Operational Use of Household Bleach to "Crash and Release" *Aedes aegypti* Prior to *Wolbachia*-Infected Mosquito Release

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ABSTRACT Dengue (family *Flaviviridae*, genus *Flavivirus*, DENV) remains the leading arboviral cause of mortality in the tropics. *Wolbachia pipientis* has been shown to interrupt DENV transmission and is presently being trialled as a biological control. However, deployment issues have arisen on methods to temporarily suppress wild mosquito populations before *Wolbachia*-infected mosquito releases. By suppressing wild populations, fewer *Ae. aegypti* releases are required to achieve a sustainable *Wolbachia* density threshold. Furthermore, public distress is reduced. This study tests the application of domestic bleach (4% NaClO) to temporarily "crash" immature *Aedes* populations in water-filled containers. Spray application NaClO (215 ppm) resulted in a mean 48-h mortality of 100, 100, 97, and 88% of eggs, second-instar larvae, fourth-instar larvae, and pupae, respectively. In the field, NaClO delayed ovipositing by 9 d in cooler months, and 11 d in hotter months, after which oviposition resumed in treated receptacles. We found bleach treatment of pot-plant bases did not cause wilting, yellowing, or dropping of leaves in two ornamental plants species. Domestically available NaClO could be adopted for a "crash and release" strategy to temporarily suppress wild populations of *Ae. aegypti* in containers before release of *Wolbachia*-infected mosquitoes. The "crash and release" strategy is also applicable to other mosquito species, e.g., *Aedes albopictus* (Skuse), in strategies using released mosquitoes.

KEY WORDS *Aedes aegypti*, dengue, *Wolbachia*, mosquito control, arbovirus

Dengue (family *Flaviridae*, genus *Flavivirus*, DENV) has emerged as the leading cause of arboviral morbidity in the tropics in the past few decades (World Health Organization 2009). In 2001–2002, a large epidemic of DENV 3 in Brazil caused over 750,000 cases. The primary vector of dengue, *Aedes aegypti* (L.), feeds almost exclusively on humans (Scott et al. 1993) and as such, has a close association with urban settings, using water-filled artificial containers such as tires, buckets, birdbaths, and pot-plant bases as larval habitat (Barker-Hudson et al. 1988, Montgomery and Ritchie 2002, Williams et al. 2008). *Aedes aegypti* is present in Australia in far north Queensland (FNQ) and DENV is initiated by imported viremic travelers (Hanna et al. 2001, Vazquez-Prokopec et al. 2010). Since the 1980s, outbreaks in FNQ have occurred in most years (Ritchie et al. 2002), and these have increased in number and magnitude recently (Vazquez-Prokopec et al. 2010). To address the increased risk of dengue outbreaks, *Wolbachia pipientis* is currently being trialled as a biological control against dengue in FNQ (Hoffmann et al. 2011). If successful, this will mark the beginning of a long-term dengue control era for endemic and seasonally introduced areas.

Wolbachia-infected *Ae. aegypti*, when interbred with wild *Ae. aegypti* populations, produce predominantly *Wolbachia*-infected offspring (Hancock et al. 2011a) (Rasgon 2011). To maximize *Wolbachia* transmission and fixation, *Wolbachia*-infected mosquitoes are released at repeated time intervals, representing pulse immigration (Hancock et al. 2011b). Smaller introductions are required during periods of low population density, and larger releases are required when wild population densities are high (Hancock et al. 2011b).

One strategy to increase the likelihood of successful *Wolbachia* establishment and reduce the required numbers of mosquitoes released, is to suppress local *Ae. aegypti* populations before release of *Wolbachia*-infected mosquitoes. This strategy of "crash and release" would additionally reduce total *Ae. aegypti* numbers, and thus reduce mosquito biting to the public. Control should include the largest potential source of uninfected individuals, eggs, and larvae within flooded containers; so it is crucial that it kill these stages without residual effects that would impact oviposition and larval development by released *Wolba-*

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chia-infected mosquitoes. Thus, efficacy must be temporary, with known duration. Simulations of *Ae. aegypti* populations using a version of CIMSiM validated for Cairns (Williams et al. 2008) suggest that 97.9% of *Ae. aegypti* population consisted of immatures in containers, with 47 and 48% as eggs and larvae, respectively.

