A COMPARISON OF OVIPOSITION PREFERENCE IN THE PRESENCE OF THREE AQUATIC PLANTS BY THE MOSQUITOES *CULEX ANNULIROSTRIS* SKUSE AND *CULEX QUINQUEFASCIATUS* SAY (CULICIDAE: DIPTERA) IN LABORATORY TESTS

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Summary

Constructed wetlands are becoming more common in New South Wales as they are a key element of Water Sensitive Urban Design within new residential and industrial developments. As well as providing waste-water management, wildlife conservation, or improved amenity, they may also inadvertently enhance local habitats for mosquitoes. The diversity and abundance of aquatic macrophytes has been identified as a predictor of mosquito abundance but there is a paucity of information on species-specific mosquito-plant associations. The aim of this study was to determine whether two pest mosquito species, *Culex annulirostris* and *Culex quinquefasciatus* exhibited an ovipositional preference when exposed to three aquatic plant species (*Salvinia molesta*, *Eichhornia crassipes*, and C*yperus haspens*) in laboratory tests. Significantly more egg rafts were laid in association with *S. molesta* than either *E. crassipes* or *C. haspens* by *Cx. annulirostris*. This result suggests that control of *S. molesta* may reduce the suitability of habitats for mosquitoes. There was no significant difference in the mean number of egg rafts laid by *Cx. quinquefasciatus* in association with the three plant species. These results highlight the need for appropriate management of aquatic weeds in wetlands to ensure the environmental and human health risks are minimised.

Keywords: Mosquito, *Culex annulirostris*, *Culex quinquefasciatus*, vector, disease, nursery, plant, transport, Sydney.

INTRODUCTION

Water Sensitive Urban Design (WSUD) principles have become an increasingly common component of new urban developments within Australia (Melbourne Water 2009). As well as the installation of rainwater tanks, one of the key elements in WSUD is the use of constructed wetlands for waste-water treatment (Melbourne Water 2005). These wetlands also provide the additional benefit of improved aesthetics, passive recreation areas and wildlife refuge. However, concern has been raised as to the potential nuisance-biting and public health risks associated with mosquito populations that may colonise constructed wetlands (Russell 1999).

The most common mosquito-borne diseases in NSW are caused by Ross River virus (RRV) and Barmah Forest virus (BFV) with an average of approximately 800 notified cases per year between 1994/95-2007/08 in coastal regions (Doggett *et al.* 2009). However, it is thought that the number of notified cases is an underestimate of the total number of infections that occur each year (Russell and Kay 2004). Although generally considered diseases associated with regional areas, the incidents of disease caused by these mosquito-borne viruses are increasingly occurring closer to urban areas (Russell and Kay 2004). In addition, the potentially fatal Murray Valley encephalitis virus (MVE) has re-emerged in south-eastern Australia in recent years (Knox *et al.* 2012).

There are over 100 mosquito species known from NSW but only a relatively small number of those species associated with freshwater habitats pose a significant public health or nuisance biting risk (Russell 1993). There is a suite of mosquito species that may be associated specifically with constructed wetlands that pose a pest or public health risk (Russell 1999). However, the greatest risks in NSW is most likely posed by *Culex annulirostris* Skuse, a known nuisance-biting pest that has been shown to transmit RRV and BFV in laboratory trials and has had RRV, BFV and MVE isolated from field collected specimens (Russell 1998, Doggett *et al.* 2009). In addition, *Culex quinquefasciatus* Say is a known pest species closely associated with highly polluted freshwater habitats that has had RRV isolated from field collected specimens (Russell 1998, Doggett *et al.* 2009).

Although effective control agents of pest and vector mosquitoes are currently available (Russell and Kay 2008) and pre-emptive mosquito control activities have been shown to assist in the prevention of mosquito-borne disease (Tomerini *et al.* 2011), there are no broadscale mosquito control programs in NSW directed towards freshwater mosquito populations. With regard to constructed freshwater wetlands, the design, construction and maintenance of these habitats remain the key strategies to ensure that mosquito populations are kept to a minimum (Walton and Workman 1998, Russell 1999).

