Scientific Note

A tool for sampling mosquito larvae from phytotelmata

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The literature on mosquitoes inhabiting phytotelmata (bodies of water impounded by plants) is relatively extensive, particularly due to bromeliad-breeding species and their public health significance in tropical regions (e.g., Cunha et al. 2002, Forattini et al. 1998, Olano et al. 1997). Due to the nature of New Zealand's native flora, phytotelm-forming plants seem to be relatively rare, and, considering the amount of information available overseas, the records of mosquitoes in phytotelmata in this country are scarce (Derraik 2005). As part of a wider research project, an extensive investigation of the Culicidae inhabiting phytotelmata was carried out in Wellington (Derraik 2005), the southernmost region on New Zealand's North Island.

Native forests in Wellington appeared to have a limited availability of phytotelm habitats, the major exception being the native epiphyte *Collospermum hastatum* (Liliaceae) (Figure 1) (Derraik 2005). This plant is commonly found in coastal and lowland forests throughout the North Island (Dawson and Lucas 2000), and individual plants are capable of harbouring mosquito larvae within leaf axils. *Collospermum hastatum* is the main breeding habitat for the endemic *Culex (Culex) asteliae* Belkin (Belkin 1968), but larvae of the introduced *Aedes (Finlaya) notoscriptus* (Skuse) have also been recorded in its leaf axils (e.g., Derraik 2004, Dumbleton 1968). As an epiphyte, *C. hastatum* is abundant in the canopy of native trees (Derraik 2005).

As a result, a sampling tool was devised to sample mosquito larvae in the water contents of phytotelmata, in particular from the leaf axils of *C. hastatum*. The tool consisted of a 55 cm long endoscopic tube with a diameter of 5 mm, and an opening of approximately 4 mm, which was attached to the long snout of a 50 ml syringe (Figure 1). In order to assess the reliability of this sampling tool, an investigation was conducted prior to the field study (Derraik 2005) to assess its accuracy in detecting and quantifying larval abundance and the presence/absence of mosquito larvae in leaf axils.

A large and healthy *C. hastatum* specimen was collected in the field and taken to a laboratory. The leaf axils capable of holding water were identified, with each being tested in a progressive sequence from the innermost to the outermost axil. Sets of field-collected *Ae. notoscriptus* larvae were separated into two batches: 1st/2nd instars, and 3rd4th instars plus pupae. Trials were carried out using one, two, five, and ten larvae at a time. Leaf axils were 2/3 filled with water, to which the larvae were carefully added with a pipette so that these were not harmed in the process. The water was then removed after 30 s with the sampling tool. The number of larvae recovered was recorded and the axil was refilled with water until all larvae were withdrawn. The procedure was repeated ten times for each treatment. Larvae were replaced by new ones whenever necessary to ensure that any individuals damaged during removal would not influence the outcome of the study.

The sampling tool was found to be efficient for detecting the presence of *Ae. notoscriptus* larvae in *C. hastatum* leaf axils, which was accurately assessed 95% of the time (76/80) (Table 1). Regarding larval groups, the tool was 98% correct for 1st/2nd instars (39/40) and 93% for the larger larvae and pupa (37/40) (Table 1). All errors occurred when only one or two larvae were present, in three and one cases, respectively (Table 1).

The tool's accuracy for quantifying numbers of larvae per leaf axil was comparatively lower. It was 78% correct for 1st/2nd instars and 81% for the larger larval group (79% overall; Table 1). There was large variation in the number of larvae detected, with the worst result occurring for the set of ten 1st/2nd instars larvae, of which as few as four larvae were detected in two occasions (Table 1).

During the experiment, it was observed that the

Figure 1. Leaf axils of the epiphyte *Collospermum hastatum* being sampled for mosquito larvae in the field.

Table 1. Results from the laboratory test on the sampling tool's accuracy to detect the presence and quantify the abundance of *Aedes notoscriptus* larvae in *Collospermum hastatum* leaf axils.

