *et al*. (2026), who observed no significant effect of water temperature (25.8 to 35.8°C) on eDNA detection probability for malaria vectors at natural breeding sites in Ghana [15]. Conversely, the intensity of the eDNA signal decreases with exposure to sunlight, as reflected by the higher Ct values observed in sites exposed to direct sunlight. This pattern suggests a thermal rather than a photochemical mechanism. In an experiment where the temperature was kept constant, with only the intensity of UV radiation varying between two test groups, UV exposure had no significant effect on eDNA degradation [31]. However, several studies have shown that higher temperatures accelerate eDNA degradation through increased enzymatic activity [32,33]. Sites exposed to direct sunlight are likely to reach higher water temperatures than shaded sites. This provides a plausible explanation for the decline in the eDNA signal observed in direct sunlight, without necessarily altering the probability of detection.

This study shows that an eDNA-qPCR test targeting *Ae. albopictus* can detect this species with high sensitivity in tropical larval habitats, while detecting a higher proportion of positive sites than conventional larval monitoring methods. These results must, however, be interpreted in context, because they relate to an already established species, present at measurable larval density. On their own, however, they do not allow this approach to be extrapolated to other operational contexts. A method capable of detecting the DNA of an invasive species at very low abundances could be valuable for surveillance at entry points into a territory that has not yet been colonised. eDNA-qPCR tests targeting *An. stephensi*, the invasive vector of

malaria, have already been developed and validated under experimental conditions [21], and the feasibility of eDNA-based surveillance (qPCR and sequencing) of mosquitoes of the genus *Anopheles* in natural breeding sites has been demonstrated in the field in Ghana and Ethiopia [15,34]. Nevertheless, we have not assessed detection by qPCR at such low abundances as those expected following a recent introduction. The adaptation and validation of these tests under environmental and operational conditions therefore remain necessary. La Réunion, like other neighbouring territories in the Indian Ocean, is at risk of invasion by *An. stephensi* [35]. A recent modelling study suggests that *An. stephensi* could become established on La Réunion and colonise most of the island within five years of introduction [36]. Developing eDNA-based surveillance methods could help to detect its introduction early and support rapid intervention measures to prevent its spread. Regarding monitoring vector control interventions, the observed sensitivity suggests this method could monitor such operations, particularly the boosted sterile insect technique tested on La Réunion [37]. This potential is even more relevant when larval densities decline as a result of effective control, a scenario in which eDNA-qPCR could help confirm whether the decline in detections reflects an actual reduction in population size.

Conclusion

This study provides a field validation of an eDNA-qPCR assay *for Aedes albopictus* under natural tropical conditions. The assay detected *Aedes albopictus* at a higher proportion of breeding sites than conventional larval dipping. Overall, eDNA-qPCR detection showed fair agreement with larval surveys, while providing a significantly higher detection rate and demonstrating its potential to identify sites without detectable larvae. This method is therefore more sensitive and can detect the mosquito even after it has passed through or disappeared due to predation or other factors.

Larval density proved to be the principal driver of both detection probability and signal intensity, largely explaining the apparent effects of water volume and shading observed in the unadjusted models. However, sun exposure remained independently associated with signal intensity after adjusting for larval density, consistent with a potential effect of solar radiation and temperature on eDNA persistence. These findings pave the way for applying eDNA-qPCR to other vectors, particularly *Anopheles* mosquitoes. It could facilitate the early detection of invasive species as well as the monitoring of vector control programmes.

Abbreviations

ANSES	:	Agence nationale de sécurité sanitaire de l’alimentation, de l’environnement et du travail
Ct	:	Cycle threshold
DNA	:	Deoxyribonucleic acid
eDNA	:	Environmental DNA
GLMM	:	Generalized linear mixed model
ICC	:	Intraclass correlation coefficient
LMM	:	Linear mixed model
LOD	:	Limit of detection
LOQ	:	Limit of quantification
NPA	:	Negative percent agreement
PCR	:	Polymerase chain reaction
PPA	:	Positive percent agreement
qPCR	:	Quantitative polymerase chain reaction

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The data supporting the findings of this study are available upon reasonable request.

Competing interests

Competing interests: The authors declare no competing interests.

