Research Article

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Experimental comparison of aerial larvicides and habitat modification for controlling disease-carrying *Aedes vigilax* mosquitoes

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Abstract

BACKGROUND: Microbial and insect-growth-regulator larvicides dominate current vector control programmes because they reduce larval abundance and are relatively environmentally benign. However, their short persistence makes them expensive, and environmental manipulation of larval habitat might be an alternative control measure. *Aedes vigilax* is a major vector species in northern Australia. A field experiment was implemented in Darwin, Australia, to test the hypotheses that (1) aerial microbial larvicide application effectively decreases *Ae. vigilax* larval presence, and therefore adult emergence, and (2) environmental manipulation is an effective alternative control measure. Generalised linear and mixed-effects modelling and information-theoretic comparisons were used to test these hypotheses.

RESULTS: It is shown that the current aerial larvicide application campaign is effective at suppressing the emergence of *Ae. vigilax*, whereas vegetation removal is not as effective in this context. In addition, the results indicate that current larval sampling procedures are inadequate for quantifying larval abundance or adult emergence.

CONCLUSIONS: This field-based comparison has shown that the existing larviciding campaign is more effective than a simple environmental management strategy for mosquito control. It has also identified an important knowledge gap in the use of larval sampling to evaluate the effectiveness of vector control strategies.

Keywords: vector control; field experiments; larvicide; environmental management; Australia

1 INTRODUCTION

The control of vector populations is the first line of defence against outbreaks of vector-borne disease and their associated public health and economic impacts. Each year, millions of people die from vector-borne diseases such as malaria, dengue and yellow fever or suffer from chronic illness as a result.[1,2] While there are many ways to treat and prevent these diseases, pharmaceutical-based solutions, where they exist, ultimately become intractable during large outbreaks. Recently, the focus for vector-borne disease control has turned to 'integrated pest management', which combines the suppression of larval stages of vectors with the prevention of human contact with adult vectors via indoor residual spraying and insecticide-impregnated bed nets.[3–6]

The efficacy of chemical-insecticide-based vector control tools will be potentially compromised by the evolution of resistant vector populations;[7] however, non-chemical-insecticide-based methods that target vector larvae have had some success in reducing vector populations and the concomitant pathogens they transmit.[8] Mosquito larvae are a vulnerable part of the life cycle because they cannot easily avoid control measures given that they are confined to their aquatic breeding sites until emergence as adults. As a consequence, microbial larvicides such as *Bacillus thuringiensis* var. *israelensis* (*Bti*), which dominates current broad-scale field larval control programmes, can more effectively reduce target vector populations. Microbial larvicides are highly effective at suppressing vector numbers, are environmentally benign for non-target organisms and, owing to the complex of insecticidal proteins present, are less likely to result in resistance than chemical insecticides.[6,9–13]

The short activity persistence of larvicides such as *Bti* (2–3 days) means that, while this method is useful for immediate control of high larval densities, repeated and costly applications are

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typically required to suppress vector densities over the long term.[3,6] Source reduction, or environmental management, is another potentially cost-effective method for vector control that refers to the modification of vector habitat to discourage larval development.[6,14,15] While broad-scale, long-term, engineered changes to wetland systems can be effective at eliminating larval habitats,[15] temporary manipulation of habitat such as seasonal vegetation removal can also reduce vector populations and is not as ecologically disruptive as permanent modification (culvert removal, increased drainage, filling operations).[3,6,15–18]

Mosquitoes are sensitive to environmental changes brought about by vegetation removal because their survival, density and distribution are influenced by small changes in microclimatic conditions.[19–21] Emergent and semi-aquatic vegetation removal can create a hostile aquatic microclimate for vector larvae by allowing greater predator access, less shelter from wind and wave actions and reduced protection from extreme temperatures and evaporation.[22] There are many cases where vegetation removal has been successful in reducing larval abundance and oviposition.[14,22–25] However, vegetation removal over broader scales has also been correlated with increases in vector numbers,[21] clearly showing that this form of vector control is ecosystem and species specific, and again highlighting the need for a detailed understanding of local vector ecology to implement effective control measures.

The effectiveness of vector control methods is realised as a reduction in adult vector numbers. However, in the case of larval vector control, linking the relative size of the adult population to the effects of control at the larval stage is challenging. In most situations, larval abundance or density estimates are used as a proxy for emerging adult numbers;[26,27] however, larval sampling often underestimates true larval densities, and it does not account for pre-emergence mortality.[14,28] Although monitoring adult numbers can track local vector population dynamics and quantify population trends resulting from control, monitoring only the adult population cannot detail the absolute effect of larval control on vector numbers owing to the confounding influences of density feedback on larval survival, larval habitat availability, adult dispersion and the alternating activities of blood-meal seeking and oviposition.[29] It is therefore important to quantify the relationship between larval abundance and adult emergence before any conclusions about the effectiveness of a control measure can be drawn.

