

## Evaluation of two dipping methods for sampling immature *Culex* and *Ochlerotatus* mosquitoes (Diptera: Culicidae) from artificial containers

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**Abstract** Monitoring changes in populations of immature (larvae and pupae) mosquitoes are fundamental to determining mosquito-borne disease risk. Dipping is the most common method used to sample immature mosquitoes, but it can be biased towards particular species and instars. We aimed to assess the generality of the findings of Mori (1989),

who showed that stirring the water of artificial containers gave more consistent and accurate samples of *Ochlerotatus togoi* compared with conventional dipping without prior stirring. Five water-filled artificial containers were placed in each of pastureland, urbanland, and native forest. These containers were subsequently colonised by *Culex pervigilans* and *O. notoscriptus* and were used to compare two dipping methods using a plastic dipper. One method involved removing the container from the field and stirring the water before sampling (destructive sampling), and was compared with a conventional field dipping method without prior stirring (field dipping). Results from this study showed that when comparing samples from the two methods, destructive sampling gave estimates that had very strong and better correlations with absolute counts, and were more accurate and consistent. Field dipping generally underestimated *O. notoscriptus*, possibly because it did not sample the bottom of the container where *Ochlerotatus* larvae browse detrital sediments. When using estimates from destructive sampling compared with field dipping, the relationship between mosquito density and land use was more similar to the relationship between absolute counts and land use across all population measures used. The results suggest that destructive sampling may be a more reliable mosquito sampling method than conventional field dipping when studying questions on mosquito ecology.

**Keywords** anthropogenic; environment; collect; New Zealand; quantitative sampling

### INTRODUCTION

To undertake efficient surveillance, control, and ecological studies of vector mosquitoes, it is essential to have reliable and simple sampling methods of immature stages (larvae and pupae). In the past, often only the presence of a species was recorded from water-filled containers (such as with the Breteau index (Breteau 1954) and house index (Conner &

Monroe 1923)). To develop better survey methods, researchers are now striving to measure changes in abundance and estimate productivity from populations of immature mosquitoes (e.g., Tun-Lin et al. 1995). However, even small containers can have large populations of mosquitoes, often rendering direct counts of known numbers unfeasible, and making subsampling the only viable option.

Dipping is the most common technique used to sample immature mosquitoes because it is simple, cost-effective, and gives estimates that are often easier to standardise between replicates, compared with other sampling methods (e.g., using sweep nets) (Service 1995). Conventional dipping methods sample only the top portion of water in habitats and preferentially collect mosquitoes near the surface. Therefore, despite its benefits, dipping can be biased towards particular species and instars which are usually vertically distributed in a habitat because of aerobic constraints, alarm reactions, and feeding modes (Service 1995). Mori (1989) found that before dipping from jars holding 2 litres of water and 600 *Ochlerotatus* (formally *Aedes togoi* (Theobald), stirring the water gave more reliable and accurate samples compared with conventional dipping without prior stirring. More recently, Tun-Lin et al. (1994) suggested that creating a vortex or homogenising the water before collecting could increase the efficiency of sampling, but their comments were in reference to sampling *A. aegypti* Linnaeus using sweep nets. It is clear from the literature on mosquito sampling methods (reviewed in Service 1995) that there is the need for the evaluation of the efficiency of dipping methods across different species and habitats. In particular, since the study by Mori (1989), no studies that we know of have set out to test the proficiency of homogenising or stirring the water before dipping from containers, although the practice is common for sampling other species in the field (e.g., *Culex pervigilans* Bergroth in Lester & Pike (2003)). Because of the increasing importance of accurately sampling mosquitoes to make informed abatement decisions, we aimed to assess the generality of the findings of Mori (1989). In addition to *O. togoi* studied by Mori (1989), we studied two mosquito species that are common and widespread in New Zealand and have potential public health importance; the endemic *C. pervigilans* Bergroth and the exotic *O. notoscriptus* (Skuse).

