Spring 2009

Bromeliads as a Breeding Site for the Dengue Vector Aedes aegypti

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SIT Study Abroad

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Submitted in partial fulfillment of the requirements for Australia:
Natural and Cultural Ecology, SIT Study Abroad, Spring 2009

ABSTRACT

Dengue Fever is a major public health concern in tropical and subtropical climates worldwide, including the city of Cairns, Australia, which is currently suffering a severe outbreak. The most important vector of the Dengue virus is the predominantly urban mosquito *Aedes aegypti* (L.), which lays its eggs in both artificial and natural containers, including the ornamental bromeliad plants found in many household gardens. The ability of larvae to develop to adulthood inside bromeliads has become controversial, however, and bromeliad enthusiasts frequently refuse to have their plants treated with insecticide. The aim of this study was to determine the conditions under which bromeliads can provide a suitable breeding site for *Ae. aegypti*. A total of 110 larvae were implanted in seven bromeliads and four artificial container controls, and rates of larval mortality and successful adult emergence were compared. Adult mosquitoes emerged from four out of seven bromeliads, and although larval death rates were high overall, there was no significant difference in productivity or mortality between bromeliad and control populations. These results have important implications for the management of *Ae. aegypti* breeding sites, which is currently the most promising method for curtailing the spread of Dengue Fever.

Key words: *Aedes aegypti*, breeding, bromeliads, Dengue, larvae.

TABLE OF CONTENTS

INDEX OF FIGURES AND TABLES

1. INTRODUCTION

1.1 Dengue Fever

Dengue Fever is a mosquito-borne infection found in tropical and sub-tropical climates that in recent decades has become a major international public health concern. It causes a severe flu-like illness, and sometimes a complication called Dengue Haemorrhagic Fever, which has fatality rates exceeding 20% without proper medical treatment. Global incidence of Dengue has grown dramatically with tropical urbanization, and 2.5 billion people, or approximately two fifths of the world's population, are at risk. The World Health Organization currently estimates there may be 50 million Dengue infections worldwide every year (Dengue and dengue haemorrhagic fever [updated 2009]).

The Dengue virus is not endemic in Australia, but large outbreaks can result when international visitors introduce it, which historically has occurred at regular intervals (Ritchie and Van Den Hurk 1994). Cairns, Australia is currently experiencing a severe outbreak of Dengue Fever. There have been 865 documented cases in Cairns since December 1, 2008, with subsequent outbreaks in Townsville, Port Douglas, Yarrabah, Injinoo, Innisfail and Mareeba (Outbreak Update [updated 2009]). This outbreak also marks the first time in history that all four serotypes of the Dengue virus have been active in the far north simultaneously, increasing the probability of contracting Dengue Haemorrhagic Fever. There is no vaccine or specific treatment for Dengue, so the only way to prevent transmission of the virus is to combat its mosquito vectors (Dengue and dengue haemorrhagic fever [updated 2009]).

1.2 Biology and breeding habits of Aedes aegypti

The most important vector of the Dengue virus worldwide is the predominantly urban species *Aedes aegypti* (L.) (Diptera: Culicidae) (Dengue and dengue haemorrhagic fever [updated 2009]). *Ae. aegypti* is currently the only vector of Dengue fever present in Australia, and within Australia it only occurs in Queensland. Originally a tree-hole breeder in Africa, *Ae. aegypti* spread overseas through colonization and has since become superbly adapted to the urban environment. Most *Ae. aegypti* females oviposit, or lay eggs, within 90m of their origin (Canyon 2001), which is a short range compared to most other species of mosquito and means that their habitats are relatively localized (Bentley & Day 1989). The need for such close proximity to both human food sources and breeding sites causes them to breed exclusively in residential areas. As in Africa, they continue to oviposit in natural containers, such as palm fronds, tree holes and bromeliads, but their preferences have expanded to include artificial containers, such as water tanks, plant pot bases and discarded tires (Ritchie and Van den Hurk, 1994).

Like other members of the mosquito tribe Aedini, *Aedes aegypti* have desiccation-resistant eggs, which can remain viable for months or even years in the absence of free water (Clements 1992). *Ae. aegypti* seek out water-filled containers and oviposit just above the water line, where their eggs will lie dormant until the container is flooded by rainfall or watering and the resulting decline in dissolved oxygen concentration stimulates them to hatch (Clements 1992). The immature mosquitoes then progress through four larval stages, or instars, which are each separated by the shedding of an exoskeleton. During this time they feed on coarse particulate organic matter such as leaves, filaments of macroalgae, and dead invertebrates, often of their own kind (Merritt et. al. 1992). After the 4^{th} instar they enter a non-feeding, metamorphic pupal stage that lasts for two to three days (Ritchie and Van Den Hurk 1994), and then the adult emerges, rests on the water surface for a short period to allow its cuticle to harden, and flies away. Mosquito development is a temperature dependant process, generally restricted to 14-30°C (Clements 1992), and in the tropics, the complete progression from egg to adult can occur over the course of 7 to 13 days (Ritchie and Van Den Hurk 1994). Figure 1 illustrates the developmental stages of a mosquito.