The government of the Northern Territory of Australia currently spends approximately $A 400 000 annually on aerial-applied larvicide for mosquito population control around the city of Darwin.[30] The main aim of the control programme is to suppress emergence of the salt-marsh mosquito *Aedes vigilax* in the swamp complexes adjacent to the northern residential suburbs of Darwin. *Ae. vigilax* is recognised as a vicious biter and is also a major vector of Ross River and Barmah Forest viruses in coastal and subcoastal areas.[31,32] Therefore, effective control of a major nuisance species that is one of the primary vectors of these pathogens is a high priority for Northern Territory public health management.[32] The major method of control currently employed is ground and aerial application of the microbial insecticide *Bti*,[16,33,34] although there have been previous successful attempts to remove larval habitats permanently in some areas of Darwin through environmental modification.[16,17,34]

Ae. vigilax eggs are mainly oviposited on damp mud at the base of vegetation, and can withstand long periods of desiccation until favourable hatching conditions occur.[35] Tides and rainfall that flood the swamp complexes create ephemeral pools suitable for larvae, and high numbers of adults often emerge following extremely high spring tides and/or high rainfall after the habitats have been dry for a variable period.[34,36,37] *Ae. vigilax* oviposition and egg density are strongly correlated with the presence of vegetation,[38,39] and, previously, large-scale engineering environmental modification methods, such as drain infilling, filling and culvert removal, that aim to increase tidal flushing in coastal swamps have been used to control this species, with some success at reduction of larval habitats.[15–17] The current aerial larval control measures for *Ae. vigilax* populations in Darwin only affect the immediate rate at which the generations fill available larval habitat, and do not appear to have a long-term impact on potential population size.[34,37]

The principal aim of the *Bti* spraying programme is to reduce or dampen the emergence of adults following a breeding initiation event, such as a high tide or rainfall. Although several studies have found evidence for reductions in surveyed larvae numbers and indoor resting adult populations in conjunction with local larvicide application,[40] this outcome has never been quantified experimentally. To this end, a field experiment was designed and implemented (i) to evaluate current aerial larval control procedures across the swamp complex to the north-east of Darwin, and (ii) to examine the relative effectiveness of alternative mosquito control measures such as environmental modification. It was hypothesised that:

1. Adult *Ae. vigilax* emergence rates will vary according to different larval abundance, vegetation types and water quality; lower emergence is expected in areas of lower larval abundance, and in less brackish water.
2. Aerial application of *Bti* effectively decreases *Ae. vigilax* larval abundance and therefore adult emergence across larval habitats in the swamp.
3. Environmental manipulation (vegetation removal via shears or localised burning) is an effective vector control surrogate for aerial *Bti* application.
4. The combined effects of aerial *Bti* application and environmental manipulation (vegetation removal via shears or localised burning) will be the most effective method of reducing *Ae. vigilax* larval abundance and adult emergence.

2 METHODS

2.1 Study site

The Leanyer/Holmes Jungle swamp complex (LHJ swamp), which lies approximately 2 km to the north-east of Darwin, Northern Territory, Australia, was chosen as the study site because this area is close to dense human settlement, contains a complex of different vegetation types and is regularly surveyed and sprayed for mosquito larvae by Medical Entomology of the Northern Territory Department of Health.[16] Emergent mosquitoes were collected from four vegetation types in the LHJ swamp: (1) closed-canopy mangrove (*Avicennia marina*) forest; (2) an area where the brackish water reed *Schoenoplectus litoralis* fringes the edge of the mangrove forest; (3) an area dominated by *S. litoralis*; (4) an area dominated by the freshwater water chestnut, *Eleocharis dulcis* (Fig. 1).

2.2 Experimental design and treatments

Larval traps were established before the highest monthly high tide events in October 2007 and November 2008 when all the

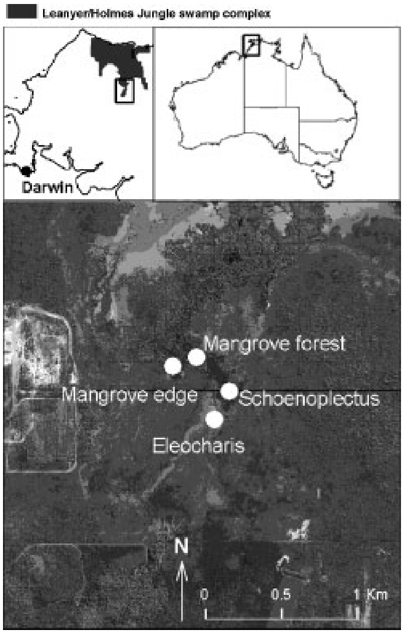

Figure 1. Location of the Leanyer/Holmes Jungle swamp complex and various *Aedes vigilax* larval habitats.