New Zealand has not yet experienced an epidemic of mosquito-borne human disease, but the past conversion of much of New Zealand into an

agro-ecosystem and current urbanisation has led to the spread of both native and, more recently, exotic vector species (Laird 1990, 1995; Cook et al. 2002). Anthropogenic land-use change has been associated with the emergence and re-emergence of mosquito-borne diseases in many parts of the world (Patz et al. 2000). Land use is thought to primarily drive disease outbreaks by altering factors associated with the immature stages of vector species. Mosquito-borne disease presents a substantial public health risk to New Zealand, with growing trade and commerce increasing the risk of viremic people entering the country (Kelly-Hope et al. 2002). Therefore, monitoring changes in populations of immature mosquitoes is fundamental to determining the influence of land use on mosquito-borne disease risk in New Zealand and overseas. *Culex pervigilans* and *O. notoscriptus* have potential public health importance and are known to prolifically breed in a range of habitats, including artificial containers (Weinstein et al. 1997). *Ochlerotatus notoscriptus* has been implicated as a potential vector of Ross River virus in Australia and the South Pacific (Maguire 1994; Watson & Kay 1998), and is increasing its distribution throughout New Zealand's North Island (Laird & Easton 1994; Laird 1995). *Culex pervigilans* is a known vector of Whataroa virus, which primarily infects native bird populations (Maguire et al. 1967). There is limited serological evidence that Whataroa virus also infects humans (Miles 1973), and *C. pervigilans* has been assessed to be a species requiring testing for its potential as a vector of other diseases (Weinstein et al. 1997).

Leisnham et al. (2004) showed that, under laboratory conditions, conventional field dipping from a 6-litre circular artificial container was likely to sample 2.5 times as many *C. pervigilans* than *O. notoscriptus* when both species were at equal initial densities. In an attempt to avoid this species bias, we developed a second method, which involved removing these containers from the field and emptying the water into a shallower container, and then stirring the water before dipping (destructive sampling). This method sampled a similar proportion of container water, thus collecting numbers of mosquitoes that can be feasibly counted and identified in studies on a larger scale (Leisnham et al. 2004). In this paper we compare the efficiency of both field dipping and destructive sampling in collecting *O. notoscriptus* and *C. pervigilans* immature mosquitoes from experimentally positioned artificial containers, and determine if they give consistent results across three sites of different land use in Paraparaumu, New Zealand.

## MATERIALS AND METHODS

### Study sites

Paraparaumu, c. 70 km north of Wellington city (40°52'S, 175°03'E), was historically a catchment of temperate lowland swamp forest, but is now dominated by a growing town (pop. c. 5000) and surrounding pastureland. Since the first European settlement in the 1840s, like most of New Zealand's lowland swamp forest, Paraparaumu has been almost entirely drained and cleared for pastureland and subsequent urban development (Maclean 1988). A c. 13-ha section of a nature reserve is the only significant patch of lowland native swamp forest remaining. This reserve, along with one pasture site and one urban site were selected for our study, and are indicative of the land-use types found throughout New Zealand. Site selection attempted to control for climate and geomorphology. All sites were within 2 km of each other, approximately of equal size (13–20 ha), <200 m a.s.l., and of similar topographies (Leisnham 2004). Consequently, the selected sites were as similar as possible in all respects except for their land use.

### Study design and materials

Random coordinates were selected for five sampling points in each land-use site using a topographical map broken into 1-m<sup>2</sup> grid squares. Coordinates were reselected if they fell inside inaccessible areas (e.g., in the middle of ponds), or inside unsecured areas (e.g., on public roads), or were within 30 m of another coordinate.

In September 2003, one 6-litre circular black plastic container (described in Leisnham et al. 2004) was secured to the ground as close to each sampling point as possible using a wooden stake. Each container was filled with 4 litres of water from the same local groundwater source filtered through a 0.5 mm mesh, and 20 g of dried pelletised sheep manure (Kiwit<sup>TM</sup>, Mount Maunganui, New Zealand; c. 3% nitrogen, 2% phosphorus, and 4% potassium) was added. Each container was then covered with 0.5 mm galvanised wire mesh (intermesh gap = 20 mm) to prevent vertebrate animals from drinking the water, and the inside surface of each container was roughened with coarse sandpaper to provide a textured surface for *Ochlerotatus* eggs to attach (Belkin 1968; Laird 1995). This type of container and initial nutrient concentration (5 g/litre) has been shown to be suitable mosquito habitat and promote high *C. pervigilans* and *O. notoscriptus* productivity (Leisnham et al. 2004). The initial water line was

marked on each container and all containers were checked for evaporation and topped up with water if needed at 2-weekly intervals from the time they were positioned until sampling.