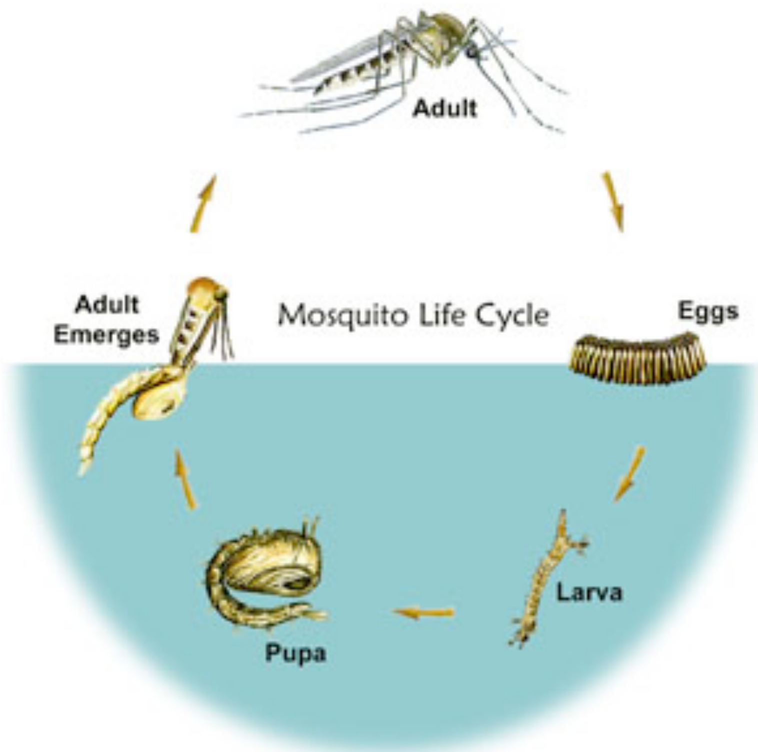

Figure 1: Developmental stages of a mosquito. Image courtesy of Omega Pest Control (http://www.omegapestcontrol.com/images/moslifecycle.jpg)

1.3 Aedes aegypti and bromeliads

Bromeliads are a popular ornamental flowering plant native mainly to the tropical Americas. The family Bromeliaceae contains over 2500 species (Frank 1990), and is divided into three subfamilies. Members of the subfamily Bromelioideae are also commonly known as "tank" bromeliads, because they possess an inferior ovary surrounded by tightly overlapping central leaves that collect rainwater. Both the central well and the four to six additional cavities formed by the outer axial leaves can hold sufficient water for mosquito larvae to complete development (Ritchie and Broadsmith 1997) (see Figure 2).

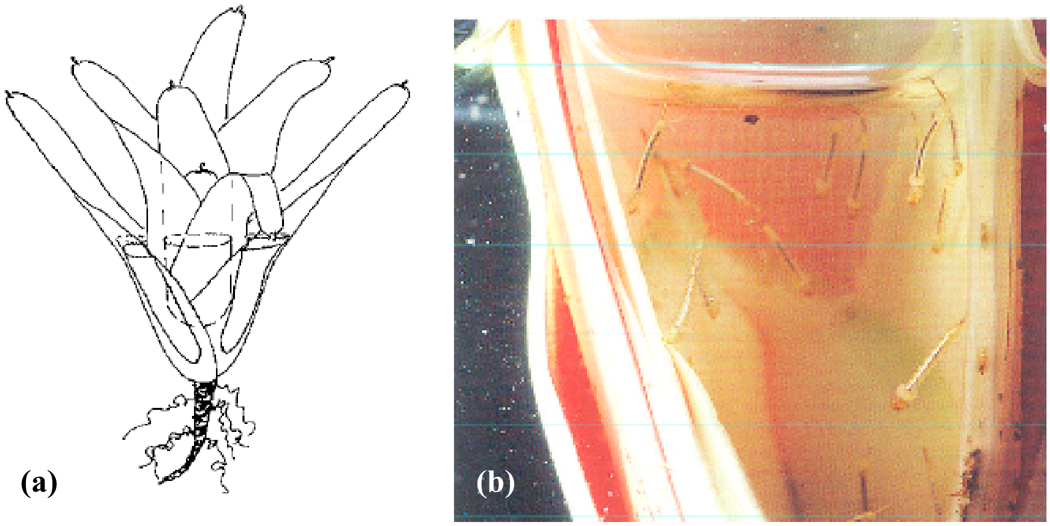

Figure 2: Bromeliads as a larval habitat. *a.* Schematic drawing showing water-impounding capacity of *Billbergia pyramidalis* (from Frank et. al. 1988) *b.* Section of the water filled tank formed by leaves of *Billbergia pyramidalis* containing *Wyeomyia* mosquito larvae (from Frank 1990)

Aedes aegypti commonly oviposit in tank bromeliads (cited in Ritchie and Broadsmith 1997): their eggs, 1st-4th instar larvae, and pupae have all been found inside bromeliads in studies conducted in Florida (Frank et. al. 1988, O'Meara et. al. 1995) and El Salvador (Varajão et. al. 2005). Here in Queensland, Tropical Population Health Services Dengue Action Response Team and James Cook University Mosquito Research staff have frequently observed larvae inside bromeliads in nurseries and domestic gardens (Johnson and Ritchie, unpublished data). During Dengue outbreaks there is no longer time to inspect individual plants, so the official policy is to treat all bromeliads, either with a residual insecticidal surface spray or slow release pellets of S-Methoprene, an artificial juvenile growth hormone that prevents larvae from progressing through the developmental stages.

1.4 Public health controversy

As a result of recent Dengue outbreaks, *Aedes aegypti* breeding in bromeliads has become a subject of some controversy. Although the policy of spray teams is to treat all bromeliads, property owners maintain the right of refusal (under the Queensland Public Health Act of 2005, Part 4: Authorised prevention and control programs). Influenced by articles in the popular press that suggest without scientific grounds that *Ae. aegypti* are incapable of breeding in bromeliads for biological reasons (see Figure 3), many bromeliad enthusiasts are refusing to have their plants treated. Discussion of the aforementioned studies by Frank and O'Meara has done little to sway the noncompliant, as that work was not based in Cairns (Long, personal communication).

THE ETERNAL DENGUE QUESTION:
Do bromeliads produce an enzyme that sterilises or digests mosquito larvae?

There is one scientific fact that bromeliads (related to pineapples), produce an enzyme called bromelain. Bromelain has undergone numerous trials to establish the claim that mozzie larvae will not survive in the water tank of a bromeliad plant. There is no apparent written evidence that this is the case, but trials suggest that it does work naturally.

Figure 3: Excerpt from an article by landscape designer and horticulturist Kim Morris in the Cairns Weekend Post (28 February 2009), implying that the proteolytic enzyme bromelain has sufficient natural insecticidal properties to prevent mosquito breeding.

A single bromeliad does not pose a significant threat, but large stocks of these plants in nurseries or the gardens of enthusiasts may cause serious problems (Ritchie and Broadsmith 1997), and any property containing a large number of untreated bromeliads puts both its owners and their neighbors at an increased health risk. Additionally, because the breeding habits of mosquitoes can change over time (Ritchie and Van Den Hurk 1994), the control of artificial containers to the neglect of natural ones may simply cause a shift in breeding site preferences, without significantly impacting the total mosquito population. If it can be conclusively proven that competent *Aedes aegypti* adults can emerge from bromeliads here in Cairns, then improved regulation of these plants could help curtail the local Dengue epidemic.

