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Infestation levels of *Culex (Phytotelmatomyia)* mosquitoes inhabiting water-filled *Eryngium pandanifolium* (Apiaceae) under different sunlight condition

Melania Teresita Junges ^{a,b}, Darío Vezzani ^{b,c} and Aníbal Eduardo Carbajo ^{a,b}

^aLaboratorio de Ecología de Enfermedades Transmitidas por Vectores, Instituto de Investigación e Ingeniería Ambiental, UNSAM, San Martín, Buenos Aires, Argentina; ^bConsejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina; ^cEcosistemas, Facultad de Ciencias Exactas, UNICEN, Tandil, Buenos Aires, Argentina

ABSTRACT

Water accumulations in vegetal structures, known as phytotelmata, serve as aquatic habitats for many insects, including mosquitoes. Among them, *Culex (Phytotelmatomyia)* is composed by a few species that are highly specialized and poorly known. The objective of this study was to evaluate the association between sunlight conditions and the abundance of immature stages of *Culex (Phytotelmatomyia)* species in the axils of *Eryngium pandanifolium*. A forest patch with a dense population of *E. pandanifolium* in the Lower Delta of the Paraná River (Entre Ríos province, Argentina) was sampled twice a month between December and May. A total of 880 plants were inspected in 220 sample zones defined by a combination of location within the patch and sunlight condition. All individuals collected ($n = 521$) belonged to *Culex (Phytotelmatomyia) hepperi* (56%), *Culex (Phytotelmatomyia) castroi* (40%), and *Culex (Phytotelmatomyia) renatoi* (4%). According to generalized linear models, the habitat availability (i.e. water presence) was positively associated with sun exposure, but not with the location within the patch. Mosquito presence and abundance were positively related only to water volume. Coexistence of *C. hepperi* and *C. castroi* was more frequent than would be expected by chance. Further studies to understand the effects of sunlight and plant architecture on water dynamics are needed.

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Introduction

Immature stages of mosquitoes (Diptera: Culicidae) are found in a wide diversity of aquatic habitats, including water collections in natural and artificial containers (Clements 1992; Service 1993). Natural containers formed by vegetal structures are known as phytotelmata; treeholes and leaf axils being the most common types (Greeney 2001). These habitats are found all around the world, but are more abundant and diverse in tropical areas, where the vegetal richness and abundant rainfall are favourable for their formation (Kitching 2001). Because of their size, limited structure and hierarchical spatial

arrangement these microhabitats allow simple and fast field tests (Srivastava et al. 2004; Srivastava 2005). Their use as a study model is more realistic than laboratory or semi-field experiments because of their natural origin and the fact that their inhabitants are exposed to the same environmental variation (Drake and Kramer 2011).

Phytotelmata dwellers generally show different levels of host-plant fidelity. Some are found in a wide variety of habitats, whereas others develop a strong association with a particular plant species or structure (Machado-Allison et al. 1986; Campos and Lounibos 1999; Greeney 2001; Delgado and Machado-Allison 2006; Campos 2010; Albicocco et al. 2011). This pattern is particularly strong in mosquito species that breed in leaf axils of plants, such as *Eryngium* L. (Dicotyledoneae: Apiaceae). This cosmopolitan genus represents the dominant native plants forming phytotelmata in Argentina (Campos 2010). In the temperate region of the country, four *Culex* spp. showed a strong association with different *Eryngium* spp. (Campos and Lounibos 1999; Campos 2010; Albicocco et al. 2011; Campos et al. 2011). These mosquitoes were recently separated in a monophyletic subgenus called *Phytotelmatomyia* based on morphological structures (Rossi and Harbach 2008).