Sodium hypochlorite (domestic bleach) can be used to control *Ae. aegypti* in water-holding containers (Lamache and Whelan 2002). A 5–10% concentration of sodium hypochlorite (NaClO) is recommended by the Australian Quarantine Inspection Service to eliminate all life stages of *Aedes* mosquitoes in water-holding containers (Lamache and Whelan 2002). At this concentration, it rapidly kills 100% of aquatic stages of mosquitoes, although ensuring that receptacles remains nontoxic to humans or animals when later refilled. Lower concentrations of NaClO (1 or 5%) have demonstrated lethality in 100% of *Ae. aegypti* eggs, larvae, and pupae when exposed under laboratory conditions (Ritchie 2001). However, a more recent study challenged 1% NaClO concentration in its ability to adequately kill 100% mosquito eggs (Shortus and Whelan 2006), and Barrera et al. 2004, found higher concentrations of NaClO were required to kill mosquito immature life stages when organic matter was present.

This paper combines four separate but linked studies to test if an operational application of commercial household bleach (4% NaClO) can be applied to as a "crash and release" strategy to reduce wild *Ae. aegypti* populations in containers without compromising oviposition and production of released *Ae. aegypti* in the field. First, under laboratory conditions, we sought to define if 4% NaClO was lethal to eggs, larvae, and pupae of container-breeding mosquitoes, alone and in the presence of organic matter (for deployment under standard field conditions). Second, under laboratory conditions, we sought to investigate any deleterious effects on common indoor plants after NaClO application to pot-plant bases, a key container for *Ae. aegypti* in north Queensland (NQ) (Williams et al. 2008). Lastly, in two field trials, we sought to establish the duration of oviposition repellency and larvicidal effect of NaClO application to water receptacles, to establish the length of time until treated containers are once again suitable for oviposition in outdoor residential areas during hot and cool season in FNQ.

Materials and Methods

Setting. Cairns, situated in the tropical region of northeastern Australia, 16° 57′ S, 145° 45′ E, has characteristic hot, humid summers averaging 31°C (December), with milder, dry winters averaging 26°C (June) (Australian Bureau of Meteorology 2011).

Laboratory Study 1: Household Bleach Effects on *A. aegypti* Eggs, Larvae, and Pupae. We tested the lethality of household bleach, (White King; Pental, Australia) 4% NaClO versus controls, on *Ae. aegypti* eggs and immature stages. Mosquito eggs, larvae, and pupae, obtained from Cairns *Ae. aegypti*, were placed in 15- by 9-liter buckets filled with 7.5 liters of H_2O. In each bucket one flannel cotton egg strip (20 plus eggs) was suspended above the water line, and 20 × second instars, 20 × fourth instars, and 20 × pupae were placed in the water. In addition, to simulate organic leaf litter (food source), 10 × dead longan (*Dimocarpus longan* Lour.) leaflets were added to six of the 12 treatment buckets. Using a Rega 5-liter pressurized sprayer, 12 buckets were sprayed with 4% NaClO, and three with H_2O (controls), until saturated, repeated once (standard deployment). Total application averaged 40 ml/bucket, creating a solution of ≈215 ppm each bucket. After treatment, egg strips were removed at 24 h and flooded in dilute yeast solution to induce hatch, then unhatched viable eggs were examined and counted. Larvae and pupae were examined and counted at 24 and 48 h.

Statistical Analysis: Laboratory Study 1. T-tests were used to compare egg count hatch proportions between treatment groups (4% NaClO versus control), and between treatment groups in the absence of the controls. The number of larvae and pupae were set at $n = 20$ each, so mortality were tested as a proportion. We compare differences across *Aedes* life stages and between treatments using binomial generalized linear models (GLM) with logit link. All analyses were performed using STATA version 11.0 (Stata Corp., College Station, TX).