1.5 Study aims and objectives

The aim of this study is to determine the conditions under which popular ornamental bromeliads can provide a suitable breeding site for the Dengue vector *Aedes aegypti*. The specific objectives that comprise this aim are:

- To monitor environmental conditions within bromeliads in a natural setting
- To determine whether or not implanted larvae can progress through developmental stages inside of a bromeliad
- To determine whether or not competent adult mosquitoes can emerge from a bromeliad
- To compare rates of immature death and adult emergence between populations of mosquitoes maintained in bromeliads and control populations maintained in artificial containers

The larger goal of this study is to contribute to the body of knowledge surrounding *Aedes aegypti* breeding sites, the elimination of which is currently the most promising method for curtailing the spread of Dengue Fever.

2. METHODS

2.1 Study organisms

2.1.1 Aedes aegypti

This study utilized mosquitoes from the Sydney strain, an established colony strain originally collected in Townsville that has been maintained at the university laboratory in Sydney for approximately ten years. Larvae were reared in the laboratory at Tropical Population Health Services in Cairns. Dried eggs were placed in 1L of water in which a pencil eraser of yeast (approximately 0.05g) had been dissolved to stimulate hatching. Clean water was gradually added to the yeast water to convert the larvae from an anaerobic to an aerobic environment, and the larvae were divided among 7L metal trays with no more than 300 larvae per tray to prevent overcrowding. The water in the rearing trays was maintained at 26-28°C, and the larvae were fed ground cat food. When the larvae reached the late 2^{nd}/early 3^{rd} instar they were transported in plastic carriers to the study site in Whitfield and implanted in the bromeliads.

2.1.2 Bromeliads

Six bromeliads of assorted genera were obtained from Riverside Bromeliads in Malanda. All of the plants were young, not in flower, had not been treated with any form of insecticide or larvicide, and possessed large tanks appropriate for mosquito breeding. Species represented included *Hohenbergia stellata*, *Nidularium leprosa*, *Billbergia pyramidalis* and hybrids within the *Neoregelia* genus. Three of the six bromeliads possessed multiple tanks, in which case the most watertight tanks were selected and the others were removed. Prior to experimentation, the central wells and leaf axils of all bromeliads were flushed with a high-pressure stream of water from a hose to remove any leaf detritus and residual eggs, larvae, or other potential contaminants. The leaves of each plant were cut to within an inch of the water level in the tank or leaf axil (see Figure 4) to allow for easier pipette access and greater visibility, and to decrease the risk of the mesh enclosures snagging on the serrated leaf margins.

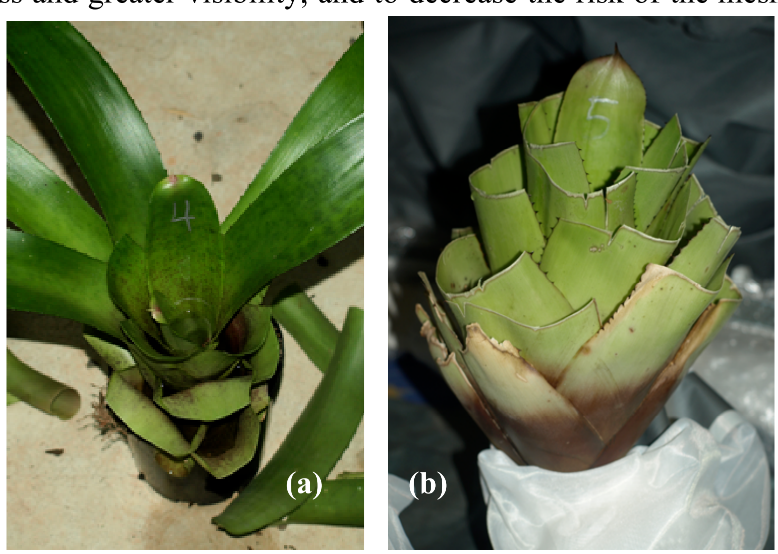

Figure 4: Trimming of bromeliad leaves as part of experimental preparations.

a. Bromeliad 4 (*Neoregelia* hybrid) in the process of being trimmed *b.* Bromeliad 5 (*Hohenbergia stellata)* after trimming

2.2 Study site

The field study was conducted at 20 McHugh Crescent in Whitfield, a suburb of Cairns (16°54’03”S, 145°43’30”E). Whitfield has had 92 confirmed Dengue cases as of 6 April 2009 (see Figure 5).

