Population dynamics between the mermithid *Strelkovimermis spiculatus* (Nematoda: Mermithidae) and the floodwater mosquito *Ochlerotatus albifasciatus* (Diptera: Culicidae) over time

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HIGHLIGHTS

► We conducted a study of population dynamics of *Strelkovimermis spiculatus* on *Ochlerotatus albifasciatus*.
► Parasitism by *S. spiculatus* occurred throughout *O. albifasciatus* populations developing during all seasons.
► Differing patterns in the infection by the nematode depended on the larval mosquito habitat.
► Flooded area correlated with *S. spiculatus* infection.
► Mosquito regulation by this parasite involved parasitism levels and infections occurring over time.

GRAPHICAL ABSTRACT
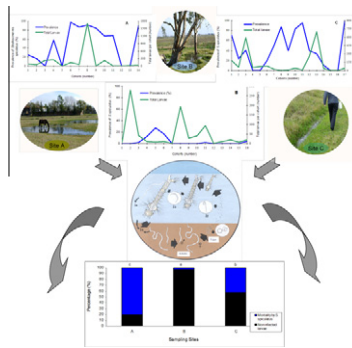
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ABSTRACT

We investigated the population dynamics of *Strelkovimermis spiculatus* in *Ochlerotatus albifasciatus* to determine the abiotic and biotic parameters involved in this host-parasite system. Weekly samplings performed from May 2007 through May 2010 in three ponds located in open areas of La Plata, Argentina revealed that parasitism by *S. spiculatus* in *O. albifasciatus* occurred throughout all seasons with differences in effectiveness among breeding habitats: A mosquito-larva reduction of 80.7% was observed in a habitat where 100% of the generations were infected by nematodes; an intermediate situation (a 41.9% reduction) involved a lower percentage of parasitism above 50% although nematode infection was likewise registered in 92.9% of the generations. Finally, a mosquito-larva reduction of 2.68% was recorded where only 46.0% of the generations were parasitized and at levels of nematode infection below 50%. We analyzed the effects of abiotic and biotic parameters on *S. spiculatus* infections. The flooded-surface area was the only variable significantly associated with percent infection. Infection prevalence correlated positively with the mean number of emerged nematodes per larva and with the *S. spiculatus* male-to-female ratio in each parasitized-mosquito generation. In conclusion, the frequency over time and levels of infection are key parameters in the regulation of mosquito populations by this mermithid.

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1. Introduction

The mermithids are a large and consequential group of nematodes that are obligate parasites of arthropods, principally insects. Mermithids occur in mosquitoes throughout much of the world, and their parasitism has been reported in at least 100 host species (Petersen and Chapman, 1979; Poinar, 1979; Becnel and Johnson, 1988). The use of mermithids for biological control involves several advantageous attributes including environmental safety, host specificity, ease of application, lethality, and the potential for a long-term recycling in the environment (Petersen, 1985; Federici, 1995; Kerry and Hominick, 2002). These nematodes have nevertheless been indicated as not constituting an ideal biological control agent because of the need for mass rearing *in vivo* and their inherent population dynamics (Federici, 1995; Kerry and Hominick, 2002). Because of the industrial infrastructure and economies of developing countries, the mass rearing of mermithids in those countries, however, would be a viable mechanism for obtaining a large-scale multiplication at a relatively low cost when compared to chemical pesticides. Examples of such systems of production that have been developed are those of *Romanomermis culicivorax* and *R. iyengari* since 1988 in Cuba (Ferraz et al., 2008) and of *R. iyengari* since that same year in Brazil (Santamarina Mijares and Bellini, 2000).

The mermithid *Strelkovimermis spiculatus* Poinar and Camino, 1986 was discovered upon its infection of larvae of the floodwater mosquito *Ochlerotatus* (*Ochlerotatus*) *albifasciatus* (Macquart) in Argentina (Poinar and Camino, 1986). *Ochlerotatus albifasciatus* is a multivoltine floodwater mosquito whose immature stages develop in shallow temporary ground pools. This mosquito species can survive dry periods (for up to 6 months) as dormant eggs that hatch when pools are flooded, and as many as eight broods a year may be produced during the rainy periods (Ludueña Almeida and Gorla, 1995). *Ochlerotatus albifasciatus* becomes a significant pest during the peaks of adult abundance that occur principally in the spring and fall (Maciá et al., 1995; Fontanarrosa et al., 2000; García and Micieli, 2000) and was also identified in 1982 as a vector of western equine encephalitis in this country (Mitchell et al., 1987). Studies on mosquito populations naturally infected by *S. spiculatus* have indicated the occurrence of parasitism in nature in five mosquito species but with epizootic infections in only *Oc. albifasciatus* (Campos et al., 1993; García et al., 1994; Maciá et al., 1995). An interesting aspect of the biology of this mermithid species is that these significant epizootics can result in prevalence and mortality rates exceeding 50% and often higher than 80% (Maciá et al., 1995; Micieli and García, 1999). Although some longitudinal studies on population naturally infected by mosquitoes are available (Micieli and García, 1999; Maciá et al., 1995), an improved understanding of the interaction among abiotic influences and the biology of the host and parasite should facilitate the development of mermithids for the biological control of mosquitoes (Platzer, 2007). Data on the dynamics of mermithid and mosquito populations over time would also be useful in deciding which biological-control strategy is likely to work using this group of mosquito antagonists.