Field dipping, destructive sampling, and absolute counts (described below) were undertaken on each container on 30 January 2004, after *O. notoscriptus* and/or *C. pervigilans* had been observed in most containers for 4 consecutive weeks (and thus appeared to have established reasonably persistent populations). The dipper used for sampling containers was a 500-ml clear polypropylene plastic cup. The cup had a depth of 62 mm and a circumference of 106 mm at its opening and 97 mm at its base. This dipper, though larger than most dippers, is affordable and commercially available, and appeared to be the most proficient at collecting mosquito larvae from the study containers in preliminary work (P. Leisnham pers. obs.).

One litre of water (c. 25% of each container) was sampled from each container by dipping the 500 ml dipper twice. Sampling approximated a conventional dipping method (Russell 1993). It involved quietly approaching the container and rapidly scooping the dipper through the water just under the water surface and across the diameter of the container so that a full amount of water (500 ml) was held in the cup.

Each litre of water was poured back into its respective container through a 0.20 mm nylon mesh. The larvae that were contained in the mesh were then placed in a lidded bottle of clean water. After sampling, each container was removed from the field and taken back to the laboratory. The dipper and mesh strainer were thoroughly rinsed with clean water before sampling the next container. Upon inspection at the laboratory, no mosquito mortality during transportation was detected. All larvae from the field samples were sorted and identified to genus under 10× magnification. Specimens were subsequently returned to the containers from which they were originally collected for subsequent destructive sampling (see below), and therefore were not killed. Because specimens were living and motile, larvae could not be identified to species level. However, specimens were assumed to be either *C. pervigilans* or *O. notoscriptus* based on the shape of the siphon and the knowledge of container breeding species present in the region (Laird & Easton 1994; Laird 1995). Larval identifications were confirmed by the results from absolute counts (detailed below).

Each container was brought back to the laboratory and sampled by pouring the entire contents into a shallow sampling tray (840 cm<sup>2</sup> × 6 cm deep). The

water was homogenised by gentle stirring before 1 litre was immediately dipped and strained as for field dipping. All collected mosquitoes were preserved in 70% ethanol for subsequent identification and counting.

### Absolute counts

To obtain absolute mosquito counts from each container, the remaining water in the sampling tray was strained and all mosquitoes were preserved for identification and counting. The laboratory procedure for destructive sampling and obtaining absolute counts was repeated for each container from each land use. The dipper, mesh strainer and tray were thoroughly rinsed with clean water before sampling the next container.

Third and fourth instar larvae were identified to species level using the key of Winterbourn et al. (2000). First and second instar larvae were keyed to genus level only. No key is available for pupae. The pupal stage is the penultimate moult before adult emergence whereas the fourth instar larval stage is the last moult that can be keyed to species (Snell 2005). Because of this, pupal density, and fourth instar density for each species were examined separately since they are the best indicators among the immature stages of total and species specific adult productivity respectively (Leisham 2004).

### Statistical analysis

The observed density estimates over replicate containers were compared between sample methods, with expected densities, and between species, using paired-sampled *t* tests (Zar 1999). Linear regression analyses were used to examine the relationship between absolute mosquito counts and density estimates from field dipping and destructive sampling (Zar 1999). Analysis of variance (ANOVA) was used to determine if density estimates reported a similar effect of land-use site on mosquito numbers as absolute mosquito counts (Zar 1999). Models of density data and absolute counts included land use as a fixed factor. Post-hoc tests were performed using the Bonferroni procedure (Zar 1999). All densities are given as means  $\pm$  1 standard error per litre of water; all tests were two-tailed and significance was assigned at the 5% level. All data were  $\log_{10}$  transformed ( $Y' = \log_{10} Y + 1$ ) before analysis and the residuals examined for normality and homogeneity of variance (Zar 1999). All statistical analyses were undertaken using the software SPSS (2003).

## RESULTS

Absolute counts revealed that only the two most common container-breeding mosquito species in New Zealand, the endemic *C. pervigilans* and the exotic *O. notoscriptus*, used the artificial containers. *Ochlerotatus notoscriptus* was the most common species of third and fourth instar larvae, constituting 64.2% ( $n = 905$ ) of the total late-instar larvae collected (*C. pervigilans*,  $n = 505$ ). Of the first and second instar larvae, 51.2% ( $n = 1199$ ) were *Culex* spp. and 48.8% ( $n = 1142$ ) were *Ochlerotatus* spp. Since *C. pervigilans* and *O. notoscriptus* were the only species of third and fourth instar larvae collected, it is likely that these first and second instar larvae were also either *C. pervigilans* or *O. notoscriptus*, making *O. notoscriptus* and *C. pervigilans* constitute 54.6% ( $n = 2047$ ) and 45.4% ( $n = 1704$ ) of the total larvae respectively.

