

IMMATURE MOSQUITOES ASSOCIATED WITH URBAN PARKLANDS: IMPLICATIONS FOR WATER AND MOSQUITO MANAGEMENT

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ABSTRACT. The aim of the present study was to compare 2 urban habitat types: pools artificially filled with water from damaged or leaking water pipes (AF) and pools naturally filled by rainwater (NF), with regard to their favorability as breeding sites for mosquitoes. Two study areas were analyzed, 1 for 5 months and the other for 9 months, covering the whole period when AF pools contained water. The AF pools held water during the entire study, and showed lower fluctuations in surface area than NF pools. The AF pools showed higher levels of total mosquitoes and of stagnant-water mosquitoes. The floodwater mosquitoes were numerically (but not significantly) more abundant in NF pools. Nine mosquito species were identified. Habitat type, temperature, and season were significant in explaining the variability in species composition according to the canonical correspondence analysis. The most abundant species were *Ochlerotatus albifasciatus* (= *Aedes albifasciatus*, predominantly in NF pools), *Culex dolosus*, and *Cx. pipiens* (mainly in AF pools). The latter 2 species differed in their temporal dynamics, with *Cx. dolosus* associated with lower temperatures and *Cx. pipiens* with higher temperatures. Overall, the results indicate that although both habitat types harbored immature mosquitoes, the AF pools were more favorable than co-occurring rain pools. Easy-to-implement management actions such as the design of adequate drainage systems and the fast repair of broken pipes will be helpful to reduce the risk of human illness associated with mosquitoes in urban green areas.

KEY WORDS Artificial filling, rain pools, urban parks, stagnant-water mosquitoes, floodwater mosquitoes

INTRODUCTION

Green spaces located in urban areas are usually perceived as having both direct and indirect beneficial effects on human health. However, these areas may also have negative effects on human health, such as the proliferation of disease vectors (Lyytimäki and Sipilä 2009). In many cases, the negative effects occur because of a deficient design or lack of proper maintenance of green spaces, and thus, prevention is possible.

Water accumulation in ground depressions with poor drainage provides appropriate conditions for the development of several mosquito species (Irwin et al. 2008, Pires and Gleiser 2010). Temporary pools filled either naturally by rainfall or artificially by damaged pipes are common in urban green spaces such as those of Buenos Aires city (Argentina). A dozen mosquito species have been recorded in rain pools (Fischer et al. 2000, Fontanarrosa et al. 2004), and their temporal dynamics and association with environmental variables have been previously described (Fontanarrosa et al. 2000; Fischer et al. 2002; Fischer and Schweigmann 2004, 2008; Fontanarrosa et al. 2009). However, although the presence of immature mosquitoes has also been recognized in artificially filled pools in other regions (Barbazan et al. 1997, Keating et al. 2004, Ageep et al. 2009, Malcolm et al. 2009), there are no studies on their temporal dynamics. For Buenos Aires in particular, no previous knowledge is available regarding the relative suitability of these habitats (as

compared to rain pools) for the breeding of local mosquito species.

Mosquito species breeding in ground pools can be classified into 2 main groups, according to their oviposition-site requirements (Bueno-Marí and Jiménez-Peydró 2011). Females of the 1st group (stagnant-water mosquitoes) lay their eggs on the surface of water, and hatching occurs immediately after embryonic development is completed (e.g., *Culex* and *Anopheles*). Females of the 2nd group (floodwater mosquitoes) deposit their eggs on moist substrates that are potentially subject to flooding (e.g., *Ochlerotatus*, *Aedes*, and *Psorophora*). Synchronized hatching of these drought-resistant eggs occurs when the substrate is covered by water (Clements 1992). Regarding the classification of the former genus *Aedes*, we follow in this article the criterion proposed by Reinert (2000), that elevated the subgenus *Ochlerotatus* (= *Aedes*) to generic level, which was confirmed by a cladistic analysis of many characters of the egg, larval, pupal, and adult stages (Reinert et al. 2009).

The aim of this study was to compare artificially filled (broken pipes) and naturally filled (rain) pools regarding their hydrology and favorability as breeding sites for different species and functional groups of mosquitoes.