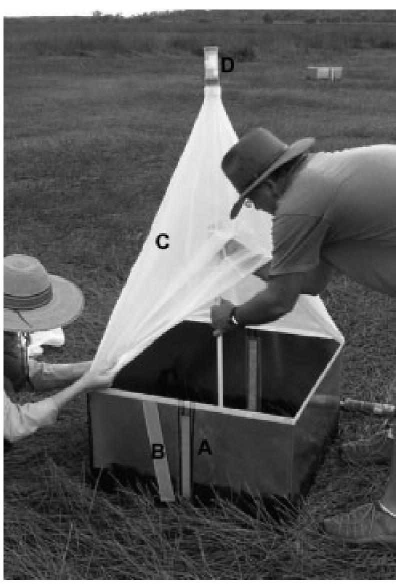

Figure 2. Emergence trap design: mesh-covered holes allow tidal flooding without larval movement (A); yellow magnets applied over holes prevent unwanted larvicide from entering the trap (B); mesh emergence tent prevents unwanted species oviposition inside the trap (C) and also catches emerging adults (D).

sites were dry. The larval traps consisted of 1 m^2 galvanised metal frames, 20 cm in height, which had vertical rectangular holes on two sides covered with fine mesh that allowed water to flood the trap but prevented the movement of mosquito larvae into or out of the trap (Fig. 2). Larval traps were dug 0.05 m into the muddy substratum, and a pyramid-shaped mosquito net was attached to the top of the traps to prevent oviposition of non-target species in the plots, and also to capture emerging adult *Ae. vigilax*. The following different treatments were applied to trap sites:

1. *Spraying*. Traps were exposed to Vectobac (*Bti* larvicide) sprayed from a jet ranger helicopter at a concentration of 1.5 L ha^{-1} at a height of approximately 2 m on 29 and 30 October 2007 to determine the effectiveness of aerial application of *Bti* at suppressing *Ae. vigilax* emergence.
2. *Burning*. Vegetation within the frames was removed via localised burning prior to the October 2007 high tide to determine whether vegetation removal by fire had a detrimental effect on *Ae. vigilax* emergence. This was achieved within the traps by igniting the vegetation using a handheld blowtorch. Vegetation was burned to ground level where possible, but charred vegetation remains were not removed from the trap.
3. *Slashing*. Vegetation within the larval traps was removed using pruning shears prior to the October 2007 high tide event to determine the effects of vegetation removal only. The vegetation was trimmed as close to ground level as possible, and it was removed from the frames.

No-spray-treatment traps were covered with plastic sheets during the spraying operation, and the holes on the side of the traps were blocked to prevent spray-contaminated water from moving inside the non-sprayed traps (Fig. 2). Treatments were only applied to traps within the vegetation types known to produce the highest numbers of emerging *Ae. vigilax* at that time of year, namely *Schoenoplectus litoralis* and *Eleocharis dulcis*.[41] Five trap replicates were placed within the *Schoenoplectus* and *Eleocharis* habitats in October 2007 for each of the three different treatments of vegetation removal (via shears or localised burning) and spraying. To test whether *Bti* application had an interactive or additive effect with either of the vegetation removal treatments (via shears or localised burning) within the *Schoenoplectus* habitat, five traps were also placed in October 2007 for each of the treatment interactions: vegetation removal via localised burning and spraying, and vegetation removal via shears and spraying. To measure uncontrolled mosquito emergence, five traps were placed in October 2007 in each of the four vegetation types as controls. Total sample size in October 2007 was therefore 60 traps within a partially orthogonal design. To examine the effectiveness of the current larval sampling procedure in relation to uncontrolled mosquito emergence, ten traps were placed in the *Schoenoplectus* habitat during the November 2008 high tide event.

2.3 Larval sampling and adult emergence

The LHJ swamp was flooded by a series of high tides during October 2007 and November 2008. In 2007, the highest tide that occurred was 7.9 m on Saturday 27 October; however, a tide capable of inundating a large area of the swamp (7.7 m in height) occurred the previous evening and on the following two days (7.9 and 7.6 m). In 2008, the highest tide (7.8 m) occurred on Friday 14 November, and tides capable of inundating the swamp (7.2 and 7.6 m) also occurred during the previous few days. The traps were monitored each day leading up to these high tide events, and, once the traps were flooded, larval sampling commenced. Each day following initial flooding, *Ae. vigilax* larval abundance was sampled in the traps using a prescribed dipping procedure: five dips were done (one in each corner of the trap and one in the centre) using a standard dipper (190 mL volume). Larvae were counted and then returned to the traps, and the final estimate of larval abundance was calculated by summing the counts of larvae across the five dips $trap^{-1}$ day^{-1}. To quantify the ability of the above sampling procedure to estimate true larval density

Table 1. (a) Numbers of larvae averaged over five sampling dips taken each day for 4 days. (b) Comparison of two models used to assess the ability of larval sampling dips to predict actual larval numbers per trap, using information-theoretic model selection. Variables included in the models are density (actual larval numbers per trap) and dip (larval numbers indicated by larval sampling using a dipper)

