ERYNGIUM SPP. (UMBELLIFERAE) AS PHYTOTELMATA AND THEIR *CULEX* (*CULEX*) INHABITANTS IN TEMPERATE ARGENTINA

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ABSTRACT. The host-plant fidelity, seasonality, and population dynamics of 3 species of *Culex* (*Culex*) were examined from monthly samples of mosquito immatures extracted from the leaf axils of *Eryngium pandanifolium* and *Eryngium cabrerae* growing sympatrically in the Punta Lara region of Buenos Aires Province, Argentina. *Culex hepperi* was collected only from *E. pandanifolium*, and abundance peaks of its larvae and pupae were asynchronous in forest and field habitats. However, the proportion of plants positive for *Cx. hepperi*, as well as larval densities and developmental success, did not differ between forest and field. *Culex renatoi* was collected only from *E. cabrerae* in the field habitat. The monthly numbers of *Cx. hepperi* and *Cx. renatoi* were not significantly correlated with the proportion of their host plants that held water. The stage-specific distributions of *Cx. hepperi* and *Cx. renatoi* numbers were highly skewed towards 1st instars and did not differ between species or, for *Cx. hepperi*, between forest and field habitats. *Culex castroi* was collected regularly from the axils of both species of *Eryngium*, but usually in lesser numbers than either *Cx. hepperi* or *Cx. renatoi*. Association analyses indicated significant positive associations between *Cx. castroi* and the other 2 culicine species in host plants. *Culex renatoi* occurred preferentially in small *E. cabrerae*, but *Cx. castroi* did not discriminate plants based on their size. These 3 species of *Culex* are known only from *Eryngium* spp. phytotelmata. We suggest that their host-plant specificity is maintained by oviposition preferences, which are more highly selective for *Cx. hepperi* or *Cx. renatoi* than for *Cx. castroi*.

KEY WORDS Phytotelmata, *Eryngium* spp., *Culex* (*Culex*) spp., seasonality, specificity

INTRODUCTION

Phytotelmata, the water-holding catchments of terrestrial plants, are important habitats for some groups of aquatic insects and, in particular, for mosquitoes (Frank and Lounibos 1983). Four hundred species of Culicidae have been estimated as inhabiting phytotelmata (Fish 1983), with many endemic or highly specialized species probably awaiting discovery. In a survey of mosquitoes inhabiting phytotelmata in a Venezuelan rain forest, Machado-Allison et al. (1985) recognized 4 of 21 species as restricted to 1 species of plant and 5 other culicid species as occurring in plants belonging to only 1 family, such as the Bromeliaceae or Araceae.

Eryngium (Umbelliferae) is a cosmopolitan genus of perennial, rhizomatous herbs that includes more than 220 species in temperate and tropical regions (Cabrera 1965). Twenty-five species of the genus are known from Argentina, 17 of which occur in Buenos Aires Province (Cabrera 1965). In several *Eryngium* spp. known from Brazil and Argentina, overlapping leaves arranged in whorls capture and retain water in their axils (Fig. 1). The aquatic fauna occurring in axils of *Eryngium pandanifolium* Cham. and Schlecht in temperate Argentina (Vucetich and Rossi 1980) and of *Eryngium floribundum* Cham. in tropical Brazil (Machado 1976) have been inventoried. Mosquitoes were recognized in these previous studies, but not identified beyond family level.

Four species of *Culex* (*Culex*) have been described from specimens collected as immatures from the axils of unidentified species of *Eryngium* in Brazil and Argentina (Lane and Ramalho 1960; Casal and Garcia 1967a, 1967b; Da Silva Mattos et al. 1978). To date, the immatures of these species of *Culex* are not known from other habitats. Herein we report on the bionomics of 3 of these species, *Culex hepperi* Casal and Garcia, *Culex renatoi* Lane and Ramalho, and *Culex castroi* Casal and Garcia, occurring in 2 species of *Eryngium* that grow sympatrically along the Río de La Plata, Argentina.