MATERIALS AND METHODS

Study area: This work was carried out in 2 parks of Buenos Aires: Plaza Sicilia (34°34'S;

Table 1. Comparison of flooding and mosquito immature densities in naturally filled (NF) and artificially filled (AF) pools of Plaza Sicilia (PSI) and Parque Pioneros de la Antártida Argentina (PAA).

| | PAA | | PSI | |
|--|------------|-------------|-------------|--------------|
| | NF | AF | NF | AF |
| Longest permanence of the water (wk) | 3 | 21 | 10 | 36 |
| Mean flooded area (m ²) | 163.5 | 511.4 | 296.2 | 128.9 |
| Coefficient of variation of flooded area (%) | 262.2 | 24.6 | 209.8 | 53.4 |
| Infested/total number of dates with water (proportions in parentheses) | 4/9 (0.44) | 21/21 (1.0) | 7/21 (0.33) | 26/34 (0.76) |
| Mean density of stagnant-water mosquitoes (no./m ²) | 11.6 | 91.6 | 0.05 | 46.7 |
| Mean density of floodwater mosquitoes (no./m ²) | 8.7 | 1.0 | 30.98 | 1.2 |
| Mean density of total mosquitoes (no./m ²) | 20.3 | 92.6 | 31.03 | 47.8 |

58°24'W) and Parque Pioneros de la Antártida Argentina (34°33'S; 58°30'W), covering areas of approximately 13 ha and 4.5 ha, respectively. Vegetation in both green spaces is composed mainly of grass, subjected to periodical cutting, and trees, which are widely distributed throughout the parks, providing differential shading conditions. Both parks have an underground pipe network, which provides water (for drinking and irrigation) through emerging faucets. The irregular relief of the land forms ground depressions where water tends to accumulate either after rainfall events or from broken water pipes.

Fieldwork: All aquatic habitats present in each park were surveyed weekly. Study periods lasted from April through August 2007 (5 months) in Parque Pioneros de la Antártida Argentina (PAA), and from May 2007 to January 2008 (9 months) in Plaza Sicilia (PSI). Every pool was classified according to the source of water as either naturally filled by rain (NF) or artificially filled by broken water pipes (AF). In either case, the flooded area was assessed weekly by multiplying the maximum length, width, and proportion of water of the resulting rectangle occupied by water. Samples of immature mosquitoes were taken from every pool in each opportunity with a hand net (10 × 12 cm, 350-μm mesh size). The size of each sample was approximately proportional to the water surface in each pool, and meters covered by the hand net were recorded. Samples were fixed in situ in alcohol 80%.

Immature mosquitoes were separated by stage and identified under a stereoscopic microscope, with the use of appropriate systematic keys and specialized literature on the local species (Darsie 1985, Almirón and Brewer 1995, Almirón and Harbach 1996, Rossi et al. 2002). First and 2nd instars, as well as pupae, were classified by genus, and 3rd and 4th instars were identified to the species level. For each sample, the number of individuals of each species and genus were summed up.

Data analysis: The total flooded area corresponding to AF and NF pools was calculated for each park and sampling date as the sum of flooded areas of the individual pools in each

filling category and study site. The fluctuations of the flooded area across the sampling dates were assessed for each filling category and park with the coefficient of variation. The flooded areas of AF and NF pools were compared within each park with the Wilcoxon test for paired samples. The infestation (number of dates with immature mosquitoes/total number of dates with water) was compared between AF and NF pools in each site with the use of the χ^2 test for independent proportions (Fleiss et al. 2003).

For the analysis of immature mosquito composition and abundance, we considered the total number of specimens collected on each sampling date, grouped by park and filling category.

Densities of each mosquito group (stagnant water and floodwater mosquitoes) and of total immature mosquitoes were calculated as the number of specimens divided by the total area covered by the net (in m²) when collecting the samples. Densities of each group were compared between AF and NF pools of each park with the Mann-Whitney *U*-test. For each filling category, only dates containing water were considered.

Mean monthly density was assessed by species as the average density on each sampling date. Based on the total number of specimens of each species collected, we calculated the percentage collected in AF and NF pools.