We conducted a study of the population dynamics of *S. spiculatus* in *Oc. albifasciatus* over a period of 3 years to determine the abiotic influences and biological parameters involved in both the host and the nematode populations of this host-parasite system in order to discover the conditions maximizing nematode infections.

2. Materials and methods

2.1. Study sites

Three characterisitic grassy pool habitats of immature *Oc. albifasciatus* were selected at random for this study. These sites

became flooded exclusively with local rainfall. The ponds were located in open areas within the Pampean Biogeographic Province (30–39°S) of the Neotropical Region, Buenos Aires province, Argentina. The climate is temperate (annual average temperature from 13 to 17 °C) with rainfall occurring during the entire year (Cabrera and Willink, 1980). Site A (34° 49′ 19.9″ S; 57° 58′ 07.5″ W), located in the coastal suburb of Punta Lara, was semipermanent, with a high variation in the water level throughout the year. The site consisted in an open field close to the Rio de la Plata River. Site B (34° 52′ 11.8″ S; 57° 57′ 28.9″ W) was also located in Punta Lara at approximately 5 km from Site A. This breeding pond was shaded and ephemeral, was likewise filled by rainwater, and lasted for from several days to a few weeks. Site C (34° 59′ 16.1″ S; 57° 59′ 43″ W) was located in the suburb of Los Hornos at 7 km from the La Plata city center. This site was a drainage ditch where water pooled as runoff from an adjacent field after its flooding by rain.

2.2. Collection and processing of samples

2.2.1. Mosquito-population data

Weekly observations were conducted during the three years extending from May 2007 through May 2010. In each pool, 10 samples, considered as a sample unit, were collected within the period between floodings until water was again detected. Each sample was taken with a standard dipper of 300-ml volume. Immature-stage mosquitoes were transported to the laboratory, where the field-collected larvae were identified according to the instar stage and transferred to plastic pans containing dechlorinated water—generally, within 2 h after samples had been removed from the ponds. The number of larvae of each instar stage and pupae in each dip sample was recorded. The sampling dates were grouped according to those where immature *Oc. albifasciatus* were found in development and those without immature mosquitoes. The number of mosquito generations that developed in each breeding site throughout the 3-year study was recorded.

2.2.2. Parasitism by S. spiculatus

The weekly design of sampling to obtain data of nematode prevalence from each mosquito generation in this study would be inacceptable since the variation in the development time of *Oc. albifasciatus* from being extremely short, especially in the summer, to quite long, in the winter, would create a bias in the prevalence data. Therefore, in order to be precise with the value of parasitism (expressed as a percent); each time the early instar stages of *Oc. albifasciatus* were detected in the field, they were followed up to when the late-third– or early-fourth–instar stage began developing in the pool. The percentage of parasitism (the fraction of parasitized hosts out of the total number examined $\times$ 100), the number of emerged nematodes per larva, and the sex ratio of mermithids were determined from each pool and from each mosquito generation by placing between 24 and 48 late-third– or early-fourth–instar larvae in the wells of spot plates and counting the number of postparasitic stages that emerged under the stereoscopic microscope. The number of mosquito larvae examined depended on the availability of immature mosquitoes in the samples. To determine the sex ratio of the mermithids, the individuals were maintained in the plates at 27 ± 1 °C within a 12:12-h (light:dark) photoperiod in an incubator up to sexual maturation before observation by phase-contrast microscopy to differentiate males from females.

The pupae from the mosquito generations were transferred to plastic containers until they emerged as adult mosquitoes. After sex determination the mosquito adults were dissected to obtain a prevalence of infection at this stage.

2.2.3. Measurements of abiotic data

The flooding level on any sampling date was quantified in the field with a measuring tape so that a flooded-surface area could be calculated for each date. The dry intervals after each of the flooded periods were also monitored regularly. Abiotic parameters, such as pH and water temperature, were registered in situ on each sampling date at all three breeding ponds in 2009 and 2010 by means of a portable pH-conductivity-temperature meter. Conductivity was registered in quadruplicate at random in each pool. Daily maximum and minimum air temperatures along with the rainfall were recorded at an atmospheric weather station situated between about 1 km (Site C) and 10 km (sites A and B) from the pools.

2.2.4. Statistical analyses

Parasitism by S. spiculatus in mosquito populations at three breeding sites.

Levels of infection by S. spiculatus in Oc. albifasciatus populations among the three breeding sites were compared through the use of restricted-maximum-likelihood models with the "gls" function of the R version 2.13.2. The data were transformed to the square-root of the arc sine of the values of the proportion infected. Because each site was observed many times throughout the entire three-year period, we included a first-order autoregressive variance-covariance structure for errors. The Akaike and Swartz's Information Criterion were used to identify the most appropriate covariance structure. A comparison between sites was performed by LSD-Fisher test.

Pearson's correlation analysis was conducted to determine for each mosquito generation the relationship between the number of emerged nematodes per larva, the number of nematode males vs. females (sex ratio), and the prevalence of infection.

