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JAMES COOK UNIVERSITY
AUSTRALIA

The Biodegradable Lethal Ovitrap as a control method for dengue in Cairns, North Queensland with a focus on post four weeks deployment.

Masters by Research
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Declaration on Ethics

The research presented and reported in this thesis was conducted in accordance with the National Health and Medical Research Council (NHMRC) National Statement on Ethical Conduct in Human Research, 2007. The proposed research study received human ethics approval from the James Cook University ethics committee, under the project title 'Development of lure and kill technologies for control of dengue vectors' (H1751).

Abstract

Dengue is a mosquito-borne flavivirus and is the leading arboviral cause of mortality and morbidity in the world. The World Health Organization estimates there are ca. 50 million dengue cases yearly, with 2 billion people at risk of contracting dengue. In Australia, 27 outbreaks of dengue have occurred in North Queensland since 2000, resulting in over 2,500 notifiable cases, including two deaths in 2004 and another dengue related death in 2009. The mosquito *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) transmits dengue in Australia.

In Australia current dengue control consists of a combination of source reduction and deployment of Lethal Ovitraps (LO). With increasing numbers and spread of dengue cases a fast, cost-effective control tool was required. This need lead to the development of the Biodegradable Lethal Ovitrap (BLO), an ovitrap made from a starch-based plastic which could be set in the field and allowed to biodegrade over time. If the BLO was to be a true "set and forget" tool against *Ae.aegypti* (and dengue) it was important to determine what happened to the BLO after the standard four week control period. The aim of this research was to determine the effectiveness of the BLO as a dengue control tool post four weeks deployment. This research also aimed to investigate what impact, if any, the BLO might have on the non-target fauna in the immediate area around where the ovitrap was set. It was also hoped that the research could also determine public acceptability of the BLO as a personal protective tool against mosquito borne diseases.

Our results suggest that the BLO is still an effective control tool against *Ae.aegypti* twenty-two weeks post deployment. The ability of *Culex quinquefasciatus* (Say) (Diptera: Culicidae) to breed in the BLOs post nine weeks deployment was an important discovery, especially if the BLOs are to be deployed in countries where *Cx.quinquefasciatus* act as disease vectors. The research did raise the question of chemical resistance becoming an issue with the BLOs in the field for such extended periods of time.

The research also found little impact on non-target fauna populations when compared against other non-target studies. Our results suggest that numerous (<90) insect Families are attracted to the BLOs with limited impact on their numbers. Further studies on specific non-targets could be of interest, especially to a broader international audience. Due to the limited number of participants in our BLO public acceptability research, our results were inconclusive, but suggested a limited acceptability. Further research into the acceptability and understanding of mosquito control tools such as the BLOs would be beneficial to mosquito control activities in the future.

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1.0 Introduction and Literature Review

Dengue is a mosquito-borne flavivirus that causes dengue fever, the leading arboviral cause of human mortality and morbidity in the world (World Health Organization 2010). The World Health Organization (WHO) estimates there are 50 million cases of dengue fever each year, with two billion people at risk of contracting the mosquito borne disease (World Health Organization 2010). Since 2000, there have been 30 outbreaks of dengue fever in North Queensland (Queensland Health unpublished data 2010). These outbreaks have resulted in over 2,500 reported cases of dengue fever and three deaths. Two of these died in 2004 (McBride 2005) and one other dengue related death occurred in 2009 (Queensland Health 2009).

1.1 Dengue Virus

Dengue viruses (DENV; Flaviviridae: Flavivirus) consists of four antigenically related serotypes (DENV 1-4) all able to cause classical dengue fever (DF) as well as dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). Dengue is the only known arthropod-borne virus (arbovirus) completely adapted to survival in humans (Mackenzie et al.2004).

The dengue virus is a single-stranded positive-sense RNA virus. Surrounding the RNA genome is a nucleocapsid of approximately 30 nanometres, covered with a lipid envelope (Leitmeyer 1999). The virus consists of approximately 10,700 base pairs which code for three structural proteins and seven non-structural proteins (Leitmeyer 1999, Ramirez and Garica 1994). The protein combinations distinguish the different serotypes as well as the unique genotypes within serotypes (Leitmeyer 1999). Mackenzie et al. (2004) suggests the differences in the viral genome are a result of genetic evolution caused by increasing viral spread between countries and transmission through different serologically primed populations.

1.2 Dengue the Disease

Historically dengue fever was a disease of tropical regions. In 2005 the International Health Regulations were revised to include dengue fever as a disease that may constitute an international public health emergency (Farrar et al. 2007). This increased risk of dengue may be a result of changes to human demographics such as population growth and urbanisation with overcrowding and inadequate water and waste management, expanding vector populations, alterations in viral virulence and cheap, or easy international travel introducing different serotypes into susceptible populations or any combination of these (LeDuc 1994, Lifson 1996).

Australia is a classic example of the increase in risk to susceptible populations because of relatively convenient, inexpensive international travel. All recent epidemics of dengue fever in North Queensland have occurred from traveller's contracting dengue from endemic areas and then arriving in North Queensland, where they are bitten by the local mosquito vector *Aedes aegypti* (Leggat 2009, Queensland Health 2011). Global warming has been suggested as a reason for the increase in risk to

people from dengue, due to the increasing spread of the mosquito vectors (Hales 1996, Jetten and Focks 1997), although this is the subject of some debate (Russell et al. 2009).

Dengue fever is a human illness that is maintained in a cycle with specific *Aedes* mosquitoes as the arthropod vector. A dengue epidemic is possible in any location where the mosquito vector lives. Historically dengue fever was considered a benign, non-fatal febrile illness of visitors to tropical regions of the world (Gubler and Kuno 1997). The exact origins of dengue fever are not clear, and there is strong circumstantial evidence for a tropical Asian origin. Dengue-like diseases were reported from China as early as 265-420 AD, but the first documented case of the disease was reported by Benjamin Rush from Philadelphia in 1780. Almost simultaneous epidemics were reported in 1779 and 1780 from Asia and Africa (Gubler and Kuno 1997). These almost simultaneous epidemics show that the virus and the mosquito vector (s) had a global distribution even then (Centres for Disease Control and Prevention 2010).

Typical dengue fever symptoms include: sudden onset of a fever, painful headaches especially behind the eyes and severe muscle and joint pain, hence the old name of 'Break-bone Fever'. Other symptoms can include: a lack of appetite, vomiting and diarrhoea, petechial rash, minor bleeding from the nose and/or gums and fatigue (Gubler and Kuno 1997, Ramirez-Ronda and Garica 1994, Thein et al. 1994, Farrar et al. 2007, WHO 2009).

1.2.1 Classical Dengue

Most cases of Classical Dengue have the non-specific, almost flu-like symptoms of; sudden onset of fever, headache, nausea, vomiting, and muscle and joint pain. Presenting with common 'flu-like' symptoms can make clinical diagnosis of dengue fever difficult (Gubler and Kuno 1997, Ramirez-Ronda and Garica 1994). The less commonly observed petechial rash, taste aberrations, dizziness and photophobia are useful in the clinical diagnosis of dengue (Hayes and Gubler 1992). There have been records of patients suffering from neurological complications such as 'short term memory loss' and 'seizure' (Row et al. 1996).

1.2.2 Dengue Haemorrhagic Fever and Dengue Shock Syndrome

Complications with classical dengue can lead to more severe dengue haemorrhagic and shock syndromes where patients can suffer from vascular permeability, and evidence of vascular collapse. The WHO classifies Dengue Hemorrhagic Fever (DHF)/ Dengue Shock Syndrome (DSS) into four levels of severity (Ramirez-Ronda and Garica 1994, WHO 2009):

1. Fever with non –specific constitutional symptoms and a positive tourniquet test,
2. Level 1 symptoms plus spontaneous bleeding,
3. Circulatory failure manifested by rapid weak pulse, narrowing of pulse pressure or hypotension, with the presence of cold clammy skin and restlessness,
4. Profound Shock with undetectable blood pressure and pulse,

Why and how dengue haemorrhagic fever and dengue shock syndrome occur in humans is still not clearly understood, and research continues to improve the dengue pathogenesis knowledge and understanding (Bhakdi 1990, Gubler and Kuno 1997, Ramirez-Ronda and Garica 1994, WHO 2009).

It is generally agreed the more severe forms of dengue usually occur after a secondary infection with a different serotype of the virus (Halstead 1990, 2003). Infection with one serotype of the virus generally provides lifelong immunity to that serotype (Kuno and Gubler 1997, WHO 2009). While infection with a second serotype increases the chances of the dengue fever infection worsening into dengue haemorrhagic or shock (DHS/DSS). The WHO (2009) estimate there are approximately 500 000 people hospitalised with dengue haemorrhagic or shock each year. Without medical intervention the mortality rate of the sever forms of dengue fever is high, about 2.5% of those hospitalised.

Figure 1.1 Geographical regions at risk of dengue fever transmission. Courtesy of WHO, Denguenet (http://www.who.int/denguenet)

1.3 Treatment of Dengue

Treatment for any type of dengue (primary or secondary infection) is supportive, with fluid replacement and pain relief. Aspirin is not recommended for pain relief because of its anticoagulant action (Hayes and Gubler 1992). In DHF/DSS cases if the correct intensive support therapy is initiated, mortality may be reduced to less than 1% of cases (WHO 2010).

The best treatment for dengue is prevention, which is why great efforts have been invested into dengue vaccine development. Currently there is no successful vaccine against all four serotypes of the dengue virus. Vaccines against single serotypes of dengue have been developed as early as the 1940's (Gubler and Kuno 1997, Hombach 2007). However, the complicated interaction between serotypes and the human immune response system has slowed the development of a tetravalent vaccine. It is generally agreed that tetravalent dengue vaccine development difficulties are affected by inadequate understanding of dengue pathogenesis (Thein et al. 1994, McBride and Bielefeldt 2000, Halstead and Deen 2002, Halstead 2003, Stephenson 2005, Hombach 2007, Coller and Clements 2011). As greater understanding of dengue pathogenesis and improved experimental study techniques are developed a successful tetravalent vaccine that protects against all serotypes and genotypes is becoming more of a possibility.

Several research groups are working on different approaches for vaccine development (Hombach 2007). These focuses are:

1. Live Attenuated Vaccines- some research showed unacceptable high reactogenicity in adult test subjects, while others showed mixed results requiring further study
2. Live Recombinant Vaccines- some research successful and further clinical trials are planned
3. Subunit and Inactivated Vaccines- which use envelope proteins to activate an antibody responses and clinical trials are under way.

One vaccine that has been showing great promise is the Sanofi Pasteur's tetravalent, live, attenuated, recombinant chimeric vaccine which is currently undergoing stage three trials (Guy et al. 2011)

1.4 Transmission of dengue

A person becomes sick with dengue fever after being bitten by a dengue infected female mosquito vector. The virus is transmitted to the person via the mosquito's saliva. A vector mosquito that bites a person viraemic with dengue, ingests the virus with the blood meal. As the mosquito digests and then incubates the blood meal before searching for a suitable site to lay her eggs (oviposition). During this time dengue virus will have multiplied and migrated from the mosquito stomach into other tissues including the salivary glands, approximately 12-30 days, after which transmission to humans can occur. This period is known as the Extrinsic Incubation Period (EIP). The mosquito is now able to transmit the dengue virus via the saliva when she takes a blood meal. The duration of the EIP is determined by several factors including ambient temperature, viraemia level in the infected person and the viral serotype (Gubler and Kuno 1997). Some dengue serotypes are more virulent than others and transmission (human- mosquito- human) occurs more readily than with other serotypes, the reason for this is not yet fully understood (Gubler and Kuno 1997).

Once a person has been infected, the virus replicates within the person's monocytes and B cells (Hase et al. 1992, Lin et al. 2002). This period is referred to as the Intrinsic Incubation Period (IIP). The IIP lasts between four and twelve days depending on the virus serotype and the person's health (Gubler and Kuno 1997). Approximately 18-24 hours prior to the onset of symptoms, the persons viraemia (amount of virus circulating in a person's blood) is high enough to be transmitted to biting vector mosquitoes, this asymptomatic viraemia period is important in prevention and control of the virus spread, as the person is unaware of the risk they are exposing other people too. A person's symptoms may last between one and fourteen days, depending on the serotype and the health of the person infected. An infected person is considered viraemic for approximately two weeks (Hanna et al. 2001) after which time the person is immune to that viral serotype.
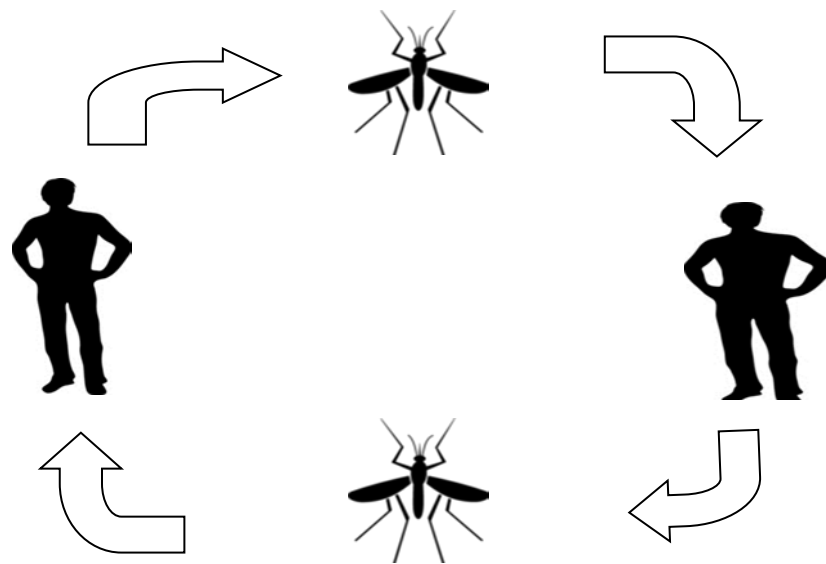
Viraemia	Extrinsic Incubation Period	Intrinsic Incubation Period
3-12 days	8-10 days	3-12 days
(Average 4-5 days)		(Average 4-5 days)

Figure 1.2 Dengue fever transmission cycle.

1.5 Dengue Vectors

Aedes aegypti (L.) (Diptera: Culicidae) and *Aedes albopictus* (Skuse) (Diptera: Culicidae) are considered the two main vectors of dengue. There is some evidence to suggest that other mosquitoes from the *Aedes scutellaris* complex such as *Aedes polynesiensis* may be vectors of the dengue virus in the absence of *Ae. aegypti* and *Ae. albopictus* (Gubler 1999, Prakash et al. 2001, Moore et al. 2007). Globally, *Ae. aegypti* is considered the primary vector of dengue (PAHO 1994, Gubler and Kuno 1997, Reiter and Gubler 1997, Morrison et al. 2007, WHO 2009) mainly due to *Ae. aegypti* females feeding almost exclusively on humans (Harrington et al. 2001, Ponlawat and Harrington 2005, Siriyasatien et al. 2010).

1.5.1 *Aedes aegypti*

Aedes aegypti have evolved to feed almost exclusively on humans for survival (Harrington et al. 2001, Ponlawat and Harrington 2005, Siriyasatien et al. 2010). Found in and around human habitation *Ae. aegypti* have distinctive behaviours making these mosquitoes one of the more complex species to study. There are numerous published journal articles discussing *Ae. aegypti* and its various behavioural characteristics, most of which are locally specific, and differ between countries and towns within a country. Some examples of behavioural variations is the discussion of the flight range of *Ae. aegypti* (Edman et al. 1998, Muir and Kay 1998, Ordonez-Gonzalez et al. 2001, Reiter et al. 1995, Russell et al. 2005). Each article compares distances female *Ae. aegypti* will fly: each produces different results (between 50 and 360 metres), and these results are explained by different environmental conditions or trapping methods used in the experiments. Another example of the geographical differences observed in *Ae. aegypti* is from Williams et al. (2006) who compared different kairomone blends to attract female *Ae. aegypti*, they found that F1 *Ae. aegypti* collected from Australia and Brazil differed significantly in their responses to various olfactory cues.

However, most basic behaviours of *Ae. aegypti* are the same worldwide. Female *Ae. aegypti* are easily disturbed while feeding, characteristically taking many interrupted blood meals per gonotrophic cycle, preferring to harbour in people's homes, close to their source of blood. Due to their preference for seeking multiple human blood meals, it is possible for multiple cases of dengue fever to occur in the same house during dengue epidemics (Rodriguez-Figueroa et al. 1995). When females have ingested enough blood, and are ready to lay eggs, they leave the relative safety of the indoors and often fly outside where they search for suitable site (s) to lay their eggs (Dibo 2005). Again there are contentions between different research groups, this time, as to whether *Ae. aegypti* lay all their eggs in the one container or, in a process referred to as skip-oviposition, lay their eggs in multiple containers (Colton et al. 2003). Harrington and Edman (2001) found no evidence for *Ae. aegypti* skip-oviposition whereas Reiter (2007) suggests that skip-oviposition is one of the major causes for the spread of dengue fever through a community. These differences maybe geographically or experimentally based, but most studies conclude that *Ae. aegypti* prefer to feed inside premises and lay their eggs in containers of water with minimal organic matter and are not found breeding in ponds, streams or swamps.

1.5.2 *Aedes albopictus*

Most of reports indicate that *Ae. albopictus* is a poorer dengue virus vector, mainly due to the unspecific nature of its blood feeding habits (Hawley 1988,Gubler and Kuno 1997, Gratz 2004, Enserink 2008, Lambrechts et al. 2010). There is however, a growing amount of conflicting literature on the vector competency of *Ae. albopictus* in the transmission of dengue virus. Degallier et al. (2003) found no dengue virus in field collected samples of *Ae. albopictus* during a Dengue 1 epidemic in Brazil, but did find Dengue 1 virus in a smaller sample of *Ae. aegypti* collected during the same period. While the results from the research by Paupy et al. (2010) in Gabon suggests that *Ae. albopictus* was a better vector for dengue virus than *Ae. aegypti*. Mitchell et al. (1987) and Gubler and Kuno (1997) suggest that *Ae. albopictus* is very susceptible to oral infection with dengue virus, meaning that in areas where *Ae. albopictus* is found there is potential for low level endemic dengue that could lead to maintenance cycles of dengue with sporadic, mild or even asymptomatic human cases.