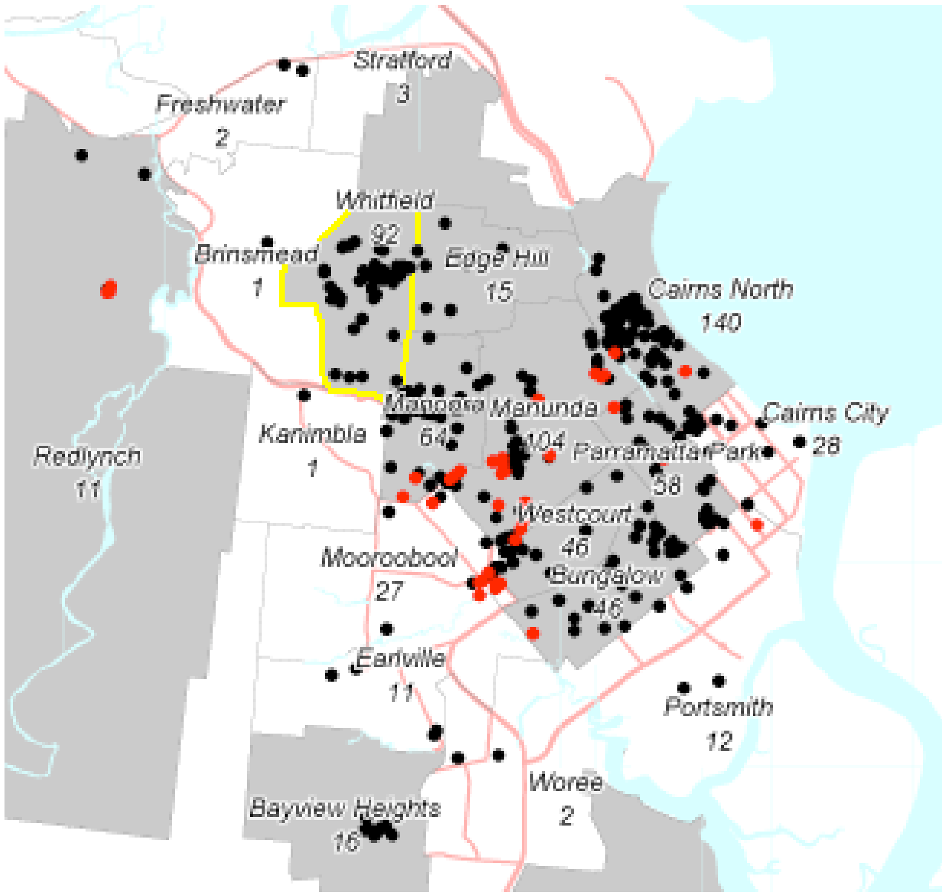

Figure 5: Location of Whitfield among the suburbs of Cairns. Grey areas indicate suburbs in which the Dengue virus is currently active. Black dots represent confirmed cases since 1 December 2008, and red dots represent confirmed cases since 18 March 2009. Numbers indicate the total number of Dengue cases in each suburb as of 6 April 2009. Image courtesy of Tropical Population Health Services, Queensland Health.

The bromeliads were maintained in a typical back yard garden environment, the suitability of which was indicated by the fact that there were already numerous bromeliads present. The experimental bromeliads were kept on a covered patio that was sheltered from rain and direct sunlight.

2.3 Experimental protocol

2.3.1 Experimental setup

Each bromeliad tank was contained within a fine mesh that secured around the base of the plant with elastic and could be opened and closed with a tie at the top (see Figure 6). The tanks were overfilled and then allowed to stand for 24 hours, at which point the volume of water remaining in each tank was noted as the standard capacity. The central well of each bromeliad was implanted with ten mosquito larvae, and 7cm of dead longan leaf were provided as food. Four additional populations of ten mosquitoes were maintained in paper cups and selectively subjected to the same feeding, watering and daily monitoring schemes as bromeliad populations to control for any negative impacts these aspects of the experimental design may have had. Table 1 outlines the treatment of each control.

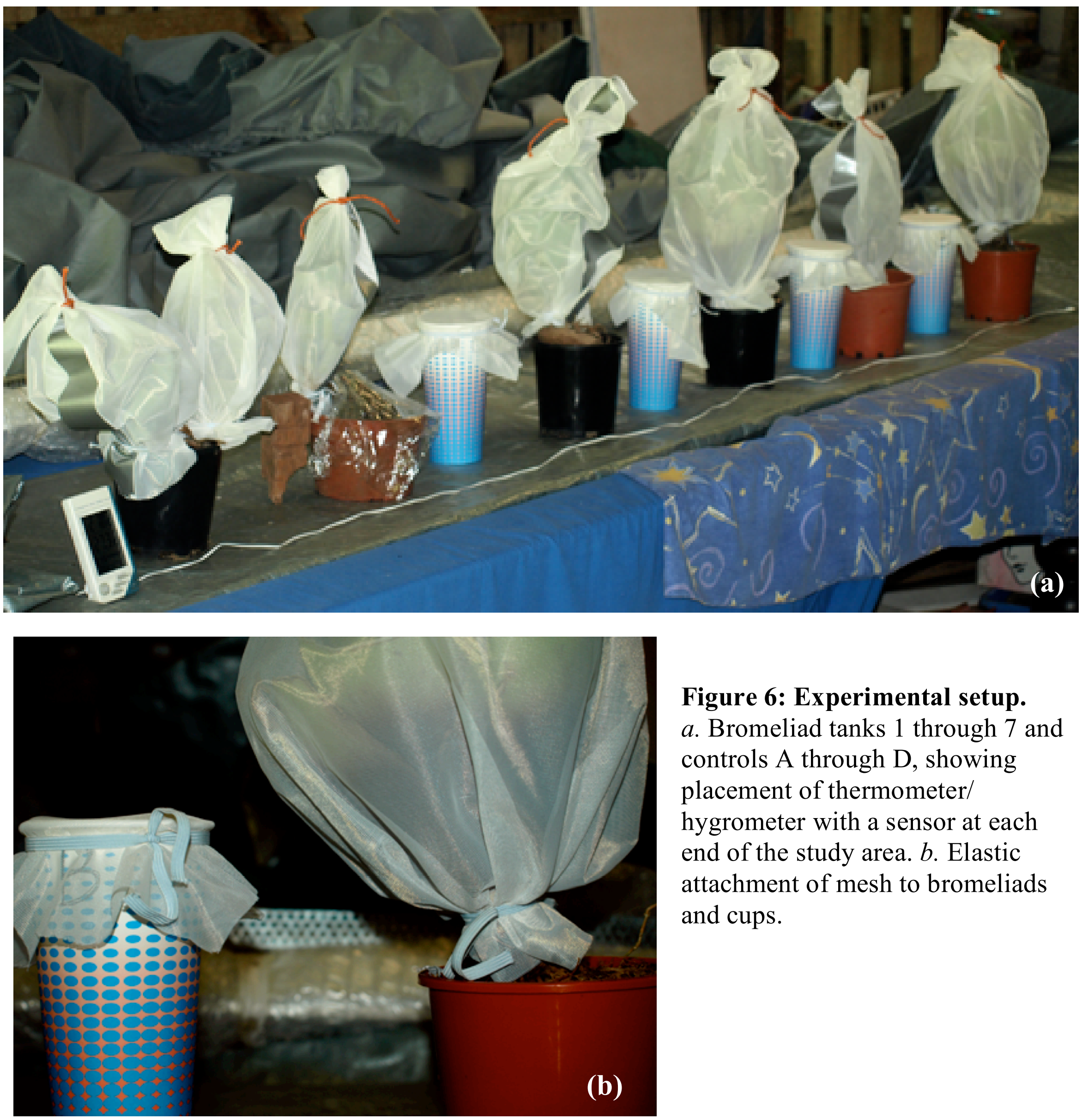

Figure 6: Experimental setup. *a.* Bromeliad tanks 1 through 7 and controls A through D, showing placement of thermometer/ hygrometer with a sensor at each end of the study area. *b.* Elastic attachment of mesh to bromeliads and cups.