We considered the prevalence of infection as equivalent to mortality on the assumption that each infected larva would eventually die from the nematode infection at the fourth-instar stage (Petersen, 1973; Campos and Sy, 2003). The number of dead individuals of a given generation was estimated on the basis of the observed prevalence of infection (from a sample ranging from 24 to 48 larvae) and extrapolated to the entire generation (e.g., the total number of fourth-instar mosquito larvae per sampling unit). Accordingly, the total number of dead immature stages per larval habitat was calculated from the mortality per generation over the entire study period. A contingency table was constructed to compare the number of mosquito-larva dead through nematode infection between sites.

2.3. Effects of abiotic and biotic parameters on the levels of infection

The effects of these parameters on the levels of S. spiculatus infection were analyzed and the data grouped according to mosquito generation. Thus, the mean value of each variable was calculated considering both the periods with and without immature stages of Oc. albifasciatus before performing the statistical analysis. Generations of mosquitoes of incomplete development (i.e., short of the fourth-instar stage) as a result of the drying-out of their habitat were not included in the analysis. Correlations were investigated through the use of Pearson's coefficient (R) and simple regression. A significant difference was considered to be at the level of p ⩽ 0.05. Accordingly, the independent variables examined were:

(1) The abiotic parameters were: the maximum and minimum air temperatures for each period (° C), the rainfall for each period (mm), the cumulative precipitation for the days between the sampling dates (mm), the lengths of the dry periods (days), the area of the flooded surface (m2), the pH, and the water temperature (°C).

(2) The biotic parameters were: the mean number of larvae and pupae per sampling unit from each generation, the development time of Oc. albifasciatus larvae per generation, and the percentage of nematode females in the previous generation (i.e., from the female-to-male sex ratio).

The statistical analyses described above were done after an arc-sine (X/100) transformation of the percentage-infection values and a $\sqrt{(x+1)}$ transformation of both the number of emerged nematodes per host and the mean ratio of nematode males to females. Statistical analyses were conducted using InfoStat version 2010 (Di Rienzo et al., 2010).

3. Results

3.1. Parasitism by S. spiculatus in mosquito populations at three breeding sites

The three ponds were shallow depressions in the ground, ranging in area from 150 to 1200 m^2 (surface measurements), with maximum depths of about 0.35 m., that were filled by rainfall and remained with water for periods ranging from several days to up to weeks.

Site A reached a maximum area of 1200 m^2. Flooding by rain occurred 14 times during the three years of study with a generation of immature Oc. albifasciatus being detected in each of those rainfalls. During 4 of those times, however, the site became dried up before the larvae could reach the fourth-instar stage (Fig. 1). Other mosquito species were also registered during the study period. Psorophora cyanescens (Coquillett), Psorophora ciliata (Fabricius), Culex dolosus (Lynch Arribalzaga), and Uranotaenia sp. were sampled and infection by S. spiculatus was found in Cx. dolosus and Uranotaenia sp. only on one occasion each during the spring and fall, respectively. In contrast, S. spiculatus was found to have infected a majority of the immature Oc. albifasciatus mosquitoes, where 100% (n = 10) of the generations that had reached the fourth-instar stage were parasitized, 8 with prevalences ranging from 58.0% to 97.7% and 2 with values at 25.0% and 16.6%, to give an average infection rate of 68.6%. Five Oc. albifasciatus generations were detected during the four seasons of the first year, with S. spiculatus infections occurring at prevalences of 25% in winter, 16.6% in spring, and 58% in summer. In the second year, only 2 infected mosquito generations, both by S. spiculatus, were registered in the winter (97.7%) and the spring (87.0%). In the final year, 7 infected generations were detected in all seasons, with most of the infections occurring at levels higher than 50% (Table 1).

Site B attained a maximum area of 150 m2. This site became flooded by rain 16 times, with 16 Oc. albifasciatus generations resulting during those events (Fig. 1). On three occasions, however, the site dried up before the larvae could reach the fourth-instar stage. The nematode S. spiculatus was found infecting 46% (n = 13) of these mosquito generations, always at parasitism levels ranging from 2.0% to 28%, at an average infection rate of 5.0% (n = 13). No infection by S. spiculatus was registered in other mosquito species such as Ps. cyanescens, Ps. ciliata, Cx. dolosus, and Uranotaenia sp. breeding in this site. During the first year, 4 mosquito generations of Oc. albifasciatus were sampled, but nematode infection was registered in only 2 of them, at an S. spiculatus prevalence of 2.0% in spring and 13% in summer. During the second year, infections by S. spiculatus were recorded in winter (28%) and in spring (17%) from the 3 generations developing in this pool, while during the third year, the infection by this nematode was registered in 2 mosquito generations, in spring (2.0%) and fall (4.0%), out of 9 generations sampled during this time (Table 1).