Aquatic macrophytes play an important role in constructed wetlands by assisting in the improvement of water quality (Thullen *et al.* 2002, Thullen *et al.* 2005). However, it is well established that the aquatic microphyte zones of constructed wetlands represent potentially important mosquito habitat (Walton and Workman 1998, Russell 1999, Greenway *et al.* 2003). These macrophyte zones provide suitable habit for mosquitoes by providing refuge from wind/wave disturbance, protection from predators, organic material for nutrition and oviposition sites for adult mosquitoes. The types of plants present in constructed wetlands can be diverse in both their species richness and growth forms and may include free floating, surface floating, emergent, and submerged depending on the design and functional objectives (e.g. water treatment, water storage, wildlife habitat) of the constructed wetland. While relationships between vegetation growth and relative water depth have been investigated (Russell 1999), there is very little information available on specific plant-mosquito interactions.

The aim of this study is to determine whether two species of mosquito, *Cx. annulirostris* and *Cx. quinquefasciatus*, exhibit an oviposition preference for different types of aquatic macrophytes under controlled conditions.

MATERIALS AND METHODS

Mosquito species

Two mosquito species were used in the laboratory tests, *Cx. annulirostris* and *Cx. quinquefasciatus*. Mosquitoes were housed within the insectary of the Department of Medical Entomology, University of Sydney and Westmead Hospital under animal ethics approval number 8001/04-10 (Colonisation and maintenance of mosquito stock colonies). The insectary operates at temperature of 23-26^{o}C, relatively humidity 60-90% and with a 12:12 h light: dark regimen. Immature mosquitoes were raised in trays approximately 35x45x10 cm in size and containing approximately 4.0 L of deionized water. Larvae were fed approximately 10 mL of a stock solution, comprised of 3.5 g brewer's yeast (Brewer Yeast, Healthy Life) and 3.5 g fish flakes (Warley's Tropical Fish Food Flakes, The Hartz Mountain Corporation) in 500 mL of deionized water, daily. Pupae were transferred to smaller cups (approximately 200 mL) and placed in a mesh cage for emergence.

Three day old adult mosquitoes were provided a blood meal by placing an anesthetised rat into the cage for approximately 45 minutes. All blood fed mosquitoes were removed from the cage and placed into a new cage and housed for approximately three days to allow egg development. Gravid mosquitoes were transported from Westmead Hospital to the University of Sydney for testing.

Oviposition experiments

Oviposition choice tests were conducted at the Darlington Glasshouses at the University of Sydney. The oviposition preference of the two mosquito species were tested separately. Tests were conducted within an enclosed room under ambient lighting and kept at 21 ± 2 ºC.

Three aquatic plant species, each exhibiting a different growth form, were chosen for the experiments. *Salvinia molesta* (common name Salvinia) is a small free-floating water plant, *Eichhornia crassipes* (common name water hyacinth) is a medium sized free-floating plant and C*yperus haspens* (common name dwarf papyrus) is an emergent macrophyte (Sainty and Jacobs 2003).

Three plastic tents (length: 120 cm, width: 120 cm, height: 95 cm) were used as cages for the oviposition choice tests and placed side by side in the centre of the room. Within each of the three tents, three 5 L plastic buckets containing one of the three different plant species and 3 L of aged tap water, were placed in predetermined locations. All buckets were "conditioned" prior to experimentation by cleaning them and placing them outdoors for two weeks prior to use to ensure that there were no residual chemicals that may influence the oviposition behaviour of the mosquitoes. The configuration of bucket placements was consistent across each of the three tents. However, the position of each bucket and plant was changed between tents so that all three possible positions were included.

Approximately 80 gravid mosquitoes of the same species were released into each of the three cages and the number of egg rafts laid in each of the buckets in each of the cages was recorded daily for three days post mosquito release. After four days, any remaining mosquitoes were killed and removed from the case. The experiment was repeated twice for each species to and the data for each mosquito species was analysed separately. The mean number of egg rafts laid per bucket across the six replicates per test per species was compared using one-way analysis of variance (ANOVA) to determine statistical significance of any differences resulting from plant species.