MATERIALS AND METHODS

Field studies were conducted from December 1994 through March 1997 in the Punta Lara area (34°51'33"S, 57°52'23"W) of Río de La Plata in Buenos Aires Province. Annual rainfall in this region was 828.6 mm in 1995 and 857.3 mm in 1996. Mean daily temperatures range from 9°C in July to 28°C in January. A gallery forest, composed of native trees and the introduced *Ligustrum lucidum* Ait., borders the river in this area and, under natural circumstances, is flooded daily at high tide. However, the artificial containment of tides by man-made levees has curtailed daily flooding and allowed for the invasion of exotic plant species in this forest. On the other side of the gallery forest was an open field.

Eryngium cabrerae Pontiroli (Fig. 1) occurred only in the field but *E. pandanifolium* was common in both the field and gallery forest. Both species have a similar growth form, but the maximum height of *E. pandanifolium* (2.5 m) is much greater than that of *E. cabrerae* (0.6 m) (Cabrera 1965).

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Fig. 1. *Eryngium cabrerae* at the field site in Punta Lara area, Buenos Aires Province, Argentina. (Left) A mature plant in flower. (Right) Top view of nonflowering plant, showing arrangement of leaves in whorls.

The axils of both species catch and accumulate rainwater and, under special circumstances, flood-waters when the surrounding terrain is inundated. Because axils overlapped (Fig. 1) and were not discrete habitat units, the entire aquatic contents of individual plants were pooled during sampling. Water-holding *E. cabrerae* at the study site contained a mean of 23.5 ml (range 1–145 ml, $n = 341$) of fluid.

Fluid contents were extracted with a pooter (aspirator) attached to a lift pump. To estimate extraction efficiency, axils were washed and reextracted 3 successive times with clean tap water. In 40 plants tested, 92.1% of mosquito larvae and pupae ($n = 372$) were recovered in the original siphoning, and 99.8% were collected after 2 washes. Therefore, for routine sampling purposes, after the initial extraction plants were flushed twice with tap water and the aquatic contents reextracted after each wash.

Once per month, for 28 successive months for *E. pandanifolium* and for 14 successive months for *E. cabrerae,* 60 plants of the former species (30 in the field and 30 in the forest) and 30 of the latter species were selected haphazardly for sampling. To avoid sampling from the same plant on successive months, recently sampled zones were excluded for a period of at least 3 months before revisiting.

Samples from individual plants were returned in separate jars to the laboratory where fluid volume of the original extract was measured. Mosquito larvae were staged to instar by relative head capsule widths and reared individually in vials provided with flaked fish food (Tetramin®, TetraWerke, Melle, Germany). Mortality during rearing was low (<1%), allowing mosquitoes to be identified to species in the adult stage with keys (Darsie and Mitchell 1985) and species descriptions (Lane and Ramalho 1960; Casal and Garcia 1967a, 1967b).