Site C attained a maximum area of approximately 60 m2. In almost every flooding cycle (i.e., some 19 times) a generation of
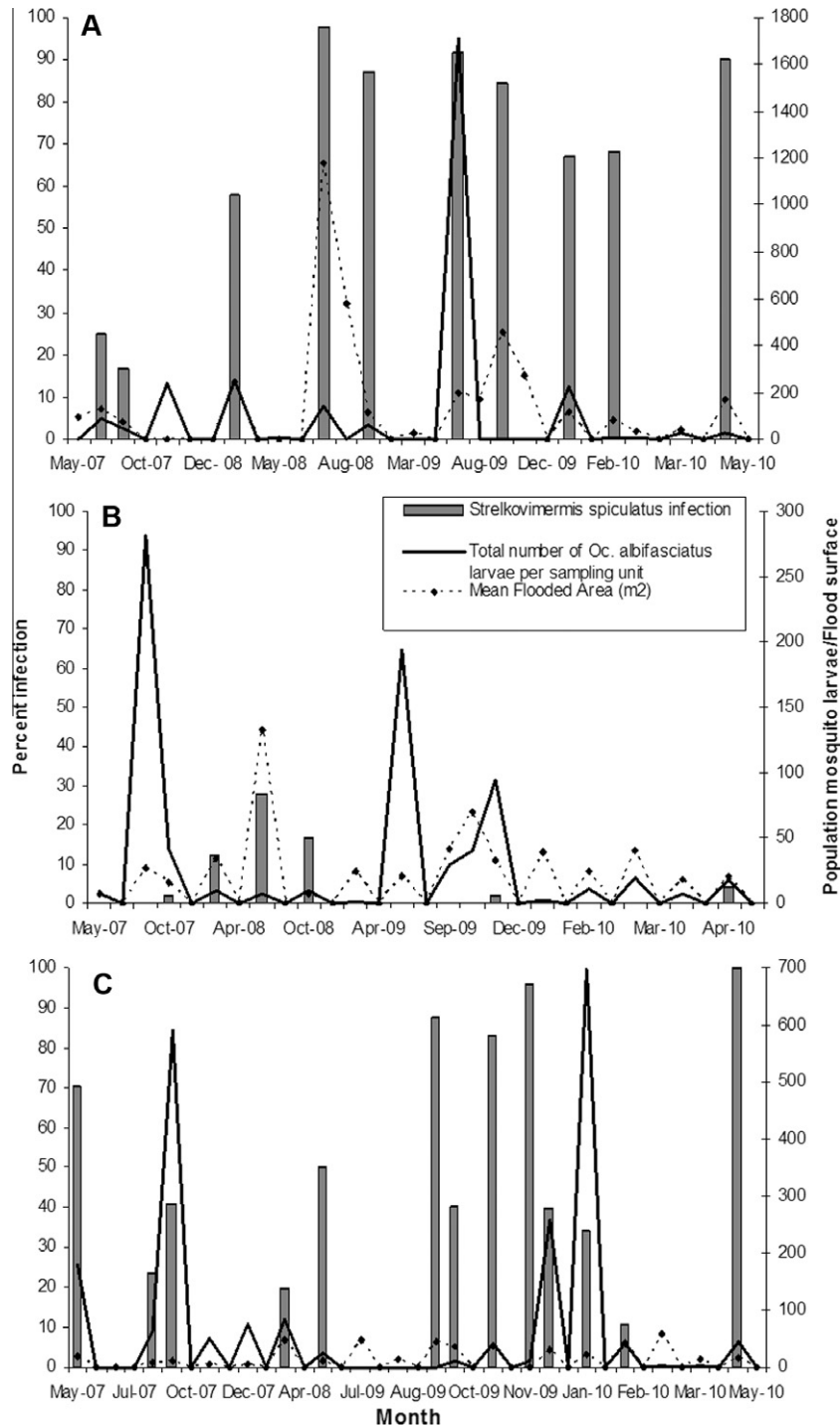
Fig. 1. Total number of *Ochlerotatus albifasciatus* larvae and the percentage of parasitism by *Strelkovimermis spiculatus* related to mean flooded area at the three sampling sites over time.

immature stages of *Oc. albifasciatus* was recorded with the only two exceptions occurring in July and August of 2009. On three occasions, however, during the spring and fall, the site dried up before the larvae could reach the fourth-instar stage (Fig. 1). The nematode was found in 93% ($n = 14$) of these generations, with prevalences ranging from 50% to 100% in 6 generations and from 0% to 41% in 8 (Fig. 1) to give an average value for infection of 50% ($n = 14$). During the first year, 6 generations of *Oc. albifasciatus* were detected with *S. spiculatus* infections at prevalences of 70% in the fall, 24% in the winter, 41% in the spring, and 20% in the

summer. In the second year, 2 mosquito generations were recorded, and both were infected by the parasite in winter time (at prevalences of 50% and 88%). During the third year, 9 mosquito generations were reported with infections in spring, summer, and fall at prevalences ranging from 11% to 100% (Table 1). *Ps. ciliata*, *Cx dolosus*, and *Anopheles albitarsis* (Lynch Arribalzaga) were also collected and scrutinized, although infection was found in only *Cx. dolosus* on two occasions, one in the fall and one in the spring.

Since in our study *S. spiculatus* was found at different abundances in the *Oc. albifasciatus* generations, we cannot consider

Table 1
Strelkovimermis spiculatus parasitism in Ochlerotatus albifasciatus populations during the 3-year study period at three larval habitats. Data for prevalence of infection, number of emerged nematodes per larva, and sex ratio in each mosquito population.