RESULTS

There was a significant difference ($F=25.49$, $df=2,17$, $p<0.01$) between the mean number of egg rafts laid in each plant species by *Cx. annulirostris* (Figure 1). Significantly greater numbers (mean±SE) of egg rafts were laid in association with *S. molesta* (33.50±1.55 egg rafts per bucket) compared to both *E. crassipes* (13.33±1.67 egg rafts per bucket) and *C. haspens* (17.83±2.20 egg rafts per bucket).

There was no significant difference ($F=0.54$, $df=2,17$, $p=0.59$) between the mean number of egg rafts laid by *Cx. quinquefasciatus* in association with *S. molesta* (13.00±1.55 egg rafts per bucket), *E. crassipes* (15.17±1.28 egg rafts per bucket) and *C. haspens* (15.17±2.17 egg rafts per bucket) (Figure 2).

Figure 1. Oviposition (mean ± SE) by *Culex annulirostris* in the presence of three aquatic macrophytes in laboratory tests. ** Indicates a statistically significant difference ($p<0.05$).

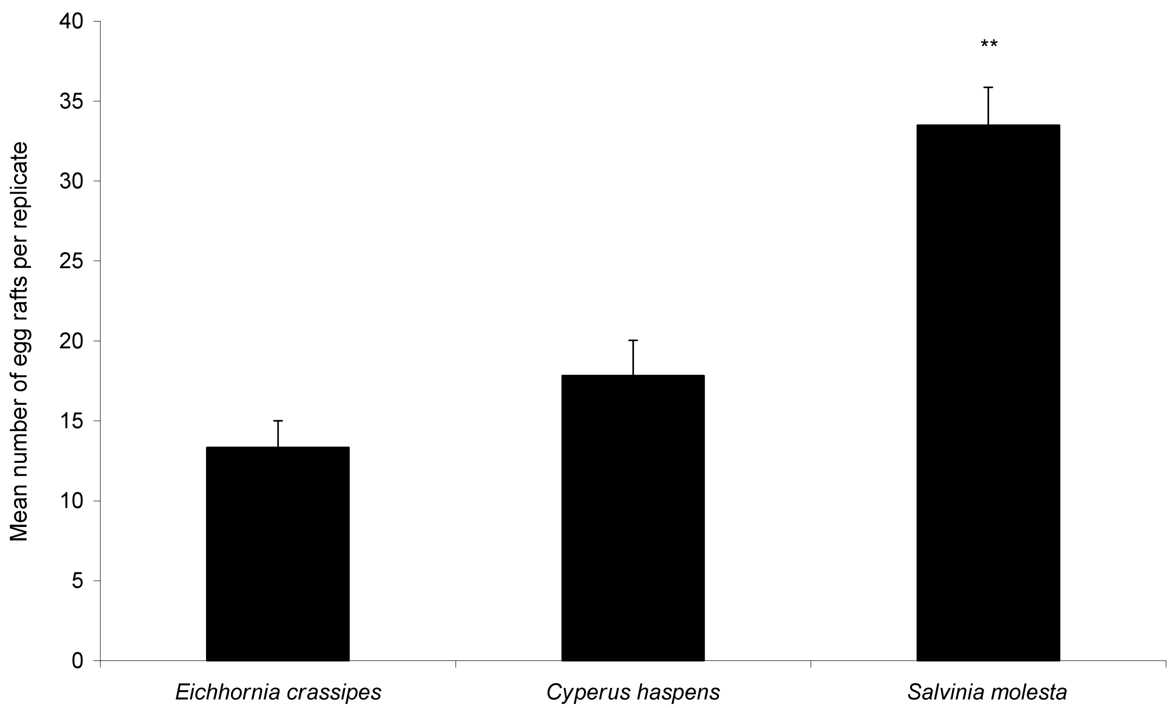
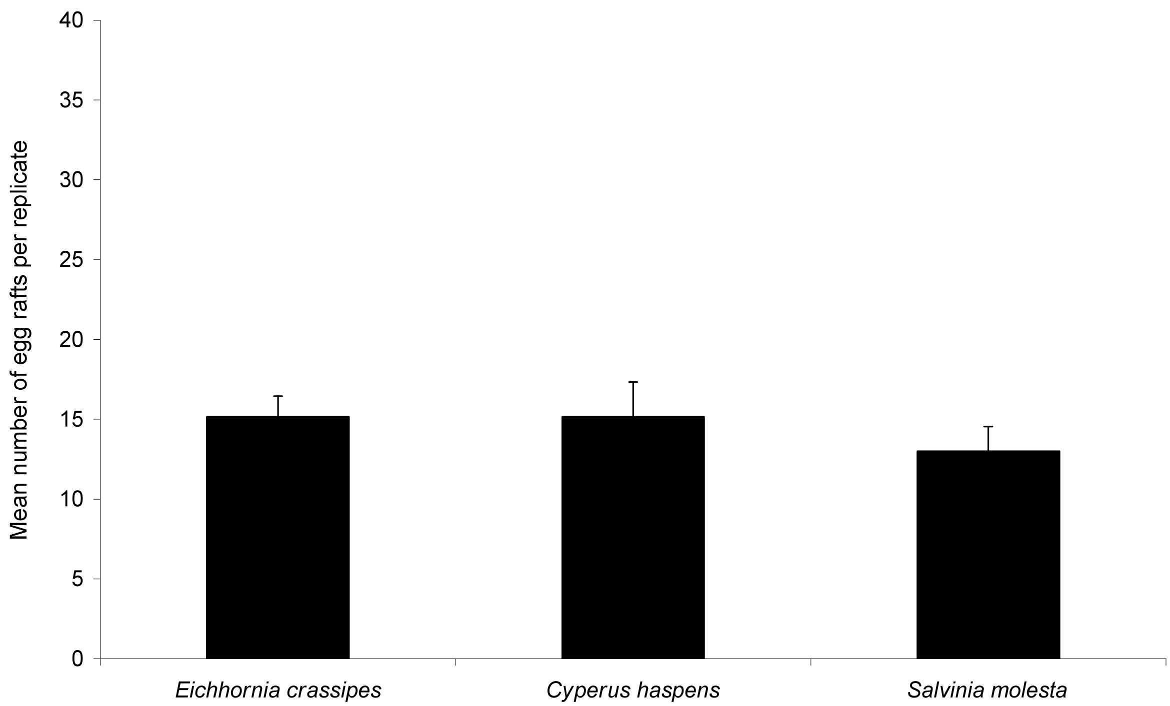