Another confounding factor in the viral competency of *Ae. albopictus* is the ecologically diverse habitats of this species. *Aedes albopictus* are often found breeding in forests and heavily vegetated urbanized areas, where they seek blood meals from any available source. Common breeding sites in the forest habitats include tree holes, leaf axils and rock pools, while in more urbanized areas *Ae. albopictus* breed in most water holding containers (Hawley 1988, Gubler and Kuno 1997). The ability for the eggs of this species to overwinter at subzero temperatures (Nawrocki et al. 1987, Adhami and Reiter 1998) and adults to survive at over 30 degrees Celsius (Gubler and Kuno 1997) enables this mosquito species to survive in a diverse variety of habitats. *Ae. albopictus* can survive in a wide temperature range, this combined with the diverse ovipostional behaviour, could result in dengue fever epidemic occurring in areas previously free of the disease. *Aedes albopictus* is a very competitive mosquito species and is capable of completely eliminating other container breeding mosquito species from areas where it has been introduced (Hawley 1988, Knudsen 1995, Gubler and Kuno 1997, Adhami and Reiter 1998, Enserink 2008) this has been shown in the Torres Straits of Australia since the discovery of *Ae. albopictus* in 2005 (Ritchie et al. 2006). These features of *Ae. albopictus* mean that if introduced into a new area, it could be extremely difficult to eradicate.

1.6 Surveillance and Control

Without a vaccine, control of dengue is contingent on vector control. With *Ae. aegypti* generally accepted as the main vector, control and prevention programs focus on this species. Most control measures used for *Ae. aegypti* are also relevant for *Ae. albopictus* with a few minor adjustments. *Aedes albopictus* control requires additional consideration of potential forest and semi-urban breeding sites and harbourage of the adults. *Aedes albopictus* also has slightly different oviposition cues that they find attractive and respond to. These cues can be applied as lures for trapping purposes (Kawada et al. 2005) especially in locations where the vector is widely dispersed.

1.6.1 Surveillance

Surveillance of dengue vectors is an important part in the control and prevention of dengue fever epidemics. Successful surveillance requires an understanding of the mosquito population in the area of interest. Surveillance is essential in dengue fever prone areas as it is the only available means of preventing or limiting a dengue epidemic. A base line of data needs to be established, which vector control staff can refer to when determining when and where preventative measures should be implemented. There are two general methods of monitoring dengue vector numbers, the first is via adult numbers and the second via immature numbers.

Historically the "Gold standard" of mosquito monitoring was the man-biting collection (Service 1993), but in dengue fever endemic areas this is not safe, as the collectors are at risk of contracting dengue. Because *Ae. aegypti* prefers to feed on humans and is active by day, the use of animal baited traps is of little use, as are night-time light-traps, which are commonly used for collecting nocturnal mosquito species. There have been several traps designed to capture the adult dengue vector. Most of these traps used the visual attraction of contrast between black and white with limited success reported (Fay and Prince 1970, Freier and Francy 1991, Schoeler et al. 2004). Thus monitoring of adult dengue vectors relies on skilled vector control officers, who can work a sweep net or a Centers for Disease Control and Prevention (CDC) backpack aspirator (Clark et al. 1994) and can accurately collect any adults resting in a particular location (Service 1993). Historically, collections of adult dengue vectors was labour intensive and tedious work.

In 2006 the BioGents Sentinel (BGS) trap was shown to be an effective trap for both *Ae. aegypti* and *Ae. albopictus* (Maciel-De-Freitas et al. 2006, Williams et al. 2006, Williams et al. 2007). The BGS works by mimicking air currents made by human breath to attract mosquitoes from a distance. Once the mosquitoes are within sight of the BGS the trap uses the mosquitoes attraction to the contrast between black and white to attract mosquitoes to the trap where the mosquitoes are then sucked into the collection bag (Geier et al. 2006). The BGS trap is quite expensive and not as robust as other mosquito traps. This has hindered the BGS trap from being used more frequently by dengue control programs around the world.

Prior to the BGS trap, and in locations where the BGS is unaffordable or not suitable, surveillance of dengue vectors was primarily of the immature stages. Using immature stages as a measure of the vector population has lead to the development of numerous surveillance indices, none of which truly define the point over which dengue transmission will occur (Tun-Lin et al. 1995, Tun-

Lin et al. 1996, Chan 1985). The most commonly quoted index is the Breteau Index (BI), which is defined as the number of containers positive for *Ae. aegypti* larvae and pupae per 100 houses. The BI was originally developed as a threshold index for yellow fever and its use has expanded to dengue because the vectors are the same (Gubler and Kuno, 1997). Other indices include: the House (Premise) Index- the percentage of houses positive for vector mosquito immatures, the Stegomyia Index- the number of positive containers per 1000 people and the Container Index- the mean number of positive containers per premise (Tun-Lin et al. 1996).

Unfortunately most of these indices don't quantify larval activity, thus they provide an equal Index for a container containing a single larva, with a container containing hundreds of larvae (Tun-Lin et al. 1996). Another drawback of these indices, is that they only report on larvae, not adult mosquito populations. This may give an overestimation of the mosquito population, as not all larvae survive to adulthood.

Focks and Chadee (1997) were the first to develop an index that accounted for container productivity. The index developed by Focks and Chadee assessed the number of pupae in a container, thus providing a more realistic estimate of the potential adult mosquito population (since the percentage of pupae surviving to adulthood is much higher than for larvae). In 2000, Focks et al. used the Pupae per person Index and proposed a transmission threshold of 0.5-1.5 pupae per person over which dengue transmission would occur.

The 0.5 -1.5 pupae per person has since been considered an exceedingly low number, but it does give dengue control programs a level to strive for. Unfortunately, few vector control units in dengue prone areas have time to conduct as thorough inspection to determine this Pupae per person Index.

1.6.1.1 Trapping

The majority of dengue prone areas use oviposition traps (ovitraps) to collect eggs. This provides a presence/ absence or egg number comparison between areas. It is faster and less labor intensive than the 'index' methods (Gubler and Kuno 1997). Ovitraps usually consist or a plastic or glass container either black or red (mosquitoes see red as black- Muir et al. 1992) which contains a wooden or masonite paddle, or a strip of paper or fabric that the mosquitoes oviposit on. The trap is then partly filled with tap water or more suitably, a plant-based infusion to attract gravid mosquitoes (mosquitoes looking for somewhere to lay their eggs) and set for four to seven days (Gubler and Kuno 1997, Reiter et al. 1991). Most infusions are grass based where the grass (hay) is left to soak (ferment) in the water for a week or more, this produces a very smelly liquid which when added to ovitraps quickly attracts mosquitoes to lay their eggs. (Gubler and Kuno 1997, Kawada et al. 2007, Ritchie 2001, Santana et al. 2006). The infusion needs to replaced with fresh infusions every couple of weeks, to maintain maximum attractiveness to gravid mosquitoes. Many dengue control programs lack the time or resources to maintain an infusion. Furthermore, the transport these infusions during dengue epidemics can also be problematic. An alternative to the infusion is the use of protein rich animal feeds that are readily purchased in pellet form (Ritchie 2001). These feed pellets can be added to the ovitrap when set. As the pellet dissolves it produces an infusion attractive to gravid mosquitoes. The use of animal feed pellets increases the rate of ovitrap deployment considerably, allowing for more

traps to be deployed in a similar time frame. Regardless of how many traps are set in the community, the traps need to be set in suitable areas to be attractive to gravid mosquito and work effectively. There are numerous published articles about the most suitable locations for setting ovitraps (Dibo et al. 2005, Gubler and Kuno 1997, Williams et al. 2006). Most authors agree that suitable sites for ovitraps include areas outdoors which are cool and dark but readily visible to mosquitoes. Ovitraps are generally not set indoors because of access constraints and smell (Sithiprasana et al. 2010, Williams et al. 2006)

The deployment and collection of ovitraps as a surveillance method for dengue vectors is usually a compromise between the area of surveillance and the resources available. With standard ovitraps there is a lag period between setting the trap and the identification of the mosquito species, as the eggs collected in the ovitrap need to be incubated for 3 days, hatched and the larvae reared for identification. The extended period between setting traps and identification of mosquito species in an area can be shortened by the use of modified ovitraps that collects adult mosquitoes. Ritchie et al. (2003) and Facchinelli et al. (2007) have shown that an ovitrap containing a polybutylene adhesive (non-drying glue) was just as effective as a standard ovitrap and it saved days as the caught mosquitoes could be easily identified on the adhesive, eliminating the requirement of rearing larvae. Another advantage of sticky ovitraps over standard ovitraps, is that the mosquitoes collected on the glue can be used for virus testing and other research projects (Ritchie et al. 2004, Facchinelli 2007). As the sticky traps are also removing adult mosquitoes from the population the sticky ovitraps are also useful in a disease prevention and control capacity (Ritchie et al. 2003).

1.6.2 Control

One of the simplest forms of dengue fever control is personal protection. The majority of people who are at risk of dengue see the responsibility of dengue control as a governmental issue (Gubler and Kuno 1997, Spiegel et al. 2005, Yap et al. 1994) and fail to perform the most basic of control measures: to protect themselves against mosquito bites. Personal protection consists of creating physical barriers against mosquito bites. Wearing suitable clothing to avoid being bitten by the dengue vector, if sleeping during the day, using a mosquito net or applying an effective and suitable insect repellent. Physical control of the dengue vectors also includes the removal of containers that the vectors could breed in, or if removal is not possible then rendering the container mosquito proof with mesh screens, sand, etc. This removal or rendering of breeding sites unattractive to mosquitoes is often referred to as 'source reduction', as the source (water receptacle) of the mosquitoes breeding cycle is being reduced. This forms the backbone of dengue control and prevention programs.

Source reduction is considered the minimal requirement of a dengue control program. There have been numerous articles published on various methods, some less successful, on effectively and efficiently conducting source reduction with minimal resources (Chadee 2004, Gubler 1989, Moloney et al. 1998, Tun-Lin et al. 1995). Additionally, there have been several articles published that dispute the benefit of source reduction as a means of dengue control. These authors raise concerns that the

removal of potential breeding sites will cause the infected mosquito to fly further in search of an oviposition site, and this would lead to a larger dispersion, and not a reduction of dengue cases (Harrington and Edman 2001, Reiter 2007). Removal of harbourage sites (i.e. vegetation) is another effective and relatively inexpensive method of dengue prevention, particularly in areas with high populations of *Ae. albopictus*. After physical control, the more resource depleting methods of control include biological and chemical control.

1.6.2.1 Biological Control

For the purposes of this literature review, biological control is the control of dengue vectors by means exclusive of chemical (insecticides) and/or physical (source reduction). Historically, adult biological control was not considered an effective method of dengue control. There have been experiments that used fungi to control adult mosquitoes (Scholte 2006, Darbro et al. 2012) but so far no large scale field experiments with dengue vectors have been completed. With a continually growing understanding of genetics, it has been suggested that genetic manipulation may one day be a means of dengue control (Crampton et al. 1990, Scott et al. 2002). Crampton et al. (1990) has suggested that the mosquito genome could be altered to render the mosquito susceptible to certain chemical or environmental factors, or even render the mosquito unable to transmit the virus. Sterile males have been trialed as a control method (Phuc et al. 2007, Alphey 2010) for *Ae. aegypti*, however it is thought that this form of biological control would be very expensive and not appropriate in areas with large *Ae. aegypti* populations and endemic dengue fever.

Currently, there are experiments being conducted to determine if the bacteria *Wolbachia* will successfully reduce the lifespan of dengue vectors (Townson 2002, Rasgon 2003) or interrupt virus replication in the mosquito and consequently limit the transmission of the dengue virus. The results of these experiments are very promising (Moreira et al. 2009, Hoffman et al. 2011) however it will be some time before *Wolbachia* infected *Ae. aegypti* are released as a global means of dengue fever control.

Immature biological control is more common and has a history of greater success as a method of control. There have been many, less successful, attempts targeting immature mosquitoes as a biological control, such as the release of *Toxorhynchites splendens* (Wiedemann) larvae into water storage containers (Annis et al. 1989) and the introduction of exotic and native fish species (Russell et al. 2001). Both these control measures worked initially, but were not suitable long-term solutions. The *Tx. splendens* died of starvation once all the larvae in the containers were eaten, and this allowed re-infestation mosquito larvae into the containers (Annis et al. 1989). The native fish preferentially ate other fish or other aquatic organisms, such as frog tadpoles, over mosquito larvae (Russell et al. 2001). Nonetheless the successes of immature biological control far outweigh the failures. Immature biological control can be further divided into bacterial/microbial larvicides (Federici 1981, Quiroz-Martinez and Rodriguez-Castro 2007) such as *Bacillus thuringiensis* var *israelensis* (Bti) and the more "classical" biological control achieved by the copepods Mesocyclops spp (Lee 1986, Nam 1998, Kay et al. 2002). Bti has successfully been used to control mosquito populations in swamps and lakes for over 20 years and there is a current trend towards trialing Bti either alone or in combination with other

organisms as a method of control against container breeding mosquitoes (Riviere et al. 1987, Chansang et al. 2004, Kosiyachinda et al. 2003, Ritchie et al. 2010). In large water storage containers, like those found in Thailand and Vietnam and in some disused wells and mine shafts in Australia copepods have been a great success as a dengue vector control method (Riviere et al. 1987, Russell et al. 1996, Nam et al. 1998, Kay and Nam 2005, Nam et al. 2005). However there are still issues to be resolved with the application of Bti and copepods especially in countries like Australia and Singapore where the primary breeding site of the dengue vectors are smaller containers which may dry out, thus requiring continued reapplication of the control organism.

1.6.2.2 Chemical Control

While strictly a chemical, Insect Growth Regulators (IGR) often fall between the two classifications of biological and chemical control. These compounds mimic hormones produced by the immature mosquitoes and inhibit the emergence of adults from the treated breeding sites (Nelson et al. 1985). IGRs are very effective and specific, but expensive. Due to the specific action of IGRs, there is little impact on non-target organisms. The ability to use IGRs such as (*s*)-methoprene, in and around private premises make IGRs essential to Queensland Health vector control staff, who often treat drinking water in rainwater tanks and pet bowls (S.Long personal observation, 2009).

There can be problems when using IGRs as they don't immediately kill larvae. The presence of larval mosquitoes in a treated container may lead to unnecessary extra treatments or in some cases, infringement notices being issued to premise occupiers (Yap 1982), these scenarios are costly and time consuming, especially in a dengue epidemic where money, time and good public relations are precious commodities. Another control method that falls between the biological and insecticidal control was published by Romi et al. (2000) where they successfully controlled *Ae. albopictus* by adding metallic copper to breeding sites.

Generally mosquito control whether it be control of adults or the immature stages has relied strongly on traditional pesticides. From *Dichlorodiphenyltrichloroethane* (DDT) to Malathion and to the synthetic pyrethroids used today there has been a wide spectrum of insecticides applied and corresponding physiological resistance to the insecticides developed in the mosquitoes treated. Today there is a greater understanding of the methods of insecticide resistance and most vector control programs use a diverse integrated management program to limit resistance issues.

With the discovery of DDT and the widespread application of the chemicals there was a brief flare of hope for dengue elimination, when *Ae. aegypti* was considered eradicated from 22 countries in the American and Mediterranean regions (Gubler and Kuno 1997). After developing resistance to DDT and other related organochlorine insecticides, *Ae. aegypti* quickly re-infested areas where it was previously eradicated (Gubler and Kuno 1997). The failure of organochlorine chemicals to control *Ae. aegypti* on a long term basis resulted in a move towards organophosphate insecticides becoming more commonly used. Mosquito resistance to the organophosphate chemicals has begun to appear (Melo-Santos et al 2010, Hemingway et al. 2002). This resistance coupled with a greater understanding of the mode of action of organophosphorous chemical to mammals, particularly humans, has caused a shift toward the less mammalian toxic synthetic pyrethroids. With the evolution of the synthetic pyrethroids

into some of the lest harmful to mammal insecticides available today, a variety of 'novel' control methods have also been developed.

1.6.2.3 Chemical Application Methods

The use of chemicals for dengue control includes the use as insect repellents such as diethyl(meta)toluamide (DEET) and more recently Picardin that is applied directly to the body. There are chemicals , such as Metofluthrin, that are applied to other substrates, such as bed nets and mosquito coils, which are then used as control products, as they are able to protect against host seeking mosquitoes for long periods of time (Itoh 1993, Gupta et al. 1990, Argueta et al. 2004, Katsuda et al. 2008). A more recent chemical application, is the use of vaporising mats or control towers, which release chemicals such as Allethrin or Metofluthrin into the air to act as either a repellent or an adulticide (Chadwick and Lord 1977, Kawada et al. 2005). Edman et al. (1997) proposed using mosquito resting boxes with non repellent synthetic pyrethroid treated material attached to the inner box walls as a potential means of control. Historically, successful control of mosquitoes in a broad scale, involved the use of thermal fogging machines or Ultra Low Volume (ULV) misters mounted on a vehicle. Aerial ULV applications of chemicals have been reported to work as a means of dengue mosquito control, but with limited effectiveness during dengue epidemics (Perich et al. 1990). Nevertheless the most common dengue mosquito control application is fogging or mist application via a truck mounted spraying. The vehicle is driven up and down the streets of the community, spraying controlling agent over all objects within range. In communities with open design housing and *Ae. albopictus* as the vector, successful dengue control could be obtained using this method (Gratz 1991, Gubler and Kuno 1997) as the misted product enters houses and covers vegetation. However, in countries with *Ae. aegypti* as the principle vector and/or a more enclosed house design, fogging or misting from the street is less effective, as the *Ae. aegypti* are mostly safe indoors where the products cannot reach them.

To overcome this barrier Perich et al. (2001) showed that the application of fog/mist inside people's homes could be quite successful as a means of emergency dengue control. However, this is dependent on social acceptability, and in Queensland, misting inside people's homes is generally considered unacceptable. There are concerns about potential dangers of indiscriminate application of mist/fog to electrical equipment inside homes, exposure to misting products on the occupiers and their pets too (Moretto 1991). This means that dengue control in Queensland, and other similar locations, can't rely on the "classical dengue control methods"(Gubler and Clark 1996) as described above.

The alternative was a novel method, where ovitraps were used as a control method. Zeichner and Perich (1999) modified the classical ovitrap by exchanging the oviposition paddle with a heavy-weight velour paper strip that had been treated with a non-repellent residual surface spray. The smooth sides of the trap limited the visiting mosquitoes to the treated strip. As the mosquito laid her eggs she treated herself with a lethal dose of chemical and then flew off and died within the hour. The advantage of this control method is in the speed with which vector control officers can respond to a dengue case. Sithiprasasna et al. (2003) held reservations about the effectiveness of the Lethal Ovitrap (LO); the reported experiment had no source reduction, so the trap had stiff competition from other

potential breeding sites. In comparison Perich et al. (2003) showed the lethal ovitrap to be a successful means for controlling for both *Ae. aegypti* and *Ae. albopictus* again without the requirement for source reduction.