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Authors' contributions

Design and implementation of the study: I.T, W.D, TB, H.M, and J.B. Laboratory activities: I.T, N.B. and M.E.A. Field sampling: I.T and C.V. Taxonomic identification: C.V. Analysis and interpretation of data: I.T. Critical writing and revision of the article to extract its important intellectual content: I.T, W.D, C.V, N.B, M.E.A, T.B, A.D, J.K, H.M, and J.B. Final approval of the version to be submitted: I.T, W.D, C.V, N.B, M.E.A, T.B, A.D, J.K, H.M, and J.B.

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Figures

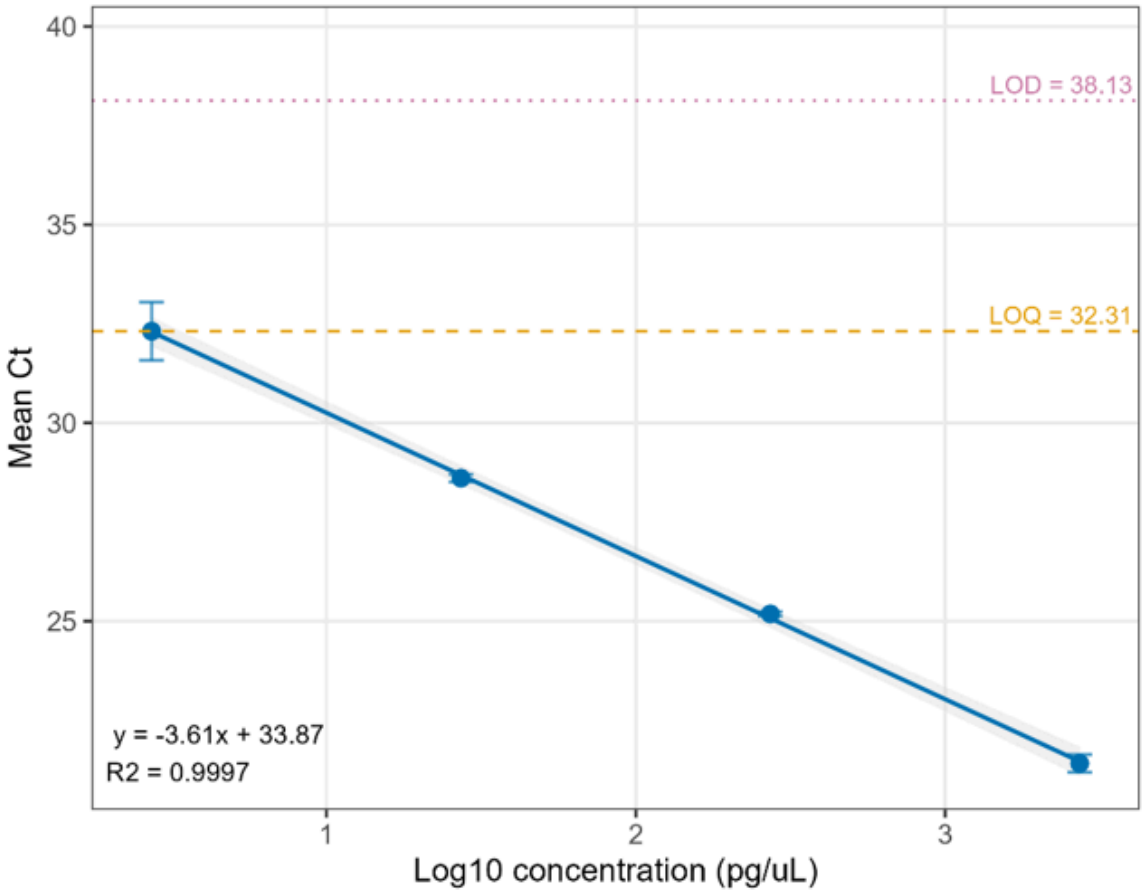


Figure 1

Standard curve of the *Aedes albopictus* eDNA-qPCR assay. Ct values are plotted against the decimal logarithm of the number of copies per reaction. The regression line and 95% confidence interval are shown. Dashed horizontal lines indicate the limit of quantification (orange) and limit of detection (red).