In an effort to measure selectivity of *Culex* spp. for *E. cabrerae* of different sizes, 4 field plots, each 2×2 m, were haphazardly chosen in which all plants were sampled and measured. Plant height was not a reliable indicator of size, because plants grew at various angles relative to the ground. Instead, plant size was estimated from the average width of 5 basal leaves, each measured at its widest point. For *E. cabrerae,* average basal leaf width was significantly correlated with 2 other size measures, namely, plant diameter ($r_s = 0.65$, $P < 0.01$, $n = 20$) and volumetric capacity ($r_s = 0.41$, $P <$
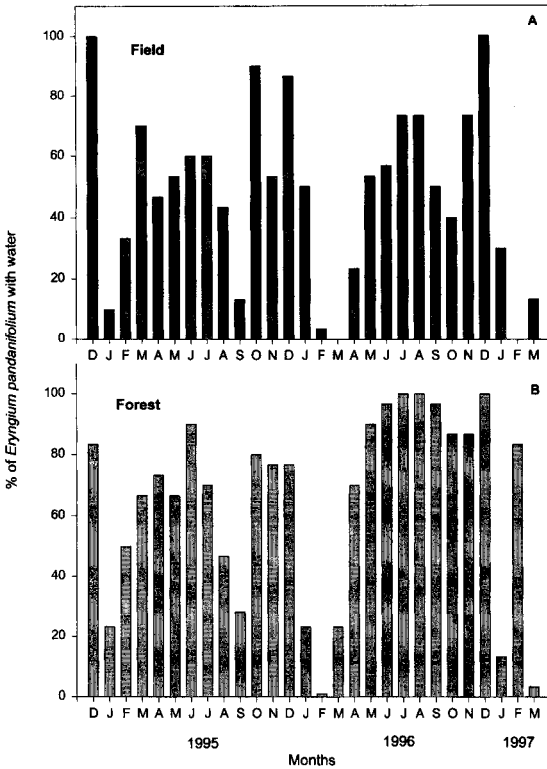
Fig. 2.   The monthly percentages of 30 *Eryngium pandanifolium* that contained measureable water (≥1 ml) in 28 months of sampling field and forest sites.
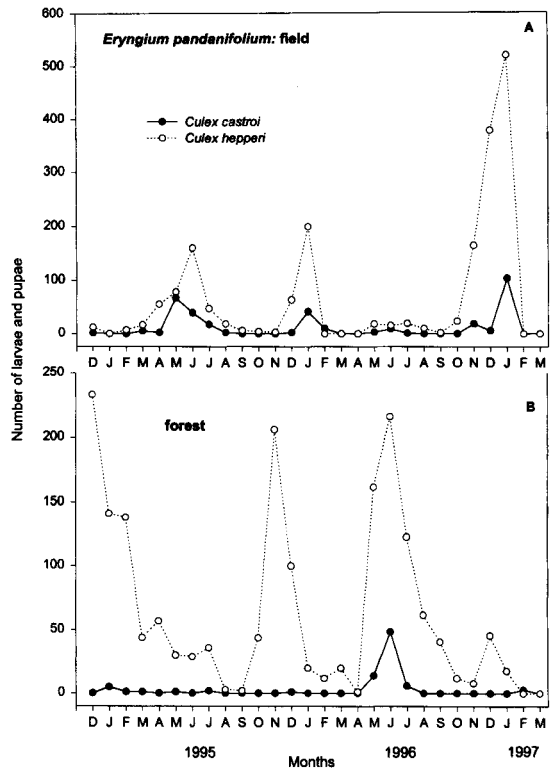

Fig. 3.   The total numbers of individuals of 2 *Culex* species identified from monthly samples of 30 *Eryngium pandanifolium* in the forest and field sites.

0.05, n = 20). Based on these width measurements, plants were categorized as either small (0.8–2.3 cm), medium (2.4–3.7 cm), or large (3.8–5.2 cm).

RESULTS

Eryngium pandanifolium and its culicid inhabitants

The proportion of plants with standing water fluctuated seasonally (Fig. 2). Plants were most likely to be dry between January and March when, on 2 occasions, no *E. pandanifolium* in the field held measurable water. The average monthly proportion of samples with water in the forest (64.5 ± 6.0 SE) was significantly greater than that for *E. pandanifolium* in the field (48.6 ± 5.5 SE) (Wilcoxon signed ranks T_s = 62.5, P = 0.002).

Both *Cx. hepperi* and *Cx. castroi* were identified from *E. pandanifolium* phytotelmata, with *Cx. hepperi* more abundant in both field and forest collections (Fig. 3). Abundance peaks of both species occurred in either the May–June or December–January periods, but did not coincide in the field and forest. Moreover, the number of larvae collected and the proportion of plants with water were not significantly correlated in either the field (r = 0.21, P = 0.27, df = 27) or forest (r = 0.33, P = 0.08,

df = 27), indicating that habitat availability per se is not the major determinant of *Culex* abundance in these phytotelmata. For the largest observed abundance peak of *Cx. hepperi*, 55.7% of immatures in December 1996 and 74% in January 1997 were 1st instars.