		1st and 2nd instars					3rd, 4th instars and pupa					
Trial	No. larvae	1	2	5	10	Total	1	2	5	10	Total	Overall
1		1	2	5	8		1	2	5	10		
2		1	1	5	7		1	2	3	9		
3		1	2	3	4		1	2	3	9		
4		0	1	5	5		1	1	4	10		
5		1	2	5	4		1	2	5	6		
6		1	2	4	10		0	0	5	7		
7		1	2	5	8		1	2	3	4		
8		1	1	5	8		1	1	4	9		
9		1	2	5	7		0	2	3	10		
10		1	2	4	8		1	2	4	8		
% detection of larval presence		90	100	100	100	**98**	80	90	100	100	**93**	95
% larvae collected		90	85	92	69	**78**	90	80	78	82	**81**	79

morphology of individual leaf axils seemed to influence the results to a certain extent. Some axils were more than 6 cm wide while the tip of the endoscopic tube was only 5 mm cross. Once the water was drawn out, some larvae would stick to inner sides of the leaf axil away from the tip of the tube, probably leading to most of the observed errors in larval counts. It was necessary to refill the axils with water to collect them, which in many cases had to be done three or four times before all larvae were removed. It was also observed that small larvae would occasionally "leak" from one axil to a lower (outer) one.

The 5 mm diameter of the sampling tube appeared to be optimal for the plants examined here. A narrower tube was tried but it was frequently blocked by debris present in the leaf axils, such as seeds, leaves, and dirt. Wider tubes, in contrast, noticeably pushed the leaf axils apart, not only damaging them but apparently leading to increased leakage of water and larvae.

Overall, the sampling tool was deemed efficient for the sampling of mosquito larvae from the leaf axils of *Collospermum hastatum*. It seems, therefore, that this sampling tool would be appropriate for sampling different phytotelm habitats, in particular to answer questions regarding presence/absence. As the results indicated, it may lead to some degree of error in the data on larval abundance, and ideally plants should be sampled from the inner-most axil outwards to ensure that any larvae carried by water leakage to adjacent axils are collected.

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REFERENCES CITED

Belkin, J.N. 1968. Mosquito Studies (Diptera: Culicidae) VII. The Culicidae of New Zealand. Contrib. Am. Entomol. Inst. 3: 1-182.

Cunha, S.P., J.R.C. Alves, M.M. Lima, J.R. Duarte, L.C.V. Barros, J.L. Silva, A.T. Gammaro, O.S.M. Filho, and A.R. Wanzeler. 2002. Presence of *Aedes aegypti* in Bromeliaceae and plant breeding places in Brazil. Rev. Saú. Públ. 36: 244-245.

Dawson, J. and R. Lucas. 2000. *Nature Guide to the New Zealand Forest*. Godwit. Auckland.

Derraik, J.G.B. 2004. A survey of the mosquito (Diptera: Culicidae) fauna of the Auckland Zoological Park. New Zealand Entomol. 27: 51-55.

Derraik, J.G.B. 2005. Mosquitoes breeding in phytotelmata in native forests in the Wellington region, New Zealand. New Zealand J. Ecol. 29: 185-191.

Dumbleton, L.J. 1968. A synopsis of the New Zealand mosquitoes (Diptera Culicidae) and a key to the larvae. Tuatara 16: 167-179.

Forattini, O.P., G.R.A.M. Marques, I. Kakitani, M. Brito, and M.A.M. Sallum. 1998. Significado epidemiológico dos criadouros de *Aedes albopictus* em bromélias. Rev. Saú. Públ. 32: 186-188.

Olano. V., G. Carrasquilla, and F. Méndez. 1997. Transmisión de la malaria urbana en Buenaventura, Colombia: aspectos entomológicos. Rev. Panamericana Salud Publ. 1: 287-294.