

Container surface area and water depth influence the population dynamics of the mosquito *Culex pervigilans* (Diptera: Culicidae) and its associated predators in New Zealand

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Received 24 February 2003; Accepted 25 July 2003

ABSTRACT: The density of larval mosquitoes in containers is related to adult mosquito ovipositional preferences and to other factors such as the abundance of predators. We examined the effects of anthropogenic container size and water depth on the population dynamics of mosquitoes and their macroinvertebrate predators in Wellington, New Zealand. *Culex pervigilans* was the only mosquito species observed in these containers. With the exception of one sampling date, throughout the year *Cx. pervigilans* larvae, egg rafts, and pupal exuviae were observed. The highest densities of *Cx. pervigilans* were observed in the containers with the smallest surface area. A multiple regression analysis was used to analyze the effects of container surface area and depth on the mean density of mosquito larvae in each container. This analysis showed larval densities significantly decreased with increasing container surface area, for two-thirds of the year. Although fewer *Cx. pervigilans* were generally observed in similarly sized containers with greater depths, this effect was not statistically significant. The significant effect of container surface area appeared to be related to adult ovipositional preferences, as few observations of predators were made in any of the container treatments. Of the predators that were observed, damselfly larvae and diving beetles tended to be more common in the larger containers. Our results suggest that mosquitoes may have strong preferences based on container size alone that influence larval population dynamics, irrespective of the abundance of aquatic predators. *Journal of Vector Ecology* 28 (2): 267-274. 2003.

Keyword Index: Anthropogenic containers, mosquito, phenology, macroinvertebrate predators.

INTRODUCTION

Artificial containers are a major source of breeding habitat for mosquitoes worldwide. These containers include tires, stock troughs, bottles, and cans. The number of mosquitoes that are vectors of human disease can be proportional to the availability of such artificial larval habitats (Wang et al. 2000).

New Zealand currently has only 16 species of mosquitoes and only one identified avian and human arbovirus known to be vectored by the two native species *Culex pervigilans* and *Culiseta tonnoiri* (Maguire et al. 1967, Miles 1973). The mosquito *Cx. pervigilans* is New Zealand's most common native mosquito species observed in artificial containers (Graham 1929, 1939, Belkin 1968, Laird 1990, 1995). *Culex pervigilans*, a vector of the human and avian Whataroa virus (Maguire et al. 1967, Miles 1973), is also suggested to be a vector of avian malaria (Holder et al. 1999) and has been highlighted as a species requiring further investigation

for its potential to vector exotic arboviruses (Weinstein et al. 1997). Several other container-dwelling species that are also able to vector disease are present in New Zealand, such as the Australian invaders *Culex quinquefasciatus* and *Ochlerotatus notoscriptus* (Weinstein et al. 1995). These known arbovirus vectors have arrived in New Zealand during the last two centuries and appear to be increasing in their distribution (Weinstein et al. 1997). While all these species have been observed in a range of container types (Laird 1990, 1995), the importance of container size and water depth has not previously been investigated.

Invertebrate predators can also directly and indirectly influence mosquito population dynamics. For example, the backswimmers *Notonecta* spp. (Hemiptera: Notonectidae) are highly predaceous, consuming large numbers of mosquito larvae and thereby structuring aquatic communities (Murdoch et al. 1984, Blaustein et al. 1995). In addition, the presence of backswimmers within a water body can significantly reduce oviposition

by adult mosquitoes (Chesson 1984, Blaustein et al. 1995). Other commonly observed larval predators of mosquitoes in New Zealand include the diving beetle *Rhantus pulverulosus* and damselfly larvae (Graham 1939). These predators are often observed in natural and anthropogenic water containers, but their effects on mosquito population dynamics have not previously been examined. Previous research has indicated that many aquatic predators show a preference for larger container sizes (Sunahara et al. 2002). The behavior of both the mosquitoes and their predators is thus likely to influence the abundance of mosquitoes in containers.