(a) Average larval density

Trap ID	15/11/08	16/11/08	17/11/08	18/11/08	Final density[a]
12	0	1.8	0.8	1.2	73
14	0.2	9.8	1.6	2.2	71
16	0	1.8	1.6	1	45
18	0	2.4	0	0.6	111
20	0	3.4	1	–	91

(b) Model comparison[b]

Model	AIC_c	ΔAIC_c	$wAIC_c$	%DE
Density ∼1	−165.87	0.00	<0.999	0
Density ∼ dip	−130.44	35.44	>0.001	8.2

[a] Final densities were estimated by removing all the water in the traps and counting total larval numbers. This occurred on 18/11/08 for traps 12 to 18, and on 17/11/08 for trap 20.
[b] The explanatory variable is the average larvae per dip; ΔAIC_c is the difference between the model AIC_c and the minimum AIC_c in the set of models; $wAIC_c$ is the AIC_c weight for each model; %DE is the percentage deviance explained.

reliably, all larvae within each of the five traps were removed and counted according to the methods outlined by Service[42] once the larvae in the traps had reached the third instar in November 2008. The water was removed by bailing with buckets and sieved through fine mesh. Larvae were carefully removed to smaller storage containers for counting in the lab. Bailing continued until two or more buckets that contained no larvae were sieved, and no larvae were observed rising to breathe at the surface of the remaining water in the traps.

After observing emerging adults in emergence traps, larval sampling was ceased in October 2007. Emerging adults were caught manually by sucking them into sampling containers using a small aspirator. This was repeated daily until emergence within the traps had ceased. During November 2008, the five control traps to test for numbers of emerging adults failed owing to an unexpected spraying event, and unfortunately no adults were collected that year.

2.4 Larval sampling

To assess whether the larval sampling procedure, used accurately, measures true larval density, two Gaussian-distributed (identity link function) linear mixed-effects models were compared. The response variable was final trap density (Table 1), and the explanatory variable was average larvae per dip. Trap ID was included as a random effect.

2.5 Water chemical and physical properties

Water pH, conductivity and dissolved oxygen were measured in each trap daily using a Horiba U10 water meter (HORIBA Ltd, Kyoto, Japan). Temperature 5 cm above the water surface was measured using Dallas DS1923 Hygrochron iButtons (Maxim Integrated Products, Sunnyvale, CA). Temperature readings were taken every 5 min, and from these the mean for the daylight hours of each day was calculated.

2.6 Larval presence and adult emergence across habitat types

To examine whether local environmental conditions affected larval presence or adult emergence, the authors developed statistical model sets including pH, conductivity ($mS\ cm^{-1}$), dissolved oxygen ($mg\ L^{-1}$) and mean daily temperature (°C) as variables in binomial error-distribution (logit link function) generalised linear mixed-effects models (GLMMs) for larval presence, and Poisson (logit link function) GLMMs for adult emergence. Random effects included in the larval presence GLMMs were trap and vegetation type (trap was nested within vegetation), and vegetation was also included as a random effect in the adult emergence models. From these model comparisons, the water qualities that were the most important drivers of larval presence and adult emergence were identified, and these were then combined via a principal components analysis (PCA). The first principal component, representing the key environmental conditions affecting larval presence or adult emergence, was included in further analyses.

To examine the differences in larval presence and adult emergence across different habitat types, binomial (logit link function) GLMMs of larval presence and Poisson (log link function) generalised linear models (GLMs) of adult emergence were developed. The explanatory variables included in the models were vegetation type (mangrove forest, mangrove edge, *Schoenoplectus* and *Eleocharis*), local environmental conditions (first principal component from the PCA of pH, conductivity, dissolved oxygen and mean daily temperature) and trap water depth (mm) to control for the confounding effects of variable water depths between traps. Median larval presence was also included in all the adult emergence models to control for the confounding effects of varied larval numbers among traps.

2.7 Spraying and vegetation removal (via shears or localised burning) as control methods

For the two habitat types that had traps exposed to spraying and vegetation removal treatments (*Schoenoplectus* and *Eleocharis*), a before-after/control-impact (BACI) experimental design was used to examine the effectiveness of these different methods at reducing larval presence.[43] Larvae were sampled in (i) control traps, (ii) traps that had vegetation removed (via shears or localised burning) and (iii) traps exposed to *Bti* for 2 days before and 4 days after the 'impact' (the spraying event). Larval presence was the response variable for sets of binomial (logit link function) GLMMs, and the variables included in the models were vegetation treatment (removal via localised burning, removal via shears or control), spray treatment (*Bti* application or shielded), time period (before or after spray event), local environmental conditions (first principal component representing environmental conditions) and water depth (mm). Random effects included in the GLMMs were trap and vegetation type (*Schoenoplectus* or *Eleocharis*); trap was nested within vegetation. Statistical evidence for an interaction between time period (before or after) and spray treatment (*Bti* application or shielded) indicates an effect of spraying on larval presence.