Destructive sampling gave density estimates that were 1.0–11.2 times higher than field dipping across all population measures (Table 1). Paired-samples *t* tests revealed that the ratio of destructive sampling to field dipping was significantly higher than 1:1 for fourth instar densities of both species (*O. notoscriptus*:  $t_6 = -4.01$ ,  $P = 0.007$ ; *C. pervigilans*:  $t_6 = -3.30$ ,  $P = 0.013$ ), and nearly significantly higher for total *O. notoscriptus* ( $t_6 = -2.40$ ,  $P = 0.053$ ) (Table 1). Mean percentage recovery rates from destructive sampling were closer to the expected recovery rate (25%) than field dipping for all population measures except total *C. pervigilans* (Table 1). Density estimates from field dipping were significantly underestimated for four out of six population measures: total *O. notoscriptus* ( $t_6 = -2.57$ ,  $P = 0.042$ ), pupae ( $t_8 = 2.64$ ,  $P = 0.030$ ), fourth instar *C. pervigilans* ( $t_7 = -3.95$ ,  $P = 0.006$ ) and fourth instar *O. notoscriptus* ( $t_6 = -4.83$ ,  $P = 0.003$ ) (other population measures:  $t_{10-12} = -0.80$ – $1.98$ ,  $P$  values = 0.072–0.443) (Table 1). Density estimates from destructive sampling were significantly different from expected densities for only two population measures: pupae (higher) ( $t_8 = 4.90$ ,  $P = 0.001$ ) and fourth instar *O. notoscriptus* (lower) ( $t_6 = -4.45$ ,  $P = 0.004$ ) (other population measures:  $t_{6-12} = -2.06$ – $0.39$ ,  $P$  values = 0.086–0.876) (Table 1).

Mean percentage recovery rates show that field dipping is 2.4 times more likely to sample *C. pervigilans* than *O. notoscriptus*; 5.1 times more likely for fourth instars in particular (Table 1). Overall, destructive sampling collected each species relatively more evenly than field dipping, being only 1.5 and 1.4 times more likely to sample total and

fourth-instar *C. pervigilans*, respectively. *Culex pervigilans* and *O. notoscriptus* were found together in only five containers and fourth instars of both species in only two. Because of the low sampling power, all paired-samples *t* tests comparing densities between species yielded non-significant results ( $t_{1-4} = 0.19-1.87$ , *P* values = 0.134–0.879).

Regression analyses showed that estimates from destructive sampling were very strongly positively related with absolute counts of mosquitoes for all population measures (Table 2). Estimates from destructive sampling were also more highly related with absolute counts than field dipping. Field dipping was significantly positively related with

absolute counts, except for pupae and fourth instar *O. notoscriptus*. Destructive sampling gave lower coefficients of variation than field dipping for all population measures (Table 2). Both sampling methods were equally reliable at detecting mosquitoes. Containers with mosquitoes ( $n = 13$ ) were never erroneously recorded as having no mosquitoes when destructively sampling and only once when field dipping.