We compared the proportion of *E. pandanifolium* containing immatures of *Cx. hepperi* in forest and field habitats. The proportion of forest plants positive (Fig. 4A) for *Cx. hepperi* (20.2%) was not significantly different from the proportion of field plants positive (15.6%) (G_{adj} = 0.16, P > 0.50). Further, the mean density of *Cx. hepperi* in mosquito-positive plants in the forest (0.86 ± 0.03 SE) did not differ significantly from that of the field habitat (0.85 ± 0.04 SE) (t_s = −0.21, P = 0.84, df = 299).

To determine whether developmental success of *Cx. hepperi* was greater in the forest or field, we compared instar distributions of the immature stages collected from these 2 habitats (Fig. 4A). First instars predominated in both sets of samples, and the stage-specific distributions of *Cx. hepperi* did not differ significantly when tested by a Kolmorogov–Smirnov 2-sample test (D_{max} = 0.20, P > 0.1, n = 5).
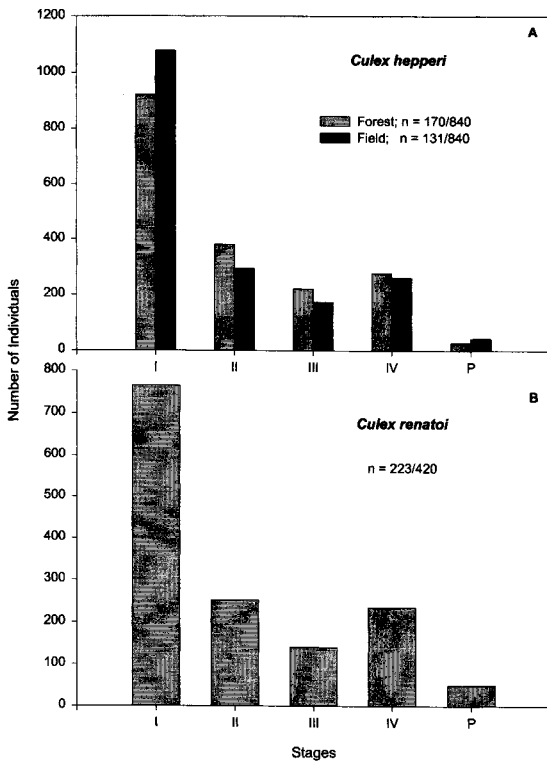
To evaluate whether *Cx. hepperi* and *Cx. castroi*

Fig. 4. (A) Numbers of *Culex hepperi* in each immature stage from all collections of *Eryngium pandanifolium*. Inset shows proportions of samples positive for *Cx. hepperi* in forest and field. (B) Numbers of individuals in each immature stage of *Culex renatoi* collected from *Eryngium cabrerae*.

0.5 and a highly significant chi-square value (corrected for continuity) of 52.5 ($P < 0.001$) for the association analysis indicated that these 2 species were highly likely to co-occur in the same plant samples.

Eryngium cabrerae and its culicid inhabitants

More than 50% of plants of this species held water on every sampling date except March 1997 (Fig. 5). On 5 of 14 occasions, 100% of the sampled plants contained measurable water.

The mosquitoes *Cx. castroi* and *Cx. renatoi* were collected on all 14 sampling dates (Fig. 5). On 2 occasions after the field had flooded from excessive rainfall, larvae and pupae of the ground-water species *Aedes albifasciatus* Macquart were recovered from many *E. cabrerae* axils. Because the presence of *Ae. albifasciatus* in this phytotelm was regarded as accidental, this species was not considered further in the present study.

On 11 of 14 occasions *Cx. renatoi* was more abundant than *Cx. castroi* in monthly collections (Fig. 5). The monthly numbers of mosquito immatures were not significantly correlated with the proportion of *E. cabrerae* with water for either *Cx. renatoi* ($r = 0.16$, $P = 0.59$, df = 13) or *Cx. castroi* ($r = -0.15$, $P = 0.60$, df = 13).