Figure 2. Oviposition (mean ± SE) by *Culex quinquefasciatus* in the presence of three aquatic macrophytes in laboratory tests. ** Indicates a statistically significant difference ($p<0.05$).



DISCUSSION

There is a range of factors that influence the oviposition behaviour of mosquitoes. Studies have demonstrated that water quality (Kramer and Mulla 1979), water depth (Dhileepan 1997) and predator (i.e. fish) presence (Walton *et al.* 2009, Hurst *et al.* 2010) can influence oviposition behaviour of *Culex* spp. However, there is a paucity of information on species-specific plant-mosquito interactions. In this study, we have demonstrated that, while *Cx. quinquefasciatus* showed no plant-specific oviposition preference, *Cx. annulirostris* laid significantly more egg rafts in buckets where *S. molesta* was present compared to either *E. crassipes* or *C. haspens*.

Culex annulirostris most commonly lays egg rafts in freshwater habitats where vegetation is present (Russell 1999). The habitats where immature *Cx. annulirostris* is found can range from ephemeral ground pools to semi-permanent ponds to flood grassland, each of these different habitats providing a different suite of environmental characteristics that influence the mosquito's survivorship and development (Mottram and Kettle 1997). The role of vegetation in determining the suitability of habitats for *Cx. annulirostris* has mostly concentrated on the abundance or development time of immature stages (Greenway *et al.* 2003). While studies have investigated either the attraction or repellency of water containing various infusions made from specific plant species for oviposition of *Culex* spp. (Allen *et al.* 2005, McPhatter *et al.* 2007), few have investigated species-specific oviposition responses and our results are the first that report on a preferential response by *Cx. annulirostris* to *S. molesta*. However, further study is required to elucidate the actual biological, chemical or physical factors that contribute to this result.