Study of the factors associated with the presence and abundance of species contributes to understanding their geographic distribution. Among those factors, it is known that the habitat size determines community composition. Particularly for phytotelmata, several studies found a positive correlation between water volume and the abundance and richness of insects in bamboos (Campos 2013), bromeliads (Armbruster et al., 2002; Jabiol et al. 2009; Torreias et al. 2010) and treeholes (Yanoviak et al. 2006). In addition, the water volume in containers is affected by the sunlight exposure. The effects of sun exposure on the water level and mosquito assemblages were previously assessed in artificial containers (e.g. Vezzani et al. 2005; Vezzani and Albicocco 2009) and some phytotelmata, especially bromeliads (Frank and O'Meara 1985). In the latter, sunlight condition affects water temperature, plant morphology and community productivity. Plants exposed to the sun have higher water volume but less detritus (Cavallero et al. 2009; Montero et al. 2010). As a consequence, more mosquito immatures were found in shaded bromeliads due to lower temperature and higher detritus content (Lopez and Rios 2001; Brouard et al. 2012).

Even though bromeliads have been well studied, knowledge of the ecology of *Eryngium* dwellers is limited. The objective of the study was to assess the effect of sunlight condition on habitat availability and on the presence and abundance of immature stages of *Culex* (*Phytotelmatomyia*) species in water-filled *Eryngium pandanifolium* Cham. and Schlecht.

Materials and methods

The Lower Delta is the terminal area of the Paraná Delta Ecoregion in Argentina (Figure 1). The mean annual precipitation and temperature are 1000 mm and 16–18°C, respectively. The zone is characterized by the presence of sandy ridges, with a height difference < 0.5 m. Vegetation is dominated by low grasses, with small forest patches of *Acacia caven* (Molina) Molina (Fabaceae) or *Erythrina crista-galli* L. (Fabaceae) and *Celtis tala* Gillies ex Planch. (Ulmaceae). The moisture gradient allows the appearance of wet grassland in some areas and permanent water in the more depressed areas (Kandus et al. 2006).

The study was performed near the locality of Ceibas (Entre Ríos province), in an elliptically shaped forest patch of 5 ha (500 m long and 50–80 m wide) located over a



Figure 1. Study area and *Eryngium pandanifolium* specimens.

ridge with northeast–southwest direction (33.5°S, 58.7°W). Its vegetation was dominated by *Erythrina crista-galli* accompanied by *Celtis tala*, and characterized by an abundant population of *Eryngium pandanifolium* (Figure 1).

The forest was visited twice a month between December 2011 and March 2012, and once in April and May 2012. Sampling effort remained constant per visit by fixing the time spent inside the forest. Access points were randomly selected, measured as a distance to the northeast end of the patch. From each access point, four sample zones were identified, representing the combination of location (border/centre) and sunlight condition (sunlit/shaded). Location was considered to discard possible edge effects, including wind and other variables not included in this study. In concordance with forest width, sample zones < 5 m from the edge were considered as ‘border’ and those between 10 and 30 m as ‘centre’. Sunlight condition was categorized as ‘shaded’ if it did not receive direct sunlight due to the canopy, and ‘sunlit’ for exposed zones.

In each sample zone, the fluid content of four plants was extracted and measured volumetrically. A non-destructive method was used for the extraction, using pipettes or plastic tubes for low and high volumes, respectively (Silver 2008). The water was returned at least twice to collect all specimens. Although this method can be inefficient for studying the entire community, it has been proved to be effective for mosquito larvae from phytotelmata (Derraik 2009; Jocque et al. 2010). To achieve independent sampling, sampled plants were different on consecutive dates. To reduce the potential effect of plant size, only those with leaves between 1.2 and 1.5 m long were included. All immature mosquitos from each plant were fixed in 70% ethanol. In the laboratory, third- and fourth-instar larvae were identified with dichotomous keys and original descriptions (Lane and Ramalho 1960; Casal and Garcia 1967a, 1967b; Rossi and Harbach 2008).

Habitat availability per sample zone was estimated as presence (1) or absence (0) of water in any of the four *E. pandanifolium* inspected, and also as the proportion of plants with water within each zone. The presence of immatures per sample zone was characterized for any and for each mosquito species identified. The abundance of mosquitoes per sample zone was indicated with the proportion of plants with water harbouring immatures, and with the number of third- and fourth-instar larvae of any and of each mosquito species found per plant.