Our aim in this study was to examine the preferences and phenology of mosquitoes in anthropogenic containers with varying surface areas and water depths. We also wanted to gain information on the mosquito predators that would utilize these containers and on the interaction between these predators and the mosquito fauna.

MATERIALS AND METHODS

We examined the mosquito population dynamics in five container sizes ranging from 1.3 to 300 L, at two different water depths (Table 1). All the containers were circular and made of plastic. The inside surface of the containers was roughened with 40 grit sandpaper to provide mosquitoes with a textured surface on which to grip the side of the container while ovipositing. The containers were also painted matt black on the interior surface with Plastikote enamel spray paint (Plastikote color T-2) to control for container color as a factor in attracting mosquitoes and their predators. A 10mm diameter hole was drilled in the side of the containers at an appropriate position to maintain the desired water level in each container despite rainfall. Containers were also monitored for any reduction in water levels due to evaporation.

Containers were placed in the field on 17 December 2001 at three sites at the Karori Wildlife Sanctuary, Wellington (WGS84 datum, latitude 41°18.000 and longitude 174°45.000). This area contains native forest, with the nearest urban development being ~500 m from any of the study sites. Sites were approximately 20 m from the lake edge at the reserve and were separated from each other by 150-400 m. At each site, each of the ten different treatments were represented. The water added to the containers was sourced from the reservoirs adjacent to the sites. Before adding the water to the containers, it was filtered through two layers of a fine nylon mesh (intermesh gap = 0.5 mm) to remove any existing macroinvertebrates. Nutrients were added to the water in the form of "Kiwi" brand pelletised sheep

manure (Cryovac, Rotorua, New Zealand) at a rate of five g/L of water, containing approximately 3% nitrogen, 2% phosphorus, and 4% potassium. This type and rate of nutrient addition was found to be ideal for mosquito populations in a nearby experiment (Leisnham et al., submitted for publication). To test the efficacy of the filtration method for removing invertebrates or their eggs, an additional five 10 L samples were filtered as above and then stored in the laboratory for four wks. No evidence of any mosquitoes or mosquito predators were subsequently found in these samples.

Each field container was sampled over the following year (from 3 January 2002 to 7 January 2003) at intervals ranging between one wk and one mo depending on season and mosquito larval abundance. On each sampling occasion the containers were first visually examined for mosquito larvae and their aquatic predators. Mosquitoes and predators such as *Rhantus* spp. larvae and adults were easily observed as they came to the water surface to breathe. A flat white plastic panel was then placed on the bottom of the containers, which provided a contrasting surface on which to observe insects swimming. Predators were identified as much as possible by *in situ* visual inspection, as they were generally in low densities and we wanted to retain these in the containers to interact with the mosquitoes. To estimate mosquito densities, the water in each container was mixed by stirring before drawing out a water sample. A 1 L sample was taken in the 10 to 300 L containers, and a 100 ml sample was taken from the 1.3 L containers. Samples were passed through nylon 0.5 mm fine mesh to filter out mosquitoes that were then preserved in 70% ethanol for identification in the laboratory.

Climate data were provided by the New Zealand National Institute for Water and Atmospheric Research in Kelburn, which is approximately 2 to 2.5 km from the study sites at Karori Wildlife Sanctuary. Data were the monthly total rainfall, average temperature, and average minimum and maximum temperature for the year 2002.

Data analysis

We analyzed the data using multiple regression. The independent variables were the surface area and water depth in each container treatment. The dependent variable was mosquito larval density, which is likely to be a result of the combined adult abundance and oviposition preference, and subsequent effects of predators, competitors, etc, from within containers. For purposes of analysis, data were broken into three equal sections: an initial phase covering the later summer and warmer autumn conditions (from January to the end of March 2001), the cool southern hemisphere winter months (May to the end of August), and the spring and

early summer months (September until the end of December). The densities in each replicate container over each sampling duration were averaged. The multiple regression model used these replicate average densities. All numerical data were log transformed prior to analysis and the residuals examined for normality and homogeneity of variance. The container surface area was also log transformed to fit the linear model. A two-way ANOVA approach was not used because the containers differed in water depth across treatments (Table 1). All mosquito densities are given as mean \pm standard error per L of water.