A canonical correspondence analysis (CCA) (ter Braak and Smilauer 1998) was used to summarize the relationships between species composition and the following environmental variables: season (winter, spring, summer, fall), temperature (weekly average), rainfall (weekly accumulation), days since the previous rainfall, flooding (estimated by the water index), filling category (artificially or naturally filled), and study site (PAA, PSI). Log-transformed weekly abundance values were used, and rare species down-weighted. Categorical variables such as season, filling category, and study site were transformed into dummy variables. Effects of each variable on mosquito composition were evaluated with the use of forward selection and a Monte Carlo resampling procedure with 999 permutations.

RESULTS

Artificially flooded pools never dried out during the whole study period and showed lower fluctuations in water surface than NF ones in both study sites (Table 1). In PAA, the flooded area of AF pools was significantly greater than that of NF pools ($z = 3.18$, $n = 21$, $P < 0.002$), whereas in PSI differences in the flooded area did not differ significantly between both filling categories ($z = 1.00$, $P = 0.32$) (Table 1).

Immature mosquitoes were recorded in both AF and NF pools, although infestation was higher in AF than in NF both in PAA ($\chi^2 = 14$, $df = 1$, $P < 0.001$) and in PSI ($\chi^2 = 10.01$, $df = 1$, $P < 0.005$).

Total mosquito density was higher in AF than in NF pools in both PAA (Mann–Whitney U -test, $P < 0.01$) and PSI (Mann–Whitney U -test, $P < 0.02$). The density of stagnant water mosquitoes showed the same pattern as total mosquitoes (Table 1), with significant differences in both PAA (Mann–Whitney U -test, $P < 0.001$) and PSI (Mann–Whitney U -test, $P < 0.002$). Although the densities of floodwater mosquitoes showed slightly higher values in NF than in AF pools in both parks (Table 1), differences were not significant (Mann–Whitney U -test, $P = 0.73$ for PAA, and $P = 0.53$ for PSI).

Nine mosquito species belonging to 4 genera were identified (Table 2). All 9 species were recorded in April (fall), and the richness was lowest in August (winter) and December (spring/summer). Among the most abundant species, *Culex dolosus* (Lynch Arribáizaga) and *Cx. pipiens* L. were collected mainly in AF, and *Ochlerotatus albifasciatus* (Macquart) (= *Aedes albifasciatus*) was recorded mainly in NF pools. *Culex chidesteri* Dyar and *Anopheles* sp. were exclusively recorded in AF, and *Psorophora varipes* (Coquillett) exclusively in NF pools. These 3 species showed the lowest abundance values.

The 1st 2 axes of the CCA accumulated 68.3% of variance in species composition. In decreasing order of importance, the significant variables for the ordination in these axes were habitat type (AF, NF), season (fall, spring), temperature, and flooding (Fig. 1), which explained 91.5% of the variance in species composition. Three groups of species can be distinguished according to their association with environmental variables (Fig. 1). The 1st group, associated with NF pools, included 2 floodwater mosquito species: *Oc. albifasciatus* and *Ps. varipes*. The remaining 2 groups were associated with AF pools. The 2nd group, composed of *Cx. pipiens*, *Cx. maxi* (Dyar), *Cx. tatoi* Casal and García, *Cx. chidesteri*, and *Oc. scapularis* (Rondoni) (= *Ae. scapularis*), was related to large flooding levels and high temperatures in fall. The 3rd group included *Cx. dolosus*

and *Anopheles* sp., and was associated with smaller flooding levels and lower temperatures.

DISCUSSION

The AF pools analyzed in this study showed marked differences in the flooding dynamics as compared with NF pools, despite the morphological similarities between both filling categories. The longer permanence and lower fluctuations in the flooding levels of AF pools could be explained by the continuous supply of water throughout the period when the pipes remained broken. The higher infestation and mosquito immature densities recorded in AF pools indicate that these are more favorable for mosquito development than the co-occurring rain pools, although such results could change with other climatic conditions.

The species composition observed in artificially and continuously filled pools showed a close agreement with the reports of previous studies performed in rain pools from the same region (Fischer et al. 2000, Fontanarrosa et al. 2009). Thus, the chemical characteristics of the water leaking from pipes seem not to affect the community of breeding mosquitoes.