Model sets were also constructed to examine the effects of spraying, vegetation removal treatment and environmental conditions on adult emergence. Total adult emergence per trap was the response variable of sets of Poisson (log link function)

GLMMs, and the explanatory variables were vegetation treatment (removal via localised burning, removal via shears or control), spray treatment (*Bti* application or shielded), median water depth (mm) and median larval abundance. Vegetation type (*Schoenoplectus* or *Eleocharis*) was included as a random effect.

2.8 Combined effects of control methods

To examine whether the combined effects of vegetation removal via localised burning and spraying or vegetation removal via shears and spraying are the most effective methods of reducing larval presence and adult emergence, data from the *Schoenoplectus* traps were analysed, as this was the only habitat to receive the combined treatments.

Again, a BACI design was used where larvae were sampled in control traps, in traps that had vegetation removed (via shears or localised burning) and in traps exposed to *Bti* for 2 days before and 4 days after the spraying event. Larval presence was the response variable for sets of binomial (logit link function) GLMMs, and the variables included in the models were vegetation treatment (removal via localised burning, removal via shears or control), spray treatment (*Bti* application or shielded), time period (before spray event or after spray event), local environmental conditions (first principal component from the PCA of pH, conductivity, dissolved oxygen and mean daily temperature) and trap water depth (mm). Trap was coded as a random effect.

To examine the effects of combined vector control methods on adult emergence, sets of Poisson (log link function) GLMs were constructed, with total adult emergence per trap as the response variable and vegetation treatment (removal via localised burning, removal via shears or control), spray treatment (*Bti* application or shielded), median water depth (mm) and median larval presence as the explanatory variables.

2.9 Model comparison

To rank and weight models, Akaike's information criterion was used, corrected for small samples (AIC_c), as an estimate of Kullback–Leibler (K–L) information loss[44] (i.e. statistical likelihoods that have been bias corrected to account for the number of parameters fitted, somewhat akin to a measure of model parsimony). The difference between the model's AIC_c and the top-ranked model's (ΔAIC_c) was calculated, as well as the relative model weights ($wAIC_c$).[44] Thus, the strength of evidence ($wAIC_c$) for any particular model varies from 0 (no support) to 1 (complete support) relative to the entire model set, and evidence is not assessed on the basis of some arbitrary probability of making a type-I error (i.e. rejecting a null hypothesis when, in fact, it is true). The amount of variance in the response variable captured by each model (i.e. structural goodness of fit) was assessed as the percentage deviance explained (%DE) relative to the null deviance.

An individual variable-ranking method was used to determine the relative importance of different predictor variables in separately and jointly explaining deviance in adult emergence.[45] Firstly, each predictor variable individually was dropped from the saturated model (the model containing all possible predictor variables), and the change in %DE was assessed. Secondly, each predictor variable individually was added to the null model, and again %DE was measured. The changes in %DE relative to the saturated and null model were calculated, and they were then summed as the total variable deviance. The total variable deviance was rescaled to sum up to 1 (relative deviance), and variables were ranked according to the relative deviance explained. All analyses were done using the R Package v.2.9.0.[46]

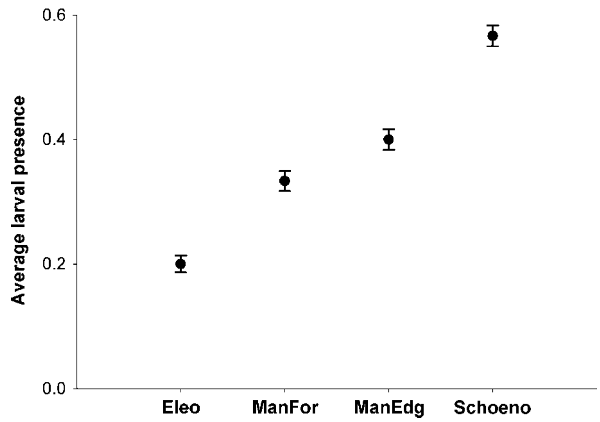

Figure 3. Average *Aedes vigilax* larval presence sampled across different habitat types (where 0 = no larvae and 1 = larvae present) with standard error bars. Habitat types are: Eleo – an area dominated by the freshwater reeds, *Eleocharis dulcis*; ManFor – closed-canopy mangrove (*Avicennia marina*) forest; ManEdg – an area where *Schoenoplectus litoralis* reeds fringe the edge of the mangrove forest; Schoeno – an area dominated by the brackish water reeds, *Schoenoplectus litoralis*.

3 RESULTS

3.1 Larval sampling, larval presence and adult emergence across habitat types

There was no statistical evidence for a relationship between average number of larvae per dip and final larval density, indicating that this form of larval sampling is not an accurate measure of mosquito production from a given habitat; rather, it simply provides an approximate measure of larval presence or absence (Table 1).