Testing the sampling method

The sampling method was examined for its ability to accurately estimate the density of mosquitoes in different sized containers. The 1.3, 10, and 100 L containers were filled with water in the laboratory and field collected *Cx. pervigilans* added to achieve a mean density of 20 larvae/L. Five samples were then taken from the containers using the above sampling method. Each sample was returned to the container before taking the next sample, so as not to deplete mosquito numbers. The observed number of mosquitoes sampled in each container was then tested for significance against the expected value of 20/L.

RESULTS

Mosquito population dynamics throughout the year

The only mosquito species that was observed to utilize the containers during the entire study was *Culex pervigilans*. Larvae of this species were observed in high numbers two wks after starting the experiment in December 2001. The first sample in January 2002 showed densities of up to 1410 larvae per L. Larval densities continued to increase in most treatments, peaking during the end of January at a mean of 4,380 \pm 1,112/L in the 1.3 L treatment containers (Figure 1).

January was the warmest month and also the second wettest month for 2002 (Figure 2). Densities declined during autumn and winter, but on only one sampling occasion (1 September 2002) were no *Cx. pervigilans* larvae observed in any of the containers. On this date, ice had to be broken to enable sampling in some of the treatments. However, climate data indicated that this was not the coldest month for 2002, which was July (Figure 2). Mosquito exuviae were periodically observed in the containers throughout the year including on the 1 September sample, indicating that the mosquito larvae observed in the 5 October sample following this date may have been recently oviposited. Larval densities increased after October with warmer weather during spring and summer, but did not reach the densities observed during January (Figure 1), possibly due to the cooler spring and summer conditions than those that had been observed during January 2002 (Figure 2).

Laboratory tests showed that our mosquito larvae sampling method was a reasonably effective method for estimating mosquito densities. No significant difference was observed between the observed density of mosquitoes for the 1.3, 10, or 100 L containers and the expected density of 20 larvae/L. Estimated mean densities from these trials were 15.0 \pm 5.3 (\pm standard error), 19.4 \pm 7.6, and 25.0 \pm 3.5, for the respective container sizes, each of which falls within the 90% confidence interval containing 20 larvae/L.

Effect of container surface area and depth

The highest larval densities were observed in the smaller containers (Figure 1). The multiple regression analysis indicated decreased density of mosquitoes with increasing container surface area, for two of the three sampling periods. Even for the third sampling period the regression coefficient was negative (Table 2). Varying responses observed throughout the year are indicative of the varying role of surface area in influencing mosquito abundance with changing seasons. For all three

Table 1. Container sizes for the experimental treatments; dimensions are diameter \times height. Containers were filled to either a "high" or "low" depth, influencing the amount of water available for mosquito larvae. Three replicates of each container size and depth were used in the experiment.

Container size	Surface area	Water at high depth	Water at low depth
1.3 L (9 \times 17cm)	0.006 m	1.1 L (15cm deep)	0.4 L (depth 6cm)
10 L (25 \times 25cm)	0.049 m	8 L (22cm deep)	3 L (depth 9cm)
30 L (45 \times 24cm)	0.159 m	22 L (19cm deep)	8 L (depth 8cm)
100 L (67cm \times 8cm)	0.353 m	66 L (24cm deep)	22 L (depth 9cm)
300 L (110 \times 37cm)	0.950 m	200 L (22cm deep)	66 L (depth 8cm)