The result has implications for the management of *S. molesta* in natural or constructed wetlands as it may predispose those habitats to a greater abundance of pest mosquitoes. This plant was first introduced into Australia in the 1950s and now infests many water ways with the potential to cause serious environmental impact (Howard and Harley 1998, Sainty and Jacobs 2003) and biological control programs using the salvina weevil, *Cyrtobagous salviniae* Calder and Sands, have been undertaken (Sullivan *et al.* 2011). While the potential to enhance the suitability of a wetland for mosquitoes would be of concern, some caution should be noted in that the actual productivity of mosquitoes in a complex natural system may be driven by a range of factors besides the presence of *S. molesta* alone.

Although a higher number of egg rafts were laid in the presence of *S. molesta*, in no replicate test were all egg rafts laid exclusively in the presence of that species. This result suggests that, although the physical or chemical qualities of *S. molesta* provide a more suitable oviposition location, *Cx. annulirostris* does not have a species-dependent relationship with that species. Further investigation is required to determine if a similar oviposition preference is displayed with other small free-floating aquatic plants such as *Azolla* spp, *Lemna* spp or *Ricciocarpus* spp. or to these species at various densities. Perhaps free-floating plants such as *S. molesta* provide a more suitable perch for female *Cx. annulirostris* to engage in oviposition.

Culex quinquefasciatus generally lays rafts of eggs in sites containing high levels of organic matter (Russell 1993). These habitats more often include waste-water ponds or polluted ground pools. This result may explain why there was no observed ovipositional preference by this species. Due to the nature of the habitats in which this species is most commonly found, water quality may be of much higher importance in determining oviposition than the presence of plant species. Studies have shown that *Cx. quinquefasciatus* displays a preference for habitats containing elevated nutrient levels (Chaves *et al.* 2009) although there is some debate surrounding the specific chemical or biological factors that make nutrient rich habitats the preferable oviposition site.

Although the three species tested would not generally be considered for plantings within constructed wetlands due to their weed status (Sainty and Jacobs 2003), there are implications for plantings and vegetation management in these habitats. The ovipositional preference of *Cx. annulirostris* for *S. molesta* highlights the need to manage this aquatic weed in urban constructed wetlands. There is a number of design and/or construction approaches that can reduce the mosquito productivity of constructed wetlands (Walton *et al.* 2012, Walton and Workman 1998, Russell 2001). These approaches typically make conditions unfavourable for mosquitoes to lay their eggs and increase the success of predators which prey upon the immature mosquitoes (Knight *et al.* 2003). However, the introduction and spread of *S. molesta* (or similar floating weed species) may negate the effectiveness of those strategies. Studies have found that where immature stages of *Cx. annulirostris* are protected from predators and wind/wave action, survivorship increases (McDonald and Buchanan 1981, Rae 1990, Mottram and Kettle 1997).

Aquatic plants such as those used in this study, are commonly used in backyard ornamental ponds and their presence may enhance conditions for nuisance-biting species. The demand for aquatic plants required for constructed wetlands, WUSD elements and ornamental backyard ponds may facilitate the movement of mosquitoes between nurseries and other locations. A survey by Durre (1998) on the mosquito abundance in nursery sites, found that only 12.8% of nurseries experienced significant mosquito breeding. However, surveys by Webb *et al.* (2010) found that nurseries can provide suitable habitats for mosquitoes. While *Aedes* spp. were the most likely mosquitoes to be found in association with nurseries, the potential for other species to be transported with large amounts of plant material and/or aquatic weeds such as *S. molesta* requires careful consideration. In particular, mosquito species with immature stages that attach to aquatic plants, such as *Mansonia* spp. and *Coquillettidia* spp., may be more likely to be transported than *Culex* spp. whose larvae require free standing water. Consideration should be given to the use of control agents (Russell and Kay 2008) to minimise the risk associated with the presence and proliferation of mosquitoes and the subsequent transport and dispersal of these vectors for disease.

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