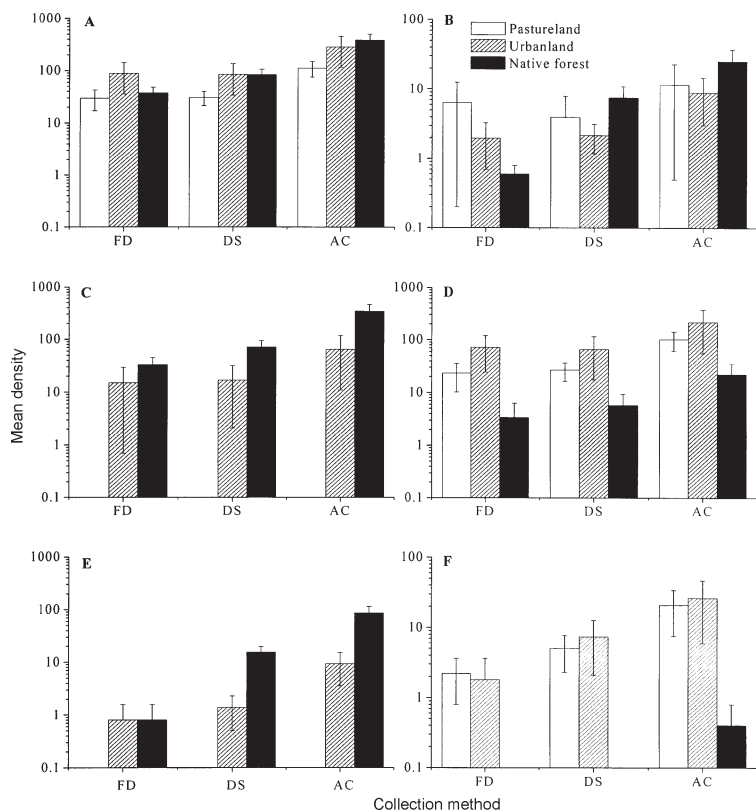
The relationship between mosquito density and land use was more similar to absolute mosquito counts across all population measures when using estimates from destructive sampling compared with field dipping (Fig. 1). Absolute counts revealed that

**Table 1** Mean mosquito numbers ± SE of population measures from absolute counts (4 litres) and two dipping methods: field dipping (FD) and destructive sampling (DS) (both density/litre). Recovery rates as percentages of absolute counts are in parentheses. Results of paired *t* tests comparing estimated densities with expected densities are shown with recovery rates, and those comparing densities between dipping methods are shown with ratios. (TM, total mosquitoes; ON, total *Ochlerotatus notoscriptus*; CP, total *Culex pervigilans*; ON4, fourth instar *O. notoscriptus*, CP4 = fourth instar *C. pervigilans*.)

	TM	ON	CP	Pupae	ON4	CP4
Absolute counts	265.5 ± 72.7	136.5 ± 56.7	113.6 ± 55.4	15.5 ± 5.8	32.2 ± 13.8	15.8 ± 8.05
Field dipping	52.1 ± 18.7	16.1 ± 6.8	32.9 ± 56.7	3.0 ± 2.1	0.5 ± 0.4	1.3 ± 0.7
Recovery rate	(19.6)	(12.0)	(29.0)	(19.4)	(1.6)	(8.2)
		<i>P</i> < 0.05		<i>P</i> < 0.05	<i>P</i> < 0.01	<i>P</i> < 0.01
Destructive sampling	67.1 ± 19.0	29.4 ± 11.7	33.1 ± 16.9	4.6 ± 1.8	5.6 ± 2.3	4.1 ± 2.0
Recovery rate	(25.3)	(21.5)	(29.0)	(30.0)	(17.4)	(25.9)
				<i>P</i> < 0.001	<i>P</i> < 0.01	
Ratio DS : FD	1.3 : 1.0	1.8 : 1.0	1.0 : 1.0	1.5 : 1.0	11.2 : 1.0	3.2 : 1.0
					<i>P</i> < 0.001	<i>P</i> < 0.01

**Table 2** Adjusted *R*<sup>2</sup> coefficients (and coefficients of variation) for numbers of different population measures of immature *Culex pervigilans* and *Ochlerotatus notoscriptus* recovered by field dipping compared with destructive sampling, in relation to the absolute counts of larvae and pupae.

Population measure	Field dipping	Destructive sampling
Total mosquitoes	0.474 (41.8%) <i>P</i> = 0.01–0.05	0.936 (8.8%) <i>P</i> < 0.001
Total <i>O. notoscriptus</i>	0.502 (50.5%) <i>P</i> = 0.01–0.05	0.962 (10.9%) <i>P</i> < 0.001
Total <i>C. pervigilans</i>	0.320 (56.0%) <i>P</i> = 0.01–0.05	0.988 (50.2%) <i>P</i> < 0.001
Pupae	0.188 (68.6%)	0.845 (45.3%) <i>P</i> < 0.001
IV-instar <i>O. notoscriptus</i>	0.052 (176.1%)	0.960 (22.1%) <i>P</i> < 0.001
IV-instar <i>C. pervigilans</i>	0.651 (140.5%) <i>P</i> = 0.01–0.05	0.970 (62.1%) <i>P</i> < 0.001



**Fig. 1** Mean mosquito numbers ( $\pm$  SE) of population measures from absolute counts (AC) (4 litres) and two dipping methods: field dipping (FD) and destructive sampling (DS) (both density/litre). **A**, total mosquitoes; **B**, pupae; **C**, total *Ochlerotatus notoscriptus*; **D**, total *Culex pervigilans*; **E**, fourth instar *O. notoscriptus*; **F**, CP4 = fourth instar *C. pervigilans*. Note that the y axis is on a log<sub>10</sub> scale.