Laboratory Study 2: Household Bleach Effects on Common Indoor Plants. Pot-plant bases are small saucers placed under potted plants to capture water overflow. They are one of the most common larval habitats of *Ae. aegypti* in NQ, Australia (Barker-Hudson et al. 1988) as well as in Bangkok, Thailand (Southwood et al. 1972). We investigated the impact of NaClO application to flooded pot-plant bases on two common ornamental plant species, *Spathiphyllum* sp. and *Whitfieldia longifolia* T.Anderson. Twenty individual plants were potted in 22-cm-diameter, 18-cm-high plastic pots with 30-cm-wide plastic pot-plant bases that were flooded with tap water. For each plant species, five plant bases were not sprayed (controls) and five were treated by spraying the exposed water of the pot plant bases with 20 ml of 4% NaClO by using a multipurpose hand held sprayer. Owing to the small relative volume of water held within the base (water volume ≈0.16 liters), this concentrated the dose to ≈4,800 ppm, 20 times the lethal treatment dose applied in study 1. Leaves of each plant were examined, counted, and inspected for signs of stress such wilting or yellowing of leaves, on days 0, 3, 7, 14, 21, and 28 posttreatment.

Statistical Analysis: Laboratory Study 2. Counts of leaves were compared between treatment groups (treatment versus controls) by using a cross-sectional random effects Poisson regression model to control for time (day_n), plant species, and grouping structure (individual plants). In this way, each plant was tested against itself in the model. Leaf counts were compared for each plant species separately.

Field Studies 1 and 2: Household Bleach Effects on *Aedes* Oviposition and Pupation. We examine the duration of effect of 4% NaClO, on *Aedes* oviposition,

Table 1. Mean (95% CI), 48-h mortality of *Ae. aegypti* in 9-liter buckets treated with commercial bleach (40 ml 4% NaClO added to 7.5-liters water for final solution 215 ppm)

	Mean percent mortality (95% CI)			
	Eggs	Second-instar larvae	Fourth-instar larvae	Pupae
Control	0.08 (−0.11–0.28)[a]	0.0 (0–0)	1.7 (−5.5–8.8)	0.0 (0–0)
NaClO (4%)	100.0 (100.0–100.0)	100.0 (100.0–100.0)	96.7 (88.1–105.2)	87.5 (74.3–100.7)
NaClO (4%) + longan leaflets	100.0 (100.0–100.0)	90.8 (67.3–114.4)	78.3 (46.0–110.6)	69.2 (20.6–117.8)

[a] 92% hatched.

when deployed under standard shaded field conditions in residential Cairns. Duplicates of buckets (two × treatment and two × control) were each set at five Cairns residences during winter (12 May–1 June 2010; field study 1) and again in late spring (4 October–1 November 2010; field study 2). Red flannel strips (25 by 30 cm) were placed in each bucket; these were examined for oviposition cumulatively (eggs were not disturbed) and bucket water was examined for larvae and pupae, every 2–3 d. Leaf litter was not set, but allowed to accumulate if it naturally occurred. All detected specimens of larvae and pupae were moved to the laboratory in 70-ml plastic sample jars with sticky panel lid to capture emerging adults that were identified to species level. NAClO levels were not measured throughout the study, as mosquito emergence acted as a surrogate for viable levels. Average maximum daily temperatures and total daily rainfall were provided by the Australian Bureau of Meteorology (BOM) station at Cairns International Airport located 2 km north of the field sites.

Statistical Analysis: Field Studies 1 and 2. Outcome data (eggs and pupae) were counts, so a cross-sectional random effects Poisson regression model was chosen that would control for repeated measure (time [day_n]), bucket location, duplicate buckets, and grouping structure (individual buckets). Models first were performed on all data (single analysis with repeated measures), and then individually when separated by day, to determine the duration effect of NaClO treatment. Incidence rates ratios (IRR) indicate the strength of association, by treatment choice.

Results

Laboratory Study 1: Household Bleach Effects on *Aedes* Eggs, Larvae, and Pupae. Sodium hypochlorite applied by a Rega pump sprayer to a partially flooded bucket killed almost all *Ae. aegypti* eggs, larvae, and pupae (Table 1). Almost all (92%) of untreated (control) *Ae. aegypti* eggs hatched (Table 1) (*t*-test, $P < 0.01$). All immature stages (second- and fourth-instar larvae and pupae) had higher average mortality rates at 48 h across treatment groups (NaClO treatment ± leaflets), and treatment was equally effective across life stages. There were no statistically significant differences in effect between life stages (binomial generalized linear model [GLM], $P = 0.344$, data not shown), but there was a significant difference between treatment groups compared with untreated controls (binomial GLM, $P < 0.01$, data not shown). When comparing between treatment groups (NaClO [4%] ± leaflets) in the absence of the untreated (controls), there was no difference in mortality between second-instar larvae, fourth-instar larvae, or pupae proportions at 48 h posttreatment (*t*-tests: $P = 0.34$, $P = 0.19$, $P = 0.37$, respectively).