1.7 Dengue Control in North Queensland

While dengue is currently only a problem in North East Queensland, particularly Townsville north to the Torres Straits, dengue transmission has been recorded as far south as Gosford, New South Wales, Carnarvon in Western Australia and regions in between (Russell etal. 2009). For a variety of reasons *Ae.aegypti* has disappeared from all regions of Australia except North Queensland (Russell et al. 2009). Queensland has a long history of dengue outbreaks, caused by viraemic people entering the country (Kay et al. 1984, Ritchie et al. 2001, 2002, 2004, Montgomery et al. 2005, Ritchie 2005). Through campaigns to remove breeding sites and extensive, expensive responses to imported dengue cases, dengue is not yet endemic in North Queensland. Even though *Ae.aegypti* is endemic and the threat from *Ae. albopictus* is growing, vector control staff strive to prevent dengue from becoming established. If dengue were to become endemic in North Queensland there is potential for numerous deaths and the impact on the economy (the health system, Tourism etc) could be far reaching. Since the reappearance of dengue in North Queensland in 1981, Queensland Health vector control staff have worked towards developing and improving responses to dengue cases.

Following the shown successes of treating the interior of premises (Farrar et al. 2007, Hanna et al. 2001, Moretto 1991, Perich et al. 2001), Ritchie et al. (2002) began selective indoor residual spraying in North Queensland. Selective indoor residual spraying involved the application of a synthetic pyrethroid via a pneumatic hand held sprayer to undersides and backs of furniture in locations where *Ae. aegypti* was known to rest, such as under tables, chairs, couches, behind cupboards and dark areas in the laundry (Queensland Health 2005).

The idea of the interior residual spraying and source reduction was to form a dengue vector-free barrier around the viraemic person preventing the person from spreading the dengue virus to vector mosquitoes and the wider human community. Where possible, every premise within a 100 metre radius of the dengue fever case was treated with an interior residual spray, along with source reduction of any potential mosquito breeding sites. This 'response' was applied to every premise where it was thought that the viraemic person might have either obtained or spread the dengue virus (Montgomery et al. 2005, Ritchie 2005).

The use of interior residual surface spray and source reduction were successful in limiting and preventing dengue fever epidemics in North Queensland for several years (Montgomery et al. 2005, Ritchie 2005). Unfortunately interior spraying in the manner described by Ritchie et al. (2002) is time-consuming, an undesirable effect during an explosive dengue epidemic. A faster, easier and equally effective method of dengue control was required to prevent dengue becoming endemic in Queensland, and elsewhere in the world.

1.7.1 Lethal Ovitrapping

In North Queensland the use of lethal ovitraps as an adjuvant to larval control and limited interior residual spraying has successfully controlled *Ae. aegypti* and dengue since 2004 (Hanna et al. 2006, Montgomery et al. 2005, Ritchie 2005, Williams et al. 2006 ,Williams et al. 2007, Ritchie et al. 2009, Rapley et al. 2009). The lethal ovitrap currently used by Queensland Health vector control staff consists of a small 1.2 Litre plastic bucket with a flannelette material strip (5 x 15cm) (ovistrip) treated with a synthetic pyrethroid, usually Bifenthrin (Williams et al. 2007, Ritchie et al. 2008, Ritchie et al. 2009). The traps are baited with a 0.5 gram pellet of compressed lucerne (Ritchie 2001), filled with tap water and placed in locations around a house or building considered optimal for attracting mosquitoes (Williams et al. 2006). The use of the lethal ovitrap has decreased the time needed for vector control staff to complete a dengue control intervention, when compared with earlier interventions (Ritchie 2005). Other advantages in using lethal ovitraps include the reduction in insecticide use, operational costs and unwanted effects on non-target organisms (Williams et al., 2007).

However, the initial time saved to respond to a new outbreak has been counteracted by the increase in work needed post dengue response. The lethal ovitraps need to be collected before the insecticide and the ovistrip decay and become *Ae. aegypti* breeding sites themselves. To allow for lethal ovitrap collection, vector control staff must record the specific location and date the traps are set. This information then needs to be managed in a database to ensure the retrieval of the lethal ovitrap within the accepted timeframes. If the dengue intervention is remote or spread over a wide distance, managing the lethal ovitrap recovery can become costly and labour intensive (Gubler 1989, Montgomery et al. 2005, Ritchie 2005, Ooi et al. 2006) both unacceptable during dengue epidemics. To combat this Ooi et al. (2006) suggested members of the community are given responsibility of their own ovitraps, which they hope would also second as a reminder of the risk of dengue and joint responsibility of its management in their community.

To combat these obstacles Ritchie et al. (2008, 2009) developed a biodegradable lethal ovitrap (BLO). BLO buckets are made from thermoplastic starch (60%) (Plantic Technologies Ltd. Altona, Victoria, Australia), known as Enpol instead of 100% plastic (Ritchie et al. 2008). They are designed to be set and allowed to degrade over time, removing the need for trap retrieval (Ritchie et al. 2008, Rapley et al. 2009). A chemically treated material strip is added to the ovitrap in a similar manner as the current lethal ovitrap. The biodegradable ovitrap does not require an infusion as it produces its' own infusion as the starch in the bucket breaks down. The biodegradable ovitrap is designed to start breaking down in the field, before the chemically treated material strip is no longer effective.

The use of a biodegradable bucket instead of the current plastic bucket would resolve many of the current disadvantages of using lethal ovitraps for dengue control and prevention. A biodegradable lethal ovitrap can decay in the field and not become a potential breeding site. Thus it is not essential that the biodegradable lethal ovitrap be retrieved, as they will breakdown if householders choose to not dispose of the remains, after they are no longer effective (post four weeks) (Ritchie et al., 2008). Using biodegradable lethal ovitraps in dengue control would be an environmentally safe alternative to current

dengue control methods. Community acceptability would enable the biodegradable trap to become the latest tool in the fight against dengue, in Australia and around the world (Ritchie et al. 2008, 2009).

The research in this thesis aims to help direct future research into the BLO, by determining what happens to the BLO after four weeks. Does the BLO become a mosquito breeding site or does it degrade over time? How long does it take for the trap to degrade and while it is degrading is it causing any harmful effects on the non-target fauna around it? And would the public be interested in using the BLO as a type of personal protection where by premise occupiers set the BLOs themselves, leaving vector control staff free to focus on more urgent dengue cases or other control activities?

1.8 Aims of Thesis

The aims of this research is to determine the effectiveness of the BLO as a dengue control tool, for up to four weeks post-deployment. This research also aims to investigate what impact, if any, the BLO might have on the non-target fauna in the immediate area of the set ovitrap, and lastly, this research aims to determine public acceptability of the BLO as residential mosquito reduction tool, to protect against mosquito-borne diseases.

2.0 Effective longevity of a biodegradable lethal ovitrap used for control of *Ae. aegypti* in Cairns, Australia

2.1 Introduction

In North Queensland the use of lethal ovitraps (LOs) as an adjuvant to larval control and limited interior residual spraying has successfully controlled *Aedes aegypti* and dengue since 2004 (Montgomery et al. 2005, Ritchie 2005). The LO currently used by Queensland Health vector control staff consists of a small 1.2L plastic bucket with a flannelette strip (5 x 15cm) (ovistrip) treated with a synthetic pyrethroid, usually bifenthrin (Williams et al. 2007, Ritchie et al. 2008, Ritchie et al. 2009). The traps are baited with a 0.5g pellet of compressed lucerne (Ritchie 2001), filled with tap water and placed in locations around a house or building that are optimal for attracting mosquitoes (Williams et al. 2006). The use of the LO has decreased the time needed for vector control staff to complete a dengue control intervention when compared with earlier interventions (Ritchie 2005).

However, the initial time saved to respond to a new outbreak has been counteracted by the increase in work needed post dengue response, as the LOs need to be collected before the insecticide and the ovistrip decay and become *Ae. aegypti* breeding sites themselves. To allow for LO collection, vector control staff must record the specific location and date the traps are set. This information then needs to be managed in a database to ensure the retrieval of the LO occurs within the accepted timeframes. If the dengue intervention is remote or spread over a wide distance, managing the LO recovery can become costly and labour intensive with potentially hundreds of LOs set (Montgomery et al. 2005, Ritchie 2005.).

The use of the Biodegradable Lethal Ovitrap (BLO) removes the need for managing location and setting data and allows for a 'set and forget' strategy. With the need to return to a premise to

collect the ovitrap removed, vector control staff can spend more time and resources preventing further cases of dengue. Ritchie et al. (2008, 2009) reported on the development of the BLO, its acceptability to ovipositing mosquitoes and its ability to degrade under standard composting conditions. The following experiments are designed to answer some of the questions raised by Ritchie et al. (2009), specifically length of time the BLO can act as a potential mosquito breeding site under field conditions, the attractiveness of the older BLO to mosquitoes as a potential breeding site and the potential for the BLO to become a mosquito breeding site.

2.2 Materials and Methods

2.2.1 Experiment One: BLO longevity and their properties over time

Ritchie et al. (2008, 2009) have shown that the biodegradable ovitrap is successful in controlling *Ae. aegypti* for the standard dengue response period - four weeks. This experiment was designed to determine the outcome of the biodegradable lethal ovitrap after the four week period.

Four types of traps were used in the experiments; three types of BLOs produced in 3 different batches by the manufacturers, Plantic Technologies Ltd. (Altona, Victoria, Australia), and a standard lethal ovitrap (LO) as a control (Ritchie et al. 2009) (Table 2.1)

Table 2.1. Biodegradable Lethal Ovitrap batch numbers and their corresponding plastic concentration

Ovitrap Label	LO	BLO A	BLO B
Batch Number	n/a	88	89
Plastic Concentration	100%	40%	30%

A combination of BLOs and LOs were set at 15 residential premises in the Cairns area. At each premise, two of each of trap types "LO ", "BLO A" and "BLO B"(differing by plastic content) were set (Table 2.1). One of each trap type was positioned on a solid substrate (e.g. concrete, tile) and one on a porous substrate (e.g. dirt, mulch).
The BLOs were deployed with a Bifenthrin-treated lethal ovistrip and 1 Litre of water, and the LOs with a Bifenthrin-treated lethal ovistrip, 1 L of water and a 0.5 gram pellet of lucerne as per Ritchie et al. (2008).

The experiment was continued until the all traps 'failed'. A BLO 'failed' when it was no longer able to hold water. For LOs, 'failure' was the complete degradation of the lethal ovistrip.
When the water level in any trap fell below 200 millilitres (ml) the traps were refilled with tap water. Traps were inspected weekly and the presence of eggs on the lethal ovistrip, trap integrity, lethal ovistrip integrity and smell were assessed. The smell of the traps was assessed to determine if odour problems experienced in previous versions of the BLO (Ritchie SA, personal communication, 2007) were resolved with the new batches (Table 2.2).

Table 2.2. Rankings used in determining smell of the BLO

Rank	Trap odour
0	Minimal or no obvious smell
1	Faint smell
2	Strong smell
3	Extreme smell/ putrid

Lethal ovistrip integrity was ranked as the percentage of the ovistrip (above the waterline) covered in organic matter (e.g. fungi) that could prevent mosquitoes landing on the lethal ovistrip.

Table 2.3. Rankings used in determining lethal ovistrip integrity

Rank	Visual impression
0	Ovistrip looks clean and new
1	< 25% of exposed ovistrip covered in organic matter
2	25 – 75 % of exposed ovistrip covered in organic matter
3	> 75% of exposed ovistrip covered in organic matter

SPSS 15 Grad Pack (SPSS Inc., Chicago, IL) was used to perform data analysis. The ovitrap condition, numbers of eggs at trap failure and trap odour were all descriptively compared. An analysis of variance (ANOVA) was used to compare means of trap longevity and qualities across the trap types and across setting surface types. The egg data were log transformed to normalise their distribution allowing for parametric testing. Further analysis of the data was performed using Levene's test for equality of variance, before 2-sided independent t-tests. The ANOVA results are displayed using the untransformed data alongside the means and medians for ease of interpretation.

2.2.2 Experiment Two: BLO longevity under 'set and forget' conditions

This experiment was conducted to determine if handling the BLOs on a weekly basis had a significant impact on the longevity of the BLOs, when in the field.

This experiment used BLOs from a new batch of BLOs (#198- 40% Plastic - BLO C) as no BLOs from the earlier batches remained. The BLOs were set at sixteen backpacker hostels in the Cairns City area (Figure 2.3). Each hostel had three BLOs set on the property (n= 48). The BLOs were set with a bifenthrin-treated lethal ovistrip and 1L of tap water in locations protected from the weather according to the standard procedures used during a dengue response (Ritchie 2005, Williams et al. 2006). A weekly inspection of the BLO was conducted where the trap was visually inspected but left undisturbed. However, if the trap was found dry, it was refilled. The trial continued until all traps failed by not being able to hold water.

Experiment 2 results were compared with the BLO results from Experiment 1, again using SPSS 15 Grad Pack statistical software.

2.2.3 Experiment Three: Larval survival in BLOs

This experiment was designed to determine if *Ae. aegypti* larvae could establish in BLOs once the BLOs were left in the field over four weeks.

This experiment used the same BLOs as experiment two (#198- 40% Plastic - BLO C). To minimise the risk of BLO destruction by chewing insects, all of the traps in this experiment were kept in large, open plastic crates that had been sprayed with a residual synthetic pyrethroid. The crates were set at one house and in locations protected from direct rain and sunlight, as close to normal ovitrap procedures as possible. Oviposition by wild mosquitoes was not excluded. Ten BLO and ten LOs were set with bifenthrin-treated lethal ovistrips. For controls, five BLO and 5 LOs were set with untreated ovistrips. The LOs also contained one lucerne pellet. The traps were filled with tap water to within 1

cm of the trap top. Every seven days, the ovitraps were checked and any visible larvae removed with a disposable pipette unique to each ovitrap, then 10- 3rd instar *Ae. aegypti* were added to each ovitrap. To assess survival to adulthood, the larvae were collected out of the ovitraps after 24 hours, placed in 75 ml jars with fresh water and monitored for 72 hours. The water in the ovitraps was topped up each week after the larvae were removed. The data was collated, graphed and t-tests performed using Microsoft Excel 2003 (Microsoft Corp, Redmond, WA).

2.3 Results

2.3.1: Experiment 1: BLO longevity and their properties overtime

The week of trap failure, total number of eggs collected and lethal strip integrity scale were all highly significantly different ($p<0.01$) between the LOs and the BLOs (Table 2.4). There was also a significant difference ($p<0.01$) between the average number of eggs per week calculated at time of trap failure (Table 2.4). Of the 60 BLOs set in this experiment, 57 failed from holes being chewed in them by arthropods (most likely cockroaches), leaving only three traps to fail by splitting. There was no difference ($p=0.83$) in the smell scale of the traps (Table 2.4).

The LOs were also excluded for the analysis of the BLO trap types by substrate type (Table 2.5). There was no difference between the longevity of BLO A and BLO B, although there was a trend towards significance ($p= 0.07$). The total number of eggs collected in each BLO ($p= 0.34$) and the strip integrity scale at time of trap failure ($p= 0.16$) did not differ significantly. There was a significant difference between the weeks at which the traps failed ($p<0.01$) depending on the surface on which the BLOs were set. When the BLOs were set on a solid substrate there was a median of 5 weeks until trap failure (mean $7.40 \pm$ SE 1.16), while the BLOs set on a porous substrate had a median week of failure of 3 (mean $4.30 \pm$ SE 0.70). The mean number of eggs per week laid in BLOs set on porous substrates (4.60 ± 1.97) was higher than in BLOs set on solid substrates (2.30 ± 0.54) ($p= 0.05$). There was no difference between the total eggs collected and the lethal strip integrity scale at time of trap failure.

Table 2.4 Results of analysis of variance (ANOVA) comparing ovitrap LO (n=30), BLO A (n=30) and BLO B (n=30) longevity and qualities. Raw data are displayed to provide more meaningful interpretation.

Variable compared	Trap type	Raw data comparison		ANOVA	
		Mean	Median (Range)	F	Significance
Week at which trap failed	LO	10.37	11.00 (3.00-15.00)	9.06*	<0.01
	BLOA	6.27	4.00 (1.00-25.00)		
	BLOB	4.57	3.00 (1.00-13.00)		
Total number of eggs on ovistrip counted at time of trap failure	LO	58.00	41.00 (5.00-158.00)	20.41*	<0.01
	BLOA	14.14	7.00 (0.00-110.00)		
	BLOB	9.75	5.00 (0.00-50.00)		
Average eggs per week calculated at time of trap failure	LO	8.15	3.43 (0.50-40.70)	3.59*	0.03
	BLOA	4.44	0.73 (0.00-55.00)		
	BLOB	2.54	1.00 (0.00-10.00)		
Scale of trap smell at time of trap failure	LO	1.23	1.00 (0.00-3.00)	0.19	0.83
	BLOA	1.10	1.00 (0.00-3.00)		
	BLOB	1.27	1.00 (0.00-3.00)		
Scale of lethal strip integrity	LO	2.67	3.00 (1.00-3.00)		

at time of trap failure	BLOA	1.67	2.00 (0.00-3.00)	14.07	<0.01
	BLOB	1.20	1.00 (0.00-3.00)		

*log transformed

2.3.2: Experiment 2. BLO longevity under 'set and forget' conditions

The median week of trap failure for the three trap types differed significantly with the BLO C traps set under 'set and forget' conditions lasting for a median of 9 weeks (Table 2.5). There was a difference in the total number of eggs collected in the trap types between the experiments (p= 0.04), but not between the average number of eggs laid in each trap type per week (Table 2.5).

