Container aperture size and nutrient preferences of mosquitoes
(Diptera: Culicidae) in the Auckland region, New Zealand

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ABSTRACT: Ovitraps are a widely used tool for mosquito vector surveillance and population monitoring. In the Auckland region, New Zealand, the oviposition preferences of mosquitoes were assessed in artificial breeding containers in relation to container aperture size and water nutrient levels. Only three mosquito species were recorded: the endemic Culex pervigilans and the exotic Culex quinquefasciatus and Ochlerotatus notoscriptus. Both Culex species were somewhat rare and would not readily utilize the ovitraps. Ochlerotatus notoscriptus displayed a significant preference for ovipositing in containers with low organic load, but mixed results were obtained in regards to container size. A linear regression analysis showed that mean daily temperature in the three weeks prior to container inspection explained almost all the variation in the larval densities of Oc. notoscriptus in ovitraps ($r^2 = 0.93$), with which it was positively associated. Journal of Vector Ecology 30 (1): 73-82. 2005.

Keyword Index: Aperture size, containers, mosquitoes, nutrient, oviposition.

INTRODUCTION

Although New Zealand has a relatively poor mosquito fauna consisting of 12 native species and four exotic species (Derraik 2004), little information is available on their bionomics, especially of the indigenous species. New Zealand is under a serious risk of a mosquito-borne disease outbreak (Derraik and Calisher 2004), and more research is urgently needed to fill the extensive knowledge gap regarding the ecology of culicids in this country.

One widely used tool for mosquito vector surveillance and population monitoring is the ovitrap (Bellini et al. 1996, Fay and Eliason 1966, Focks 2003). Leiser and Beier (1982) showed that numbers of Culex egg rafts in ovitraps were positively correlated with Culex adult catches from New Jersey light traps, indicating that both methods provide comparable results for mosquito population monitoring. Many authors suggest that ovitraps are efficient, easy to use, cheap, and a sensitive technique for mosquito monitoring (Fay and Eliason 1966, Leiser and Beier 1982, Madder et al. 1980). However, to be able to draw any conclusions regarding presence/absence or relative abundance of a mosquito species, it is fundamental to have information on its particular habitat preferences.

Nutrient concentration is an important factor determining female mosquito site selection (Clements 1999), as the products of decaying organic matter often provide important chemical cues for mosquito oviposition (Mulla 1979). Mattingly (1969), for instance, described a progressive replacement of mosquito species as the organic content of the water within coconut shells diminished and dilution by rainwater occurred. Container aperture size is also an important feature determining the attractiveness of a particular habitat to mosquito species (Becker 1995). Such preferences are somewhat variable, and while some culicids prefer small containers others favor larger recipients (Sota 1998, Sunahara et al. 2002).

In the North Island of New Zealand, three species are known to be dominant: the endemic Culex (Culex) pervigilans Bergroth, and the exotic Ochlerotatus (Finlaya) notoscriptus (Skuse) and Culex (Culex) quinquefasciatus Say (Hearnden et al. 1999, Laird 1990, 1995). Culex pervigilans is New Zealand’s most common and widespread species, which seems to be a result of its ability to exploit a wide range of larval mosquito habitats (Belkin 1968, Laird 1990, 1995). This species is a vector of New Zealand’s only described arbovirus (Whataroa virus), which infects birds (Miles 1973), and it has been suggested to be a possible vector of avian malaria (Holder et al. 1999).

The two dominant exotic species are known vectors of numerous diseases (Derraik 2004). Culex quinquefasciatus was recorded in New Zealand in 1848 (Laird et al. 1994), and it is, for instance, an efficient vector of West Nile virus (Turell et al. 2001) and Wuchereria bancrofti (lymphatic filariasis) (Zagaria and Savioli 2002), a vector of Japanese encephalitis virus (van den Hurk et al. 2003) and Murray Valley encephalitis virus (Russell 1995, Russell 1998). Culex quinquefasciatus is also a vector of Plasmodium relictum (avian malaria) and avian pox viruses, and the introduction of this mosquito to Hawaii led to the extinction of native bird species (van Riper III et al. 1986). Avian malaria has been
recorded in New Zealand, and Cx. quinquefasciatus is likely to have been the vector responsible for the outbreaks (Derraik, in press).