The evident differences in mosquito composition and abundance between the 2 pool categories analyzed could be explained mainly by the disparity in the permanence of the water. The predominance of floodwater species (mainly *Oc. albifasciatus*) observed in the ephemeral pools filled by rain was coincident with our expectations because of the known association of this species with fluctuations in flooding levels (Gleiser et al. 2000) and its ability to exploit the presence of short-lasting water pools (Fischer and Schweigmann 2008). On the other hand, the increased infestation and density of stagnant-water mosquitoes in artificially filled pools is consistent with the general pattern observed in mosquitoes, according to which increasing proportions of species lacking adaptations to drought are recorded in more permanent habitats (Wiggins et al. 1980, Laird 1988, Schneider and Frost 1996).

The long-lasting presence of water in shallow ground pools provided the opportunity to study the seasonal dynamics of the mosquito community independently of the fluctuations in flooding caused by rain. For the 2 most abundant species in artificially filled pools in particular (*Cx. dolosus* and *Cx. pipiens*), our results showed a temporal differentiation related to temperature. The apparent association of *Cx. dolosus* with intermediate temperatures and of *Cx. pipiens* with higher temperatures is coincident with previous results from rain pools in the region (Fischer and Schweigmann 2004). The existence of this pattern is also supported by laboratory studies of survival

Table 2. Mean surface area, average monthly density of mosquito larvae, and total number and percentage of each species collected from artificially filled (AF) and naturally filled (NF) pools.

| Species | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Number collected by habitat type | % by habitat type |
|------------------------------------|-------|-------|-------|-------|-------|-------|--------|-------|-------|-------|-------------------------------------|----------------------|
| AF (mean surface) | 330.1 | 373.2 | 353.0 | 398.4 | 333.3 | 158.3 | 155.2 | 78.5 | 94.7 | 96.2 | | |
| <i>Psorophora</i> | — | — | — | — | — | — | — | — | — | — | 0 | 0 |
| <i>varipes</i> ¹ | 3.98 | 0.32 | — | 0.13 | 0.08 | 5.28 | 1.05 | — | — | — | 66 | 4.1 |
| <i>Ochlerotatus</i> | — | — | — | — | — | — | — | — | — | — | — | — |
| <i>albifasciatus</i> | 0.88 | — | — | — | — | — | — | — | — | — | 7 | 77.8 |
| <i>Oc. scapularis</i> | 86.74 | 30.89 | 0.69 | 0.13 | — | — | — | 0.26 | 2.08 | 27.59 | 1,945 | 97.0 |
| <i>Culex pipiens</i> | 5.95 | 1.77 | — | — | — | — | — | — | — | — | 115 | 98.3 |
| <i>Cx. maxi</i> | 29.79 | 6.93 | 0.06 | — | — | — | — | — | — | 0.62 | 444 | 99.1 |
| <i>Cx. tatoi</i> | 3.79 | 41.24 | 17.97 | 8.15 | 4.67 | 0.28 | 0.52 | 60.55 | 22.60 | 3.64 | 2,797 | 99.9 |
| <i>Cx. dolosus</i> | 0.17 | — | — | — | — | — | — | — | — | — | 2 | 100 |
| <i>Cx. chidesteri</i> ¹ | 0.08 | 0.30 | 0.20 | — | — | — | — | — | — | — | 17 | 100 |
| <i>Anopheles</i> sp. ¹ | — | — | — | — | — | — | — | — | — | — | — | — |
| NF (mean surface) | 943.2 | 10.4 | 403.0 | 3.0 | 306.6 | 720.5 | 520.8 | 3.7 | 4.3 | 3.1 | | |
| <i>Ps. varipes</i> ¹ | 0.09 | — | — | — | — | — | — | — | — | — | 1 | 100 |
| <i>Oc. albifasciatus</i> | 5.67 | 5.95 | — | — | — | 5.28 | 138.48 | 2.08 | — | — | 1,540 | 95.9 |
| <i>Oc. scapularis</i> | 0.04 | — | — | — | — | — | 0.02 | — | — | — | 2 | 22.2 |
| <i>Cx. pipiens</i> | 2.96 | 7.05 | — | — | — | 0.08 | 0.02 | — | — | — | 61 | 3.0 |
| <i>Cx. maxi</i> | — | 0.79 | — | — | — | — | — | — | — | — | 2 | 1.7 |
| <i>Cx. tatoi</i> | 0.18 | 0.14 | — | — | — | — | — | — | — | — | 4 | 0.9 |
| <i>Cx. dolosus</i> | — | 0.47 | — | — | — | — | 0.02 | — | — | — | 3 | 0.1 |
| <i>Cx. chidesteri</i> | — | — | — | — | — | — | — | — | — | — | 0 | 0 |
| <i>Anopheles</i> sp. ¹ | — | — | — | — | — | — | — | — | — | — | 0 | 0 |