Table 2.5. Comparison of the three different BLO types used in Experiments 1 and 2

Variable compared	Trap	Raw data comparison Median (Range)	ANOVA F	Significance
Week at which trap failed	BLO A	4.00 (1.00-25.00)		
	BLO B	3.00 (1.00-13.00)	7.31	<0.01
	BLO C	9.00 (1.00-23.00)		
Total eggs collected in trap counted at time of trap failure	BLO A	7.00 (0.00-110.00)		
	BLO B	5.00 (0.00-50.00)	3.50	0.04
	BLO C	3.00 (0.00-629.00)		
Mean number of eggs per week calculated at time of trap failure	BLO A	0.73 (0.00-55.00)		
	BLO B	1.00 (0.00-10.00)	0.57	0.57
	BLO C	0.44 (0.00-44.90)		
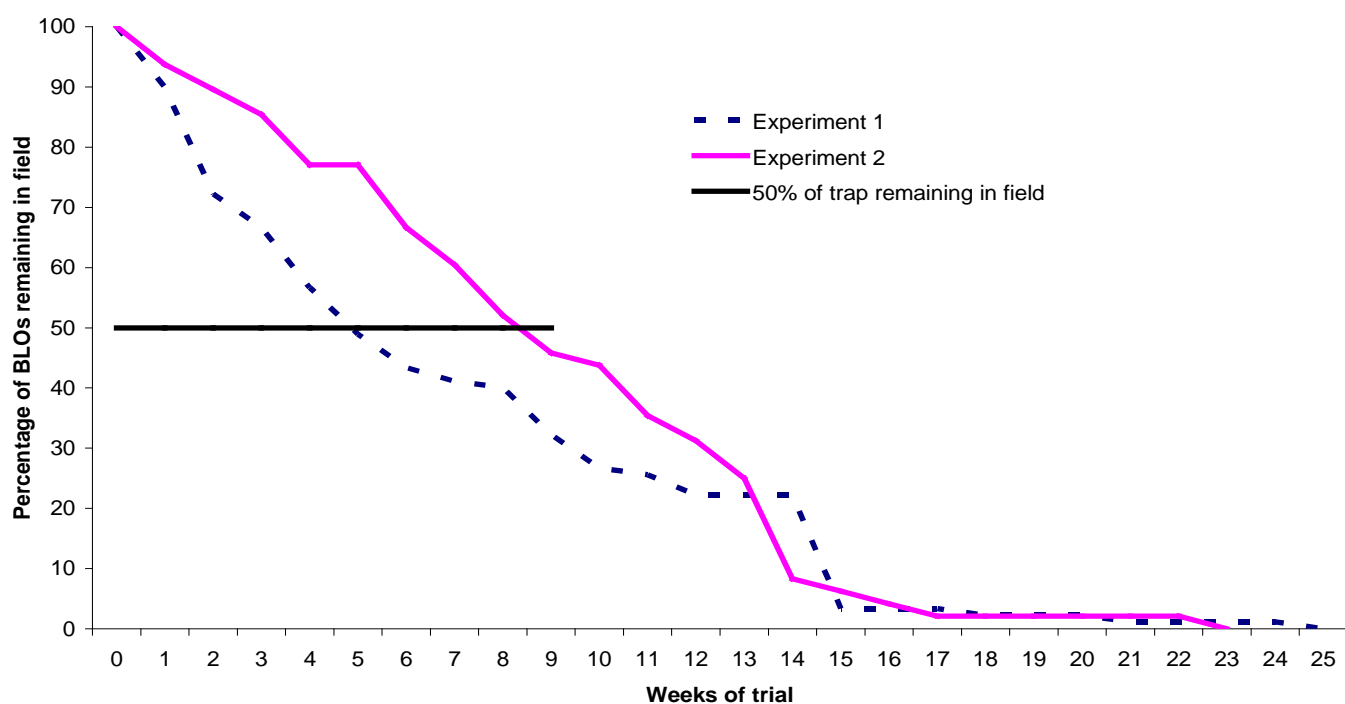
Figure 2.1 Comparison of the percentage of BLOs remaining in the field during the two longevity experiments in Cairns, QLD. Experiment 1 is the combined results of trap types "A" and "B" and when traps were physically examined each week. Experiment 2 used trap type BLO C and these were visually inspected but left undisturbed each week

The two longevity experiments both lasted beyond 22 weeks (Figure 2.1). In Experiment 1 (BLO A & B), a greater rate of BLO failure occurred during the early stages of the experiment in comparison with the BLOs used in Experiment 2 (BLO C). However, Experiment 2 saw the failure of all the BLOs earlier than Experiment 1.Of the 48 BLOs set in experiment two, 46 failed, due to holes created by insect chewing and the remaining two could not be found, believed to have been discarded by hostel guests.

Figure 2.2 Invertebrate chewing on a biodegradable lethal ovitrap set under field conditions in the Cairns area for 1 week set on a nonporous surface
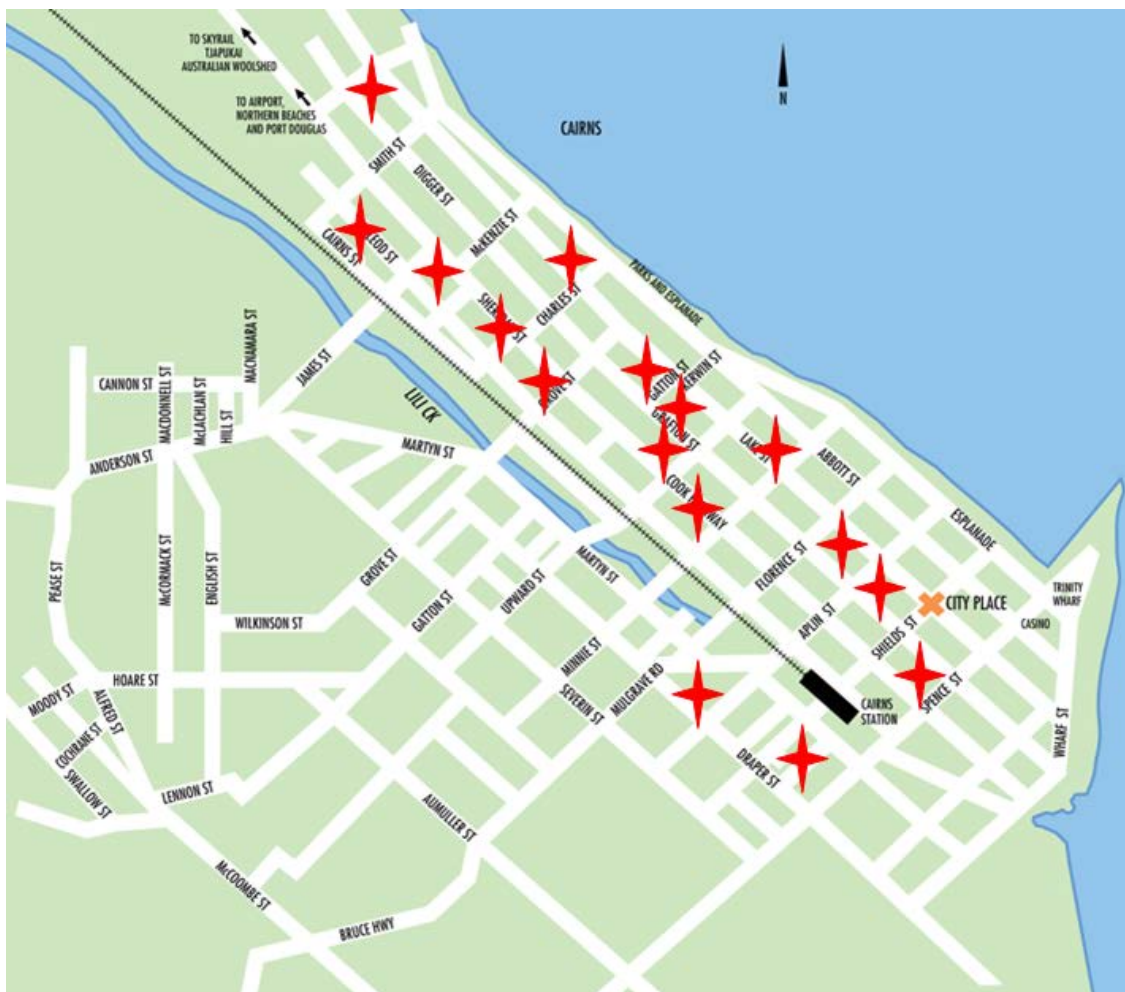
Figure 2.3 Locations of hostels used in BLO longevity and non-target experiments, Cairns, North Queensland

2.3.3 Experiment Three: Larval survival in BLOs
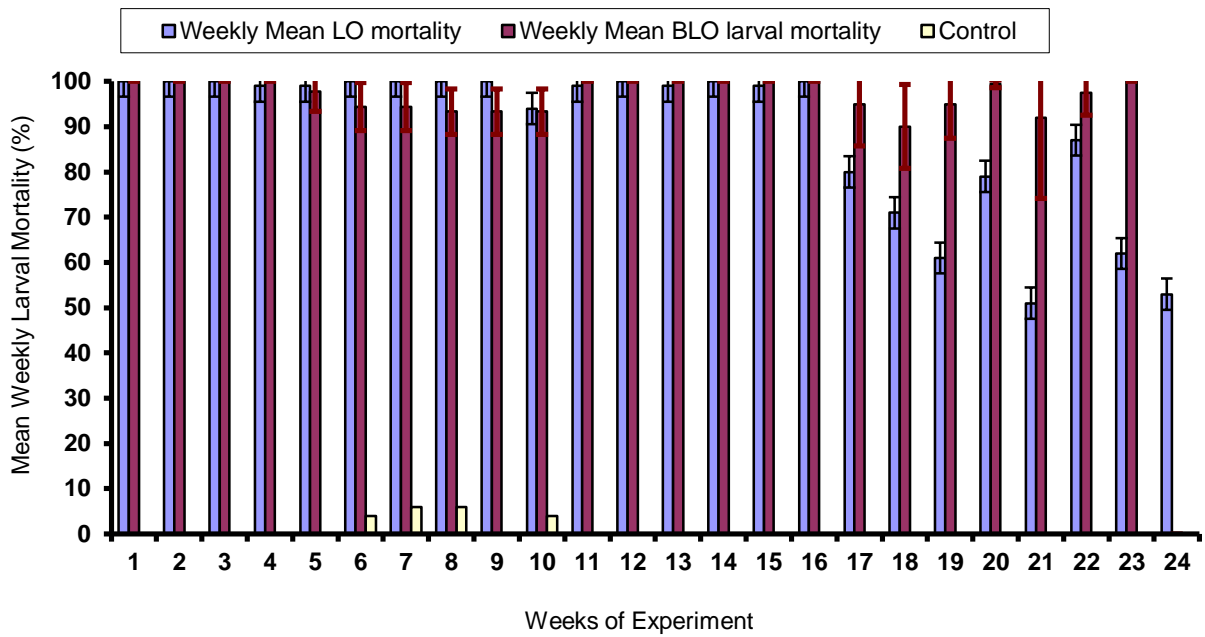
Figure 2.4 Mean larval mortality (± SE) of 3rd instar *Ae. aegypti* larvae exposed to bifenthrin treated lethal ovistrips in BLOs and LOs over the time BLOs can survive under field conditions.

The mean weekly larval mortality was calculated from the functional traps each week. Mortality rates of 3rd instar *Ae. aegypti* in the BLOs remained over 90% for the life of the BLOs (Figure 2.4), while larval mortality in the LOs remained over 90% only until week 17. Mortality rates of larvae in untreated control ovitraps (BLOs and LOs) was either zero or less than 10%. By week 9, all the traps (LOs and BLOs) had *Culex quinquefasctiatus* adults emerging from them.

By week 21, 50% of the BLOs had failed due to insect chewing and were no longer holding water. By week 24 all the BLOs had failed (Figure 2.4). Larval mortality in the BLOs was not statistically significantly throughout the experiment. Larval mortality in the LOs was not statistically significant up to week 17. After week 17 there was statistically significant (t-test, p=0.01) differences in the larval mortality in the LOs (Figure 2.4)

2.4 Discussion

These experiments confirm that a Bifenthrin treated BLO will not become an *Aedes* mosquito breeding site if left in the field longer than four weeks. Zeichner and Perich (1999) demonstrated that a Deltamethrin LO exposed to field conditions for 6 months killed 89% of *Ae.aegypti* larvae. Similarly, the larval mortality experiment we conducted shows the BLO to be active against *Ae. aegypti* larvae beyond 23 weeks. However, the BLO may become a breeding site for *Culex* mosquitoes if still holding water beyond 9 weeks. This is due to different larval feeding behaviour between *Culex* and *Aedes* mosquitoes. The active insecticide in the lethal ovistrip is consumed by the grazing *Aedes* larvae but not by the filter feeding *Culex* (Clements 1992, Merritt et al. 1992). While *Cx. quinquefasciatus* do not regularly feed on humans, they will if alternative food sources are unavailable. Thus BLOs could potentially become a prolific 'nuisance'-mosquito breeding site, and this needs to be considered when trying to gain public support. The BLO breeding *Culex* mosquitoes could also be a serious problem in other countries, such as the USA where other mosquito borne diseases are transmitted by *Culex* mosquitoes, such as West Nile virus (Zinser et al. 2004).

The results presented here suggest that BLOs with a higher plastic concentration, as suggested by Ritchie et al. (2008), do last longer in the field. These results also suggest that the BLO has a longer field life when set on a solid substrate (median week to failure = 5) than on a porous substrate (median week to failure = 3). These results are different to those reported by Ritchie et al. (2009) who found no difference in the survival time of the BLOs regardless of the substrate the BLOs were set on. After four weeks Ritchie et al. (2009) had 51% of their BLOs with holes from probable insect chewing, whereas the experiments reported here all had greater than 95% of the BLOs with holes chewed after four weeks. The apparent increase in BLO survival when set on non-porous surfaces such as tile and concrete can be attributed to the decrease in interaction between cellulose feeding organisms with the BLO. The problem is that many of the non-porous surfaces available to set the BLO can be stained from the water weeping out of the BLO (S. Long personal observation 2009). While this staining doesn't occur with each batch of BLOs, it does occur often enough to be of concern and must be consideration when setting the BLOs on private property. Setting the BLOs on nonporous surfaces also limits where you can set the BLO for mosquito control. Setting the BLO in locations to ensure survival of the trap can potentially decrease the effectiveness of the BLO in dengue prevention and control- defeating the purpose of the BLO. It would be possible to have a plate on which the BLO could sit but this would also defeat the idea of the BLO as a practical "set and forget" trap. The BLO is designed to be an environmentally friendly LO that can degrade in place, or be thrown into a compost heap. The presence of a plate (paper or plastic) would decrease the environmentally friendly aspect of the BLO and would require the premise occupier to dispose of the plate. Care would also be needed to make sure that the plate couldn't breed mosquitoes but had high enough sides to deter the non-target organisms. Furthermore, vector control staff would need to carry additional equipment during dengue prevention or control, reducing practicality.

Use of BLOs for dengue responses, has provided strong evidence that the survival of the BLO is partially dependent on the chewing-insect population in the response area. In two dengue

interventions using BLOs, all the BLOs set within a 100 m radius of a dengue contact address had leaked water from holes caused by chewing within 7 days, while other responses saw >95% of BLOs still functioning after three weeks (S. Long personal observation 2009).

The risk of the BLOs failing, as observed in the longevity experiments, means that there is potential for a gap in the control barrier (around the dengue case) that could allow dengue infected mosquitoes to break through vector control barriers and cause dengue cases in untreated areas. This potential risk would be essential for vector control staff to be aware of.

The suggestion by Rapley et al. (2009), that the BLOs should be trialed in an area before deployment, is a good idea, especially in light of the variations in BLO survival. However, due to time constraints, it might not be realistically possible to pre-test during dengue responses. Each suburb would have to be trialed prior to vector control staff deploying BLOs for dengue response and the results may change from season to season and year to year.

While statistically the BLO would seem to be a suitable alternative for dengue control, as approximately 50% of the traps would survive beyond 4 weeks, and the traps are designed to be set and forgotten, the unpredictable rate of the BLOs failure make most vector control staff nervous and hesitant to rely on BLOs as a control measure. It would be possible to apply an insecticide to the BLO or substrate prior to setting the BLO, but this would increase the chemical exposure to people, non-target fauna and provides no guarantees that invertebrate chewing will be prevented. Applying an insecticide to the BLO or substrate prior to setting, would counteract the benefits of using the LO techniques for dengue control. There is also some risk that the BLOs may play a role in chemical resistance by mosquitoes being exposed to non-lethal doses of insecticide from lethal ovistrips that are no longer 100% effective. So until a reliable invertebrate consumption resistant BLO is developed, vector control requires additional time and resources required to respond to dengue using the LOs.

3.0 The diversity of non-target fauna found in ovitraps set in the field, Cairns, - North Queensland

3.1 Introduction

Public awareness of personal risk and ecosystem risk, has contributed to the undertaking of many studies assessing mosquito control impacts on non-target organisms. Research on the impacts on non-target organisms can be divided into: larvicides (Didia et al. 1975, Mulla et al. 1979, Yap et al. 1982, Clark et al. 1989, Mortimer et al. 1995, Brown et al. 1996, Stevens et al. 2011), adulticides (Coldburn and Langford 1970, Flexner et al. 1986, Milam et al. 2000, Davis et al. 2007) and chemical application techniques (Jensen et al. 1999, Boyce et al. 2007, Kwan et al. 2009, Oberhauser et al. 2009). However, there is limited information available on the impacts 'mosquito traps' have on non-target fauna.

Pucci et al. (2003) found that 49.6% of all specimens collected in field trials for the Mosquito Magnet Pro® were non-targets. Chironomidae (non-biting midges), Psychodidae (moth flies) and Cecidomyiidae (gall midges) were the main Dipterans collected by the Mosquito Magnet Pro®, with undetermined Coleoptera and Hymenoptera also abundant. In a small study by Frick and Tallamy (1996) on electric insect traps, "Zappers" in suburban Newark, Delaware, they stated the zappers were "worthless for biting fly reduction and probably counterproductive to homeowners and other consumers" and the traps were "anything but benign" to the non-target population. Biery (1974) reported on entomological impact of ultra low volume aerial spraying at Robins Air Force Base, Georgia. He discussed the changes in the numbers of non-target arthropod orders identified pre and post application of Dibrom 14 insecticide (85% Naled). He used 0.5 m^2 aluminium foil covered cardboard squares coated with "Stickum" to monitor the knockdown of flying insects. Biery's report also discusses the changes in the number of arthropod orders collected in CDC Miniature and New Jersey light traps. He concluded that results from the New Jersey light traps were inconclusive because of irregularities in the pre-spray sampling, while the results from the CDC miniature light trap suggest the aerial spray application had no substantial toxic effect on non-target arthropods. Burkett et al. (1998) testing mosquito attractiveness to different coloured Light Emitting Diodes (LEDs) on mosquito traps. They found that all colours tested were equally attractive to the tabanid *D. ferrugatus*, while chaoborid flies (Corethrella spp.) showed significant preference for white and blue light over other colours.