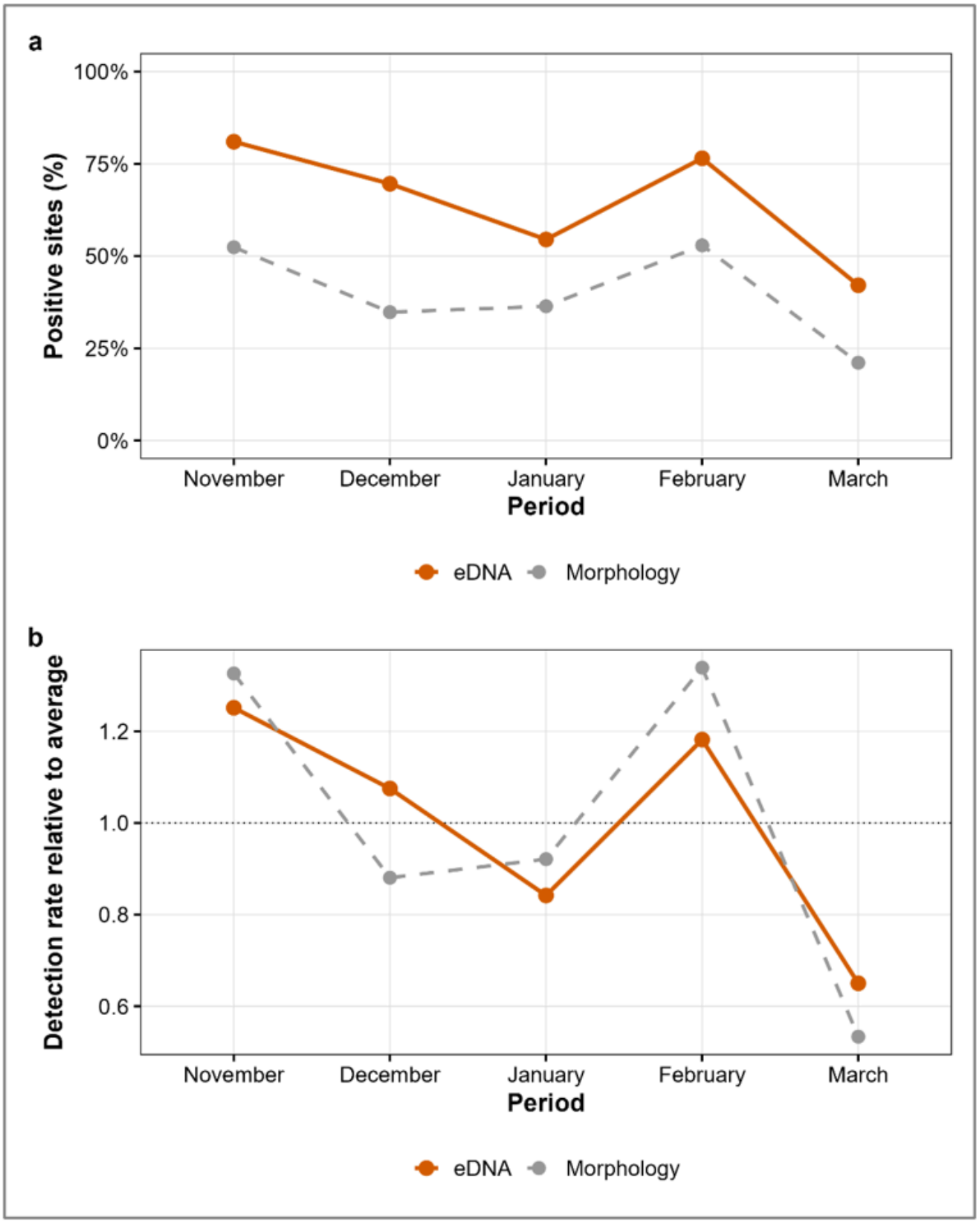


Figure 2

Temporal dynamics of eDNA and larval surveys detection rates across sampling periods. (a) Positive sites (%) detected by each method per period. (b) Detection rates expressed relative to each method's own average, factoring out the overall difference in sensitivity between methods.