Laboratory Study 2: Impact of Bleach Treatment of Pot-Plant Bases on Ornamental Plants. Treatment of pot-plant bases with bleach induced no significant yellowing of leaves or wilt for the two plant species. There was no significant leaf loss for *Spathiphyllum* sp. and no significant difference of leaf counts in treated or control plants throughout the study (Table 2, model 1). For *W. longifolia*, again there was no statistically significant leaf loss, and no difference between individual plants in the study (Table 2, model 2). There was a slight decline in *W. longifolia* leaf number but this occurred in parallel with the control plants, indicating extrinsic factors of association rather than NaClO effect; furthermore, this decline failed to reach statistical significance (Table 2, model 2; Fig. 1a and b).

Field Study (1), Household Bleach Effects on *Aedes* Oviposition and Pupation. Mosquitoes failed to oviposit in exposed buckets (treated and controls) at two residential locations during the study period; these were removed from the analysis, leaving 3 properties and 6 pairs of buckets. For the remaining study sites, oviposition in bleach treated buckets was significantly lower than in controls ($P < 0.01$) (Table 3, model 1). The incidence of eggs increased by a rate ratio of 1:1 for each day of inspection. Location and bucket pair were not statistically significant (Table 3, model 1). There were two eggs laid in one treated bucket on day 9; after this point in time, there was no longer a statistical difference between treated versus control

Table 2. Poisson regression results for NaClO (4%) effects on counts of *Spathiphyllum* sp. and *W. longifolia* leaves (4,800 ppm)

Model 1: *Spathiphyllum* sp.				
Counts of leaves≈	IRR	P	95% CI	
Treatment	0.93	0.74	0.60	1.43
Individual plant variation	1.00	0.63	1.00	1.00
Model 2: *W. longifolia*				
Counts of leaves≈	IRR	P	95% CI	
Treatment	0.86	0.15	0.69	1.06
Individual plant variation	1.00	0.02	1.00	1.00