There are no published studies on lethal ovitrap impact on non-target organisms. Lethal ovitraps exploit the ovipositing behaviour of *Aedes* mosquitoes while limiting chemical exposure to vector control staff, the public and non-target organisms (Zeichner and Perich 1999, Ritchie 2005, Perich et al. 2003). Queensland Health vector control staff in North Queensland use small black 1.2 L buckets, with a flannelette cloth strip (for ovipostion) treated with bifenthrin as a lethal ovitrap (Ritchie 2005, Williams et al. 2007). Since the development of the BLO in 2008 (Ritchie et al. 2008, Rapley et al. 2009) vector control staff in Cairns, North Queensland, have been using the BLOs in their response to dengue notifications. As the BLO buckets are made from thermoplastic starch (Ritchie et al. 2008), they are prone to invertebrate consumption around the base (Rapley et al. 2009).

This consumption of the starch based plastic creates holes that render the ovitrap unviable: unable to hold water, which is required to attract ovipositioning mosquitoes.

After piloting the BLOs, the discovery of holes from invertebrate eating raised the question of the impact of lethal ovitraps on the non-target fauna. To quantify the impact of lethal ovitraps on the non-target fauna, we aimed to assess the number and variety of non-targets that visit lethal ovitraps over a period of one year in Cairns, North Queensland.

3.2 Materials and Methods

The Standard Sticky Ovitrap (SSO) was used as a proxy for the LO and BLO to sample the non-targets visiting the ovitrap. The SSO used in these experiments consists of a small 1.2 L bucket with two -15 x 5.5 cm plastic strips coated in polybutylene adhesive (UVR-32, Atlantic Paste and Glue, Brooklyn, NY) fastened to the opposite inner walls of the bucket with 50 mm paperclips. The traps were filled with tap water to the base of the glue strips and a 0.5 g lucerne pellet was added. A hole was drilled into the side of the bucket, above the water level, to prevent rainfall from overflowing the bucket and subsequently wetting the glue strips.

The BLO also contained two glue strips attached with thumbtacks, tap water was filled to the base of the glue strips, but lucerne was omitted.

The classification of the non-targets insect follows that described in The Insects of Australia (2000), published by CSIRO.

3.2.1: Experiment 1: Standard sticky ovitrap as a proxy for Biodegradable Lethal Ovitraps

This experiment was designed to determine if the SSO was a suitable proxy for the LO and BLO to sample the non-targets that would visit ovitraps.

In a field study at 16 Backpacker hostels in the Cairns area, two SSO and two BLO sticky (BLO-S) were set at each property. The ovitraps were set in discrete locations protected from the weather and out of sight of the hostel guests. The ovitraps were inspected weekly- over five weeks and the glue panels replaced at each inspection. The water in the ovitraps was sieved through a fine nylon colander (Retail Australia Pty Ltd, North Ryde, NSW). The non-targets in the water were collected and stored in 70% ethanol for subsequent identification in the medical entomology laboratory.

Data analysis consisted of counts of Orders/Family were collated and graphed using Microsoft Excel 2003 (Microsoft Corp, Redmond, WA).

3.2.2: Experiment 2: Non-targets in sticky ovitraps over one year

This experiment was designed to use the SSO as a confirmed proxy for the BLO and LO to discover what non-targets visited ovitraps over a 12 month period.

SSOs were set at 16 Backpacker Hostels in the Cairns area. The traps were set in discrete locations protected from the weather and out of sight of the hostel guests. Every week the glue strips were replaced and returned to the lab where the non-targets collected were identified.

The data was collated, graphed and Chi square tested using Microsoft Excel 2003 (Microsoft Corp, Redmond, WA).

3.2.3: Experiment 3: Impact of Lethal Ovistrips on visiting non-targets

This experiment was designed to determine if the lethal ovistrip caused any impact on the non-targets that visit the ovitraps.

Due to a limit on the number of available BLOs, this experiment was conducted at only nine of the 16 Backpacker Hostels in the Cairns area. Each hostel had a BLO and a LO set as per a dengue response in a location protected from the weather but visible to the mosquitoes. Each BLO/LO had two glue panels (5 x 15 cm) and a red flannelette lethal ovistrip (5 x 15 cm, treated with bifenthrin) attached between the two glue panels. The LO also had a 0.5 g lucerne pellet. The traps were filled to the base of the glue panels with tap water.

The traps were inspected twice weekly and the glue panels changed at each inspection visit. The water in the traps was sieved to collect any non-targets dead in the water. The non-targets collected from the trap water were stored in 70% Ethanol. The glue panels and sieved non-targets were returned to the lab where they were identified. After seven days the trap locations were swapped and the water level was topped up to the base of the glue panels as required.

Data analysis consisted of counts of Orders/Family which were collated and graphed using Microsoft Excel 2003 (Microsoft Corp, Redmond, WA). Family count data were log (x+1) transformed and a t-test was used to compare mean counts by family between SOs and BLOs.

3.3 Results

Due to the poor condition of some specimens stuck in the glue and the skill of the identifier, not all non-targets could be identified past order but where possible specimens were identified to family. Any vertebrates collected were identified to species where possible. Those specimens identified as Micro refer to any specimen smaller than 2 mm that could not be identified further.

3.3.1: Experiment 1: Standard sticky ovitrap as a proxy for Biodegradable Lethal Ovitraps
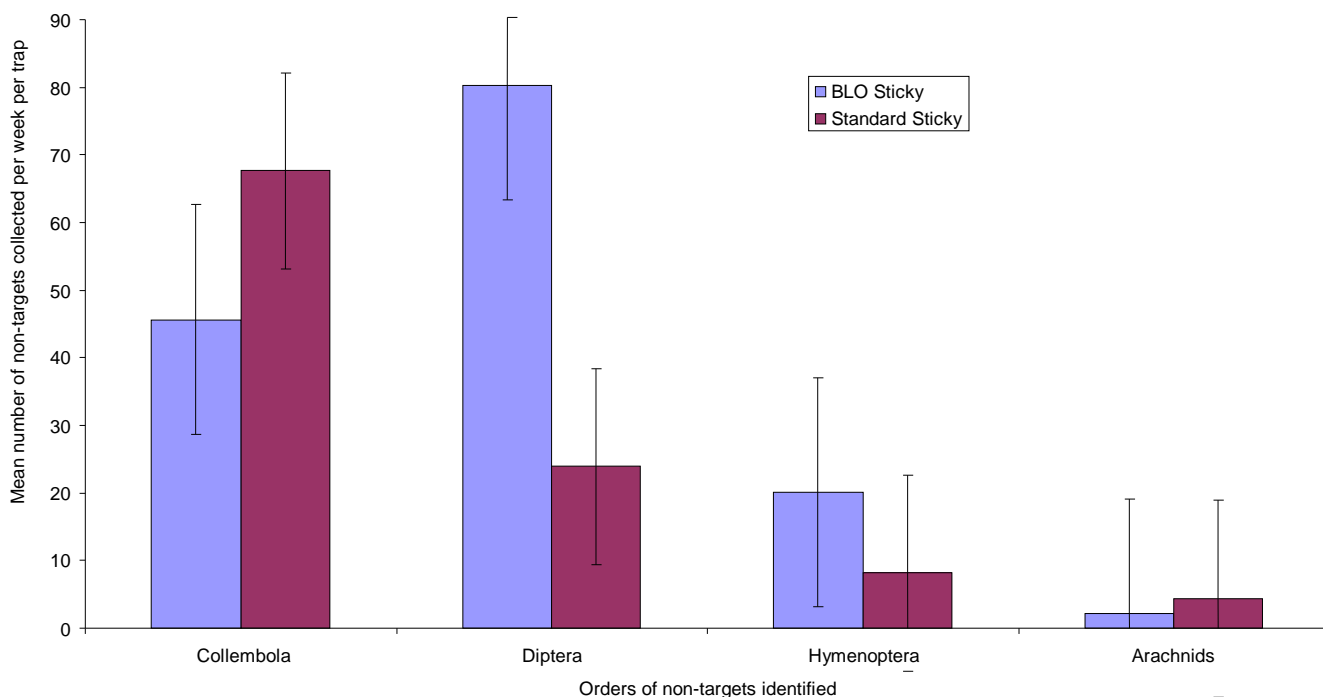
Figure 3.1 Comparison of the mean number of specimens collected per week/trap (±SE) of the major non-target orders collected on the glue panels in the standard sticky ovitrap versus biodegradable lethal ovitrap sticky, over five weeks
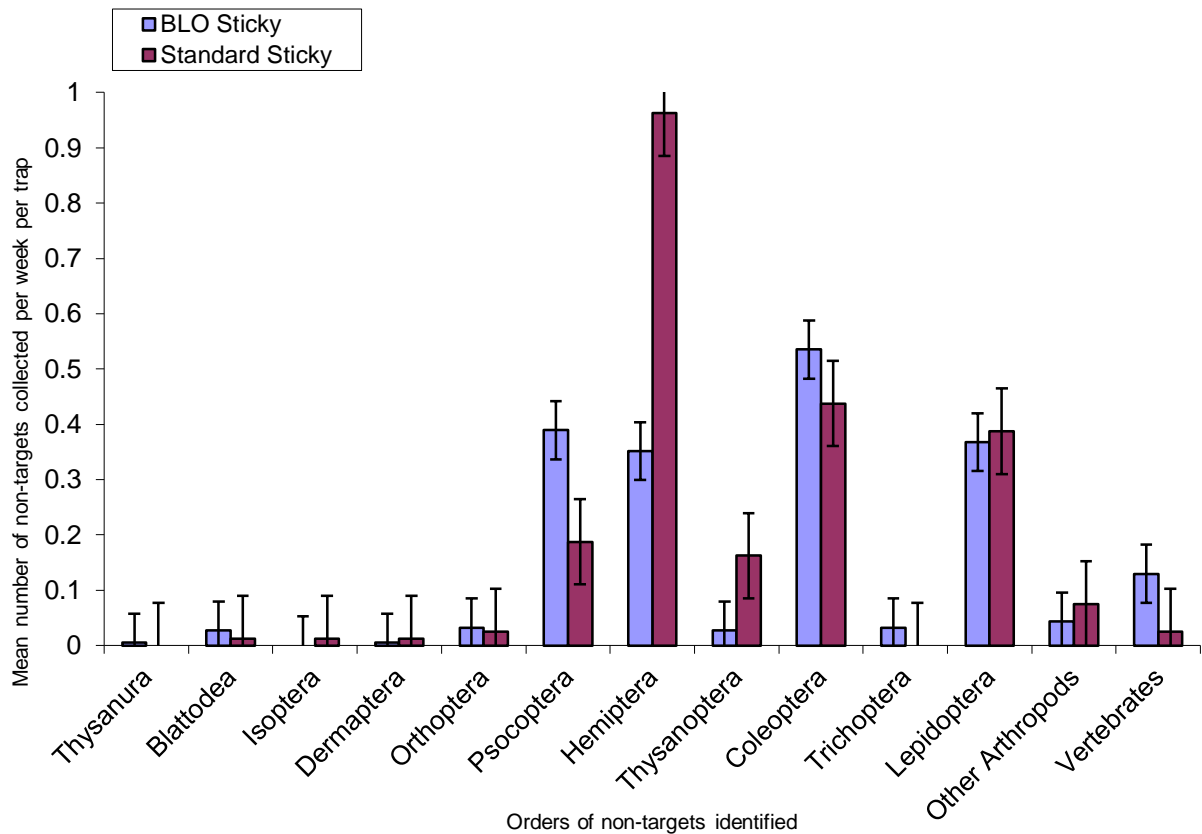
Figure 3.2 Comparison of the mean number of specimens collected per week per trap(±SE) of minor non-target orders collected on glue panels, standard sticky ovitrap versus BLO-S, over five weeks

The SSO collected the same orders and families of non-targets as the BLO-S except for Trichoptera (Caddis-flies) which were collected in the BLO but not in the SO (Fig. 3.2). The difference in the mean number of non-targets collected per week in the traps is a result of trap location variation rather than differences between the trap types. For example, during week one a SO collect 10 aphids while its paired BLO-S caught one. In week two of the trial, three BLO-Ss each collected over 100 Muscidae, while their paired SSO collected fewer than 20 specimens combined. These differences in numbers account for the differences in the mean number of non-targets collected per trap per week in Figures 3.1 and 3.2.

3.3.2: Experiment 2: Non-targets in sticky ovitraps over one year

A total of 44,132 non-target specimens were collected in the year long survey of non-targets collected in SOs. Only 19 (0.04%) were completely unidentifiable. Collembola made 44.2% of the non-targets, while Diptera and Hymenoptera made up 36.8% and 10.5%, respectively. Interestingly the ovitraps collected 4 termites (Isoptera), 3 web-spinners (Embioptera), 1 Flea (Siphonaptera), 1 Pseudoscorpion (Pseudoscorpiones) and 11 Lizards (Order Squamata) (Table 3.1).
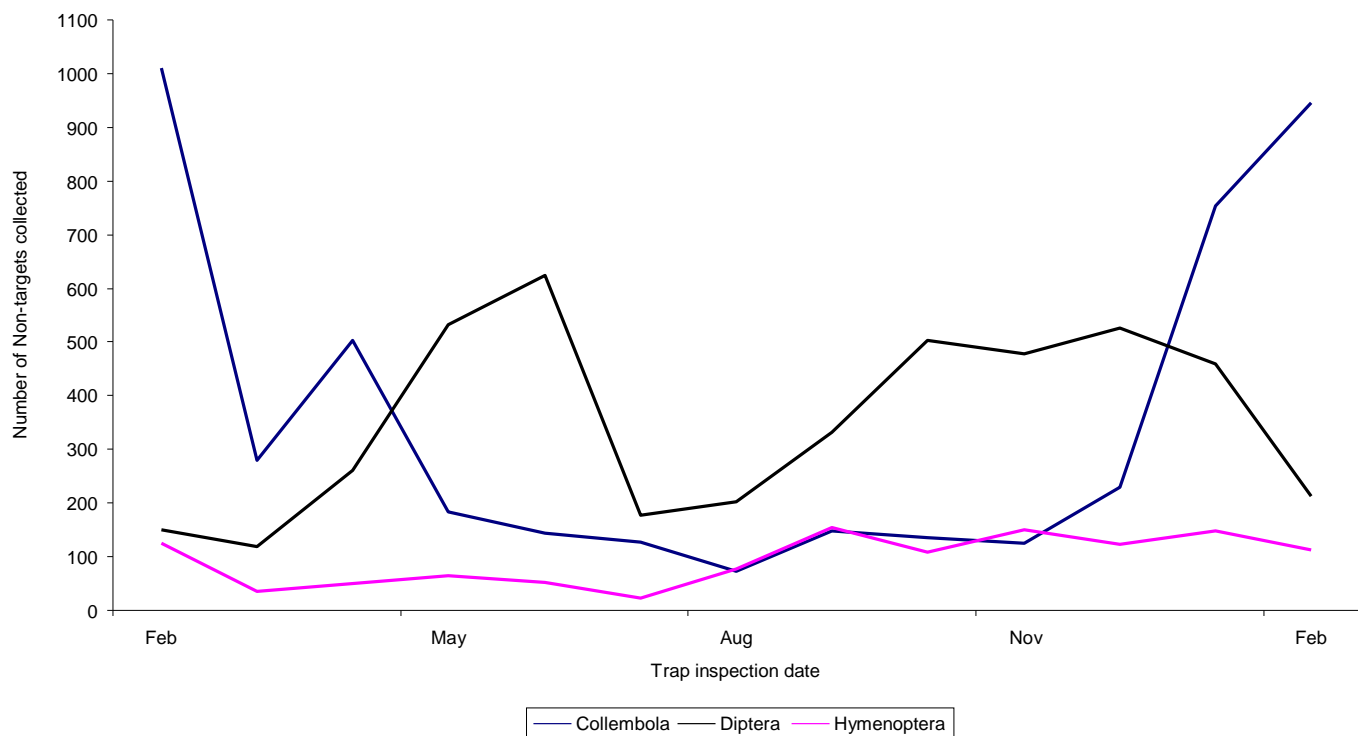
Figure 3.3 Yearly distributions of the three major Orders of non-targets collected in SSO in Cairns hostels

Table 3.1 Order and when possible Family identification of non-target fauna collected from Standard Sticky ovitraps (n=16) set in Backpacker Hostels in the Cairns area, over one year

Order	Family	No. of specimens	Order	Family	No. of specimens
Collembola		19513		Bibionidae	7
Thysanura		1		Cecidomyiidae	1055
Blattodea	Blattellidae	6		Ceratopogonidae	22
Isoptera		4		Chironomidae	313
Dermaptera	Forficulidae	4		Dolichopodidae	12
Orthoptera	Super/F Grylloidea	9		Drosophilidae	105
	Acrididae	5		Empididae	1
Embioptera		3		Heleomyzidae	1
Psocoptera		189		Muscidae	25
Hemiptera	Aphididae	32		Mycetophilidae	31
	Aphrophoridae	15		Neriidae	2
	Cercopidae	7	Diptera	Phoridae	9957
	Cicadellidae	32		Psychodidae	1317
	Super/F Coccoidea	118		Sarcophagidae	10
	Coreidae	1		Scatopsidae	375
	Cydnidae	28		Sciaridae	2906
	Delphacidae	5		Simuliidae	11
	Diaspididae	4		Tachinidae	7
	Eurymelidae	15		Tephritidae	40
	Enicocephalidae	6		Tipulidae	6
	Flatidae	6		Xenasteiidae	11
	Lygaeidae	91		Juvenile/other	27
	Pentatomidae	9		Micro	1
	Veliidae	1	Trichoptera	Ecnomidae	10
	Juvenile/other	234		Noctuidae	9
	Micro	2		Tineidae	23
Thysanoptera	Phlaeothripidae	23	Lepidoptera	Super/F Gelechioidea	242
	Thripidae	51		Juvenile/other	17
Neuroptera		2		Micro	1