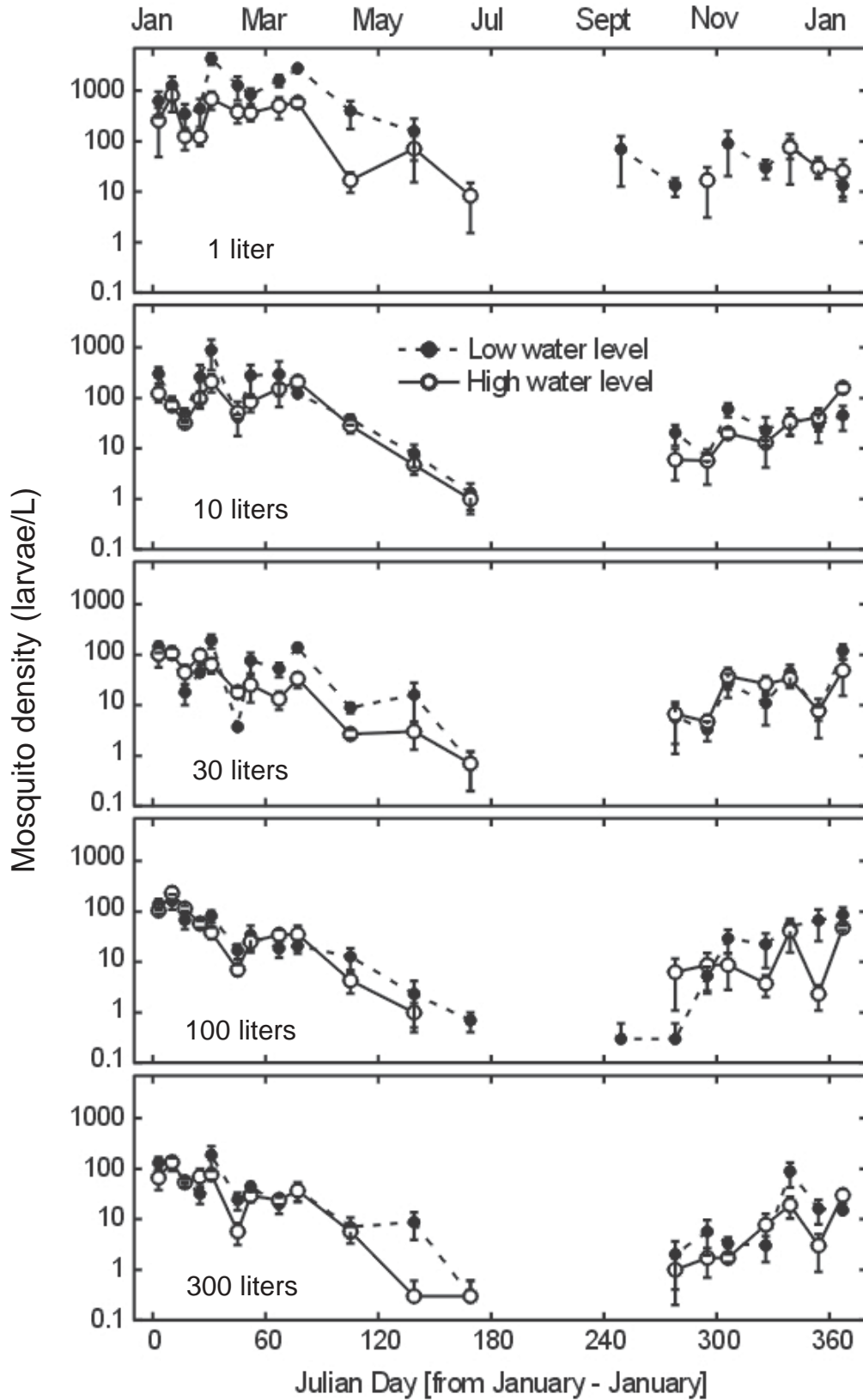


Figure 1. The phenology of *Culex pervigilans* in the different containers and depth treatments in Karori Wildlife Sanctuary during 2002. Note that the y-axis is on a log scale, result in zero values not being plotted. For all graphs, $n = 3$ for each treatment; error bars are 1 standard error.