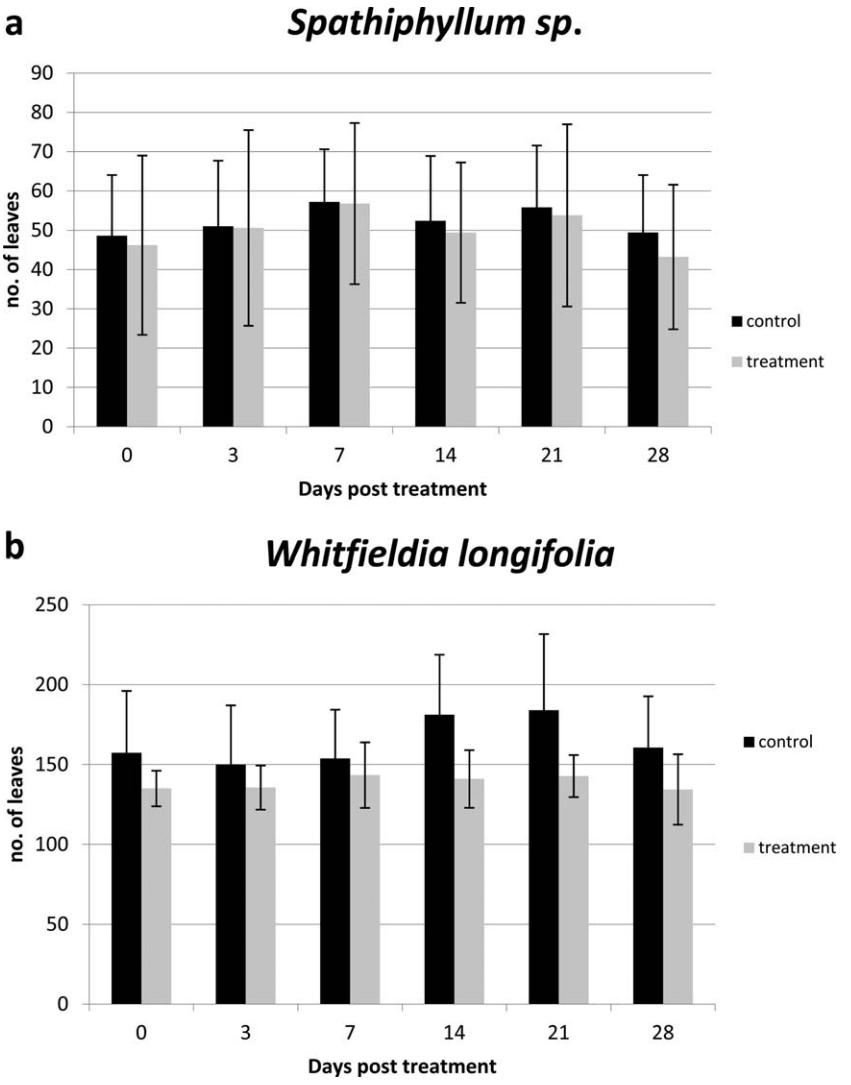

Fig. 1. a and b. Average number of leaves, 95% CI for (a) *Spathiphyllum* sp. and (b) *Whitfieldia longifolia.*

buckets for *Aedes* oviposition, even when controlling for location (IRR = 0.01, $P = 0.16$, 95% confidence interval [CI]: 0.00–5.2; Fig. 2a).

Table 3. NaClO (4%) effects on counts of *Ae. aegypti* eggs and pupae, May (dry season)

Model 1: eggs				
Counts of eggs≈	IRR	P	95% CI	
Treatment	0.26	<0.01	0.10	0.65
Pair	0.89	0.81	0.35	2.25
Residential location	0.88	0.66	0.50	1.55
Day	1.10	<0.01	1.10	1.11
Model 2: pupae				
Counts of pupae≈	IRR	P	95% CI	
Treatment	0.14	<0.01	0.06	0.36
Pair	1.22	0.68	0.49	3.04
Location	0.71	0.22	0.40	1.23
Day	1.08	<0.01	1.07	1.09

Results of bleach treatment on *Aedes* pupation were similar to that of oviposition. Pupae were not detected in treatment buckets until day 21 versus day 12 for the controls ($P < 0.01$, Table 3, model 2; Fig. 2b). The incidence of pupae increased by IRR 1.08 for each day of inspection (Table 3, model 2), after day 21, pupae developed in treated buckets at the same rate as controls (IRR = 0.13, $P = 0.26$, 95% CI: 0.00–4.31; Fig. 2b). Adult emergence revealed that most species were *Aedes*, 26% were *Ae. aegypti*, and 69% were *Aedes palmarum* (Edwards) (Table 4). Weather data provided by the BOM, indicated that mean maximum daily temperatures were 28.15°C, with 16 mm of rainfall at Cairns Airport throughout field study 1.

Results: October, Field Study (2), Household Bleach Effects on *Aedes* Oviposition and Pupation. One residential location remained free of *Aedes* oviposition during the study period, so this study site was excluded from the analysis. The number of residential