¹ Species that were exclusively collected in a single category of water bodies.

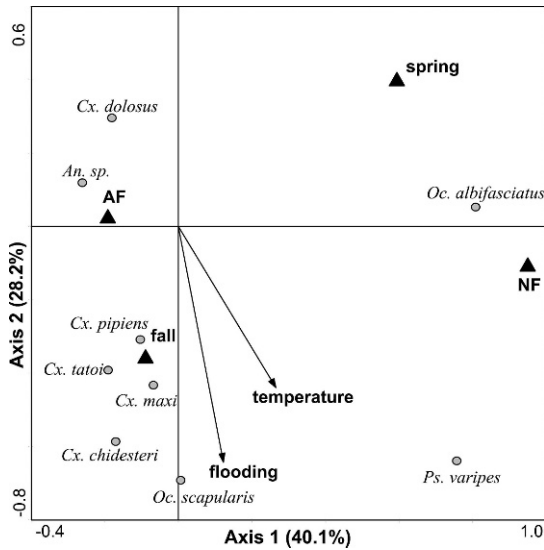


Fig. 1. Canonical correspondence ordination diagram for mosquito species and environmental variables. Triangles indicate categorical variables, arrows indicate quantitative variables, and circles indicate species.

during development at different temperatures (Loetti et al. 2011a, 2011b). Intermediate temperatures corresponding to the fall or spring seasons in Buenos Aires would be more favorable for *Cx. dolosus* (synonymous of *Cx. eduardoi*) (Loetti et al. 2011a), whereas higher temperatures characteristic of the summer season would be more favorable for *Cx. pipiens* (Loetti et al. 2011b).

Some of the mosquito species recorded in the present study are epidemiologically important both for humans and domestic animals (Sabattini et al. 1998). In particular, *Cx. pipiens*, *Oc. albifasciatus*, and *Oc. scapularis* have been implicated in the transmission of several encephalitis viruses. *Culex pipiens* is a recognized vector of West Nile virus (Hamer et al. 2008) and Saint Louis encephalitis virus (Mitchell et al. 1985). *Ochlerotatus albifasciatus* has been incriminated in the transmission of the western equine encephalomyelitis virus, because the virus has been isolated from wild specimens caught during the outbreak registered in 1982–1983 (Mitchell et al. 1987), and under laboratory conditions this species has proved to be a competent vector of the virus (Avilés et al. 1990). *Ochlerotatus scapularis* is suspected to transmit Rocio encephalitis virus in Brazil (Mitchell and Forattini 1984), and Venezuelan equine encephalitis virus in Argentina (Mitchell et al. 1985). Thus, their presence in green areas close to human dwellings involves direct risks for human health. The ability of the remaining species found in the present study to transmit diseases is not known, because species endemic to southern South America have not

been intensively studied as compared to more widespread species.

The results of this study show that the poor maintenance of urban green spaces may favor the formation of suitable aquatic environments, which are recognized and colonized by mosquitoes in a short time, even in those sites with no previous history of flooding. Management actions such as ground leveling, the design of an adequate drainage system, and the fast repair of broken or leaking water pipes would contribute to preventing the formation of such aquatic habitats and the consequent mosquito breeding. In most cases, such actions would be easy to implement and would attain efficient results, although it should be noted that long-lasting water accumulations are usually not considered a public health problem by local authorities. The results of the present article contribute to the knowledge of the risks involved in the presence of mosquito breeding sites in public areas, and should taken into account when management priorities are assigned.

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