The Australian Oc. notoscriptus has been in New Zealand since at least 1920 (Laird and Easton 1994). It is presently one of the most abundant and widespread species in the North Island, and it appears to be particularly well established in urban and peri-urban areas. The mosquito is a vector of Dirofilaria immitis (canine heartworm) (Russell and Geary 1996) and a potential vector of Barmah Forest virus (Doggett and Russell 1997, Watson and Kay 1999). Ochlerotatus notoscriptus is most likely a vector of Ross River virus in urban areas in Australia (Doggett and Russell 1997, Russell 1995, Russell 1998, Watson and Kay 1997), which is the disease thought to pose the greatest threat to public health in New Zealand (Derraik and Calisher 2004).

Nonetheless, knowledge on the bionomics of these potential disease vectors in New Zealand is still scarce. This particular study, therefore, aimed to assess some aspects regarding the oviposition preferences of these three dominant mosquito species in northern New Zealand, more specifically in relation to container aperture size and water nutrient levels. In addition, we aimed to assess the usefulness of a particular ovitrap design for population monitoring of such species due to their human and animal health significance.

MATERIALS AND METHODS

This study was carried out in the Auckland region, North Island of New Zealand (c. 36° 30' S, 174° 35' E), as part of a larger investigation into the ecology of mosquitoes. Oviposition preferences were investigated throughout nine sites in the region, one of which was an anthropic habitat (Auckland Zoological Park) while the remaining eight locations were native forest habitats of various sizes (from 3 to 1,500 ha). The latter were selected to address macro-ecological questions through between-site differences in mosquito species assemblages. This particular study aimed to address ecological questions at the microhabitat scale.

The ovitraps were transparent plastic containers whose inner and outer surfaces were made rougher using 60-grit sandpaper, as rough surfaces seem to favor mosquito oviposition (Beckel 1955, O’Gower 1957a, O’Gower 1957b, Wilton 1968). Plastic seems to be the standard material used for ovitraps due to its ease of use in the field, and at least one study has given some indication that container breeders prefer plastic ovitraps over those made of glass or metal (Bellini et al. 1996). All containers had the outer surfaces coated with matt black paint.

Each set of ovitraps consisted of four such containers of two different aperture sizes, 35.3 and 103.9 cm², whose respective volumes were approximately 262 and 706 ml. All containers were filled up to 1 cm below the containers’ rim with two different sheep manure solutions in tap water at 0.5 g/L and 5.0 g/L (dry weight). Manure pellets were placed in boiling water from a standard household jug in order to form a slurry solution. Nutrient concentrations of nitrogen, phosphorus and potassium were approximately 0.015, 0.010, and 0.020 g/L, respectively, for the 0.5 g/L solution and 0.15, 0.10, and 0.20 g/L, respectively, for the 5.0 g/L solution.

The four containers in each individual set were arranged in a latin square and clamped together to prevent tampering by animals, strong wind gusts, or falling debris. At each site two ovitraps were placed at least 200 m apart at ground level, in full shade, and directly against the bases of tree trunks. The latter factor has been shown to favor oviposition by container breeding species, in contrast to traps set away from tree trunks (Jordan 1991, Jordan and Hubbard 1991). It seems that the presence of tree trunks adjacent to the ovitraps increases the chances of a gravid female finding them, in comparison to those not associated with trees (Jordan 1991).

Ovitraps were checked every three weeks between December 2002 and June 2003. The pre-inspection of sites prior to the placement of ovitraps identified few or no competing container habitats in the vicinity of individual sets. Containers were checked eight times in four sites, seven times in other three and at the Zoo they were checked nine times. A total of 552 container inspections were consequently carried out. Each time a container was checked for mosquito larvae, it was emptied and the manure solution replaced. All contents were taken to the laboratory, where larvae (and pupal cases) were counted and identified. Many larvae were reared to adults, with voucher specimens of both stages sent to Amy Snell (Wellington School of Medicine and Health Sciences, University of Otago) for confirmation and/or identification.