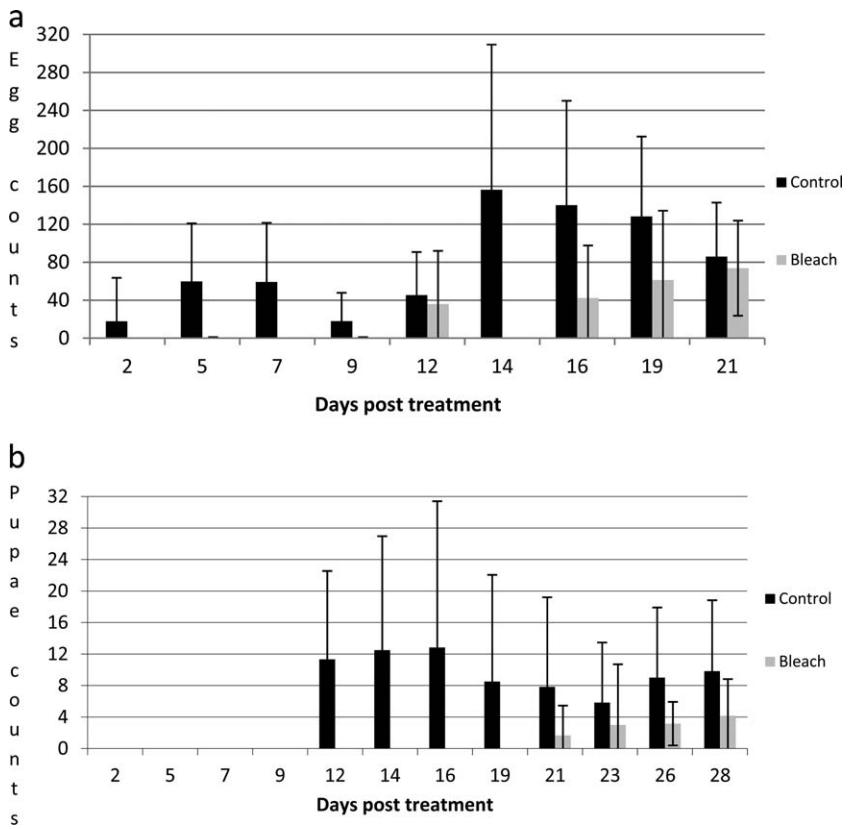

Fig. 2. a and b. May field study (1), undiluted household bleach (4% NaClO) effects on (a) *Aedes* oviposition and (b) pupation, with 95% CI.

locations included in the analysis was four, with a total of eight bucket pairs. There was no statistically significant difference in egg counts between treatment (4% NaClO) and control buckets in the October trial overall (all days combined), ($P = 0.44$; Table 5, model 3). However, when modeled by day, there were significantly more eggs laid in control buckets compared with treated bucket strips on days 7 (IRR = 0.07, $P < 0.01$, 95% CI: 0.02–0.25) and 9 (IRR = 0.10, $P < 0.01$, 95% CI: 0.03–0.35), respectively (data not shown), after which, there was no difference in oviposition between treatment groups.

As with oviposition, there was no statistically significant difference between pupae numbers in treatment (4% NaClO) versus control buckets when controlling for residential location, bucket pair, and day ($P = 0.13$; Table 5, model 4). When modeled by day, there was a difference between versus control pupae development in buckets on day 16 (IRR = 0.04, $P = 0.02$, 95% CI: 0.00–0.55, data not shown). After this day, there was no difference between pupae development in treated buckets compared with controls, until day 23, after which treated buckets produced more pupae than controls, peaking on day 28 at over 12 times the number of pupae compared with the control (IRR = 12.05, $P < 0.01$, 95% CI: 2.45–59.08) (data not shown).

Adult emergence revealed pupae consisted of predominantly *Aedes;* 10% were *Ae. aegypti* and 68% *Ae. palmarum* when controls and bleach treated buckets

Table 4. Identification of pupae collected from buckets treated with 4% NaClO versus untreated control, n (%), May field study

Species	*Ae. aegypti*	*Ae. palmarum*	*Ae. notoscriptus*	*Cx. quinguefasciatus*
Control	110 (36.7%)	187 (62.3%)	3 (1.0%)	0
Bleach	10 (6.4%)	126 (80.3%)	12 (7.6%)	9 (5.7%)

Table 5. NaClO (4%) effects on counts of *Ae. aegypti* eggs and pupae, October (buildup to wet season)

Model 3: eggs				
Counts of eggs≈	IRR	P	95% CI	
Treatment	0.89	0.44	0.67	1.19
Pair	0.73	0.03	0.55	0.97
Residential location	0.84	0.00	0.76	0.92
Day	1.07	0.00	1.07	1.07
Model 4: pupae				
Counts of pupae≈	IRR	P	95% CI	
Treatment	1.68	0.13	0.86	3.27
Pair	0.49	0.03	0.25	0.95
Residential location	0.86	0.20	0.68	1.08
Day	1.12	0.00	1.10	1.13

Table 6. Identification of pupae collected from buckets treated with 4% NaClO versus untreated control, n (%), October field study