All data were analysed with generalized linear models, which allow for non-normal errors and count or proportional data (Crawley 2007). Water availability and volume models included sunlight condition and location as explanatory variables. For immature presence and abundance, the logarithm of water volume was also included as an explanatory variable. Binomial error was considered for habitat availability, quasi-binomial for the proportion of plants per sample zone with water, normal for logarithm of water volume and quasi-Poisson for immature abundance. The link function of the models was logit for habitat, identity for volume and log for abundance. Significance was evaluated using a *t*-test on the parameter/standard deviation with residuals degrees of freedom. To estimate the final parameters the models were adjusted without the non-significant terms and bootstrapped (1000 replications). Coexistence between the most common species was evaluated with a frequency analysis and a χ^2 test. Analyses were performed using the open-source software R 3.1.2 (R Core Team 2005) and Infostat (InfoStat group 2014).

Results

Habitat availability and water volume

A total of 220 sample zones (83.6% with at least one water-filled *E. pandanifolium*) were visited and 880 plants (47.8% with water) were inspected. Habitat availability, both presence and proportion of plants with water, was related to sunlight condition but not to location within the forest patch (Table 1). More water-filled plants were found in sunlit zones, regardless of whether the zone was on the edge or in the centre of the forest. At least one water-filled plant was found in 90.8% of the sunlit zones, and in 76.7% of the shaded ones. Also, the mean proportion of plants with water per sample zone differed between sunlight conditions (sunlit: 0.525 ± 0.316 ; shaded: 0.432 ± 0.321).

Table 1. Generalized linear models for habitat availability index and water volume per site.

Initial models	HAI _{p-a} ^a		HAI _{pro} ^a		Log(Vol) ^b	
	Parameter	T	Parameter	t	Parameter	T
Ordinate	1.750	8.62	-0.086	-0.99	2.534	43.60
Sunlight condition	-0.563	-2.77*	-0.205	-2.35*	-0.034	-0.58
Location	0.007	0.36	0.051	0.59	0.037	0.60
Sunlight condition × location	-0.161	-0.79	-0.011	-0.14	-0.018	-0.31
Chosen models	Parameter	SE	Parameter	SE	Parameter	SE
Ordinate	1.771	0.205	-0.085	0.086	NC	NC
Sunlight condition	-0.579	0.207	-0.208	0.086	NC	NC

^a HAI = Habitat availability index ($n = 220$); HAI_{p-a}: presence/absence of water; HAI_{pro}: proportion of plants with water.

^b Vol = Water volume (153 df).

* Significant term ($p < 0.05$).

The chosen models show the bootstrapped parameters with the significant terms only.

Water volume was not significantly related to the studied variables (Table 1). Plants accumulated between 5 and 270 ml of water (median = 10 ml; Q₁ = 5 ml; Q₃ = 25 ml).

Immature presence and abundance

A total of 521 third- and fourth-instar larvae of mosquitoes were collected, all belonging to *Culex* (*Phytotelmatomyia*). Immature mosquitoes were found in 36.4% (67/184) of the sample zones with water. Three species were identified as follow: *Culex* (*Phytotelmatomyia*) *hepperi* Casal and García (56% of the specimens), *Culex* (*Phytotelmatomyia*) *castroi* Casal and García (40%), and *Culex* (*Phytotelmatomyia*) *renatoi* Lane and Ramalho (4%). Water volume was the only variable related to the presence of the subgender *Culex* (*Phytotelmatomyia*) and of each species (Table 2). The model described a direct relation of water volume with the probability of finding an immature mosquito, and with the proportion of plants with mosquitoes. The presence of mosquito immatures did not differ by plant location or sunlight condition. Water volume was also related positively with immature abundance (Table 3). Mosquito abundance varied between 1 and 45 specimens per plant (median = 4; Q₁ = 1; Q₃ = 8).