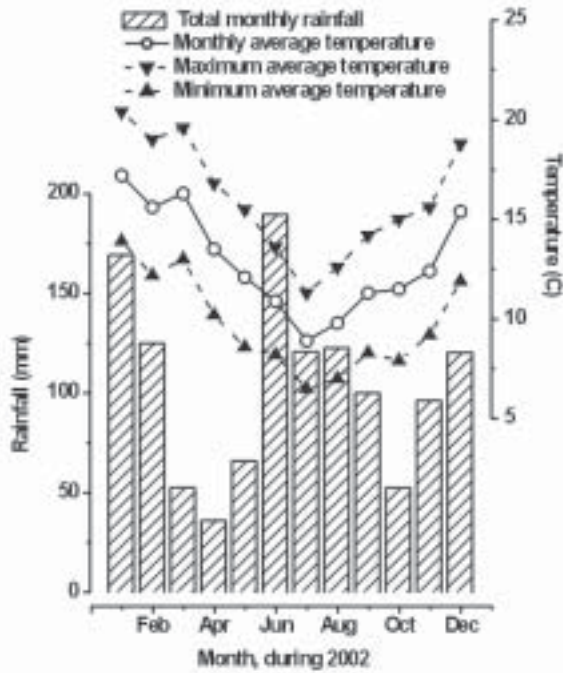


Figure 2. Climate data from Kelburn, approximately 2–2.5 km from the study sites. Rainfall estimates are for the total amount of rainfall for the entire month. Mean temperatures are the average for each month in 2002. Minima and maxima are the monthly mean of the daily extremes.

analyses, a log transformation for both mosquito density and surface area approximately linearized the relationship between these two variables (Figure 3).

Generally, more mosquitoes were observed in the

containers with lower water levels. This result was observed for all containers but was most apparent in the 1.3 L container treatments, in which containers with 2.8 times as much water held on average 3.6 times more mosquito larvae (Figure 1). In the 300 L containers, the low water treatment only had an average of 1.4 times more mosquitoes than in the high water treatment. Consequently, there was always a negative sign on the depth coefficient in the multiple regression analysis (Table 2). However, the effects of depth were not significant in the multiple regression analysis ($P = 0.103-0.225$).

The container size and water depth treatments were chosen so as to allow specific comparison of mosquito dynamics when the same amount of water was presented but in different container depths and surface areas. Paired treatments that contained the same amount of water were the “10 L high level” and the “30 L low level”, the “30 L high level” and the “100 L low level”, and the “300 L low level” and the “100 L high level” (Table 1). Similar numbers of mosquitoes were observed in these treatments (Figure 3).

Observations of mosquito predators

Surprisingly few aquatic macroinvertebrate predators were observed during the course of the year-long experiment. However, the only treatment in which no mosquito predators were observed was the 1.3 L containers. This was unlikely to be a sampling effect as the 1.3 L containers were the easiest to examine for predators, due to the small water volume within these containers. In the 10 L container treatments only one predator, the backswimmer *Anisops* spp., was observed only once over the duration of the experiment. The only