Larval habitat	Ochlerotatus albifasciatus populations	Prevalence of infection (%)	Emerged nematode per host (number)	Ratio ♂:♀	Larval habitat	Ochlerotatus albifasciatus populations	Prevalence of infection (%)	Emerged nematode per host (number)	Ratio ♂:♀	Larval habitat	Ochlerotatus albifasciatus populations	Prevalence of infection (%)	Emerged nematode per host (number)	Ratio ♂:♀
A	1[a] (W)[b] 26[c]	25.00	1	0:1	B	1 (F) 0	0.00	0	0:0	C	1 (F) 19	70.20	1.60	3:1
	2 (Sp) 10	16.60	1	0:1		2 (W) 16	0.00	0	0:0		2 (W) 16	23.50	1	0:1
	3 (Sp) 0	0.00	0	0:0		3 (Sp) 9	2.00	1	1:0		3 (Sp) 9	40.90	2.10	1:1
	4 (S) 4	58.00	1	0:1		4 (S) 4	12.50	1	1:0		4 (Sp) 0	0.00	0	0:0
	5 (F) 0	0.00	0	0:0		5 (W) 17	27.70	3	7:1		5 (Sp) 0	0.00	0	0:0
	6 (W) 17	97.70	2.40	1.38:1		6 (Sp) 8	17.00	1	0:1		6 (S) 4	19.70	1.16	1:6
	7 (Sp) 14	87.00	2.80	1.6:1		7 (S) 7	0.00	0	0:0		7 (W) 17	50.00	1.5	1:2
	8 (W) 24	91.60	3	1.5:1		8 (W) 0	0.00	0	0:0		8 (W) 14	87.50	2.85	2.5:1
	9 (Sp) 10	84.60	2.45	1.8:1		9 (Sp) 10	0.00	0	0:0		9 (Sp) 11	40.00	1.25	1:1
	10 (S) 6	67.10	1.84	2.45:1		10 (Sp) 8	0.00	0	0:0		10 (Sp) 11	83.30	1.58	3:1
	11 (S) 5	68.18	3.80	1.2:1		11 (Sp) 8	2.00	1	0:0		11 (Sp) 8	95.80	2.30	2.3:1
	12 (S) 0	0.00	0	0:0		12 (S) 6	0.00	0	0:0		12 (Sp) 9	39.58	1.78	6.6:1
	13 (F) 0	0.00	0	0:0		13 (S) 5	0.00	0	0:0		13 (S) 6	34.00	1.25	2.3:1
	14 (F) 9	90.00	3	6.3:1		14 (S) 4	0.00	0	0:0		14 (S) 6	11.00	1	1:7
						15 (F) 0	0.00	0	0:0		15 (S) 4	0.00	0	0:0
						16 (F) 9	4.00	1	1:0		16 (F) 0	0.00	0	0:0
											17 (F) 9	100.00	2.90	1:0

[a] Number of mosquito population.
[b] Season: W: winter, Sp: spring, S: summer, F: fall.
[c] Development time (days): from 1st to 4th instar larvae. Zero means development incomplete due to the habitat became dried up before the larvae could reach the fourth-instar stage.

any particular level of S. spiculatus infection as being typical. A percentage of infection of about 50%—this figure considered a high level of parasitism—was found in 14 instances among all the mosquito generations, whereas a low level of parasitism (<50%) was registered in 15 generations.

Significant differences were detected in the mean percentages of infection by S. spiculatus at each site during the entire study period (F value = 7.08; df, 2/34; P = 0.0022). Site B showed the lowest mean rate of infection (9.0 ± 0.07%) (p < 0.05), whereas no significant differences in the rates of infection were found between sites A and C (80 ± 0.25% and 69 ± 0.23%, respectively). The abiotic parameters pH (F = 2.296; df, 1/140; P = 0.1044) and water temperature (F = 2.99; df, 1/140; P = 0.0533) among the sites showed no significant differences. The conductivity did, however, exhibit differences among the pools (F = 16.14; df, 2/9; P = 0.0011), with significantly lower values being registered at sites B and C compared to site A (Table 2). The contingency table of immature stages killed by nematode infection—it based on the assumption that each infected larva would die—showed significant differences among the three breeding sites (Chi square = 579.71; df 2; P < 0.00001). The highest value for mortality in Oc. albifasciatus larvae was reached at Site A, where 1271 fourth-instar larvae from all the generations (80.7%; n = 1575) were hypothetically killed by nematode infection, compared to Site C, where 290 larvae could have been killed (41.9%; n = 693). The lowest larval mortality was accordingly calculated for Site B (4 dead larvae; 2.68%; n = 145). Although we had considered the prevalence of infection as equivalent to mortality, nevertheless infected female and male adult mosquitos (previously collected as pupae) were recorded in 5 generations from two different sampling sites, once each year, at a prevalence ranging from 3.3 to 25%, with most (80%) being found in coincidence with a high level of parasitism at the larval stage (Table 3).