The two most common container breeding species on the North Island, the native Cx. perpugilans and the exotic Oc. notoscriptus, belong to two genera with distinct oviposition strategies. Aedes, and its former subgenus Ochlerotatus, oviposit eggs singly on moist substrata near bodies that are water-filled or likely to be flooded (Clements 1999), such as the inner walls of tree-holes and containers. The respective eggs hatch with the rising water level after, for instance, a rainfall event (Buxton and Hopkins 1927). Culex spp. and a number of unrelated genera in contrast, lay eggs in a raft formation on the water surface (Bates 1949, Clements 1999).

The most accurate method of quantifying oviposition per se would be to check the containers for the respective eggs. However, this is labor-intensive and time-consuming in comparison to larval counts, particularly when dealing with a large number of ovitraps at any given time. Larval counts are unreliable for comparing relative abundances of those genera with different oviposition strategies such as Ochlerotatus and Culex, since it would be considerably biased towards the latter group, particularly in the absence of a significant rainfall event. In this particular study, containers of all aperture sizes should, in theory, receive the same amount of rain per unit area, and the number of eggs lost to overflow or reached by the rising water level was likely to be independent of container surface area. Thus, it was decided that counting only larvae would be a good indicator of oviposition, as it would not affect between-
treatment comparisons. Moreover, as a result of the very large number of ovitraps handled, larval counts were considered the logistically viable method for this particular experiment.

Oviposition preferences for both water nutrient level and container aperture size were analysed based on the presence or absence of larva-positive ovitraps and larval density (number of larvae per cm²) using a general linear model with calculations performed using a regression approach. The variations in the data over time and between sites were included as factors in the model. Simple regressions were used to compare environmental variables to larval abundance. Dependent variables were $\sqrt{+0.5}$ transformed to stabilize the variance, and the significance level was set at $P<0.05$. Environmental data were provided by the National Institute of Water and Atmospheric Research (NIWA) and the Auckland Regional Council.

The species occupancy of ovitraps varied considerably throughout the experiment, and a large number of containers were larva-negative. Therefore, the data were subsampled by eliminating the ovitraps that at any given period had all four containers larva-negative, as a large number of zeros would lead to high levels of statistical ‘noise’ and possibly obscure existing patterns.

RESULTS

From the 552 container inspections carried out, 188 (34%) were positive for culicids, yielding a total of 5,822 mosquito larvae. It appeared that no larvae completed their life cycle during the three-week period between ovitraps checks, as no pupal cases were found. Only the three species known to be dominant in the region were recorded: Cx. pervigilans, Oc. notoscriptus, and Cx. quinquefasciatus. Surprisingly, only 179 specimens were of the endemic Cx. pervigilans (3%) while all the remaining 97% were made up by the exotic mosquitoes. Ochlerotatus notoscriptus was the most abundant species (5,352; 92%), while Cx. quinquefasciatus made up 5% with 291 larvae. Dominance by Oc. notoscriptus was also demonstrated by its presence in 181 ovitraps (33%), while Cx. pervigilans and Cx. quinquefasciatus were recorded in 11 (2%) and 10 (2%), respectively. Only two triple infestations were recorded, while Oc. notoscriptus was recorded in double infestations with both Cx. pervigilans (nine times) and Cx. quinquefasciatus (six times). The two Culex species were not recorded co-infesting any containers. Ochlerotatus notoscriptus was the sole species collected in 164 ovitraps, while, in contrast, single infestations occurred only twice for Cx. pervigilans and on four occasions for Cx. quinquefasciatus.

Cx. quinquefasciatus was recorded in three medium and seven large containers whose mean larval densities were 0.07 and 0.15 per cm², respectively. This species was more commonly found in containers with high nutrient solutions, as larvae were collected in eight such ovitraps in comparison to two with low nutrients whose larval densities were 0.19 and 0.03, respectively. Culex pervigilans larvae were present in three medium and eight large ovitraps with mean larval densities of 0.06 and 0.10 per cm², respectively. Larvae of this species were recorded in five low and six high nutrient ovitraps with larval densities of 0.03 and 0.13, respectively. The scarcity of Cx. pervigilans and Cx. quinquefasciatus larvae meant that no reliable inferences could be made regarding their oviposition preferences. This study consequently has focused on the bionomics of Oc. notoscriptus which was somewhat widespread and abundant. Following the subsampling of the data, the subsequent results and analyses were based on data from 276 ovitraps.