Control	Food	Pipetting
A	Yes	Yes
B	No	No
C	No	Yes
D	Yes	No

Table 1: Treatment of control populations for effects of feeding and pipetting.

2.3.2 Daily monitoring

Mosquito populations were monitored every afternoon from 18 April through 3 May 2009. Emergent adults were counted, collected with an aspirator, and ejected onto sticky paper. Larvae and pupae were removed from the bromeliads with a turkey baster or small pipette, as appropriate, and placed in a cup for closer inspection. The number of live larvae and pupae were noted, and dead larvae, pupae, or adults were counted and discarded. Any water remaining in the bromeliad was tipped out into a tray, and the total water volume was recorded. The pH of the water was measured with a fish tank pH kit accurate for fresh water pHs between 6.2 and 7.4 (The Wardley Corporation, USA). All the original water and surviving larvae and pupae were replaced into the central well of the bromeliad, and water was added to compensate for evaporation or pH changes as necessary.

Control populations were treated in exactly the same manner, except for those designed to control for the stress of monitoring, which were observed without being removed from the cups, and for which water volume was never directly measured.

The containers were reassigned to new positions within the study area every 24 hours, which were determined by random numbers generated in Microsoft Excel. The relative humidity and the ambient temperature at each end of the study area were measured in the morning, afternoon and evening.

2.4 Statistical analysis

The total number of deaths and adult emergences occurring in bromeliad and control populations were compared using the Chi-square test for significant difference between multiple populations. This test was appropriate because the data were discrete and nominal, and no expected value was less than 5.

3. RESULTS

Of the 110 larvae implanted in bromeliads and control containers combined, 58 individuals died and were found, 19 presumably died but were not found, 17 survived but had not completed development by the end of the study period, and 16 emerged as competent adults. Larvae developed successfully to adulthood in 4 out of 7 bromeliads and 3 out of 4 controls. Table 2 and Figure 7 illustrate the fate of larvae implanted in each container.

STATUS OF *Aedes aegypti* LARVAE 15 DAYS AFTER IMPLANTATION AS 2nd/3rd INSTARS

	Container	Number of individuals (out of 10 implanted)		
		Dead (# missing in parentheses)	Still in imm. stages	Emerged adults
1	*Hohenbergia stellata*	10 (4)	0	0
2	*Nidularium leprosa*	10 (4)	0	0
3	*Neoregelia* hybrid	8 (4)	1	1
4	*Neoregelia* hybrid	6	0	4
5	*Hohenbergia stellata*	4 (2)	4	2
6	*Billbergia pyramidalis*	8 (2)	2	0
7	*Neoregelia* hybrid	6 (3)	1	3
A	food, pipet	6	1	3
B	no food, no pipet	6	3	1
C	no food, pipet	10	0	0
D	food, no pipet	3	5	2

Table 2: Status of *Aedes aegypti* larvae 15 days after implantation as 2nd/3rd instars.

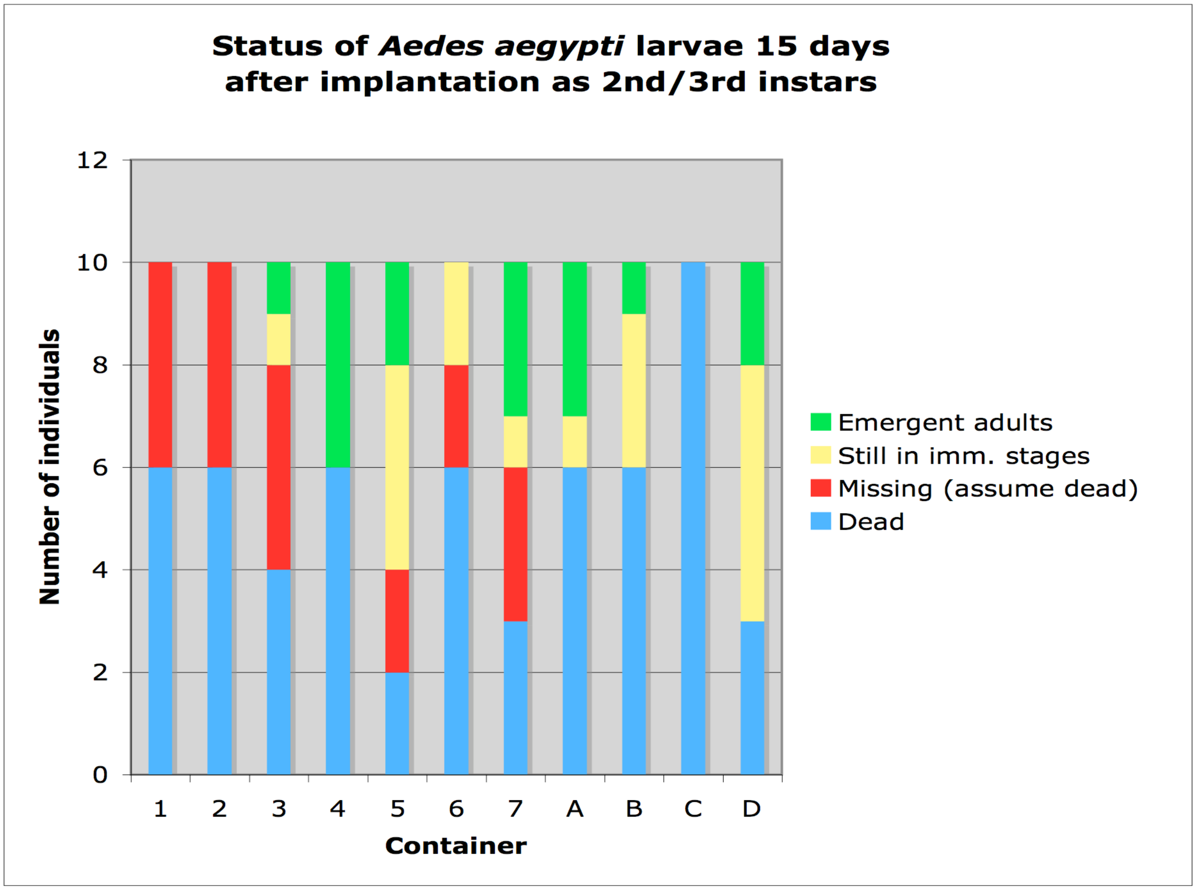

Figure 7: Status of *Aedes aegypti* larvae 15 days after implantation as 2nd/3rd instars.