**Table 3** ANOVA analyses testing the relationship between land use and mosquito numbers across various population measures: comparing data from field dipping (FD), destructive sampling (DS), and absolute counts (AC). Degrees of freedom are: error, 12; land use, 2. Significant post-hoc comparisons (Bonferroni) are shown. (Nf, native forest; Pa, pastureland; Ur, urbanland; TM, total mosquitoes; ON, total *Ochlerotatus notoscriptus*; CP, total *Culex pervigilans*; ON4, fourth instar *O. notoscriptus*, CP4 = fourth instar *C. pervigilans*.)

	F statistics and P values of population measures					
	TM	ON	CP	Pupae	ON4	CP4
Field dipping	0.272	4.850 <i>P</i> < 0.01	1.160	0.241	0.500	1.010
Post hoc tests of FD		Nf > Pa				
Destructive sampling	0.900	12.350 <i>P</i> < 0.001	0.961	1.050	14.460 <i>P</i> < 0.001	2.281
Post hoc tests of DS		Nf < Pa, Ur			Nf > Pa, Ur	
Absolute counts	0.150	14.170 <i>P</i> < 0.001	1.894	0.977	13.808 <i>P</i> < 0.001	2.133
Post hoc tests of AC		NF > Pa, Ur			Nf > Pa, Ur	

female *O. notoscriptus* mainly oviposited in native forest containers and never in pastureland. ANOVA analyses using estimates from destructive sampling gave similar results to ANOVA analyses using absolute count data across all population measures (Table 3). There was a significant land-use effect on

total *O. notoscriptus* and fourth instar *O. notoscriptus*, with significantly higher numbers in native forest compared to pastureland and urbanland (Table 3). Analyses using estimates from field dipping found no significant effect of land use on fourth instar *O. notoscriptus* (Table 3). Field dipping found

a significant effect of land use on total *O. notoscriptus*, with native forest having significantly lower numbers than pastureland but not urbanland ( $P < 0.05$ , Table 3).

## DISCUSSION

Destructive sampling is the more proficient method of sampling *C. pervigilans* and *O. notoscriptus* from artificial containers, compared with field dipping. Estimates of mosquito density from destructive sampling were strongly positively related with absolute counts. They were also better related with absolute counts, and were more accurate and consistent when compared with field dipping. These results are in accordance with the findings from Mori (1989), who found that stirring the water before dipping jars gave more reliable and accurate samples of *O. togoi* compared with conventional dipping without prior stirring.

Destructive sampling better indicated true mosquito numbers than field dipping probably because the alarm reactions from larvae and pupae were annulled owing to the procedure of stirring the water before dipping. Field dipping, which is similar to most conventional dipping methods, is likely to sample mosquitoes just under the water surface and in the middle of the container (Service 1995). Immature mosquitoes commonly dive to the bottom or sides of containers in response to optical (e.g., shadows) and mechanical (e.g., vibrations) stimuli, thus lowering their probability of being collected and increasing the variability between samples (Clements, 2000).

Although destructive sampling better estimated pupae and fourth instar *O. notoscriptus* compared with field dipping, it still yielded estimates that were significantly different from expected recovery rates based on absolute counts. There may be multiple hypotheses for this result. One hypothesis may be increased natural sampling variation as a result of small population sizes. However, the same result was not seen for fourth instar *C. pervigilans*, which also existed in low numbers. A hypothesis to explain lower than expected fourth instar *O. notoscriptus* densities may be that, compared with early instars and *C. pervigilans*, fourth instar *O. notoscriptus* are better able to cope with water currents created by stirring and thus evade being sampled (P. Leishnam pers. obs.)