The ratio of S. spiculatus males/females that emerged in each mosquito generation was positively correlated with the mean number of emerged nematodes per larva ($R = 0.73$; $R^2 = 0.53$; $P < 0.00001$; $n = 47$) as well as with the prevalence of infection ($R = 0.57$; $R^2 = 0.33$; $P < 0.00001$, $n = 47$). A significant correlation was likewise detected between the mean number of emerged nematodes per larva and the prevalence of infection ($R = 0.84$, $R^2 = 0.71$; $P < 0.00001$, $n = 47$) upon consideration of all the mosquito generations from the three larval sites (Table 1).

3.2. Effects of abiotic and biotic parameters on the levels of infection

We considered sites A and C as a separate group (Site A–C) from Site B in order to find associations between parasitism by S. spiculatus and biotic and/or abiotic parameters. Abiotic parameters such as pH and water temperature, weather data, and desiccation periods as well as certain biotic conditions (number of total larvae: from first- to fourth-instar stage, number of pupae per sampling unit per mosquito generation, the development time of Oc. albifasciatus larvae per generation, and the percentage of nematode females in the previous generation) were not statistically associated with the rate of infection by S. spiculatus, as demonstrated by both simple regressions and the correlation coefficients (Table 4). The area of the flooded surface (Fig. 1) was, however, significantly correlated between sites A-C and B although with poor fit between the data and the respective regression equations ($y = 33.8 + 0.04x$, $r = 0.40$, $P = 0.05$; $y = -0.58 + 0.08x$, $r = 0.56$, $P = 0.046$) (Table 4).

4. Discussion

The three pools under study were transient habitats subject to periodic flooding from local rainfall and as such constituted typical breeding sites of immature Oc. albifasciatus. Other mosquito

Table 2
Abiotic parameters at the three natural sites of *Ochlerotarus albifasciatus*, the natural host of *Strelkovimermis spiculatus*. Means followed by different letters were significantly different.

Sites	pH	Water temperature (°C)	Conductivity (μmhos/cm)
A	7.1 ± 0.4[*] ($n = 52$)[a]	19.7 °C ± 5.8 ($n = 52$)[a]	480.0 μmhos/cm ($n = 4$)[a]
B	6.8 ± 0.2 ($n = 30$)[a]	18.1 °C ± 5.1 ($n = 30$)[a]	240.5 μmhos/cm ($n = 4$)[b]
C	7.0 ± 0.2 ($n = 28$)[a]	19.1 °C ± 5.8 ($n = 28$)[a]	260.5 μmhos/cm ($n = 4$)[b]

[*] Mean ± standard deviation.

Table 3
Infection by *Strelkovimermis spiculatus* of mosquito adults previously collected as pupae.

Site	Date	Parasitism in larval stage %	Infected adult female %	Infected adult male %
Site A	July 08	97.70	16.60 (6)	3.30 (30)[1]
	August 09	91.60	25.00 (4)	0.00
	January 10	67.10	6.25 (16)	0.00
Site C	October 09	83.30	0.00	25 (4)
	February 10	11.10	0.00	4.70 (21)

[1] Number of *Oc. albifasciatus* adults examined.

Table 4
Effects of abiotic and biotic parameters on levels of infection by *S. spiculatus* evaluated by simple linear regression and correlation analysis.

Parameter	Sites	df	F	R	R²	P
Flooded area (m²)	A–C	1; 22	4.26	0.402	0.162	0.050
	B	1; 11	5.03	0.560	0.314	0.046
Ratio of *S. spiculatus* females from previous Generation (%)	A–C	1; 22	1.78	0.273	0.075	0.196
	B	1; 11	3.38	0.510	0.261	0.074
Development time of *Oc. albifasciatus* generations	A–C	1;22	0.98	0.206	0.042	0.333
	B	1;11	2.20	0.408	0.167	0.166
Number of larvae per generation per sampling unit	A–C	1; 22	0.20	0.095	0.009	0.657
	B	1; 11	0.75	−0.253	0.064	0.404
Number of pupae per generation per sampling unit	A–C	1; 22	3.02	−0.347	0.120	0.096
	B	1; 11	0.03	−0.053	0.0003	0.863
Maximum temperature (°C)	A–C	1; 22	2.55	−0.322	0.103	0.124
	B	1; 11	0.33	−0.169	0.028	0.580
Minimum temperature (°C)	A–C	1; 22	2.38	−0.312	0.097	0.137
	B	1; 11	3.34	−0.482	0.233	0.095
Rainfall (mm)	A–C	1; 22	2.99	−0.346	0.119	0.097
	B	1; 11	0.37	−0.180	0.003	0.555
Accumulated rainfall (mm)	A–C	1; 22	0.16	−0.085	0.007	0.690
	B	1; 11	1.46	−0.342	0.117	0.252
Dry periods (days)	A–C	1; 22	0.33	0.121	0.014	0.572
	B	1; 11	2.21	0.408	0.167	0.165
Water pH	A–C	1; 16	1.18	0.262	0.069	0.293
	B	1; 7	0.08	−0.104	0.011	0.789
Water temperature (°C)	A–C	1; 16	1.52	−0.295	0.087	0.235
	B	1; 7	0.10	−0.117	0.014	0.764

species were also registered at low densities, and the nematode was sporadically observed in their natural populations at an enzootic level. Unlike that circumstance, parasitism by *S. spiculatus* in *Oc. albifasciatus* occurred throughout all seasons with differing patterns in the infection of this mosquito population by the nematode depending on the larval habitat. Within this scenario, the wide host range of *S. spiculatus* for mosquito species that had been previously reported (Becnel and Johnson, 1998; Achinelly et al., 2004; Maciá et al., 1995; Micieli and García, 1999; Campos and Sy, 2003; Rodríguez-Rodriguez et al., 2003) can be beneficial for the persistence of this parasite in nature. Pathogens with efficient transmissions can increase their epidemic outbreaks through the possibility of having multiple hosts (Dobson, 2004).