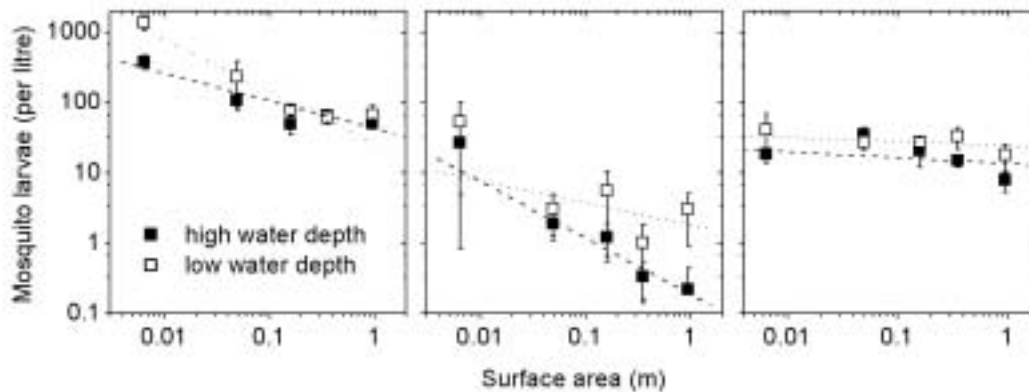


Figure 3. The relationship between container surface area and average larval mosquito density, for containers with high and low water treatments. The densities in each replicate container over the sampling duration were averaged, and each point on the graph represents the overall mean of the three replicate containers in each treatment ± one standard error.

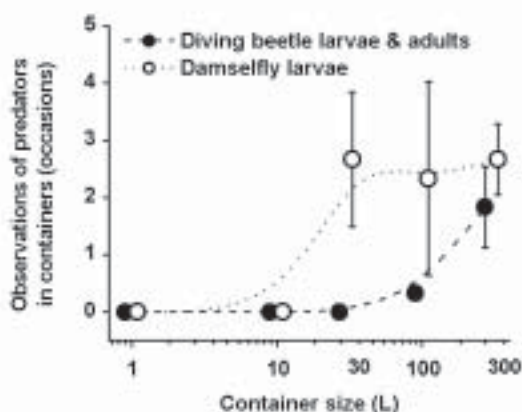


Figure 4. The average number of occasions when predators were recorded in each container size treatment, from the entire years observations. Only data for diving beetle larvae (*Rhantus* spp.) and damselfly larvae are shown. $n = 126$ for each treatment, error bars are 1 standard error.

other occasion an *Anisops* spp. was observed was in a 300 L container on the 14 February sample.

Damselfly larvae and diving beetles were more commonly observed in the containers. Two species of damselflies were likely to be present, *Austrolestes colenstonis* (White) and *Xanthocnemis zealandica* (McLachlan). Adults of both species appeared to form territories over the containers during the summers. Larvae of these predators were observed to occur between one and eight times out of the twenty sampling occasions for each container in the 30–300 L treatments. Diving beetles were less common than damselflies and were most commonly observed in the 300 L containers (Figure 4).

DISCUSSION

The only mosquito species observed in this study was *Culex pervigilans*. This species is often cited as New Zealand's most common mosquito species, utilizing a wide variety of larval habitat types (Graham 1939, Belkin 1968, Laird 1990, 1995). New Zealand currently has only 16 mosquito species, many of which have much more specific larval habitat requirements (Belkin 1968). For example the other common container-dwelling species *Ochlerotatus* (formally *Aedes*) *notoscriptus* appears to prefer containers that are not in direct sunlight and is only rarely found in containers such as stock troughs (Lee et al. 1982, Laird 1990, 1995). Early studies on *Cx. pervigilans* in the warmer, northern parts of New Zealand showed ovipositing adults and larvae to be present in containers throughout the year, with larvae even alive under ice (Graham 1929, 1939). We found similar results, with only the September sample completely lacking *Cx. pervigilans*. Mosquito larvae, pupal exuviae, and egg rafts from adults were found in the coldest winter month of 2002 (July).