Table 2. Generalized linear models for mosquito presence and proportion of *Culex* spp. in breeding plants.

Initial models	<i>Culex</i> spp. ^a		<i>Cx. hepperi</i> ^a		<i>Cx. castroi</i> ^a		Cx _{pro} ^b	
	Parameter	t	Parameter	t	Parameter	t	Parameter	t
Ordinate	-2.147	-6.14	-2.856	-6.94	-2.691	-7.08	-2.082	-9.60
Location	-0.060	-0.31	-0.350	-1.46	0.019	0.08	-0.085	-0.62
Sunlight condition	-0.183	-0.95	-0.242	-1.03	0.031	0.13	-0.168	-1.23
Mean volume	0.084	4.65*	0.072	4.40*	0.044	3.39*	0.037	4.88*
Location × Sunlight condition	-0.016	-0.08	-0.131	-0.55	0.152	0.64	-0.107	-0.79
Chosen model	Parameter	SE	Parameter	SE	Parameter	SE	Parameter	SE
Ordinate	-2.178	0.341	-2.821	0.412	-2.709	0.417	-2.419	0.287
slope(Mean volume)	0.087	0.019	0.072	0.017	0.048	0.019	0.047	0.013

^a n = 160.

^b Cx_{pro} = proportions of plants with water breeding *Culex* spp (159 df).

* Significant term (p < 0.05).

The chosen models show the bootstrapped parameters with the significant terms only.

Table 3. Generalized linear models for number of immatures per site.

Initial models	<i>Culex</i> spp. ^a		<i>Culex hepperi</i> ^a		<i>Culex castroi</i> ^a	
	Parameter	T	Parameter	t	Parameter	t
Ordinate	0.473	1.90	-0.017	-0.05	-0.541	-1.76
Location	0.168	0.91	0.005	0.02	0.367	1.63
Sunlight condition	-0.130	-0.71	-0.196	-0.77	0.025	0.11
Log(Vol)	0.008	4.70*	0.007	2.91*	0.009	4.25*
Location × Sunlight condition	-0.034	-0.18	-0.037	-0.14	-0.023	-0.10
Chosen model	Parameter	SE	Parameter	SE	Parameter	SE
Ordinate	0.357	0.297	-0.057	0.381	-0.705	0.346
slope(Log(Vol))	0.029	0.009	0.024	0.009	0.034	0.011

^a n = 160.

* Significant term (p < 0.05).

The chosen models show the bootstrapped parameters with the significant terms only. All double and triple interactions were tested.

Culex hepperi was found in 21% of the sample zones with water, followed by *C. castroi* (19%). Of all breeding habitats, 23 contained only *C. castroi* and 30 contained only *C. hepperi*. Immatures of both species coexisted in 11 out of 364 water-filled plants, being more than expected by chance ($\chi^2 = 14.67$; $df = 1$; $p < 0.001$). *Culex renatoi* was only collected during December in 2.7% of the sample zones with water; low values precluded statistical analysis.

Discussion

Culex mosquitoes of the subgenus *Phytotelmatomyia* embrace a small group of poorly known phytotelmata dwellers, with four valid species and two others under study (Rossi and Harbach 2008). In the present research, the infestation levels and some characteristics of the aquatic habitat of three of these species in temperate Argentina were exposed.

Eryngium pandanifolium, a plant in which these mosquitoes develop during pre-imaginal states, retained water more frequently in sunlit than in shaded plants. This result was not expected because of sunlight's influence over evaporation levels (Kitching 2001). However, other authors observed that sun exposure may affect plant architecture and biomass allocation. In bromeliads, the number of leaves and the sheath/leaf area were affected by sun exposure, influencing water dynamics, with higher water content under sunlit conditions (Cavallero et al. 2009; Montero et al. 2010). For *Eryngium* spp. it was observed that number of leaves was positively correlated with water volume (Campos 2010), but there are no studies dealing with insolation effects on plant architecture.