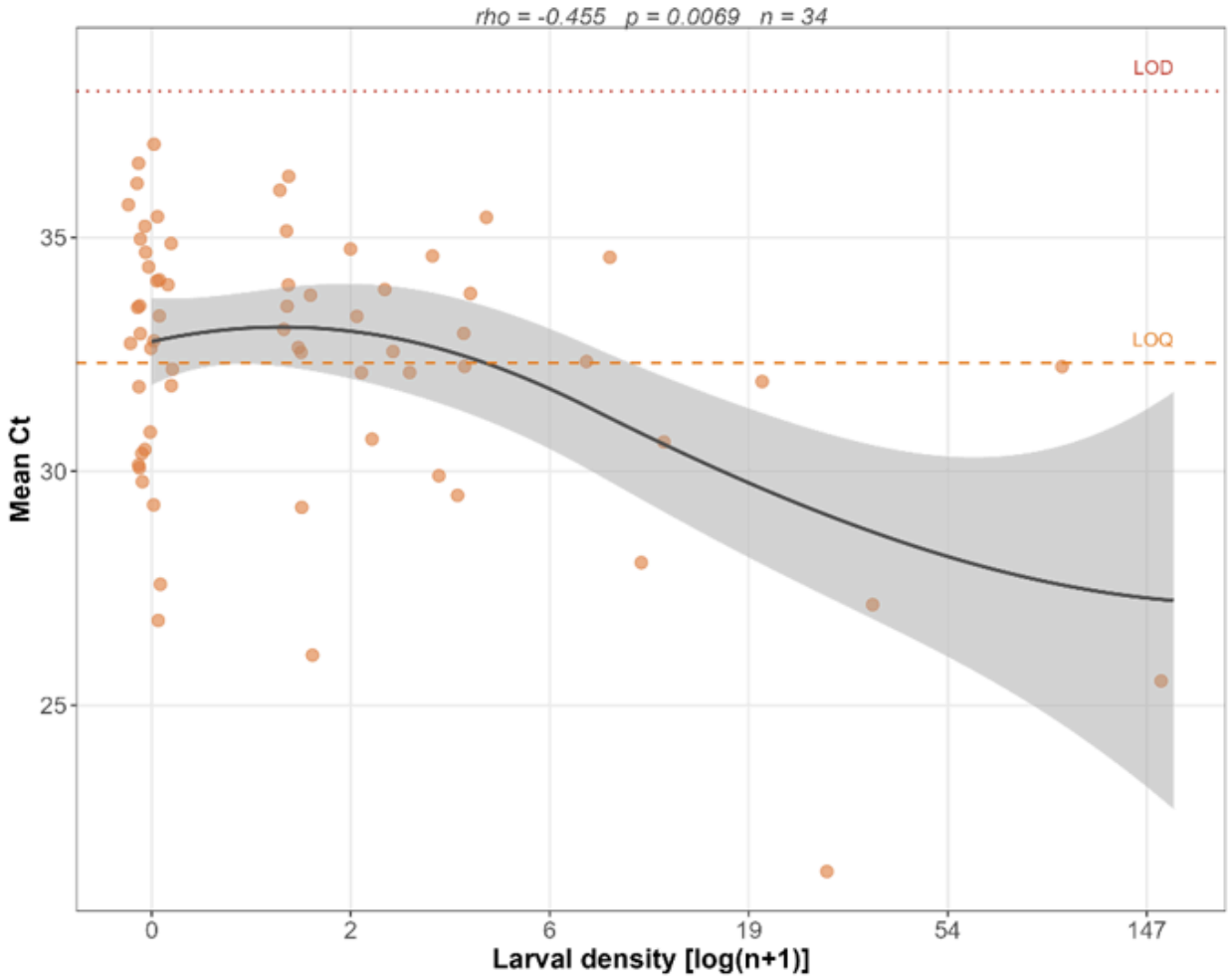


Figure 3

Correlation between larval abundance and eDNA qPCR cycle threshold (Ct) across positive breeding sites. Points show individual eDNA-positive samples, and larval density is expressed as log(n+1). The solid line shows a LOESS smoother, and the grey shaded area represents the 95% confidence interval. Horizontal dashed lines mark the limit of quantification (LOQ; orange) and the limit of detection (LOD; red). Spearman correlation: rho=-0.455, p=0.007.