Ecological studies on the lethal capacities of the natural enemies of insects have frequently demonstrated that the mortality of a given host is dependent on its density, although this effect of density dependence must be defined within the dimensions of both space and time (Fuxa and Tanada, 1987). In our study, the absence of a relationship between the percentage of hosts infected and the total number of immature stages (larvae and pupae) per sampling unit per generation would suggest that this parasite can act independently of host density. One parameter possibly involved in the low level of parasitism by *S. spiculatus* registered in habitat C, however, could be related to a significantly low total number of larvae per sampling unit of *Oc. albifasciatus* present at that site. On the basis of this single observation, however, we

cannot conclude whether or not the mortality caused by this parasite is dependent on host density.

The seasonality in the parasitism by *S. spiculatus* may be related to the existence of an optimal season for the populations of the host *Oc. albifasciatus* rather than a period maximizing either the presence or the infectivity of the parasite. Maciá et al. (1995) observed that in the temperate climate of the Buenos Aires province, Argentina, *Oc. albifasciatus* occurs from the spring through the fall with even occasional hatches in the winter. Indeed, in this study generations of mosquito larvae were recorded during all four seasons. The parasitism of *S. spiculatus* in *Oc. albifasciatus* has been observed to be seasonal, with peaks restricted to spring and fall (Maciá et al., 1995; Micieli and García, 1999). Campos and Sy (2003) reported parasitism by *S. spiculatus* in *Oc. abifasciatus* generations to have developed in fall, summer, and spring. In this study, we observed *S. spiculatus* in mosquito generations throughout the four seasons.

In order to understand the development of this disease, data on the parasite, the host, and the environment were included in the analyses. In the *S. spiculatus*–*Oc. albifasciatus* system, we were unable to find a correlation between the levels of *S. spiculatus* infection and any climatic parameters or other abiotic variables analyzed, such as the pH and the temperature of the water. The pHs registered during this study in all three pools ranged from 6.8 to 7.1, values considered as optimal for *S. spiculatus* under laboratory conditions (Camino and García, 1991). Brown and Platzer (1978), however, had reported that parasitism by *Romanomermis culicivorax* occurred over a pH range of 3.6 to 8.6. The effects of temperature on parasitism have been well documented for a number of mermithid species. Brown and Platzer (1977) noted parasitism by *R. culicivorax* in the laboratory at 12 °C; whereas Galloway and Brust (1977) observed only limited parasitism in field releases at 10 °C. Our results suggested that water temperature has no affect on the infectivity of the J2 stage of *S. spiculatus* under field conditions since we recorded parasitism at temperatures from 9 °C to 28 °C. Accordingly, Achinelly and García (2003), measuring the effect of temperature (from 4 °C to 27 °C) on the longevity and infectivity of preparasites of *S. spiculatus* in the laboratory, found that although longevity decreased at higher temperatures, the infectivity exceeded 70% at each temperature. The area of the flooded surface giving rise to the larval habitats was the only independent variable significantly associated with the prevalence of mosquito-generation infection. The water flooding the breeding sites of *Oc. albifasciatus* arises mostly from rain. The correlation between the area of the flooded surface and the prevalence of *Oc. albifasciatus* infection could be related to an activation of nematode juvenile stages quiescent in the sediment and/or the hatching of nematode eggs that could remain unhatched but viable for several months. The number of days registered for the dry periods between mosquito generations was hypothesized to be a condition that could impact upon the viability of the eggs present in the substratum of the mosquito pools. Nonetheless, no association between those two variables was found. The periods of dessication in the larval habitats in between mosquito generations registered during this study reached a maximum of 126 days. Laboratory research on the hatching patterns of batches of *S. spiculatus* eggs maintained in damp sand under different experimental conditions have shown that eggs have the potential to remain viable at even 120 days after oviposition (Micieli, data not shown). We therefore concluded that, even when a habitat appears to have dried out, sufficient moisture must remain to maintain the parasite populations until the time when the area once again becomes flooded, with the result that such ostensible desiccation does not become a serious limiting condition for *S. spiculatus* survival.