When the number of larval, pupal and adult deaths and the number of successful adult emergences were compared, there was found to be no significant difference between populations raised in bromeliads and populations raised in controls (Chi-square test: critical value 3.84, experimental value 0.15).

The average number of deaths occurring in bromeliad populations (including missing individuals) was 7.43. The average number occurring in control populations was 6.25. Among the control groups, populations that were provided with food and not subjected to pipetting had the lowest mortality rate, while populations subjected to pipetting and not provided with food had the highest. Mortality rates for populations with both food and pipetting or neither of the above were intermediate. Figure 8 illustrates larval mortality among the control populations.

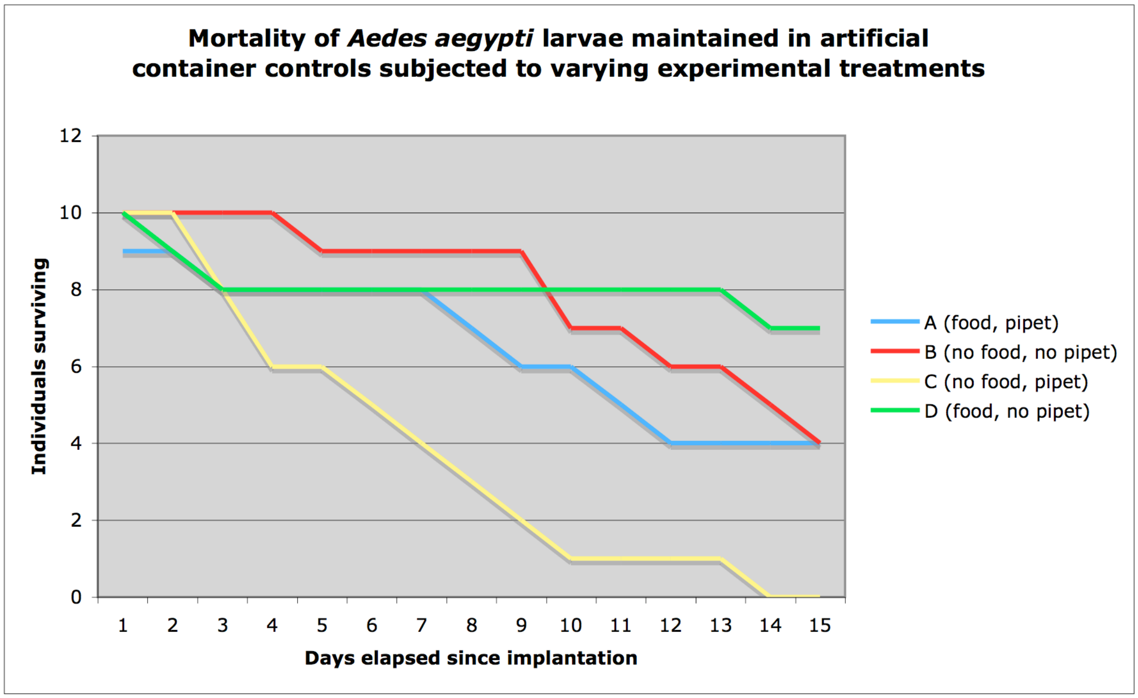

Figure 8: Mortality of *Aedes aegypti* larvae maintained in artificial container controls subjected to varying experimental treatments.

Rates of mortality and adult emergence varied between individual bromeliads, but there were insufficient data to make significant distinctions between species. There were also insufficient data to distinguish between the influence of species differences and environmental factors. The average ambient temperature in the study area was 25.1°C, with observed minima and maxima of 20.1 and 28.0°C. Relative humidity ranged from 42 to 87%, with an average of 65%. All water used in this experiment came from an ordinary garden tap, and even after sitting for 24 hours it maintained a slightly basic pH of 7.4. Artificial control containers remained at a basic pH, while water kept in bromeliad tanks became consistently more acidic. Bromeliad water ranged from average individual pHs of 6.2 to 7.3, with a group average of 6.8. Relative humidity affected all containers equally. There was no clear relationship between larval mortality and water pH, and there were too many variables to discern a relationship between larval mortality and localized temperature.

4. DISCUSSION

4.1 Adult emergence and larval mortality

A total of 10 adult mosquitoes emerged successfully from 4 out of the 7 bromeliads, which disproves the popular notion that mosquito larvae found in bromeliads cannot develop completely. There was also potential for adults to emerge from a fifth bromeliad given more time; environmental conditions were not ideal and low nocturnal temperatures suppressed the rate of larval development in all containers.

Mortality rates among both bromeliad and control populations were abnormally high. According to Bar-Zeev 1957, the average mortality rate for *Aedes aegypti* larvae is between 3 and 10 out of a cohort of 30, given an unlimited food supply (cited in Focks et. al. 1993). Applied to the cohorts of 10 utilized in this study, the Bar-Zeev mortality rate would translate to an expected 1 to 3.33 deaths per container. The average number of deaths observed in bromeliad populations (missing individuals included) was 7.43, and the average number occurring in control populations was 6.25. Although mortality in bromeliads was high, these deaths cannot be conclusively attributed to any properties of the bromeliads themselves, because there was also high mortality among control populations. That the differences between bromeliad and control populations in terms of mortality and adult production were statistically insignificant (Chi-square value 3.69 below critical) suggests that under less harsh conditions, bromeliads may provide equally auspicious breeding sites.