The mean density of Oc. notoscriptus larvae was higher in medium-size containers (0.40/cm²; SE = 0.06) than in the larger ones (0.24/cm²; SE = 0.03), though the reverse occurred regarding infestation of ovitraps with 87 and 94 being larva-positive, respectively (Figure 1). Although the latter difference was not statistically significant ($P = 0.308$), the comparison between larval densities lies on the significance threshold ($P = 0.052$) suggesting that Oc. notoscriptus had some bias towards the medium-size ovitraps (Figure 1).

In relation to water nutrient contents, Oc. notoscriptus displayed preference for the nutrient solutions with low

![Figure 1](image1.png)  
**Figure 1.** Number of larva-positive ovitraps and mean larval density per cm² for Ochlerotatus notoscriptus in the Auckland region, in relation to container size ($n = 276$). Aperture size of medium and large containers was 35.3 and 103.9 cm², respectively. Error bars represent the standard error for the mean.

![Figure 2](image2.png)  
**Figure 2.** Number of larva-positive ovitraps and mean larval density per cm² for Ochlerotatus notoscriptus in the Auckland region ($n = 276$), according to water nutrient levels (0.5 and 5.0 g manure/L). Error bars represent the standard error for the mean.
Figure 3. Mean larval density per cm² and number of larva-positive ovitraps for *Ochlerotatus notoscriptus*, recorded at seven sites in the Auckland region, according to water nutrient levels and container aperture size. The number of ovitraps per field site based on the subsampled data is shown in brackets. Standard errors for the means are not shown. Two and zero specimens were collected at two sites, which were consequently not included in figure.
Figure 4. *Ochlerotatus notoscriptus* mean larval density per cm² recorded in ovitraps in two sites in the Auckland region (Zoo & Wainui), according to water nutrient levels and container aperture size. Standard errors for larval density are shown. Solid gray and black lines represent mean daily rainfall and temperature, respectively.
organic load (Figure 2). A total of 108 containers with the 0.5 g/L solution were larva-positive for this species, in contrast to 73 with the 5.0 g/L solution ($P < 0.001$; Figure 2). The same highly significant difference occurred based on larval densities ($P < 0.001$), as a mean of 0.39 larvae per cm$^2$ ($SE = 0.05$; total of 3405 specimens) was recorded in low manure solutions, in comparison to 0.25 larvae per cm$^2$ ($SE = 0.05$; 1888) collected from high manure solutions (Figure 2). Note that there were no significant interactions between container aperture size and nutrient level, with high $P$-values obtained for both container occupancy and larval density ($0.884$ and $0.464$, respectively).

Figure 3 shows the treatment comparisons observed at each individual site, except Cascade and Tapu Bush, where only two and zero specimens were recorded, respectively. Regarding container size, larval densities were consistently higher in medium-size ovitraps at all sites (except Pohuehue), while container occupancy was biased towards the large containers (Figure 3). For water nutrient contents, larval densities were higher at all sites in the treatments with low organic load (0.5 g/L). Of interest was the very marked preference of *Oc. notoscriptus* for the low nutrient solutions at Pohuehue (Figure 3). The bar graphs (Figure 3) indicated that the overall patterns observed for the Auckland region (Figures 1 and 2) were present to a large extent at the individual sites.

Between-treatment differences, however, were neither constant over time nor varied consistently among the different field sites, as illustrated by a comparison of the results obtained at the two sites with the highest larval abundances (Figure 4). At both Auckland Zoo and Wainui, the larval densities of *Oc. notoscriptus* recorded in medium and large ovitraps were relatively similar during most of the experiment. However, in both sites a marked bias towards medium-size containers was registered when the mosquito population at the two sites peaked between mid-February and early March (Figure 4). The same did not occur in regards to water nutrient contents, where no marked between-treatment differences were observed at any particular time (Figure 4).

A common pattern that was observed for the population of *Oc. notoscriptus* was that larval densities in all treatments peaked between mid-February and mid-March and afterwards declined until densities apparently stabilized at very low densities (< 0.1 larvae/cm$^2$) (Figure 4). These patterns were thought to be linked to environmental variables, and mean daily temperature and rainfall were consequently plotted in Figure 4. The resulting comparisons indicated that the mosquito population decline appeared to be associated with a decrease in mean temperature. Interestingly, the *Oc. notoscriptus* population peaked in most treatments in mid-February following a three-week long drought (Figure 4).