Smaller containers had higher densities of *Cx. pervigilans*. Although not previously reported in the literature on this mosquito species, we expected this result from our preliminary observations. It is possible that smaller containers may have had more *Cx. pervigilans* due either to adult ovipositional preferences for smaller containers or mortality factors influencing the survival of eggs and larvae. Other mosquito species have been observed to display strong ovipositional preferences (Williams et al. 1999, Pavlovich and Rockett 2000). The presence of chemicals from vertebrate or invertebrate mosquito predators can significantly reduce mosquito oviposition (Blaustein et al. 1995, Blaustein 1998, Angelon and Petranka 2002, Spencer et al. 2002). Some previous studies in New Zealand have suggested that aquatic predators can strongly influence the survival of larval *Cx. pervigilans in situ*. Graham (1939) noted that

Table 2. Results from multiple regression analysis of mean mosquito densities, for mean densities over three periods of the experiment. Surface area and mosquito abundance were log transformed prior to analysis. Data each coefficient of the multiple regression analyses are presented \pm the standard error. Data were log transformed prior to analysis. *, $P > 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Coefficient	January - April	May - August	September - December
Constant	4.107 \pm 0.381 ***	1.016 \pm 0.627	3.109 \pm 0.358 ***
Surface area	-1.114 \pm 0.175 ***	-0.653 \pm 0.287*	-0.240 \pm 0.164
Depth	-0.033 \pm 0.0198	-0.043 \pm 0.033	-0.023 \pm 0.018
R^2	0.65	0.24	0.15

some stock troughs with the backswimmer *Anisops assimilis* were free of mosquitoes, while puddles in depressions surrounding the troughs caused by stock footprints contained "an energetic mosquito activity."

The complete lack of predators in some of the intermediate sized containers, combined with their significantly reduced densities of *Cx. pervigilans* compared to densities in smaller containers, suggests that these mosquitoes were choosing habitat based solely on habitat size. *Culex pervigilans* is known to spend some time flying around the water body, apparently evaluating it as an oviposition site (Graham 1929). Container size could be a criterion in such a selection process. It is possible to theorize how such behavior could be positively selected over several generations if larger containers did generally contain more predators.

Predators, although not common to all treatments, did appear to be more abundant in the larger containers of our study. Damselfly larvae and juvenile and adult diving beetles were observed in the larger containers, often in high densities. However, we observed few *Anisops* spp. in any container treatment. The absence of predators in small containers or higher abundance of predators in larger containers has been observed elsewhere (Sunahara et al. 2002). Some authors have attributed this result to an island biogeographic effect, wherein mosquito predators are more likely to find larger habitat patches (Washburn 1995). Perhaps the lack of *Anisops* spp. in our larger containers may have been quite fortuitous. Had *Anisops* spp. been present in the larger containers we might have assumed that it was the direct or indirect presence of these predators that caused the reduction in mosquito densities relative to smaller container treatments. Our results indicated that some containers had no or few predators whatsoever and yet still had significantly lower mosquito densities than in the 1.3 L treatment.

The lack of a significant effect of water depth in the containers was surprising considering the densities in the lower water treatment were generally higher than in the high water depth. This trend was reflected in the multiple regression results, wherein the depth coefficient was always negative, but not significantly so. High variation and too few replicates may have led to this result. Other workers have found water depth influences mosquito population dynamics, but this effect is thought to occur because shallow pools can easily dry up (Fischer et al. 2002). Our containers did not dry out during the experiment.

Mosquito utilization of different sized containers may be influenced by the presence of predators directly (e.g. consumption) or indirectly (e.g. the presence of predator chemicals) (Murdoch et al. 1984, Blaustein et

al. 1995). However, our results suggest that mosquitoes may have strong preferences based on container size alone that influence population dynamics. While the relationship between container size, predators, and mosquito abundance is becoming better understood (e.g. Sunahara et al. 2002), further understanding is likely to be achieved by experiments which manipulate the abundance of predators and container size. Such experiments are likely to aid mosquito management by identifying highly productive containers and container sizes unlikely to be influenced by macroinvertebrate predators.

Acknowledgments

We thank Raewyn Empson and the staff of Karori Wildlife Sanctuary for hosting this study, Elaine Fouhy and the National Institute of Water & Atmospheric Research (NIWA) for climate data, and Jo Love for assistance in the field. We also thank Stephen Hartley and two anonymous referees who reviewed this manuscript. This research was funded by Victoria University of Wellington.

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