Table continues over page

Table 3.1 Continued

Order	Family	No. of specimens	Order	Family	No. of specimens
Coleoptera	Anthicidae	311	Hymenoptera	Agaonidae	44
	Anthribidae	7		Apidae	4
	Bostrichidae	25		Braconidae	6
	Carabidae	27		Chalcididae	347
	Chrysomelidae	20		Diapriidae	406
	Coccinellidae	2		Evaniidae	33
	Corylophidae	7		Encyrtidae	279
	Curculionidae	9		Formicidae	2871
	Dytiscidae	3		Ichneumonidae	7
	Elateridae	2		Mymaridae	169
	Hydrophilidae	16		Pergidae	11
	Languriidae	13		Pteromalidae	95
	Ptiliidae	11		Scelionidae	348
	Nitidulidae	40		Vespidae	7
	Salpingidae	47		micro	21
	Scarabaeidae	22	Acari		1471
	Scydmaenidae	4	Araneae		304
	Sphindidae	3	Pseudoscorpiones		1
	Staphylinidae	17	Unidentifiable		19
	Tenebrionidae	68	Stylommatophora		12
	Juvenile/other	17	Isopoda		21
	Micro	8	Amphipoda		5
Siphonaptera	Pulicidae	1	Vertebrates	*Hemidactylus frenatus*	8
				Scincidae	1
				Gekkonidae	2
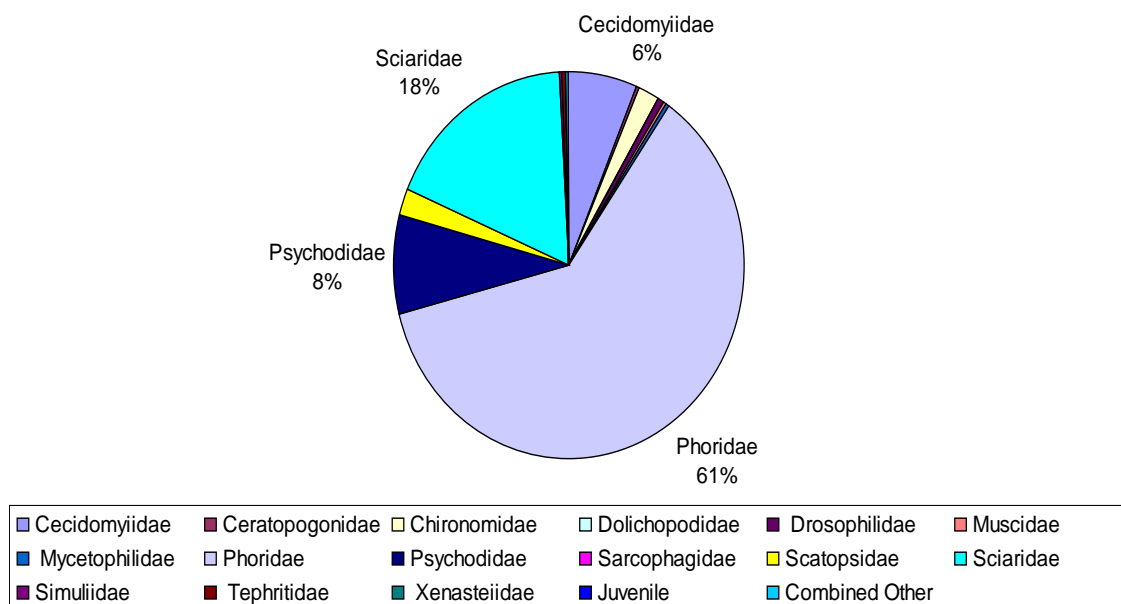
Figure 3.4 Dipteran details collected in sticky ovitrap in Cairns Hostels (n=16), over one year

Dipteran non-targets made up 36.8% of the total non-targets collected. Of the Dipterans collected 61% were from the Family Phoridae (22.5% of the total non-targets) (Figure 3.4) which was statistically significant (x^2;df=16; p=0.01). Juvenile Dipterans consisted of maggots found stuck in the

glue, usually near Muscidae or Sarcophagidae adults. The 'Combined Other' in Figure 3.4 is families of dipterans which had less than 10 specimens collected over the year (Table 3.1).
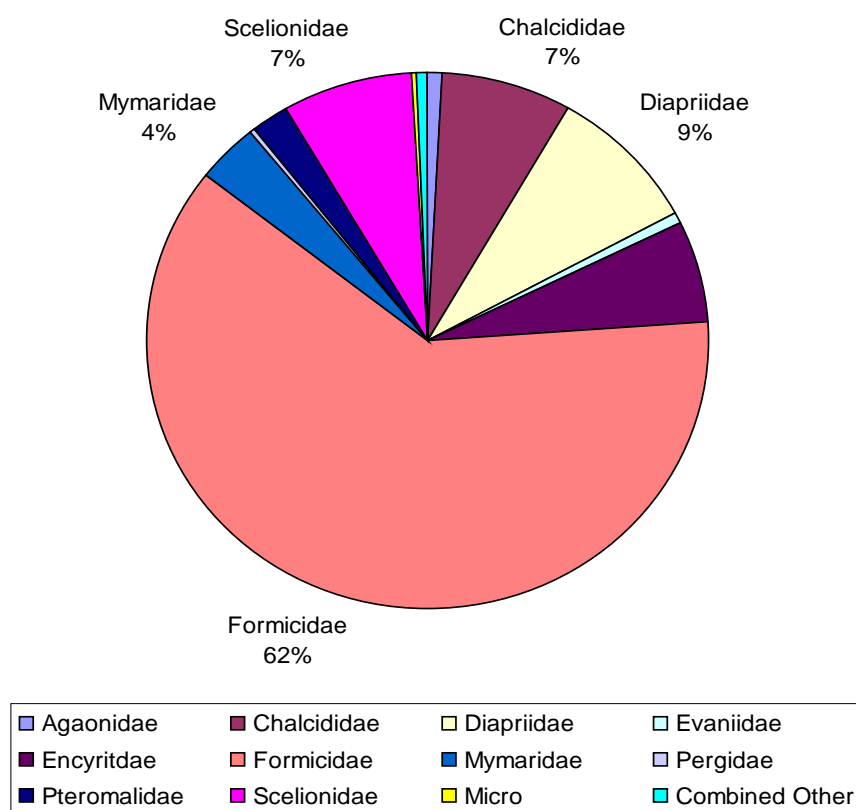
Figure 3.5 Hymenopteran details collected in sticky ovitraps in Cairns Hostels (n=16), over one year

Hymenopteran non-targets made up 10.5% of the total non-targets collected. Sixty- two percent (62%) of the Hymenopterans collected were from the Family Formicidae (6.5 % of the total non-targets) (Figure 3.5) which was statistically significant (x^2, p=0.01). The 'Combined Other' in Figure 3.5 is families of hymenopterans which had less than 10 specimens collected over the year (Table 3.1).

3.3.3: Experiment 3: Impact of Lethal Ovistrips on visiting non-targets

The number of Formicidae collected in the BLO was significantly different to the LO (t-test, p=0.01) (Figure 3.6). There was also a trend towards significance in the Family Scelionidae between the BLO and the LO (t-test, p=0.06). The BLO collected more Collembola and Diptera but no single Family was collected in numbers significantly different between the trap types (Figure 3.6).
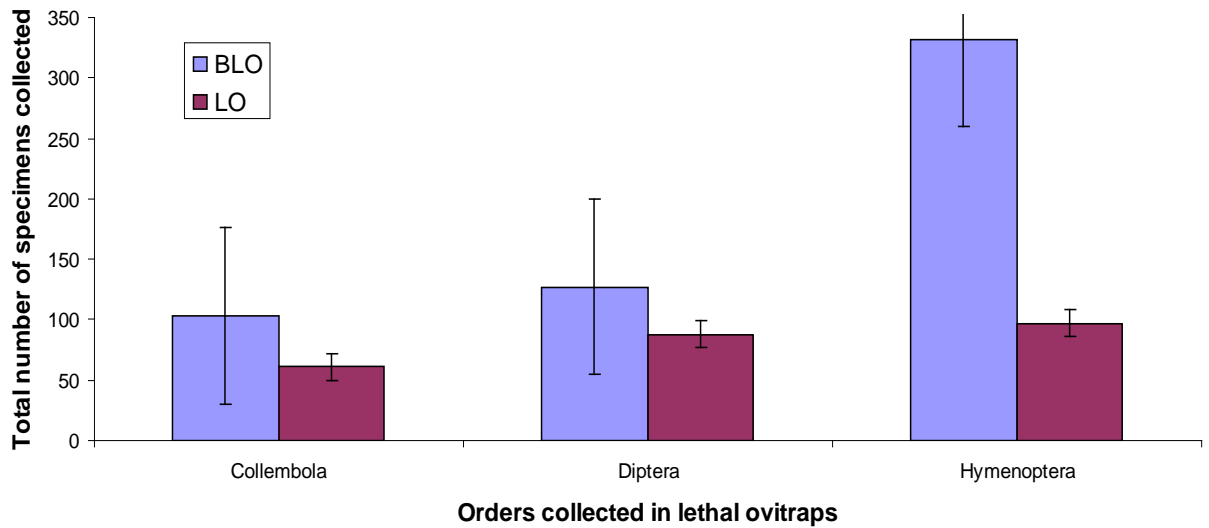
Figure 3.6. Total number(±SE) of specimens collected from the Major Orders collected in Biodegradable (BLO) and Lethal (LO) ovitraps, over 2 weeks
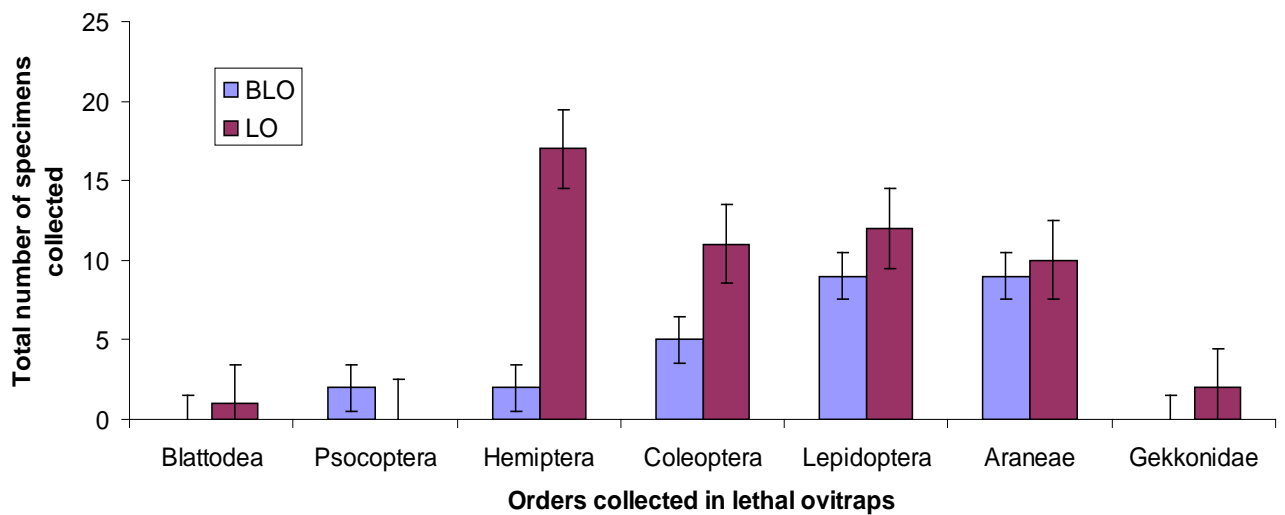
Figure 3.7. Total number of specimens (±SE) of the minor Orders collected in Biodegradable (BLO) and Lethal (LO) ovitraps, over 2 weeks

There was consistently more Hemiptera and Coleoptera collected in the LOs than in the BLOs (Figure 4.7). The Hemipteran Family Cicadellidae were significantly greater (t-test, p=0.03) in the LO than in the BLO (Data not shown). And the Coleopteran Family Ptiliidae (t-test, p= 0.04) were greater in the LO than in the BLO (Data not shown). In this experiment the LO also caught one Blattellidae (Ground cockroach) and two Gekkonidae (Asian house gecko, *Hemidactylus frenatus* (Schlegel) while the BLO caught none (Figure 3.7). The BLO caught two Psocoptera (Bush psocid) while none were collected in the LO (Figure 3.7).

Figure 3.8. Examples of the condition and variety of non-target fauna collected in Sticky Ovitraps during the one year study. Clockwise from top left: *Hemidactylus frenatus***, Phoridae, Formicidae, Hemiptera nymph, Cecidomyiidae, unknown Hymenoptera, Pseudoscorpion, Siphonaptera, Embioptera and another Cecidomyiidae.**

3.4 Discussion

With dengue control moving away from broad-scale application towards more specific, targeted applications there is a need for studies on non-target organisms that could be impacted by the new techniques. This is especially relevant to the lethal ovitraps, both LO and BLO. As the BLO consists of 60% corn starch this is an attractive, easily digested food for numerous non-target organisms in gardens around residential homes. Ritchie et al. (2009) found that 62% of their recovered BLOs had bite marks while 42% of the holed BLOs failed within the 4 weeks of their trial. They suggested that the chewing was probably from cockroaches although they did find ants, isopods and amphipods feeding on the BLOs. Before a large proportion of BLOs are set in the field, it is important to determine if BLOs are environmentally benign to non-vertebrates if consumed.

This study reports that many non-target animals visit the ovitraps. There are many possibilities on why non-targets visit the traps. Some may have been in search of water, others may have been attracted to the readily available food in the ovitrap, (organic matter, Lucerne or fungi), others may have been attracted to the food contained within the substrate of the ovitrap (corn starch), others may have been attracted to other non-targets already caught in the ovitrap.

The sticky ovitrap acted as a suitable proxy for the BLO (and the LO) as the ovitraps collected similar numbers and types of non-targets. Using the sticky ovitrap as a proxy allowed us to obtain a continuous flow of data throughout the one year study. The data collected suggests that the BLO is more attractive to certain families of non-targets.

There was a large number of Collembola collected in the ovitraps over the study. Initial thoughts were that the BLOs could impact on Collembola population densities, but in comparison to findings from (Rusek 1998) that Collembola population densities may reach up to several million individuals per m^2, the ~20 000 specimens collected in this research seem trivial. The high densities of Collembola commonly found in garden soils suggest that the ovitraps were not as attractive to Collembola as first thought. The comparison between the sticky ovitrap and the lethal ovitraps suggests that numerous Collembola may visit the ovitraps but few are killed.

Of the Diptera families collected most are considered nuisance insects and would have been attracted to the ovitraps by the organic materials (decaying vegetation in the water and fungi) that occur in the ovitraps. Some specimens would have accidently flown or fallen into the ovitraps from the vegetation immediately adjacent or above the ovitraps. Several of the Families collected have species that are considered health risks as they have the potential to contaminate human and pet food with bacteria and other disease-producing organisms (Naumann 2000, Disney 2008). Without further study into the specific species collected it is not possible to determine if any of the specimens we collected were those considered a potential health risk. Other Families (e.g. Cecidomyiidae- Diptera, Scatopsidae- Diptera, Super Family Coccoidea- Hemiptera,) are pest insects which damage agriculturally important plants by forming scale and galls, or their larvae feed on new roots, or on fungi spores (such as the oyster mushroom, *Pleurotus ostreatus* (Jacquin) (Naumann 2000, Kwang-Ho 2000).

The majority of non-target hymenopteran families collected were parasitic of other insects, laying their eggs in the eggs or larval stage of their hosts (Naumann 2000, Noyes 2010). These non-targets are generally considered beneficial and numerous species have been used in biological control of pest insects (including Hemiptera, Psocoptera, Coleoptera, Diptera, and Orthoptera) in agriculture (Trjapitzin 2010, Galloway and Austin 1984). It is possible that the beneficial, parasitic hymenoptera collected in the ovitraps were attracted to the other non-targets already caught in the ovitraps. If the ovitraps had not contained the glue strips, it is likely that very few specimens from these beneficial Families would have been collected.

The numbers of non-target Hymenoptera caught in the ovitraps during the study are not thought to be significant enough to have an impact on the ecology around the ovitraps, but more research would need to be conducted to confirm this. Our study collected only four native bees (*Trigona* sp) and no honey bees (*Apis mellifera*)(Linnaeus) making it difficult to compare the impact of ovitrapping with other mosquito control techniques which have used bees as their primary non-target (Coldburn and Langford 1970, Davis et al. 2007). Since only four native bees were collected it does suggest that ovitrapping is much less harmful than other mosquito control techniques. Many of the mosquito control non-target studies described in the literature focus on either the impact of insecticides pre and post application (Boyce et al. 2007, Kwan et al. 2009, Breidenbaugh and De Szalay 2010), or on a more select variety of non-targets (Brown et al. 1996, Stevens et al. 2011).

One limitation from this experiment is that it did not focus our attention on specific non-targets, or try to collect data pre- and post ovitrap deployment, thus we had difficulty comparing our results with other experiments. In a broad sense we are able to compare our results with Breidenbaugh and De Szalay (2010) who used Malaise traps and pan traps (disposable yellow plastic plates of water with 5% detergent which sample flying insects attracted to reflected light) to monitor the impact of aerial applications of Naled, an organophosphate insecticide. Breidenbaugh and De Szalay (2010) had Diptera and Hymenoptera as their dominant orders collected with Dolichopodidae as their most abundant non-target Family. Our results also had Diptera and Hymenoptera as dominant orders. Collembola were the most abundant non-target animal collected in our study followed by Phoridae. While we were able to collect a large volume of interesting data without further studies to compare against we can only make general interferences about the environmental impact of ovitraps, specifically BLOs. It is recommended that further research into ovitrap impacts on non-targets occurs. The research would need to be focus on a smaller more select number of Families or species or have a focus on the numbers of non-targets pre and post application of the ovitraps.

4.0 Public Acceptability of the Biodegradable Lethal Ovitrap as a Do-it-yourself control method against dengue, Cairns, North Queensland: A pilot study

4.1 Introduction

As the vaccine for dengue is still some years away (Hombach 2007, Coller and Clements 2011) dengue control must focus on prevention, such as personal protection and the control of vectors. Effective and sustainable dengue control and preventionis only possible if the local community share the responsibility , as discussed by Gubler and Clark (1994). A combined effort between government and the community is essential if dengue control is to be successful (Arunachalam et al. 2010, Baly et al. 2007, Parks and Lloyd 2004, Lloyd 2003). However, there are numerous barriers to successful engagement between government and communities, primarily keeping the community engaged in the project, cost of running the project and the time required to keep up the engagement between the government and the community (Gubler 1989, Lloyd 1992, Kroeger et al. 1995, Chiaravalloti et al. 1998, Renganathan 2003, Parks 2004, Horstick et al. 2010). Successful community based interventions, like those by Kittayapong et al. (2006) with measureable reduction in mosquito numbers, are predominantly reported from Asian countries where community structures are strong.