Species	*Ae. aegypti*	*Ae. palmarum*	*Ae. notoscriptus*	*Cx. pullus*	*Cx. quinquefasciatus*
Control	51 (32.9%)	94 (60.6%)	0	1 (0.6%)	9 (5.8%)
Bleach	0	330 (99.7%)	1 (0.3%)	0	0

were combined (Table 6). More adults emerged from bleached buckets in total, but only after day 21 (Fig. 3b). BOM data indicated that mean maximum daily temperatures were 29.8°C, with 137 mm of rainfall at Cairns airport during field study 2 (Australian Bureau of Meteorology 2011).

Discussion

Combined laboratory and field study results indicate that domestic bleach (4% NaClO) can be used to rapidly "crash" immature populations of *Ae. aegypti* in artificial containers. When applied to flooded containers to create a concentration of 215 ppm, NaClO kills eggs, larvae, and pupae. It also inhibits *Aedes* oviposition and viability of larvae and pupae for approximately 2 wk during the cooler months of the year, and 1 wk during hotter months. This finding supports the denaturing quality of NaClO as demonstrated when exposed to heat, ultraviolet light, contaminants, or all three, especially when ambient heat increases over 21°C (Odyssey Manufacturing Company 2007). When used for mosquito control, NaClO treatment of flooded pot-plant bases did not cause leaf loss, yellowing, or wilting in two ornamental domestic plant species. Receptacles treated with NaClO for mosquito control showed no residual effects, allowing subsequent *Aedes* oviposition and pupal production to return to normal levels once NaClO was denatured. Furthermore, after ovicidal application of NaClO, receptacles are safe for potable water use and animal drinking, including pet water bowls and bird baths (Ritchie 2001, Lamache and Whelan 2002).

When applied around gardens and houses, routine deployment of NaClO would reduce the number of eggs, larvae, and pupae of container-breeding mosquitoes to effectively "crash" the wild *Aedes* population before the "release" of *Wolbachia* infected *Ae. aegypti.* Reduction of the native uninfected *Ae. aegypti* population should enhance the spread of *Wolbachia*

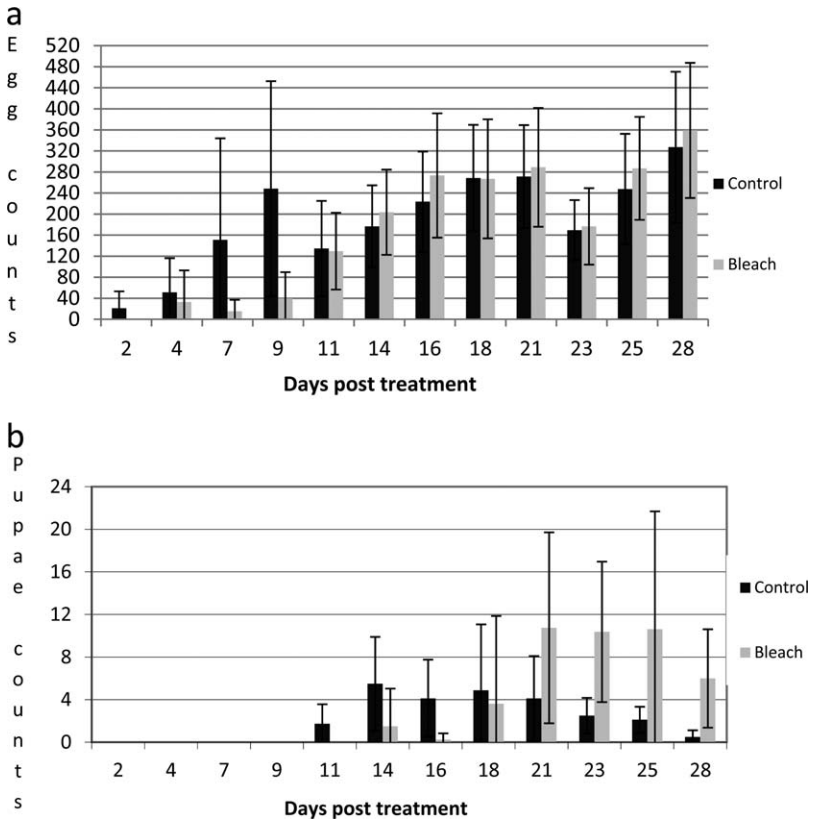

Fig. 3. a and b. October field study (2), undiluted household bleach (4% NaClO) effects on (a) *Aedes* oviposition and (b) pupation, with 95% CI.