In order to identify the environmental variables affecting the larval densities of *Oc. notoscriptus* in the ovitraps, linear regressions were run, comparing the transformed relative abundances recorded in all containers at any given period to the cumulative rainfall and mean daily temperature recorded during the three wk preceding ovitrap inspections (Figure 5). The rainfall data were very scattered (Figure 5), and the fact that the analysis gave a significant result ($P = 0.022$) is likely to have been an artifact as demonstrated by the very low $r^2$ (0.04). There was, in contrast, a strong positive association ($r^2 = 0.93$) between temperature and the abundance of *Oc. notoscriptus* ($P < 0.001$) (Figure 5).

**DISCUSSION**

The scarcity of *Cx. pervigilans* larvae (3%) in the ovitraps inspected was surprising. A number of surveys carried out on the North Island have shown that this species is the dominant culicid when all artificial larval habitats are accounted for (Browne 1995, Hearnden et al. 1999, Laird 1990). According to Belkin (1968), however, *Cx. pervigilans* is not a container breeder *per se*, and it is possible that the ovitraps used were too small due to their relative sizes in comparison to larger artificial breeding habitats such as drinking troughs, drums, guttering pipes, etc. If much larger container types had been used in this study, *Cx. pervigilans* may have been more abundant in the containers surveyed.

The same explanation could apply for *Cx. quinquefasciatus*, as Becker (1995), for instance, found that aperture size was a significant covariant influencing oviposition selection of this species, which preferred the largest containers (300-3000 cm$^2$). The lower boundaries of this preferred range are nearly three times bigger than the aperture size of the largest container employed in this study.
In Auckland City, the main larval habitats for this species seem to be underground stormwater systems (Laird 1990) and other large abandoned containers such as metal drums (Belkin 1968).

In relation to nutrient contents, both Cx. quinquefasciatus and Cx. pervigilans would be expected to prefer organically richer solutions. Culex pervigilans has a wide tolerance to water quality levels and is capable of breeding in both clean and contaminated waters, having been found even in liquid manure (Graham 1929). Culex quinquefasciatus, in particular, is known to prefer organically rich and often polluted waters (Dumbleton 1968, Graham 1939, Urbain 2001), and in New Zealand Cx. quinquefasciatus has been recorded in oxidation ponds in a sewage treatment plant (Belkin 1968). This species has been recorded elsewhere thriving in septic tanks (Barnish 1984, Russell 1993), and organically enriched ovitraps have been shown to stimulate oviposition by Cx. quinquefasciatus females (Rodcharoen et al. 1997). It is interesting to point out that the co-infections observed in this experiment reflected the patterns obtained in other studies. Cx. quinquefasciatus and Cx. pervigilans seem to be rarely recorded together, while the latter species is frequently found co-infecting container habitats with Oc. notoscriptus (Belkin 1968, Laird 1995).

The results for Oc. notoscriptus indicated that larval densities were higher in medium-size ovitraps than in large ones, even though contrasting patterns were obtained in regards to container occupancy (Figures 1 and 3). Moreover, between-treatment differences varied throughout the experiment and among sites (Figure 4). At Pohuehue in particular, the number of larva-positive large containers was considerably larger than medium-size ones (Figure 3). These results contrasted to some extent with the findings from a previous experiment in the Wellington region, where Oc. notoscriptus demonstrated a very marked preference for identical medium-size ovitraps1. Little information seems to be available on Oc. notoscriptus oviposition preferences regarding container size, apart from the species’ ability to breed in a very wide range of containers (e.g. Hamlyn-Harris 1929, Lee and Bugledish 1999). Hamlyn-Harris (1929) found that Oc. notoscriptus preferred to oviposit in wide-mouthed containers, but since the author did not quantify the latter it is not possible to compare it to our data.