Factors that contribute to larval mortality are generally lack of food, overcrowding, and other sources of environmental stress. In the context of this experiment it seems unlikely that food shortage or overcrowding were responsible, as the longan leaves should have provided abundant food, and the optimal density for *Aedes aegypti* production is as high as 0.5 larvae per mL of medium (cited in Peters and Barbosa 1977). The patterns of mortality observed among control populations suggest that experimental treatment had a significant impact on larval health. The presence of food appears to decrease mortality rates within a population, while subjection to daily pipetting increases them. Therefore, the worst conditions for larval development were found in control C, which was subjected to pipetting and contained no food. The best conditions were found in control D, which was exempt from pipetting and did contain food. Controls A and B each received one positive and one negative treatment (i.e. food and pipetting, or no food and no pipetting), and were therefore intermediately hospitable. Mortality rates observed in all four controls accurately reflect these conditions.

Another factor that may have contributed to high larval mortality was the sensitivity of the Sydney strain. The larvae used in this experiment were more than the hundredth generation of a mosquito colony that has been in culture at the university laboratory in Sydney for approximately ten years. The Sydney strain has never been exposed to larvicides or insecticides of any kind, and therefore individuals of this strain possess no drug resistance and are more sensitive to environmental disturbances than wild mosquitoes. For this reason, they serve as a fine experimental barometer to determine if a breeding site is in some way unsuitable. In general, wild mosquitoes developing in bromeliads can be expected to be hardier and have lower mortality rates.

Mortality totals among bromeliad populations were significantly increased by the number of missing larvae (an average of 2.7 per bromeliad, accounting for 36.5% of all bromeliad deaths). It is possible that these individuals swam away from the central well and became stuck in the leaf axils, as mosquito larvae tend to accumulate farthest from the source of light (Ritchie and Van Den Hurk 1994), and travel within a bromeliad is largely unidirectional. Under natural circumstances there would have been food available in the axils, but because the experimental bromeliads had been flushed of leaf detritus and any resident invertebrates, food availability was limited to the central well. Several investigators have observed scavenging by *Aedes aegypti* larvae on carcasses of their own species (cited in Merritt et al 1992), and for larvae in leaf axils isolated from the provided food source, scavenging would explain why some carcasses were never found. Dead larvae in controls were easily spotted and were removed within a maximum of 24 hours, presumably before complete scavenging could take place.

The results of this study suggest that some species of bromeliad may provide better breeding sites than others, but further investigation will be required for confirmation, as there were insufficient data to make statistically significant comparisons between species, or to clearly distinguish between species differences and the many environmental variables involved. In the meantime, there are a few general factors that influence whether an individual bromeliad is a good candidate for mosquito breeding, some of which bromeliad owners may be able to control.

4.2 Additional considerations for bromeliads in natural settings

A bromeliad only poses a risk if mosquitoes are likely to oviposit in it. For *Aedes aegypti,* the selection of an oviposition site is based upon the complex interaction of multiple chemical and physical factors, including the color, optical density, temperature, reflectance and humidity of the site, as well as tactile, chemotactile and olfactory stimuli (Bentley & Day 1989). Some of these factors require further investigation with regard to bromeliads: for example, mosquitoes can be discouraged from oviposition by some plant extracts (cited in Bentley & Day 1989), and in the bromeliad *Tillandsia utriculata*, gravid *Wyeomyia vanduzeei* mosquitoes appear to be repelled by a chemical change associated with the blooming of the plant, as that particular species of bromeliad tends to die soon after flowering (cited in Bentley & Day 1989). If flowering also discourages oviposition of *Ae. aegypti*, then some species of bromeliad may only be a threat in certain life stages.

Other factors influencing breeding site quality are more straightforward. Although larvae and pupae are difficult to displace, mosquito eggs can be flushed out of bromeliads by heavy rain or excessive watering (Frank and Curtis 1977). Additionally, the maximum temperature limit for embryonic development is 35ºC (Farnesi et. al. 2009), which in this study was easily exceeded in direct sunlight. In the Farnesi study, the greatest percentage of larvae emerged successfully from their eggs at 25ºC. In general, bromeliads in shade are better breeding site candidates than those in sunlight. Some bromeliad species may provide better breeding sites than others by virtue of being better suited to shady environments. The limitations that sun and rain exposure impose on mosquito breeding are promising, as bromeliad owners can take these factors into consideration when selecting planting sites.

Competition presented by other species of mosquito or additional resident organisms will also affect the quality of a breeding site. Within Australia, the following mosquito species are commonly found in the same containers as *Aedes aegypti*: *Aedes scutellaris, Aedes notoscriptus, Aedes katherinensis, Aedes tremulus, Culex halifaxii, Culex quinquefasciatus* and *Toxorhynchites speciosus* (Ritchie and Van Den Hurk 1994). Larvae can out-compete one another through depletion of a limited food source or excretion of deleterious chemicals. None of the considerations enumerated above guarantee exemption from mosquito breeding, however, as depleted nutritional reserves can force gravid females to oviposit in poor or overcrowded habitats (Bentley & Day 1989).

4.3 Suggestions for further inquiry

4.3.1 Potential improvements to study design

The primary improvement that could be made to this study would be to conduct it in a warmer environment, or in the same environment during a warmer part of the year. Warmer temperatures would cause larval development to proceed more quickly, allowing a greater number of replications and time for a 'full trial' beginning with the implantation of dry eggs, as originally intended. The other major improvement would be the refinement of experimental parameters to make daily sampling less stressful for the larvae. Sealing off the central well so that larvae cannot escape into the leaf axils, and lighting the bromeliads strongly from below so that larvae are enticed to swim upwards would make them sufficiently easy to recover that perhaps it could be done more gently. Measuring the water volume in each bromeliad by weight would eliminate the need to overturn the bromeliads, which frequently led to the rather violent ejection of larvae that had eluded the pipette. Alternatively, daily sampling of larvae could be removed from the procedure entirely, and the number of immature deaths could simply be calculated once all the adults have emerged. Bromeliads could also be dissected at the end of each study in a final search for dead larvae. Utilizing homogenous groups of bromeliad species and controlling environmental variables more carefully (by selecting a study area without a temperature gradient; measuring the temperature of the water itself, as larvae can dive to thermoregulate; maintaining the bromeliads' natural pH) would make it easier to distinguish between the influences of each. A greater number of control populations that receive the same experimental treatment as bromeliad populations (i.e. feeding and pipetting) would allow a more significant comparison between the two. Finally, enclosing the containers in a green mesh instead of white would make emergent adults more visible.