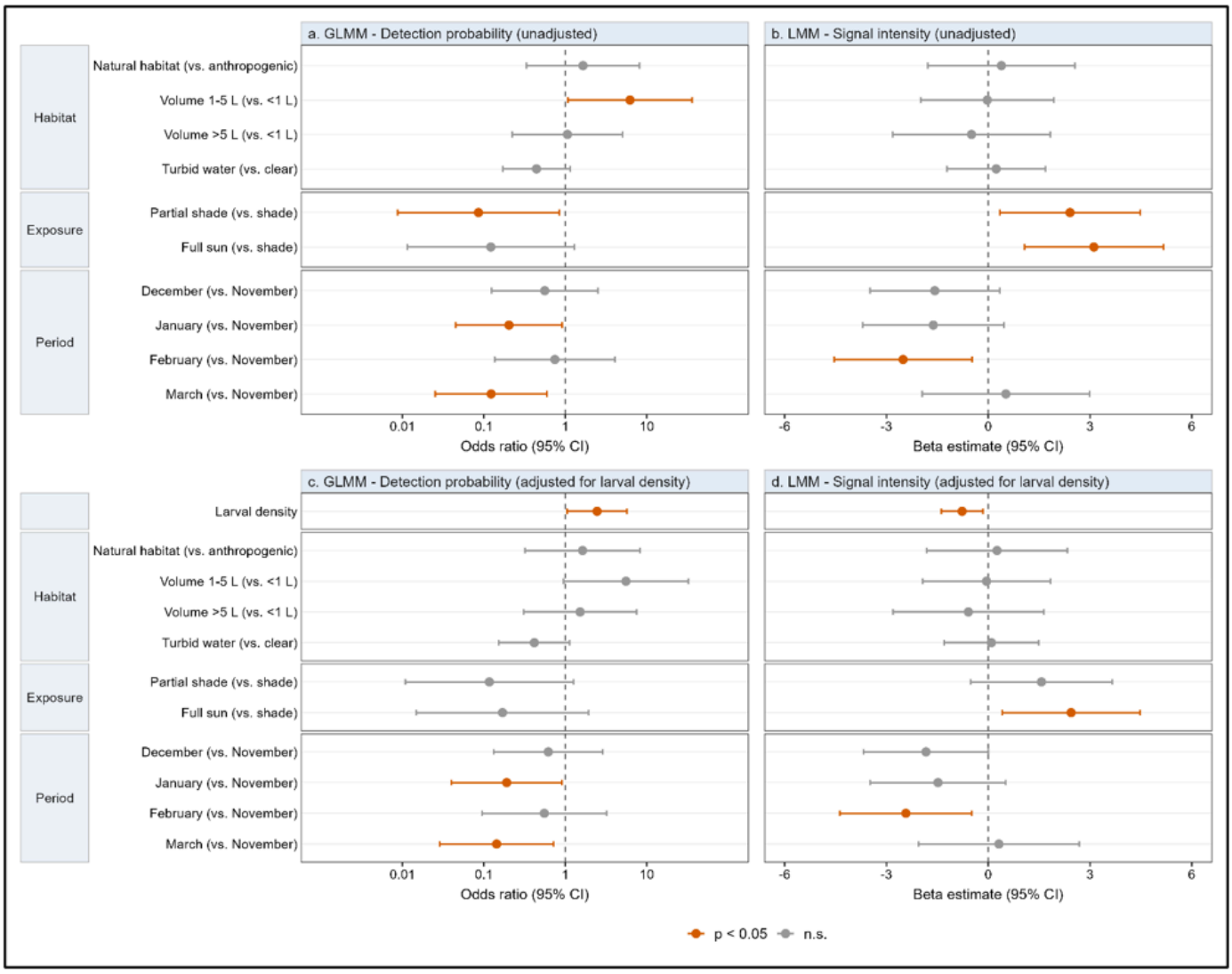


Figure 4

Odds ratios for *Aedes albopictus* eDNA detection and estimated changes in Ct associated with environmental and sampling-related factors, before and after adjustment for larval density. (a) Unadjusted GLMM for eDNA detection. (b) Unadjusted LMM for eDNA signal intensity. (c) Larval-density-adjusted GLMM for eDNA detection. (d) Larval-density-adjusted LMM for eDNA signal intensity. Points show parameter estimates and horizontal bars represent 95% confidence intervals. Dashed lines indicate the null value: an odds ratio of 1 in panels (a) and (c), and a change in Ct of 0 cycles in panels (b) and (d).