In many studies, however, oviposition preferences in relation to container size are carried out on a per volume basis rather than surface area, but we believe that the latter is the most suitable variable as mosquitoes are not likely to be capable of assessing container depth. Browne2, for instance, observed that mosquitoes showed no preference for deep water over shallow water when ovipositing in test buckets, with females ovipositing in the containers irrespective of whether the containers were full or nearly empty, which he suggested as evidence that mosquitoes were incapable of assessing water depth. Alternatively, tree hole breeding species seem to select containers with a higher optical density of water (i.e. darker) (Wilton 1968). Even though mosquitoes do not seem capable of assessing water depth, they appear to utilize other habitat characteristics as surrogate measures of the water holding capacity and permanence of a container habitat. These are potentially advantageous as deeper larval habitats are less likely to dry out (Braddock and Holzapfel 1983, Wilton 1968), and containers with a larger storage capacity are less likely to overflow, which can reduce egg and larval mortality (Frank and Curtis 1977, Washburn and Anderson 1993) and lead to resource depletion by removal of nutrients (Dieng et al. 2003). Nonetheless, despite the apparent advantages to larval survivorship provided by larger container habitats, surface area preferences are somewhat variable among mosquito species (e.g. Sota 1998, Sunahara et al. 2002).

Regarding water nutrient levels, Oc. notoscriptus displayed a significant preference for ovipositing in low nutrient solutions (Figure 2), which was rather consistent throughout the field sites. This was exemplified at Pohuehue where there appeared to be an actual aversion to the containers with high organic load (Figure 3). It is possible that the observed pattern at this site was a result of phenotypic or genotypic variation within this particular population. Williams et al. (1999) for instance, obtained variable oviposition preferences regarding nutrient content among different populations of Oc. notoscriptus, and while mosquitoes in one site were not selective for water type, in other sites the species displayed a significant bias towards manure slurry water in comparison to pond water.

Foot (1970) also carried out an experiment with Oc. notoscriptus in Auckland, and the species showed a pronounced preference for ovipositing in organically enriched water versus clean tap water. Here, in contrast, Oc. notoscriptus showed a marked preference for the solutions with lower organic load (0.5 versus 5.0 g/L). However, Foot (1970) did not describe the nutrient contents of the solution used, only mentioning that it consisted of a “larval rearing medium with a bottom layer of soil” (p. 24). As a result, it is not possible to compare her results to this study, since our low nutrient solution might have been comparatively organically rich.

Reiter et al. (1991) showed that solutions with a high organic load can provide olfactory stimuli for long-range attraction, even though particular mosquito species may prefer more diluted solutions where choice is available. In regards to our experiment, due to the methodology employed it is not possible to assess whether that was the case for Oc. notoscriptus. There is no doubt, however, that this species tolerates a wide range of water quality environments, from very clean water to highly putrid solutions1. Oviposition preferences for the species may vary among different populations, although whether these are genotypic or phenotypic is unknown (Williams et al. 1999). As suggested by the variation in oviposition preferences throughout the experiment in the two sites displayed in Figure 4, it seems that differences could actually be phenotypic and triggered by environmental cues. The strong association between larval abundance and mean daily temperature (Figure 5) indicates that the latter may likely be the main environmental factor

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affecting species bionomics at any given time. Although one would expect temperature to be an important factor, the highest larval densities recorded during the three-week drought was surprising and counter-intuitive, as rising water level should have been a determinant for egg hatching.

It is of interest that two of the most common mosquitoes in northern New Zealand were rarely recorded in the ovitraps utilized in this study. Based on evidence from this study and that from a recent survey in the Auckland region, we believe that ovitraps are not a suitable technique for population monitoring of *Cx. pervigilans* and *Cx. quinquefasciatus*. Neither seems particularly attracted to container habitats, and adult traps are likely to be the most appropriate tools for the monitoring of these species in the New Zealand scenario, as the use of considerably larger containers would be logistically difficult.

In relation to *Oc. notoscriptus*, this species displayed significant preference for ovipositing in low nutrient solutions, although mixed results were obtained in relation to container size. However, the ecology of individual populations is likely to vary between different localities. The data also suggested that populations of this species should peak in February at the height of summer, which is the warmest month of the year in the Auckland region (NIWA 2004). Although more research is needed to understand the ecology of this species in New Zealand, such data are relevant from a public health perspective. *Ochlerotatus notoscriptus* seems to have become the most common container breeder in northern New Zealand, both in native habitats and anthropic environments. With many authors warning that an arbovirus outbreak is likely to occur sooner rather than latter in this country (Crump et al. 2001, Weinstein 1996), this species could indeed play an important role in urban epidemics of, for instance, Ross River virus (Derraik and Calisher 2004).

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