Further, taken across all habitats, the present analyses did not reveal statistically meaningful differences in the effects of local environmental conditions for either larval presence or adult emergence, even though there was some variation across habitat types. The ranges of environmental conditions experienced by the developing larvae were: pH 6.69–4.39, dissolved oxygen 5.91–5.35 mg L^{-1}, salinity 71.48–67.4 mS cm^{-1} and mean daily temperature 35.49–32.34 °C. The most parsimonious model explaining the variation in *Ae. vigilax* adult emergence included median larval presence and trap water volume, although there was also support for models including local environmental conditions and habitat type (*Schoenoplectus*,*Eleocharis*, mangrove forest, mangrove edge) [see supporting information Table S1, (a)]. The individual variable ranking revealed that median larval presence and habitat type (*Schoenoplectus*,*Eleocharis*, mangrove forest, mangrove edge) explained 81.9 and 14.3% relative deviance in adult emergence respectively [Table 2, (a)].

Both water depth and habitat type were the best predictors of differences in larval presence [see supporting information Table S1, (b)]. Larval presence was highest in *Schoenoplectus*, then mangrove forest and mangrove edge, and lowest in *Eleocharis* (Fig 3).

3.2 Spraying and vegetation removal (via shears or localised burning) as control methods

In the traps that were exposed to aerially applied *Bti*, 97% fewer adults emerged than in unexposed traps (Fig. 4a). Statistical evidence was also found for an effect of vegetation removal (via shears or localised burning) on adult emergence numbers [see supporting information Table S2, (a)]. For this model set, the individual variable ranking revealed that exposure to aerial

Table 2. Individual explanatory strength of predictor variables for different model sets of *Aedes vigilax* adult emergence[a]

Predictor variables	%DE deletion	%DE addition	Relative deviance
(a) Habitat type models			
median larval presence	21.6	26.8	81.9
habitat type	0.9	7.6	14.3
trap water volume	0.1	1.0	1.8
local environmental conditions	0.1	1.2	2.1
(b) Spraying or vegetation removal models			
exposure to aerial *Bti* application	22.6	27.2	46.9
median larval presence	14.4	21.6	33.9
vegetation removal (via shears or burning)	6.5	1.4	7.4
local environmental conditions	4.8	3.0	7.3
trap water volume	4.3	0.5	4.6
(c) Combined control method models			
exposure to aerial *Bti* application	25.0	52.6	60.0
median larval presence	4.7	34.4	30.2
trap water volume	7.0	0.3	5.6
vegetation removal (via shears or burning)	2.2	1.8	3.0
local environmental conditions	0.4	0.6	0.8
combined *Bti* and vegetation removal	0.2	0.2	0.3

[a] Habitat type = mangrove forest, mangrove edge, *Schoenoplectus*, *Eleocharis*.

Bti (spray), median larval presence and vegetation removal explained 46.9, 33.9 and 7.4% relative deviance in adult emergence respectively [Table 2, (b)]. The present models predict that, with all other variables held equal, vegetation removal via localised burning will reduce adult emergence by 41% (95% confidence interval 22–55%), and vegetation removal via shears will reduce adult emergence by 57% (95% confidence interval 44–66%).

The larva models showed no evidence for any effect of vegetation removal (via shears or localised burning) on larval presence, and hardly any evidence for the interaction term between time and impact (spraying) [see supporting information Table S2, (b)].

3.3 Combined effects of control methods

In the model set examining the effects of combined vector control methods on adult emergence, although there was strong evidence for the separate effects of aerial *Bti* application and vegetation removal (via shears or localised burning), there was no statistical evidence that combining these different control measures would further reduce adult emergence numbers [see supporting information Table S3, (a)]. Individual variable ranking revealed that exposure to aerial *Bti* (spray) explained 60.0%, median larval presence explained 33.9%, trap water volume explained 5.6% and vegetation removal explained 3.0% of the relative deviance in adult emergence [Table 2, (c)]. Adult emergence was lowest in the traps that had the treatments of vegetation removal via localised burning and *Bti* exposure, vegetation removal via shears and *Bti* exposure and just *Bti* exposure than in the traps that had vegetation removal via shears but no *Bti* exposure. Emergence was highest in the traps that had vegetation removal via localised burning and the control traps (Fig. 4b).

In the model sets examining the effects of combined vector control methods on larval presence, there was no evidence that combining vegetation removal via shears and *Bti* exposure or vegetation removal via localised burning and *Bti* exposure was more effective at reducing larval presence than just *Bti* exposure alone [see supporting information Table S3, (b)].