The stage-specific distribution of *Cx. renatoi* indicated a prevalence of 1st instars (Fig. 4B). To determine whether the distribution of *Cx. renatoi* immatures by stage differed from that of field-collected *Cx. hepperi* in *E. pandanifolium* (Fig. 4A), the 2 were compared with a Kolmorogov–Smirnov 2-sample test, which indicated no significant difference between the distributions of the 2 species in the 2 plants ($D_{max} = 0.20$, $P > 0.1$, $n = 5$).

The co-occurrence of *Cx. castroi* and *Cx. renatoi* in the same *E. cabrerae* plants was positive in the 2 × 2 association analysis, which yielded a signif-
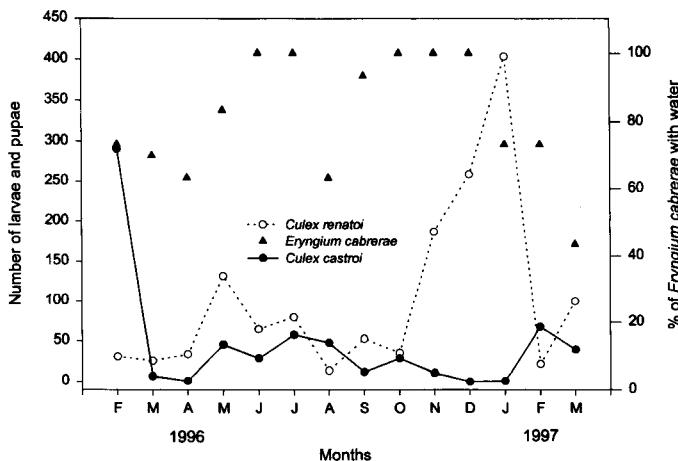
co-occurred in the same *E. pandanifolium* plants more often than chance would predict, we performed a 2 × 2 association analysis and calculated the C_8 coefficient of interspecific association, which varies from +1 to -1 (Hurlbert 1969). The C_8 of

Fig. 5. The monthly percentages of 30 *Eryngium cabrerae* that contained measureable water (≥ 1 ml) and the total numbers of 2 *Culex* species in these samples for 14 consecutive months.
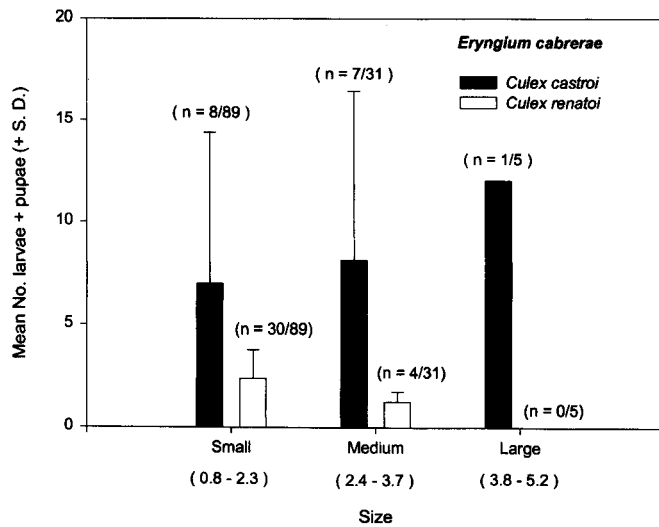
Fig. 6.   The mean numbers of *Culex castroi* and *Culex renatoi* immatures in *Eryngium cabrerae* plants positive for these mosquito species, segregated into size categories by width measurements of basal leaves.

icantly positive chi-square value (corrected for continuity) of 3.86 ($P < 0.05$). However, the C_8 value for interspecific association between these 2 species was <0.01, indicating a relatively weak association.

The sampling of all *E. cabrerae* plants in the 2 × 2-m plots yielded 5 large, 31 medium, and 89 small plants with measureable water. The average density of *Cx. renatoi* was highest in small plants, and no immatures were recovered in the large plants (Fig. 6). Significantly more small plants were occupied by *Cx. renatoi* than were medium-sized *E. cabrerae* ($G_{adj} = 4.19$, $P < 0.05$).