On the other hand, water volume was not related to sunlight condition in the study area. Both variables, water presence and water volume, are affected by the relationship between precipitation and evaporation, and therefore by plant architecture, which is probably modelled by sunlight condition. An effect of structure over water dynamics was previously described for other phytotelmata. The overlapping structure of *Scirpus giganteus*, which also accumulates water in leaf axils, delays evaporation, explaining the absence of a relation between water presence and precipitation levels (Marti et al. 2007). Another example is the banana leaf, for which the close structure of the axil allows water retention during dry periods (Lang and Ramos 1981). Hence, our findings might be mainly related to differences in plant structure between sun and shade and future works should be focused in that direction.

Eryngium axils retained low water volume (median 10 ml), as was previously described for the genus (Campos 2010; Albicocco et al. 2011). Small habitats are associated with unpredictable changes, so it is more likely to find specialist dwellers. In plants of this genus, only species of *Culex* (*Phytotelmatomyia*) were previously found, indicating a strong association between these plants and mosquitoes (Campos and Lounibos 1999; Albicocco et al. 2011). The three mosquito species recorded here were previously described inhabiting *Eryngium* in temperate Argentina, but with different relative abundances (Campos and Lounibos 1999; Campos 2010; Albicocco et al. 2011). For example, *C. hepperi* was the most frequent in Entre Ríos province but absent in the south of Buenos Aires province (Campos 2010). In contrast, *C. renatoi* was dominant in Buenos Aires (Campos 2010) and less frequent in the present study

in Entre Ríos. These differences in species composition and abundances could indicate a latitudinal and climatic influence over mosquito bionomics and/or phytotelmata characteristics.

Water volume explained *Culex* presence and immature abundance. The relation between habitat availability and community structure was described for other phytotelmata, such as bamboo (Campos 2013), bromeliads (Armbruster et al., 2002; Jabiol et al. 2009; Torreias et al. 2010) and treeholes (Yanoviak et al. 2006). However, this was not previously observed in *Eryngium* spp. and *S. giganteus*, probably due to flood influence and heavy rainfall wash (Campos and Lounibos 1999; Marti et al. 2007). This is not the case in the forest studied here, in which flooding has very low frequency and the upper stratum could reduce rain wash effects.

The two other variables assessed, *Eryngium* location and sunlight condition, seem to not affect *Culex* presence and abundance. For bromeliads, more *Culex* immatures were found in shaded habitats when there was a difference in water temperature (Lopez and Rios 2001). However, inside a forest, where plants were not fully exposed to sun and no water temperature difference was detected, species richness was similar in shaded and sunlit plants, despite the differences of litter mass observed (Montero et al. 2010; Brouard et al. 2012). Differences registered between *Eryngium* and bromeliads could be explained once again by plant structure.

Culex hepperi and *C. castroi* coexisted more than would be expected by chance. A positive association was described for species dwellers of these phytotelmata in other temperate wetlands (Campos and Lounibos 1999; Campos 2010; Albicocco et al. 2011), and for others habitats, such as banana leaf axils (Lang and Ramos 1981). Although it cannot be concluded directly from this study, this pattern would suggest that, in the search for oviposition habitats, some water characteristics (e.g. pH, nutrients, temperature) are more important than interspecific competition.

In brief, presence of water in the axils of *E. pandanifolium* seems to be highly influenced by sunlight condition, and the presence and abundance of *Culex* (*Phytotelmatomyia*) spp. were associated only with water volume. It is necessary to include the study of *Eryngium* structure under different sunlight conditions to achieve a more comprehensive approach.

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Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Melania Teresita Junges  <http://orcid.org/0000-0002-6435-5033>

Darío Vezzani  <http://orcid.org/0000-0002-6124-4418>

Anibal Eduardo Carbajo  <http://orcid.org/0000-0002-8792-7276>

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