4 DISCUSSION

Clear evidence was found that larval presence and numbers of emerging adult *Ae. vigilax* were reduced – by an average of 95% – after the aerial application of *Bti* larvicide, across a range of different vegetation types and water qualities. Applications of *Bti*-based products can be highly effective at reducing larval densities over a range of mosquito vectors including *Ae. vigilax*.[3,11,12,47] It has also been shown that other control methods based on vegetation removal, via either shears or localised burning, can reduce adult emergence in *Schoenoplectus* and *Eleocharis* habitats by around 50%. Uniquely, the authors compared several different forms of control for *Ae. vigilax*, separately and in combination (aerial larvicide application and environmental manipulation), and in so doing provided direct confirmation that larvicide application is the most effective control method (of those considered at these experimental scales) for reducing adult emergence of this species from a range of larval habitats. It has also been shown that the experimental larval sampling methods do not accurately measure larval abundance or adult emergence numbers. This is important for vector control, as larval surveys are commonly used to determine areas of high or low density for control procedures.[26,27]

4.1 Larvicide control of vectors

Monthly tidal inundation of breeding habitats during the dry season stimulates desiccation-resistant *Ae. vigilax* eggs to hatch, resulting in the new generation hatching, growing and emerging at the same time. Therefore, *Bti* application during the larval stage will ensure suppression of the entire generation, making the timely application of this method relatively cost effective for this species. By contrast, species that breed in semi-permanent or standing

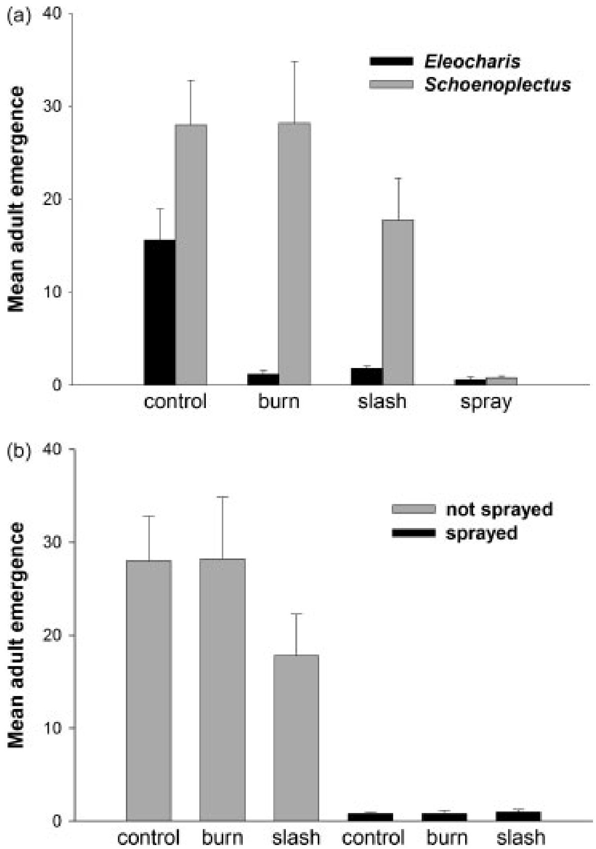

Figure 4. (a) Mean number of adult *Aedes vigilax* that emerged from traps in *Schoenoplectus litoralis* and *Eleocharis dulcis* habitats with standard error bars. Treatments applied to traps include: control – no manipulation; burn – vegetation burnt; slash – vegetation mowed; spray – trap exposed to aerial larvicide application. (b) Mean number of adult *Aedes vigilax* that emerged from traps in *Schoenoplectus litoralis* habitat with standard error bars. Treatments applied to traps include: control, burn, slash. Black columns indicate traps that were exposed to aerial larvicide application, and grey columns indicate traps that were shielded from aerial larvicide application.

water, and slowly build population density over longer periods rather than exhibiting monthly peaks in emergence, will require repeated applications of *Bti* over at least fortnightly intervals to control each generation, and will therefore be more expensive. *Culex annulirostris*, the mosquito species that dominates the LHJ swamp once it is inundated with fresh water during the wet season, exhibits this pattern of breeding.[34,37,48] This might be why studies of *Cx. annulirostris* population dynamics have revealed little to no long-term effects of opportunistic *Bti* larvicide application on this species.[34,37]

4.2 Environmental manipulation for vector control

Vegetation removal can lead to a decrease in larval numbers, presumably by increasing exposure to higher temperatures and removing protection from predators, wind and wave action. Removal can also facilitate control in areas where larvicide application or diffusion is impeded by thick, emergent vegetation.[14,22,23,49,50] It was found that the removal of *Eleocharis* or *Schoenoplectus* reeds (either via shears or localised burning) reduced emergence of *Ae. vigilax*, but was not as effective as aerial *Bti* application. One possible reason relates to the traps that were designed to exclude predators and thereby reduce the confounding effects of predation on larvicide-induced mortality. The traps might have provided some shade, wind and wave protection, thus negating the effects of vegetation removal. Another factor influencing the present results might be the timing of vegetation removal; the vegetation was removed in the experiments just prior to inundation and hence after egg deposition had occurred following a previous inundation. It is possible that oviposition, larval hatching and adult emergence would have been appreciably less in the burned or sheared plots if the vegetation removal had occurred prior to egg laying. This would have provided a less attractive egg-laying environment with more direct sunlight, and, by allowing the ground surface to dry much sooner, it might have more quickly become unsuitable for continued egg laying.[35] Further study involving traps that allow predator access to the larvae, or broad-scale (i.e. over hundreds of metres) treatment plots without treatment frames, would make it possible to explore more fully the benefits of vegetation removal as a larval control measure for this species. Broad-scale plots with unburnt areas and areas burnt prior to any egg deposition episodes would also allow aspects such as the time of burning, open water areas and wave action and predator effects to be examined in more detail. Larger-scale vegetation manipulation would likely restrict the distribution of larvae to marginal areas, such that the total area of breeding would be smaller and hence easier to survey and control.