Comparative success of *Cx. castroi*

Because *Cx. castroi* occurred in both *E. pandanifolium* and *E. cabrerae*, it was of interest to com-
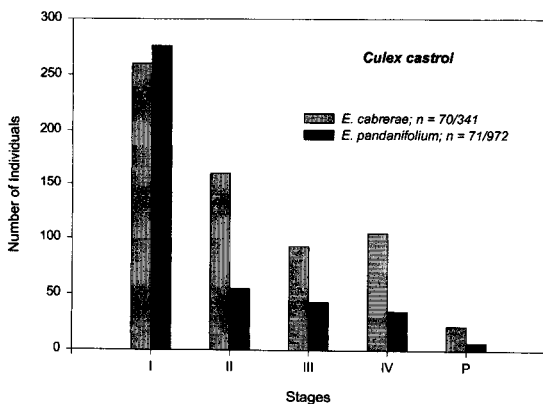

Fig. 7.   The numbers of individual *Culex castroi* in different immature stages collected from the 2 species of *Eryngium*. Inset indicates proportions of samples of the 2 plants that were positive for *Cx. castroi*.

pare the occupancy of the 2 phytotelmata by this species. The proportion of *E. cabrerae* positive (Fig.7) for *Cx. castroi* (0.203) was significantly higher than the proportion of *E. pandanifolium* that were positive for this species (0.073) ($G_{adj} = 39.8$, $P < 0.001$). Distributions of instars (Fig. 7) did not differ between plant species (Kolmorogov–Smirnov 2-sample test, $D_{max} = 0.60$, $P > 0.1$, $n = 5$).

In the 2 × 2-m plot of *E. cabrerae*, *Cx. castroi* occurred in plants of all 3 sizes (Fig. 6). A *G*-test for independence indicated no significant differences in the proportion of plants of the 3 size classes occupied by *Cx. castroi* ($G_{adj(2)} = 3.72$, $P > 0.1$).

DISCUSSION

Thirty-seven species of Culicidae are known from the Punta Lara area (Ronderos et al. 1992, Campos 1994), but only 7 species from 5 genera (*Culex, Haemagogus, Wyeomyia, Runchomyia, Toxorhynchites*) have been recovered from phytotelmata. Larvae of the 3 *Culex* species of the present study are known only from *Eryngium* spp. axils. Interestingly, *Culex dolosus* Lynch Arribalzaga, which was recovered from *Eryngium* axils in Minas Gerais State, Brazil (Da Silva Mattos et al. 1978), was not observed in Argentinian *Eryngium*, despite the abundance of *Cx. dolosus* in temporary and permanent ponds along the Río de la Plata (Maciá et al. 1996). *Culex spinosus* Lutz has been reported from the axils of an unidentified *Eryngium* in Paraná State, Brazil (Lozovei and Luz 1976), but this culicid is not known from phytotelmata in other parts of its range, which includes Argentina (Darsie and Mitchell 1985).

Thus, 3 levels of host-plant specificity may occur among the 6 species of *Culex* known from *Eryn-*

gium axils. *Culex hepperi* and *Cx. renatoi* are the most host-specific, occurring only in 1 species of *Eryngium. Culex machadoi*, described from an unidentified species of *Eryngium* from Minas Gerais State, Brazil (Da Silva Mattos et al. 1978), may fall in this category or the next. *Culex castroi* represents a 2nd level of specificity, insofar as it occurs in more than 1 species of *Eryngium* but is not known from any other larval habitat. Finally, *Cx. dolosus* and *Cx. spinosus*, both of widespread distribution in South America, represent a class of habitat generalists that occupy phytotelmata, including *Eryngium*, only occasionally.

Habitat specificity of phytotelm-inhabiting mosquitoes is frequently mediated by plant-produced chemical cues for oviposition (Fish 1983). For example, fluid from the bracts of *Heliconia caribaea* stimulates oviposition by the specialist *Wyeomyia ulocoma* (Theobald) (Lounibos and Machado-Allison 1993), and a water-soluble extract from *Sarracenia purpurea* induces oviposition by its host-specific inquiline, *Wyeomyia smithii* Coq. (Istock et al. 1983). The selectivity of gravid *Cx. hepperi* and *Cx. renatoi* for *E. pandanifolium* and *E. cabrerae*, respectively, suggests the use of host-specific chemical cues by these mosquito species to locate their host plants. *Culex castroi*, on the other hand, may use less specific chemical cues, if any.