The sex of mermithid nematodes emerging from parasitosis is influenced by the diet; the level of infection; and the sex, species, and size of the host (Petersen, 1972). High levels of infection caused by certain mermithid parasites can produce multiple parasitism so as to increase the number of males and potentially reduce the production of J2 stages (Petersen, 1972; Camino, 1988; Camino and Reboredo, 1994), as confirmed by several laboratory assays. Thus, in laboratory trials with *S. spiculatus*, Camino (1988) obtained a sex ratio of 1:1 when the number of postparasites emerged per mosquito larva was between 1 and 5. When this number was increased to 6 or more, the fraction of male nematodes became progressively greater until only males were observed at 10 postparasites per larva. In *R. culicivorax*, when the average number of emerged nematodes exceeded 3, the male-to-female ratio increased rapidly (Petersen, 1972). In spite of these observations, no data are available about this relationship in the field. In these studies, we have shown in this instance, under field conditions, that a positive correlation exists among the number of emerged nematodes per host, the mean proportion of *S. spiculatus* males/females, and the prevalence of infection in each parasitized mosquito generation.

We observed *S. spiculatus* infections in all of the sampled mosquito habitats. Nevertheless, the spatial distribution of the parasite within a given area containing breeding habitats for this mosquito is still unknown. Since parasitized *Oc. albifasciatus* adults have the capacity to disperse *S. spiculatus* nematodes (Camino and Reboredo, 1994), this property will necessarily exert a critical influence on the spatial distribution of the disease. Accordingly, at different levels of parasitism, we found infections by *S. spiculatus* in both female and male adult mosquitoes—previously collected as pupae once each year from two different sampling sites. Campos and Sy (2003) also reported both sexes of adult *Oc. albifasciatus* parasitized by *S. spiculatus* in generations with high and low parasitism and observed that uninfected and infected *Oc. albifasciatus* adults have the same potential to migrate to new pools.

The range of disease prevalence can have very different impacts on the host populations. Because other natural enemies—such as *A. albifasciati* and certain fungi (Maciá et al., 1995; Micieli et al., 2001)—as well abiotic conditions affect *Oc. albifasciatus* generations (Maciá et al., 1995; Fontanarrosa et al., 2000; García and Micieli, 2000; Gleiser et al., 2000), the impact of *S. spiculatus* parasitism *per se* is difficult to assess. Campos and Sy (2003) determined the mortality attributable to *S. spiculatus* under field conditions, estimated a value for its killing power (K) using life tables, and registered the maximum K value when the highest level of parasitism was reached in the mosquito generation. We assume that each larva infected by this nematode is likely to die at the fourth-instar stage although a certain percentage of parasitized larvae are, in fact, able to molt to adults before doing so. These authors found that fewer than 2% of the infected larvae molted to adults under field conditions. These infected adults arose from only third- and fouth-instar larvae collected from the field, while no infected first- or second- instar larvae had suvived to the adult stage. Whereas behavioral resistance to the mermithid *R. culicivorax* was reported in the mosquito *Culex quinquefasciatus* under laboratory conditions imposing a selective pressure after 300 generations (Petersen, 1978), no instances of resistance in response to selective pressure over time have been reported in any nematode species (Shelton et al., 2007). The exposure of preparasites to the mosquito late-instar stage as the consequence of a new flooding of the habitat carrying recent hatchings of *S spiculatus* could result in the eventual manifestation of infection in the adult mosquito. Considering the percentage of infection by *S. spiculatus* as the only cause of mortality for *Oc. albifasciatus* generations, we observed three different situations. First, the highest mosquito-larval reduction was observed in the habitat where the nematode infected 100% of the generations. Second, an intermediate situation was associated with the occurrence of a lower percentage of

high-level parasitism, although nematodes were nevertheless registered in 93% of the generations as well. Finally, the lowest mosquito-larval killing was observed in a habitat where 46% of the generations were infected by only a low level of nematode parasitism. Abiotic parameters such as pH and water temperature were equivalent among the sites, so that the conductivity remained the only parameter significantly different among the breeding pools. Although variations in this parameter were found among the sites, no statistical differences were detected in trials under laboratory conditions carried out to evaluate the infectivity of S. spiculatus preparasite stages in Ae. aegypti larvae in samples of the water from the same breeding sites (Achinelly and Micieli, unpublished data). Nevertheless, future laboratory assays aimed at evaluating the possible effect of ions on the infectivity of S. spiculatus in nature—as has already been observed by Becnel et al. (2001) to play a significant role in the infectivity of Culex nigripalpus nucleopoliedrovirus in Cx nigripalpus Theobald and Cx. quinquefasciatus (Say) immature—could provide some useful information. Although we were not able to explain the differences observed in the parasitism levels recorded among the sites, the physical variation among those habitats (e. g., topography, soil type), the host abundances, and also the chemical features of each pond may have been involved. We conclude that both the occurrence of infections over time and the levels of those infections play a critical role in the regulation of mosquito populations by this nematode. Although numerous field trials have demostrated the effectiveness of R. culicivorax and R. iyengari against mosquito larvae after field releases (Petersen et al., 1978; Walker et al., 1985; Santamarina and Perez Pacheco, 1997, 2007; Perez Pacheco et al., 2005, 2009), nothing is known about the effectiveness of augmentative releases of S. spiculatus into already established nematode populations to improve the natural regulation of mosquito larvae by this nematode. Our results would point to S. spiculatus as a suitable candidate for release into floodwater-mosquito breeding sites as an alternative approach to mosquito control by enhancing the natural action of this mermithid in the field.

Acknowledgments

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