The inability of field dipping to sample the bottom of containers was the likely reason for it to severely

underestimate *O. notoscriptus*. In relation to destructive sampling, field dipping collected similar densities of total *C. pervigilans* but nearly half the total *O. notoscriptus* and over 10 times fewer fourth instar *O. notoscriptus*. These findings were probably because of behavioural differences between the species. Whereas *Culex* larvae usually suspend from the water surface to filter-feed, some *Ochlerotatus* larvae often browse submerged surfaces and bottom sediments (Merritt et al. 1992, 1996), thus likely lowering their probability of collection by field dipping. Likewise, in this study, we anecdotally noticed the aggregation of *C. pervigilans* under the water surface and *O. notoscriptus* near the bottom before field dipping.

Along with fourth instar *O. notoscriptus*, density estimates of fourth instar *C. pervigilans* were also significantly lower (3.2 $\times$ ) for field dipping compared with those from destructive sampling. These results were somewhat unexpected since most previous laboratory and field studies show higher recovery rates of late instar larvae when dipping other species (Zhen & Kay 1993; Tun-Lin et al. 1994; Service 1995). Increased recovery of late instar larvae are likely to be a result of their larger size, decreased responsiveness to alarm stimuli, and decreased capacity for cutaneous respiration (Service 1995; Clements 2000). However, there is still relatively little literature on the differences in recovery rates for different instars, species, and habitat types to yield a consensus on the subject (Service 1995). Further research on the associations between feeding behaviours, alarm responses, instars, and the vertical distribution of *O. notoscriptus* and *C. pervigilans* in containers is likely to provide answers for the differential recovery rates from sampling methods.

*Ochlerotatus notoscriptus* is usually collected in shaded container habitats, using artificial varieties in modified areas (e.g., tyre casings, buckets, tanks) as well as natural sites in forest areas (e.g., tree-holes, and leaf axis of astelias and palms) (Belkin 1968; Lee et al. 1988). Absolute counts from this study showed that *O. notoscriptus* constituted the majority of mosquitoes in artificial containers placed in native forest. Because of this and a field dipping bias against collecting *O. notoscriptus*, average mosquito densities from native forest were generally underestimated across all population measures by field dipping. However, of all population measures, field dipping estimates only yielded an erroneous result for total and fourth instar *O. notoscriptus*.

The proficiency of our destructive sampling method compares well with other methods that have

sampled different species in container habitats. For example, the destructive sampling method gives higher correlation coefficients, lower coefficients of variation, and similar recovery rates, when compared with sweeping and dipping methods that sampled *A. aegypti* from 200-litre metal drums (Tun-Lin et al. 1994) and 3-, 4-, and 28-litre tyres (Zhen & Kay 1993). Although comparisons between studies that have tested different methods of sampling various larval mosquito habitats are of limited use, they still suggest that destructive sampling appears to accurately sample *Culex* and *Ochlerotatus* species in small artificial containers.

Dipping is usually undertaken to sample from existing larval habitats, creating indices of larval abundance by taking a number of dips and calculating the mean number of mosquitoes per dip or unit volume. In general, this procedure encounters many known but unavoidable problems, including the location and type of habitat, the variable and fluctuating size of habitats, unequal larval dispersion within habitats, and variable vegetation cover around habitats (Russell 1993). All of these issues make it difficult to standardise between replicates and monitor changes in immature populations over seasons or between treatments.

Artificial containers avoid the potentially confounding variables associated with existing habitats, have the benefit of being easily standardised and replicatable, and can be positioned anywhere. However, they have the potential problem of misrepresenting relative abundances of species in environments if the responses of species to them are not representative of that to other habitats in the surrounding environment, and therefore should not be used in isolation for investigating species presence or abundance. In general, the combination of beneficial qualities of artificial containers makes them amenable to studies involving experimental manipulation of container-using species (Drake et al. 1996; Yanoviak 1999). By extension, artificial containers may be useful for comparing the influence of land use and associated variables on relative densities of larvae and pupae of mosquito species when they are destructively sampled because of the benefits already mentioned and because that absolute population estimates are not required but rather comparisons of mean numbers.

As well as being proficient at quantifying mosquitoes, mosquito-sampling techniques primarily need to be simple, cost-effective, commercially available everywhere, and accepted by the community. Destructive sampling fulfils these

requirements for container-using species. Destructive sampling requires more time to undertake than conventional field dipping, but the results of this study show that it provides a better reflection of true mosquito numbers and patterns between land uses and species. We recommend destructive sampling as a practical and proficient way to sample co-occurring populations of *C. pervigilans* and *O. notoscriptus* in artificial containers, as well as examine ecological questions for these species and possibly others.

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