The occupancy patterns of immatures in plants of 3 size classes is consistent with the expectation from chemical stimulation of oviposition by the most specialized mosquito species. The youngest phytotelm habitats, such as freshly opened *Sarracenia* leaves or *Heliconia* bracts, are the most attractive to specialist mosquitoes, such as *Wy. smithii* or *Wy. ulocoma*, lured by host-plant chemicals (Fish and Hall 1978, Machado-Allison et al. 1983). Thus, it is not surprising that *Cx. renatoi* should significantly favor oviposition in the smallest *E. cabrerae*, which distinguishes it in host choice from the less-specific *Cx. castroi* (Fig. 6).

The distribution of immature stages of all 3 species of *Culex* was highly skewed towards younger, especially 1st, instars (Figs. 4 and 7), suggesting high mortality early in larval life. Causes of this mortality remain to be resolved. Unlike *E. floribundum* in Minas Gerais, Brazil, where inquiline odonate larvae prey on mosquitoes (Machado 1976, Corbet 1983), the axils of *E. pandanifolium* and *E. cabrerae* did not harbor predators of mosquito larvae (Vucetich and Rossi 1980; Campos, personal observations). On the other hand, a fungus (López Lastra 1997) and a microsporidan (Micieli 1996), both isolated from *Cx. renatoi*, are capable of contributing to mortality of larval mosquitoes.

The absence of a correlation between larval abundance and habitat availability, as observed for *Cx. hepperi* and *Cx. castroi*, is common to other axil-inhabiting mosquitoes. Heavy rainfall is known to wash mosquito eggs from axils (e.g., Pajot 1976, Frank and Curtis 1977), which together with the occasional floods that washed *Ae. albifasciatus* into axils may make the rainiest periods less propitious for the *Culex* colonists.

REFERENCES CITED

Cabrera, A. L. 1965. Colección científica: flora de la Provincia de Buenos Aires. IV. Secretaría de Estado de Agricultura y Ganadería de la Nación, Instituto Nacional de Tecnología Agropecuaria (INTA), Buenos Aires, Argentina.

Campos, R. E. 1994. Presencia de *Toxorhynchites (Lynchiella) theobaldi* (Diptera: Culicidae) en la Provincia de Buenos Aires, Argentina. Rev. Soc. Entomol. Argent. 53:120.

Casal, O. H. and M. García. 1967a. *Culex (Culex) castroi*, nueva especie de la República Argentina (Diptera, Culicidae). Physis 26:451–457.

Casal, O. H. and M. García. 1967b. *Culex (Culex) hepperi*, nueva especie del Delta Bonaerense del Río Paraná (Diptera, Culicidae). Physis 27:87–94.

Corbet, P. S. 1983. Odonata in phytotelmata, pp. 29–54. *In:* J. H. Frank and L. P. Lounibos (eds.). Phytotelmata: terrestrial plants as hosts for aquatic insect communities. Plexus, Medford, NJ.

Darsie, R. F. and C. J. Mitchell. 1985. The mosquitoes of Argentina: parts I and II. Mosq. Syst. 17:153–360.

Da Silva Mattos, S., A. Da Silva Guedes and S. Hamilton Xavier. 1978. Uma espécie nova de *Culex (Culex)* que cria em Umbelífera (Diptera, Culicidae). Rev. Bras. Biol. 38:31–35.

Fish, D. 1983. Phytotelmata: flora and fauna, pp. 1–27. *In:* J. H. Frank and L. P. Lounibos (eds.). Phytotelmata: terrestrial plants as hosts for aquatic insect communities. Plexus, Medford, NJ.

Fish, D. and D. W. Hall. 1978. Succession and stratification of aquatic insects inhabiting the insectivorous pitcher plant *Sarracenia purpurea*. Am. Midl. Nat. 99:172–183.