In Western societies,such as North Queensland, where communities are generally less close-knit, it can be more difficult to maintain a community-based dengue control program. The current dengue control program in Cairns, North Queensland has vector control staff visiting every property with a 100m radius of a dengue case (Queensland Health 2010). The vector control staff inspect the grounds (gardens and open areas) of the premise, treating any potential mosquito breeding sites they find (this process is known as source reduction) and they also set a minimum of two lethal ovitraps (LOs). If the premise occupier is home or the vector control staff think that the premise is high risk for dengue transmission they will try to gain access to the interior of the premise where a residual synthetic pyrethroid is applied to surfaces where *Ae.aegypti* are known to rest (Queensland Health 2010, Ritchie 2005). This process of source reduction and interior spraying can be extrememly time consuming, especially if there are numerous potential mosquito breeding sites. It would be more economical financially and time wise if the vector control staff could focus on just interior residual spraying and treatment of mosquito breeding sites. An environmental and user friendly means of protection against dengue, that thegeneral public could set or control would both decrease the cost of the dengue response (no longer need to pay extra vector control staff to set LOs) and increase the awareness of the public to the health risks (associated paperwork delivered with traps would inform and educate).

With the development of the Biodegradable Lethal Ovitrap (BLO) it would be possible to deliver an environmentally friendly control and prevention tool that could be easily used by the premise occupier and could provide long term protection against dengue with little effort. This study aimed to increase our understanding of public acceptability of the BLO, and if acceptable would the

utilise the BLO as a method of residential mosquito control, to protect their householdfrom dengue transmission.

4.2 Materials and Methods

4.2.1 Materials:

4.2.1.1 Biodegradable lethal ovitrap "Do-it-yourself" Kit

The kit, as it will be referred to from now on, consisted of

- Biodegradable plastic bag clearly labelled as "Dengue mosquito do-it-yourself kit"
- Two biodegradable lethal ovitraps, each with a 5 x 15 cm flannelette strip treated with bifenthrin, stapled to the inner wall
- One pair of disposable gloves
- One pamphlet on dengue, including symptoms and what to do around the yard to help prevent *Ae. aegypti* from breeding
- One pamphlet on the biodegradable lethal ovitrap with instructions on how to set the ovitraps

4.2.1.2 Questionnaire

The questionnaire consisted of 50 questions divided into four sections

A-Experience of dengue fever

B-Knowledge of dengue fever

C-Engagement with and uptake of the written material

D- Socio-demographic data

4.2.1.3 BLO Information Sheet

The BLO information sheet included information about the pilot study informing the participant of their rights concerning the pilot study and any risks that might occur from participating in the pilot study. This information sheet is a mandatory requirement of the James Cook University ethics committee (Appendix 1)

4.2.1.4 BLO Informed Consent Form

Written informed consent was obtained from each participant before commencing the questionnaire form (JCU ethics committee requirement), (Appendix 2).

4.2.2 Methods:

4.2.2.1 Participants

Participants for the pilot study were gathered from people who attended a James Cook University on-campus Open day. As members of the public visited the *Tropical Medicine* Display Marque, they were asked if they would like to participate in a dengue mosquito control experiment. Those that said 'yes' recorded their name and contact phone number. One week later the participants were contacted and a time made to deliver the kit. The kits were all delivered on the same day. Two

weeks after kit delivery, the participants were again contacted and an appointment was made to visit their homes to complete the questionnaire, inspect the ovitraps and answer any questions that might have arisen.

4.2.2.2 The Questionnaire

When the participants were visited, they were asked to read the BLO Information sheet and read and sign the BLO consent forms. The participants were interviewed (digitally recorded) following the structure of the questionnaire form. Interviews were transcribed and data entered into a spreadsheet. To ensure confidentiality, participants names were replaced with reference numbers. These were used in the data analysis. The participants were asked about their exposure to dengue, if they or someone they knew has ever had dengue. They were then asked about their general knowledge of dengue, including questions about the breeding behaviour of *Ae. aegypti*. They were asked to comment on the information in the pamphlets delivered as part of the kit. They were then asked a series of questions about the BLO and their acceptability of measures for controlling dengue-carrying mosquitoes (not specifically *Ae. aegypti*). The participants also answered questions about their age, sex, time living in North Queensland, level of education and experience with pest control activities. At the conclusion of the interview the ovitraps were inspected and comments recorded about each location and condition of the ovitrap. The data was recorded, collated and analysed using Microsoft Excel 2003 (Microsoft Corp, Redmond, WA).

4.3 Results

Of the 234 people asked to participate in the dengue mosquito control experiment, only 47 (20%) agreed to be contacted further. The majority of people declined to participate in the experiment due to time constraints. Of the 47 people who agreed to be contacted further, 27 (57%) agreed to set the traps and were willing to be interviewed. Of these 27 participants, only 14 (52%) completed the pilot study by setting the ovitraps and being interview. The remaining 13 (48%) withdrew from the pilot study. Reasons for withdrawal included, people moving interstate (3), people moving to different cities where *Ae. aegypti* are not present (e.g.Tolga) (3), or no longer responding to attempts to contact them (7). Of the total 234 people approached about the pilot study only 14 (6%) completed the study. Twenty nine percent of the participants had either themselves or someone in their immediate family suffer from dengue. Two of the participants (14%) knew of a friend or work associate who had suffered from dengue.

Table 4.1. Participant Socio-demographic factors

Category		Males n (%) (n=7)	Females n(%) (n=7)
Age Group	18-24	2 (14.3%)	0 (0.0%)
	25-39	1 (7.14%)	2 (14.3%)
	40-49	3 (21.42%)	1 (7.14%)
	50-59	0 (0.00%)	3 (21.42%)
	60-74	1 (7.14%)	0 (0.00%)
	75+	0 (0.00%)	1 (7.14%)
Highest education level achieved	Post Graduate Degree	0 (0.00%)	1 (7.14%)
	Graduate Diploma/Certificate	0 (0.00%)	1 (7.14%)
	Bachelor	1 (7.14%)	2 (14.3%)
	Advance Diploma/ Diploma	2 (14.3%)	0 (0.00%)
	Certificate	1 (7.14%)	0 (0.00%)
	Secondary	3 (21.42%)	3 (21.42%)
Years spent living in North Queensland	2 or less	0 (0.00%)	3 (21.42%)
	3-10	0 (0.00%)	2 (14.3%)
	11-20	3 (21.42%)	1 (7.14%)
	21-30	2 (14.3%)	0 (0.00%)
	31-40	0 (0.00%)	0 (0.00%)
	41-50	0 (0.00%)	0 (0.00%)
	51-60	1 (7.14%)	0 (0.00%)
	61+	1 (7.14%)	1 (7.14%)

The socio-demographic indicators collected from participants were similar between the sexes (Table 4.1), however participant numbers were not large enough to conduct a statistical comparison. The majority of the participants had between Secondary and Advance Diploma levels of education and had lived in North Queensland less than 30 years (Table 4.1). When questioned about past experiences in pest control activities, 43% had no experience, 22% had experience in pest control activities other than mosquito control (e.g. treating of building foundations for termites and spraying sugarcane for pest control), 14% of the participants had specific mosquito control experience (as an environmental health officer in Alice Springs and mosquito control officer) and 21% claimed some general mosquito control experience where they sprayed commercially available aerosols around their place of work or burnt coconut husks around their homes as a fogging technique. And in one participant's case they claimed pest control experience as they spent one day trying to sell pest control door-to-door.

Table 4.2. Participant responses to key interview questions, post biodegradable trap delivery

Questionnaire Category	Question	Participants
Experience with Dengue	Know anyone who had dengue	6 (42.86%)
	List any symptoms	14 (100.00%)
	Minimal to no personal risk in next 12 months (0-4 scale)	12 (85.71%)
Knowledge relating to dengue fever	Understanding that mosquitoes transmit dengue to humans	14 (100.00%)
	Dengue-carrying mosquitoes live and breed in water holding containers around the home	14 (100.00%)
	Dengue-carrying mosquitoes most likely to bite during the day	10 (71.43%)
Engagement with and uptake of the written material	Information clear and easy to understand	12 (85.71%)
	Questioned the truth of the flyer	3 (21.43%)
	More motivated to set trap during dengue	12 (85.71%)
Engagement with and uptake of biodegradable trap	Check on traps once they were set	14 (100.00%)
	Disliked the trap	6 (42.86%)
	Would consider purchasing trap if commercially available	10 (71.43%)

All participants were able to name some symptoms of dengue (Table 4.2). While almost half (42.86%) of the participants identified fever, aches/pains and lethargy as the main symptoms of dengue (Appendix 8.4); 29% identified rash and vomiting/nausea as other symptoms common in dengue-related illness and only 21% recognized headache as a main symptom of dengue (Appendix 8.4).

Only one participant considered their risk of experiencing dengue in the next 12 months was above medium level (Appendix 8.4). The majority of the participants (85.71%) felt that they had little or no risk of experiencing dengue in the next year (Table 4.2) and that dengue mosquitoes were not found in their suburb.

When questioned about their understanding of dengue and dengue carrying mosquitoes, all the participants stated that humans became sick after being bitten by a dengue mosquito (Table 4.2). When asked to describe the dengue mosquito 64% said that it was the mosquito with stripy legs. Interestingly one participant commented that the dengue mosquito larvae could be identified "As they swam like they were crazy". One participant who had lived in North Queensland for over 15 years identified *Ae. aegypti* as the "green mosquito with black dots". All participants said that dengue carrying mosquitoes live and breed in water holding containers (Table 4.2). Twenty one percent also said that dengue-carrying mosquitoes would live in freshwater creeks and ponds under the right circumstances, usually if the water was not flowing and there were few predators in the water.

When questioned about the BLO, 57% of the participants said that the trap was simple and easy to use (Table 4.2). Of the participants who liked the trap, 21% said that they liked the trap because it was environmentally friendly and 14% liked the trap simply because it was a good idea. The remaining 43% thought that the trap was unsightly and difficult to set or move if required (Table 4.2). When asked if they felt confident that the trap was safe, 50% said 'no', as they has concerns about the ovitrap being dangerous to children and animals. Concern was also raised about chemical contamination of the surrounding area if/when the ovitrap began degrading. When asked if they would consider purchasing a commercially available ovitrap, 29% said 'no', as you could not see the ovitrap working nor were there enough mosquitoes around to bother, 43% were 'undecided', stating they

would consider purchasing the ovitrap if dengue in their immediate neighborhood and the ovitraps were cheap and readily available.

Table 4.3. Responses to question "Rank in terms of acceptability to you the following measures for controlling dengue-carrying mosquitoes (1 highly acceptable - 5 not acceptable)

Control Method	People thought control measure unacceptable
Spraying insecticides in and around house	13 (92.86%)
Use of a Bacterial agent that would reduce the mosquitoes capacity to pass on dengue	8 (57.14%)
Use of a Biological agent or natural enemy of mosquitoes that would reduce the dengue-carrying mosquito population	6 (42.86%)
Use of household insect sprays	13 (92.86%)
Use of personal insect repellent	11 (78.57%)
Biodegradable trap	2 (14.29%)

Only 14% of the participants felt that any type of chemical control, including the biodegradable lethal ovitrap was completely unacceptable for the control of dengue-carrying mosquitoes (Table 4.3). The majority (93%) of the participants said that spraying insecticides in and around the house was completely unacceptable and use of personal insect repellent only slightly less so. Of the control measures mentioned the BLO was considered the best option (Table 4.3).

4.4 Discussion

This experiment aimed to understand public acceptability of BLOs and mosquito control techniques. Due to the small number of responding volunteers and the limited timeframe, we could only complete a pilot study, even though the study was designed as a larger stand alone research topic.

The public acceptability of mosquito control techniques has been the focus of many research projects around the world. Many research projects report a commonality of difficulty in gaining community participation. This lack of interest or willingness to participate is especially obvious in Western society where communities are less close-knit compared with those in Eastern societies. Numerous published articles discuss outcomes (successes and failures) of community based mosquito control programs (Baly et al. 2007, Chiaravalloti et al. 1998, Fernandez 1998, Gubler and Clark 1994, Gubler and Clark 1996, Kay 1994, Kroeger et al. 1995, Leontsini et al. 1993, Lloyd et al. 1992, McNaughton et al. 2010, Parks et al. 2004, Parks and Lloyd 2004). The programs considered 'successful' were those that maintained ongoing community engagement , over the life of the program. The programs that were not successful, reported problems with engaging the community, or keeping the community engaged in the project. Our pilot study was similar to less successful programs. When first approached, most people were keen to take part, however this interest waned when the study was due to start two weeks later. This short-term lack of interest is often amplified when a project is long-term. This lack of interest is common in many community based projects and has been noted as one of the major causes of project failure.

Another factor that limited our study was the lack of participant knowledge about dengue and dengue vectors. While conducting interviews with participants, participant lack of understanding of

dengue and dengue mosquitoes became evident. The participants considered their knowledge to be accurate about dengue and the mosquitoes capable of transmitting the virus. However, when questioned in detail, many participant assumptions about dengue and dengue mosquitoes were incorrect. Furthermore, these incorrect assumptions influenced participants behaviour in the experiment and their attitudes towards the BLOs and mosquito control in general. This is similar to the findings by McNaughton et al. (2010) who found that an average lay person held various assumptions which were counter-productive to public health messages and appeared to be placing themselves at risk from dengue. McNaughton et al. (2010) argues that instead of dismissing lay understanding as ignorance, concerted effort is required to understand where the assumptions originate and health authorities must find strategies to better educate the lay population. In our pilot study we found that if a participant held incorrect assumptions, it was a simple matter of explaining the correct information in a manner which the participant could understand, this was not necessarily the same for each participant however. While this study had a small participant sample, the demographics were a good representation of the broader community with an even spread of sexes, ages and dengue knowledgeParks and Lloyd (2004) suggest that even though more people are becoming educated and aware of dengue, they are not taking action to protect themselves. Parks and Lloyd (2004) state "Regrettably, an informed and educated individual is not necessarily a behaviourally responsive individual". This was evident in our pilot study when we went to observe the locations which our participants had set the BLOs. Often the locations were unsuitable and when asked why the traps were set in these locations, the participants said that it was convenient. On further discussion the participants themselves realised that if they had thought more carefully about the location of the BLO before they set it, there would have been more of an observable effect (fewer mosquitoes) from the BLO. Claro et al (2006) also found that knowledge about the vector mosquito and the disease, including symptoms, was not enough to cause people to change their behaviour. In our pilot study we had participants who were well educated about dengue and its mosquito vector, who had mosquito breeding sites in their gardens, clearly visible, from where they had set the BLOs. It is possible that even if the public were given the BLOs to set with information regarding the BLOs and dengue prevention they would not progress to the point where they actively searched their premises for mosquito breeding sites and attempt to deal with these sites.

There is clearly a gap in the understanding of the lay person around dengue and dengue control. Until this break between education and activation can be bridged, research like our pilot study will continue to show poor responses. In community-based programs similar to those run in Vietnam and Thailand (Kittayapong et al 2006, Nam et al 2005) community engagement is on a one-to-one level and the participants are encouraged to be heavily involved in the activities. This more personal and long term support appears to be the only way to successfully gain community participation in dengue control and prevention.

Like all community engagement research , our pilot study was subject to volunteer bias, where by only people who are interested in the research agreed to participate. The low number of participants in our pilot study combined with the growing number of dengue cases in the Cairns region (despite

increasing health promotion activities) clearly demonstrates that there is a disconnection between knowledge and activity in regards to dengue control and pevention.   Without a clearer understanding of dengue and ways to activate the public, a successful community- based dengue prevention program is less likely to become established. Until greater community awareness , understanding and activity is achieved, and tested, the implementation of BLOs as a tool in a community-based program would result in the BLOs being misused and underutilized.

5.0 General Discussion

The BLO was designed to be a fast, easily deployed control tool for use during dengue outbreaks where time and resources were limited. As such the experiments described here form a solid base from which further development of the LO and BLO can occur. A significant number (>95%) of the BLOs used in the longevity studies failed due to what we believe to be invertebrate chewing (most likely cockroachs). This result is very different from those obtained by Rapley et al (2009) who had only 51% of their BLOs chewed. Our results also differed from Rapley et al. (2009) in regards to the survival time of the BLOs when set on different substrates. Our results clearly demonstrate that BLOs set on solid substrates such as tile or brick last longer than BLOs set on porous substrates such as dirt or garden mulch. The difference in survival time on the different substrates could have a serious impact on the choice of locations for BLO deployment during dengue control. If vector control staff are concerned that the BLO will fail before the minimum control period (currently four weeks, Williams et al. 2005) when set on porous surfaces, they may look for suitable BLO setting sites instead of suitable mosquito control sites.

While it would be possible to deploy greater numbers of BLOs, theoretically overcoming the failure rate, this may only serve to increase the difficulties vector control staff face when trying to finding suitable locations for the BLOs. Trialing the BLOs in an area before deployment could be suitable for remote areas where returning to retrieve LOs is costly. However, blanket trialing of the BLOs prior to deployment would counteract the whole time saving aspect of the BLO, be extremely expensive as twice the number of BLOs would be required and the results could change with time.

The ability of *Cx. quinquefasciatus* to breed in the BLOs after nine weeks is also concerning. There is the potential that the public would see the BLO breeding mosquitoes and become concerned that the trap was producing potentially harmful mosquitoes. This assumption could lead to poor relationships between the public and vector control staff, an outcome that needs to be avoided. Further research into methods of preventing *Culex* mosquitoes from breeding in the BLO would need to be conducted before long-term (post nine weeks) deployment of BLOs occurred. Ritchie et al (2008) suggested that a long lasting pyrethroid treated mesh cover for the BLO could be used instead of the current lethal ovistrip, while Kroger et al. (2007) trialed sachets of slow releasing larval growth inhibitors (LGI) in water holding containers. Unfortunately Kroger et al.(2007) had poor community acceptance rates for the LGI in drinking water vessels, but this idea, or similar, could be trialed in the BLOs. Our results confirm that further research into a longer lasting, more effective, chemical delivery system also needs to be conducted before BLOs are deployed for extended periods.