Ultimately, the efficacy and long-term cost effectiveness of any control measure depends on how well the intervention is matched to the ecology of the species targeted. Environmental manipulation might be an effective vector control method in some cases, but this strategy can also negatively affect non-target species.[22] Also, incomplete control procedures that reduce larval densities rather than exterminating all larvae allow the survivors greater access to resources, with the corollary that they will emerge as larger adult mosquitoes potentially capable of surviving longer, dispersing farther and infecting more people.[51–53]

4.3 Larval sampling, larval presence and adult emergence across habitat types

The end goal of any vector control programme is the reduction or, if possible, elimination of the adult vector population, and therefore, by logical extension, the reduction in incidence of vector-transmitted pathogens and disease. An essential step that is often missed in examining the effectiveness of larval control methods, however, is quantifying the direct effects of larval control on adult emergence.[40] However, adult emergence trap results are not necessarily indicative of adult numbers caught in CO_2 traps because emergence cages remove the problems associated with partial or incomplete spraying in landscape control operations and adult dispersal from outside control areas.

The present experiment aimed to quantify larval abundance by counting all larvae captured in single traps, and showed that the experimental larval sampling (dipping) procedures do not adequately quantify larval abundance or adult emergence. At best, the sampling method only measures larval presence or absence, and there was little evidence for a correlation between larval abundances and adult emergence numbers. This is an important result because it suggests that the current practice of using larval surveys to determine where and when larval control should be applied might not quantify larval abundance or density (only presence or absence). The implication for mosquito control is that managers may instead opt to apply larvicide in all likely habitats and forego larval sampling altogether. Otherwise, habitat-specific models predicting larval vector population abundances would need to be developed for a wider suite of species and habitats.

It is important to have a thorough understanding of the ecological features of vector breeding sites to implement effective and efficient control within resources available, and certain larval habitat environmental conditions have been previously identified as important in determining larval habitat suitability. These include water salinity, pH, dissolved oxygen, ambient temperature, vegetation presence, habitat and type.[19–21,54–56] For *Ae. vigilax* it was found that, although there was some influence of different vegetation types and water qualities, no single environmental attribute was identified as the principal correlate of larval development or adult emergence. *Ae. vigilax* are able to breed effectively in a relatively wide range of temperatures and salinities,[35,57] and the maxima and minima of the microclimate environmental parameters measured fell well within these ranges. During the spring tides, the LHJ swamp acts as an ideal *Ae. vigilax* larva incubating environment; none of the water qualities affects larval survival, so generation times will be as rapid as developmental instar progression allows.

It is imperative that effective control be implemented in environments such as these, where human occupation is so close to large populations of insect vectors. The present results showed that, although there were some combined effects of local environmental conditions and vegetation removal (via shears or localised burning), aerial larvicide application remained the most effective control method for reducing adult vector emergence across all habitats. Previous studies have not identified an effect of different larval habitat environmental conditions on the effectiveness of larvicide at suppressing larval densities.[9,13] Vegetation type was not found to add some statistically useful explanatory capacity to models of larval presence; however, *Ae. vigilax* larval presence was highest in mixed mangrove forest edge and *Schoenoplectus* reed and pure *Schoenoplectus* reed habitats, and lowest in the *Eleocharis* reed habitat. This is further evidence that the tidally flooded *Schoenoplectus litoralis* reed beds of the coastal swamps, which are highly productive habitats for *Ae. vigilax*, are indeed an important target for control.[41]

5 CONCLUSIONS

The current control method of aerial larvicide application is effective at suppressing adult emergence of *Ae. vigilax* across a range of habitat types, and vegetation removal can also be an effective control alternative for this species in some habitats, but this conclusion should be investigated with larger-scale experiments. The trade-off in terms of cost, negative environmental consequences of environmental manipulation versus the risk of eventual resistance[58] and other longer-term negative effects of larvicide application remain unclear. However, this area warrants more investment, as comparative approaches such as this study are rarely done. An important knowledge gap in evaluating the effectiveness of larval vector control strategies has also been identified.

Quantification of the relationship between larval sampling measures, larval abundance and/or density and adult vector emergence is essential to determine whether control methods are indeed reducing the productivity of larval habitats. Larval sampling is routinely used to direct control efforts; therefore, extreme care needs to be taken when interpreting larval sampling surveys. Supplementing larval sampling surveys with models of the climatic, environmental and intrinsic factors that drive vector population dynamics to determine optimum control strategies can assist, with the corollary that good predictive models based on the ecology of local vector populations might eventually supplant expensive and time-consuming larval surveys.

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