Frank, J. H. and G. A. Curtis. 1977. On the bionomics of bromeliad-inhabiting mosquitoes. IV. Egg mortality of *Wyeomyia vanduzeei* caused by rainfall. Mosq. News 37:239–245.

Frank, J. H. and L. P. Lounibos. 1983. Phytotelmata: terrestrial plants as hosts for aquatic insect communities. Plexus, Medford, NJ.

Hurlbert, S. H. 1969. A coefficient of interspecific association. Ecology 50:1–9.

Istock, C. A., K. Tanner and H. Zimmer. 1983. Habitat selection by the pitcher-plant mosquito, *Wyeomyia smithii*: behavioral and genetic aspects, pp. 191–204. *In:* J. H. Frank and L. P. Lounibos (eds.). Phytotelmata: terrestrial plants as hosts for aquatic insect communities. Plexus, Medford, NJ.

Lane, J. and G. E. Ramalho. 1960. A new neotropical *Culex*. Rev. Bras. Entomol. 9:173–176.

López Lastra, C. C. 1997. Primera cita de *Smittium culisetae* y *S. culicis* (Trichomycetes: Harpellales) en larvas

de mosquitos (Diptera: Culicidae) de la República Argentina. Bol. Soc. Argent. Bot. 33:43–46.

Lounibos, L. P. and C. E. Machado-Allison. 1993. Field test of mosquito ovipositional cues from Venezuelan phytotelmata. Fla. Entomol. 76:593–599.

Lozovei, A. L. and E. Luz. 1976. Diptera culicidae em Curitiba e arredores. I. Ocorrência. Arq. Biol. Tecnol. 19:25–42.

Machado, A. B. M. 1976. Fauna associada a água das folhas de umbelíferas com observações sobre a ninfa de *Roppaneura beckeri* Santos (Odonata—Protoneuridae). Ciênc. Cult. 28:895–896.

Machado-Allison, C. E., D. J. Rodríguez, R. Barrera R. and C. Gómez Cova. 1983. The insect community associated with inflorescences of *Heliconia caribaea* Lamarck in Venezuela, pp. 247–270. *In:* J. H. Frank and L. P. Lounibos (eds.). Phytotelmata: terrestrial plants as hosts for aquatic insect communities. Plexus, Medford, NJ.

Machado-Allison, C. E., R. Barrera, J. H. Frank, L. Delgado and C. Gómez-Cova. 1985. Mosquito communities in Venezuelan phytotelmata, pp. 79–93. *In:* L. P. Lounibos, J. R. Rey and J. H. Frank (eds.). Ecology of mosquitoes: proceedings of a workshop. Florida Medical Entomology Laboratory, Vero Beach, FL.

Maciá, A., J. J. García and R. E. Campos. 1996. Variación estacional de tres especies de *Culex* (Diptera: Culicidae) y sus parásitos en Punta Lara, provincia de Buenos Aires, Argentina. Rev. Biol. Trop. 44/45:267–275.

Micieli, M. V. 1996. Estudios referentes a los ciclos biológicos de microsporidios heterospóricos (Protozoa, Microspora) patógenos de culícidos de la provincia de Buenos Aires. Doctoral thesis. Universidad Nacional de La Plata, La Plata, Argentina.

Pajot, F.-X. 1976. Contribution a l'étude écologique d'*Aedes* (*Stegomyia*) *simpsoni* (Theobald 1905) (Diptera, Culicidae). Observations concernant les stades preimaginaux. Cah. ORSTOM Ser. Entomol. Med. Parasitol. 14:31–48

Ronderos, R. A., J. A. Schnack and A. Maciá. 1992. Composición y variación estacional de una taxocenosis de Culicidae del ecotono subtropical Pampásico (Insecta, Diptera). Graellsia 48:3–8.

Vucetich, M. C. and J. B. Rossi. 1980. Estudio preliminar de la fauna fitotélmica de *Eryngium pandanifolium* Cham. et Schlecht. Limnobios 1:403–409.