The length of time the BLOs are in the field may impact on the non-target fauna exposed to the BLO. As part of further research into the impact of BLOs on non-targets, consideration should be made to what impact both the biodegradable bucket and the lethal ovistrip could have on non-target fauna. It is possible that the broad scale use of BLOs could cause an increase in pest non-targets especially those that feed on the BLOs. It is hoped that the impact the BLOs have on non targets is limited in scope, similar to the results found by Breidenbaugh and Szalay (2010). Breidenbaugh and Szalay (2010) found that large-scale aerial spraying, had a short term impact on insect orders

(Dolichopodidae, Sarcophagidae, Syrphidae, Tachinidae) but over a year there were no significant changes in numbers of insect orders or specimens collected, while the mosquito numbers declined dramatically. Further research into BLO and LO impacts on the environment around them would be beneficial to all vector control staff who use lethal ovitrapping as a control or prevention method. Further research into non-target impacts would also be useful in educating the public, as evidence that the ovitraps are safer and more environmentally sensitive, so fewer people would be hesistant or concerned about having the ovitraps on their property

The concern members of the public feel about the safety of the LO and BLO could clearly be seen in our pilot study of public accepatbility. While the pilot study was quite small, in comparison to other community engagement research, the trends seen in the pilot study do appear to mirror the broader community as a whole. While the pilot study did not give resounding public approval or acceptance of the BLO it must be noted that use of the BLO during dengue responses in North Queensland have resulted in almost no complaints or comments from the public (Personal Observation 2009). Similarly the results from Ritchie et al. (2009) where public acceptance was measured as the number of traps retained by residents suggest that the BLOs are actually acceptable to the general public as a whole. It could be argued that similarly to the poor response from our pilot study that the public are reluctant to exert themselves and accept the BLOs by default. Lloyd et al (1992) found that premise occupiers in Merida, on Mexico's Yucatan Peninsula thought that emptying buckets and throwing out water holding containers was pointless if the public health authorities continued to do nothing about nearby cesspools. These same premise occupiers scored highly when questioned about their awarenss of dengue fever, a fact which clearly demonstrates lack of true understanding about dengue and dengue control.

There is antedotal evidence that suggests that given the choice most people in North Queensland would prefer not to have mosquito traps on their property but once the traps are set they leave the traps alone (Personal Observation 2009). Whether the public would be interested in using the BLO as a type of personal protection/ control measure is still undetermined. I believe the health authorities, including the vector control staff, need to increase their efforts to engage with the Cairns community and once they have established a strong repor, only then, will the BLO and dengue prevention at a community level truely have a longterm chance at success.

5.1 Limitations

There was several limitations to the research in this thesis. The BLO longevity trials were not replicated, and any environmental or seasonal variations to BLO survial were no considered. Also the two longevity trials were conducted in different types of premises. The initial trial was in residential permises and the second was conducted in Hostels, business premises. There are strict regulations placed on business to ensure regular pest control is carried out, not so in private residences. This difference may have impacted on the BLO longevity by changing the number of interactions the BLOs would have had with cellulose feeding invertebrates, fewer cellulose feeding invertebrates means that the BLOs will last longer. Also the initial longevity study had the BLOs being physically disturbed on a weekly basis, as they were picked up and inspected. While none of these activities appeared to have an impact on the results it would be good scientific methodology to repeat these experiments to confirm no impact.

Another limitation was in the non-target experiments. While our study did not identify any obvious environmental impacts, it must be noted that the research was not designed to identify anything but a major and very obvious impact. No attempt was made to determine if there was a change to the non-target fauna pre and post BLO deployment and there was no effort made to determine if there was a broader environmental impact from the BLOs being in place for extended periods of time. There was no control aspect to the year long non-target identification experiment, any changes to non-target numbers was assumed to be seasonal. The research conducted in this series of experiments was very preliminary and non specific.

The other major limitation to this thesis was in the pilot study of public acceptability. Originally this experiment was to be larger (over 200 participants) and more focused on premise occupiers in the central Cairns city area. Due to a lack of available BLOs and other resources and the overwhelming lack of interest from the public, the experiment was cut back. It was suggested that the pilot study be removed from the thesis, due to the limited scope. It remains in the thesis as an obvious area for further research into dengue control and prevention in Cairns, North Queensland.

6.0 Overall Conclusions

The key results from these experiments show that the BLO is attractive to *Ae. aegypti* mosquitoes for as long as it holds water and *Ae. aegypti* mosquitoes do not breed in the BLO while the lethal ovistrip is present. Fifty percent (50%) of the BLOs deployed survive to nine weeks and BLOs set on porous surfaces don't last as long as BLOs set on non porous surfaces. Under certain conditions BLOs can remain in the field up to twenty weeks. Unfortunately *Culex* mosquitoes are able to breed in BLOs from approximately nine weeks and due to non-target chewing of the BLOs there is a potential risk that the traps on a property will fail before the minimum control period is over. This failure could lead to potential mosquito and dengue break through into untreated areas.

The LO and BLO appear to have a minimal impact on the non-target population, although the ovitraps are visited by a wide variety of invertebrates and other non-target fauna. Ovitraps appear to be very attractive to Collembola, Phoridae, Sciaridae, and Formicidae, while having minimal attraction to Apidae and other commonly monitored non-targets.

These results indicatethere remains a large gap in public knowledge about dengue and dengue mosquitoes. There appears to be a general lassitude from the public where dengue control and prevention is concerned. This lassitude to dengue control and prevention is not unique and occurs in response to most mosquito control and prevention activities. The behaviour of the general public towards mosquito control and prevention should be of great concern to health officials and further research into methods of educating and getting the public proactive against mosquitoes must occur.

If BLOs are to become a standard tool for vector control officers it is important to determine a better and more cost effective "set and forget" lethal ovitrap that does not require trials in an area before being deployed for dengue control. It would also be neccessary to determine if there was an insecticide which could be applied to the BLO that would prevent *Culex* mosquitoes from breeding in the BLO, while still working to kill *Aedes* mosquitoes. The chemical would also have to be tested to determine if a decreasing exposure level caused by time in the field would lead to the potential development of chemically resistant mosquitoes.

Other areas for future investigation involve impacts on non-target populations pre and post deployment of ovitraps. Experiments could be conducted comparing if non-target organisms impacted by ovitraps are at sufficient numbers to be detrimental to non-target populations or their environments. Also, a test could be developed to assess the difference in the non-target populations between residential properties and businesses locations .

This thesis has great potential to become an integral part of vector control activities in North Queensland. Further research and development is essential but the current BLO appears to be a solid prospect from which to progress.

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8.0 Appendix

8.1 Information Sheet

ADMINISTRATIVE DOCUMENTATION HAS BEEN REMOVED

Pilot Study - Public Acceptance of BLO - October 2008

Address: …………………………………Suburb:………….…………………………

Date: .. / .. / ..

Interviewer: ……………………

A: Experience of dengue fever

I'd like to begin with a few questions about your own exposure to dengue fever.

1. Have you, a member of your immediate family or any of your friends or associates had any sort of dengue fever?

    Yes   □   No   □   Not sure □

Who _____(ie. self, parent, child etc)

(if known please state which - Dengue Fever or Dengue Hemorrhagic Fever)

2. What do you understand to be the main *symptoms* of dengue related illnesses?

    ………………………………………………………………………………….
    ………………………………………………………………………………….

3. On a scale of 1 to 10 – where 1 means 'no risk' and 10 means 'extreme risk' - how would you rate your own risk of experiencing dengue-related illness over the coming 12 months?

    <u>0   1   2   3   4   5   6   7   8   9   10</u>
    NO RISK   EXTREMELY HIGH RISK

4. True or False? Dengue fever is a mild disease that does never cause serious illness:

True □   False □   Not sure □
Comments:…………………………………………………………………………………
………………………………………………………………………

B: Knowledge relating to dengue fever

I'd now like to ask a few questions about your knowledge of the nature and causes of dengue fever.

5. How does a human become infected with dengue fever?
………………………………………………………………………………….
………………………………………………………………………………….
………………………………………………………………………………….

Note: if the respondent's answer indicates that s/he does not know that dengue is transmitted by mosquitoes, DO NOT ask the remaining questions in this section, as they are not applicable.

6. (A) Have you heard of the *Aedes aegypti* mosquito? Yes □   No   Not sure □

(B) Can you describe the appearance of the dengue carrying mosquito?
…………………………………………………………………………………………….
…………………………………………………………………………………………….

7. In which if the following places would you find dengue-carrying mosquitoes living and breeding?

Water holding containers around the home-garden	Yes □	No □	Not sure □
Swamps and lagoons	Yes □	No □	Not sure□
Freshwater Creeks or ponds	Yes □	No □	Not sure□
Beaches, mangroves, brackish water	Yes □	No □	Not sure□

Other (specify) ………………………..

8. At what time of day are dengue-carrying mosquitoes most likely to bite you:
During the day □
Around dusk   □
At night   □
Day and night   □

I would now like to ask you some questions about the flyer we dropped off with the Biodegradable trap.

C: Engagement with and uptake of the written material

9. Did you read the flyer that we provided with the trap?
 Yes □   No □   Some parts □

(b) ("No") Can you tell me why you didn't read the written material?
………………………………

(c) ("Some parts") Can you tell me which parts you read?
……………………………………………

10. Have you read or re-read the flyer within the last 48 hours?
    Yes □   No □

11. What do you think were key messages of the flyer?
……………………………………………………………………………………………
………
……………………………………………………………………………………………
………

12. What did you **like** about the flyer and the information it provided?

……………………………………………………………………………………………
…….

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13. What **didn't you like** about the flyer and the information it provided?
……………………………………………………………………………………………………
……………

(if they have not already stated how easy or difficult it was to understand ask the following)
14. Do you think the flyer was
(a) clear and easy to understand or
(b) confusing and difficult to understand?
15. Can you point out the sections you found confusing or difficult to understand?

……………………………………………………………………………………………...……
.

16. Was there anything in the flyer that was hard to believe, anything that you questioned the truth of?
Yes □   No □

(b) If Yes, what
………………………………………………………………………………………

(c) Why…………………………………………………………………………………………

17. Did the written information convince you that the trap would reduce the number of *Aedes aegypti* (dengue) mosquitoes around your home?   Yes □   No □

18. Can you tell me why it did or did not convince
you?..

19. Did you notice a reduction in the numbers of *Aedes aegypti* (dengue) mosquitoes while the trap was at your home?   Yes □   No □   Not sure □

20. If this trap was delivered free to your door, would the information in the flyer motivate you to set out a trap?   Yes □   No □

(a) (Can you tell me why)
……………………………………………………………………………

21. Would you be more or less motivated to set out the trap if it was delivered during a dengue fever outbreak?   More □   Less □

22. After reading the flyer did you have any other questions that were not answered by the information we provided?

(b) if Yes. Identify these
…………………………………………………………………………………..

23. So what is the biodegradable dengue mozzie trap made from?
…………………………………

24. How does the trap work?
…………………………………………………………………..

25. Did you set the trap out? Yes ☐   No ☐

(a) If No, can you tell us why?
………..

(if they have not set out the traps – move to question…38.)

26. How long after the traps were delivered did you set them out?
………
……

27. Can you describe the steps you took when setting up the traps?
………
…

28. Did you use the gloves?   Yes ☐   No ☐

29. Where did you place the traps?...
……

30. Why did you place it in this location? …………………………………………………………
……

31. Did you check the trap one it was in place?   Yes ☐   No ☐
(b) If Yes, how often did you check it?...

32. What did you like about the
trap?..
....
.............33. What didn't you like about the
trap?..
………
…………
(b) (if they haven't mentioned it ask) Were the instructions on setting the trap,
1. easy to follow,
2. suitable
3. not that easy to follow
    4. difficult to follow

34. Did you feel confident that the trap was safe?   Yes ☐   No ☐
        (b) Can you tell us
why?...

35. Did you have any further questions about the trap that were not covered in the information
provided?   Yes ☐   No ☐
(b) If Yes, What were they?…………………………………………………………………...

        (*record here after the interview, if they called US with a question*) Yes ☐   No ☐

36. If this trap was made available commercially would you consider purchasing it? Yes ☐
No ☐
………
………..

37. Have you disposed of the trap?   Yes ☐   No ☐

………

……………

Do you have any suggestions on how we could improve the public's use or acceptance of the biodegradable

trap?..

....

....

....

Rank in terms of acceptability to you the following measures for controlling dengue-carrying mosquitoes (1 highly acceptable - 5 not acceptable)

    a) Spraying chemical insecticides both inside the house and outside

    b) Release of a bacterial agent that would reduce the mosquitoes capacity to pass on dengue fever

    c) Release of a biological agent or natural enemy of mosquito that would reduce the dengue mozzie population

    d) Use of household insect sprays

    e) Use of personal insect repellents

    f) Biodegradable trap

D: Socio-demographic data

I'd like to finish wind the interview by asking you for some background information about you and your household.

40. Gender: Male ☐ Female ☐   Other (specify)…………………………….

41. Length of time spent living in North Queensland……………………………………………….

42. Do you identify yourself as belonging to any particular cultural or ethnic group?
  No ☐   Yes Aboriginal ☐   TSI ☐   Other: (specify)……………………

43. Age Group (tick box)

18-24 years		25-39 years		40-59 years		60-74 years	

  Other (specify) ………………………..

  44. What is the highest Education Level you have achieved?

    Postgraduate Degree level   ☐

    Graduate Diploma or Graduate Certificate level   ☐

    Bachelor Degree level   ☐

    Advanced Diploma and Diploma level   ☐

    Certificate level   ☐

Secondary education   □

Primary education   □

Pre-primary education   □

Other education   □

48. Do you have any past experience in pest control activities?...……………………………………………… ……………………………………………………………
49. In the last 5 years have you volunteered your time/ resources or made any donations to an environmental organisation or group?

Yes □   No □

Which groups?: ....

Thank you for your time and your support

50. Would it be possible to see the trap?

(Note: where they placed it, if there is shade, if it is safe, if it has water in it, if it's

breaking down etc)

………………………………………………………………………………………………
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8.3 Informed Consent

JCU JAMES COOK UNIVERSITY
JAMES COOK UNIVERSITY

TOWNSVILLE Queensland 4811 Australia Telephone: (07) 4781 4111

INFORMED CONSENT FORM

PRINCIPAL INVESTIGATOR	**Dr Scott Ritchie**
PROJECT TITLE:	**Development, deployment and public acceptance of the Biodegradable Lethal Ovitrap (BLO)**
SCHOOL	**School of Public Health, Tropical Medicine and Rehab. Services**

I understand the aim of this research study is to develop, deploy and determine public acceptance of a Biodegradable Lethal Ovitrap. I consent to participate in this project, the details of which have been explained to me, and I have been provided with a written plain language statement to keep.

I understand that my participation will involve an interview or questionnaire and I agree that the researcher may use the results as described in the plain language statement. I understand that the interview will be audio taped.

I acknowledge that:

- any risks and possible effects of participating in the interview or questionnaire have been explained to my satisfaction;

- taking part in this study is voluntary and I am aware that I can stop taking part in it at any time without explanation or prejudice and to withdraw any unprocessed data I have provided;

- that any information I give will be kept strictly confidential and that no names will be used to identify me with this study without my approval;

(Please tick to indicate consent)

I consent to be interviewed		Yes	No
I consent for the interview to be audio taped		Yes	No
I consent to complete a questionnaire		Yes	No

Name: *(printed)*	
Signature:	**Date:**

8.4 Complete Data from Public Acceptability of BLOs

Table 8.1 Complete data from the Public Acceptability Pilot study

	Questionnaire Section	Response n (%)		Questionnaire Section	Response n(%)
Dengue Experience	IMMEDIATE FAMILY HAD DENGUE	4 (28.57%)	**Socio-Demographics**	MALE	7 (50.00%)
	KNOW OTHERS WHO HAD DENGUE	2 (14.29%)		FEMALE	7 (50.00%)
	IS A SERIOUS ILLNESS	12 (85.71%)		LENGTH TIME LIVING IN NQ	
	SYMPTOMS			2 years or less	3 (21.43%)
	Fever	6 (42.86%)		3-10 yrs.	2 (14.29%)
	Aches/pains	6 (42.86%)		11-20 yrs.	3 (21.43%)
	Lethargy	6 (42.86%)		21-30 yrs.	3 (21.43%)
	All three major symptoms	2 (14.29%)		31-40 yrs.	0 (0.00%)
	RISK			41-50 yrs.	0 (0.00%)
	0 - no risk	0 (0.00%)		51-60 yrs.	1 (7.14%)
	10 - Extreme risk	0 (0.00%)		61-70 yrs.	1 (7.14%)
	Average Risk	2.64/10		70 + yrs.	1 (7.14%)
Dengue Knowledge	HUMANS INFECTED BY MOSQUITOES	14 (100.00%)		CULTURAL/ ETHNIC GROUP	
	HEARD OF *AE.AE*	11 (78.57%)		None/ Australian	11 (78.57%)
	APPEARANCE OF *AE.AE*			Aboriginal/TSI	0 (0.00%)
	Stripy legs	9 (64.29%)		North Queenslander	1 (7.14%)
	Black	4 (28.57%)		Other	2 (14.29%)
	No idea	4 (28.57%)		AGE GROUP	
	AE.AE LIVE/BREED WHERE?			18-24	2 (14.29%)
	water holding containers around home-garden	14 (100.00%)		25-39	3 (21.43%)
	swamps/lagoons	2 (14.29%)		40-59	7 (50.00%)
	Freshwater creeks/ponds	3(21.43%)		60-74	1 (7.14%)
	Beaches/Mangroves/	1 (7.14%)		75+	1 (7.14%)
	BITE WHEN?			HIGHEST EDUCATION	
	during day	4 (28.57%)		Post Grad Degree	1 (7.14%)
	around dusk	5(35.71%)		Grad Diploma/certificate	1 (7.14%)
	at night	0 (0.00%)		Bachelor	3 (21.43%)
	day and night	5 (35.71%)		Advanced Diploma/Diploma	2 (14.29%)

Material Engagement		
READ INFO PROVIDED	12 (85.71%)
RE-READ IN LAST 48 HRS	0 (0.00%)
KEY MESSAGE	
Prevent mosquito Breeding	6 (42.86%)
Free trap/ new trap to try	2 (14.29%)
put traps out	1 (7.14%)
INFO CLEAR AND CONCISE	12 (85.71%)
QUESTIONED TRUTH OF INFO	3 (21.43%)
IF TRAPS DELIVERED DURING DENGUE OUTBREAK	
more motivated to set	12 (85.71%)
less motivated to set	0 (0.00%)
no change to motivation	2 (14.29%)

Certificate	1 (7.14%)
Secondary	6 (42.86%)
PREVIOUS PEST CONTROL ACTIVITIES	
Yes	8 (57.14%)
No	6 (42.86%)
DONATE TO ENVIRONMANTAL ORG.	
Yes	6 (42.86%)
No	6 (42.86%)