for a fixed release number (Hancock et al. 2011b). Fewer releases of laboratory reared mosquitoes will cost less, and be more favorably received by the public, who risk receiving fewer mosquito bites if the population density is lower (Hancock et al. 2011b). Indeed, in 2012 releases of the *w*Melpop strain of *Wolbachia* in Cairns, commercial bleach was used to treat a large sump pit and a flooded boat to kill large numbers of larvae and potential eggs (S. Ritchie 2012, personal communications).

Barrera et al. trialled household bleach with reapplication to control *Ae. aegypti* populations long-term, and found varying strengths were required to kill different life stages of *Ae. aegypti* (Barrera et al. 2004). Lethal concentrations of NaClO in the presence of organic matter were 16 ppm for first instars, 64 ppm for second instars, and 250 ppm for third and fourth instars, and a single NaClO treatment of 250 ppm (poured using two tablespoons per 5 liters of water) killed all larvae for 12–17 d (Barrera et al. 2004). In our study, we investigated 215 ppm NaClO spray application (as standard deployment method) to determine the duration of effect, under wet and dry season conditions, until receptacles were then receptive to *Wolbachia* infected mosquitoes for oviposit. We found 215 ppm controlled all life stages for less than 2 wk, which is shorter than Barrera et al., but better suited for temporary population control (Barrera et al. 2004).

Commercial bleach can also be used for household-initiated mosquito control. This can dramatically minimize community exposure to potentially infected mosquitoes, although again reducing the workload of mosquito eradication teams in critically understaffed outbreak situations (Gubler 1989, Gubler and Clark 1994). Household-level source reduction is one step toward the integration of community-based strategies for *Ae. aegypti* control with the public (Sherman et al. 1998). In the event of a dengue outbreak, domestic bleach can be purchased and applied at the recommended rate without specialist licensing or supervision. Furthermore, readily available household products are perceived as more acceptable to householders than biological larvicides (Fernandez et al. 1998). Field experiments in Honduras indicate familiarity with products, such as household bleach and detergent. They also ensure increased use, with higher confidence when subsequently applied for mosquito control (Fernandez et al. 1998, Sherman et al. 1998).

This study did not address the capacity of NaClO when applied at sublethal levels to indirectly control mosquito immatures through the destruction of microorganisms that provide nutrients to developing larvae (Merritt et al. 1992, Barrera et al. 2004). In addition, this study did not incorporate the addition of detergent with bleach, and the potential deleterious effects of the combined product on pot plants. Findings from others suggest that when detergent is added, lethality is increased but oviposition recommenced after 11.1 d (standard deviation (SD) 5.8) (Sherman et al. 1998), which was similar to findings from this study. The addition of laundry detergent to NaClO was discovered to greatly improve the lethality of 5.25% NaClO when applied at concentration 5:1 (Sherman et al. 1998). Detergent is inherently lethal to mosquito larvae independent of NaClO, and combined with undiluted 10% NaClO is the current recommendation for exotic mosquito eradication on international shipping vessels arriving in the Northern Territory of Australia (Shortus and Whelan 2006). However, detergent could deter oviposition longer than bleach alone, and could negatively impact the success of released mosquitoes. One final limitation to this study was the investigation of longer-term effects of repeated application of bleach on common pot plants may require further research.

In conclusion, the use of domestic bleach at an application rate of 215 ppm has been demonstrated to effectively inhibit container-breeding mosquitoes without residual effect. In the field, oviposition resumed within 2 wk of treatment as NaClO was denatured, as quantified by successful oviposition. Furthermore, no deleterious effects were observed on common outdoor pot plants. However, commercial bleach should not be used in fishponds and aquariums containing fish because of potential toxicity to fish (Barrera et al. 2004). These findings enable NaClO to be incorporated into a "crash and release" strategy for a larger scale internationally supported *Wolbachia* integration programs.

Acknowledgments

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