4.3.2 Questions remaining for future study

Further inquiry is required to determine whether some bromeliad species provide higher quality breeding sites than others. Studies to this end should also take into account the effects of varying sun exposure, flushing intensity, larval density, bromeliad pH, bromeliad blooming, and competition among resident invertebrate species. It would likely be enlightening to conduct further larval surveys of bromeliads in gardens around Cairns and note the influence of these factors in addition to designing controlled studies.

Numerous laboratory and field studies have already been conducted on the ovipositional preferences of *Aedes aegypti*; these studies could be easily redesigned to evaluate the preference of *Ae. aegypti* for natural vs. artificial containers.

5. CONCLUSIONS

Previous laboratory studies have suggested that the competence of an adult mosquito to vector disease (i.e. the internal physiological factors that govern the infection of human pathogens in a mosquito) varies with the quality of the larval environment (cited in Merritt et. al. 1992). This study demonstrated that common ornamental bromeliads provide an environment in which the Dengue vector *Aedes aegypti* can successfully breed. The absolute quality of bromeliads as a larval environment will require further investigation to determine, but this study suggests that it is not significantly below that of an artificial container. This conclusion has powerful implications for Dengue management within the suburbs of Cairns: if artificial containers are removed but large quantities of bromeliads remain untreated, the ovipositional preferences of local *Ae. aegypti* will likely shift back toward natural containers, as they originally were in Africa. If the treatment of artificial and natural containers can be approached with equal seriousness, not only by local authorities but most importantly through the conscientiousness of bromeliad owners, we will stand a better chance of curtailing the spread of the Dengue virus.

ACKNOWLEDGEMENTS

Many people contributed to the success of this project. I am greatly indebted to my advisor Sharron Long for all her guidance and generosity with her time. I would also like to thank everyone at Topical Population Health Services for being welcoming and helpful, particularly Dr. Scott Ritchie and Mr. Brian Montgomery; Brad Milligan for connecting me with TPHS, and both he and his wife Rominy Balodis for letting me stay in their house and experiment in their backyard; Steve Ward of Riverside Bromeliads for supplying the bromeliads, and his wife Pam for delivering them; Lynn Hudson of the Cairns Bromeliad Society for her additional help with bromeliad identification; Jack Grant and Tony Cummings for developing my project in its infancy; my family for their support, and my father in particular for the idea of working on a Dengue-related problem.

LITERATURE CITED

Bentley MD. 1989. Chemical Ecology and Behavioral Aspects of Mosquito Oviposition. Ann Rev Entomol 34:401-21.

Canyon DV. 2001. A review of the dengue mosquito, *Aedes aegypti* (Diptera: Culicidae), in Australia. Rural and Remote Environmental Health I:27-36.

Clements AN. 1992. The Biology of Mosquitoes Vol. 1: Development, Nutrition and Reproduction. London: Chapman & Hall. p. xiv, 72, 153.

Dengue and dengue haemorrhagic fever. [Internet]. Fact Sheet No. 117 [updated 2009 March] World Health Organization; [cited 2009 April 28]. Available from: http://www.who.int/mediacentre/factsheets/fs117/en/print.html

Farnesi LC, Martins AJ, Valle D, Rezede GL. 2009. Embryonic development of *Aedes aegypti* (Diptera: Culicidae): influences of different constant temperatures. Mem Inst Oswaldo Cruz, Rio de Janeiro 104(1): 124-126.

Focks DA, Haile DG, Daniels E, Mount GA. 1993. Dynamic life table model for *Aedes aegypti* (Diptera: Culicidae): simulation results and validation. J Med Entomol 30(6): 1018-1028.

Frank JH. 1990. Bromeliads and Mosquitoes. Entomology Circular No 331. Fla Dept Agric & Consumer Serv Division of Plant Industry

Frank JH, Curtis GA. 1977. On the Bionomics of Bromeliad-inhabiting Mosquitoes. IV. Egg Mortality of *Wyeomyia vanduzeei* Caused by Rainfall. Mosq News 37: 239-245.

Frank JH, Stewart JP, Watson DA. 1988. Mosquito larvae in axils of the imported bromeliad *Billbergia pyramidalis* in southern Florida. The Florida Entomologist 71:(1) 33-43.

Johnson P and Ritchie SA. 2008. Unpublished data.

Merritt RW, Donald RH, Walker ED. 1992. Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. Annu Rev Entomol 37: 349-374.

Morris, K. (2009, February 28). The eternal dengue question: do bromeliads encourage or discourage mozzies? The Cairns Weekend Post Weekender, p 10.

O'Meara GF, Evans LF Jr., Gettman AD, Patteson AW. 1995. Exotic Tank Bromeliads Harboring Immature *Aedes albopictus* and *Aedes bahamensis* (Diptera: Culicidae) in Florida. Journal of Vector Ecology 20(2): 216-224.

Outbreak update - Current outbreaks in north Queensland. [Internet]. [updated 2009 April 23] Cairns (QLD): Queensland Health; [cited 2009 April 28]. Available from: http://www.health.qld.gov.au/dengue/outbreak_update/current.asp

Peters TM, Chevone BI, Greenough NC, Callahan RA, Barbosa P. 1969. Intraspecific competition in *Aedes aegypti* (L.) larvae. I. Equipment, techniques, and methodology. Mosq News 29: 667-74.

Queensland Public Health Act 2005, Reprint No. 2B. 2008. Office of the Queensland Parliamentary Council.

Ritchie SA, Broadsmith G. 1997. Efficacy of Altosid® Pellets and Granules Against *Aedes aegypti* in Ornamental Bromeliads. Journal of the American Mosquito Control Association 13(2): 201-202.

Ritchie SA, Van Den Hurk A. 1994. Basic Mosquito Biology and Surveillance with emphasis on *Aedes aegypti*. Unpublished.

Varajão JBM, Santos CB dos, Rezende HR, Bevilacqua LC, Falqueto A. 2005. *Aedes* (Stegomyia) *aegypti* (Linnaeus, 1762) breeding sites in native bromeliads in Vitória City, ES. Revista da Sociedade Brasileira de Medicina Tropical.

PERSONAL COMMUNICATIONS

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